THE DIATOM *PSEUDO-NITZSCHIA* (PERAGALLO) IN IRISH WATERS.

Volume 1 (of 2)

A thesis submitted to the National University of Ireland for the degree of Doctor of Philosophy

by

Caroline K. Cusack B.MrSc.

Marine Microbiology Laboratory,
The Martin Ryan Institute,
National University of Ireland,
Galway

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Head of Department

Professor Emer Colleran,
Department of Microbiology,
National University of Ireland, Galway,
Ireland.

Research Supervisors

Doctor John W. Patching & Doctor Robin Raine,
Marine Microbiology Section,
The Martin Ryan Science Institute,
National University of Ireland, Galway,
Ireland.

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OBJECTIVES OF THIS STUDY

1. Determine which *Pseudo-nitzschia* species are present in Irish waters using light microscopy, scanning and transmission electron microscopy.

2. Investigate the hydrographic conditions that relate to the distribution of the genus *Pseudo-nitzschia*.

3. Determine which species are capable of producing domoic acid in unialgal cultures.

4. Compare SSU and ITS1 and part of the 5.8S ribosomal DNA sequences of Irish *Pseudo-nitzschia* isolates with available sequences of *Pseudo-nitzschia* isolates from other geographic regions.

5. Examine the efficacy of existing oligonucleotide probes to aid identification of *Pseudo-nitzschia* strains in Irish waters.

CHAPTER 1

GENERAL INTRODUCTION

This project is concerned specifically with *Pseudo-nitzschia*, a genus of the marine diatoms, class Bacillariophyceae (Haeckel 1878 as cited in Skov et al. 1999). *Pseudo-nitzschia* is cosmopolitan having a worldwide distribution (Cupp 1943; Hasle 1965, 1972, Hasle et al.1996; Villac et al. 1993; Hallegraeff 1994; Fryxell et al. 1997) and has become an attractive choice for research by many scientists in recent years, for the simple reason that some species within the genus can produce a toxin (domoic acid) responsible for Amnesic Shellfish Poisoning in humans.

More than ten classes of unicellular algae contribute to marine phytoplankton. Of these, the diatoms (Bacillariophyceae) are one of the most common, representing a large component of the microalgal flora, with approximately 1,500 planktonic (drifting) diatom species in the sea (Sournia et al. 1991). Diatoms can assume either solitary or colonial forms and typical photosynthetic pigments include chlorophyll a, c, ß–carotene, fucoxanthin, diatoxanthin and diadinoxanthin.

Diatoms characteristically have a rigid cell wall that is chiefly composed of silica (silicon dioxide). It is the symmetry of this heavy siliceous exoskeleton, referred to as the frustule that divides diatoms into the Centrales (Centric diatoms) and the Pennales (pennate diatoms) following Karsten (1928). Pennate diatoms are further separated into two subgroups, the araphid pennate diatoms that possess a sternum, and the raphid pennate diatoms in which both a sternum and raphe[†] are present. *Pseudo-nitzschia* is placed within the pennate diatoms (elongated rod shaped diatoms). As a diatom, the frustule of *Pseudo-nitzschia* consists of two morphologically composite units (opposing surfaces) called valves, each connected by adjoining structures referred to as girdle elements. The older valve with its girdle elements (epitheca) fits over the younger valve (hypotheca), comparable to the way a “pill box” fits together (Figure 1a). Under inspection *Pseudo-nitzschia* can present two views, the vale view or the girdle view, and its symmetry is based around a central line (Figure 1b). The three planes of symmetry of

[†] A glossary of all terms used may be found in Appendix 1.

the *Pseudo-nitzschia* frustule are portrayed in Figure 2.

Figure 1. Frustule structure where a.) is a diagrammatic drawing showing the frustule components in diatoms (after Hasle and Syvertsen 1997, Fig. 4) and b.) is a diagrammatic drawing of *Pseudo-nitzschia* in valve and girdle view (after Hasle and Syvertsen 1997, Fig. 9).

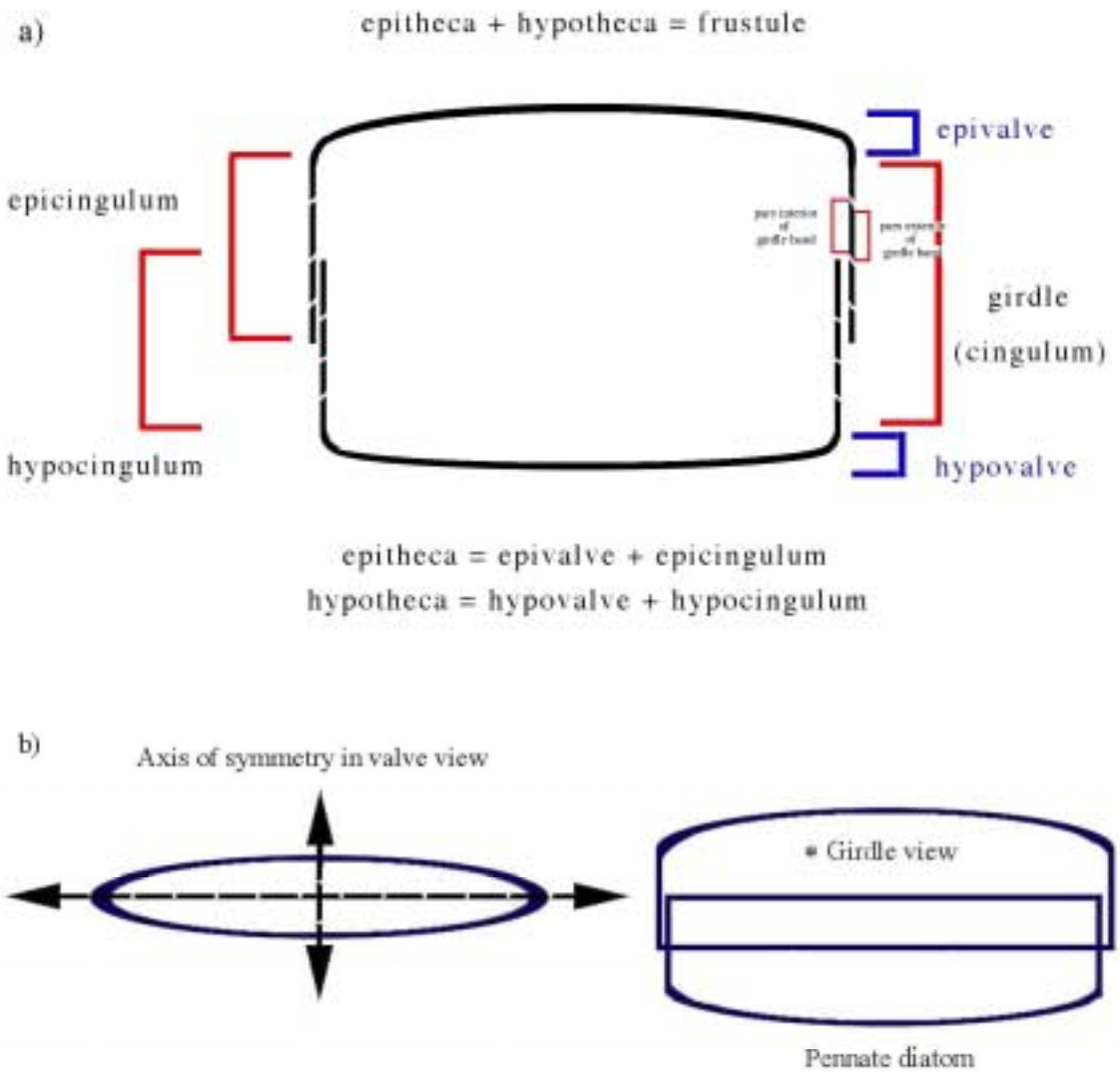
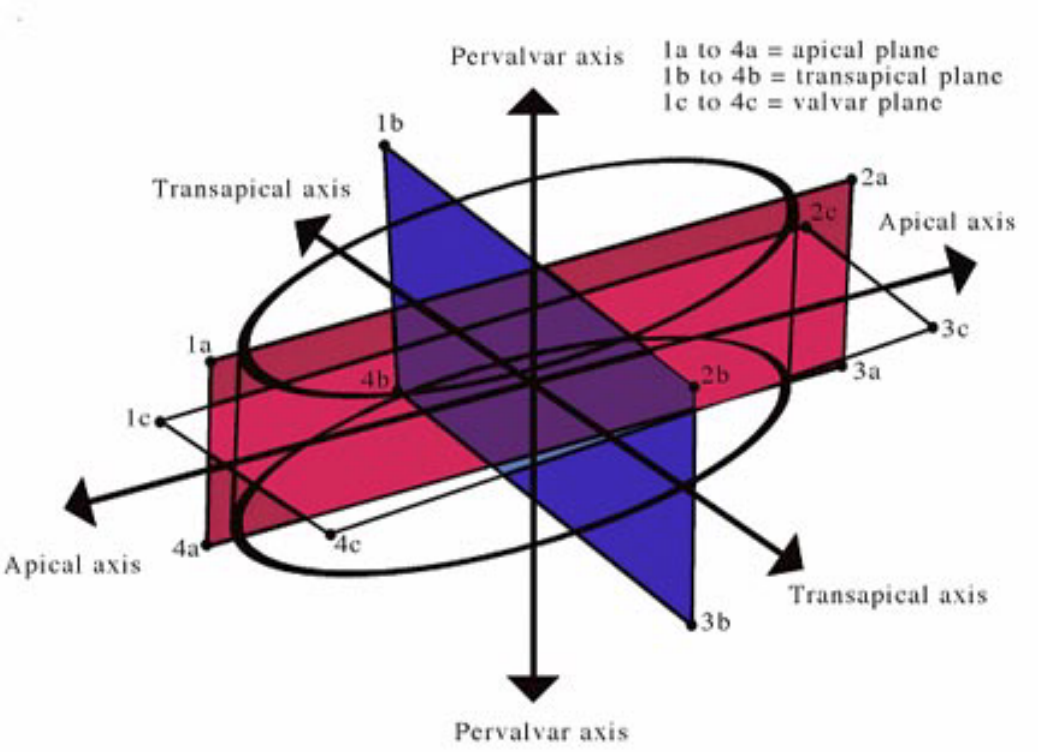


Figure 2. Axis and planes of symmetry of *Pseudo-nitzschia* (after Barber and Haworth 1981, Fig. 3)



1. THE GENUS *PSEUDO-NITZSCHIA*

1.1 NOMENCLATURE

Class: Diatomophyceae (*Rabenhorst 1864) / Bacillariophyceae (*Haeckel 1878)
Order: Bacillariales Hendy 1937 / Pennales *Karsten 1928 (pennates)
Family: Bacillariaceae *Ehrenberg 1831
Genus: *Pseudo-nitzschia* H. Peragallo 1900
Lectotype: *Pseudo-nitzschia seriata* (Cleve) H. Peragallo (in H. and M. Peragallo 1897–1908)
* Cited in Skov et al. (1999)

The nomenclature history of the diatom *Pseudo-nitzschia* begins with the erection of the genus for the planktonic diatoms *Nitzschia seriata* Cleve, *N. fraudulenta* Cleve and *N.*

sicula Castracane (Peragallo, H. and Peragallo, M. 1897-1908). This decision was based on the fact that the cells of at least *N. seriata* and *N. fraudulenta* were associated in long filaments (cellules associées en long filaments, Peragallo, H. and Peragallo, M. 1897-1908, p. 300). *Pseudo-nitzschia* was later reduced to a section of the large genus *Nitzschia* (Hassall) by Hustedt (1958) based on the limited reduction of the raphe, as. some motility of cells must be required to form the step-like colony formation. Finally, Professor Grethe Hasle (1994) clarified its position by reinstating it to the rank of genus after carrying out detailed morphological investigations. Douglas and his colleagues (1994) have supported her decision based on molecular work they carried out on the small subunit rRNA gene of *Pseudo-nitzschia, Nitzschia* and other diatoms.

Today the genus *Pseudo-nitzschia* includes 22 described species, 1 forma and 2 varieties (Tables 1–2).

Table 1. Nomenclature of the "Pseudo-nitzschia seriata" group (valve width 3 µm or more, *sensu* Hasle 1965). *Pseudo-nitzschia* H. Peragallo in Peragallo and Peragallo 1900, emend. Hasle 1993.

	"P. seriata" group	Basionym	Synonym
1a	*P. seriata* (Cleve) H. Peragallo in Peragallo and Peragallo1900	*N. seriata* Cleve 1883	
1b	*P. seriata* f. *obtusa* (Hasle) Hasle 1993	*N. seriata* f. *obtusa* Hasle 1974	
2	*P. australis* Frenguelli 1939		*N. pseudoseriata* Hasle 1965
3a	*P. pungens* (Grunow ex Cleve) Hasle 1993	*N. pungens* Grunow ex Cleve 1897	
3b	*P. pungens* var. *cingulata* Villac and Fryxell 1998		
4	*P. multiseries* (Hasle) Hasle 1995	*N. pungens* f. *multiseries* Hasle 1974	*P. pungens* f. *multiseries* (Hasle) Hasle 1993
5	*P. pungiformis* (Hasle) Hasle 1993	*N. pungiformis* Hasle 1971	
6	*P. fraudulenta* (Cleve) Hasle 1993	*N. fraudulenta* Cleve 1897	*P. seriata* var. *fraudulenta* (Cleve) Peragallo in Peragallo and Peragallo 1900.
7	*P. subfraudulenta* (Hasle) Hasle 1993	*N. subfraudulenta* Hasle 1974	
8	*P. heimii* Manguin 1957	*N. heimii* (Manguin) Hasle 1965	*N. pacifica* Cupp 1943 sensu Hustedt 1958 "nomen confusum"
9	*P. subpacifica* (Hasle) Hasle 1993	*N. subpacifica* Hasle 1974	
10	*P. sinica* nom. Prov.? Qi (1994)		
11	*P. antarctica* Manguin 1957		
12	*P. multistriata* (Takano) Takano 1993	*N. multistriata* Takano 1993	

Table 2. Nomenclature of the "Pseudo-nitzschia delicatissima" group (valve width of 3 µm or less, *sensu* Hasle 1965). *Pseudo-nitzschia* H. Peragallo in Peragallo and Peragallo 1900, emend. Hasle, 1993.

	"P. delicatissima" group	Basionym	Synonym
13	*P. delicatissima* (Cleve) Heiden in Heiden and Kolbe 1928	*N. delicatissima* Cleve 1897	*N. actydrophila* Hasle 1965
14	*P. lineola* (Cleve) Hasle 1993	*N. lineola* Cleve 1897	*N. barkleyi* Hustedt 1952
15	*P. prolongatoides* (Hasle) Hasle 1993	*N. prolongatoides* Hasle 1965	*N. prolongata* Manguin 1957 non *Nitzschia prolongata* Hustedt 1938
16	*P. turgidula* (Hustedt) Hasle 1993	*N. turgidula* Hustedt 1958	
17	*P. turgiduloides* (Hasle) Hasle 1995	*N. turgiduloides* Hasle 1965	*P. barkleyi* var. *obtusa* Manguin 1960
18	*P. cuspidata* (Hasle) Hasle 1993	*N. cuspidata* Hasle 1974	
19	*P. inflatula* (Hasle) Hasle 1993	*N. inflatula* Hasle 1974	
20	*P. pseudodelicatissima* (Hasle) Hasle 1993	*N. pseudodelicatissima* Hasle 1976	*N. delicatula* Hasle 1965 non *N. delicatula* Skvortzow 1946
21a	*P. granii* (Hasle) Hasle 1993	*N. granii* Hasle 1974	
21b	*P. granii* var. *curvata* Hasle 1993	*N. granii* var. *curvata* Hasle 1974	
22	*P. subcurvata* (Hasle) Fryxell in Fryxell et al. 1991	*N. subcurvata* Hasle 1974	

1.2 TAXONOMY

1.2.1 MORPHOLOGY

The genus *Pseudo-nitzschia* is recognised by the unique formation of its colonies, which appear in step-like chains formed by the overlapping of cell ends. Members of this genus are characterised by the 'spindle-shaped', pointed fusiform valves. The arrangement of two chloroplasts per cell, situated on either side of the centrally placed nucleus, is evident in water mounts observed under a light microscope (LM).

Successful identification of *Pseudo-nitzschia* requires examination of the shape and structure of the silica frustule. Conventional brightfield light microscopy can provide a guide in discriminating between *Pseudo-nitzschia* species. It cannot however, reveal all of the morphological structures needed to positively identify *Pseudo-nitzschia* to species level. Therefore this technique must be combined with the use of electron microscopy. Scientific literature on phytoplankton distribution based on LM studies in the past have rarely differentiated between *Pseudo-nitzschia* species.

Pseudo-nitzschia may be assigned to one of two groups (see Figure 3) the "P. seriata" group (Table 1) representing wide cells (valve width 3µ or more) and the "P. delicatissima" group (Table 2), the narrow cells (valve width 3µ or less). In many earlier studies these were listed as “Nitzschia seriata” and “Nitzschia delicatissima” respectively (Hasle 1965). Detailed historical reports of the distribution of *Pseudo-nitzschia* species are thus rare.

The most reliable keys for the identification of *Pseudo-nitzschia* are those of Hasle et al. (1996) and Hasle and Syvertsen (1997).

The morphological features of the diatom *Pseudo-nitzschia* Peragallo visible using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are outlined below. By and large these follow the criteria of Hasle (1965, 1995), Mann (1978), Hasle and Fryxell (1995), Hasle et al. (1996) and Hasle and

Syvertsen (1997). The terminology used here to describe the siliceous structures of the diatom frustule follows Anonymous (1975), Ross et al. (1979) and Barber & Haworth (1981). A table explaining the terminology used can be found in Appendix 1. Appendix 2 describes the *Pseudo-nitzschia* species occurring in Irish waters during the present study. This follows the standardised descriptions of Hasle (1965, 1972, 1995), Hasle & Fryxell (1995), Hasle et al. (1996) and Hasle and Syvertsen (1997). Appendix 3 displays a large number of micrographs taken of *Pseudo-nitzschia* species observed under a light, scanning electron and transmission electron microscopes.

The features of the diatom *Pseudo-nitzschia* that are initially apparent to the observer are firstly the size of the cell in question (large or small) and secondly the shape and symmetry of the cell. Figure 4 shows a simple diagram displaying different shapes seen in *Pseudo-nitzschia*. Morphological features and characteristics of *Pseudo-nitzschia* cells using light and electron microscopy are outlined in Tables 3 – 7 below.

Figure 3. Diagram showing the "P. seriata" and "P. delicatissima" groups along with the terminology used to describe some of the siliceous morphological structures.

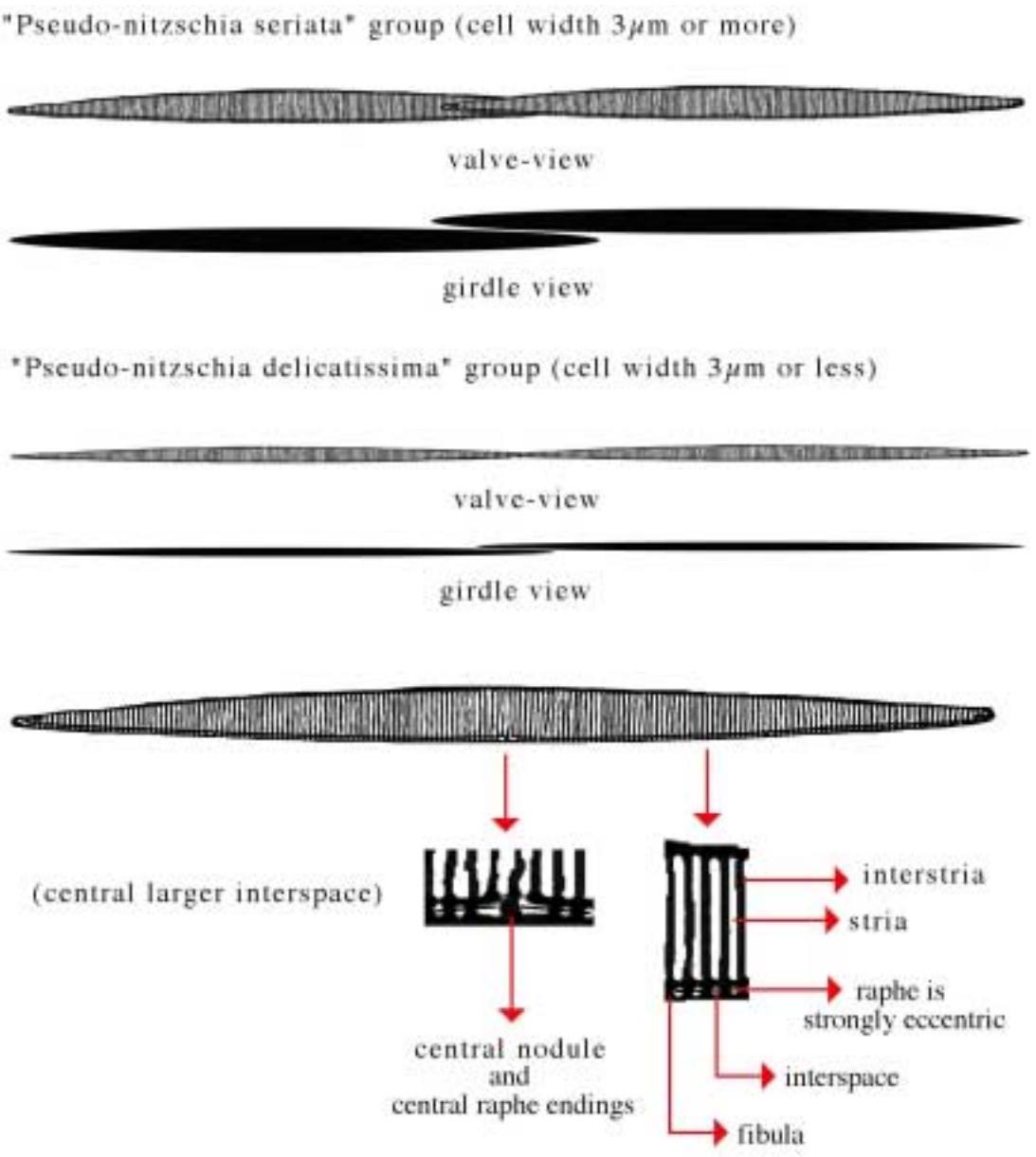
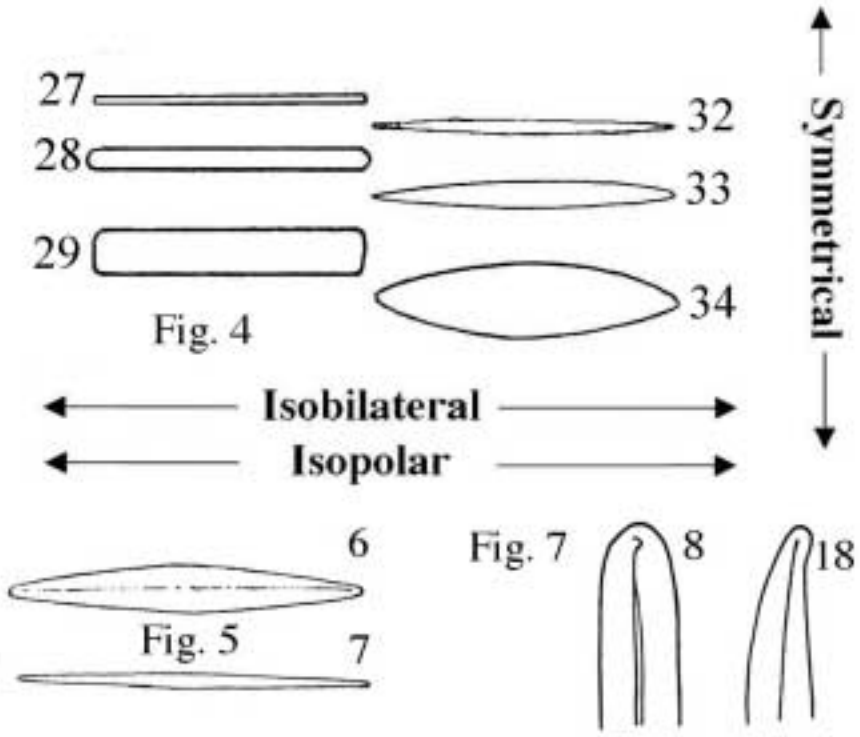


Figure 4. A simple diagram displaying the geometric extensions of the valve and girdle outline, redrawn from Barber & Haworth (1981, Fig. 4; Fig. 5, Fig. 7).

Barber & Haworth (1981, Fig. 4)	Barber & Haworth (1981 Fig. 5)	Barber & Haworth (Fig. 7)
27: Linear	6: Lanceolate, narrow	8: Ends broadly rounded
28: Linear	7: Lanceolate, fusiform	18:Ends rostrate
29: Linear		
32: Acicular (or spindle shaped)		
33: Fusiform		
34: Lanceolate		



Water samples containing *Pseudo-nitzschia* cells are initially counted using the cell size group differentiation based on the width of the valve (Hasle 1965), a drop of sample is then mounted onto a glass slide and further examination of the raw material is carried out. *Pseudo-nitzschia* colonies more often than not appear in the girdle view where the typical generic characteristics are clearly visible, however accurate species identification is not possible for many *Pseudo-nitzschia* species at this stage of the investigation.

Table 3. Distinct features visible in vegetative cells of *Pseudo-nitzschia* in water mounts (phase contrast, light microscopy).

DISTINCT FEATURES OF VEGETATIVE *PSEUDO-NITZSCHIA* CELLS.	
VALVE OUTLINE:	MARGINS
	Curved, straight or one margin curved the other straight.
VALVE SYMMETRY:	BILATERAL SYMMETRY ABOUT THE APICAL PLANE
	Symmetrical with respect to the apical plane
	= transapical axis isopolar.
	Asymmetrical with respect to the apical plane
	= transapical axis heteropolar.
DIMENSIONS:	PROPORTION BETWEEN LENGTH AND WIDTH OF CELL
	Length of the apical (diameter) axis.
	Length of the transapical axis (width).
CHLOROPLAST ARRANGEMENT:	*PSEUDO-NITZSCHIA* CELLS CONTAIN 2 PLATE–LIKE CHROMATOPHORES
	These are positioned symmetrically about the median transapical plane.
COLONY FORMATION:	The "STEP-LIKE" chains characteristic of *Pseudo-nitzschia* colonies can be observed in the girdle view. These colonies are formed by the overlapping of cell ends that attach over a short distance on the valve face. The length of overlap seems to be consistent within each species but differs between species.

Since the organic contents of vegetative cells complicate the image of the valve and its processes, it is imperative that this is removed to allow further morphological analysis of the silica structure. The siliceous elements of the frustule often break apart during the cleaning treatment separating the girdle elements (bands) and the valves. Several cleaning methods exist in the literature and some of these are discussed in Appendix 4. Cleaned specimens are mounted onto glass slides and viewed under the light microscope.

Table 4. Siliceous features that can be observed in watermounts of acid cleaned *Pseudo-nitzschia* valves viewed under a light microscope (oil immersion LM; phase contrast or darkfield illumination).

DISCERNABLE FEATURES OF ACID CLEANED *PSEUDO-NITZSCHIA* VALVES IN WATER MOUNTS	
FIBULAE, INTERSTRIAE AND CENTRAL LARGER INTERSPACE:	Visibility of the FIBULAE, INTERSTRIAE and CENTRAL LARGER INTERSPACE (if present).
THE RAPHE:	In all *Pseudo-nitzschia* species the RAPHE is strongly eccentric (marginal raphe) and is not raised above the general level of the valve (flush with the valve).

Since the organic matter has been removed from the cell, the chloroplast arrangement cannot be seen. Colony formation cannot be observed if the frustule elements have come apart. Some *Pseudo-nitzschia* species have a more coarsely structured valve then others. Consequently, resolution of finite structures such as the striae and fibulae are more discernible in the robust valves then in the more delicately formed ones when examined in watermounts. However, it is always much easier for the observer to view the heavily silicified structures when the diatom material is embedded in a permanent mount. In cleaned water mounts there is very little contrast between the diatom and it's surrounding medium to allow recognition of structural detail. This is because the refractive index (RI) of the silica wall (~1.15) is similar to that of water (~1.3). To resolve this and increase the contrast the specimens are embedded in a medium of a higher refractive index (such as Naphrax, RI = 1.72).

Table 5. Additional siliceous structures that can be observed in acid cleaned *Pseudo-nitzschia* valves mounted in a medium of high refractive index and viewed under a light microscope (oil immersion LM).

ADDITIONAL FEATURES OF ACID CLEANED VALVES THAT BECOME VISIBLE WHEN THE *PSEUDO-NITZSCHIA* SPECIMENS ARE EMBEDDED IN A MEDIUM OF HIGH REFRACTIVE INDEX (OIL IMMERSION LM)	
FIBULAE AND INTERSTRIAE:	The LINEAR DENSITY of the interstriae and fibulae (theses structures may not be visible in all *Pseudo-nitzschia* species examined under these conditions).
POROIDS:	SHAPE AND NUMBER OF ROWS OF POROIDS PER STRIA / striae structure. This is only seen in *Pseudo-nitzschia pungens* where the two rows of poroids of the striae are sometimes visible. This is one of the few *Pseudo-nitzschia* species that can be clearly identified in the valve view under a LM.
GIRDLE STRUCTURE:	The CINGULUM has a varied number of perforated bands (ribbed), generally three with some unperforated bands. The girdle bands are open, one apices of a band can be seen to form a loop while at the other apices the band splits to form two pointed ends. The bands are thicker in the midpoint of the cell and they progressively taper towards the poles (Figure 5a).

Table 6. Morphological structures required for species identification that may not be resolved or are difficult to discern during light microscopy investigations.

FEATURES OF ACID CLEANED VALVES THAT MAY OR MAY NOT BE DISCERNABLE WHEN THE *PSEUDO-NITZSCHIA* SPECIMENS ARE EMBEDDED IN A MEDIUM OF HIGH REFRACTIVE INDEX (OIL IMMERSION LM)	
FIBULAE AND INTERSTRIAE:	The LINEAR DENSITY of the interstriae and fibulae (discernible in some species).
STRIAE STRUCTURE:	STRIAE STRUCTURE of the valve face and the girdle bands.
CENTRAL LARGER INTERSPACE:	GREATER DISTANCE (IN STRIAE) BETWEEN THE TWO MEDIAN FIBULAE, the central nodule and central raphe endings when present.

When identifying *Pseudo-nitzschia* to species level, electron microscopy provides additional information to the structures distinguished under the LM. The taxonomic features (ornamentation of the valve) that are difficult or impossible to discern using a LM can be observed and described in detail using an electron microscope. A distinction can be made between species with similar valve outlines such as *P. pungens* and *P. multiseries*, *P. delicatissima* and *P. pseudodelicatissima* or *P. seriata* and *P. australis* (smaller forms). However, valve outlines should be examined as this feature helps to discriminate between species with matching characteristics such as the striae structure found in *P. multiseries* and *P. seriata*. Morphometric numerical data (e.g. number of fibulae or striae in 10µm) can often be of significant value when comparing between species that have similar morphological features (*P. subpacifica* and *P. heimii*). Electron microscopy[†] is essential as an additional tool to LM to identify *Pseudo-nitzschia* to species.

[†] SEM gives a 3d image (shows the curvature of the valve). TEM gives a 2d image (valve looks flattened) with high resolution.

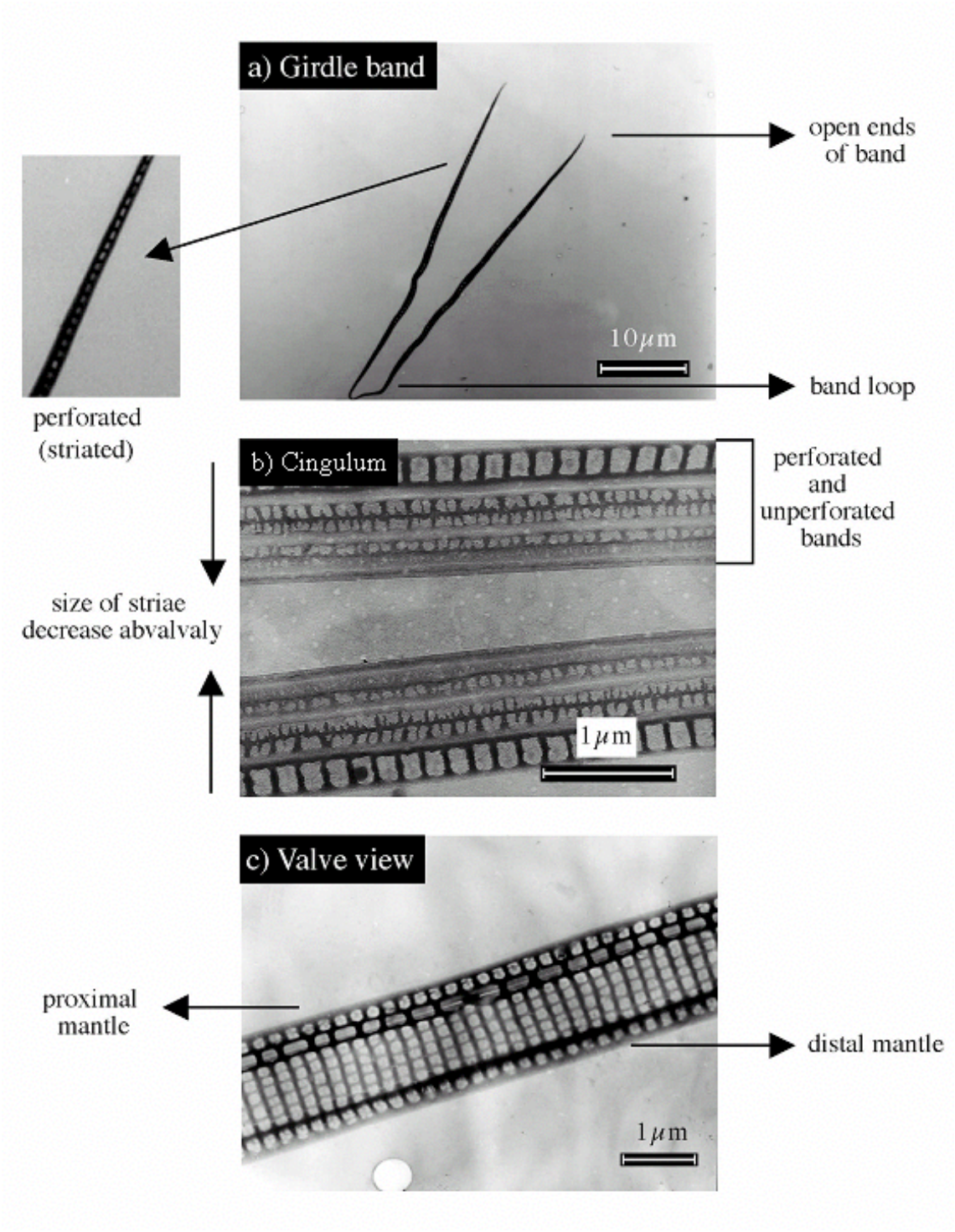
Table 7. Features of acid cleaned valves and girdle bands that are clearly visible when observed under an Electron Microscope (SEM and TEM). Ultrastructural study of the diatom frustule.

VISIBLE FEATURES OF ACID CLEANED FRUSTULES WHEN VIEWED UNDER AN ELECTRON MICROSCOPE	
FIBULAE:	DENSITY OF FIBULAE in 10µm.
INTERSTRIAE:	DENSITY OF STRIAE in 10µm. The exterior valve face is smooth, the striae are therefore level with the interstriae on the valve exterior in all *Pseudo-nitzschia* species.
STRIAE STRUCTURE:	STRUCTURE OF THE STRIAE (axial and central area), NUMBER OF ROWS OF POROIDS PER STRIAE. Vela are close to the valve exterior in all *Pseudo-nitzschia* species.
AREOLATION:	SHAPE of the areolae/poroids. LINEAR DENSITY OF POROIDS in 1µm, measured transapically at the center region of the valve face (across the width of the valve).
CENTRAL RAPHE ENDINGS:	PRESENCE OR ABSENCE of central raphe endings. In some *Pseudo-nitzschia* species the raphe consists of an unbroken longitudinal slit extending the length of the valve. While in other species the raphe is made up of two longitudinal slits. At the mid–region of the valve this "broken" raphe produces two central raphe endings, one on either side of the central nodule (together contained in the central larger interspace). The raphe is not raised above the general level of the valve and the raphe canal walls do not exhibit poroids in any *Pseudo-nitzschia* species.
CENTRAL LARGER INTERSPACE:	NUMBER OF STRIAE per central larger interspace.
GIRDLE BANDS:	NUMBER OF STRIATED GIRDLE BANDS in a fully developed cingulm. The MORPHOLOGICAL STRUCTURE (perforation/striation) of girdle bands. The striae structure decreases in size abvalvarly see Figure 5(b).
MANTLE STRUCTURE:	Figure 5(c). The distal mantle is similar to the valve face in striae structure and alignment and there is a strip of nonperforated silica between this structure and the valve face in all *Pseudo-nitzschia* species. The proximal mantle is nearly always similar to the valve face in striae structure and can be aligned with the fibulae, the interspaces or the valve

Electron photomicrographs of specimens are used to record morphological measurements such as the number of fibulae in 10µm. Errors can arise if acid cleaning does not remove all the organic matter thereby obscuring valve structures, specimens are fragmented or an aberrant form of a species is present. The main disadvantage resulting from electron microscopy studies is that quantitative methods are not applicable.

Pseudo-nitzschia cultures can at times exhibit unusual growth tendencies. The cell structure often undergoes morphological changes during the vegetative growth stage resulting in the formation of aberrant cells displaying unusual characteristics such as swellings along the valve margin ("lobed" cells) or an undulation of the frustule. The resulting modifications to the silica frustule structure become a permanent trait in some clones (Fryxell unpublished, Subba Rao and Wohlgeschaffen 1990, Garrison et al. 1992, pers. obser.). This type of observation is common in diatom cultures and may be a result of chance plasmolysis as suggested by Round et al (1990) or perhaps because of the malformation of delicately silicified cells during cell division (Garrison et al. 1992). Hasle (1965, Plate 2, Fig. 9) shows an example of lobed valve margins in a sickly culture of *P. cuspidata*. The formation of aberrant cells is not however selective to a particular species, anomalous cells of *P. multiseries* (Subba Rao and Wohlgeschaffen 1990), *P. pungens* (Takano and Kikuchi 1985) and *P. australis* (Garrison et al. 1992, pers. obser.) have also been documented. Deformities are not unique to *Pseudo-nitzschia* cultures, they have also been observed in natural populations of *Pseudo-nitzschia* in oligotrophic waters and during bloom conditions (Takano and Kikuchi 1985, Subba Rao and Wohlgeschaffen 1990). Although, such anomalies in the structure of the silica frustule may add to difficulties in the identification of affected *Pseudo-nitzschia* cells, verification of the species identity should not be completely retarded if other typical and distinct morphological features are persevered (Hasle and Syvertsen 1997).

Figure 5. Diagram showing morphological features of girdle bands (a and b) and the valve mantles (c).



1.2.2 MOLECULAR BIOLOGY

For centuries the taxonomy and systematics of diatoms have relied heavily on morphological criteria. However, comparative morphological studies can, at times, be difficult, particularly when strains or ecotypes of a particular species differ only in size range and other minor morphological details from the designated "type strain"[†]. The classification and phylogeny (evolutionary history and relationships) of diatoms have been based on comparisons of shared morphological characters of fossil and extant species together with life cycle experiments, biochemical research and cytological studies (Sorhannus et al. 1995).

Recent advances in molecular biology (Polymerase Chain Reaction (PCR), cloning, sequencing etc.) have complimented the classical techniques used in the study of diatoms including *Pseudo-nitzschia* (Manhart et al. 1995, Douglas et al. 1994, Scholin et al 1994).

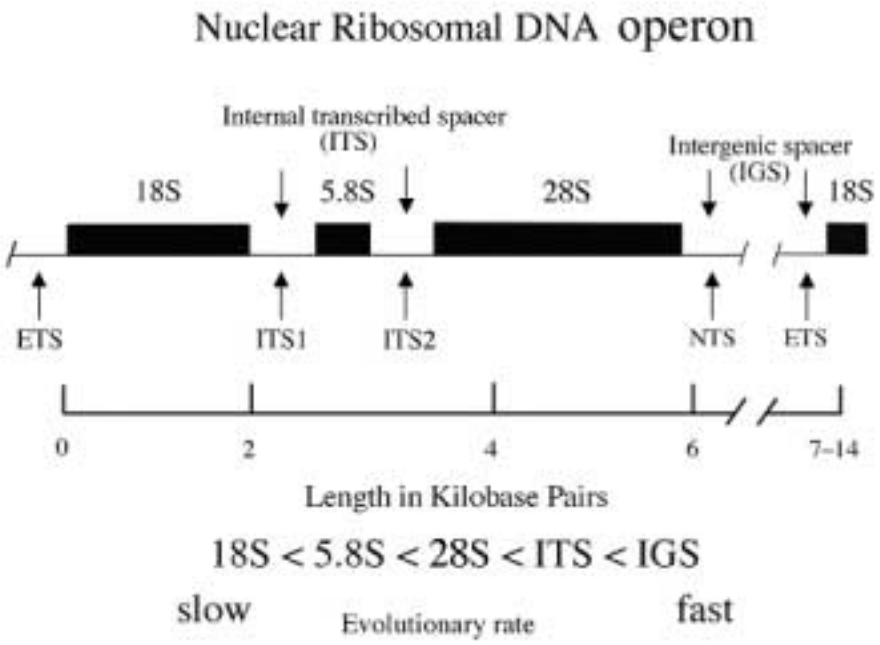
The molecular approach involves the study of genetic information, stored in biological macromolecules, nucleic acids and proteins, to understand the systematics and evolutionary history of organisms. Ribosomal DNA (rDNA) is a molecule that scientists working on *Pseudo-nitzschia* have focused their attention on to determine phylogenetic relationship between its species and with other diatoms (Douglas et al. 1994, Scholin et al 1994). Ribosomal DNA encodes for ribosomal RNA (rRNA) and so it plays an important role in the production of proteins essential for the structure, function and regulation of the cells, tissues, and organs in all living organisms (Woese 1987). One of the main reasons this molecule is used in phylogentic studies is because large databases (e.g. Ribosomal Database Project) exist that contain thousands of rDNA sequences for multiple organisms and these can be used to compare results. Ribosomal DNA is also present in the nucleus of all living things and organelles. The molecule has functional consistency and certain regions of the genes are universally conserved while

[†] "Type strain" or "Reference strain": This is usually the first strain of a species to be studied. The specimen is well characterised and deposited in an international publicly accessible collection.

other domains are highly variable and unique to a given species which in principle makes it suitable for analyses of evolutionary relationships (Gerbi 1985). Ribosomal DNA nucleotide sequences are also sufficiently long enough to provide statistically meaningful comparisons between organisms (Woese 1987).

In diatoms the rDNA operon (Figure 7) typically contains an external transcribed spacer (ETS), the 18S small subunit (SSU) rDNA, internal transcribed spacers (ITS1 and ITS2) found on either side of the 5.8S rDNA. The large subunit (LSU) rDNA contains two moieties, the 28S rDNA and the 5.8S rDNA (Gerbi 1985).

Figure 7. Schematic layout of the nuclear ribosomal genes (rDNA) based on Gerbi (1985). The rDNA operon includes the 18S, 5.8S and 28S ribosomal genes. These are separated by the internal transcribed spacers (ITS1 and ITS2). The rDNA tandem repeats are separated by the intergenic spacer (IGS) that is composed of a non–transcribed spacer (NTS) and an external transcribed space (ETS).



Studies on *Pseudo-nitzschia* have used rDNA nucleotide sequences to distinguish between morphologically similar species such as *P. multiseries* and *P. pungens*

(Manhart et al. 1995, Douglas et al. 1994, Scholin et al. 1994) by using PCR and restriction fragment length polymorphism (RFLP).

Phylogenetic analysis of sequences can be carried out using a variety of different methods (See Appendix 5 for background information on methods relating to this project). Scholin et al. (1994) and Douglas et al. (1994) have used distance and/or parsimony based methods to discern the genetic relatedness of *Pseudo-nitzschia* species with each other and with other diatoms including *Nitzschia* species on the basis of SSU and LSU rDNA sequences. These results have reinforced morphological data that separated the genus *Pseudo-nitzschia* from *Nitzschia* (Douglas et al. 1994).

The work described above has led to the development of fluorescently labelled oligonucleotide probes designed from variable regions of the LSU rDNA to discriminate between *Pseudo-nitzschia* species (Miller and Scholin 1996). Oligonucleotide probes are short nucleic acid strands designed to anneal to a complementing unique sequence in a target organism. A label (e.g. fluorescent, radioactive tracer molecules) is attached to the probe to allow for its detection. The development and application of *in situ* methods using rRNA targeted nucleic acid probes have been used successfully in the past to discriminate between bacterial populations in the wild (see reviews by Amann and Kühl 1998, Amann and Ludwig 2000). Such methods offer an alternative research tool for studies of harmful algae. For example Scholin (1998) describes in detail how to develop and apply probe technology for use in monitoring harmful microalgae including *Pseudo-nitzschia*. Many of these techniques are still at the fine-tuning stages of their development but within the next decade or so, some, at least, will become be an integrated part of monitoring programs for harmful algae. Ribosomal RNA targeted species-specific gene probes for *Pseudo-nitzschia* designed by Chris Scholin and his co-workers have proven to be very useful in the harmful phytoplankton surveillance programme operating in New Zealand (Rhodes et al. 1998). Scholin et al. are currently carrying out field trials using a novel tool called the Environmental Sample Processor (ESP) designed to process environmental samples *in situ*. The prototype can process and analyse water samples using species-specific molecular probes in addition to collecting and preserving phytoplankton samples for examination

using more conventional methods such as light and electron microscopy (Chris Scholin pers. commun.). The system can also collect and process other environmental data and samples (e.g. toxins) required for a particular study.

When using probes it should be remembered that small differences within *Pseudo-nitzschia* species are possible at the nucleotide sequence level and that these variations may not be apparent when identifying the species using morphological methods (Scholin et al. 1994). It is also possible that the nucleotide sequences of other marine aquatic microalgae that have not yet been documented may have a similar genetic code to the probes. This may lead to unsuccessful applications of the species-specific oligonucleotide probes or unwanted cross reactions with non-target organisms. Species-specific probes must be tested against a number of cultures of microalgae including different clones of the target species, preferably from different biogeographic regions, and fine tuned to eliminate any cross reactivity that may be encountered. Field testing is also essential to determine if cross reactivity exists between the probe and non-target organisms in the wild.

1.3 *PSEUDO-NITZSCHIA* GLOBAL DISTRIBUTION

Since phytoplankton play a significant role in marine ecosystems, accurate identification of individual species is therefore important for the various types of studies carried out. Such investigations can range from the study of the structure and distribution of phytoplankton communities in time and space, through to toxic algal monitoring programmes.

In the past there have been relatively few publications with detailed speciation of the diatom *Pseudo-nitzschia*. However, this is rapidly changing with the discovery that some *Pseudo-nitzschia* species can produce the toxin domoic acid. Its widespread distribution threatens the aquaculture industry worldwide. Along with the intensive studies of Grethe Hasle on this genus, Greta Fryxell, Celia Villac and many others have

contributed largely to the increasing records on the global distribution of *Pseudo-nitzschia* species (Table 8).

Information on the distribution of *P. delicatissima, P. fraudulenta, P. heimii* (particularly abundant in the sub Antarctic), *P. inflatula, P. lineola, P. multiseries, P. pseudodelicatissima,* P. *pungens* and *P. turgidula* from the literature demonstrate that these species are relatively cosmopolitan. However, *P. delicatissima* is a species that appears to show a variety of morphotypes and it is expected that a taxonomic revision of this species will be carried out presently (Nina Lundholm pers. commun.). When this is completed, it is highly likely that the geographic distribution of this species will be altered slightly. Southern cold water species include *P. prolongatoides, P. subcurvata* and *P. turgiduloides* while *P. seriata* f. *obtusa* is a more northern cold water species (Hasle and Syvertsen 1997). The record of *P. seriata* tentatively identified from Chilean waters (South east Pacific) by Rivera et al. (1985) needs to be confirmed. It is likely that the specimens of *P. seriata* forma *obtusa* observed were *P. australis*. *Pseudo-nitzschia pungiformis* thus far can be found in warm waters, Fryxell et al. (1997) reported this species in warm to temperate waters off the west coast of America. At present, information on the distribution of *P. multistriata* is relatively scarce and is restricted to Chinese waters. This is because *P. multistriata* has only been recently defined (Takano 1993).

Pseudo-nitzschia australis (recently recorded from waters in the North Pacific and North Atlantic), *P. subfraudulenta, P. subpacifica,* and *P. cuspidata* show a warm to temperate water distribution (Hasle and Syvertsen 1997). Records of *P. pungens var.* cingulata (warm to temperate waters) are limited to the West coast of the United States (Fryxell et al. 1997), but reports will probably increase as more detailed studies using electron microscopy are carried out worldwide. Finally, *P. seriata* f. seriata and *P. granii* seem to be more northern cold to temperate water species (Hasle and Syvertsen 1997).

Table 8. Global distribution of species from the diatom *Pseudo-nitzschia*.

(? = tentative identification)

DISTRIBUTION	SPECIES	REFERENCE
North America (east coast)		
Canada (Bay of Fundy, New Brunswick; Prince Edward island)	*P. delicatissima*?; *P. multiseries*; *P. pungens*; *P. pseudodelicatissima*	Bates et al. 1989; Martin et al. 1990; Smith et al. 1990
Greenland	*P. delicatissima*	Skov et al.1999
North America (west coast)		
Canada (British Columbia)	*P. australis*; *P. multiseries*; *P. pungens*; *P. seriata f. seriata*	Bates et al. 1989; Forbes & Denman 1991; Taylor & Haigh 1996
USA (California)	*P. australis*; *P. cuspidata*; *P. delicatissima*; *P. fraudulenta*; *P. heimii*; *P. inflatula*?; *P. lineola*; *P. multiseries*; *P. pungens*; *P. pungens var. cingulata*; *P. pungiformis*?; *P. pseudodelicatissima*; *P. seriata f. seriata*?; *P. subfraudulenta*; *P. subpacifica*;	Fryxell et al. 1997
USA (Oregon)	*P. australis*; *P. fraudulenta*; *P. heimii*; *P. pungens*; *P. pungens var. cingulata*; *P. multiseries*; *P. pseudodelicatissima*	Fryxell et al. 1997
USA (Washington)	*P. australis*; *P. pungens*; *P. multiseries*; *P. pseudodelicatissima*;	Fryxell et al. 1997
Central America		
Gulf of Mexico	*P. pseudodelicatissima*	Dortch et al. 1997
Mexico (Pacific)	*P. pungens*	Hernández–Becerril 1998
South America		
Argentina (Atlantic)	*P. australis*; *P. multiseries*; *P. pungens*; *P. pseudodelicatissima*	Hasle 1965; Ferrario et al. 1999
Brazil (Atlantic)	*P. delicatissima*; *P. heimii*; *P. multiseries*?; *P. pungens*; *P. pseudodelicatissima*; *P. subfraudulenta*?	Villac & Tenenbaum 2000
Chile (Pacific)	*P. cuspidata*; *P. delicatissima*; *P. fraudulenta*; *P. inflatula*; *P. pungens*; *P. pseudodelicatissima*; *P. seriata f. seriata*?; *P. seriata* f.*obtusa*?; *P. subpacifica*;	Rivera 1985
Uruguay (Atlantic)	*P. multiseries*	Hasle 1965
Africa		
Northwest Africa (Atlantic)	*P. subpacifica*; *P. delicatissima*	Hasle, 1965; Hasle et al. 1996
Mediterranean		
Algeria (Algiers)	*P. multiseries*	Skov et al. 1999
Turkey (Gulf of Annaba)	*P. multiseries*	Skov et al. 1999

Table 8 cnt. Global distribution of species from the diatom *Pseudo-nitzschia*.
(? = tentative identification)

DISTRIBUTION	SPECIES	REFERENCE
Europe		
Spain	*P. australis; P. cuspidata; P. delicatissima*; *P. fraudulenta; P. multiseries*; *P. pseudodelicatissima; P. pungens*; *P. subpacifica*	Fraga et al. 1998
Portuguese waters	*P. subpacifica*	Hasle et al. 1996
Northern Europe		
Denmark	*P. delicatissima*; *P. fraudulenta*; *P. multiseries*; *P. pungens*; *P. pseudodelicatissima*; *P. seriata f. seriata*	Lundholm et al. 1994; Lundholm et al. 1997; Lundholm pers. commun;
Skagerrak	*P. delicatissima*; *P. fraudulenta*; *P. heimii*; *P. multiseries*; *P. pseudodelicatissima*; *P. pungens*; *P. seriata f. seriata*;	Hasle et al. 1996
South coast of Norway	*P. heimii*; *P. multiseries*	Hasle 1965; Hasle et al. 1996
West coast of Norway and northwards	*P. seriata* f. *obtusa*	Hasle et al. 1996
Russia east coast	*P. multiseries*; *P. pungens*; *P. pseudodelicatissima*	Orlova et al. 2000
Scotland east coast (off Shetland)	*P. heimii*	Hasle 1965
Scotland west coast	*P. australis*; *P. delicatissima*; *P. fraudulenta*; *P. multiseries*; *P. pungens*; *P. seriata f. seriata*	Gallacher et al. 2000
Australasia		
Australia	*P. cuspidata*; *P. delicatissima*; *P. heimii*; *P. subfraudulenta*	Lapworth et al. 2000
North and north west Australia (Gulf of Capentaria, Arafura sea)	*P. lineola*	Hallegraeff 1994; Hallegraeff 1995;
North east Australia (Coral sea)	*P. lineola*; *P. turgidula*	Hallegraeff 1994
East, southeast Australia (Victoria, New South Wales, Tasmania)	*P. australis*; *P. fraudulenta*; *P. multiseries*; *P. pungens*; *P. pseudodelicatissima*; *P. subpacifica*; *P. turgidula*	Hallegraeff 1994; Lapworth et al. 2000
Indonesian waters	*P. pungens*	Sidabutar et al. 2000
Japan	*P. delicatissima*?; *P. fraudulenta*; *P. multiseries*; *P. pungens*; *P. pseudodelicatissima*; *P. subfraudulenta*; *P. turgidula*?	Takano & Kuroki 1977; Takano & Kikuchi 1985; Kotaki et al. 1999
New Zealand	*P. australis*; *P. delicatissima*; *P. fraudulenta*; *P. heimii*; *P. multiseries*; *P. pungens*; *P. pseudodelicatissima*; *P. turgidula*	Rhodes 1998
Asia		
China (South China sea)	*P. delicatissima*; *P. multistriata*; *P. multiseries*; *P. pungens*; *P. pseudodelicatissima*; *P. subfraudulenta*; *P. subpacifica*; *P. turgidula*;	Yang pers. commun.; Takano, 1993; Dickman & Glenwright 1997; Weijian 2000
South Korea (East China sea)	*P. multiseries*; *P. pungens*	Lee 1994; Lee & Baik 1997

1.4 DOMOIC ACID AND TOXIC INCIDENTS OF AMNESIC SHELLFISH POISONING (ASP)

Coastal areas in all parts of the world often experience periodic phytoplankton blooms. The term "red tide" is often applied to describe such events since water discolouration is frequently visible. This natural proliferation of algae, where cell concentrations can reach millions of cells per litre is, more often than not, harmless. Nonetheless, extensive blooms can sometimes be noxious, indiscriminately killing marine life forms when anoxic conditions arise as the bloom subsides and decomposition begins.

Other harmful algal events (HAE[†]) can arise when phytoplankton present have the ability to produce potent chemical toxins. The toxin can accumulate and concentrate in the tissues of bivalves through filter–feeding, and transfer through the food chain to humans, generating a public health risk to consumers. Some phytoplankton species can produce toxic effects at low cell concentrations.

The occurrence of red tides and toxic species are of major concern worldwide since such events can have large impacts on seafood quality (cultured and wild fisheries), aquaculture markets and tourism. It is difficult if not impossible to prevent the development of a harmful algal event (HAE). However, a toxic event that poses a threat to public health through the consumption of contaminated seafood can be avoided if early warning systems are set up. Effective methods to ensure seafood safety such as the regular monitoring of the flesh of fish/shellfish for the presence of toxins through chemical analysis and bioassays, coupled with the monitoring of the water column for potentially toxic phytoplankton producers (non-chemical means) are now common place in locations where aquaculture operations exist.

Up until the late 1980's, species from the class Pyrrophyceae, the dinoflagellates, were considered to be the main cause of red tides and toxic events. It should be noted that a

[†] HAB vs. HAE: The term HAE is used here preferentially to the more commonly applied HAB (Harmful algal bloom). This is due to confusion that can sometimes arise regarding the term "bloom". This literally means a proliferation of cells and such a proliferation need not always occur during a HAE.

red tide (not always strictly red) is linked to the colour expression from the chromatophores in the causative organisms, in this instance a red pigment called peridinin hence the name "red tide". The most common illnesses in humans resulting from the consumption of seafood products contaminated with toxin are Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP) and Ciguatera Fish Poisoning (CFP). The causative organisms include species from the genera *Alexandrium*, *Gymnodinium* and *Pyrodinium* (PSP), *Dinophysis* and *Porocentrum* (DSP), *Ptychodiscus* (NSP) and *Gambierdiscus* (CFP) respectively. These along with numerous other nuisance algae are well documented in the literature (Shumway 1990, Wright 1995, UNESCO 1995).

A new type of seafood poisoning associated with the consumption of shellfish was encountered in Canada during November 1987. People became ill after ingesting cultured blue mussels (*Mytilus edulis*) harvested from Prince Edward Island, Eastern Canada. Over 100 cases of poisoning including 3 deaths were linked to the incident (Perl et al. 1990, Todd 1993). The toxicological symptoms ranged from mild – gastrointestinal (nausea, vomiting, anorexia, diarrhoea, abdominal cramps and headaches) to severe – excessive bronchial secretions, difficulty in breathing, loss of balance/disorientation, seizures, coma, and sometimes death (Perl et al. 1990, Todd 1993). Some of the afflicted individuals suffered permanent short-term memory loss. As a result, the illness became known as Amnesic Shellfish Poisoning (ASP). A toxin extracted from the contaminated mussel tissue was identified as domoic acid (Bird et al. 1988, Wright et al. 1989). This was not the first documentation of this natural toxin. It had previously been isolated from a red macro alga, *Chondria armata* (Kütz) Okamura, by researchers in Japan (Takemoto and Daigo 1958). The Japanese name for this plant is "Hanayanagi" or "Domoi" hence the name "domoic acid". In the past the Japanese used this alga as a natural remedy for treating and expelling stomach worms. Takemoto and Daigo (1958) found that small doses of domoic acid (20 mg) extracted from the plant had such an antihelminthic result. Iverson and Truelove (1994) stated that persons who ingested infected mussel containing low levels of domoic acid (~20 mg) during the crisis in Prince Edward Island did not suffer the clinical symptoms of ASP. Although

domoic acid (DA) was known to be a neuroexcitant, at the time of the shellfish poisoning there had been no previous reports of human toxicity of this kind. Further investigations revealed that the pennate diatom *Pseudo-nitzschia multiseries* was the source of the toxin. A phytoplankton bloom at the time of the outbreak was primarily made up of this organism. Cultures isolated from the area showed that *P. multiseries* had the ability to produce DA (Bates et al. 1988). This was the first time a diatom was shown to produce a harmful toxin. Other DA producers present at the time of the incident, but in low cell concentrations, were *P. delicatissima* and a benthic diatom *Amphora coeffaeformis* (Bates et al. 1988).

Since the genus *Pseudo-nitzschia* is a cosmopolitan genus, the discovery of its toxic potential led to concerns that a similar outbreak to that experienced in Prince Edward Island might recur either locally or abroad. A comprehensive monitoring programme was set up in Canada to provide an early warning system that would prevent a reoccurrence of ASP. The following year (1988) *P. pseudodelicatissima* was recorded in high cell densities in plankton samples in the Bay of Fundy, eastern Canada (Martin et al. 1990). Commercial blue mussels and soft-shell clams were found to be unfit for human consumption as they were tainted with DA. Phytoplankton samples tested gave a positive DA result. Cultures of *P. pseudodelicatissima* later isolated from the area also produced this toxin (Martin et al. 1990).

Since 1987, there have been numerous reports of ASP in other parts of the world. In September 1991 an ASP event arose for the first time in the United States. Along the Californian coast many of the local seabirds: brown pelicans (*Pelecanus occidentalis*) and brant cormorants (*Phalacrocorax penicillatus*) fell ill or died after feeding on the herbivorous fishes, anchovies (*Engraulis mordax*) (Work et al. 1993). After analysing the stomach contents of these fishes it was discovered that DA was present in high concentrations. Analysis of their digestive system also revealed that the diatom *P. australis* was their main food source. A bloom of this phytoplankton species was also evident from analysis of water samples taken at the time of the outbreak (Fritz et al. 1992).

Amnesic Shellfish Poisoning reports north of Monterey Bay, California, were documented in November 1991. A number of people showed minor symptoms of ASP after the consumption of softshell razor clams (*Mya arenaria*) (Horner and Postel 1993, Todd 1993). Investigators discovered DA in the clams and also in Dungeness crabs (*Cancer magister*) in California and Washington. It is not known what the source of the toxin was, but *P. australis* was suspected.

In Europe, DA was first detected in October 1994 in mussels harvested in Galicia, northwest Spain. This episode followed a bloom of *Pseudo-nitzschia australis* (Míguez et al. 1996). To establish if any *Pseudo-nitzschia* species observed in Spanish waters had the ability to produce DA, strains of *P. australis*, *P. fraudulenta*, *P. cuspidata*, *P. pungens* and *P. delicatissima* were isolated and the cultures tested for DA. Screening studies revealed that *P. australis* could indeed produce the toxin (Fraga et al. 1998).

More recently in 1999, an ASP event was experienced off the west coast of Scotland, though no cases of human toxicity were reported. The scallop (*Pecten maximus*) fisheries were forced to close with consequential large financial losses (Gallacher et al. 2000). The causative organism remains uncertain, although potential DA producing *Pseudo-nitzschia* species such as *P. pungens*, *P. multiseries*, *P. seriata*, *P. australis* and *P. delicatissima* have been identified in water samples from inshore areas where DA was not detected (Gallacher et al. 2000).

The phenomenon can now be referred to as Domoic Acid Poisoning (DAP) since mammals other than humans have shown to be affected by this potent marine neurotoxin including birds and sea lions. The toxin can also be transferred through the food–web by vectors other than shellfish (Work et al. 1993, Scholin et al. 2000). Other mammals that have tested positive for symptoms of acute DA poisoning in the laboratory include rats and monkeys (Tryphonas et al. 1990).

Since the first DAP incident in eastern Canada, global investigations have reported that several *Pseudo-nitzschia* species are potential DA producers. Presently we know of

seven *Pseudo-nitzschia* species that can produce DA. These along with other DA producers are listed in Table 9. Additional toxigenic species will probably be encountered since research on this diatom is still ongoing. Stephen Bates (1998) reported that the level of toxicity can be variable in each species (having the same external morphology) in cultures from different global localities and also within the same geographical region. Culture studies carried out in the last decade testify that some *Pseudo-nitzschia* species, for example *P. multiseries*, significantly produce DA when under physiological stress resulting from nutrient limitation, such as silicate and phosphate (Bates 1998).

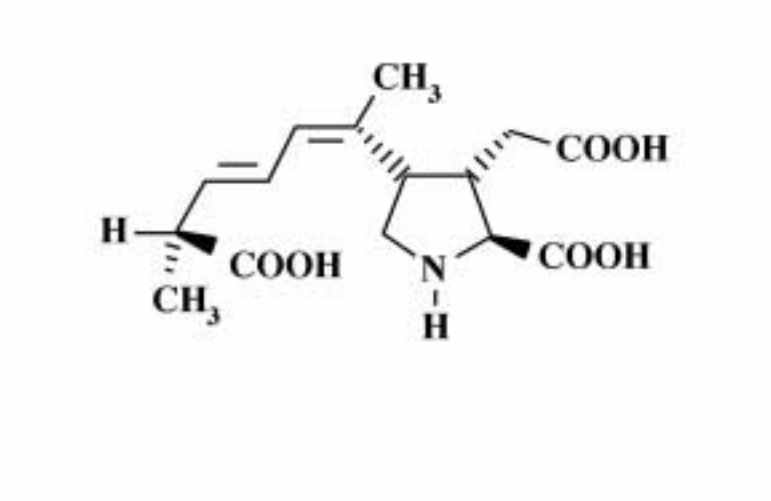
Table 9. Organisms that produce domoic acid in culture, some strains of the species indicated by a * have been found to be non-toxigenic in other culture studies (Bates et al. 1998 modified). PEI = Prince Edward Island, BF = Bay of Fundy, CA = California, MB = Monterey Bay NA = Narranagansett Bay CB = Coos Bay, RI = Rhode Island, OB = Ofunato Bay, GB = Galveston Bay, TX = Texas, M = Massachusetts Bay.

***Pseudo-nitzschia* species**	**Biogeographical area**	**Reference**
****P. multiseries***	Canada (PEI)	Subba Rao et al. 1988; Bates et al. 1989; 1991; 1993; 1995; 1996
	USA (GB, TX)	Fryxell et al. 1990
	USA west coast (MB, CA)	Villac et al. 1993
	USA west coast (CA)	Fritz et al. 1992
	USA (NA, RI)	Hargraves et al. 1993
	Japan (OB)	Kotaki, et al. 1999
	Southern Korea	Lee and Baik 1997
	*USA (M)	*Villareal et al. 1993
**P. pungens*	New Zealand	Rhodes et al. 1996
	* Canada east coast	*Smith et al. 1990
	*USA (GB, TX)	*Fryxell et al. 1990
	*Gulf of Mexico	*Villac et al. 1993
	*USA (NA, RI)	*Hargraves et al. 1993
	*Denmark	*Lundholm et al. 1994
	*New Zealand	*Mackenzie et al. 1993
**P. australis*	USA west coast (MB, CA)	Garrison et al. 1992; Buck et al. 1992; Villac et al. 1993
	USA west coast (CB, CA)	Villac et al. 1993; *Villac et al. 1993
	USA west coast (Iwaco CA,)	Villac et al. 1993; Dickey et al. 1992; Fritz et al. 1992
	New Zealand	Rhodes et al. 1996
	Spain	Míguez et al. 1996
**P. seriata*	Denmark	Lundholm et al. 1994
	*Canada east coast (PEI)	Bates et al. 1989
**P. fraudulenta*	New Zealand	Rhodes et al. 1998
	*USA (NB, RI)	*Hargraves et al. 1993
	*USA west coast (MB, CA)	*Villac et al. 1993
**P. delicatissima*	Canada (east coast)	Smith et al. 1990
	New Zealand	Rhodes et al. 1998
	Denmark	Lundholm et al. 1997; *Lundholm et al. 1997
	*USA west coast (MB, CA)	*Villac et al. 1993
**P. pseudodelicatissima*	Eastern Canada (BF)	Martin et al. 1990
	Denmark	Lundholm et al. 1997
	*Denmark	Lundholm et al. 1997
	*USA (GB, TX)	*Reap 1991;
	*USA (M)	*Villareal et al. 1993
	*Australia	*Hallegraeff 1994
	*USA (MB)	*Walz et al. 1994; Villac et al. 1993
P. turgidula	New Zealand	Rhodes et al. 1996
Other diatoms:		
**Amphora coeffaeformis*	Canada (PEI)	Shimizu et al. 1989; Maranda et al. 1990
	*Canada (PEI)	*Bates et al. 1989
Nitzschia navis-varingica	Vietnam (shrimp-culture pond)	Kotaki et al. 2000
Red algae family *Rhodomelaceae*		
Chondria armata	Japan	Takemoto and Daigo 1958
Chondria baileyana	Canada (Southern Nova Scotia and PEI)	Bird et al. 1988; Laycock et al. 1989
Alsidium corallinum	Mediterranean (Sicily)	Impellizzeri et al.1975
Amansia glomerata		Sato et al. 1996
Digenea simplex		Sato et al. 1996
Vidalia obtusiloba		Sato et al. 1996

1.4.1 DOMOIC ACID

Domoic acid (DA) is a naturally occurring crystalline water–soluble amino acid (Figure 6). This heat stable biotoxin belongs to the kanoid group of compounds and is similar in structure to kainate. More detailed information of its chemical and physical properties are given by Takemoto and Daigo (1958, 1960) and Wright (1989). Domoic acid is considered to be a restricted form of glutamic acid, and can serve as a competitor to glutamate (neurotransmitter) in the brain and central nervous system if ingested in large amounts. It binds to the kainate receptor proteins present in the central nervous system where the neurons are stimulated continuously until they are impaired; damage of the neurons in the hyppocampal region of the brain follows (Quilliam and Wright, 1989).

Figure 6. Chemical structure of domoic acid (redrawn from Wright and Quilliam 1995).



METHODS OF DOMOIC ACID DETECTION

The most characteristic indication displayed by mice injected with DA is the scratching of their shoulders with a hind leg, followed by spasms and occasionally death (Tasker et al. 1991). A mouse bioassay was used during the first outbreak of domoic acid poisoning in Canada. A safety limit of 20 $\mu g.g^{-1}$ was recommended by Iverson and Truelove (1994) and has subsequently been adopted as the regulatory limit in many countries such as the United States, Spain and other EU countries including Ireland.

Since the mouse bioassay only shows effects at > 40 $\mu g.g^{-1}$ (Wright and Quilliam 1995), alternative highly sensitive chemical analytical methods are often preferred. One such testing procedure is the FMOC–HPLC method that detects DA in water samples with a detection limit of 0.1 ng $DA.mL^{-1}$ (Pocklington et al. 1990). When a new DA biotoxin producer is identified, confirmation of DA production is required; this can be determined using tandem mass spectrometry (Bates 1998).

1.5 DOMOIC ACID PRODUCTION BY *PSEUDO-NITZSCHIA* IN CULTURE AND IN THE FIELD.

Ever since the first account of an ASP event and with it the knowledge that *P. multiseries* could produce the marine biotoxin domoic acid (DA), many isolates of the diatom *Pseudo-nitzschia* have been cultured from different geographic regions around the world. Several studies have suggested that various factors (biological, chemical and physical) can influence DA production in these organisms (see review by Bates 1998). Information on toxic and non-toxic strains has been compiled (Table 9) along with the understanding that DA production can vary in clones of the same species growing under seemingly similar conditions (Bates et al. 1989). Various factors have been found to influence DA production. These include bacterial association, nutrients, temperature and light.

In a recent review Bates (1998) suggested that bacterial association and/or genetic variability between *Pseudo-nitzschia* isolates could be a possible explanation for the differences in the production of DA observed in various studies. Douglas and Bates (1992) discovered that xenic[†] cultures of *P. multiseries* can produce DA at the same level or up to 20 times greater than axenic cultures. Bates et al. (1995) have shown that several species of bacteria can enhance DA production of *P. multiseries* by factors of between 2 – 115 when added to xenic cultures. This shows that although bacteria are not essential for the production of DA, their presence seems to influence its production (Douglas and Bates 1992, Bates et al. 1993).

[†] xenic cultures = bacterial flora present, axenic cultures = bacteria free

One of the first batch culture investigations of *P. multiseries* carried out by Bates et al. (1989) showed that DA was produced during the stationary phase of the growth cycle, where silica was the limiting nutrient. Lundholm et al. (1994) discovered that a small amount of DA was produced by 3 isolates of *P. seriata* (batch culture) during late–exponential phase, with most of the DA detected during the stationary phase of growth. A time–course study on a batch culture of *P. australis* showed that this species produced DA earlier, in mid–exponential phase (Garrison et al.1992).

Further culture studies revealed that *P. multiseries* could indeed produce DA in mid–exponential and late–exponential phase. The DA production began when cell division started to decline (resulting from a controlling factor such as silica or phosphate limitation), in the presence of extracellular nitrate and an adequate light source (Bates et al. 1991; 1993; 1998, Pan et al. 1996). Silica (Si) and phosphate (P) controlled continuous culture studies have also shown that when either of these nutrients are limiting, DA is produced by *P. multiseries* (Bates et al 1996, Bates 1998). Bates et al. (1991) found that *P. multiseries* would only produce DA when nitrate was present and cell division had begun to decline in a nitrate limited batch culture study. As with all amino acids this nutrient is required for DA synthesis.

Lewis et al. (1993) found that *P. multiseries* was tolerant to a wide temperature range (5 ºC to 25 ºC). During their investigations, DA production was positively correlated with increasing temperatures. In contrast, Lundholm et al. (1994) discovered that *P. seriata* (an organism common in colder waters) isolates in culture produced more DA at a temperature of 4 ºC than at the higher temperature of 15 ºC.

Work with *P. multiseries* batch cultures show that cell division rates decrease with decreasing photon flux densities (Bates et al. 1991). Lewis et al. (1993) found similarities in the division rates and DA production of a *P. multiseries* culture grown at 80 and 180 µmol.photons.m^{-2}.s^{-1}. Bates and Léger (1992) found that DA continued to increase up to a light intensity of 100 µmol. m^{-2}.s^{-1}, but below 35 µmol.m^{-2}.s^{-1} DA production decreased dramatically. Hargraves et al. (1993) carried out an experiment

that tested the affects of UV exposure to three *Pseudo-nitzschia* species namely *P. fraudulenta, P. pungens* and *P. multiseries*. The results showed that *P. multiseries* did not show any obvious affects from exposure to UV light. However, the growth rates of *P. fraudulenta* seriously declined while the cell division rates of *P. pungens* were initially inhibited by UV exposure.

An important physiological observation in *Pseudo-nitzschia* cell culture studies to date has been the production of domoic acid (in the presence of nitrogen) when division rates begins to decline as a result of some form of environmental stress (for e.g. a decrease in the availability of an essential nutrient required for growth).

Although culture work may not exactly replicate *in situ* environmental conditions, it has been a very useful technique for studying DA production. It has helped to elucidate various physiological conditions favouring growth of different *Pseudo-nitzschia* species along with confirming DA production of some species. When optimum conditions for growth are elucidated and the ranges or tolerances to temperature, salinity, light and various nutrients are recorded for each species then results can be compared to observations noted in the field. However, it is important to note that observations of environmental factors in unialgal cultures have their limitations and may not always reflect *in situ* observations. For example the tolerances a microalgal culture exhibits to changes in salinity, temperature, light and other environmental factors are more generally wider in range than those recorded in the wild, therefore interpretations of experimental culture work must be considered carefully. Unialgal cultures have been useful when molecular studies of *Pseudo*-nitzschia are carried out to determine phylogenetic relationships between species and strains of the same species.

Most field studies concerning the diatom *Pseudo-nitzschia* have concentrated on either localised areas hit by domoic acid outbreaks or waters in the vicinity of Universities or marine research laboratories. Concerns about public health, and the negative effect an ASP outbreak can have on the aquaculture industry has led to many studies investigating the factors responsible for the development of *Pseudo-nitzschia* blooms.

Initially, a lot of research, both culture and field studies concentrated on *P. multiseries*, since this species was the first of many *Pseudo-nitzschia* species found to be capable of producing domoic acid.

In the main, the theme of field and culture investigations has been the study of variation and interrelationships between the physical (temperature, salinity, run–off, upwelling, stratification, seasonal circulation patterns, meteorological events, water transparency) and chemical (macronutrients, nutrient supply, oxygen) environments on bloom formation and DA production. However, to date no single causative stimulus has been identified.

Field studies have tended to focus on the influence of nutrient supply, in particular a change in the nutrient environment caused by

a) Variations in nutrient input
b) Upwelling, particularly after extended periods of nutrient depletion
c) Natural variation resulting from the spring bloom
d) Meteorology

Meteorological events may modify the physical and chemical properties of the water column thus leading to more favourable conditions for growth of certain phytoplankton species. Attempts have been made to correlate *Pseudo-nitzschia* bloom events with periodic cycles of weather and meteorology. For example, in 1987 the *P. multiseries* bloom implicated in the first ASP outbreak in the estuaries of Cardigan Bay (Prince Edward Island, eastern Canada), occurred after a long and dry summer followed by periods of heavy rain in the autumn (Smith et al. 1990). Smith et al. (1990) suggested that sufficient concentrations of a limiting nutrient from fresh water runoff contributed to this bloom as *Pseudo-nitzschia* cell concentrations were positively correlated with pulses in nitrate. In 1988, the region again experienced a dry summer but with less rainfall in the autumn then the previous year. A toxic bloom of *P. multiseries* was evident at this time but the size of the bloom was less intense. Smith (1993) reported

that turbulent storms experienced in Prince Edward Island in 1990 had a direct effect on a *P. multiseries* bloom as it was carried offshore and dispersed.

Lange et al. (1994) carried out a preliminary study on the occurrence and abundance of *Pseudo-nitzschia* species in waters off Southern California. High numbers of *P. australis* occurred sporadically between February and August although no DA outbreaks were reported. Proliferation of this organism was associated with a drop in water temperature and an increase in nutrients. It was thought that the change in the water column structure might have been a result of upwelling. In Monterey Bay (California) blooms of *P. australis* accompanied by the presence of DA, are associated with the end of the upwelling season. On these occasions, the water column is thermally stratified and nutrients in the surface layer are limiting. During the spring when coastal upwelling is evident *Pseudo-nitzschia* blooms seemed to be less developed and shorter in their duration (Buck et al. 1992; Walz et al. 1994). The complex oceanography of Monterey Bay is discussed by Garrison et al. (1992). Some shallow parts of the Bay have long water residency times. In these areas phytoplankton blooms and high concentrations of ammonia are often recorded, and may be due to local regeneration processes.

It should be noted that other studies have found no obvious relationship associated with nutrients and *Pseudo-nitzschia* bloom formation. *Pseudo-nitzschia pseudodelicatissima* dominated the phytoplankton assemblage in the Bay of Fundy in the autumn of 1987 when DA was detected in commercial shellfish. There was no obvious correlation between nutrient concentrations and *P. pseudodelicatissima* cell numbers in the water column. However, observations did show an elevation of water temperature at the time of the bloom (Martin et al. 1993).

Investigations into the preferences of *Pseudo-nitzschia* species for different environmental parameters may be an important factor in understanding its synecology. Fryxell et al. (1990) noted that although *P. multiseries* and *P. pungens* sometimes co-occur in the phytoplankton population in the Gulf of St. Lawrence (Galveston, Texas),

P. multiseries was more abundant in well mixed waters, with *P. pungens* in high cell densities in stratified waters.

Investigations are needed to elucidate the chemical, physical and biological factors responsible for the collapse of a *Pseudo-nitzschia* bloom. It was speculated that the decline of *P. multiseries* in the estuaries of Cardigan Bay (eastern Canada) during 1992 was the result of parasitic chytrid fungi (see review by Bates et al. 1998). Since pathogenic fungi/viruses may influence the *Pseudo-nitzschia* community structure, studies of these types of associations in cultures would provide valuable information.

Most warning systems in place today rely primarily on the detection of DA in commercial shellfish tissue. However, the 1991 Monterey Bay domoic acid incident, affecting brant cormorants and brown pelicans, has shown that DA outbreaks are not exclusive to commercial shellfish products (Buck et al. 1992; Fritz et al. 1992; Work et al. 1993). Therefore more intense experimental and field studies are needed to understand the hydrography, ecology, and oceanographic conditions controlling population dynamics of potentially toxic *Pseudo-nitzschia* blooms and the introduction of DA into the marine foodweb. Further investigations (both culture and field) will probably provide a better understanding of the bloom dynamics and the conditions required for DA production by *Pseudo-nitzschia* species.

The work presented in this thesis set about to undertake such an approach in studying the distribution of the diatom *Pseudo-nitzschia* in Irish waters and to test the ability of cultured isolates to produce DA.

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CHAPTER 2

SPECIES OF THE DIATOM *PSEUDO-NITZSCHIA* PERAGALLO IN IRISH WATERS.

Caroline Cusack

Marine Institute Phytoplankton Laboratory, Martin Ryan Institute, National University of Ireland, Galway, Ireland

Robin Raine and John W. Patching

Martin Ryan Institute, Marine Microbiology Department, National University of Ireland, Galway, Ireland

ABSTRACT

The presence of the potentially toxic diatom *Pseudo-nitzschia* was investigated using net material acquired during several research cruises carried out off the south, southwest and west coasts of Ireland between 1993 and 1997. Examination of phytoplankton net samples showed that *P. pungens*, *P. multiseries*, *P. fraudulenta*, *P. australis*, *P. delicatissima* and *P. pseudodelicatissima* were regularly present. Two other *Pseudo-nitzschia* species provisionally identified as *P. seriata* (*P. seriata* cf. *seriata* and *P. seriata* cf. *obtusa*) and *P. subpacifica* were also observed though less frequently.

Key index words: Pseudo-nitzschia, Irish waters, spatial and temporal distribution.

INTRODUCTION

Pseudo-nitzschia (Peragallo) is a widely distributed marine planktonic diatom (Hasle 1965, 1972, Hallegraeff 1994, Fryxell et al. 1997). The step-like colony formation and the spindle shape of the pennate frustule readily identify this genus. At present, there are no detailed records of *Pseudo-nitzschia* in Irish coastal waters, because of the difficulty in discriminating among species using the light microscope. Existing records of *Pseudo-nitzschia* in Irish waters are limited to identification at the generic size-group level; a distinction based on the width of the cell valve (Hasle 1965). Reported instances have referred to either "Nitzschia seriata" (= "P. seriata") or "Nitzschia delicatissima" (= "P. delicatissima") (Roden et al. 1981, Raine et al. 1990). Identification of individual *Pseudo-nitzschia* species requires examination of the fine structural detail of the valve features under an electron microscope.

The importance in discriminating between *Pseudo-nitzschia* species is emphasised by the fact that some species have the ability to produce the potent neurotoxin domoic acid. Contamination of shellfish with this toxin has caused serious illness or even death in humans and certain marine birds (see review by Bates et al. 1998), with the syndrome referred to as Amnesic Shellfish Poisoning (ASP) or Domoic Acid Poisoning (DAP).

This paper presents results of the taxonomic and morphological identification of *Pseudo-nitzschia* spp. sampled around the Irish coast.

MATERIALS AND METHODS

The study area encompassed shelf and coastal waters off the south, southwest and west coasts of Ireland (Fig. 1). Samples were collected on board the Irish national research vessels *Lough Beltra* until June 1997 or the *Celtic Voyager* after this date. Samples were also obtained on board the *Heinke*. Sampling stations generally ranged from within a few kilometers of the coastline out as far as ca. 75 kilometers offshore. Bottom depths ranged from 40 meters at the coastal stations to up to 230 meters at stations located further

offshore. Occasional samples were taken from estuaries such as Waterford Harbour, where the stations were shallower with water depths of between 25-30 m, or the Kinsale estuary (11 m).

Material used in this study was selected from phytoplankton net samples collected during research cruises that took place between 1993 and 1997. These cruises were designed to investigate phytoplankton ecology in relation to the physical and chemical oceanographic features of the region. Samples were for the most part collected during the summer and autumn months. Further material from the sample archive within the Microbiology Department, National University of Ireland, Galway, was also used for morphological examination.

Qualitative phytoplankton samples were taken at each sampling location using a 35 µm mesh plankton net hauled vertically from depths of up to 100 m to the surface. Lugol's iodine or neutralised formalin were added to preserve the phytoplankton samples. On occasion, samples were preserved with acidic iodine or acidic formaldehyde (Hasle and Syvertsen 1997). Where possible samples were stored in the dark at 4°C, otherwise they were stored in the dark at room temperature until analysis. Lugol's iodine was replenished when necessary in samples stored in plastic bottles.

At stations where *Pseudo-nitzschia* was found to be one of the dominant taxa, 5 mL of net sample was rinsed three times with distilled water to remove preservatives and salt. The organic matter of the cells was then removed by acid cleaning. Concentrated nitric acid was added and the samples were heated in a water bath at 80˚C for 30 minutes (cf. Boyle et al. 1984). The cleaned samples were then rinsed with distilled water and stored in absolute alcohol until further use. Prior to the examination of specimens under the electron microscope, water and strewn permanent mounts were observed under a Nikon Optiphot-2 oil immersion, phase contrast light microscope. Black and white light micrographs were taken with a Nikon Microflex UFX-DX camera and Ilford 100 ASA, 150 ASA or 400 ASA film. Samples examined under the scanning electron microscope (SEM) were mounted in triplicate. The SEM stub surface was wiped with acetone and a

drop of colloidal silver was used to cement a clean coverslip onto the stub and allowed to air dry. A drop of sample was pipetted onto the coverslip and left to dry overnight. Alternatively, the sample was filtered onto 1-3 µm Nucleopore filters, air dried and mounted onto metal stubs. Specimens were examined under a Leica S430 scanning electron microscope with an accelerating voltage of 15 kV or under a Hitachi S-570 scanning electron microscope with an accelerating voltage of 20 kV. Black and white scanning electron micrographs were taken with a Mamiya camera (Tokyo, Japan) and Kodak 100 ASA film. Where possible, thirty valves from each stub were randomly selected and morphometric measurements were recorded for each one. In addition to SEM analysis transmission electron microscopy (TEM) analysis was carried out on the phytoplankton net material. A drop of cleaned net material was pipetted onto a formvar carbon-coated 50 or 75-mesh (hexagonal lines per inch) TEM copper grid (Agar Scientific). A minimum of 3 grids were used per sample. The grids were left to air dry and then checked under a LM before being viewed under a Hitachi-7000 transmission electron microscope at an accelerating voltage of 75 kV. Black and white transmission electron micrographs were taken with a Mamiya camera (Tokyo, Japan) and Kodak 100 ASA film.

Some of the photomicrographs taken were examined by Professor G.R. Hasle (Department of Biology, Marine Botany Section, University of Oslo, Oslo, Norway) in order to confirm the *Pseudo-nitzschia* species identity. Nomenclature is in accordance with Hasle (1974, 1993), Hasle et al. (1996), Hasle and Syvertsen (1997), Fryxell et al. (1997).

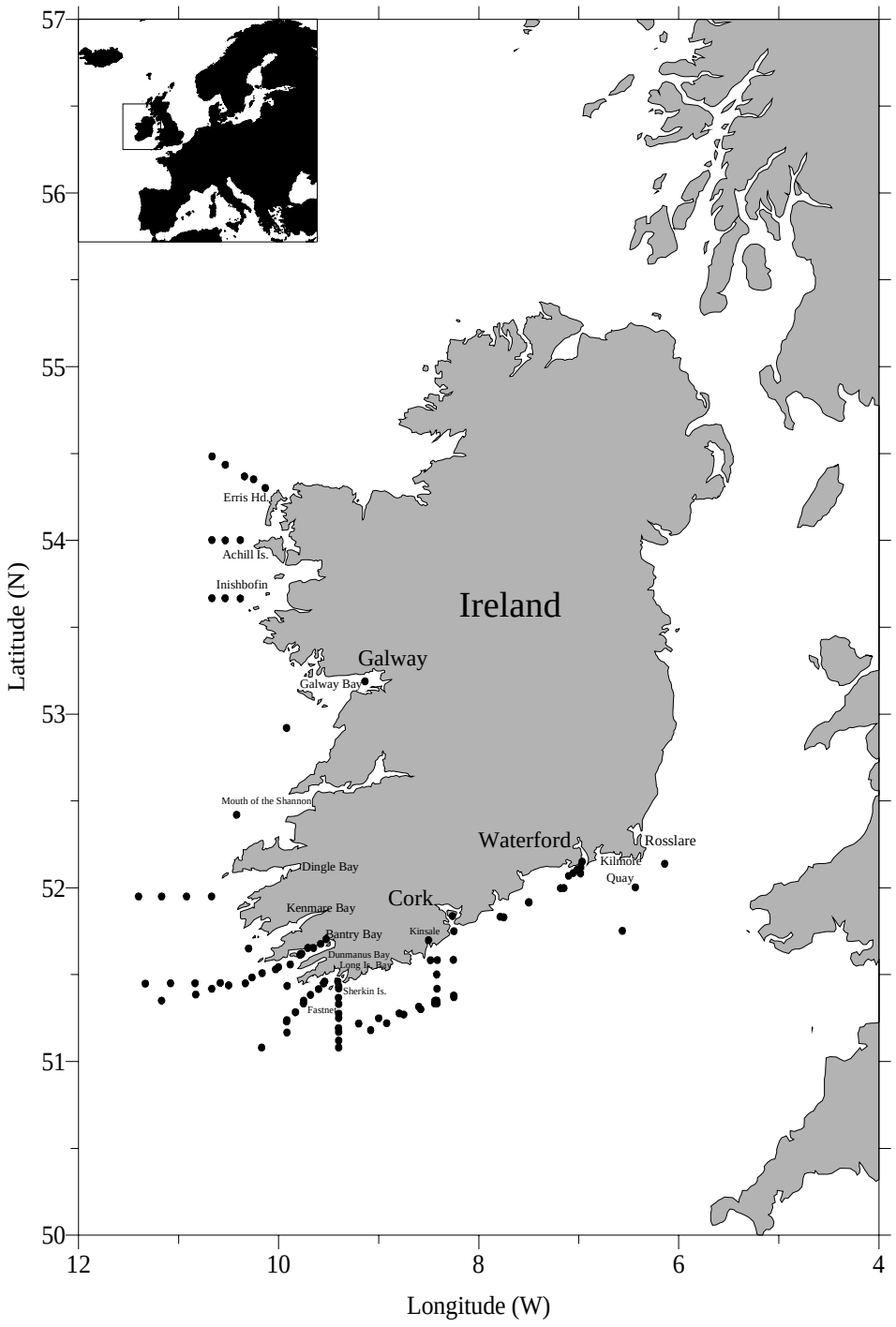

Figure 1. Map of study area showing the location of stations sampled off the coast of Ireland (1993-1997).

RESULTS

Morphological analysis

Over the course of these surveys six *Pseudo-nitzschia* species were unambiguously identified. These were *P. pungens*, *P. multiseries*, *P. australis*, *P. fraudulenta*, *P. delicatissima* and *P. pseudodelicatissima* (Table 1, Plates 1-6, Appendices 3 and 6). Detailed species descriptions have already been discussed in Hasle (1965, 1972, 1995), Hasle & Fryxell (1995), Hasle et al. (1996) and Hasle and Syvertsen (1997) (Appendix 2). Tentative identification was made of a further two species (*P. subpacifica* and 2 forms of *P. seriata*) based on valve shape and morphometric data recorded under the SEM. Only a small number of valves (<4) of the provisionally identified species were examined (Table 2, Plates 7-8, Appendices 3 and 6). *Pseudo-nitzschia* species labelled A-D are tentatively identified as *P. seriata* f. *seriata*, E-F as *P. seriata* f. *obtusa* and G-H are provisionally identified as *P. subpacifica* (Table 2). Recent data from Hasle et al. (1996) and Hasle and Syvertsen (1997) were used for comparative purposes and it is evident that nearly all of the measurements are extremely close to published morphometric records. In general only the valve structure was examined as girdle elements became dissociated from the frustule during the acid cleaning procedure. Good quality micrographs of *Pseudo-nitzschia* valves tentatively identified as *P. seriata* f. *seriata* (4 valves observed) and *P. seriata* f. *obtusa* (2 valves observed) were not obtained, as the specimens were either not completely cleaned of their organic matter or silica dissolution was apparent after acid cleaning. The tentatively identified *P. seriata* valves had more or less rounded valve ends, showed asymmetry with respect to the apical axis (Plate 7) and exhibited a pattern of striae (the number of rows of poroids per stria) similar to that reported for this species in the literature. The provisionally identified *P. seriata* f. *seriata* valves had 3-4 rows of poroids per stria while only 2 rows of poroids per stria were apparent in valves identified as *P. seriata* f. *obtusa*.

Table 1. Morphometric data recorded under the SEM for *Pseudo-nitzschia* species observed in Irish waters. n = number of valves measured. Figures in brackets refer to data in Hasle et al. (1996) and Hasle and Syvertsen (1997) and are added for comparative purposes.

Species	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Poroids (in 1 µm)	No. of striae per central nodule
P. pungens	(74–142)	(2.9–4.5)	(9–15)	(9–15)	(3–4)	
n=97	61–156	2.2–5.4	10–16	10–16	2–4	–
P. multiseries	(68–140)	(3.4–5.0)	(10–15)	(10–15)	(4–6)	
n=17	80–128	2.8–4.6	12–16	12–16	6–7	–
P. australis	(75–144)	(6.5–8.0)	(12–18)	(12–18)	(4–5)	
n=130	63–143	5.3–8.0	15–19	15–19	3.5–6	–
P. fraudulenta	(64–117)	(4.5–6.5)	(12–24)	(18–24)	(4–5)	(3–4)
n=152	65–164	4.0–8.0	21–25	21–25	4.5–7	2.5–4
P. delicatissima	(40–76)	(1.0–ca. 2)	(19–25)	(36–40)	(10–12)	(3–3.5)
n=79	24–64	1.2–2.4	20–26	39–44	9–13	3–4
P. pseudodelicatissima	(59–140)	(1.3–2.5)	(16–26)	(30–46[?])	(4–5)	(4)
n=37	27–85	1.0–1.9	22–26	39–44	4–6	3–4.5

Table 2. Morphometric data recorded under the SEM of field specimens (single valves) taken from Irish waters. The identity of these *Pseudo-nitzschia* species requires confirmation. Figures in brackets refer to data in Hasle et al. (1996), Hasle and Syvertsen (1997) and are added for comparative purposes.

Species	Tentatively identified as	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Poroids (in 1 µm)	No. of striae per central nodule
P. seriata f. seriata		(91–160)	(5.5–8.0)	(14–18)	(14–18)	(7–8)	–
P. species A	*P. ser* f. *seriata*	131	6.2	15	17	8	–
P. species B	*P. ser* f. *seriata*	108	6.0	20	20	7	–
P. species C	*P. ser* f. *seriata*	113	5.8	20	20	8	–
P. species D	*P. ser* f. *seriata*	153	5.5	20	20	8	–
P. seriata f. *obtusa*		(61–100)	(4.5–5.5)	(15–20)	(15–20)	(7–8)	–
P. species E	*P. ser* f.*obtusa*	126	5.7	18	18	6	–
P. species F	*P. ser* f.*obtusa*	65	5.5	19	18	8	–
P. subpacifica		(33–70)	(5.0–7.0)	(15–20)	(28–32)	(9–10)	
P. heimii		(50–120)	(4.0–6.0)	(11–18)	(19–28)	(7–8)	(4–5)
P. species G	*P. subpacifica*	62.5	4.5	18–19	30–31	9.5	3
P. species H	*P. subpacifica*	63	4.9	18	27	9	3

Plate 1. *Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle.

1a-c LM.

Clonal culture, strain WW_3 isolated from Waterford Harbour, 8th October 1997. 1a. Chain of cells in girdle view: overlap of cell ends ca. 1/4 of total cell length. 1b. Acid-cleaned frustule mounted in Naphrax; coarsely structured valve with pointed cell ends. Valve margins are symmetrical. 1c. Acid-cleaned valve mounted in Naphrax; note that the two rows of poroids per stria are just visible.

1d-i TEM.

1d-g. Specimens in net material collected in Bantry Bay between the 20-21st July, 1996. 1d. Part of the valve with raphe and proximal mantle. The fibulae and striae are present in equal number. Poroids of the valve face and proximal mantle are hymenate (see enlargement). The striae are perforated with two rows of poroids, sometimes with a single poroid or extra partial row of poroids between the rows. 1e. Valve face; note irregular shaped poroids. 1f-g. Two cell ends from the same valve taken from the wild. 1h-i. Clonal culture, strain SL isolated from Clifden Bay, west coast of Ireland, 7th August 1997. Two ends of one valve from a clonal culture: note the overall shape of cell ends.

Scale bars 10 µm (1a, 1b); 1 µm (1c, 1e, 1f, 1g, 1h); 0.5 µm (1d)

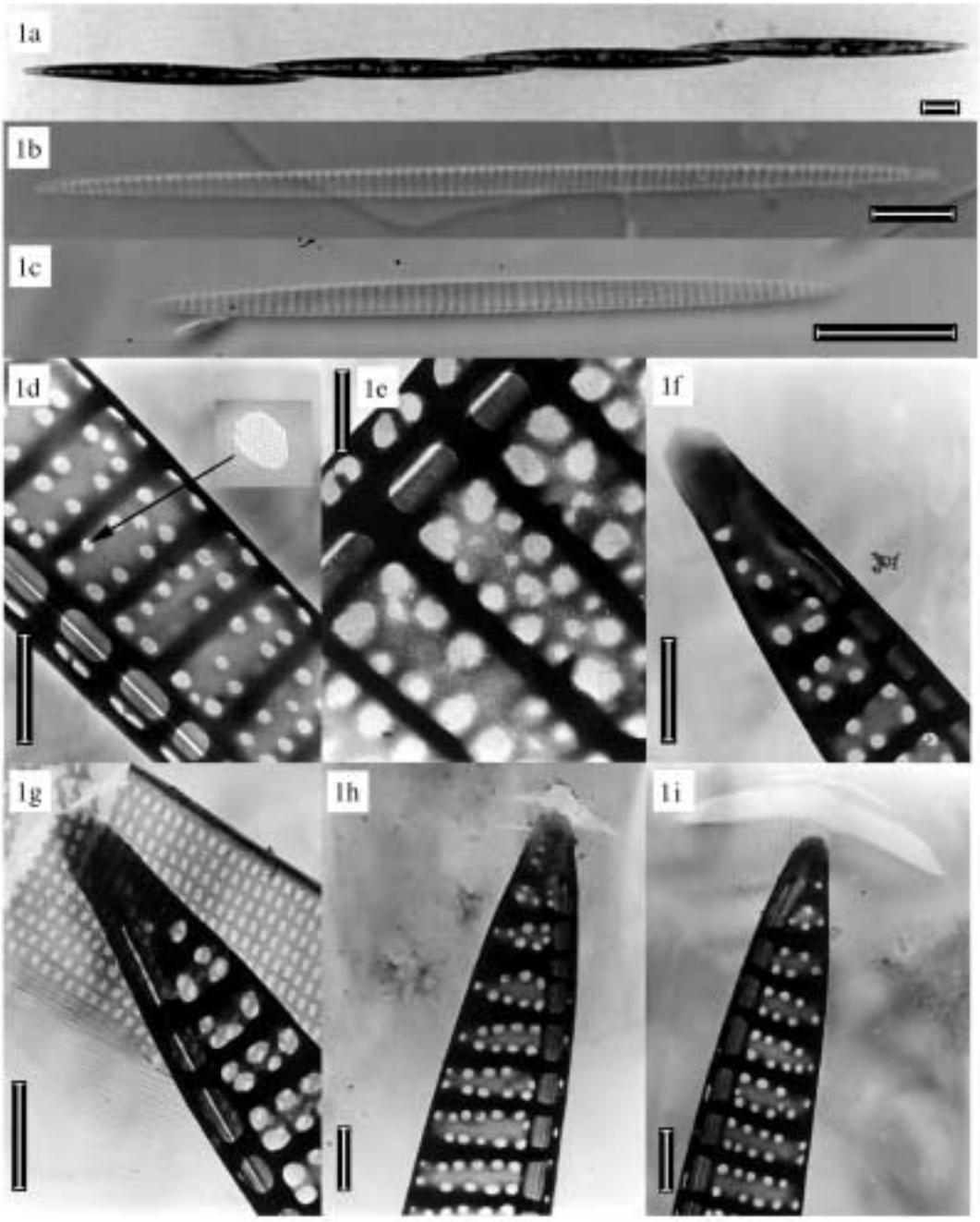

Plate 1. *Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle.

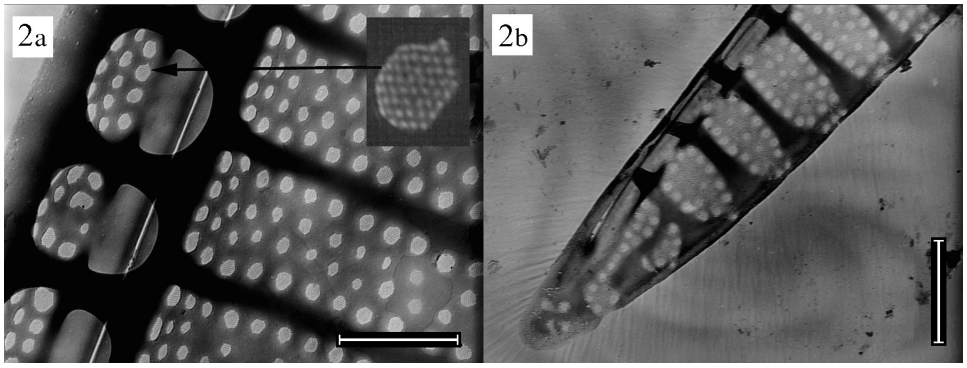

Plate 2. *Pseudo-nitzschia multiseries* (Hasle) Hasle.

2a-b TEM.

Specimens in net material collected southwest of Long Island Bay, 23rd July 1996. 2a. Close-up of valve showing the hymenate velae of the poroids: 4 rows of poroids are evident per stria. 2b. Pointed valve end showing branching of the interstriae towards the tip.

