MITOTIC PERIODICITY AND RELATED CYTOLOGICAL OBSERVATIONS IN TWO SPECIES OF INTERTIDAL RED ALGAE RHODOMELA LARIX (TURNER) C. AGARDH AND PORPHYRA LANCEOLATA (SETCHELL AND HUS) G. M. SMITH

BY

JOHN DOUGLAS PRINGLE

B.Sc. University of Victoria

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biology

Accepted for the Faculty of Graduate Studies

May 13, 1969

We accept this thesis as conforming to the required standard

JOHN DOUGLAS PRINGLE

UNIVERSITY OF VICTORIA

AUGUST 1968

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ABSTRACT

An investigation was initiated in September of 1966 to ascertain to what affect certain environmental conditions have on the diurnal distribution of mitosis in meristematic areas of intertidal red algae.

Rhodomela larix, a mid-shore plant, and Porphyra lanceolata, a high shore plant, handled well with the chosen technique and thus were selected as initial plants from a number of possible species.

Eight stages of karyokinesis were defined for both species and quantitative data on mitosis were attained.

Statistically significant rhythms in karyokinesis were present in both species but the diurnal pattern of the rhythms differed in each species. R. larix plants collected in July maintained a statistically significant, asynchronous rhythm of mitosis throughout a 14 hour period, but those collected in February showed fluctuations over a 24 hour period which were not statistically significant. Nuclei of P. lanceolata plants maintained a phased rhythm of mitosis over 24 hour periods in November and January; karyokinesis was initiated between 1430 and 1630 hours in November and between 1230 and 1430 hours in January but in both monthly collections the maximum peaks in the seven earliest stages of karyokinesis occurred at 1630 hours. From this work it is suggested that light intensity may play a significant role in bringing about a periodicity in mitosis in P. lanceolata.

P. lanceolata was maintained under varying laboratory

conditions in January in an attempt to discern which, if any, environmental factor(s) triggered karyokinesis. Plants kept submersed under constant light exhibited a statistically significant peak in karyokinesis at 1630 hours.

Aspects on the life history and cytology of P. lanceolata were observed and plants collected in November were observed to contain three distinct types of asexual spores. Plants collected in January exhibited different types of spores to the November plants and are thought to be α and β spores. Sexual reproduction including the fertilization process is described and illustrated as is the post-fertilization process.

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ACKNOWLEDGMENTS

I thank Dr. A.P. Austin for encouraging me to enter graduate school, for suggesting a very interesting thesis problem and for being so understanding throughout the study. Dr. Austin's willingness to listen to, and implement, new ideas added interest to the work.

I sincerely thank: Dr. Marc Bell for encouragement and wise council; Dave Barber, Sharon Brown, Marlene Roberge, Rik Warrington and Joan Pringle for their generous aid on various field trips; Dr. Gordon Robinson and Dr. Kathleen Cole for assistance in solving certain cytological problems; Dr. A. Austin, Dr. M. Bell, Dr. J. Owens and Dr. L. Saunders for their critical analysis of the manuscript; Dr. A. Austin and Dr. T. Algard for aid in developing suitable photographic techniques; Mr. Fred Duncan for his excellent technological work; my fellow graduate students Bob Dunnill, Bob Otto, Betty Westerborg and especially Sharon Brown for invaluable discussion periods and the office staff for their typing of manuscripts.

Sincere and deepest thanks are given to my wife for patiently attending the thankless position as the wife of a graduate student and for typing the final draft of this thesis.

PART I

MITOTIC PERIODICITY IN *RHODOMELA LARIX* COLLECTED *IN SITU*

ABSTRACT

A review of the literature indicated a lack of quantitative data on cell division in macroscopic algae in the laboratory and particularly *in situ*. By means of SCUBA techniques selected red algae were collected from their *in situ* location on a 24-hour basis through the seasons of the year. The varied oceanographic and meterological conditions on the southern tip of Vancouver Island provided shore habitats of adequate contrast. *Rhodomela larix* (Turner) C. A. Agardh, was found to be a suitable test species for quantitative work and an exploration of differentials in mitotic activity in the growing parts of the plant was effected. Using squash techniques a method of quantitative estimation of mitotic activity employing a point counting method was developed. It was found necessary to define the limits of mitotic phases in *R. larix* to facilitate quantitative analysis which was carried out over 24-hour periods both in July and February. The cells from the apical tips of *R. larix* plants collected in July exhibited a periodicity in nuclear division which was statistically significant at the five percent level. Fluctuations in nuclear activity throughout the day were observed in cells from the February plants but the peaks were not statistically significant. The distribution of mitotic activity throughout the day was similar both in July and February despite the dissimilarity of photoperiod, temperature and tidal factors which suggests the presence of a circadian rhythm. This preliminary work is being continued by further *in situ* investigations correlated with laboratory cultures under controlled environmental condition.

INTRODUCTION

Despite the variety and extremes of conditions experienced by most littoral plant species little attention has been paid to the relation between environmental conditions and cell division in these organisms. The latter may be immersed or emmersed for varying periods over a large spectrum of conditions of light, darkness, temperature, humidity, insolation and so on in different localities at different seasons. Scagel (1961, 1963) drew particular attention to the considerable influence that the coincidence of low water, daylight and season has upon the algal vegetation of the Pacific Northwest, where the present work is being conducted, and it is generally accepted that local meterologic and oceanographic conditions have a variety of effects upon intertidal biota.

In some instances it has been shown that seashore organisms have rhythms correlated with rhythmic changes in their surroundings. Naylor (1958), and Fingerman (1955), have shown a tide-linked rhythm in crabs (Brachyonotus and Callinectus respectively), Rao (1954), found a diurnal rhythm in water propulsion rates of Mytilus californianus and showed, under laboratory conditions, the rhythm was linked with the tidal cycle. Likewise Bennett (1954) showed a tide linked rhythm in Venus mercenaria.

Little information seems to be available regarding the relationship between the frequency of cell division or mitotic activity in intertidal organisms and gross environmental factors. Farmer and Williams (1898), felt that environmental conditions influenced

mitosis to a large extent. Working on the Fucaceae, these authors stated the only time they could procure mitotic figures in the field was just after the plants had been covered by the flood tide. Evans (1962), also working with Fucus sp. throughout neap, spring, flood and ebb tides, stated that no fluctuations in incidence were found. Iyengar and Balakrishnan (1950), sampled Polysiphonia platycarpa at one hour intervals on a 24-hour basis both in the field and in the laboratory. Without giving any statistical evidence they stated that nuclear divisions were more abundant between the hours of 9 p.m. and 12 a.m. Hoyt (1927), studying the production of reproductive cells of Dictyota sp. showed a definite relationship between occurrence of sexual structures and tidal and meterologic conditions. Friedmann (1963), working on life cycles of Prasiola stipitata found that the occurrence of meiosis was governed by tidal conditions.

Friesner (1920), Winter (1929), and more recently Zeuthen (1966), and Tamiya (1966), review much of the work done on mitotic periodicity of higher plants and of organisms in culture. Leedale (1959), working with the mitotic rhythms of Euglena in culture, also reviews previous work on mitotic periodicity in animals and higher plants as well as the algae. More recently a compendium on algal chromosomes (Godward 1966), includes Leedales work but no further data are presented with regard to mitotic activity of algae in situ. It appears that most work in marine algal cytology, including much of the most recent, is based upon observations of mitotic figures seen in preparations of material collected and fixed when

the plants become accessible on the sea shore, viz, at the time of low water. In order to obtain mitotic figures, in what must be assumed to be material which had too few divisions occurring in material collected and fixed as mentioned above, some authors killed and fixed material at various intervals subsequent to collection, the plants being held under unspecified conditions in the laboratory. Yabu and Tokida (1963a), working on Arthrethamnus bifidus and Yabu (1964), working on Agarum cribosum and Alaria praelonga made fixations at midnight of material kept in the laboratory; Yabu and Tokida (1963b), working on species of Porphyra made fixations at a variety of times from material held in the laboratory, however no evidence of greater or lesser mitotic activity at these times of fixation is presented. Magne (1964), working on a wide spectrum of red algae collected and fixed all his material at the time of low water, which in his area was around mid-day, but presents no evidence to support his statement that time of day is without effect upon the incidence of mitoses in the material. Certain authors state that fixation of material during night time is important (Wolfe 1904, Lewis 1909, Svedelius 1911, Iyengar and Balakrishnan 1950, Mullahy 1952, and Austin 1959), whilst others feel that division is independent of the hour of day or night (Westbrook 1928, Drew 1934, Rao 1956, Evans 1962, and Magne 1964). Of these only Evans (1962) reports upon mitotic activity, over daily and seasonal cycles of plants kept in the sea.

The present work attempts to provde a framework of information on the incidence of mitotic divisions in some common macroscopic marine algae of the Pacific Northwest, in situ, at

different times of day and night, tidal cycle and season. This programme has expanded into a limited study of the effects of some environmental factors upon growth, as reflected in mitotic activity, of the species involved and is concurrent with a phenological survey of these species the whole being designed to facilitate programmes of cytological and life history work on the Rhodophytes in this region; both latter aspects will be reported elsewhere.

MATERIALS AND METHODS

The plants used throughout the work were taken directly from their natural habitat and fixed with a minimum of delay. For a limited period before and after low water most of the plants, or their fronds, could be collected and fixed at once i.e. plucked off the shore and deposited directly in freshly combined 1 : 3 acetic alcohol mixture. At other periods, when the lowest plants were covered by two or more feet of water, SCUBA equipment was employed. The diving techniques used were standard except that a shore- or boat-based member was employed to fix specimens on receipt from the diving member. Furthermore, underwater lamps such as Scubalites or Ekelites together with shore based beacon lamps were employed during overnight diving collection periods. These lamps together with certain safety precautions were necessary during darkness particularly over prolonged winter night-diving periods. Wetsuits were found necessary at all times except during the warmest summer weather conditions. A tent

in summer or a mobile trailer in winter was necessary accommodation for most overnight diving work, the diving member or members requiring some shelter when removing wet suits and for drying these suits for re-attire. The planning of night time diving programmes required weather prediction data from appropriate agencies (Anon 1967), together with thorough familiarity with the shore profiles over which the collections were made. The selection of suitable shore locations is important and were chosen in the present instance to embrace the following prerequisites:

i) The presence of all the selected species within as limited an area as possible in order to reduce movement over distances through water and time taken locating plant groups and removing samples. This is necessary in order that all the plants are fixed as close to the same time as practicable.

ii) Accessibility by landbased vehicle and divable under almost any conditions.

iii) Possession of conditions of contrast with other selected shores regarding conditions of aspect, (i.e. exposure to wave action), nature of water mass in the area, (i.e. different sediment loads, proximity to pollution sources, etc.), and local climatic differences, in particular rainfall, atmospheric humidity, and cloud-cover; the south end of Vancouver Island being, fortunately, quite variable with regard to all these features.

The problem of repeatedly locating marked areas on the shore particularly underwater, and during periods of darkness, has been solved in this instance partly by complete familiarity with the natural features of the shore worked, (aided by the regular photographic work

connected with the phenological survey), and partly by marking the shores with pegs and/or paint. The former would constitute "major" marks and the latter "minor" marks of a "major-minor" underwater marking system (Austin unpub). The paint used was white "Chlorubite" a preparation used for lining swimming pools and of high adhesive and durable qualities. This paint withstands a year of weathering even at mid-intertidal positions. These marks were more successful on some shores than the more laborious pegging method (Austin 1960) of driving ½" x 8" lengths of steel rod (Plate 1 Fig. 1) into an approximately 4" deep hole drilled in the rock using a portable power drill, the power source for which had to be carried onto the shore. At some locations these pegs were lost during stormy seas which moved large logs over the pegged area; plants were damaged only in a few instances. Other "minor" mark methods, employed successfully in deeper water (Austin unpub), including the use of fluorescent plastic materials formed into samll "labels" which clip round the lower parts of the plants or fronds, were not successful in the intertidal zones.

The above-described field procedures were all aimed at reducing to the shortest possible duration the time interval between removal of the plants, or parts of plants, from their <u>in situ</u> field position and their fixation. In no case in the present investigation has this amounted to more than 1-2 minutes, this interval occurring whilst the specimens were submerged under transportation by the diver from the growing site to the shore or boat-based member for fixation.

The fixed material was sometimes processed to squashes within twenty-four hours of collection but where this was not possible the fixative was washed out using 100% ethyl alcohol for 10 - 15 minutes and then stored in 70% ethyl alcohol. Material stored in this fashion for as long as 14 months yielded excellent preparations.

The technical difficulties of demonstrating the chromosomes of the Rhodophyta have often been acknowledged(Bold 1951), and relatively recent compendia in algal cytology (Magne 1964 and Godward 1966), are without photographic evidence of a reduction in this problem, the illustrations of red algal nuclei in Godward having been prepared by the senior author in 1955 (Austin 1960). The present authors found little difficulty in staining and spreading the chromosomes of the species listed here employing an iron-alum aceto-carmine technique (Austin 1958), with the addition of some simple refinements including the use of plexiglass "squashers" to produce intense pressure at localised spots on the cover slip without breaking same. The "squashers" were made of 3 mm by 4 mm by 150 mm plexiglass strip with the ends ground and honed to a perfectly level chisel edge. Permanent preparations were made according to the method by Bradley (1948), otherwise the preparation was ringed with glycerine jelly which resulted in a semi-permanent mount.

A detailed report of the technical aspects of this work will be reported separately but a brief summary of each species will be given below. Of a considerable number of species explored only the most easily handled were chosen (Table 1). All but Odonthalia

floccosa gave good fixation; the latter species exhibited a number of pycnotic nuclei. Rhodemela larix (Turner) C.A. Agardh, Laurencia spectabilis and Endocladia muricata fixed, squashed and stained extremely well. Ceramium spp., Cryptosiphonia woodii, Microcladia borealis, Porphyra spp. and Pterosiphonia bipinnata all fixed well and squashed and stained satisfactorily. Halosaccion glandiforme and Odonthalia floccosa were difficult to squash and the latter appeared difficult to stain. However, preliminary modifications of the technique indicated that demonstration of the nuclear detail in difficult material is possible.

Previous work by one of the present authors (Austin 1955) on the chromosomes of the Rhodophyta demonstrated nuclei of a relatively large size in Rhodomela subfusca. The common local species R. larix (Turner) C. A. Agardh, (Pl. I, Fig. 1 & 2) was thus explored at an early date and adopted as the initial species for quantitative work due to its technical ease of handling, presence on almost all local shores and frequent abundance where conditions were suitable. Figure 1 illustrates its midlittoral position on the shore and this was considered an advantage since any pattern or trends in mitotic activity exhibited by such a species could be explored further by investigating plants upshore and down shore from this experimental specimen. Furthermore R. larix is a perennial of unknown growing season and longevity, (the elucidation of which is part of the present work), and could therefore be followed throughout all seasons. The location of the university, less than one-half mile from a large variety of seashores, makes such all year-round, all weather-work possible.

