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Size Reduction, Reproductive Strategy and the Life Cycle of a Centric Diatom

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Size reduction, reproductive strategy and the life cycle of a centric diatom

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CONTENTS

SUMMARY

The life cycle of *Aulacoseira subarctica* (O. Müller) Haworth in Lough Neagh, Northern Ireland, is described. Cell numbers can reach up to 17 000 per millilitre in spring. Most cells sediment to the bottom after silica limitation and go into a resting state during summer. The inoculum in autumn partly comes from resuspension, with the surviving cells (0.5–5%) continuing to grow through the winter, doubling every one to two weeks. The population goes through a size reduction and regeneration cycle linked to sexual reproduction. Gametes are only produced in narrower cells (3.8–7.4 μm diameter), usually after interruptions in growth caused by low light conditions (surface irradiance 100–150 $\mu E\ m^{-2}\ s^{-1}$), but availability of nutrients, especially silica and nitrogen, is also important. Even the highest densities of auxospores ($20\ ml^{-1}$) represent only a small proportion of the total cells present (0.16%). Size regeneration results in initial cells with diameters (14.8 ± 2 μm) about three times those of the parent. Larger parent cells usually give rise to larger initial cells. Subsequently, cell division leads to a decrease in population diameter, because of the way new valves are laid down below the girdle bands. Reductions are largest in broader cells (0.32 μm per division) and gradually decrease as cells get narrower. Occasionally large reductions, up to 1 μm, follow periods of environmental stress. By combining these results with studies of changes in cell size (width, length and volume) in related individuals along filaments, it was possible to explain why there have been difficulties in applying the MacDonald–Pfitzer hypothesis to natural populations. Theoretically, the life cycle in L. Neagh might extend over 100 divisions or 15 years but, in practice, cells reach a sexually inducible size in 4–6 years. The discrepancy is because environmental factors (e.g. sedimentation, resuspension, parasitism, etc.) are also important in size selectivity. The interaction of these factors, when combined with intermittent sexual reproduction at

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low frequencies, results in a relatively stable population size distribution, where there are always some cells in the size range in which sexual differentiation can be induced. Overall, the results demonstrate, that for a full understanding of diatom population dynamics, it is important to quantify events over complete life cycles.

1. INTRODUCTION

The study of centric diatom life cycles in natural planktonic populations has been largely overlooked, with only a few reports and these limited to relatively short periods (see French & Hargraves 1986; Kustenko 1986, 1987). Consequently we know little about the frequency of sexual reproduction that is so important for understanding evolutionary processes (see Lewis 1984), in a group that is believed to contribute 20–25% of the world's net primary production (Werner 1977). It also means that information on recruitment, which is usually the starting point for most plant and animal population studies (see Boero 1990), is unknown for centric diatoms. In order to improve our understanding of population dynamics, we need to know more about the significance of events over complete life cycles.

In contrast to the paucity of information on sexual reproduction in natural populations, evidence from cultures has been accumulating gradually since the first description of oogamy in a centric species by von Stosch (1950, 1951). For example, a wide range of factors have been shown to induce sexual stages in centric diatoms, including a variety of light and temperature conditions (Bruckmayer–Berkenbusch 1954; Drebes 1966; Holmes 1966; Roshchin 1976; Mizuno 1977; Furnas 1985; Vaulot & Chisholm 1987; Ambrust, Chisholm & Olson 1990), a range of nutrients (Steele 1965; Davis *et al.* 1973; French and Hargraves 1985, 1986) and salinity changes (Schultz & Trainor 1968, 1970). Even a shift in temperature (Werner 1971) or transfer of cells to new culture media (Manton & von Stosch 1966; Mizuno 1977) may enhance gamete production. However, environmental cues alone are insufficient to induce sexual differentiation in diatoms. From culture studies, it has been established that cell size has to be below a certain threshold, usually when the cell diameter is reduced to less than 30–40% of the maximum (see Drebes 1977). Excellent summaries of this and other details of sexual reproduction are given in reviews by Geitler (1932, 1973), von Stosch (1965), Drebes (1977), Mann (1988) and Round *et al.* (1990) for both centric and pennate diatoms. They leave little doubt that sexual reproduction is linked to the cycles of size reduction and regeneration seen in most diatom populations. The time to complete a cycle is largely determined by the rate at which size reduction takes place.

In centric species, there is usually a decrease in diameter of the valve equally along all radii. This was first described in the last century, along with proposals of how the girdle bands might influence the size of daughter cells (see, for example, Pfitzer 1869, 1871, 1882; MacDonald 1869). Since then considerable discussion has taken place, as to whether or not diameter reduction is an inevitable consequence of having a silica frustule (see Hustedt 1967; Hutchinson 1967; Margalef 1969; Round 1972; Crawford 1980; Mann 1988). If it is unavoidable, then size restitution, via sexual reproduction, might be seen as an 'escape' but there have been some reports that diameter reduction can be circumvented, albeit in specialised conditions (see Stosch 1965; Hutchinson 1967; Round 1972; Gallagher 1983). Also, some of the more complex shaped marine centric diatoms show greater reduction along one axis (e.g. *Eucampia antarctica*, R. Jordan personal communication), as do many pennates (see, for example, Geitler 1932). In two stimulating articles, Lewis (1983, 1984) discussed how diameter decrease might be used, when combined with a size threshold for sex inducement, as a way of solving the problem of 'clocking' sex in unicellular organisms that live in habitats where it is difficult to find a reliable environmental cue for periods beyond one year. He also suggested that it was a mechanism for reducing the cost of sex, by preventing it occurring too often, and speculated as to how the 'costs' might be balanced against the potential benefits resulting from recombination during sexual reproduction. The rarity with which sex has been observed in nature (see Mann 1988; Sommer 1988) tended to suggest the costs outweighed the benefits but that does not seem to fit with the enormous adaptive radiation of the diatoms (see Round & Sims 1980; Round *et al.* 1990) and as Lewis (1983) points out 'Since sex is difficult to document in algae, ignorance may well exaggerate our impression of pervasive asexuality in algae'.

The study presented here aims to show how the process of sexual reproduction is incorporated into the ecology of a filamentous centric diatom, *Aulacoseira subarctica* (O. Müller 1906) Haworth. Particular emphasis is placed on how environmental factors interact with 'in-built' mechanisms of size reduction (i.e. girdle band arrangment) to influence the length of the life cycle. *A. subarctica* is one of the most suitable species to answer these questions as it does not separate after division but forms long chains, which facilitates study of changes in cell size (length, width and volume) of related individuals.

There are twenty years of work on different aspects of the biology of *A. subarctica* in L. Neagh (Gibson *et al.* 1970; Gibson 1981, 1984; Gibson & Foy 1987, 1988; Gibson & Fitzsimmons 1990; Jewson *et al.* 1981; Fairburn *et al.* 1987). It has been a dominant diatom species in the lake for centuries (Battarbee 1978) and even millenia, as it is easily the most common species in diatomite deposits dating back over 8000 years (see Jessen 1949; Battarbee 1977; Woodman 1977). It is also a species widely known from the classic seasonal

studies of Lund (1954, 1955, 1971), who recorded its presence in a number of lakes in England, Ireland and Scotland. It was first described, in a perceptive paper on cell sizes, as early as 1906 by Müller from Thingvallavatn in Iceland. It was then called *Melosira italica* subspecies *subarctica* O. Müller. Recently, the genus *Melosira* was split, with many of the freshwater planktonic species being put in the genus *Aulacoseira* (see Simonsen 1979, Crawford 1981, 1988; Haworth 1988). Of the relatively limited reports of sexual reproduction, a high proportion come from the old *Melosira* genus, including the first clear description in a centric species by von Stosch (1950, 1951) of *M. varians*, followed by *M. nummuloides* (Bruckmayer–Berkenbusch 1954; Erben 1959) and *M. moniliformis* (von Stosch 1958; Migita 1967; Mizuno 1977; Kustenko 1986).

2. SITE

The sampling site was in the middle of L. Neagh (5 km south of Portlee Point and 7 km east of Kinturk Flats). This gave a good representation of phytoplankton population changes, as the lake has a well-mixed water column and a relatively simple morphomety (see Jewson 1976). Samples were collected with a 1 litre Ruttner sampler at depths of 0, 5 and 10 m. The water depth was 13 m. Temporary periods of stratification do occur but rarely last more than a few days. The depth of the photosynthetic zone (1% light level) is relatively constant throughout the year, at between 2 to 3 m (see Jewson 1976, 1977). The mean depth of the lake is 8.9 m, which is relatively shallow for a lake of 383 km^2, and means most of the bottom can be disturbed by wave action (Jewson & Hueston 1992). The lake is eutrophic (see Gibson, Smith & Stewart 1988). Phosphate removal at the major sewage works was started in the early 1980s (Gibson 1986) but the lake showed only limited recovery over the period of this study (see Jewson & Zlinszky 1991). The lake has frozen over completely only once this century (1947).

3. METHODS

Preserved phytoplankton samples were fixed in Lugol's iodine or glutaraldehyde within an hour of collection. During periods of calm weather vertical heterogeneity in the population can develop, so all estimates of cell concentration and measurements were based on averaging the results from three depths (surface, 5 m and 10 m). Samples were sedimented in 0.25 ml chambers and then counted and measured (to the nearest 1 μm with an eyepiece graticule) on an Olympus inverted microscope (model IMT) at a magnification of × 500 (SPlan 40 objective and × 1.25 AH-NA intermediate tube). More detailed checks of size used a dry Leitz EF × 63 objective. On most occasions the dimensions of all cells in the three sedimentation chambers were measured. This usually exceeded 500 cells. When population concentrations rose above this, then a representative proportion of cells was measured from each chamber. At low concentrations (below 200 cells per millilitre), 1 ml sedimentation chambers were used to give sufficient numbers. On a few occasions, when concentrations fell below 50 cells per millilitre, then only 100 cells were measured.

Sexual stages were observed in a water mount (with the cover slip sealed by Vaseline) using a × 100 SPlan oil immersion lens and differential interference contrast. For less detailed but longer periods of observation, cells were placed in a 0.5 ml Perspex sedimentation chamber that could be left open to the atmosphere and viewed from underneath using the × 63 dry objective fitted to the inverted microscope. Material for the stereoscan (JEOL, model JSM-840) was air-dried directly onto a brass stub and sputter coated with gold. The size reductions in the valve mantle were measured from high definition photographic prints viewed under an Olympus SZH stereo microscope. With 100 graticule divisions per micrometre, this allowed a discrimination of 0.01 μm.

4. RESULTS

(*a*) *Population characteristics*

A. subarctica is the only major planktonic species that manages to grow (i.e. has a net increase in cell numbers) through most of the winter in the turbid environment of L. Neagh. The other main species are two diatoms (both species of *Stephanodiscus*) and two Cyanobacteria (both *Oscillatoria*) (see Gibson 1981; Gibson and Fitzsimons 1982). Only details of *A. subarctica* are shown in figure 1. Population changes of the other species will be published later, including the life cycles of the *Stephanodiscus* sp. (Jewson 1992) and evidence of factors affecting the resting cells in the sediments and their resuspension. The surface irradiance and concentrations of nutrients are also shown in figure 1 as a guide to the conditions under which events in the life cycle of *A. subarctica* take place.

In brief, the spring growth period usually ends in late March or early April when silica is depleted, although occasionally it may be as early as the first week in February (e.g. 1989). *A. subarctica* is the first diatom species to stop growing. This means that it is in a good condition to survive on the bottom during the summer months when nutrient availability is low in the water (see Jewson *et al.* 1981). On the bottom, cells go into a resting state but this is not the morphologically distinct stage that is seen in many marine diatoms. The cytoplasm is withdrawn around the chloroplasts in a way first described by Lund (1954) and cells are able to survive in this form for up to 18 months. This is nearly 12 months longer than the other main planktonic diatoms in the lake (e.g. *Stephanodiscus* sp.). After resuspension, the cells recover sufficiently to start dividing again in about 6 days. Phosphorus and nitrogen concentrations remain low during the summer, because of the high biomass of Cyanobacteria, mainly *Oscillatoria agardhii*. *A. subarctica* only starts growing again in autumn, when all these nutrients are once more available (see figure 1). As the autumn is a time of decreasing solar radiation,

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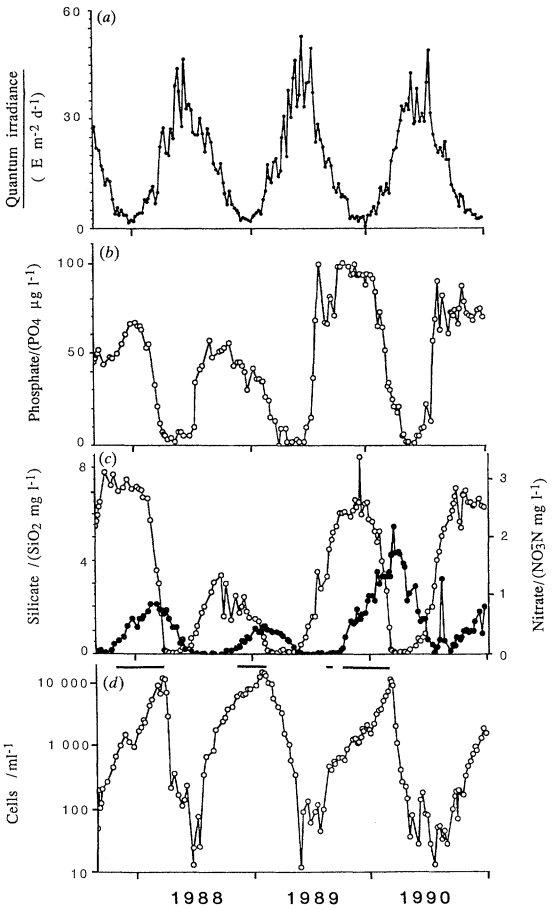

Figure 1. The seasonal changes in light, nutrient concentrations and cell numbers of *A. subarctica* in L. Neagh, from September 1987 until December 1990. (*a*) Surface quantum irradiance (400–700 nm), averaged over 7 days; (*b*) soluble reactive phosphate; (*c*) nitrate (filled circles) and silicate (open circles) concentrations; (*d*) cell numbers, expressed on a logarithmic scale. The black bars show the periods over which auxospores have been found. The chemical data was supplied by Dr C. E. Gibson.

the rate of increase in cell numbers is slow. This situation continues through the winter, with the number of cells only doubling once every one or two weeks (see figure 1). During periods of poor irradiance, increase in cell numbers may cease altogether but this does not usually last for more than one or two weeks.

