

On the life history of the marine plankton diatom *Stephanopyxis palmeriana*

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KURZFASSUNG: Zur Entwicklungsgeschichte der marinen Planktondiatomee *Stephanopyxis palmeriana*. Im Frühjahr 1964 wurde die zentrische Planktondiatomee *Stephanopyxis palmeriana* (GREV.) GRUNOW – eine aus japanischen Gewässern stammende Warmwasserform und unserer *S. turris* sehr nahe verwandt – in Kultur genommen. Schon nach kurzer Zeit war es möglich, den gesamten Formwechsel dieser Alge in seinen Grundzügen kennenzulernen. Mit Hilfe von Wasserimmersionen konnten in direkter Lebendbeobachtung Zellteilung, Meiosis, Befruchtung und Auxosporenbildung mit ihren metagamen Mitosen sowie die Bildung und Keimung der Dauersporen studiert werden. Morphologisch stimmt der Formwechsel mit dem von *S. turris* (v. STOSCH & DREBES 1964) überein; in der Abhängigkeit von Umweltfaktoren bestehen jedoch Unterschiede. So werden Dauersporen bei dieser zwischen 19 und 156 μ Zellbreite (= Transversaldurchmesser) vegetativ existierenden Alge nur von schmalen, unter 90 μ breiten Zellen in Gegenwart von Phosphatmangel (spärlich manchmal auch bei Nitratmangel) differenziert. Eine Temperatur von 12° C wirkt dabei sehr begünstigend. Die Keimung der Dauersporen setzt nach Beseitigung des Phosphatmangels ein. Zur Sexualisierung sind nur unter 60 μ breite Zellen fähig; sie findet statt nach sprunghafter Steigerung der Beleuchtungsstärke (von 400 auf 4000 Lux) sowie einer Temperaturerhöhung von 15° auf 21° C. Wegen der Dickchaligkeit bereitet die Änderung der Zellgröße auf künstlichem Wege durch SiO₂-Mangel mit nachfolgender Schalenregeneration einige Schwierigkeiten. Diese Manipulationen sind jedoch notwendig, wenn ein Klon ohne Veränderung seines Idiotypus über die Auxospore für unbegrenzte Dauer zur Verfügung stehen soll.

INTRODUCTION

After many years of investigation we succeeded in manipulating the complete life cycle of *Stephanopyxis turris* under controlled laboratory conditions (v. STOSCH & DREBES 1964), thus paving the road for culturing other species of the same genus. If the few species described by GRUNOW (1884) from the Arctic Ocean near Franz Josef Land, which are difficult to obtain, are excluded, there are three remaining species available as study objects: the warm-water form *S. palmeriana*, *S. orbicularis* from the coastal waters of New Zealand and *S. nipponica* from the northern North Pacific. Fortunately, a culture of *S. palmeriana* was kindly put at my disposal by Dr. H. TAKANO of Tokyo.

¹ The studies were carried out in the Botanical Institute of the University, Marburg.

S. palmeriana does not occur in northern Europe and is very seldom found along the southern European coasts. Since this species is often confused with the closely related species *S. turris*, its distribution is not clearly known. According to HUSTEDT (1930) *S. palmeriana* seems to be restricted to the warm seas, the Indian Ocean and the Pacific. Culturing *S. palmeriana* offered interesting insights into morphological and physiological features of a warm-water species.

MATERIAL AND METHODS

Stephanopyxis palmeriana (GREV.) GRUNOW was obtained from a plankton sample dated November 1, 1963, taken by Mr. OMORI at 33° 20' N, 139° 00' E, on board the "Tansei-maru", a ship owned by the Oceanographical Institute of the Tokyo University. Dr. TAKANO isolated the alga and kept it in a fully synthetic medium, where it grew very well. On April 21, 1964, the alga arrived at my previous laboratory in the Botanical Institute of the University of Marburg. The immediate transfer into our half-synthetic nutritional medium (Table 1) was successful. The composition of this medium is identical with the one used for *S. turris*.