The species chosen for the present work are listed in Table 1 a and b, and are common northwestern Pacific Rhodophytes selected to represent and have some ecological significance in the three main littoral regions. The levels occupied by these species were determined by a simple staff-levelling method using local tidal predictions (Anon 1966a and 1966b) as reference; the results are presented in Table 1 and Figure 1.

On the southern tip of Vancouver Island three shores were chosen which adhered to the previously outlined prerequisites and which supported the selected species. Two of these shores were located at Victoria and one at Port Renfrew and opposed each other to a certain extent with regard to oceanographic and meteologic conditions.

The shore location at Port Renfrew lies 70 miles to the west of Victoria situated on a large inlet and has a northerly aspect; the area has very little settlement compared to the other (Victoria) shores. The mouth of the inlet lies about a mile from the shore station sampled and is exposed directly to the open Pacific Ocean. Port Refrew receives approximately 70 more inches of rain per year than does Victoria. Widdowson (1965), did a horizontal and vertical distribution study of plants in the intertidal zone between Victoria and Port Renfrew. This author monitored salinity, temperature and tidal rhythms and felt that the only environmental factor which appeared to have a regular gradient in the region studied was rainfall and he felt that this coast line was a transitional area between the open ocean and the more sheltered waters of the Victoria area. The

present authors feel that meteorlogical conditions play a large role in the vertical distribution of the plants on the shore as well as in growth with regard to the two areas and have recorded such data in collections other than that presented in this preliminary report.

The shore station at Ogden Point lies on the southwest edge of Victoria, a city of 150,000 population. The community of plants sampled here lie on the exposed side of the Breakwater; this constitutes a semi-exposed shore. The proximity of sewage outlets to the area sampled suggests that the plants receive a good deal of dissolved nutrients. R. larix, sampled here have an abundance of epiphytes, usually diatoms, which are not found to any extent on the plants at Port Renfrew where nutrients, due to domestic effluent are negligible.

The second shore station in Victoria was at Ashlee Bay which has a southeast aspect and is situated on the east coast of the city. The ecological conditions of the two Victoria stations vary little except for prevailing winds. The Ashlee Bay site is protected from the summer northwesterly which the Breakwater receives, and both shores receive the full force of the winter southeasterly. Thus, during the summer springs, which appear in the early to late morning, there is likely to be more desiccation of the intertidal plants at the Breakwater.

Defining the various stages of mitosis in R. larix was necessary before any quantitative work could begin. Dixon (1966) points to the lack of descriptive work on the nuclei and chromosomes

of the Rhodophyta, the bulk of the literature in this area of cytology being represented by fragments of larger life history studies. The authors, following Grundmann (1966) and personal communication with Cole and Robinson delineated seven stages of mitosis (Plate II) for quantitative purposes. Laughlin (1919) carrying out similar studies in Allium cepa illustrated and counted eleven stages. According to Hughes (1952), the earliest sign of mitosis is difficult to discern and is the area where error may be introduced in calculating degree of mitotic activity. Similarly Dodge (1963) claims that the chromosomes in the Dinophycean nucleus remain condensed at interphase and resemble the various stages of meta- and ana-phase found in higher plants. In tabulating the mitotic frequency of a Dinoflagellate Dodge (1964), counted only meta-, ana- and telo-phase; the same stages were used by Jacobs and Morrow (1961), working with the shoots of Coleus sp. and Winter (1929), using roots of Gladiolus sp. The inclusion of only the later phases of mitosis indicated a difficulty in delimitation of prophase stages. Although this was an early problem with our material it was felt by counting only meta-, ana- and telo-phases valuable data, such as stage index (Laughlin 1919), and possibly mitotic rate (Brown and Rickless 1949), would be lost. Furthermore increased cytological experience with R. larix revealed fairly clear differences between the various stages. These are illustrated on Plate II figs. 1-9.

Cytokinesis in R. larix, described in detail in a separate paper, appears to lag behind karyokinesis and furthermore in some cases the nuclei progress toward interphase at differing rates. This

can result in a situation where one of the nuclei in a binucleate cell, might be in typical telophase whilst the other nucleus may appear quite different. This type of cell was recorded as telophase as long as one of the nuclei conformed with typical telophase characteristics. As would be expected, other binucleate cells of the same size appeared with neither nuclei in typical telophase. These were registered as telophase only in the data presented in Table 2 A. These cells were mitotically active and were recorded as such, but on a re-evaluation of their status, for data presented in all other tables, it was decided to classify them on the basis of one of the nuclei i.e. the nucleus that was in the later stage of mitosis.

The methods of preparing material, by various authors, for quantitative mitotic work are few, Gray and Scholes (1951), Hejnowicz and Morrow (1961) used material embedded in paraffin which was then sectioned and stained. Brown and Rickless (1949) macerated the root apex of Cucurbita in a known volume of fluid and a sample of the suspended cells was counted with the aid of a haemocytometer. Austin (1955 and 1960), studying the anatomy and reproductive structures of certain red algae using a squash technique (Austin 1959), found that the cells spread and stained extremely well in most of the species he worked with. It was felt that a quantitative study of mitotic activity in meristematic tissue of red algae could be made, using a squash technique, if the species concerned showed the following prerequisites:

1) fixed well

2) squashed well and uniformly with very little overlapping of cells (Plate III figs. 1-4)

3) the nuclear phase should be distinguishable in no less than 90% of the cells. (Plate III figs. 1-4)

Mitotic periodicity is, in the present work understood to refer to the distribution of mitotic activity with time, usually the time of day. Mitotic index refers to the summation of cell phases of mitosis (i.e. mitotic activity) expressed as a percentage of the total number of nuclei (Rayns and Godward 1965). Mitotic activity was assessed from the squashes by means of a point counting method employing a Kpl 8 x Zeiss Integrating Eyepiece I Test Point Graduated. The counts were made using a 100 x oil immersion planapochromat Zeiss objective and each cell lying on a point of the test point lattice was counted (Plate III fig. 2); the counts being split up into the appropriate mitotic phase and recorded on a key-punch tally register (Table 3).

A preparatory quantitative evaluation involving point counting every field (7,330 cells) from one complete squash using the integrated eye piece gave a value for the mitotic index (Table 2). This process took 1 ½ days to complete and it was obvious that a more rapid method of determining the mitotic index of each squash was necessary. This was done by counting a number of random fields of this same squash and comparing the two mitotic index values. Table 2 shows the mitotic index of five random fields as compared to the total. There is an error of less than 9% and as the mitotic index values for the major fixation times of July and February were far higher than

this figure, even at the lowest mitotic index (Table 8 a, b, c, d), it was decided to use five random fields per squash. These five fields were in roughly the same position in each squash and initially located using the low power 10 x objective so that nuclear activity could not be discerned and introduce choice error.

Using the above described methods quantitative data was procured and presented in the following manner. The mitotic index per ramulus was calculated by summing the values of the five fields and averaging the total. The mitotic index of the four ramuli were summed and averaged to give a value for each plant. The mitotic index for each minor fixation time was calculated by summing and averaging the indices for the three plants. The mitotic index for each plant and for each minor fixation time is shown respectively in Table 8 a & c, and b & d.

All quantitative data from the point counting procedure as well as that of position of squash on ramulus (Table 4), size of the latter (Table 5), and plant size (Table 6), were statistically analysed. Statistical significance between the various factors were carried out using an analysis of variance. Ranking of treatment means (Table 8, b & d) was done through Duncan's New Multiple Range Test. To avoid any interpretative bias all material squashed and counted was coded and the correlation between collection time and mitotic activity was not possible until final assembly of data.

OBSERVATIONS AND RESULTS

All plants and plant parts were handled under a Tri-X Olympus Trinocular stereomicroscope which facilitated photography of the site of origin of material from which squash preparations were made as well as the measuring of the lengths of plant parts.

The morphology of R. larix, fragmentary knowledge of seasonal growth pattern (Austin unpub.) and literature on Rhodomelaceae (Kylin 1956 and Fritsch 1945) suggested most growth takes place in the apical region. Dissection of the latter revealed an apical cup (Plates IV, fig. 2) consisting of rather irregularly arranged tapering, finger-like ramuli, all curving toward the central axis, the smaller (1 mm. - 3 mm.) internal, pale pink ramuli being surrounded by larger (4 mm. - 7 mm.) darker tan-brown ramuli often covered with encrusting epiphytes (Plate IV, figs. 1 & 2). The epiphytes appear to grow from February to July, being conspicuous in the latter month. These epiphytes are not visible on the smaller ramuli of the inner part of the cup (Plate IV, figs. 1 & 2). The inner and outer ramuli of the apical cup contrast greatly with those of the thallus below the cup (Plate I fig. 2 and Plate IV figs. 1 & 2). These ramuli are black in color with the encrusting epiphyte very conspicuous the year round and sometimes on the ramuli of the extreme lower portion of the thallus, cover the entire ramulus.

On comparison of the external features and color of the above described ramuli it was obvious that the tissue where maximum mitotic

index would likely be found was at the tips of the pink, tender looking ramuli which make up the inner portion of the apical cupule (Plate IV figs. 1 & 2). To test this theory tips were squashed and counted from ramuli taken at random locations on the thallus. Statistical analysis of this data was planned but found unnecessary as it was clear that the inner ramuli of the apical cupule were suitable.

It was then necessary to decide which part of any given ramulus was maximally active. Table 4 presents data on the mitotic index of tissues from different positions on the ultimate ramuli. The ultimate 0.5 mm. tip was removed as was the penultimate 0.5 mm. tip and they were stained, squashed and counted. An analysis of variance was carried out and there was no significant difference between the two positions on the ramulus. Further sequential sections were taken down the length of the ramulus, below the penultimate tip, and counted. The mitotic index of this tissue showed an obvious trend in decreasing mitotic activity compared with that found in the ultimate and penultimate tips but there was not enough material counted for a statistical analysis.

Due to the presence of large numbers of very small deeply staining cells in the extreme apical 250 microns this length was discarded and the squashing procedure was performed throughout the work (on this plant) upon the penultimate 500 microns of the ramulus apex. The absence in this plant of a quiescent region at the extreme apex (Lance, 1952 working on Vicia faba) conforms with a good deal of work on apical meristems reviewed by Clowes (1961).

The Apical cup of R. larix varies in the numbers of inner tender ramuli from winter to summer. In the summer material there is usually no more than six to seven ramuli from 1-3 mm. in length whereas in the winter material there is anywhere from 12-14 ramuli of this size. When choosing ramuli from the same plant those of a similar size were taken. This was easily accomplished in the winter material due to the abundance of tender ramuli (Plate I fig. 2 and Plate IV figs. 1 & 2), but in the summer material there was usually a variance in length, anywhere from 1.5 mm. - 3 mm. Because of this variation in size it was thought it might affect the statistical validity of the mitotic index values. Karsten (1915) and Friesner (1920) collecting quantitative data on mitosis in the roots of higher plants found they had to relate the number of mitoses found per root to a unit area of root tip as each root they used varied a great deal in size. Winter (1929) doing a similar study with the roots of Gladiolus sp. showed that he did not have to relate mitotic figures to the size of the root as the material was extremely uniform in size. Since the ramuli of R. larix used here were so uniform in size that such a procedure was also unnecessary in this work.

Table 5 presents the mitotic index of ramuli 1.5 mm., 2.0 mm. and 2.5 mm. in length. The analysis of variance of this data showed an insignificant F value thus it was concluded that there is no variance in the mitotic index between ramuli of these lengths.

The possibility of a differing mitotic activity in ramuli from plants of different size, and presumably age, as reported for higher plants, (Jensen and Kavaljian 1958) was explored. Table 7

presents the results which indicate no significant difference for ramuli of equal length taken from plants of 2 cm., 3 cm. and 4 cm.

After having taken these preliminary precautions a program of squashes of the penultimate 500 u of four inner apical cup ramuli from each of three plants at every minor fixation time could proceed. This number of squashes made and plants sampled was designed to attempt to give statistically sound data. The main aim was to compare mitotic activity between plants fixed at different times of day and season. Firstly, however, variance in the sample of four ramuli per plant was tested. This is done in Table 7 and there appeared to be no appreciable variation between these ramuli.

Although the growing season of R. larix is not fully known it was thought that a preliminary quantitative estimation of mitotic activity at about mid summer and mid winter might lend support to our phenological data. The months chosen were July and February which, for reference in this paper, will be designated major fixation times. The collections for July 27 were done at a time of warm weather, the tidal cycle being the end of a neap period. The collections were made about every three hours but did not cover a full 24 hour period, a gap existing from 1600 hrs. to 2300 hrs. The plants were emerged from about 0630 hrs. to about 0900 hrs. and during this state were fixed once at 0845 hrs. The plants received direct sunlight from about 0715 hrs., thus, almost the entire period the plants were emerged they received direct sunlight.

The major fixation period on February 22 was initiated at

2045 hrs. and was terminated at 1910 hrs. on the 23, the tide was in a spring cycle at this time. Low tide occurred at 1950 hrs. on the 22, and the plants were emerged at 1745 hrs., the hour of sunset. The flood tide covered the plants at 2200 hrs. thus the plants fixed at 2045 hrs. and 2140 hrs. were emerged. Low tide was at 2030 hrs. on the 23, the plants fixed at 1830 hrs. were emerged with exception of the occasional wave passing over them. The plants fixed at 1910 hrs. were emerged. The temperature of the water was approximately 8.5°C and the temperature of the air ranged from 5.0°C to 9.5°C. A gap in the sampling exists between the hours of 0030 hrs. and 0745 hrs.

DISCUSSION AND CONCLUSIONS

Bruce (1964), reviewing work on cell cycle time in various organisms felt it was more than a coincidence that this time was about 24 hrs. in many organisms. A circadian mitotic periodicity appears to occur in most higher plants explored. Friesner (1920), using excised roots in culture claimed a periodicity of mitotic rhythm however Winter (1929) growing roots of Gladiolus both in natural and culture conditions found no variation of mitotic activity. More recently Jensen and Kavaljian (1958) found, in the same species (Allium cepa), two maxima of cell division one at noon and one at midnight. Most of these above mentioned authors report a periodicity linked with external conditions although Friesner reports that the periodicity is apparent only when conditions are uniform.

Regarding the presence of mitotic rhythms in higher plant parts other than root tips Jacobs and Morrow (1961) failed to find mitotic periodicity in the apices of Coleus but reported a periodicity for the initiation of leaf primordia. Kaufman (1965) also failed to find any mitotic periodicity in the elongating internodes of Avena sativa. However, Lance (1952), working on Vicia faba apices found a rhythm with a peak at 0900 hrs. and another smaller peak at 2100 hrs. Speese (1939) sampling Smilax leaves (grown in the field) claimed 2 peaks of division shortly after midnight and shortly after noon.

Ample proof of unicellular aquatic plants having periodic fluctuations in mitotic index is given in the literature; Friesner (1920) and Winter (1929) give adequate reviews of previous work. More recent work is reviewed by Leedale (1959) and Tamiya (1964) and the results of their own studies in the lab show that unicellular algae divide during darkness when subject to diurnal fluctuations of light and temperature (Sorokin 1965) which agrees with most of the other work done on the Chlorophyceae. However Rayns and Godward (1965) studying the mitotic periodicity of Eudorina elegans under fluctuating light (16 hours light and 8 hours darkness), showed a primary peak about two hours before the end of the light period with subsidiary peaks at other times, particularly during the dark phase.