A. subarctica does sometimes occur as a single cell (most frequently in autumn following grazing over the summer) but is usually found with filaments containing between two and 16 cells. Occasionally much longer filaments occur, sometimes exceeding 45 cells but the number of cells per filament varies over the growing season. Figure 2 shows the distribution of cells per filament at the beginning of growth in autumn, when the population average drops to about two cells per filament, and at peak numbers the following spring, when the average rises to about eight to ten cells per filament. This is fewer than might be seen in other species of *Aulacoseira*. One of the main reasons for this is that *A. subarctica* is unusual in only producing end (i.e. separation) cells (Round *et al.* 1990, p. 170). So, there is a greater tendency for cells to 'separate' from each other as filaments get longer.

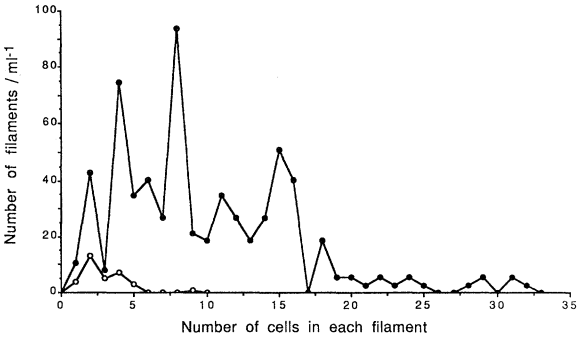

Figure 2. The variation in the numbers of living cells per filament in the *A. subarctica* population of L. Neagh at the beginning (17 September 1990; open circles) and end (19 February 1991; filled circles) of the growing season. Note the predominance of 2, 4, 8, 12 and 16 cells per filament in the February sample.

(*b*) *Cell diameters*

In *A. subarctica* diameters can vary from 3 to 14 µm (see figure 3) but very occasionally up to 18 µm. However, most of the population (90–95%) usually has diameters in a very narrow size range between 4 and 7 µm (figure 4). In weekly measurements over 3 years, there was little change in the modal class (5–6 µm), although the total population numbers (i.e. cells per millilitre) increased and decreased by over three orders of magnitude each year (see also figure 1). The lowest average population diameter recorded was 5.0 µm in June 1989, in cells remaining in the water column after silica limitation (population numbers were under 100 per millilitre). The highest average population diameter was 6.8 µm in August 1990 after resuspension of cells from the sediment.

Figure 3. (*a*) An initial cell of *A. subarctica* with one valve of the parent cell attached (plus another valve of the adjacent cell). The membrane of the auxospore is still intact. (*b*) Higher magnification of (*a*) to show the surface structure. (*c*) A broad filament with the hemispherical valve of an initial cell still attached (note steps in the mantles of two valves). In contrast, a narrow cell of 4 µm diameter can be seen to the right. Two Cyanobacteria *Oscillatoria agardhii* filaments are lying across the larger cells. (*d*) A hemispherical initial cell valve cleaned with hydrogen peroxide. (*e*) A higher magnification to show the surface of the initial cell wall. (*f*) Examples of a broad (12·7 µm) and a narrow filament (4 µm). The broad filament has steps in both valves but the narrow valve does not. It has snapped apart to show the end wall and has left the tips of two spines in place on the upper cell. The narrow cell has an *Oscillatoria* wrapped around it. (*g*) The cingulum is not one element but made up of several copulae. The other valve is a small *Stephanodiscus*.

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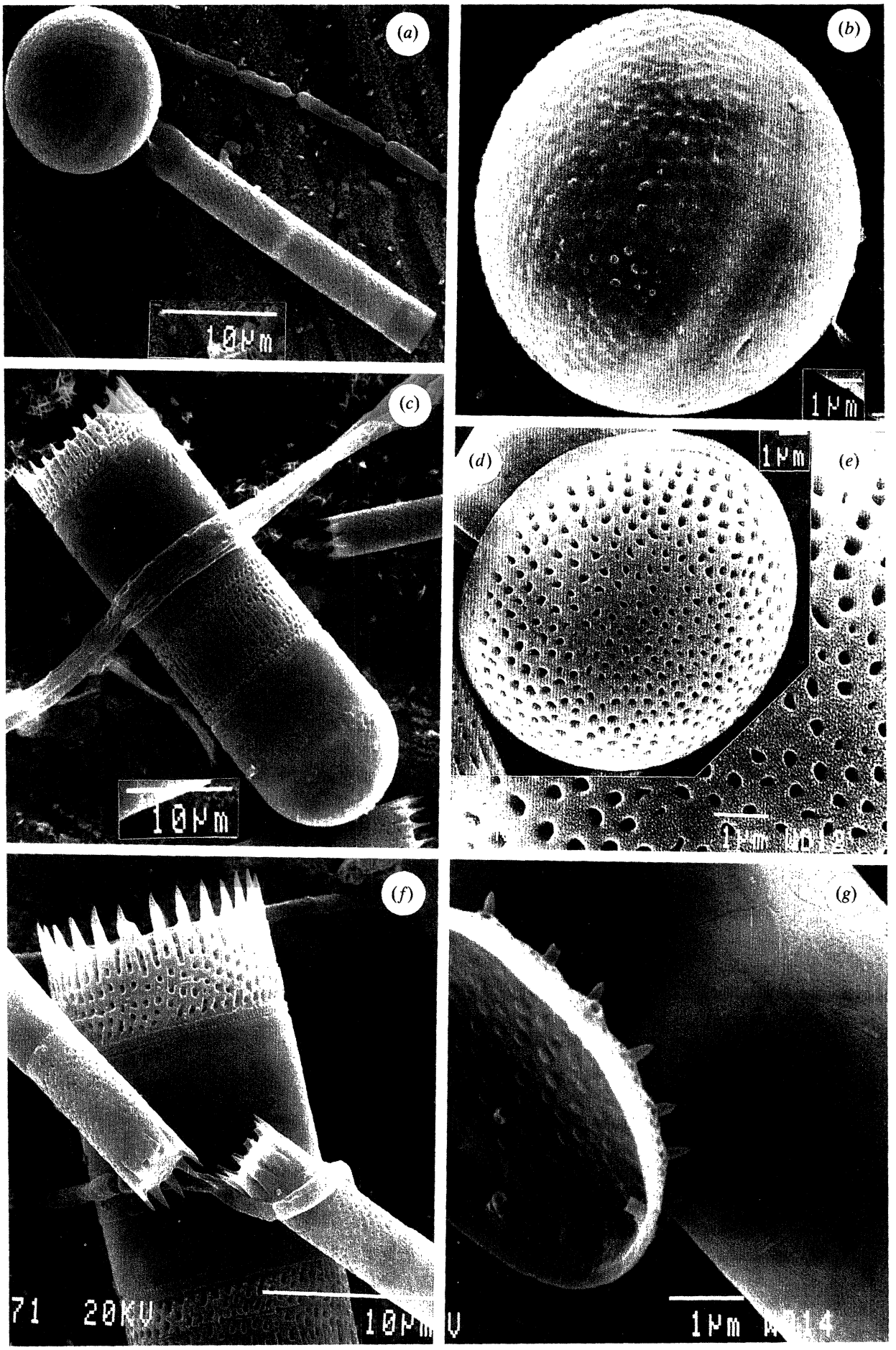

Figure 3. For description see opposite.

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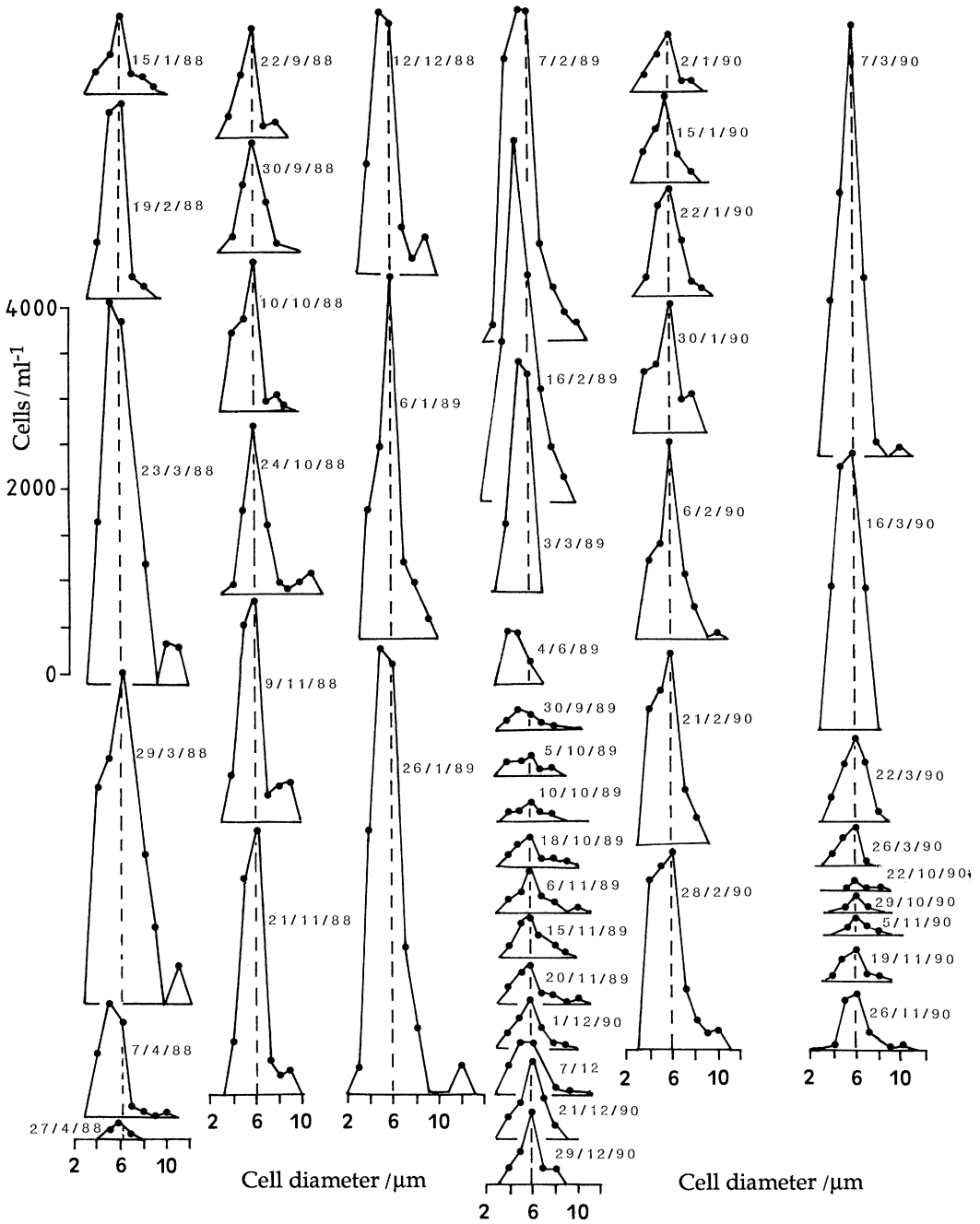

Figure 4. Size distribution of diameters of *A. subarctica* population over three years from 1988 to 1990. The dashed line, inserted at 6µm, indicates the upper limit of sexually inducible cells.

Such extremes exist for relatively short periods. For ninety five per cent of the time during those three years, the population average was 5.99 ± 0.6 µm, i.e. in a narrow size band between 39 and 47% of the maximum diameter. This apparent consistency masks considerable fluxes that are the result of a complex interaction between internal controls of cell size and selective environmental pressures (especially differential sedimentation of filaments of different diameter), as well as loss of cells through death and sexual reproduction. During these changes, the shape of the size distribution curves may deviate from normal (and become skewed) but soon reverts back again (see figure 4). Essentially the losses from one size class are replaced by cells from the one above. The factors causing these shifts are discussed in more detail below, starting with changes to individual cells.