Scale bars 1 µm (2b); 0.5 µm (2a)

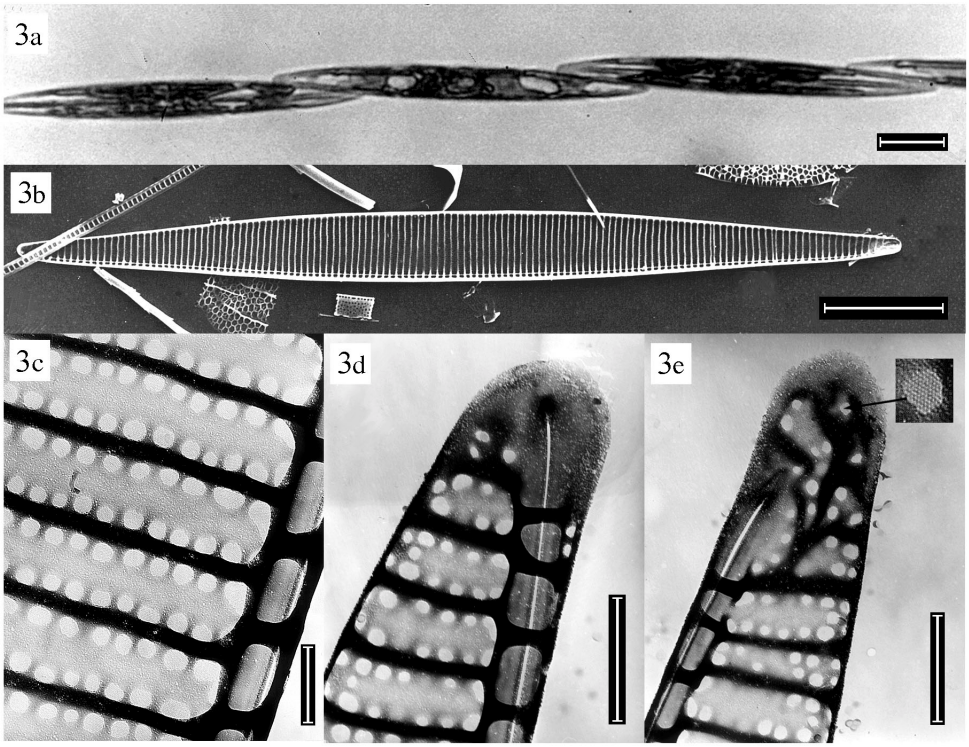

Plate 3. *Pseudo-nitzschia australis* Frenguelli.

3a LM.

Clonal culture, strain WW_4 isolated from Waterford Harbour, 8th October 1997. Colony in girdle view: overlap of cell ends ca. 1/4 of total cell length.

3b SEM.

Specimen in net material collected west of the Shannon Estuary, 3rd September 1996. Acid-cleaned valve showing the fibulae and interstriae. Valve symmetrical with respect to the apical axis; margins are parallel at the middle part of the valve.

3c-e TEM.

Specimen in net material collected west of the Shannon Estuary, 3rd September 1996. 3c. Part of valve showing two rows of hymenate poroids (see enlargement) with a third poroid sometimes present between these two rows towards the valve margin. 3d-e. Rounded cell apices of the same valve with one end bearing branched-like interstriae.

Scale bars 10 µm (3a, 3b); 1 µm (3d, 3e); 0.5 µm (3c).

Plate 4. *Pseudo-nitzschia fraudulenta* (Cleve) Hasle.

4a-b LM.

Clonal culture, strain 2011 isolated from a station off Erris Head, west coast, 3rd May 1997. 4a. Vegetative cells in girdle view: overlap is ca. 1/8 of total cell length. 4b. Acid-cleaned valve mounted in Naphrax: the central nodule and fibulae are more discernable than the interstriae. Valve symmetrical with respect to the apical axis with pointed ends; valve margins curved.

4c-f TEM.

4c-d. Specimens in net material collected south of Cork, 6th October 1997. 4c. Part of the valve showing the raphe and central interspace. Fibulae and striae are found in equal numbers. 4d. Close-up of the poroid structure, which is divided into sections with hymenate velae. Note also that sometimes there is a third row or partial row between the two rows of poroids. 4e. Specimen in net material collected south of Waterford, 7th October 1997. 4e. Two valve ends of the same frustule; one valve end has one or two oblique rows of poroids that are not present on the other. 4f. Clonal culture, strain 2011 isolated from a station off Erris Head, west coast, 3rd May 1997. 4f. Part of a valve of an aberrant cell from a clonal culture.

Scale bars 10 µm (4a, 4b); 5 µm (4c); 1 µm (4e, 4f); 0.5 µm (4d)

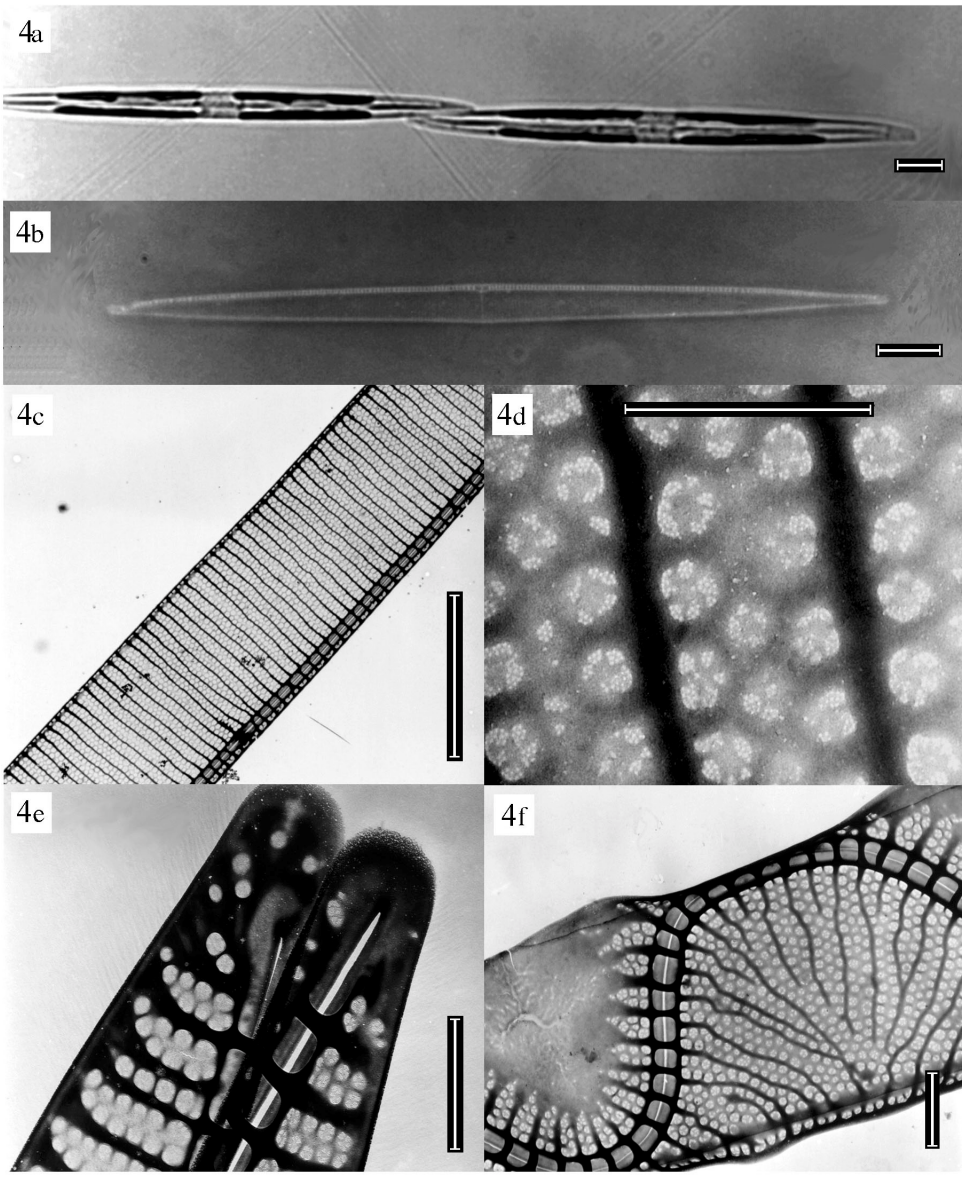

Plate 4. *Pseudo-nitzschia fraudulenta* (Cleve) Hasle.

Plate 5. *Pseudo-nitzschia delicatissima* (Cleve) Heiden.

5a-e TEM.

5a. Specimen in net material collected southwest of Bantry Bay, 15th August 1993. 5a. Part of valve showing the raphe and the central larger interspace. There are an uneven number of fibulae to striae. 5b. Specimen in net material collected southwest of Bantry Bay, 22nd July 1996. 5b. Close-up of the valve face showing 3 rows of striae per central larger interspace. Note the two rows of triangular to hexagonal hymenate poroids per stria. 5c-e. Specimens in net material collected in Galway Bay, 6th May 1996. 5c-d. Rounded cell apices from the same valve with no difference in structure. 5e. Girdle showing 3 areolated and 1 non-areolated bands.

Scale bars 1 µm (5a, 5e); 0.5 µm (5b, 5c, 5d)

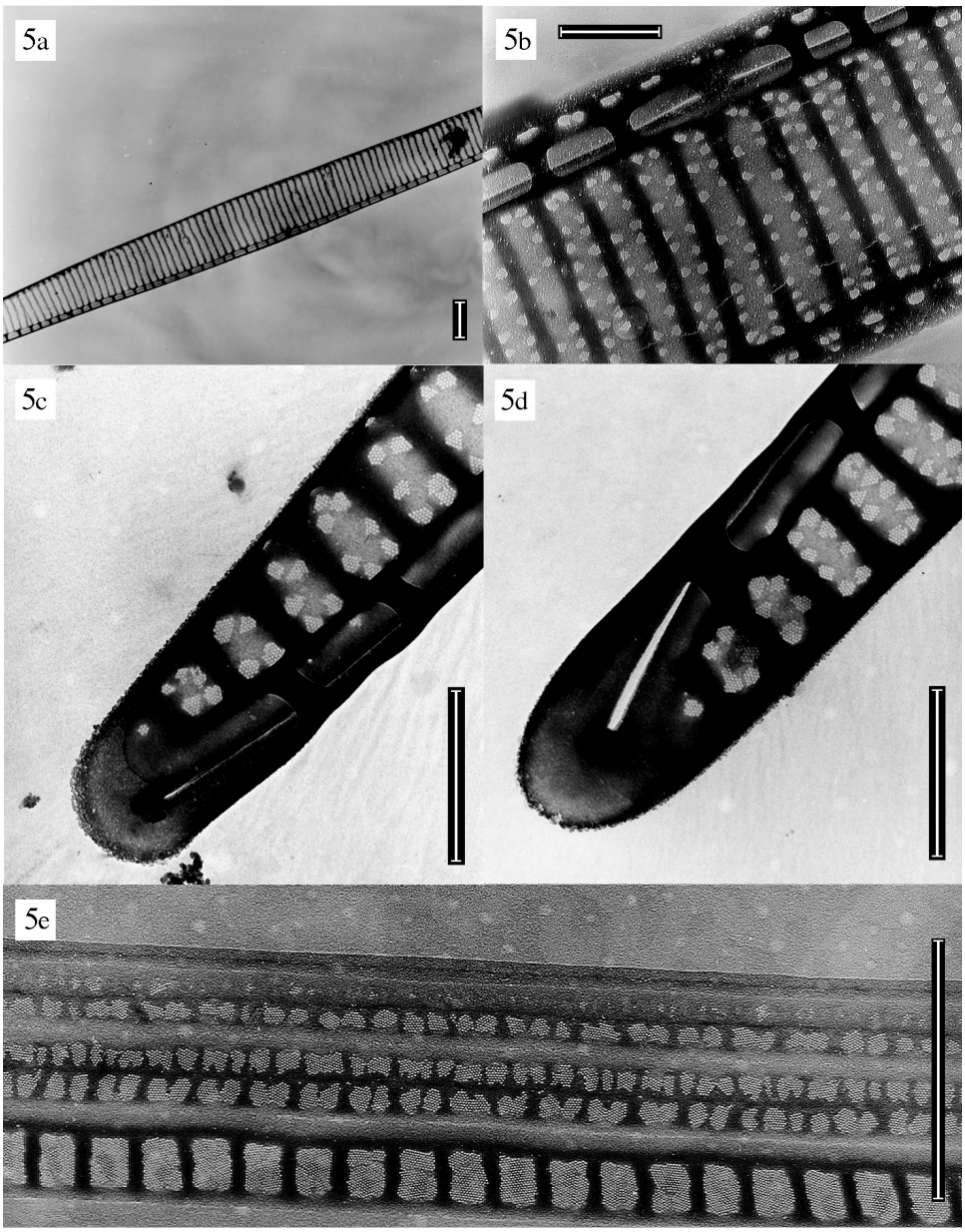

Plate 5. *Pseudo-nitzschia delicatissima* (Cleve) Heiden.

Plate 6. *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle.

6a-d SEM.

Specimens in net material collected southwest of Dunmanus Bay, 21st July 1996. 6a. Whole valve with pointed valve ends. 6b. Part of the valve face showing the central larger interspace, the central nodule (in the upper part of the micrograph) and the striae with a single row of poroids. There are an uneven number of fibulae to striae. 6c-d. Two ends of the same valve, similar in structure.

6e-f TEM.

Specimens in net material collected south of Kinsale, 22nd July 1996. 6e. Valve face showing the proximal and distal mantle, the central larger interspace with central raphe endings. Note the squarish structure of the valve face poroids. 6f. The poroid structure consists of divided sectors with hymenate velae (see enlargement). There are 4 rows of striae per central larger interspace.

Scale bars 1 µm (6b, 6c, 6d, 6e, 6f); 3 µm (6a)

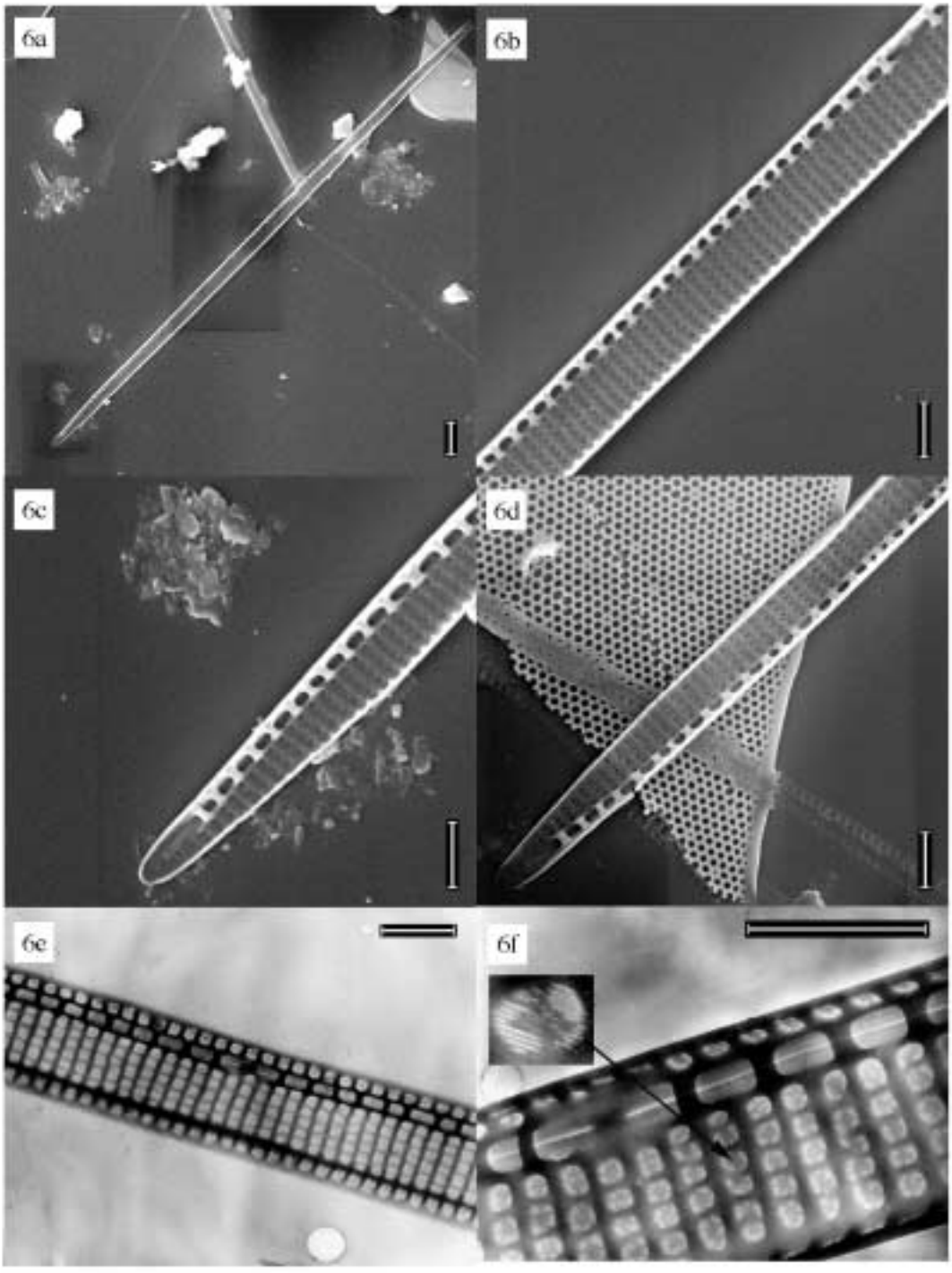

Plate 6. *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle.

Plate 7. *Pseudo-nitzschia* cf. *seriata* (Cleve) H. Peragallo (species A, Table 2).

7a-c SEM.

Specimen in net material collected southwest of Dunmanus Bay, 21st July 1996. 7a. Whole valve with one visible pointed end. There are slightly more striae than fibulae (see Table 2). 7b. Close-up of part of the valve face towards one of the cell ends; note that the sample is not completely cleaned of its organic material. Enlargement shows some of the striae where the valve is more visible; there are 3 rows of circular poroids per striae. 7c. One of the valve ends.

Scale bars 10 µm (7a); 1 µm (7b, 7c)

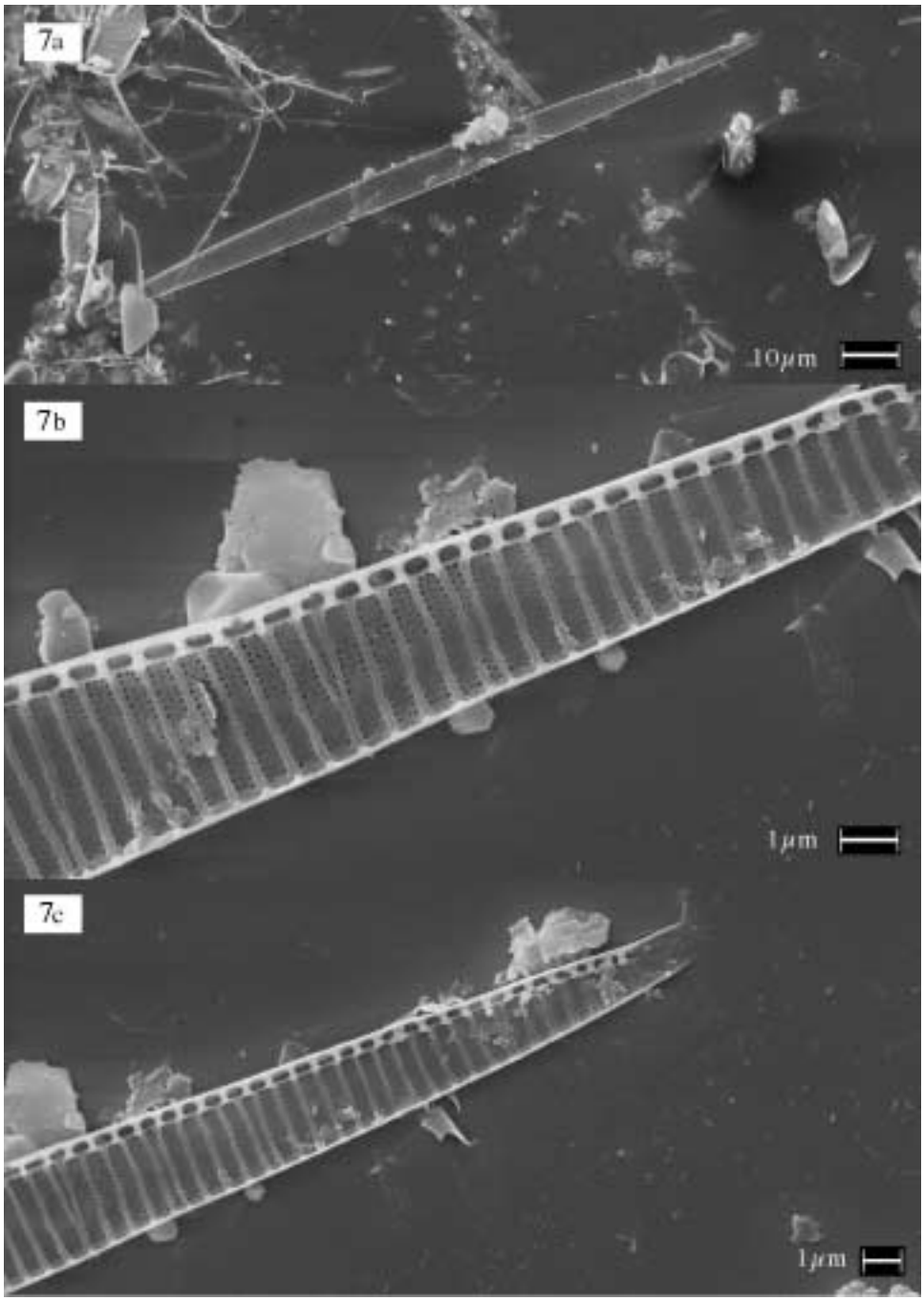

Plate 7. *Pseudo-nitzschia* cf. *seriata* (Cleve) H. Peragallo (species A).

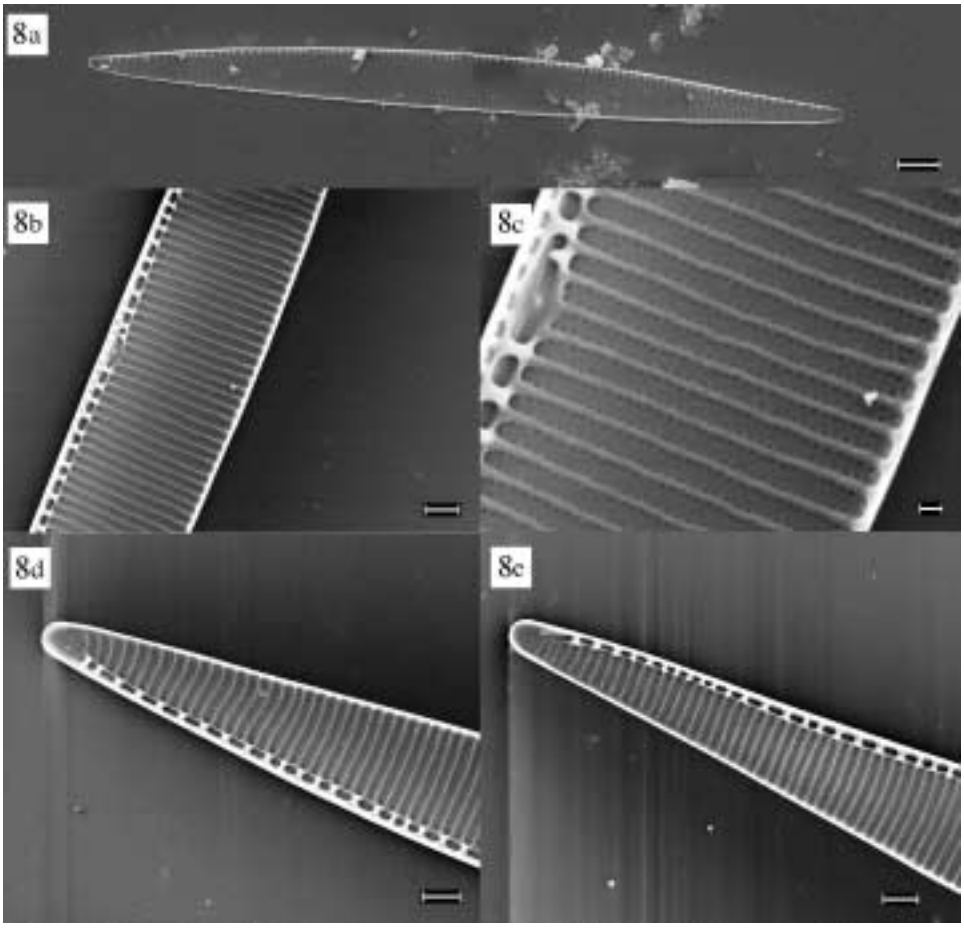

Plate 8. *Pseudo-nitzschia* cf. *subpacifica* (Hasle) Hasle (species G and H, Table 2).

8a-e SEM.

8a. Specimen in net material collected southwest of Long Island Bay, 21st July 1996. 8a. *Pseudo-nitzschia* species G. Whole valve showing asymmetry of the two valve margins: lower margin curved, upper margin straight towards the centre. Valve ends are pointed. Fibulae and striae found in unequal numbers. 8b-e. Specimen present in net material collected south of Cork, 10th October 1997. 8b-e. *Pseudo-nitzschia* species H. 8b-c. Centre of the valve showing the central larger interspace with raphe endings and a central nodule. The striae have 2 rows of minute poroids. 8d-e. Ends of the same valve, similar in structure.

Scale bars 3 µm (8a); 1 µm (8b, 8d, 8e); 0.2 µm (8c)

Spatial and Temporal distribution of Pseudo-nitzschia

Maps were plotted to summarise the spatial distribution of *Pseudo-nitzschia* species described (Figs. 2-3). Even though most of the sampling stations were located around the south and southwest coast of Ireland, the following distributional patterns can be tentatively suggested. *Pseudo-nitzschia fraudulenta*, *P. delicatissima* and *P. pseudodelicatissima* appeared at the more northerly stations whereas other species did not (Fig. 2). *Pseudo-nitzschia* cf. *seriata* and *P.* cf. *subpacifica* appeared to be confined to waters off the southwest coast (Fig. 3).

An overview of the presence of the different *Pseudo-nitzschia* species found in phytoplankton net material during each cruise is presented in Table 3. From the results on species presence or absence on an individual station basis the most commonly occurring species recorded were *P. pungens*, present in 82% of the samples examined, *P. fraudulenta* (76%), *P. australis* (67%), *P. delicatissima* and *P. pseudodelicatissima* (49%), and *P. multiseries* in 41% of the samples. The species least often observed were *P. seriata* (12%) and *P.* cf. *subpacifica* (4%).

Pseudo-nitzschia pungens and *P. multiseries* were present in net material in June, July, August, September and October, *P. australis* in June, July, August, September and October, *P. fraudulenta* in May, June, July, August, September and October, *P. delicatissima* in May, June, July, August and September, *P. pseudodelicatissima* in May, July, August, September and October, while *P.* cf. *seriata* was evident in samples examined in July and August and *P.* cf. *subpacifica* in July and October.

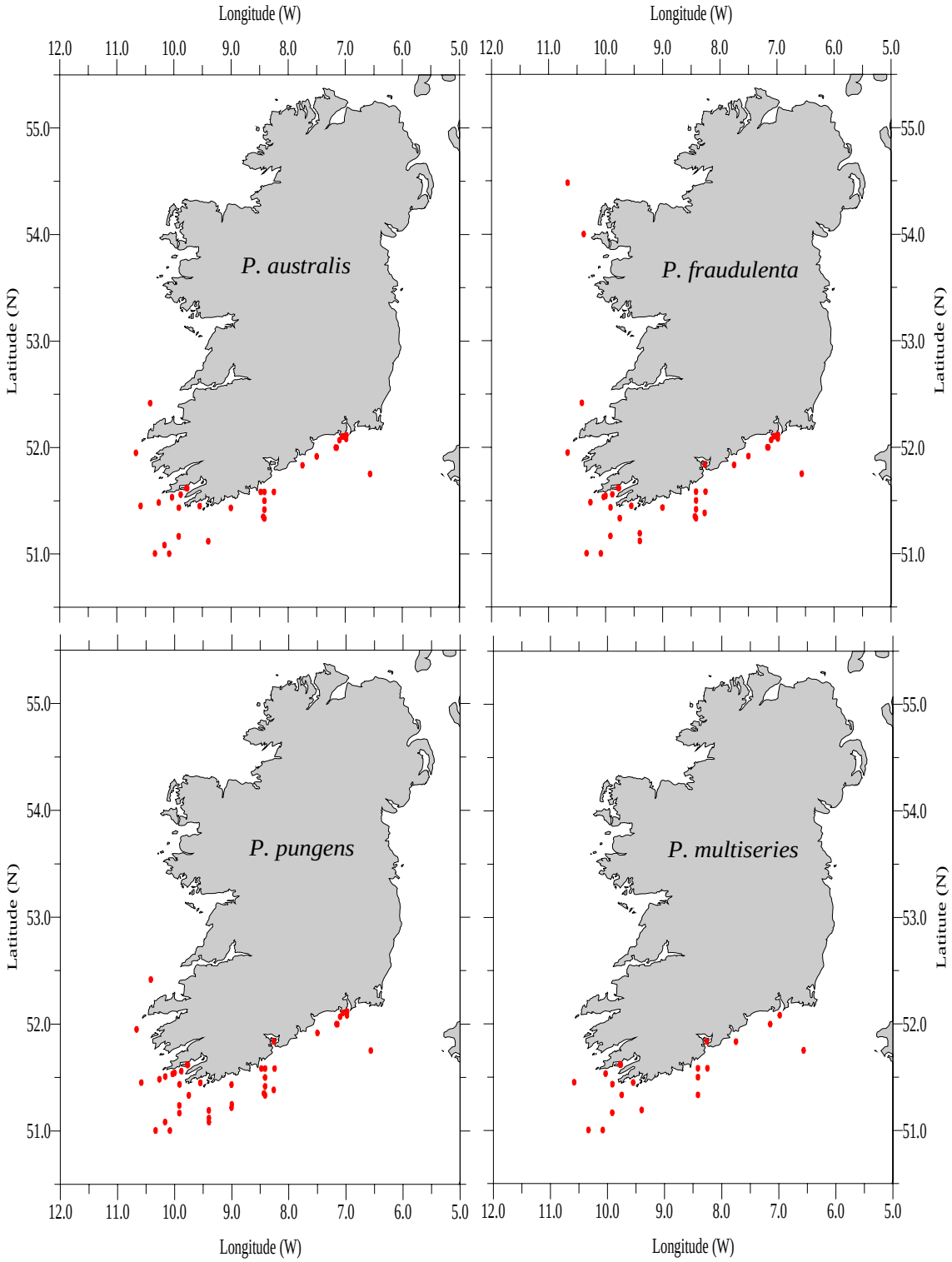

Figure 2. Spatial distribution of *P. australis*, P. *fraudulenta*, *P. pungens* and *P. multiseries*. Red dots indicate stations where species were recorded from net haul samples taken off the south, southwest and west coast of Ireland (1993-1997).

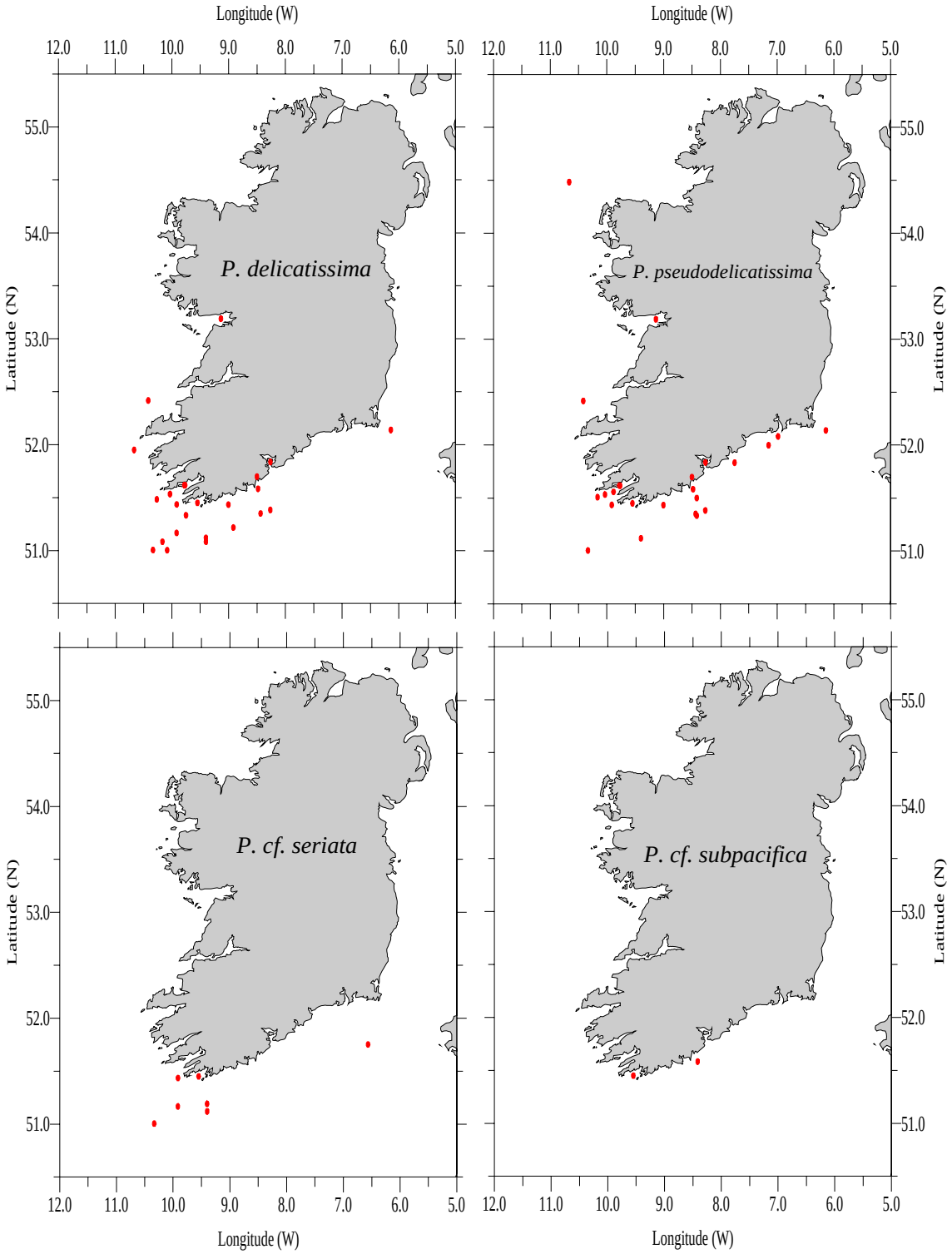

Figure 3. Spatial distribution of *P. delicatissima, P. pseudodelicatissima*, *P.* cf. *seriata* and *P.* cf. *subpacifica*. Red dots indicate stations where species were recorded from net haul samples taken off the south, southwest and west coast of Ireland (1993-1997).

Table 3. Number of stations where individual *Pseudo-nitzschia* species were recorded from phytoplankton net material collected off the south, southwest and west coasts of Ireland (1993-1997). n= number of stations where samples were examined using electron microscopy.

Date	No. of stations examined	*P. pungens*	*P. multiseries*	*P.* cf. *seriata*	*P. australis*	*P. fraudulenta*	*P.* cf. *subpacifica*	*P. delicatissima*	*P. pseudo-delicatissima*
Surveys with >3 stations examined									
Aug 1993	n=10	10	3	1	3	6	0	3	2
July 1996	n=15	13	9	5(?)	12	11	1	11	13
Sept 1996	n=8	6	2	0	5	5	0	8	4
Oct. 1997	n=12	10	5	0	12	12	1	0	3
Surveys with <3 stations examined									
June 1995	n=1	1	1	0	1	1	0	1	0
May 1996	n=1	0	0	0	0	0	0	1	1
May 1997	n=2	0	0	0	0	2	0	0	1
Total occurrence	n=49	40	20	6	33	37	2	24	24

DISCUSSION

Morphological analysis

Ultrastructural examination of samples collected during the course of this investigation provided the first identification records of the diatom *Pseudo-nitzschia* down to species level in Irish waters. At least six *Pseudo-nitzschia* species were abundant during certain times of the year in waters off the south, southwest and west coasts of Ireland. These included *P. pungens*, *P. multiseries*, *P. fraudulenta*, *P. australis*, *P. delicatissima* and *P. pseudodelicatissima*. The morphometric data recorded for these species also complies with well known taxonomic guides for *Pseudo-nitzschia* (Hasle et al. 1996, Hasle and Syvertsen 1997). During the morphological investigations a small number of *Pseudo-nitzschia* valves examined showed similarities to *P. seriata* (*P.* cf. *seriata* and *P.* cf. *obtusa*) and *P. subpacifica.* Confirmation of the identity of these uncommon species requires further investigation because it is very difficult to unambiguously verify their presence based on observations of less than 4 valves per species. This is especially true

if the specimens examined like the *P.* cf. *seriata* valves in this study are not completely stripped of the organic components of the cell wall or if silica dissolution is apparent. However, the morphometric measurements of the *P.* cf. *seriata* valves observed (see Table 2) appear to be in keeping with the literature. Although very similar in structure, the most obvious feature distinguishing the two forms of *P. seriata* is the number of rows of poroids per stria. *Pseudo-nitzschia seriata* f. *seriata* has 3-4 rows of poroids while *P. seriata* f. *obtusa* has only 2. Other structural differences which distinguish *P. seriata* f. *obtusa* from the nominate form include the fact that its valve ends are more rounded and the interstriae of the valve face are placed closer together (Hasle et al. 1996). However, *P. australis* can be easily confused with *P. seriata* f. *obtusa* (see morphological data in Tables 1 and 2) as it is quite common to see valves from smaller cells of *P. australis* with an asymmetrical outline. Both species have two rows of hymenate poroids per stria. *Pseudo-nitzschia seriata* f. *obtusa* however, has more poroids (7-8) in 1 µm than *P. australis* (4-5). In this study 6 out of 127 valves identified as *P. australis* had 6 poroids in 1 µm. It is therefore possible that the tentatively identified *P.* cf. *seriata* f. *obtusa* valve, species E, in Table 2 was an asymmetrically shaped *P. australis* valve as its length, 126 µm is more in keeping with records for *P. australis* then *P. seriata* f. *obtusa*. One of the best ways to differentiate between these two species is by observing the girdle bands. The band striae of *P. australis* normally have more rows of poroids then those found in *P. seriata* (Hasle et al. 1996). However, this type of confirmation can be very difficult when working with field samples containing a mixture of *Pseudo-nitzschia* species since the girdle elements can break apart after acid cleaning and this information is lost. In this study the band structure was not observed for any of the tentatively identified *Pseudo-nitzschia* species. What is known is that the presence of *P. seriata* in Irish waters is questionable and needs to be confirmed.

The morphological data recorded for the two tentatively identified *P. subpacifica* valves from net material collected in July 1996 and October 1997 are in keeping with previously published data, with the exception of the valve width (see Table 2). This raises uncertainty concerning its identification. Distinguishing between *P. subpacifica* and *P.*

heimii can be somewhat problematic and identification can be easily confused as they are very similar in structure (Hasle 1972). Both species are asymmetrical with respect to the apical axis, they possess a central interspace and have an unequal density of interstriae to fibulae. Differences are very subtle; *P. subpacifica* is not as heavily silicified, can have a different number of fibulae and striae in 10 µm and the valves are shorter while the width is wider than reported for *P. heimii* (Hasle et al. 1996). Hasle (1972) reported that some specimens that were examined from the Gulf of California off San Diego and in the North Pacific showed similarities to both *P. subpacifica* and *P. heimii*. Her observations of *P. heimii* from the North Pacific differed in the shape of the valve from specimens of the subantarctic and Antarctic. Future studies combining morphological and molecular biological data might help resolve why different morphotypes are recorded.

Temporal and Spatial Distribution

The seasonal patterns displayed by *Pseudo-nitzschia* species in Irish waters correspond well with other studies in European temperate waters (Hasle et al. 1996, Míguez et al. 1996, Campbell et al. 2001).

Pseudo-nitzschia pungens, a cosmopolitan species present in coastal waters worldwide (Hasle and Fryxell 1995) is often present throughout the year and is found in high cell densities during autumn in northern European waters (Hasle et al. 1996). This species was especially abundant in the *Pseudo-nitzschia* flora off the south and southwest coasts of Ireland during July (1996) and August (1993). *Pseudo-nitzschia pungens* was also present, though less abundant, in June (1995) September (1996) and October (1997). This species and *P. multiseries* regularly co-occurred in the net samples although *P. multiseries* was observed only as a minor component of the *Pseudo-nitzschia* population. This type of co-occurrence has been reported in other studies where *P. pungens* is the more predominant of the two species in summer and autumn when thermal stratification is evident and is replaced by *P. multiseries* during colder times of the year from late autumn to spring (Fryxell et al. 1990, Dickey et al. 1992, Hasle et al. 1996). As most of the sampling in this study was carried out during the summer and autumn months when thermal stratification was particularly evident offshore, the predominance of *P.* pungens

over *P. multiseries* lends further support to this view. The sparse occurrence of *P. multiseries* is most likely due to the fact that none of the sampling cruises were carried out in the colder months (November to March) of the year. Since *P. multiseries* blooms have the potential to produce high concentrations of domoic acid and this species was the causative organism in toxic outbreaks of Amnesic Shellfish Poisoning during the winter months off the east coast of Canada (Bates et al. 1989), it is imperative that further investigations into its distribution off the Irish coast are carried out, particularly during the winter and spring.

Off the south and southwest coasts of Ireland *P. australis* was regularly abundant especially in autumn (September and October) when the thermocline was well developed. In Europe, blooms of *P. australis* have been recorded during September (1994) along the Iberian Shelf in the North Atlantic (Míguez et al. 1996) and it was observed in net material during August and October 1999 in northwestern Scottish waters (Campbell et al. 2001). In the North Pacific, this species is a regular component of the marine flora off the west coast of America during spring and autumn (Buck et al. 1992, Villac et al. 1993). The observation of *P. australis* around Ireland is significant and its occurrence is important as this species is potentially very toxic and has been implicated as the source of domoic acid in shellfish from other European countries including Spain and Scotland (Míguez et al. 1996, Campbell et al. 2001). Blooms of this diatom have also been responsible for poisoning sea lions and marine birds off the coast of California at times when relaxed periods of upwelling are evident (Scholin et al. 2000).

Pseudo-nitzschia fraudulenta was frequently observed in net material off the Irish coast from spring to autumn but was especially predominant in October 1997. It is thought that this species has no particular seasonal pattern (Hasle 1972). Hasle et al. (1996) recorded this species as a smaller component of the *Pseudo-nitzschia* population from samples examined over 13 years, from 1980 to 1993, in north European waters. High cell concentrations were however observed during November 1989. It has also been recorded from Scottish waters in August and October (Campbell et al. 2001). *Pseudo-nitzschia*

fraudulenta has at times been shown to be the predominant *Pseudo-nitzschia* species present in high concentrations off the west coast of America, after *P. australis* blooms in autumn (Fryxell et al. 1997). Although most investigations of this species in culture have shown that *P. fraudulenta* does not produce domoic acid above the levels of detection, a culture study of an isolate from New Zealand confirmed that this species could produce low levels of the toxin (Rhodes 1998).

The smaller species, *P. pseudodelicatissima* and *P. delicatissima*, were the only representatives from the "P. delicatissima" group found in Irish waters. In northern European waters *P. delicatissima* displays a winter-spring temporal distribution while *P. pseudodelicatissima* frequently occurs throughout the year from June to December (Hasle et al. 1996). Results from this study show that both species are regular components of the phytoplankton community from May to October, and were predominant in July 1996. *Pseudo-nitzschia delicatissima* was also abundant in May and September of this year. Although these species do not have a large cell biovolume, blooms of toxic *P. pseudodelicatissima* lasting 2 months in well mixed waters have caused DA build-up in shellfish in Canada (Martin et al. 1990).

Pseudo-nitzschia seriata f. *seriata* is characteristic of winter and spring in northern European waters although it has also been recorded in the Skagerrak during autumn (Hasle et al. 1996). The presence of this cold water to temperate species in Irish waters needs to be confirmed since cultures of *P. seriata* f. *seriata* isolated from Danish waters have shown to produce domoic acid at similar levels to those recorded for *P. multiseries* cultures from Canada (Lundholm et al. 1994). Neither *P. seriata* f. *obtusa*, nor *P. subpacifica* were recorded in the extensive records of Hasle et al. (1996) for the Skagerrak, North Atlantic and adjacent waters. *Pseudo-nitzschia seriata* f. *obtusa* is regarded by Hasle and Syvertsen (1997) as a northern cold water species. *Pseudo-nitzschia subpacifica* is apparently an important element of the plankton of the Atlantic south of 50˚N with a northern most limit of 51˚N (Hasle 1972). Several dinoflagellates that have a warm temperate to sub-tropical distribution are frequently recorded off southwestern Ireland. These are common on the outer side of the Irish shelf front, but

can be mixed across the front into coastal water (Roden 1984, Raine and Joyce 1996). It has been proposed that the presence of these dinoflagellate species is wholly or in part due to transport with the northward slope current (Raine, White and Dodge in press). The presence of *P. subpacifica* off Ireland could also be due to a similar transport mechanism.

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CHAPTER 3

HYDROGRAPHIC CONDITIONS RELATING TO THE DISTRIBUTION OF THE DIATOM *PSEUDO-NITZSCHIA* IN IRISH WATERS

Caroline Cusack,
Marine Institute Phytoplankton Laboratory, Martin Ryan Institute, National University of Ireland, Galway, Ireland

Robin Raine, Shane O'Boyle and John W. Patching
Martin Ryan Institute, Marine Microbiology Department, National University of Ireland, Galway, Ireland

ABSTRACT

The presence and abundance of the potentially toxic diatom *Pseudo-nitzschia* was investigated in relation to physical and chemical oceanographic features off the Irish coast using data collected from research cruises carried out between 1993-1997. *Pseudo-nitzschia* populations were found to be most abundant in moderate to well stratified waters off the south and southwest coasts with cell densities in the order of 10^3 cells.mL^{-1} recorded during August 1993, July 1996 and October 1997. These blooms were only evident on the coastal side of the Irish Shelf Front positioned off the southwest coast of Ireland although in May 1997 low concentrations (18 cells.mL^{-1}) of *Pseudo-nitzschia* were recorded on the oceanic side of this thermohaline front. An exceptional bloom of *P. delicatissima* occurred in the surface brackish waters of Galway Bay off the West coast in May 1996. In general, no obvious relationship between nutrients, temperature or salinity and the presence of *Pseudo-nitzschia* was observed, most likely due to the fact that an array of species were often present in the water column at any one time. Two distinct groups of *Pseudo-nitzschia* species were recorded on either side of the tidally driven Celtic Sea Front off the southeast coast of Ireland. Overall, *Pseudo-nitzschia* species were found in warm nutrient depleted waters with the "P. delicatissima" group observed over a wider range of salinity and temperature than the "P. seriata" group. In general, the appearance of large populations of *Pseudo-nitzschia* spp. in Irish waters occur when environmental conditions reflect the interlude between the well mixed waters associated during the spring bloom period and the deep seasonal stratification that materialises in summer.

Key words: Pseudo-nitzschia, distribution, Ireland, coastal waters

INTRODUCTION

Numerous surveys have attempted to investigate the biogeography and abundance of *Pseudo-nitzschia* spp. (see reviews by Hallegraeff 1994, Hasle et al.1996, Skov et al. 1999). These investigations in the main have resulted from the increased interest in this genus since *P. multiseries* was found to produce the amnesic shellfish toxin domoic acid (DA) during the late 1980s. Only a few studies have, however, tried to relate local hydrographic and environmental factors to the temporal and spatial variability of *Pseudo-nitzschia* (Buck et al. 1992, Zou et al. 1993, Fryxell et al. 1997, Dortch et al. 1997, Scholin et al. 2000).

It is now thought that water column stability and nutrient availability play important roles in promoting the growth of *Pseudo-nitzschia*. Dortch et al. (1997) found that *Pseudo-nitzschia* was more abundant in warm weak to moderately stratified shelf waters of the Gulf of Mexico than in the adjacent Terrebonne Bay estuary. Highest cell densities were recorded in the spring with smaller blooms evident in the autumn. Upwelling events and rough weather conditions in the Gulf were thought to be important processes in influencing the onset of *Pseudo-nitzschia* blooms, since these events result in the destabilisation of the water column allowing nutrient entrainment from deeper waters below the pycnocline (Dortch et al. 1997). Taylor et al. (1994) found that *P. pungens* was the predominant *Pseudo-nitzschia* species during summer in the Sechelt Inlet, Western Canada and its presence was associated with stratified waters depleted in ammonia and nitrate.

In Monterey Bay, USA *Pseudo-nitzschia* blooms were found during the spring, a time when advection of water offshore results in large scale upwelling in the region (Buck et al. 1992, Walz et al. 1994). Persistent blooms dominated by *P. australis* have been reported here in late summer and autumn when nutrient concentrations are indicative of post-upwelling events, surface waters are warm (>13.0°C) with low salinity (33.3-33.5 ppt) and the water column is moderately stratified (Buck et al. 1992, Walz et al. 1994).

In the Bay of Fundy (E. Canada) *P. pseudodelicatissima* is present all year round with blooms peaking in late summer when the surface water temperature averages at approximately 10.0°C. In autumn smaller blooms occur when the surface temperature increases up to 18.0°C (Martin et al. 1993). Here, high tidal velocities result in short flushing times of the bay and it is thought that nutrients are not a limiting factor for phytoplankton growth.

Riverine input has also been suggested as one of many possible conditions that govern the seasonal changes of *Pseudo-nitzschia* abundance at inshore stations in the Gulf of Mexico, but since neritic environments are very complex, often a result of local hydrography and tidal influences, this alone could not explain the seasonal differences observed (Dortch et al. 1997). Nutrient loading (NO_3 and PO_4) from rivers has been shown to enhance the biomass of *Pseudo-nitzschia* species (*P. multiseries*) in other parts of the world (Zou et al. 1993). In Canada, dense populations of *P. multiseries* have been recorded when nitrate pulses were associated with heavy rainfall after very dry summers (Bates et al. 1998). During 1998 in early summer, Scholin et al. (2000) observed the development of a *P. australis* bloom in Monterey Bay and thought it might have been linked with a water body that had increased levels of silicate suggestive of freshwater runoff.

The present study examined the distribution of the diatom *Pseudo-nitzschia* in relation to local oceanographic conditions in Irish waters. The sampling cruises extended over a relatively large area covering both shelf and coastal environments. As discussed by Raine and McMahon (1998) and McMahon et al. (1995), the oceanography off the Irish coast is complex. A weak clockwise coastal current, the Irish Coastal Current (ICC) flows around the south, southwest and western coasts and phytoplankton distribution in these areas is chiefly governed by advective processes. Phytoplankton populations are often advected into the bays along the route, caused by entrainment via wind driven mechanisms (Raine and McMahon 1998). Off the west and southwest coasts a "surface-to-bottom" salinity driven thermohaline front (a type I front as described by Hill and Simpson 1989) separates this coastal water from water of more oceanic character (salinity

>35.30), lying approximately 30 km away from the coastline. Seasonal thermal stratification develops over the entire region in summer, with the exception of a narrow band of mixed water, ca. 2 km wide, formed by coastal mixing. Diatom populations are common both in the mixed coastal water and following upwelling events off the southwest coast when nutrients are replenished from the deeper oceanic waters (Raine and Joyce 1996). Off the southeast coast of Ireland the dominant hydrographic feature is the Celtic Sea Front (CSF), a tidal front (a type II front as described by Hill and Simpson 1989) that separates Celtic Sea water from the Irish Sea. Biological investigations in this region have shown that diatom populations are typical of the microflora on the Irish Sea side of the front. When the water column of the Celtic sea is well stratified during the summer, the phytoplankton is typically characterised by dinoflagellate communities (Raine and McMahon 1998).

As a thriving shellfish industry exists along the south, southwest and west coasts of Ireland (McMahon et al. 1998) the work presented here also intended to assess the possible implications of potentially toxic blooms of *Pseudo-nitzschia* for the aquaculture industry.

MATERIALS AND METHODS

Data was collected from several research cruises that took place off the coast of Ireland between 1993-1997. Figures 1-5 present the locations of stations sampled during each cruise. Additional samples were taken off the southwest coast of Ireland (June 1995) and in Galway Bay (May 1996).

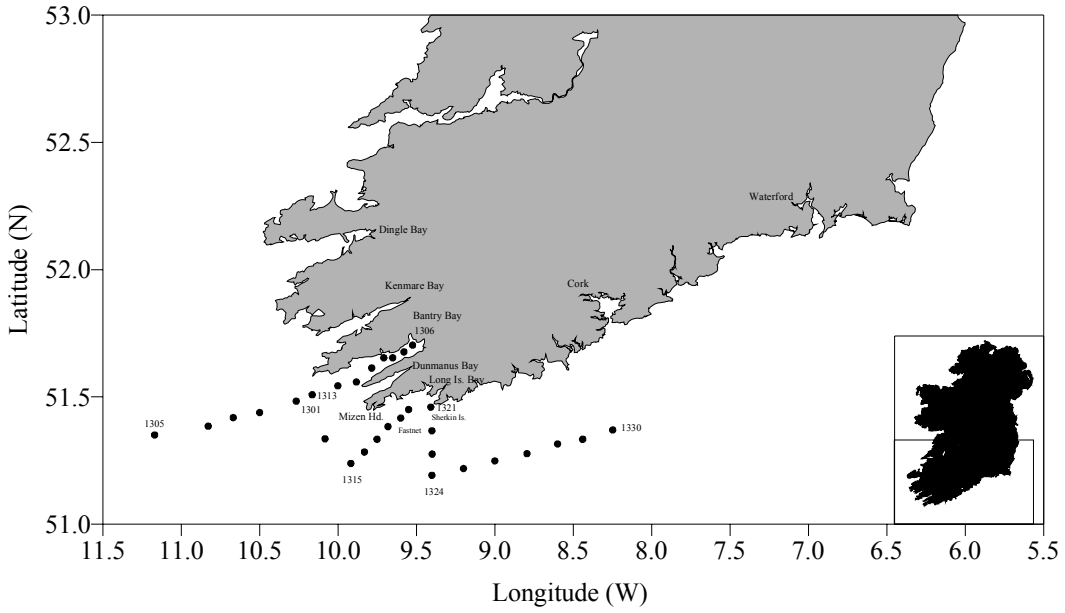

Figure 1. Location of stations sampled off the southwest coast of Ireland, between August 8th-18th 1993. Station details can be found in Appendix 7.

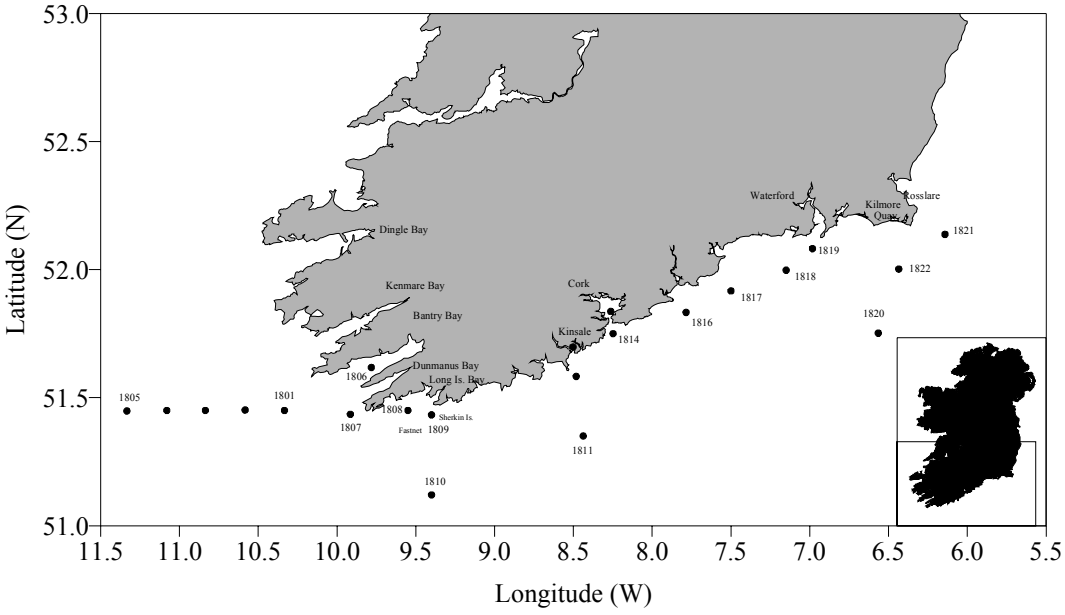

Figure 2. Location of stations sampled off the southeast, south and southwest coasts of Ireland between July 20th-25th 1996. Station details can be found in Appendix 7.

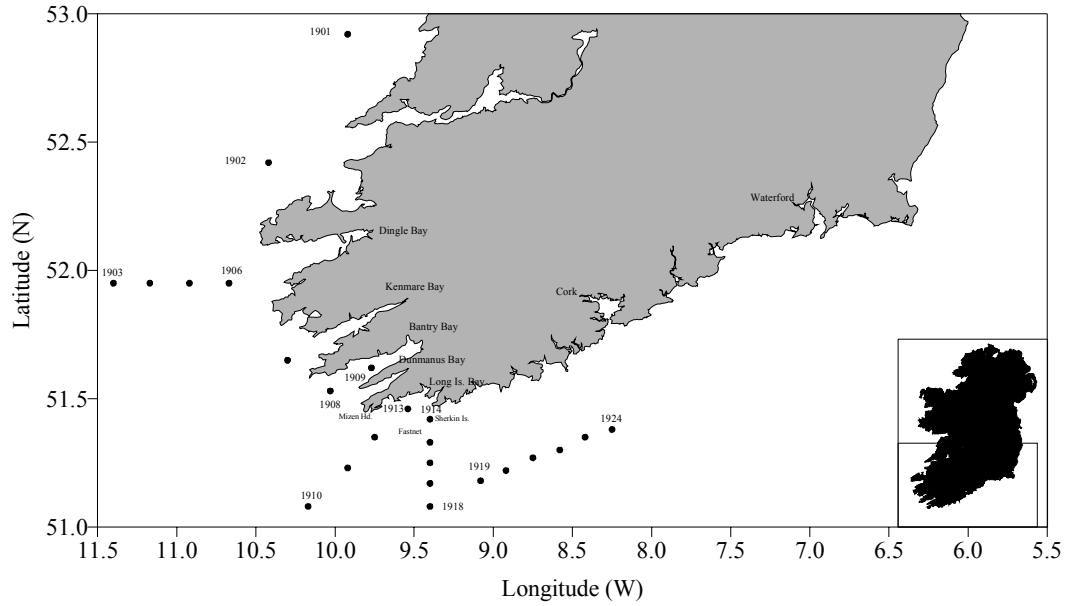

Figure 3. Location of stations sampled off the southwest coast of Ireland between September 3rd-8th 1996. Station details can be found in Appendix 7.

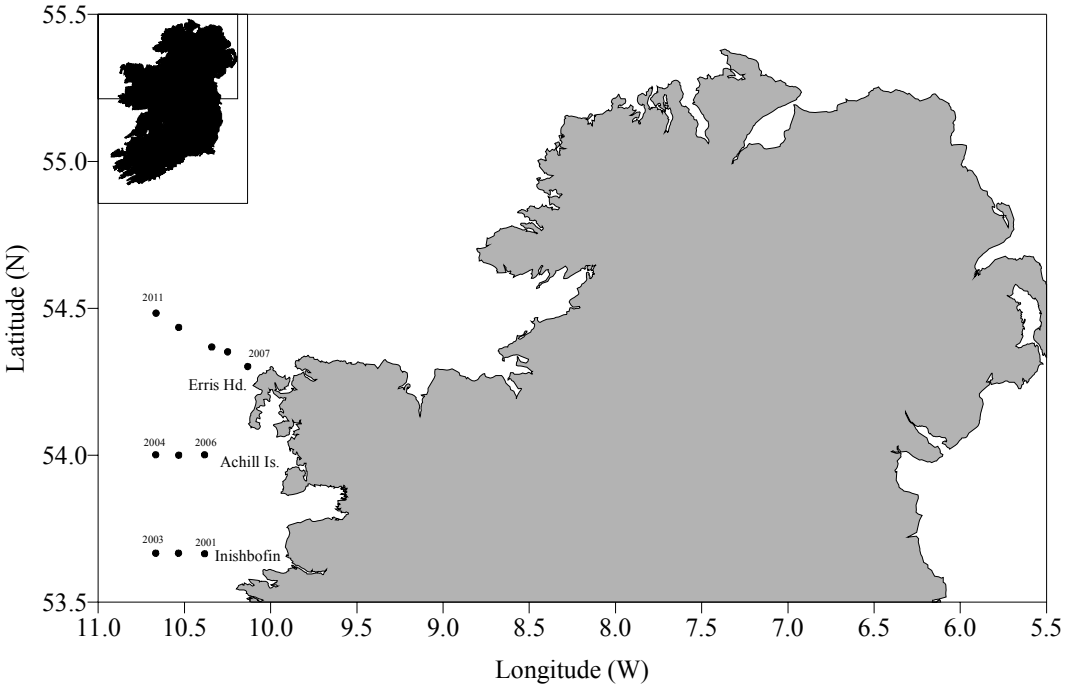

Figure 4. Location of stations sampled off the northwest coast of Ireland between May 1st-5th 1997. Station details can be found in Appendix 7.

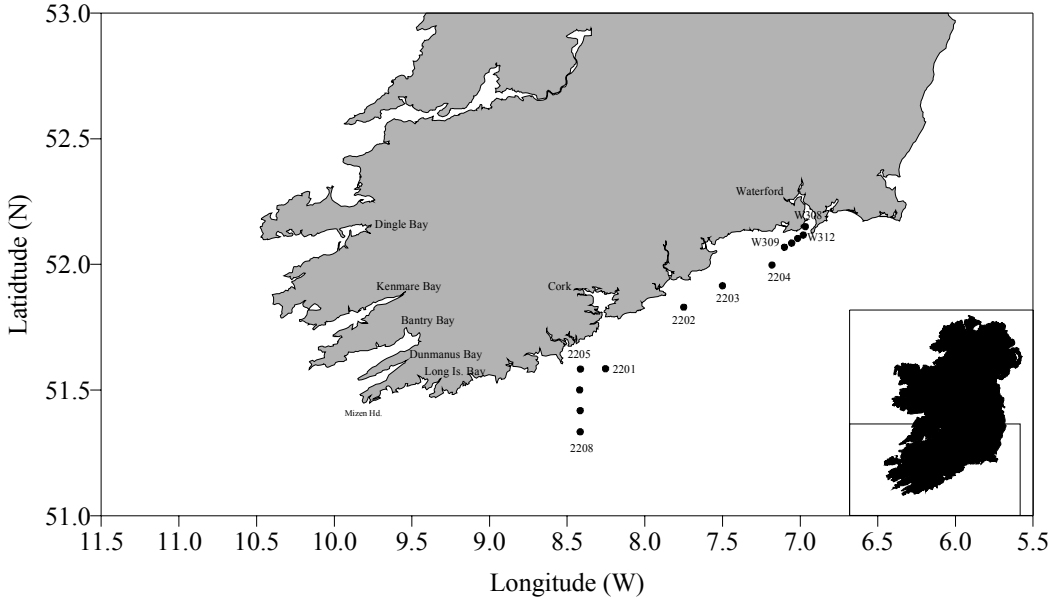

Figure 5. Location of stations sampled off the south coast of Ireland, between October 7th-12th 1997. Station details can be found in Appendix 7.

During each cruise, surface parameters such as chlorophyll fluorescence (Turner-Design Model 10 fluorometer) and salinity (Sea-Bird SBE 20 Thermosalinograph) were measured continuously on water pumped from a depth of 2 meters. Temperature was measured by a sensor (Furuno) located in the ship's hull. Output signals from the instruments were interfaced with the Data Acquisition System (DAS) on board the research vessel. The records of date, time, position (GPS) and depth were included in the logged data files. Vertical profiles of temperature and salinity were made at each station using a Sea-Bird SBE-19 CTD deployed off a hydrographic winch. Attached to the CTD was an *in situ* fluorometer (Sea Tech, Oregon) providing chlorophyll fluorescence data. Both instruments were multiplexed to a host microcomputer giving real-time displays. Temperature readings were calibrated using reversing thermometers attached to 2.5 L Hydro-Bios (Kiel, Germany) water sampling bottles. Salinity readings were calibrated against discrete water samples, whose salinity was measured using a high precision salinometer (Guildline Autosal Salinometer Model 8400B) at the Central Marine Services Unit, National University of Ireland, Galway. Salinities were

determined on the practical salinity scale, UNESCO 1978 (UNESCO. 1981) and are hereafter quoted without units. The samples were collected using 200 mL plastic screw-cap borosilicate glass bottles with plastic seal inserts. The bottles were rinsed 3 times with seawater from the sample bottle before being filled to the neck. To prevent salt crystal build-up on the plastic insert and screw cap lid these were wiped with damp tissue paper before closing the bottle.

Discrete depths were chosen from the CTD and fluorescence profiles for sampling with water bottles. Typically these were:

a) The surface
b) The mixed layer above the pycnocline
c) The sub-surface chlorophyll *a* maximum
d) The bottom mixed layer

Extra depths were included at stations depending on water column depth and the detail in the CTD output. Surface water samples were collected using a clean plastic bucket. Subsurface samples were taken using the water bottles, from which sub-samples were taken for analysis of salinity, nutrients, chlorophyll and phytoplankton cell counts. Water samples for nutrient analysis were filtered through Whatman GF/C filters (0.45 μm pore diameter) to remove particulate matter. The filtrates were transferred into acid-washed 500 mL plastic bottles and stored at –20.0ºC. The frozen water samples were thawed and analysed for nitrate, phosphate and silicate using the methods described by Grasshoff (1976). 250 mL aliquots of seawater for chlorophyll *a* analysis were filtered through Whatman GF/F glass fibre filters (nominal 0.22 μm pore diameter). The filters were immediately stored in 15 mL plastic centrifuge tubes (Sarstedt, Germany) in the dark at –20.0ºC to prevent photodegradation of the pigments. Chlorophyll *a* was determined fluorometrically after pigment extraction with 90% acetone following the procedure in Tett (1987). The fluorescence signal was measured using a Turner Designs Model 10 fluorometer, calibrated with a stock chlorophyll solution of known (HPLC-derived) concentration. Secchi depth was recorded at all stations sampled during daylight.

Quantitative phytoplankton counts were carried out within 3 to 4 weeks of sampling. Phytoplankton samples were allowed to settle overnight in 60 mL tissue culture bottles (Sterilin, U.K.) or 25 mL sedimentation chambers (Hydro-Bios, Kiel). *Pseudo-nitzschia* cell numbers (cells with intact chloroplasts) were counted using a modified version of Utermöhl's method (Hasle 1978). This was carried out under phase contrast light microscopy (LM) using a Nikon Diaphot or Nikon TMS inverted LM with a 20x dry phase contrast (numerical aperture, NA 0.4) objective lens. *Pseudo-nitzschia* cell concentrations were divided into two groups, "P. seriata" (width >3 μm) and "P. delicatissima" (width <3 μm) following Hasle (1965). Net samples containing high cell concentrations of *Pseudo-nitzschia* were acid cleaned (cf. Boyle et al. 1984) and examined under a scanning electron microscope (Leica S430; accelerating voltage 15 kV or Hitachi S-570; accelerating voltage 20 kV) and a transmission electron microscopy (Hitachi-7000, accelerating voltage 75 kV).

The potential energy anomaly, phi (Φ) an index of bulk water column stratification was calculated using the equation (1) after Simpson et al. (1982):

$$\Phi = \frac{1}{h}\int_{-h}^{0}(\overline{\rho} - \rho)g.zdz : \overline{\rho} = \frac{1}{h}\int_{-h}^{0}\rho dz \qquad (1)$$

where h is the water column depth (m), $\overline{\rho}$ is the mean seawater density (Kg.m^{-3}) between 0 and h metres, ρ is the water density at depth z metres and g is the acceleration due to gravity (m.s^{-2}). Values of Φ <10 J.m^{-3} indicate that the water column is well mixed, Φ = 10-30 J.m^{-3} indicate that the water column is transitionally (weakly-moderately) stratified and Φ > 30 J.m^{-3} indicate that the water column is well stratified. Water clarity scaled with depth was expressed by λ.h, where λ is the diffuse attenuation coefficient for downwelling irradiance. Attenuation coefficients, λ, were calculated from 1.7/secchi depth (Parsons *et al*., 1984). Where stations were deeper than 100 m, the 100 m value has been used as the water column depth.

RESULTS

Distribution of Pseudo-nitzschia in relation to local Hydrographic conditions

Summaries of local hydrographic features and *Pseudo-nitzschia* distribution derived from each research cruise are presented below. For ease of interpretation these are presented in consecutive order. The first set of samples used in this study were taken in August 1993 and a series of surveys followed up until October 1997.

Full Station data, Secchi depth readings, temperature, salinity, inorganic nutrients, chlorophyll, *Pseudo-nitzschia* cell concentrations at discrete depths, *Pseudo-nitzschia* species identification, calculated values for phi, (Φ) and Delta-t are presented in tables in Appendix 7. Figures showing the vertical profiles of nutrients, chlorophyll and *Pseudo-nitzschia* cell concentrations are also included in Appendix 7. Pseudo-nitzschia species recorded from net material at a number of stations are also presented in Appendix 7.

August 1993

A phytoplankton and hydrographic survey in shelf waters off the south and southwest coast of Ireland took place between August 13th-22nd 1993. Temperatures at all stations (1301-1330, Figure 1) ranged from a maximum of 16.1-16.2°C at the surface of stations 1308 off the southwest coast and 1330 positioned south of Cork to 9.3°C at a depth of 91 m at station 1330. The overall range in salinity was from 34.7 at the surface of station 1330 to 35.4 at depth (>54 m) at station 1315. Thermal stratification was evident throughout the survey region but was more pronounced at stations away from the coast with temperature differences of up to 4.0°C across the thermocline situated between 20-35 m. At some of the coastal stations (1320-1321) stratification was less well developed as a result of increased tidal stirring associated with the shallower stations (Figure 6).

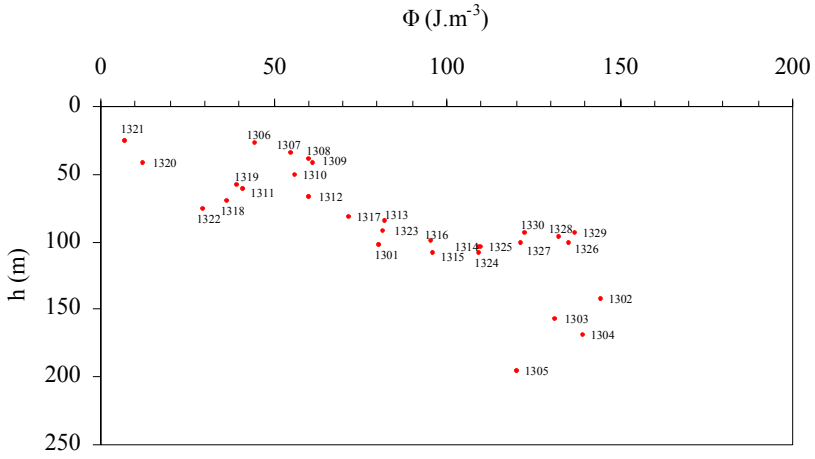

Figure 6. Plot of stations against water column depth, h, and water column stability expressed as the stratification parameter, Φ, off the south and southwest coasts of Ireland between August 8th-18th 1993. Station positions are shown in Figure 1.

The distribution of temperature and salinity from this survey has already been described in detail by Raine and McMahon (1998). In summary the Irish Shelf Front, a surface to bottom salinity front separating NE Atlantic water from Irish coastal water was represented by the position of the 35.3 isohaline (Figure 7). Its surface expression was just inshore of station 1305 at 11º 05'W. The front takes on an S-shape configuration in summer and its bottom boundary was found close to the coast at station 1301. The bottom boundary was also evident off the south coast beneath the thermocline where the water body was more saline (>35.3) and warmer (>10.0°C) to the west than to the east where salinity values were <35.1 and temperature was <9.5°C (Figure 8). The latter water body, referred to by Raine and McMahon (1998) as Northern Celtic Sea Water (NCSW) circulates to a greater or lesser extent clockwise around southwest Ireland. This feature was observable as a mid-water jet from the TS record at 50 m at station 1316 (Figure 9). This circulation pattern could be the reason why the thermocline was so shallow (20-25 m depth) southwest of Fastnet Rock, which may have resulted in the uplift in the thermocline outside Bantry Bay (station 1301).

Nitrate, phosphate and silicate concentrations were relatively depleted in the surface layer at nearly all stations while concentrations increased below the thermocline. At stations 1301, 1312, 1313 and 1314 however, surface nutrient concentrations were moderate to high. Nitrate concentrations ranged from below the limit of detection at the surface of stations 1325 and 1328 up to 10.2 μM at 50 m at station 1325. Similarly phosphate ranged from 0.08-0.89 μM and silicate from 0.6-8.8 μM. Chlorophyll data (Appendix 7) showed subsurface maxima associated with the thermocline, the highest being recorded at station 1313 with a value of 12.2 $mg.m^{-3}$ chlorophyll *a*. Surface waters showed low chlorophyll concentrations ranging from 0.5-1.5 $mg.m^{-3}$ while in bottom waters >60 m levels were low with typical concentrations of 0.01 $mg.m^{-3}$.

In general, diatoms including *Pseudo-nitzschia* spp. (Figure 10) were widely distributed throughout the area sampled. *Pseudo-nitzschia* were numerically dominant at stations 1312 and 1314 within the subsurface chlorophyll maxima where chlorophyll levels were 9.6 $mg.m^{-3}$ at 18 m (1312) and 11.5 $mg.m^{-3}$ at 15 m (1314). *Rhizosolenia hebetata* was however, the dominant phytoplankton in terms of biomass (up to 75%) at these two stations. This diatom was numerically dominant at stations 1313, 1301 and 1315. Off the south coast, along stations 1326-1330, *Leptocylindrus danicus* predominated. It is likely that the diatom dominance resulted from previous coastal upwelling.

Pseudo-nitzschia cell numbers in relation to temperature, salinity and chlorophyll are also shown in Figures 7-9. *Pseudo-nitzschia* was not detected at stations 1304, 1303 and 1302 (no phytoplankton data are available from station 1305). Cells were however, recorded at the surface and in the pycnocline at stations 1301, 1313 and 1312.

The upper mixed layer at station 1301 showed 1.5-60 $cells.mL^{-1}$ of "P. seriata" (*P. pungens, P. australis* and *P. fraudulenta*[†]) and 0.2-6.3 $cells.mL^{-1}$ of "P. delicatissima" (*P. delicatissima*). The high cell numbers of *Pseudo-nitzschia* in the top 20 m was associated with a rise in the position of the 12.0°C isotherm (Figure 7). Concentrations of nitrate (5.4 μM), phosphate (0.45 μM) and silicate (4.4 μM) at this depth were high. At station

[†] The *Pseudo*-nitzschia species referred to were those observed in net material.

1313 (12m), "P. seriata" (*P. pungens*) was recorded at cell densities of 135 cells.mL^{-1} and "P. delicatissima" (*P. pseudodelicatissima*) at 5 cells.mL^{-1}. High chlorophyll levels (12.2 mg.m^{-3}) were recorded at the thermocline (20 m, 12.0°C isotherm) at station 1313 where "P. seriata" (*P. pungens*) and "P. delicatissima" (*P. pseudodelicatissima*) cell densities were 95 cells.mL^{-1} and 2 cells.mL^{-1}, respectively. At station 1312, 400 cells.mL^{-1} of "P. seriata" (*P. pungens* and *P. fraudulenta*) were found within the subsurface chlorophyll maximum (18 m depth). "Pseudo-nitzschia delicatissima" was also recorded at this depth (330 cells.mL^{-1}) but was not observed in the net material.

Station 1314 (data not shown), southwest of Dunmanus Bay, was thermally stratified with a surface to bottom temperature difference of 4.0°C. The thermocline was shallow (15-20 m) and a sample taken from 15 m was high in nutrients and chlorophyll. Highest cell densities of *Pseudo*-nitzschia were also found here with 300 cells.mL^{-1} of "P. seriata" (*P. pungens*, *P. multiseries*, *P. fraudulenta* and *P. australis*) and 400 cells.mL^{-1} of "P. delicatissima" (*P. delicatissima*).

Pseudo-nitzschia cell numbers increased in a westward direction (1330-1324) with the exception of a sample taken at the subsurface fluorescence maximum (25 m) at station 1326 where the NE Atlantic seawater and NCSW met (Figure 8). Here, cell densities of up to 180 cells.mL^{-1} of "P. delicatissima" and 360 cells.mL^{-1} of "P. seriata" were found. *Pseudo-nitzschia pungens* was the only species identified from net samples. At stations 1324 and 1325, at a depth of between 10-20 m, 20-40 cells.mL^{-1} of both the "P. seriata" (*P. pungens*, *P. fraudulenta*, *P. multiseries* and *P.* cf. *seriata*) and "P. delicatissima" (species not observed in net samples) groups were recorded.

High cell concentrations of *Pseudo-nitzschia* were recorded above the thermocline at stations 1315-1319 (Figure 9; no phytoplankton data are available for station 1320) where chlorophyll levels ranged from 0.5-1.5 mg.m^{-3}. Light microscopy examination of the surface samples showed cell concentrations of 31-413 cells.mL^{-1} of the "P. seriata" group. Ultrastructural examination of net samples taken at stations 1315 and 1317 showed that *P. pungens*, *P. fraudulenta*, *P. multiseries* and *P. delicatissima* were present.

Representatives from the "P. delicatissima" group were only observed at the outer station (1315) with levels of 11 cells.mL^{-1} recorded at the surface although *P. pungens* was the only species observed in the net material examined at this station.

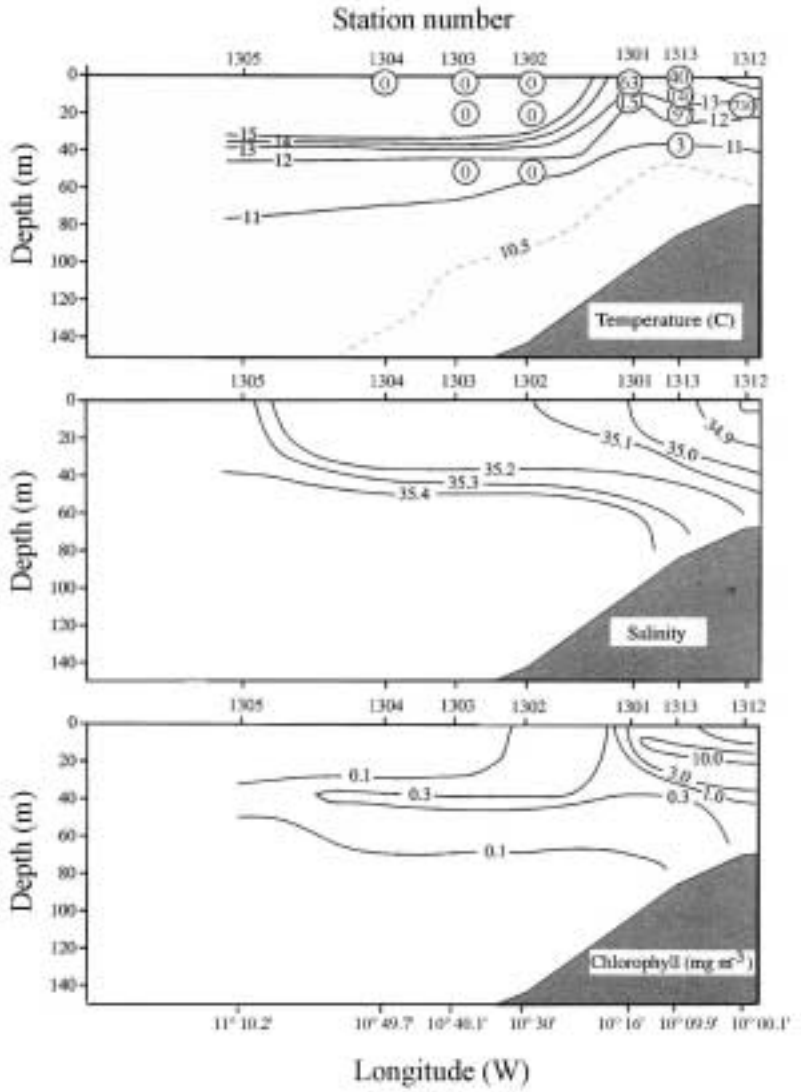

Figure 7. Distributions of temperature (°C), salinity and chlorophyll (mg.m^{-3}) at stations 1301-1305 and 1312-1313 off the southwest coast of Ireland, southwest of Bantry Bay sampled between August 15th-16th 1993 (modified from Raine and McMahon 1998). Station positions are shown in Figure 1. Included in the temperature plot are the total cell densities of *Pseudo-nitzschia* recorded at discrete depths (i.e. the sum of "P. seriata" + "P. delicatissima" shown as cells.mL^{-1}, 0 = not detected).

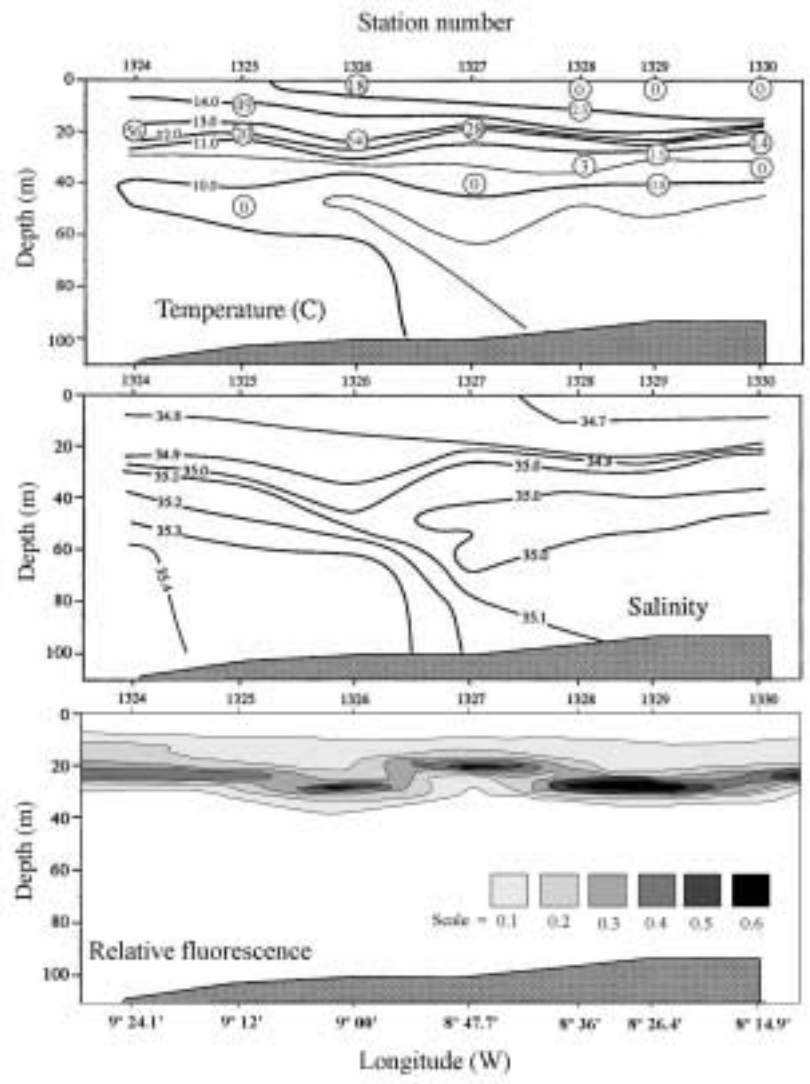

Figure 8. Distributions of temperature (°C), salinity and relative chlorophyll fluorescence along a station transect (1324-1330) off the south coast of Ireland from Sherkin Island to south of Cork sampled on August 18th 1993 (modified from Raine and McMahon 1998). Station positions are shown in Figure 1. Included in the temperature plot are the total cell densities of *Pseudo-nitzschia* recorded at discrete depths (i.e. the sum of "P. seriata" + "P. delicatissima" shown as cells.mL^{-1}, 0 = not detected).

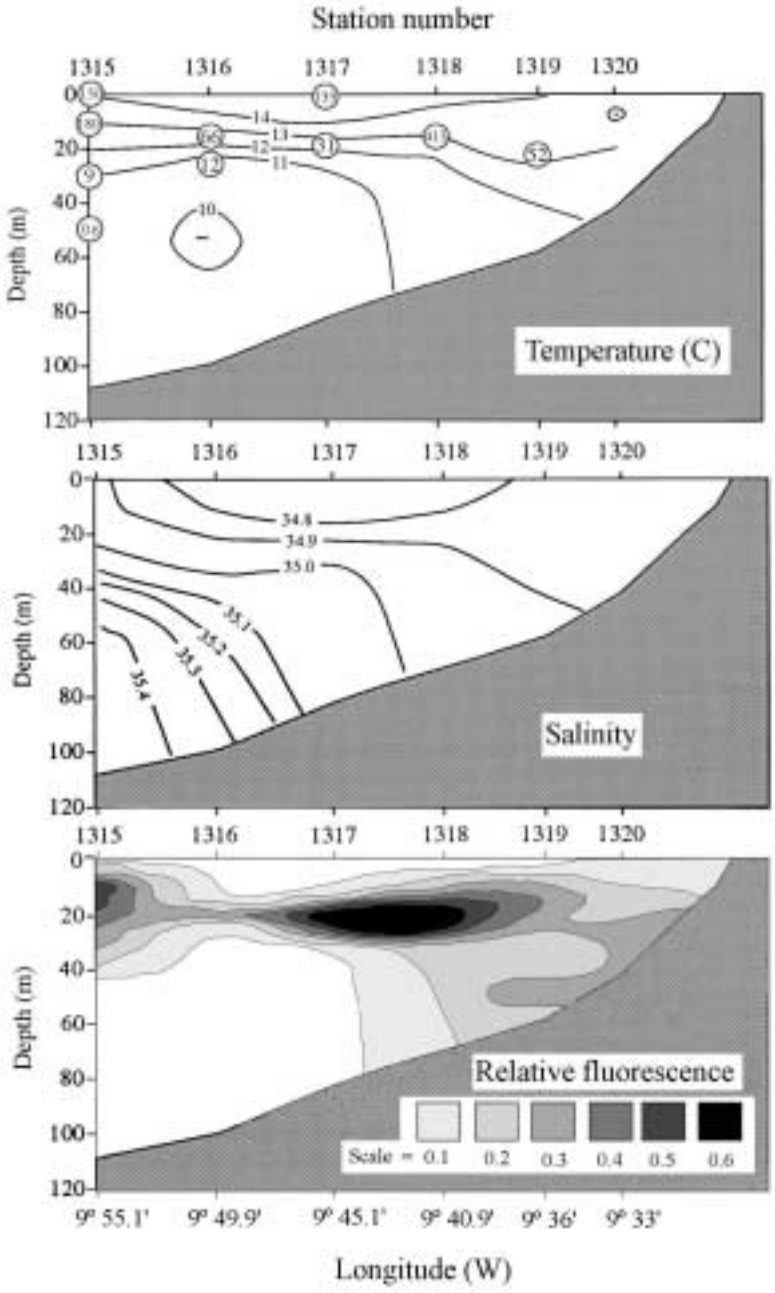

Figure 9. Distributions of temperature (°C), salinity and relative chlorophyll fluorescence along a station transect (1315-1320) from inside Long Island Bay to out past Fastnet Rock sampled on August 17th 1993 (modified from Raine and McMahon 1998). Station positions are shown in Figure 1. Included in the temperature plot are the total cell densities of *Pseudo-nitzschia* recorded at discrete depths (i.e. the sum of "P. seriata" + "P. delicatissima" shown as cells.mL^{-1}).

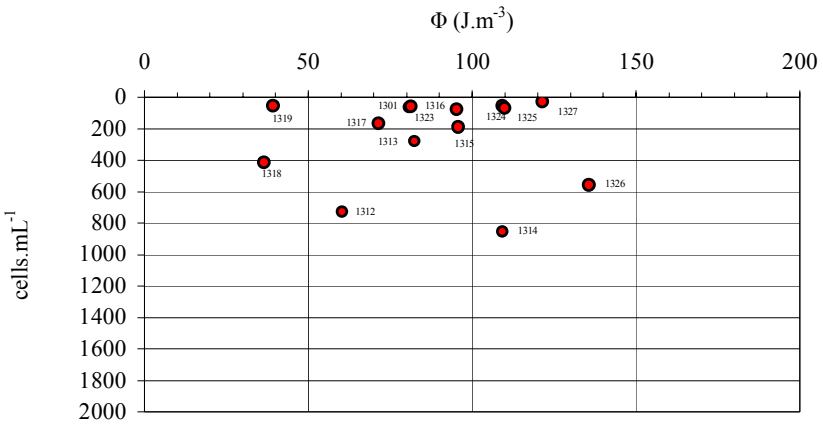

Figure 10. Plot of stations against mean integrated *Pseudo-nitzschia* concentrations (>10 cells.mL^{-1}) and water column stability expressed as the stratification parameter, Φ, off the south and southwest coasts of Ireland between August 8th-18th 1993.

June 1995

During the course of a grid survey of 30 stations on the southwestern continental shelf carried out between 49° 30' N and 51° 30' N and extending from 9° 00' W to 13° 00' W between June 2nd-8th 1995, *Pseudo-nitzschia* cells dominated the net flora at only one station close to the coast at 51° 10' N, 9° 55' W. No quantitative samples were taken but the net sample examined comprised mainly of *P. pungens*, with lesser amounts of *P. delicatissima*, *P. fraudulenta*, *P. australis* and *P. multiseries*.

May 1996

An exceptional bloom of *Pseudo-nitzschia* occurred between May 6th-8th 1996 in Galway Bay (west coast of Ireland), when biomass levels in the top meter were high enough to cause water discolouration. Salinity and temperature at the water surface was <16.0 and 10.5°C. Salinity increased markedly with depth, at 2.5 m a salinity value of 32.9 was recorded. Secchi disc readings within the bloom were 3.5 m to 4.5 m. Again, no quantitative samples were taken, but examination of net material revealed that the bloom comprised primarily of *P. delicatissima*, although *P. pseudodelicatissima* was also present.

July 1996

Between the 20th-25th July 1996, hydrographic conditions around southwest Ireland were similar to those observed in August 1993. Most stations examined were moderate to well stratified (Figure 11). Offshore, the water column was thermally stratified with temperature differences of up to 5.0°C across the thermocline. The minimum sea surface temperature (15.4°C) was recorded at station 1801 southwest of Bantry Bay and a maximum surface temperature of 17.0°C was observed at the inshore station 1813 in the Kinsale estuary. Otherwise surface values (13.7-16.7°C) along the southwest averaged at 15.7°C. The lowest temperature, 9.5°C, was recorded at station 1811 (40 m) off the south coast. Off the southeast coast, stations 1820-1822 straddled the Celtic Sea Front, a tidal front separating tidally mixed (1821) and thermally stratified water (1820).

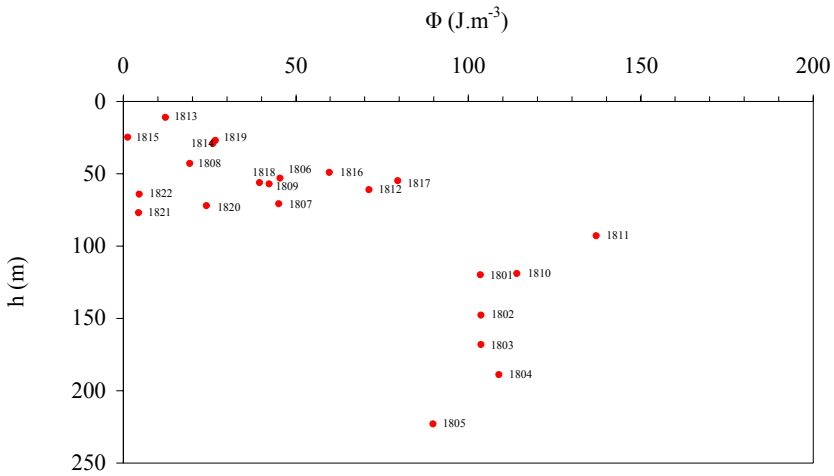

Figure 11. Plot of stations against water column depth, h, and water column stability expressed as the stratification parameter, Φ, off the southeast, south and southwest coasts of Ireland between July 20th-25th 1996. Station positions are shown in Figure 2.

Inorganic nutrients distributions in July 1996 were not as high as those recorded in August 1993. Depleted concentrations in the surface mixed layers as a consequence of phytoplankton activity were apparent and concentrations increased markedly with depth at stations located further offshore (e.g. 1801, 1802, 1805, 1810 and 1811). Nitrate values ranged from below the limit of detection (e.g. 1802-1804, 1806, 1809 and 1811)

up to a maximum of 7.4 µM at stations 1810 and 1811. Similarly phosphate values ranged from 0.02 µM (10-15 m at stations 1806 and 1803) to 0.58 µM (40 m at station 1811) and silicate from 0.2 µM (11-13 m, stations 1816 and 1820) to 2.8 µM at station 1811 (40 m).

Pseudo-nitzschia spp. concentrations within the euphotic zone ranged from below the limit of detection to very high cell densities >100 cells.ml^{-1} in the discrete water samples examined (Figure 12). When present, cell concentrations of between 3-140 cells.mL^{-1} of the "P. seriata" group were recorded. Cell densities of the "P. delicatissima" group were higher, with cell counts of up to 850 cells.mL^{-1} recorded at station 1810.

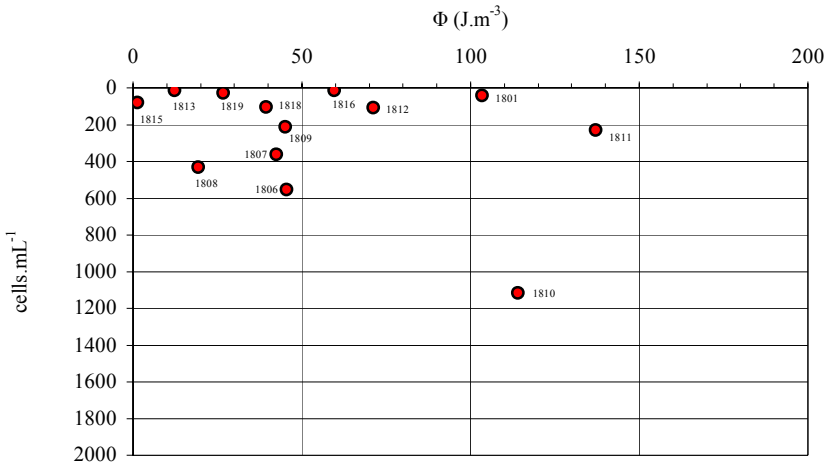

Figure 12. Plot of stations against mean integrated *Pseudo-nitzschia* concentrations (>10 cells.mL^{-1}) and water column stability expressed as the stratification parameter, *Φ,* off the southeast, south and southwest coasts of Ireland between July 20th-25th 1996.

Highest cell concentrations of *Pseudo-nitzschia* species were recorded at stations 1806, 1808, 1809 and 1810 (Figure 13). Vertical profiles of temperature, salinity and relative chlorophyll fluorescence show that the water column was well mixed at inshore shallow stations 1808 and 1809. The water column was however, thermally stratified at station 1810 further offshore (Figure 13). Water clarity recorded by Secchi depth was 10 m at station 1808, 10.5 m at station 1809 and 11.5 m at station 1810. Subsurface chlorophyll *a*

values ranged from 2.6 $mg.m^{-3}$ (35 m) at station 1808 to 2.3 $mg.m^{-3}$ (35 m) at station 1810.

Phytoplankton net material examined from station 1808 was predominated by *P. pseudodelicatissima* but *P. fraudulenta*, *P. pungens*, *P. delicatissima*, *P. australis*, *P. multiseries*, *P. seriata* cf. *seriata* were also present. A tentatively identified *P. subpacifica* valve was also noted in this sample. Water samples collected at 15 m and 35 m at this station showed cell densities of up to 240 $cells.mL^{-1}$ of the "P. delicatissima" group. The "P. seriata" group was found at cell densities ranging from non-detectable to 10 $cells.mL^{-1}$ at these depths. *Pseudo-nitzschia pseudodelicatissima* predominated the net material of stations 1809 and 1810. Other *Pseudo-nitzschia* species present at these stations included *P. fraudulenta*, *P. australis*, *P. pungens* and *P. delicatissima*. Cell concentrations of *Pseudo-nitzschia* ("P. seriata" and "P. delicatissima" groups) recorded at discrete depths of stations 1808-1810 ranged from 5-850 $cells.mL^{-1}$ (Figure 13). Material examined from 15 stations using electron microscopy showed that *P. pseudodelicatissima* predominated at six sites (stations 1807-1810, 1815 and 1821); *P. pungens* predominated at six locations (stations 1801, 1802, 1806 and 1818-1820) and *P. delicatissima* at a further three (stations 1811-1812). Other species noted were *P. australis*, *P. fraudulenta*, *P. multiseries*, *P.* cf. *subpacifica*, *P.* cf. *seriata* f. *seriata* and a tentatively identified *P. seriata* f. *obtusa*.

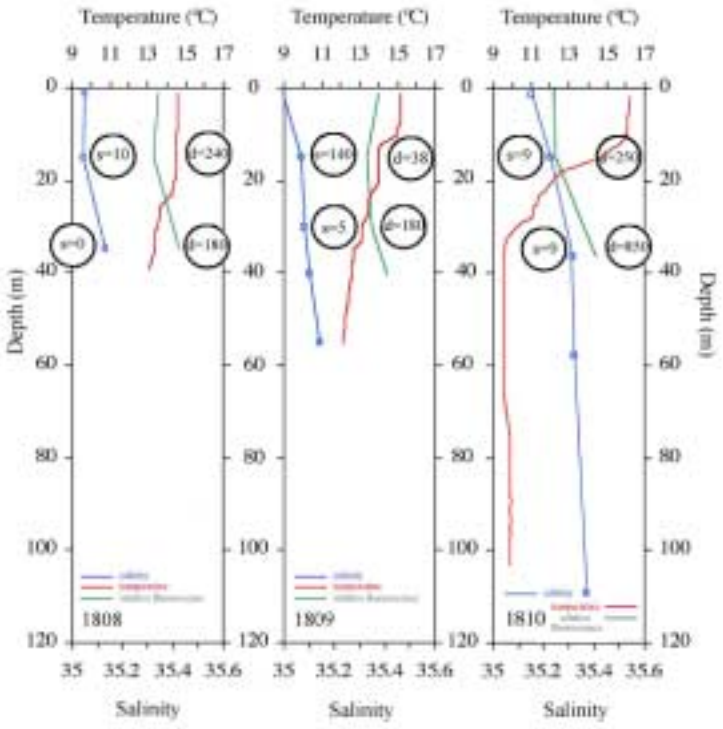

Figure 13. Vertical profiles of temperature (°C), salinity and relative chlorophyll fluorescence recorded at stations 1808-1809 (south of Long Island Bay) and 1810 (south of Sherkin Island) sampled between July 21st-22nd 1996. Station positions are shown in Figure 2. Results from salinity and chlorophyll measurements from discrete samples only are shown as the output from the CTD was faulty and an *in situ* fluorometer was not used. Included in the plot are the cell densities of the two groups of *Pseudo-nitzschia* recorded at discrete depths (s = "P. seriata" and d = "P. delicatissima" shown as cells.mL^{-1}, 0 = not detected).

Off the east coast of Ireland temperature records clearly showed that stations 1820 (14.8°C) and 1821 (13.8°C) were located on either side of the Celtic Sea Front (Figure 14). On the stratified side of the CSF, *Pseudo-nitzschia* cells were in found in low numbers (3 cells.ml^{-1}, 15 m) at station 1820, south of Kilmore Quay although a large number of species were observed in the phytoplankton net material. *Pseudo-nitzschia pungens* was the predominant *Pseudo-nitzschia* species with *P. multiseries*, *P. australis*, *P. fraudulenta* and *P. seriata* cf. *seriata* also present. In spite of the fact that

phytoplankton cell counts were not determined for station 1821, electron microscopy analysis of the net sample showed that the *Pseudo-nitzschia* population present composed of *P. pseudodelicatissima* and *P. delicatissima*. Representatives from the "P. seriata" group were not present in the net haul sample at this station. The vertical distribution of temperature, salinity and relative chlorophyll fluorescence at these stations showed that the water column of station 1820 was weakly stratified with a temperature difference of 1.5°C across the thermocline (surface to bottom temperature difference of 2.2°C). The water column at station 1821 was well mixed with a surface to bottom temperature and salinity difference of 0.6°C and 0.03 respectively (Figure 14). Chlorophyll *a* concentrations of 1.3 $mg.m^{-3}$ were recorded at the surface of both stations and water clarity measured using secchi depth was 13 m and 15 m for station 1820 and 1821 respectively. Nutrient concentrations at station 1820 increased with depth.

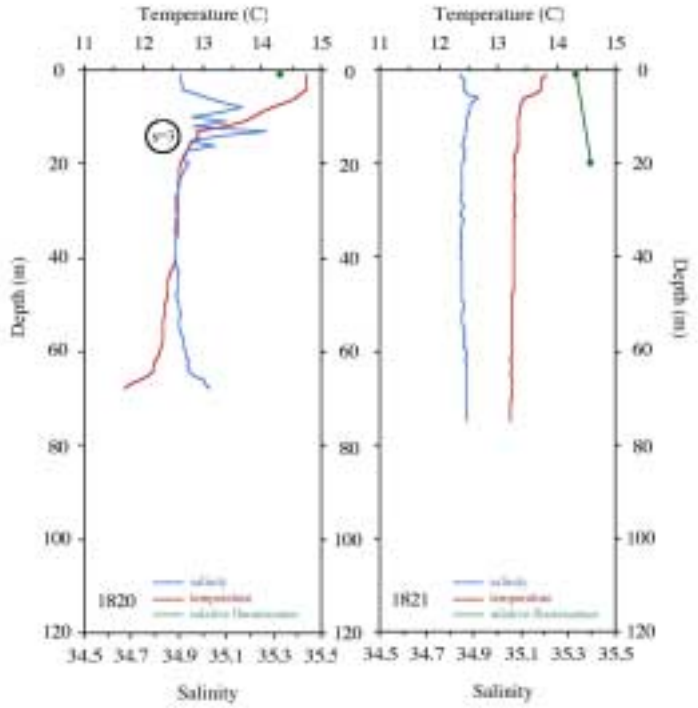

Figure 14. Vertical profiles of temperature (°C), salinity and relative chlorophyll fluorescence recorded at stations 1820 and 1821 sampled on July 24th 1996 (southwest to southeast of Rosslare on either side of the Celtic Sea Front). Results from chlorophyll measurements from discrete samples only are shown, as an *in situ* fluorometer was not used. "Pseudo-nitzschia seriata" cell concentrations recorded at 15m are presented for station 1820 (s = "P. seriata" in cells.mL^{-1}). The "P. delicatissima" group was not detected in the discrete sample examined using light microscopy. Station positions are shown in Figure 2.