Mitotic index studies of higher aquatic plants are extremely limited. Savelkoul (1957) having sampled Elodea densa every three hours from green house pools found mitotic fluctuations but apparently they were not statistically significant.

Workers interested in periodic phenomena of plants have virtually neglected the marine environment. There appears to be no statistical treatment of mitotic activity carried out on multicellular plants either from the marine environment and kept in laboratory conditions or sampled *in situ*. The few cytologists working with the seaweeds rely on chance in carrying out their sampling methods to acquire dividing nuclei. Evans (1962) is the only worker who has carried out a sampling program designed to reveal a time of day and/or a time of year in which species of intertidal *Fucus* would yield more dividing nuclei than that found by indiscriminate sampling.

In *R. larix*, throughout the July (major) fixation sampling period a periodicity in cellular division was found (Table 8 a & b, Fig. 2). The fluctuations are statistically significant (Table 8 b). There were two minima and one maximum period of activity; the former being at 0100 and 1100 hrs., the latter at 0845 hrs. (Fig. 2).

Mitotic indices of plants sampled varied with the time of day fixed. At periods of low mitotic activity there are considerable differences in the mitotic indices between plants. The largest standard deviations occurred in plants fixed at 0100 and 1100 hrs., this material is also the least mitotically active. At the time of day when the mitotic activity is high there was found to be very little difference in the mitotic indices between plants sampled. Those fixed at 0845 and 1445 hrs. had the lowest standard deviations; this material is also the most active.

In the February (major) fixation sampling period the standard

deviations were calculated for the mitotic indices of three plants per minor fixation time (Table 8 c). The standard deviation ranged from ±3.37 to ±21.65 with seven out of the 12 values between ±11.18 and ±14.68. Thus, the mitotic activity of plants fixed at the same time of day varies considerably in most cases. However, even though the standard deviations are high, they are approximately equal (Table 8 d), hence a comparison of the mitotic index across fixation times is legitimate. Two of the lowest standard deviations are found at two of the four peaks of mitotic activity (Tables 8 c & d); a similar trend to the July data, where the variation in mitotic activity between plants is small when the material is actively dividing.

The mitotic activity fluctuates throughout the 24 hour period sampled; the mitotic indices range from 49% to 75% (Table 8 c & d). There are four times of day when the mitotic index is higher than than found at the other eight fixation times but there is no statistical significance at the 5% level in this data. Nevertheless definite peaks of mitotic activity are present (Fig. 3).

Because of the possibility of increased mitotic index just before emergence and just after submergence (Farmer & Williams (1898) plants were fixed at ½ hourly and hourly intervals respectively at these times of tide. The four half-hour interval fixations, from 1730 to 1900 hrs., revealed a steady state in mototic activity (Table 6 d) with no fluctuation more than 5% (ranging between 61.63% and 66.54%). This plateau in mitotic activity, is unique in this major fixation sequence. Two, possibly three, of the environmental conditions changed between 1730 and 1910 hrs. The sunset at 1748 hrs.,

15 minutes after the first of this series of samples was taken and the plants had been emerged for ½ hour when the fourth sample was fixed, 1910 hrs. The temperature dropped from 8.0°C - 5.0°C. None of these ecological changes seemed to have an effect on the mitotic index of the 12 plants sampled, (Fig. 3).

One of the higher peaks of mitotic activity occurred while the plants were emerged and had been emerged for a period of about three hours. The lowest trough came less than an hour later, the plant having been emerged for almost four hours. The flooding tide covered the plants at 2200 hrs. and by 2230 hrs. the mitotic index had started an upward trend which by 2330 hrs. had reached the highest value in the entire major fixation time. One plant at this hour had a mitotic index of 90.99% (Table 8 c).

A significant decrease in mitotic index occurred at 1100 hrs. when the plants had been submerged for 13 hours. The highest tide of the day took place at 1150 hrs. so at this minor fixation time the plants were covered with seven feet of water which is almost the maximum amount for the entire tidal regime (Fig. 3).

The most outstanding feature of the two sets of data is the occurrence of the mitotically active and inactive periods at the same time of day (Fig. 4). They both show a low mitotic index just after midnight and another at 1100 hrs., or late morning. The high mitotic indices occurred about mid morning, 0800 hrs., with a second peak in mitotic activity in the early afternoon. The coincident fluctuations took place in R. larix despite very dissimilar ecological conditions. Seven months separated the two collection times (July and

February), these collections were made at different locations and the meterological and oceanographic conditions differed. Since little work has been done on the influence of environmental conditions in nature upon mitosis in plants (Winter, 1929 and Farmer and Williams, 1898) a brief consideration of the ecological conditions to which R. larix was subject at the times of 0800, 1100 and 1400 hrs., (the times of similar mitotic activity) in both seasons is pertinent. The temperature of the water in February was 9°C while in July it was 13°C. The light period in July was 15.17 hours while in February it was 10.40 hours, therefore at 0800 hours the sun had been up three hours longer in July than at the same time in February. The plants collected at 0800 hrs. (a peak) in February had been submerged for about ten hours and were covered with five feet of water while those collected at the same time in July had been emerged for two hours. At 1100 hrs. (low activity) the plants in February were covered with seven feet of water and had been submerged for 13 hours whilst in July these had been submerged for about an hour and had about one foot of water over them. The July plants at 1400 hrs. (a peak) had been submerged for four hours and were under four feet of water whilst in February they were subjected to the ebbing tide, had been submerged for 16 hours and were covered with about three feet of water.

In these data the ecological conditions of tides (emergence, submergence and depth of water covering the plants), temperature of the water and meterology seem to have little effect on mitotic periodicity.

The high rate of mitotic activity while the plants were emerged was surprising, especially when, in each case, they had been emerged for two hours or more. The authors felt that desiccation at low tide would result in a decrease of mitotic activity in intertidal algae. The explanation may be that the plants were emerged at a time of day (early morning and evening) when very little desiccation of the plant would take place.

A feature of the distribution of activity in R. larix is the sudden decrease in mitotic activity after a period of increased divisions (Fig. 4). The only time there was a levelling of mitotic activity was between 1700 and 1910 hrs. (February data Fig. 3) when there was a fluctuation of no more than 5% for two hours.

Despite the less complete data for July, it appears that mitotic activity is low but very variable in the July material and high but more steady in the February material. From Fig. 4 it is clear that one peak of activity in the July material is higher than any of the February values but soon decreases to a lower value than any of the February values. Furthermore, the standard deviations for values taken in February were much higher than those of July and this, together with a lack of significance (5% level) between the peaks and troughs in February, indicate a trend towards more uniform activity in cell division throughout the 24 hour period in February.

The data suggests a parallelity of mitotic rhythm within the 24 hour period both at July and February, but with a greater amplitude

in the summer period than in the winter period. Although the curves are for data taken from slightly different tidal cycles it has already been suggested (page 25) that tidal rhythm may not be of prime importance, however if desiccation is a factor influencing cell division at least in the summer period, one would expect a greater incidence of mitosis during neap tides. Furthermore some untreated data on material fixed through a spring tide sequence in July gave numerous mitotic divisions just after the plants were covered by the flood tide, whilst material collected at low water gave very few divisions and throughout this fixation sequence divisions were relatively infrequent.

The suggestion that mitotic activity and growth, insofar as the latter is related to the former (Sorokin 1965), is more intense and more evenly spread or consistant throughout the 24 hour period during the winter may be supported by general ecological observations. Clearly R. larix is subjected to desiccating influences from April to September (Scagel 1961 & 1963) due to its lower mid-intertidal location and daytime low water periods.

Although, as mentioned, mitotic periodicity may exist in R. larix, there is no sign of mitotic synchrony. Cell synchrony, a mitotic periodicity where all the cells are in the same stage of mitosis at any given time, was found as early as 1952 (Aach) and Tamiya et al (1953) in cultures of Chlorella. Mattingly (1966) reviews works on induced synchrony in the roots of higher plants using five amino-uracil. Løvlie (1964), working on Ulva in Føyn culture medium, showed that this plant produced complete synchrony in cell division every night.

Synchrony of cell division in both unicellular and multicellular organisms can be brought about by varying the culture conditions such as light and temperature. In R. larix, during periods of increased mitotic activity every phase of mitosis was found and at no time were all the cells in one phase. The cells in the roots of Allium cepa enter division synchronously in the form of waves (Laughlin 1919), but Hughes (1952) claims the latter is not typical, if mitotic periodicity exists in multicellular organisms it is typical to find greater proportions of cells dividing at certain times, at other times mitosis will be found but to a lesser degree.

From the results of the present work it is somewhat surprising that Evans (1962), working with species of Fucus, did not find, at least a seasonal difference in mitotic activity even though a rhythm based upon a 24 hour cycle was not evident. The latter work was carried out between July 1960 and April 1961, a period in which environmental conditions would have been changing both diurnally and over the seasonal period; however, no influence upon cell division could be detected. These results are contrary to those of Farmer and Williams (1898), who used a boat and a cutting instrument to sample plants of Fucus from their in situ positions; their material revealed an increased mitotic activity with the flood tide. Evans, on the other hand, suspended his plants on an undescribed pulley apparatus and could emerge sample and resubmerge the plants regardless of the state of the tide. Furthermore he measured fronds over a 16 day period during which they increased in length from between 0.35 cm. to 1.85 cm. with no fluctuations in the number of dividing cells. It would have been useful if a comparison could have been made between

these data and those of a similar 16 day period when a different rate of growth could be reasonably assumed. Evans did not give his methods for quantitative estimation of mitotic activity and it is the present authors experience that the latter may be easily overlooked without the proper precautions. Moreover the Fucus plants on the pulley arrangement were not strictly in situ, although it is difficult to see why this should have the effect of levelling up any differences in mitotic activity. Since it is generally supposed that upshore Phaeophytes such as Pelvetia and Fucus, may be physiologically adapted to withstand desiccation these plants may be typical with regard to environmental influences upon cell division. However, Winter (1929), measuring roots of Gladiolus spp. found that they increased 9 cm. in length in six days but no periodicity of cell division was found and at no time was there more than 2% of the cells dividing. Cell divisions in Fucus may resemble Gladiolus and divide at a low but steady rate.

A mitotic periodicity unaffected by obvious environmental conditions appears to occur in R. larix. The periodicity was remarkably similar both in February and July even though photoperiod, temperature and tidal factors were all dissimilar, suggesting the presence of a circadian rhythm in cell division. This is being investigated further by cultural work under controlled environmental conditions together with thorough exploration of R. larix fixed through spring and neap tidal periods for much of the year. Other species from high shore and low shore as well as subtidal habitats have been selected and are at present being investigated. Any rhythm in mitotic activity correlated with conditions attendent to tidal rhythm will, it is thought, become clearer with extension

of the work in this way. Furthermore the present data are being processed to extract information with regard to mitotic rate and stage index.

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TABLE 1.

Species involved in this program and their respective vertical position, expressed as feet above or below tidal datum level.

a) Nontidal pool

Species	SHORE LEVELS								
	Port Renfrew			Breakwater			Ashlee Bay		
	Highest level	Most abundant	Lowest level	Highest level	Most abundant	Lowest level	Highest level	Most abundant	Lowest level
Ceramium	4'7"	4'1"	3'7"						
Cryptosiphonia Woodii	*4'0"	3'6"	3'0"	3'3"	3'	*10"			
Endocladia muricata	6'1"	4'9"	2'9"	7'6"			8'11"	6'0"	3'4"
Halosaccion glandiforme	4'11"	4'3"	3'1"	not present			2'10"	7"	7"
Laurencia spectabilis	not present			2"	0.0	-9"	not present		
Microcladia borealis									
Odonthalia floccosa				*3'		2"			
Odonthalia washingtoniensis	not present			2"	-3'	-3'3"	not present		
Porphyra sp.				7'6"					
Pterosiphonia bipinnata				6'6"	*3'	3'3"?	not present		
Rhodomela larix	6'10"	4'7"	3'7"	3'2"	2'5"	1'	2'10"	2'10"	2'0"

b) Tidal pool

Location of tidal pool	Location above sea level	Ceramium	Endoclaida	Rhodomela larix	Cryptosiphonia woodii
Ashlee Bay	6'9"	x	x	x	
Port Renfrew	4'1"			x	x

* estimated level

TABLE 2.

Comparison of two methods of quantitative determination of the mitotic index of one squash.

(The minimum area for quantiative estimation was arrived at in two ways:

A - by counting the entire area of the squash field by field;

B - by counting five random fields; their respective mitotic indices are remarkably similar.

The point counting lattice was used throughout.)

	Interphase	Prophase			Metaphase	Anaphase	Telophase	Total No. of Cells Counted	Total No. of Dividing Cells	Mitotic Index
		Early	Mid	Late						
A	3,149	2,929	849	86	66	17	*234	7,330	4,181	57.04%
B	34	25	9				8	76	42	52.26%

* See discussion p. 12.

TABLE 3.

Comparison of mitotic counts made during "active" and "inactive" periods in July and February. (Numbers of nuclei in each mitotic phase are given for two "active" and two "inactive" periods in February. Since only one active period occurs in July this is given along with one inactive period. (Text fig. 4)).

Season of Fixation	Amount of Activity	Time of Day	PHASES OF MITOSIS							Total No. of Cells Counted	Total No. of Dividing Cells
			Interphase	Prophase			Metaphase	Anaphase	Telophase		
				Early	Mid	Late					
February	Active	2330 hrs.	375	843	239	21	11	0	1	1,490	1,115
		0745 hrs.	427	825	183	34	14	3	7	1,493	1,066
	Inactive	1100 hrs.	696	660	57	10	11	5	0	1,439	743
		2140 hrs.	753	631	91	2	14	0	0	1,491	738
July	Active	0845 hrs.	217	648	264	24	15	1	15	1,184	967
	Inactive	1100 hrs.	768	471	39	13	10	1	7	1,309	541

TABLE 4.

A comparison of the mitotic indices of the ultimate and penultimate 500 microns of tissue from the same ramulus at each minor fixation time.

Fixation Season	Minor Fixation Time	Mitotic Index of Ultimate 500 u	Mitotic Index of Penultimate 500 u
July	0500 hrs.	73.21	82.20
July	0500 hrs.	45.35	71.13
July	0845 hrs.	85.71	94.38
July	0845 hrs.	84.34	82.60
July	0845 hrs.	80.24	71.11
July	1100 hrs.	46.32	33.98
July	1100 hrs.	53.13	47.00
July	1430 hrs.	52.38	74.39

TABLE 5.

The mitotic indices of the penultimate 500 microns of ramuli of different lengths taken from the same plant.

Fixation Season	Minor Fixation Time	Length of Ramulus 1.5 mm	2.0 mm	2.5 mm
February	2030 hrs.	75.81	70.66	63.41
February	2030 hrs.	69.35	75.00	86.29
February	2330 hrs.	66.39	65.85	81.80
February	1415 hrs.	74.17	83.95	73.72
February	1415 hrs.	52.63	66.66	48.27
February	1730 hrs.	80.00	57.65	81.15

TABLE 6.