(*c*) *Diameter reduction of cells*

The top two cells illustrated in figure 5 show a plan, suggested by Crawford (1980), of the most likely arrangement of girdle bands and valve walls in *M. arenaria* (now *Ellerbeckia*, see Crawford 1988). I have developed this further in figure 5 to help explain the arrangement of valves and girdle bands found along filaments of *A. subarctica*. The drawings are diagrammatic and are not to scale. Terminology follows that of Round *et al.* (1990). The girdle comprises an epicingulum and a hypocingulum. For simplicity,

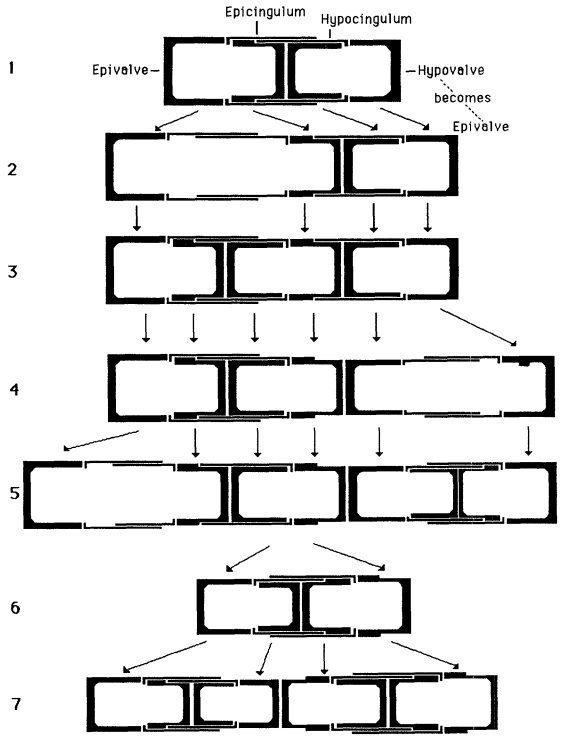

Figure 5. A diagrammatic representation of the valve and girdle band arrangement in *A. subarctica*. The top row is based on a plan of Crawford (1981) for *Ellerbeckia* (previously *M. arenaria)*. Rows 2 to 5 show the sequence in which cells divide. Rows 6 and 7 show how the arrangement of valves and girdle bands seen in figure 6*g* could be obtained, where the left cell has no steps and the right cell has one on each valve. The close fitting of the hypocingulum into the step shown in Rows 2 and 3, can also been seen in figure 6. The drawings are not to scale. For full explanation see text.

each cingulum is represented as a single element but is composed of several sections called copulae (see figure 3*f*). The epicingulum is connected to the older and broader valve (epivalve) and the hypocingulum to the narrower valve (hypovalve).

To understand reduction in diameter, it is necessary to follow the sequence of events over several divisions. The explanation that follows is based on the diagrams in figure 5 and photographs in figures 3 and 6. Before division the cell increases in length, so that new valves of the daughter cell can be formed. The girdle bands give protection at this time. In the example shown in figure 5, the left cell of row 1 divides first. A new hypocingulum is formed as the cell lengthens, growing out from the edge of the newly formed hypovalve (row 2). I have never seen, in this species, an example of where the smaller daughter cell (i.e. the one on the right in row 1) divides first, that would require the hypocingulum to be withdrawn from under the epicingulum. In the offspring, the parent epicingulum becomes the epicingulum of one of the daughter cells (the left one in row 3, figure 5) and the hypocingulum will become the epicingulum of the other (right cell, row 4). It is important to remember that the epicingulum cannot be added to after it is formed. The production of the hypocingulum below it, means that the epicingulum becomes isolated from the areas of silica deposition in the cell. It is then passed on, intact, at each subsequent division.

Crawford's (1980) plan predicts that the hypocingulum will leave a distinct step in the mantle (side wall) of the hypovalve of the larger daughter cell as it is laid down. Such steps occur in *A. subarctica*, although not in the narrowest filaments below 5 μm (figure 3, 6). To confirm Crawford's idea, which implied that these steps give a decrease in diameter not an increase as originally believed (Müller 1883, 1884), I have shown in figure 5 how subsequent divisions can give the arrangement of steps seen along *A. subarctica* filaments. One case needs special mention. It is similar to a pattern suggested by Crawford (1973) for *M. nummuloides*, based on earlier work of Müller (1883). In approximately 10% of cells of *A. subarctica* an interim stage can be observed, where the pattern of valves and girdle bands is similar to that in the second and third rows in figure 5 (see also figure 6*d*, *e*). The left daughter cell has finished dividing but the right one has not started yet. This leaves the old hypocingulum in place, fitting tightly into the mantle of the hypovalve of the left cell. The right cell now takes its turn to divide. As it lengthens, the old hypocingulum (which will now become the epicingulum of the new cell) pulls away, leaving the mantle and step visible (row 4). The new valves are then laid down below the girdle bands and this results in another step being formed in the new valve (row 5). The next cell to divide would be the one on the left of row 4. Again a new hypocingulum would be formed as the cell lengthened (row 5). After this, it would be the centre cell of row 5 that divided and so on. Under normal conditions, *A. subarctica* cells will always divide in this sequence.

The validity of this design can be tested further by trying to explain patterns of steps and cell diameters that occur along a filament. A recurring pattern, that was initially difficult to explain, is shown in figure 6*a*. The division sequence necessary for this is shown in rows 5, 6 and 7 of figure 5. Essentially, if the middle cell of the fifth row was to divide twice more, then the arrangement of valves and girdle bands would be as shown in the bottom line with one cell having steps on both valves and the other none. The girdle bands also overlap in the correct way.

The steps left in the mantle by the girdle bands vary with cell size, becoming less distinct as the diameter of the cells decreases (see figure 6). To quantify this, the height of the step was measured in cells of different diameters (figure 7). This proved easier and more relevant for assessing the degree of constriction, than measuring the thickness of the girdle bands directly. The steps were deepest in the broadest cells (i.e. those with the thickest cingula), which explains why size reductions are greater in the broader cells. For example, the deepest step was 0.16 μm (i.e. diameter reduced by 0.32 μm). Whereas for the most common modal class of 5.5 to 6 μm (see figure 4), it was 0.075 μm (i.e. a 0.15 μm diameter reduction). Below 5.5 μm, steps become very faint or are undetectable. It appears that as cell diameter decreases, girdle bands

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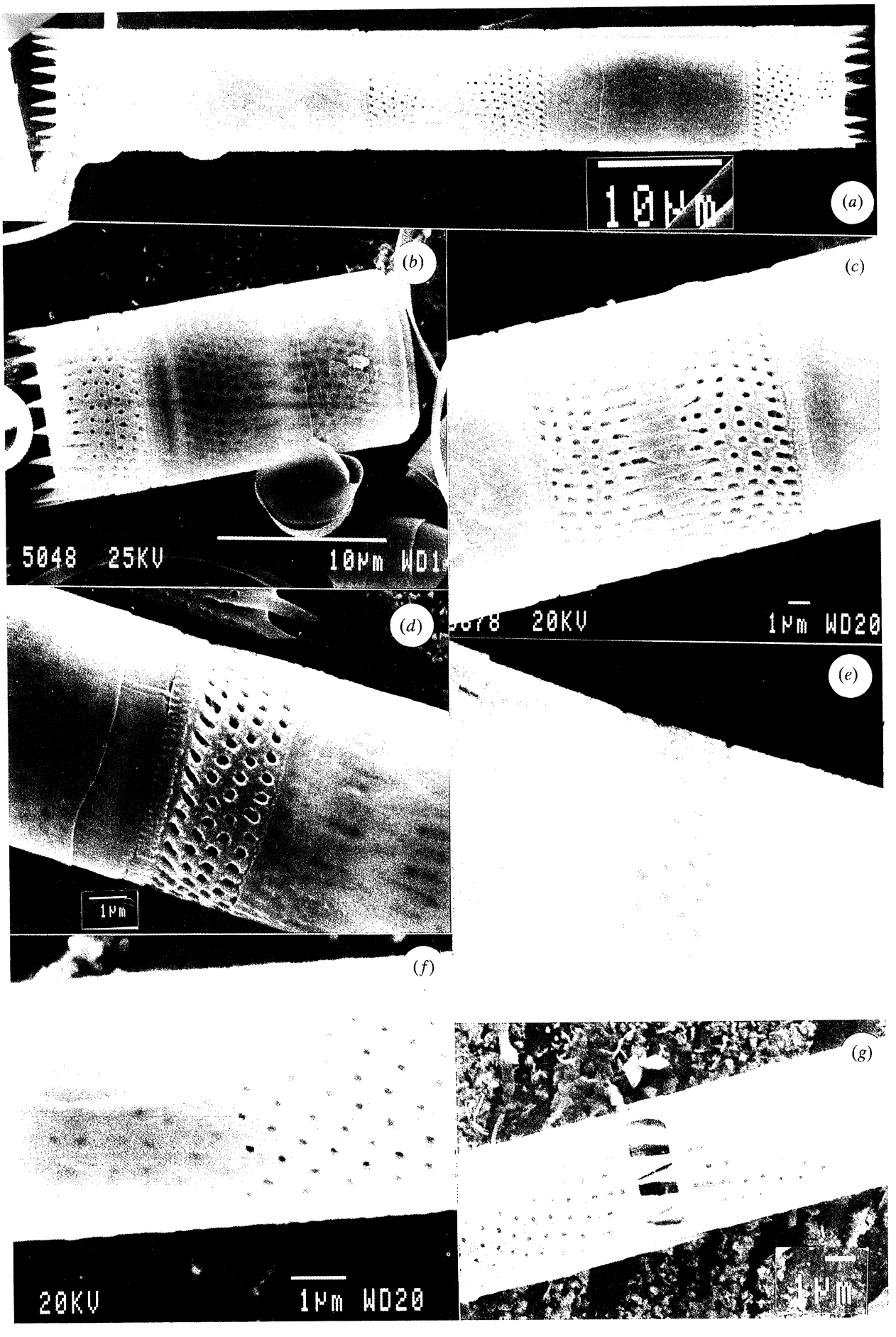

Figure 6. For description see opposite.

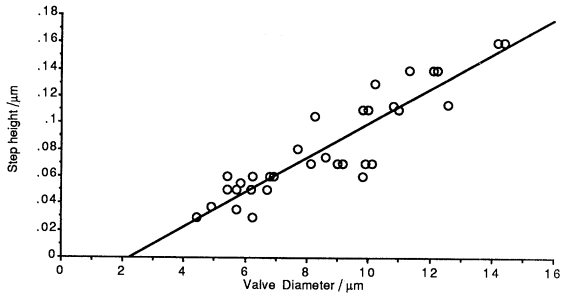

Figure 7. The height of the steps in the valve mantle, over the size range of diameters found in L. Neagh, for *A. subarctica* cells. Regression statistics: $y = 0.013$, $x - 0.028$, $r^2 = 0.838$.

are thinner, more flexible and less restrictive (compare figure 6*f* with other cingula shown in figures 3 and 6). Only two steps were found below 5.5 µm (see figure 7), amongst the many cells of this diameter that were inspected. The results in figure 7 indicate a considerable variation in step thickness at any given diameter. More measurements should establish whether this is a natural variation within the population or an artefact created by measuring from photographs, where only one axis can be viewed.

The next stage was to look at the pattern of valve diameters along individual filaments of different widths (figure 8). For sufficient resolution, the narrower filaments were measured on the scanning electron microscope (SEM). Unfortunately this means that newly formed daughter valves cannot be seen clearly under the girdle bands and measurements are restricted to half the number of cells that can be used under the light microscope. Most valves showed reductions that are in line with the changes expected from the constrictions caused by the girdle bands described above. However, there are a number of size decreases that are larger than expected, e.g. the end cell on 6–7 µm filament in figure 8. Even larger reductions, over 1 µm, have been found in routine measurements under the light microscope (figure 9). The reasons for this are not fully understood but they appear (along with reduced valve length) more often during or just after periods of environmental stress, for example, in those filaments kept in suspension during low nutrient concentrations in summer or at times of low light in November or December. There are usually only one or two cells like this in the longer filaments and so these distortions reflect the periods of stress in its history.

By using the information on the likely range and frequency of decreases in diameter, it is possible to estimate the reductions that might result in one

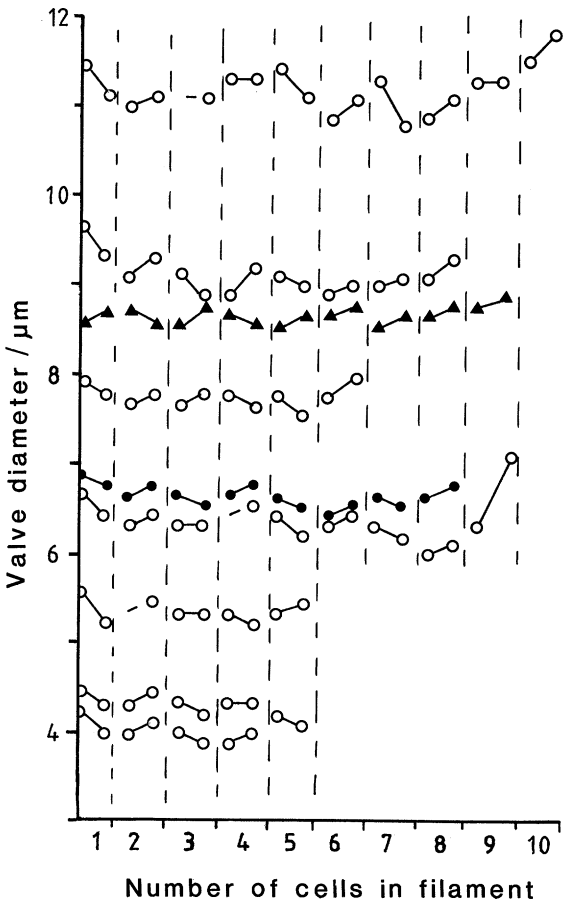

Figure 8. Variations in the diameters of valves along filaments (between 4 µm and 12 µm width) of *A.subarctica*. All measurements were made on the stereoscan. Separate cells are indicated by the vertical lines. An idealized pattern, predicted if all cells divide at the same rate, is shown for a 7 µm wide filament (see line with filled circles).

season. If the largest reductions were to occur at every division, then the diameters of some daughter cells could come down from 14 to 10 µm in a growing season of six divisions (i.e. 64 daughter cells from an original parent cell) and, if this were to continue, some cells could reach reproductive size in three years. However, as the adverse conditions that produce such large decreases would only be experienced by a filament perhaps once or twice in any one season (see figure 6) they will only affect one or two divisions at most. More typically, results from figure 8 suggest that it is possible for some of the offspring of broader cells (above 10 µm diameter) to reduce by 2–3 µm per season and the middle sized ones (6–8 µm diameter) about 1 µm. In the narrower cells below 5.5 µm,

Figure 6. Girdle bands and steps in the mantle of *A. subarctica*. (*a*) The arrangement of girdle bands and steps in these two cells corresponds to the plan in row 7 of figure 5. (*b*) The valves in this picture are from a sample cleaned with hydrogen peroxide. A step can be seen in the mantle of the valve (diameter 10·6 µm). The epicingulum has become torn and is coiled at the side of the cell. The newly forming valves can be seen, below the girdle band. Note the relatively large pore size, indicating an early stage of silicification. (*c*) The step in the mantle of this cell is clearly visible. The tight interlocking of the spines contrasts with the cells separating in (*f*). (*d*) This shows a cingulum still fitting tightly into the step on the mantle. (*e*) In this case the cingulum is just pulling away. (*f*) In narrow filaments the cingulum is less rigid and thinner. This one has become wrinkled and the mantle below it is clearly visible. (*g*) This shows two narrow valves pulling apart.