September 1996

Hydrographic conditions off south and southwest coast of Ireland between 3rd-8th, 1996 showed a similar structure as in previous summer cruises with the water column at some stations sampled close to the coast being well mixed (1901, 1913) to moderately stratified (1909 and 1914). At stations positioned further offshore stratification was well developed (Figure 15). Sea surface temperature ranged from 14.0°C at station 1907 southwest of Kenmare Bay to 17.1°C off the south coast (station 1922). At stations 1901 (93m), 1920 (100m) and 1922 (97m) the lowest bottom temperature value (9.5°C) was

recorded while the maximum surface to bottom temperature difference of 7.5°C was recorded at station 1922. A representative section of the TS distributions is shown in Figure 17. The overall range in salinity was from 34.9 at the surface at station 1907 to 35.5 (40 m) at the outer most station (1903) west of Dingle bay. At the stratified stations a nutrient depleted surface layer and nutrient rich bottom was evident. Inorganic nutrients at the thermocline varied in concentration with nitrate values of between 0.2-2.7 µM, phosphate between 0.10-0.47 µM, and silicate from 0.6 µM to 1.2 µM. Lowest values for nitrate and phosphate were found at station 1906 but inorganic silicate at this station was higher then at the other stations. Nutrients increased with depth at this station. Highest values of nitrate, phosphate and silicate below the thermocline (80 m) at station 1906 were 2.8 µM, 0.42 µM and 4.6 µM respectively.

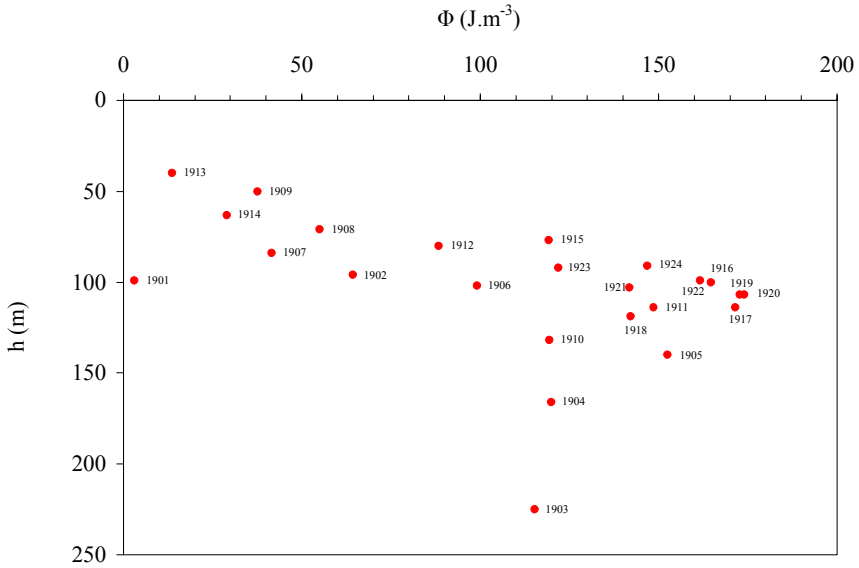

Figure 15. Plot of stations against water column depth, h, and water column stability expressed as the stratification parameter, *Φ,* off the south and southwest coasts of Ireland between September 3rd-8th, 1996. Station positions are shown in Figure 3.

Pseudo-nitzschia was observed in much lower cell densities then those found in previous surveys (Figure 16). Species were however present at 50% of the stations (1901-1924) sampled. The "P. seriata" group was found at concentrations of <1 cells.mL^{-1} at all stations with the exception of station 1902 where 92 cells.mL^{-1} was recorded at 15 m.

The "P. delicatissima" group was more abundant and recorded at 10 stations (1906-1910, 1914-1915, 1922-1923 and 1924) with cell concentrations of between 1-78 cells.mL^{-1}.

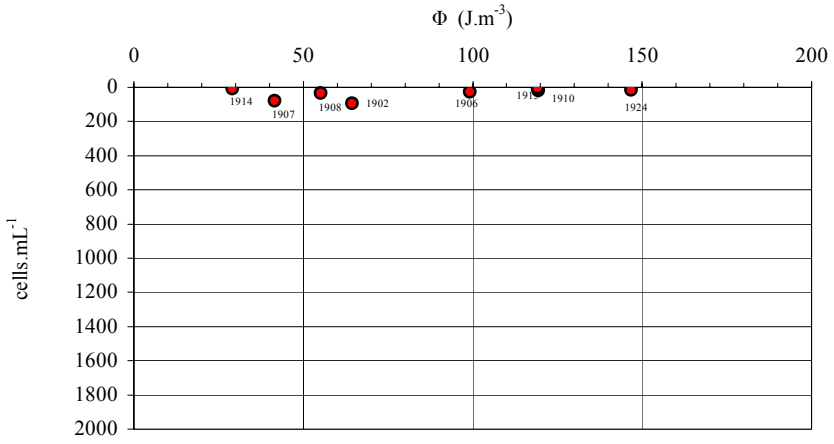

Figure 16. Plot of stations against integrated *Pseudo-nitzschia* concentrations (>10 cells.mL^{-1}) and water column stability expressed as the stratification parameter, *Φ,* off the south and southwest coasts of Ireland between September 3rd-8th 1996.

Light microscopy examination of samples showed that *Pseudo-nitzschia* was not detected at stations 1903, 1904 and 1905 in samples taken at the subsurface chlorophyll maximum (between 20-60 m). *Pseudo-nitzschia* cell counts were observed however at station 1906 (20 m) with 25 cells.mL^{-1} of the "P. delicatissima" group. No cells from the "P. seriata" group were recorded. Net haul material at this station showed that *P. delicatissima* predominated the sample. Representatives from the "P. seriata" group were also present and included *P. australis*, *P. fraudulenta* and *P. pungens*. Chlorophyll *a* concentrations along the Dingle transect were not exceptionally high (<1.8 mg.m^{-3}).

Figure 18 shows the vertical temperature and salinity profile at station 1902 positioned north of Dingle Bay (52° 25.0' W; 10° 25.0' N). Subsurface phytoplankton samples taken at this station from 15 m contained 92 cells.mL^{-1} of "P. seriata" (*P. australis*, *P. pungens* and *P. fraudulenta*) and the chlorophyll *a* measurement at this depth was 0.5 mg.m^{-3}. Other *Pseudo-nitzschia* species present in the net material examined were *P. delicatissima* and *P. pseudodelicatissima*. However, the "P. delicatissima" group was not

observed in the phytoplankton sample taken at a depth of 15m. In the surface mixed layer nutrient concentrations were exhausted, probably as a result of biological uptake.

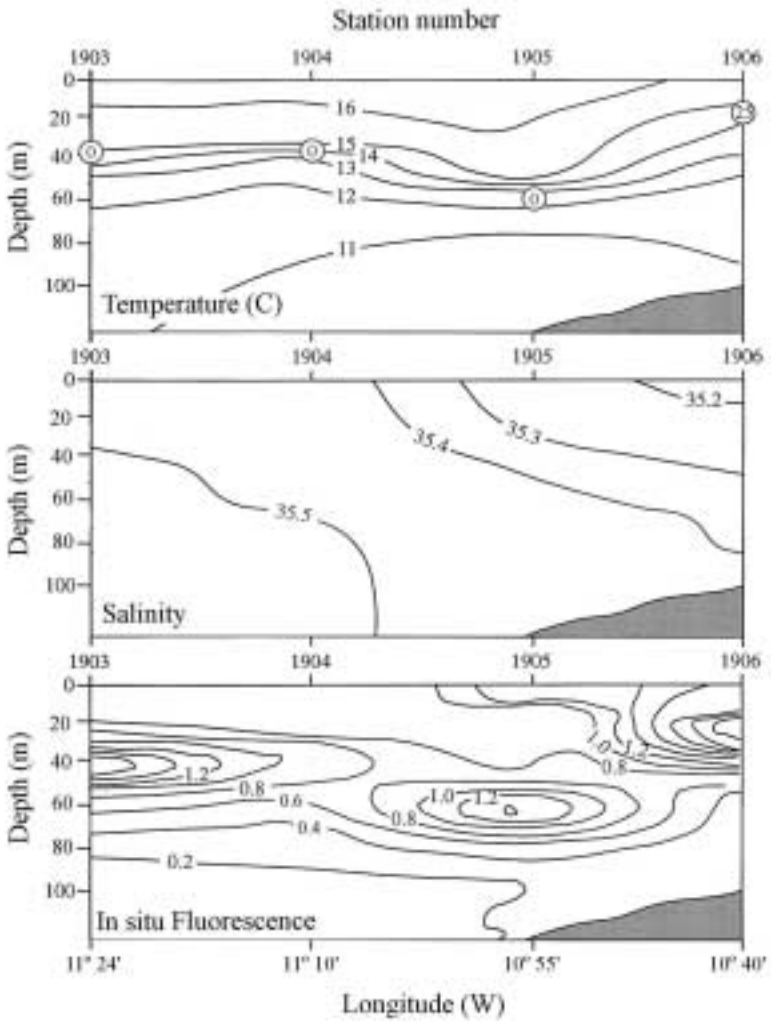

Figure 17. Distributions of temperature (°C), salinity and *in situ* fluorescence (units are relative fluorescence) along a station transect (1903-1906) running in an east west direction, outside Dingle Bay sampled on September 4th 1996. Station positions are shown in Figure 3. Included in the temperature plot are the total cell densities of *Pseudo-nitzschia* recorded at discrete depths (i.e. the sum of "P. seriata" + "P. delicatissima" groups are shown as cells.mL^{-1}, 0 = not detected). The surface position of the Irish Shelf Front can be seen between stations 1904 and 1905.

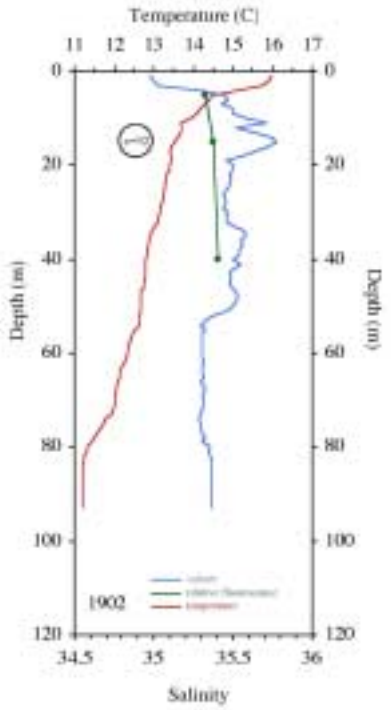

Figure 18. Vertical profiles of temperature (°C), salinity and relative fluorescence recorded at station 1902 (north of Dingle Bay at 52º 25.0' N, 10º 25.0' W). Results from chlorophyll measurements from discrete samples only are shown as an *in situ* fluorometer was not used. Included in the plot are the total cell densities of *Pseudo-nitzschia* recorded at 15m (s = "P. seriata"). The "P. delicatissima" group was not detected in the discrete sample at this depth. Station position is shown in Figure 3.

May 1997

During a survey in shelf waters off the west coast of Ireland carried out between May 1st-5th 1997, temperatures at all stations (2001-2011) ranged from a maximum of 13.7°C at the surface off Erris Head (station 2008) to 9.1°C at a depth of 80 m off Inishbofin Island (station 2001). The overall range in salinity was from 34.9 at the surface of station 2006 (inshore station off Achill Island) to 35.5 (200 m depth) at station 2011 (furthest station offshore). In general both temperature and salinity increased due west, offshore. The low salinity values recorded at the inshore stations (2001, 2006 and 2007), was probably a result of freshwater runoff. Near surface thermosalinograph data (Figure 19) revealed that the surface signature of the ISF was most pronounced along the Erris Head

transect and positioned close to the coast (~15 km offshore) at station 2008 (54° 21.1' N 10° 15.0' W). Stations 2003, 2004 and 2009-2011 were situated on the oceanic side of the ISF front. Fluctuating values of temperature and salinity towards the coast along the Inishboffin and Achill Island transects may be a results of the presence of eddies inshore of the ISF. Water depths ranged from 97 m at the coastal stations to ~ 229 m at the stations further offshore. The water column was weakly stratified at stations 2002, 2203, 2005-2208 and 2010, off Inishboffin, Achill Island and Erris Head (Figure 20). The remaining stations were either well stratified (2001, 2009, 2011) or vertically mixed (2004).

Nutrient concentrations at the surface were depressed at all stations except the outermost stations of the Erris head transect (2010 and 2011) where nitrate concentrations were >4.5 μM and phosphate values were 0.38 μM. Silicate in the surface water ranged between 0.6 μM at 2201 to 2.6 μM at station. 2010. Chlorophyll levels were highest in the surface water of the outermost station (1.8 mg m^{-3}) along the Inishbofin transect, at station 2005 (3.3 mg m^{-3}) along the Achill Island transect and the innermost station (2.4 mg m^{-3}) of the Erris Head transect. *Pseudo-nitzschia* was not present in very high cell densities at any of the stations sampled (Figure 21). Highest cell densities of *Pseudo-nitzschia* (19 cells.mL^{-1} of the "P. delicatissima" group) were recorded at 30 m depth at the outermost station (2011) west of Achill Island (Figure 22). At this station *Pseudo-nitzschia* co-dominated with the diatom *Thalassionema frauenfeldii*. At all other stations "P. delicatissima" cell densities ranged from 0.08-14 cells.mL^{-1}. The "P. seriata" group was only present in very low cell numbers (<1 cells.mL^{-1}) at only 3 (2201, 2204 and 2205) out of the 11 stations sampled. Two net samples (station 2006 and 2011) were selected and the *Pseudo-nitzschia* component was examined using electron microscopy. *Pseudo-nitzschia fraudulenta* predominated both samples with *P. pseudodelicatissima* also present at station 2011. Vertical profiles of salinity, temperature and chlorophyll for stations 2006 and 2011 showed that at the inshore station 2006, a layer of fresher water was present above water with more saline characteristics, probably as a result of freshwater runoff. Station 2011 positioned further offshore was more saline and thermally stratified (Figure 23).

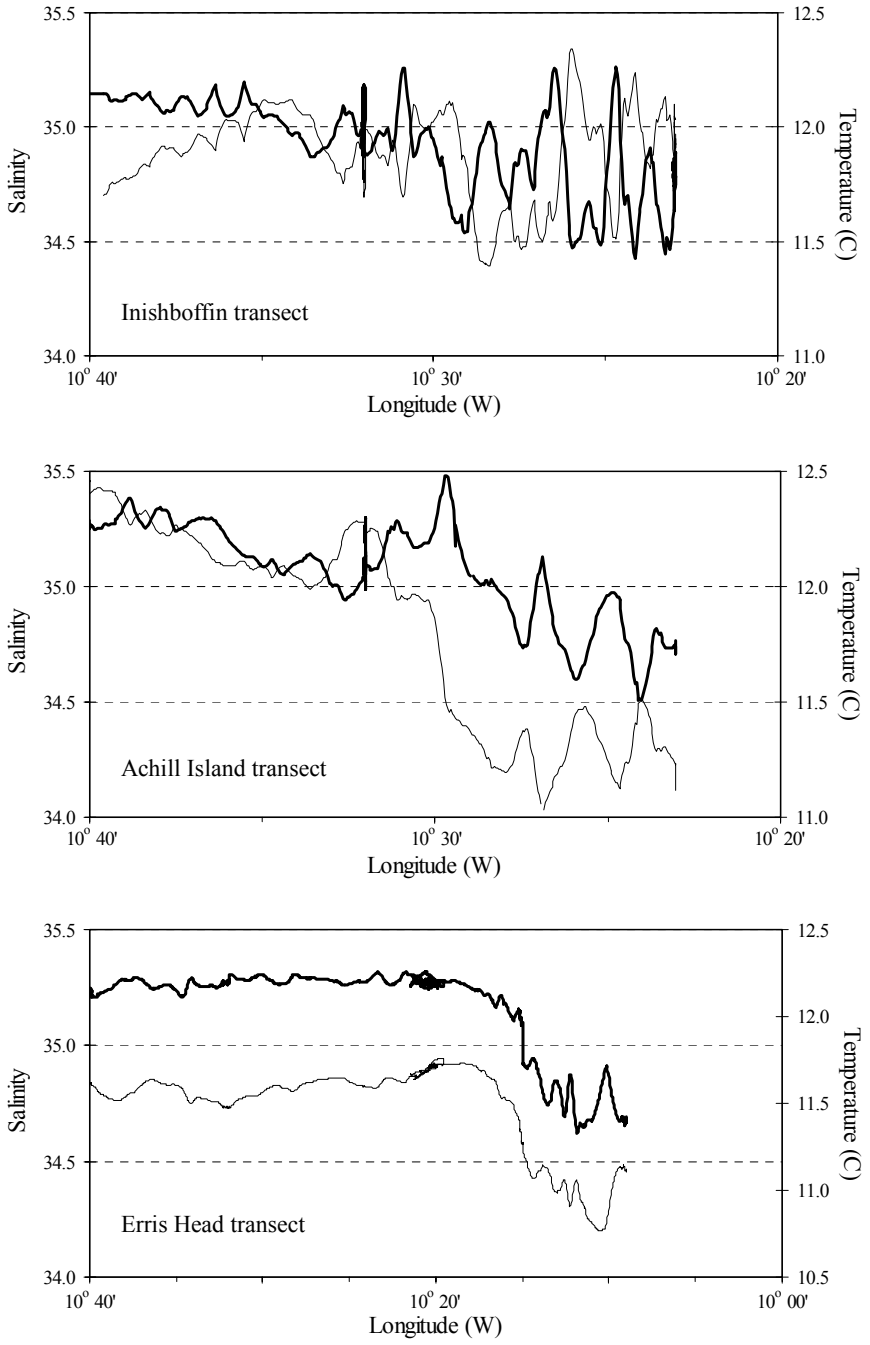

Figure 19. Underway surface distribution of temperature (thin line) and salinity (dark line) along transects sampled off the west coast of Ireland between May 1st-5th 1997.

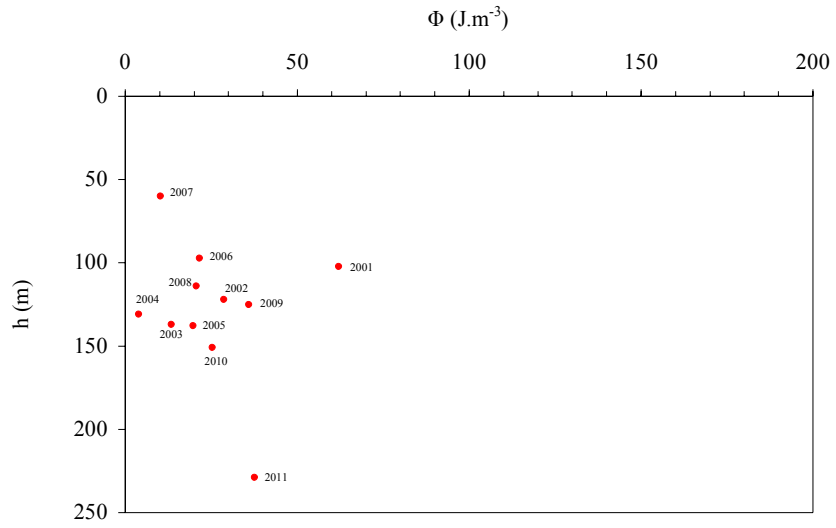

Figure 20. Plot of stations against water column depth, h, and water column stability expressed as the stratification parameter, *Φ,* off the west coast of Ireland between May 1st-5th 1997. Station positions are shown in Figure 4.

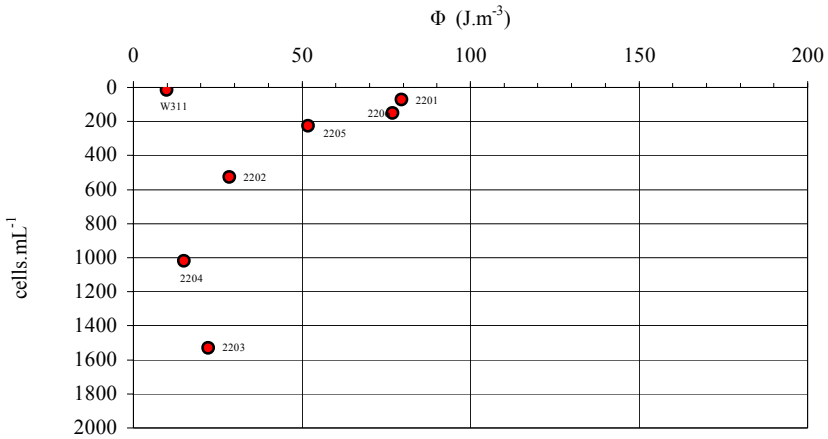

Figure 21. Plot of stations against integrated *Pseudo-nitzschia* concentrations (>10 cells.mL^{-1}) and water column stability expressed as the stratification parameter, *Φ,* off the west coast of Ireland between May 1st-5th 1997.

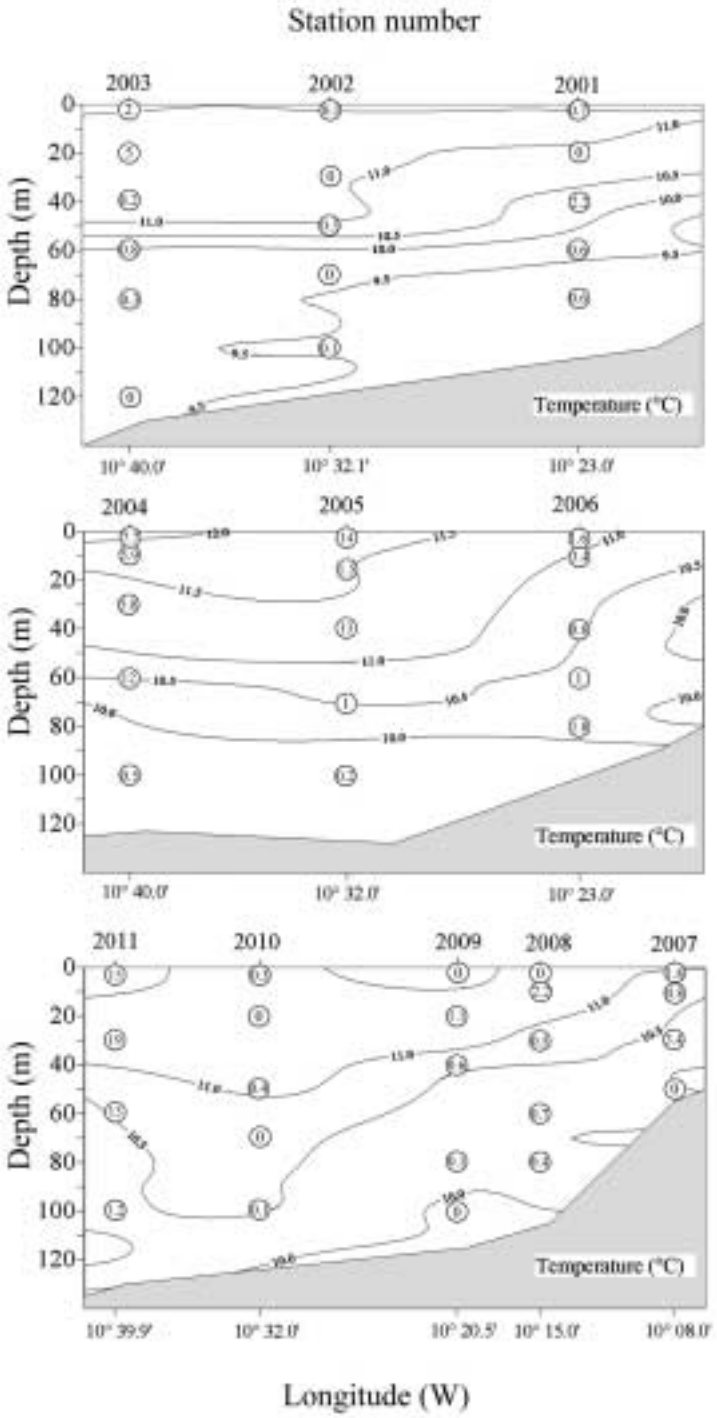

Figure 22. Temperature (°C) distributions off the west coast of Ireland May 1st-5th 1997. Included in the temperature plot are the total cell densities of *Pseudo-nitzschia* recorded at discrete depths (i.e. the sum of "P. seriata" + "P. delicatissima" groups are shown as cells.mL^{-1}, 0 = not detected). Station positions are shown in Figure 4.

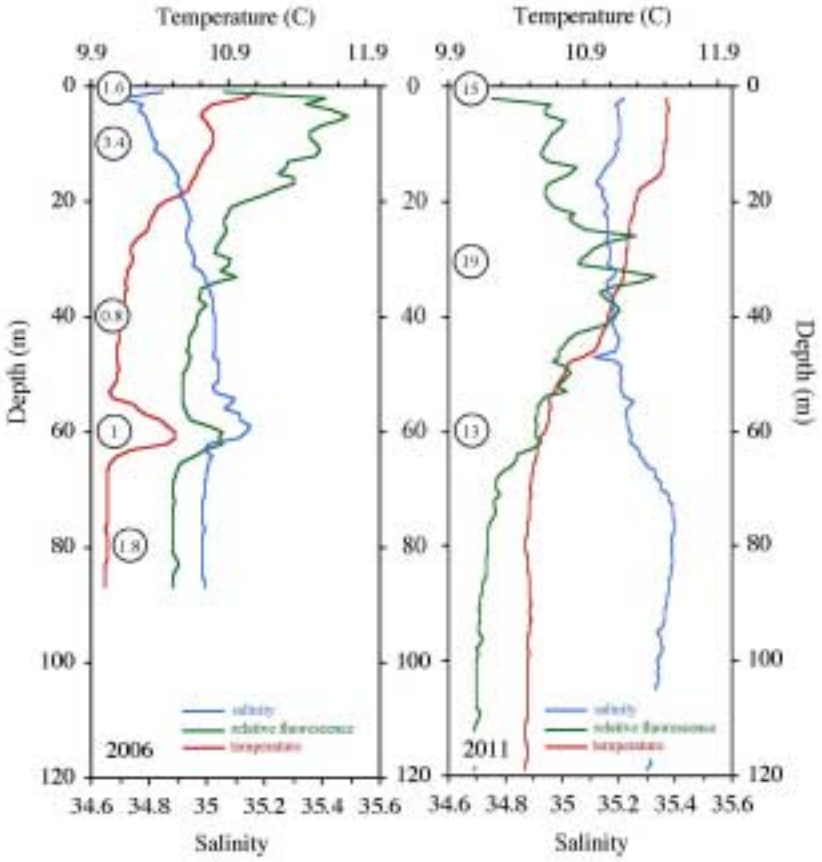

Figure 23. Vertical profiles of temperature (°C), salinity, relative fluorescence and "P. delicatissima" cell concentrations ("P. delicatissima" cells.mL^{-1}, 0 = not detected) recorded at discrete depths at stations 2006 and 2011 sampled on May 3rd 1997. The "P. seriata" group was not detected in discrete samples examined using light microscopy. Station positions are shown in Figure 4.

October 1997

During a survey carried out off the south coast of Ireland between October 6th-10th 1997 temperature ranged from a maximum of 15.7°C at the surface of station 2201 to 9.7°C at 70 m at station 2208. Overall range in salinity was 35.1 at the surface to 35.3 at depth (m) at stations 2205 and 2206 respectively. Thermal stratification was more pronounced at the offshore stations then at those nearer the coast. Water depths ranged from 55 m at the coastal stations to ~ 95 m at the more stratified stations further offshore (Figure 24).

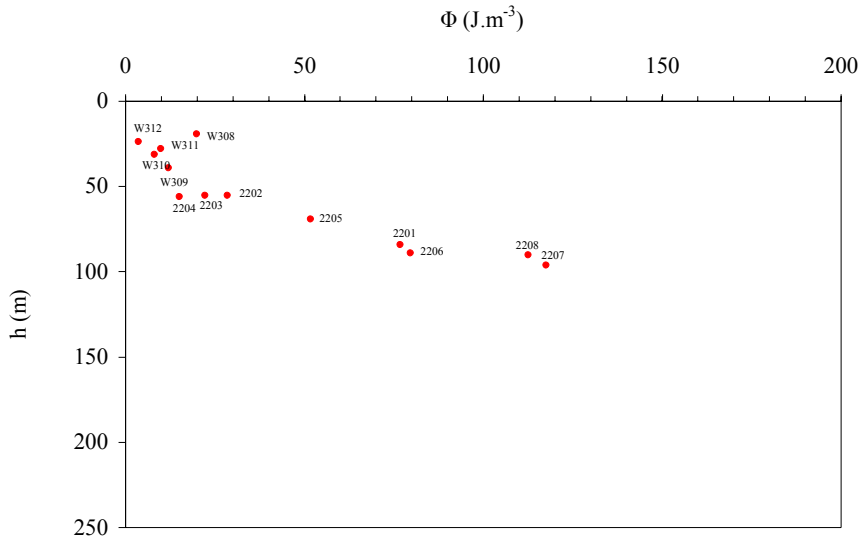

Figure 24. Plot of stations against water column depth, h, and water column stability expressed as the stratification parameter, Φ, off the south coast of Ireland between October 6th-10th 1997. Station positions are shown in Figure 5

Highest abundances of between 1-2,000 cells.mL^{-1} of the "P. seriata" group were recorded off the south coast (Figure 25). The species observed most frequently were *P. fraudulenta* and *P. australis*, although *P. pungens*, *P. multiseries* and *P.* cf. *subpacifica* were also detected using electron microscopy. *Pseudo-nitzschia pseudodelicatissima* was the only representative from the "P. delicatissima" group identified at 3 stations (2202, 2206 and 2208) using electron microscopy. Levels of between 1-14 cells.mL^{-1} of this group were, however, recorded at stations 2201-2206, 2208 and W309-W312 analysed using light microscopy.

The vertical profiles of temperature, salinity and *in situ* fluorescence recorded at several stations (2201-2204 and 2206) running parallel to the south coast from Cork to Waterford showed that the more western stations (2206 and 2201) were moderately stratified with surface to bottom temperature differences of ~3.4-5.0°C (Figure 26). The depth of the upper mixed surface layer was approximately 25m. Thermal stratification of the water column was weak at the more coastal stations (2202-2204) to the east with maximum temperature differences of 1.0°C across the thermocline. However the water temperature

at these stations was warm (>15.0°C). A subsurface chlorophyll maximum was observed at about 20 m depth. Chlorophyll *a* concentrations (Figure 26) increased towards the east with a maximum value observed of >5 mg.m^{-3} at station 2204 (15 m). Secchi depth readings decreased from 13 m at station 2206 to 8.5 m at station 2204 and 8 m recorded at station 2203.

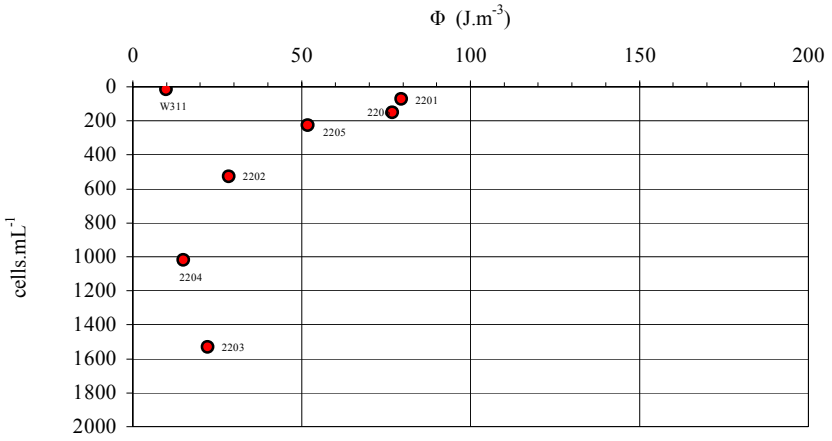

Figure 25. Plot of stations against integrated *Pseudo-nitzschia* concentrations (>10 cells.mL^{-1}) and water column stability expressed as the stratification parameter, *Φ*, off the south coast of Ireland between October 6th-10th 1997.

Light microscopy results showed that surface cell concentrations of the "P. seriata" group increased from 2 cells.mL^{-1} at station 2206 to 550 cells.mL^{-1} at station 2204. Cell densities at the chlorophyll maximum also increased in a similar manner from 65-450 cells.mL^{-1}. Highest counts for this group were found at station 2203 (730 cells.mL^{-1}) at the surface. Electron microscopy analysis of net material showed that *P. fraudulenta* predominated the water column at all stations (2206, 2201-2204) except for station 2206 where it co-occurred with *P. australis*. Other species recorded were *P. pungens* (2201, 2203, 2204 and 2206) and *P. multiseries* (2201, 2202 and 2206). *Pseudo-nitzschia pseudodelicatissima* was observed in 2 of the 5 stations (2201 and 2206), although light microscopy revealed the "P. delicatissima" group in 4 of the stations (2202, 2203, 2204 and 2206). Cell densities ranged from 1-8 cells.mL^{-1} at the surface and 2-12 cells.mL^{-1} at the subsurface chlorophyll maximum. Nitrate concentrations in the surface mixed

layer were low, between 0.1-0.6 µM. Phosphate values in the surface layer were also depleted and ranged from 0.06-0.16µM (2204-2202 respectively). Silicate distributional patterns in the upper water column ranged from 1.1 µM at station 2203 to 1.4 µM at station 2201. Nutrient concentrations increased with depth. Highest levels of nitrate were found in the bottom mixed layer at station 2206 (8.9 µM).

A second transect of stations running offshore, south of Cork (stations 2205-2208) was examined (Figure 27). The structure of the water column at these stations was moderately stratified at the outer stations (2206-2208) with a 3.0°C surface to bottom temperature difference. At the inshore stations the water column became more weakly stratified, probably a result of turbulent mixing. Chlorophyll *a* concentrations in the upper water column increased from 0.1-0.5 $mg.m^{-3}$ at the outer stations to between 1.6-2.5 $mg.m^{-3}$ at the inner stations. This was also reflected in *Pseudo-nitzschia* cell counts recorded at these stations. The presence of *Pseudo-nitzschia* species at the outer most stations (2207 and 2208) was scant with only "P. delicatissima" recorded at <2 $cells.mL^{-1}$ at 60 m, 80 m and 85 m. Weather conditions at the time of sampling this station transect may have influenced these results as strong southwesterly winds reaching up to force 8 were blowing and this may have resulted in the advection of surface water shoreward. Electron microscopy observations of net material from station 2208 (2207 not examined) showed that *P. pseudodelicatissima*, *P. fraudulenta P. australis*, *P. pungens* and *P. multiseries* were present in the water column. Stations 2205 and 2206 were rich in "P. seriata" cell numbers in the top 25 m. Cell concentrations of 65-120 $cells.mL^{-1}$ were found at 20-25 m while only 2-8 $cells.mL^{-1}$ of the "P. delicatissima" group were recorded in the samples examined. *Pseudo-nitzschia australis* predominated the net samples taken at both stations. Additional species observed were *P. fraudulenta*, *P. pungens*, *P. multiseries* (station 2205 and 2206), *P. pseudodelicatissima* (station 2206) and a tentatively identified *P. subpacifica* (station 2205). Nutrient distributions were similar to those described above for the other stations. Highest nitrate levels of 0.6 µM were recorded at the surface layer at station 2205 and 11.1 µM below the thermocline in the bottom water at station 2207. Phosphate values ranged from 0.09-0.19 µM (station 2206 and 2205) and silicate values

of 0.7-1.6 (station 2207 and 2208) in the surface mixed layer. Bottom nutrients ranged from 5.7-11.1 µM for nitrate, 0.24-0.82 µM for phosphate and 5.1-6.6 µM for silicate.

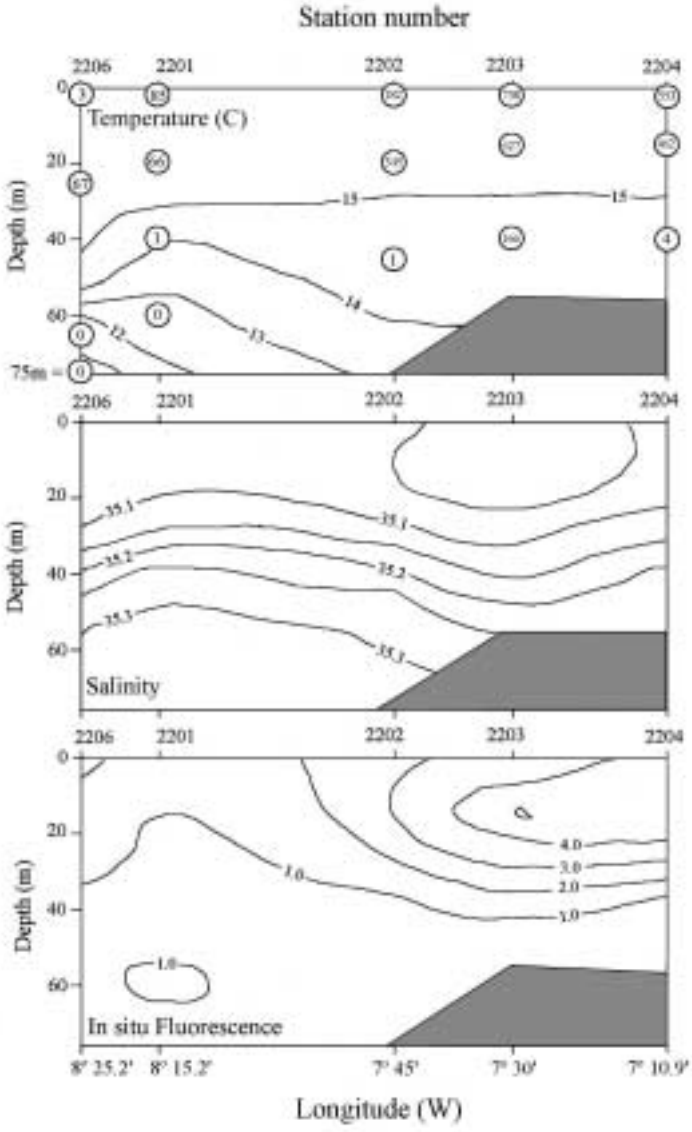

Figure 26. Distributions of temperature (°C), salinity and *in situ* fluorescence (units are relative fluorescence) along a station transect (2206, 2201-2204) running in a northeast direction between Cork and Waterford sampled between 7th-11th October 1997. Station positions are shown in Figure 5. Included in the temperature plot are the total cell densities of *Pseudo-nitzschia* recorded at discrete depths (i.e. the sum of "P. seriata" + "P. delicatissima" shown as cells.mL^{-1}, 0 = not detected).

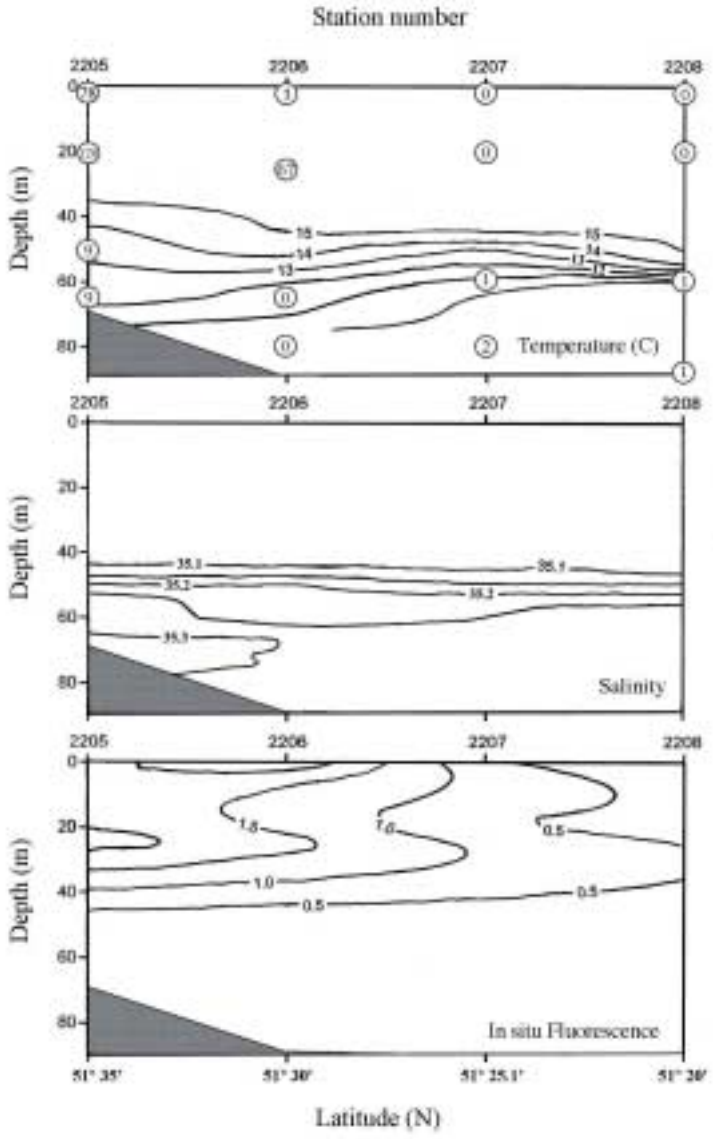

Figure 27. Distributions of temperature (°C), salinity and *in situ* fluorescence (units are relative fluorescence) along a station transect (2205-2208) south of Cork sampled on the 11th October 1997. Station positions are shown in Figure 5. Included in the temperature plot are the total cell densities of *Pseudo-nitzschia* recorded at discrete depths (i.e. the sum of "P. seriata" + "P. delicatissima" shown as cells.mL^{-1}, 0 = not detected).

Presence of Pseudo-nitzschia in relation to environmental data

In an effort to relate the presence of *Pseudo-nitzschia* species to environmental variables, their occurrence was firstly compared with the temperature and salinity distribution, as well as the degree of stratification, represented by differences between the surface and bottom (or 100 m depth) TS values (Table 1). With the exception of *P. delicatissima* and *P. pseudodelicatissima* occurring at stations with lower salinities no other relationships between *Pseudo-nitzschia* species and these environmental factors were evident. Stations with lower salinities were sampled in Galway Bay (May 1996) and the Kinsale Estuary (July 1996).

The relationship between *Pseudo*-nitzschia, water column stability (stratification) and water clarity scaled with depth was also investigated. Water column stability was expressed by means of the potential energy anomaly, phi (Φ), which represents the amount of energy in $J.m^{-3}$, required per unit volume to completely mix the water column and is directly proportional to the strength of stratification (Simpson et al. 1982).

The ordination of *Pseudo-nitzschia* cell numbers in the quantitative samples and the predominant species in the net hauls were plotted on a 2-dimensional graph of the stratification parameter (Φ) against the optical depth ($\lambda.h$). The distribution of *Pseudo-nitzschia*, for both cell numbers and dominant species, falls on areas of the graph that have been shown to be occupied by diatom and dinoflagellate communities (Figures 28-30, Jones and Gowen 1990).

Table 1. Ranges in the surface and bottom temperature and salinity values, together with surface to bottom differences of these parameters for *Pseudo-nitzschia* species recorded in Irish waters (n = 46 stations). If stations were deeper than 100 m, the 100 m value has been used.

	P. pungens	*P. multiseries*	*P. fraudulenta*	*P. australis*	*P. delicatissima*	*P. pseudodelicatissima*
Surface T (ºC)						
average	15.2	15.2	15.0	15.3	15.5	15.3
range	13.5-16.8	14.0-16.5	11.1-16.8	13.5-16.7	13.5-17.0	11.6-17.0
median	15.2	15.3	15.2	15.3	15.5	15.4
s.d	0.8	0.6	1.2	0.7	1.1	1.2
T (ºC) at depth						
average	11.5	11.7	11.8	12.0	11.4	11.6
range	9.5-15.2	9.5-15.2	9.5-15.2	9.5-15.0	9.5-16.0	9.5-16.0
median	11.0	11.7	11.3	11.8	11.0	11.2
s.d	1.7	1.5	1.8	1.7	1.7	1.8
Surface S						
average	35.0	35.0	34.9	35.0	34.9	34.9
range	34.1-35.4	34.1-35.4	34.1-35.3	34.1-35.4	*33.7-35.3	*33.7-35.3
median	35.0	35.0	35.0	35.0	35.0	35.0
s.d	0.2	0.3	0.2	0.3	0.3	0.3
S at depth						
average	35.2	35.2	35.2	35.2	35.1	35.1
range	34.8-35.5	34.8-35.5	34.6-35.5	34.6-35.5	34.3-35.5	34.3-35.5
median	35.2	35.3	35.2	35.2	35.2	35.2
s.d	0.2	0.2	0.2	0.2	0.3	0.3
Delta T (ºC)						
average	3.7	3.6	3.2	3.3	4.1	3.7
range	0.2-7.2	0.7-5.9	0.0-7.2	0.0-7.2	0.6-7.5	0.6-7.2
median	3.8	3.8	3.4	3.4	4.2	3.8
s.d	1.9	1.5	2.0	2.0	2.1	2.1
Delta S						
average	0.2	0.2	0.2	0.2	0.2	0.2
range	0.0-0.8	0.0-0.8	0.0-0.9	0.0-0.8	0.0-0.6	0.0-0.8
median	0.2	0.2	0.2	0.2	0.2	0.2
s.d	0.2	0.2	0.2	0.2	0.2	0.2

* surface salinity measurements at a station in Galway Bay May 1996 had a salinity value of <16.

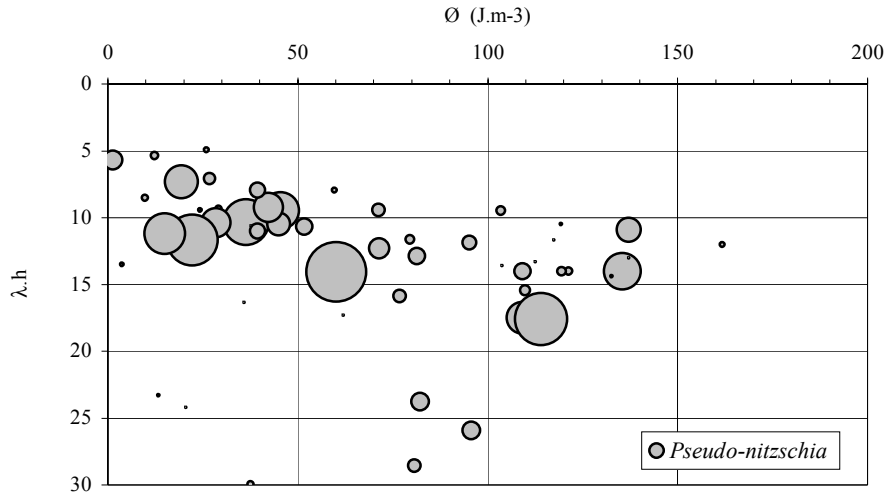

Figure 28. Mean abundance of *Pseudo-nitzschia* cell numbers (74 stations) plotted against the stratification parameter, *Φ*, and the dimensionless optical depth (λ.h) for stations sampled off the south, southwest and west coast of Ireland, between 1993-1997. Size of bubble markers reflects *Pseudo-nitzschia* cell concentrations, which ranged from 0.1 cells.mL^{-1} to 728 cells.mL^{-1}.

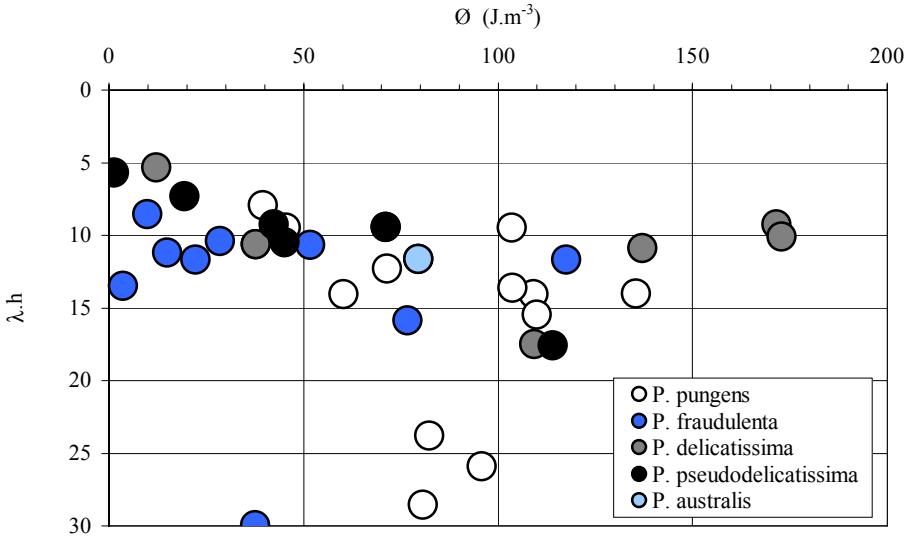

Figure 29. Predominant *Pseudo-nitzschia* species present in vertical phytoplankton net hauls (34 stations) plotted against the stratification parameter, *Φ*, and the dimensionless optical depth (λ.h) for stations sampled off the south, southwest and west coasts of Ireland, between 1993-1997.

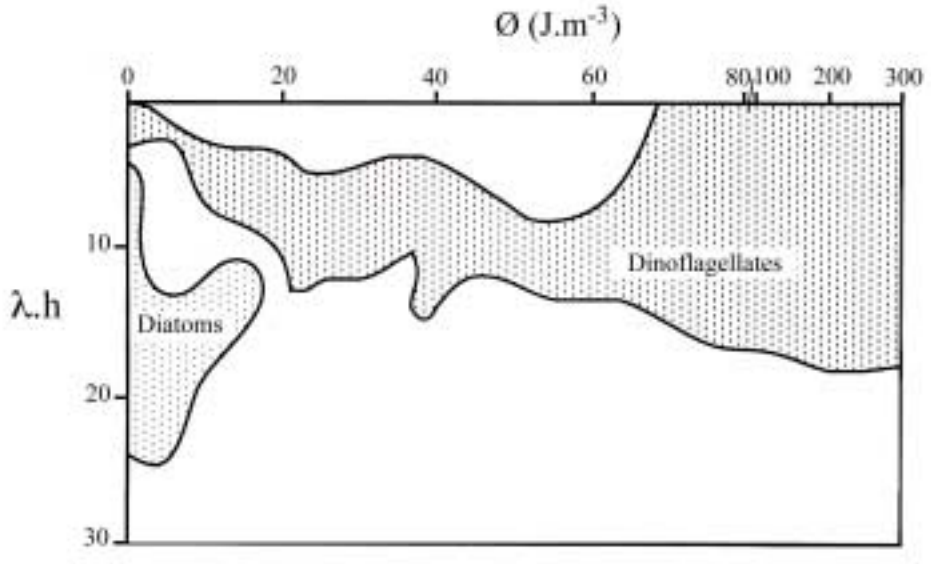

Figure 30. Areas of a plot of stratification (Φ) and the dimensionless optical depth (λ.h) considered to be occupied by dominant diatom or dinoflagellate communities (after Jones and Gowen 1990).

When *Pseudo-nitzschia* cell concentrations were plotted directly against nutrients (Figure 31), no obvious relationship was evident between cell numbers and levels of nitrate. Where there was measurable phosphate, the "P. seriata" group was more abundant at low (<0.2 μM) phosphate levels. Not surprisingly, numbers of both groups tended to be maximal when silicate was in the range of 1-4 μM. There was no relationship between cell numbers and chlorophyll concentrations measured, due to the presence of other phytoplankton (Figure 31).

Quantitative (LM) data was plotted on a temperature-salinity diagram (Figure 32). It is apparent that the "P. delicatissima" group occurred more frequently over a wider range of both temperature and salinity than the "P. seriata" group. The "P. delicatissima" and the "P. seriata groups occurred over temperature and salinity values of 9.1-17.0°C, 33.8-35.5 and 9.6-15.7°C, 34.69-35.3 respectively. A similar analysis was carried out with inorganic nutrients (Figure 33). The ratio of nitrate (NO_3) to phosphate (PO_4), and silicate (SiO_4) to phosphate (PO_4) taken from these plots were 14N:1P:7Si with r^2 values of 0.86 and 0.53 respectively. The interrelationships of these nutrients are lower then

what might be expected for phytoplankton micronutrient requirements (106C:16N:1P by atoms) as suggested by the Redfield ratio (Redfield 1963) and the uptake ratio of silicate in western N. Atlantic waters (15N:1P:16Si by atoms, Richards 1958). Most of the observations of *Pseudo-nitzschia* occurred at lower nutrient concentrations, probably as a result of biological uptake that would be typical of the euphotic surface layer of stratified water. This is more evident in plots of nutrients against temperature (Figure 34), which also suggest that warm (14.0-16.0ºC) stratified water containing measurable nutrients is where one tends to find more species from the "P. seriata" group then "P. delicatissima" group.

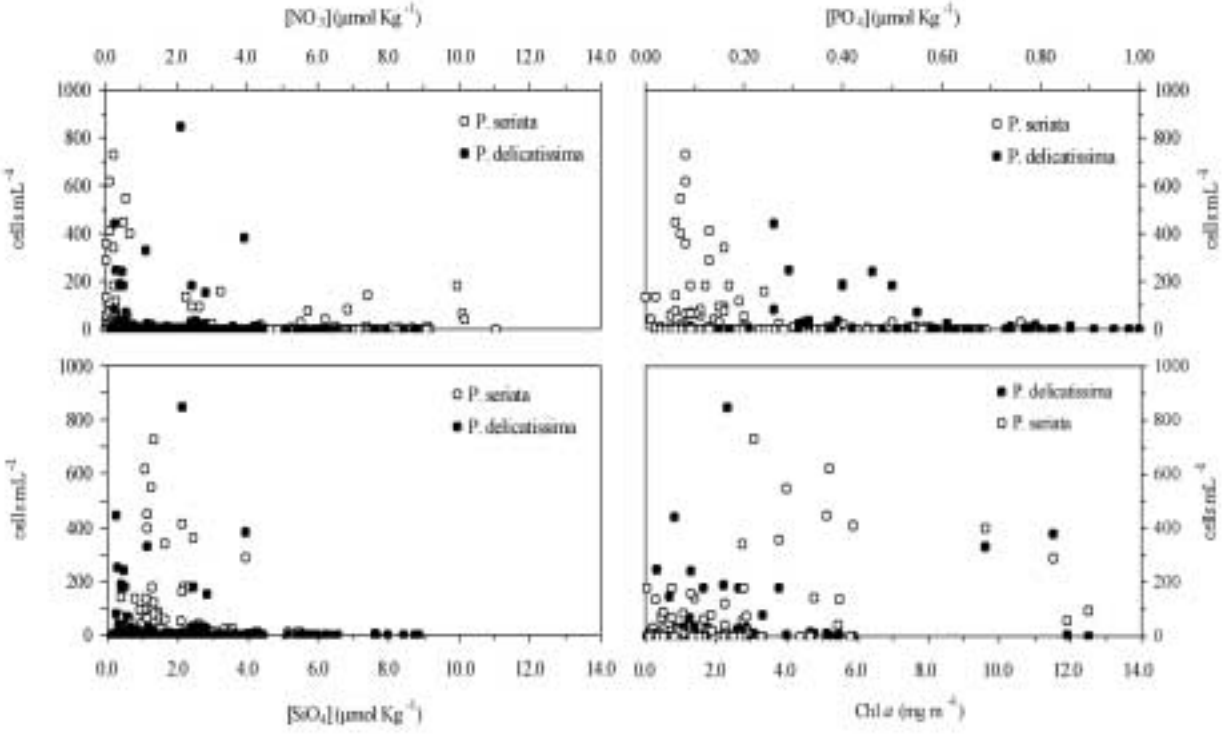

Figure 31. Scatter plots of nitrate, phosphate, silicate and chlorophyll against the "P. seriata" and "P. delicatissima" groups in cells.mL^{-1}. Samples from all depths and times throughout the study containing all the above variables were used.

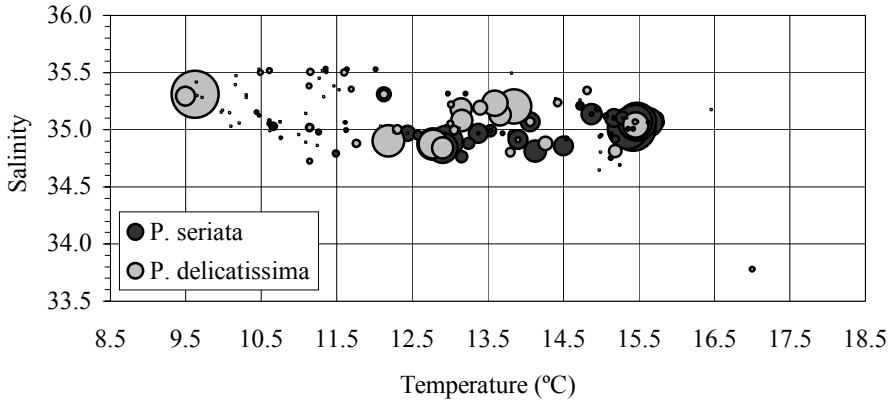

Figure 32. Cell numbers of the "P. seriata" and "P. delicatissima" groups plotted against temperature and salinity. Samples from all depths and times throughout the study containing all the above variables were used. Size of bubble markers reflects *Pseudo-nitzschia* cell concentrations. The "P. seriata" group ranged from 0.1 cells.mL^{-1} to 730 cells.mL^{-1} and the "P. seriata" group ranged from 0.1 cells.mL^{-1} to 850 cells.mL^{-1}.

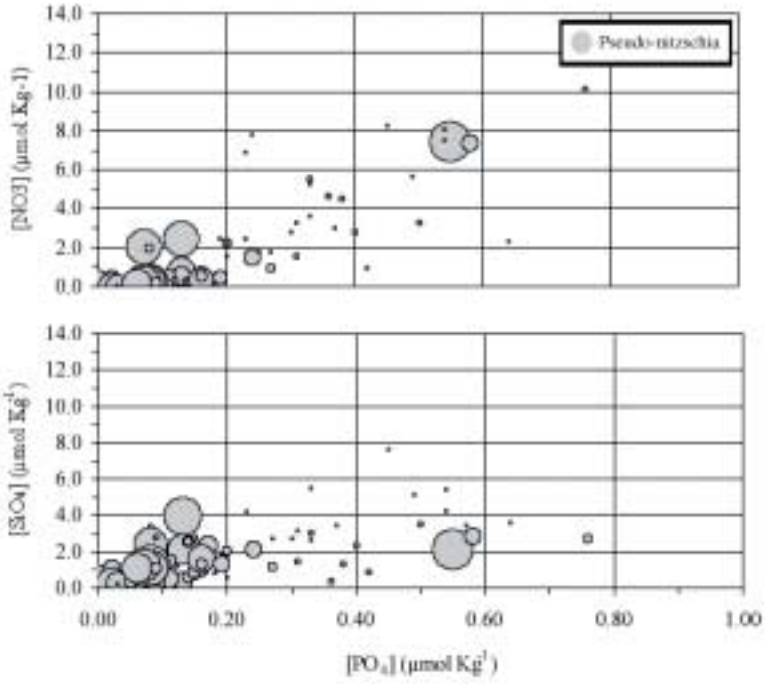

Figure 33. *Pseudo-nitzschia* cell numbers plotted against inorganic nutrients (nitrate, phosphate and silicate). Samples from all discrete depths and times throughout the study containing the above variables were used. Size of bubble markers reflects *Pseudo-nitzschia* cell concentrations, which ranged from 0.1 cells.mL^{-1} to 860 cells.mL^{-1}.

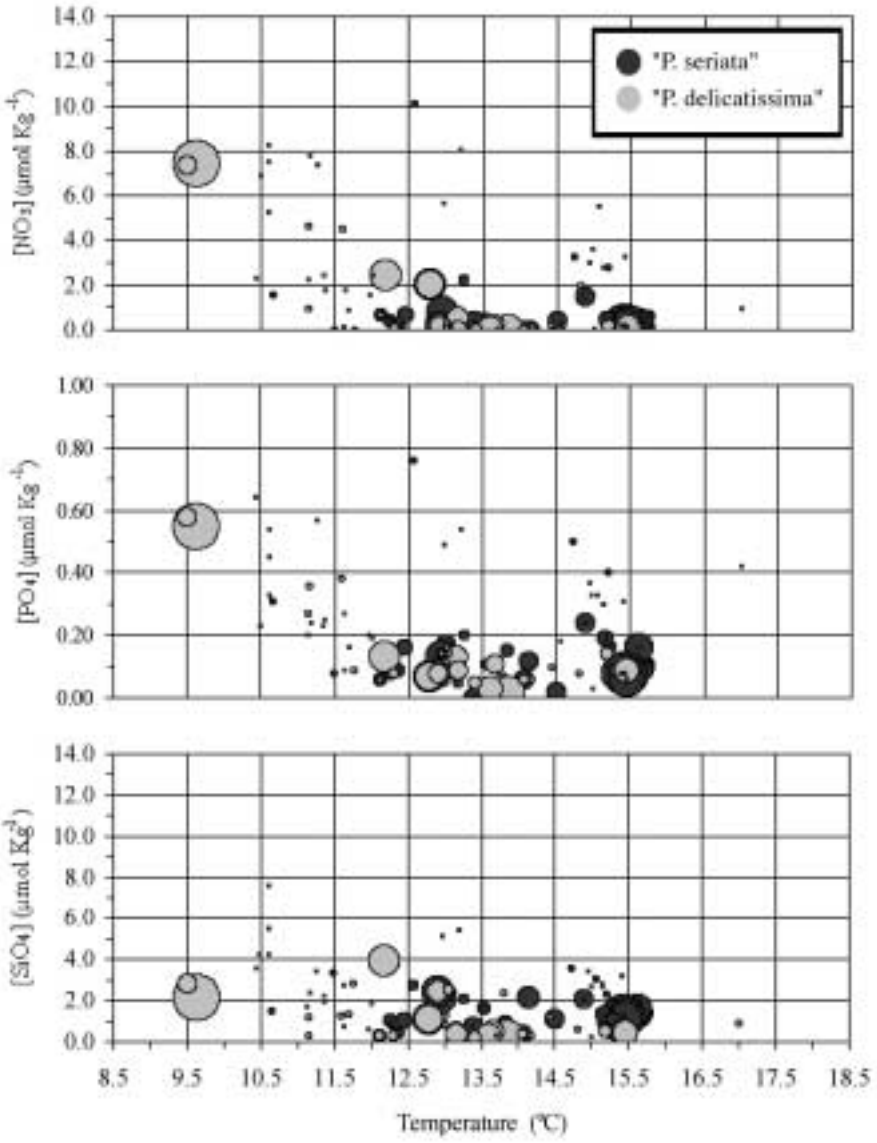

Figure 34. Cell numbers of the "P. seriata" and "P. delicatissima" groups plotted against temperature and inorganic nutrients (nitrate, phosphate and silicate). Samples from all discrete depths and times throughout the study containing all the above variables were used. Size of bubble markers reflects *Pseudo-nitzschia* cell concentration. Size of bubble markers reflects *Pseudo-nitzschia* cell concentrations. The "P. seriata" group ranged from 0.1 cells.mL^{-1} to 730 cells.mL^{-1} and the "P. seriata" group ranged from 0.1 cells.mL^{-1} to 850 cells.mL^{-1}.

DISCUSSION

Pseudo-nitzschia is a regular and abundant component of the phytoplankton in the coastal and shelf waters off Ireland. This is reflected in its presence in 67 of the 86 samples examined in this study. The physical environment where *Pseudo-nitzschia* spp. were observed is typical of a temperate zone with surface water temperatures of between 12.6-17.1°C. Moreover, the distribution of these species is in broad agreement with previously published data on *Pseudo-nitzschia* from other temperate waters (Hasle and Syvertsen 1997).

The earliest account of *Pseudo-nitzschia* in Irish waters is from data collected at 4 sites off the southwest, southeast and northeast coasts in 1904 by Gough (1906). Although the results presented by Gough are qualitative, *Pseudo-nitzschia*, referred to at the time as *N. seriata*, was present but rare off the southwest coast in the vicinity of Sherkin Island during August when surface water temperature recorded was low (12.0-13.0ºC). Raine et al. (1990a) re-examined Gough's daily records of surface water temperature in August 1904 and inferred that the area may have been under the influence of a local upwelling system. Gough also documented that *N. seriata* was present but very rare along the southeast coast in March and June and present but rare in August and September off the northeast coast of Ireland. Surface water temperature records were between 7.0-13.0ºC and salinity values ranged from 34.1-34.7 ppt.

More recent data on this genus in Irish waters has shown it to be a common component of the marine flora in coastal waters to the west of Ireland (Dooley 1973, Roden 1984, Roden et al. 1987, Raine et al 1990b, Roden and Raine 1994, Pybus 1996), eastern Atlantic shelf waters (McMahon et al. 1995), south western coastal waters (Roden et al. 1980**,** Raine et al. 1990a, 1990b**,** 1993a, Raine and Joyce 1996, Raine and McMahon 1998) and in the Celtic Sea (Raine and McMahon 1998).

Distribution of Pseudo-nitzschia in relation to local hydrographic conditions

Phytoplankton distributions off the west and southwest of Ireland are governed by the physical separation of coastal water and shelf water of more oceanic characteristics (Atlantic seawater) by the Irish Shelf Front (ISF) (McMahon et al. 1995, Raine and McMahon 1998). In spring, the seaward side of the ISF supports high levels of phytoplankton biomass and chlorophyll compared to the surrounding waters but does not at other times of the year (McMahon et al. 1995). McMahon et al. (1995) noted two distinct diatom communities on either side of the western ISF in April 1992. A transition across the front from *Pseudo-nitzschia* spp. and *Thalassiosira* spp. in coastal waters to *Thalassionema frauenfeldii* or *Chaetoceros decipiens* populations offshore was evident (McMahon et al. 1995). In early May 1997, the sampling area in the shelf waters off the west coast was well-mixed inshore with the development of thermal stratification becoming evident further offshore. Sea surface temperatures were relatively low (11.6-12.1°C) and the position of the ISF was close to the coast (~15 km) probably due to the strong to moderate W/SW winds that prevailed prior to the cruise. Although *Pseudo-nitzschia* spp. were only found at relatively low cell concentrations (non-detectable to 19 cells.mL^{-1}) throughout the region, the genus was present on either side of the ISF. *Pseudo-nitzschia* also increased in abundance across the ISF where nutrient levels were highest. At the furthest station offshore along the Erris Head transect *Pseudo-nitzschia* spp. were numerically dominant and co-occurred with the oceanic diatom *Thalassionema frauenfeldii* (O'Boyle 2002). Ultrastructural examination of 2 selected samples from either side of the front showed that while *P. fraudulenta* was present on both sides of the ISF; *P. pseudodelicatissima* was only evident on the oceanic side of the front.

Raine and McMahon (1998) found that the phytoplankton composition off the southwest coast changed markedly in relation to the position of the ISF. Here the transition in the type of phytoplankton communities on either side of the ISF was most evident during the summer. Small armoured dinoflagellates (e.g. *Heterocapsa rotundatum*), microflagellates and some summer diatoms (e.g. *Leptocylindrus mediterraneus*) accumulated offshore in water of more oceanic characteristics and a

greater diversity and a high standing crop of diatoms persisted on the coastal side of the front (Raine and McMahon 1998). Overall, results observed in this region during August 1993, July 1996 and September 1996 showed that *Pseudo-nitzschia* populations were only evident on the coastal side of the ISF.

Another major frontal system, the Celtic Sea Front (CSF), exists at the southern approach to the Irish Sea off the southeast coast of Ireland. The position of the CSF can be determined by a strong horizontal gradient in surface temperature (~1.0°C per kilometre) during summer. At this time of year the Celtic Sea, a region of low tidal energy, is thermally stratified while tidally generated turbulence in the Irish Sea prevents the development of the seasonal thermocline. Diatoms are the typical flora present in the Irish Sea while dinoflagellates are generally more characteristic of the Celtic Sea in late summer. In July 1996, a horizontal transition in the composition of *Pseudo-nitzschia* species was evident across the CSF. This front seemed to form a physical barrier that separated two distinct *Pseudo-nitzschia* populations. *Pseudo-nitzschia pseudodelicatissima* and *P. delicatissima* were the only *Pseudo-nitzschia* representatives recorded on the Irish Sea side of the front. On the thermally stratified side of the CSF, an array of *Pseudo-nitzschia* species from the "P. seriata" group (*P. pungens*, *P. multiseries*, *P. australis*, *P. fraudulenta* and *P.* cf. *seriata*) were present.

Off the southwest coast of Ireland strong wind-driven coastal upwelling occurs periodically. The resulting advection of deeper nutrient rich water, coupled with the destabilisation of the water column can promote the growth in summer of diatom species characteristic of the spring bloom (Raine et al. 1990a). Sea surface temperature can fall by up to 5.0°C within 5 days during these periodic events of upwelling, and so these events can be physically characterised by low surface water temperatures of <11.0°C (Raine et al. 1990a). In the absence of upwelling events, stratification is more pronounced in this region during summer and high-density populations of dinoflagellates including the "red-tide" forming dinoflagellate, *Karenia mikimotoi* (formerly known as *Gyrodinium aureolum*) are found associated with the subsurface chlorophyll maximum (Raine et al. 1993a). In August 1985, Raine et al. (1993a) noted that a pattern in

phytoplankton succession was apparent during an upwelling event off the southwest coast as one moved seaward. Diatom species typical of the spring bloom proliferated at the cooler well-mixed inshore stations while summer diatoms such as *Pseudo-nitzschia* (up to 460 cells.mL^{-1}) became more abundant in the adjacent weakly stratified waters outside the mouth of Bantry Bay. Roden et al. (1981) also referred to a sudden drop in surface water temperature from 15.0ºC to 11.0ºC in the vicinity of Fastnet Rock (southwest Ireland) in mid July 1979, and noted that the 13.0ºC isotherm provided a good indication of the transition zone from stratified to mixed waters. At this time *Pseudo-nitzschia* spp. predominated the phytoplankton community with cell densities of up to 1,300 cells.mL^{-1} on the inshore side of the mixed/stratified zone where surface temperature was 12.6ºC. Dinoflagellates became more common after this period and during August 1979, oceanic waters containing *K. mikimotoi* were advected shoreward displacing the established populations of *Pseudo-nitzschia*. Raine et al (1993a) concluded that the observations made by Roden et al. (1981) were probably a result of coastal upwelling rather than a tidal front separating inshore and offshore waters. Weak coastal upwelling activity also occurs from time to time around the southwest coast of Ireland in summer. This physical process causes the subsurface seasonal thermocline to shallow, transporting its associated phytoplankton population towards the surface to improved irradiance conditions (Raine et al. 1990b).

Observations along the Bantry Bay transect in August 1993 reflected similar conditions with up to 730 cells.mL^{-1} of *Pseudo-nitzschia* (predominately *P. pungens*) recorded at the mouth of the bay where an uplift of the thermocline isotherms was evident. Surface temperature recorded (12.0-13.0ºC) was at least 2.0-3.0ºC cooler then waters inshore and the well-stratified shelf water further off the coast. *Pseudo-nitzschia* was however, not evident in enumerated samples in adjacent shelf waters southwest of Bantry Bay at the time, suggesting that the physical environment observed might have reflected a post upwelling event. Local hydrographic conditions involving upwelling of colder nutrient rich water seem to play a large role in the distributional patterns of *Pseudo-nitzschia* populations in other parts of the world. (Buck et al. 1992, Walz et al. 1994, Dortch et al. 1997). For example, off the west coast of America in Monterey Bay *Pseudo-nitzschia*

spp. have a tendency to increase in cell numbers at times of post upwelling events (Fryxell et al. 1997).

On the continental shelf off the south and southwest coasts of Ireland, the typical situation in early summer is a thermally stratified water column with a fully developed seasonal thermocline located between 35-40 m. Temperature differences across the thermocline of 6.0ºC are typical in these waters during the summer and nutrient depletion in the upper mixed layer occurs as a result of biological activity. Raine and McMahon (1998) have shown that the abundance of *Pseudo-nitzschia* off the south and southwest coasts of Ireland can vary in summer from year to year. For example in this region during July 1991, July 1992 and August 1993 cell densities of >5 cells.mL^{-1} of *Pseudo-nitzschia* spp. were recorded off the south coast of Ireland but it was not detected in August 1994 or August 1995 (Raine and McMahon 1998). In this study, *Pseudo-nitzschia* was abundant with up to 860 cells.mL^{-1} in thermally stratified shelf waters off the south and southwest coasts during summer and autumn. These populations were concentrated in the top 25-40 m of the surface mixed layer during August 1993 (predominantly *P. pungens*), July 1996 (predominantly *P. pseudodelicatissima*) and October 1997 (predominantly *P. fraudulenta*) while very low concentrations of *Pseudo-nitzschia* spp., (non-detectable to 16 cells.mL^{-1}) were evident in September 1996.

Close to the Irish coast, the turbulent kinetic energy resulting from tides is strong enough to prevent the development of the thermocline in summer and so the water column remains relatively mixed all year round. Roden and Raine (1994) found that the entrainment of subpycnocline nutrient rich water helps sustain phytoplankton growth off the Connemara coast, northwest of Galway Bay. Although present in the spring, *Pseudo-nitzschia* appears to be more abundant and widespread in the bays and inshore waters off the west coast during summer and autumn (Roden et al. 1987, Raine et al. 1990b, Roden and Raine 1994, Pybus 1996). Highest cell densities of *Pseudo-nitzschia* ("P. delicatissima" group) of up to 567 cells.mL^{-1} have been reported in this region in June (Raine et al. 1990b). In the present study, a bloom of *Pseudo-nitzschia* comprising

primarily of *P. delicatissima* was observed in Galway Bay in early May 1996. This seems to be in agreement with Pybus's account of the seasonal patterns of phytoplankton in Galway Bay, where the diatom spring bloom in March/April is succeeded by a mixed assemblage of summer diatoms including, at times, large populations of *Pseudo-nitzschia* spp. (Pybus 1996).

The seasonal patterns of phytoplankton community composition in Irish waters are strongly linked to water column stability in addition to the availability of light and nutrients (Raine et al. 1993b). Diatoms are typically the first phytoplankton group to appear during spring when surface irradiance dramatically increase and vertical stratification of the water column as a result of solar heating deepens the critical depth for growth below the surface mixed layer. As the summer season progresses dinoflagellate populations tend to dominate the flora as the water column becomes well stratified. It is now known that *Pseudo-nitzschia* is often present and occasionally abundant off the west coast of Ireland during May. Off the southwest coast of Ireland the substantial numbers of *Pseudo-nitzschia* recorded at coastal stations in autumn 1993 may have been a consequence of coastal upwelling activity resulting in the elevated levels of nutrients especially silicate (>1μM) observed in surface waters. Such physical conditions would be consistent with those expected off the west coast in May when the spring bloom has subsided and water column stability increases.

Results observed off the south coast have also shown, however, that *Pseudo-nitzschia* cell densities in excess of >90 cells.mL^{-1} can, on occasions, be situated at depths of up to 25- 40 m. For example, in August 1993, large numbers of *P. pungens* (540 cells.mL^{-1}) were found at the thermocline (25 m) at a shelf station off the south coast. Cell densities of *Pseudo-nitzschia* spp. of between 150-860 cells.mL^{-1} were also observed at 35-40 m at a couple of stations off the south coast in July 1996. Nutrient concentrations were lower in these warmer nutrient depleted surface waters further off the Irish coast and one possible reason for the presence of these *Pseudo-nitzschia* populations at the seasonal thermocline may have been because the environmental conditions (light and nutrients) at this depth were well suited to growth. It is well documented that the

thermocline is generally a region where phytoplankton are exposed to sufficient irradiance levels from above and to moderate rates of nutrients from below, while cells are only gradually removed from this beneficial regime by turbulent diffusion (Tett 1981). In a review on the distribution of *Pseudo-nitzschia* off the west coast of America by Fryxell et al. (1997), the authors proposed that the distinctive behaviour of this genus in culture might explain the observation of subsurface populations in thermally stratified water. A chain forming planktonic stage was evident when nutrients were abundant, but cells became disassociated into singlets or doublets during stationary growth and sank to the bottom of the culture chamber. When additional nutrients were reintroduced into the cultures the same growth behaviour recurred. It remains unclear if these subsurface thermocline populations of *Pseudo-nitzschia* are the result of some form of physical accumulation process, *In situ* growth at these depths, or the settling out of cells from the water column until they reach a strong vertical density gradient at the seasonal thermocline.

Presence of Pseudo-nitzschia in relation to environmental data

The current study shows that the genus *Pseudo-nitzschia* is most abundant in Irish waters when thermal stratification is beginning to develop or is well established and nutrients are more limiting. This seems to be especially true for the "P. seriata" group. The "P. delicatissima" group was also present in more mixed waters and at times when the thermocline was less well developed. The ordination of *Pseudo-nitzschia* on plots of stratification and water clarity scaled with depth did not conform to the generalised pattern of diatoms and dinoflagellates presented by Jones and Gowen (1990). Instead it would appear that *Pseudo-nitzschia* occupies a transitional zone or ecological niche between these two groups. Other diatom species such as the weakly silicified *Leptocylindrus mediterraneus* and *Rhizosolenia alata* have also shown similar patterns in their distributions (Raine and Joyce 1996).

Populations of *Pseudo-nitzschia* in Irish waters were found over a wide range of temperature (9.1-17.0 ºC), salinity (33.8-35.5) and nutrient concentrations. Overall, the "P. delicatissima" group was present over a wider range of salinity then the "P. seriata"

group. The "P. seriata" group was most abundant in warmer waters during July, August and October. Levels of micronutrients ranged from non-detectable to 9.9 µM for nitrate, non-detectable to 0.76 µM for phosphate and 0.2 to 8.8 µM for silicate. Although the presence of high cell densities of *Pseudo-nitzschia* at silicate levels of between 1-2 µM reflects a variety of depths within the photic zone this observation requires further investigation since most diatoms are thought to grow best at silicate concentrations of >2 µM (Egge and Aksnes 1992). It is difficult to make direct relationships between these variables when a suite of *Pseudo-nitzschia* species are considered as a single entity. Future studies should concentrate on identifying the species composition at discrete depths in order to get a better understanding of the factors that govern the ecology of individual *Pseudo-nitzschia* species. Competitive culture studies of individual *Pseudo-nitzschia* species and other diatoms typical of the spring bloom might also help to elucidate what environmental factors, such as the relative concentrations of inorganic nutrients, in particular silicate, favour the growth of this diatom during early summer. One such culture study carried out by Sommer (1994), using a mixture of 12 phytoplankton species, showed that the relative abundance of *P. multiseries* increased with increasing silicate:nitrate ratios and it became the dominant species at low light irradiance (33 µmol photons.m^{-2}.s^{-1}) and at a silicate:nitrate ratio of 31:1.

Threat posed to the Aquaculture Industry by toxic Pseudo-nitzschia spp.

Martin et al. (1993) reported *P. pseudodelicatissima* at cell densities of 1,000 cells.mL^{-1} when shellfish were contaminated with domoic acid in the Bay of Fundy in 1988. Villac et al. (1993) subsequently considered harmful bloom concentrations of *Pseudo-nitzschia* to be of this order. More recently, however, Bates et al. (1998) noted that even 100 cells.mL^{-1} of a toxic species could contaminate shellfish with domoic acid at levels above the permissible concentration (20 µg DA g^{-1} wet weight of tissue). The current study shows that *Pseudo-nitzschia* populations off the Irish coast can reach cell densities above estimated trigger levels set in other countries for testing shellfish for the presence of domoic acid. These concentrations are comparable to earlier records of Roden et al. (1981) and Raine et al. (1990a). In Ireland, the Marine institute (MI) is the regulatory authority responsible for monitoring biotoxins in shellfish. Although harvesting closures

are solely based on the presence of these toxins in shellfish, an estimated number of 50 cells.mL^{-1} of *Pseudo-nitzschia* spp. has been set as a trigger level for flesh testing of shellfish (Joe Silke pers. comm.).

In the field, harmful events resulting from domoic acid production have been associated with *P. multiseries* and *P. pseudodelicatissima* in Eastern Canada (Subba Rao et al. 1988, Bates et al. 1989, Martin et al. 1990) and with *P. australis* along the Californian coast and in European waters (Buck et al. 1992, Fritz et al. 1992, Míguez et al. 1996, Campbell et al. 2001). Environmental and physiological conditions resulting in these events are still not completely resolved, but there are indications that physiological stress such as silica or phosphorus limitation may promote domoic acid production, at least in *P. multiseries* (Bates et al. 1991, 1996, Bates 1998). Walt et al. (1994) suggested that one possible explanation for the detection of domoic acid during Spring in shallow areas of Monterey Bay, a time when active upwelling is common, might be the result of physiological stress on *Pseudo-nitzschia* populations due to biological induced nutrient depletion as water in these areas are often retained due to the local hydrography of the bay. Culture studies have also been carried out to ascertain if other *Pseudo-nitzschia* species have the ability to produce the amnesic shellfish toxin, domoic acid and to investigate the physiological attributes involved in the production of the toxin (see Bates 1998). From these studies we now know that at least 7 *Pseudo-nitzschia* species can produce this toxin and that domoic acid is produced at times when division begins to decline in the absence of essential nutrients required for growth such as silicate and phosphate. Nitrate, however, is an essential nutrient for the production of amino acids such as domoic acid.

Seven out of the eight species observed in this study, including those tentatively identified, have been shown to be capable of producing domoic acid in laboratory cultures. These are *P. pungens* (Rhodes et al. 1996), *P. multiseries* (Subba Rao et al. 1988, Bates et al. 1989, Fryxell et al. 1990), *P. seriata* (Lundholm et al. 1994), *P. australis* (Garrison et al. 1992), *P. fraudulenta* (Rhodes et al. 1998), *P. delicatissima* (Smith et al. 1990) and *P. pseudodelicatissima* (Martin et al. 1990, Lundholm et al.

1997). Isolates of *P. pungens*, *P. fraudulenta*, *P. australis* and *P. delicatissima* from Irish waters have also been tested for domoic acid production. To date, the only isolate from Irish waters, *P. australis* has produced this toxin in culture (see chapter 4). This is cause for concern since this species was very abundant during October 1997 and was also present in net hauls taken during other times of the year.

Although the exact mechanisms that promote and control *Pseudo-nitzschia* blooms in Irish coastal waters are still unclear, this study has shown that localized blooms of this diatom have been recorded off the west and south coasts. Given that the Irish coastal current plays an important role in the advection of dinoflagellates populations from the south coast around to the bays of the southwest in summer it is reasonable to assume that *Pseudo-nitzschia* blooms would be transported in a similar manner (Raine and McMahon 1998).

Since the species composition and seasonal succession patterns of phytoplankton off the Irish coast can vary greatly from year to year, future research should involve the continued collection of biological, physical and chemical information over a time series to provide a better insight into the processes that influence *Pseudo-nitzschia* species shifts and distributions off the Irish coast. The application of species specific probes similar to those used in Monterey Bay for *Pseudo-nitzschia* (Scholin et al.2000) would greatly enhance the resolution of *Pseudo-nitzschia* species present at discrete depths and within different water bodies. The use of these novel techniques in conjunction with a time and depth series of environmental data would allow the tracking of *Pseudo-nitzschia* populations and the investigation of the relationship between environmental conditions that influence the development, maintenance and decline of these blooms.

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CHAPTER 4

CONFIRMATION OF DOMOIC ACID PRODUCTION BY *PSEUDO-NITZSCHIA AUSTRALIS* (BACILLARIOPHYCEAE) ISOLATED FROM IRISH WATERS.

Caroline K. Cusack

Marine Institute, Martin Ryan Marine Science Institute, Marine Microbiology Department, National University of Ireland, Galway, Ireland

Stephen S. Bates

Fisheries and Oceans Canada, Gulf Fisheries Centre, P.O. Box 5030, Moncton, NB E1C 9B6, Canada

Michael A. Quilliam

Institute for Marine Biosciences, National Research Council of Canada, 1411 Oxford St., Halifax, NS, B3H 3Z1, Canada

John W. Patching and Robin Raine

Martin Ryan Marine Science Institute, Marine Microbiology Department, National University of Ireland, Galway, Ireland

ABSTRACT

A non-axenic isolate of the potentially toxic diatom *Pseudo-nitzschia australis* (Frenguelli) from Irish waters was tested in two separate batch culture experiments. When grown under a low irradiance (~12 µmol photons.m^{-2}.s^{-1}; 16:8 h L:D cycle) for up to 40 days, the culture produced only trace amounts of the neurotoxin domoic acid (DA) during late stationary phase. Growth at a higher irradiance (~115 µmol photons.m^{-2}.s^{-1}; 12:12 h L:D cycle) resulted in DA production starting during late exponential phase and reaching a maximum concentration of 26 pg DA.$cell^{-1}$ during late stationary phase. Liquid chromatography coupled to mass spectrometry (LC-MS) was used to confirm the identity of DA in the culture. Irradiance and photoperiod could be important factors that contribute directly or indirectly to the control of DA production in *P. australis*. This is the first record of a DA-producing diatom in Irish waters and results indicate that *P. australis* may have been the source of DA that has recently contaminated shellfisheries in this area.

Key index words: domoic acid; electron microscopy; irradiance; Irish waters; mass spectrometry; *Pseudo-nitzschia australis*

INTRODUCTION

The pennate diatom genus *Pseudo-nitzschia* Peragallo has attracted much attention in recent years because some species within this genus have the potential to produce the neurotoxic amino acid, domoic acid (DA). Domoic acid was identified as the causative toxin in an episode of amnesic shellfish poisoning (ASP) in eastern Canada during November to December 1987 (Wright et al. 1989). Three people died and over 100 became ill after consuming blue mussels (*Mytilus edulis* Linnaeus) contaminated with DA (Todd 1993). The organism responsible for this toxic event was the pennate diatom *Pseudo-nitzschia multiseries* (Hasle) Hasle, which dominated a phytoplankton bloom at the time of the outbreak (Bates et al. 1989). Subsequently, other investigations have reported a total of nine species within the genus *Pseudo-nitzschia*, and one within the genus *Nitzschia* Hassall, that are potential DA producers (Bates 2000). These include, in decreasing amounts of DA per cell, *P. australis* (Frenguelli), *P. seriata* (Cleve) H. Peragallo, *P. multiseries*, *Nitzschia navis-varingica* Lundholm et Moestrup, *P. pseudodelicatissima* (Hasle) Hasle, *P. multistriata* Takano (Takano), *P. fraudulenta* (Cleve) Hasle, *P. pungens* (Grunow *ex* Cleve) Hasle, *P. delicatissima* (Cleve) Heiden, and *P. turgidula* (Hustedt) Hasle. Additional toxigenic species, and even genera, may be encountered as more countries establish monitoring programs for routine testing of DA in shellfish tissue samples.

The toxicity of *Pseudo-nitzschia* strains may differ within a given species. Toxigenic and non-toxigenic strains have been described for most of the *Pseudo-nitzschia* species listed above (Bates et al. 1998). For example, an isolate of *P. seriata* from Cardigan Bay, eastern Canada, did not produce DA (Bates et al. 1989), whereas one from Danish waters was toxigenic in culture (Lundholm et al. 1994). It can be difficult to compare toxicity among strains, because cultures are not always grown under the same conditions and optimum conditions for DA production are not necessarily known. Furthermore, isolates of the same species (e.g. *P. multiseries*) vary in the concentration of DA produced under seemingly similar conditions (Bates et al. 1989).

Attention was first drawn to *P. australis* after it was identified as the source of DA that killed Brandt's cormorants (*Phalacrocorax penicillatus* Brandt) and brown pelicans (*Pelecanus occidentalis* Ridgway) in Monterey Bay, California in 1991 (Fritz et al. 1992). The presence of DA was demonstrated conclusively, using liquid chromatography coupled to mass spectrometry (LC-MS), in a plankton tow that contained *P. australis*, in the anchovy vector (*Engraulis mordax* Girard), and in the pelican stomach contents (Fritz et al. 1992, Work et al. 1993). In 1999, *P. australis* was found to be responsible for the deaths of over 400 California sea lions (*Zalophus californianus* Lesson) (Scholin et al. 2000). *Pseudo-nitzschia australis* was also suspected to be the source of DA in New Zealand scallops (*Pecten novaezealandiae* Reeve) in 1993 and 1994 (Rhodes et al. 1996). In European waters, *P. australis* was first suspected as the source of DA in cultured mussels from Spain in 1994 (Míguez et al. 1996). This species was again the suspected source of DA that contaminated scallops (*Pecten maximus* Linnaeus and *Chlamys opercularis* Linnaeus) in Scottish waters in 1999 and 2000, and resulted in a closure of shellfish harvesting sites (Gallacher et al. 2001). In Ireland, DA was first detected in king scallops (*P. maximus*) above the regulatory limit of 20 µg DA.g^{-1} wet weight in 1999 (Mc Mahon and Silke 2000). Testing of mussels (*M. edulis*), oysters (*Crassostrea gigas* Thunberg and *Ostrea edulis* Linnaeus), and razor clams (*Ensis siliqua* Linnaeus) has shown low concentrations of DA (J. Silke, Marine Environment & Health Services Division, Marine Institute, Abbotstown, Dublin, Ireland, personal communication). Again, *P. australis* was found among the phytoplankton population in Irish waters (Cusack et al. submitted).

The production of DA by a newly suspected source organism should be tested by verifying the identity of DA produced by a unialgal culture by some unequivocal method, such as mass spectrometry (Bates 2000). Isolates of *P. australis* from Monterey Bay, New Zealand, Spain and Scotland have been reported to produce DA in culture (Garrison et al. 1992, Villac et al. 1993, Rhodes et al. 1996, Fraga et al. 1998, Campbell et al. 2001). However, it seems from these publications that the DA was analyzed by HPLC alone, a technique that relies on the coincidence of retention times

for the unknown and authentic DA peaks in the HPLC chromatograms. It is possible that other compounds may appear with the same retention time as DA, therefore leaving some doubt as to the true identity of the compound.

The aims of the present study were to isolate *P. australis* from Irish waters and to test it for the ability to produce DA in laboratory culture. Furthermore, the identity of DA produced in the *P. australis* cultures would be confirmed using LC-MS.

MATERIALS AND METHODS

A unialgal non-axenic culture of *P. australis* (strain WW_4) was isolated on 8 October 1997, from a net sample collected off the south coast of Ireland (52º 04.10' N; 07º 06.10' W, in the vicinity of Waterford harbour). A stock culture was maintained at 15ºC, under an irradiance of ~12 µmol photons.m^{-2}.s^{-1} (16:8 h L:D cycle) in sterile filtered sea water (salinity of 35.5), fortified with f/2 nutrients (Guillard and Ryther 1962) and silicon (250 µM). Aliquots (10 mL) of a 4-day old (exponential phase) *P. australis* stock culture were inoculated into six 500-mL Erlenmeyer flasks containing 300 mL of f/2 medium and silicon (250 µM). Initial cell densities were ~1,000 cells.mL^{-1}. Prior to inoculation, aliquots of culture medium, sea water and inoculum were shown to contain no detectable DA (see below). A separate sample was analysed for nutrients present in the culture medium using standard techniques (Grasshoff and Koroleff 1983). This gave concentrations of 760 µM NO_3, 40 µM NO_2, 20 µM NH_4, 20 µM PO_4 and 250 µM SiO_4. Four of the flasks containing *P. australis* (referred to throughout as Cultures 1, 2, 3 and 4) were incubated under the same conditions as the stock culture (i.e. at 15ºC, under an irradiance of ~12 µmol photons.m^{-2}.s^{-1}; 16:8 h L:D cycle). The remaining two flasks (Cultures 5 and 6) were incubated in a different growth chamber at 15ºC under an irradiance of ~115 µmol photons.m^{-2}.s^{-1} (12:12 h L:D cycle). Irradiance was measured with a Li-Cor Quantum Meter (model L1-1000). Variation in irradiance among the cultures in each growth chamber was minimized by alternating the culture positions daily; the cultures were also swirled daily.

Subsamples were taken from each culture every 1 – 3 days until stationary phase, after which the sampling interval was extended to every 4 – 6 days. Aliquots (15 mL) taken from Cultures 1 and 2 were filtered through membrane filters (Nuclepore 1-µm pore size). Filtrate and cells collected on the filter (resuspended in 10 mL of freshly filtered DA-free sea water) were stored at -20ºC until analysis for DA. Aliquots (15 mL) from Cultures 3 – 4 and 5 – 6 were used to analyse DA present in the "whole culture" (i.e. cells plus medium, cf. Bates et al. 1991).

Prior to DA analysis, *Pseudo-nitzschia* cells in 5-mL aliquots were sonicated for 1 min at 100 W, using a 1-cm diameter probe, to disrupt the cells. The debris was then removed by membrane filtration (Nuclepore 1-µm pore size).

Domoic acid was analyzed using the fluorenylmethoxycarbonyl (FMOC) derivatization and HPLC-fluorescence method (Pocklington et al. 1990), with the following modifications. The chromatographic system consisted of a Beckman System Gold HPLC (Beckman Coulter Canada Inc., Mississauga, ON, Canada) equipped with a 126 solvent delivery system, 507 autosampler (injection volume 20 µL) with built-in column heater (column temperature 38°C), and a Shimadzu RF-535 fluorometric detector (269 nm excitation; 311 nm emission) connected to a Beckman 406 interface module. Separations were performed on a Beckman ODS Ultrasphere column (25 cm x 4.6 mm i.d., Beckman Coulter Canada Inc., Mississauga, ON, Canada). Gradient elution was programmed linearly from 37.5% to 55% acetonitrile over 15 min, followed by an increase to 90% acetonitrile over 6 min, which was maintained for 6 min before programming back to initial conditions over 2 min. Initial conditions were maintained for a further 9 min, resulting in a total cycle time of 38 min. Calibration standards were prepared from pure DA obtained from Diagnostic Chemicals Ltd. (Charlottetown, PEI, Canada), with final concentrations from 3 ng $DA.mL^{-1}$ to 380 ng $DA.mL^{-1}$. The detection limit was 0.5 ng $DA.mL^{-1}$.

To confirm the identity of DA, culture samples were analysed using LC-MS (Quilliam et al. 1989). Analyses were conducted on an API-165 quadrupole mass spectrometer

with nebulizer-assisted electrospray ion source (PE-Sciex, Concorde, Ontario, Canada) interfaced with an Agilent (Palo Alto, CA) HP1100 HPLC. Separations were performed on a Keystone Scientific (Bellefonte, PA) column (5 cm x 2 mm i.d.) packed with 3 µm Hypersil-BDS C_8-silica, using 0.2 $mL.min^{-1}$ acetonitrile/water (9:1) containing 50 mM formic acid and 2 mM ammonium acetate. Detection was afforded by selected ion monitoring of the $[M+H]^+$ ion, *m/z* 312, and three confirmatory ions, *m/z* 266, 248 and 220, using 250 ms dwell times. The detection limit for DA was 50 ng $DA.mL^{-1}$. Calibration was performed using DACS-1C, a certified reference material provided by the NRC Certified Reference Material Program (Halifax, NS, Canada).

Culture aliquots (2 mL) were preserved in Lugol's iodine for direct visual counts of vegetative cells, using an improved Neubauer haemocytometer (Labkem Ltd., Dublin). The mean of 6 counts was reported. Only healthy cells (chloroplasts still intact) were counted. Specific growth rates were estimated by linear regression of log-transformed cell concentrations determined on 4 occasions in exponential phase.

Ultrastructural examination was carried out after the cells were acid cleaned (70% nitric acid; Boyle et al. 1984). Valves were examined under a Leica S430 scanning electron microscope (SEM) and a Hitachi 700 transmission electron microscope (TEM). Characterization of the *Pseudo-nitzschia* species was made according to the criteria of Hasle (1965), Hasle et al. (1996), and Hasle and Syvertsen (1996). Thirty *P. australis* cells from each culture were randomly selected under the LM and morphometric measurements of the valve and girdle dimensions recorded. Estimated cell volume was calculated using the equation for *Pseudo-nitzschia* in Hillebrand et al. (1999). An estimate of the DA cell content in "whole cultures" (i.e. cells plus medium) of Cultures 3 - 6 was calculated by dividing the concentration of DA recorded in each sample by the number of cells present (cf. Bates et al. 1991).

RESULTS

Ultrastructural examination revealed the cultured isolate as *P. australis* (Plate 1). Morphometric measurements of *P. australis* WW_4 are presented in Table 1, as well as morphological information on *P. australis* from wild samples taken off the Irish coast and data from Hasle et al. (1996) and Hasle and Syvertsen (1996). The development of aberrant (deformed) cells or "lobate" silica frustules was evident. Such changes in the frustule structure of *Pseudo-nitzschia* have previously been reported to be common in cultures, although less so in natural samples (Subba Rao and Wohlgeschaffen 1990, Garrison et al. 1992). The mean cell volume of *P. australis* WW_4 was 750 μm^{-3} ± 140, SD (n = 30, day 0).

Table 1. Morphometric data for *Pseudo-nitzschia australis*, recorded using electron microscopy. Measurements are presented as the mean ± SD; the range of measurements is shown in parentheses and n = the number of separate valves measured. Data from Hasle et al. (1996) and Hasle and Syvertsen (1996) are included for comparison.

Origin	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Poroids (in 1 µm)
Field samples	95 ± 17 (63-143) n = 124	6.6 ± 0.5 (5-8) n = 127	17 ± 1 (15-19) n = 127	17 ± 1 (15-19) n = 127	5.0 ± 0.5 (3.5-6) n = 125
Strain WW_4	51 ± 3 (46-55) n = 30	5.9 ± 0.4 (5-7) n = 30	17 ± 1 (16-18) n = 10	17 ± 1 (16-18) n = 10	5.1 ± 0.6 (4-6) n = 8
Literature	(75-144)	(6.5-8.0)	(12-18)	(12-18)	(4-5)

Cultures 1 – 4 (under an irradiance of ~12 µmol photons.m^{-2}.s^{-1}; 16:8 h L:D cycle) remained in exponential growth until day 6 (Figure 1). Highest cell concentrations recorded during stationary phase were 183,000 - 200,000 cells.mL^{-1}. The specific growth rates were 0.71, 0.71, 0.90 and 0.82 d^{-1} for Cultures 1, 2, 3 and 4, respectively. Domoic acid was not detected until late stationary phase in these four cultures (Figure 1). Extracellular DA was noted in the filtrate of Culture 1 on day 29 (23 ng DA.mL^{-1}). The toxin was not detected in the cell fraction until day 40 (0.20 pg DA.$cell^{-1}$), when ~ 89% of the total DA present was found in the culture medium (160 ng DA.mL^{-1}). For Culture 2, DA was also first detected in the filtrate on day 40 (16 ng DA.mL^{-1}); the toxin was not detected in the cell fraction of this culture during the experiment, indicating that intracellular DA may not be retained in the cells for long. Domoic acid was first observed in the "whole culture" (cells plus medium) of Culture 3 on day 40 (20 ng DA.mL^{-1}, 0.21 pg DA.$cell^{-1}$) and in Culture 4 on day 29 (26 ng DA.mL^{-1}, 0.17 pg DA.$cell^{-1}$). At this low irradiance, the highest DA concentration was found in Culture 4 on day 40 (120 ng DA.mL^{-1}, 0.98 pg DA.$cell^{-1}$).

Cultures 5 and 6, grown under a higher irradiance (~115 µmol photons.m^{-2}.s^{-1}; 12:12 L:D cycle), approached stationary phase on day 6 (Figure 2), as did Cultures 1 – 4. However, they attained a lower plateau, with a maximum cell density of 73,000 and 97,000 cells.mL^{-1}, respectively. Specific growth rates were 0.73 and 0.94 d^{-1} for Cultures 5 and 6, respectively. Domoic acid in the "whole culture" was first detected during the late exponential phase in the cultures grown at the higher irradiance (Figure 2). For Culture 5, this was on day 5 (14 ng DA.mL^{-1}, 0.24 pg DA.$cell^{-1}$). During early stationary phase, the DA concentration remained low until day 10 (36 ng DA.mL^{-1}, 0.65 pg DA.$cell^{-1}$), after which it rose to 260 ng DA.mL^{-1} (26 pg DA.$cell^{-1}$) on day 30. Culture 6 started to produce DA on day 4, during the exponential phase (13 ng DA.mL^{-1}, 0.63 pg DA.$cell^{-1}$). The cellular DA concentration remained relatively constant during the early stationary phase, after which it rose steadily from 31 ng DA.mL^{-1} (1.2 pg DA.$cell^{-1}$) on day 9 to 260 ng DA.mL^{-1} (4.8 pg DA.$cell^{-1}$) on day 19. It is pertinent to note that DA production increased dramatically in Cultures 5 and 6 on day 9 when cell densities decreased by 60%. Approximately 50 ng DA.mL^{-1} was produced per day

(0.20-1.87 pg DA.cell^{-1}.day^{-1}) until day 13 when DA production decreased and there was a slight increase in cell numbers. The fact that this was reflected in both cultures suggests that a common environmental factor, such as a sharp change in light intensity or temperature may have affected the cultures. No changes in environmental conditions were however, recorded over this period. The net result was that much lower cell densities were recorded in Cultures 5 and 6 in contrast to Cultures 1 – 4 on day 9 (Figures 1 and 2).