Comparison of mitotic indices of similar tissue taken from plants of different lengths and presumably age.

(Each value is a mean of the mitotic indices of penultimate 500 microns of four similar ramuli from one plant.)

Fixation Time of Month & Day		Length of Thalli 2.0 cm	3.0 cm	4.0 cm
February	2140 hrs.	51.26	30.52	66.31
February	2230 hrs.	68.63	79.74	60.56
February	0745 hrs.	88.28	65.18	61.04
July	0030 hrs.	36.41	52.91	82.93
July	0500 hrs.	56.28	22.06	48.46

TABLE 7.

Range of mitotic index exhibited by the penultimate 500 microns of ramuli from the same plant.

(The data was chosen at random from material already processed quantitatively. It can be seen that there is very little variation in mitotic index in ramuli from the same plant.)

Season	Minor Fixation Time	Plant	Mitotic index of 4 ramuli from one plant			
			1	2	3	4
February	2030 hrs.	1	75.81	63.41	70.61	73.98
February	2140 hrs.	2	30.65	30.40	33.60	27.42
February	2230 hrs.	3	60.33	63.28	53.60	65.04
February	2430 hrs.	4	65.85	68.39	65.57	81.60
February	0030 hrs.	5	34.43	33.61	27.20	41.60
February	1415 hrs.	6	84.87	85.90	93.40	80.90

TABLE 8.

Statistical data for the July and February *in situ* fixations.

a) July fixation data showing the time of day the plants were fixed, the mitotic index mean for each plant and the standard deviation for each fixation time.

Fixation Time	Mitotic index per plant per fixation time	Standard Deviations for 3 plants per fixation time
0100 hrs.	1. 62.93 2. 53.91 3. 37.41	±12.94
0500 hrs.	1. 75.13 2. 59.12 3. 70.24	± 8.20
0845 hrs.	1. 75.71 2. 83.78 3. 87.52	± 5.50
1100 hrs.	1. 53.34 2. 41.53 3. 32.82	±10.20
1445 hrs.	1. 63.37 2. 74.50 3. 68.36	± 5.57

TABLE 8

b) July fixation data, showing the mean values for three plants per fixation time and its rank in relationship to other fixation times. Statistical significance between means at the 5% significance level is shown.

Time of Day	Mitotic Index per Fixation Time (Three-Plant Mean)	
1100 hrs.	42.56	a
0100 hrs.	51.42	a
0500 hrs.	68.16	b c
1445 hrs.	68.75	b c
2045 hrs.	82.67	c *

* those followed by the same letter are not significantly different at the 5% level.

TABLE 8.

c) February fixation data showing the time of day the plants were fixed, the mitotic index mean for each plant and the standard deviation for each fixation time.

Fixation Time	Mitotic Index per Plant per Fixation Time	Standard Deviation per Fixation Time
2030 hrs.	1. 70.96 2. 77.26 3. 76.20	± 3.37
2140 hrs.	1. 66.31 2. 51.26 3. 30.52	±17.97
2230 hrs.	1. 60.56 2. 79.74 3. 68.63	± 9.63
2330 hrs.	1. 69.85 2. 64.76 3. 90.99	±13.91
0030 hrs.	1. 34.21 2. 73.04 3. 70.22	±21.65
0745 hrs.	1. 61.04 2. 65.18 3. 88.28	±14.68
1100 hrs.	1. 69.48 2. 39.10 3. 55.46	±11.18
1415 hrs.	1. 74.40 2. 60.48 3. 86.28	±12.91
1730 hrs.	1. 73.78 2. 69.18 3. 49.48	±12.90
1800 hrs.	1. 63.18 2. 55.98 3. 65.72	± 5.05
1830 hrs.	1. 58.47 2. 60.54 3. 80.60	±12.22
1910 hrs.	1. 56.51 2. 79.87 3. 59.27	±12.77

TABLE 8.

d) February fixation data, showing the mean value for three plants per fixation time and its rank in relation to other fixation times. Lack of statistical significance between means at the 5% significance level is shown.

Time of Day	Mitotic Index * per Fixation Time (Three-Plant-Mean)
2140 hrs.	49.36
1100 hrs.	51.68
0030 hrs.	59.16
1800 hrs.	61.63
1730 hrs.	64.15
1900 hrs.	65.25
1830 hrs.	66.54
2230 hrs.	69.64
0845 hrs.	71.50
1415 hrs.	73.72
2030 hrs.	74.81
2330 hrs. *	75.20

* No statistical significance between the means at the 5% level.

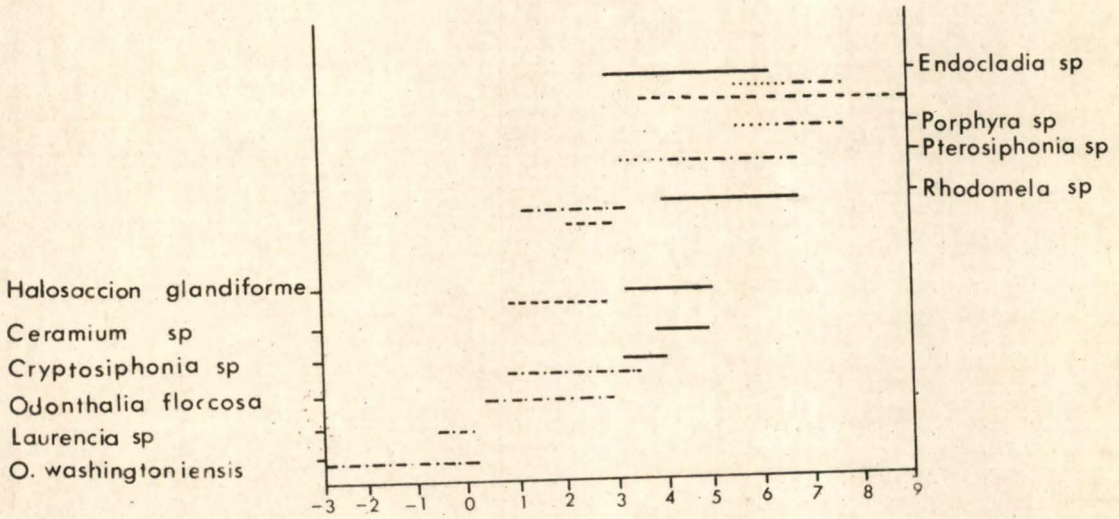


Fig. 1 Vertical distribution, on the three shore stations on Southern Vancouver Island, of the intertidal red algae selected for this programme (tidal pool species are excluded). __, Port Renfrew; __ . __, breakwater; _ _ _, Ashlee Bay; ....., estimated distribution.

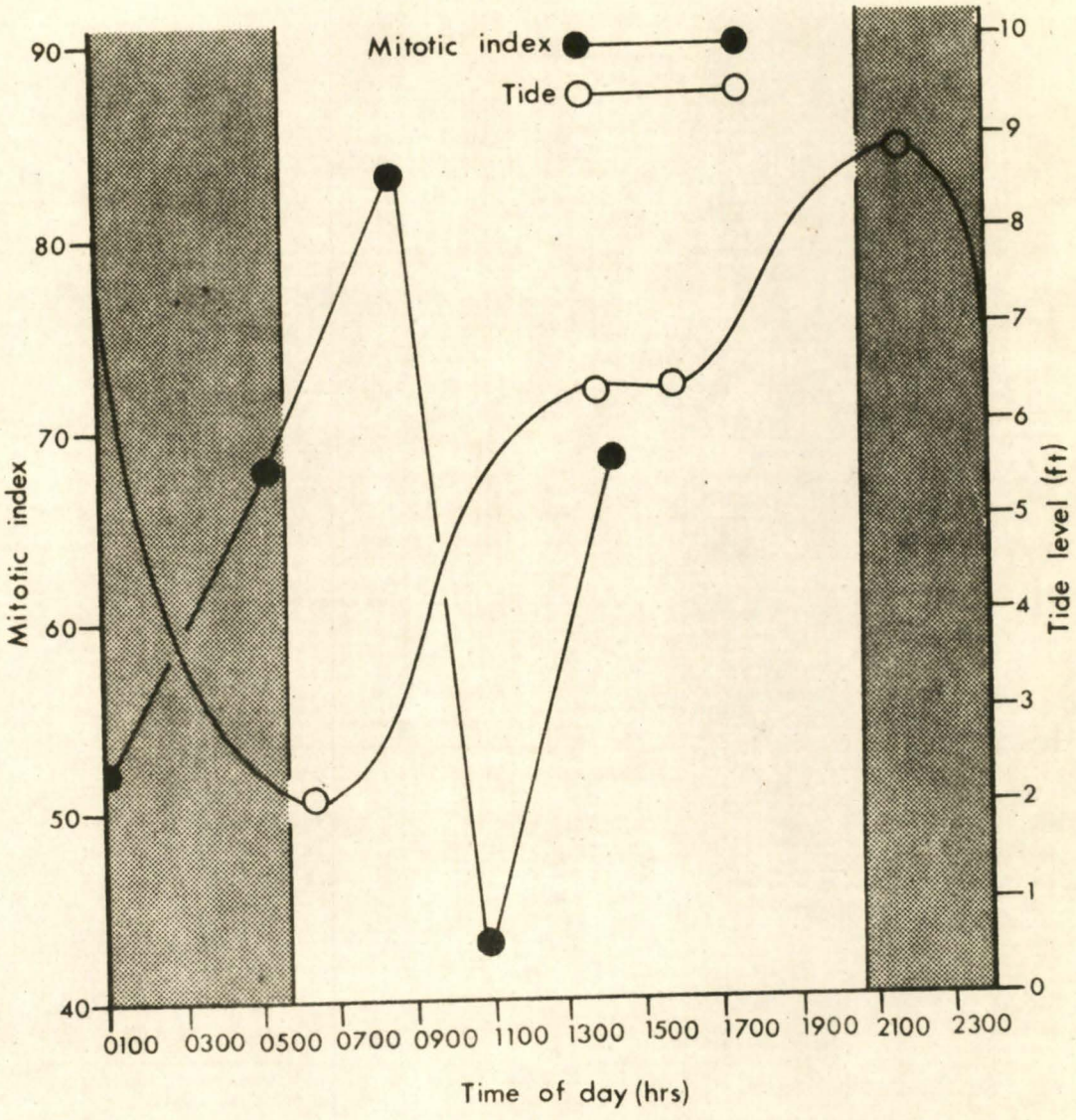


Fig. 2 Distribution of mitotic activity, tide levels and photoperiod throughout the 24 hour period sampled in July.

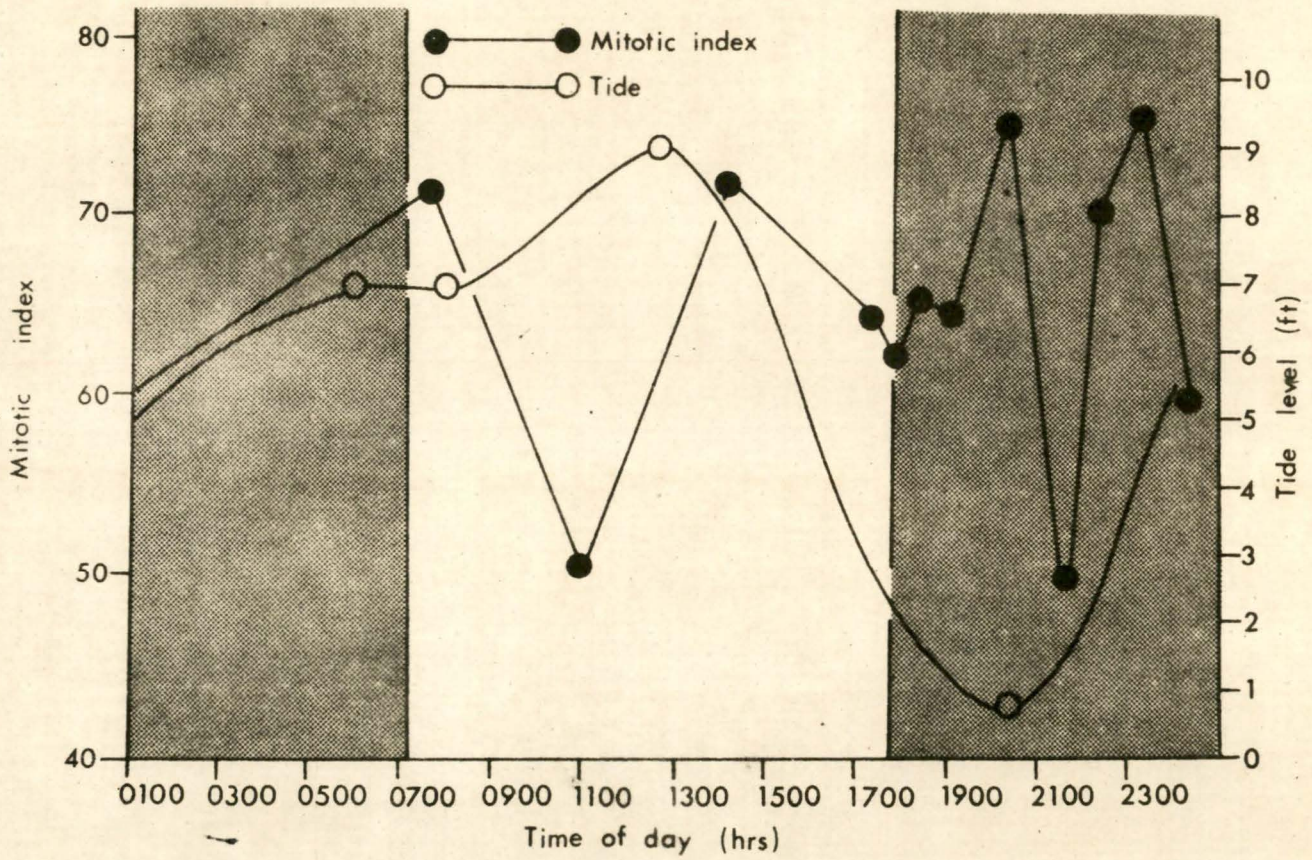


Fig. 3 Distribution of mitotic activity, tide levels and photoperiod throughout the 24 hour period sampled in February.