Table 1
Nutritional medium (after v. STOSCH & DREBES 1964) used in experiments

Composition	Weight (mg)	Portion of most important elements (μ g)
Sea water (North Sea)	1020000	—
Na NO ₃	42.5	7000 N
Na ₂ HPO ₄ \times 12 H ₂ O	10.75	930 P
FeSO ₄ \times 7 H ₂ O	0.278	55.8 Fe
MnCl ₂ \times 4 H ₂ O	0.0198	5.9 Mn
SiO ₂	12	5.6 Si
Na ₂ EDTA \times 2 H ₂ O	3.72	
Vitamin B ₁₂	0.0007	

No test was undertaken to see whether or not *S. palmeriana* exhibits vitamin B₁₂ auxotrophy. The alga was kept as a unialgal culture at the vegetative stage in small Jena Glass Petri dishes (diameters of 5 or 6 cm) exposed to a constant temperature of 15° C at a light intensity of 400 Lux; in addition, other constant temperatures between 9° and 24° C were offered in order to take the alga through its complete life cycle. The light-dark rhythm was 16:8 hours at 15° C and 14:10 hours (light phase: 8 a.m. to 12 midnight and 8 a.m. to 10 p.m. respectively) at all other temperatures. The observation in vivo was done with a microscope (having a sea water proof immersion objective, 50:1, N. A. 1.00, Leitz-Wetzlar), which was dipped into the culture dishes. For a discussion of nutritional requirements and detailed methods, see v. STOSCH & DREBES (1964).

MORPHOLOGY AND MANIPULATION OF CELL SIZE

The frustule structure of this alga as seen through a light microscope (HUSTEDT 1930) clearly distinguishes it from *S. turris*. In addition, the wall and structure of the frustule are thicker and more strongly developed in *S. palmeriana* than in *S. turris*.

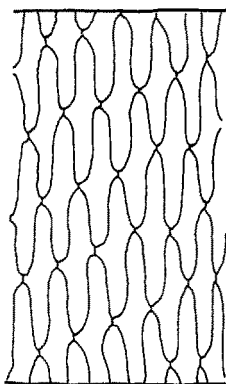


Fig. 1: Structure of the pleura of *Stephanopyxis palmeriana*, drawn from a photograph (510 \times)

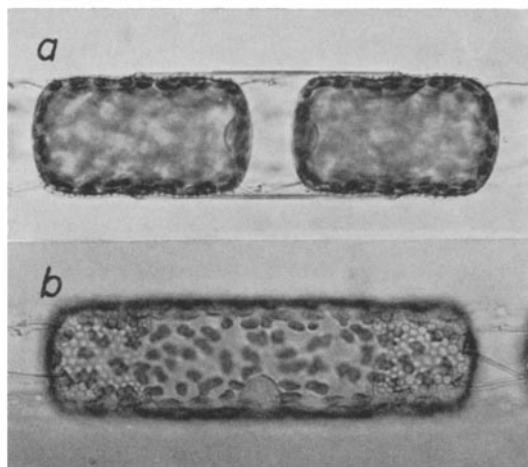


Fig. 2: *S. palmeriana*. (a) A pair of cells with interphasic nucleus located in the discus of the hypovalva; (b) elongated cell shortly before cell division, nucleus in the equator of the cell (450 \times)

As has been the case earlier in *S. turris* (v. STOSCH & DREBES 1964), the pleural structure of *S. palmeriana* was unknown. It was made visible after skeletonization in Eau-de-Javelle, washing in distilled water and treating with 0.1 % crystal-violet solution (Fig. 1). Such treatment revealed the same structural properties as in *S. turris* and *S. orbicularis*, in which CASSIE & BERTAUD (1960) observed a pleural fragment

using electronoptical techniques. This new type of pleural structure seems to be a characteristic of the genus *Stephanopyxis*.

Another characteristic of *Stephanopyxis* is the interphasic location of the cell nucleus in the discus of the hypovalva (Fig. 2a). A "nucleus central" in *S. palmeriana* had been reported earlier by GRAN & ANGST (1931), as shown in Figure 2b. However, this stage corresponds to the stretch phase just before cell division, when the inner girdle (hypopleura) is simultaneously formed. The interphasic nucleus is known for

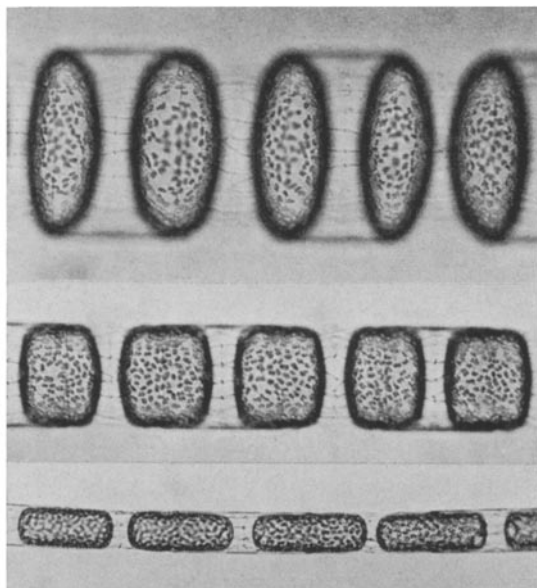


Fig. 3: *S. palmeriana*. Three cell chains of various cell diameters (200 \times)