The identity of DA in the *P. australis* isolate was confirmed by conducting selected ion monitoring LC-MS analyses on several samples that had been analyzed previously by the FMOC-HPLC method. Electrospray ionization in conjunction with a high orifice voltage (50 V) afforded four ions: $[M+H]^+$ at *m/z* 312, $[M+H-HCOOH]^+$ at *m/z* 266, $[M+H-HCOOH-H_2O]^+$ at *m/z* 248, and $[M+H-2HCOOH]^+$ at *m/z* 220. Confirmation of DA was achieved by the coincident detection of all four ions in the same relative abundance and retention time as standard DA. Although the objective of the LC-MS experiment was primarily qualitative, fairly good agreement of quantitative determinations by the HPLC and LC-MS methods was also achieved for most samples. For example, the LC-MS determination of DA concentration in one sample (Figure 3) was 230 ng DA.mL^{-1}, whereas the HPLC analysis of that same sample gave 260 ng DA.mL^{-1}.

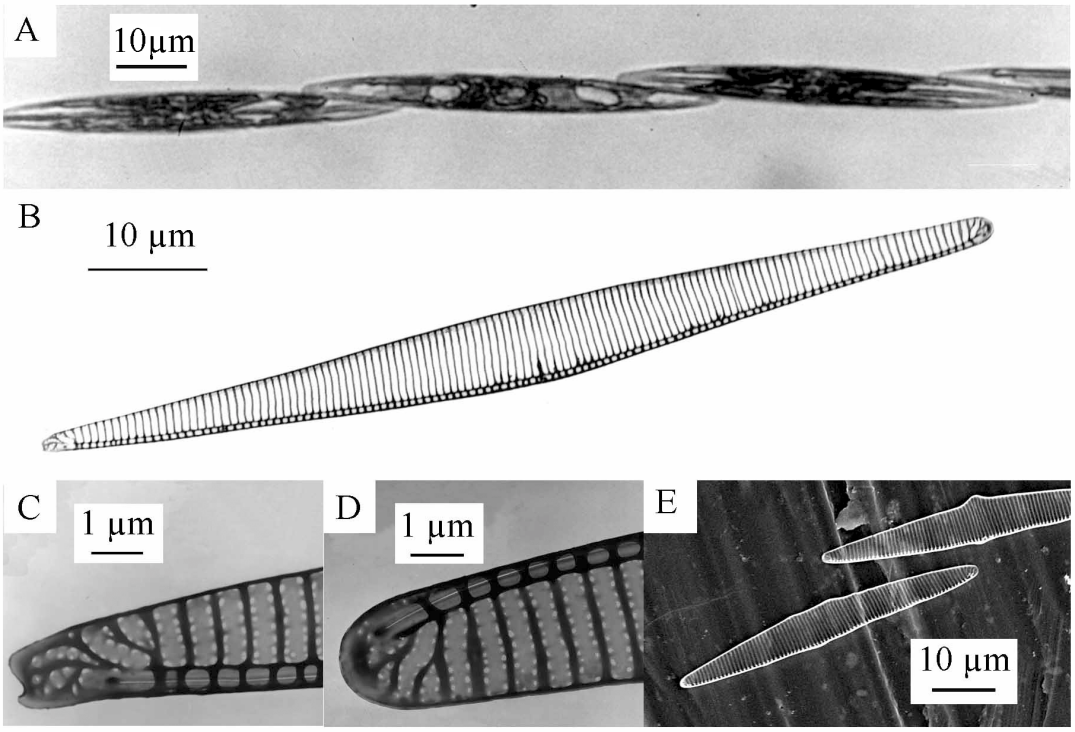

Plate 1. *Pseudo-nitzschia australis* strain WW_4. Light micrograph (A) showing vegetative cells in girdle view; overlap of cell ends ~1/4 of total cell length. Cell length ~60 µm and width ~5 µm. Transmission electron micrograph (B) of an acid-cleaned valve; 82 µm in length and 6.5 µm in width. The valve has an equal number of fibulae to interstriae (16 in 10 µm). Two rows of hymenate poroids are present per stria, with five poroids in 1 µm. Central interspace is absent. Transmission electron micrographs (C and D) showing the rounded valve apices. Scanning electron micrograph (E) of two acid-cleaned valves; lower valve is 50 µm in length and 6 µm in width. The upper valve is aberrant ("lobed" cell); this type of silica structure was frequently observed in the *P. australis* WW_4 isolate (see text).

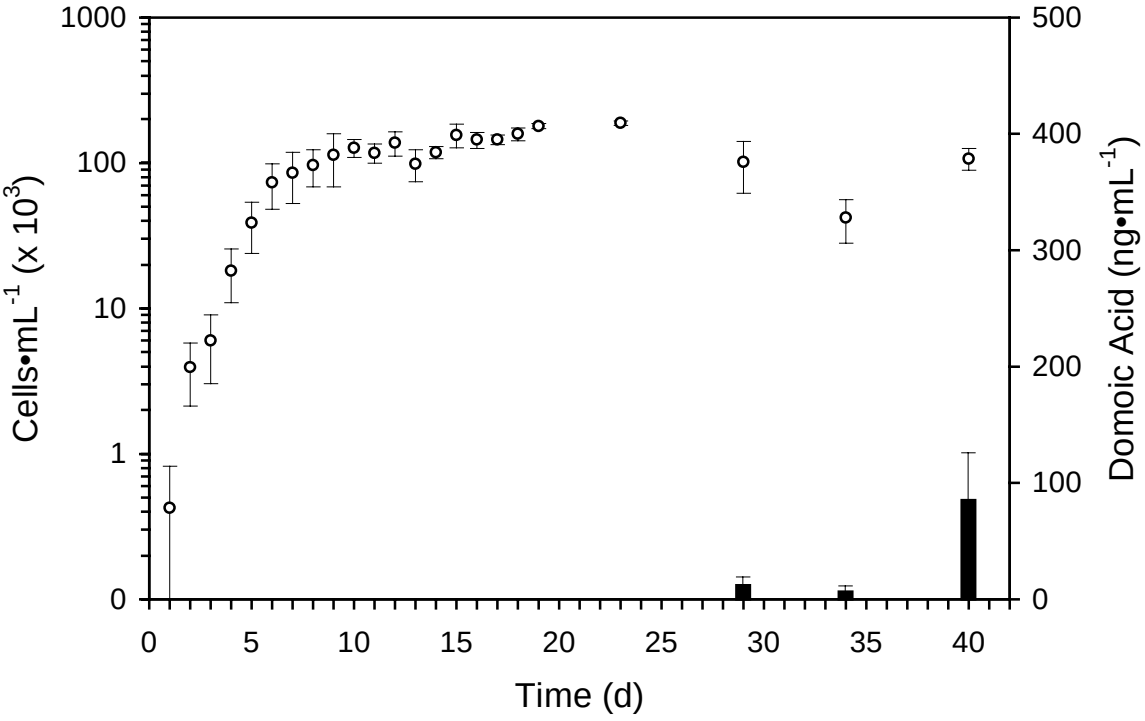

Figure 1. Average cell growth (○) and domoic acid content in Cultures 1 - 4 of *Pseudo-nitzschia australis* strain WW_4, grown at an irradiance of ~12 µmol photons.m^{-2}.s^{-1} (16:8 h L:D cycle). Domoic acid concentration in the "whole culture" (cells plus medium) expressed per mL (black bars). n = 4, ± SE.

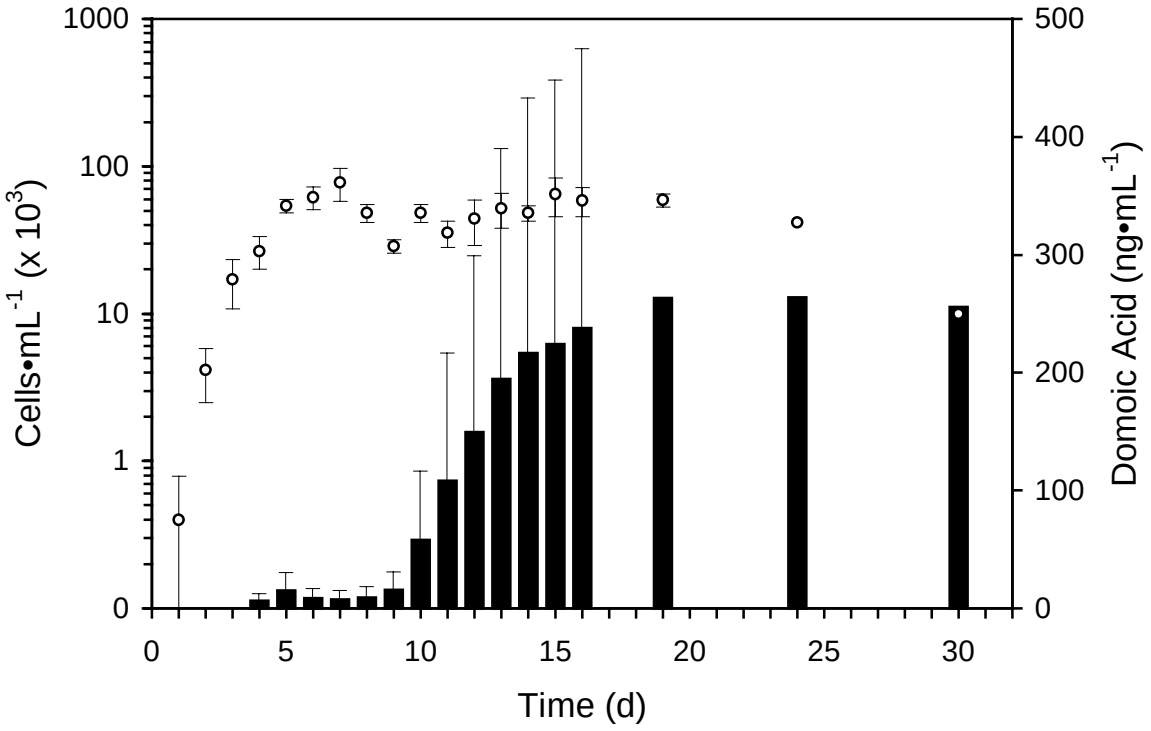

Figure 2. Average cell growth (○) and domoic acid content in Cultures 5 and 6 of *Pseudo-nitzschia australis* strain WW_4, grown at an irradiance of ~115 µmol photons.m^{-2}.s^{-1} (12:12 h L:D cycle). Domoic acid concentration in the "whole culture" (cells plus medium) expressed per mL (black bars). No samples were available from Culture 6 after day 19. n = 2, ± SE.

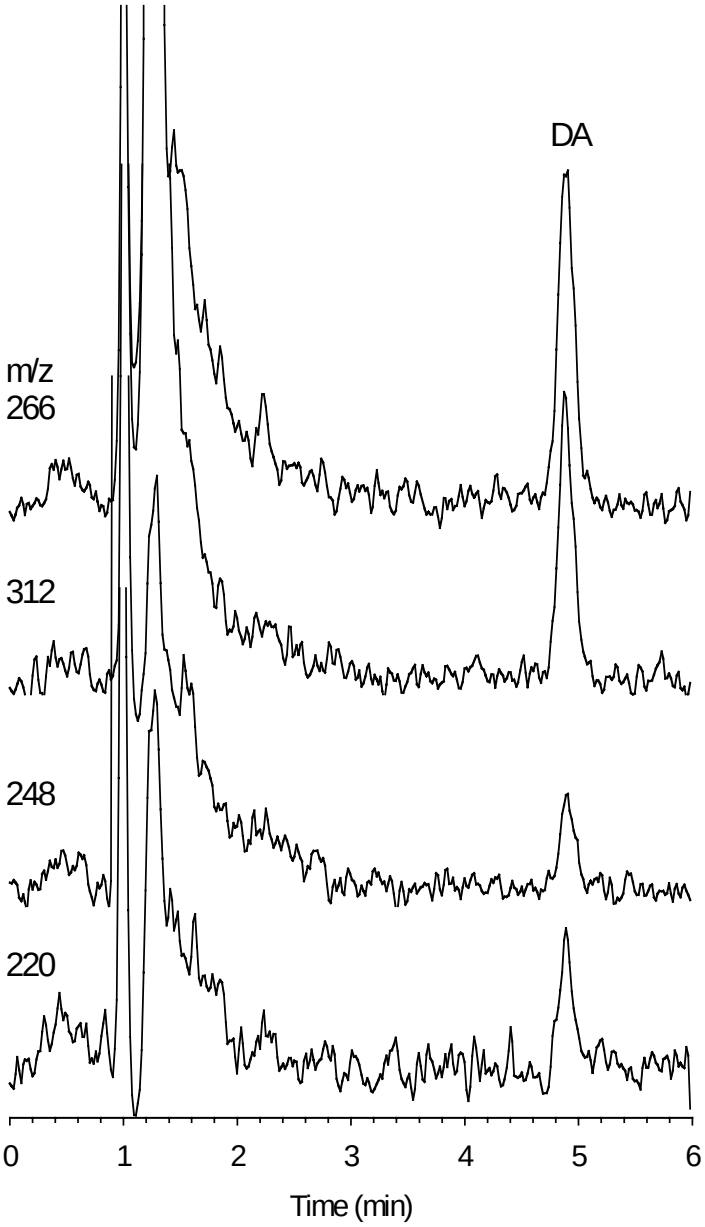

Figure 3. LC-MS analysis of Culture 5 (day 30; 1,000 cells.mL^{-1}) for domoic acid (DA). Selected ion monitoring was used in conjunction with high orifice voltage, which induced fragmentation of the $[M+H]^+$ ion, *m/z* 312, to three confirmatory ions, *m/z* 266, 248 and 220.

DISCUSSION

Diatoms of the genus *Pseudo-nitzschia* are a regular component of the marine microflora in Irish waters, with cell concentrations reaching >1,000,000 cells.L^{-1} (Roden et al. 1981). There is also clear evidence that at least one potentially toxigenic *Pseudo-nitzschia* species, *P. australis*, is present in these waters. This species is a common component of the marine flora during autumn off the south coast of Ireland (Cusack et al. submitted) and has also been recorded in waters off the west coast of Scotland (Campbell et al. 2001) and further to the south, off north west Spain (Míguez et al. 1996). These investigations show that the biogeography of *P. australis* in NE Atlantic waters is a lot more extensive than previously thought (Hasle 1972). In addition to this species, a further five potentially toxigenic *Pseudo-nitzschia* species (*P. multiseries, P. pseudodelicatissima, P. fraudulenta, P. pungens,* and *P. delicatissima*) have to date been identified in field samples collected off the Irish coast (Cusack et al. submitted). Although cultured isolates of *P. fraudulenta* (2 clones), *P. pungens* (1 clone), and *P. delicatissima* (5 clones) from Irish waters have tested negative for DA production (personal observation)

In this study, we have provided solid evidence that an isolate of *P. australis* from Irish waters produces DA. Furthermore, it is the first recorded verification of DA production by a *P. australis* culture using LC-MS. Cultures grown at high irradiance, 12:12 h L:D cycle (Cultures 5 and 6) contained amounts of DA (up to 26 pg DA.$cell^{-1}$, whole culture) similar to those reported by Garrison et al. (1992) (12 and 37 pg DA.$cell^{-1}$, actual cellular DA) for *P. australis*. Cultures grown at low irradiance, 8:16 h L:D cycle (Cultures 1 – 4) contained DA at amounts between non-detectable and 0.2 pg DA.$cell^{-1}$ (Cultures 1 - 2, actual cellular DA) and non-detectable to 0.98 pg DA.$cell^{-1}$ (Cultures 3 - 4, whole culture), comparable to the values measured by Villac et al. (1993) (non-detectable to 0.4 pg DA.$cell^{-1}$, actual cellular DA), Rhodes et al. (1996) (2 pg DA.$cell^{-1}$, actual cellular DA), Fraga et al. (1998) (non-detectable to 30 ng DA.mL^{-1}) and Campbell et al. (2001) (1.20 to 1.32 pg DA.$cell^{-1}$, actual cellular DA). These comparisons should, however, be treated with caution in view of the fact that

calculations of the DA cell content in Cultures 3 – 6 was estimated from "whole cultures". In addition to this, samples were taken on different days in batch culture, and the culture conditions were not the same across all of the studies. For example, the initial silicate concentration in the medium at the start of the experiment reported here was relatively high (250 µM), in contrast to the substantially lower level (39 µM) in the culture medium used by Garrison et al. (1992). Although nutrient measurements were only carried out at the beginning of this experiment it would seem likely that either phosphate or trace metals were limiting during stationary growth when highest amounts of domoic acid were detected in the cultures. Domoic acid content measured in Cultures 1 and 2 showed that highest concentrations were present in the media. Although the reasons for the release of DA into the surrounding medium remain unclear, Rue et al. (2000) have suggested that one possible biochemical function of DA is to assist the uptake of trace metals such as iron (Fe).

Irradiance can affect cellular DA levels because photosynthetic energy is required for DA production (Bates et al. 1991). Cultures of *P. multiseries* produced more DA at 100 than at 35 µmol photons.m^{-2}.s^{-1} (Bates 1998). In this study, cultures produced approximately 4 times more DA when grown under higher irradiance, most likely because of the greater availability of photosynthetic energy, as has been the case in studies on *P. multiseries* (Bates 1998). Production of DA was substantially lower and delayed until late stationary phase in the low irradiance cultures. This supports the hypothesis that energy for cell growth and maintenance competes with that required for DA biosynthesis (Pan et al. 1998). Different light:dark cycles may also have contributed to the amount of DA produced by our *P. australis* isolate. Villac et al. (1993) reported no detectable DA in clones of *P. australis* grown under continuous light, but DA was produced by the same isolate when the photoperiod was changed to 12:12 h L:D.

The results presented here emphasize the importance of culture conditions in determining when (exponential or stationary phase) DA production is triggered in batch culture. The literature shows some discrepancies in this regard. For *P. multiseries*,

many studies have shown that DA production begins slowly in late exponential phase and continues more rapidly into stationary phase (Bates 1998). Similar results were found for *P. seriata* (Lundholm et al. 1994) and *Nitzschia navis-varingica* (Kotaki et al. 2000). Recent results for *P. pseudodelicatissima* are not entirely consistent. Pan et al. (2001) reported DA production during most of the exponential phase and not during stationary phase, whereas Adams et al. (2000) showed that DA was produced during the late exponential as well as stationary phase. A similar discrepancy is found for *P. australis*. Garrison et al. (1992) reported DA production during most of the exponential phase and not during stationary phase. This contrasts with the present study, which shows that DA production can begin in late exponential phase and continue into stationary phase. The largest amounts of DA are, however, produced when cell division has either stopped or dramatically declined, consistent with *P. multiseries* (Bates 1988). The studies by Villac et al. (1993), Rhodes et al. (1996) and Fraga et al. (1998) did not report DA production curves in batch culture, but only single values, which makes comparisons dubious. Our results demonstrate the importance of irradiance level in determining when DA production begins in batch culture. Alternatively, discrepancies may be explained by differing physiological behavior among strains or isolates of presumably the same species of *Pseudo-nitzschia* (cf. Bates 2000).

The *P. australis* isolate WW_4 was in culture for almost a year when these growth and DA production experiments were carried out. During that time, the cell apical length, and therefore cell volume, had decreased (to 750 μm^3 ± 140, SD, as a result of vegetative division) relative to recorded morphometric measurements of *P. australis* in field samples (1770 μm^3 ± 450, SD; personal observation). Other field studies have estimated the biovolume of *P. australis* to range from 1834 μm^3 (Buck et al. 1992) to 4084 μm^3 (Walz et al. 1994). The production of DA by *P. multiseries* has been shown to decrease over time in cultures (Bates et al. 1999). This decrease is greater than can be explained simply by a reduction in cell size and volume. It is not yet known if *P. australis* exhibits this same tendency to decrease its production of DA over time in culture (see Villac et al. 1993).

The cell size of *P. australis* is larger than many other *Pseudo-nitzschia* species, including *P. multiseries*. An estimated size ratio of these two species is ~ 2:1 (Walz et al. 1994). Along with cell concentration, the larger cell volume of *P. australis*, and therefore its greater potential toxicity per cell, is an important consideration for determining how rapidly filter-feeding organisms can become toxic.

Results of this study support the supposition that *P. australis* is a source of DA in scallops from Irish waters. A detailed phytoplankton monitoring program to identify *Pseudo-nitzschia* to species level and to screen more *Pseudo-nitzschia* species isolates for their ability to produce DA is needed to fully elucidate the abundance and number of DA-producing organisms in these waters.

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CHAPTER 5

IDENTIFICATION OF *PSEUDO-NITZSCHIA* CULTURED ISOLATES USING MOLECULAR TECHNIQUES.

Caroline Cusack
Marine Institute Phytoplankton Laboratory, Martin Ryan Institute, National University of Ireland, Galway, Ireland

Donal Eardly
Martin Ryan Institute, National University of Ireland, Galway, Ireland

Christopher Scholin, *Peter Miller*
Monterey Bay Aquarium Research Institute, USA

Robin Raine and John W. Patching
Martin Ryan Institute, National University of Ireland, Galway, Ireland

ABSTRACT

Ribosomal DNA (SSU, ITS1 and a portion of the 5.8S) of three *Pseudo-nitzschia* species from Irish waters were amplified by PCR and the genetic relationship of the generated SSU rDNA sequences was evaluated to access their taxonomic position with published sequence data of *Pseudo-nitzschia* and other diatom genera whose sequence information was retrieved from the Ribosomal Database Project (RDP). Percentage similarity between the *Pseudo-nitzschia* SSU rDNA sequences was determined using a simple pairwise-distance method (uncorrected-p). Phylogenetic reconstruction using Maximum-Parsimony, LogDet/paralinear distances and Maximum-Likelihood analysis generated phylogenetic trees that separated a clade comprising of *Pseudo-nitzschia* species from the other diatom genera investigated.

Species specific LSU rRNA–targeted fluorescent oligonucleotide probes designed for a limited set of *Pseudo-nitzschia* strains isolated off the west coast of America were screened against several *Pseudo-nitzschia* cultures to investigated possible applications in the Irish biotoxin monitoring programme. Successful species specific responses were recorded for the *P. pungens*, *P. australis,* and *P. fraudulenta* probes. The *P. australis* isolate exhibited slight labelling reactions with gene probes designed for *P. multiseries*, *P. pungens* and *P. delicatissima*. Slight cross–reactions were observed of the *P. fraudulenta* isolates with the *P. pungens* specific probe. *Pseudo-nitzschia delicatissima* isolates exhibited no fluorescent signal when hybridized with the probe designed for *P. delicatissima*, but were positively labelled with the probe designed for *P. fraudulenta*.

Species identity of each *Pseudo-nitzschia* isolate was confirmed by examination of morphological features using the conventional method of electron microscopy.

INTRODUCTION

The potentially toxic marine diatom, genus *Pseudo-nitzschia* is made up of more then 20 species, all of which are difficult to discriminate between when viewed under a conventional light microscope (LM). Because some species can produce the neurotoxin domoic acid (DA), which causes amnesic shellfish poisoning (ASP) in humans (Wright et al. 1989, Todd 1993), it is important that an easy detection method is used in biotoxin monitoring programmes. Counting the number of *Pseudo-nitzschia* cells alone cannot determine if a *Pseudo-nitzschia* bloom is toxic or not and so identification down to species level is necessary to detect the presence or absence of toxigenic species. Because this requires some expertise on the morphology of these organisms, several molecular based methods have been investigated as alternative approaches to the traditional methods used for identification purposes. These molecular tools seem promising and include lectin binding assays (Fritz 1992), immunofluorescence assays (Bates et al. 1993), and distinctions between species based on differences in ribosomal DNA nucleic acid sequences (Scholin et al. 1994, Douglas et al. 1994, Manhart et al. 1995). Ribosomal DNA coding regions can also help to infer the phylogenetic relationships with other taxa (Douglas et al. 1994, Manhart et al. 1995). In fact these investigations have supported the decision of Hasle (1995) to raise *P. pungens* f. *multiseries* to the rank of species (*P. multiseries*) separate to *P. pungens* based on ultrastructural morphological examination. In order to examine this further, SSU rDNA sequences of 3 *Pseudo-nitzschia* species (*P. pungens, P. delicatissima* and *P. fraudulenta*) from Irish waters were compared with the sequence data of *Pseudo-nitzschia* in the literature (Manhart et al. 1995, Douglas et al. 1994).

Short complimentary rRNA-targeted molecular probes can be designed for specific target species using variable and conserved evolutionary regions of rDNA sequences. Probe specificity to the target organism can then be evaluated with cultures and environmental samples. Work carried out by Scholin et al. (2000) showed that species-specific rRNA-targeted oligonucleotide probes for *Pseudo-nitzschia* could be used as an alternative rapid quantitative technique in phytoplankton monitoring programmes. In

fact, in New Zealand, the Cawthron Institute uses these gene probe assays in conjunction with domoic acid testing to monitor potentially toxic blooms of *Pseudo-nitzschia* in their waters (Rhodes et al. 1998). The probes have also helped gather evidence linking a *P. australis* bloom to sealion deaths in Monterey Bay, California (Scholin et al. 2000).

Since this reliable and simple approach has proved to be an invaluable and cost-effective method in other routine phytoplankton monitoring programmes, a trial was set up to evaluate the specificity of LSU rRNA-targeted gene probes, already designed (Miller and Scholin 1996), using several cultured *Pseudo-nitzschia* isolates from Irish waters. This study investigated the possible application of these probes in aquaculture monitoring programmes in Ireland.

MATERIALS AND METHODS

Cultured Isolates

Non-xenic unialgal *Pseudo-nitzschia* cultures were isolated from vertical net haul samples collected off the coast of Ireland (Table 1). Cultures were maintained in a controlled growth chamber at 15ºC, under a photon flux density of ~12 $\mu E.m^{-2}.s^{-1}$ with a 16:8 h L:D cycle. Cultures were grown in sterile filtered seawater fortified with f/2 nutrients (Guillard and Ryther 1962) and silica. 5 mL aliquots of each culture were treated using concentrated nitric acid, heated in a water bath at 80ºC for 30 min (cf. Boyle et al. 1984). The cleaned frustules were rinsed with distilled water and stored in absolute ethanol until further use. Acid cleaned valves were examined under a Leica S430 scanning electron microscope (SEM) with an accelerating voltage of 15 kV or a Hitachi-700 transmission electron microscope (TEM), accelerating voltage of 75 kV. Identification of each culture was determined using the description keys outlined in Hasle et al. (1996) and Hasle and Syvertsen (1997).

Table 1. *Pseudo-nitzschia* species isolated from net material collected off the Irish coast. The identities of all the isolates were confirmed by examination of silica frustule using EM. $^{+}$*Pseudo-nitzschia* isolates screened with LSU rDNA probes, **Pseudo-nitzschia* isolates from which rDNA was extracted and sequenced.

SPECIES	REF.	DATE ISOLATED	LATITUDE (N)	LONGITUDE (W)	LOCAL DESCRIPTION
*P. delicatissima**$^{+}$	1913	6-Sept 1996	51º 27.50'	09º 32.40'	Long Island Bay, SW coast
*P. delicatissima**$^{+}$	1917	6-Sept 1996	51º 10.00'	09º 24.00'	Off Fastnet rock, SW coast
P. delicatissima^{+}	1924-3	7-Sept 1996	51º 23.00'	08º 15.90'	Shelf position off Cork, S coast
P. delicatissima^{+}	1424-4	7-Sept 1996	51º 23.00'	08º 15.90'	Shelf position off Cork, S coast
*P. fraudulenta**$^{+}$	W_2	8-Oct. 1997	52º 04.09'	07º 06.05'	Waterford Harbour, S coast
P. fraudulenta^{+}	2011	3-May 1997	54˚ 29.00'	10˚ 39.90'	Off Erris Head, Sligo, W coast
*P. pungens**$^{+}$	WW_3	8-Oct. 1997	52º 04.09'	07º 06.05'	Waterford Harbour, S coast
P. australis^{+}	WW_4	8-Oct. 1997	52º 04.10'	07º 06.10'	Waterford Harbour, S coast

DNA manipulation

Double stranded genomic DNA was extracted from *P. delicatissima* (strains 1913, 1917), *P. fraudulenta* (strain W2) and *P. pungens* (strain WW_3) using a modified version of the CTAB method of Doyle and Doyle (1987). The target rDNA was amplified by means of the polymerase chain reaction (PCR) technique carried out in a Perkin–Elmer 480 DNA thermal cycler. Oligonucleotide primers, PSEUD-1 and PSEUD-2 (Table 2) were designed from a published alignment of the 18S SSU, ITS1 and a fragment of the 5.8S rDNA of *P. Pungens* and *P. multiseries* (Manhart et al. 1995). Each PCR amplification reaction contained ~ 10–15 ng of template genomic DNA, 1/10 10x *Taq* buffer (Bioline NH_4 buffer (10x): 160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl (pH 8.8 at 25°C), 500 mM KCl, 0.1% Tween-20, 1.5 mM $MgCl_2$ (Bioline), 200 μM dNTPs (Bioline), 100ng of each primer, 1.0 U of *Taq* DNA polymerase (Bioline) in a total volume of 100 μL aliquots (profiles of the PCR reaction conditions are present in Table 2). All PCR reactions included a negative control where sterile water was used instead of template DNA. The PCR products were assayed by electrophoresis on a 1% agarose gel containing 10 $\mu g.mL^{-1}$ Ethidium Bromide (Boehringer–Mannheim) and visualised by UV excitation.

Gel purification of the PCR product, cloning and transformation was carried out using a pCR®-XL-TOPO® cloning kit (Invitrogen). Clones were grown overnight in LB broths containing 50 μg/ml kanamycin and the plasmid DNA extracted using QIAprep Miniprep kits (Qiagen) according to the manufacturers instructions. To verify that the plasmids contained an inserted PCR product, a restriction enzyme, *EcoR1*, (Boehringer–Mannheim) digest was carried out. This was confirmed by PCR amplification of the insert directly from the clones using the oligonucleotide primers $M13_{(-20)}$ forward and M13 reverse (Table 2) which flank the vector multiple–cloning site. Plasmid DNA was then ethanol precipitated and centrifuged at top speed for 15 minutes. The resulting pellet was air-dried before being sent to the Recombinant DNA group (NUI,Galway) or MWG-Biotech (Milton Keynes, England) for automated sequencing. Sequencing was carried out using an ABI Prism 310 or ABI Prism 377 (Applied Biosystems, California). Nucleotide sequencing was determined using dRhodamine dye terminator cycle sequencing ready reaction kits (Perkin Elmer, California) or Bigdye mix version 3 kits (Macherey & Nagel, UK) as recommended by the manufacturers instructions. 250 ng of plasmid DNA product was used per read in each 20 μL sequencing reaction (NUI,Galway) or 1500 ng of plasmid DNA product was used per read in each 3 μL sequencing reaction (MWG-Biotech). Sequences were generated in a 5'-3' direction using the M13 sequencing primers and sequence specific primers (PSEUD-2 and PSEUD-3 designed from the published sequences alignment in Manhart et al. 1995, Table 2) and with a final accuracy of 96% (NUI,Galway) to >99% (MWG-Biotech).

Vector specific sequences were removed from both ends of the resultant sequences. Partial sequences were linked together in overlapping regions using the manual sequence alignment editor, GeneDoc (Nicholas et al. 1997). To verify the origins of the PCR products, a GenBank BLAST search was conducted (Altschul *et al*. 1990) and showed significant sequence homology with other *Pseudo-nitzschia* rDNA sequences. Selected SSU rDNA sequences of *Pseudo-nitzschia pungens* (GenBank accession #U18240), *P. multiseries* (#U18241), *Nitzschia apiculata* (#M87334), *Bacillaria paxillifera* (#M87325), *Cylidrotheca closterium* (#M87326), *Thalassiosira rotula* (#X85397), *Skeletonema costatum* (#X85395), *Stephanopyxis broschii* (#M87330) and *Rhizosolenia*

setigera (#M87329) were retrieved with gaps preserved (showing information on secondary structure) from the Ribosomal Database Project (RDP) SSU Eukaryotes Data Set using the Hierarchy Browser (Maidak et al. 2001). The SSU rDNA sequence data of *P. pungens* (strain BRUNDC-X), *P. multiseries* (strains 13CC, NPARL, POM-X) and *P. australis* (PSEUD-X) published in Douglas et al. (1994) and the SSU rDNA sequenced portion of the Irish isolates were manually aligned with these sequences. Internal transcribed spacer 1 fragments and the portion of the 5.8S region sequenced from the Irish isolates were excluded during phylogenetic analysis because of alignment difficulties with the ITS1 region (sequences were ambiguously aligned) and the lack of data on these regions for *Pseudo-nitzschia* in GenBank. Overall similarities between the SSU coding region of the *Pseudo-nitzschia* species were calculated by converting the data into a pairwise-distance matrix (PAUP* ver. 4.0b10) (Swofford 1996) using the uncorrected-pairwise difference distance method (all characters and all transformations weighted equally).

Phylogenetic analysis

Phylogenetic reconstruction was performed using PAUP* ver. 4.0b10 (Swofford 1996). Phylogenetic trees were inferred and constructed from the aligned sequences with Maximum-Parsimony (MP), distance and Maximum-Likelihood (ML) based methods. *Stephanopyxis broschii* (#M87330) and *Rhizosolenia setigera* (#M87329) were used as outgroups to root the derived trees. The SSU rDNA sequence alignment contained 1762 characters (including gaps) of which 1633 characters were considered unambiguous and used in the analysis (see Appendix 5). Differences in character states in very conserved regions and ambiguities observed in the base composition of the SSU within each species were omitted during phylogenetic analysis because of the possibility of sequencing errors or potential micro-heterogeneity within the 18S rRNA gene. For example, base differences observed in *P. delicatissima*, isolates 1913 and 1917, at character positions (Appendix 5) 879 (A↔G), 893 (T↔C), 1016 (G↔A), 1078 (C↔T) and 1484 (A↔C) and removed prior to phylogenetic investigation.

Maximum-Parsimony trees were generated using heuristic searches based on 1000 random sequence addition replicates and a branch-swapping algorithm, tree-bisection reconnection (TBR). All characters were treated as unordered (allows the possibility of any base to change into another) and with equal weight, gaps were treated as a "fifth base". Branches collapsed (creating polytomies) if maximum branch length was zero. Multistate taxa were interpreted as uncertain and starting tree(s) were obtained via stepwise addition.

Table 2. Oligonucleotide primers and amplification profiles used in PCR^{+} amplification and sequencing* reactions of rDNA from *Pseudo-nitzschia delicatissima* (1913, 1917), *P. fraudulenta* (W2) and *P. pungens* (WW3).

NAME	PRIMERS	POSITION	PCR amplification profile
$^{+}$PSEUD-1 forward	5'-CAGTAGTCATACGCTCGTCT-3'	1-20	25 cycles
$^{+}$PSEUD-2 reverse	5'-GGATGTCTAGGTTCCCACAA-3'	1913-2064	94 ºC for 1 min. (denaturation)
			56 ºC for 1 min. (primer annealing)
			72 ºC for 1 min. (extension)
			final extension step of 72 ºC for 7 min.
$^{+}$*PSEUD-3 forward	5'-GTGCCAGCAGCCGCGG-3'	532-547	1 cycle
$^{+}$*PSEUD-4 reverse	5'-GTACACACCGCCCGT-3'	1606-1619	94 ºC for 5 min. (denaturation)
			25 cycles
			94 ºC for 1 min. (denaturation)
			50 ºC for 1 min. (primer annealing)
			72 ºC for 1 min. (extension)
			final extension step of 72 ºC for 7 min.
$^{+}$*$M13_{(-20)}$ forward	5'-GTTTTCCCAGTCACGAC-3'		25 cycles
$^{+}$*M13 reverse	5'-CAGGAAACAGCTATGAC-3'		94 ºC for 30 sec (denaturation)
			52 ºC for 1 min. (primer annealing)
			72 ºC for 1 min. (extension)
			final extension step of 72 ºC for 5 min.

Trees generated using the distance-based method followed the optimum criterion of minimum evolution (selects the tree that minimises the sums of branch lengths). Pair-wise distances were calculated using LogDet/paralinear (Lockhart et al. 1994) and neighbor-joining (Saitou and Nei 1987) analysis.

Modeltest ver. 3.06 (Posada and Crandall 1998) was used to determine the DNA substitution model with the highest log-likelihood for the dataset. The derived parameters were then imputed for the ML searches (see results for details).

Statistical support for phylogenetic groupings on individual taxa was accessed by bootstrap analysis. Bootstrap analysis (resampling all characters) was carried out on the trees generated from the three methods used, 1000 replicates for parsimony and distance trees and 100 replicates for the ML tree.

Application of LSU rRNA-targeted oligonucleotide probes

Each culture was screened (x3) with large-subunit ribosomal RNA (LSU rRNA) targeted oligonucleotide fluorescent probes (Table 3) labelled with fluorescein isothiocyanate (FITC) using the whole cell (*In situ*) hybridization technique outlined in Miller and Scholin (1998). The LSU rRNA target location and sequences of the probes can be found in Miller and Scholin (1996). The relative signal intensity observed on stained cells after the hybridization procedure was given a score following Miller and Scholin (1996). A score of -- was given to the negative probe (LSU rRNA-targeted for North American strains of *Alexandrium tamerense*) and ++ to the positive control (small subunit rRNA-targeted universally conserved sequence). Test cells were compared to these and scored accordingly. When the signal intensity was seen to be brighter than the negative control but not as bright as the positive control a value of +- was given. A no probe control was used to gauge the extent of the cells natural autofluorescence.

Table 3. LSU rRNA–targeted oligonucleotide probes and their target species (Miller and Scholin 1996).

Probe	Target species
uniC	positive control (all organisms).
uniR	negative control.
muD1	*Pseudo-nitzschia multiseries* (++).
muD2	*P. multiseries* (++), *P. pseudodelicatissima* (++).
puD1	*P. pungens* (++).
auD1	*P. australis* (++).
frD1	*P. fraudulenta* (++), *P. delicatissima* (+-), *P. heimii* (+-).
deD1	*P. delicatissima* (++).
heD2-2	*P. heimii* (++).
amD1	*Nitzschia americana* (++). *N. americana* is no longer considered to be part of the genus *Pseudo-nitzschia* (Hasle and Syversten 1997)

1 mL of mid-exponential growth cultured cells was gently collected onto 25 mm, 1 µm Nucleopore polycarbonate membrane filters in a custom filter manifold. 5 mL of saline EtOH (70%) fixative was added to each tube immediately and allowed to fix for 2 hours. Samples were then rinsed a second time with the fixative to reduce any autofluorescence and washed twice with 1 mL hybridization buffer to prevent any precipitates forming that could interfere with the assay. 400 µl of hybridization buffer was then added to each tube (to resuspend the cells) after which 12 µL (200 ng.μL^{-1}) of either the positive, negative or species specific probe was aliquoted to each filter. Hybridization was carried out in a pre-heated darkened waterbath and maintained at the appropriate hybridization temperature (muD1 @ 55˚C, all other probes @ 45˚C) for 2 hours. Finally each filter was washed twice with pre-warmed hybridization buffer (incubated at 45-50˚C) and left to stand for 5 minutes to remove excess unbound probe (eliminates any non-specific background staining) on the filter**.** The filter was mounted on a standard microscope slide and 20 µl of Prolong antifade (Molecular Probes, Europe) was added before mounting the coverslip. The slides were protected from bright light and stored at -20ºC to maintain the fluorescence stability of the probe.

Samples were viewed under a Nikon Optiphot-2, epifluorescent light microscope. The microscope was fitted with a fluorescein filter set containing a narrow band-pass

excitation filter (excitation 470-490nm), a dichroic beam-splitting mirror (≥ 510 nm at 50% of the maximum transmission), and a long band-pass emission filter (≥ 515 nm). The light source used was an ultra high-pressure Short Arc mercury lamp HBO (100 W). Colour light micrographs were taken with a Nikon Microflex UFX-DX camera and Kodak Gold 400ASA film. All images were recorded at an exposure time of 32.36s. The printing process was also kept constant to allow comparison of epifluorescent intensity ranges on the cells hybridized with fluorescently labelled negative and positive controls and the species-specific probes.

RESULTS

Morphology

Electron microscopy analysis confirmed that the *Pseudo-nitzschia* species in culture were *P. delicatissima* (1913, 1917, 1924-3, 1924-3), *P. fraudulenta* (W2, 2011), *P. pungens* (WW3) and *P. australis* (WW4) (Plate 1). Morphological identification of the cultures to species level was based on the criteria outlined in Hasle and Syvertsen (1996).

Ribosomal DNA analysis

Ribosomal DNA coding regions 18S SSU, ITS1 and a fragment of the 5.8S of three *Pseudo-nitzschia* species were sequenced and aligned (Figure 1). A high sequence identity of 98.12-99.94% was recorded between characters positions of the SSU coding region within the genus *Pseudo-nitzschia* (the total number of character positions after alignment including gaps was 1747, Table 4, Appendix 5). The two *P. delicatissima* isolates showed significant similarities (99.71%), as did *P. multiseries* (99.66-99.83%) and *P. pungens* (99.54-99.94%). The ITS1 region of *P. delicatissima* (strain 1913, 1917), *P. fraudulenta* (W2), *P. pungens* (WW3, F310: Manhart et al. 1994) and *P. multiseries* (TKA-2: Manhart et al. 1994) showed high variability in sequence length and base composition between species. The length in nucleotides of the ITS1 of *P. delicatissima* (1913 and 1917) was 249 bp, 286 bp in *P. fraudulenta* (W2), 259-260 bp in *P. pungens* (F310, WW3) and 227 bp in *P. multiseries* (TKA-2). The sequenced portion of the 5.8S fragment (43-44 bp) was very conserved between species.

Phylogenetic analysis

Based on the alignment used to infer the phylogenetic relationship of the genus *Pseudo-nitzschia* and other diatom genera (Appendix 5), a tree with a comparable overall branching topology was found with all the molecular phylogenetic methods examined (Figures 2-4). The *Pseudo-nitzschia* sequences formed a monophyletic group with a high bootstrap support (100%). Within this group several clusters formed and the branch lengths between these ingroups were very short, reflecting the close relationship within the genus. The *Pseudo-nitzschia* isolates fell into distinct species complexes, but the branching order of each clade varied according to the phylogenetic method used to create the tree. *Pseudo-nitzschia delicatissima* (always clustered together with a bootstrap support of 100) and *P. fraudulenta* formed a monophyletic group that was moderately (70-84%) supported. *Pseudo-nitzschia multiseries* formed a monophyletic clade well supported by bootstrap analysis (86-100%) and all *P. pungens* isolates grouped together in a well-supported clade (94-99%). The branching order of *P. australis* in relation to other *Pseudo-nitzschia* species however, was not well resolved and the placement of this species could not be determined with any confidence. This taxa formed a polytomy in ML analysis indicating that *P. australis* may be part of a separate lineage to the other *Pseudo-nitzschia* species examined. The distance and parsimony methods placed *P. australis* as a deep branching lineage of *P. pungens* although bootstrap support for this topology was very low (51-54%) and the *australis/pungens* cluster formed a collapsed branch during bootstrap analysis. This could have been an effect of taxon sampling, since the data set examined contained only one sequence from this species. Additional sequence data of several strains of *P. australis* is required to investigate this further.

Of the 1633 characters used in the MP analysis, 1321 characters were constant/conserved, 159 variable characters were considered uninformative and 153 characters were considered informative. One parsimonious tree of 456 steps was generated. The generated tree gave a consistency index of 0.829, a retention index of 0.797 and a rescaled consistency index of 0.661.

The total number of rearrangements tried in LogDet/paralinear analysis was 2160 with the best tree(s) score of 0.45316. The tree topology was similar to that obtained by MP analysis.

The ML tree was obtained by a general time reversible model of DNA substitution with the base frequencies (A=0.2757 C=0.1898 G=0.2531 T=0.2812), gamma shape parameter (1.077684) and proportion of invariable sites (0.56493) estimated by PAUP. This model of DNA substitution had the highest – log-likelihood using the Modeltest program and yielded a tree with a score of 4586.1895.

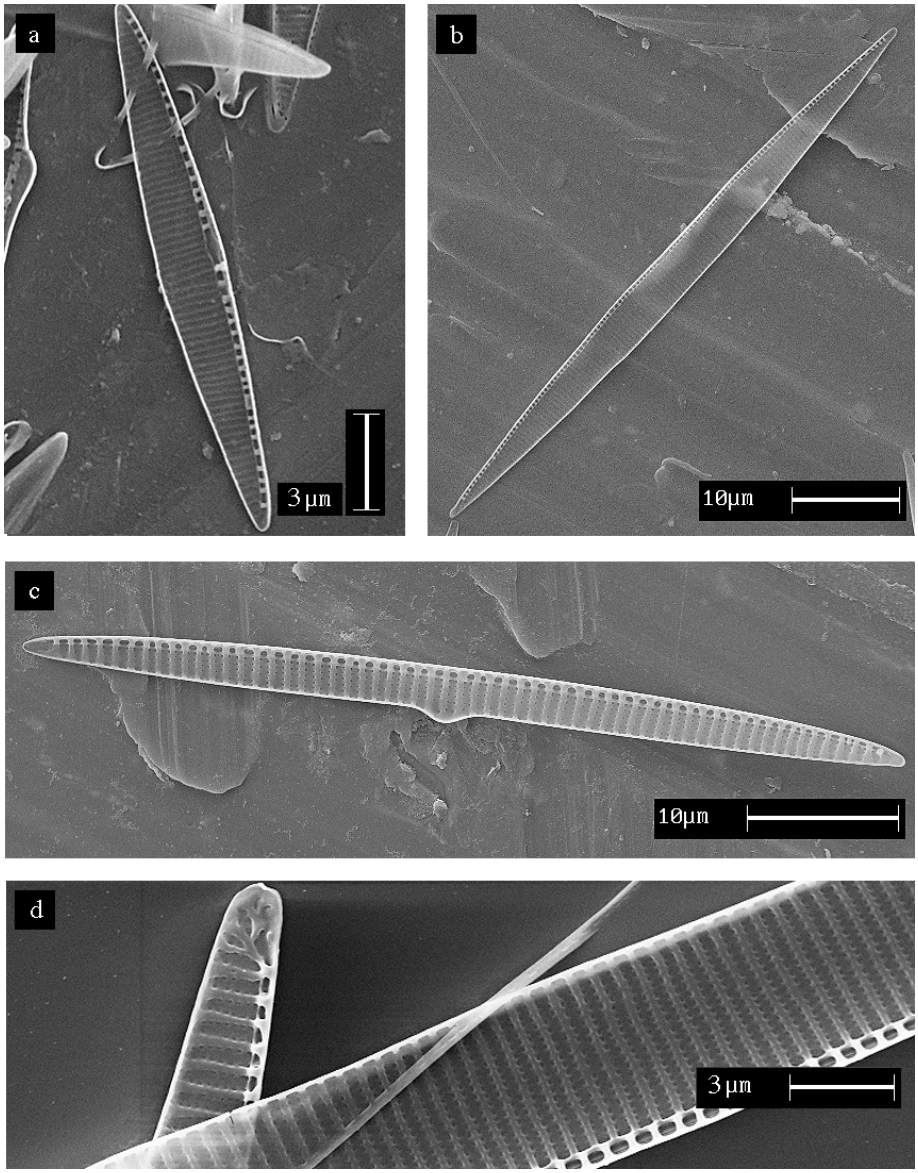

Plate 1. Scanning electron micrographs electron micrographs of *Pseudo-nitzschia* species selected for molecular analysis. (a) *Pseudo-nitzschia delicatissima* (isolate 1913: length = 16.5 µm, width = 2 µm, striae in 10 µm = 40, fibulae in 10 µm = 26, poroids in 1 µm = 8, 2 rows of poroids per stria and 4 striae per central interspace). (b) *Pseudo-nitzschia fraudulenta* (isolate 2011: length = 65.5 µm, width = ~ 4 µm, striae and fibulae in 10 µm = 22, poroids in 1 µm = 5, 2 rows of poroids per stria and 3.5 striae per central interspace). (c) *Pseudo-nitzschia pungens* (isolate WW3: length = 56.4 µm, width = 2.7 µm, striae and fibulae in 10 µm = 11, poroids in 1 µm = 3 and 2 rows of poroids per stria). (d) *Pseudo-nitzschia australis* (isolate WW4: length = ~ 45 µm, width = 5.4 µm, striae in 10 µm = 17, fibulae in 10 µm = 16, poroids in 1 µm = 5-6 and 2 rows of poroids per stria).

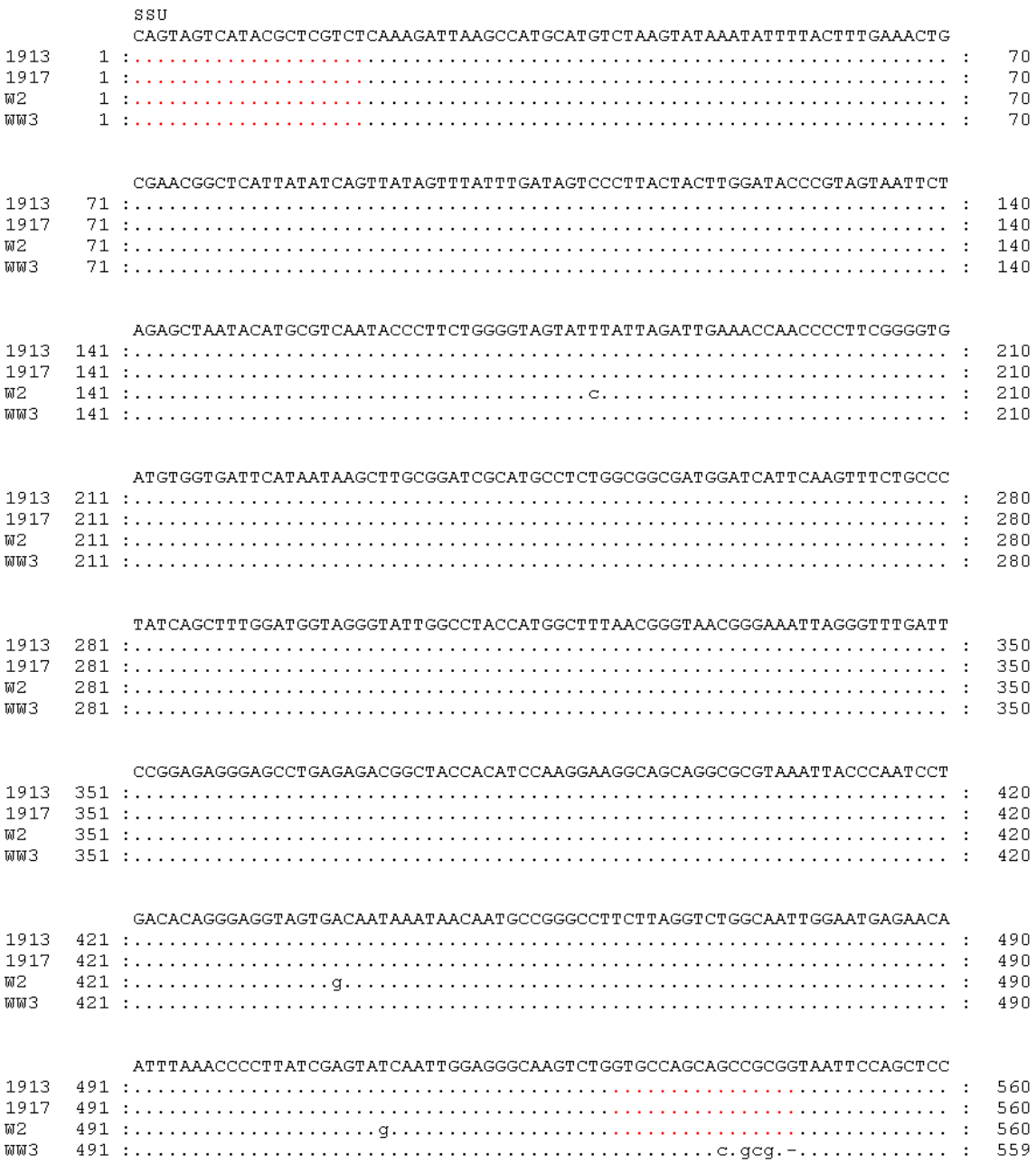

Figure 1. Multiple DNA sequence alignment of the 18S SSU (1745 bp), ITS1 (286 bp) and 5.8S (44 bp) rDNA of 4 Irish *Pseudo-nitzschia* isolates. The black dots represent conserved character positions, hyphens represent insertion/deletion events and the red dots represent the primer annealing sites. Taxa abbreviations in the left hand column are as follows: 1913 and 1917 = *P. delicatissima*, W2 = *P. fraudulenta* and WW3 = *P. pungens*.

```
                AATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTGTGGTGTGTCCAGTCGACCTT
1913  561 :.......................................................................... :  630
1917  561 :.......................................................................... :  630
W2    561 :.......................................................................... :  630
WW3   560 :..........................................................g.... :  629

                TGCTCTTTGAGTGATTGTGTTGTACTGGTCTGCCATGTTTGGGTGGAATCTGTGTGGCATTAAGTTGTCG
1913  631 :.......................................................................... :  700
1917  631 :.......................................................................... :  700
W2    631 :.....................................a.................................... :  700
WW3   630 :.......c.........c.c.................................................... :  699

                TGCAGGGGATGCCCATCGTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCTTATGCCGTTGAATATA
1913  701 :.......................................................................... :  770
1917  701 :.......................................................................... :  770
W2    701 :.......................................................................... :  770
WW3   700 :.a.t.a..g.................................................................. :  769

                TTAGCATGGAATAATGATATAGGACCTTGGTACTATTTTGTTGGTTTGCGCACTAAGGTAATGATTAAGA
1913  771 :.......................................................................... :  840
1917  771 :.......................................................................... :  840
W2    771 :.......................................................................... :  840
WW3   770 :.......................................................................... :  839

                GGGACAGTTGGGGGTATTTGTATTCCATTGTCAGAGGTGAAATTCTTGGATTTTTGGAAGACAAACTACT
1913  841 :.....................................g.............c.................. :  910
1917  841 :.......................................................................... :  910
W2    841 :.......................................................................... :  910
WW3   840 :.......................................................................... :  909

                GCGAAAGCATTTACCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATTAGATA
1913  911 :.......................................................................... :  980
1917  911 :.......................................................................... :  980
W2    911 :.......................................................................... :  980
WW3   910 :.......................................................................... :  979

                CCATCGTAGTCTTAACCATAAACTATGCCGACAAAGGATTGGTGGAGTCTCGTTTCGTCTCCATCAGCAC
1913  981 :.......................................................................... : 1050
1917  981 :.....................................g.................................... : 1050
W2    981 :.......................................................................... : 1050
WW3   980 :.................................g.......c...................g....... : 1049

                TTTGTGAGAAATCATAAGTCTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTG
1913 1051 :.............................t............................................ : 1120
1917 1051 :.......................................................................... : 1120
W2   1051 :.......................................................................... : 1120
WW3  1050 :c.................................-........................................ : 1118

                ACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTTACCAGGTCC
1913 1121 :.......................................................................... : 1190
1917 1121 :.......................................................................... : 1190
W2   1121 :.....................a.................................................... : 1190
WW3  1119 :.......................................................................... : 1188

                AGACATAGTGAGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTT
1913 1191 :.......................................................................... : 1260
1917 1191 :.......................................................................... : 1260
W2   1191 :...............c.......................................................... : 1260
WW3  1189 :.............................c........................................... : 1258
```

Figure 1 continued.

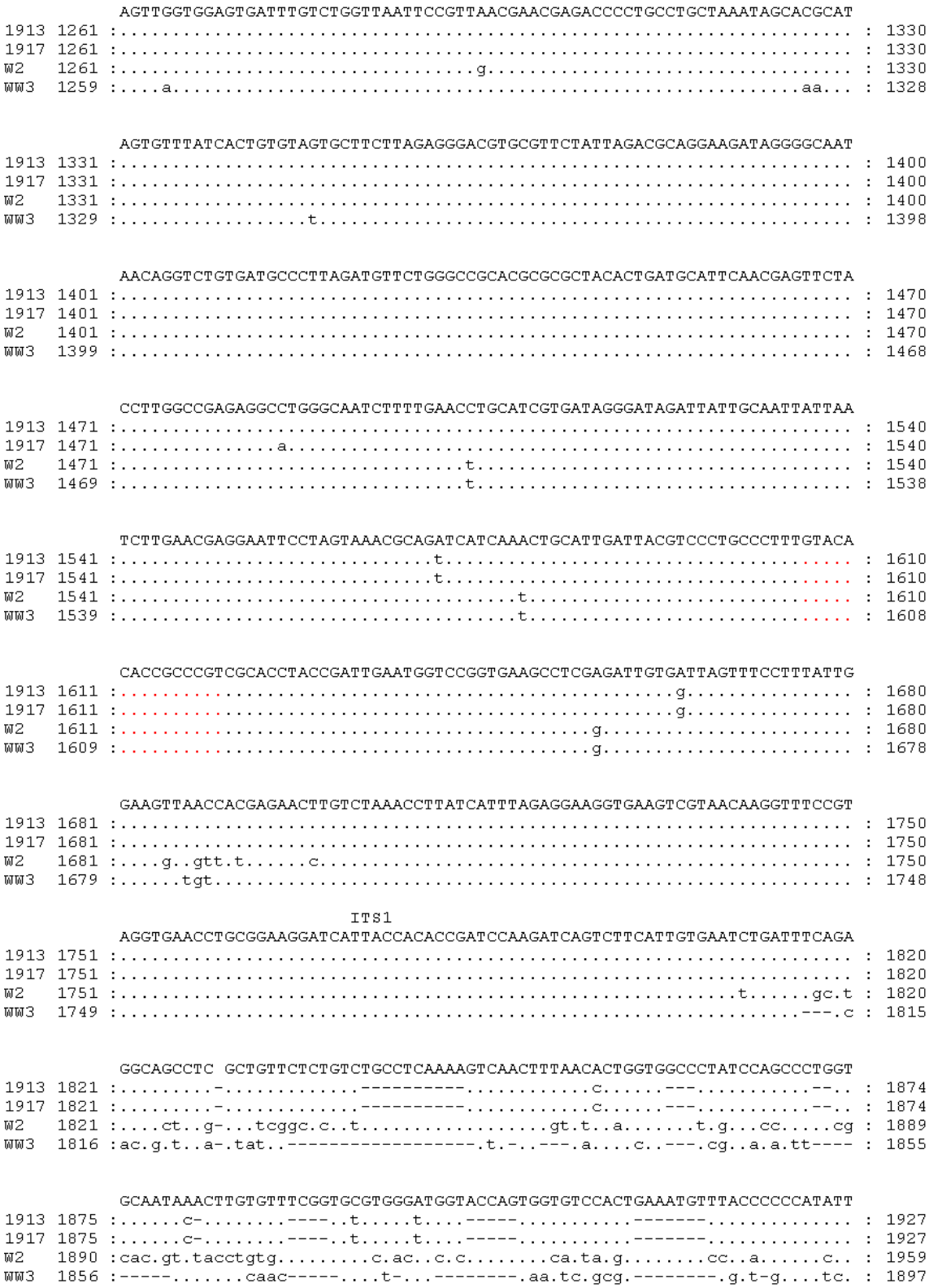

Figure 1 continued.

```
           CTACAATTGGAAACTGGAAAGAACCAAATGACCTAAAGCTGAAAGGATGCAGTGGGTCGGAGTGCTGAGC
1913 1928 :...t.--.................................-....--......t....t.-...t..c.- : 1990
1917 1928 :...t.--.................................-....--......t....t.-...t..c.- : 1990
W2   1960 :t...g..c..t....t..t.....................t....--........tc...-caa.....- : 2025
WW3  1898 :t...g.....t..........................ta....t.........tt......c....... : 1967

                                                                          5.8S
           GGGCGTCTTAAGTATTAGATGCACCTTGTGCCAACACTATTGCCCGCCCTGTACACAAAACCCTAATGAC
1913 1991 :-----------------------.............-------..............-...-....... : 2028
1917 1991 :-----------------------.............-------..............-...-....... : 2028
W2   2026 :-----------------------.........gg.g------....t.......t......-....... : 2065
WW3  1968 :.......................taccaaa..g..t.......tg..a...c...tt.c........aca : 2037

           TTACAACTTTCAGCGGTGGATGTCTAGGTTCCCACAA
1913 2029 :.....................................- : 2064
1917 2029 :.....................................- : 2064
W2   2066 :.....................................- : 2101
WW3  2038 :..................................... : 2074
```

Figure 1 continued.

1913: Length = 2064 base pairs, G+C content = 45.25%, A+T content = 54.75%, 1917: Length = 2064 base pairs, G+C content = 45.20%, A+T content = 54.80%, W2: Length = 2101 base pairs, G+C content = 45.60%, A+T content = 54.40%, WW3: Length = 2074 base pairs, G+C content = 44.74%, A+T content = 55.26%.

Table 4. Percentage similarity of the 18S SSU rDNA between *Pseudo-nitzschia* isolates calculated using the uncorrected-pairwise distance method. 1 = *P. delicatissima* (1917), 2 = *P. delicatissima* (1913), 3 = *P. fraudulenta* (W2), 4 = *P. australis* (PSEUD-X), 5 = *P. multiseries* (TKA-2), 6 = *P. multiseries* (13CC), 7 = *P. multiseries* (NPARL), 8 = *P. multiseries* (POM-X), 9. *P. pungens* (WW3), 10. *P. pungens* (BRUDC-X) and 11. *P. pungens* (F310).

	1	2	3	4	5	6	7	8	9	10	11
1	-										
2	99.71	-									
3	98.85	98.8	-								
4	98.40	98.34	98.4	-							
5	98.91	98.85	98.8	98.85	-						
6	98.74	98.68	98.62	98.68	99.83	-					
7	98.74	98.68	98.62	98.68	99.83	99.66	-				
8	98.74	98.68	98.62	98.68	99.83	99.66	99.77	-			
9	98.22	98.16	98.11	98.34	98.85	98.68	98.68	98.68	-		
10	98.68	98.62	98.57	98.80	99.26	99.08	99.08	99.08	99.60	-	
11	98.68	98.63	98.45	98.68	99.20	99.03	99.03	99.03	99.54	99.94	-

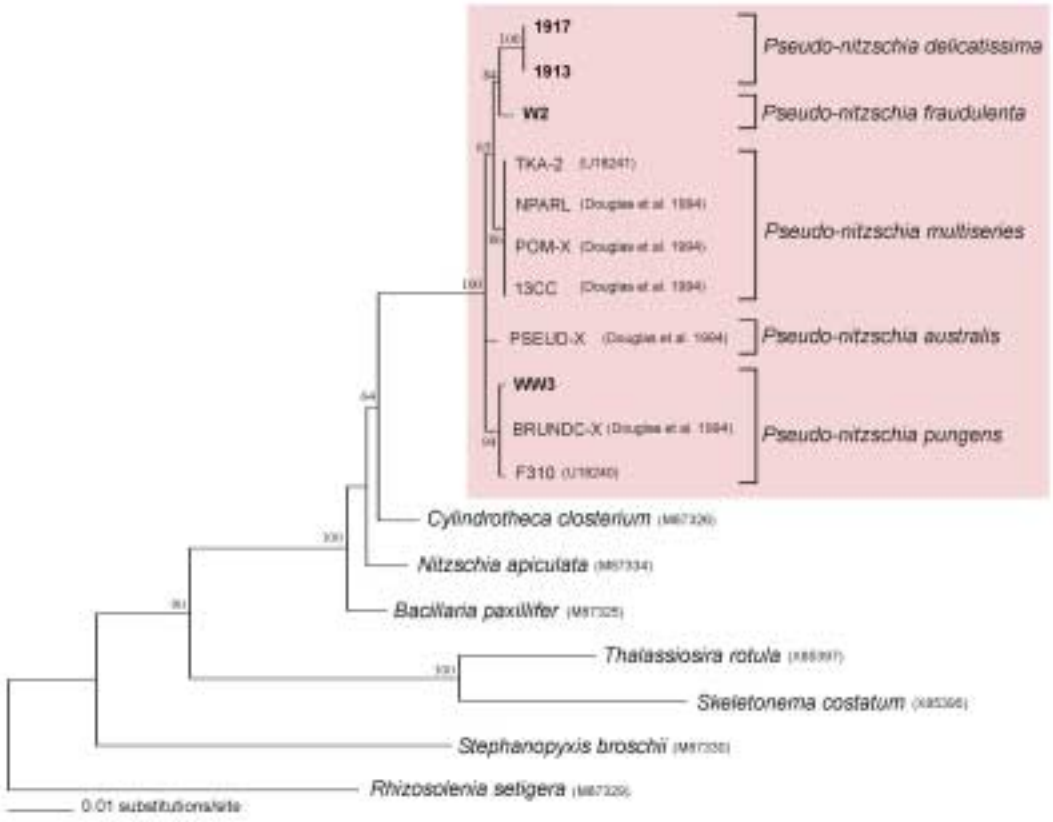

Figure 2. Phylogenetic representation of eighteen diatom specimens including *Pseudo-nitzschia* based on sequence comparisons of the SSU rDNA sequences (1732 characters used). The tree was constructed using Maximum-Likelihood method. *Stephanopyxis broschii* (#M87330) and *Rhizosolenia setigera* (#M87329) were used as outgroups. Numbers at the internal nodes are the inferred bootstrap values for 100 bootstrap resamplings, only bootstrap values above 50 are shown. The scale below the tree indicates the branch length corresponding to 0.01 substitutions per site.

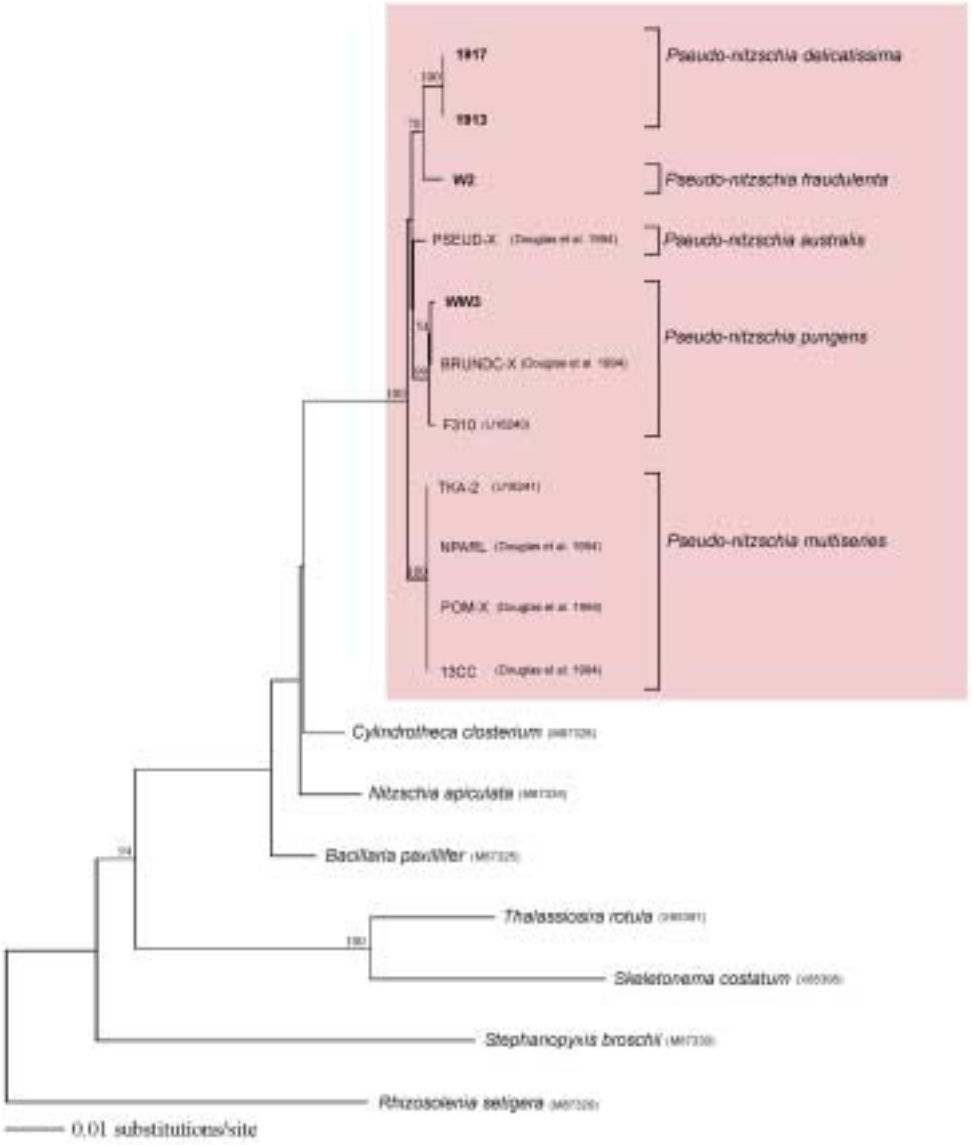

Figure 3. Phylogenetic representation of eighteen diatom specimens including *Pseudo-nitzschia* based on sequence comparisons of the SSU rDNA sequences (1732 characters used). The tree was constructed using LogDet/paralinear distances. *Stephanopyxis broschii* (#M87330) and *Rhizosolenia setigera* (#M87329) were used as outgroups. Numbers at the internal nodes are the inferred bootstrap values for 1000 bootstrap resamplings, only bootstrap values above 50 are shown. The scale below the tree indicates the branch length corresponding to 0.01 substitutions per site.

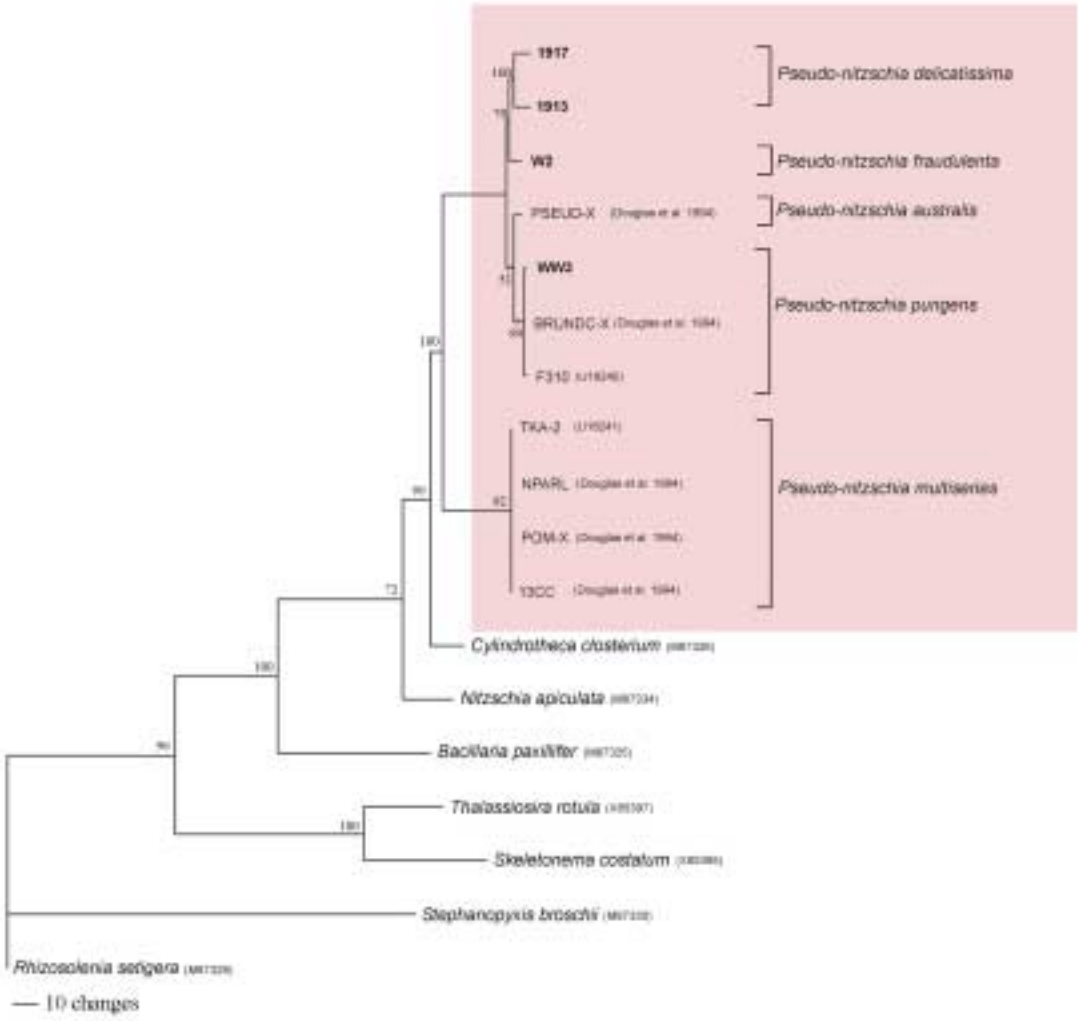

Figure 4. Phylogenetic representation of eighteen diatom specimens including *Pseudo-nitzschia* based on sequence comparisons of the SSU rDNA sequences (1732 characters used). The tree was constructed using a Maximum Parsimony method (tree probability = 4586.1895). *Stephanopyxis broschii* (#M87330) and *Rhizosolenia setigera* (#M87329) were used as outgroups. Numbers at the internal nodes are the inferred bootstrap values for 100 bootstrap resamplings, only bootstrap values above 50 are shown. The scale below the tree indicates the branch length corresponding to 10 changes per 100 nucleotide positions.

Application of rRNA-targeted oligonucleotide probes

Labelling intensities varied between the *Pseudo-nitzschia* isolates screened with LSU rRNA targeted probes (Table 5, Plate 2). Cells hybridized with the positive control probe showed bright green fluorescent signals. No positive signal (i.e. bright green cells) was evident in cells labelled with the negative control probe or when no probe was applied.

Successful *Pseudo-nitzschia* species-specific responses of hybridized cells distinctly labelled (++) were recorded for the puD1, auD1 and frD1 fluorescent probes. The puD1 probe showed a disparity in its labelling intensity of the *P. pungens* isolate, ranging from a bright signal (++) to a less intense but positive signal (+-) it also exhibited weak cross reactions with the *P. fraudulenta* and *P. australis* isolates. The auD1 probe showed slight labelling reaction (+-) to one of the *P. delicatissima* isolates, while the frD1 probe labelled all *P. delicatissima* hybridized cells with an intense signal (++) similar to that observed with the positive control. Other probes that had weak labelling reactions (+-) with non–target species were muD1 (*P. australis* and *P. delicatissima*) and muD2 (*P. australis*). The deD1 probe did not label (--) the species-specific target *P. delicatissima* isolates and also exhibited some cross reactivity with the *P. pungens* and *P. australis* isolates (+-).

Table 5. Reactivity of whole cell hybridization gene probe trials on *Pseudo-nitzschia* cultures.

		Fluorescent oligonucleotide probe										
Species	strain	uniC	uniR	No probe	muD1	muD2	puD1	auD1	frD1	deD1	he2-2	amD1
P. delicatissima	1913	++	-	-	+-	-	-	+-	++	-	-	-
P. delicatissima	1917	++	-	-	+-	-	-	-	++	-	-	-
P. delicatissima	1924-3	++	-	-	-	-	-	-	++	-	-	-
P. delicatissima	1924-4	++	-	-	-	-	-	-	++	-	-	-
P. pungens	WW-3	++	-	-	-	-	++/+-	-	-	+-	-	-
P. australis	WW-4	++	-	-	+-	+-	+-	++	-	+-	-	-
P. fraudulenta	2011	++	-	-	-	-	+-	-	++	-	-	-
P. fraudulenta	W-2	++	-	-	-	-	+-	-	++	-	-	-

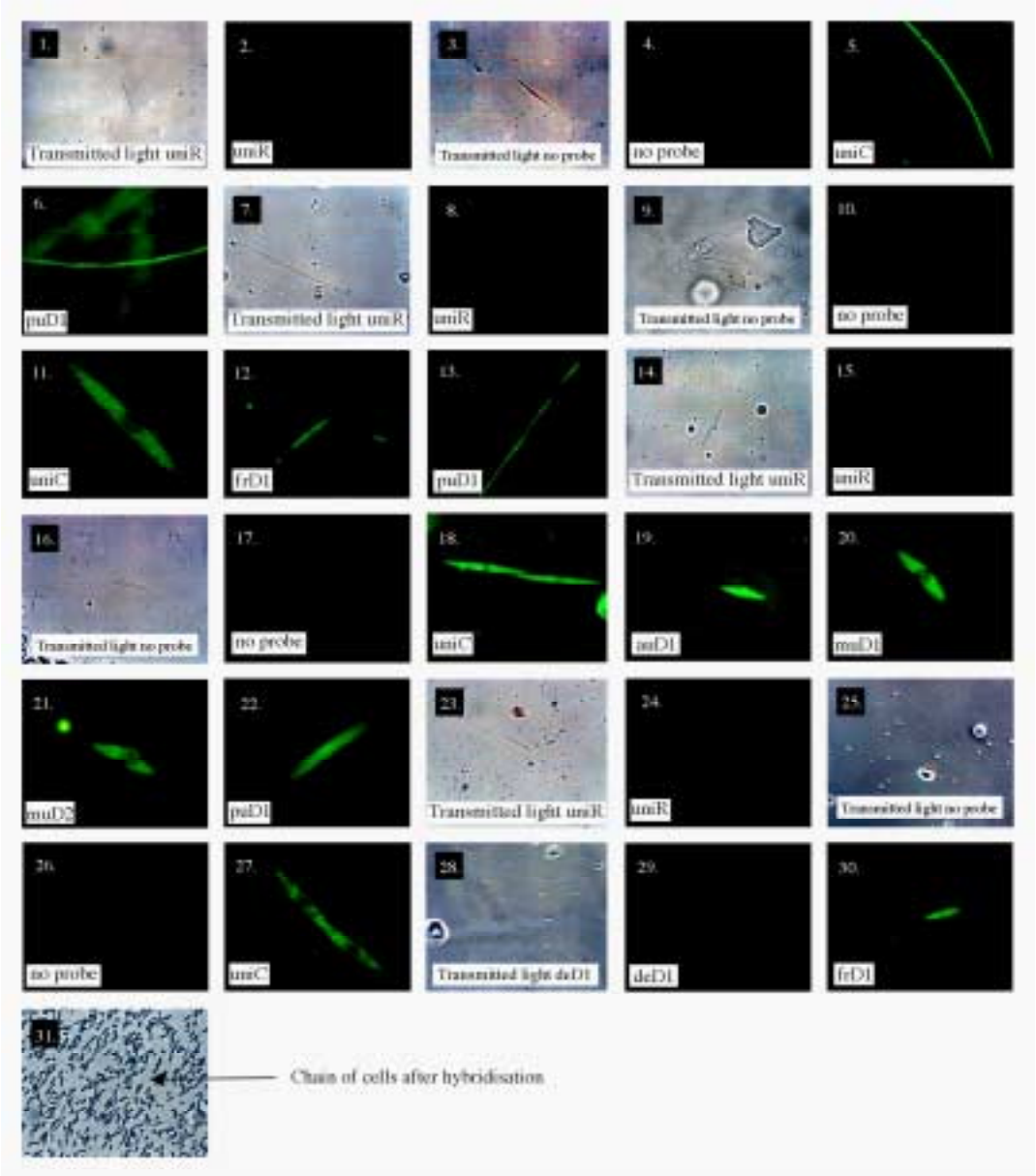

Plate 2. Photomicrographs of *Pseudo-nitzschia* cultures hybridized with fluorescent oligonucleotide probes (negative:UniR, positive:UniC and species specific:PuD1, FrD1, AuD1, MuD1, MuD2 and DeD1). *Pseudo-nitzschia pungens* plate 2(1–6), *P. fraudulenta* plate 2(7–13), *P. australis* plate 2(14-22) and *P. delicatissima* plate 2(23–30). Hybridized *Pseudo-nitzschia* cells under transmitted light are difficult to visualise when a 1 µm Nucleopore polycarbonate membrane filter is used, plate 2(31).

DISCUSSION

Since traditional techniques used to identify *Pseudo-nitzschia* to species level are fraught by enumeration difficulties and are not really appropriate for monitoring programmes, molecular technology has great potential to assist in high frequency sampling (spatially and temporally) programmes. Molecular techniques may also help us understand how and why a single species can suddenly bloom and result in toxic events.

Ribosomal DNA and Phylogenetic analysis

Pseudo-nitzschia species defined by morphological criterion can also be identified by comparison of rDNA sequence information. Manhart et al. (1995) noted a high level of variability within the ITS1 rDNA of the genus *Pseudo-nitzschia* and used this to further support the species designation of *P. multiseries* separate to *P*. pungens. Since the base composition and sequence length of the ITS1 rDNA was conserved at the *Pseudo-nitzschia* species level and highly divergent between species in this study also, future investigations could use this region to examine closely related species and possibly strains of the same species in populations studies. Since the use of ITS sequences as molecular marker relies on the mechanisms of concerted evolution to ensure the rDNA array is evolving as a single molecule, this must first be established before this fragment of rDNA can be used in such studies.

Phylogenetic analysis of the 18S SSU rDNA showed that the genus *Pseudo-nitzschia* formed a monophyletic group separate to the other pennate and centric diatoms. This is in agreement with Douglas et al. (1994), although the branching order in this study was less well resolved between the *Pseudo-nitzschia* species investigated. For example, in this study *P. multiseries* was placed at the base of the *Pseudo-nitzschia* cluster and did not form as close relationship to *P. pungens* as shown by other SSU (Douglas et al. 1994) and LSU rDNA studies (Scholin et al. 1994), and the morphological investigations of Hasle (1995). When the sequence data from this study and those published in Manhart et al. (1995) were excluded during phylogenetic analysis, the

generated tree had the same topology as Douglas et al. (1994). This suggests that differences in the branching order noted in this study may have arisen due to the lack of 18S sequence data on *P. australis*. Additional sequence information on other genes of the species from this genus with more closely related diatom genera (e.g. araphid diatoms) is probably required to provide the essential data for complete phylogenetic resolution within *Pseudo-nitzschia*.

The rDNA sequence data determined in this study was retrieved from clones containing a single amplified PCR copy of the genes of interest and so any mutations introduced during PCR would have manifested in the sequence of that clone. Future work investigating species and strain differences (polymorphisms) of *Pseudo-nitzschia* should employ a minimum of 3 isolates of each *Pseudo-nitzschia* species and amplified PCR products should be sequenced directly in both directions (to confirm the fidelity of the derived sequences i.e. prevent errors from reading sequences and reveal any micro-heterogeneities) to generate a consensus sequence for phylogenetic analysis. Alternatively multiple clones could be pooled before sequencing to prevent technical errors.

As already demonstrated by Scholin et al. (1994), Douglas et al. (1995) and Manhart et al. (1995), restriction enzyme digest assays such as RFPs (Restriction Fragment Patterns) can help resolve species-specific, strain-specific and allele dependant (micro-heterogenity in gene copies of the SSU rDNA) nucleotide signatures. The use of such a method as a diagnostic tool to discriminate between *Pseudo-nitzschia* species is useful in culture studies when screening large numbers of isolates since it can help define polymorphisms that arise from base substitutions and compensatory base changes within a species. This type of analysis, however, requires specialised equipment and personnel. The amount of time needed to prepare the amplified target region is equivalent to that required for traditional microscopy methods, so it is unlikely that such a method would be incorporated into routine phytoplankton monitoring programmes (Scholin et al. 1994). Ribosomal DNA sequence data generated in molecular studies, however, can be very valuable since this type of information can be used to construct species-specific

probes (Scholin et al. 1994). Although it is difficult to design an oligonucleotide probe from a selected sequence that will specifically hybridize only to the target organism, several potential sites unique to the SSU rDNA gene (Appendix 5) for each *Pseudo-nitzschia* species could be investigated further to design oligonucleotide probes complimentary to these regions and tested their specificity on a large number of *Pseudo-nitzschia* isolates. Potential polymorphisms and/or micro-heterogeneities, however, need to be confirmed within each target species and avoided when designing probes.

Application of LSU rRNA-targeted oligonucleotide probes

Optical examination of fluorescent probes can be an important tool in combating the problems of identifying and enumerating species from the genus *Pseudo-nitzschia* when the quality of fluorescent staining shows a clear distinction between the species targeted and other non-target species. This probe based cell detection technique has already been successful in obtaining near real–time data on harmful *Pseudo-nitzschia* blooms in New Zealand where it has been integrated into routine harmful algal bloom (HAB) surveillance programmes (Rhodes et al. 1998). Recent investigations by Chris Scholin and his co-workers (2000) have demonstrated that the whole cell (*In situ*) hybridization assay could be a better indication of DA in the plankton entering the marine food web rather than just relying exclusively on the detection of DA in commercial shellfish tissue (Scholin et al. 2000).

Some of the Irish *Pseudo–nitzschia* isolates showed cross–reaction with certain species–specific LSU rRNA–targeted fluorescent probes. Direct visualisation of the *P. delicatissima* strains after hybridization with the deD1 probe exhibited no florescent signal from the target cells. Nina Lundholm (personnel communication) has also reported a similar reaction with this gene probe on *P. delicatissima* strains isolated from northern European waters (off the coast of Denmark). One explanation for this is that there may be some form of genetic variation within the LSU rRNA target sequence between the European *P. delicatissima* isolates and the reference strain. The annealing ability of the species–specific rRNA–target probe depends on the nucleotide composition of the probe and that of the target sequence. Although the outward

appearance (morphotype) of the *P. delicatissima* cultures tested in both studies are in keeping with that described in the literature, cells from different biogeographic localities may differ in LSU rRNA sequence in regions that affect binding or access of the probe. Miller and Scholin (1996) found that the frD1 probe had a weak binding ability towards strains of *P. delicatissima* and *P. heimii* isolated from the west coast of America. The frD1 gene probe has also been reported to bind slightly with *P. subpacifica* (Miller and Scholin, 1998). When this gene probe was applied to Irish *P. delicatissima* strains in this study, a strong labelling intensity similar to that detected with the positive control was observed. This also suggests that rRNA of Irish strains differs from western North American strains of *P. delicatissima*. The frD1 probe could however be used to identify this species in Irish waters since *P. fraudulenta* is easily distinguished from *P. delicatissima* by observing the cell dimensions in valve view, there is a significant difference in the cell width of *P. fraudulenta* (>3µm) and *P. delicatissima* (<3µm) cells (Hasle 1965).

Other results in this preliminary study showed that the *P. australis* strain exhibited slight labelling reaction with the gene probes muD1, muD2, puD1 and deD1. Nevertheless, enumeration of *P. australis* cells from wild samples has some potential. The overall gross morphology of *P. australis* (> 4µm in width, spindle to lanceolate shape and rounded ends) in association with the bright labelling intensity of this species by the auD1 probe can assist in distinguishing between *P. australis* and other non–target *Pseudo-nitzschia* species. The disparity in the labelling strength of the puD1 probe with the *P. pungens* strain has been documented by Miller and Scholin (1998) on other *P. pungens* strains, even after repeated applications of this probe to the same clone of *P. pungens*. This type of anomaly in the labelling efficiency of puD1 requires some care when examining samples. Even though slight cross–reactions (+-) of the *P. australis* and *P. fraudulenta* strains by the puD1 oligonucleotide probe were observed, differentiation between these two species and *P. pungens* can be made. Their cell outlines are clearly different; *P. pungens* is a more linear in it's valve view while the other two have a wider cell width and exhibit a more spindle to lanceolate overall shape.

The fluorescently labelled probes used in this study were originally designed for a limited set of *Pseudo-nitzschia* strains isolated off the west coast of America. Since the LSU rRNA contains universally conserved sequences with some variable regions unique to a given species, chosen short length sequences were used to synthetically design oligonucleotide probes which would compliment the target sequence and discriminate between closely related *Pseudo-nitzschia* species (Miller and Scholin 1996). That is, these short nucleic acid strands contain a unique sequence to the given species they targeted, those species being isolated from the central Californian coast.

Future work should concentrate on investigating genetic variations between the Irish *Pseudo-nitzschia* strains and their western American counterparts. The most informative method to clarify this would be to sequence the D1-D2 region of the LSU rRNA (rDNA) of all, including any cross-reacting strains and compare their genetic signatures to the reference sequences (Miller and Scholin 1996). This would distinguish any regional genetic variation and allow the necessary "fine tuning" or redesigning of the probe sequences for successful application on Irish *Pseudo-nitzschia* strains.

Several other elements can influence the labelling proficiency of the *Pseudo-nitzschia* species–specific probes. Miller and Scholin (1998) found that the labelling ability of the probes was effected when a natural water sample containing large amounts of particulate material was analysed, non-specific binding and background fluorescent interference was observed and the incorporation of the probes into target organisms was reduced with positively labelled cells exhibiting weaker signals. Samples must be processed and analysed as quickly as possible since the fluorescence of positively labelled cells fade over time (Miller and Scholin 1998). Difficulties can also be encountered if the salt concentration of the hybridization buffer is either too high or too low thus reducing the labelling intensity of the probe. The hybridisation buffer must also be kept at room temperature, if stored in the fridge, precipitation of salts can occur and this will result in non-specific binding of the probe (Chris Scholin, personnel communication). Slight changes in hybridization temperature will have the same effect (Chris Scholin, personnel communication). If the fluorochrome fluorescein

isothiocyanate (FITC) is used to label the probes then samples should be stored in the dark, exposure to light will affect the labelling intensity and reduce the signal. Optimised conditions for labelling with FITC require a slightly basic pH. If there is contamination of materials and reagents with RNase, a lower signal strength or no signal of the applied probe will be the final effect, since this enzymes digest RNA (John Tyrell, personnel communication). An important stage during the assay is rinsing out excess probe after hybridization since this eliminates background fluorescence and any weak labelling reactions. If too much buffer is used during the rinsing step this will reduce the hybridization signal of the target organisms. The choice of membrane filters may effect labelling, Millipore 'Isopore' and Whatman 'cyclopore' filters have been reported to show less background fluorescence then when Nucleopore polycarbonate membrane filters are used in the assay (Chris Scholin personnel communication). Whatman 'cyclopore' filters are optically transparent and therefore allow visualisation of a sample under transmitted LM (Miller and Scholin 1998). Other filter types such as the Nucleopore polycarbonate membrane filters used in this study cause interference in this regard and make it difficult to view and photograph cells under transmitted light microscopy as shown in Plate 2(31). A final factor that can affect background fluorescence on the filter is the length of time hybridization is allowed. The Cawthron institute allow 30 minutes for hybridization and in California (MBARI) samples are generally hybridized for 1 hour (John Tyrell, personnel communication). If the hybridization time is allowed to continue for a longer time, the background fluorescence noise may increase dramatically. The optimal reaction conditions need to be determined empirically if further research is to be carried out in Irish waters with the LSU rRNA–targeted *Pseudo-nitzschia* probes, for example a hybridization time-series should be performed to compare signal outputs and background fluorescent levels on the filter and on non-target cells. A change in the type of filter used is also recommended to eliminate visualisation problems of cells under transmitted light. A barrier filter with a narrow bandwidth would be a useful alternative to the long band–pass filter used in this study, as it would eliminate a larger fraction of cells natural autofluorescence. The removal of chlorophyll fluorescence prior to analysis would also help to alleviate this kind of interference.

If the probes are to be used as a monitoring tool it is crucial that the person employed to carry out the assay has the utmost confidence in their technique. It was proposed by Chris Scholin at the HABTech 2000 workshop (New Zealand, 2000) that universal standard reagents and test strains to aid in training probe operators was the best possible solution to this problem. Consequently, any unwanted cross–reactions resulting from applying the probes in a non-stringent fashion would be eliminated. Finally, it should be stated that, given the results described above, there is great potential for the use of LSU rRNA–targeted oligonucleotide probes for monitoring potentially harmful *Pseudo-nitzschia* blooms in Irish waters. However, before this can be achieved successfully, further investigations are necessary to confirm and, if required, rectify any irregularities such as the unwanted cross–reactions exhibited by the gene probes with non–target organisms.

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CONCLUDING REMARKS

CONCLUDING REMARKS

This project was prompted by the recognition that certain species within this genus are capable of producing the shellfish neurotoxin, domoic acid (DA). During the study six potentially toxic *Pseudo-nitzschia* species (*P. pungens*, *P. multiseries*, *P. fraudulenta*, *P. australis*, *P. delicatissima*, and *P. pseudodelicatissima*) were unambiguously identified from vertical phytoplankton net haul samples collected from a series of research cruises off the Irish coast between 1993–1997. In separate investigations worldwide, isolates of the above species have been found to produce DA (Rhodes et al. 1996, Bates et al. 1989, Rhodes et al. 1997, Garrison et al. 1992, Smith et al. 1990 and Martin et al. 1990 respectively). The presence of these *Pseudo-nitzschia* species alone, signals a need for a more detailed phytoplankton monitoring programme in Irish coastal waters, especially in the vicinity of intensive shellfish farming.

Two *Pseudo-nitzschia* species provisionally identified as *P. subpacifica* and *P. seriata* (*P. seriata* f. *seriata* and *P. seriata* f. *obtusa*) were also noted in some of the field samples examined during this investigation. A more detailed survey off the Irish coast is required to confirm the presence of *P. seriata* f. *seriata* as some strains of this species can produce domoic acid (Lundholm et al. 1994).

The cruises undertaken in this study were carried out between spring and autumn and were primarily concentrated off the south and southwest coasts. The seasonal occurrence of each *Pseudo-nitzschia* species in Irish waters was in keeping with the literature (Hasle and Syvertsen 1997). *Pseudo-nitzschia* species that are abundant from October to April may have been missed, however, due to the timing of the cruises.

No obvious relationship between environmental data (e.g. nutrients, temperature and salinity) and the presence of the genus *Pseudo-nitzschia* was observed. These diatoms did, however, seem to be more abundant in the summer months following the spring bloom and in late summer. One possible reason for the lack of association between environmental variables and the presence of *Pseudo-nitzschia* could be that individual

species were not identified at discrete depths. In future studies, high sampling frequency collecting physical, chemical and biological data will be necessary to ascertain the synecology of these diatoms in Irish waters.

Pseudo-nitzschia australis is becoming an important source of DA in Europe. Blooms of this organism resulted in the closure of the shellfish industry in Galicia, Spain, during September 1994 (Míguez et al, 1996). This species has also been implicated in DA outbreaks in Monterey Bay (California, USA) resulting in the deaths of local seabirds and sealions (Buck et al. 1992; Fritz et al. 1992; Work et al. 1993, Scholin et al. 2000). *Pseudo-nitzschia australis* is a regular component of the microalgae in Irish waters, often predominating vertical phytoplankton net samples.

Irish isolates of *P. pungens*, *P. fraudulenta* and *P. delicatissima* tested negative for domoic acid production. However, it is recommended, that additional *Pseudo-nitzschia* species are isolated from Irish waters into unialgal cultures to assess exactly how many toxic species are present. Screening for toxic and non-toxic strains within the same species also needs to be addressed. This type of information could become important if phytoplankton monitoring is used as an aid in early warning systems in HAB programmes in Ireland.

The importance of continuing further work from both research and aquacultural viewpoints is further highlighted by the fact that DA has been identified in Irish shellfish (Terry Mc Mahon personal communication). Since the completion of the practical work carried out for this thesis, there have been several incidents of DA contamination in Irish shellfish. A biotoxin monitoring programme for DA began in Ireland after concerns were raised when an ASP event led to large scale closures of the scallop fisheries in Scotland in May 1999. The Irish authorities began to test for the presence of this toxin in mussels (*Mytilus edulis*) and scallops (*Pecten maximus*) collected from all commercial shellfish sites around the Irish coast. In early December 1999, the first detection of DA in Irish scallops was reported. Analysis of scallop tissue samples at the Marine Biotoxin Unit, Marine Institute has shown consistently that maximum levels of DA are concentrated in

the hepatopancreas (HP) region of this mollusc. Highest concentrations of up to 3,679 µg DA.g^{-1} (HP) have been reported. To date, the toxin has been found in concentrations above the regulatory limit of 20 µg.g^{-1} more frequently in scallops although testing of mussels, oysters (*Crassostrea gigas* and *Ostrea edulis*) and razor clams (*Ensis siliqua*) have also shown low concentrations of DA (Joe Silke personal communication). Phytoplankton samples taken soon after the first ASP incident showed no indication of a *Pseudo-nitzschia* bloom (personal observation). However, if there were high cell densities of *Pseudo-nitzschia* in the affected areas, it is quite possible that the bloom had subsided before phytoplankton samples were collected for examination. The causative organism remains uncertain.

Today the turnaround time to obtain detailed information on individual *Pseudo-nitzschia* species in a sample can be up to 48 hours using traditional methods. This requires identification skills since this genus *Pseudo-nitzschia* is difficult to identify down to species level. Molecular biological techniques are advancing rapidly and these alternative methods of identification may well ensure a more efficient response in detecting and enumerating species of *Pseudo-nitzschia*. Sequencing results from this study showed that the genus *Pseudo-nitzschia* formed a monphyletic group, although the relationships between individual species need to be investigated further with a larger sequencing data set. Results from species-specific gene probe application tests on *Pseudo-nitzschia* cultures suggest that there may be some genetic variation between Irish *Pseudo-nitzschia* populations and those off the west coast of the USA. The LSU ribosomal DNA of the Irish isolates need to be sequenced to allow for direct comparisons with the published data of Scholin et al. (1994).

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THE DIATOM *PSEUDO-NITZSCHIA* (PERAGALLO) IN IRISH WATERS.

Volume 2 (of 2)

A thesis submitted to the National University of Ireland for the degree of Doctor of Philosophy

by

Caroline K. Cusack B.MrSc.

Marine Microbiology Laboratory,
The Martin Ryan Marine Science Institute,
National University of Ireland,
Galway

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APPENDIX 1

Appendix I

Terminology used to describe the morphological features of the siliceous frustule in the diatom *Pseudo-nitzschia*. The terminology follows Anonymous (1975), Ross et al. (1979) and Barber & Haworth (1981).

TERMINOLOGY IN ACCORDANCE WITH ANONYMOUS 1975, ROSS ET AL. 1979 AND BARBER & HAWORTH, 1981.

APICAL AXIS: The long axis of a bilateral diatom, the axis between the two poles of the cell.

APICAL PLANE: This plane is perpendicular to the transapical axis.

AREOLA(E): (poroid(s)) Regular repeated perforations through the basal siliceous layer, which are occluded by a velum (multi–perforated silica membrane). One or more rows of areolae can be found in *Pseudo-nitzschia*.

BILATERAL: (valve shape) The valve outline is dissimilar either side of the apical axis, the dorsi–ventral side being narrower.

CANAL RAPHE: The raphe runs along this channel. There is a tubular passage running along its inner side and it is separated from interior frustule by siliceous elements called the fibulae. The spaces in between the fibulae are called interspaces.

CENTRAL LARGER INTERSPACE: This is seen as the greater distance between the two media fibulae.

CENTRAL NODULE: (pseudonodulus) This structure is more robust then other siliceous features of the valve and is positioned between the two central raphe endings. The central nodule is located in the central larger interspace.

CINGULUM: The girdle elements associated with one valve, usually the older valve.

DISTAL MANTLE: The mantle that is positioned furthest away from the raphe.

EPICINGULUM: (girdle elements) This overlaps the hypocingulum and is attached to the epivalve, together forming the hypotheca.

TERMINOLOGY IN ACCORDANCE WITH ANONYMOUS 1975, ROSS ET AL. 1979 AND BARBER & HAWORTH, 1981 CNT.

EPITHECA:	Epivalve + epicingulum (composed of one or more girdle bands).
EPIVALVE:	Larger and older of the 2 valves.
FIBULA(E): (keel punctum(a))	A band of silica that forms an arch across the raphe and raphe canal. Between each fibula are openings called the interspaces.
FRUSTULE:	The siliceous cell wall of the diatom cell. Epitheca + hypotheca = Epivalve + girdle + hypovalve.
FUSIFORM:	The shape of a valve with rostrate ends (attenuated ends).
GIRDLE:	The part of the frustule which contains the epicingulum and hypocingulum.
GIRDLE BAND:	A single element of the girdle. Girdle bands = copulae.
HETEROPOLAR : (valve symmetry)	If the transapical axis is heteropolar, this means that the valve is asymmetrical with respect to the apical plane. Heteropolar on its own means that the ends of a valve differ in shape or size.
HYMENATE VELUM:	A type of velum that is very fine and sieve-like in structure.
HYPOCINGULUM: (girdle elements)	This is attached to the epivalve together forming the hypotheca. This part of the frustule is laid down by the daughter cell after cytokinesis.
HYPOTHECA:	Hypovalve + hypocingulum (composed of one or more girdle bands).
HYPOVALVE:	Smaller and younger/newer of the 2 valves.
INTERSTRIA(E): (transapical costa(e))	Non perforated strip of silica between two striae on the valve face. They run transapically in the diatom *Pseudo-nitzschia*.

TERMINOLOGY IN ACCORDANCE WITH ANONYMOUS 1975, ROSS ET AL. 1979 AND BARBER & HAWORTH, 1981 CNT.

ISOBILATERAL: (valve shape) The outline of the valve is similar on either side of the apical axis.

ISOPOLAR: (valve symmetry) If the transapical axis is isopolar, this means that the valve is symmetrical with respect to the apical plane.

MARGINAL RIDGE: A ridge between the valve face and valve mantle.

PERVALVAR AXIS: The axis through the centre point of the two valves (height) the size varies with the number of girdle bands formed.

PROXIMAL MANTLE: The mantle that is positioned nearest the raphe.

RAPHE: An elongated slit through the valve wall along the apical axis. The raphe in *Pseudo-nitzschia* valves is found either as an unbroken line running along the length of the valve margin or in two parts separated in the central area by the central nodule. Raphe terminals (ends, nodules) occur at the apices (poles) of the valves. Where the raphe is split into two parts, raphe terminals can also be found in the central larger interspace. The raphe usually has a shape like a V on its side < in a cross section of a valve. In the diatom *Pseudo-nitzschia* the raphe on opposite valves of the one frustule are positioned diagonally to each other.

RAPHE ECCENTRIC: The raphe does not run along the centre of the valve. In the diatom *Pseudo-nitzschia* the raphe is said to be eccentric, this means that the raphe is found running along one of the valve margins.

ROSTRATE: (usually referring to the valve ends) Following Sim and Ross, 1985.
Prolonged distal parts of the valves (drawn out ends), more or less sharply delimited from the central part of the valve. The term projection is sometimes used instead of rostrate.