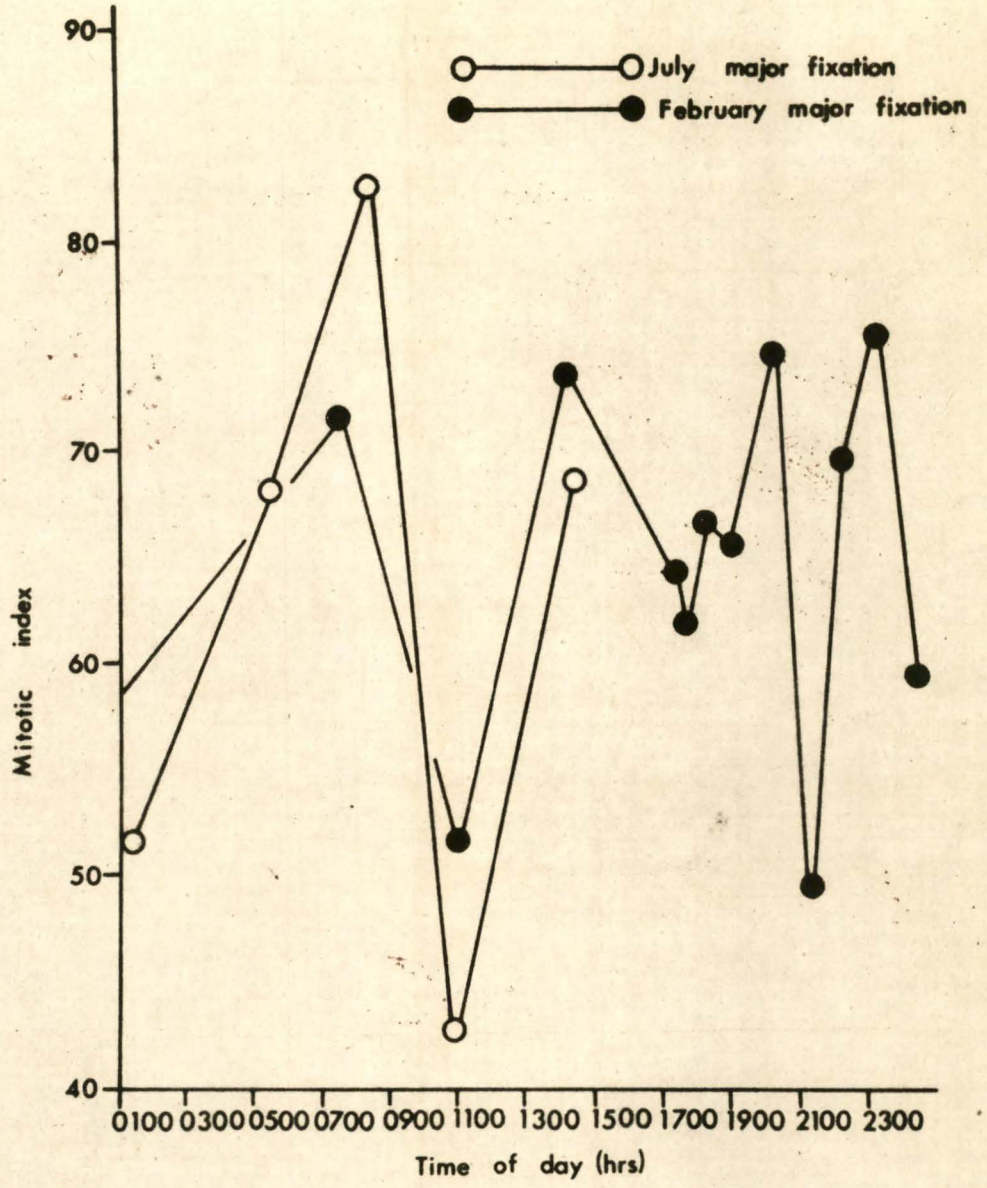
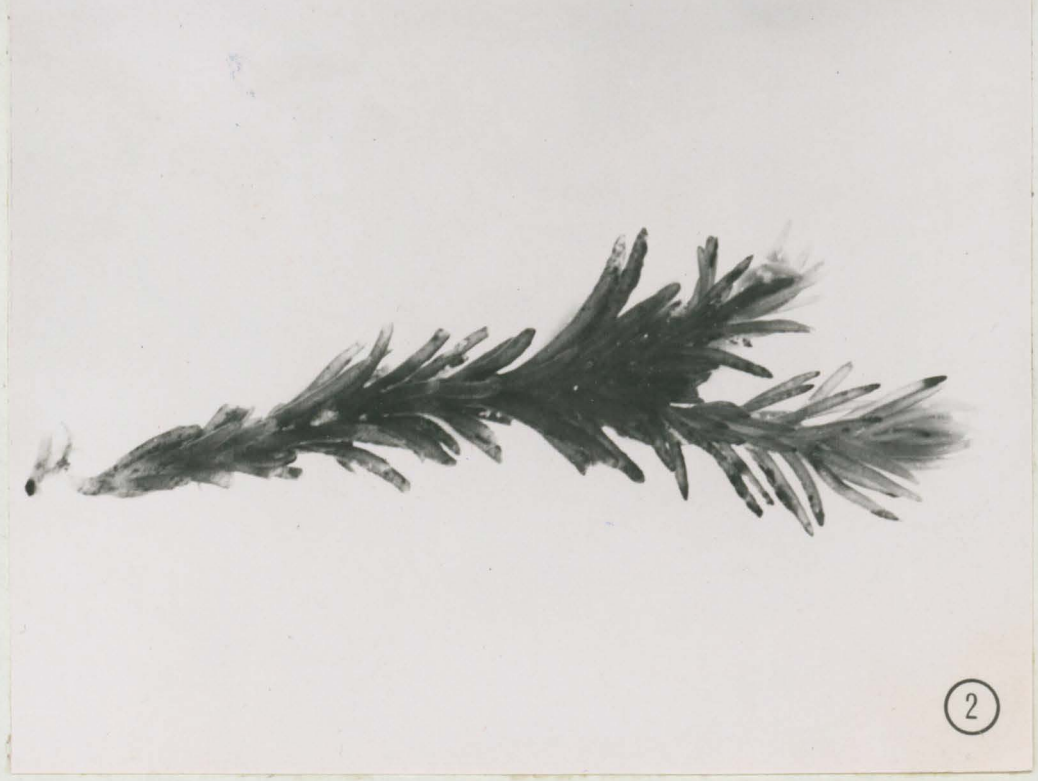


Fig. 4 Comparison of the distribution of mitotic indices for July and February.

PLATE I

Fig. 1 A group of Rhodomela larix (lower left of photo) marked by a peg which has two "spore collecting plates" attached. (X1/2)

Fig. 2 Plant of Rhodomela larix to show form of plant, mode of branching and different sized unbranched laterals or ramuli. At the apex these ramuli are clustered into a cup or cupule which protect the inner younger ramuli. Young ramuli are lighter in color and epiphytes upon some of them can be seen as dark patches.



(2)

PLATE II

Illustrations of typical mitotic figures in meristematic regions of *R. larix* employed in this quantitative work. Figures 4 and 5 are both late prophase and prometaphase (fig. 6) was incorporated in the plate but was not used for counting purposes. (X3000)

Fig. 1 interphase.

Fig. 2 early prophase.

Fig. 3 mid prophase.

Fig. 4 late prophase in a large cell.

Fig. 5 late prophase in a small cell.

Fig. 6 prometaphase.

Fig. 7 metaphase.

Fig. 8 early anaphase figure with an interphase nucleus close by.

Fig. 9 telophase figure with an interphase nucleus close by.

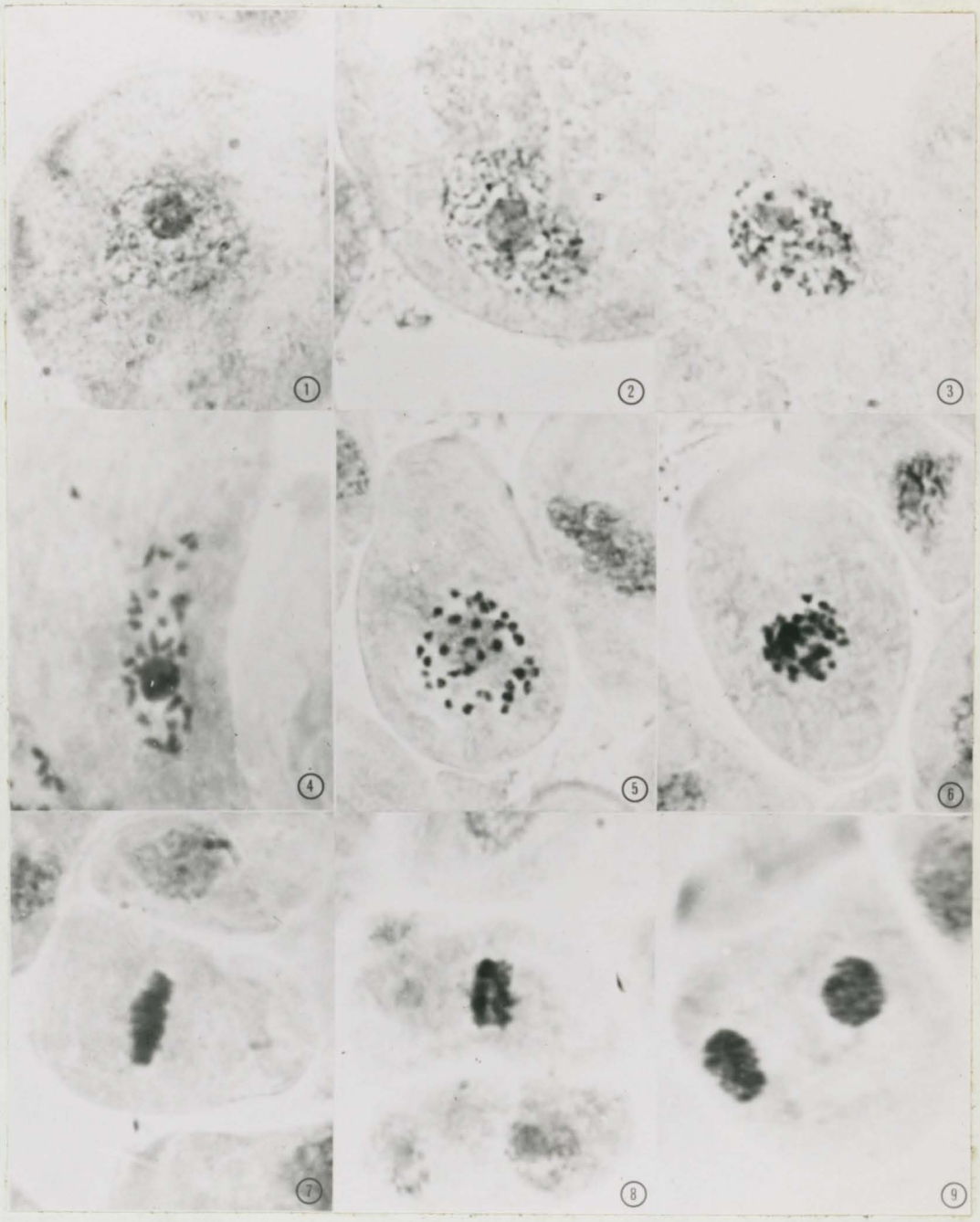

1
2
3
4
5
6
7
8
9

PLATE III

Fig. 1-4 Comparison of numbers of dividing nuclei seen in typical squashes made at periods of high and low mitotic activity in both summer and winter collection sequences. Arrows indicate dividing nuclei. (X500)

Fig. 1&2 are fields of summer active and inactive material respectively.

Fig. 3&4 are fields of winter active and inactive material respectively.

The high percentage of dividing nuclei in fig. 1 & 2 contrast with low percentage activity in fig. 2 & 4. The highest activity can be seen in winter active material whilst the summer active material has a lower proportion of dividing nuclei.