certain to lie in the discus of the epivalva in the closely related genus *Melosira* (v. STOSCH 1951, 1958, DREBES unpublished) and after further investigations, also in *Podosira montagnei*, *Pyxidicula mediterranea* and *Hyalodiscus spec.* (DREBES unpublished). All these genera, including *Stephanopyxis*, have been placed in the sub-family *Melosirinae* by HUSTEDT (1930). He gives the cell diameter for *S. palmeriana* as 35 to 150 μ , and CUPP (1943) 27 to 71 μ . In culture the diatom divided down to 19 μ wide cells; in measuring cells of freshly germinated auxospores, the maximum diameter was found to be 156 μ . Figure 3 shows three cell chains of various cell diameters.

Since the life cycle of diatoms, the formation of auxospores and also of resting spores are a function of cell size (GEITLER 1932, v. STOSCH & DREBES 1964), an attempt was made to obtain as quickly as possible "subclones" of different cell widths from a 100 μ -clone during the vegetative phase. Very old cultures containing cells already partly deformed received a fresh nutritional solution. Thereafter, elongated, sandglass-shaped strangulated cells were often able by continuous cell division to form cell chains of smaller diameters. Such regenerates were isolated as new subclones. Thus within

two months, cell material with the following diameters were made available: 100 μ , 66 μ , 56 μ and 36 μ . When smaller cells were cultured in SiO₂-deficient solution, they often could enlarge by plasma protrusion; in order to get frustule regeneration after enlargement, SiO₂ solution was added again. This procedure, however, caused some difficulties; according to the previously mentioned degree of frustule thickness, the regenerated algal cells require a very long time to reach normal shape again. For a detailed discussion of the problem and technique of cell size manipulation during the vegetative phase, see v. STOSCH (1965).

SEXUAL REPRODUCTION AND AUXOSPORE FORMATION

Aside from an observation by GRAN & ANGST (1931), who only once found a single cell with eight "microspores", nothing is known about sexual processes and auxospore formation. As the findings about the main characteristics of *S. turris* (v. STOSCH & DREBES 1964) agree with those of *S. palmeriana*, a short discussion with a few illustrations should be adequate.

As was expected, only cells in the lower part of cell size range are capable of sexualization. Their diameter lies between 19 μ and about 60 μ . Taking into consideration the cell size as an endogenous, non-genetic factor, abundant formation of gametes occurs only if *S. palmeriana* – previously kept at 15° C and 400 Lux – is exposed for at least 1.5 to 2 days to light intensities between 3000 and 5000 Lux and a temperature of 21° C. In contrast to *S. turris*, the increase in light intensity alone is not sufficient to initiate sexualization in *S. palmeriana* (in only one case was a single cell containing spermatogonia found at 15° C). Thus the warm-water living *S. palmeriana* exhibits significantly higher temperature requirements for sexual reproduction.

Like all centric diatoms studied up to now, *S. palmeriana* is monoecious, one clone producing male and female gametes. With the beginning of the 14-hour light phase, the male determined cell, in two or three depauperizing mitoses, forms four, six or eight spermatogonia, which are enclosed in the mother cell (spermatogonangium). Each spermatogonium is surrounded by two rudimentary valves. Figure 4 shows some of the stages of spermatogonia formation. By undergoing meiosis the spermatogonia swell, a process which usually opens the spermatogonangium (Fig. 4d). The moment in which the meiosis begins depends on the number of cell divisions during the formation of the spermatogonia. After only two depauperizing mitosis meiosis begins earlier than after three (Fig. 5b). The metaphase I of meiosis becomes visible by a shrinkage of the vacuole and is followed by shrinkage of the whole protoplast (Fig. 5). Both meiotic nuclear divisions are not accompanied by cytokinesis. The flagella develop during the interkinesis as in all other Centrales so far studied. When the reduction divisions are completed, the four nuclei of a spermatocyte, which are surrounded only by a small amount of cytoplasm, detach themselves as unflagellate, colorless sperms (Fig. 5). The remaining mass of cytoplasm and all plastids form a dying rest-body. This type of spermatogenesis is termed *merogenous* (*Melosira* type).