TERMINOLOGY IN ACCORDANCE WITH ANONYMOUS 1975, ROSS ET AL. 1979 AND BARBER & HAWORTH, 1981 CNT.

STRIA(E): (intercostal membrane)	Row or rows of areolae (pores). Groups of poroids.
STRIAE/FIBULAE-FREQUENCY:	This is the number (density) of striae or fibulae in 10µm, measured from the central area of the valve face towards the valve poles.
STRIATED BANDS: (ribbed)	Perforated girdle bands as seen under a light microscope. They are often seen as a row of rectangular openings in *Pseudo-nitzschia*.
THECA:	Valve with it's associated girdle elements.
TRANSAPICAL AXIS:	The imaginary axis that runs perpendicular to the apical axis.
TRANSAPICAL PLANE:	This plane is perpendicular to the apical plane or axis.
VALVE: (face + mantle)	One of two opposing distal plates in the frustule. A valve is generally more or less flattened or convex in shape.
VALVE FACE:	Part of the valve that is surrounded by the mantle. Oriented to the valvar plane.
VALVE MANTLE:	The marginal part of the valve differentiated by a slope. Oriented to the apical plane.
VALVAR PLANE:	This plane is parallel to the valves where cell division causes a split between them; it is perpendicular to the pervalvar plane or axis.
VALVOCOPULA:	This is the first girdle band. It is the element adjacent to the valve.

APPENDIX 2

Appendix II

Morphological descriptions of *Pseudo-nitzschia* species recorded from Irish waters. The morphological criteria follow Hasle (1965, 1972, 1995), Hasle & Fryxell (1995), Hasle et al. (1995) and Hasle & Syvertsen (1996).

Pseudo-nitzschia pungens

(Robust: v heavily silicified)

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

1. The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/4 to 1/3 or more of the total cell length.

LIGHT MICROSCOPY (LM) ACID CLEANED VALVES

2. The frustule is heavily impregnated with silica.
3. VALVE VIEW:
   a) The valve is symmetrical with respect to the apical axis.
   b) The shape of the valve is linear in large valves and lanceolate to fusiform in smaller valves.
   c) Valve ends are pointed.
   d) Valve margins are straight to gently curved.
   e) Central larger interspace is absent.
   f) The density of fibulae to interstriae is more or less equal.

      The interstriae are visible in watermounts. The fibulae are sometimes seen as a continuation of the interstriae in water mounts. The fibulae and the interstriae are visible in permanent mounts where the fibulae can be easily distinguished from the interstriae.

   g) Perforation of the striae can be observed occasionally in acid cleaned valves mounted in a medium of high refractive index under phase contrast (100x) and oil immersion (100x) LM.
4. GIRDLE VIEW
   h) *Pseudo-nitzschia pungens* can be identified in the girdle view by the coarse fibulae and interstriae. The interstriae are visible in watermounts.
   i) The girdle shape is linear to lanceolate (or fusiform).
   j) Deep pervalvar axis (wide in girdle view).
   k) The girdle ends are sharply pointed.

l) The girdle margins are curved.

m) Girdle bands have a ribbed–like appearance, at the centre of the valve the ribs of the girdle bands are more widely spaced than at the poles.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

5. The fibulae and striae are present in more or less equal numbers. The fibulae are generally aligned with the interstriae, although sometimes they are slightly displaced from each other.
6. The striae are perforated by two rows of medium sized circular poroids ("biseriate") often with a single poroid or extra partial row of poroids between the two rows. The space between the two rows of poroids is more often then not non–perforated.
7. Poroid structure of the valve face and proximal mantle are round and are occluded by a finely perforated velum of a hymenate type (very fine and sieve-like).
8. Valve ends differ in that one end has a fewer number of poroids per striae than the other.
9. Valve mantle is one poroid high. The proximal mantle has one to two rows of poroids that are usually aligned with a fibula or an interspace, very rarely aligned with the striae.

The cingulum consists of at least three bands, each band has one transverse row of large hymenate poroids, the areas toward the apices lack poroids. The valvocupula is a lot wider then the other bands. The size of the poroids reduces abvalvarly.

Pseudo-nitzschia multiseries

(Robust, heavily/coarsely silicified)

This species when observed under the LM is very similar to *Pseudo-nitzschia pungens* in both the valve and girdle view.

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

1. The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/4 to 1/3 or more of the total cell length.

DIFFERENCE BETWEEN *P. MULTISERIES* AND *P. PUNGENS*

2. Valve view
   a) *Pseudo-nitzschia multiseries* is not as heavily silicified (coarse) as *P. pungens*.
   b) The ratio of the width:length of smaller to medium sized cells of *P. multiseries* are usually wider then similar sized *P. pungens* valves.
   c) The fibulae are more distinguishable that the interstriae in cleaned valves mounted in a high refractive medium such as Naphrax.
   d) In LM observations the perforations of the striae of P. multiseries cannot be seen even under oil immersion LM.
   e) The perforated bands are narrower and more delicate in cleaned mounted specimens of *P. multiseries*.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

Pseudo-nitzschia multiseries can only be distinguished from *P. pungens* by observing the striae structure, the structure of the valve ends and girdle bands under an electron microscope. The valve shape of *P. pungens and P. multiseries* is also very like that of *P. pungiformis* under the LM. A distinctive feature that separates *P. pungiformis* from the other two species is that this species possesses a central larger interspace.

Pseudo-nitzschia multiseries is also similar to *P. seriata* in the structure of the valve face striae, valve mantle and bands. They differ in their overall gross morphology (valve shape and valve ends).

3. The fibulae and striae are present in roughly in equal numbers.
4. The fibulae are generally positioned beside or slightly displaced with the interstriae.
5. The striae are perforated by three to five closely spaced rows of small poroids ("multiseriate") - not as big as those seen in *P. pungens*. The poroids directly adjacent to the interstriae are slightly larger that the poroids of the intermediate rows.
6. The poroids of the valve face and proximal mantle are round and are covered by a finely perforated velum of a hymenate type (very fine and sieve-like).
7. The valve ends differ in that one end bears more branched-like interstriae than the other (different to *P. pungens*).
8. Valve mantle is two to three poroids high.
9. The proximal and distal mantles have a similar striae structure to the valve face, sometimes with less poroids and more irregular poroid rows. The mantle striae are aligned with the raphe interspaces.
10. The valvocopula can have four to five hymenate poroids per striae in the pervalvar direction, the number of poroids decreases to one or no poroids abvalvarly in the other bands and the band apices.

Pseudo-nitzschia seriata f. *seriata*

(Lightly to moderately silicified)

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

1. The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/3 to 1/4 of the total cell length.

LIGHT MICROSCOPY (LM) ACID CLEANED VALVES

2. The frustule is weakly to moderately silicified.
3. VALVE VIEW
   a) The valve is asymmetrical with respect to the apical axis.
   b) The valve shape is lanceolate to fusiform.
   c) The transapical axis is broad.
   d) The valve ends are slightly rostrate (elongated) with rounded poles.
   e) One valve margin is curved and the other is more or less straight, this is easily observed at the centre of the valve.
   f) The canal raphe can be situated on either the straight or the curved margin.
   g) Central larger interspace is absent.
   h) The density of fibulae to interstriae is equal.

      Interstriae are visible in the more silicified valves in watermounts (dry lens). Fibulae are not discernible in watermounts. Fibulae are more distinguishable than the interstriae in permanent mounts. Oil immersion is not required to see interstriae or fibulae.
4. GIRDLE VIEW
   i) The girdle shape is linear to lanceolate or fusiform.
   j) The girdle ends are pointed.
   k) The interstriae are visible in vegetative cells in watermounts.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

When a fragment of a *P. seriata* valve is viewed under the electron microscope it is

difficult to distinguish from a fragmented *P. multiseries* valve. However, *P. seriata* may have poroids that are smaller in size to poroids found in *P. multiseries*. Observation of the overall valve shape and valve ends is necessary to differentiate these two species. *Pseudo-nitzschia seriata* is quite similar in the shape of its valve to smaller forms of *P. australis*. The dimensions and the overlap of cell ends are other features that these two species share in common. Differences in the valvar structure discriminate between the two species. Electron microscopy examination reveals that *P. seriata* has 3 to 4 rows of poroids per stria, while *P. australis* has only 2. The girdle bands of these two species also differ in structure (*P. australis* usually has more rows of poroids).

5. The fibulae and striae are present in more or less equal numbers.
6. The fibulae are generally positioned beside or slightly displaced with the interstriae.
7. The striae are perforated by three to five rows of poroids "multiseriate".
8. The rows of poroids adjacent to the interstriae have a larger poroid structure and the poroids are more consistent in appearance then those found in the other rows.
9. The poroids of the valve face and mantle are round and covered by a finely perforated velum of the hymenate type (very fine and sieve-like).
10. The structure of the valve ends differ, one end bears more branched-like interstriae.
11. The valve mantle is two to three poroids high and wide, with a striae structure similar to the valve face.
12. The striae of the proximal mantle are aligned with the raphe interspaces.
13. Three to four girdle bands are known. The first 3 bands are perforated while the fourth band is a narrow and nonperforated band. The striae structure is well formed on the first two bands with 2 rows of poroids (sometimes with a third poroid between the outer two poroids) with hymenate velae. At the centre region of the valve the valvocopula is four to six poroids high in the pervalvar direction, this decreases towards the apices where one to two poroids can be seen. The second band is about half the height of the first band and the third band is one poroid in height.

Pseudo-nitzschia seriata f. *obtusa*

(Lightly to moderately silicified)

This form of *Pseudo-nitzschia seriata* when viewed under a LM is quite similar to the nominate form (*P. seriata* f. *seriata*). LM observation reveals that *P. seriata* f. *obtusa* is smaller in size (length and width), it has more rounded, blunter (obtuse) ends and the striae are more densely spaced than the fibulae. Discrimination between *P. seriata* f. *obtusa* and its nominate form can also be made by observing the striae structure.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

14. The striae are perforated by one to two rows of hymenate poroids.

Pseudo-nitzschia australis

(Lightly to moderately silicified)

Pseudo-nitzschia australis shows great similarity to *P. seriata* in the shape of the valve particularly the slightly rostrate valve ends. *Pseudo-nitzschia australis* (large representatives) is symmetrical while *P. seriata* is asymmetrical with respect to the apical axis in valve view. Electron microscopy is required to confirm the identification of these two species. *Pseudo-nitzschia australis* is generally larger and more coarsely silicified than *P. seriata*. The striae and band structure are additional features that can help to differentiate these two species.

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

1. The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/4 to 1/3 of the total cell length.

LIGHT MICROSCOPY (LM) ACID CLEANED VALVES

2. The frustule is moderately silicified.
3. VALVE VIEW
   a) The valve is symmetrical with respect to the apical axis, this is difficult to see in smaller valves (these can sometimes look asymmetrical).
   b) The valve shape is linear-lanceolate or fusiform.
   c) The valve ends are slightly rostrate (elongated or pinched) in the larger specimens, all *P. australis* species have rounded poles.
   d) The valve margins are slightly curved and the middle part of the valve has more or less straight parallel sides (this can be difficult to discern in smaller forms).
   e) The density of fibulae to interstriae is roughly equal. The interstriae can be discerned in water mounts. The fibulae are more distinct than the interstriae in permanent mounts (oil immersion LM).
   f) A central larger interspace is absent.
4. GIRDLE VIEW

g) The frustule is linear to lanceolate or fusiform in shape in the girdle view.

h) The girdle ends are pointed.

i) The bands (intact frustules) of the girdle are strongly silicified with transverse ribs that are more densely packed then the interstriae of the valve face.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

5. The striae are perforated by two rows of small poroids ("biseriate"). The poroids are positioned close to the interstriae leaving a space of non–perforated silica between the two rows. The poroids of *P. australis* are circular in structure with hymenate velae and are larger in size than those found on *P. seriata* valves. Sometimes an extra poroid is visible towards the valve margins, positioned between the other two rows of poroids.
6. The structure of the valve ends differ, one end bears more branched-like interstriae than the interstriae at the other apices.
7. The valve mantle is one to three poroids high and two to three poroids wide, with striae structurally similar to the valve face. The striae of the proximal mantle are aligned with the raphe interspaces although they can sometimes be slightly displaced.
8. Girdle bands: there are three perforated associated bands, at times with a fourth nonperforated narrow band attached. The band striae have more than two rows of poroids with hymenate vela. The band striae of *P. australis* differ to those of *P. seriata* in shape and there are usually more rows of poroids in the band striae of *P. australis*. Transverse striae of the intercalary bands are strongly silicified and somewhat more closely spaced than those found on the valve face (Villac et al. 1993).

Pseudo-nitzschia fraudulenta

(Lightly silicified)

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

1. The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/8 to 1/6 of the total cell length.

LIGHT MICROSCOPY (LM) ACID CLEANED VALVES

2. The frustule is lightly (weakly) silicified.
3. VALVE VIEW:
   a) The valve is symmetrical with respect to the apical axis.
   b) The valve shape is lanceolate to fusiform.
   c) The valve ends are pointed.
   d) The valve margins are curved to straight.
   e) A central larger interspace is present.
   f) The fibulae and interstriae are in equal numbers. The fibulae are not discernible in watermounts. The interstriae are sometimes visible in water mounts. In permanent mounts, the fibulae and central nodule are visible (LM oil immersion), while the interstriae are barely discernible. The central larger interspace is visible in dark field illumination of water mounts and in permanently mounted slides.
4. GIRDLE VIEW
   g) The frustule is linear to lanceolate or fusiform.
   h) More heavily silicified specimens can be identified in the girdle view as the fibulae and interstriae are visible.
   i) The girdle ends are pointed.
   j) The band structure is not visible.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

5. The number of fibulae to interstriae differs slightly, generally with more interstriae

then fibulae. The fibulae are aligned with the valve face interstriae and often more irregularly with the striae or the interstriae of the proximal mantle.

6. Two rows of fairly large, closely packed circular to squarish poroids are visible per stria. The rows of poroids are also closely aligned. Occasionally there is an extra partial row of poroids between these 2 rows.
7. This species has a different poroid vela structure than many of the other *Pseudo-nitzschia* species in that the vela is divided into compartments, with nonperforated sections (delicate bands of silica) that radiate out from the centre and separate the hymenate sectors.
8. The central larger interspace spans three to four valve face striae.
9. The valve mantle is one to two poroids high
10. The valvocopula exhibits rectangular striae with two rows (sometimes a third row of smaller poroids) of a varying number of irregularly shaped poroids, ~ 8 to 10 in the pervalvar direction. These hexagonal to triangular poroids are similar in structure and size of the hymenate sectors of the valve face poroids. A second narrow and indistinctly structured band may be present and an unknown number of non–perforated bands.

Pseudo-nitzschia subpacifica

(Lightly silicified)

Pseudo-nitzschia subpacifica is very like *P. seriata* under a LM except it has a narrower valve width and possesses a central nodule. It is also very like *P. heimii* although it is smaller in size and the ratio of width:length is larger in *P. subpacifica*. These three species are alike in that all three are asymmetrical with respect to the apical axis.

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

1. The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/5 to 1/6 of the total cell length.

LIGHT MICROSCOPY (LM) ACID CLEANED VALVES

2. The frustule is weakly silicified (structure is delicate), *P. subpacifica* valves are comparatively broader than *P. heimii*.
3. VALVE VIEW:
   a) The valve is asymmetrical with respect to the apical axis.
   b) The valve is lanceolate to fusiform in shape.
   c) The valve ends are rostrate (elongated) with pointed ends.
   d) One side of the valve margin is convex the other margin is more or less straight this is most evident in the middle part of the valve.
   e) A central larger interspace and nodule is present and can be seen in permanent mounts under oil immersion LM.
   f) The density of fibulae to interstriae is unequal (~ 1:2). The interstriae are barely visible when viewed under a LM. The fibulae can be seen under oil immersion LM.
4. GIRDLE VIEW
   g) The girdle margins are convex.
   h) The girdle ends are sigmoid and obliquely truncated at the tips.
   i) The fibulae and interstriae are discernible in permanent mounts.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

The fine structure of the valve face striae resembles *P. australis* and *P. heimii*. *Pseudo-nitzschia subpacifica* is also similar to *P. seriata* in the outline of the valve, but *P. subpacifica* is narrower, it has a central larger interspace and approximately twice as many interstriae as fibulae.

5. The number of fibulae to interstriae is unequal, with more interstriae present then fibulae. The fibulae are aligned with the valve face interstriae and often more irregularly with the striae or the interstriae of the proximal mantle.
6. Two rows of minute circular poroids with hymenate velae are visible per stria. The rows of poroids are also closely aligned to the interstriae leaving a space of non–perforated silica between the two rows.
7. The central larger interspace spans the length of three valve face striae.
8. The valve mantle is one to two poroids high.
9. The valvocopula exhibits rectangular striae with two rows of poroids ~ 8 to 10 in the pervalvar direction (mid region of the band). There are an unknown number of perforated bands.

Pseudo-nitzschia heimii

(Lightly silicified)

This *Pseudo-nitzschia* species is very like *P. subpacifica* in overall structure including the striated bands. *Pseudo-nitzschia heimii* is however more heavily silicified, has a longer apical axis and is narrower than *P. subpacifica.* In water mounts the valve outline is similar to *P. seriata* since *P. heimii* is also asymmetrical. *Pseudo-nitzschia heimii* differs to *P. seriatai in that it possess* a central larger interspace and has a higher density of interstriae to fibulae. These features can be resolved in acid cleaned specimens mounted in a medium of high refractive index. *Pseudo-nitzschia heimii* can be distinguished from *P. fraudulenta* in LM by observing the valve symmetry and the less distinct but more densely spaced fibulae in *P. fraudulenta*. Electron microscopy confirms species designation especially when the poroid structure is included in the identification process.

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

1. The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/6 to 1/4 of the total cell length.

LIGHT MICROSCOPY (LM) ACID CLEANED VALVES

2. Frustule is weakly silicified.
3. VALVE VIEW:
   a) The valve is asymmetrical with respect to the apical axis.
   b) The valve shape is linear to lanceolate, larger valves are almost fusiform, the valve is comparatively narrow compared to *P subpacifica*.
   c) The valve ends are broadly rounded (more rounded than *P. subpacifica*) the smaller forms generally have broad rounded, obtuse ends, often with a constriction near the poles.
   d) One side of the valve margin is convex, the other margin more or less straight.
   e) The raphe and fibulae together can be situated on either margin.

f) A central larger interspace is present. The central larger interspace and central nodule can be seen when viewed under oil immersion LM.

g) The density of fibulae to interstriae is unequal, there is more than one stria to each fibula. These features can be seen when a specimen is viewed oil immersion LM.

h) The fibulae and central larger interspace are visible in permanent mounts of cleaned specimens (oil immersion LM).

i) The interstriae although visible in cleaned specimens mounted in Naphrax (oil immersion LM) are not as distinct as the fibulae.

4. GIRDLE VIEW

j) The girdle shape is linear tapering towards the poles.

k) The girdle apices are to some extent sigmoid with obliquely truncated tips.

l) More heavily silicified specimens can be identified in the girdle view where the fibulae and interstriae are visible.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

5. The valve face striae bear 1–2 rows of closely spaced (transapically) hymenate poroids (round in shape) with a wide non–perforated layer of silica between the two rows.
6. The central larger interspace is wide and spans 4 to 5 valve face striae.
7. The valve mantle is 3 to 4 poroids high with two rows of poroids as seen in the striae structure of the valve face. The striae of the proximal mantle are slightly displaced from those on the valve face.
8. The valvocopula has rectangular striae with two rows of 3 to 4 hymenate poroids in the pervalvar direction. There are an unknown number of girdle bands

Pseudo-nitzschia delicatissima

(Lightly silicified)

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

1. The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/8 to 1/9 of the total cell length.

LIGHT MICROSCOPY (LM) ACID CLEANED VALVES

2. The frustule is weakly delicately silicified.
3. VALVE VIEW:
   a) The valve is symmetrical with respect to the apical axis
   b) The valve length is short.
   c) The valve is narrow, linear to spindle in shape, wide in middle part of the valve in larger specimens and slightly rhomboid or sublinear in smaller valves.
   d) The valve is drawn out at the apices with valve ends that are slightly rounded and blunt.
   e) The valve margins are linear or sometimes curved.
   f) A central larger interspace is present.
   g) The fibulae can be resolved in watermounts.
   h) The fibulae and occasionally the central larger interspace can be seen under oil immersion LM in permanent mounts.
   i) The interstriae are not visible in LM.
4. GIRDLE VIEW
   j) Linear and narrow in girdle view with slightly sigmoid ends.
   k) The girdle ends are cut-off (truncated).
   l) The band structure cannot be resolved with the LM.

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

5. The striae are perforated by two transapical rows of minute triangular to round poroids with hymenate vela. There is a non–perforated area between the 2 rows of poroids.
6. The density of fibulae to interstriae is not equal (fibulae:striae ~1:2).
7. There are ~3 striae per central larger interspace.
8. The valve ends do not differ in structure.
9. The valve mantle is one poroid high.
10. The striae of the valve mantle have one row of large poroids (which can sometimes be divided in two) with hymenate velae.
11. The striae of the proximal mantle are aligned with the valve face striae or valve face interstriae.
12. Three striated bands are found, sometimes wit a fourth narrow non–perforated band attached. The valvocopula has one row of large rectangular poroids with hymenate velae. Each poroid is about the same width as a valve face stria. The second band has a silicified rib running along the its length separating the band into two halves. Each half consists of striae with 1 or 2 irregular poroids. The bottom half of the band has smaller poroids then the upper half. The third band is again divided into two halves by a silicified rib. The upper part of the band consists of 1 to 2 irregular shaped poroids per striae. The lower part of the band is unperforated for the most part. In some of the specimens examined very small poroids (scattered) were evident along the lower part of the third band. The fourth band is unperforated and narrow.

Pseudo-nitzschia pseudodelicatissima

(Lightly silicified)

This species is very similar to *P. delicatissima* when observed under a LM. However, the centre part of the valve is straighter in *P. pseudodelicatissima* than in *P. delicatissima* and the cell ends differ.

LIGHT MICROSCOPY (LM) VEGETATIVE CELLS

The girdle view of whole cells shows that the overlapping of cell ends can be as much as 1/9 to 1/8 of the total cell length .

LIGHT MICROSCOPY (LM) ACID CLEANED VALVES

The frustule is weakly silicified .

1. VALVE VIEW:
    a) The valve is symmetrical with respect to the apical axis.
    b) The valve is narrow, straight and needle in shape.
    c) The valve tapers towards pointed ends.
    d) The valve margins are linear to almost linear, more so then the margins of *P. delicatissima*.
    e) A central larger interspace and nodule is present, these features can be seen when a *P. pseudo-delicatissima* specimen is mounted in a medium of high refractive index..
    f) The fibulae can be seen in water and permanent mounts.
    g) The interstriae can sometimes be observed in heavily silicified valves mounted in a medium of high refractive index.
2. GIRDLE VIEW
    h) The girdle ends are pointed.
    i) The girdle is linear to almost linear

ELECTRON MICROSCOPY (EM) ACID CLEANED VALVES

3. The striae are perforated by one row of large circular to squarish poroids, partly occluded by a membrane with hymenated sectors. The poroids are more developed near the valve apices.
4. The valve ends are similar in structure.
5. The density of fibulae to striae is unequal (fibulae:striae, ~1:2)
6. The valve mantle is approximately one poroid high with one apical row of poroids that are squarish to rectangular in shape. The velae of the mantle poroids are similar in structure to the poroids of the valve striae.
7. The striae of the proximal mantle are generally aligned with the valve face striae.
8. The distance between the two fibulae at the centre of the valve (the central interspace) is equivalent to approximately 4 striae.
9. The cingulum consists of at least three striated bands and a non–perforated band. The valvocopula is wider than the other bands. The striae of the first three bands have 2 to 3 rows of 4 to 6 poroids in the pervalvar direction. The poroids are similar in shape and structure as the sectors of the poroids on the valve face striae.

APPENDIX 3

Appendix III

Micrographs of several *Pseudo-nitzschia* species observed in samples collected from Irish waters. The specimens were examined under light, scanning electron and transmission electron microscopes. Permanent mounts refer to a specimen mounted in Naphrax medium.

Pseudo-nitzschia pungens

(Figures 1 – 55)

Figs. 1–18 LIGHT MICROGRAPHS (LM).

Figs.1–17 Light micrographs (LM) of a clonal culture, strain WW_3 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Figs. 1–4 LM, OIL IMMERSION, WATER MOUNTS.

Fig. 1 Girdle view of vegetative cells in chain formation, overlap ~1/4 of total cell length, 90 µm long.

Fig. 2 Valve view of dead cells in chain formation, 58 µm long, 3 µm wide.

Fig. 3 Valve view of vegetative cells in chain formation, 94 µm long, 4 µm wide.

Fig. 4. Valve view of an acid cleaned valve, 115 µm long, 3.5 µm wide.

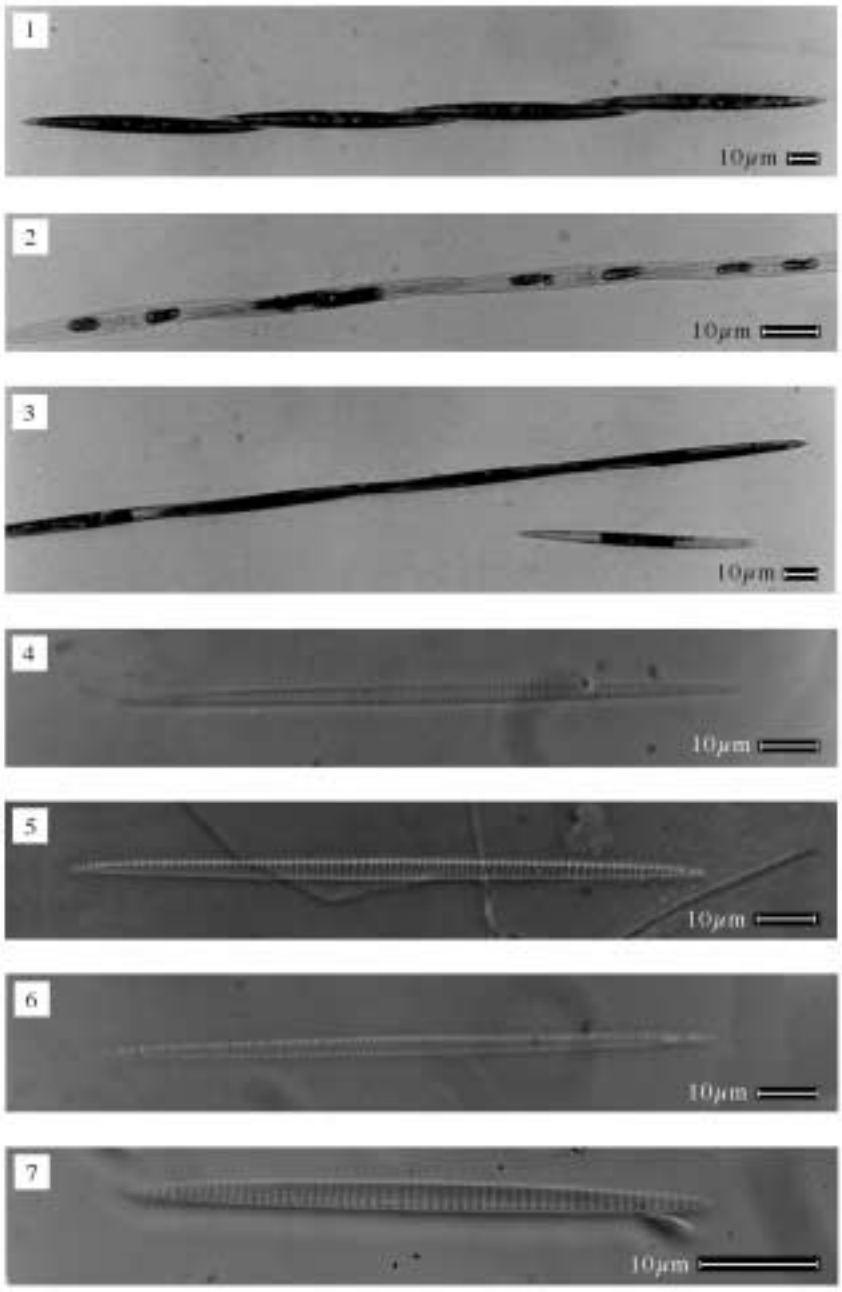
Figs. 5–16 LM, OIL IMMERSION, NAPHRAX MOUNTS.

Figs. 5–7 ACID CLEANED WHOLE VALVES IN PERMANENT MOUNTS.

Fig. 5 106 µm long, 3.55 µm wide.

Fig. 6 112 µm long, 3 µm wide.

Fig. 7 52 µm long, 2.4 µm wide.


1
10μm
2
10μm
3
10μm
4
10μm
5
10μm
6
10μm
7
10μm

Figs. 1–18 LIGHT MICROGRAPHS (LM).

Figs.1–17 Cont'd. Light micrographs (LM) of a clonal culture, strain WW_3 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Figs. 8–10 SHOWS DIFFERENT PARTS OF THE SAME VALVE (MOUNTED IN NAPHRAX).

Fig. 8 Valve end with16 interstriae and 16 fibulae in 10 µm.

Fig. 9 Other end with15 interstriae and 15 fibulae in 10 µm.

Fig. 10 Middle part of the valve showing14 interstriae and 14 fibulae in 10 µm.

Figs. 11–12 SHOWS DIFFERENT PARTS OF THE SAME VALVE (MOUNTED IN NAPHRAX).

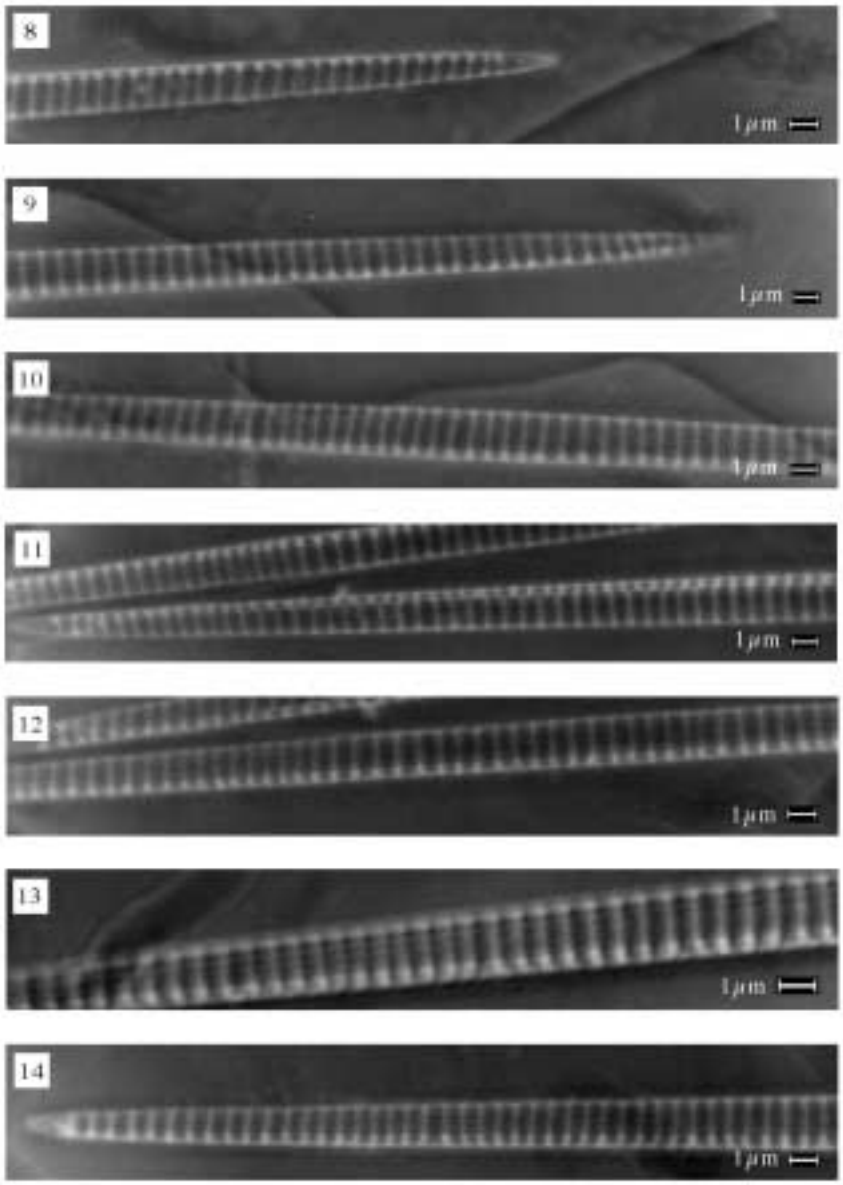
Fig. 11 Valve end with15 interstriae and 15 fibulae in 10 µm.

Fig. 12 Middle part of the valve showing15 interstriae and 15 fibulae in 10 µm.

Figs. 13–14 SHOWS DIFFERENT PARTS OF THE SAME VALVE (MOUNTED IN NAPHRAX).

Fig. 13 Middle part of the valve showing13–14 interstriae and 13–14 fibulae in 10 µm.

Fig. 14 Valve end with 15 interstriae and 15 fibulae in 10 µm.


8
1 μm
9
1 μm
10
1 μm
11
1 μm
12
1 μm
13
1 μm
14
1 μm

Figs. 1–18 Cont'd. LIGHT MICROGRAPHS (LM).

Figs.1–17 Cont'd. Light micrographs (LM) of a clonal culture, strain WW_3 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Figs. 15–17 ACID CLEANED WHOLE VALVES.

Fig. 15 Valve view of an acid cleaned valve, 86 µm long, 3.5 µm wide.

Fig. 16 Valve view of an acid cleaned valve, 109 µm long, 3.7 µm wide.

Fig. 17 Valve view of an acid cleaned valve, 73 µm long, 2.2 µm wide (phase contrast LM, Naphrax mount).

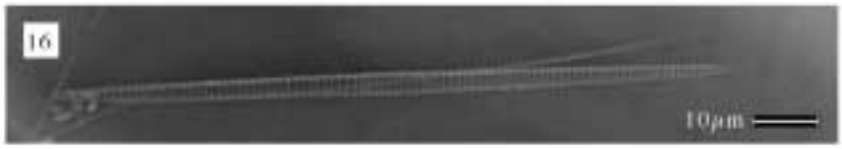
Fig. 18 VALVE VIEW OF AN ACID CLEANED VALVE.
Southwest Ireland (51˚10'N; 09˚ 55'W) June 1995, 132 µm long, 4.5 µm wide (LM, oil immersion, Naphrax mount).

Figs. 19–40 SCANNING ELECTRON MICROGRAPHS (SEM)

Fig. 19 Specimens from a Danish culture, kindly provided by Øjvind Moestrup (University of Copenhagen).

Fig. 20 Specimen taken from the south coast of Ireland, station: 1324 (51˚ 11.5'N; 09˚ 24.1'W), August 18th 1993. 3 µm wide with 3–4 poroids in 1 µm.


15
10μm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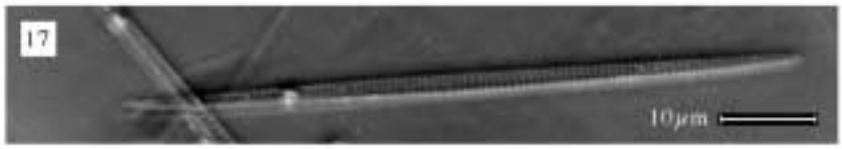
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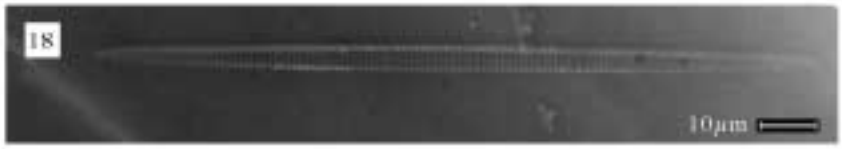
10μm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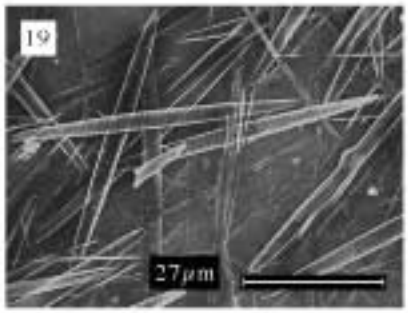
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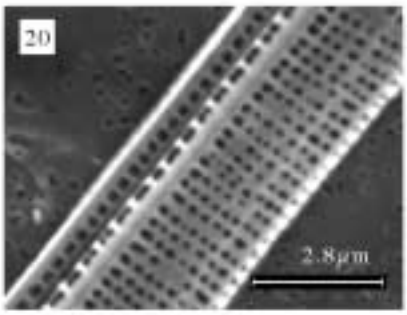
27μm


20
2.8μm

Figs. 19–40 Cont'd. SCANNING ELECTRON MICROGRAPHS (SEM).

Figs. 21–23 Specimen from the southwest coast of Ireland, station: 1801 (51˚ 27'N; 10˚ 20'W), July 20th 1996. 3.3 µm wide, 12 striae, 12 interstriae, 12 fibulae in 10 µm, 3 poroids in 1 µm.

Fig. 21 Middle part of the valve.

Fig. 22 Valve end.

Fig. 23 Other end.

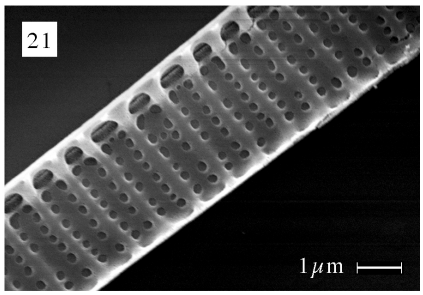
Fig. 24 Specimen from the southwest coast of Ireland, station: 1801 (51˚ 27'N; 10˚ 20'W), July 20th 1996. Middle part of a valve with girdle bands.

Fig. 25 Specimen from the southwest coast of Ireland, station: 1902 (52˚ 55'N; 10˚ 25'W), September 3rd 1996, middle part of a valve, 12 interstriae in 10 µm, 3 poroids in 1 µm.

Figs. 26–30 Scanning electron micrographs (SEM) of a clonal culture, strain WW_3 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Fig. 26 Valve end. 4 poroids in 1 µm.

Figs. 27–28 Valve ends from the same valve, 3–4 poroids in 1 µm.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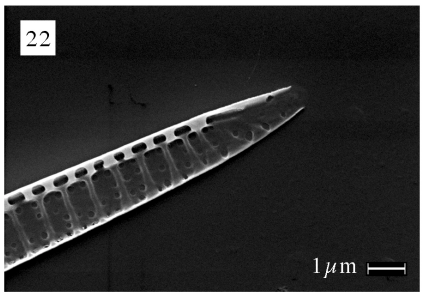
1μm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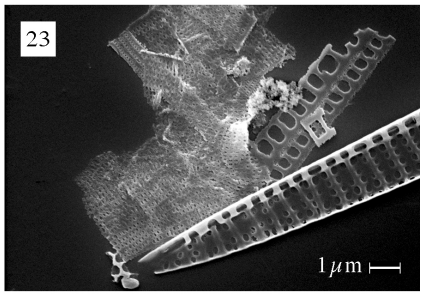
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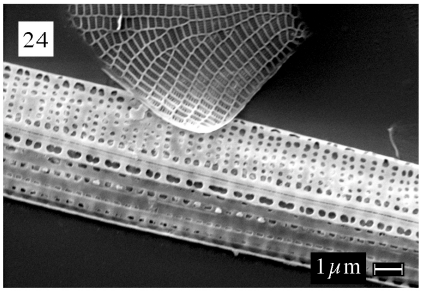
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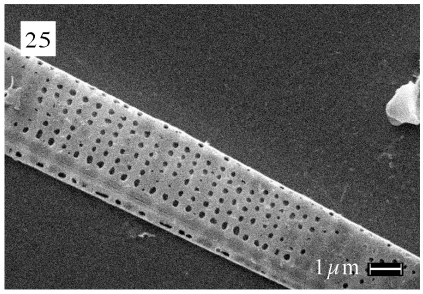
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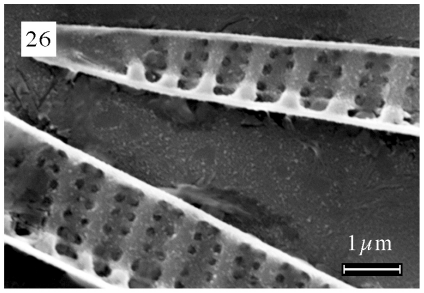
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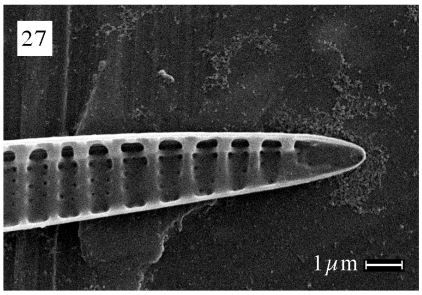
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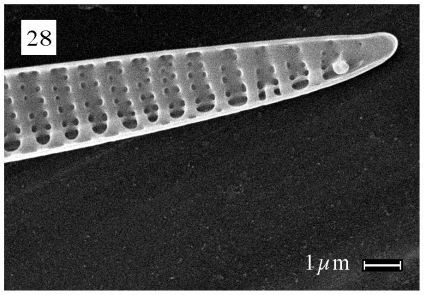

28
1μm

Figs. 19–40 Cont'd. SCANNING ELECTRON MICROGRAPHS (SEM).

Figs. 26–30 Cont'd. Scanning electron micrographs (SEM) of a clonal culture, strain WW_3 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Fig. 29–30 Valve ends from the same valve, 3 poroids in 1 µm.

Figs. 31–32 Specimen from a Danish culture, kindly provided by Øjvind Moestrup (University of Copenhagen).

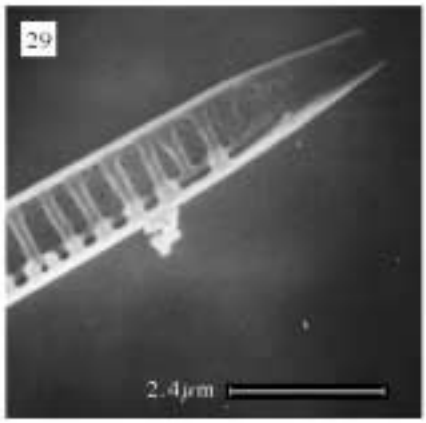
Fig. 31 Middle part of a valve, 16 striae, 16 interstriae and 15 fibulae in 10 µm, 4 poroids in 1 µm.

Fig. 32 Middle part of a valve, 12 striae, 12 interstriae and 11 fibulae in 10 µm, 4 poroids in 1 µm.

Figs. 33–34 Scanning electron micrographs (SEM) of a clonal culture, strain WW_3 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Fig. 33 Whole valve, 56.4 µm long, 2.7 µm wide.

Fig. 34 Valve end.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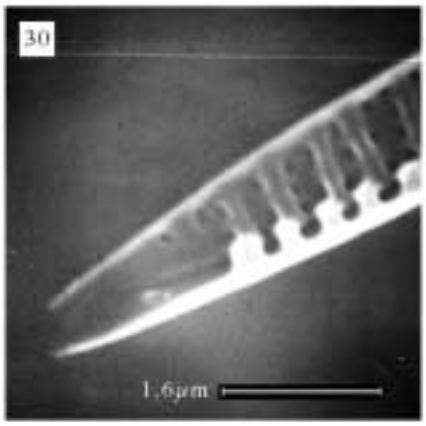
2.4μm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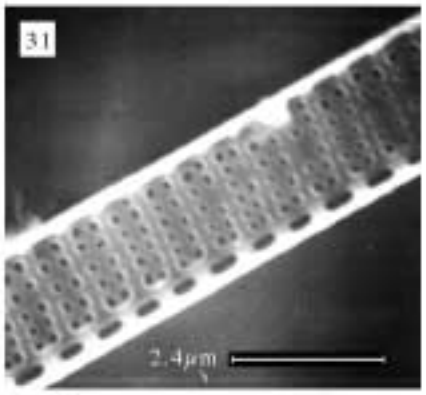
1.6μm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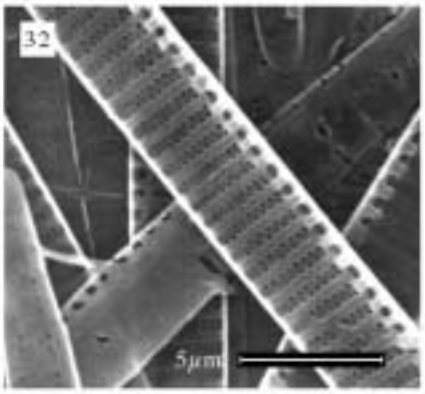
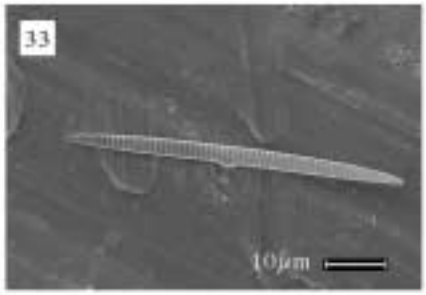
2.4μm


32
5μm


33
10μm


34
1μm

Figs. 19–40 Cont'd. SCANNING ELECTRON MICROGRAPHS (SEM).

Figs. 35–38 Specimens from a Danish clonal culture, kindly provided by Øjvind Moestrup (University of Copenhagen).

Fig. 35 Middle part of a valve, 12 striae, 12 interstriae and 11 fibulae in 10 µm, 3–4 poroids in 1 µm.

Fig. 36 Valve end, 4 poroids in 1 µm.

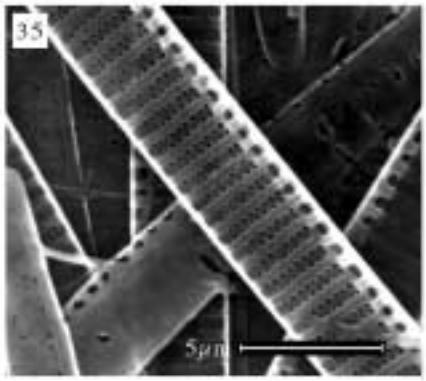
Fig. 37 Middle part of a valve, 14 striae and 14 interstriae in 10 µm, 4 poroids in 1 µm.

Fig. 38 Valve end, 4 poroids in 1 µm.

Fig. 39–40 Specimen from the south coast of Ireland, station: 1324 (51˚ 11.5'N; 09˚ 24.1'W), August 18th 1993.

Fig. 39 Whole valve, 115 µm long, 3.4 µm wide, 16 interstriae and 16 fibulae in 10 µm.

Fig. 40 Middle part of a valve, 3.4 µm wide, 4 poroids in 1 µm.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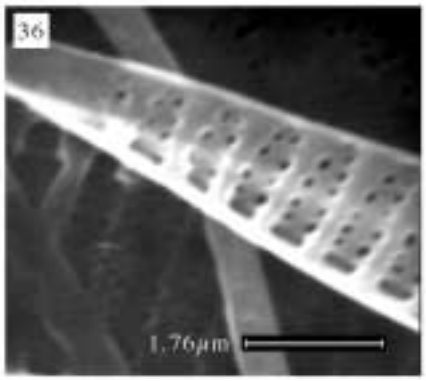
5μm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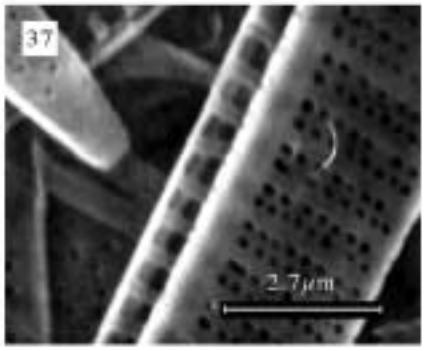
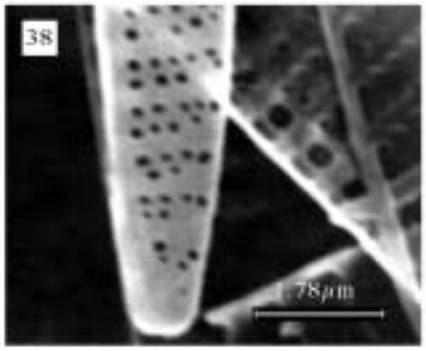
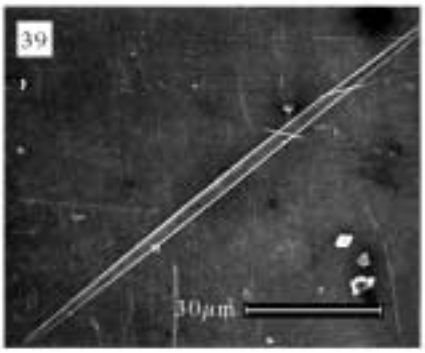
1.76μm


37
2.7μm


38
1.78μm


39
30μm

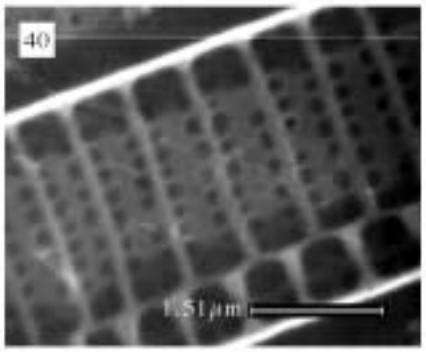

40
1.51μm

Fig. 41–55 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Fig. 41–43 Transmission electron micrographs (TEM) of a clonal culture, strain S_1 isolated from Salt lake (53˚ 28.4'N, 10˚ 01'W), west coast of Ireland, August 7th 1997.

Fig. 41 Whole valve, 58.8 µm long, 3.1 µm wide, 12 striae, 13 interstriae and 13 fibulae in 10 µm, 3–4 poroids in 1 µm.

Fig. 42 Whole valve, 58 µm long, 3 µm wide, 11 striae, 12 interstriae and 12 fibulae in 10 µm, 3–4 poroids in 1 µm.

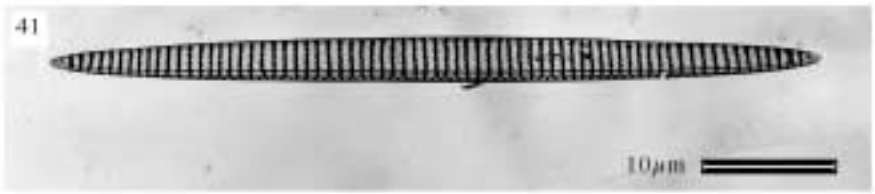
Fig. 43 Whole valve, 57.8 µm long, 3.3 µm wide, 12 striae, 12 interstriae and 12 fibulae in 10 µm, 3–4 poroids in 1 µm.

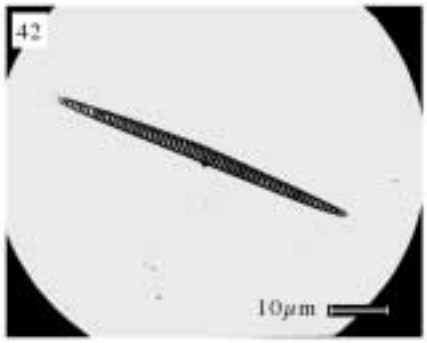
Fig. 44 Specimen from the southwest coast of Ireland, station: 1806 (51˚ 37.1'N, 09˚ 46.9'W), July 21st 1996. Part of the valve face, 4 poroids in 1 µm.

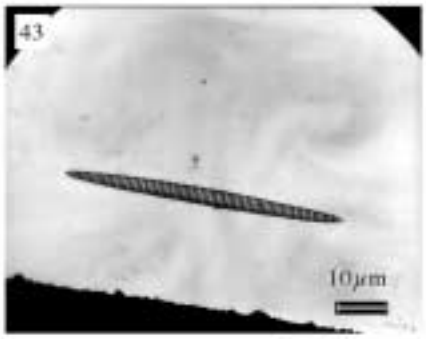
Fig. 45 Transmission electron micrograph (TEM) of a clonal culture, strain S_1 isolated from Salt lake (53˚ 28.4'N, 10˚ 01'W), the west coast of Ireland, August 7th 1997. 2.9 µm wide, 12 striae, 13 interstriae and 13 fibulae in 10 µm, 3–4 poroids in 1 µm.

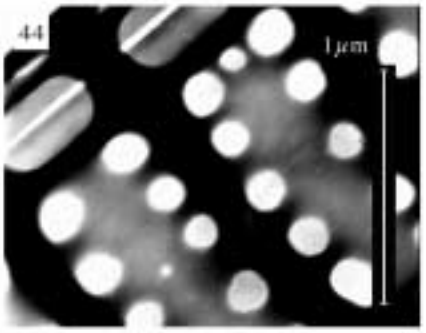
Fig. 46 Specimen from the southwest coast of Ireland, station: 2206 (51˚ 30.03'N, 08˚ 25.17'W), October 11th 1997. Middle part of a valve, 3–4 poroids in 1 µm.

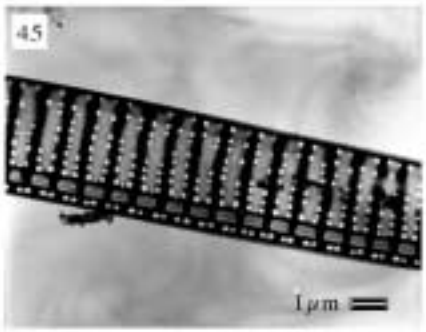
Fig. 47 Specimen from the southwest coast of Ireland, station: 1801 (51˚ 27.0'N, 10˚ 20.0'W), July 20th 1996, part of a valve, 2 poroids in 1 µm.

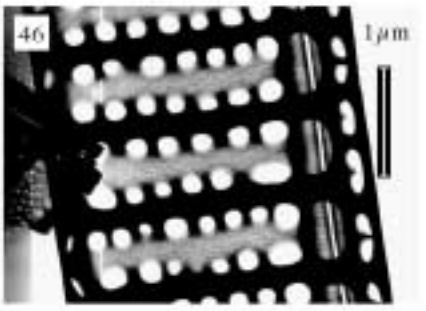

41
10μm


42
10μm


43
10μm


44
1μm


45
1μm


46
1μm


47
1μm

Fig. 41–55 Cont'd. TRANSMISSION ELECTRON MICROGRAPHS (TEM).

Figs. 48–49 Transmission electron micrographs (TEM) of a clonal culture, strain S_1 isolated from Salt lake (53˚ 28.4'N, 10˚ 01'W), west coast of Ireland, August 7th 1997.

Fig. 48 Valve end.

Fig. 49 Other end, 2 poroids in 1 µm.

Fig. 50 Specimen from the southwest coast of Ireland, station: 1314 (51˚ 20.1'N, 10˚ 05.0'W), August 17th 1993. Valve end, 4 poroids in 1 µm.

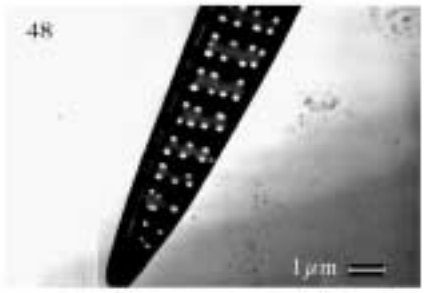
Fig. 51–52 Specimen from the southwest coast of Ireland, station: 1802 (51˚ 27.1'N, 10˚ 35.0'W), July 20th 1996. Part of a valve and valve end, 13 striae, 15 interstriae and 13 fibulae in 10 µm, 3–4 poroids in 1 µm.

Fig. 53–55 Specimen from the southwest coast of Ireland, station: 1806 (51˚ 37.1'N, 09˚ 46.9'W), July 21st 1996.

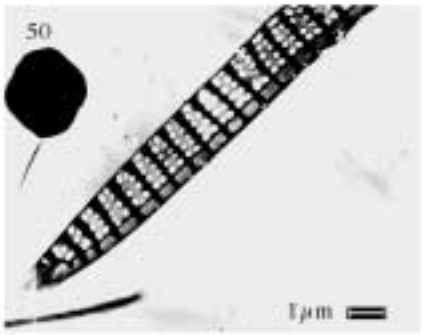
Fig. 53 Part of a girdle band showing the stria structure of the simple hymenate type.

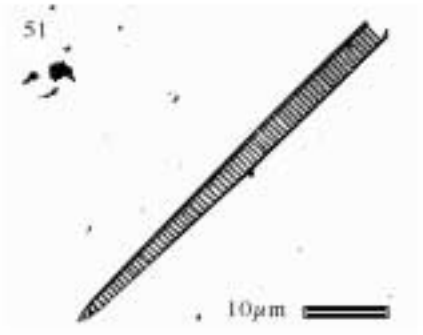
Fig. 54 Close-up of a poroid with hymenate stria on a girdle band.

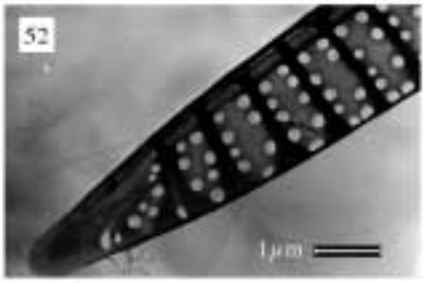
Fig. 55 Close-up of a hymenate poroid of the valve face stria.

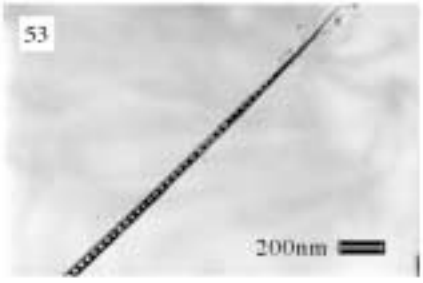

48
1μm

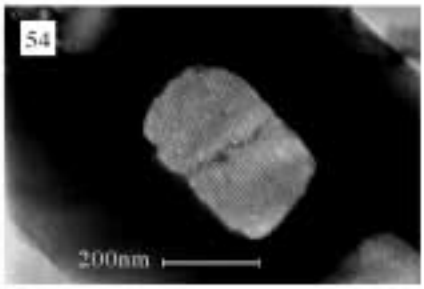

49
1μm

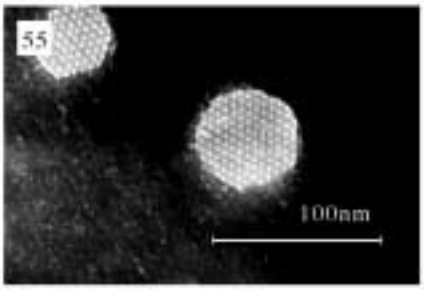

50
1μm


51
10μm


52
1μm


53
200nm


54
200nm


55
100nm

Pseudo-nitzschia australis

(Figures 56 – 154)

Figs. 56–86 LIGHT MICROGRAPHS (LM).

Figs. 56–82 Light micrographs (LM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Fig. 56 – 74 LIGHT MICROGRAPHS, WATER MOUNTS.

Fig. 56 – 73 LIGHT MICROGRAPHS OF VEGETATIVE CELLS IN WATER MOUNTS.

Fig. 56 Girdle view of Vegetative cells in chain formation, overlap ~1/4 of total cell length, 60 µm long, 5 µm wide (oil immersion).

Fig. 57 Vegetative cell in valve view, 48 µm long, ~5 µm wide (oil immersion).

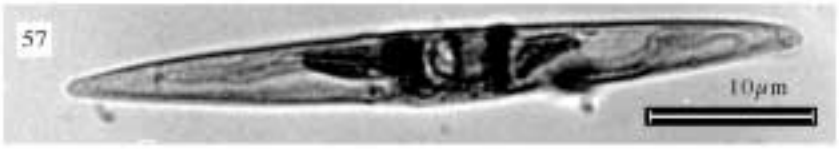
Fig. 58 Vegetative cell in valve, 55 µm long, ~6 µm wide (oil immersion).

Fig. 59 Vegetative cell in girdle view, 45 µm long, 4 µm wide. (oil immersion).

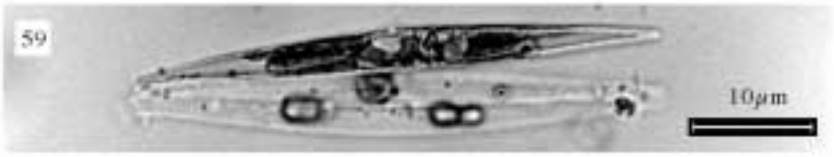
Fig. 60 Two Vegetative cells in valve view, 69 µm long, 5.95 µm wide (phase contrast).

Fig. 61 Two Vegetative cells in valve view, 77 µm long, ~7.8 µm wide (oil immersion).

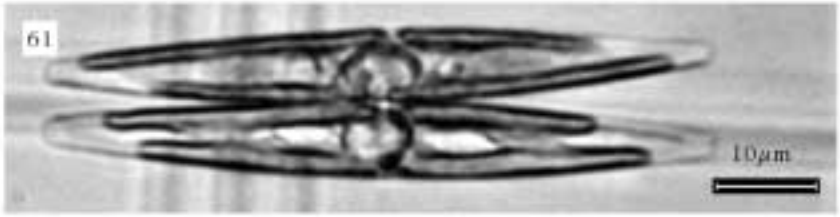

56
10μm


57
10μm


58
10μm


59
10μm


60
10μm


61
10μm

Figs. 56–86 Cont'd. LIGHT MICROGRAPHS (LM).

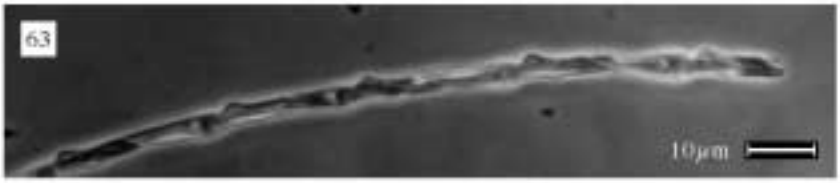
Figs. 56–82 Cont'd. Light micrographs (LM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Fig. 56 – 74 Cont'd. LIGHT MICROGRAPHS, WATER MOUNTS.

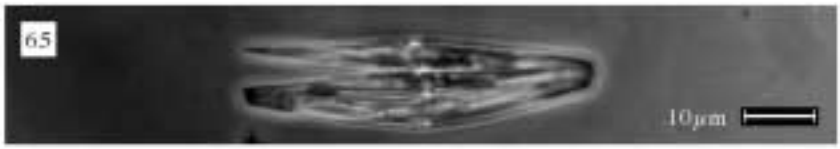
Fig. 56 – 73 Cont'd. LIGHT MICROGRAPHS OF VEGETATIVE CELLS IN WATER MOUNTS.

Fig. 62–67 ABERRANT FORMS OF *Pseudo-nitzschia australis*.


62
10μm


63
10μm


64
10μm,


65
10μm


66
10μm

Figs. 56–86 Cont'd. LIGHT MICROGRAPHS (LM).

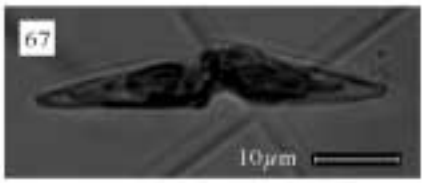
Figs. 56–82 Cont'd. Light micrographs (LM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

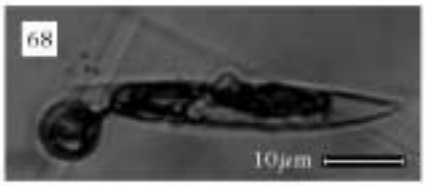
Fig. 56 – 74 Cont'd. LIGHT MICROGRAPHS, WATER MOUNTS.

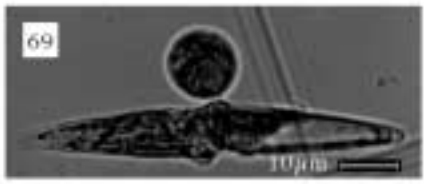
Fig. 56 – 73 Cont'd. LIGHT MICROGRAPHS OF VEGETATIVE CELLS IN WATER MOUNTS.

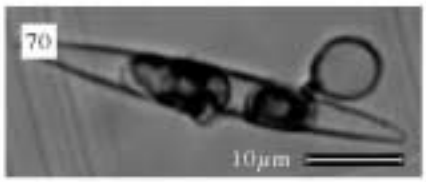
Fig. 67 ABERRANT FORM OF A VEGETATIVE P. *australis* cell.

Figs. 68–73 In old or slightly damaged diatoms, the cytoplasm frequently leaves the cell to form a spherical structure (Stefanie Kuehn pers. comm.).

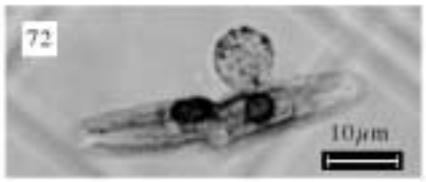

67
10μm

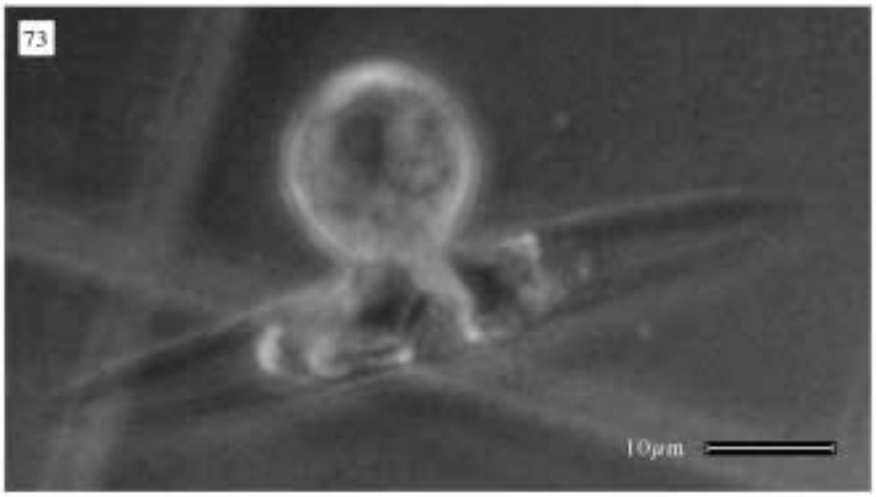

68
10μm


69
10μm


70
10μm


71
10μm


72
10μm


73
10μm

Figs. 56–86 Cont'd. LIGHT MICROGRAPHS (LM).

Figs. 56–82 Cont'd. Light micrographs (LM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Fig. 56 – 74 Cont'd. LIGHT MICROGRAPHS, WATER MOUNTS.

Figs. 74–86 ACID CLEANED VALVES.

Fig. 74 Water mount, 131 µm long, ~7 µm wide.

Figs. 75–86 NAPHRAX MOUNTED ACID CLEANED SPECIMENS.

Fig. 75 79 µm long, 5.4 µm wide.

Fig. 76 74 µm long, 4.5 µm wide.

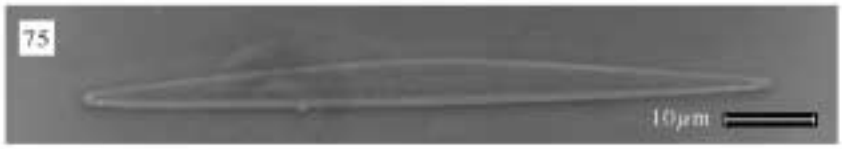
Figs. 77–79 PARTS OF THE SAME VALVE

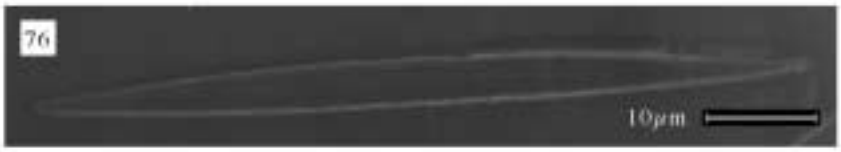
Fig. 77 Valve end.

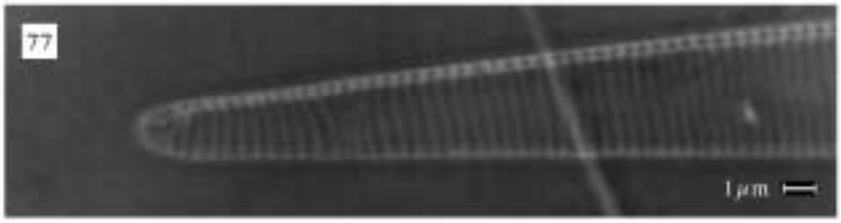
Fig. 78 Centre of valve, 5.35 µm wide, 19 striae, 19 interstriae and 19 fibulae in 10 µm.

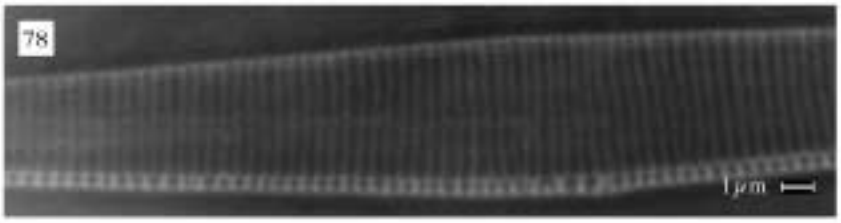
Fig. 79 Other end.

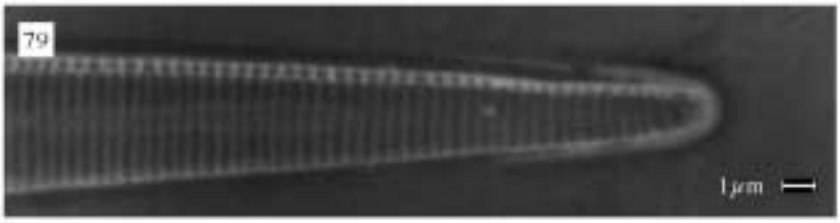

74
10μm


75
10μm


76
10μm


77
1μm


78
1μm


79
1μm

Figs. 56–86 Cont'd. LIGHT MICROGRAPHS (LM).

Figs. 56–82 Cont'd. Light micrographs (LM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Figs. 75–86 NAPHRAX MOUNTED ACID CLEANED SPECIMENS.

Figs. 80–82 PARTS OF THE SAME VALVE

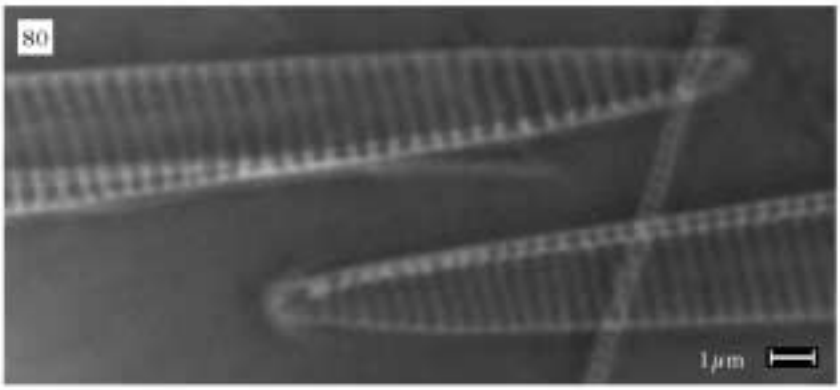
Fig. 80 Valve ends (two separate valves). 19 striae, 19 interstriae and 19 fibulae in 10 µm.

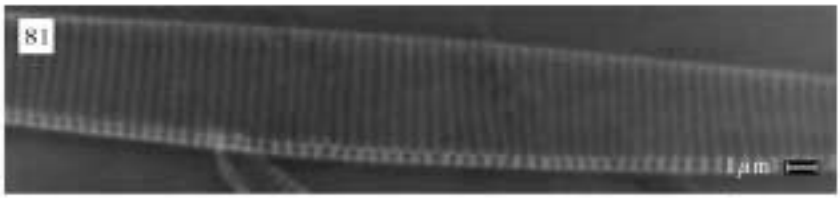
Fig. 81 Centre region of valve, 4.7 µm wide, 18 striae, 18 interstriae and 18 fibulae in 10 µm.

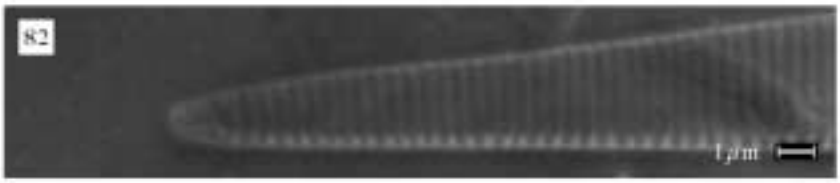
Fig. 82 Other end 19 striae, 19 interstriae and 19 fibulae in 10 µm.

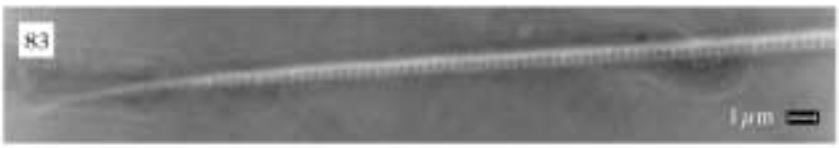
Fig. 83 Specimen from the south coast of Ireland, station: 2204 (51˚ 59.85'N, 07˚ 10.88'W) October 7th 1997, part of a girdle band.

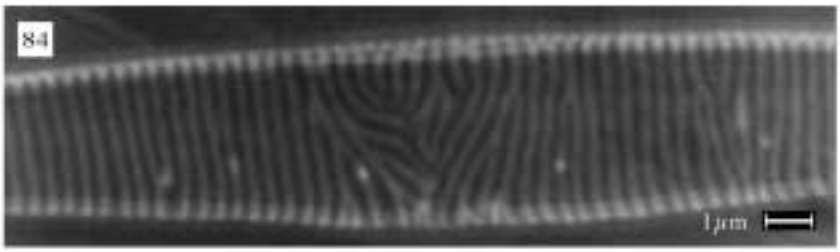
Fig. 84 Light micrographs (LM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997. Part of an aberrant valve.


80
1μm


81
1μm


82
1μm

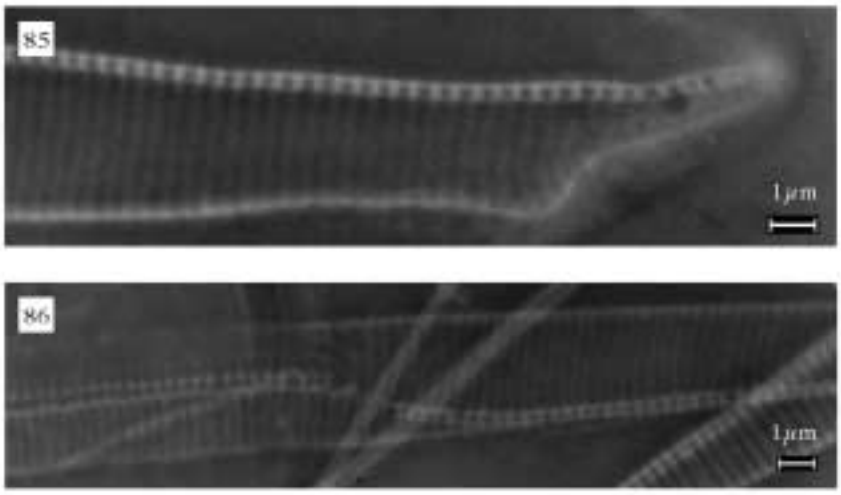

83
1μm


84
1μm

Figs. 56–86 Cont'd. LIGHT MICROGRAPHS (LM).

Figs. 75–86 NAPHRAX MOUNTED ACID CLEANED SPECIMENS.

Figs. 85–86 Light micrographs (LM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997. Parts of aberrant valves.


8S
1 μm

8S
1 μm

Figs. 87–105 SCANNING ELECTRON MICROGRAPHS (SEM).

Figs. 87–89 A specimen from the southwest coast of Ireland, station: 1801 (51˚ 27.0'N, 10˚ 20.0'W) July 20th 1996.

Fig. 87 Whole valve , 79.4 µm long, ~7.4 µm wide.

Fig. 88 Middle part of valve, 18 striae, 18 interstriae and 18 fibulae in 10 µm.

Fig. 89 Valve end, 16 striae, 17 interstriae and16 fibulae in 10 µm.

Figs. 90–92 A specimen from the southwest coast of Ireland, station: 1801 (51˚ 27.0'N, 10˚ 20.0'W) July 20th 1996.

Fig. 90 Whole valve , 76.9 µm long, 6.9 µm wide.

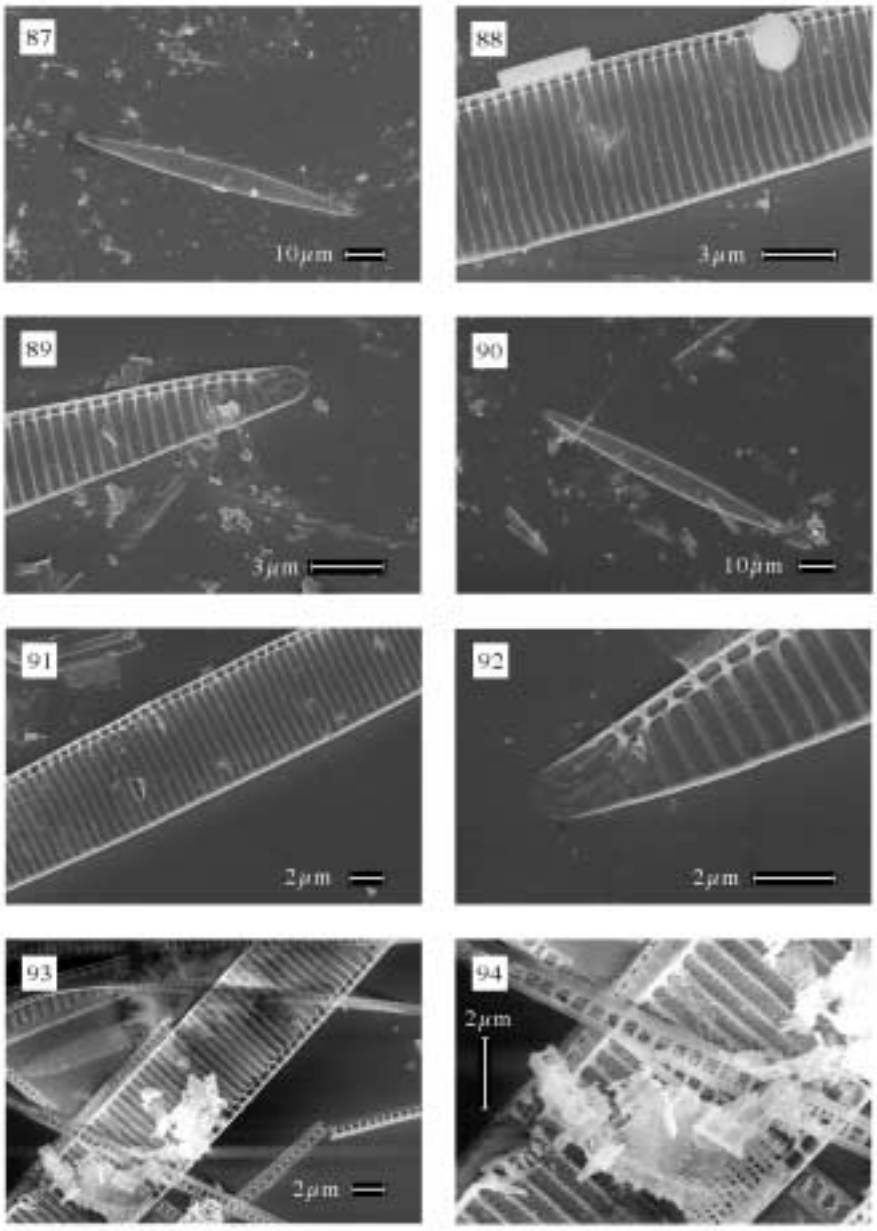
Fig. 91 Middle part of valve, 15 striae, 16 interstriae and 15 fibulae in 10 µm.

Fig. 92 Valve end, 14 striae, 16 interstriae and 14 fibulae in 10 µm.

Figs, 93–94 A specimen from the southwest of Ireland, station:1902 (52˚ 55'N; 10˚ 25'W), September 3rd 1996.

Fig. 93 Some girdle bands and the middle part of a valve , 17 striae, 17 interstriae and 17 fibulae in 10 µm.

Fig. 94 Close-up of girdle bands and valve face, 5 poroids in 1 µm.


87
10μm
88
3μm
89
3μm
90
10μm
91
2μm
92
2μm
93
2μm
94
2μm

Figs. 87–105 SCANNING ELECTRON MICROGRAPHS (SEM).

Fig. 95–102 Scanning electron micrographs (SEM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Fig. 95 Middle pat of a valve, 5.4 µm wide, 17 striae, 17 interstriae and 16 fibulae in 10 µm, valve end with branched interstriae at the pole.

Fig. 96 Middle part of a valve, 17 striae and 17 interstriae in 10 µm, 5 poroids in 1 µm.

Fig. 97 Valve end, 6 poroids in 1 µm.

Fig. 98 Whole valve, 48.7 µm long, 5.2 µm wide, 19 striae, 19 interstriae and 19 fibulae in 10 µm.

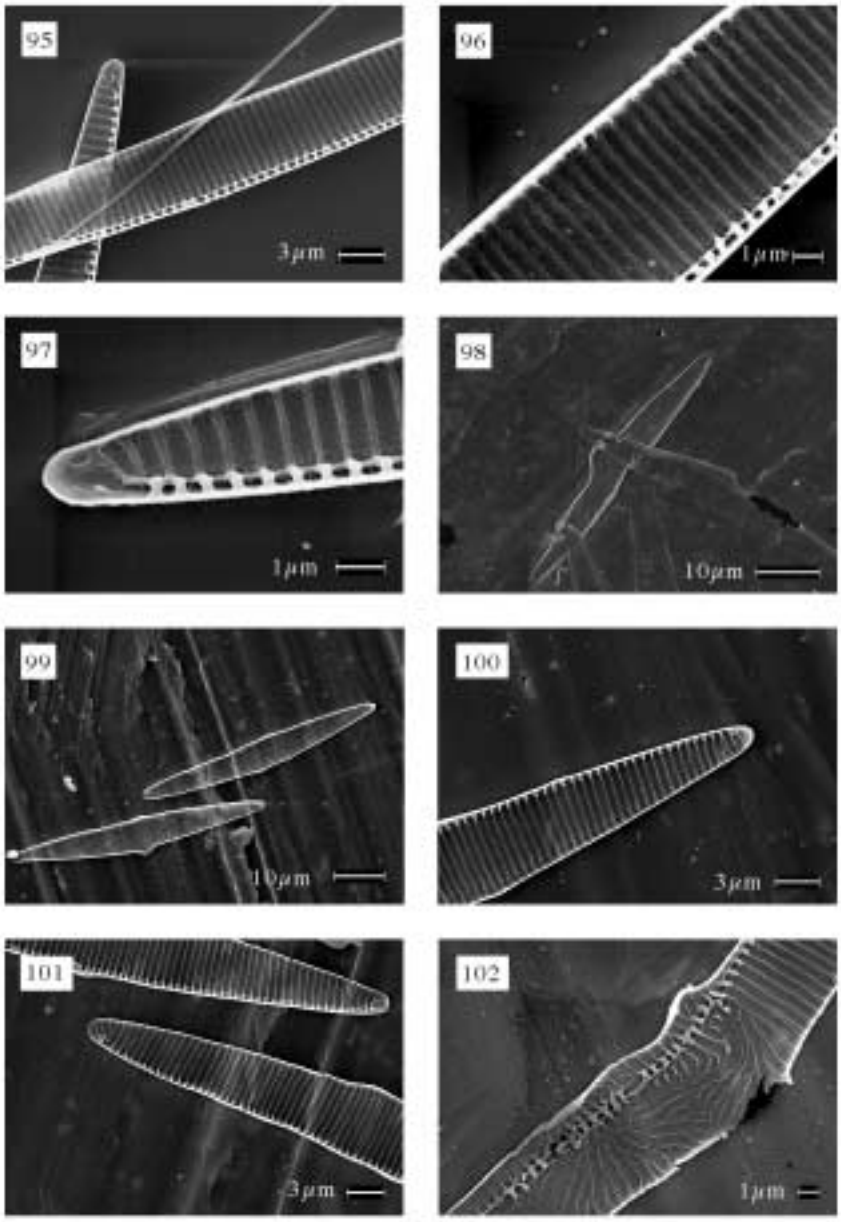
Figs. 99–101 PARTS OF THE SAME VALVE

Fig. 99 Whole valves, upper valve: 48.9 µm long, 6.1 µm wide, 17 striae, 17 interstriae and 17 fibulae in 10 µm. Lower valve: 50.6 µm long, 6.1 µm wide, 17 striae, 17 interstriae and 17 fibulae in 10 µm.

Fig. 100 Upper valve end, 17 striae, 17 interstriae and 17 fibulae in 10 µm, 5 poroids in 1 µm.

Fig. 101 Upper valve other end, 17 striae, 17 interstriae and 17 fibulae in 10 µm, 5 poroids in 1 µm.

Fig. 102 Part of an aberrant valve.


95
3 μm
96
1 μm
97
1 μm
98
10 μm
99
10 μm
100
3 μm
101
3 μm
102
1 μm

Figs. 87–105 SCANNING ELECTRON MICROGRAPHS (SEM).

Figs 103–105 Specimen from the southwest coast of Ireland, station: 1902 (52˚ 25'N, 10˚ 25'W) September 3rd 1997.

Fig. 103 Polar end of a valve, 5 poroids in 1 µm

Fig. 104 Middle part of a valve, 18 striae, 18 interstriae and 18 fibulae in 10 µm, 6 poroids in 1 µm

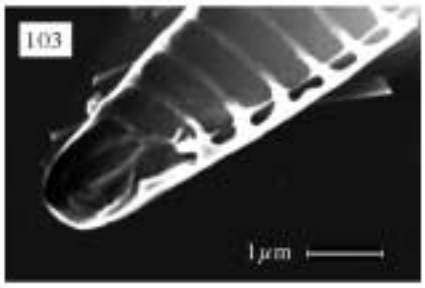
Fig. 105 Whole valve, 74.3 µm long, 5.7 µm wide, 18 striae, 18 interstriae and 18 fibulae in 10 µm

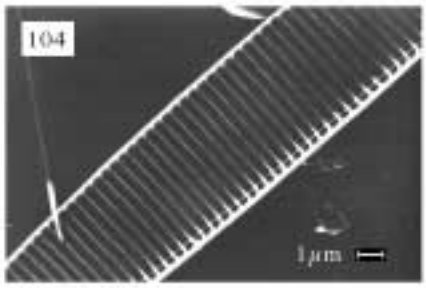
Figs. 106–154 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

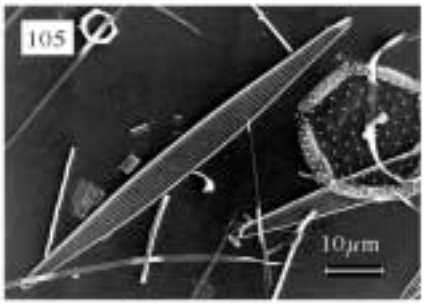
Figs. 106–107 Transmission electron micrographs (SEM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

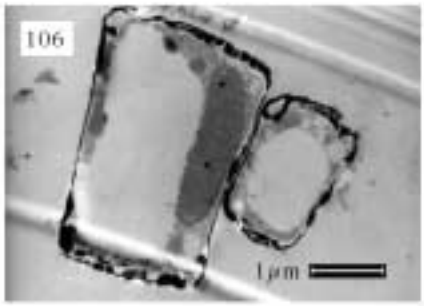
Fig. 106. Transect of two cells.

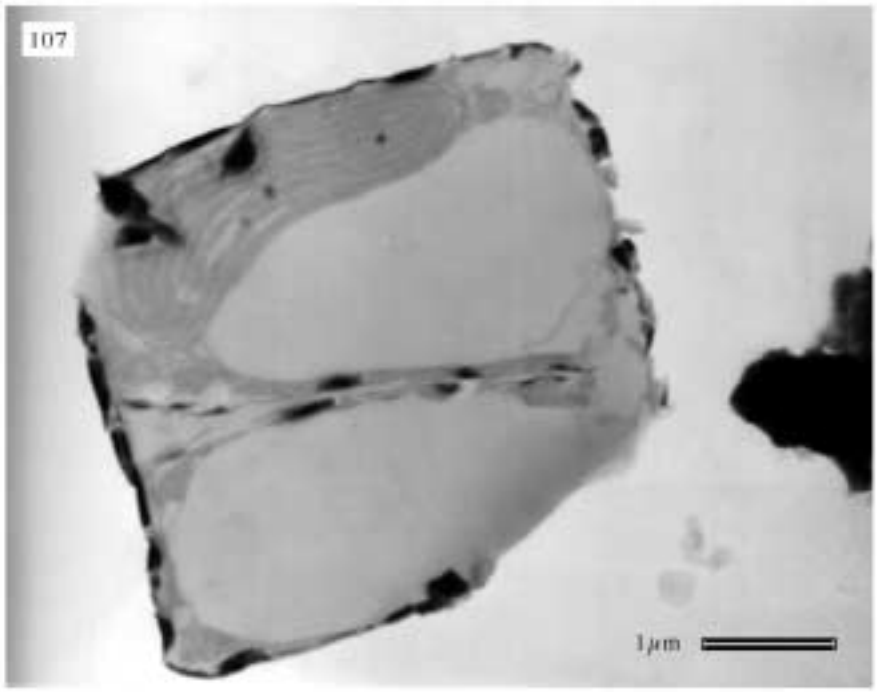
Fig. 107 Transection of a vegetative cell.


103
1μm


104
1μm


105
10μm


106
1μm


107
1μm

Figs. 106–154 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 108–111 Specimen from the southwest coast of Ireland, station: 1902 (52˚ 25'N, 10˚ 25'W) September 3rd 1997.

Fig. 108 Part of the valve, 19 striae, 19 interstriae and 19 fibulae in 10 µm, 5 poroids in 1 µm.

Fig. 109 Valve end, 19 striae, 19 interstriae and 16 fibulae in 10 µm.

Fig. 110 Middle part of the valve, 18 striae, 19 interstriae and 19 fibulae in 10 µm.

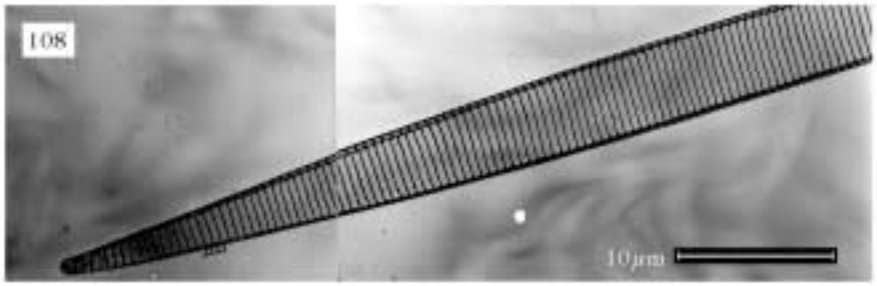
Fig. 111 Other end of the valve, 19 striae, 19 interstriae and 17 fibulae in 10 µm.

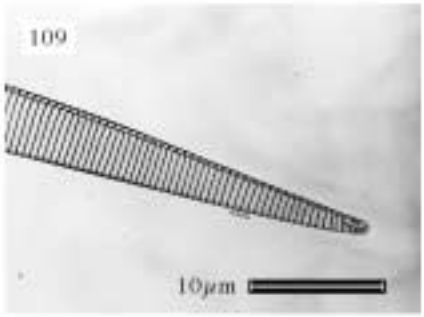
Figs. 112–114 Specimen from the southwest coast of Ireland, station: 1806 (51˚ 37.1'N, 09˚ 46.9'W), July 21st 1996.

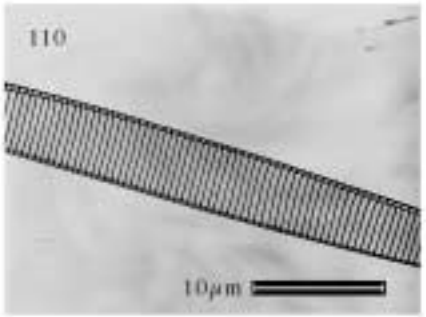
Fig. 112 5.7 µm wide, 16–18 striae,16–18 interstriae and 16–18 fibulae in 10 µm, 5–6 poroids in 1 µm.

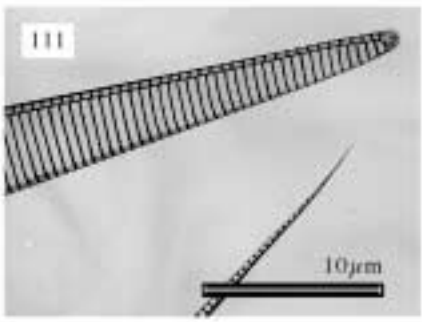
Fig. 113 Valve end.

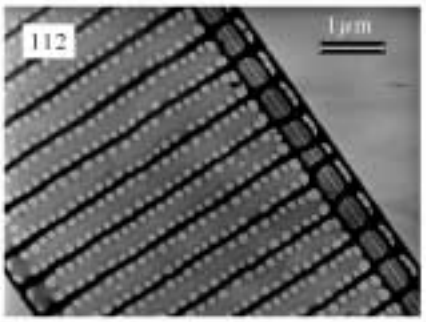
Fig. 114 Other end.

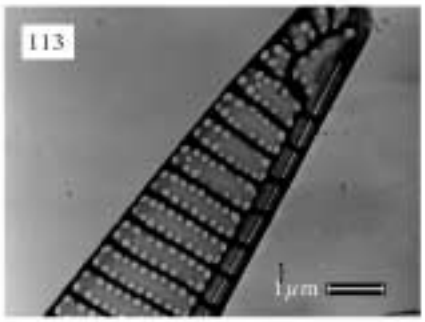

108
10μm

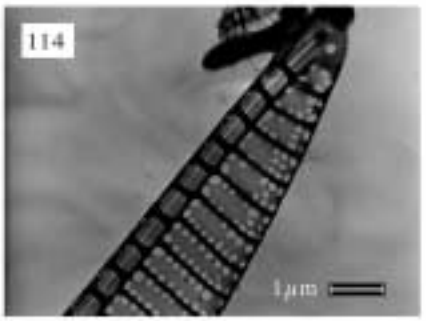

109
10μm


110
10μm


111
10μm


112
1μm


113
1μm


114
1μm

Figs. 106–154 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 115–118 Specimen from the southwest coast of Ireland, station: 1902 (52˚ 25'N, 10˚ 25'W) September 3rd 1997.

Fig. 115 Whole valve, 60 µm long, 6 µm wide.

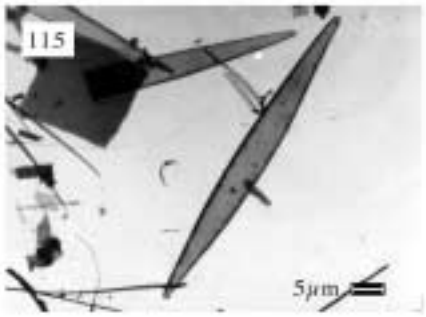
Fig. 116 Valve end.

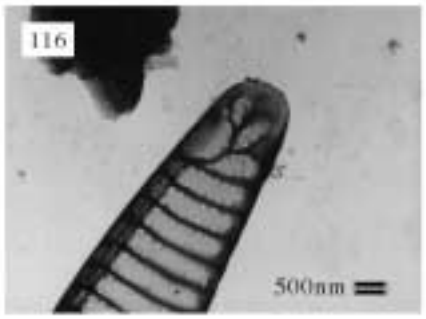
Fig. 117 Other end.

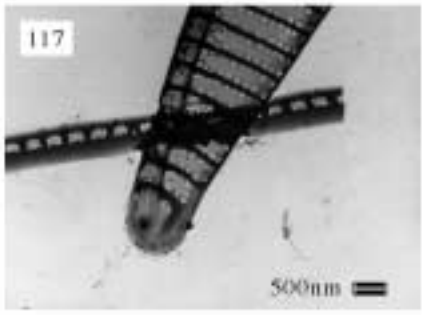
Fig. 118 Middle part of the valve, 18 striae, 19 interstriae and 17 fibulae in 10 µm, 6 poroids in 1 µm.

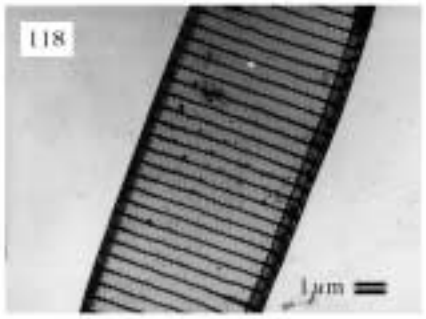
Fig. 119 Specimen from the southwest coast of Ireland, station: 1902 (52˚ 25'N, 10˚ 25'W) September 3rd 1997.

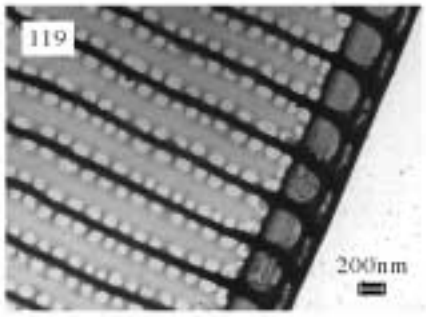
Fig. 120 Specimen from the southwest coast of Ireland, station: 1902 (52˚ 25'N, 10˚ 25'W) September 3rd 1997.

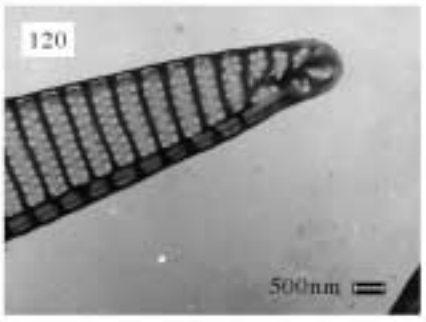

115
5μm


116
500nm


117
500nm


118
1μm


119
200nm


120
500nm

Figs. 106–154 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 121–124 Specimen from the southwest coast of Ireland, station: 1908 (51˚ 32'N, 10˚ 02'W) September 4th 1997.

Fig. 121 Whole valve, 62.6 µm long, 5.5 µm wide, 17 striae, 17 interstriae and 17 fibulae in 10 µm, 5–6 poroids in 1 µm.

Fig. 122 Valve end, 5–6 poroids in 1 µm.

Fig. 123 Other end, 5 poroids in 1 µm.

Fig. 124 Close up of part of the proximal mantle.

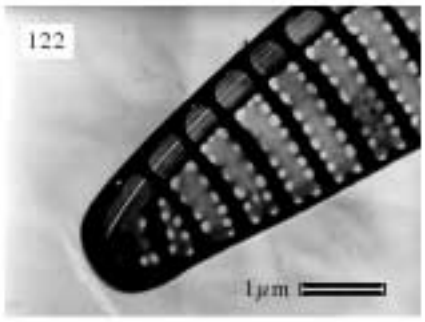
Figs. 125–127 Specimen from the southwest coast of Ireland, station: 1908 (51˚ 32'N, 10˚ 02'W) September 4th 1997.

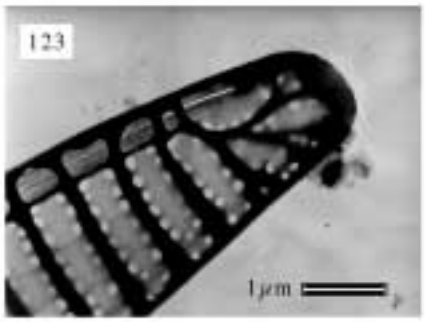
Fig. 125 Valve end, 6 poroids in 1 µm.

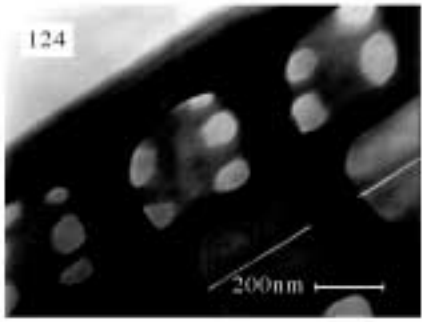
Fig. 126 Middle part of the valve, 17 striae, 17 interstriae and 17 fibulae in 10 µm.

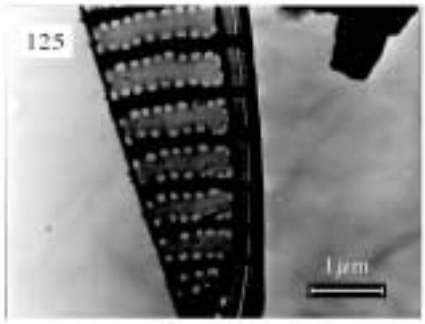
Fig. 127 Other end.

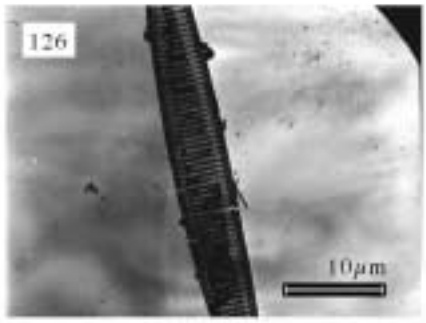

121
10μm


122
1μm


123
1μm


124
200nm


125
1μm


126
10μm


127
1μm

Figs. 106–154 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 128–129 Specimen from the southwest coast of Ireland, station: 1801 (51˚ 27'N; 10˚ 20'W), July 20th 1996.

Fig. 128 Valve end, 5 poroids in 1µm

Fig. 129 Other end, 6 poroids in 1µm

Figs. 130–131 Specimen from the south coast of Ireland, Waterford Harbour, station: W309 (52˚ 4.09'N, 07˚ 6.05'W) October 8th 1997, clonal culture, strain WW_4 girdle band.

Fig. 130 Girdle band, 41.3 µm long (from open end to the closed end).

Fig. 131 Close up of the stria structure on the girdle band.