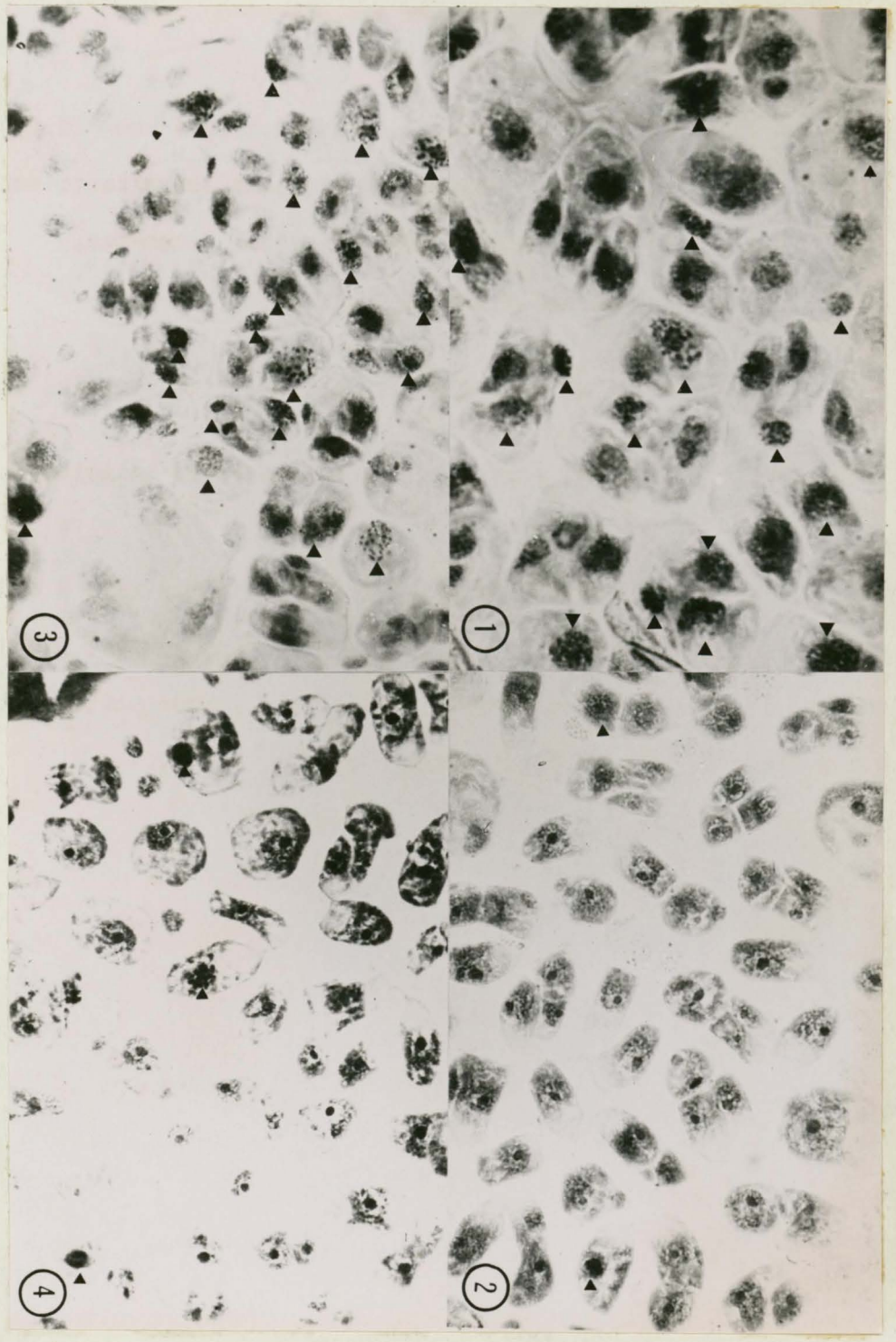
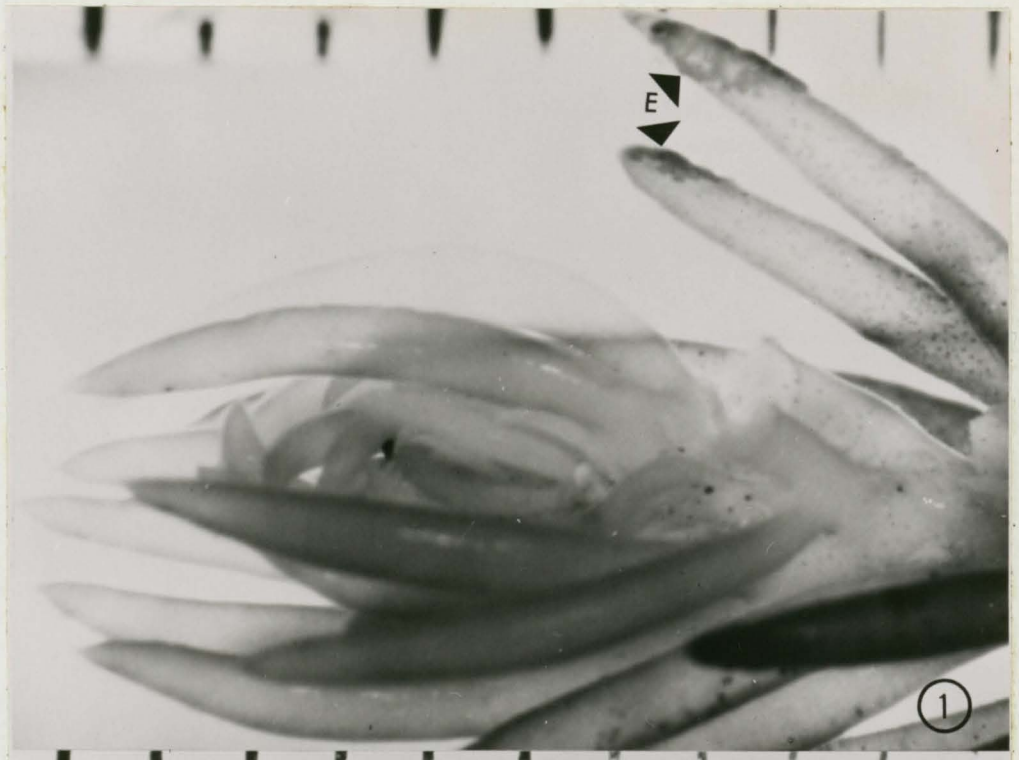
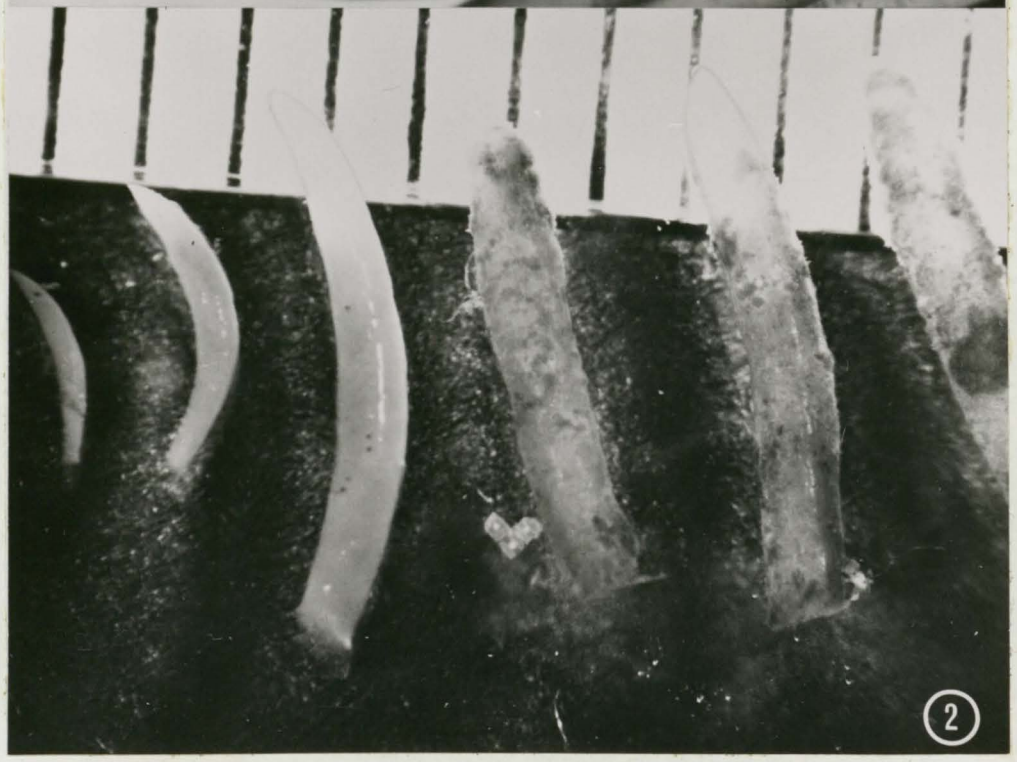

3
1
4
2

PLATE IV

Fig. 1 Close up of apical cluster or cupule which has had some of the outer ramuli removed in order to show inner smaller ramuli. Epiphytes in the older ramuli are clearly seen (E). The scale to the right is marked in mm.

Fig. 2 A series of ramuli of different lengths, the first two from the inner and the 3rd from the outer part of the apical cup whilst 3, 4 & 5 are from various positions below the apex, Epiphytes can be seen on the latter three, scale in mm.


E
1


2

PART II

MITOTIC PERIODICITY IN *PORPHYRA* (PSEUDO) *LANCEOLATA* COLLECTED BOTH *IN SITU* AND UNDER CERTAIN LABORATORY CONDITIONS

INTRODUCTION

The previous paper of this series dealt with aspects of the mitotic periodicity of *Rhodomela larix* (Turner) C. Agardh, a mid-intertidal red alga of Northeast Pacific shores. The plants used were collected from separate locations in the months of July and February, and were thus subject to quite different oceanographic and meterological conditions. A statistically significant periodicity in cell division was found in July and evidence was also given of a marked periodicity in February but there was little indication that tidal rhythm influenced the time of day at which cell division took place in these collections of *R. larix*.

Intertidal organisms exhibit various rhythms which have been related, directly or indirectly, to tidal regimes. Certain physiological and behavioural rhythms of marine invertebrates have been shown to be associated closely with tidal patterns, the latter sometimes functioning as the external synchronizer in cases of endogenous rhythms (Rao 1954; Bennett 1954; Fingerman 1955) and certain rhythms found in marine algae have been correlated to tidal regimes. Species of *Euglena* and diatoms exhibit an endogenous rhythm in their vertical migration through sediment surfaces (Round and Palmer 1966). A diurnal and lunar endogenous rhythm of oxygen consumption in a species of *Fucus* (Brown et al 1955), as well as the liberation of reproductive structures in various other intertidal seaweeds have been linked to tidal regimes, (Page and Kingsbury 1968; Vielhaben 1963; Smith 1947; Hoyt 1927).

Furthermore meiosis, in certain species of green algae, has been observed to be affected by environmental conditions connected, directly or indirectly, to tidal conditions. Friedman, (1963) correlated distribution of sexual plants of Prasiola stipitata with the periodicity of water cover the plants received, in that the latter governed the incidence of meiosis. In addition Thiadens and Zeuthen (1967) showed that in cultured plants of Ulva mutabilis ("slender"), cell division was transformed from presumptive mitosis to presumptive meiosis by changing the nutrient content of the culture media.

The pattern of daily mitotic indices observed by the present authors' in midshore R. larix prompted an investigation into the karyokinetic pattern of a supra-littoral species, Porphyra lanceolata (Setchell and Hus) G. M. Smith. Though the plant is economically important (Iwasaki 1965, Tseng and Chang 1955), very little detailed cytological work has been carried out on it or upon other members of the Bangiaceae, (Dixon 1966). The workers that have studied the cytology of Porphyra, have not carried out a critical study of the incidence of karyokinetic figures correlated with environmental factors either in the lab or in situ. Krishnamurthy (1959), fixed Porphyra umbilicalis at unstated intervals throughout the day, both in the laboratory and in the field, and found that karyokinesis takes place at any time of day and light was not required, for cell division figures were seen in material fixed in the laboratory at 2200 hours. Yabu and Tokida (1963), fixed Porphyra yezoensis at unstated times of day and night at two shore locations and also from vats of seawater in the laboratory. Without stating whether a

particular hour or tidal condition yielded more cell division figures they based further work on laboratory cultured specimens of P. yezoensis and P. onoi, thereby inferring that they found more nuclei in karyokinesis in plants kept under lab conditions than under in situ conditions. Other workers (Farmer and Williams 1898, and Rao 1956), indicate, but give no statistical evidence, that intertidal algae have a greater number of cells in karyokinesis during periods of high tide and state generally that tides and cell division are related phenomena. The present paper is a further study using methods outlined in paper I of this series (Austin & Pringle 1968) upon a plant which occurs at a very different intertidal location. A more complete review of the relevant literature is included in the previous paper.

MATERIALS AND METHODS

In the present work P. lanceolata, was fixed under both lab and field conditions. The plants employed occurred, between October and March, 7.5 feet above sea level in exposed and semi-exposed locations on the Ogden Point Breakwater in Victoria, B.C. where two major fixation series, (i.e. the overall twenty-four hour period in which plants are fixed), were carried out. A description of the field station is given in the previous paper of this series. Plants were fixed every two hours, (minor fixation time), for a twenty-four hour period on November 2-3, 1967 and January 12-13, 1968 and at these times certain environmental conditions were also recorded. In conjunction with the January major fixation series plants were placed under various culture conditions in the laboratory and fixations made in partial synchrony with field fixations.

The plants were cultured in a circulating saltwater system in 10 gallon plexiglass aquaria which were placed in a simple light-tight, environmental chamber constructed of plywood, 8' x 3' x 3' and divided into two compartments, each being supplied with water and light. The banks of lights were connected to timing mechanisms set to give one compartment constant illumination and the other an alternating photoperiod. The water flow was sufficient to effect circulation yet slow enough to maintain the plants in one location.

Plants were gathered for culture on January 11, 1968 from precisely the same community as those fixed in situ and transported

in buckets of seawater which kept them close to the sea temperature until placed in the culture chamber in the laboratory some 15 minutes later. In an attempt to explore which, if any, of the gross environmental parameters influenced mitotic incidence in P. lanceolata the plants were cultured under conditions of constant submersion with both constant and alternating photoperiod, and constant emersion with constant photoperiod. The constant illumination was initiated at 2000 hrs. January 11, but the plants exposed to the alternating light conditions did not receive light until 0930 hrs. January 12 and were given an 8/16 light/dark photoperiod. The plants under laboratory conditions were fixed every two hours for twenty-four hours beginning at 0830, January 12.

Simulation of low tide was effected by arranging plants upon an enamel pan inverted over the mouth of a glass aquarium filled with sea-temperature seawater. The surface of the pan was wetted and seawater was allowed to run constantly around the concave rim of the pan though not coming in direct contact with the plants. These measures were taken in order to keep the temperature of the emersed plants on the pan similar to that of the plants in the aquaria (i.e. at sea temperature) and also to maintain a high and constant humidity. This apparatus was set up in the chamber which was subjected to continuous light and at 1730 hrs., January 12, 35 plants were transferred from aquaria under such conditions and layed out flat on the surface of the enamel pan. These plants were fixed every hour from 1730 hrs. until 0530 hrs., January 13 and the relative humidity was recorded.

It was planned to collect and fix plants simultaneously *in situ* and from lab conditions but, due to extremely stormy seas, *in situ* collections were not initiated until 1830 hrs., January 12, ten hours behind the lab fixations but synchronous two hourly fixations were then carried out until 0600 hrs.

During the collections and fixations various environmental parameters were measured. Under the lab conditions light intensity and humidity were recorded only once as they were thought to be relatively constant. In the field, temperature and the presence or absence of water around the plants were recorded at every minor fixation period. Other environmental variables were measured at appropriated periods; light intensity during the day and humidity at times when the plants were emersed. Meterological information including amounts of precipitation and time of sunrise and sunset were obtained from published recordings (Anon 1967/68)

The equipment used for monitoring environmental conditions were as follows: light intensity was recorded using a Kahlsico photometer and readings were converted to foot candles; temperature was measured by a mercury hand thermometer; pH of water was obtained using a Rigosha colorimeter and relative humidity determined using a sling psychrometer manufactured by Weksler Instruments Corporation.

The procedures of field sampling throughout all tidal phases together with aspects of fixing and storing *P. lanceolata* were the same as that used and reported for *R. larix*, (Austin & Pringle 1968). Each plant sampled was sketched and measurements of width and length

were recorded (Table 1).

Quantitative data for mitotic activity was obtained by observing cells from rectangles of thallus 3/4 by 2 mm. removed, using iris scissors, from various locations on the plant. The occurrence of reproductive structures on plants collected in January made it necessary to remove material at a different location from those plants collected in November. Throughout any one major fixation period material was removed from about the same location on each plant sampled.

Workers attempting to gain quantitative data on mitosis in higher plants knew approximately the location of the meristematic tissue in their particular plants, (Lance 1952, Jensen and Kavaljian 1958). In an alga with the morphology of P. lanceolata (Fig. 1 and in Part III, fig. 45-47) which is assumed to have diffuse growth, it is difficult to assess where maximum cell division is taking place at any given time. In an attempt to ascertain where maximum cellular division occurred on November plants rectangles of cells were removed squashed and stained from various locations on plants from most minor fixation periods. This preliminary explorative sampling and squashing did not yield any cells that were in an obvious phase of karyokinesis, however some binucleate cells were seen as well as pairs of cells in close approximation to each other (Part III, fig. 5). The latter were similar to the ones Krishnamurthy (1959), illustrated as having just completed cell division in P. umbilicalis. The largest numbers of this type of cell were present mid-way along the peripheral portion

of the thallus. It was thus decided to sample this area in an attempt to gain data on mitotic periodicity in P. lanceolata. To do this rectangles of tissues were removed, squashed and stained from three plants per minor fixation time and each squash was quickly scanned for cells in stages of karyokinesis. Cell division was found to be actively taking place at certain times of day and this material was very carefully analysed for stages of karyokinesis.

The location and number of rectangles of tissue removed per plant, at the respective major fixation period are illustrated in Fig. 1 A-F. In November, four rectangles of material per plant were removed from three plants per minor fixation time (Fig. 1 A). Fig. 1 B shows where material was removed in an attempt to sample vegetative cell division in January field material. The number of plants and rectangles of thallus sampled were the same as in November, and in addition from the same major fixation period four rectangles were removed from the reproductive area (Fig. 1 D) of one plant per minor fixation period. From each laboratory experiment two plants per minor fixation period were sampled and rectangles of material were removed from the locations shown in Fig. 1 C, E and F. Seven rectangles per plant were removed from plants under conditions of constant light (Fig. 1 C & F) and five from those under the alternating photoperiod.

Material removed was stained following the procedure of Austin (1959), with some minor modifications. The latter together with detailed aspects of nuclear cytology and photomicrography will be reported elsewhere.