In contrast to the formation of spermatogonia by depauperizing mitoses, the

oogonia develop directly from vegetative cells. Increased multiplication of plastids, considerable elongation of the cell and the swollen nucleus (Fig. 6a), observable under a dissection microscope ($40\times$), make it possible to distinguish the oogonia easily from the vegetative reproduction phases. The meiotic divisions take place without cyto-

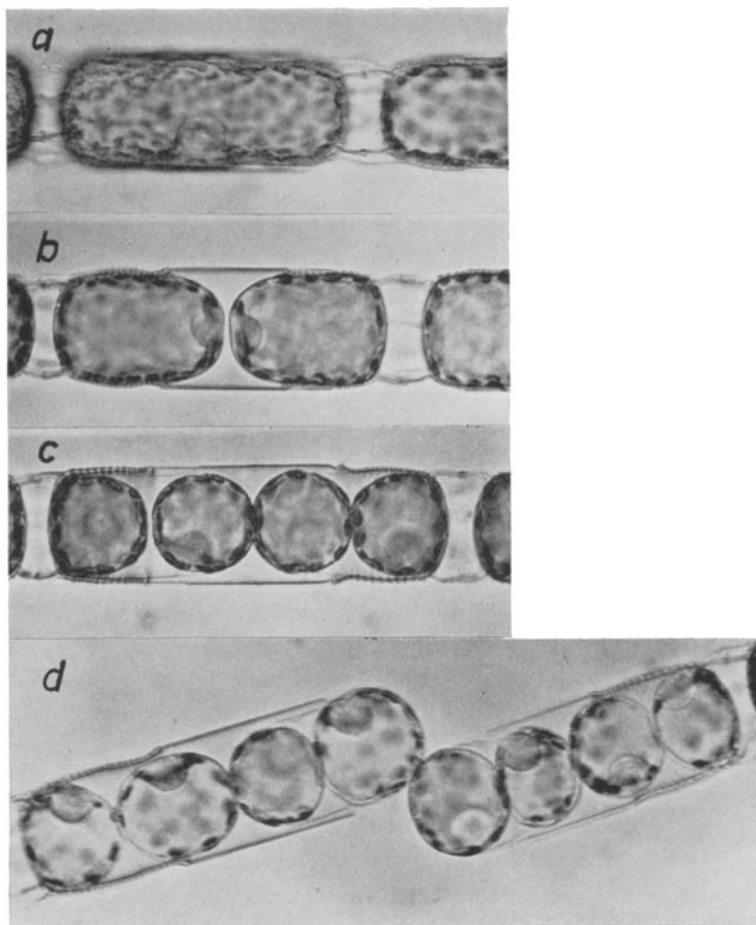


Fig. 4: Some stages of spermatogonia formation in *S. palmeriana*. (a) Cell just before the first, (b) after the first, (c) after the second, (d) after the third depauperizing mitosis; in (d) the swollen spermatogonia undergoing meiosis have opened the spermatogonangium ($450\times$)

kinesis; after each nuclear division one nucleus becomes pycnotic. The peculiar process of nuclear abortion has been recorded in detail for *S. turris* (v. STOSCH & DREBES 1964). At the end of this process the mature egg contains one haploid nucleus and two pycnotic nuclei (Fig. 6b). The meiosis occurs simultaneously with that of the spermatocytes at the end of the 14 hour light phase. In comparison to *S. turris* the formation of gametes in *S. palmeriana* is completed about two hours earlier.

Following meiosis the oogonium stretches until its pleurae totally separate; this action leads to a slight bending of the cell (Fig. 6b, c). Between the openings of the two pleurae the partially exposed female protoplast comes into contact with the external medium. In this way penetration of the sperm into the egg is made possible; during penetration the motile organelle of the sperm, the flagellum, is discarded. After passing through the plasmalemma, the small, homogenously colored sperm nucleus