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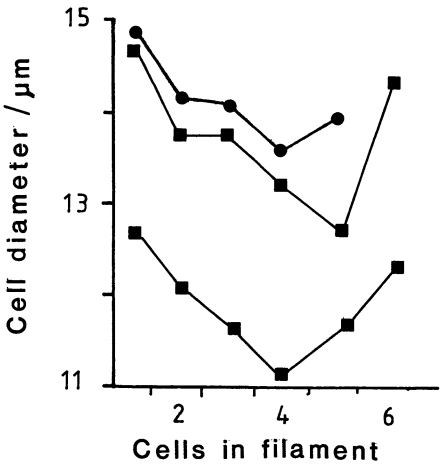

Figure 9. Variations in the diameters of the broader cells of *A. subarctica*, measured by light microscopy. Diameters are averages of two readings from each valve. These filaments were chosen to illustrate the largest changes that have been recorded.

diameter decreases are smaller. With the light microscope they are usually missed. From SEM measurements (figure 8), it appears that no detectable change could be found in 25% of cells, changes of 0.1 µm were most common (50%), with a few of 0.2 µm (less than 20%) and rarely 0.3 µm (less than 5%). In one growing season, reductions could then be between 0.6 and 1.0 µm. Effectively, these smaller reductions mean that the rate of diameter decline is lessened once the reproductive size range is reached (between 4 and 6 µm).

(*d*) *Diameter reduction and the length of the life cycle*

To help later comparisons with size selection due to environmental factors (e.g. sedimentation, etc.), it is useful to estimate more precisely what the potential rate of diameter reduction in the population over prolonged periods might be. A number of workers in the past have used a binomial relationship (see Pfitzer 1882; Hustedt 1930; Hutchinson 1967) where size reduction is predictable in terms of girdle band thickness. It assumes that one daughter cell will have the same dimensions as the parent. In the other cell, the old hypovalve of the parent becomes the epivalve of the daughter cell and the new hypovalve will be smaller by 2 times the thickness of the cingulum (see, for example, figure 5). However, size reductions estimated in this way only seem to explain the pattern in a few natural populations (Hustedt 1929; Hutchinson 1967). Part of the problem is that a binomial progression predicts an increase in variance, whereas most populations are found with relatively narrow size classes.

One of the most important points that has been largely overlooked hitherto (except Mann 1988, p. 400), is allowance for the limited lifetime of individual valves. Evidence from natural populations of *Stephanodiscus* species (Jewson 1992), where it easier to follow the loss of specific size classes, suggests an epivalve and its cingulum only last through about six to eight divisions. I have not been able to directly measure it in *A. subarctica* yet but I believe it is similar, so in the calculations that follow, a value of seven divisions has been assumed for *A. subarctica*.

To establish the rate of diameter decline in the population, it is necessary to know the number of divisions that cells undergo in a year. This must include an allowance for cells lost due to sedimentation, grazing, etc. (see below). During this study, the number of divisions over four growth periods starting in autumn 1987 and ending in 1991 were eight, six and a half, six and seven (see figure 1). Over the previous decade it ranged from five to eight. On this basis, the mode of the population diameter of one generation (after auxospore production) would probably take over 100 divisions or over 15 years (if seven divisions per year) to reduce to the optimum size for sexual reproduction. This has been plotted in figure 10 (curve A). The horizontal scale can be read either in terms of number of divisions since auxosporulation or in terms of years. In the later case, the two most common division rates per annum over the last decade have been shown. All calculations include an assumption that epivalves only last through seven divisions. The diameter decrease in curve A of figure 10 was based on the regression analysis of results in figure 7.

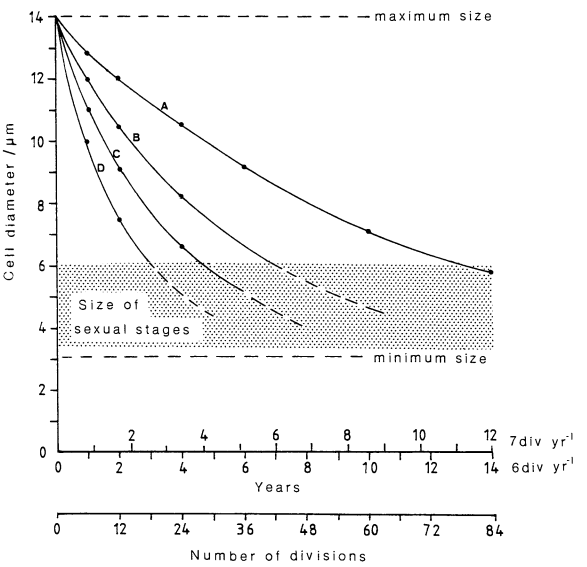

Figure 10. The decline in the mode of the population diameter of *A.subarctica* with time. The horizontal scale is expressed in three different formats. The top two give the number of years it would take to reach a given diameter, assuming either six or seven divisions per year. These are typical for L. Neagh but other rates of division can be estimated by using the lowest scale, which represents age as the number of divisions. Curve A is for a theoretical population where there are no losses from environmental factors. Curve B assumes some loss of broader cells and represents the longer lifetimes expected in the lake. Curve C incorporates all mortality factors and is representative of the most likely rate of decline. Curve D represents a case based on a rate of decline with the largest reductions recorded for each width and where only the narrowest cells are selected for. The size range over which sexual induction can take place is shaded.

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It was also assumed that all cells divide at the same rate. As early as 1884, Müller suggested that this might not be true for all species, based on evidence from a filamentous diatom, *Melosira arenaria* (now *Ellerbeckia arenaria*, see Crawford 1988), in which the smaller daughter cell took twice as long to divide as the larger. Mann (1988) confirmed this finding for the same species. In *A. subarctica* the smaller cell does divide later (as can be predicted from the girdle band arrangement described above in figures 5 and 8) but it does not normally skip a generation (see further discussion below in cell length changes). I have compared both the normal binomial progression and Müller's adapted scheme and found, for this species the former seems to fit the results better (e.g. compare measured and predicted diameter distribution in figure 8). However, this rate of division is likely to vary between species, depending on the girdle band and valve arrangements and needs further study.

The fastest possible rate of decline (curve D in figure 10) was calculated assuming that at each division, cells underwent the largest reductions that have been measured for each diameter. Even with this, it would take 3 years to reach the optimum size for female gametes. This would be a rare occurrence as it assumes poor growth conditions throughout. The most probable rates of decline in L. Neagh are closer to the middle curves in figure 10 (B and C). In the natural population, allowances must be made for environmental size selection pressures (see below).

(*e*) *Diameter reduction and sedimentation*

One of the most important environmental size selective factors is the preferential sedimentation of broader cells under certain conditions. This can be seen by comparing the size distribution of cells in the water column and surface of the mud at 10 m depth in November 1988 (figure 11). The surface irradiance was low and the population stopped growing. Under these conditions, there was a greater sedimentation of broader diameter cells. This altered the size distribution early in the growth period, as the sedimented cells were not resuspended. At other times of the year, with an actively growing population, this differential settling has not been so obvious (Jewson & Hueston 1992). An increase in sedimentation rate has been recorded for this species following silica limitation but the study did not include an analysis of cell size (Gibson 1984).

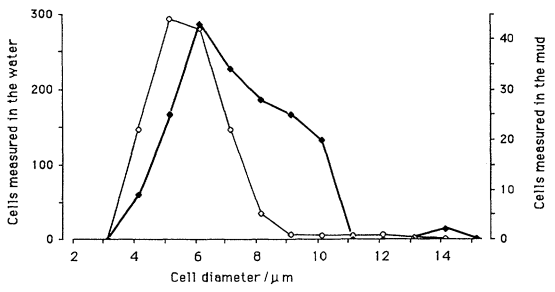

Figure 11. The size distributions of diameters of *A. subarctica* in the water (open circles) and in the surface layers of the mud (at 10 m) (filled symbols) in L. Neagh on the 7 December 1989, after two weeks calm weather.

(*f*) *Parasitism and diameter selectivity*

Another size-selective factor is parasitism by the chytrid *Zygorhizidium melosirae*. The percentage of cells infected over the three years varied between 2 and 8%. The highest levels occurred in the winter of 1990–1991. All cells can be infected but if the infestation is expressed as a percentage of the individuals in each size class then the size range most likely to produce gametes is also the most prone to parasitism, with the rate nearly doubling when the diameter drops from 6 to 5 µm (figure 12*a*). The size distribution in the two previous years also gave a similar result (at this time of year) but later in the season the percentage infestation decreased (figure 12*b*) and the relative difference between sizes was less obvious. It is possible that the less rigid and poorer fitting girdle bands in the narrower cells, discussed above (see figure 6*f*), makes the cells more vulnerable to parasitic attack.

(*g*) *Diameter increase from resuspension*

A size selection that works in the opposite direction (i.e. against narrower cells) is the increase in population diameter that occurs when cells are resuspended from the bottom of the lake. After silica limitation in spring (see figure 1), the population divides into two, most cells sink to the bottom and go into a resting state (see Jewson *et al.* 1981). The others (approximately 1%) remain in the water column during the summer but during this time may undergo several

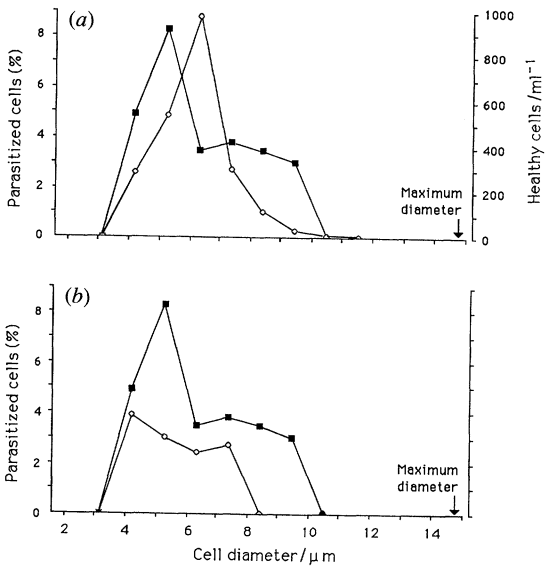

Figure 12. The size distribution of parazitized cells in the *A. subarctica* population of L. Neagh. (*a*) A comparison between the numbers of healthy cells per millilitre (open symbols) and the percent of the total population parasitized (filled symbols) in December 1990; (*b*) a comparison between the size distribution of parasitized cells (%) at the beginning of the growing season, in December 1990 (filled symbols), and at the maximum population concentration, in February 1991 (open circles).

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periods of settlement and resuspension from shallow inshore areas. The average population diameter may decrease to 5.5 μm. In dry years, with little inwash of silica, cells often become distorted and are much shorter than during the growing season. In the sediments, most of the population is gradually eaten by benthic invertebrates (chironomids and oligochaetes) (Jewson *et al.* 1981; Jewson & Hueston 1992). All cell sizes can be found but as the summer progresses broader cells tend to survive longer. The surviving water and sediment populations are brought back together by late summer and autumn storms (see steep rises in population in figure 1), which can disturb surface sediments down to water depths over 10 m. If there is insufficient silica or nitrogen most cells sink again. Cells can sediment and be resuspended several times, as a result of mid-summer calm and storm periods. It is only if nutrient conditions are suitable that the population starts to grow again.