The squashed and stained preparations were sampled quantitatively using, in most cases, the method developed for sampling squashes of *R. larix*, (Austin & Pringle 1968). Five microscope (high power) fields per squash and 25 cells per field were examined, each cell being placed in one of the nine distinct phases of mitosis delimited for this work, (Part III, fig. 27-35).

Mitotic indices for each plant and each minor fixation time in the November material were determined by the method developed for *R. larix* (Austin & Pringle 1968) using three rectangles per plant. In the January field and culture material the mitotic index for rectangles numbered two, three and four of each plant were determined. The most peripheral rectangle removed from the thallus was discarded. These values were then totalled and averaged to give a mitotic index for each plant. The mitotic indices for the two plants sampled per minor fixation were then totalled and averaged to give a minor fixation period mitotic index. In tables 9, 11, 14 and 15 the plant mitotic index was derived by counting cells from rectangles two, three and four only. When necessary the quantitative data were treated statistically using an analysis of variance and then the treatment means were ranked and significance at the five percent level was demonstrated using Duncan's New Multiple Range Method, (Li, 1964).

OBSERVATIONS AND RESULTS

Structures which were probably reproductive in nature were found in a few plants in November (Part III of thesis) but the characteristic signs indicating the presence of reproductive structures were not readily obvious. However, in January some plants had an obvious creamy white edge (Part III, fig. 46) which consisted of packets of β spores. Other plants (Part III, fig. 47) did not contain these packets of spores nor possess the obvious white border, but did have larger reproductive structures which may be a stage of a spore formation (Conway 1964 and 1967, Edelstein and McLauchlin 1966). There are other obvious differences between the plants that give rise to β spores and those that give rise to α spores but details of this will be published elsewhere.

The literature available on the detailed process of mitosis in marine macroscopic red algae is very limited (Dixon 1966) and although various authors (Wolfe 1904, Mullahy 1952, Yabu and Kawamura 1959, Austin 1956 & 1960, and Magne 1964), have shown certain phases of mitosis in genera of red algae no author has systematically outlined and illustrated, using photomicrographs, all the stages of mitosis in any one species. A few workers (Kito 1966, Yabu and Tokida 1963, Krishnamurthy 1959, Ishikawa 1921), have worked on various species of Porphyra and, combining their interpretations of mitosis with that generally accepted for other organisms (Grundmann 1966), the present authors' delimited nine phases of mitosis for P. lanceolata and these are illustrated in Part III, fig. 27-35. A critical determination of

mitotic phases was carried out for both diploid and haploid cells. Cytological detail will be included in a later paper but of interest here is the extremely prolonged period between karyokinesis and cytokinesis which accounts for the very long, late-telophase stage (Fig. 2). In this paper for ease of discussion only, the three phases of prophase will be referred to as the "early-stage" of karyokinesis; prometaphase, metaphase, anaphase and early telophase will be referred to as the "mid-stage" and late telophase the "late-stage" of karyokinesis.

November Material

Mitotic indices, per minor fixation time, were calculated for the major fixation period of November and are shown in Table 2. Two major peaks in cell division were present; the first one at 1630 hrs. is made up of the "early- and mid-stages" of karyokinesis, the second at 0230 hrs. consisting mainly of the late telophase stage (Fig. 2).

The quantitative data were treated statistically and from Table 3 it can be seen that the minor fixation periods can be grouped into three classes of mitotic activity which are significant at the five percent level. The mitotic index of each plant, per minor fixation time, and the corresponding standard deviations are given in Table 4. The standard deviations are extremely low, thus there is very little difference in mitotic activity between plants at any one minor fixation time.

Of the 13 consecutive minor fixations made only two did not have plants with nuclei in late telophase, but the other phases of karyokinesis were found only at specific times of day (Table 2 and 4). Furthermore cells containing nuclei in prophase were not seen earlier in the day than 1630, (Fig. 2), and were found in more cells at this time than at any other during the major fixation period, 9.42%, the peak being statistically significant at the five percent level, (Table 5). The prophase stages rapidly decreased in frequency from 1630 hrs. to 2230 hrs. when only one nucleus was recorded in prophase; a "minor" peak of nuclei in prophase occurred at 0230. Thus, under the in situ environmental conditions of November 2-3 (Table 8) karyokinesis was initiated sometime between the hours of 1430 and 1630. The "mid-stage" of karyokinesis were initially seen at the same minor fixation time as the "early stage" (Table 2 and Fig. 2.) and the major peak in the former also occurred at 1630 hours and is statistically significant at the five percent level, (Table 6). The number of cells counted in "mid-stage", when combined are very similar to, but a little more than, the number found in the three stages of prophase and their minimum peaks occurred slightly later in the evening.

The late telophase stage of karyokinesis appeared to have the longest phase time of any of the karyokinetic stages (Fig. 2). The evidence for this is that more nuclei were found in this stage than any of the other stages of karyokinesis. The peak of nuclei in the late telophase stage is statistically significant at the five percent level (Table 7).

During the period of sequential fixations in this work certain environmental conditions were monitored, (Table 8, and fig. 8). The plants were emersed at the initial collection of 0830 hrs. and were submersed between 1030 and 1430 hrs. The tide then receded resulting in emersion again between 1630 and 0230 hrs. Between the minor fixation times of 0430 and 0830 hrs. the plants were not continually submersed but were occasionally covered by waves. The plants were subject to a ten hour light period during which the light intensity varied throughout. The temperature of the air was lower than the water thus at times of submersion the plants were subject to considerably higher temperatures. Relative humidity ranged from 100%, when the plants were submerged, to 64% at 2230; at 1630 it was 66%. The pH of the seawater was recorded only during times of submersion and did not vary to any extent.

January Material

The striking results obtained with the November material prompted another *in situ* major fixation period in January and in an attempt to discern which, if any, of the environmental factors initiated or synchronized cell division, the plants were placed under the laboratory conditions described earlier.

The plants collected in January were thought to be near the end of their growth period and possessed prolific reproductive structures but it was felt that vegetative cell division would very likely be taking place at some location on the plant. Plants that possessed β spores were chosen, as preliminary squashes revealed that

cell division could be found at any time of day on the extreme outer edge of the white coloured area, (Part IV, fig. 46), in all the plants sampled. Since quantitative data on mitosis in vegetative cells were desired rectangles of material were taken from just inside the peripherally located white strip (Fig. 1). Three plants per minor fixation time were sampled in this manner and no cells were observed with nuclei in a stage of karyokinesis. Consequently, sampling of the white reproductive tissue seemed necessary and consecutive pieces of material, 3/4 x 2 mm., were removed from the extreme edge of the strip inward toward the middle of the thallus to ascertain if a transitional or intercalary meristematic zone, made up of vegetative cells and sporangium mother cells, may be present. If the latter existed then any mitotic periodicity that might have been present in the vegetative cells could possibly be present here.

The four rectangles of material removed from the January (shore) material (Fig. I D), revealed a gradient in the number of cells per packet from approximately 130 in rectangle 1 (outermost) to single cells and packets of 2, 3, and 4 cells in rectangle 4 (innermost). As well a gradient in the mitotic indices was evident becoming more marked progressing from rectangles 2, 3, to 4 at most minor fixation times with the exception of both 1630 and 2030 hours. Rectangle 2 showed karyokinetic activity at all minor fixation times but one, whilst rectangle 3 had three nonactive periods and rectangle 4 had seven such periods. Two of the three minor fixation times where rectangle 4 revealed a high mitotic index were at 1630 hrs., 19.20% and 2030 hrs., 24.00% (Table 9). Under the environmental conditions prevalent during this January major fixation time a

periodicity in mitosis was present. Between the hours of 0030 and 1230 hrs. no cells were observed with a nuclei in the "early and mid-stage" of karyokinesis with the exception of one nucleus at 1230 (Table 9). Nuclei in the initial stages of karyokinesis were most abundant at 1430 and 1630 hrs., but none were observed at 1830 hrs. Minimum peaks in the "early- and mid-stages" of karyokinesis are present at 2030 hrs. and the late telophase stage showed a maxima at 2030 hrs. and a minima at 1630 hrs., (Fig. 3).

Sea conditions were extremely rough through most of the January major fixation period which increased the height of the tides, submerging the plants for longer periods than they would have been under less stormy conditions. Between the hours of 0230 and 1230 hrs., January 13, the plants were covered with at least one foot of water while at 1030 hrs. they were submerged under three feet of water, the maximum amount for the 24 hour period. The temperature varied between a low of 7.5°C at 1030 hrs. and a high of 9.0°C at the three minor fixation periods of 1630, 2030 and 2230 hrs. with the plants thus under warmer conditions at times of submersion (Table 10). The sun rose at 0803 hrs. and set at 1640 hrs. giving a light period of approximately 8.5 hours and the light intensity increased gradually throughout the morning reaching a peak of 1207 foot candles at 1230, dropping off rapidly in the afternoon. The pH of the seawater was steady throughout at 7.8 and the plants were subject to a relative humidity of 100% throughout the entire major fixation period, for it rained at times of emergence.

January Laboratory Material

Plants kept submersed under constant light.

Quantitative data on the mitotic activity of the plants kept under constant conditions of submersion, photoperiod, temperature and pH in the laboratory are presented in Table II. Figure 1 F illustrates where the seven rectangles of material were removed from each of the plants used. Cell division was not evident at any time of day or night in the rectangles removed from the center of the thallus but mitosis was evident in the rectangles removed from the peripheral edge or reproductive area. Cells were found in most stages of karyokinesis fromas early as 0630 hrs. through to 2030 hrs. but the maximal peaks in the "early- and mid-stages" of karyokinesis occurred at 1630 hrs. and are statistically significant at the five per-cent level, (Tables 12 and 13). A "minimum peak" in the "early- and mid-stages" of karyokinesis occurred at 0630 hrs.

Thus, under conditions of constant submersion and constant light, most stages of karyokinesis are seen in seven out of the 13 minor fixation periods, and "minimum peaks" of karyokinesis occurred at 1030, 1430 and 0630 hrs. with the "maximum peak" at 1630 hrs. (Fig. 4).

Plants kept emersed under constant light.

Quantitative data on mitosis for the plants kept in the laboratory under conditions of constant light and emergence between the hours of 1830 to 0530 are presented in Table 14. A few cells were

found in the "early and mid-stages" of karyokinesis at most minor fixation periods but small peaks in cell division occurred at 1930, 0130, 0430 and 0530 hrs. (Fig. 5). Cells with nuclei in the late telophase stage showed peaks at 0130 and 0230 hrs. A distinct gradient in mitotic activity occurred from the outer edge of the plants inward with a higher and steadier mitotic index evident on the outer edge as opposed to the inner material.

Plants kept submersed with an 8/16 light/dark photoperiod.

Quantitative data for those plants kept under laboratory conditions of constant submergence and alternating light are presented in Table 15. Cells with nuclei in various stages of karyokinesis are found at the majority of minor fixation times (Fig. 6), throughout the major fixation period. The pattern of "early- and mid-stages" of karyokinesis fluctuated very little and were seen in a few cells at most of the minor fixation times with the exception of 0230 and 0430 hrs.

The 8 hour photoperiod began at 0930 hrs. and ended at 1730 hrs. ; the temperature fluctuated between 8.7°C and 10.0°C; light intensity and pH were constant. Under these environmental conditions the only stage of karyokinesis that fluctuated was late telophase with a "maximum peak" at 2030 hrs. (Fig. 6).

DISCUSSION & CONCLUSION

Certain individual species of higher plants, under conditions of an alternating photoperiod, have peaks in cell division in both the light and dark period (Fig. 7 and Table 16), although the greater majority of unicellular algae investigated divide during the dark period.

Very little work has been carried out on mitotic periodicity in the macroscopic algae, but in the few species that have been observed a nocturnal peak in karyokinesis (Fig. 7 and Table 16) is common.

When a periodicity in mitosis exists the degree of regimentation of cell division may vary in different plants. In meristematic tissue of higher plants (Fig. 7) cells are typically found in various stages of mitosis at any given time of day or night (Hughes 1952 and Schrader 1953), but a peak in the number of nuclei in karyokinesis may be seen at a certain time throughout a 24 hour period (Kellicott 1904, Laughlin 1919, Speese 1939, Brown 1951, Lance 1952 and Jensen and Kavaljian 1958) which may be termed an asynchronous rhythm of cell division. Synchrony in cell division, which is the occurrence of every mitotically active nucleus in the same phase of mitosis at any one time (Hastings and Sweeney 1964), had not as yet been found in naturally occurring plant populations whether unicellular or multicellular. However, certain unicellular algae exhibit a synchrony in cell division when given the proper culture conditions (Tamiya 1966).

A type of karyokinetic regimentation similar to synchronous cell division is evident in a few unicellular algae where cell division takes place at a specific time every 24 hours in only a certain percentage of the mitotically active cells. Hastings and Sweeney (1964) have termed this type of periodicity "phased" cell division and it is the result of cells having a mitotic generation time longer than 24 hours.

In a naturally occurring population of P. lanceolata, under the environmental conditions of November 2-3 (Table 8), a statistically significant rhythm in karyokinesis was present. It was not the type of cell division rhythm found in higher plants, for cells in certain stages of karyokinesis were found only at a specific time of day, however it does follow the type of regimentation outlined for the marine dinoflagellate, Gonyaulax polyedra (Hastings and Sweeney 1964) which is the "phased" type of cell division rhythm. In P. lanceolata the peak in the "early- and mid-stages" of karyokinesis was found at 1630 hrs. with a mitotic index of 9.42%. The peak in the late telophase stage was seen ten hours later at 0230 hrs. and had a similar peak, 9.33%, to the early stage of karyokinesis. At the minor fixation time of 1430 hrs. there were no cells seen with nuclei in any of the stages of karyokinesis (Fig. 2) suggesting that, under the environmental conditions of this major fixation period, karyokinesis from early prophase to late telophase takes approximately 10 hours and the total time for karyokinesis and cytokinesis, approximately 20 hours. Late telophase had the longest phase time of the eight stages of karyokinesis. Temperature may have been the factor contributing to this

prolonged stage, for each stage of mitosis has its own temperature coefficient (Schrader 1953).

P. lanceolata, collected in situ both in November and January showed a periodicity in cell division with peaks at similar times of day, (Fig. 9 a & b). This striking similarity is remarkable since the major fixation periods were separated by two months, environmental conditions differed, and the plant material sampled was slightly dissimilar. The plants had grown an average of seven cm. between November and January (Table 1) and had developed reproductive structures from which the quantitative data for mitosis was acquired in the January material. In the latter specimens the gradation in mitotic periodicity is quite marked going from the edge of the thallus toward the centre (Table 9), from rectangle two to rectangle four, in the reproductive material (Fig. 1 D). Even though the mitotic index per plant (in the January material) was derived by combining the quantitative data from rectangles two, three and four, the initiation of karyokinesis occurred approximately at the same time of day as it did in November (Fig. 10). In the January in situ material karyokinesis was first observed at 1430 hrs. in rectangles two and three but in section four it occurred at 1630 hrs. which is the same time of day as karyokinesis was initiated in the November material. The occurrence of the "early-stage" of karyokinesis two hours earlier in rectangles two and three in January than in November material (Fig. 9 a & b) could be the result of the evident gradation of the mitotic periodicity from section two to four (Table 9). However there are other variables such as tidal pattern, photoperiod and light intensity, which must be considered before

even a tentative conclusion on the nature of the rhythm, based on this material, can be made.