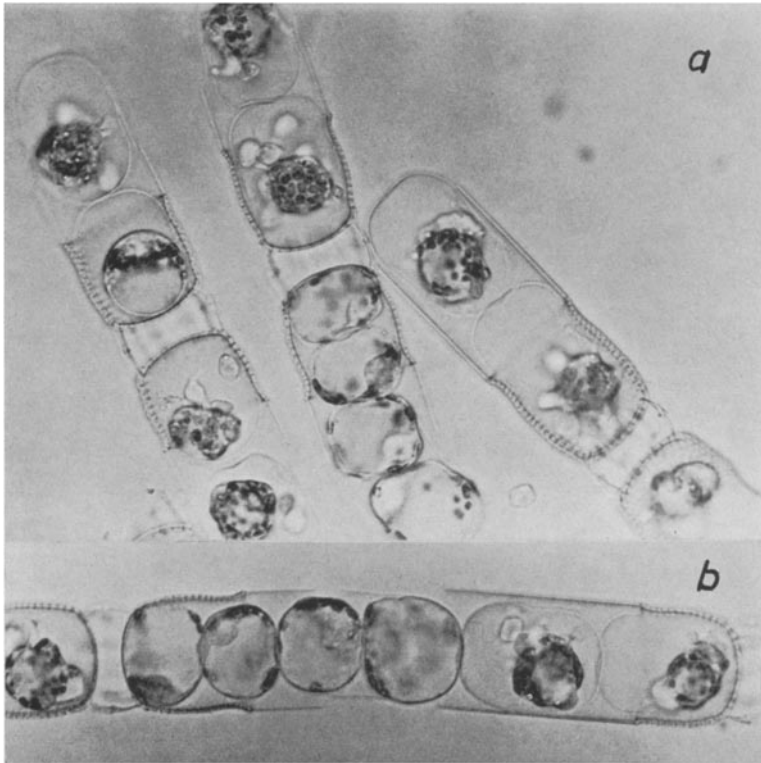


Fig. 5: *S. palmeriana*. (a) Various stages of meiosis with formation of colorless sperms. Note the rudimentary, thin and spineless valvae of the spermatogonia. (b) Spermatogonangium containing six spermatogonia, the four at the left formed by three, the two at the right by only two depauperizing mitoses. The four spermatogonia on the left side started meiosis later (450 \times)

slowly approaches within 2 to 6 minutes the great transparent egg nucleus, which contains 1 to 2 nucleoles, and attaches itself to it. Meanwhile a contraction of the protoplast closes the oogonium again. In the course of the next half hour the male nucleus swells, becomes reticulate, and finally a thread-like structure of chromosomes (Fig. 6d) appears. Then the male chromosomes – wandering along the periphery of the female nucleus – enter the oogon nucleus from all sides. On the basis of cytological data and observations on living material, v. STOSCH & DREBES (1964) had already assumed that this fundamental process took place in a similar manner in *S. turris*. In

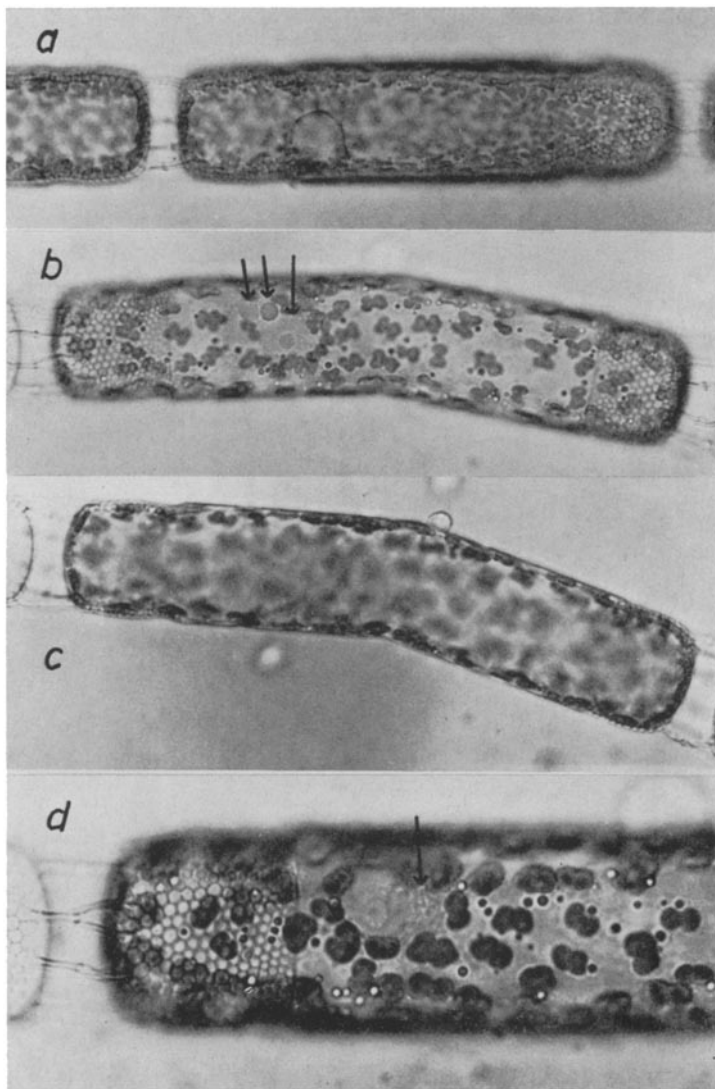


Fig. 6: *S. palmeriana*. Formation of the female gamete and fertilization. (a) Oogonium in meiotic prophase (swollen nucleus); (b) mature egg containing one surviving haploid nucleus (right arrow) with a central nucleolus and two small pycnotic nuclei (two left arrows); (c) slightly bent, opened oogonium with sperm on its surface; (d) stage of karyogamy. Arrow points to the chromosomes of the male nucleus (a to c: 450 \times ; d: 750 \times)