The proportions of cells resuspended from the sediment in the years of this study were 53% in 1987, 92% in 1988, 79% in 1989 and 80% in 1990. These figures are estimated from increases in cell concentrations following storms in the late summer period when favourable nutrient conditions (of silica, nitrogen and phosphorus) return (see figure 1). A full description of this is to be published elsewhere (Jewson & Hueston 1992) but as a guide, the later in the year the resuspension occurs, the greater is the proportion of broader cells resuspended. This is mainly because there is a shift in where cells are resuspended from. The later in the year, the stronger the winds and the more likely cells will be resuspended from deeper, muddy deposits, where broader cells survive better than those below 5.5 μm diameter. However, in summer resuspension is more likely from sandy, inshore areas. For example, when the storms occured in July (1988) little change in population cell diameter was recorded. After August storms (1989), there was an increase of 6.1 to 6.4 μm but the largest increase followed September storms in 1987 (from 5.9 to 6.5 μm). Therefore, in these three years, the increases in mean population diameter varied from 0 to 0.6 μm. These may seem small but would be equivalent to size reduction from several divisions, during normal growth conditions, described above (see figures 7 and 8). Exchanges with the sediment surface occur throughout the winter but as the population numbers in the water increase, the relative proportion of cells recruited by resuspension decreases and so there is less impact on population diameter changes.

(*h*) *Cell length*

In *A. subarctica*, cells lengthen rapidly just before division. The appearance of the two new valve faces is one of the last stages in the cell cycle. For length measurements (pervalvar depths), their appearance was taken as the point in time when parent and daughter cells could be distinguished. There is considerable overlap in lengths of cells of different diameters but, in general, as diameter decreases so cells tend to be longer. Ignoring rapidly elongating cells, if length is plotted against diameter (figure 13), it shows that there there is a linear relationship until below 4 μm, when some cells become longer than expected. Cells of this size do not appear to survive very long in the lake and are probably prone to breakage. The results in figure 13 are based on actively growing, spring crops. At times of nutrient and especially light limitation (in mid-winter), shorter, distorted cells can be found.

If the individual lengths along a filament are compared (figure 14), the number of cells in the period of rapid elongation before division can be seen. In actively growing populations (e.g. February and March), there is considerable variation, with cells on the same filament (i.e. derived from the same parent) at different lengths and, therefore, at different stages in the cell cycle. Such an arrangement can be expected because of the necessity to divide in the sequence shown in figure 5. This may be a mechanism to ensure that there is asynchrony of cell division in the filament. It is unusual to find more than two or three cells at the rapid elongation stage on one filament (e.g. there are 5 out of 18 on one filament in figure 14*b*) The number of dividing cells in the whole population can vary from below 1% in the summer months through to a maximum of 12.5% during spring growth (i.e. one in eight cells).

In mid-winter, if light is too low even for sexual reproduction, then all cells on one filament gradually assume the same length (figure 14*a*). It is interesting that this may be different for filaments of similar diameter. This suggests that for each diameter there may be a genetically determined range of sizes. If so, this would be another example of spreading the risk, as there are so many environmental pressures acting on cell size.

(*i*) *Cell Volume*

The decrease in cell diameter is not compensated for by increased length (ignoring dividing cells), so cell volume decreases as the cells get narrower (figure

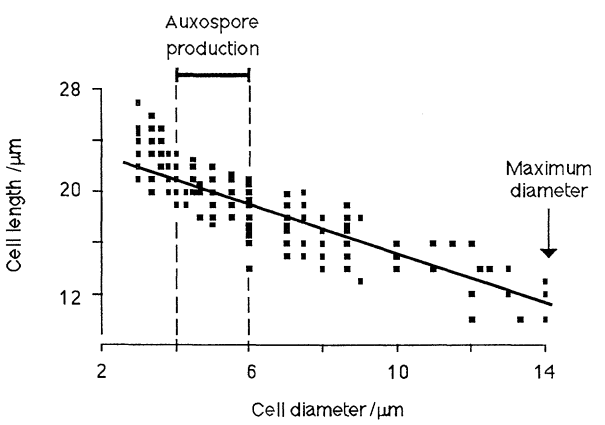

Figure 13. The variation in cell length, during normal growing conditions, over the range of cell diameters found in *A. subarctica* populations. The size limits of sexually inducible cells are shown. Regression statistics: $r^2 = 0.72$, $x = 12.46 - 0.97y$.

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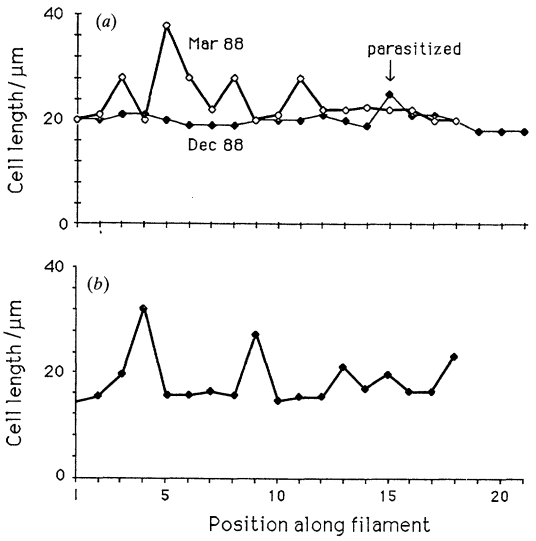

Figure 14. The lengths of cells along individual filaments. (*a*) The variation between seasons is illustrated with filaments (diameter 4.8 μm) from December 1988, when the population had ceased growing, and March 1988 when the population was growing rapidly (five cells are elongating). (*b*) Cell length in a filament (diameter 5.3 μm) from February 1991, showing five cells at different stages of elongation before division.

15). By the time the diameter has decreased to the width coinciding with the maximum production of auxospores, the volume is 22% of that of the initial cells after auxosporulation (i.e. 14.8 μm diameter cells). The range of diameters in which sexual differentiation can be induced (3.8–6.3 μm) lies between 26 and 43% of the maximum (14.8 μm), whereas the corresponding values for the volume are 14 and 28%. Below the lower threshold, only a few cells are found with volumes of 150 μm^3 (sexual differentiation has not been observed in cells of this size).

(*j*) *Sexual stages and auxospore production*

A. subarctica, like other centric diatoms, is oogamous and reproduction is similar to the *Melosira* type of Drebes (1977). Usually there are two or four spermatogonia, produced after differentiating mitoses, with the parent cell showing a gradual reduction in plastids compared to the normal vegetative cells. It is only in these later stages that sex cells can be identified with any certainty. Cell diameters were usually between 4 and 5 μm. Oogonia are generally broader, with a mean of 5 μm but a range of 3 to 7 μm has been found (figure 16). Cells elongate slightly (by 6–8 μm) before fertilization. Some cells seem capable of remaining like this for considerable periods. After fertilization, the zygote (auxospore) rapidly expands to about three times the original parent diameter, before starting to silicify (figure 3). One or both of the parent valves may remain attached to the auxospore for some time (figure 3*a*). No small siliceous scales, such as the kind found in *Melosira*, have so far been observed on the outer membrane. After the zygote is fully enlarged, initial cells are formed with hemispherical valves that are very heavily silicified (figure 3*d*) and are usually only seen in the water for a relatively short time, disappearing after a few days, but occasionally hemispherical valves may remain attached for several divisions (see figure 3*c*).

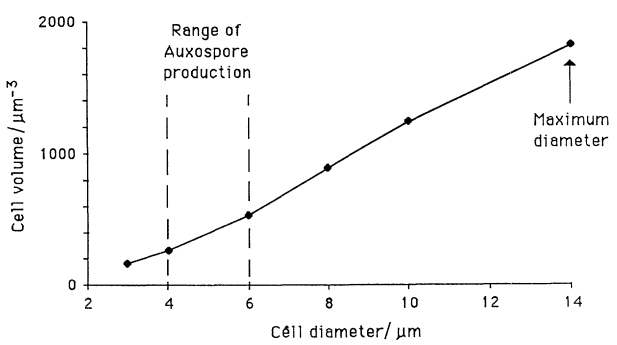

Figure 15. The variation in the cell volume (based on the regression analysis of figure 13) for different cell diameters of *A. subarctica*. The size limits of sexually inducible cells are shown.

(*k*) *Sizes of females and auxospores*

If analysis of size variation of female cells had to rely on first differentiating them from vegetative cells (as with male cells), it would be tedious and error-prone. In this genus, it is easier than in most others, as one or both frustules of the female parent cell often remain attached to the auxospore after size regeneration (see figure 3*a*). Consequently, it is possible to compare the diameter of the parent valve with the size of regenerated initial cells. In figure 16 the size distribution of the diameters are shown for all female cells with an attached auxospore seen in L. Neagh between 1987 and 1990. The mode of the female cells is 5.3 μm but the size of all observed cells is in a range of 20 to 47% of the mean diameter of the fully expanded auxospore (14.8 μm). Initial cells range between 12.7 and 19.0 μm. To establish whether the size of the parent cell has any influence on the size of regeneration, the diameters of the initial cell are plotted against the diameters of their attached parent cells in figure 17. The results suggest that bigger parents do produce bigger offspring. However, the values for two days in different seasons are highlighted. In the first (August 1989), some of the largest auxospores ever found were produced. This was probably because the high irradiance at that time (except on the days of sexual induction) ensured a

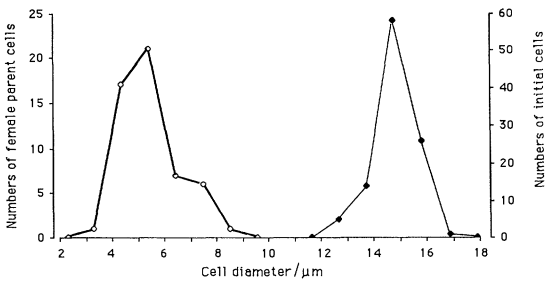

Figure 16. The size distribution of all the oogonia (open symbols) and initial cells (filled symbols) of *A. subarctica* found in L. Neagh between 1987 and 1990.

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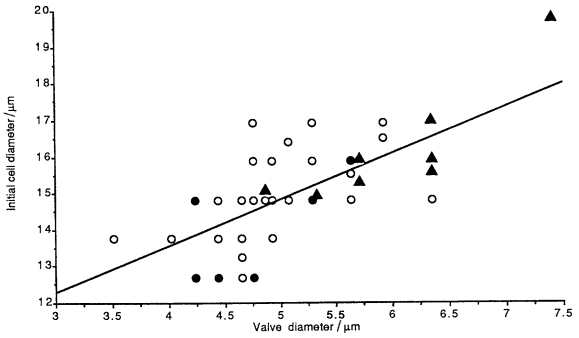

Figure 17. The relationship between the size of the initial cell and the size of the parent cell in *A. subarctica*. The size range for 7 February (filled circles) and for August 1989 (filled triangles) are highlighted. Regression statistics: $y = 1.273x + 8.463$; $r^2 = 0.488$.

maximum size was reached. In the other example (February 1989), the sizes of the auxospores were smaller and they show much variation, so it is probable that internal resources (i.e. previous light and nutrient history) of a cell at the time of differentiation are also a determinant. In the case of L. Neagh, it is unlikely that cells will have had a similar light history, due to the combination of limited light penetration and turbulent water circulation patterns. One result of this variability, is that initial cells in natural populations will have a relatively broad range of diameters (see figure 16 and 17). The extremes of this range could represent the equivalent of two years of diameter decline. In most years, this means there is an overlap in size with subsequent auxosporulations.

In figure 18, the size distribution of female cells is compared with the normal size distribution of the population (on 7 February 1989). The overlap shows the elegance of the solution that keeps some of the population at a point where sex stages can be induced but still ensures some of the cells remain as an inoculum to continue vegetative growth.

(*l*) *Cue for reproduction*

The exact cue for production is still not known but by looking at the times when the highest percentages are recorded, the most likely cue does seem to be a check in growth caused by low irradiance, about 100–150 μE m^{-2} s^{-1} of photosynthetically available radiation (400–700 nm) at the lake surface. However, various other conditions must also be fulfilled, especially sufficient nutrients. In autumn, it seems to be the supply of nitrogen that is most important, as phosphate and silica are usually plentiful at this time (see figure 1). In most cases of auxospore production, the nitrate concentration was above 0.15 mg l^{-1}. In only one case of large numbers of auxospore production, 23–28 August 1989, was it below this. However, there had been a large nutrient release from the sediments in the weeks before, with ammonia reaching 228 mg l^{-1} on 1 August. Although uptake by the algae had reduced this back down to undetectable levels in the week the auxospores were produced, it is probable that cells still had sufficient nitrogen. Surface irradiances low enough to induce the development of sexual stages did occur on 14 and 24 August but it is also possible some of these cells may have already undergone sexual induction in February and were just continuing development following resuspension (see below).

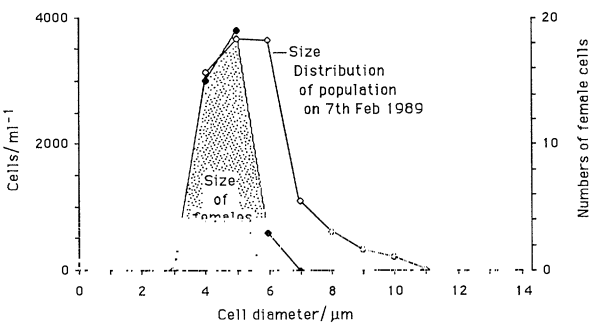

Figure 18. The size distribution of all female (oogonia) cells measured in this study compared to the size distribution of the population of *A. subarctica* on 7 February 1989.

Some growth (i.e. a net increase in population numbers, after losses are taken into account) is possible when surface irradiance falls to between 100–150 μE m^{-2} s^{-1} but it is very slow (see figure 1 and 19). If surface irradiance falls below this, growth ceases altogether (i.e. elongating cells are not found) and cell numbers start to decline. No auxospore production is seen in these circumstances but males and females do still seem to be present, presumably continuing their development when irradiance improves, which is usually in two to three days.