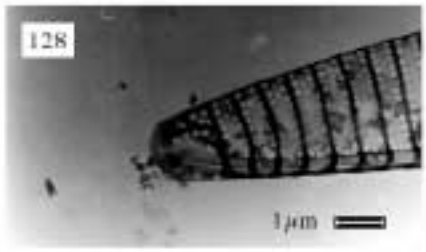
Figs. 132–134 Specimen from the southwest coast of Ireland, station: 1809 (51˚ 26'N; 09˚ 24'W), July 21st 1996.

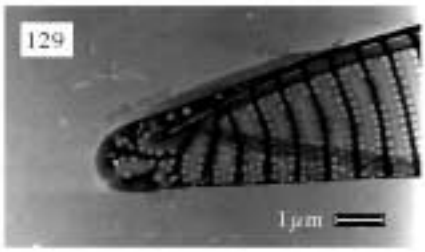
Fig. 132 Valve end, 5–6 poroids in 1µm.

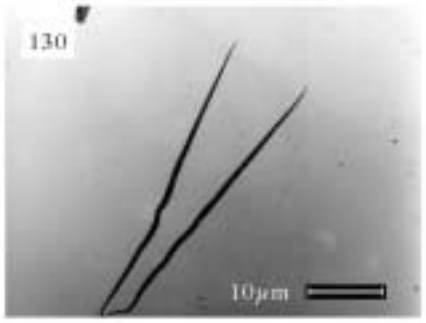
Fig. 133 Middle part of the valve, 19 striae, 19 interstriae and 19 fibulae in 10 µm, 6–7 poroids in 1 µm.

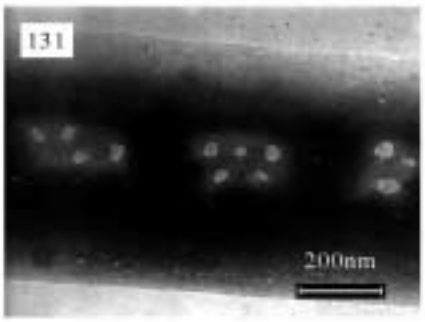
Fig. 134 Other end, 6 poroids in 1 µm.

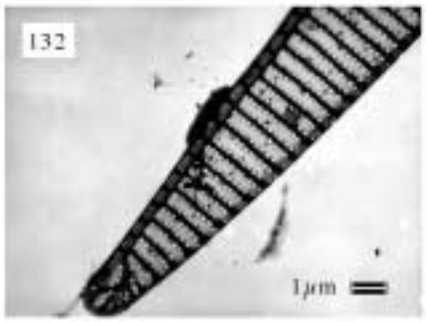
Fig. 135 Specimen from the southwest coast of Ireland, station: 1806 (51˚ 37.1'N; 09˚ 46.9'W), July 21st 1996, whole valve, 70 µm long, 5.5 µm wide, 17 striae, 17 interstriae and 17 fibulae in 10 µm.

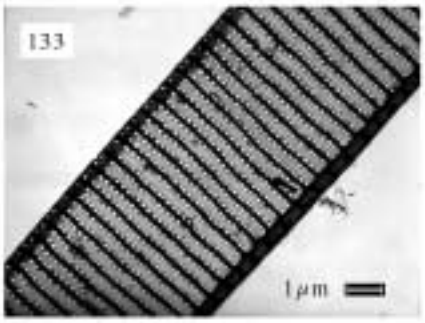

128
1μm

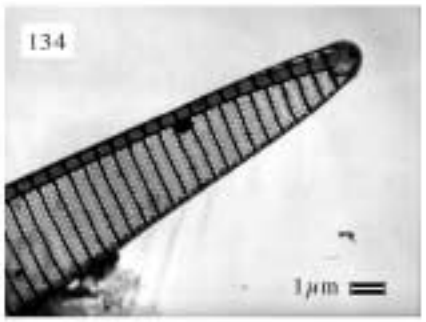

129
1μm

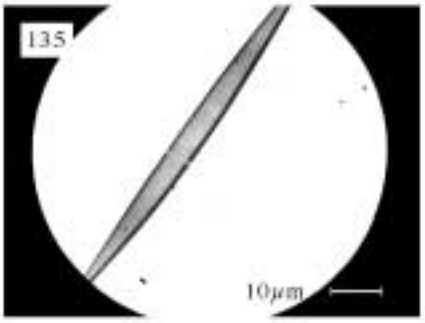

130
10μm


131
200nm


132
1μm


133
1μm


134
1μm


135
10μm

Figs. 106–154 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 136–137 Specimen from the south coast of Ireland, station: 1810 (51˚ 07.2'N; 09˚ 24.0'W), July 22nd 1996.

Fig. 136 Part of the valve face, 6 poroids in 1 µm.

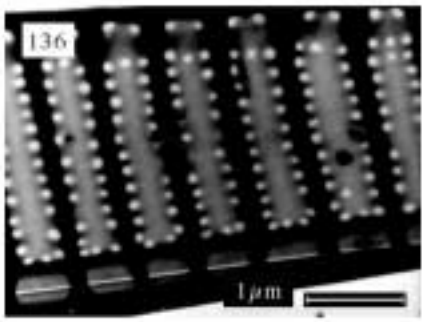
Fig. 137 Valve end, 6 poroids in 1 µm.

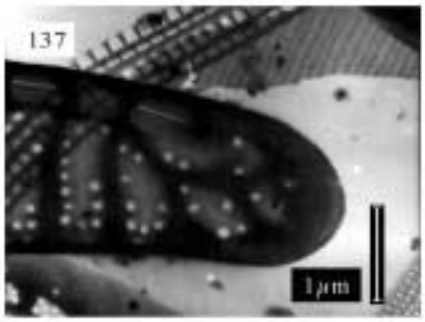
Figs. 138–140 Specimen from the southwest coast of Ireland, station: 2206 (51˚ 30.03.2'N; 08˚ 25.17'W), October 11th 1997.

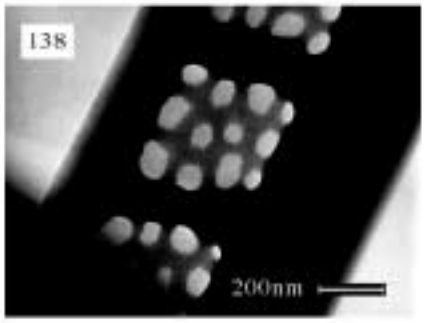
Fig. 138–139 Parts of a girdle band.

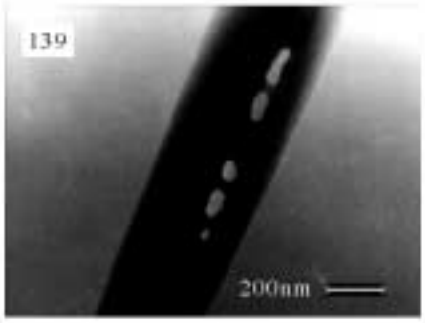
Fig. 140 Part of the valve face, 6 poroids in 1 µm.

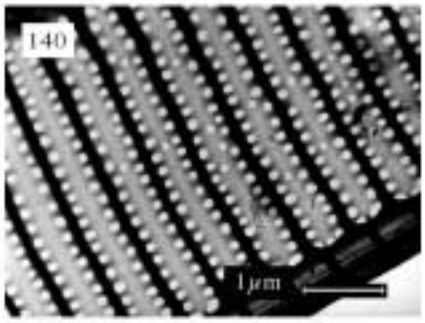
Fig. 141 Specimen from the southwest of Ireland, station: 1902 (52˚ 25'N, 10˚ 25'W) September 3rd 1997, part of a girdle band.

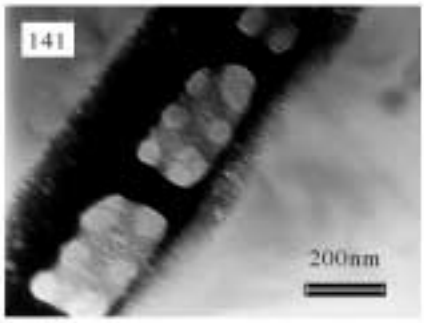

136
1μm


137
1μm


138
200nm


139
200nm


140
1μm


141
200nm

Figs. 106–154 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 142–154 Transmission electron micrographs (TEM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Figs. 142–145 DIFFERENT PARTS OF THE SAME VALVE

Fig. 142 Whole valve, 47.7 µm long, 5 µm wide, 17 striae, 18 interstriae and 18 fibulae in 10 µm.

Fig. 143 Vale end, 6 poroids in 1 µm.

Fig. 144 Middle part of the valve, 6–7 poroids in 1 µm.

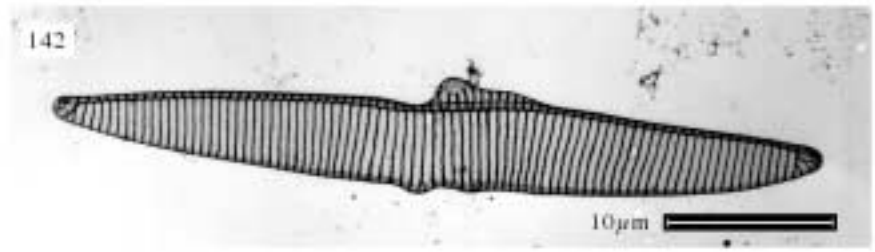
Fig. 145 Other end, 6–7 poroids in 1 µm.

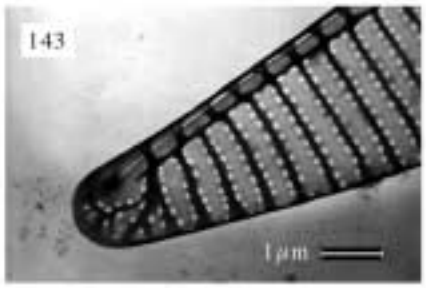
Fig. 146 Part of a valve end, 5 poroids in 1 µm..

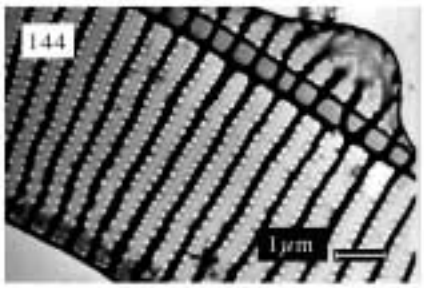
Figs. 147–150 DIFFERENT PARTS OF THE SAME VALVE.

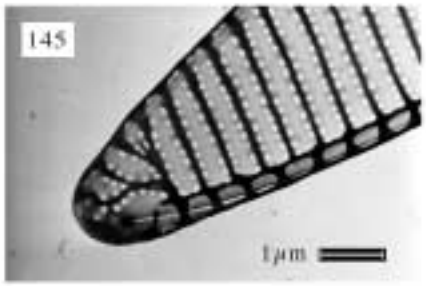
Fig. 147 Most of the valve, ~ 50 µm long, 5.5 µm wide, 17 striae, 18 interstriae and 18 fibulae in 10 µm.

Fig. 148 Valve end, 6 poroids in 1 µm.

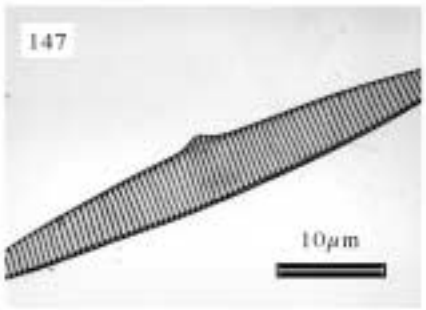

142
10μm

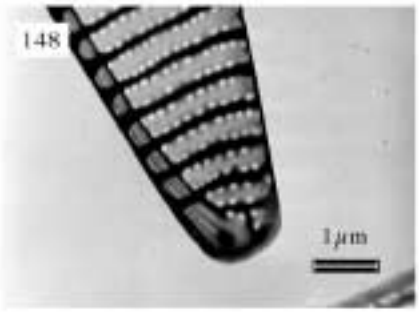

143
1μm


144
1μm


145
1μm


146
1μm


147
10μm


148
1μm

Figs. 106–154 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 142–154 Cont'd. Transmission electron micrographs (TEM) of a clonal culture, strain WW_4 isolated from Waterford Harbour, south coast of Ireland, station: W309 (52˚ 4.09'N, 07˚ 6.05'W), October 8th 1997.

Figs. 147–150 Cont'd. DIFFERENT PARTS OF THE SAME VALVE.

Fig. 149 Part of the valve face, 5 poroids in 1 µm.

Fig. 150 Other end, 6 poroids in 1 µm.

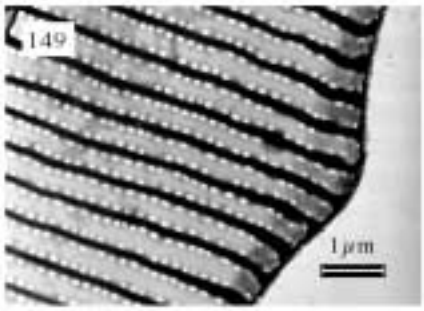
Figs. 151–154 DIFFERENT PARTS OF THE SAME VALVE.

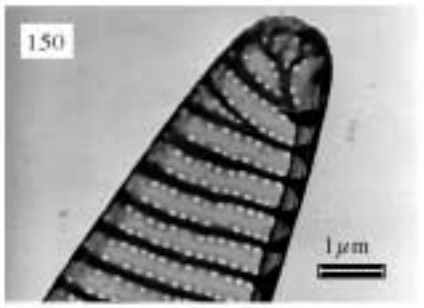
Fig. 151 Most of the valve, ~47 µm long, 5.9 µm wide, 16 striae, 17 interstriae and 17 fibulae in 10 µm.

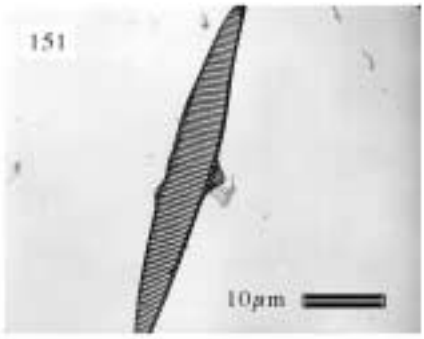
Fig. 152 Valve end, 4–5 poroids in 1 µm.

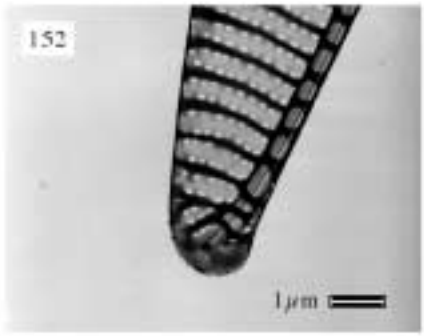
Fig. 153 Other end, 5 poroids in 1 µm.

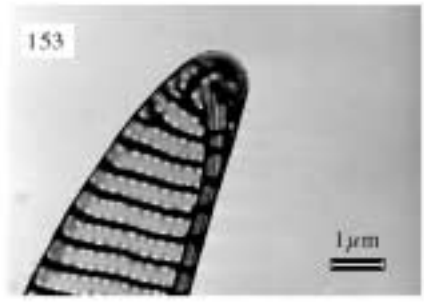
Fig. 154 Part of the valve face, 4–5 poroids in 1 µm.

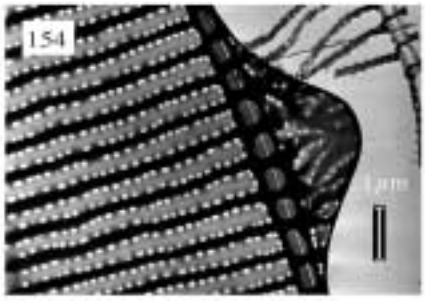

149
1 μm


150
1 μm


151
10μm


152
1 μm


153
1 μm


154
1 μm

Pseudo-nitzschia fraudulenta

(Figures 155 – 227)

Figs. 155–168 LIGHT MICROGRAPHS (LM).

Figs. 155–157 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997, light micrographs (LM) of clonal culture, strain 2011

Fig. 155 114 µm long, 5.2 µm wide (OIL IMMERSION)

Figs. 156–157 In old or slightly damaged diatoms frequently the cytoplasma leaves the cell and forms a sperical structure. Dr. Stefanie Kuehn, University of Bremen (pers. commun.)

Fig. 156 105 µm long, 6.9 µm wide (OIL IMMERSION)

Fig. 157 Close up of the sperical structure (OIL IMMERSION, PHASE CONTRAST)

Fig. 158 West coast of Ireland, St. Hawk (53˚ 289.1'N 10˚ 04.4'W) August 7th, 1997, light micrograph (LM) of a clonal culture, strain H_2, 54 µm long, 5.6 µm wide (OIL IMMERSION)

Figs. 159–164 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997, light micrographs (LM) of clonal culture, strain 2011

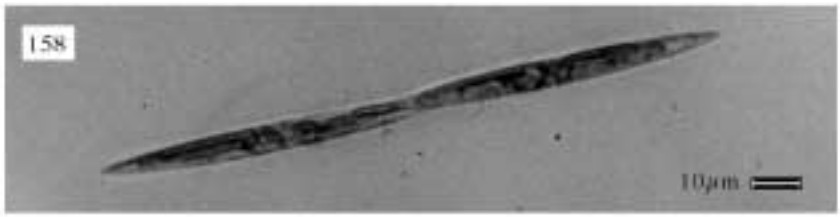
Fig. 159 137 µm long (OIL IMMERSION)

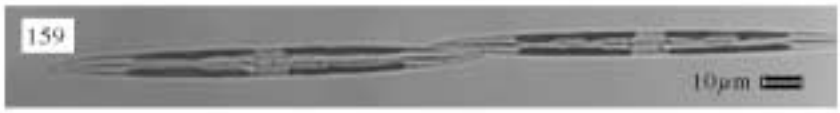
Fig. 160 134 µm long, 6 µm wide (OIL IMMERSION)

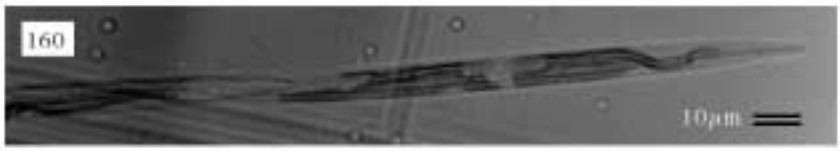

155
10μm


156
10μm


157
1μm


158
10μm


159
10μm


160
10μm

Figs. 155–168 Cont'd. LIGHT MICROGRAPHS (LM).

Figs. 159–164 Cont'd. West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997, light micrographs (LM) of clonal culture, strain 2011

Fig. 161 142 µm long, ~7 µm wide (OIL IMMERSION, NAPHRAX MOUNT)

Fig. 162 60.6 µm long, 4.2 µm wide (OIL IMMERSION, WATER MOUNT)

Fig. 163 140 µm long, 6 µm wide (OIL IMMERSION, NAPHRAX MOUNT)

Fig. 164 140 µm long, 6.5 µm wide (OIL IMMERSION, NAPHRAX MOUNT)

Fig. 165 South coast of Ireland, St. 2204 (51˚ 59.85'N, 07˚ 10.88' W) October 7th 1997, 110 µm long, ~8 µm wide (OIL IMMERSION, NAPHRAX MOUNT)

Figs. 166–168 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997, light micrographs (LM) of clonal culture, strain 2011 (OIL IMMERSION, NAPHRAX MOUNTS)

Fig. 166 Middle part of the valve, ~4 µm wide

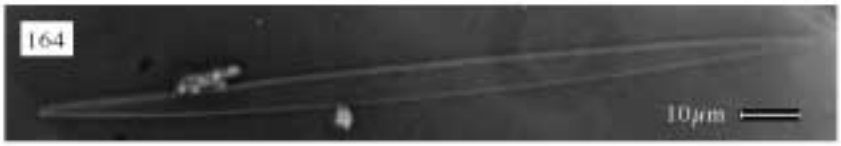
Fig. 167 Valve end

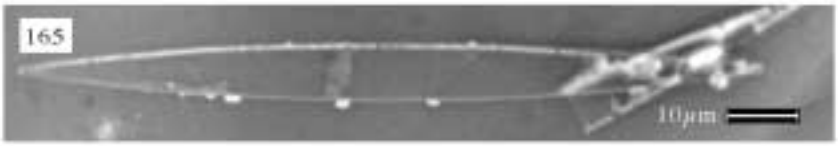
Fig. 168 Other end

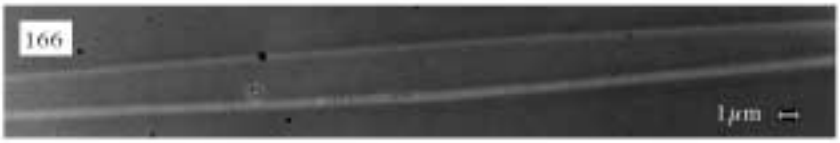

161
10μm

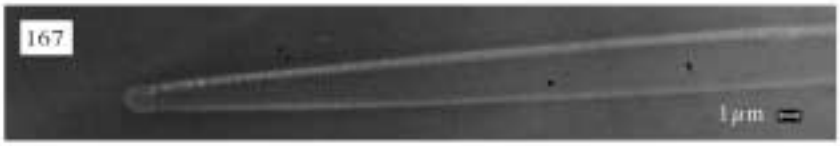

162
10μm

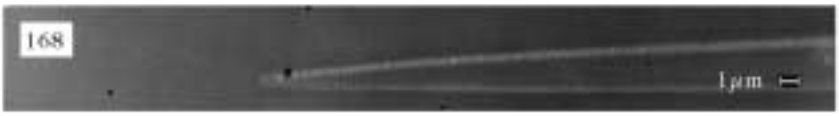

163
10μm


164
10μm


165
10μm


166
1μm


167
1μm


168
1μm

Figs. 169–210 SCANNING ELECTRON MICROGRAPHS (SEM) OF ACID CLEANED SPECIMENS

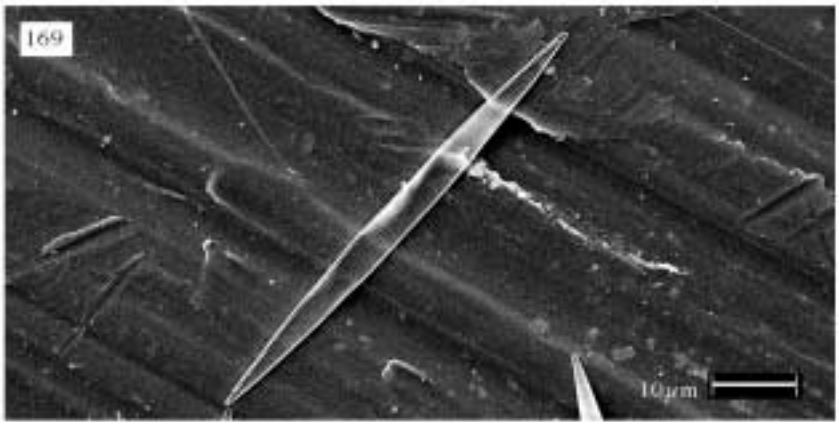
Figs. 169–172 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997, clonal culture, strain 2011

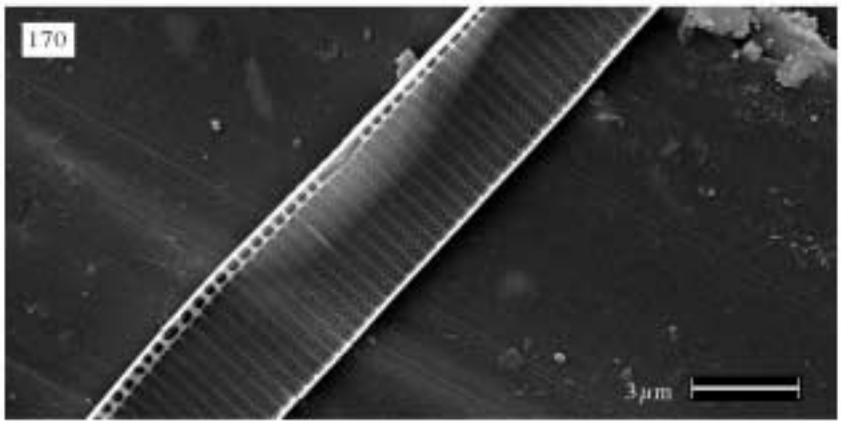
Fig. 169 Whole valve, 61.5 µm long, ~4 µm wide

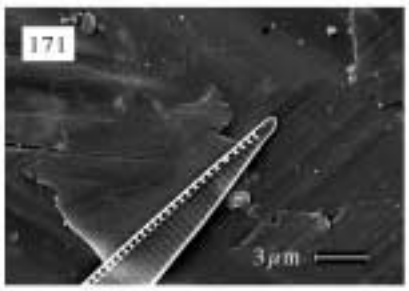
Fig. 170 Middle part of the valve, 22 striae, 23 interstriae and 22 fibulae in 10 µm, 5 poroids in 1 µm

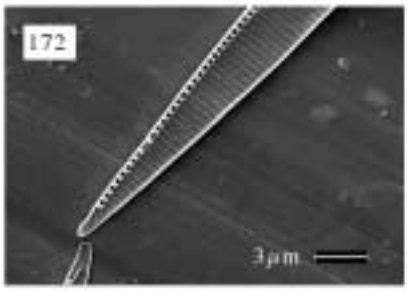
Fig. 171 Valve end

Fig. 172 Other end


169
10μm


170
3μm


171
3μm


172
3μm

Figs. 169-210 Cont'd. SCANNING ELECTRON MICROGRAPHS (SEM) OF ACID CLEANED SPECIMENS

Figs. 173–177 South coast of Ireland, St. 1314 (51˚ 20.1'N; 10˚ 05.0'W), August 17th 1993

Fig. 173 Two valves, upper valve: 104 µm long, 4.9 µm wide, lower valve: 91.8 µm long, 4.9 µm wide

Figs. 174–177 Lower valve (positioned diagonally from the bottom left hand corner to the upper right hand corner of the micrograph)

Fig. 174 Valve end, 6 poroids in 1 µm

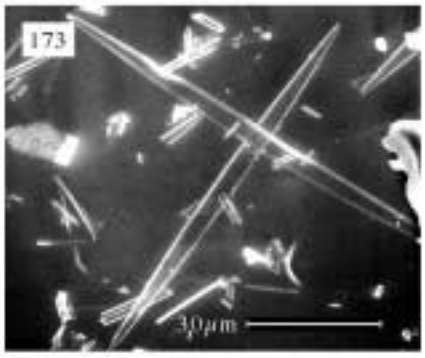
Fig. 175 Other end

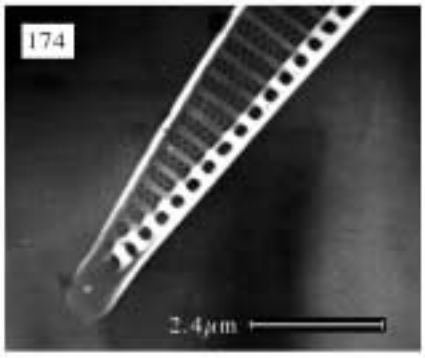
Fig. 176 Middle part of the valve, 25 striae in 10 µm

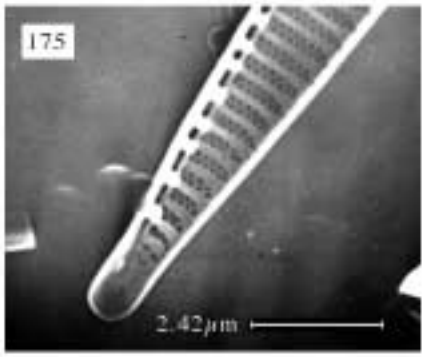
Fig. 177 Close-up of the valve face, 6 poroids in 1 µm

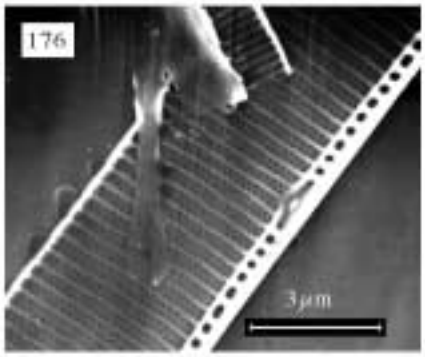
Figs. 178–181 South coast of Ireland, St. 1324 (51˚ 11.5'N; 09˚ 24.1'W), August 18th 1993

Fig. 178 Whole valve, 90.7 µm long, 4.4 µm wide

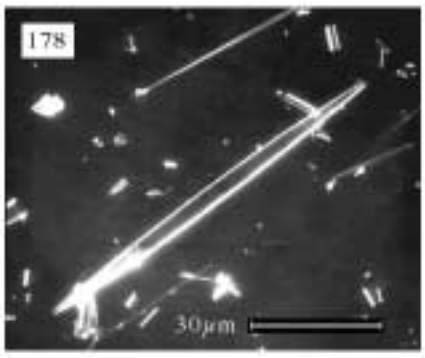

173
3.0μm


174
2.4μm


175
2.42μm


176
3μm


177
1.42μm


178
30μm

Figs. 169-210 SCANNING ELECTRON MICROGRAPHS (SEM) OF ACID CLEANED SPECIMENS Cont'd.

Figs. 178–181 South coast of Ireland, St. 1324 (51˚ 11.5'N; 09˚ 24.1'W), August 18th 1993 Cont'd.

Fig. 179 Valve end, 5 poroids in 1 µm

Fig. 180 Other end, 5 poroids in 1 µm

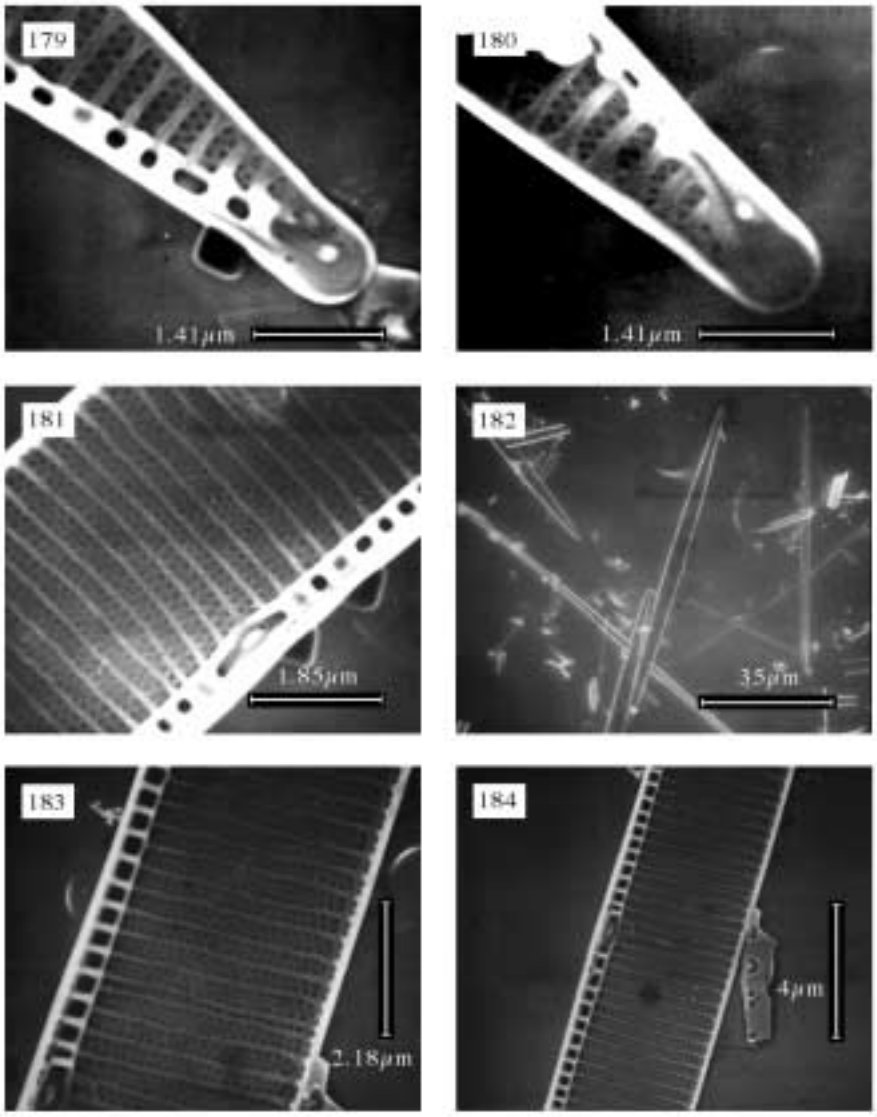
Fig. 181 Middle part of the valve, ~25 striae in 10 µm

Figs 182–186 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997

Fig. 182 Whole valve, 89.7 µm long, 4.5 µm wide

Fig. 183 Close up of the middle part of the valve , 4.5 µm wide, 5 poroids in 1 µm

Fig. 184 Middle part of the valve, ~ 26 striae, ~ 26 interstriae and ~ 26 fibulae in 10 µm


179
1.41μm
180
1.41μm
181
1.85μm
182
35μm
183
2.18μm
184
4μm

Figs. 169-210 Cont'd. SCANNING ELECTRON MICROGRAPHS (SEM) OF ACID CLEANED SPECIMENS

Figs 182–186 Cont'd. West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997

Fig. 185 Valve end

Fig. 186 Other end

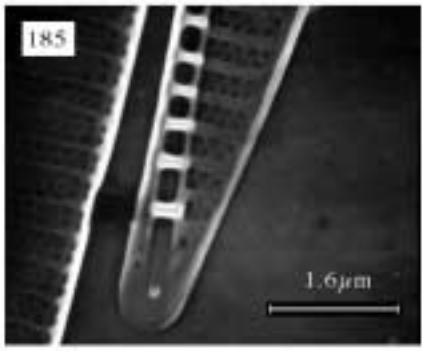
Figs. 187–189 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997,

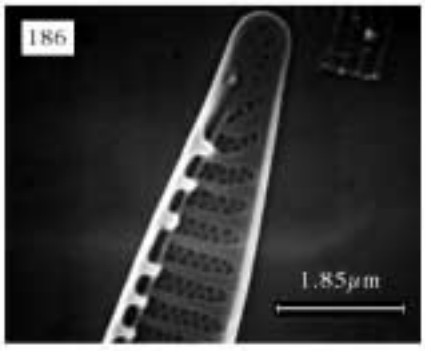
Fig. 187 Whole valve, 91.3 µm long, ~4.5 µm wide

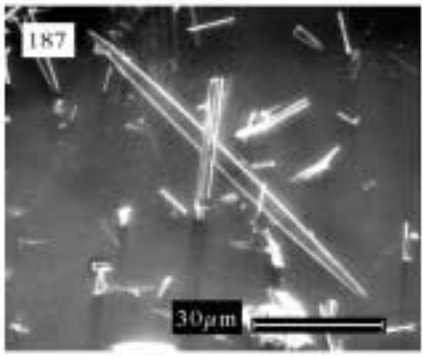
Fig. 188 Valve end

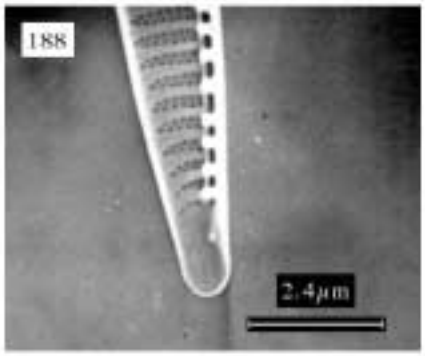
Fig. 189 Middle part of the valve, 4.5µm wide, ~ 26 striae, ~ 26 interstriae and ~ 26 fibulae in 10 µm, 5 poroids in 1 µm

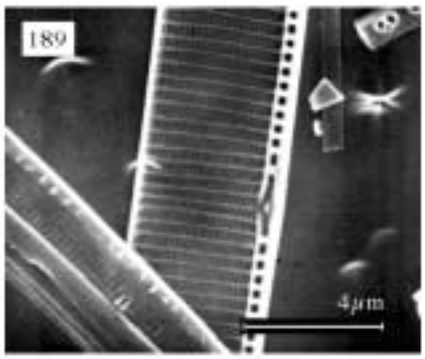
Fig. 190 South coast of Ireland, St. 1812 (51˚ 35'N; 8˚ 28.9'W), July 22nd 1996, close-up of a valve face, 6–7 poroids in 1 µm

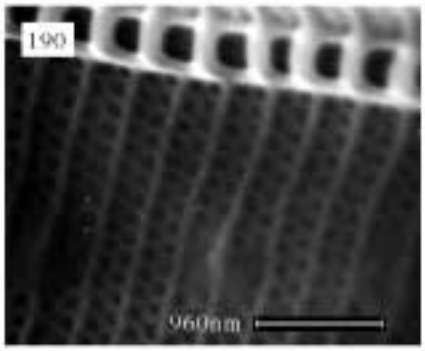

185
1.6µm


186
1.85µm


187
30µm


188
2.4µm


189
4µm


190
960nm

Figs. 169-210 SCANNING ELECTRON MICROGRAPHS (SEM) OF ACID CLEANED SPECIMENS Cont'd.

Fig. 191 South coast of Ireland, St. 1324 (51˚ 11.5'N; 09˚ 24.1'W), August 18th 1993, polar end of a valve, 5 poroids in 1 µm

Figs. 192–196 South coast of Ireland, St. 1324 (51˚ 11.5'N; 09˚ 24.1'W), August 18th 1993

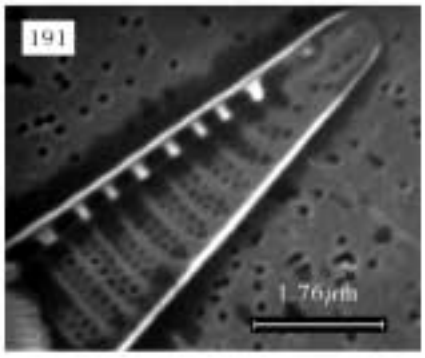
Fig. 192 Whole valve, 76.8 µm long, ~5 µm wide

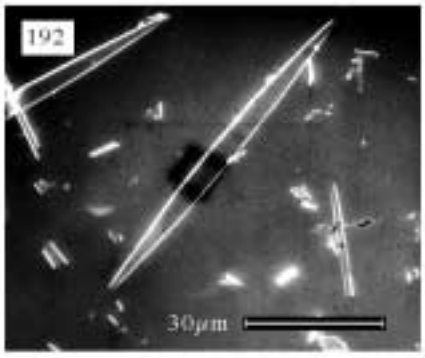
Fig. 193 Valve end, 5–6 poroids in 1 µm

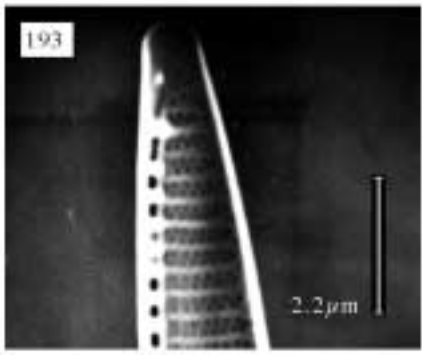
Fig. 194 Middle part of the valve, 5.1µm wide, ~26striae and ~26 interstriae in 10 µm, 5–6 poroids in 1 µm

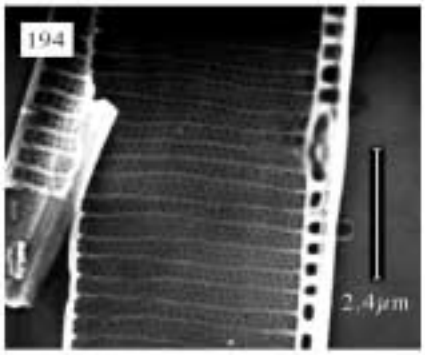
Fig. 195 Other end, 6 poroids in 1 µm

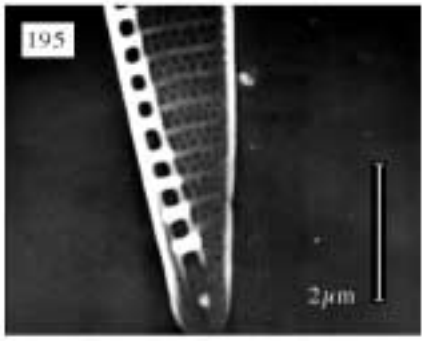
Fig. 196 Close-up of the valve face, 6 poroids in 1 µm

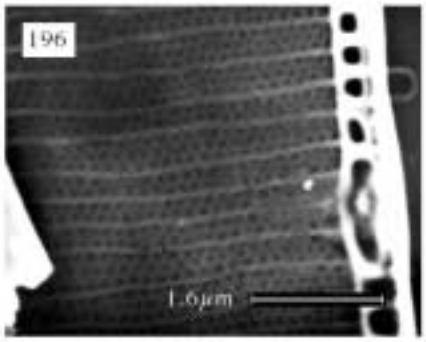

191
1.76μm


192
30μm


193
2.2μm


194
2.4μm


195
2μm


196
1.6μm

Figs. 169-210 SCANNING ELECTRON MICROGRAPHS (SEM) OF ACID CLEANED SPECIMENS Cont'd.

Fig. 197–198 South coast of Ireland, St. 1324 (51˚ 11.5'N; 09˚ 24.1'W), August 18th 1993

Fig. 197 Whole valve, 88.8 µm long, 4.8 µm wide, 25 striae, 25striae and 25 fibulae in 10 µm

Fig. 198 Valve end, 5 poroids in 1 µm

Figs. 199–226 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 199–201 South coast of Ireland, St. 2204 (51˚ 59.85'N, 07˚ 10.88' W) October 7th 1997

Fig. 199 Valve end, 6 poroids in 1 µm

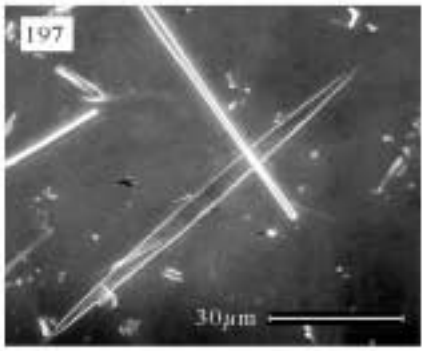
Fig. 200 Other end, 6 poroids in 1 µm

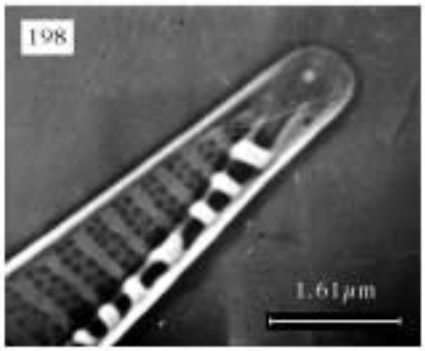
Fig. 201 Middle part of the valve, 5.1 µm wide, 6–7 poroids in 1 µm

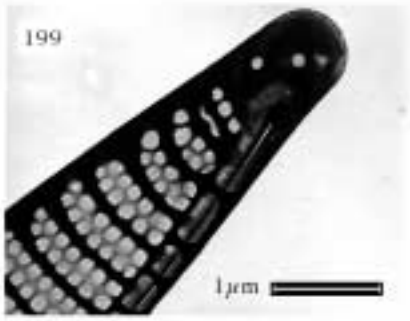
Fig. 202 South coast of Ireland, St. 2206 (51˚ 30.03'N, 08˚ 25.17' W), October 11th 1997, part of a girdle band

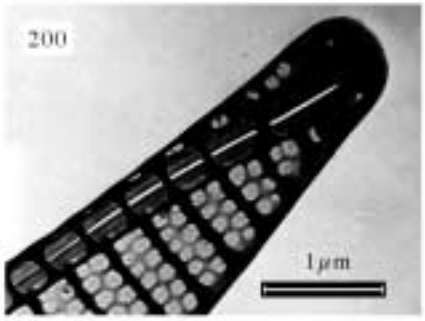
Fig. 203 South coast of Ireland, off Waterford, St. W312 (52˚ 7.02'N, 06˚ 58.81' W) October 8th 1997, part of a valve showing the proximal mantle, 7 poroids in 1 µm

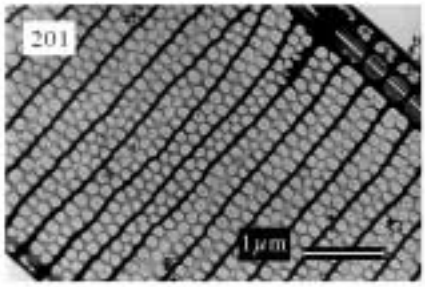
Fig. 204 South coast of Ireland, St. 2204 (51˚ 59.85'N, 07˚ 10.88' W) October 7th 1997, part of a girdle band, 20 poroids in 1 µm

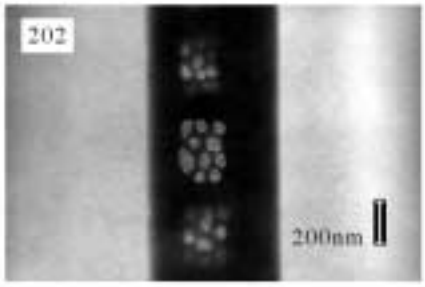

197
30μm

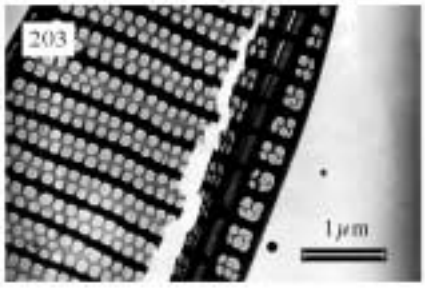

198
1.61μm

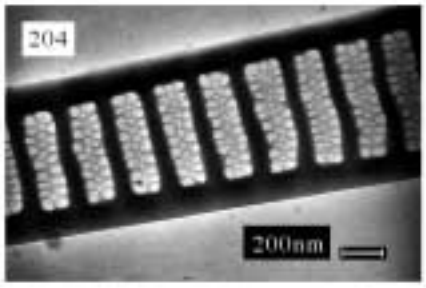

199
1μm


200
1μm


201
1μm


202
200nm


203
1μm


204
200nm

Figs. 199–226 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

Figs. 205–208 South west coast of Ireland, St. 1819 (52˚ 04.9'N; 06˚ 59.0'W), July 23rd 1996

Fig. 205 Whole valve, ~70 µm long, ~4 µm wide, 25 striae, 25 interstriae and 25 fibulae in 10 µm

Fig. 206 Middle part of the valve, 3 striae per central nodule, 6 poroids in 1 µm

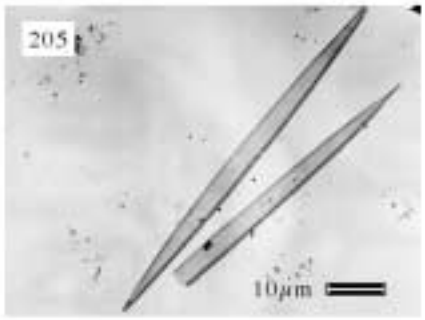
Fig. 207 Valve end, 6 poroids in 1 µm

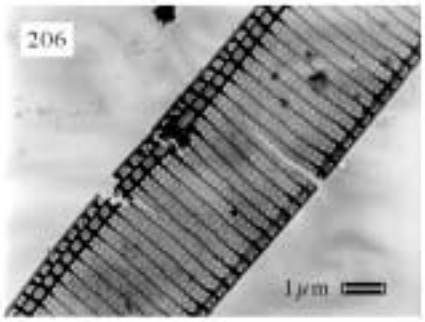
Fig. 208 Other end, 7 poroids in 1 µm

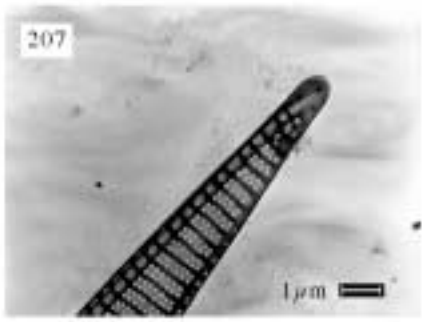
Fig. 209 South coast of Ireland, St. 1808 (51˚ 27.00'N; 09˚ 33.00'W), July 21st 1996, valve end, 5–7 poroids in 1 µm

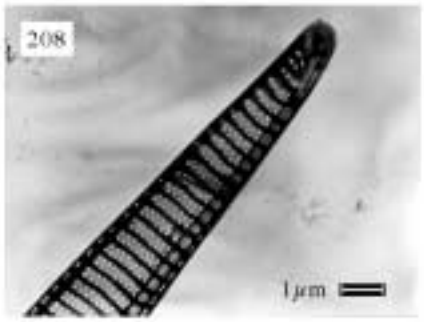
Fig. 210 South coast of Ireland, St. 1809 (51˚ 26.00'N; 09˚ 24.00'W), July 21st 1996, part of a valve with the proximal and distal mantles visible, 25 striae, 25 interstriae and 25 fibulae in 10 µm, 7 poroids in 1 µm

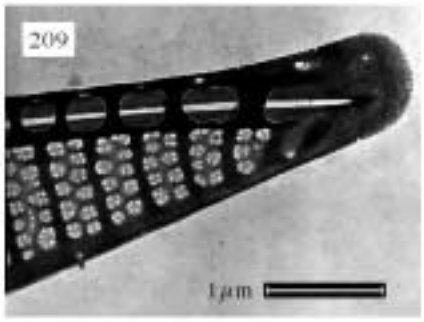
Fig. 211 South coast of Ireland, St. 2204 (51˚ 59.85'N, 07˚ 10.88' W) October 7th 1997, part of a girdle band

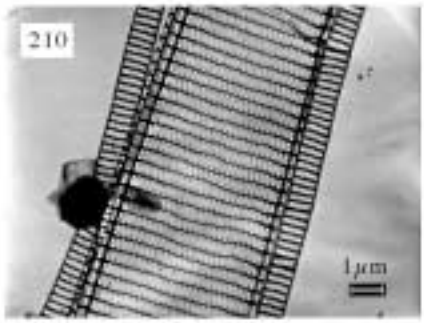

205
10μm


206
1μm


207
1μm


208
1μm


209
1μm


210
1μm


211
200nm

Figs. 199–226 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

Figs. 212–214 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997, clonal culture, strain 2011

Fig. 212 Middle part of the valve, 4.4 µm wide, 4 striae per central larger interspace, 24 striae and 24 interstriae in 10 µm, 6 poroids in 1 µm

Fig. 213 Valve end, 5–6 poroids in 1 µm

Fig. 214 Other end, 6 poroids in 1 µm

Figs. 215–217 South west coast of Ireland, St. 1801 (51˚ 27'N; 10˚ 20'W), July 20th 1996

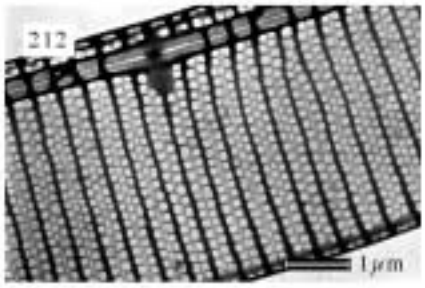
Fig. 215 Part of a valve, 5 µm wide, 25 striae, 25 interstriae and 25 fibulae in 10 µm

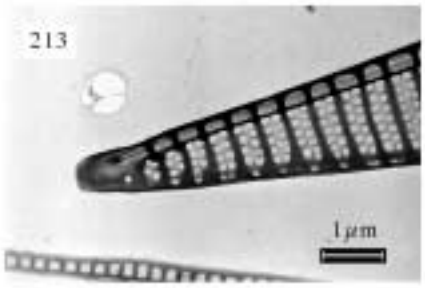
Fig. 216 Valve end

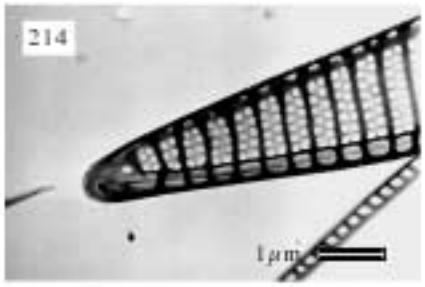
Fig. 217 Middle part of the valve, 5.4 µm wide, 4 striae per central larger interspace, 24–25 striae and 24–25 interstriae in 10 µm, 6 poroids in 1 µm

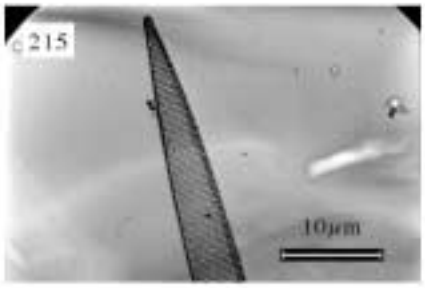
Fig. 218 South west coast of Ireland, St. 1801 (51˚ 27'N; 10˚ 20'W), July 20th 1996, close up of the valve face and distal mantle

Fig. 219 South coast of Ireland, St. 2208 (51˚ 20.04'N, 08˚ 25.00' W), October 11th 1997, part of a girdle band

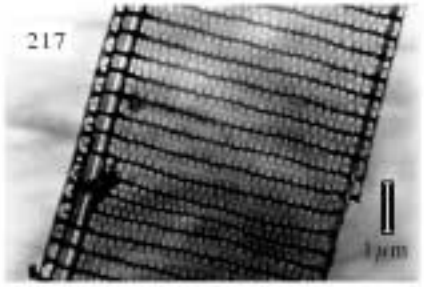

212
1μm

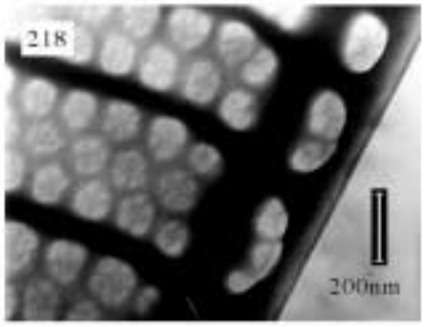

213
1μm

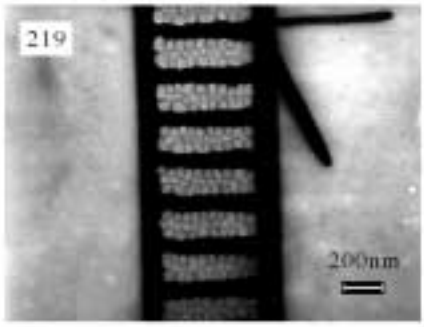

214
1μm


215
10μm


216
1μm


217
1μm

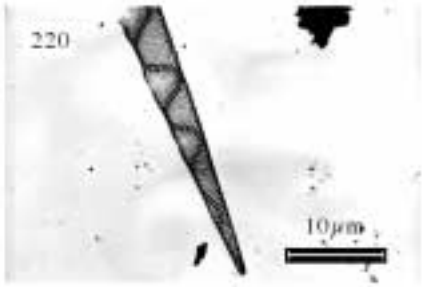

218
200nm

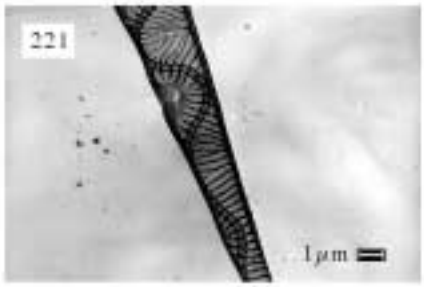

219
200nm

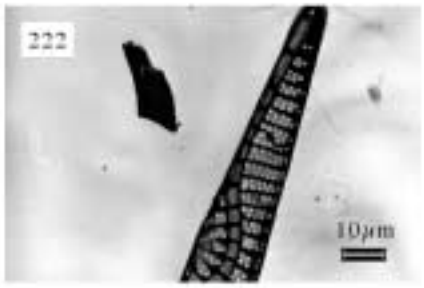
Figs. 199–226 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

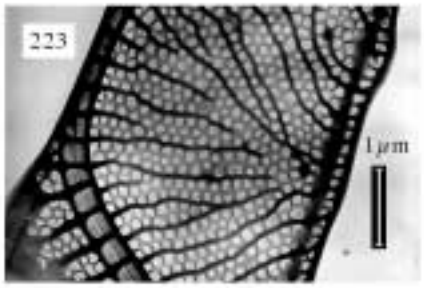
Figs. 220–226 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997, clonal culture, strain 2011, aberrant morphological structures

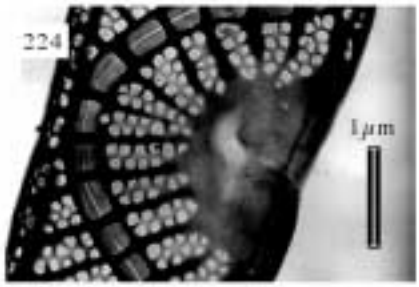
Fig. 227 West coast of Ireland, off Eris Head, st 2011 (54˚ 29.0'N 10˚ 39.9'W) May 3rd 1997, clonal culture, strain 2011, part of a girdle band

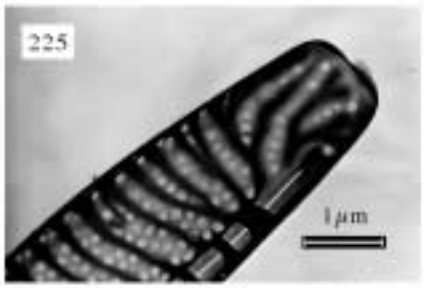

220
10μm

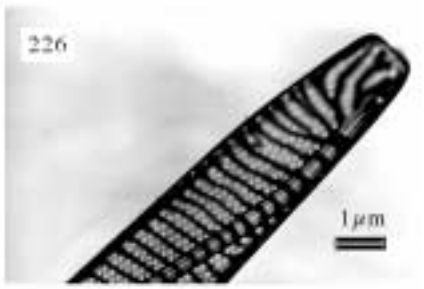

221
1μm

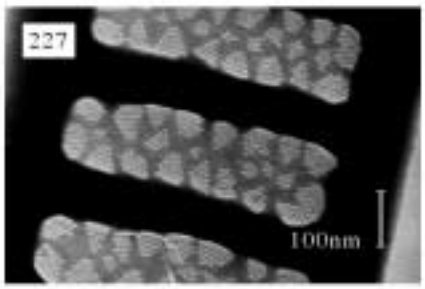

222
10μm


223
1μm


224
1μm


225
1μm


226
1μm


227
100nm

Pseudo-nitzschia cf. *subpacifica*

(Figures 228 – 236)

Figs. 228–236 SCANNING ELECTRON MICROGRAPHS (SEM)

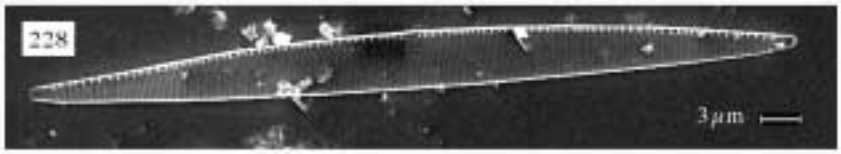
Figs. 228–231 South coast of Ireland, St. 1808 (51˚ 27.00'N; 09˚ 33.00'W), July 21st 1996

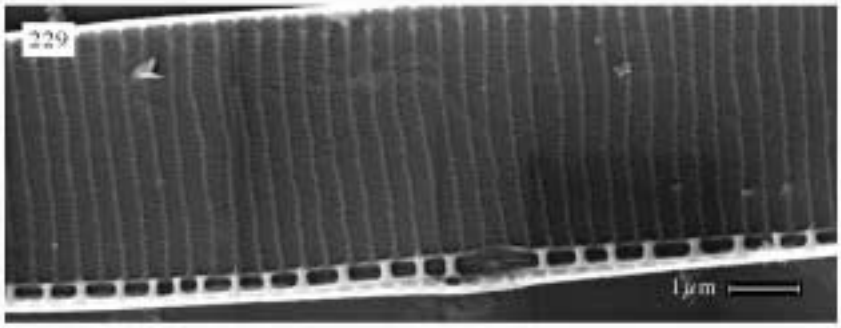
Fig. 228 Whole valve , 59.6 µm long, 4.4 µm wide, 31striae, 31 interstriae and 19 fibulae in 10 µm

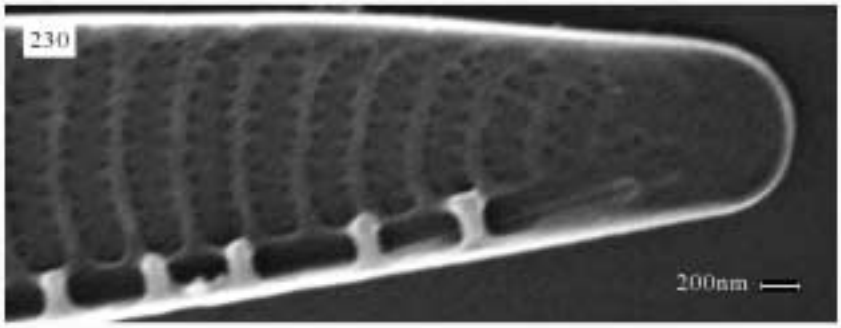
Fig. 229 Middle part of the valve, 9 poroids in 1 µm, 3 striae per central larger interspace

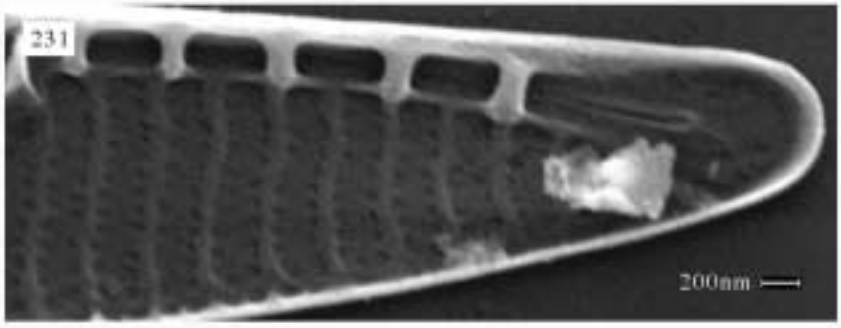
Fig. 230 Valve end, 9 poroids in 1 µm

Fig. 231 Other end, 9 poroids in 1 µm


228
3μm


229
1μm


230
200nm


231
200nm

Figs. 228–236 SCANNING ELECTRON MICROGRAPHS (SEM)

Cont'd.

Figs. 232–236 South coast of Ireland, St. 2205 (51˚ 35.02'N, 08˚ 24.84'W), October 11th 1997

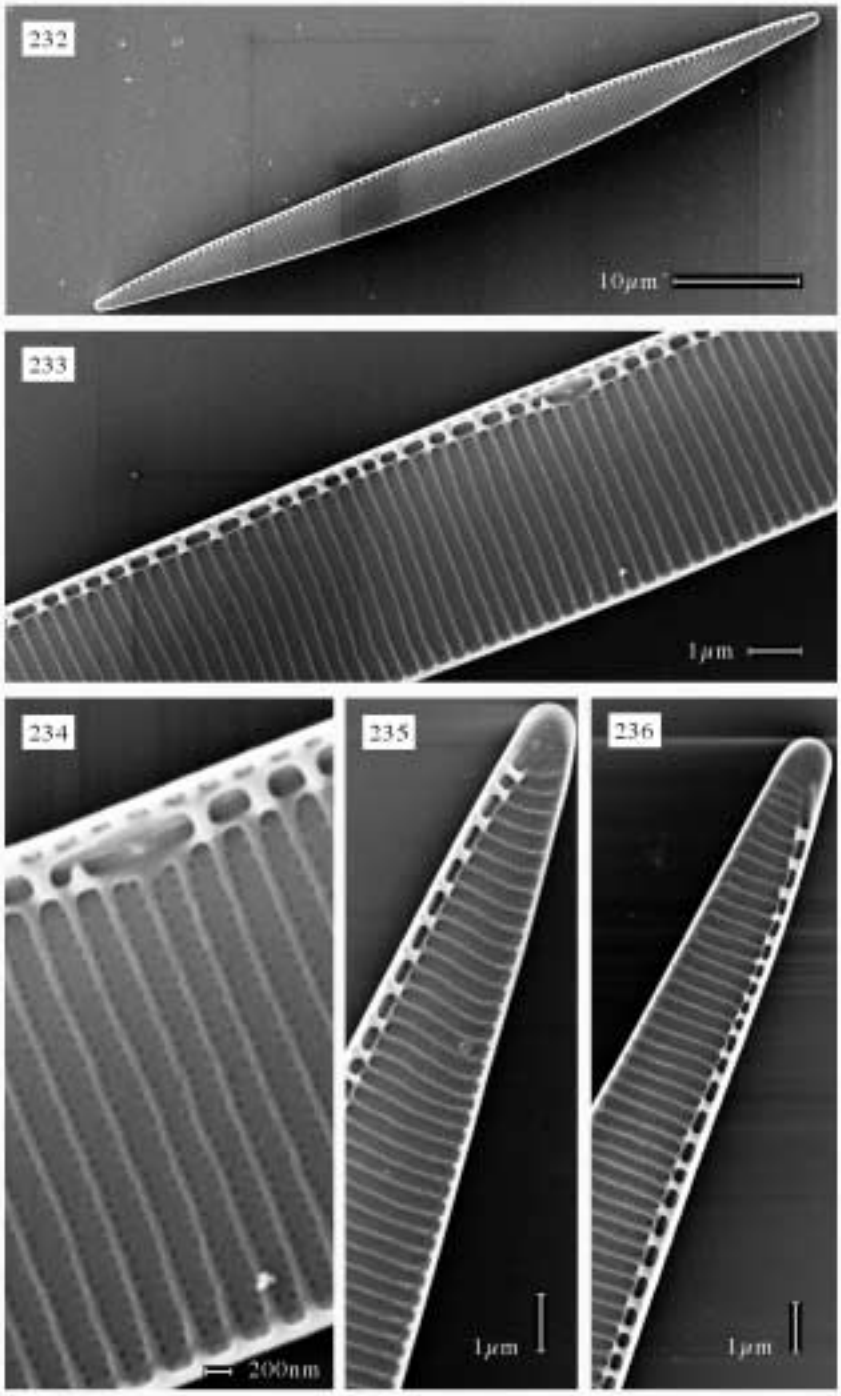
Fig. 232 Whole valve , 62.6 µm long, 4.6 µm wide, 30striae, 30 interstriae and 19 fibulae in 10 µm

Fig. 233 Middle part of the valve, 9 poroids in 1 µm, 3 striae per central larger interspace

Fig. 234 Close-up of the valve face

Fig. 235 Valve end, 9 poroids in 1 µm

Fig. 236 Other end, 9 poroids in 1 µm


232
10μm
233
1μm
234
200nm
235
1μm
236
1μm

Pseudo-nitzschia delicatissima

(Figures 237 – 334)

Figs. 237–247 LIGHT MICROGRAPHS (LM)

Figs. 238–241 Scale bar not available

Fig. 237 West coast of Ireland, St. Inner Galway Bay (53˚ 10.57' N, 09˚ 12.07'W), May 8th 1996, girdle view of vegetative cells in chain formation, overlap ~1/9 of total cell length, ~49 µm long, 2.2 µm wide (water mount, phase contrast)

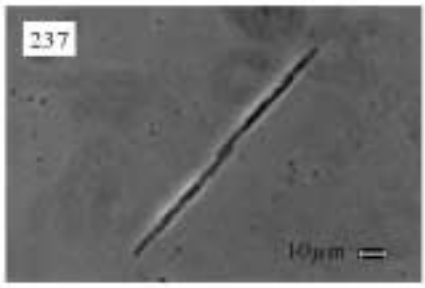
Fig. 238–241 South west coast of Ireland, St. 1909 (51˚ 37.00' N, 09˚ 46.00'W) September 6th 1996, culture strain 1909 (water mounts, phase contrast)

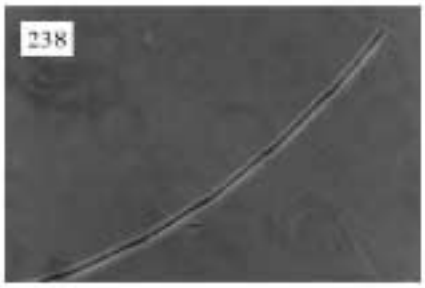
Fig. 238 Girdle view of vegetative cells forming a spiral-like chain : a common observation in *Pseudo-nitzschia* cultures

Fig. 239 Dividing cells in girdle view

Fig. 240 Vegetative cells in girdle view

Fig. 241 Culture in stationary phase, this type of clumping of cells is often observed in *Pseudo-nitzschia* cultures


237
10μm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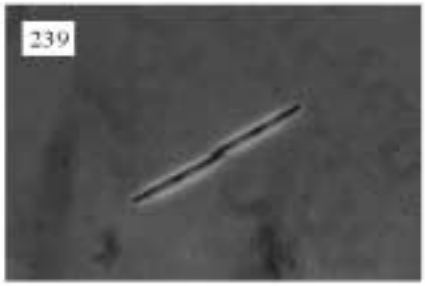
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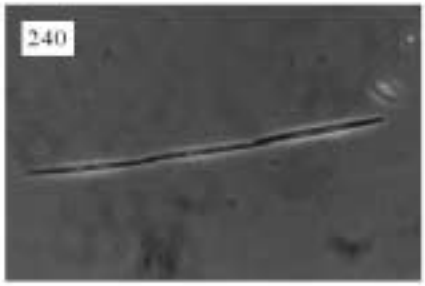
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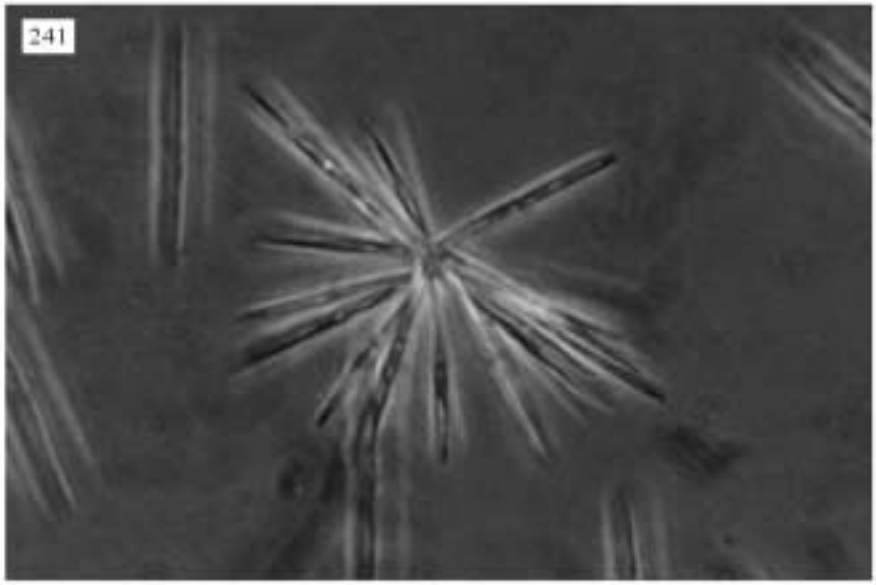

241

Figs. 237–247 Cont'd. LIGHT MICROGRAPHS (LM)

Figs. 242–244 South coast of Ireland, continental shelf off Cork, St. 1924 (51º 23.0'N 08º 15.9'W), September 7th 1996, culture strain 1924_6

Fig. 242 Vegetative cell in valve view, 28.6 µm long, 2.2 µm wide (water mount, oil immersion)

Fig. 243 Vegetative cell in valve view, 28.5 µm long, 2.89 µm wide (water mount, oil immersion)

Fig. 244 Acid cleaned valve in valve view mounted in Naphrax, 21 µm long, 1.9 µm wide

Figs. 245–246 South coast of Ireland, St. 1919 (51º 11.00'N 09º 05.00'W), September 7th 1996, culture strain 1919

Fig. 245 Acid cleaned valve, 34.7 µm long, 1.6 µm wide (Naphrax mount , oil immersion)

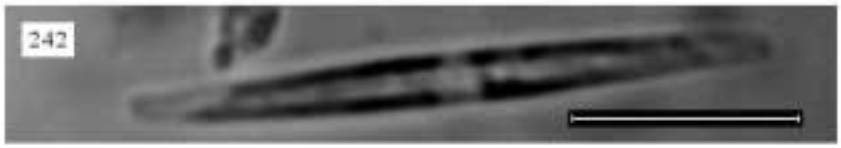
Fig. 246 Acid cleaned valve in valve view, 34.7 µm long, 1.6 µm wide (water mount, phase contrast)

Fig. 247 South coast of Ireland, St. 1917 (51º 10.00'N; 09º 24.00'W), September 7th 1996, culture strain 1917, 29 µm long, 1.7 µm wide (Naphrax mount, oil immersion)

Figs. 248–249 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

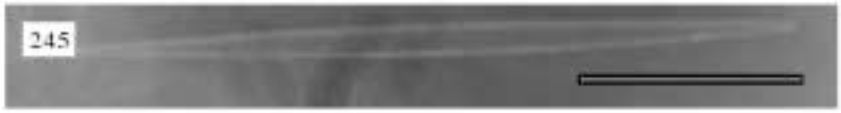
Fig. 248 South coast of Ireland, St. 1917 (51º 10.00'N; 09º 24.00'W), September 7th 1996, culture strain 1917. Acid cleaned valve, 31.4 µm long, 2.2 µm wide, 43 striae and 27 fibulae in 10 µm

Fig. 249 South coast of Ireland, St. 1913 (51º 11.00'N 09º 05.00'W), September 7th 1996, culture, strain 1913. Acid cleaned valve, 15.8 µm long, 2 µm wide, 44 striae and 28 fibulae in 10 µm, 3 striae per central larger interspace


242


243


244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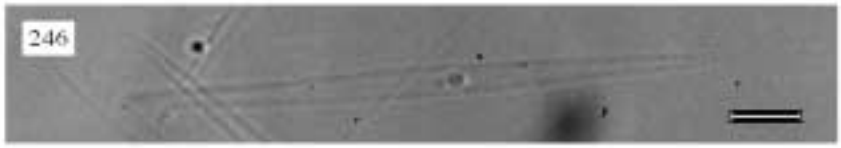
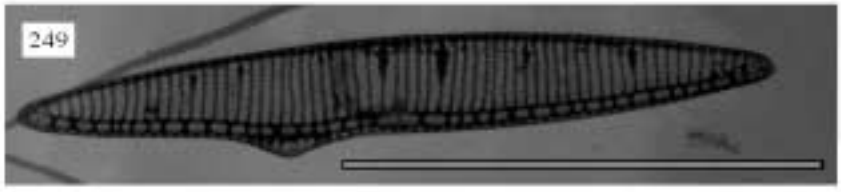

246


247


248


249

Figs. 250–261 SCANNING ELECTRON MICROGRAPHS (SEM)

Figs. 250–253 South west coast of Ireland, St. 1801 (51˚ 27'N; 10˚ 20'W), July 20th 1996

Fig. 250 Whole valve, 28.6 µm long, 1.8µm wide, 40striae, 40 interstriae and 26 fibulae in 10 µm

Fig. 251 Middle part of the valve, 1.8 µm wide, 9 poroids in 1 µm

Fig. 252 Valve end

Fig. 253 Other end

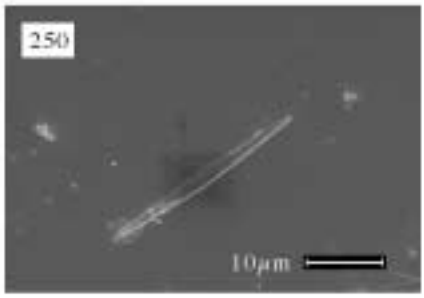
Figs. 254–256 South west coast of Ireland, St. 1807 (51˚ 26.10'N; 09˚ 54.9'W), July 21st 1996

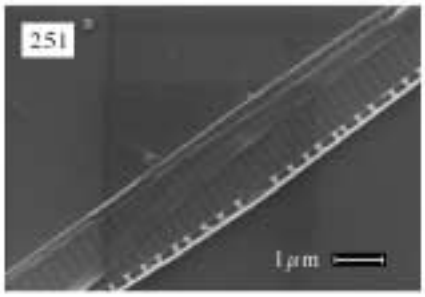
Fig. 254 Whole valve, 45.9 µm long, 1.1µm wide, 39 striae, 39 interstriae and 23 fibulae in 10 µm

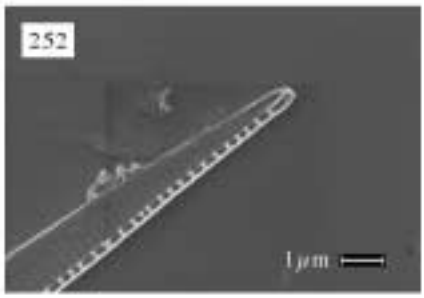
Fig. 255 Middle part of the valve, 9–13 poroids in 1 µm, 3 striae per central larger interspace

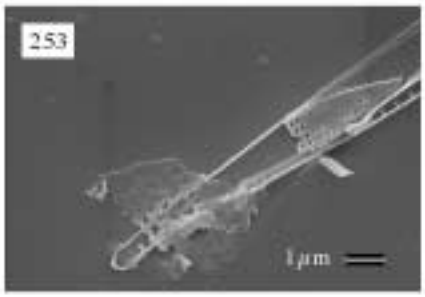
Fig. 256 Valve end

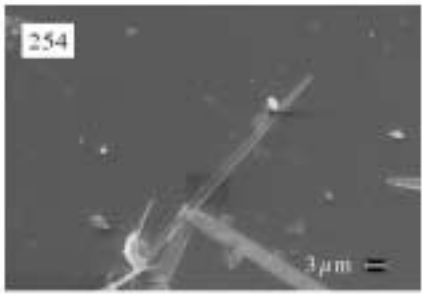
Fig. 257 West coast of Ireland, St. Inner Galway Bay (53˚ 10.57' N, 09˚ 12.07'W), May 8th 1996, 1.6 µm wide , 9 poroids in 1 µm

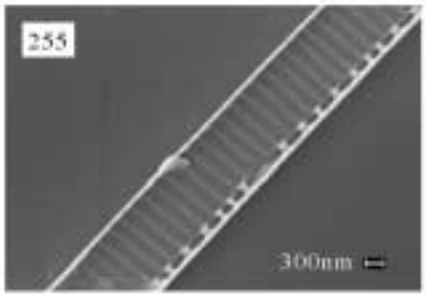

250
10μm

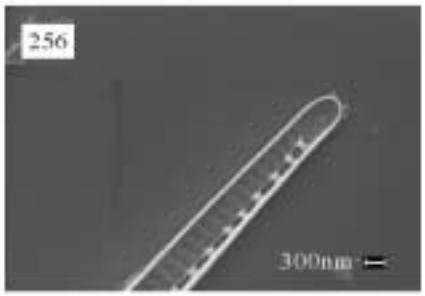

251
1μm

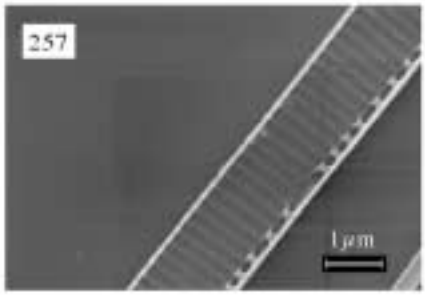

252
1μm


253
1μm


254
3μm


255
300nm


256
300nm


257
1μm

Figs. 250–261 SCANNING ELECTRON MICROGRAPHS (SEM)
Cont'd.

Figs. 258–261 South coast of Ireland, St. 1913 (51º 11.00'N 09º 05.00'W), September 7th 1996, culture, strain 1913

Fig. 258 Whole valve, 16.5 µm long, 2 µm wide, 40 striae, 40 interstriae and 26 fibulae in 10 µm

Fig. 259 Middle part of the valve, 2 µm wide, 4 striae per central larger interspace, 8 poroids in 1 µm

Fig. 260 Valve end

Fig. 261 Other end

Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 262–265 West coast of Ireland, St. Inner Galway Bay (53˚ 10.57' N, 09˚ 12.07'W), May 8th 1996

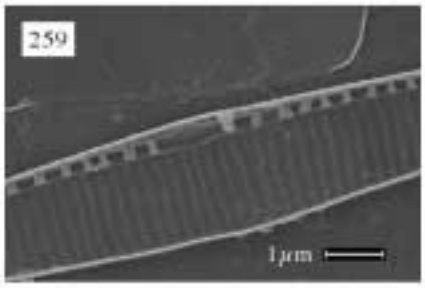
Fig. 262 Valve end

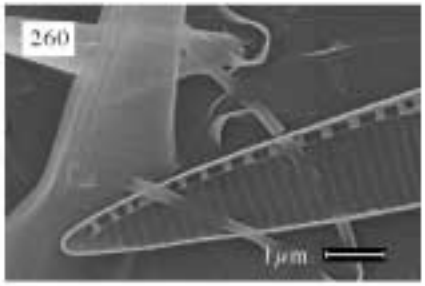
Fig. 263 Middle part of the valve, 1.3 µm wide, 9–12 poroids in 1 µm

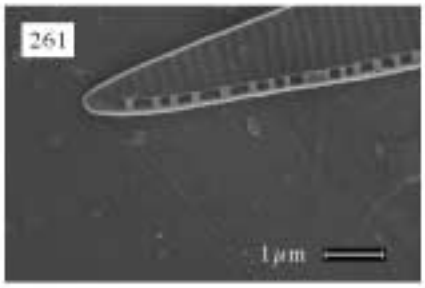
Fig. 264 Other end

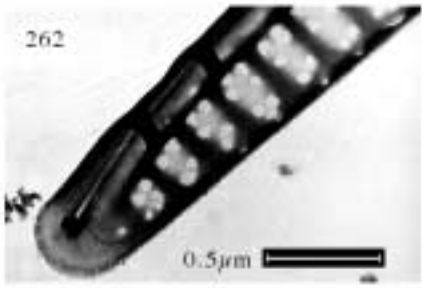
Fig. 265 Part of the valve with another partial *Pseudo-nitzschia delicatissima* valve along side, this valve shows the part of the proximal and distal mantles along with some girdle bands. Upper valve shows 40 striae, 40 interstriae and ~26 fibulae in 10 µm, Lower valve shows 41 striae, 41 interstriae and ~25 fibulae in 10 µm

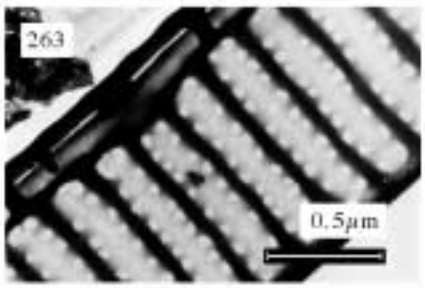

258
3μm

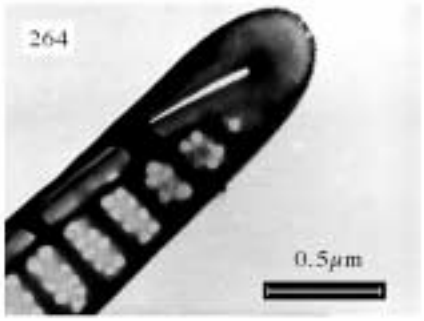

259
1μm

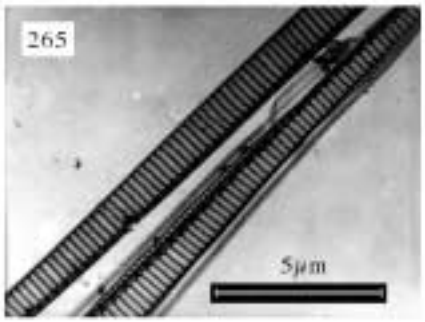

260
1μm


261
1μm


262
0.5μm


263
0.5μm


264
0.5μm


265
5μm

Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

Fig. 266 South coast of Ireland, St. 1811 (51˚ 21.00'N; 08˚ 26.20'W), July 22nd 1996, close-up of the central larger interspace with the central raphe endings, there are 3 striae to the central larger interspace

Fig. 267 South coast of Ireland, St. 1810 (51˚ 07.2'N; 09˚ 24.0'W), July 22nd 1996, close-up of the valve face striae

Figs. 268-269 South coast of Ireland, St. 1811 (51˚ 21.00'N; 08˚ 26.20'W), July 22nd 1996, valve end and close-up of valve face, 10 poroids in 1 µm

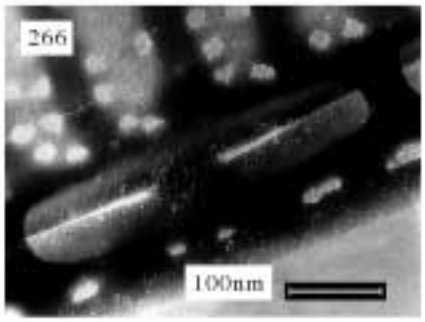
Figs. 270–271 South coast of Ireland, St. 1811 (51˚ 21.00'N; 08˚ 26.20'W), July 22nd 1996

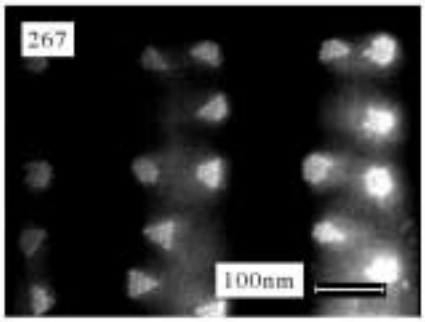
Fig. 270 Most of a valve, ~39.5 µm long, 1 µm wide, 46 striae, 46 interstriae and 25 fibulae in 10 µm,

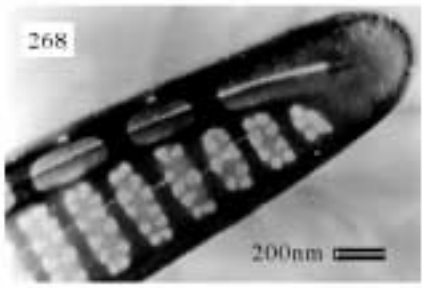
Fig. 271 Close-up of the valve face with the central larger interspace and central raphe endings 3 striae per central larger interspace, ~10 poroids in 1 µm

Fig. 272 West coast of Ireland, St. Inner Galway Bay (53˚ 10.57' N, 09˚ 12.07'W), May 8th 1996, Close-up of the valve face, 1.3 µm wide, 9–12 poroids in 1 µm, 4 striae per central larger interspace

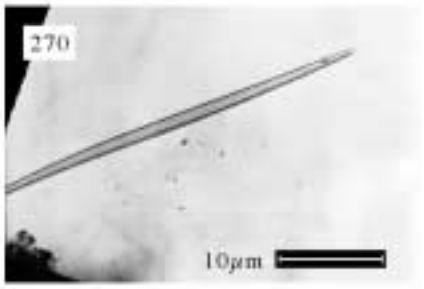
Fig. 273 South west coast of Ireland, St.1906 (51˚ 57.00'N; 10˚ 40.00'W), September 3rd 1996, close-up of the central larger interspace, the central raphe endings, the proximal mantle and the valvocopula

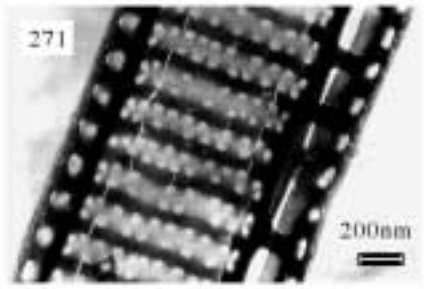

266
100nm

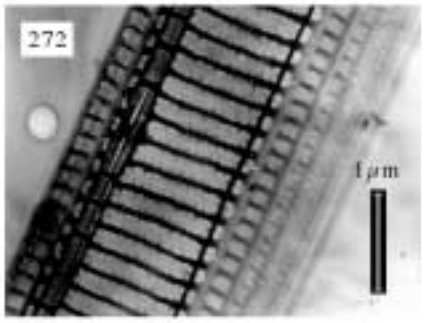

267
100nm

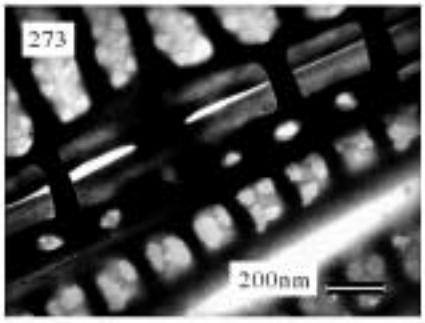

268
200nm


269
200nm


270
10μm


271
200nm


272
1μm


273
200nm

Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

Figs. 275–276 South coast of Ireland, St. 1812 (51˚ 35'N; 8˚ 28.9'W), July 22nd 1996

Fig. 274 Whole valve: 63 µm long, 1.1 µm wide, 44 striae, 44 interstriae and 27 fibulae in 10 µm

Fig. 275 Part of the valve, 1.5 µm wide, 9–10 poroids in 1 µm, 37 striae, 37 interstriae and 20 fibulae in 10 µm

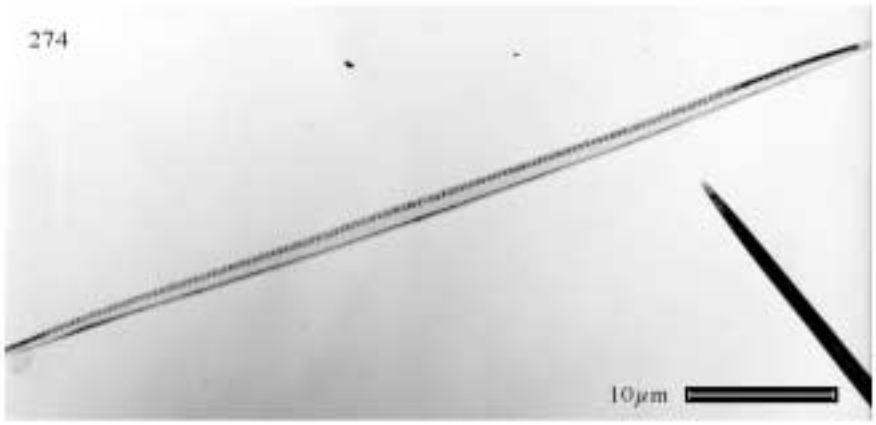
Fig. 276 Central part of the valve with 3 striae to the central larger interspace, 8–10 poroids in 1 µm

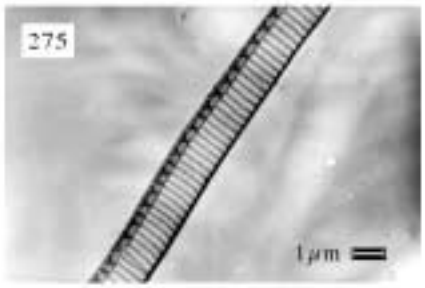
Figs. 277–279 South coast of Ireland, St. 1810 (51˚ 07.2'N; 09˚ 24.0'W), July 22nd 1996

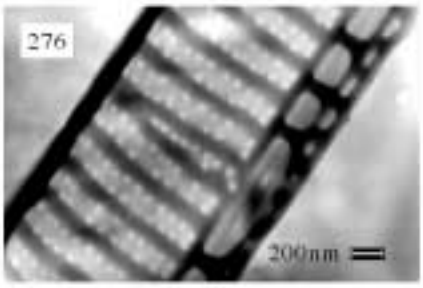
Fig. 277 Whole valve, 50 µm long, 1 µm wide, 39 striae, 39 interstriae and 23 fibulae in 10 µm

Fig. 278 Valve end

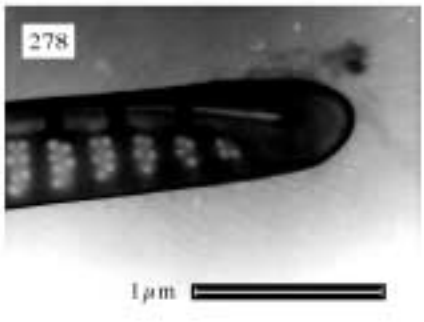
Fig. 279 Middle part of the valve showing 3.5 striae per central larger interspace, ~ 11 poroids in 1 µm

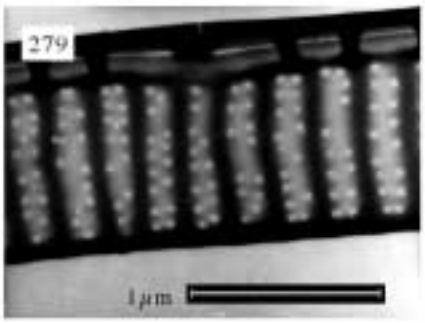

274
10μm


275
1μm


276
200nm


277
10μm

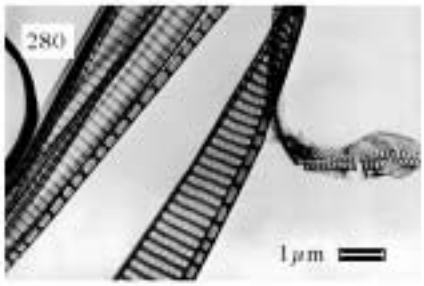

278
1μm

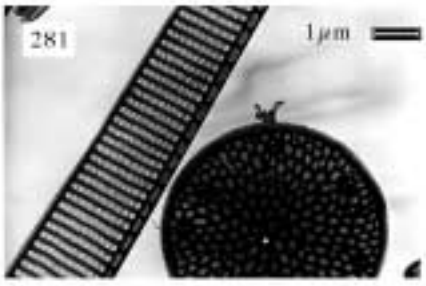

279
1μm

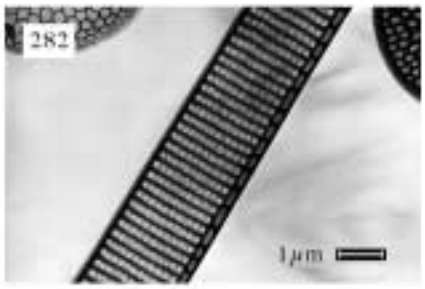
Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

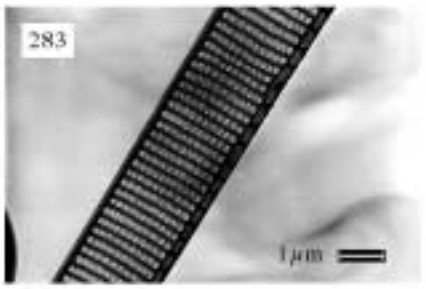
Figs. 280–290 South coast of Ireland, St. 1810 (51˚ 07.2'N; 09˚ 24.0'W), July 22nd 1996, rough culture 1810_1 ***Pseudo-nitzschia* cf. *delicatissima***. Although the poroid structure at the valve ends are similar to that seen in *Pseudo-nitzschia* delicatissima (ie. small rounded with a simple hymenate vela) if one looks at the poroid structure towards the centre of the valve there is a slight similarity in the poroid structure to that seen in *Pseudo-nitzschia pseudodelicatissima*

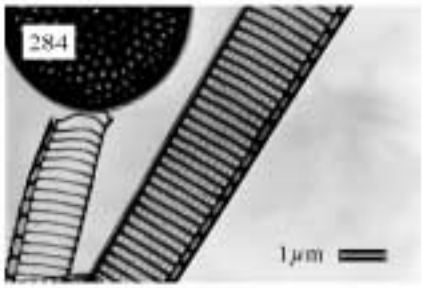
Figs. 280–287 Parts of the valve, 2.3 µm wide, 6 poroids in 1 µm, 3 striae to the central larger interspace

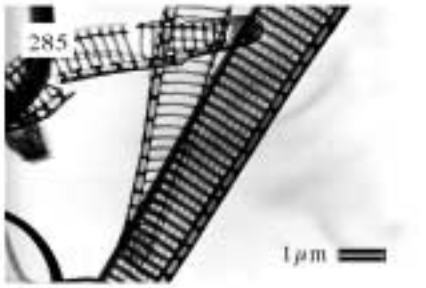

280
1μm

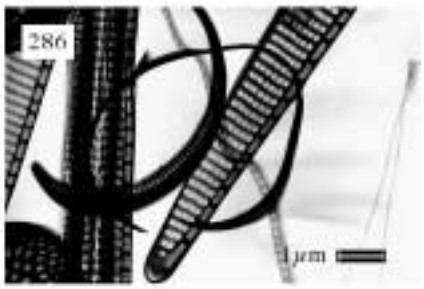

281
1μm

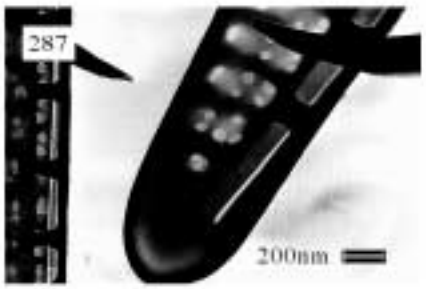

282
1μm


283
1μm


284
1μm


285
1μm


286
1μm


287
200nm

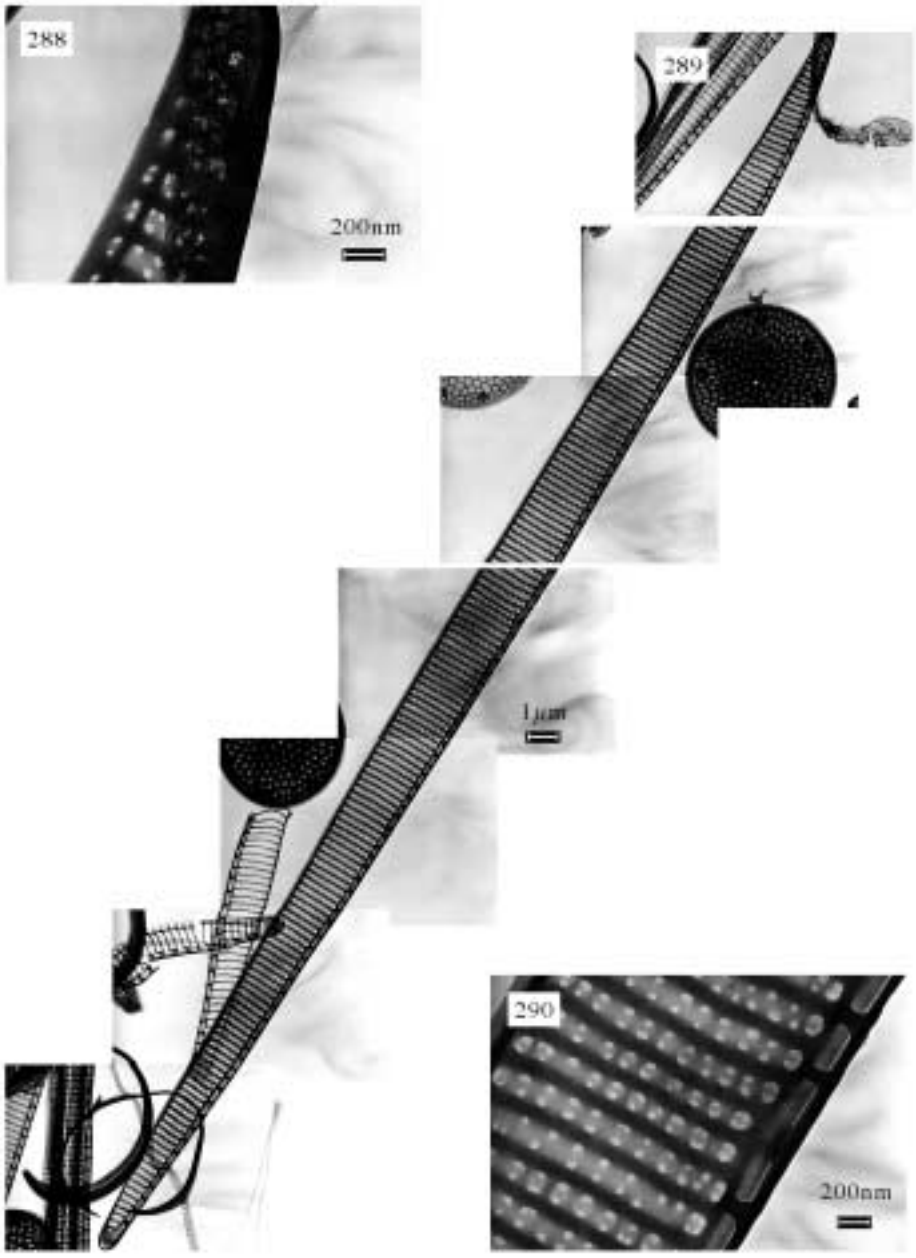
Figs. 262–334 Cont'd. TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 280–290 Cont'd. South coast of Ireland, St. 1810 (51˚ 07.2'N; 09˚ 24.0'W), July 22nd 1996, rough culture 1810_1 ***Pseudo-nitzschia* cf. *delicatissima***

Fig. 288 Vale end, detail obscured by a girdle band

Fig. 289 Mosaic of micrographs, 50 µm long, ~ 2.4 µm wide, 36 striae, 36 interstriae and 23 fibulae in 10 µm

Fig. 290 Central part of the valve, 5–6 poroids in 1 µm


288
200nm
289
1 μm
290
200nm

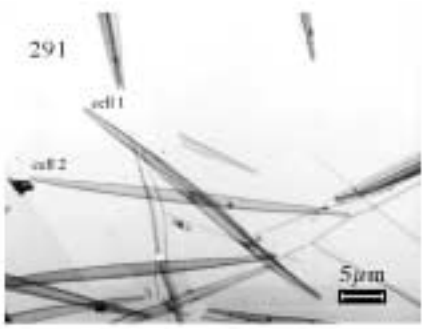
Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

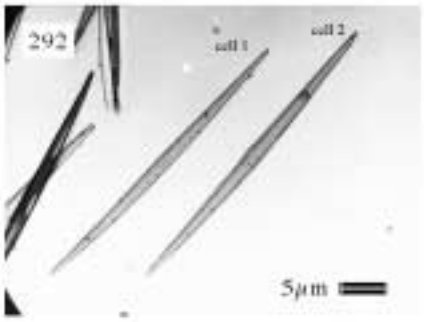
Figs. 291-301 South coast of Ireland, St. 1919 (51º 11.00'N 09º 05.00'W), September 7th 1996, culture strain 1919

Fig. 291 Whole valves
cell 1: 36.4 µm long, 1.6 µm wide, 42 striae, 42 interstriae and 29 fibulae in 10 µm
cell 2: 42.9 µm long, 1.43 µm wide, 40 striae, 40 interstriae and 27 fibulae in 10 µm