S. palmeriana it was now possible to clearly observe the whole process of karyogamy in vivo.

Preceding the subsequent light phase, the zygote contracts and then – increasing in size and assuming the shape of a sphere with two protrusions (Fig. 7) – the auxo-

spore is formed. During the light phase the auxospore forms its first two valvae; these are secreted in two subsequent steps, the second beginning some 7 hours after the first one, and in each case preceded by a metagamic mitosis. The resulting valvae lack the girdles and the circlets of spines. During the stretching initiating the first cell division,

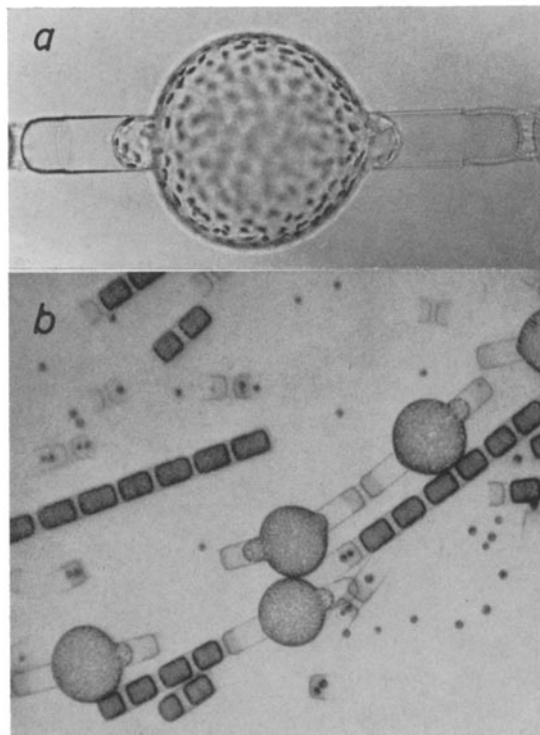


Fig. 7: *S. palmeriana*. (a) The zygote has inflated to an auxospore; (b) four auxospores, vegetative cell chains and empty valvae with small rest bodies left over after spermatogenesis (a: 216 \times ; b: 55 \times)

both an epipleura and a hypopleura are developed. The epipleura, somewhat shorter and of abnormal structure, has an irregular, polygonal areolation, which was already designated as a "praepleur" in *S. turris* (v. STOSCH & DREBES 1964). During the first cell division the fertilization membrane (auxospore membrane) bursts, and thus a new clone of larger cells is born.

RESTING SPORES

In *S. palmeriana* resting spores are known to exist in nature and have been described by GRAN & ANGST (1931). Their formation takes place in two differentiating divisions, in the same manner as in *S. turris*. Observations on my cultures showed

that in the early stage the first cell division does not differ from a normal vegetative reproduction division. The character of a heterovalvate division, which indicates resting spore formation, becomes apparent only during the secretion of the new daughter valvae, which are thicker, shorter and more strongly areolated. Before the