So far, only surface irradiance has been considered but in L. Neagh periods of low light are usually associated with low pressure weather systems (depressions) and are also very windy. In these cases, there is also the likelihood of reduced light penetration (see, for example, Secchi discussion in figure 19). Even in normal conditions in L. Neagh, light penetration varies relatively little with season; usually the depth of 1% light penetration is around 2 m (see Jewson 1977). In this situation, a cell circulating in L. Neagh in the euphotic zone on an overcast day would receive, on average, about a tenth of the surface irradiance. This would be about 10–15 μE m^{-2} s^{-1} (400–700 nm) at times of auxospore production. The total irradiance received in one day obviously also depends on the daylength. At the time of the most frequent auxospore occurrences, before and after the winter equinox, daylength is about 7–8 hours. So, a cell might receive 0.29 E m^{-2} in one day, if it remained in the euphotic zone. Some of the cells will inevitably exceed this dosage and others receive less, because of the circulation patterns in L. Neagh (Jewson & Hueston 1992). A cell circulating through the whole water column (mean depth 8.9 m), including the aphotic depths, would receive considerably less, about 0.06 E m^{-2} in one day.

(*m*) *Frequency of sexual reproduction*

In this species, sexual stages are produced relatively frequently during the winter months but the percent-

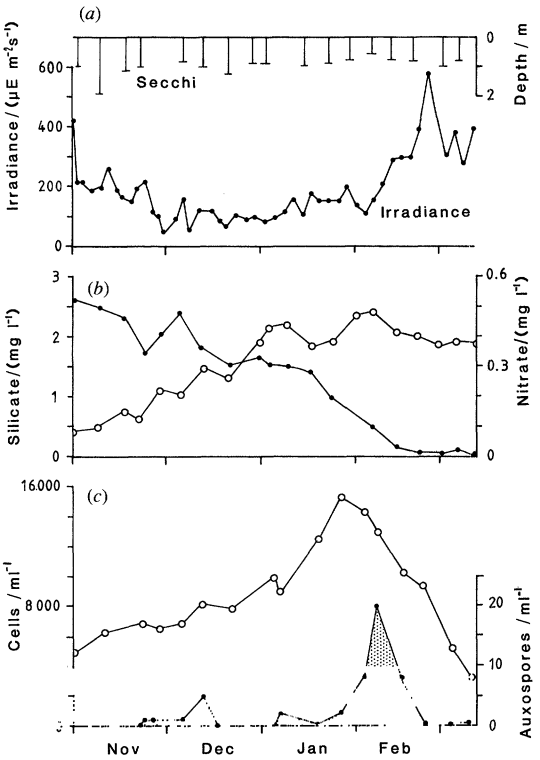

Figure 19. (*a*) The seasonal occurrence of auxospores of *A. subarctica* during the winter of 1988–89. The main environmental controlling factors are also shown. The available light is expressed as quantum irradiance (400–700 nm) and is the mean of surface values between sunrise and sunset (μE m^{-2} s^{-1}) averaged over 3 days. Secchi measurements represent the approximate depth at which light is reduced to 10% of the surface value in L. Neagh. (*b*) The water chemistry data were supplied by Dr C. E. Gibson (filled symbols, silicate; open symbols, nitrate). (*c*) Seasonal changes in the concentration of *A. subarctica* cells are also shown (open symbols, cells; filled symbols, auxospores).

age of cells involved at any one time is normally quite low and easily missed in normal counting. For example, the highest concentration of auxospores recorded was 20 per millilitre on 7 February 1989 (see figure 19). This was in water at all depths (0, 5 and 10 m). They represented only 0.16% of the cells present. The periods over which auxospores have been found in the water are shown in table 1. These are not expressed as a concentration. They are the sum of auxospores found in all observations, including concentrated material from net hauls. They are included here to show how the production is not limited to one particular time but if averaged over several years then they can be found for 8 months of the year. The single one in May 1988 comes from resuspension. The high value in August 1989 are also probably linked to resuspension. It was a wet year in which nutrients became available earlier than usual (see figure 1). Resuspension was from a Force 7, S.E. wind on 14 August. The auxospores were probably formed from the remnant of cells in which sexual differentiation had started in spring but where sedimentation had interrupted development (see below). Considerable numbers of auxospores were found in surface sediments at 10 and 15 m in February. The auxospores in August were among the largest found, with a mean of 15.8 μm and a range of 14.8–17.6 μm, in 12 individuals measured. The parent cells ranged from 5.3 to 7.4 μm (mean 6.0 μm), which is also larger than found at other times. Concentrations of auxospores up to five per millilitre were found between 23 and 28 August.

The auxospores represent successful completion of sexual reproduction, it is less easy to estimate the number of cells that differentiate but are not successful. On 7 February 1989 when the highest number of developing auxospores were found, 18 male cells could be identified in a count of 1000; female cells were eight in 500 (1.8 and 1.4% respectively). On all other occasions it has been much less than this (usually below 0.1%). The concentration of 20 auxospores per millilitre represents 0.16% of the total population.

(*n*) *Timing of reproduction*

The interaction of factors controlling the timing of reproduction is shown for the winter of 1988–1989 (see figure 19), as sufficient auxospores were present to enable quantitative estimates. It was not a typical year, as the population reached it's peak early in February rather than March or April (see figure 1). The previous summer of 1988 had been very wet and nutrients were available earlier than usual which meant crops could grow while irradiance was still adequate. Large populations were able to build up in the early winter (see figure 1 and 19). Auxospores were produced (up to two per millilitre) after checks in growth due to low irradiance in late November and early December of 1988. They were not produced before this, as irradiance was too high and nitrogen concentrations too low. Another small burst of auxospore production occurred in early January following a similar check in growth but the highest concentrations were found in late January, reaching a peak in early

Table 1. *Seasonal distribution of A. subarctica auxospores*

(The values are the total auxospores observed in each month, not concentrations.)

	J	F	M	A	M	J	J	A	S	O	N	D
1987									0	1	5	7
1988	25	5	7	0	1	0	0	3	1	3	6	9
1989	10	23	2	0	0	0	0	12	0	3	4	3
1990	1	6	8	0	0	0	0	0	1	3	0	2
1991	0	0	2	5	0	0						
total	36	34	19	5	1	0	0	15	2	10	15	21

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February. They could still be found for up to a week after this, on the 10, 15 and 16 February during very windy weather. The unusually large numbers produced on this occasion were the result of a combination of low irradiance (as the peak was early in the year) and a reduced growth rate caused by low silica concentrations. *A. subarctica* is unusual in that it stops growing while there is still a relatively high concentration of silica (see Jewson *et al.* 1981). Its sinking rate increases (Gibson 1984) and so cells reach the bottom in good condition. It probably means that on this occasion more cells were checked at the point in the cell cycle that determines whether development continues with mitosis or meiosis (see Chisholm *et al.* 1984; Ambrust *et al.* 1990)

The need for the coincidence of sufficient nutrient supply, the right light requirements (combined with the presence of sufficient numbers of cells of the right size), explains the very different periods of auxospore production in the three years (compare figure 1 and table 1).

Although the numbers of auxospores appears low at any one time, if the whole lake is considered, it means a very large number can be produced. For example, on 7 February 1989 it would have been 7×10^{16}, assuming 20 million per cubic metre, a mean depth of 8.9 m and surface area of 383 km^2.

(*o*) *Summary of the life cycle*

Individual cells have a limited life expectancy, as 98% of the cells die within one year. They are either eaten by benthic invertebrates or become buried too deep in the muddy sediments to return via resuspension (see Jewson *et al.* 1981; Jewson & Zlinszky 1991). An analysis of the inoculum at the beginning of the growth period in autumn (see figure 1) over the last 12 years, gives a mean value of 2.2% (s.d. $\pm$ 1.36) of cells surviving from the spring maximum. The highest was 5.1% in 1985 and the lowest 0.5% in 1980.

Each year the population then divides between five and eight times to regain it's spring maximum (figure 1). The combination of the size decreases associated with the girdle band arrangement and the environmental size selection, described above, means that the most likely rate of decline of cell diameters lies between curves B and C in figure 10, depending on environmental conditions. This suggests about 4 years (on average) to reach the optimum size for gametes. There is likely to be a considerable range (depending on environmental conditions and time in the sediment) but few cycles would be completed in less than 3 years (curve D). It is possible for cells to survive 1 or 2 years in the diameter range in which sexual stages can be induced but few cells seem able to survive for long once the diameter falls below 4 μm.

As a summary, a diagrammatic representation of the life cycle is shown in figure 20. The resting state is a physiological 'phase' (see Jewson *et al.* 1981; Gibson & Fitzsimons, 1990) and should not be confused with the resting spores seen in many marine diatoms (see French & Hargraves 1985, 1986). Cells may pass through the guts of invertebrates and some still remain viable but, overall, there is a reduction in numbers and cells per filament during the summer period spent in the sediment on the bottom of the lake.

5. DISCUSSION

For phytoplanktonic organisms living in an environment where losses from sedimentation, grazing, parasitism, etc., can be very high and where there are limited resources of light and nutrients (see Jewson *et al.* 1981; Reynolds 1984, 1988; Sommer 1988), any interruption of vegetative growth is likely to cause a loss of competitive advantage. Unfortunately, sexual reproduction (if it has been considered at all) has for a long time been thought of in such terms. In two stimulating papers, Lewis (1983, 1984) drew attention to this, outling the problems for sex in single cells from a theoretical viewpoint. He discussed what the costs of disruption by sex might be, highlighting the production of males and the time lost in the mechanics of sexual reproduction. Such costs, he stressed, must be set against the possible benefits associated with improved fitness. The results from studies, such as the one presented here on *A. subarctica*, can go a long way to answering the conundrums raised by Lewis (1983, 1984).

The first question concerns the cost of producing males. One group of diatoms, the pennates, has managed to avoid this altogether, through isogamy. They were able to do this because they have motile vegetative cells, which enhances the chance of finding a partner (see Geitler 1932; Mann 1988; Round *et al.* 1990). Planktonic life is more suited to oogamy (see Drebes 1977) but there are many risks. French & Hargraves (1985) described how male gametes can get tangled in detritus. *A. subarctica* sperms were also observed to get trapped in this way in samples from L. Neagh concentrated for inspection. The problem is that male gametes only have a limited duration before becoming moribund (see Schultz & Trainor 1968). The second major cost is based on the idea that completing the sexual reproduction process (from initial differentiation, through fertilisation to zygote production) is likely to take the time of several vegetative divisions (with a potentially exponential rate of increase this might otherwise have been eight or 16 cells) whereas only one initial cell will be produced in the same time. French & Hargraves (1985, 1986), in one of the few descriptions of a complete life cycle of a planktonic centric diatom, even speculated that some marine coastal species might be evolving away from sexuality in favour of re-enlargement by vegetative means (see, for example, Gallagher 1983), so circumventing these apparent disadvantages. In the long term, they suggest, such a policy would obviously slow the rate of evolution. To answer such questions on the costs and benefits, it is necessary to quantify the various steps in the life cycle, especially how frequently sexual reproduction occurs. A review of the literature has produced no satisfactory examples from natural populations of planktonic centric diatoms. So, is sex rare or has it been missed?

Mann (1988) discusses several possible reasons why

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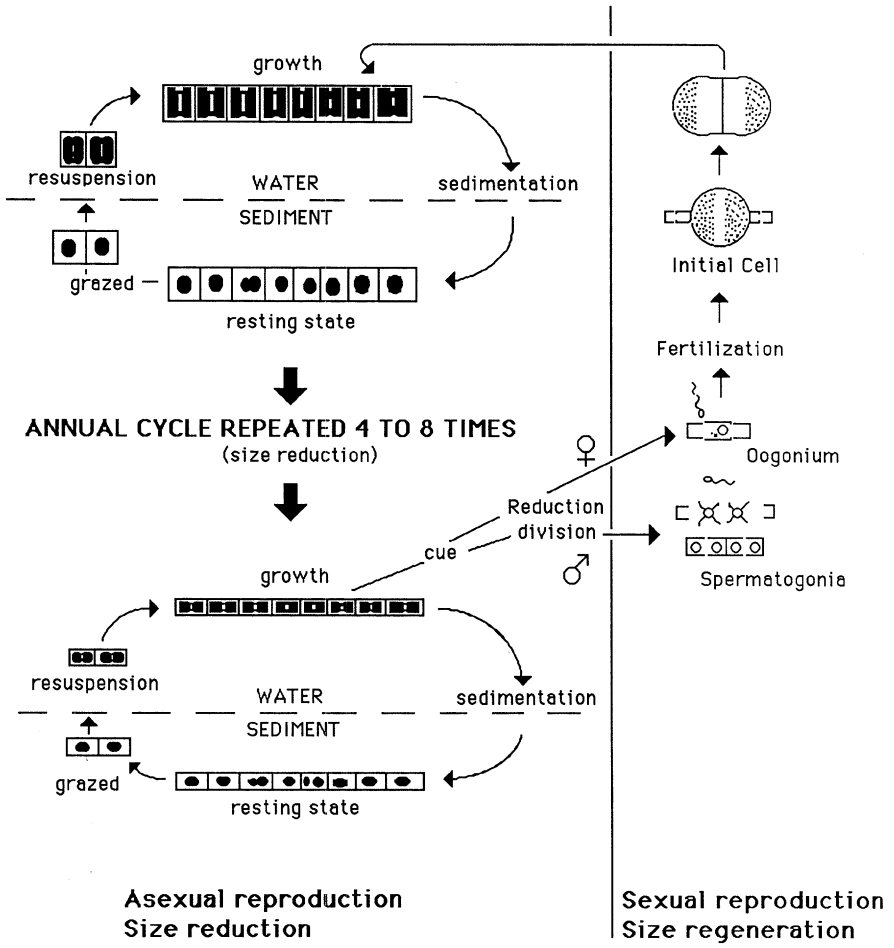

Figure 20. Diagrammatic representation of the life cycle of *A. subarctica* in L. Neagh.

sexual reproduction might be so rarely reported in natural populations. One is the lack of recognition of sexual cells on the part of the observer. This might seem obvious but was certainly a contributory factor in this case. Looking back through preserved samples, it is now possible to see that sexual cells were present in most years but at concentrations low enough to be seen only very infrequently in routine counting and therefore ignored as insignificant. In *A. subarctica* sexual differentiation of cells occurs after checks in growth during low irradiance (see figure 19). From the population's point of view, these are good times to reproduce. Low irradiance means that vegetative growth is slow and so there is less cost in switching at least some of the population over to sexual production. The new cells are also in a good position to have the maximum effect when growth conditions improve.