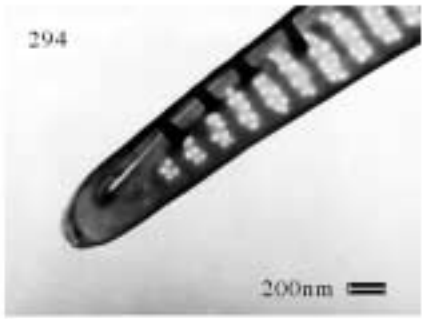
Fig. 292 Whole valves
valve 1: 41.4 µm long, 1.3 µm wide, 42–43 striae, 42–43 interstriae and 26–28 fibulae in 10 µm
valve 2: 38.2 µm long, 1.6 µm wide, 42 striae, 42 interstriae and 26 fibulae in 10 µm

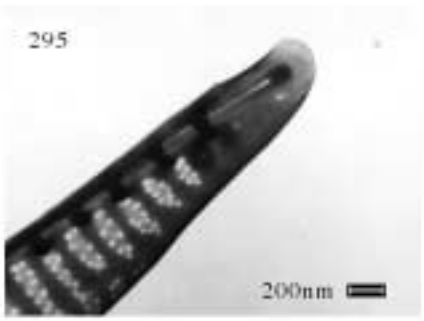
Figs. 293–297 Close-up of valve 2 from fig. 292
- Fig. 293 Valve 2
- Fig. 294 Valve end
- Fig. 295 Other end
- Fig. 296 Close-up of the central larger interspace and central raphe endings, 4 striae to the central larger interspace
- Fig. 297 Part of the valve and the proximal mantle, 10–11 poroids in 1 µm

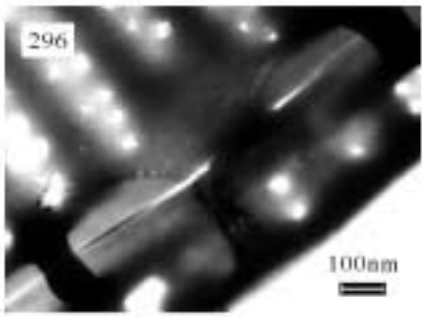

291
cell1
cell2
5μm

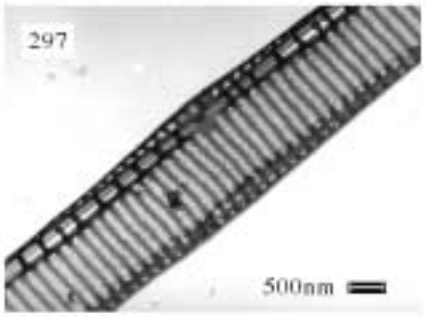

292
cell1
cell2
5μm


293
5μm


294
200nm


295
200nm


296
100nm


297
500nm

Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

Figs. 291-301 South coast of Ireland, St. 1919 (51º 11.00'N 09º 05.00'W), September 7th 1996, culture strain 1919

Figs. 298–301 Close-up of valve 1 from fig. 292

Fig. 298 Valve 1

Fig. 299 Valve end

Fig. 300 Middle part of the valve, 10–12 poroids in 1 µm, 4 striae to the central larger interspace

Fig. 301 Other end

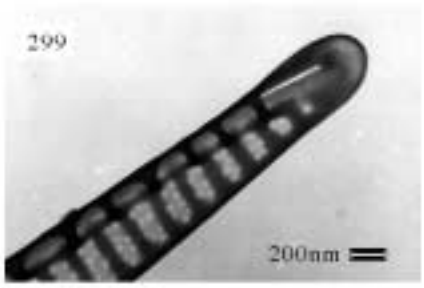
Figs. 302–304 West coast of Ireland, St. Inner Galway Bay (53˚ 10.57' N, 09˚ 12.07'W), May 8th 1996

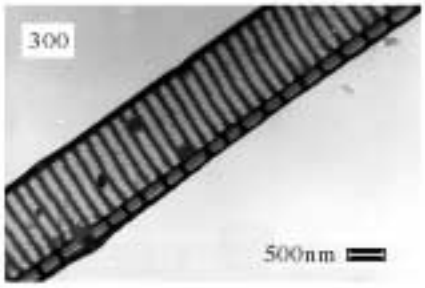
Fig. 302 Whole valve, 47.1 µm long, 1.2 µm wide, 41 striae, 42 interstriae and 26 fibulae in 10 µm

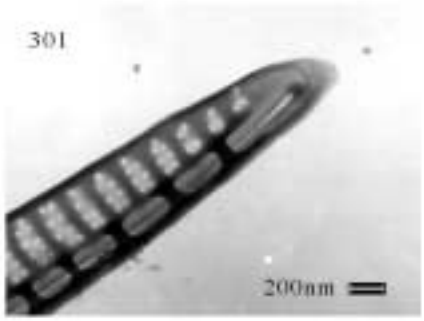
Fig. 303 Valve end

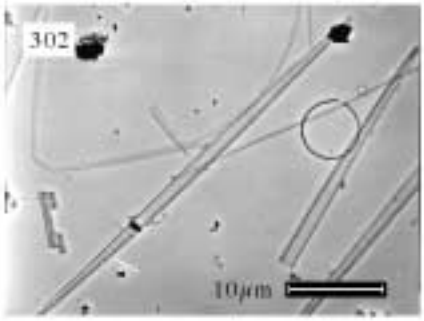
Fig. 304 Other end

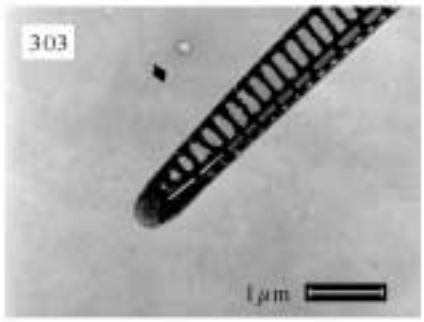

298
5μm

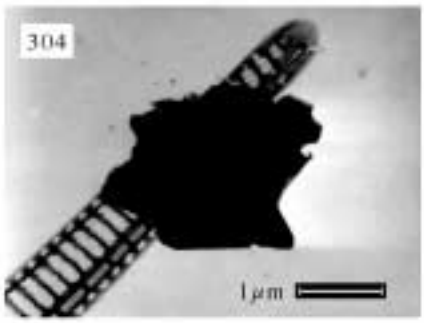

299
200nm


300
500nm


301
200nm


302
10μm


303
1μm


304
1μm

Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

Figs. 305–308 South west coast of Ireland, St. 1301 (51˚ 29.00'N; 10˚ 16.00'W), August 15th 1993

Fig. 305 Whole valve, 31.9 µm long, 1.9 µm wide, 45 striae, 46 interstriae and 28 fibulae in 10 µm

Fig. 306 Valve end

Fig. 307 Other end

Fig. 308 Middle part of the valve, 9–10 poroids in 1 µm, 3 striae to the central larger interspace

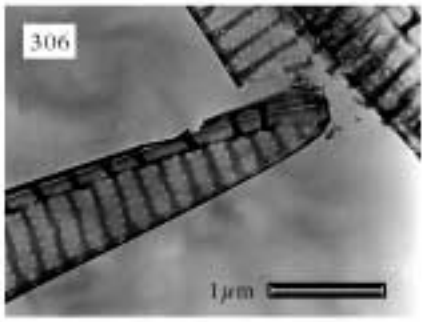
Figs. 309–311 South west coast of Ireland, St. 1301 (51˚ 29.00'N; 10˚ 16.00'W), August 15th 1993

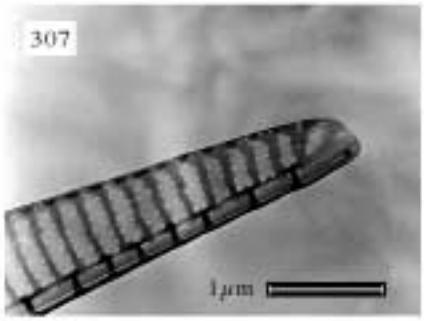
Fig. 309 Valve end

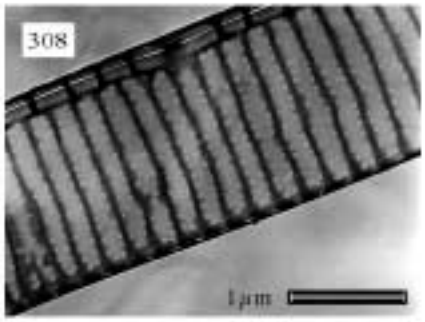
Fig. 310 Other end

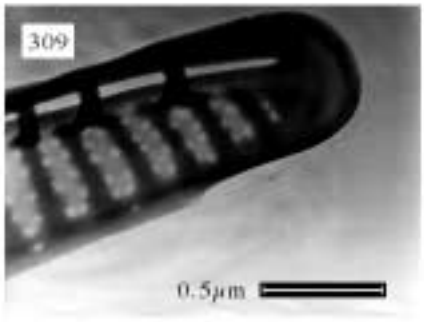
Fig. 311 9–11 poroids in 1 µm, 3.5 striae to the central larger interspace

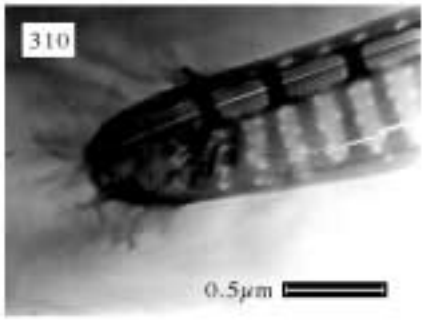

305
10μm

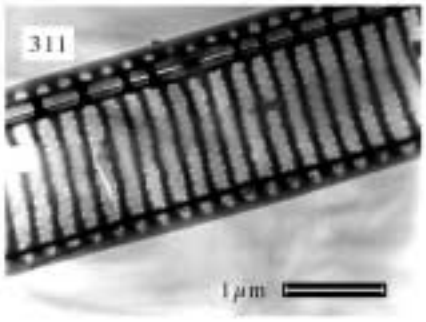

306
1μm


307
1μm


308
1μm


309
0.5μm


310
0.5μm


311
1μm

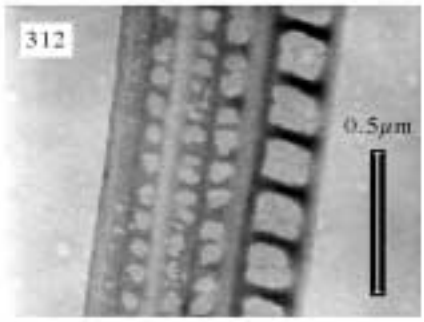
Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

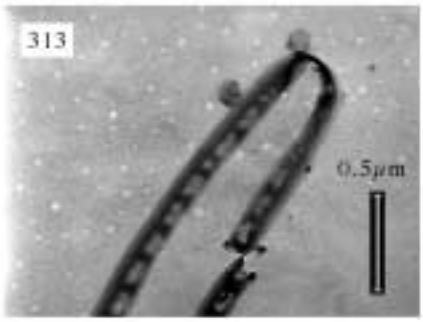
Figs. 312–327 Girdle bands

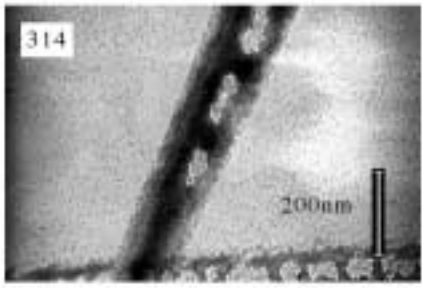
Figs. 312–317 West coast of Ireland, St. Inner Galway Bay (53˚ 10.57' N, 09˚ 12.07'W), May 8th 1996

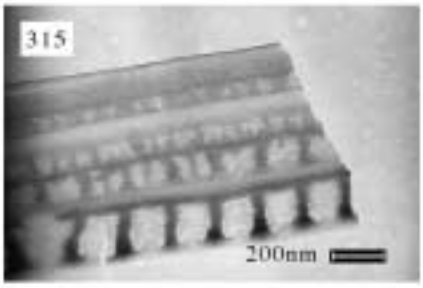
Fig. 318 South coast of Ireland, St. 1810 (51˚ 07.2'N; 09˚ 24.0'W), July 22nd 1996

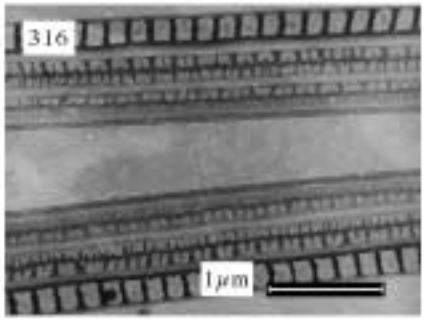
Fig. 319 West coast of Ireland, St. Inner Galway Bay (53˚ 10.57' N, 09˚ 12.07'W), May 8th 1996

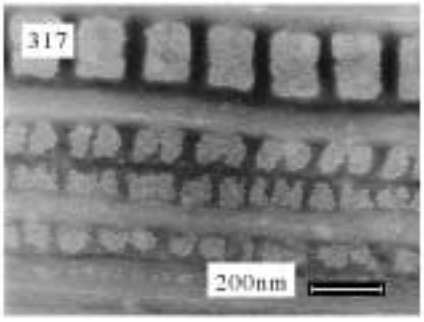

312
0.5μm

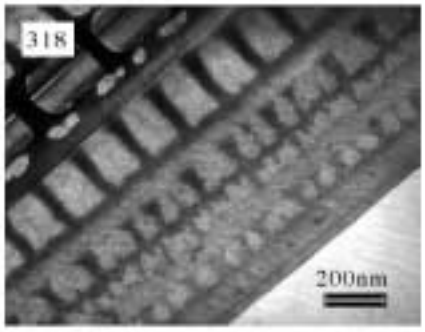

313
0.5μm

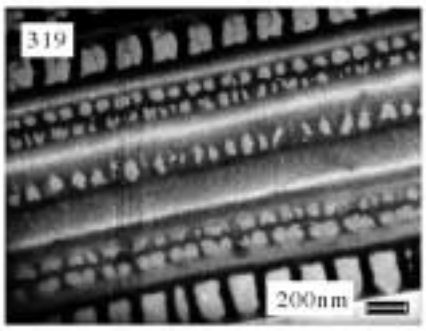

314
200nm


315
200nm


316
1μm


317
200nm


318
200nm

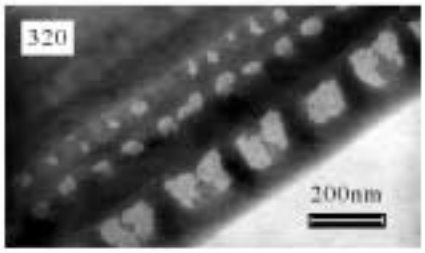

319
200nm

Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

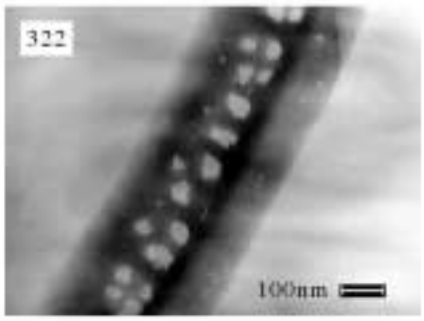
Figs. 320–324 South coast of Ireland, St. 1811 (51˚ 21.00'N; 08˚ 26.20'W), July 22nd 1996

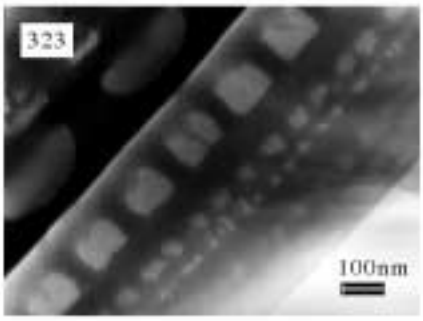
Fig. 325 South west coast of Ireland, St. 1809 (51˚ 26'N; 09˚ 24'W), July 21st 1996

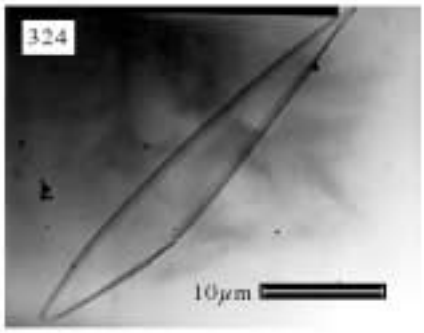
Figs. 326–327 West coast of Ireland, St. Inner Galway Bay (53˚ 10.57' N, 09˚ 12.07'W), May 8th 1996

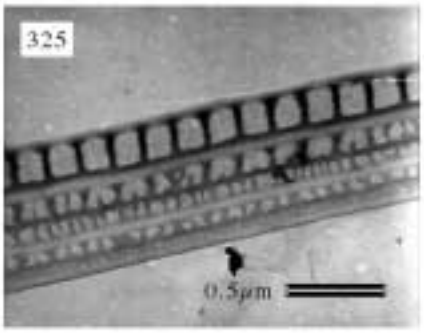

320
200nm

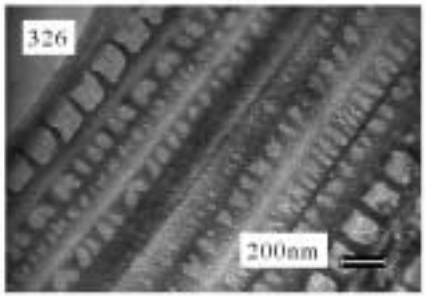

321
100nm

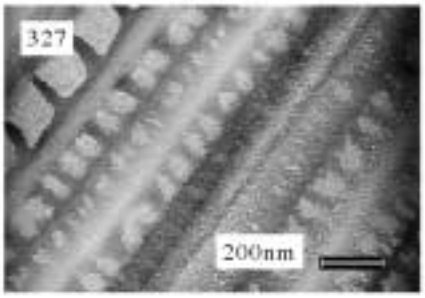

322
100nm


323
100nm


324
10μm

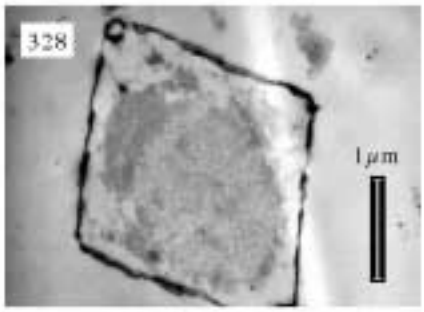

325
0.5μm

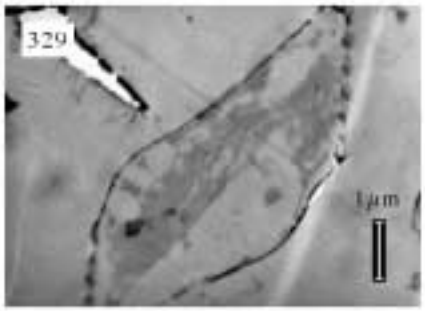

326
200nm

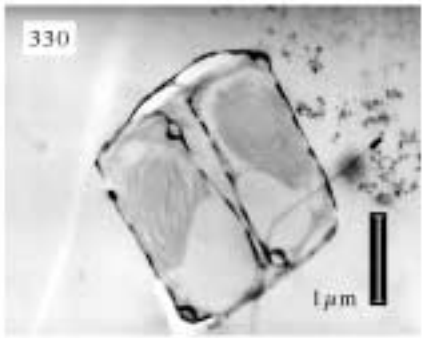

327
200nm

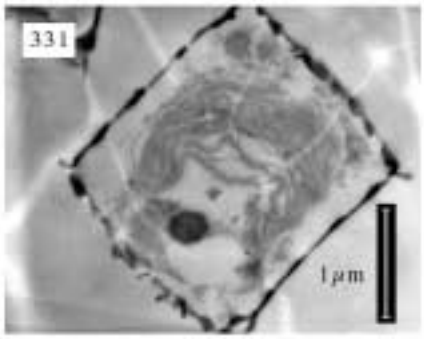
Figs. 262–334 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

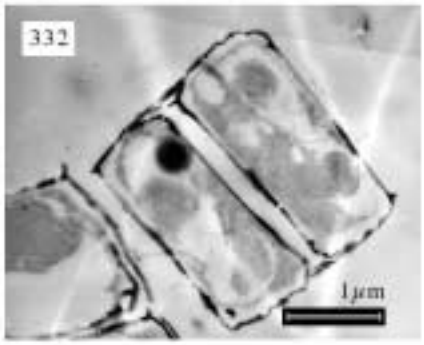
Figs. 328–333 South coast of Ireland, continental shelf off Cork, St. 1924 (51º 23.0'N 08º 15.9'W), September 7th 1996, culture strain 19246, cross section of vegetative cells

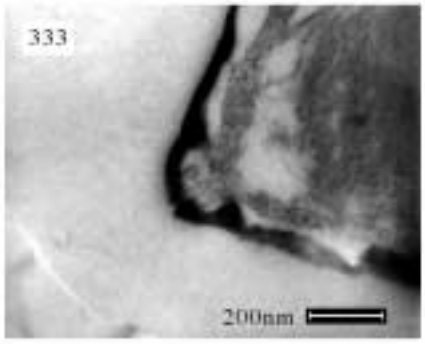

328
1 μm


329
1 μm


330
1 μm


331
1 μm


332
1 μm


333
200nm

Figs. 262–334 Cont'd. TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Fig. 334 South coast of Ireland, continental shelf off Cork, St. 1924 (51º 23.0'N 08º 15.9'W), September 7th 1996, culture strain 19246, cross section of vegetative cells


334
5μm

Pseudo-nitzschia pseudodelicatissima

(Figures 335 –361)

Figs. 335–351 SCANNING ELECTRON MICROGRAPHS (SEM)

Figs. 335–240 South west coast of Ireland, St. 1807 (51˚ 26.10'N; 09˚ 54.9'W), July 21st 1996

Fig. 335 Whole valve, 51.1 µm long, 1.5 µm wide, 39 striae, 39 interstriae and 21 fibulae in 10 µm

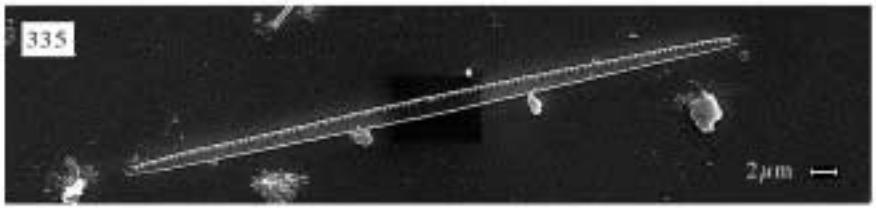
Fig. 336 Middle part of the valve, 4–6 poroids in 1 µm, 4.5 striae to the central larger interspace

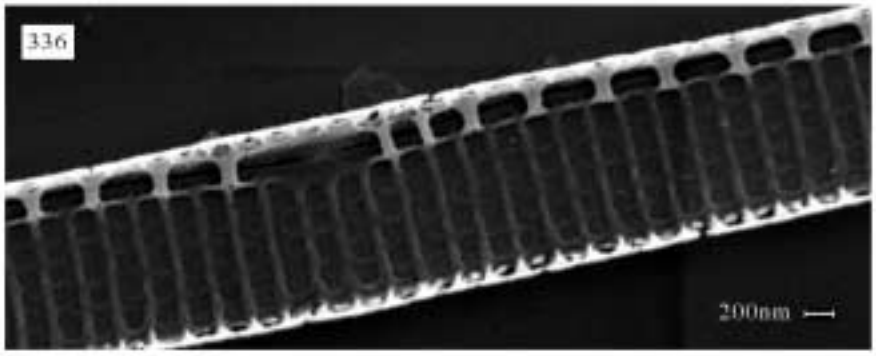
Fig. 337 Valve end

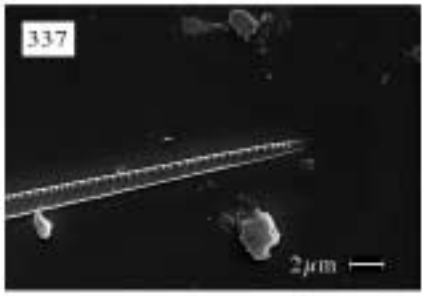
Fig. 338 Close-up of the valve end

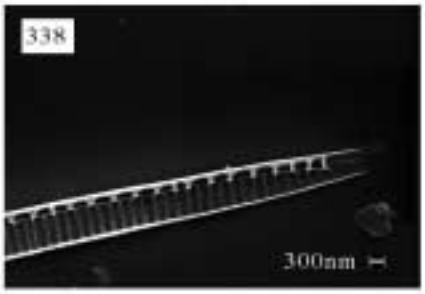
Fig. 339 Other end

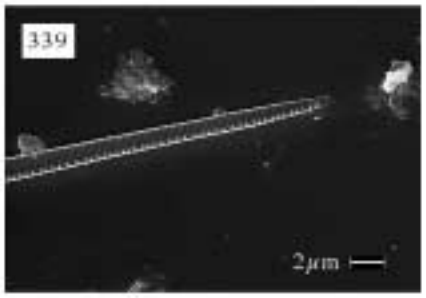
Fig. 340 Close-up of the other polar end of the valve

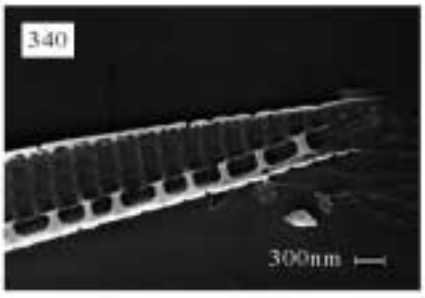

335
2μm


336
200nm


337
2μm


338
300nm


339
2μm


340
300nm

Figs. 335–351 SCANNING ELECTRON MICROGRAPHS (SEM)
Cont'd.

Figs. 341–346 South west coast of Ireland, St. 1807 (51˚ 26.10'N; 09˚ 54.9'W), July 21st 1996

Fig. 341 Whole valve, 60 µm long, 1.7 µm wide, 37 striae, 37 interstriae and 22 fibulae in 10 µm

Fig. 342 Middle part of the valve, 5–6 poroids in 1 µm

Fig. 343 Close-up of the central larger interspace with central raphe endings, corresponding to 3.5 striae

Fig. 344 Close-up of the valve face striae, roundish to squarish poroids containing sections of hymenate velae

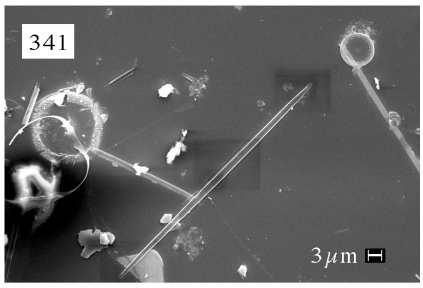
Fig. 345 Valve end

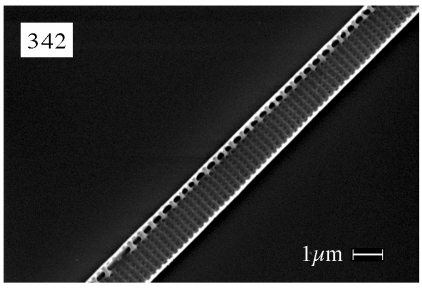
Fig. 346 Other end

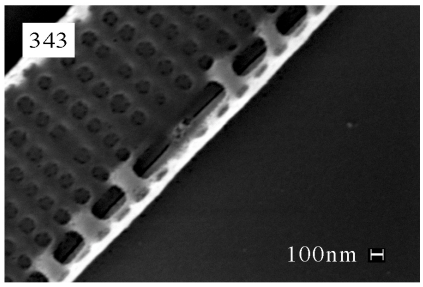
Figs. 347–348 South west coast of Ireland, St. 1807 (51˚ 26.10'N; 09˚ 54.9'W), July 21st 1996

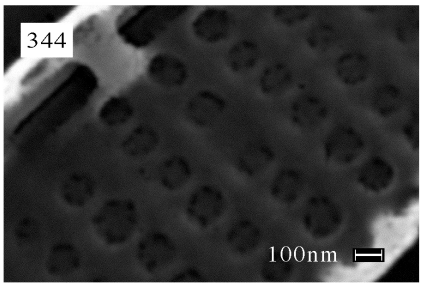
Fig. 347 Centre of tf the valve, 5 poroids in 1 µm, 5 striae to the central larger interspace

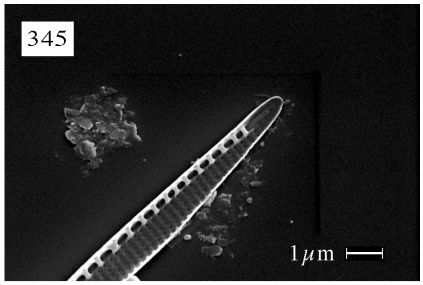
Fig. 348 Valve end

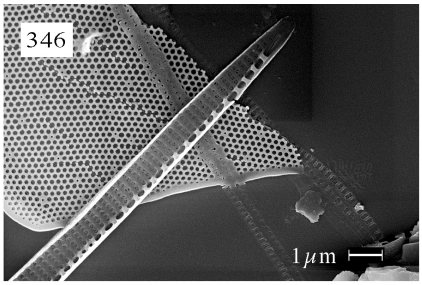

341
3μm

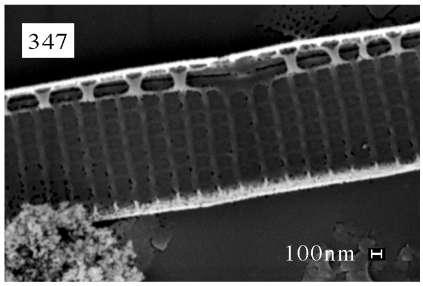

342
1μm

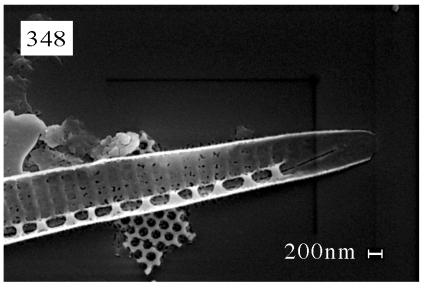

343
100nm


344
100nm


345
1μm


346
1μm


347
100nm


348
200nm

Figs. 335–351 SCANNING ELECTRON MICROGRAPHS (SEM)
Cont'd.

Fig. 349 South west coast of Ireland, St.1906 (51˚ 57.00'N; 10˚ 40.00'W), September 3rd 1996, valve end

Fig. 350 South west coast of Ireland, St.1906 (51˚ 57.00'N; 10˚ 40.00'W), September 3rd 1996, middle part of a valve showing 5 striae corresponding to the central larger interspace, ~ 6 poroids in 1 µm

Figs. 351–352 South coast of Ireland, St. 1808 (51˚ 27.00'N; 09˚ 33.00'W), July 21st 1996

Fig. 351 Whole valve, 73.5 µm long, 2 µm wide, 39 striae, 39 interstriae and 23 fibulae in 10 µm

Fig. 352 Close-up of a valve showing the proximal mantle and poroid structure

Figs. 353–361 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

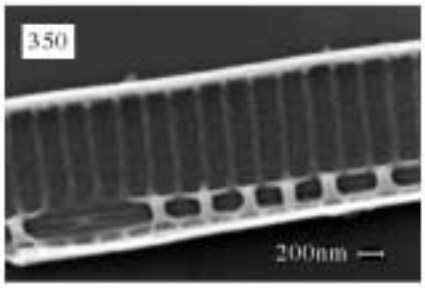
Figs. 353–354 South coast of Ireland, St. 1812 (51˚ 35'N; 8˚ 28.9'W), July 22nd 1996, rough culture 1812

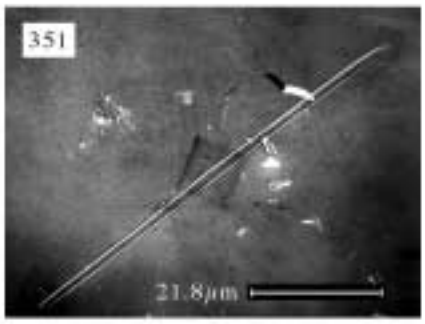
Fig. 353 Valve end

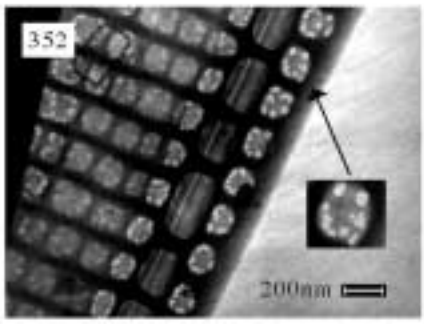
Fig. 354 Part of the valve showing the proximal and distal mantles, 5 poroids in 1 µm

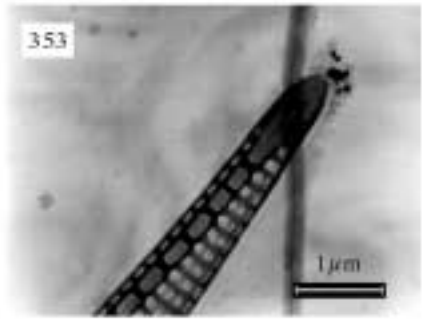
Fig. 355 South coast of Ireland, St. 1812 (51˚ 35'N; 8˚ 28.9'W), July 22nd 1996, rough culture 1812, part of a valve, ~54 µm long, 1.4 µm wide, 44 striae, 45 interstriae and 25 fibulae in 10 µm

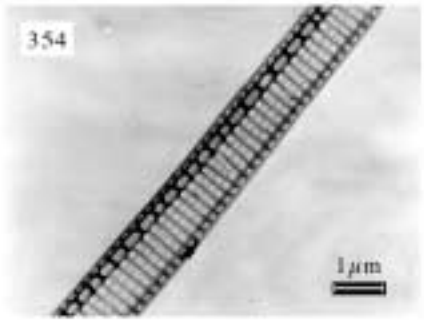

349
1μm


350
200nm


351
21.8μm


352
200nm


353
1μm


354
1μm


355
10μm

Figs. 353–361 TRANSMISSION ELECTRON MICROGRAPHS (TEM)
Cont'd.

Fig. 356 South west coast of Ireland, St. 1807 (51˚ 26.10'N; 09˚ 54.9'W), July 21st 1996, part of the valve showing the stria structure, 3 striae to the central larger interspace, 1.2 µm wide, 6–7 poroids in 1 µm

Fig. 357 South west coast of Ireland, St. 1311 (51˚ 20.1'N, 10˚ 05.0'W), August 17th 1993, valve end of *Pseudo-nitzschia* cf. *pseudodelicatissima*, 7–8 poroids in 1 µm

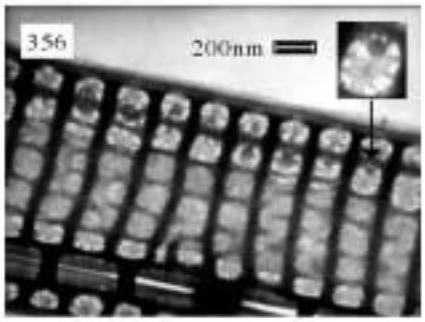
Figs. 358–361 South coast of Ireland, St. 1812 (51˚ 35'N; 8˚ 28.9'W), July 22nd 1996, rough culture 1812

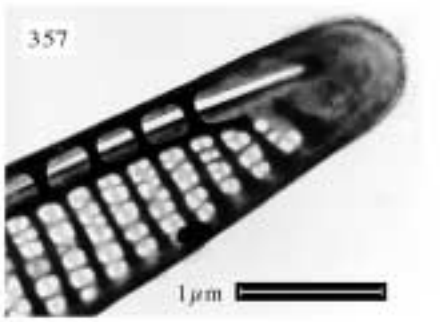
Fig. 358 Part of a valve, 1.4 µm wide, ~6 poroids in 1 µm, 4 striae to the central larger interspace

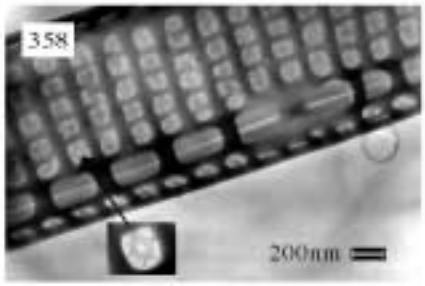
Fig. 359 Part of a valve end

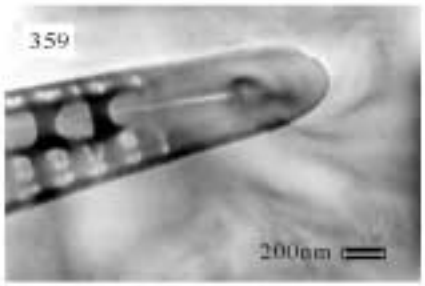
Fig. 360 Middle part of a valve showing the proximal and distal mantles, 3.5 striae to the central larger interspace, 1.0 µm wide, ~44 striae and ~45 interstriae 10 µm

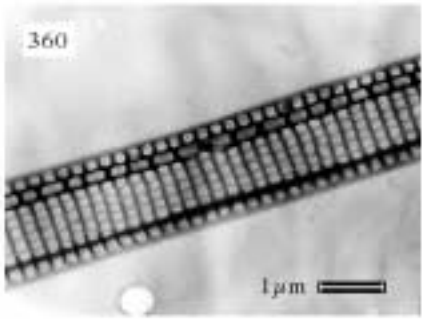
Fig. 361 Whole valve, 66 µm long, 1.3 µm wide, 41 striae, 41 interstriae and 26 fibulae in 10 µm

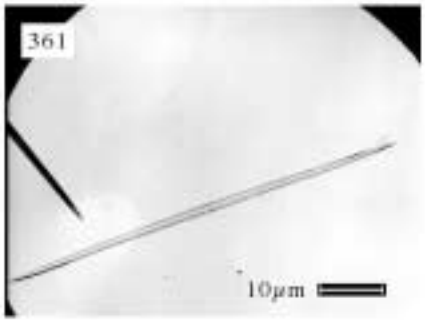

356
200nm


357
1μm


358
200nm


359
200nm


360
1μm


361
10μm

Pseudo-nitzschia cf. *multiseries*

(Figures 362 –377)

Figs. 362–364 SCANNING ELECTRON MICROGRAPHS (SEM)

Figs. 362–363 Specimen from the southwest coast of Ireland, station: 1807 (51˚ 26.10'N; 09˚ 54.9'W), July 21st 1996.

Fig. 362 Part of a valve showing the striae structure, 4.1 µm wide, ~10 striae, ~10 interstriae and ~10 fibulae in 10 µm, 5–7 poroids in 1 µm.

Fig. 363 Valve end.

Fig. 364 Specimen from the south coast of Ireland, station: 1808 (51˚ 27.00'N; 09˚ 33.00'W), July 21st 1996, part of a valve, ~3 µm wide, ~14 striae, ~14 interstriae and ~14 fibulae in 10 µm, 6 poroids in 1 µm.

Figs. 365–377 TRANSMISSION ELECTRON MICROSCOPY (TEM)

Figs. 365–366 Specimen from the southwest coast of Ireland, station: 1314 (51˚ 20.1'N, 10˚ 05.0'W), August 17th 1993.

Fig. 365 Valve end.

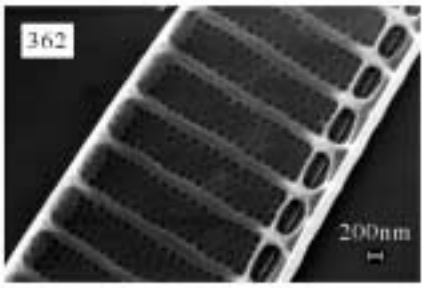
Fig. 366 Middle part of the valve, ~2.9 µm wide, ~12 striae, ~12 interstriae and ~12 fibulae in 10 µm, 6 poroids in 1 µm.

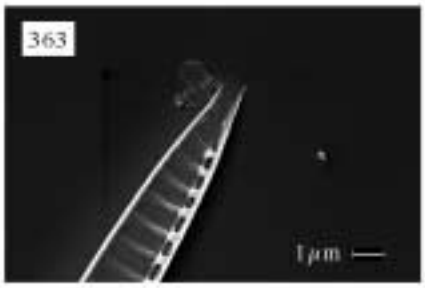
Figs. 367–369 Specimen from the south coast of Ireland, station: 1815 (51˚ 50.2'N; 08˚ 15.8'W), July 23rd 1996.

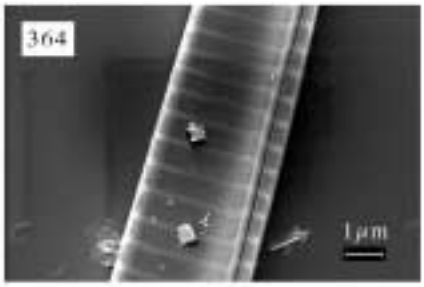
Fig. 367 Close-up of the valve face, 7 poroids in 1 µm.

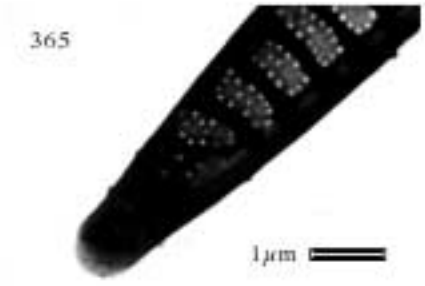
Fig. 368 Part of the valve, ~ 3 µm wide, 11 striae, 11 interstriae and 12 fibulae in 10 µm.

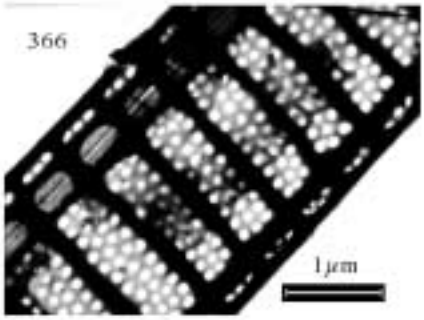
Fig. 369 Valve end.

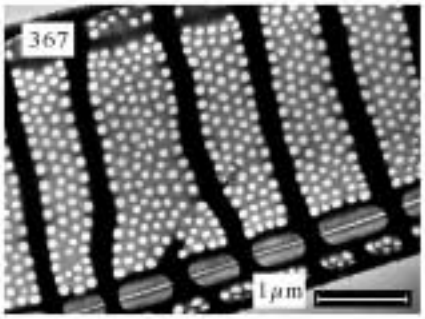

362
200nm

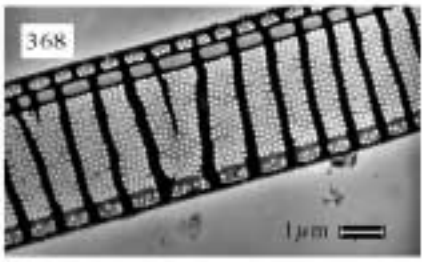

363
1μm

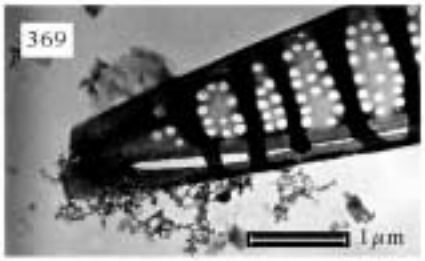

364
1μm


365
1μm


366
1μm


367
1μm


368
1μm

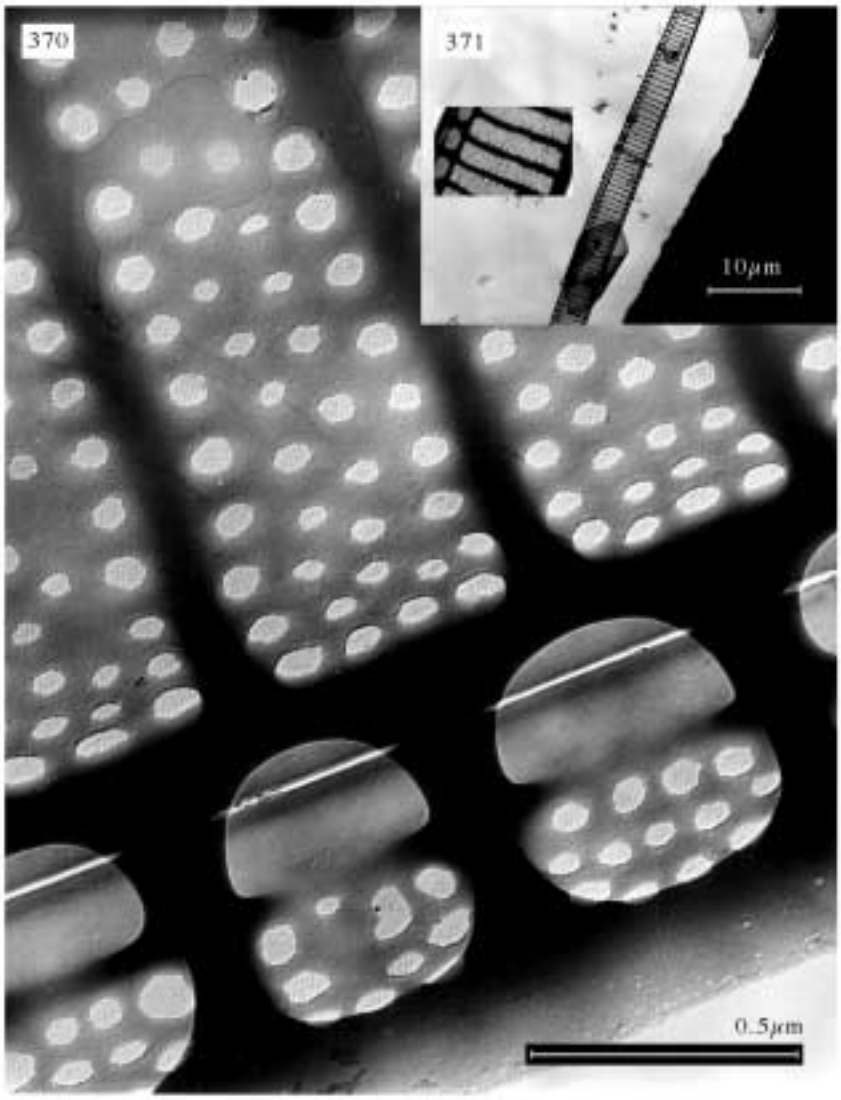

369
1μm

Figs. 365–377 TRANSMISSION ELECTRON MICROSCOPY (TEM)

Figs. 370–371 Specimen from the south coast of Ireland, station: 1815 (51˚ 50.2'N; 08˚ 15.8'W), July 23rd 1996.

Fig. 370 Close-up of the valve face striae and proximal mantle, 9 poroids in 1 µm.

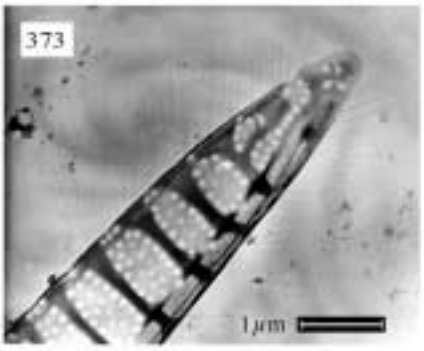
Fig. 371 Part of the valve, ~ 3 µm wide, 15 striae, 15 interstriae and 15 fibulae in 10 µm.

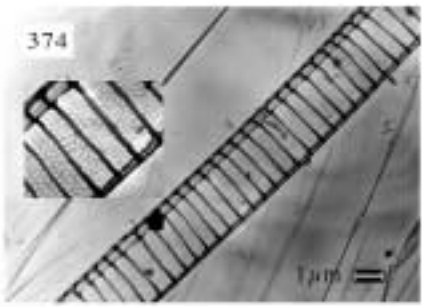

370
371
10μm
0.5μm

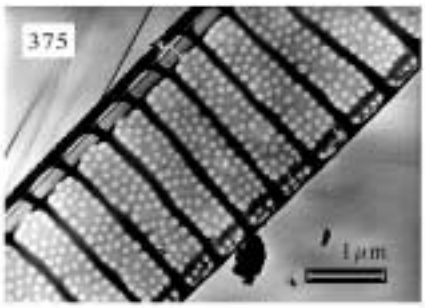
Figs. 365–377 TRANSMISSION ELECTRON MICROSCOPY (TEM)

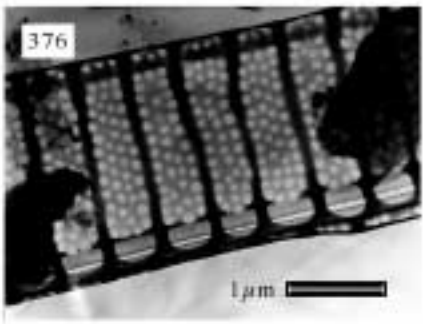
Figs. 372–377	Specimen from the southwest coast of Ireland, station: 1819 (52˚ 04.9'N; 06˚ 59.0'W), July 23rd 1996
Fig. 372	Whole valve, 93 µm long, 2.8 µm wide, 15–16 striae, 15–16 interstriae and 15–16 fibulae in 10 µm
Fig. 373	Valve end
Fig. 374	Middle part of the valve showing the striae structure, 7 poroids in 1 µm
Fig. 375	Part of the valve
Fig. 376	Part of the valve
Fig. 377	Other end


372
10μm


373
1μm


374
1μm


375
1μm


376
1μm


377
1μm

Pseudo-nitzschia cf. *seriata*

(Figures 378 –386)

Figs. 378–386 TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Figs. 378–379 Specimen from the south coast of Ireland, station: 1820 (51˚ 45.10'N; 06˚ 33.9'W), July 24th 1996.

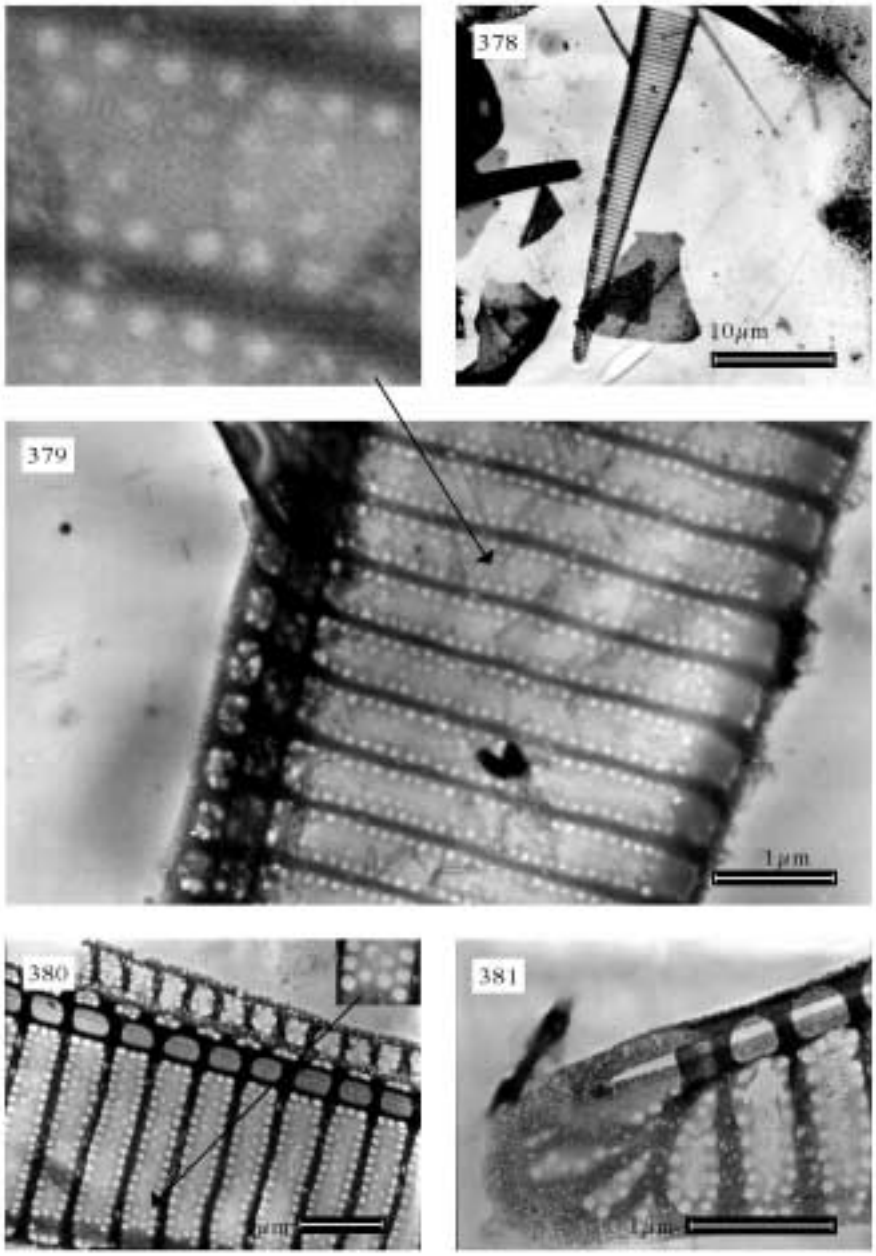
Fig. 378 Part of a valve, 20 striae, 20 interstriae and 20 fibulae in 10 µm.

Fig. 379 Part of a valve, 8–9 poroids in 1 µm.

Figs. 380–381 Specimen from the south coast of Ireland, station: 1819 (52˚ 04.90'N; 06˚ 59.00'W), July 23rd 1996.

Fig. 380 Part of a valve, 8 poroids in 1 µm.

Fig. 381 Valve end.


378
10μm
379
1μm
380
381

Figs. 378–386 Cont'd. TRANSMISSION ELECTRON MICROGRAPHS (TEM)

Fig. 382 Specimen from the south coast of Ireland, station: 1810 (51˚ 07.2'N; 09˚ 24.0'W), July 22nd 1996, part of a valve, 5.3 µm wide at this part of the valve, 7 poroids in 1 µm.

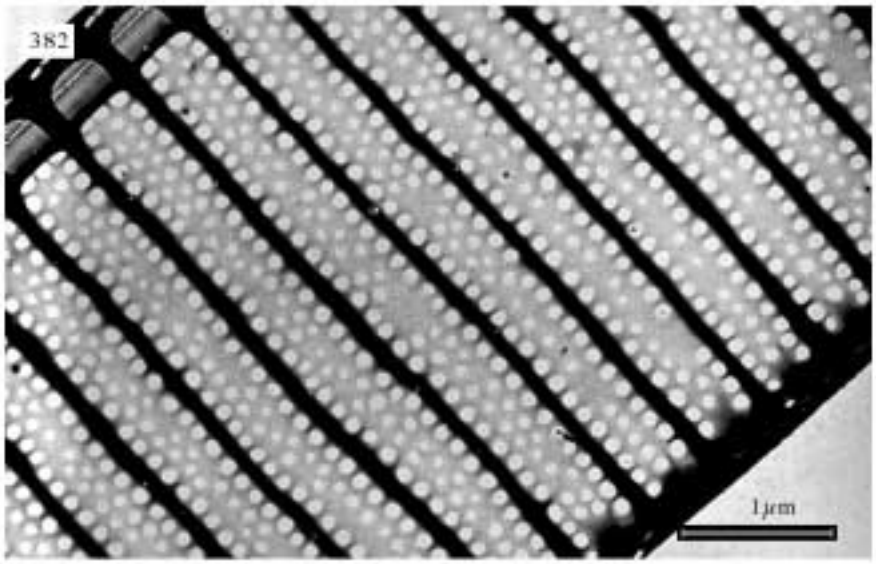
Figs. 383–386 Specimens from the coast of Ireland, station: 1820 (51˚ 45.10'N; 06˚ 33.9'W), July 24th 1996.

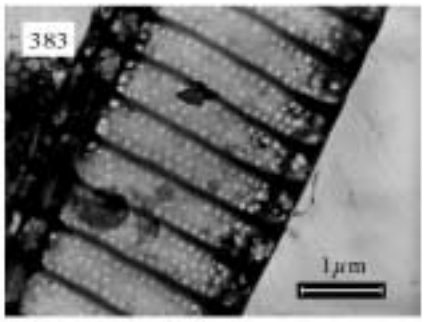
Fig. 383 Part of a valve, 8 poroids in 1 µm.

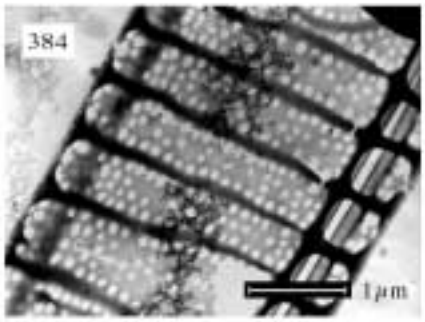
Fig. 384 Part of a valve, 8 poroids in 1 µm.

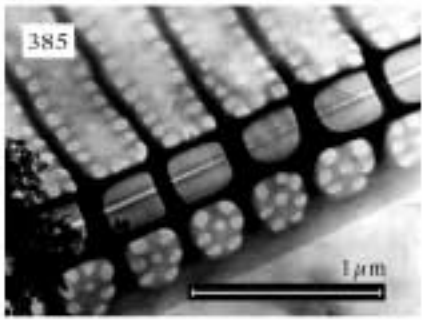
Fig. 385 Part of a valve, 9–10 poroids in 1 µm.

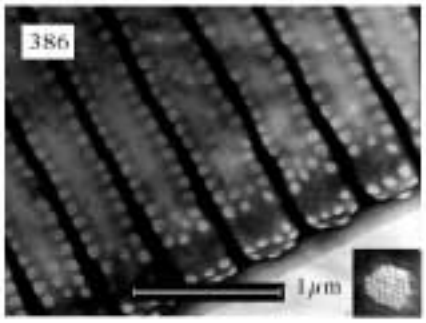
Fig. 386 Part of a valve, 9 poroids in 1 µm.


382
1 μm


383
1 μm


384
1 μm


385
1 μm


386
1 μm

APPENDIX 4

Appendix IV

A list of the oxidation (cleaning) methods used during the study to remove the organic material from the siliceous frustule of the diatom *Pseudo-nitzschia*. Included are comments on the success rate of each method carried out. Mounting cleaned *pseudo-nitzschia* specimens onto permanent slides. Preparation of thin sections of fixed embedded cultured vegetative Pseudo-nitzschia cells.

PREPARATION OF DIATOMACEOUS MATERIAL FOR LIGHT AND ELECTRON MICROSCOPY

At stations where *Pseudo-nitzschia* was found to be one of the dominant taxa, aliquots (ca. 5 mL) were taken from the net samples and the organic matter of the cells was removed by acid cleaning.

Organic contents of diatoms visually obstruct the image of the silica frustule whose structure is primarily relied upon for the species identification of many diatoms. The preparation of diatom material therefore entails using a cleaning method. Strong oxidising agents such as a concentrated acid are often applied to burn away the organic matter of the cells in order to achieve good resolution of the siliceous structures. After oxidising the organic matter from the sample, mineral substances will remain, including the siliceous skeleton of diatom cells. Many cleaning procedures exist that oxidise the organic material from diatomaceous samples. These are carried out by using physical methods or more frequently by chemical means. Several cleaning methods were attempted during the course of this study, of which the following method was used as the standard. Other variations of this method and comments on their performance are discussed below.

REMOVAL OF ORGANIC MATERIAL FROM DIATOMACEOUS MATERIAL

The following steps were carried out during cleaning preparations

1. 2–5 mL of net sample was placed in a clean test tube and resuspended in distilled water. Cells were collected by centrifuging at 4,500 rpm for 10 min. The supernatant was aspirated off (using a separate pipette for each sample to avoid cross contamination) and the remaining pellet was washed with distilled water. This was repeated three to five times to ensure no preservative or salt remained.

2. Chemical preparations were carried out under a fume hood whenever toxic chemicals were used. A vortex was used to resuspend the cells and to ensure clumping did not occur, thus allowing an increased cell surface area for chemical

activity. Cleaned samples were rinsed with distilled water to remove any chemical traces (samples were tested with pH sticks to ensure that all the acid was removed).

3. After the cleaning procedure cleaned material was rinsed with distilled water and stored in glass vials with an equal volume of absolute ethanol (this inhibits fungal growth) until further use. All sample vials were labelled. A drop of clean sample was mounted onto a slide (washed in ethanol) and checked under a light microscope for salt crystal formation or remaining organic matter (e.g. chromatophores still visible.)

Cell concentrations and the amount of particulate matter present can differ greatly between phytoplankton samples. Therefore, smaller aliquots of the more concentrated samples were used. High-grade Analar water was used for rinsing after it was discovered that the distilled water from the laboratory was contaminated with foreign particles. Test tubes were not reused, since diatom frustules can often remain attached to the glass.

NITRIC ACID METHOD (Modification of Boyle et al. 1984).

Concentrated nitric acid (70% HNO_3) was added to the sample pellet (1:1) and heated in a water bath at 80 ºC for 30 min. (the time varied depending on the amount of organic material in the sample). A marble was placed on top of each test tube to prevent loss of sample. The solution was vortexed gently every 10 min. to resuspend the sample.

This nitric acid treatment was the most suitable cleaning method for the field and cultures samples examined. Mann (1978) found that nitric acid was a very efficient oxidising agent (especially in calcium free samples, as nitrates are more soluble than a lot of the chlorides). However, he found that sediment samples with high organic contents could sometimes take a long time to clean. During this investigation, a series of time and temperature trials using a *P. australis* culture were carried out to establish

the standard method. This included variations in the length of time a sample was left in the waterbath, the temperature and a combination of HNO_3 and hydrochloric acid (HCl) treatments. When silica dissolution was apparent after chemical treatment, the cleaning procedure was repeated with fresh material and the samples were left at 80ºC for a shorter length of time. The procedure was repeated on samples that contained a lot of organic matter after the nitric acid treatment. One of the disadvantages this method has is that the siliceous frustule often disassociates into its separate components (valves and girdle elements) and discrimination between girdle bands of different species in field samples is difficult if not impossible. *Pseudo-nitzschia delicatissima* was one of the few species whose girdle elements often remained intact and attached to the valve mantle.

OTHER METHOS TESTED FOR THE REMOVAL OF ORGANIC MATERIAL FROM VEGETATIVE DIATOM CELLS.

PHYSICAL CLEANING

INCINERATION METHOD

(Zoto et al. 1973 cited in Ma and Jefferey 1978)

A small drop (fraction of a mL) of washed sample was pipetted onto a clean glass coverslip. This was placed in a muffle furnace and heated to 500°C, the temperature was maintained for a limited period of time. A time series ranging from 10 min. to 60 min. was carried out.

This method was very disappointing. While frustules remained intact, a great deal of organic matter remained, obscuring the fine siliceous structure of the frustule. This was recurrent even when the samples were left for up to 30 min. at 500 °C. When samples were left in the muffle furnace for a longer period of time (40 to 60 min.), the diatom frustules became broken or damaged. Patrick and Reimer (1966 cited in Ma and Jefferey 1978) used a similar method and a hot plate was used to burn off the organic matter instead of an oven. They found this cleaning procedure worked best only on phytoplankton samples containing primarily diatoms with little or no substrate.

CHEMICAL CLEANING

An alternative to the physical approach of stripping diatom cells of their organic contents is by chemical means. Acid attack is a popular and widely practised method used to destroy the various organic components that obscure the fine morphological detail of the diatom frustule. However, strong oxidising methods can be damaging especially to the more delicately formed species. Silica destruction can occur as a direct result of the cleaning process used, silica dissolution can also be the product of long term storage in an unsuitable preservative before any ultrastructural examination is carried out. Another possible reason for this type of observation may be the environment in which the diatoms were formed. Diatoms growing in conditions where there are low concentrations of silica may form lightly silicified frustules that will dissolve a lot faster in preserved samples and be more open to attack by chemicals. Some of the less aggressive methods may permit delicate siliceous elements such as the poroid velae to remain intact, but organic matter can remain so this kind of detail is unclear or concealed. Ma and Jefferey (1978), in a review on diatom preparation and cleaning, mentioned that the use of sulphuric acids or potassium persulphate may result in precipitation of sulphate compounds unless the working sample is pre–rinsed with weak hydrochloric acid.

HYDROGEN PEROXIDE (H_2O_2) METHOD

A variety of H_2O_2 concentrations (10%, 20%, or 30%) were added to separate phytoplankton samples. When the sample contained a lot of organic material the solution bubbled. After 5 to 10 min. distilled water was added (1:1; H_2O:H_2O_2) and the mixture was then left at room temperature for a few hours or over-night.

The hydrogen peroxide cleaning method was found to be inefficient at removing organic material in the samples used. The organic matter was poorly digested in all samples examined after the treatment. Mann (1978) found that hot hydrogen peroxide (28%) was only useful for removing epiphytic diatoms from their substrate and partially

breaking down particulate matter. He used H_2O_2 only as a pre-treatment before the addition of other oxidising agents.

HYDROCHLORIC ACID (HCl) METHOD

Concentrated HCL (36%) was added to several phytoplankton samples as with the hydrogen peroxide procedure above.

Again this treatment was unsuccessful at removing much of the organic material in the samples tested. This method would probably be sufficient if the samples contained very little organic particulate matter and the target diatoms had delicate siliceous valves. Cupp (1944) used hydrochloric acid as a pre–treatment to bring the organic material somewhat into solution and remove calcareous matter from her samples before using a more aggressive acid to oxidise the organic matter that remained. Mann (1978) also used hydrochloric acid as a rinsing pre–treatment to avoid precipitation of calcium salts (eg. calcium sulphate) when sulphuric acid (H_2SO_4) was to be added during the cleaning procedure.

HYDROCHLORIC ACID AND POTASSIUM PERMANGANATE ($KMnO_4$) METHOD

(following Simonsen 1974)

Concentrated potassium permanganate was added to the phytoplankton pellet (1:1). The solution was left to stand for 24 hours. Concentrated hydrochloric acid was then carefully added to the solution (1:2). If the sample turned a dark brown colour this indicated that the potassium permanganate was being reduced to manganese dioxide. The mixture was heated slowly (either over an alcohol lamp, a bunsen burner or a hot plate) until it boiled, the solution turned an olive-green colour and eventually a light yellow colour.

Simonsen (1974) highly recommended this method except when samples contained a lot of humic acid. In this study this method did not clean the samples adequately.

HYDROCHLORIC ACID AND POTASSIUM PERMANGANATE METHOD

(Simonsen R. 1974 modified)

Concentrated hydrochloric acid was added to the sample pellet and the sample was left overnight (1:1). An equal amount of potassium permanganate ($KMnO_4$ concentrated solution) was then added to the mixture and again left overnight (1:2). An equal amount of concentrated hydrochloric acid (HCl) was added to the solution (1:3) and heated on a hotplate until oxidation was complete.

The degree of oxidation achieved with this method was not very satisfactory, a lot of organic matter remained in the samples and cleaned siliceous elements were a rare occurrence.

NITRIC ACID (HNO_3) AND SULPHURIC ACID METHOD

(Hasle G. pers. comm.).

65% Nitric acid was added to the phytoplankton pellet (1:1; HNO_3:sample). Concentrated sulphuric acid was then added and a brownish colour was considered a good sign. The solution was heated over a bunsen burner while continuously shaking the test tube (it sometimes bubbled vigorously and spat out). When the solution turned a yellowish brown, heating continued until the total volume retreated down to the original pellet level. The sample turned a whitish-grey colour when completely oxidised. The sample was allowed to cool before rinsing with distilled water.

This method was found to be slightly aggressive on some of the samples, destroying delicate parts of the silica frustule (disappearance of the ornamentation on the poroid velae). Organic matter sometimes remained, and obscured the view of certain siliceous structures in other samples.

SULPHURIC ACID, POTASSIUM PERMANGANATE AND OXALIC ACID ($COOH_2$) METHOD

(following Hasle and Fryxell 1970)

3–5 mL of net sample or 20 mL of bottle sample was poured into a glass beaker. 20 mL of concentrated sulphuric acid (H_2SO_4) was added and agitated gently. Saturated potassium permanganate ($KMnO_4$) was then added drop by drop and agitated gently after each addition until the liquid turned a purple colour (this chemical oxidises the organic matter inside the cells). Freshly made-up saturated oxalic acid ($COOH_2$) was then added a little bit at a time and agitated gently after each addition until the solution cleared (this sometimes bubbled vigorously). The solution was then centrifuged and concentrated to 5 mL and the supernatant carefully aspirated off.

This method was aggressive on some of the samples (dissolution of the silica frustule was evident) while in other parts of the same sample organic material remained.

BIOLOGICAL WASHING POWDER

(Brian Ottway, personal communication)

The phytoplankton pellet was resuspended in warm distilled water containing a few grains of biological washing powder. The sample was vortexed to ensure it was well mixed. The mixture was then heated in a waterbath and left for a specific period of time.

SAMPLE 1: left at 40 ºC for 90 min. then the temperature was increased to 70 ºC for 20 min..

SAMPLE 2: left at 40 ºC for 90 min. then the temperature was increased to 70 ºC for 20 min. after which 15% nitric acid added and the sample was left at room temperature for 5 min..

SAMPLE 3: 40 ºC for 180 min. then the temperature was decreased to 60 ºC for a further 30 min..

SAMPLE 4: Left at room temperature.

Several ranges of temperatures and time lengths were tested all of which were ineffective at removing the organic material from the samples investigated.

FINAL NOTE: Many of the procedures described above were found unsuitable for the samples analysed. Several of the cleaning methods removed a significant amount of organic matter, but left behind a thin film that obscured the velae structure of the valve face. Other cleaning attempts were more aggressive leaving the *Pseudo-nitzschia* valves with a "ghost-like" appearance (dissolution of silica), in some instances the valves were broken and the vela silica structure had dissolved.

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MOUNTING CLEANED *PSEUDO-NITZSCHIA* SPECIMENS ONTO PERMANENT SLIDES

(Grethe Hasle pers. commun.)

Samples were mounted in triplicate. Circular coverslips number 0 (thickness of 0.13 mm) or number 1 (thickness of 0.16 mm) were cleaned with absolute alcohol. A drop of clean sample was placed on the coverslip. The sample was diluted with distilled water when it contained a high concentration of material (obvious by its cloudy appearance). The material was then evenly spread out across the coverslip using a clean glass pipette (prevents crossover contamination between samples) and left to air dry overnight. Samples were sometimes dried under an electric lamp but this tended to cause the valves to clump together (surface tension). To protect against dust particles, a covering (petridish) was put over the coverslips while the sample dried. A drop of resin (Naphrax, RI: 1.72) was placed onto a clean microscope glass slide and the coverslip was gently pressed on top making sure there were no bubbles. The slide was placed on a hot plate and heated (60ºC) to soften the Naphrax mounting agent (which would spread over the entire coverslip). A cocktail stick was used to tap the coverslip to aid in the removal of air bubbles. When this proved difficult, a small aliquot of Naphrax medium was placed at the edge of the coverslip, this would flow into and replace any remaining air pockets. The slide was left at room temperature until the resin hardened. Excess Naphrax was scraped off with a scalpel blade and the coverslip was permanently sealed with clear nailpolish (painted on the edge of the coverslip). Slides were labelled and checked under a Nikon Optiphot-2 LM using bright field and phase contrast to ensure the cleaning procedure was successful. Objective lenses used to examine the material included a 20x dry lens (NA 0.5), a 20x dry phase contrast lens (NA 0.75), a 40x dry phase contrast lens (NA 1.30), a 50x immersion oil lens (NA O.85) and a 100x immersion oil lens (NA 1.25).

PREPARATION OF THIN SECTIONS OF FIXED EMBEDDED CULTURED VEGETATIVE PSEUDO-NITZSCHIA CELLS.

Two *Pseudo-nitzschia* cultures (*P. australis* and *P. delicatissima*) were used to examine vegetative cell sections along the transapical and valvar planes. Cells were collected by gentle centrifugation at 4,500 rpm for 10 min. 10 mL aliquot of 3% gluteraldehyde in 0.1 M sodium cacodylate buffer (1 mL) at pH 7.2 was added and the sample was left to fix for 90 min. Cells were postfixed in 2 mL aliquots of 2% osmium tetroxide in cacodylate buffer for 1 hour (Osmium tetroxide acts as a preservative and stains carbohydrates and lipids). The material was washed once in 1 mL cacodylate buffer for 5 min. The sample was dehydrated in a series of 10, 30, 50, 70, 90% acetone:distilled water and 2x 100% acetone for 10 min. at each step. The cells were gradually infiltrated into Spurr's resin (Spurr 1969) first in acetone:resin (1:1) for 1 hour and then in 100% resin and left to stand overnight. Embedded samples were polymerised at 60 ºC for a period of 3 days and sectioned using an ultramicrotome ("Ultramicrotome–E", Reichert–Jung) equipped with a diamond knife. Ultra–thin (60–90 µm) sections were placed on 200–mesh copper grids and post–stained with uranyl acetate for 30 min. at 40 ºC. After rinsing in distilled water the sections were post-stained with lead–citrate for 5 min. at room temperature and finally rinsed in distilled water. All fixatives and buffer were made up in filtered seawater (0.22 µm Nucleopore filters). Specimens were viewed under a Hitachi–7000 transmission electron microscope at an accelerating voltage of 75 kV.

APPENDIX 5

Appendix V

Short description of the Phylogenetic methods used in this study. Multiple DNA sequence alignment of the 18S SSU (1745 bp) rDNA of several *Pseudo-nitzschia* isolates. Multiple rDNA alignments of character positions of the SSU considered unambiguous and analysed using Maximum-Likelihood, distance (Logdet transformation) and Maximum Parsimony methods.

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MAXIMUM PARSIMONY MODEL OF EVOLUTION

Maximum Parsimony is a character state based method where each nucleotide position in an alignment is considered. The method is based on the principal that the simplest explanation consistent with a data set should be chosen over more complex explanations (Steward, 1993). This produces a tree called a Maximum Parsimony tree. Parsimony analysis only uses informative sites. A nucleotide site is phylogenetically informative in maximum parsimony if there are at least two different kinds of nucleotide at the site, each of which is represented in at least two taxa, which allows a decision to be made regarding the topology of the tree. Indels can be regarded as a "fifth base", a major advantage that parsimony has over distance and likelihood based methods. In a sequence alignment, all the parsimony-informative sites are first identified, trees are constructed, and the tree requiring the least amount of steps is favoured. Sometimes there are many trees that are equally parsimonious but have different topologies. In this case a consensus tree can be constructed. A strict consensus tree compares the topology of all the equally parsimonious trees and resolves any conflicts in branching order by creating a multifurcation at that point. If many multifurcations are created it may comprise the structure of the tree. A 50% majority-rule consensus tree also compares the topology of all the equally parsimonious trees and the branching pattern that occurs in greater than 50% of the trees is used. This tree may not have as many multifurcations as a strict consensus tree.

DISTANCE-BASED METHODS

Distance-based methods calculate the overall similarities or dissimilarities between sequences and convert this data into a matrix of pairwise distances. The phylogenetic tree is constructed from the distance matrix. The simplest distance estimate is the p-distance, which simply reflects the actual distance between two sequences. It does not take multiple substitutions (e.g. A-G-A) into account. Other distance estimates try and correct for multiple substitutions and range from simple models like the Jukes and Cantor, one parameter model of evolution that assumes all kinds of substitutions are equally likely (Jukes and Cantor 1969) and Kimura 2, two parameter model, which assumes that the rate of transitions (changes within purines, G and A, and within

pyrimidines, C and T) differ from transversions (changes between purines and pyrimidines) (Kimura 1980), to more complex models containing more parameters (e.g. General Time Reversible). Once a distance matrix has been generated using some model of the substitution process, a phylogenetic tree construction method is implemented based on the distance calculation. Neighbor-joining (Saitou and Nei, 1987) is one such method. The principal of the neighbor-joining method is to find neighbors sequentially that minimize the total length of the tree. The method starts with a starlike tree in which there is no clustering of the OTUs. The first step is to separate a pair of OTUs from all the others, creating an internal branch. Among the possible pairs of OTUs that can be taken, the one that gives the smallest sum of branch lengths is chosen. This pair is then regarded as a single OTU, and the arithmetic mean distances between OTUs are computed to produce a new distance matrix. The next pair of OTUs that gives the smallest sum of branch lengths is calculated and the process is repeated until all internal branches have been determined. Neighbor-joining does not assume a constant rate of evolution among the lineages unlike UPGMA (unweighted pair-group method with arithmetic mean).

MAXIMUM-LIKELIHOOD MODEL OF EVOLUTION

Maximum-likelihood (ML) is a statistical method of phylogenetic reconstruction that was developed for use with DNA sequences by Felsenstein (1981). The method is made up of three ingredients: the data (DNA sequences for groups whose relationships are to be estimated), a phylogenetic hypothesis (a candidate tree to be tested) and a model of evolution (such as the Jukes and Cantor one parameter model). Maximum likelihood evaluates the tree (the branching order and branch lengths) in terms of the probability that the proposed model of evolution and the hypothesised tree would give rise to the observed data. ML is a character-state based method, like maximum parsimony, where every position in an alignment is considered. It is the most computationally intensive method of phylogenetic reconstruction and can become impossibly slow if many taxa are being considered due to the number of calculations required. It does, however, make more use of sequence data than the other two methods and generally outperforms them providing the model of evolution being used is consistent with the data.

Modeltest Version 3.0 is a programme that can be used with PAUP* to find the model of evolution that best fits the data, using 56 possible models. This is accomplished through an implementation of hierarchical likelihood ratio tests.

BOOTSTRAPPING (ASSESES SUPPORT FOR BRANCHING PATTERNS IN A TREE)

Once a phylogenetic tree has been constructed, it is important to evaluate the accuracy of the tree to see how well it reflects the data. The bootstrapping technique, random resampling with replacement, is the most commonly used method to evaluate trees since it was introduced by Felsenstein (1985). The bootstrap provides assessment of confidence for each clade of an observed tree, based on the proportion of bootstrap trees showing the same clade. The method works by randomly choosing a site in the original data set and using the site as the first site in a new data set. The chosen character is replaced and the process repeated until a new dataset is created that is the same size as the original data set. A tree is now produced from this dataset. In the new data set some sites will not be included while others can be represent more than once. The method is repeated many times (100-1000) and a majority rule consensus tree is constructed which includes the frequencies with which a given branch was found, called the bootstrap proportion. Branches that appear in more than 95% of bootstrap trees can be said to be well supported.

```
                SSU
                AGTCATACGCTCGTCTCAAAGATTAAGCCATGCATGTCTAAGTATAAATATTTTACTTTGAAACTGCGAA
1917        1 :.............................................................. :   70
1913        1 :.............................................................. :   70
W2          1 :.............................................................. :   70
PSEUD-X     1 :.............................................................. :   70
TKA-2       1 :.............................................................. :   70
13CC        1 :.............................................................. :   70
NPARL       1 :.............................................................. :   70
POM-X       1 :.............................................................. :   70
WW3         1 :.............................................................. :   70
BRUDC-X     1 :.............................................................. :   70
F310        1 :.............................................................. :   70

                CGGCTCATTATATCAGTTATAGTTTATTTGATAGTCCCTTACTACTTGGATACCCGTAGTAATTCTAGAG
1917       71 :.............................................................. :  140
1913       71 :.............................................................. :  140
W2         71 :.............................................................. :  140
PSEUD-X    71 :.............................................................. :  140
TKA-2      71 :.............................................................. :  140
13CC       71 :.............................................................. :  140
NPARL      71 :.............................................................. :  140
POM-X      71 :.............................................................. :  140
WW3        71 :.............................................................. :  140
BRUDC-X    71 :.............................................................. :  140
F310       71 :.............................................................. :  140

                CTAATACATGCGTCAATACCCCTTCTGGGGTAGTATTTATTAGATTGAAACCAACCCCTTCGGGGTGATG
1917      141 :..................-............................................. :  209
1913      141 :..................-............................................. :  209
W2        141 :..................-............c................................ :  209
PSEUD-X   141 :................t...-........a..................................... :  209
TKA-2     141 :..................-............................................. :  209
13CC      141 :.............................................................. :  210
NPARL     141 :.............................................................. :  210
POM-X     141 :.............................................................. :  210
WW3       141 :..................-............................................. :  209
BRUDC-X   141 :.............................................................. :  210
F310      141 :..................-............................................. :  209
```

Figure 1. Multiple DNA sequence alignment of the 18S SSU (1745 bp) rDNA of several *Pseudo-nitzschia* isolates. The black dots represent conserved character positions and hyphens represent insertion/deletion events. Taxa abbreviations in the left hand column are as follows, 1913 and 1917 = *P. delicatissima*, W2 = *P. fraudulenta*, PSEUD-X = *P. australis*, TKA-2 (Manhart et al 1994, Genebank accession # U18241), 13CC (TKA2-28, Douglas et al. 1994), NPARL (Douglas et al. 1994) and POM-X (Douglas et al. 1994) = *P. multiseries* and WW3, F310 (Manhart et al 1994, Genebank accession #U18240) and BRUNDC-X (Douglas et al. 1994) = *P. pungens*.

```
                TGGTGATTCATAATAAGCTTGCGGATCGCATGCCTCTTGGCGGCGATGGATCATTCAAGTTTCTGCCCTA
1917     210 :.....................................-.............................. :  278
1913     210 :.....................................-.............................. :  278
W2       210 :.....................................-.............................. :  278
PSEUD-X  210 :.....................................-.............................. :  278
TKA-2    210 :................aa.................t.............................. :  279
13CC     211 :................aa.................t.............................. :  280
NPARL    211 :................aa.................t.............................. :  280
POM-X    211 :................aa.................t.............................. :  280
WW3      210 :.....................................-.............................. :  278
BRUDC-X  211 :.....................................-.............................. :  279
F310     210 :.....................................-.............................. :  278

                TCAGCTTTGGATGGTAGGGTATTGGCCTACCATGGCTTTAACGGGTAACGGGAAATTAGGGTTTGATTCC
1917     279 :.......................................................................... :  348
1913     279 :.......................................................................... :  348
W2       279 :.......................................................................... :  348
PSEUD-X  279 :.......................................................................... :  348
TKA-2    280 :.......................................................................... :  349
13CC     281 :.......................................................................... :  350
NPARL    281 :.......................................................................... :  350
POM-X    281 :.......................................................................... :  350
WW3      279 :.......................................................................... :  348
BRUDC-X  280 :.......................................................................... :  349
F310     279 :.......................................................................... :  348

                GGAGAGGGAGCCTGAGAGACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGTAAATTACCCAATCCTGA
1917     349 :.......................................................................... :  418
1913     349 :.......................................................................... :  418
W2       349 :.......................................................................... :  418
PSEUD-X  349 :.......................................................................... :  418
TKA-2    350 :.......................................................................... :  419
13CC     351 :.......................................................................... :  420
NPARL    351 :.......................................................................... :  420
POM-X    351 :.....................................................t.................... :  420
WW3      349 :.......................................................................... :  418
BRUDC-X  350 :.......................................................................... :  419
F310     349 :.......................................................................... :  418

                CACAGGGAGGTAGTGACAATAAATAACAATGCCGGGCCTTCTTAGGTCTGGCAATTGGAATGAGAACAAT
1917     419 :.......................................................................... :  488
1913     419 :.......................................................................... :  488
W2       419 :................g......................................................... :  488
PSEUD-X  419 :.......................................................................... :  488
TKA-2    420 :.......................................................................... :  489
13CC     421 :.......................................................................... :  490
NPARL    421 :.......................................................................... :  490
POM-X    421 :.......................................................................... :  490
WW3      419 :.......................................................................... :  488
BRUDC-X  420 :.......................................................................... :  489
F310     419 :.......................................................................... :  488

                TTAAACCCCTTATCGAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAA
1917     489 :.......................................................................... :  558
1913     489 :.......................................................................... :  558
W2       489 :...................g...................................................... :  558
PSEUD-X  489 :.......................................................................... :  558
TKA-2    490 :.......................................................................... :  559
13CC     491 :.......................................................................... :  560
NPARL    491 :.......................................................................... :  560
POM-X    491 :.......................................................................... :  560
WW3      489 :.....................................................c.gcg.-................ :  557
BRUDC-X  490 :.......................................................................... :  559
F310     489 :.......................................................................... :  558
```

Figure 1 continued.

```
                TAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTGTGGTGTGTCCAGTCGGCCTTTG
1917      559 :.....................................................................a..... :  628
1913      559 :.....................................................................a..... :  628
W2        559 :.....................................................................a..... :  628
PSEUD-X   559 :............................................................................ :  628
TKA-2     560 :............................................................................ :  629
13CC      561 :............................................................................ :  630
NPARL     561 :.....................................................c...................... :  630
POM-X     561 :.....................................................c...................... :  630
WW3       558 :............................................................................ :  627
BRUDC-X   560 :............................................................................ :  629
F310      559 :............................................................................ :  628

                CTCTTTGAGTGATTGCGCTGTACTGGTCTGCCATGTTTGGGTGGAATCTGTGTGGCATTAAGTTGTCGTG
1917      629 :...............t.t........................................................... :  698
1913      629 :...............t.t........................................................... :  698
W2        629 :...............t.t.....................a..................................... :  698
PSEUD-X   629 :............................................................................ :  698
TKA-2     630 :............................................................................ :  699
13CC      631 :............................................................................ :  700
NPARL     631 :............................................................................ :  700
POM-X     631 :............................................................................ :  700
WW3       628 :.....c.....................................................................a :  697
BRUDC-X   630 :.....................................................................a..... :  699
F310      629 :.....................................................................a..... :  698

                CAGGGGATGCCCATCGTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCTTATGCCGTTGAATATATT
1917      699 :............................................................................ :  768
1913      699 :............................................................................ :  768
W2        699 :............................................................................ :  768
PSEUD-X   699 :............................................................................ :  768
TKA-2     700 :............................................................................ :  769
13CC      701 :............................................................................ :  770
NPARL     701 :............................................................................ :  770
POM-X     701 :............................................................................ :  770
WW3       698 :.t.a..g..................................................................... :  767
BRUDC-X   700 :.t.a..g..................................................................... :  769
F310      699 :.t.a..g..................................................................... :  768

                AGCATGGAATAATGATATAGGACCTTGGTACTATTTTGTTGGTTTGCGCACTAAGGTAATGATTAAGAGG
1917      769 :............................................................................ :  838
1913      769 :............................................................................ :  838
W2        769 :............................................................................ :  838
PSEUD-X   769 :............................................................................ :  838
TKA-2     770 :............................................................................ :  839
13CC      771 :............................................................................ :  840
NPARL     771 :............................................................................ :  840
POM-X     771 :............................................................................ :  840
WW3       768 :............................................................................ :  837
BRUDC-X   770 :............................................................................ :  839
F310      769 :............................................................................ :  838

                GACAGTTGGGGGTATTTGTATTCCATTGTCAGAGGTGAAATTCTTGGATTTTTGGAAGACAAACTACTGC
1917      839 :............................................................................ :  908
1913      839 :.....................................g.............c..................... :  908
W2        839 :............................................................................ :  908
PSEUD-X   839 :............................................................................ :  908
TKA-2     840 :............................................................................ :  909
13CC      841 :............................................................................ :  910
NPARL     841 :............................................................................ :  910
POM-X     841 :............................................................................ :  910
WW3       838 :............................................................................ :  907
BRUDC-X   840 :............................................................................ :  909
F310      839 :............................................................................ :  908
```

Figure 1 continued.

```
                GAAAGCATTTACCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATTAGATACC
1917     909 :.............................................................. :  978
1913     909 :.............................................................. :  978
W2       909 :.............................................................. :  978
PSEUD-X  909 :.............................................................. :  978
TKA-2    910 :.............................................................. :  979
13CC     911 :.............................................................. :  980
NPARL    911 :.............................................................. :  980
POM-X    911 :.............................................................. :  980
WW3      908 :.............................................................. :  977
BRUDC-X  910 :.............................................................. :  979
F310     909 :.............................................................. :  978

                ATCGTAGTCTTAACCATAAACTATGCCGACAAGGGATTGGTGGAGTCTCGTTTCGTCTCCATCAGCACCT
1917     979 :.................................a..g.....................................t. : 1048
1913     979 :.................................a.....................................t. : 1048
W2       979 :.................................a.....................................t. : 1048
PSEUD-X  979 :.........................................c...acga....a.......g......... : 1048
TKA-2    980 :.................................................t.......................... : 1049
13CC     981 :.................................................t.......................... : 1050
NPARL    981 :.................................................t.......................... : 1050
POM-X    981 :.................................................t.......................... : 1050
WW3      978 :.........................................c...................g......... : 1047
BRUDC-X  980 :.........................................c...................g......... : 1049
F310     979 :.........................................c...................g......... : 1048

                TGTGAGAAATCATAAGTCTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGAC
1917    1049 :.............................................................. : 1118
1913    1049 :.............................t................................ : 1118
W2      1049 :.............................................................. : 1118
PSEUD-X 1049 :.............................................................. : 1118
TKA-2   1050 :.............................................................. : 1119
13CC    1051 :........g..................................................... : 1120
NPARL   1051 :.............................................................. : 1120
POM-X   1051 :.............................................................. : 1120
WW3     1048 :.................................-............................ : 1116
BRUDC-X 1050 :.............................................................. : 1119
F310    1049 :.............................................................. : 1118

                GGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTTACCAGGTCCAG
1917    1119 :.............................................................. : 1188
1913    1119 :.............................................................. : 1188
W2      1119 :....................a......................................... : 1188
PSEUD-X 1119 :.............................................................. : 1188
TKA-2   1120 :.............................................................. : 1189
13CC    1121 :.............................................................. : 1190
NPARL   1121 :.............................................................. : 1190
POM-X   1121 :.............................................................. : 1190
WW3     1117 :.............................................................. : 1186
BRUDC-X 1120 :.............................................................. : 1189
F310    1119 :.............................................................. : 1188

                ACATAGTGAGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAG
1917    1189 :.............................................................. : 1258
1913    1189 :.............................................................. : 1258
W2      1189 :............c................................................. : 1258
PSEUD-X 1189 :.............................................................. : 1258
TKA-2   1190 :.............................................................. : 1259
13CC    1191 :.............................................................. : 1260
NPARL   1191 :.............................................................. : 1260
POM-X   1191 :.............................................................. : 1260
WW3     1187 :.............................c................................ : 1256
BRUDC-X 1190 :.............................................................. : 1259
F310    1189 :.............................................................. : 1258
```

Figure 1 continued.

```
                TTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCTGCCTGCTAAATAGCACGCATAG
1917    1259 :.......................................................................... : 1328
1913    1259 :.......................................................................... : 1328
W2      1259 :.............................g............................................ : 1328
PSEUD-X 1259 :..........................................................................a : 1328
TKA-2   1260 :.......................................................................... : 1329
13CC    1261 :.......................................................................... : 1330
NPARL   1261 :.......................................................................... : 1330
POM-X   1261 :.......................................................................... : 1330
WW3     1257 :..a.....................................................................aa..... : 1326
BRUDC-X 1260 :.......................................................................aa..... : 1329
F310    1259 :.......................................................................aa..... : 1328

                TGTTTATCACTGTGTAGTGCTTCTTAGAGGGACGTGCGTTCTATTAGACGCAGGAAGATAGGGGCAATAA
1917    1329 :.......................................................................... : 1398
1913    1329 :.......................................................................... : 1398
W2      1329 :.......................................................................... : 1398
PSEUD-X 1329 :.........t........................................................................ : 1398
TKA-2   1330 :.......................................................................... : 1399
13CC    1331 :.......................................................................... : 1400
NPARL   1331 :........g......................................................................... : 1400
POM-X   1331 :.......................................................................... : 1400
WW3     1327 :.................t........................................................... : 1396
BRUDC-X 1330 :.................t........................................................... : 1399
F310    1329 :.................t........................................................... : 1398

                CAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGCATTCAACGAGTTCTACC
1917    1399 :.......................................................................... : 1468
1913    1399 :.......................................................................... : 1468
W2      1399 :.......................................................................... : 1468
PSEUD-X 1399 :.......................................................................... : 1468
TKA-2   1400 :.......................................................................... : 1469
13CC    1401 :.......................................................................... : 1470
NPARL   1401 :.......................................................................... : 1470
POM-X   1401 :.......................................................................... : 1470
WW3     1397 :.......................................................................... : 1466
BRUDC-X 1400 :.......................................................................... : 1469
F310    1399 :.......................................................................... : 1468

                TTGGCCGAGAGGCCTGGGCAATCTTTTGAACTTGCATCGTGATAGGGATAGATTATTGCAATTATTAATC
1917    1469 :..............a..................c..................................... : 1538
1913    1469 :.............................c..................................... : 1538
W2      1469 :.......................................................................... : 1538
PSEUD-X 1469 :.......................................................................... : 1538
TKA-2   1470 :.......................................................................... : 1539
13CC    1471 :.....................................a.................................... : 1540
NPARL   1471 :.......................................................................... : 1540
POM-X   1471 :.......................................................................... : 1540
WW3     1467 :.......................................................................... : 1536
BRUDC-X 1470 :.......................................................................... : 1539
F310    1469 :.............................c..................................... : 1538

                TTGAACGAGGAATTCCTAGTAAACGCAGATCATCAATCTGCATTGATTACGTCCCTGCCCTTTGTACACA
1917    1539 :.............................t.......a.................................... : 1608
1913    1539 :.............................t.......a.................................... : 1608
W2      1539 :.......................................................................... : 1608
PSEUD-X 1539 :.......................................................................... : 1608
TKA-2   1540 :.......................................................................... : 1609
13CC    1541 :.......................................................................... : 1610
NPARL   1541 :.................................g........................................ : 1610
POM-X   1541 :.............................................................t............ : 1610
WW3     1537 :.......................................................................... : 1606
BRUDC-X 1540 :.......................................................................... : 1609
F310    1539 :.......................................................................... : 1608
```

Figure 1 continued.

```
                CCGCCCGTCGCACCTACCGATTGAATGGTCCGGTGAAGCCTCGGGATTGTGATTAGTTTCCTTTATTGGA
1917     1609 :.....................................a.......g..................... : 1678
1913     1609 :.....................................a.......g..................... : 1678
W2       1609 :.......................................................................... : 1678
PSEUD-X  1609 :.....................................................g.....c..... : 1678
TKA-2    1610 :.......................................................................... : 1679
13CC     1611 :.......................................................................... : 1680
NPARL    1611 :.......................................................................... : 1680
POM-X    1611 :.......................................................................... : 1680
WW3      1607 :.......................................................................... : 1676
BRUDC-X  1610 :.......................................................................... : 1679
F310     1609 :.......................................................................... : 1678


                AGTTTGTCACGAGAACTTGTCTAAACCTTATCATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCCG
1917     1679 :....aac.................................................................. : 1745
1913     1679 :....aac.................................................................. : 1745
W2       1679 :..g.a..t.t......c........................................................ : 1745
PSEUD-X  1679 :..g.a...........c.............................a..................... : 1745
TKA-2    1680 :.......................................................................... : 1746
13CC     1681 :.....................................................g.................... : 1747
NPARL    1681 :.......................................................................... : 1747
POM-X    1681 :.......................................................................... : 1747
WW3      1677 :.......................................................................... : 1743
BRUDC-X  1680 :....-.......................................................................... : 1745
F310     1679 :.......................................................................... : 1745
```

Figure 1 continued.