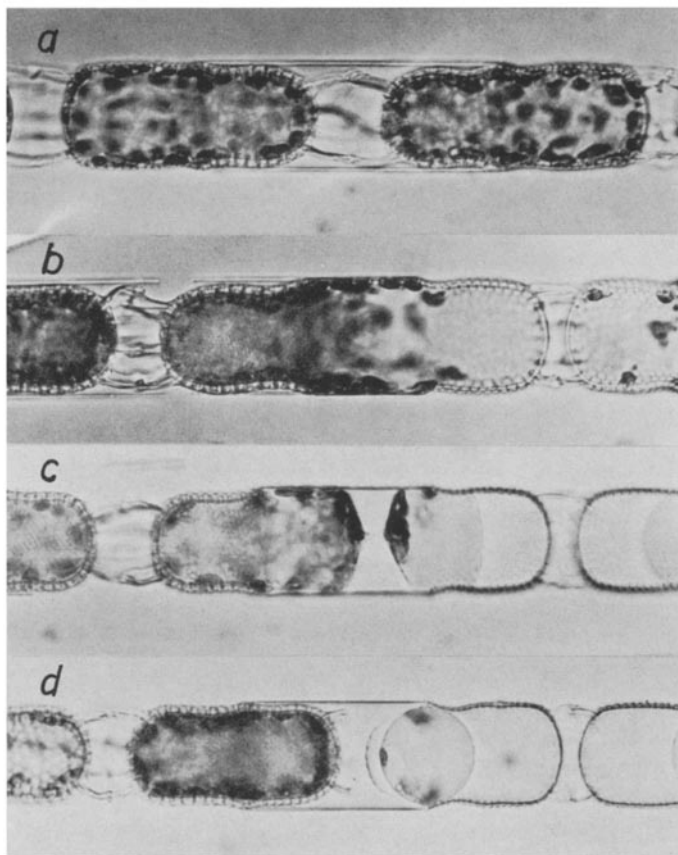


Fig. 8: Resting spore formation of *S. palmeriana*. (a) Two heterovalvate cells, each containing the first spore valva; (b) heterovalvate cell during polarization of the cell content, plastids leaving the epivalva; (c) extremely unequal cell division with (d) formation of the resting spore and a small, later disintegrating body. Note the rudimentary thin valva of this body (450 \times)

second cell division a polarization in the protoplast, which is recognizable by the migration of the plastids (Fig. 8b), occurs. Finally, after the cell content concentrates in the prospective spore-side, all the plastids vacate the entire epivalva and part of adjacent epipleura. Thus an extremely inequal cytokinesis follows (Fig. 8c), which produces the resting spore by secreting the second spore valva, and only a small body with a nucleus and a few plastids remains. This later disintegrating body still develops a rudimentary, sandglass-shaped valva (Fig. 8d). The spines of the second spore valva

do not diverge and do not taper as in *S. turris*, but are nearly parallel to the pervalvar axis and have blunt ends (Fig. 8d).

Germination of the resting spore occurs in the same way as in *S. turris*, by elongation of the cell and normal cell division. The spore valvae are used further by the germinated cells. According to MICHAILOWA (1962), who first described a resting spore germination, the spore valvae in the species *Chaetoceros lauderi* RALFS are discarded during germination.

Which conditions induce the production of resting spores in *S. palmeriana*? In order to find an answer to this important question, three series of experiments were conducted. In the first series various constant temperature levels (between 9° and 24° C) were offered. No formation of resting spores was observed. In the second series the possible influence of cell size was analyzed by using different subclones of various cell diameters. Again, this treatment did not produce any resting spores. Finally, in the third series of experiments, the possible importance of nutritive substances was tested. Because phosphate and nitrate represent two of the most important minimum factors in the sea, nutritional solutions were used without the additional doses of phosphate and nitrate, respectively. Cultures inoculated with a few short cell chains and exposed to a temperature of 12° C (light intensity of about 1200 Lux) started to form resting spores in the state of phosphate-deficiency after 6 to 7 days. In most cases nitrate deficiency did not produce resting spores at all, in a few cases only sporadically. Probably the most intensive effect on resting spore formation is created by a nutritional medium lacking both phosphate and nitrate. Among the temperature levels offered (9°, 12°, 15°, 18°, 21° and 24° C) 12° C proved to be most effective. However, resting spore formation took place, although very occasionally, at temperatures up to 24° C.

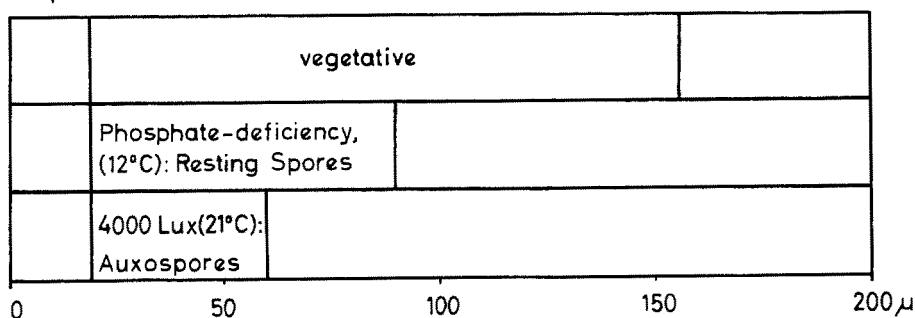
In the existing diatom literature nothing was known about the influence of cell size on the formation of resting spores. The first pertinent data on *S. turris* were published by DREBES (1964) and v. STOSCH & DREBES (1964), who found that only cells of a large diameter (30 to 97 μ) are capable of resting spore formation. According to newer information (DREBES unpublished) this range can be extended to 115 μ (Fig. 9). The relation between cell size on resting spore formation is completely different in *S. palmeriana*. In the first experiment with 100 μ and 66 μ wide cells, only the latter formed resting spores. Further studies showed resting spore formation down to 19 μ cell diameter, the lower limit of vegetative existence. A 100 μ clone, as well as a clone consisting of cells of 150 μ diameter, which originated from a just germinated auxospore, never produced any resting spores. Resting spores occurred only after the cell diameter decreased to 90 μ .