At the maximum, only a relatively few cells, in this species, are normally involved in sex at one time (i.e. usually below 0.1%, with a maximum of 4%). As in other diatoms, cells have to be the right diameter (see, Drebes 1977) and the right point in their cell cycle (see Chisholm *et al.* 1980, 1984; Vaulot & Chisholm 1987). Armbrust *et al.* (1990), working on spermatogenesis in a marine centric diatom, *Thalassiosira*, make the point that the lack of strict phasing between the cell cycle and photocycle suggests that only a small fraction of the population will be at the correct stage to induce gametogenesis. Because of the variability of the light climate which *A. subarctica* cells experience in L. Neagh, there are nearly always likely to be some cells checked. Only occasionally do conditions arise to synchronize more cells at this point and so give a larger number of auxospores. For example, when the period immediately following the onset of silica limitation coincides with low irradiance (figure 19), more cells are held at the critical stage in the cell cycle. The details of this are further expanded below, after consideration of the factors controlling size distribution, but in the case of *A. subarctica* the main point is that sexual reproduction is not as rare as it appeared and its importance to the ecology of this species has been overlooked.

One of the most remarkable things is the stability of the size distribution of diameters in *A. subarctica*. Gibson (1981), in a report of the seasonal changes of this species in L. Neagh during the 1970s, refers to the stability of the mean population diameters between 5.5 and 6 µm. Müller (1906) also gives similar sizes for *A. subarctica* in Thingvallavatn, Iceland. Such a stable size distribution is not a sign of lack of change but the result of a dynamic 'feedback', where the loss of cells from one size class is replaced with cells from the one above. It suggests that the cell dimensions are well matched to this type of cool, turbid lake habitat.

The relative constancy of the size distributions of *A. subarctica* contrast with many other examples of drifts in the modal peaks of diatom populations with time

(e.g. Nipkow 1927; Wesenberg-Lund 1908; Hutchinson 1967; Mann 1988). One of the most debated explanations of such diatom cell size changes is the Macdonald–Pfitzer hypothesis (see MacDonald 1869; Pfitzer 1869, 1871). These authors, working independently, were able to use the improvements in microscope performance, in the preceding decades, to distinguish the arrangement of the girdle bands. They then suggested how this might influence the size of the daughter cells. From early on (Tomaschek 1873, cited by Crawford 1980; Pfitzer 1882), it was suggested that size reduction should follow a binomial progression, with the degree of reduction related to the thickness of the girdle bands. Since then, there have been a number of assessments but few real tests of their observations (see Hustedt 1929, 1930; Hutchinson 1967; Round 1972; Rao & Desikachary 1970; Crawford 1980; Mann 1988). Essentially, the hypothesis predicts increased variance in population cell diameter as the population grows (see § 4*d*). In reality, most populations show relatively narrow size classes (e.g. figure 4). It took another improvement in microscopes, nearly a century later, to provide the next real advance. Crawford (1980) used electron microscopy to illustrate how size reduction might result from the arrangement of girdle bands and valves (see figure 5). His description of *Melosira arenaria* (now *Ellerbeckia arenaria*, see Crawford 1988) also fitted for *A. subarctica* and provided a basis for understanding the way in which size reduction might proceed (see figures 5, 7 and 8), along the lines predicted by the MacDonald–Pfitzer hypothesis. It was then possible to differentiate between changes in size distribution in the population caused by structural changes to valves (figures 8 and 9), as opposed to size selection resulting from the loss or gain of cells due to environmental factors (figures 10–12).

These various size-selective pressures combine to maintain a relatively narrow size distribution in the population. To show how this applies to *A. subarctica*, the factors tending to reduce diameter, by preferentially affecting the broader filaments, are discussed first. They can be grouped into four categories.

1. Wider cells decrease in diameter more at each division (0.32 μm), due to thicker cingula (figure 7).
2. Some cells show an even larger decrease in diameter under unfavourable environmental conditions (up to 1 μm, figure 8 & 9).
3. Relatively higher sedimentation of broader cells, especially if low light and calm conditions coincide (figure 11).
4. Limited lifetime of individual valves.

All of these combine to shift the size distribution lower (i.e. to the left in figure 4). The first two ensure more rapid reduction in cell size in broader filaments and their importance is discussed later, along with the length of life cycles. Their effect in the natural ecosystem is exaggerated by the increased chance of broader cells sedimenting quicker (figure 11). Davey (1986), in laboratory studies on a closely related species of similar dimensions, *A. granulata*, showed that diameter is one of a number of factors that influences sinking rate, with the widest filaments sinking at over twice the rate of the narrowest. However, in natural populations of *A. granulata* the main sedimentation events (in summer) were associated with changes in filament length (Davey 1987). This illustrates an important point that sedimentation may be influenced by different factors according to environmental conditions. In the case of L. Neagh, the preferential loss of the wider cells when the population is low in early winter (figure 11) has relatively little effect on the final spring numbers but has implications for sexual reproduction, as it shortens the length of the life cycle by speeding up the rate at which the population diameter decreases. The fourth category, listed above, is not often considered but is very important. Theoretically, as one valve (and its girdle band) is passed to each daughter, cells might be considered as eternal, assuming they are not eaten, parasitized or have sex, etc. In practice, there seems to be a limited life time. It has not been possible to determine this yet for *A. subarctica* but in *Stephanodiscus neoastrea* initial cells die within weeks and the first set of cells after this last less than a year. Presumably, there is an inbuilt senescence after so many divisions (i.e. no more than six to eight divisions for *S. neoastrea* in L. Neagh but this probably varies between species). It is logical for the larger cells to die after so many divisions, i.e. once increase in population numbers has been assured, as otherwise they will compete for resources with the new generation and they are still above the size at which sex can be induced. When this and the other influences tending to reduce the diameter are combined, we might expect to see large shifts in population sizes with time but instead there is a remarkable stability in the size distribution curves found in L. Neagh (figure 4).

This suggests that there must be other important size-selective processes, that are biased to preferentially removing the narrower diameters. The most likely are grazing, parasitism, breakage and sexual reproduction. Grazers are mainly cyclopoids and rotifers at the time the population grows. Generally, *A. subarctica* is not heavily grazed in the plankton and even if ingested, many cells remain viable (Jewson *et al.* 1981). More important for size changes is the resuspension of cells that survive grazing when they are on the bottom during the summer. All sizes can be found when cells are returned to the water column, but the larger diameters become more prevalent the longer the period before resuspension. This could be due to better reserves but it could also be linked to parasitism which seems to affect the narrower filaments more (see figure 12). The increased infestation is probably a price of the more flexible cingula which could allow easier entry for the chytrid. However, rates of parasitism are generally quite low (see Jewson *et al.* 1981; Gibson 1981) and never reach epidemic proportions. These chytrids are highly specific in host choice (Canter 1967) and it would not be in their best interest to let infestation get too high in such a slow growing species.

Another major factor, in removing narrower diameter cells, is breakage during turbulence. A culture

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of *A. subarctica* isolated by Dr C. E. Gibson (see Gibson 1988) has been kept for many years at 3 μm diameter, yet this size rarely lasts long in the lake (see figure 4). Cells of less than 4 μm also begin to deviate from the otherwise linear relationship between width and length (figure 13). The cingula (girdle bands) are extremely frail at this stage (figure 6*f*), so cells must be very prone to any torque or leverage forces. Cells of 3 μm and less are probably too small to produce auxospores successfully (see figure 17), so are of no benefit to the population. It means there is effectively an optimum size for reproduction. Too small and there are insufficient resources or too large and there is a waste. In between, there is a range within which the size of the auxospore is determined by the size of the parent but this can be modified according to the internal reserves of the cell (figure 17). Together this produces a range of size classes in the new generation (figure 16). Similar relationships between parent and initial cells were also observed for *Melosira* (*Aulacoseira*) *baicalensis* (Skabitschewsky 1929) and *M. nummuloides* (Bruckmayer–Berkenbusch 1954). The spread of diameters in *A. subarctica* is effectively 12–18 μm and for the few individuals at the extremes, could mean a difference of one or two years in the length of their life cycle (figure 8). With so many pressures on size selection this variability must mean a spreading of the risk and an improvement of the chances of survival. It also enhances cross-over between year classes, preventing a temporal isolation of populations.

Significantly, the size distribution is the result of an interaction between 'in-built' cellular mechanisms and external selective pressures. In the past, much of the emphasis has been put solely on the MacDonald–Pfitzer hypothesis as the driving force. Its importance lies in being a mechanism (discussed with figures 5, 7 and 8) over which species have some control, i.e. through the controlling rate of diameter reduction. It is a beautifully designed adaptation for controlling the length of the life cycle (figure 20). Cell numbers can be built up through the period when cells are above the size threshold for sex differentiation but then, in the later stages, kept within the sexually inducible size range for extended periods. As Lewis (1984) points out, it is a way of timing ('clocking') sex for periods longer than one year, when other cues, such as solar and lunar, would be too short.

It is strange that although the MacDonald–Pfitzer hypothesis is discussed frequently, there have been relatively few who have tried any measurements to evaluate it. One of the early testers was Müller (1884), who concluded from work on *Melosira* (*Ellerbeckia*) *arenaria* that daughter cells from the hypotheca (i.e. smaller valve) divided at half rate. This is also supported by Mann (1988) for the same species. The implication of this is a modified pattern of size distribution (see Hustedt 1930, pp. 27–31; Hutchinson 1967, pp. 920–925). However, the evidence on cell lengths (figure 14) and also the distribution of cell diameters (figure 8) in *A. subarctica* suggests that it is not half the rate of the larger cell, although it does divide later (figure 5). It is probable that the rate of division will be found to vary with species. Filamentous, benthic diatoms are more likely to be similar to *Ellerbeckia*, at least those living in habitats where slow growth is not critical to survival. Skipping a generation drastically reduces the speed at which population numbers can build up. In a planktonic environment, where so much effort is put into maintaining 'market share, it would seem a major disadvantage to divide at half the rate.

In *A. subarctica*, it seems growth is only slowed enough to ensure that cells are asynchronous, which is presumably an advantage, as in a filamentous form it prevents all cells dividing at once. This would be detrimental for a number of reasons. For instance, until the new silica walls of the daughter cells are laid down the extended parent cell is supported only by the girdle bands. A long filament with many cells would be very prone to breakage from increased leverage, predation etc. Also, a filament with all cells dividing at once would change drastically in density. So, restricting division to one or two cells at a time, seems advantageous. Asynchrony also reduces the danger of self-fertilization, as cells will be at different stages in the cell cycle (see Chisholm *et al.* 1980, 1984; Vaulot & Chisholm 1984, Ambrust *et al.* 1990). However, the extent of phasing is likely to vary between species. Among four other diatom species in L. Neagh, there is considerable variation (Jewson 1992).

The potential lengths of the life cycle under different selective pressures are shown in figure 8. The longest would be over 100 divisions or about 15 years, at the average of seven divisions per year. In L. Neagh, it is considerably shorter because of the selection against the broader cells discussed above. It is probably between 4 and 6 years. The range is quoted not because I am unsure but because it is a variable on which selection can be allowed to act. For example, in L. Neagh there has been a change in the length of *A. subarctica*'s growing season over the last decade, as the lake responds to reduced nutrient loading. Also, the range in size of initial cells means that it could take from 1 to 2 years for the largest to get down to the same diameter of the narrowest produced at the same time. It will be interesting to see if there are differences in the lengths of the life cycle (for the same species) in lakes with different morphometry, latitude and nutrient status.