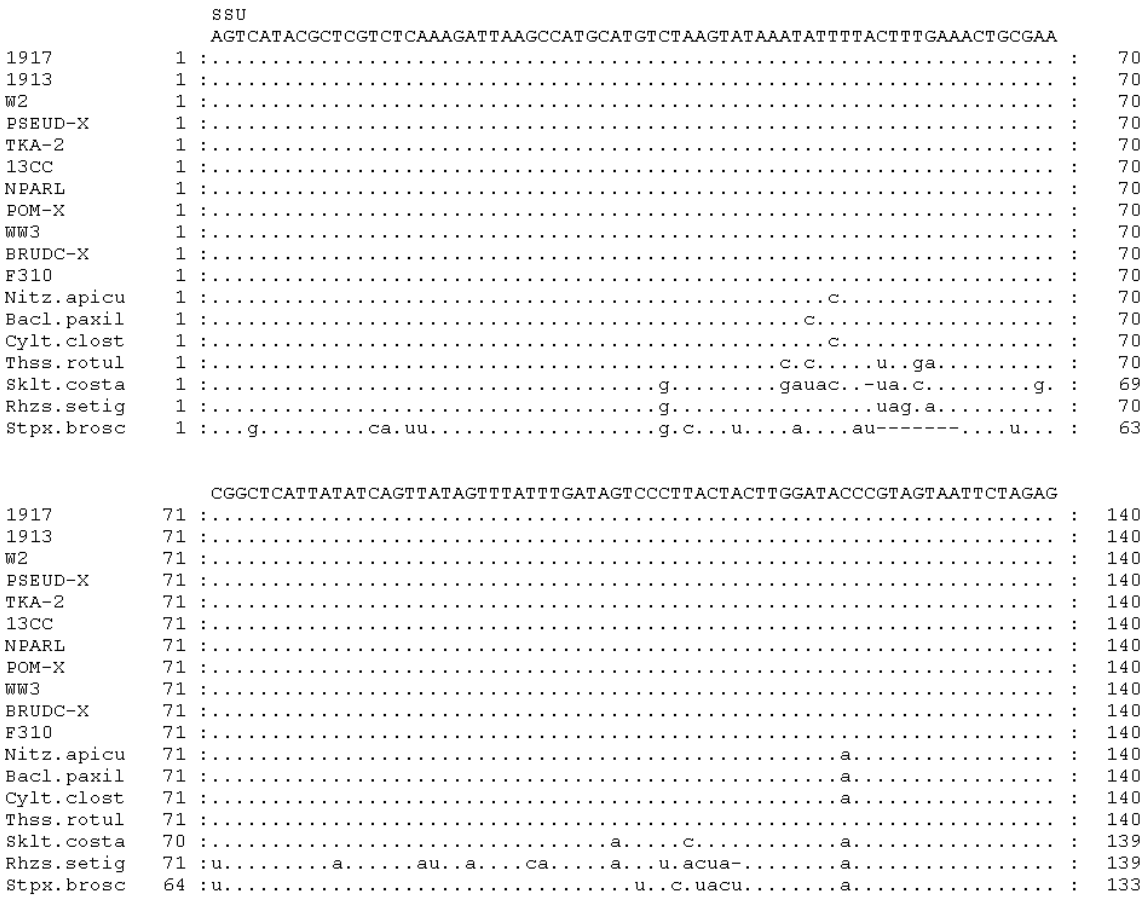

Figure 2. Multiple rDNA alignments of character positions of the SSU considered unambiguous and analysed using Maximum-Likelihood, distance (Logdet transformation) and Maximum Parsimony methods. The black dots represent conserved character positions and hyphens represent insertion/deletion events. Taxon abbreviations in the left hand column are as follows 1913 and 1917 = *P. delicatissima*, W2 = *P. fraudulenta*, PSEUD-X (Douglas et al. 1994) = *P. australis*, WW3, BRUND-X (Douglas et al. 1994) and F310 (Genebank accession #U18240) = *P. pungens*, Nitz.apicu = *Nitzschia apiculata* (#M87334), Bacl.paxil = *Bacillaria paxillifera* (#M87325), Cylt.clost = *Cylidrotheca closterium* (#M87326), Thss.rotul = *Thalassiosira rotula* (#X85397), Sklt.costa = *Skeletonema costatum* (#X85395), Rhzs.setig = *Stephanopyxis broschii* (#M87330) and Stpx.brosc = *Rhizosolenia setigera* (#M87329).

```
                    CTAATACATGCGTCAATCCCTTCTGGGGAGTATTATTAGATTGAAACCAACCCCTTCGGGGTGATGTGGT
1917        141 :.......................................................................... :  210
1913        141 :.......................................................................... :  210
W2          141 :.......................................................................... :  210
PSEUD-X     141 :.......................................................................... :  210
TKA-2       141 :.......................................................................... :  210
13CC        141 :.......................................................................... :  210
NPARL       141 :.......................................................................... :  210
POM-X       141 :.......................................................................... :  210
WW3         141 :.......................................................................... :  210
BRUDC-X     141 :.......................................................................... :  210
F310        141 :.......................................................................... :  210
Nitz.apicu  141 :.....................................................uu.....-............ :  209
Bacl.paxil  141 :.....................................................--..................... :  208
Cylt.clost  141 :.......................................................................... :  210
Thss.rotul  141 :...........a..........g-.............g.au.g.......u......a...c.u.... :  209
Sklt.costa  140 :......u....a....a.ggc..g-cc.c..g.......ua.u.....uu..u......a.....u.... :  208
Rhzs.setig  140 :...........u.a.....-c.ug--....c..........a...g...u..u......a..au.u.... :  206
Stpx.brosc  134 :...........a....g......g-...u.............ac.....u.u.u..u-a.a.ag...... :  201

                    GATTCATAATAAGCTTGCGGATCGCATGCCTCTGGCGGCGATGGATCATTCAAGTTTCTGCCCTATCAGC
1917        211 :.......................................................................... :  280
1913        211 :.......................................................................... :  280
W2          211 :.......................................................................... :  280
PSEUD-X     211 :.......................................................................... :  280
TKA-2       211 :...........aa.................u.......................................... :  280
13CC        211 :...........aa.................u.......................................... :  280
NPARL       211 :...........aa.................u.......................................... :  280
POM-X       211 :...........aa.................u.......................................... :  280
WW3         211 :.......................................................................... :  280
BRUDC-X     211 :.......................................................................... :  280
F310        211 :.......................................................................... :  280
Nitz.apicu  210 :.........................g..u..c.................................... :  279
Bacl.paxil  209 :.........................g.gua.c.................................... :  278
Cylt.clost  211 :.........................g..u..c.................................... :  280
Thss.rotul  210 :...........cu..u..a........g...c.c.................................... :  279
Sklt.costa  209 :..g.........c...u..a..u......aca.u.....a.............................. :  278
Rhzs.setig  207 :..a.........cu.............g.....c......ca.....................u..a :  276
Stpx.brosc  202 :..a....g.....u.............g...c.c......ca......au.................. :  271

                    TTTGGATGGTAGGGTATTGGCCTACCATGGCTTTAACGGGTAACGGGAAATTAGGGTTTGATTCCGGAGA
1917        281 :.......................................................................... :  350
1913        281 :.......................................................................... :  350
W2          281 :.......................................................................... :  350
PSEUD-X     281 :.......................................................................... :  350
TKA-2       281 :.......................................................................... :  350
13CC        281 :.......................................................................... :  350
NPARL       281 :.......................................................................... :  350
POM-X       281 :.......................................................................... :  350
WW3         281 :.......................................................................... :  350
BRUDC-X     281 :.......................................................................... :  350
F310        281 :.......................................................................... :  350
Nitz.apicu  280 :.......................................................................... :  349
Bacl.paxil  279 :.......................................................................... :  348
Cylt.clost  281 :.......................................................................... :  350
Thss.rotul  280 :...........u.......a.....................aauug......caa....u...... :  349
Sklt.costa  279 :..g.........u.......a.....................aauug......caa....u...... :  348
Rhzs.setig  277 :.........cu.u.......a.ag.....u....g.u.........ag.........cc.......... :  346
Stpx.brosc  272 :.............................a..............g..c.........c........ :  341
```

Figure 2 continued.

```
                    GGGAGCCTGAGAGACGGCTACCACATCCAAGGAAGGCAGCAGGGCGTAAATTACCCAATCCTGACACAGG
1917        351 :.......................................................................... :  420
1913        351 :.......................................................................... :  420
W2          351 :.......................................................................... :  420
PSEUD-X     351 :.......................................................................... :  420
TKA-2       351 :.......................................................................... :  420
13CC        351 :.......................................................................... :  420
NPARL       351 :.......................................................................... :  420
POM-X       351 :.......................................................................... :  420
WW3         351 :.......................................................................... :  420
BRUDC-X     351 :.......................................................................... :  420
F310        351 :.......................................................................... :  420
Nitz.apicu  350 :.......................................................................... :  419
Bacl.paxil  349 :.......................................................................... :  418
Cylt.clost  351 :.......................................................................... :  420
Thss.rotul  350 :.......................................................................... :  419
Sklt.costa  349 :.......................................................................... :  418
Rhzs.setig  347 :.......................................................................... :  416
Stpx.brosc  342 :..............................................c........................... :  411

                    GAGGTAGTGCAATAAATAACAATGCCGGGCCTTCTTAGGTCTGGCAATTGGAATGAGAACAATTTAAACC
1917        421 :.......................................................................... :  490
1913        421 :.......................................................................... :  490
W2          421 :.......................................................................... :  490
PSEUD-X     421 :.......................................................................... :  490
TKA-2       421 :.......................................................................... :  490
13CC        421 :.......................................................................... :  490
NPARL       421 :.......................................................................... :  490
POM-X       421 :.......................................................................... :  490
WW3         421 :.......................................................................... :  490
BRUDC-X     421 :.......................................................................... :  490
F310        421 :.......................................................................... :  490
Nitz.apicu  420 :..............................ug........................................... :  489
Bacl.paxil  419 :..............................ug.....................................u. :  488
Cylt.clost  421 :..............................ug.....................................u. :  490
Thss.rotul  420 :..............................uac....................................u. :  489
Sklt.costa  419 :..............................uac....................................u. :  488
Rhzs.setig  417 :............................u..a..a..a.............................u. :  486
Stpx.brosc  412 :..........g..u..................u.c.......................g..g.....a :  481

                    CCTTATCGAGTACAATTGGAGGGCAAGTCTGGTGCCAGCAGTAATTCCAGCTCCAATAGCGTATATTAAA
1917        491 :.......................................................................... :  560
1913        491 :.......................................................................... :  560
W2          491 :.......................................................................... :  560
PSEUD-X     491 :.......................................................................... :  560
TKA-2       491 :.......................................................................... :  560
13CC        491 :.......................................................................... :  560
NPARL       491 :.......................................................................... :  560
POM-X       491 :.......................................................................... :  560
WW3         491 :.......................................................................... :  560
BRUDC-X     491 :.......................................................................... :  560
F310        491 :.......................................................................... :  560
Nitz.apicu  490 :.......................................................................... :  559
Bacl.paxil  489 :..........g................................................................ :  558
Cylt.clost  491 :.......................................................................... :  560
Thss.rotul  490 :.......................................................................... :  559
Sklt.costa  489 :.......................................................................... :  558
Rhzs.setig  487 :..........g................................................................ :  556
Stpx.brosc  482 :..c.......g................................................................ :  551
```

Figure 2 continued.

```
                    GTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTGTGGTGTTTGAGGCCATGTTTGGTGGAATCTGTGTGGC
1917        561 :.......................................................................... :  630
1913        561 :.......................................................................... :  630
W2          561 :.......................................................................... :  630
PSEUD-X     561 :.......................................................................... :  630
TKA-2       561 :.......................................................................... :  630
13CC        561 :.......................................................................... :  630
NPARL       561 :.......................................................................... :  630
POM-X       561 :.......................................................................... :  630
WW3         561 :.....................................c.................................... :  630
BRUDC-X     561 :.......................................................................... :  630
F310        561 :.......................................................................... :  630
Nitz.apicu  560 :.....................................c..a.u......cc.........c......... :  629
Bacl.paxil  559 :.....................................c.....u......cc.........c......... :  628
Cylt.clost  561 :.....................................a..u......cc........uc......... :  630
Thss.rotul  560 :..............................c.a.c.c.cug......cc....ga..uc....u.... :  629
Sklt.costa  559 :..............................c...c.ac..u......cc....ga..uc....u.... :  628
Rhzs.setig  557 :............................a...c.......cug......cca...ga...cu.a.c.... :  626
Stpx.brosc  552 :............................a...c...c...g.u......cc....ga..gc......... :  621

                    ATTAAGTTGTCGTGCAGGGGATGCCCATCGTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCTTATG
1917        631 :.......................................................................... :  700
1913        631 :.......................................................................... :  700
W2          631 :.......................................................................... :  700
PSEUD-X     631 :.......................................................................... :  700
TKA-2       631 :.......................................................................... :  700
13CC        631 :.......................................................................... :  700
NPARL       631 :.......................................................................... :  700
POM-X       631 :.......................................................................... :  700
WW3         631 :.............a.u.a..g.................................................... :  700
BRUDC-X     631 :.............a.u.a..g.................................................... :  700
F310        631 :.............a.u.a..g.................................................... :  700
Nitz.apicu  630 :....g.........................a.......................................... :  699
Bacl.paxil  629 :....g.........................a.......................................... :  698
Cylt.clost  631 :.......................................................................... :  700
Thss.rotul  630 :........r...g.........a.................................u.................. :  699
Sklt.costa  629 :............g.........aa.................................u.................. :  698
Rhzs.setig  627 :............g.uu..ca..cuu....u.......................................... :  696
Stpx.brosc  622 :............g..g......c..................g............................. :  691

                    CCGTTGAATATATTAGCATGGAATAATGATATAGGACCTTGGTACTATTTTGTTGGTTTGCGCACTAAGG
1917        701 :.......................................................................... :  770
1913        701 :.......................................................................... :  770
W2          701 :.......................................................................... :  770
PSEUD-X     701 :.......................................................................... :  770
TKA-2       701 :.......................................................................... :  770
13CC        701 :.......................................................................... :  770
NPARL       701 :.......................................................................... :  770
POM-X       701 :.......................................................................... :  770
WW3         701 :.......................................................................... :  770
BRUDC-X     701 :.......................................................................... :  770
F310        701 :.......................................................................... :  770
Nitz.apicu  700 :...........g..............a.g.....................................c.... :  769
Bacl.paxil  699 :............................a.g.....................................cg... :  768
Cylt.clost  701 :............................agg.....................................c.... :  770
Thss.rotul  700 :............................a.g.......u.c..a.....................uuacg.a. :  769
Sklt.costa  699 :............................a.g.......u...agu.....................aguc..a. :  768
Rhzs.setig  697 :............................a.g.......ua.....................u....c.u.. :  766
Stpx.brosc  692 :....c......c................a.g.....................................uc.... :  761
```

Figure 2 continued.

```
                    TAATGATTAAGAGGGACAGTTGGGGGTATTTGTATTCCATTGTCAGAGGTGAATTCTTGGATTTTGGAAG
1917        771 :.......................................................................... :  840
1913        771 :.......................................................................... :  840
W2          771 :.......................................................................... :  840
PSEUD-X     771 :.......................................................................... :  840
TKA-2       771 :.......................................................................... :  840
13CC        771 :.......................................................................... :  840
NPARL       771 :.......................................................................... :  840
POM-X       771 :.......................................................................... :  840
WW3         771 :.......................................................................... :  840
BRUDC-X     771 :.......................................................................... :  840
F310        771 :.......................................................................... :  840
Nitz.apicu  770 :..........a...........u.......c.................................... :  839
Bacl.paxil  769 :..........u...................c.................................... :  838
Cylt.clost  771 :..........u...................c.....u.............................a... :  840
Thss.rotul  770 :..........u...................c.....u.g.........................c.a... :  839
Sklt.costa  769 :..........u...................c.....u.........................c.a... :  838
Rhzs.setig  767 :..........u...................c.................................a..... :  836
Stpx.brosc  762 :..........u...................c......a.........................c..... :  831

                    ACAAACTACTGCGAAAGCATTTACCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGA
1917        841 :.......................................................................... :  910
1913        841 :.......................................................................... :  910
W2          841 :.......................................................................... :  910
PSEUD-X     841 :.......................................................................... :  910
TKA-2       841 :.......................................................................... :  910
13CC        841 :.......................................................................... :  910
NPARL       841 :.......................................................................... :  910
POM-X       841 :.......................................................................... :  910
WW3         841 :.......................................................................... :  910
BRUDC-X     841 :.......................................................................... :  910
F310        841 :.......................................................................... :  910
Nitz.apicu  840 :..g........................................................................ :  909
Bacl.paxil  839 :..g........................................................................ :  908
Cylt.clost  841 :..g........................................................................ :  910
Thss.rotul  840 :..g........................................................................ :  909
Sklt.costa  839 :..g........................................................................ :  908
Rhzs.setig  837 :..g.....................................................a.................. :  906
Stpx.brosc  832 :..g.....................................................g.................. :  901

                    TGATTAGATACCATCGTAGTCTTAACCATAAACTATGCCGACAAGGGTTGGCGGAGTCTCCGTCAGCACC
1917        911 :.....................................a......u.........a.......u :  980
1913        911 :.....................................a......u.........a.......u :  980
W2          911 :.....................................a......u.........a.......u :  980
PSEUD-X     911 :.......................................................................... :  980
TKA-2       911 :.....................................................u.........a........ :  980
13CC        911 :.....................................................u.........a........ :  980
NPARL       911 :.....................................................u.........a........ :  980
POM-X       911 :.....................................................u.........a........ :  980
WW3         911 :.......................................................................... :  980
BRUDC-X     911 :.......................................................................... :  980
F310        911 :.......................................................................... :  980
Nitz.apicu  910 :.....................................................................c..... :  979
Bacl.paxil  909 :.......................................................................... :  978
Cylt.clost  911 :.....................................................u.........a........ :  980
Thss.rotul  910 :.....................................uc....c..a..u.a....u..g..... :  979
Sklt.costa  909 :.....................................uca.........u.a.............u :  978
Rhzs.setig  907 :.....................................a..u..a..c...a.u.a.a.u...g...u. :  976
Stpx.brosc  902 :.....................c.................a..u......a....u.a......u...... :  971
```

Figure 2 continued.

```
                    TTGTGAGAATCATAAGTCTTTGGGTTCGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGAAATTGACGG
1917        981 :.......................................................................... : 1050
1913        981 :.......................................................................... : 1050
W2          981 :.......................................................................... : 1050
PSEUD-X     981 :.......................................................................... : 1050
TKA-2       981 :.......................................................................... : 1050
13CC        981 :.......................................................................... : 1050
NPARL       981 :.......................................................................... : 1050
POM-X       981 :.......................................................................... : 1050
WW3         981 :.......................................................................... : 1050
BRUDC-X     981 :.......................................................................... : 1050
F310        981 :.......................................................................... : 1050
Nitz.apicu  980 :..a.........c.................................................................. : 1049
Bacl.paxil  979 :..a.........c.................................................................. : 1048
Cylt.clost  981 :..a.........c.................................................................. : 1050
Thss.rotul  980 :g.a.........-.................................c.g.......................... : 1048
Sklt.costa  979 :g.a.........-.................................................................. : 1047
Rhzs.setig  977 :..a.........-.................................................................. : 1045
Stpx.brosc  972 :..a.........-.................................................................. : 1040

                    AAGGGCACCACCAGGATGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTTACCAGGTCCAGACA
1917       1051 :.......................................................................... : 1120
1913       1051 :.......................................................................... : 1120
W2         1051 :.......................................................................... : 1120
PSEUD-X    1051 :.......................................................................... : 1120
TKA-2      1051 :.......................................................................... : 1120
13CC       1051 :.......................................................................... : 1120
NPARL      1051 :.......................................................................... : 1120
POM-X      1051 :.......................................................................... : 1120
WW3        1051 :.......................................................................... : 1120
BRUDC-X    1051 :.......................................................................... : 1120
F310       1051 :.......................................................................... : 1120
Nitz.apicu 1050 :.......................................................................... : 1119
Bacl.paxil 1049 :.......................................................................... : 1118
Cylt.clost 1051 :.......................................................................... : 1120
Thss.rotul 1049 :.....................a.-.................................................... : 1117
Sklt.costa 1048 :.....................a...................................................... : 1117
Rhzs.setig 1046 :.....................-...................................................... : 1114
Stpx.brosc 1041 :.....................-...................................................... : 1109

                    TAGTGAGGATGACAGATTGAGAGCCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGTG
1917       1121 :.......................................................................... : 1190
1913       1121 :.......................................................................... : 1190
W2         1121 :.......................................................................... : 1190
PSEUD-X    1121 :.......................................................................... : 1190
TKA-2      1121 :.......................................................................... : 1190
13CC       1121 :.......................................................................... : 1190
NPARL      1121 :.......................................................................... : 1190
POM-X      1121 :.......................................................................... : 1190
WW3        1121 :.......................................................................... : 1190
BRUDC-X    1121 :.......................................................................... : 1190
F310       1121 :.......................................................................... : 1190
Nitz.apicu 1120 :.......................................................................... : 1189
Bacl.paxil 1119 :.......................................................................... : 1188
Cylt.clost 1121 :.......................................................................... : 1190
Thss.rotul 1118 :.......................u.................................................... : 1187
Sklt.costa 1118 :.......................u.................................................... : 1187
Rhzs.setig 1115 :.......................................................................... : 1184
Stpx.brosc 1110 :.......................................................................... : 1179
```

Figure 2 continued.

```
                    GAGTGATTTGTCTGGTTAATTCCGTTACGAACGAGACCCCTGCCTGCTAAATAGTAGTGTTTATCCTGTT
1917       1191 :.............................................................. : 1260
1913       1191 :.............................................................. : 1260
W2         1191 :.............................................................. : 1260
PSEUD-X    1191 :.....................................................a........u.... : 1260
TKA-2      1191 :.............................................................. : 1260
13CC       1191 :.............................................................. : 1260
NPARL      1191 :.............................................................. : 1260
POM-X      1191 :.............................................................. : 1260
WW3        1191 :.............................................................. : 1260
BRUDC-X    1191 :.............................................................. : 1260
F310       1191 :.............................................................. : 1260
Nitz.apicu 1190 :.....................................................a..u....... : 1259
Bacl.paxil 1189 :.....................................................c....a.gu..... : 1258
Cylt.clost 1191 :.....................................................aa.u....... : 1260
Thss.rotul 1188 :.....................................g.c.............c.a.cg..u.gu.... : 1257
Sklt.costa 1188 :.....................................g.c.............c.a.ag..u.uu.... : 1257
Rhzs.setig 1185 :..u..........c......g.................c............a..a..a.....u.a.. : 1254
Stpx.brosc 1180 :.....................................c..............a..a..u..u.a.. : 1249

                    CTTAGAGGGACGTGCGTTCTATTAGACGCAGGAAGATAGGGGCAATAACAGGTCTGTGATGCCCTTAGAT
1917       1261 :.............................................................. : 1330
1913       1261 :.............................................................. : 1330
W2         1261 :.............................................................. : 1330
PSEUD-X    1261 :.............................................................. : 1330
TKA-2      1261 :.............................................................. : 1330
13CC       1261 :.............................................................. : 1330
NPARL      1261 :.............................................................. : 1330
POM-X      1261 :.............................................................. : 1330
WW3        1261 :.............................................................. : 1330
BRUDC-X    1261 :.............................................................. : 1330
F310       1261 :.............................................................. : 1330
Nitz.apicu 1260 :..............a..........u..................................... : 1329
Bacl.paxil 1259 :..............a..........u..................................... : 1328
Cylt.clost 1261 :..............a..........u..................................... : 1330
Thss.rotul 1258 :............u.a.....ca...u.a........g.c.....................u... : 1327
Sklt.costa 1258 :............u.a.....ca...u.a........g.c..................... : 1327
Rhzs.setig 1255 :...........u.uu.g.-g.c..ac.aa......u.g..................... : 1323
Stpx.brosc 1250 :...........u.u..g.-g.c..ac..a......u.g..................... : 1318

                    GTTCTGGGCCGCACGCGCGCTACACTGATGCATTCAACGAGTTCTACCTTGGCCGAGAGGCGGGAATCTT
1917       1331 :.............................................................. : 1400
1913       1331 :.............................................................. : 1400
W2         1331 :.............................................................. : 1400
PSEUD-X    1331 :.............................................................. : 1400
TKA-2      1331 :.............................................................. : 1400
13CC       1331 :.............................................................. : 1400
NPARL      1331 :.............................................................. : 1400
POM-X      1331 :.............................................................. : 1400
WW3        1331 :.............................................................. : 1400
BRUDC-X    1331 :.............................................................. : 1400
F310       1331 :.............................................................. : 1400
Nitz.apicu 1330 :..c.........................u...........u..................... : 1399
Bacl.paxil 1329 :..c.....................................ua.-.c....u....a....-..... : 1396
Cylt.clost 1331 :..c.................................................c.u..... : 1400
Thss.rotul 1328 :..c..............u...........c........cauaua.c.............u..... : 1397
Sklt.costa 1328 :..c..............u...........c........cauaua.c.............u..... : 1397
Rhzs.setig 1324 :.....................................c.uau.................... : 1393
Stpx.brosc 1319 :..c......g.............................a..cauaua.c.............u..... : 1388
```

Figure 2 continued.

```
                    TTGAACTTGCATCTGATAGGGATAGATTATTGCAATTATTAATCTTGAACGAGGAATTCCTAGTAAACGC
1917        1401 :......c.................................................................. : 1470
1913        1401 :......c.................................................................. : 1470
W2          1401 :......................................................................... : 1470
PSEUD-X     1401 :......................................................................... : 1470
TKA-2       1401 :......................................................................... : 1470
13CC        1401 :......................................................................... : 1470
NPARL       1401 :......................................................................... : 1470
POM-X       1401 :......................................................................... : 1470
WW3         1401 :......................................................................... : 1470
BRUDC-X     1401 :......................................................................... : 1470
F310        1401 :......c.................................................................. : 1470
Nitz.apicu  1400 :......g.................................................................. : 1469
Bacl.paxil  1397 :......g.................................................................. : 1466
Cylt.clost  1401 :......c.................................................................. : 1470
Thss.rotul  1398 :g.u.....................................................................u... : 1467
Sklt.costa  1398 :g.u.....................................................................u... : 1467
Rhzs.setig  1394 :..a...g.................................................................. : 1463
Stpx.brosc  1389 :.ga...g.................................................................. : 1458

                    AGATCATCATCTGCATTGATTACGTCCCTGCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGG
1917        1471 :..u......a............................................................... : 1540
1913        1471 :..u......a............................................................... : 1540
W2          1471 :......................................................................... : 1540
PSEUD-X     1471 :......................................................................... : 1540
TKA-2       1471 :......................................................................... : 1540
13CC        1471 :......................................................................... : 1540
NPARL       1471 :......................................................................... : 1540
POM-X       1471 :......................................................................... : 1540
WW3         1471 :......................................................................... : 1540
BRUDC-X     1471 :......................................................................... : 1540
F310        1471 :......................................................................... : 1540
Nitz.apicu  1470 :......................................................................... : 1539
Bacl.paxil  1467 :......................................................................... : 1536
Cylt.clost  1471 :......................................................................... : 1540
Thss.rotul  1468 :g.u......a.c...a.......................................................... : 1537
Sklt.costa  1468 :..u......a.....a.......................................................... : 1537
Rhzs.setig  1464 :......................................................................... : 1533
Stpx.brosc  1459 :.....................................................................u : 1528

                    TCCGGTGAAGCCTCGGGATTGTGCCTTTTTGGTGTCACGAGAACTTGTCTAAACCTTATCATTTAGAGGA
1917        1541 :..............a..................ac.................................... : 1610
1913        1541 :..............a..................ac.................................... : 1610
W2          1541 :.....................................u.u......c.......................... : 1610
PSEUD-X     1541 :.............................................c.......................... : 1610
TKA-2       1541 :......................................................................... : 1610
13CC        1541 :......................................................................... : 1610
NPARL       1541 :......................................................................... : 1610
POM-X       1541 :......................................................................... : 1610
WW3         1541 :......................................................................... : 1610
BRUDC-X     1541 :......................................................................... : 1610
F310        1541 :......................................................................... : 1610
Nitz.apicu  1540 :.....................................g................................... : 1609
Bacl.paxil  1537 :.....................................g................................... : 1606
Cylt.clost  1541 :............................c........g................................... : 1610
Thss.rotul  1538 :........g.a..............c.....ac.g.............cg..................... : 1607
Sklt.costa  1538 :........g.ag...a..................uc.g.......c.c..c..................... : 1607
Rhzs.setig  1534 :........g..............u-.......acugu......c....c..................... : 1602
Stpx.brosc  1529 :........g.a..........au..........a.a.u.c.....c...c..................... : 1598
```

Figure 2 continued.

```
                  AGGTAGTCGTAACAAGGTTTCCG
1917       1611 :....................... : 1633
1913       1611 :....................... : 1633
W2         1611 :....................... : 1633
PSEUD-X    1611 :....................... : 1633
TKA-2      1611 :....................... : 1633
13CC       1611 :....................... : 1633
NPARL      1611 :....................... : 1633
POM-X      1611 :....................... : 1633
WW3        1611 :....................... : 1633
BRUDC-X    1611 :....................... : 1633
F310       1611 :....................... : 1633
Nitz.apicu 1610 :....................... : 1632
Bacl.paxil 1607 :....................... : 1629
Cylt.clost 1611 :....................... : 1633
Thss.rotul 1608 :....................... : 1630
Sklt.costa 1608 :....................... : 1630
Rhzs.setig 1603 :....................... : 1625
Stpx.brosc 1599 :....................... : 1621
```

Figure 2 continued.

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APPENDIX 6

Appendix VI

Raw data of the morphometric analysis of *Pseudo-nitzschia* species recorded under the scanning electron microscope.

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)
1	120.00	3.15	10	10	10	3.0
2	81.66	3.44	11	11	11	3.0
3	98.32	2.26	11	11	11	3.0
4	146.64	2.81	11	11	11	3.0
5	95.30	2.99	11	11	11	3.0
6	151.45	2.73	11	11	11	3.0
7	102.60	2.71	11	11	11	3.0
8	86.64	3.19	11	11	11	3.0
9	95.68	2.96	11	12	12	3.0
10	130.12	3.65	11	11	11	3.0
11	115.69	3.73	12	12	12	3.0
12	118.52	3.64	12	12	12	3.0
13	125.41	2.95	12	12	12	3.5
14	119.86	3.07	12	12	12	3.0
15	88.54	3.43	12	11	12	2.0
16	120.21	3.36	12	12	12	2.5
17	104.84	3.78	12	12	12	3.0
18	119.50	3.20	12	12	12	3.5
19	101.63	3.52	12	12	12	3.0
20	113.84	3.60	12	12	12	3.0
21	110.00	4.20	12	12	12	3.0
22	61.00	3.50	12	12	12	3.0
23	90.62	3.17	12	12	12	3.0
24	144.87	3.43	12	12	12	3.0
25	146.37	3.40	12	12	12	3.0
26	139.10	2.91	12	12	12	3.0
27	122.69	2.92	12	12	12	3.0
28	148.30	2.70	12	12	12	3.0
29	109.94	2.58	12	12	12	3.0
30	100.85	3.69	12	12	12	4.0
31	98.50	2.93	12	12	12	3.0
32	102.22	3.05	12	12	12	3.0
33	145.86	3.22	12	12	12	3.0
34	85.64	3.69	12	12	12	3.0
35	75.90	3.42	12	12	12	3.0
36	96.97	3.70	12	12	12	3.0
37	96.34	3.07	12	12	12	3.0
38	94.48	2.46	12	12	12	3.0
39	100.49	3.59	12	12	12	3.0
40	117.10	3.03	12	12	12	3.0
41	120.00	3.00	12	11	12	4.0
42	117.00	3.40	12	12	12	n/d
43	107.65	3.28	13	13	13	3.0
44	89.36	3.01	13	13	13	3.0
45	143.35	3.67	13	13	13	3.0
46	94.00	3.60	13	13	13	3.0
47	99.00	3.80	13	13	13	3.0
48	85.00	4.00	13	13	13	3.0
49	99.00	4.05	13	12	12	3.0
50	91.23	3.08	13	13	13	3.0

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)
51	88.54	2.85	13	13	13	3.0
52	155.66	3.84	13	13	13	3.0
53	157.77	4.27	13	13	13	3.0
54	109.33	3.33	13	13	13	4.0
55	95.37	3.04	13	13	13	3.5
56	106.65	2.85	13	13	13	3.0
57	94.18	2.99	13	12	13	3.0
58	93.47	3.25	13	13	13	3.0
59	152.68	2.79	13	13	13	3.0
60	90.81	3.00	13	13	13	3.0
61	94.87	3.14	13	13	13	2.5
62	n/d	2.80	13	13	13	4.0
63	106.39	3.41	13	13	13	3.0
64	101.13	3.28	13	13	13	2.5
65	90.64	3.44	13	13	13	2.5
66	85.75	3.01	13	13	13	3.0
67	81.16	2.22	13	13	13	3.5
68	95.08	2.59	13	13	13	3.0
69	116.67	3.51	13	13	13	2.5
70	85.00	3.90	13.3	13.3	13.3	2.5
71	111.00	3.00	13.33	13.33	13.33	3.0
72	99.00	3.12	13.33	13.33	13.33	3.0
73	108.00	3.40	13.33	13.33	13.33	3.0
74	125.00	4.56	13.9	13.9	13.9	2.0
75	94.50	4.00	14	14	14	3.0
76	87.50	3.80	14	16	14	3.0
77	120.00	5.40	14	14	14	4.0
78	125.40	4.00	14	14	14	3.0
79	117.80	3.00	14	12	12	3.0
80	n/d	2.25	14	13	14	4.0
81	87.93	3.35	14	14	14	3.0
82	74.90	2.77	14	13	14	3.0
83	87.11	2.88	14	14	14	4.0
84	89.25	2.74	14	14	14	3.0
85	102.18	3.10	14	13	14	3.0
86	109.88	3.29	14	14	14	3.0
87	117.00	4.00	14	14	14	3.0
88	135.63	2.73	14	14	14	3.5
89	108.00	2.60	14	14	14	3.0
90	108.00	2.97	14	14	14	3.0
91	102.00	4.30	16	14	16	3.0
92	89.10	4.00	16	15	15	3.0
93	n/d	2.87	16	14	16	4.0
94	107.90	3.75	16.5	16.5	16.5	3.4
95	123.00	3.50	n/d	n/d	n/d	n/d
96	98.00	4.00	n/d	n/d	n/d	n/d
mean	107.51	3.30	12.76	12.65	12.73	3.06
STDEV ±	20.25	0.54	1.17	1.09	1.13	0.37

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstria (in 10 µm)	No. of poroids (in 1 µm)
1	10.0	10.0	10.0	–
1	16.5	15.0	15.0	–
1	–	16.0	16.5	–
1	–	16.5	–	–
2	16.0	–	16.0	2.0
4	13.3	13.3	13.3	–
6	–	–	–	2.5
8	–	–	–	3.5
8	–	–	11.0	4.0
9	11.0	–	–	–
10	–	11.0	–	–
14	–	14.0	–	–
16	–	–	14.0	–
18	14.0	–	–	–
26	–	–	13.0	–
27	13.0	–	–	–
28	–	13.0	–	–
32	12.0	–	–	–
34	–	12.0	–	–
35	–	–	12.0	–
71	–	–	–	3.0

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)
1	127.64	4.14	12	12	12	6
2	80.48	4.08	12	12	12	6
3	94.66	3.68	13	13	13	6
4	86.00	4.20	13.33	13.33	13.33	7
5	109.98	3.75	14	15	15	6
6	98.00	4.00	14	14	14	7
7	98.00	4.00	14	14	14	6
8	80.20	3.89	14	14	14	7
9	85.75	4.23	14	14	14	6
10	96.00	4.80	14.8	14.8	14.8	6
11	91.01	4.56	15	15	15	n/d
12	93.00	4.00	15.78	15.78	15.78	6
13	n/d	2.70	16	15	16	7
14	92.00	3.00	16	15	16	7
15	n/d	2.83	16	16	16	7
16	n/d	2.83	16	16	16	7
mean	94.82	3.79	14.37	14.31	14.43	6.47
STDEV ±	12.69	0.63	1.37	1.26	1.37	0.52

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstria (in 10 µm)	No. of poroids (in 1 µm)
1	13	13	13	–
1	13.3	13.3	13.3	–
2	12	12	12	–
3	15	16	–	–
4	–	–	14	–
4	–	–	15	–
5	14	14	–	–
5	16	15	16	–
7	–	–	–	7
8	–	–	–	6

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)
Cell #	Length (µm)	Width (µm)	Fibulae	Striae	Interstriae	Poroids
1	130.7	6.16	15	17	17	8
2	153.34	5.54	20	20	20	8
3	113.3	5.83	20	20	20	8
4	108	6	20	20	20	7
mean	126.34	5.88	18.75	19.25	19.25	7.75
STDEV ±	20.45	0.27	2.50	1.50	1.50	0.50

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstria (in 10 µm)	No. of poroids (in 1 µm)
1	15	17	17	7
3	20	20	20	8

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)
1	65	5.5	19	18	19	8
2	125.88	5.73	18	18	18	6
mean	95.44	5.615	18.5	18	18.5	7
STDEV ±	43.05	0.16	0.71	0.00	0.71	1.41

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstria (in 10 µm)	No. of poroids (in 1 µm)
1	19	-	19	8
1	18	-	18	6
2	18	18	-	-

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)
1	128.25	6.52	14	17	17	5.0
2	114.15	7.06	14	16	16	5.0
3	103.22	6.54	15	15	15	5.0
4		5.80	15	18	18	6.0
5	123.75	7.44	15	16	16	5.0
6	80.69	7.25	15	15	15	5.0
7	113.62	6.03	15	17	17	5.0
8	114.28	6.56	15	16	16	5.0
9	118.70	6.28	15	14	15	5.0
10	124.62	6.06	15	15	15	5.0
11	83.79	6.61	15	17	17	5.0
12	110.74	6.98	15	16	16	5.5
13	91.00	6.63	15	16	16	5.0
14	120.45	7.12	15	15	15	5.0
15	104.02	6.35	15	15	15	5.0
16	107.40	7.24	15	15	15	4.0
17	87.17	7.42	15	16	16	5.0
18	108.90	7.06	15	15	15	5.0
19	109.61	6.03	16	16	16	5.0
20	102.75	6.52	16	16	16	5.0
21	81.09	6.58	16	16	16	5.0
22	85.25	6.42	16	16	16	5.0
23	79.22	6.85	16	16	16	5.0
24	77.44	6.99	16	16	16	4.0
25	80.00	5.66	16	16	16	5.0
26	82.55	5.30	16	16	16	4.0
27	78.90	7.27	16	16	16	6.0
28	93.28	5.82	16	15	16	5.5
29	84.70	6.98	16	17	18	5.0
30	113.39	6.99	16	16	16	5.0
31	118.49	6.20	16	16	16	5.0
32	74.22	6.90	16	17	17	5.0
33	112.40	6.29	16	15	16	5.0
34	101.37	6.58	16	17	17	5.0
35	76.97	6.17	16	16	16	5.0
36	124.15	6.29	16	16	17	5.0
37	143.09	5.71	16	15	16	5.0
38	96.83	5.56	16	17	17	5.0
39	114.77	6.16	16	17	17	5.0
40	68.04	6.18	16	16	16	5.0
41	80.20	6.71	16	17	17	5.0
42	79.69	6.35	16	16	16	5.0
43	83.12	6.20	16	16	16	5.0
44	92.63	6.16	16	16	16	5.0
45	65.00	6.40	16	16	16	5.0
46	106.29	6.80	16	16	16	5.0
47	113.74	7.52	16	16	16	5.0
48	94.80	6.55	16	16	16	5.0
49	111.84	6.92	16	16	16	4.0
50	108.57	6.61	16	16	16	5.0
51	94.47	6.22	16	17	16	5.0
52	94.98	6.79	16	17	18	5.0
53	92.40	6.14	16	16	16	4.5
54	108.59	6.25	16	16	16	5.5
55	92.21	7.36	17	18	18	5.0
56	87.18	6.01	17	17	17	5.0

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)
57	81.57	6.70	17	17	17	5.5
58	86.15	6.09	17	17	17	6.0
59	88.38	7.25	17	16	17	4.0
60	84.01	6.52	17	17	17	5.0
61	83.06	5.79	17	16	17	5.0
62	78.32	6.56	17	17	17	5.0
63	112.61	6.59	17	18	18	5.0
64	80.97	6.98	17	17	17	5.0
65	89.09	6.96	17	16	17	5.0
66	82.96	6.99	17	16	16	5.0
67	118.21	6.72	17	17	17	5.0
68	114.49	7.65	17	17	17	5.0
69	78.90	6.26	17	17	17	5.0
70	122.99	6.83	17	17	17	5.0
71	117.07	6.55	17	17	17	5.6
72	74.54	6.43	17	17	17	5.0
73	62.69	6.11	17	17	17	5.0
74	79.83	5.89	17	17	17	5.0
75	72.47	6.38	17	17	18	4.5
76	83.09	6.03	17	17	17	5.5
77	69.75	6.80	17	17	17	5.0
78	78.78	5.42	17	17	17	5.5
79	57.50	6.25	17	17	17	5.0
80	90.87	6.89	17	17	17	5.0
81	106.51	6.72	17	16	17	5.0
82	88.08	7.10	17	16	17	5.5
83	107.03	7.43	17	16	17	5.0
84	85.68	7.46	17	17	17	5.0
85	106.33	6.69	17	17	17	5.0
86	88.00	6.76	17	17	17	5.0
87	94.22	6.56	17	17	17	5.0
88	95.23	6.98	17	17	17	5.0
89	88.00	7.48	17	17	17	5.0
90	92.49	6.24	17	17	17	4.0
91	91.84	7.35	17	17	17	4.0
92	89.84	6.70	17	17	17	4.0
93	101.85	6.82	17	17	17	5.0
94	113.06	6.57	17	17	17	5.0
95	98.56	6.08	17	17	17	5.0
96	99.98	6.44	17	17	17	5.0
97	90.39	5.95	17	16	17	
98	84.05	7.12	18	18	18	6.0
99	71.86	6.74	18	18	18	5.5
100	136.24	6.49	18	18	18	6.0
101		6.00	18	18	18	
102	90.00	5.60	18	18	19	5.5
103	76.00	5.75	18	18	18	5.5
104	126.44	6.92	18	18	18	5.0
105	116.88	6.71	18	18	18	5.0
106	112.91	6.61	18	18	18	5.0
107	81.71	6.43	18	18	18	5.0
108	77.82	6.98	18	18	18	5.0
109	80.54	6.77	18	17	17	6.0
110	91.96	6.20	18	18	18	5.0
111	79.57	6.66	18	18	18	6.0
112	72.95	6.87	18	18	18	4.0

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)
113	72.33	6.61	18	18	18	5.0
114	79.49	6.39	18	17	18	5.5
115	91.24	6.74	18	18	18	5.0
116	92.63	6.94	18	17	18	5.0
117	102.05	7.79	18	18	18	5.0
118	90.78	7.17	18	18	18	5.0
119	109.39	7.08	18	18	18	5.0
120	115.81	7.02	18	18	18	5.0
121		6.80	19	18	19	6.5
122	96.62	6.84	19	19.2	19.5	5.0
123	91.81	6.91	19	19	19	5.0
124	96.61	6.50	19	19	19	4.5
125	88.42	7.28	19	19	19	4.0
126	113.05	6.82	19	19	19	3.5
127	63.00	6.00	20	19	20	5.0
mean	94.89	6.60	16.72	16.80	16.95	5.01
STDEV ±	17.04	0.50	1.14	1.02	1.03	0.50

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstri (in 10 µm)	No. of poroids (in 1 µm)
1	20	14	20	3.5
1	–	–	–	6.5
2	14	–	–	–
3	–	–	–	4.5
6	19	19	–	–
7	–	–	19	6.0
8	–	–	15	–
10	–	15	–	4.0
12	–	–	–	5.5
14	–	–	–	–
16	15	–	–	–
23	18	–	–	–
24	–	18	–	–
27	–	–	18	–
35	–	–	16	–
36	16	–	–	–
39	–	16	–	–
43	17	–	–	–
47	–	17	–	–
49	–	–	17	–
91	–	–	–	5.0

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)	No. of striae central interspace
1	90.82	7.20	17	17	17	5.0	n/d
2	77.36	5.82	21	21	21	5.0	n/d
3	79.91	5.03	21	22	22	n/d	n/d
4	80.06	6.58	21	22	22	5.0	4.0
5	86.68	6.53	21	23	23	5.0	3.5
6	73.59	5.59	22	22	22	5.5	4.0
7	78.84	5.74	22	22	22	5.5	3.0
8	74.47	5.69	22	22	22	5.0	4.0
9	72.59	5.04	22	22	22	6.0	4.0
10	72.33	4.88	22	22	22	5.0	3.0
11	76.65	6.04	22	22	22	5.0	3.0
12	n/d	4.75	22	26	26	6.0	4.0
13	66.00	6.50	22	22	22	6.0	4.0
14	84.40	5.56	22	22	22	5.0	3.0
15	72.36	5.22	22	22	22	5.5	3.0
16	102.13	6.28	22	22	22	6.0	4.0
17	n/d	6.00	22	22	22	6.0	3.5
18	82.78	5.86	22	22	22	5.0	3.0
19	81.36	6.44	22	23	22	5.0	4.0
20	81.53	5.79	22	22	22	6.0	4.0
21	75.01	6.35	22	23	22	5.0	4.0
22	76.64	6.90	22	22	22	6.0	4.0
23	78.83	5.97	22	22	22	5.0	4.0
24	78.10	5.42	22	24	24	5.0	4.0
25	78.44	5.35	22	22	22	5.0	4.0
26	75.68	5.94	22	24	25	6.0	4.0
27	81.27	6.40	22	22	22	6.0	3.0
28	81.09	8.22	22	22	22	5.0	4.0
29	70.90	6.09	22	22	22	5.0	4.0
30	85.01	6.53	23	23	23	5.0	3.0
31	84.82	5.94	23	22	23	6.0	4.0
32	82.14	6.30	23	22	23	5.5	4.0
33	82.75	6.87	23	22.5	23	5.0	4.0
34	78.06	6.49	23	22	23	5.5	4.0
35	70.78	5.48	23	23	23	6.0	3.0
36	80.45	6.55	23	23	23	5.0	4.0
37	79.02	5.67	23	23	23	5.5	3.0
38	86.00	5.59	23	22	23	5.0	4.0
39	80.19	5.71	23	23	23	5.5	4.0
40	75.66	5.67	23	23	23	5.0	4.0
41	77.66	5.99	23	23	23	6.0	3.0
42	75.47	5.63	23	23	23	5.0	3.0
43	78.82	6.31	23	23	23	n/d	4.0
44	85.45	6.91	23	23	23	n/d	3.0
45	70.60	5.66	23	22	23	5.0	3.0
46	77.48	5.71	23	23	23	5.0	4.0
47	76.09	5.88	23	23	23	5.0	4.0
48	94.00	5.30	23	24	25	6.0	4.0
49	n/d	5.40	23	24	24	6.0	4.0
50	77.53	4.82	23	23	23	5.0	3.0
51	93.63	5.45	23	23	23	5.0	3.0
52	83.34	5.38	23	23	23	5.0	n/d
53	74.30	5.45	23	23	23	5.0	n/d
54	81.62	5.39	23	23	23	6.0	3.0
55	84.28	4.91	23	23	23	6.0	3.0
56	78.72	5.95	23	23	23	5.0	4.0
57	73.40	6.25	23	23	23	7.0	4.0

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)	No. of striae central interspace
58	86.63	6.41	23	23	23	6.0	4.0
59	78.13	6.32	23	23	23	5.0	3.0
60	80.76	5.38	23	23	23	5.0	3.0
61	75.40	5.08	23	23	23	5.0	3.0
62	85.89	5.14	23	23	23	5.0	4.0
63	98.66	5.23	23	23	23	7.0	3.0
64	101.78	5.03	23	23	23	5.5	3.0
65	99.93	4.07	23	23	23	5.0	4.0
66	85.34	5.12	23	24	24	5.0	3.0
67	102.86	4.72	23	23	23	5.0	4.0
68	81.16	5.30	23	23	23	5.0	4.0
69	77.92	5.40	23	23	23	n/d	3.0
70	80.50	5.61	23	23	23	n/d	3.0
71	73.65	6.05	23	24	24	n/d	3.0
72	73.76	6.06	23	23	23	5.0	3.0
73	75.76	5.83	23	23	23	n/d	4.0
74	75.89	5.57	23	23	23	5.5	3.5
75	93.53	5.46	23	24	24	6.0	n/d
76	74.47	5.94	23	24	24	5.5	4.0
77	81.41	5.70	23	23	23	5.5	4.0
78	80.39	5.91	23	22	23	5.0	4.0
79	79.36	6.17	23	23	23	5.0	3.0
80	73.23	6.38	23	24	23	5.0	3.0
81	80.91	6.06	23	22	23	5.5	3.0
82	81.52	6.40	23	22.5	23	5.5	4.0
83	76.14	5.89	23	23	23	5.0	4.0
84	77.73	5.66	23	23	23	5.0	4.0
85	81.48	5.39	23	23	24	5.0	3.0
86	79.69	6.14	24	24	24	6.0	3.0
87	80.35	5.81	24	23	24	6.0	3.0
88	57.20	5.90	24	24	24	6.0	3.0
89	57.00	6.30	24	24	24	6.0	4.0
90	67.50	5.00	24	24	24	6.0	4.0
91	66.24	5.01	24	24	24	6.0	3.0
92	70.00	4.75	24	24	24	5.5	4.0
93	80.38	5.53	24	24	24	5.0	3.0
94	70.40	6.00	24	24	24	n/d	3.0
95	75.29	5.57	24	24	24	6.0	4.0
96	99.62	5.42	24	24	24	5.0	4.0
97	75.86	5.39	24	23	24	5.0	4.0
98	102.21	5.22	24	24	24	6.0	3.0
99	81.94	5.45	24	24	24	5.0	4.0
100	79.83	5.60	24	24	24	5.0	3.0
101	78.54	5.78	24	24	24	4.5	3.0
102	70.77	5.60	24	24	24	5.0	4.0
103	78.47	5.65	24	24	24	6.0	n/d
104	75.16	5.71	24	24	25	6.0	4.0
105	69.97	4.94	24	24	25	6.5	3.0
106	70.58	5.16	24	24	24	6.0	3.5
107	70.67	6.60	24	24	24	6.0	4.0
108	75.90	5.65	24	24	25	6.0	4.0
109	74.74	5.05	24	24	24	6.0	3.0
110	71.96	6.49	24	24	24	n/d	4.0
111	80.13	5.35	24	23	24	5.0	3.0
112	72.86	5.56	24	24	24	6.0	3.0
113	74.15	5.56	24	24	24	5.0	3.0
114	74.77	6.41	24	24	24	5.5	3.0

Cell no.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)	No. of striae central interspace
115	74.98	5.98	24	24	24	6.0	3.0
116	77.13	5.77	24	23	24	5.5	4.0
117	75.34	6.00	24	24	24	5.5	3.0
118	75.78	5.60	24	23	24	5.0	4.0
119	106.50	5.84	24	24	24	5.0	4.0
120	76.53	5.20	24	24	24	5.0	4.0
121	80.02	5.92	24	24	24	5.0	4.0
122	75.50	6.22	24	24	24	5.0	4.0
123	70.79	5.89	24	23	24	6.0	4.0
124	78.87	6.47	24	24	24	5.5	4.0
125	81.39	6.01	24	24	24	5.0	3.0
126	76.94	5.71	24	24	24	5.0	3.5
127	76.42	6.02	25	25	25	6.0	3.0
128	74.15	6.23	25	24	25	5.5	3.0
129	n/d	5.00	25	25	26	6.0	4.0
130	n/d	5.58	25	25	25	n/d	3.0
131	74.86	4.88	25	25	25	6.0	3.0
132	90.00	5.40	25	24	25	7.0	4.0
133	71.75	5.97	26	23	23	6.0	4.0
134	69.00	6.00	n/d	n/d	n/d	n/d	n/d
135	67.00	6.90	n/d	n/d	n/d	n/d	n/d
136	100.00	6.00	n/d	n/d	n/d	n/d	n/d
137	111.89	4.75	n/d	n/d	n/d	n/d	n/d
138	74.90	5.76	n/d	n/d	n/d	5.0	3.0
139	75.58	5.73	n/d	n/d	n/d	n/d	n/d
140	75.98	6.28	n/d	n/d	n/d	n/d	3.0
mean	79.29	5.77	23.14	23.15	23.30	5.45	3.5
STDEV ±	8.81	0.58	1.06	1.02	1.08	0.52	0.5

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstria (in 10 µm)	No. of poroids (in 1 µm)
1	17.0	17.0	17.0	4.5
1	26.0	21.0	21.0	6.5
1	–	26.0	–	–
2	–	22.5	26.0	–
3	–	–	–	7.0
4	21.0	25.0	–	–
6	25.0	–	–	–
10	–	–	25.0	–
19	–	–	–	5.5
24	22.0	–	–	–
23	–	–	22.0	–
28	–	22.0	–	–
39	–	–	–	6.0
41	24.0	–	–	–
45	–	–	24.0	–
46	–	24.0	–	–
50	–	23.0	–	–
51	–	–	23.0	–
56	23.0	–	–	–
61	-	–	–	5.0

Cell No.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)	No. of striae central interspace
1	62.62	4.64	19	30	30	9	3
1	59.61	4.39	19	31	31	9.5	3
mean	61.12	4.52	19.00	30.50	30.50	9.25	3.00
STDEV ±	2.13	0.18	0.00	0.71	0.71	0.35	0.00

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstria (in 10 µm)	No. of poroids (in 1 µm)
1	–	30	30	9
1	–	31	31	9.5
2	19	–	–	–

Cell No.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)	No. of striae per central interspace
1	n/d	1.5	20	38	38	10	3
2	41.61	1.59	22	40	40	12	3
3	53.47	1.42	22	39	39	10	3
4	54.99	1.34	22	39	39	10	3
5	37.45	1.48	22	39	39	12	3
6	32.5	1.68	22.5	41	41	10	n/d
7	36.51	1.84	23	40	40	11	3
8	35.76	1.68	23	40	40	12	3.5
9	35.47	2.23	23	40	40	11	3
10	31.94	1.87	23	40	40	12	3
11	60.79	1.49	23	39	39	**6**	3
12	28.12	1.54	23	40	40	12	3
13	47.5	1.5	23	40	44	10.5	n/d
14	49.5	1.37	23	40	40	12	3
15	47.91	1.51	23	39	39	10	3
16	51.89	1.39	23	40	40	11	3.5
17	75.04	1.72	23	40	40	n/d	3
18	39.08	1.42	23	40	40	11	3
19	51.51	1.45	23	39	39	10	3
20	48.61	1.48	23	39	39	11	2
21	48.28	1.27	23	41	41	10	3
22	42.58	1.44	23	40	40	12	3
23	52.2	1.39	23	40	40	10	3.5
24	52.95	1.56	23	40	40	10	3
25	42.49	1.54	23	39	39	9	3
26	n/d	n/d	23	39	39	12	3
27	33	2.25	24	44.5	44	8.5	3.5
28	35.36	2.38	24	39	39	11	4
29	36.08	1.89	24	40	40	12	3
30	35	2.03	24	39	39	12	3
31	34.88	2.13	24	40	40	12	3
32	35.01	1.87	24	40	40	12	3
33	34.31	2.06	24	40	40	11	3
34	47.54	1.35	24	40	40	n/d	3
35	47.41	1.47	24	39	39	12	3.5
36	28.97	1.86	24	44	44	n/d	3.5
37	27.03	1.54	24	40	40	12	3
38	25.14	1.64	24	40	40	12	3
39	26.27	1.82	24	40	40	12	3
40	26.05	1.62	24	39	39	12	3
41	25.06	1.91	24	41	41	12	3
42	28.02	1.63	24	40	40	11	3
43	30.6	1.79	24	40	40	12.5	3
44	45.52	1.52	24	40	40	12	3.5
45	42.47	1.53	24	40	40	11	3
46	38.68	1.64	24	40	40	12	3
47	67.76	1.72	24	39	39	11	3
48	57	1.66	24	40	40	n/d	3.5
49	34.9	1.74	24	40	40	10	3
50	35.9	1.4	24	40	40	12	3
51	46.37	1.41	24	39	39	10	3
52	39.76	1.45	24	40	40	10	3
53	n/d	1.7	24.5	41	42	11.5	n/d
54	27.21	1.7	25	40	40	12	3
55	27.51	1.95	25	44	44	11	3
56	23.54	1.83	25	41	41	n/d	3
57	26.87	1.92	25	41	41	n/d	3

Cell No.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)	No. of striae per central interspace
58	49	1.55	25	40	40	11	3
59	49.71	1.56	25	40	40	11	3
60	49.06	1.43	25	40	40	12	3.5
61	49.35	1.57	25	40	40	12	3
62	50.33	1.34	26	39	39	10	3
63	32.5	1.75	26	40	43	10	n/d
64	32.75	1.75	26	40	40	10	3
65	64.12	1.52	26	41	41	11	3.5
66	24.61	1.83	26	41	41	13	3
67	42	1.2	27	44	48	10	3
68	n/d	1.29	27	40	40	10.5	n/d
69	41.43	1.57	27.5	45	46	11	n/d
70	42.8	2	28	46	46	9	n/d
71	28.82	2.16	29	40	40	10	3
72	n/d	1.95	n/d	40	47	8.5	3
73	25.74	1.9	n/d	n/d	n/d	n/d	n/d
74	27.06	1.91	n/d	n/d	n/d	n/d	n/d
75	25.96	1.75	n/d	n/d	n/d	n/d	n/d
76	41	1.33	n/d	40	40	n/d	n/d
mean	40.05	1.66	24.05	40.24	40.51	10.94	3.08
STDEV ±	11.36	0.25	1.48	1.46	1.94	1.20	0.25

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstria (in 10 µm)	No. of poroids (in 1 µm)
1	20.0	38.0	38.0	6.0
1	22.5	44.5	42.0	11.5
1	24.5	45.0	43.0	12.5
1	25.5	46.0	47.0	13.0
1	28.0	–	48.0	–
1	28.0	–	–	–
2	27.0	–	46.0	8.5
2	–	–	–	9.0
2	–	–	–	10.5
3	–	44.0	–	–
4	22.0	–	44.0	–
5	26.0	–	–	–
7	–	–	41.0	–
8	25.0	41.0	–	–
15	–		–	11.0
16	–	39.0	39.0	–
17	–	–	–	10.0
20	23.0	–	–	–
24	–	–	–	12.0
26	24.0	–	–	–
39	–	–	40.0	–
42	–	40.0	–	–

Cell No.	Length (µm)	Width (µm)	Fibulae (in 10 µm)	Striae (in 10 µm)	Interstriae (in 10 µm)	Poroids (in 1 µm)	No. of striae central interspace
1	82.92	1.58	22	41	41	4	5
2	56.42	1.62	23	42	42	5	3
3	66.4	1.21	23	43	43	4.5	3
4	55.55	1.23	23	42	42	5	3.5
5	53.78	1.52	23	43	43	5	3
6	71.25	1.32	23	42	42	4.5	n/d
7	58.1	1.54	23	42	42	4	n/d
8	70.82	1.12	23	43	43	4	5
9	48.86	1.3	23	42	42	5	4
10	47.43	1.44	23	44	44	5	4
11	60.79	1.49	23	39	39	6	3
12	63.44	1.37	23	42	42	4	4.5
13	52.92	1.37	24	42	42	4	4
14	69.36	1.25	24	41	41	5	3.5
15	78.66	1.32	24	43	43	4	n/d
16	59.46	1.43	24	42	42	5	n/d
17	59.83	1.34	24	42	42	5	3
18	84.64	1.25	24	44	44	5	3
19	62.71	1.14	24	42	42	4.5	4
20	42.22	1.17	24	40	40	4	4
21	48.71	1.4	24	43	43	5	4.5
22	57.81	1.25	24	41	41	5	4
23	27.41	1.89	25	40	40	4	3
24	60.31	1.55	25	42	42	4.5	4
25	76.89	1.29	25	43	43	4	4
26	70.24	1.13	25	40	40	4	4
27	53.81	1.3	25	43	43	5	3.5
28	61.24	1.28	25	43	43	4	4.5
29	52.06	1.28	25	42	42	5.6	3.5
30	63.05	1.34	26	43	43	5	3.5
31	58.86	1.36	26	42	42	4	4
32	65.14	1.32	26	44	44	5	3
33	n/d	1.35	26	43	44	7	n/d
34	56.14	1.05	26	43	43	4	3.5
35	66.22	1.64	26	44	44	4	4.5
36	n/d	n/d	30	40	40	6	n/d
37	62.44	1.21	n/d	n/d	n/d	n/d	n/d
mean	60.74	1.35	24.33	42.14	42.17	4.68	3.77
STDEV ±	11.33	0.17	1.49	1.25	1.28	0.71	0.61

No. of Cells	No. of fibulae (in 10 µm)	No. of striae (in 10 µm)	No. of interstria (in 10 µm)	No. of poroids (in 1 µm)
1	22	39	39	5.6
1	30	–	–	7.0
2	–	–	–	6.0
3	–	41	41	–
4	–	40	40	4.5
4	–	44	–	–
5	–	–	44	–
6	26	–	–	–
7	25	–	–	–
10	24	–	43	–
11	23	43		–
13	–	42	42	–
14	–	–	–	4.0
14	–	–	–	5.0

APPENDIX 7

Appendix VII

Data on station positions, depth, temperature, salinity, chlorophyll, inorganic nutrients, *Pseudo-nitzschia* cell concentrations ("*P. seriata*" and "*P. delicatissima*" groups), *Pseudo-nitzschia* identification, secchi depth, the vertical attenuation coefficient, λ, the dimensionless optical depth (λ .h), stratification parameter, Φ, and surface to bottom water temperature difference, Delta-t, along transects sampled during separate cruises off the west coast of Ireland in May 1997, and the south and southwest coasts of Ireland in August 1993, July 1996, early September 1996 and October 1997 .

Table 1. Station positions, temperature and salinity values recorded at discrete depths relating to archived samples, cruise # 13, southwest coast of Ireland, August 8th-18th 1993.

Station Number	Date (1993)	Latitude (N) dec. deg	Longitude (W) dec. deg	Depth (m)	Temperature (°C)	Salinity (PSU)
1301	15-Aug-93	51.48	10.27	5	13.54	34.99
				15	11.60	35.06
1302	15-Aug-93	51.44	10.50	0	15.00	34.74
				20	15.40	35.22
				50	11.25	35.39
1303	15-Aug-93	51.42	10.67	0	15.00	35.24
				20	15.35	35.21
				50	11.44	35.21
1304	15-Aug-93	51.39	10.83	45	12.12	35.22
1312	15-Aug-93	51.54	10.00	18	12.78	34.87
1313	16-Aug-93	51.51	10.17	0	13.84	34.95
				12	13.38	34.97
				20	12.44	34.97
				40	11.62	35.06
1314	17-Aug-93	51.34	10.08	0	14.13	34.81
				15	12.18	34.90
				30	10.61	35.08
				50	10.30	35.30
1315	17-Aug-93	51.24	9.92	0	13.90	34.91
				10	13.00	34.95
				30	10.61	35.05
				50	10.30	35.28
1316	17-Aug-93	51.28	9.83	18	12.25	34.90
				28	11.26	34.98
1317	17-Aug-93	51.33	9.75	0	14.50	34.85
				20	12.57	34.95
1318	17-Aug-93	51.38	9.68	15	12.95	34.89
1319	17-Aug-93	51.42	9.60	25	13.25	34.88
1323	18-Aug-93	51.28	9.40	20	13.16	34.76
1324	18-Aug-93	51.19	9.40	20	12.93	34.81
1325	18-Aug-93	51.22	9.20	12	13.80	34.80
				22	11.49	34.79
				50	9.86	35.18
1326	18-Aug-93	51.25	9.00	0	15.13	34.75
				25	12.90	34.84
1327	18-Aug-93	51.28	8.80	20	11.76	34.88
				40	11.35	35.04
1328	18-Aug-93	51.32	8.60	0	15.70	34.69
				10	15.25	34.69
				35	10.75	35.07
1329	18-Aug-93	51.33	8.44	0	15.80	34.75
				27	11.24	34.86
				40	10.10	35.03
1330	18-Aug-93	51.37	8.25	0	16.20	34.80
				23	11.14	34.72
				33	11.10	35.09

Table 2. Station positions, temperature and salinity values recorded at discrete depths, cruise # 18, south coast of Ireland, July 20th-25th 1996. n/d = not determined.

Station Number	Date (1993)	Latitude (N) dec. deg	Longitude (W) dec. deg	Depth (m)	Temperature (°C)	Salinity (PSU)
1801	20-Jul-96	51.45	10.33	20	14.81	35.34
				40	10.58	35.48
1802	20-Jul-96	51.45	10.58	25	13.82	35.49
				55	11.45	35.52
1803	20-Jul-96	51.45	10.84	10	15.54	35.51
				30	14.06	35.53
1804	20-Jul-96	51.45	11.08	15	15.73	35.55
				35	14.32	35.54
1805	20-Jul-96	51.45	11.33	30	13.16	35.54
				50	11.79	35.55
1806	21-Jul-96	51.62	9.78	15	13.85	35.20
				35	12.12	35.31
1807	21-Jul-96	51.44	9.92	20	13.15	35.18
				40	11.97	n/d
1808	21-Jul-96	51.45	9.55	15	15.46	35.04
				35	13.67	35.13
1809	21-Jul-96	51.43	9.40	15	14.06	35.07
				30	13.16	35.08
1810	22-Jul-96	51.12	9.40	15	13.60	35.23
				35	9.62	35.31
1811	22-Jul-96	51.35	8.44	20	13.40	35.19
				40	9.50	35.29
1812	22-Jul-96	51.58	8.48	18	12.30	35.00
				30	11.14	35.02
				50	10.66	35.03
1813	22-Jul-96	51.70	8.50	6	17.00	33.78
1814	23-Jul-96	51.75	8.25	15	13.70	34.97
1815	23-Jul-96	51.84	8.26	20	15.19	34.81
1816	23-Jul-96	51.83	7.79	13	15.00	34.95
				23	11.63	35.00
1817	23-Jul-96	51.92	7.50	22	13.08	34.95
1818	23-Jul-96	52.00	7.15	20	12.36	n/d
				40	12.26	34.98
1819	23-Jul-96	52.08	6.98	10	14.17	n/d
1820	24-Jul-96	51.75	6.57	15	14.00	n/d

Table 3. Station positions, temperature and salinity values recorded at discrete depths, cruise # 19, south coast of Ireland, September 3rd-8th 1996. n/d = not determined.

Station Number	Date (1993)	Latitude (N) dec. deg	Longitude (W) dec. deg	Depth (m)	Temperature (°C)	Salinity (PSU)
1901	03-Sep-96	52.92	9.92	30	13.19	35.22
1902	03-Sep-96	52.42	10.42	15	13.83	35.23
1903	04-Sep-96	51.95	11.40	40	12.86	35.52
1904	04-Sep-96	51.95	11.17	40	13.43	35.46
1905	04-Sep-96	51.95	10.92	60	11.36	35.44
1906	04-Sep-96	51.95	10.67	20	14.43	35.24
1907	05-Sep-96	51.65	10.30	0	14.27	34.88
1908	05-Sep-96	51.53	10.03	25	13.05	34.99
1909	05-Sep-96	51.62	9.77	20	13.12	35.01
1910	06-Sep-96	51.08	10.17	25	13.01	35.05
1911	06-Sep-96	51.23	9.92	25	11.63	35.10
1912	06-Sep-96	51.35	9.75	25	13.96	34.94
1913	06-Sep-96	51.46	9.54	10	14.65	34.94
1914	07-Sep-96	51.42	9.40	43	14.55	34.92
1915	07-Sep-96	51.33	9.40	40	11.09	35.14
1916	07-Sep-96	51.25	9.40	35	13.20	35.12
1917	07-Sep-96	51.17	9.40	32	12.22	35.14
1918	07-Sep-96	51.08	9.40	30	n/d	35.17
1919	07-Sep-96	51.18	9.08	35	10.01	35.17
1920	07-Sep-96	51.22	8.92	35	10.40	35.18
1921	07-Sep-96	51.27	8.75	20	13.06	35.12
1922	07-Sep-96	51.30	8.58	25	10.44	35.15
1923	07-Sep-96	51.35	8.42	20	16.46	35.17
1924	07-Sep-96	51.38	8.25	30	13.01	35.22

Table 4. Station positions, temperature and salinity values recorded at discrete depths, cruise # 20, west coast of Ireland, May 1st-5th 1997. n/d = not determined.

Station Number	Date (1993)	Latitude (N) dec. deg	Longitude (W) dec. deg	Depth (m)	Temperature (°C)	Salinity (PSU)
2001	02-May-97	53.67	10.38	0	12.08	35.03
				20	10.88	35.00
				40	9.99	35.17
				60	9.26	35.19
				80	9.11	35.27
2002	02-May-97	53.67	10.54	0	11.85	35.27
				30	10.35	35.22
				50	9.47	35.28
				70	9.39	35.34
				100	9.38	35.34
2003	02-May-97	53.67	10.67	0	11.53	35.35
				20	11.36	35.50
				40	11.17	35.51
				60	9.64	35.41
				80	9.60	35.43
				120	9.58	35.42
2004	03-May-97	54.00	10.67	0	12.01	35.52
				10	11.63	35.53
				30	11.35	35.53
				60	10.49	35.50
				100	9.85	35.46
2005	03-May-97	54.00	10.53	0	11.69	35.35
				15	11.46	35.38
				40	11.13	35.38
				70	10.17	35.47
				100	9.88	35.46
2006	03-May-97	54.00	10.38	0	11.09	34.89
				10	10.76	34.93
				40	10.21	35.06
				60	10.08	35.15
				80	9.97	35.15
2007	03-May-97	54.30	10.13	0	11.00	34.96
				10	10.62	34.99
				30	10.47	35.13
				50	10.46	35.16
2008	03-May-97	54.35	10.25	0	11.16	35.38
				10	11.27	35.45
				30	10.77	35.46
				60	10.16	35.39
				80	10.09	35.37
2009	03-May-97	54.37	10.34	0	11.60	35.54
				20	11.31	35.52
				40	10.87	35.48
				70	10.14	35.38
				100	9.82	35.42
2010	03-May-97	54.44	10.53	0	11.41	35.52
				20	11.25	35.52
				50	11.04	35.53
				90	10.48	35.53
				120	9.99	35.48
				140	n/d	35.47
2011	03-May-97	54.48	10.67	0	11.60	35.50
				30	11.15	35.50
				60	10.61	35.51
				100	10.47	35.53
				150	10.31	35.51
				200	10.21	35.51

Table 5. Station positions, temperature and salinity values recorded at discrete depths, cruise # 22, south coast of Ireland, October 7th-12th 1997. n/d = not determined.

Station Number	Date (1993)	Latitude (N) dec. deg	Longitude (W) dec. deg	Depth (m)	Temperature (°C)	Salinity (PSU)
2201	07-Oct	51.59	8.25	0	15.74	35.07
				20	15.74	35.10
				40	14.40	35.27
				60	12.44	35.34
2202	07-Oct	51.83	7.75	0	15.67	35.07
				20	15.62	35.06
				45	14.72	35.26
2203	07-Oct	51.92	7.50	0	15.43	35.01
				15	15.36	35.01
				40	14.88	35.13
2204	07-Oct	52.00	7.18	0	15.48	35.07
				15	15.45	35.07
				40	15.42	35.22
2205	11-Oct	51.58	8.42	0	15.22	35.09
				20	15.17	35.10
				50	13.21	35.31
				65	12.98	35.32
2206	11-Oct	51.50	8.42	0	15.34	35.10
				25	15.28	35.10
				65	11.80	35.33
				75	11.43	35.31
2207	11-Oct	51.42	8.42	0	15.58	35.13
				20	15.54	35.11
				60	11.33	35.29
				80	9.72	35.28
2208	11-Oct	51.33	8.42	0	15.49	35.09
				20	15.43	n/d
				60	10.61	n/d
				85	9.66	35.30
W309	08-Oct	52.07	7.10	2	15.19	34.92
				30	14.73	35.21
W310	08-Oct	52.09	7.06	6	15.13	34.96
				26	14.95	35.18
W311	08-Oct	52.10	7.02	6	15.06	35.12
				26	14.98	34.65
W312	08-Oct	52.12	6.98	6	15.00	34.80
				16	14.98	34.94

Table 6. Values for Delta-t (surface to bottom temperature differences), the stratification parameter, Φ, the vertical attenuation coefficient, λ, and the dimensionless optical depth (λ .h) for station visited off the south and southwest coast of Ireland, August, 1993. Attenuation coefficients are calculated from 1.7/Secchi depth (Parsons *et al*., 1984). Where stations were deeper than 100 m, the 100 m value has been used as the water column depth.

Station number	Depth h (m)	Secchi Depth (m)	λ (m^{-1})	λ.h	Φ ($J.m^{-3}$)	Delta-t ºC
1301	102	6	0.28	28.56	81	3.3
1302	142	19	0.09	12.78	145	5.0
1303	157	20	0.09	14.13	131	5.3
1304	169	20	0.09	15.21	140	5.3
1305	195	22	0.08	15.60	120	4.9
1306	27	8	0.21	5.67	44	4.0
1307	34	8	0.21	7.14	55	4.5
1308	38	8	0.21	7.98	60	4.9
1309	41	7	0.24	9.84	61	4.8
1310	50	8	0.21	10.50	56	4.9
1311	61	10	0.17	10.37	41	3.8
1312	67	8	0.21	14.07	60	3.9
1313	85	6	0.28	23.80	82	3.6
1314	103	10	0.17	17.51	109	3.8
1315	108	7	0.24	25.92	96	3.7
1316	99	14	0.12	11.88	95	4.7
1317	82	11	0.15	12.30	72	4.4
1318	69	11	0.15	10.35	36	2.8
1319	58	9	0.19	11.02	39	3.0
1320	42	9	0.19	7.98	12	1.4
1321	25	8.5	0.20	5.00	7	1.1
1322	76	11	0.15	11.40	29	3.9
1323	92	12	0.14	12.88	81	4.9
1324	108	13	0.13	14.04	109	4.7
1325	103	11	0.15	15.45	110	4.8
1326	100	12	0.14	14.00	136	5.4
1327	100	12	0.14	14.00	121	5.9
1328	96	11	0.15	14.40	133	6.5
1329	93	12	0.14	13.02	137	6.6
1330	93	13	0.13	12.09	123	6.5

Table 7. Values for Delta-t (surface to bottom temperature differences), the stratification parameter, Φ, the vertical attenuation coefficient, λ, and the dimensionless optical depth (λ .h) for station visited off the south and southwest coast of Ireland, July, 1996. Attenuation coefficients are calculated from 1.7/Secchi depth (Parsons *et al*., 1984). Where stations were deeper than 100 m, the 100 m value has been used as the water column depth. n/d = not determined.

Station number	Depth h (m)	Secchi Depth (m)	λ (m^{-1})	λ.h	Φ ($J.m^{-3}$)	Delta-t ºC
1801	120	21.5	0.08	9.49	104	4.9
1802	148	18.5	0.09	13.60	104	5.9
1803	168	14.0	0.12	20.40	104	5.7
1804	189	13.5	0.13	23.80	109	5.7
1805	223	12.0	0.14	31.59	90	5.3
1806	53	9.5	0.18	9.48	46	4.1
1807	71	11.5	0.15	10.50	45	4.2
1808	43	10.0	0.17	7.31	19	1.5
1809	57	10.5	0.16	9.23	42	3.0
1810	119	11.5	0.15	17.59	114	6.3
1811	93	14.5	0.12	10.90	137	7.2
1812	61	11.0	0.15	9.43	71	5.2
1813	11	3.5	0.49	5.34	12	1.0
1814	29	10.0	0.17	4.93	26	3.1
1815	25	7.5	0.23	5.67	1	0.7
1816	49	10.5	0.16	7.93	60	4.9
1817	55	10.0	0.17	9.35	80	5.3
1818	56	12.0	0.14	7.93	40	4.7
1819	27	6.5	0.26	7.06	27	2.5
1820	72	13.0	0.13	9.42	24	3.1
1821	77	n/d	-	-	4	0.6
1822	64	n/d	-	-	5	0.6

Table 8. Values for Delta-t (surface to bottom temperature differences), the stratification parameter, Φ, the vertical attenuation coefficient, λ, and the dimensionless optical depth (λ .h) for station visited off the south and southwest coast of Ireland, September, 1996. Attenuation coefficients are calculated from 1.7/Secchi depth (Parsons *et al*., 1984). Where stations were deeper than 100 m, the 100 m value has been used as the water column depth. n/d = not determined.

Station number	Depth h (m)	Secchi Depth (m)	λ (m^{-1})	λ.h	Φ ($J.m^{-3}$)	Delta-t ºC
1901	99	12.0	0.14	14.03	3	7.1
1902	96	n/d	-	-	64	4.8
1903	225	n/d	-	-	115	5.4
1904	166	n/d	-	-	120	5.5
1905	140	n/d	-	-	153	6.2
1906	102	n/d	-	-	99	4.8
1907	84	5.5	0.31	25.96	42	2.1
1908	71	n/d			55	3.6
1909	50	8.0	0.21	10.63	38	3.0
1910	132	16.0	0.11	14.03	119	4.5
1911	114	15.0	0.11	12.92	149	5.9
1912	80	12.0	0.14	11.33	88	5.2
1913	40	7.0	0.24	9.71	14	1.7
1914	63	11.5	0.15	9.31	29	2.4
1915	77	12.5	0.14	10.47	119	6.2
1916	100	12.0	0.14	14.17	165	7.0
1917	114	21.0	0.08	9.23	172	7.3
1918	119	18.0	0.09	11.24	142	7.2
1919	107	18.0	0.09	10.11	173	7.5
1920	107	14.0	0.12	12.99	174	7.4
1921	103	16.0	0.11	10.94	142	7.4
1922	99	14.0	0.12	12.02	162	7.5
1923	92	n/d	-	-	122	7.2
1924	91	n/d	-	-	147	7.2

Table 9. Values for Delta-t (surface to bottom temperature differences), the stratification parameter, Φ, the vertical attenuation coefficient, λ, and the dimensionless optical depth (λ .h) for station visited off the south and southwest coast of Ireland, May, 1997. Attenuation coefficients are calculated from 1.7/Secchi depth (Parsons *et al*., 1984). Where stations were deeper than 100 m, the 100 m value has been used as the water column depth. n/d = not determined.

Station number	Depth h (m)	Secchi Depth (m)	λ (m^{-1})	λ.h	Φ ($J.m^{-3}$)	Delta-t ºC
2001	102	10.0	0.17	17.34	62	2.9
2002	122	9.5	0.18	21.83	29	2.4
2003	137	10.0	0.17	23.29	13	1.9
2004	131	n/d	-	-	4	2.2
2005	138	n/d	-	-	20	1.8
2006	97	n/d	-	-	22	1.1
2007	60	n/d	-	-	10	0.5
2008	114	8.0	0.21	24.23	21	1.2
2009	125	13.0	0.13	16.35	36	1.7
2010	151	13.0	0.13	19.75	25	1.5
2011	229	13.0	0.13	29.95	38	1.1

Table 10. Values for Delta-t (surface to bottom temperature differences), the stratification parameter, Φ, the vertical attenuation coefficient, λ, and the dimensionless optical depth (λ .h) for station visited off the south and southwest coast of Ireland, October, 1997. Attenuation coefficients are calculated from 1.7/Secchi depth (Parsons *et al*., 1984). Where stations were deeper than 100 m, the 100 m value has been used as the water column depth. n/d = not determined.

Station number	Depth h (m)	Secchi Depth (m)	λ (m^{-1})	λ.h	Φ ($J.m^{-3}$)	Delta-t ºC
2201	84	9.0	0.19	15.87	77	3.4
2202	55	9.0	0.19	10.39	29	1.1
2203	55	8.0	0.21	11.69	22	1.0
2204	56	8.5	0.20	11.20	15	0.7
2205	69	11.0	0.15	10.66	52	2.7
2206	89	13.0	0.13	11.64	80	5.1
2207	90	11.5	0.15	13.30	113	5.8
2208	96	14.0	0.12	11.66	118	5.8
W308	19	3.0	0.57	10.77	20	0.3
W309	39.1	n/d	-	-	12	0.4
W310	31	n/d	-	-	8	0.3
W311	27.6	5.5	0.31	8.53	10	0.0
W312	23.8	3.0	0.57	13.49	4	0.2

Table 11. Nutrient, chlorophyll levels and *Pseudo-nitzschia* cell concentrations (separated into the "P. seriata" and "P. delicatissima" groups) recorded at discrete depths during cruise # 13, southwest coast of Ireland, August 8th-18th 1993. n/d = not determined.

Station Number	Depth (m)	NO3 (µM)	PO4 (µM)	SiO4 (µM)	Chl a (mg m^{-3})	"P. seriata" (cells.mL^{-1})	"P. delicatissima" (cells.mL^{-1})
1301	5	0.6	0.11	1.6	11.9	57.0	6.3
	15	5.4	0.46	4.4	0.6	1.5	0.0
1302	0	0.7	0.04	1.5	0.2	0.0	0.0
	20	0.6	0.05	2.2	0.1	0.0	0.0
	50	3.0	0.36	1.9	0.3	0.0	0.0
1303	0	0.7	0.09	0.9	0.0	0.0	0.0
	20	0.6	0.04	0.8	0.0	0.0	0.0
	50	7.9	0.51	1.7	0.4	0.0	0.0
1304	45	0.7	0.05	1.1	0.6	0.0	0.0
1312	18	2.1	0.07	1.1	9.6	399.0	329.0
1313	0	0.4	0.01	1.0	5.4	40.0	0.0
	12	0.4	0.00	0.8	5.5	135.0	5.0
	20	0.7	0.16	1.1	12.5	95.0	2.0
	40	6.2	0.50	4.0	0.2	3.0	0.0
1314	0	0.0	0.12	2.1	0.8	180.0	0.0
	15	2.4	0.13	3.9	11.5	290.0	380.0
	30	8.3	0.45	7.6	0.3	4.0	0.0
	50	9.9	0.74	8.4	0.1	1.0	0.0
1315	0	n/d	n/d	n/d	4.8	140.0	11.0
	10	0.0	0.17	2.3	0.0	180.0	0.0
	30	5.3	0.33	5.5	n/d	9.0	0.0
	50	8.4	0.38	5.7	n/d	0.6	0.0
1316	18	0.4	0.08	1.1	0.5	66.0	0.0
	28	7.4	0.57	3.4	n/d	12.0	0.0
1317	0	0.4	0.02	1.1	0.3	135.0	0.0
	20	10.1	0.76	2.7	0.2	31.0	0.0
1318	15	0.8	0.13	2.1	5.9	413.0	0.0
1319	25	2.3	0.20	2.1	n/d	52.0	0.0
1323	20	0.4	0.05	0.7	2.8	57.0	0.0
1324	20	0.1	0.14	2.6	2.3	40.0	16.0
1325	12	0.0	0.07	2.4	1.8	19.0	30.0
	22	0.0	0.08	3.3	2.9	20.0	0.0
	50	10.2	0.58	8.7	0.2	0.0	0.0
1326	0	0.1	0.15	2.8	0.5	9.0	9.0
	25	0.2	0.08	2.4	3.8	360.0	180.0
1327	20	0.0	0.09	2.8	2.6	3.0	25.0
	40	9.1	0.57	7.9	0.1	0.0	0.0
1328	0	0.0	0.17	2.9	0.2	0.0	0.0
	10	0.0	0.12	3.1	0.3	1.5	0.8
	35	6.1	0.45	7.7	1.4	0.8	2.3
1329	0	0.2	0.14	2.9	0.2	0.0	0.0
	27	4.5	0.30	5.9	0.9	0.0	1.5
	40	9.2	0.52	8.8	0.1	0.0	0.8
1330	0	0.2	0.12	3.3	0.2	0.0	0.0
	23	n/d	n/d	n/d	n/d	1.5	12.3
	33	7.7	0.39	6.2	0.2	0.0	0.0

Table 12. Nutrient, chlorophyll levels and *Pseudo-nitzschia* cell concentrations (separated into the "P. seriata" and "P. delicatissima" groups) recorded at discrete depths, during cruise # 18, south coast of Ireland, July 20th-25th 1996. n/d = not determined.

Station Number	Depth (m)	NO3 (µM)	PO4 (µM)	SiO4 (µM)	Chl a (mg m^{-3})	"P. seriata" (cells.mL^{-1})	"P. delicatissima" (cells.mL^{-1})
1801	20	2.0	0.08	0.6	0.5	19.0	21.6
	40	6.9	0.50	2.2	1.7	0.0	0.0
1802	25	0.0	0.08	0.5	0.5	0.0	2.3
	55	4.4	0.33	0.7	0.2	0.0	0.0
1803	10	0.0	0.02	0.5	0.3	0.0	0.0
	30	1.1	0.15	0.6	0.3	0.0	0.0
1804	15	0.1	0.03	0.7	0.2	0.0	0.0
	35	0.0	0.06	0.6	0.3	0.0	0.0
1805	30	1.6	0.25	1.0	0.3	0.0	0.0
	50	5.3	0.43	1.4	0.3	0.0	0.0
1806	15	0.0	0.02	0.3	0.8	3.0	444.0
	35	0.7	0.06	0.3	2.8	74.0	31.0
1807	20	0.5	0.13	0.4	2.2	12.0	190.0
	40	1.5	0.20	0.6	2.2	9.0	0.0
1808	15	0.1	0.09	0.5	1.3	10.0	240.0
	35	0.3	0.11	0.5	2.6	0.0	180.0
1809	15	0.0	0.06	0.4	1.4	140.0	38.0
	30	0.0	0.09	0.4	1.6	5.0	180.0
1810	15	0.1	0.03	0.3	0.3	9.0	250.0
	35	7.4	0.55	2.1	2.3	9.0	850.0
1811	20	0.0	0.05	0.3	0.5	0.0	80.0
	40	7.4	0.58	2.8	0.7	0.0	150.0
1812	18	0.1	0.08	0.3	1.1	4.0	37.0
	30	1.0	0.27	1.2	0.7	23.0	17.0
	50	1.5	0.31	1.5	n/d	25.0	1.0
1813	6	1.0	0.42	0.9	4.6	0.0	14.0
1814	15	0.1	0.08	0.3	2.2	0.0	6.0
1815	20	0.2	0.14	0.6	1.2	11.0	69.0
1816	13	0.1	0.03	0.2	0.6	0.0	4.0
	23	0.2	0.09	0.7	0.9	0.0	9.0
1817	22	1.3	0.04	0.4	2.0	0.0	0.0
1818	20	0.1	0.09	0.4	n/d	70.0	6.0
	40	0.5	0.15	0.8	0.9	28.0	1.0
1819	10	0.1	0.06	0.3	2.9	9.0	18.0
1820	15	0.1	0.05	0.2	n/d	3.0	0.0
	60	0.59	0.12	0.31	n/d	n/d	n/d

Table 13. Nutrient, chlorophyll levels and *Pseudo-nitzschia* cell concentrations (separated into the "P. seriata" and "P. delicatissima" groups) recorded at discrete depths, during cruise # 19, south coast of Ireland, September 3rd-8th 1996. n/d = not determined.

Station Number	Depth (m)	NO3 (µM)	PO4 (µM)	SiO4 (µM)	Chl a (mg m^{-3})	"P. seriata" (cells.mL^{-1})	"P. delicatissima" (cells.mL^{-1})
1901	30	0.6	0.05	0.4	0.7	0.0	0.0
1902	15	0.2	0.15	0.9	0.5	92.0	0.0
1903	40	1.6	0.35	1.1	0.1	0.0	0.0
1904	40	0.5	0.17	0.6	0.1	0.0	0.0
1905	60	2.7	0.47	0.8	0.2	0.0	0.0
1906	20	0.2	0.10	1.2	1.8	0.0	25.0
1907	0	n/d	n/d	n/d	3.3	0.0	78.0
1908	25	0.2	0.14	2.5	0.9	0.0	33.0
1909	20	0.1	0.25	2.2	1.8	0.0	1.0
1910	25	0.2	0.12	1.3	1.4	0.0	19.0
1911	25	2.4	0.26	1.7	1.7	0.0	0.0
1912	25	0.1	0.18	1.5	0.4	0.0	0.0
1913	10	0.3	0.16	1.4	0.5	0.0	0.0
1914	43	0.3	0.18	0.8	1.0	0.0	8.0
1915	40	2.8	0.38	2.8	0.0	0.0	2.0
1916	35	0.1	0.08	0.6	0.3	0.0	0.0
1917	32	0.2	0.23	0.7	4.4	0.0	0.0
1918	30	0.2	0.11	1.7	1.1	0.0	0.0
1919	35	1.6	0.48	3.0	0.1	0.0	0.0
1920	35	0.5	0.25	1.2	5.8	0.0	0.0
1921	20	1.0	0.23	1.0	0.1	0.0	0.0
1922	25	2.3	0.64	3.6	0.3	0.0	6.0
1923	20	1.8	0.34	1.4	0.1	0.0	2.0
1924	30	0.4	0.16	0.8	0.1	0.0	16.0

Table 14. Nutrient, chlorophyll levels and *Pseudo-nitzschia* cell concentrations (separated into the "P. seriata" and "P. delicatissima" groups) recorded at discrete depths, during cruise # 20, west coast of Ireland, May 1st-5th 1997. n/d = not determined.

Station Number	Depth (m)	NO3 (µM)	PO4 (µM)	SiO4 (µM)	Chl a (mg m^{-3})	"P. seriata" (cells.mL^{-1})	"P. delicatissima" (cells.mL^{-1})
2001	0	1.5	0.07	0.8	0.7	0.0	0.7
	20	0.2	0.06	0.2	1.0	0.0	0.0
	40	4.1	0.38	2.2	1.4	0.3	1.9
	60	5.1	0.45	1.7	0.4	0.0	0.6
	80	n/d	n/d	n/d	1.0	0.0	0.6
2002	0	0.8	0.17	0.6	1.0	0.0	0.1
	30	0.7	0.13	0.7	1.0	0.0	0.0
	50	4.9	0.34	1.3	0.3	0.0	0.3
	70	6.9	0.55	3.5	0.6	0.0	0.0
	100	7.5	0.56	3.8	0.3	0.0	0.1
2003	0	0.9	0.15	1.0	1.8	0.0	2.1
	20	1.8	0.25	2.0	1.8	0.0	5.0
	40	7.8	0.24	2.4	0.9	0.0	8.2
	60	2.4	0.50	2.9	0.2	0.0	0.8
	80	6.5	0.50	4.1	0.2	0.0	0.3
	120	8.9	0.52	4.4	0.3	0.0	0.0
2004	0	2.5	0.19	1.9	1.8	0.1	7.6
	10	1.8	0.27	2.7	2.8	0.1	5.8
	30	2.5	0.23	2.2	1.6	0.0	5.8
	60	6.9	0.23	4.2	0.6	0.0	12.0
	100	7.0	0.53	3.6	0.3	0.0	0.5
2005	0	0.9	0.16	1.3	1.8	0.0	14.0
	15	1.2	0.16	1.6	3.3	0.4	1.1
	40	2.3	0.20	1.7	1.3	0.0	13.0
	70	7.0	0.29	3.6	0.4	0.0	1.0
	100	7.1	0.52	3.4	0.3	0.0	0.2
2006	0	0.0	0.09	0.9	1.8	0.0	1.6
	10	2.2	0.09	0.6	1.8	0.0	3.4
	40	2.4	0.17	1.7	0.7	0.0	0.8
	60	4.3	0.31	2.3	0.6	0.0	1.0
	80	2.8	0.26	1.5	0.7	0.0	1.8
2007	0	3.9	0.23	2.5	2.4	0.0	1.8
	10	5.1	0.42	3.0	1.7	0.0	0.8
	30	0.8	0.15	0.5	0.8	0.0	3.4
	50	1.1	0.13	2.3	0.7	0.0	0.0
2008	0	2.1	0.24	1.8	2.7	0.0	0.0
	10	2.4	0.25	1.5	2.0	0.0	2.2
	30	2.0	0.17	1.5	0.9	0.0	0.5
	60	3.0	0.17	2.0	0.4	0.0	0.7
	80	2.8	0.31	1.9	0.4	0.0	0.4
2009	0	2.5	0.25	1.9	1.9	0.0	0.0
	20	3.3	0.28	2.3	1.6	0.0	1.1
	40	3.2	0.32	2.1	0.8	0.0	0.6
	70	4.5	0.51	2.5	0.5	0.0	0.1
	100	6.6	0.49	3.3	0.4	0.0	0.0
2010	0	4.5	0.38	2.6	0.7	0.0	0.3
	20	4.5	0.36	2.9	0.9	0.0	0.0
	50	4.3	0.36	2.8	0.6	0.0	0.4
	90	8.6	0.50	3.9	0.1	0.0	0.0
	120	8.2	0.57	4.4	0.2	0.0	0.1
	140	7.4	0.54	3.5	0.2	0.0	0.0
2011	0	4.5	0.38	1.3	0.5	0.0	15.0
	30	4.7	0.36	0.3	1.0	0.0	19.0
	60	7.5	0.54	4.3	0.5	0.0	13.0
	100	7.5	0.54	3.3	0.1	0.0	1.2
	150	9.2	0.56	3.9	0.1	0.0	0.0
	200	7.2	0.52	3.5	n/d	0.0	0.0

Table 15. Nutrient, chlorophyll levels and *Pseudo-nitzschia* cell concentrations (separated into the "P. seriata" and "P. delicatissima" groups) recorded at discrete depths, during cruise # 22, south coast of Ireland, October 7th-12th 1997.

Station Number	Depth (m)	NO3 (µM)	PO4 (µM)	SiO4 (µM)	Chl a (mg m^{-3})	"P. seriata" (cells.mL^{-1})	"P. delicatissima" (cells.mL^{-1})
2201	0	0.6	0.11	1.4	1.1	85.0	0.0
	20	0.1	0.10	1.4	0.8	66.0	0.0
	40	3.6	0.34	3.0	0.2	1.0	0.0
	60	6.8	0.53	5.5	1.5	0.0	0.0
2202	0	0.3	0.09	1.3	2.8	180.0	2.0
	20	0.4	0.16	1.6	2.7	340.0	5.0
	45	4.1	0.36	3.6	0.1	1.0	0.0
2203	0	0.2	0.08	1.3	3.1	730.0	8.0
	15	0.2	0.08	1.1	5.2	620.0	7.0
	40	1.5	0.24	2.1	1.3	160.0	6.0
2204	0	0.2	0.07	1.2	4.0	550.0	3.0
	15	0.1	0.06	1.1	5.1	450.0	12.0
	40	3.3	0.31	3.2	0.2	4.0	0.0
2205	0	0.6	0.16	1.3	1.8	78.0	0.0
	20	0.5	0.19	1.3	2.2	120.0	8.0
	50	8.1	0.54	5.4	0.2	9.0	0.0
	65	5.7	0.49	5.1	0.2	8.0	1.0
2206	0	0.3	0.15	1.3	2.5	2.0	1.0
	25	0.2	0.09	1.1	1.6	65.0	2.0
	65	8.7	0.79	6.6	0.1	0.0	0.0
	75	9.0	0.82	5.9	0.1	0.0	0.0
2207	0	0.3	0.13	0.7	0.5	0.0	0.0
	20	0.2	0.12	1.6	0.5	0.0	0.0
	60	8.9	0.69	5.6	0.1	0.0	1.0
	80	11.1	0.74	6.3	0.1	0.0	2.0
2208	0	0.5	0.12	1.6	0.1	0.0	0.0
	20	0.4	0.13	1.5	0.4	0.0	0.0
	60	9.1	0.67	5.5	0.1	0.0	1.0
	85	8.8	0.77	5.4	0.1	0.0	1.0
W309	2	2.8	0.40	2.3	1.9	22.0	0.0
	30	3.3	0.50	3.5	0.5	28.0	1.0
W310	6	2.8	0.30	2.7	1.1	9.0	0.0
	26	3.0	0.37	3.5	0.4	11.0	1.0
W311	6	5.5	0.33	3.0	0.9	14.0	1.0
	26	6.5	0.38	3.3	0.4	0.0	1.0
W312	6	4.4	0.33	3.1	0.8	0.0	1.0
	16	3.6	0.33	2.7	0.5	1.0	4.0

Table 16. Nutrient levels in inshore and offshore waters during cruises carried out between 1993 and 1997 off the south, southwest and west coasts of Ireland.

Year	Month	Nitrate (μM)	Phosphate (μM)	Silicate (μM)
Surface mixed Layer				
Inshore				
1993 (cruise #13)	August	0.4-2.3	0.00-2.00	0.7-2.1
1996 (cruise #18)	July	0.0-1.3	0.02-0.42	0.2-0.9
1996 (cruise #19)	September	0.1-0.3	0.16-0.25	1.4-2.5
1997 (cruise #20)	May	0.0-5.1	0.06-0.42	0.2-3.0
1997 (cruise #22)	October	0.1-6.5	0.06-0.40	0.7-3.5
Offshore				
1993 (cruise #13)	August	0.0-2.4	0.04-0.17	0.8-3.9
1996 (cruise #18)	July	0.0-2.0	0.02-0.25	0.2-1.0
1996 (cruise #19)	September	0.2-2.4	0.01-0.64	0.9-3.6
1997 (cruise #20)	May	0.7-4.5	0.13-0.38	0.6-2.9
Year	Month	Nitrate (μM)	Phosphate (μM)	Silicate (μM)
Bottom mixed Layer				
Inshore				
1993 (cruise #13)	August	6.2-10.1	0.05-0.76	2.7-4.0
1996 (cruise #18)	July	0.0-1.5	0.06-0.31	0.3-1.5
1996 (cruise #19)	September	0.3-2.8	0.18-0.38	0.8-2.8
1997 (cruise #20)	May	0.8-5.1	0.13-0.45	0.5-2.3
1997 (cruise #22)	October	1.5-11.1	0.24-0.82	2.1-6.6
Offshore				
1993 (cruise #13)	August	0.7-10.2	0.05-0.74	1.1-8.8
1996 (cruise #18)	July	0.0-7.4	0.06-0.58	0.6-2.8
1996 (cruise #19)	September	0.7-10.2	0.05-0.74	1.1-8.8
1997 (cruise #20)	May	1.3-9.2	0.17-0.57	1.3-4.4

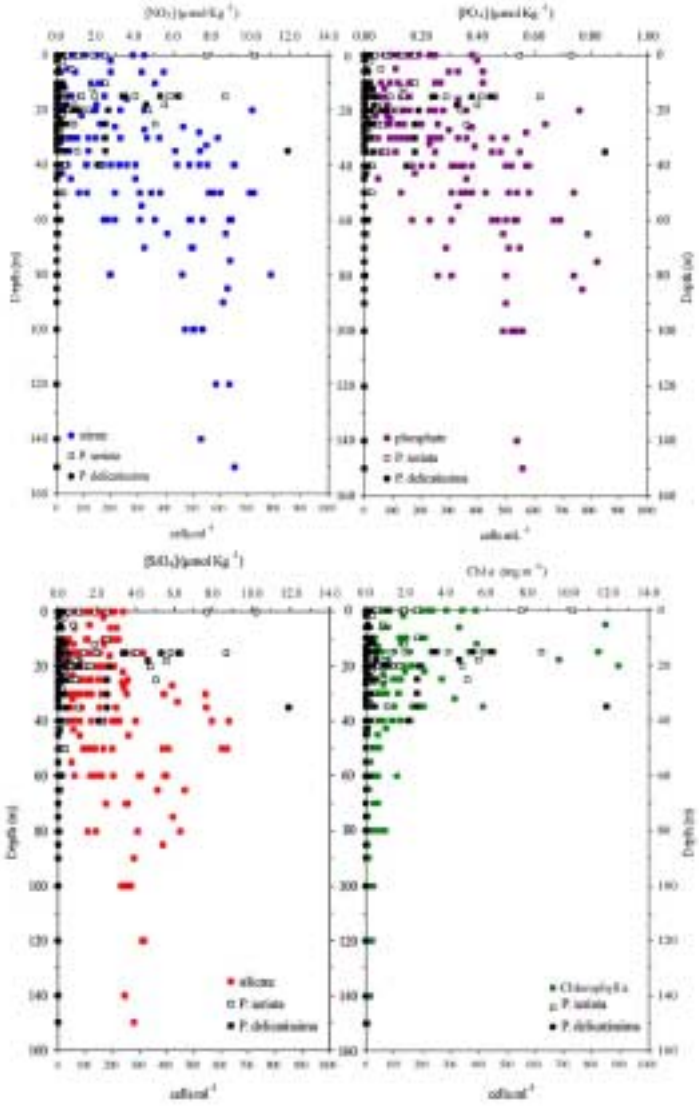

Figure 1. Vertical profiles of nitrate, phosphate, silicate and chlorophyll concentrations with depth. Included in the plot are the cell densities of the “P. delicatissima” and "P. seriata” groups recorded at discrete depths. Samples from all depths and times throughout the study (cruise #13, 18, 19, 20 and 22) containing all the above variables were used.

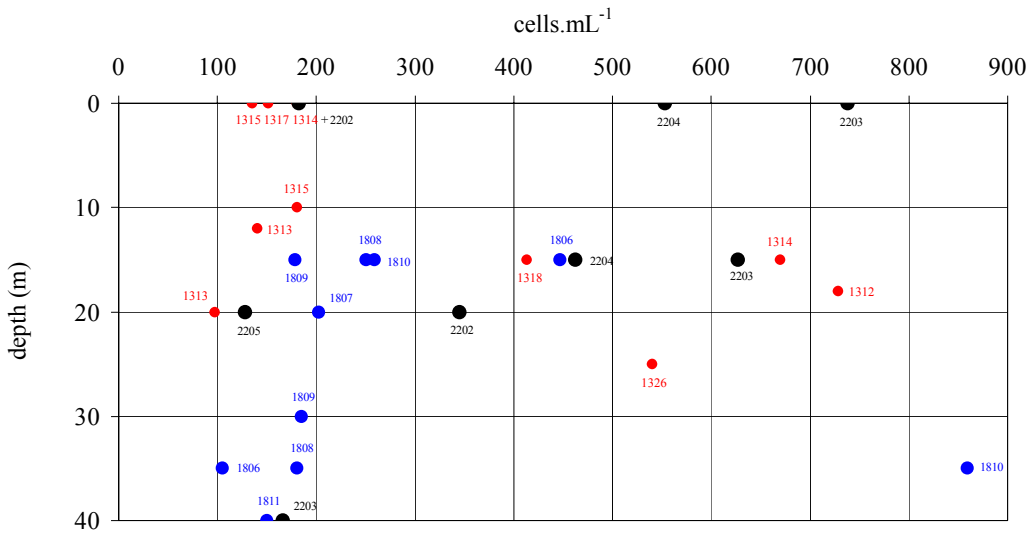

Figure 2. Stations where *Pseudo-nitzschia* cell numbers were >90 cells.ml^{-1} are plotted against depth. The data are derived from cruises #13, 18, 19, 20 and 22 carried out between 1993 and 1997. Red = August 1993, blue = July 1996 and black = October 1997. No counts of >90 cells.ml^{-1} were recorded during cruises #19 and 20.

Table 17. *Pseudo-nitzschia* species recorded in net material collected during cruise # 13, southwest coast of Ireland, August 8th-18th 1993. ++ = dominant *Pseudo-nitzschia* species in the sample, + = present, - = absent, n = number of stations were net samples were examined.

Station	*P. pungens*	*P. multiseries*	*P. seriata*	*P. australis*	*P. fraudulenta*	*P. subpacifica*	*P. delicatissima*	*P. pseudo-delicatissima*
1301	++	-	-	+	+	-	+	-
1311	+	-	-	+	+	-	-	++
1312	++	-	-	-	+	-	-	-
1313	++	-	-	-	-	-	-	+
1314	+	+	-	+	+	-	++	-
1315	++	-	-	-	-	-	-	-
1317	++	+	-	-	+	-	+	-
1324	++	+	+ (?)	-	+	-	-	-
1325	++	-	-	-	-	-	-	-
1326	++	-	-	-	-	-	-	-
n = 10	100%	30%	10%	30%	60%	0%	30%	20%

Table 18. *Pseudo-nitzschia* species recorded in net material collected during cruise # 18, south coast of Ireland, July 20th-25th 1996. ++ = dominant *Pseudo-nitzschia* species in the sample, + = present, - = absent, n = number of stations were net samples were examined. % = presence or absence on an individual station basis.

Station	*P. pungens*	*P. multiseries*	*P. seriata*	*P. australis*	*P. fraudulenta*	*P. subpacifica*	*P. delicatissima*	*P. pseudo-delicatissima*
1801	++	+	+ (?)	+	+	-	+	+
1802	++	+	-	+	-	-	-	-
1806	++	+	-	+	+	-	+	+
1807	+	+	+ (?)	+	+	-	+	++
1808	+	+	+ (?)	+	+	+ (?)	+	++
1809	+	-	-	+	+	-	+	++
1810	+	-	+ (?)	+	+	-	+	++
1811	+	-	-	+	+	-	++	+
1812	+	-	-	+	-	-	++	++
1813	-	-	-	-	-	-	++	+
1815	+	+	-	-	+	-	+	++
1818	++	+	-	+	+	-	-	+
1819	++	+	-	+	+	-	-	+
1820	++	+	+ (?)	+	+	-	-	-
1821	-	-	-	-	-	-	+	++
n = 15	87%	53%	36% (?)	73%	73%	7% (?)	73%	87%

Table 19. *Pseudo-nitzschia* species recorded in net material collected during cruise # 19, south coast of Ireland, September 3rd-8th 1996. ++ = dominant *Pseudo-nitzschia* species in the sample, + = present, - = absent, n = number of stations were net samples were examined.

Station	*P. pungens*	*P. multiseries*	*P. seriata*	*P. australis*	*P. fraudulenta*	*P. subpacifica*	*P. delicatissima*	*P. pseudo-delicatissima*
1902	++	-	-	++	+	-	++	+
1906	+	-	-	+	+	-	++	-
1907	+	+	-	++	+	-	+	+
1908	+	+	-	++	+	-	+	+
1909	++	-	-	+	-	-	++	-
1917	-	-	-	-	-	-	++	-
1919	-	-	-	-	-	-	++	-
1924	+	-	-	-	+	-	++	+
n = 8	75%	25%	0%	63%	63%	0%	100%	50%

Table 20. *Pseudo-nitzschia* species recorded in net material collected off the southwest coast of Ireland, June 2nd-8th 1995, and in Galway Bay, May 6th-8th 1996. ++ = dominant *Pseudo-nitzschia* species in the sample, + = present, - = absent, n = number of stations were net samples were examined.

Station	*P. pungens*	*P. multiseries*	*P. seriata*	*P. australis*	*P. fraudulenta*	*P. subpacifica*	*P. delicatissima*	*P. pseudo-delicatissima*
June	++	+	-	+	+	-	+	-
n = 1	100%	100%	0%	100%	100%	0%	100%	0%
May	-	-	-	-	-	-	++	+
n = 1	0%	0%	0%	0%	0%	0%	100%	100%

Table 21. *Pseudo-nitzschia* species recorded in net material collected during cruise # 20, west coast of Ireland, May 1st-5th 1997. ++ = dominant *Pseudo-nitzschia* species in the sample, + = present, - = absent, n = number of stations were net samples were examined.

Station	*P. pungens*	*P. multiseries*	*P. seriata*	*P. australis*	*P. fraudulenta*	*P. subpacifica*	*P. delicatissima*	*P. pseudo-delicatissima*
2006	-	-	-	-	++	-	-	-
2011	-	-	-	-	++	-	-	+
n = 2	0%	0%	0%	0%	100%	0%	0%	50%

Table 22. *Pseudo-nitzschia* species recorded in net material collected during cruise # 22, south coast of Ireland, October 7th-12th 1997. ++ = dominant *Pseudo-nitzschia* species in the sample, + = present, - = absent, n = number of stations were net samples were examined.

Station	*P. pungens*	*P. multiseries*	*P. seriata*	*P. australis*	*P. fraudulenta*	*P. subpacifica*	*P. delicatissima*	*P. pseudo-delicatissima*
2201	+	+	-	+	++	-	-	-
2202	-	+	-	+	++	-	-	+
2203	+	-	-	+	++	-	-	-
2204	+	-	-	+	++	-	-	-
2205	+	+	-	+	++	+ (?)	-	-
2206	+	+	-	++	++	-	-	+
2208	+	+	-	+	++	-	-	+
W308	+	-	-	+	++	-	-	-
W309	+	-	-	+	++	-	-	-
W310	+	-	-	+	++	-	-	-
W311	-	-	-	+	++	-	-	-
W312	+	-	-	+	++	-	-	-
n = 11	82%	45%	0%	100%	100%	9%	0%	27%