The resting spores germinated after they were transferred into a fresh, complete nutritional solution, which compensated for the deficiencies suffered in the previous culture medium.

COMPARISON OF VITAL DATA OBTAINED FOR *S. TURRIS*
AND *S. PALMERIANA*

Besides *S. turris*, a further test-case model, *S. palmeriana*, is presented for studying the complete life history of a marine planktonic diatom in the laboratory. The transparence of this alga allows direct observation of its fundamental nuclear processes, such as meiosis and karyogamy, under a light microscope. These two species may therefore easily be used as classroom specimens for university students and as research subjects for developmental physiology.

S. palmeriana



S. turris

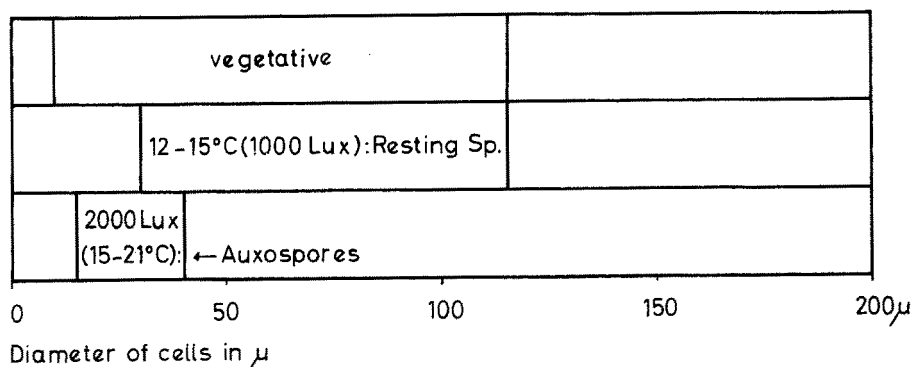


Fig. 9: Important internal and external factors controlling the life histories of *S. turris* and *S. palmeriana*. Diagrammatic illustration

The most important internal and external factors controlling the life histories of *S. turris* and *S. palmeriana* are diagrammatically illustrated in Figure 9. As can be seen from this figure cell size, temperature, light and nutrients decisively control the phases of the life cycles. A comparison of the information (only the optimal conditions are recorded here) obtained for the temperate *S. turris* and the warm-water species *S. palmeriana* is given below:

Stephanopyxis turris

Vegetative phase: cell size 10 to 115 μ ; Resting spores (middle line): cell size 30 to 115 μ , temperature 12° to 15° C, light 1000 Lux; Auxospores (lower line): cell size 15 to 40 μ , temperature 15° to 21° C, light 2000 Lux.

Stephanopyxis palmeriana

Vegetative phase: cell size 19 to 156 μ ; Resting spores (middle line): cell size 19 to 90 μ , temperature 12° C, nutrients phosphate-deficiency; Auxospores (lower line): cell size 19 to 60 μ , temperature 21° C, light 4000 Lux.

One reason for the fact that *S. palmeriana* avoids colder waters may be the high temperature requirements for sexualization. The influences of both internal and external factors on the formation of resting spores are quite different in these two species. Unfortunately, the taxonomic identity of these closely related species has frequently been misjudged, consequently, their geographical distribution is not clearly known. Further investigations are necessary in order to obtain a sound basis for a discussion of the first results obtained in laboratory cultures.

SUMMARY

1. The cell diameter of *Stephanopyxis palmeriana* ranges between 19 and 156 μ . The pleural structure is considered to be specific for the genus. The interphasic nucleus lies in the discus of the hypovalva.
2. The cell size can be manipulated during the vegetative phase through SiO₂-deficiency followed by frustule regeneration.
3. Only 19 to 60 μ wide cells are capable of forming gametes and auxospores. Differentiation begins after light intensity is suddenly increased from 400 to 4000 Lux, and temperature from 15° to 21° C.
4. Only 19 to 90 μ wide cells are capable of forming resting spores. These are produced in the presence of phosphate-deficiency; maximum production occurs at 12° C. After correction of the phosphate-deficiency, resting spores germinate.
5. Morphologically, the life history (formation of gametes, auxospores, resting spores) conforms essentially with the findings on *Stephanopyxis turris* (v. STOSCH & DREBES 1964).
6. The most important factors controlling the life cycle are cell size, temperature, light and nutrients. The effects which these factors produce in *S. turris* are compared with those observed in *S. palmeriana*.

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