Unfortunately, the length of life cycles in other centric diatoms is largely unknown. Mann (1988) reviewed the fragmentary information now available and tried to use the experience and results from his own excellent work on pennates to help make some estimates for planktonic species. He suggested that life cycles might be up to 40 years for *Aulacoseira islandica* var. *helvetica,* based on the results of cell diameter changes measured by Nipkow (1927) over 24 years in the varved sediments of Zürichsee. Although such long life cycles may well prove theoretically possible in some species, there are a number of pieces of evidence to suggest that, in this case, it is an overestimate. Firstly, Mann (1988) stressed that one hundred cells is too few to measure in order to pick up all age classes adequately. Secondly, the absence of separate size

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classes does not mean sexual reproduction has not occurred but that it might be spread out over longer periods (at low numbers), as described above. Nipkow (1927) does state that he saw auxospores throughout his observations but at a low frequency. In L. Neagh, loss of one size class is matched by recruitment from a bigger one but it appears in Zürichsee that there is probably a greater proportion of larger cells lost. This causes a slow drift in the population to smaller diameters. Large bursts of reproduction are then more likely, as the majority of the population is in the appropriate size range. In Nipkow's the largest is in 1904–1905 but there were lesser ones in 1912 and then again 1920 to 1924. Thirdly, sediments can give a misleading result because of spatial and temporal separation of size classes. This occurs in L. Neagh (Jewson & Hueston 1992). Fourthly, Nipkow mostly took only one sample (early spring) for each year, so the cells he recorded may also include older cells resuspended from elsewhere. This does not mean sediments cannot be used, it just means that results need to be related to an understanding of contemporary events in the lake system. Nipkow was aware of this and mentions the likely over-estimate of larger specimens in his results. He did look at one plankton sample in February 1905 and saw a few auxospores (with parent cells attached) but not as frequently as in the sediment. In his photographs of cells in the sediment of spring 1905, two filaments can be seen with the rounded valves of the initial cells still attached. One has undergone two divisions, the other as many as four (i.e. 16 cells). So it is likely that sexual reproduction was going on over a longer period (in 1904 and 1905) than suggested from the sediment records. Nipkow also noted that the size range of initial cells was varied, with a normal range between 16 and 22 μm but a maximum of 27 μm. So, there are many similarities with L. Neagh *A. subarctica* populations. These two species are not infrequently found together. In one of the earliest seasonal studies of phytoplankton, Ostenfeld and Wesenberg–Lund (1906) comment on the much greater frequency of auxospore production by *A. islandica* compared to *A. subarctica* (referred to as *M. italica*) in Thingvallavatn in Iceland. They include drawings of both and a record of the timing of auxospores. In summary, the length of the life cycle of *A. islandica* in Zürichsee is unlikely to be 40 years but much less, probably nearer 4 to 8 years.

In lakes, such as Zürichsee, where there is a preferential loss of larger cells and, therefore, a gradual decline in the population diameter (Nipkow 1927), there is always likely to be a higher chance of ending-up with a large proportion of the population in the right size range and so, involved in sexual reproduction at one time. This, in turn, will result in more recognizable size classes. An excellent example of this comes from one of the world's deepest lakes, L. Baikal, where the results of Skabitschewsky (1929), on the size changes of *Melosira (Aulacoseira) baicalensis*, show clear cyclical patterns in cell diameter. His results suggest life cycles of 4 to 5 years for *M. baicalensis*.

Overall, sexual reproduction is always likely to be a risky process for a dispersed planktonic population, so selection has produced a range of strategies. In *A. subarctica* a maximum of 4% of cells have been found as gametangia (at one time) with only a small proportion of these (0.2% of the population) estimated as successfully producing auxospores. It is a slow growing, filamentous, shade species that lives in cool, well-mixed lakes (see, for example, Lund 1954, 1955, 1971; Gibson 1981; Jewson *et al.* 1981); the use of asynchronous sexual reproduction (see Ims 1990), with relatively frequent but low numbers, appears most suitable in these conditions. It is not difficult to see how reproductive strategy might be different in other species, in the same way that flowering plants have a wide variety of adaptations ensuring fertilisation in their particular niche.

Therefore, adaptations of sexual reproduction and the life cycle can be seen to act at a number of levels, including the following.

1. Control of frequency; by only allowing induction of gametes in cells below a given size threshold.
2. Control of the length of the life cycle; through girdle band structure and its effect on the speed of size reduction (e.g. MacDonald–Pfitzer).
3. Timing of reproduction; through adjustment of the cell clock (Chisholm *et al.* 1980, 1984) and the nature of the cue (light, temperature, nutrient, salinity, etc.; Ambrust *et al.* 1990).
4. Linkage to resting stages (see, for example, French & Hargraves 1985, 1986)
5. Environmental pressures; resulting in size selectivity, which in turn affects the length of the life cycle.

Perhaps, it is helpful to imagine diatom populations like an extended plant, in which most cells are vegetative but where the sexual ones are only produced at times favourable for reproduction (e.g. as in the production of pollen, etc.). The cost then doesn't appear so high, especially if we draw the analogy with the percentage of biomass invested by terrestrial plants in gametes versus the productive areas of leaves etc. In *A. subarctica,* sexual reproduction takes place when environmental conditions are not favourable for vegetative growth (i.e. at times of low irradiance). In addition, as the cells which will form gametes, especially the smaller males, are at a size where they will either reproduce or die, they are not so costly (in terms of disruption to vegetative growth) as they might otherwise appear (see Lewis 1983, 1984). Added to this, it is only after several seasons that each generation breeds (albeit different cells, it is the same genetic stock), which allows selection to take place over the whole vegetative growth phase, including grazing, sedimentation, parasitism, nutrient limitation, resting stages, etc. As each of these is likely to vary in importance in different years, it is probably a considerable advantage to have a life cycle that is longer than one year in most planktonic environments. It allows the number of cells to build-up following reproduction, eventually selecting for those cells most suitably adapted to all selective pressures to

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breed. Even so, the evidence in L. Neagh is that within the population there are usually some individuals that are at the right size (age) to breed in most years, with the overlap of sizes aiding an exchange of genetic material between years.

There are still many unanswered questions. For example, what decides whether cells become male or female? Is this environmentally or genetically controlled? In some species, both sexes can be induced in the same filament (von Stosch 1951) but in many species males tend to be smaller (see Drebes 1977). It is also important, for a better understanding of the ecology of diatoms, that there is a reassessment of how cell size changes required by the 'diatom sex clock' (Lewis 1984) interact with environmental factors controlling vegetative growth, such as nutrient uptake, sinking, light reception, grazing, parasitism, etc. (see Sommer 1988; Reynolds 1988). Of all the major plant and animal groups, perhaps, diatom life cycles are the least understood. Yet, they offer a marvellous combination of structural beauty and adaptive elegance for the population ecologist to study.

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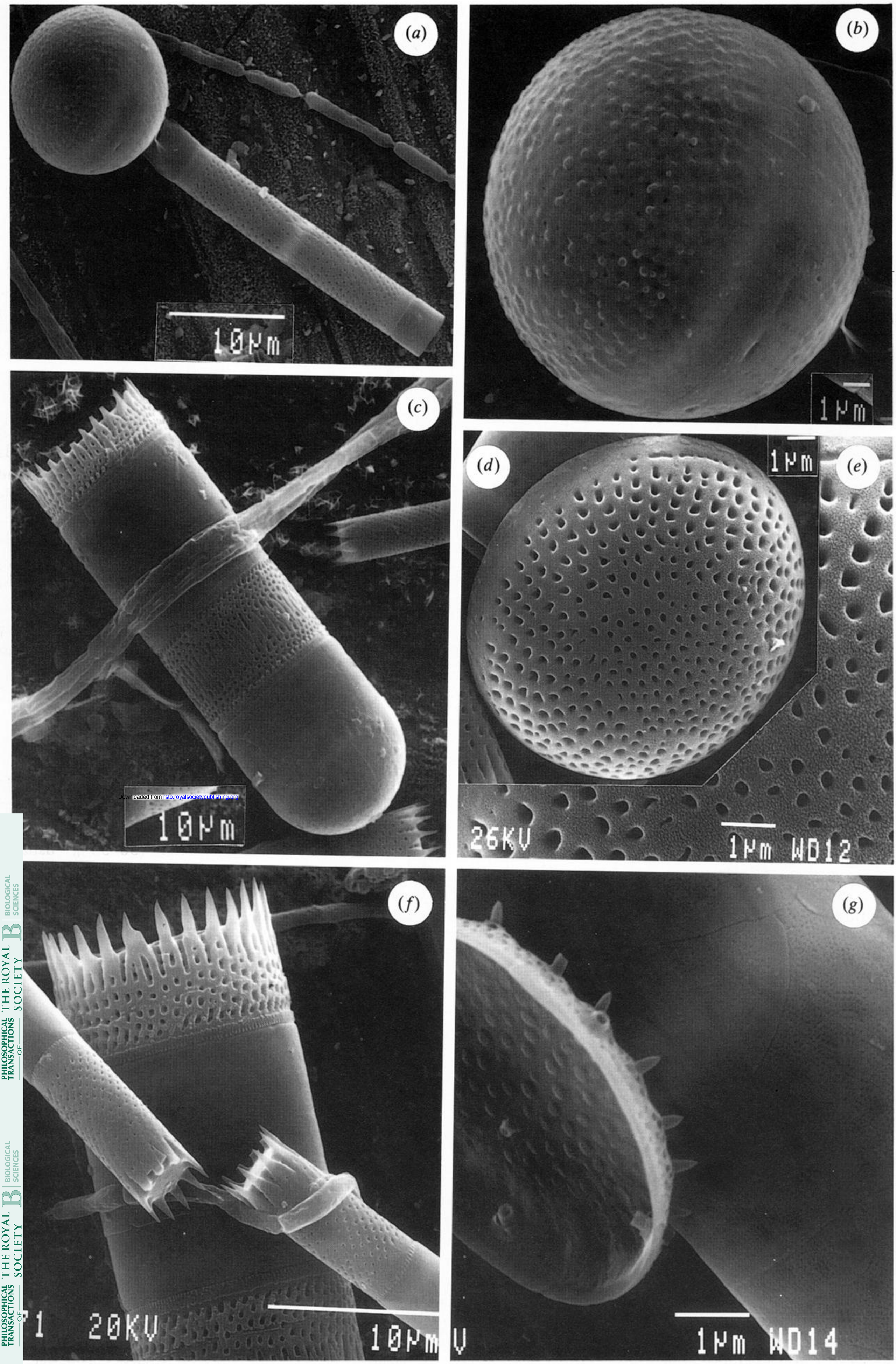

Figure 3. For description see opposite.

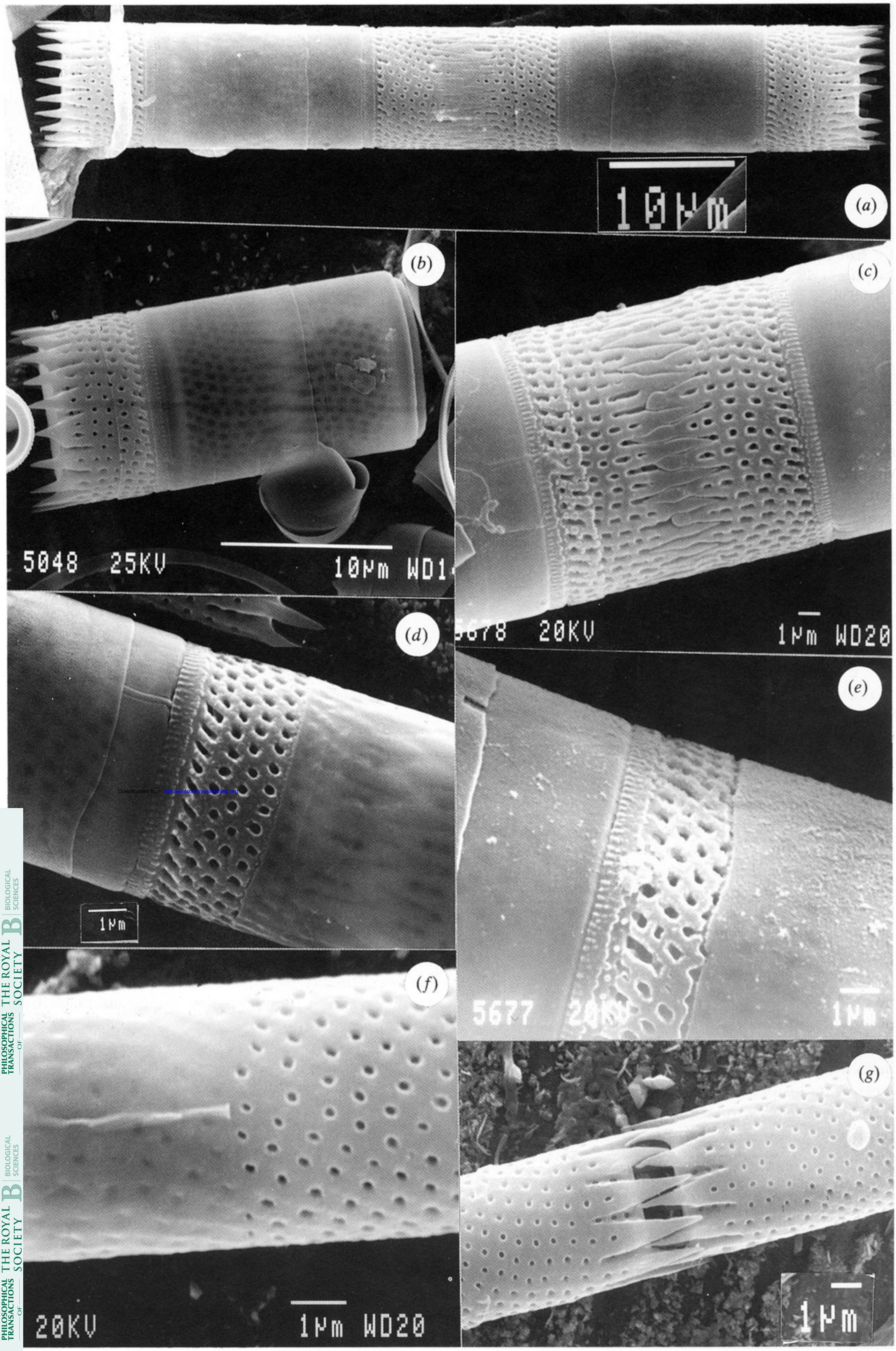

Figure 6. For description see opposite.