The daily rhythm of the tides clearly bring about significant changes in the habitat of intertidal organisms and play a large role in the distribution of intertidal algae. Along most of the Pacific Coast of North America there is a semi-diurnal rhythm in tides which, for the majority of intertidal organisms, bring about marked changes in habitat four times in 24 hours. Farmer and Williams (1898) and Rao (1956), working on the cytology of species of marine algae felt tidal patterns and the incidence of karyokinesis were related. The former workers observed that karyokinesis could only be found in plants of Fucus just after the plants had been covered by the flood tide. The latter worker would only fix plants of Polyides caprinus when they were submerged, thus implying that karyokinetic figures were more abundant under these conditions. The present authors however showed that tide level appeared to have had very little affect on presence or absence of karyokinesis in R. larix (Austin & Pringle 1968).

P. lanceolata occurs in the supra-littoral where the plants would be emersed as often as they would be submersed when seas are not stormy. Thus it was felt likely that tides may play a role in influencing phenomena, such as mitosis, in this plant although Blinks and Givan (1961), did not find a photosynthetic rhythm in P. lanceolata collected in California. In the November material karyokinesis was first observed at 1630 hrs. and must have commenced some time between 1430 and 1630 hrs., however, the plants became emersed between these

hours as the tide had been receding since 1330 hrs. Furthermore, in the material collected *in situ* in January, karyokinesis was first observed in large numbers at 1430 hrs. and high tide for the day occurred at 1015 hrs. (Fig. 10). At 1430 hrs. in January the plants were covered with six inches of water, but under less stormy conditions they may have been emersed. Since there was a two hour difference in the peaks of karyokinesis for the two *in situ* major fixation periods and a three hour difference in the occurrence of high tide (Fig. 8 and 10) there is the possibility that the tides could be the external synchronizer for mitotic periodicity in *P. lanceolata*. If the periodicity in cell division in *P. lanceolata* is an endogenous one and the external synchronizer is the time of high tide or is exogenously controlled by water cover then the shift in the initiation of karyokinesis from 1630 hrs. in November to 1430 hrs. in January can be accounted for by the shift in tides.

Other important ecological factors such as photoperiod and light intensity varied significantly throughout the day during both major fixation times of November and January. The initiation of the light period, the end of the dark period or the length of the photoperiod could initiate, in the cell, processes which lead to karyokinesis a given number of hours later. This is probably not the case in *P. lanceolata*, for the sun rose one hour earlier in November than in January and the "early-stage" of karyokinesis was observed two hours later in November.

Light intensity varied considerably throughout the day for

each major fixation period, and there was also a marked difference in total light intensity between November and January (Tables 8 and 10). Leedale (1959), found that photoperiod controlled an exogenous rhythm in mitosis in species of Euglenophyta cultured under conditions of natural light (Fig. 7 and Table 16). He reports that karyokinesis would begin one to two hours after the initiation of the dark period if the plants had been subject to 12 hours of light but would not take place under conditions of constant light, constant dark or if the plants were subjected to light one hour after the onset of darkness. In a dinoflagellate, Gonyaulax polyedra, constant light of high intensity (900 f.c.) has been found to inhibit the endogenous rhythm in mitosis (Hastings and Sweeney 1964), although under constant light of low intensity (100 f.c.) the rhythm was maintained for 10 days or more. Furthermore, the endogenous rhythm of luminescence (Hastings and Sweeney 1964), and photosynthesis in Gonyaulax polyedra, (Hastings et al 1960) was also inhibited at high light intensities. Intensities over 600 f.c. inhibited the endogenous photosythetic rhythm in a species of diatom and dinoflagellate (Palmer 1964). Rayns and Godward (1965), found an asynchronous rhythm of cell division in cultures of Eudorina elegans which, when cultured under a light intensity of 200 to 300 f.c. in a 16 hour light and 8 hour dark photoperiod would begin division about two hours before the dark period.

Thus, the evident rhythm of mitosis in P. lanceolata could very well be controlled by the fluctuation or rhythm in light intensity which occurs daily under natural conditions. The beginning of the

light period might initiate a stage (s) of mitosis leading up to karyokinesis and the high light intensity of mid-day, could inhibit a stage preceding karyokinesis, thus the cells would not enter early prophase until the light intensity had decreased. This hypothesis could account for karyokinesis in January, beginning two hours ahead of karyokinesis in November, (Fig. 9 a and b) as the light intensity throughout the day in January was considerably less; the most significant difference in light intensity between the two days being at 1430 hrs. when in November it was 1371 f.c. and in January 430 f.c. (Tables 8 and 10). A somewhat similar explanation is provided for the control of cell division in Chlorella ellipsoidea, (Tamiya 1966). Japanese workers have found a method of synchronizing cultures of C. ellipsoidea, by observing that each cell goes through seven distinct morphological changes leading up to karyokinesis. Each stage requires either light or dark to complete its particular series of events and proceed to the next stage. Thus by correlating a stage of cytokinesis with the light-dark regime, synchrony in cell division can be effected.

Figure 11 compares the distribution of cell division found both in the field and submersed under conditions of constant and alternating light carried out over the 24 hour period in January. There was no significant occurrence of karyokinesis in the field material until 1430 hrs. but in both culture experiments karyokinesis was recorded at the first minor fixation period of 0830 hrs. Under the alternating photoperiod no peak in karyokinesis was evident and cell division occurred at every minor fixation period but two which were 0230 and 0430 hrs. However the plants cultured under constant light showed a statistically significant peak in karyokinesis at 1630 hrs., January 12

and the "early- and mid-stages" of karyokinesis were not evident again until 0630 hrs., January 13 (Fig. 11).

In an attempt to relate the results from the lab material with that found in situ a review of the events leading up to their being placed in the lab is required. The plants were collected on January 11 at 2000 hrs. while they were emersed, and transported to the laboratory where they were kept submersed under conditions of either darkness or constant light. After being placed in the aquaria the plants were not sampled until 0830 hrs., January 12, thus it is not known if karyokinesis appeared in the cells during the 12 hrs. between 2000 hrs., January 11 and 0830 hrs., January 13. Karyokinesis could have been initiated during this time by either of two events to which the plants were subject, previous to the initial fixation, i.e. the sudden submergence upon collection and the exposure to light in the culture chamber. It is unlikely that karyokinesis was initiated by submergence for the plants in the field did not show any evidence that submergence initiated the process, (Tables 2, 8, 9, and 10). Placing the plants under illumination three hours into the dark period may have initiated karyokinesis. If the latter occurred sometime between 2000 hrs., January 11 and 0830 hrs., January 12, in the plants under constant light, and the pattern of division resembled that found in those under the alternating photoperiod in the laboratory then the apparent statistically significant peak in cell division described for the plants under constant light conditions may not be a rhythm at all.

If, however, the pattern of karyokinesis was similar to that in plants under alternating light (at a low but constant level) then one would expect a somewhat similar pattern of karyokinesis to be found for plants under constant light, and this was not observed (Fig. 11). Karyokinesis was not recorded again until 14 hours later, at 0630 hrs. and following the above statement it is unlikely that the significant peak at 1630 hrs. would have appeared.

The pattern of karyokinesis evident under the alternating photoperiod is considerably different to that found *in situ* or under constant light in the laboratory (Fig. 11). A comparison of environmental conditions between the plants collected *in situ* and those in the laboratory under an eight hour light and 14 hour dark photoperiod points out that two major ecological conditions were significantly different, i.e. light intensity and depth of water coverage. The plants *in situ* had water around them most of the day but at times it was only a few inches deep whereas in the laboratory the plants were under about 18 inches of water constantly. It is unlikely that the amount of water coverage over the plants has a significant effect on karyokinesis from previous results shown. Light intensity may, as discussed previously, have a significant effect on mitosis. If the plants are subject to a dark period, then a high light intensity amy be required to phase karyokinesis, otherwise a low level of karyokinesis persists throughout the day, as was found in the laboratory under alternating light conditions.

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TABLE 1

Numbers of plants used per experiment and their average dimensions.

Month Plants Collected	Experiments in January	Number of Plants Measured	Average Plant Length for each Experiment	Average Plant Width for each Experiment
January	Experiment #1	24	19.52 cm	3.72 cm
	Experiment #2	36	10.5 cm	2.76 cm
	Experiment #3	11	13.10 cm	3.42 cm
	Experiment #4	24	11.71 cm	2.78 cm
	Experiment #5	24	19.73 cm	3.65 cm
January Total		119	74.56 cm	16.33 cm
January Average			14.91 cm	3.27 cm
November		39	7.23 cm	1.13 cm

TABLE 2

Numbers of nuclei invarious staqes of karyokinesis and mitotic index per minor fixation period in plants sampled in November. Stage index shown in italics.

TABLE 2

Minor Fixation Time	Number of Cells in Various Stages of Karyokinesis								Number of Active Cells Per Minor Fixation Time	Mitotic Index Per Minor Fixation Time
	Prophase			Prometaphase	Metaphase	Anaphase	Telophase			
	Early	Mid	Late				Early	Late		
0830	0	0	0	0	0	0	0	0	0	0.0
1030	0	0	0	0	0	0	0	3 *100%*	3	0.27%
1230	0	0	0	0	0	0	0	2 *100%*	2	0.18%
1430	0	0	0	0	0	0	0	0	0	0.0
1630	17 *16.04%*	7 *6.60%*	20 *18.87%*	23 *21.70%*	10 *9.43%*	7 *6.60%*	9 *8.49%*	13 *12.26%*	106	9.42%
1830	2 *3.03%*	2 *3.03%*	17 *25.76%*	8 *12.12%*	4 *6.06%*	4 *6.06%*	3 *4.55%*	26 *39.39%*	66	5.87%
2030	5 *6.17%*	2 *2.47%*	2 *2.47%*	9 *11.11%*	4 *4.94%*	2 *2.47%*	3 *3.70%*	54 *66.67%*	81	7.20%
2230	0	0	1 *1.41%*	1 *1.41%*	0	0	1 *1.41%*	68 *95.77%*	71	6.31%
0030	0	0	0	1 *1.67%*	0	0	1 *1.67%*	58 *96.67%*	60	5.38%
0230	10 *9.52%*	1 *0.95%*	1 *0.95%*	3 *2.86%*	1 *0.95%*	1 *0.95%*	0	88 *83.81%*	105	9.33%
0430	0	0	0	0	0	0	0	29 *100%*	29	2.58%
0630	0	0	0	0	0	1 *20.00%*	0	4 *80.00%*	5	0.35%
0830	0	0	0	0	0	0	0	2 *100%*	2	0.17%
Total	34	12	41	45	19	15	17	347	530	

TABLE 3

Minor Fixation Time	Mitotic Index Per Minor Fixation Hour	Significance Rating at 5% Level
0830	0.00	a
1430	0.00	a
0830	0.17	a
1230	0.18	a
1030	0.27	a
0630	0.35	a
0430	2.58	a b
0030	5.38	b c
1830	5.87	b c
2230	6.31	b c
2030	7.20	b c
0230	9.33	c
1630	9.42	c *

November *in situ* fixation data, showing the mean values for three plants per minor fixation time and its rank in relation to other fixation times. Statistical significance between means at the 5% level is shown.

* those followed by the same letter are not significantly different at the 5% level.

TABLE 4

November *in situ* fixation data showing the time of day the plants were fixed, the mitotic index mean for each plant and the standard deviation for each fixation time.

TABLE 4

Minor Fixation Time		Mitotic Index Per Plant	Standard Deviation
	A	0.00	
0830	B	0.00	0.00
	C	0.00	
	A	0.27%	
1030	B	0.53%	approximately 0
	C	0.00	
	A	0.53%	
1230	B	0.00	approximately 0
	C	0.00	
	A	0.00	
1430	B	0.00	0.00
	C	0.00	
	A	12.53%	
1630	B	10.13%	3.51
	C	5.60%	
	A	4.80%	
1830	B	8.53%	2.32
	C	4.27%	
	A	2.13%	
2030	B	11.46%	4.71
	C	8.00%	
	A	6.13%	
2230	B	5.33%	1.08
	C	7.47%	
	A	5.60%	
0030	B	1.87%	3.40
	C	8.67%	
	A	6.40%	
0230	B	16.27%	6.03
	C	5.33%	
	A	5.33%	
0430	B	0.55%	2.48
	C	1.87%	
	A	0.53%	
0630	B	0.53%	approximately 0
	C	0.00	
	A	0.00	
0830	B	0.00	approximately 0
	C	0.53%	

TABLE 5

Minor Fixation	Mean Number of Cells in Prophase per Fixation Time	Significance Rating at 5% Level
0830	0	a
1030	0	a
1230	0	a
1430	0	a
0030	0	a
0430	0	a
0630	0	a
0830	0	a
2230	0.33	a
2030	3.00	a b
0230	4.00	a b
1830	7.00	b
1630	14.67	c *

Statistical data for the "early-stage" of karyokinesis in the November *in situ* field material.

* those followed by the same letter are not significantly different at the 5% level.

TABLE 6

Minor Fixation Time	Mean Number of Cells in the "mid-stage" of Karyokinesis per Fixation Time	Significance Rating at 5% Level
0830	0	a
1030	0	a
1230	0	a
1430	0	a
0430	0	a
0830	0	a
0630	0.33	a
2230	0.66	a
0030	0.66	a
0230	1.66	a
2030	6.00	b
1830	6.33	b
1630	16.33	c *

Statistical data for the "mid-stage" of karyokinesis in the November in situ field material.

* significant difference (5% level) at termination of each series of small letters.

TABLE 7

Minor Fixation Time	Mean Number of Cells in the late Telophase Stage per Minor Fixation Time	Significance Rating at 5% Level
0830	0	a
1430	0	a
1230	0.666	a
0830	0.666	a
1030	1.000	a
0630	1.330	a
1630	4.330	a b
1830	8.660	a b c
0430	9.660	a b c
2030	18.000	b c d
0030	19.330	b c d
2230	22.60	c d
0230	29.330	d *

Statistical data for the "late telophase stage" of karyokinesis in the November in situ field material.

* those followed by the same letter are not significantly different at the 5% level.

TABLE 8

Time of Day	Plant Submerged or Emerged	Temp. of Plant Environment	Light Intensity Sea Cell	Relative Humidity	pH	Photo Period Rise	Photo Period Set
0830	emerged	7.5°C	7.06 f.c.	75%		7:02	4:52
1030	submerged	9.5°C	30.59 f.c.	100%	7.8		
1230	submerged	9.5°C	2548.24 f.c.	100%	7.8		
1430	submerged	9.0°C	1371.76 f.c.	100%	7.7		
1630	emerged	7.0°C	not sufficient light	66%			
1830	emerged	6.5°C	dark	71%			
2030	emerged	6.0°C	dark	65%			
2230	emerged	6.5°C	dark	64%			
0030	emerged	5.0°C	dark	74%			
0230	emerged	5.0°C	dark	71%			
0430	occasional wetting	7.0°C	dark	100%			
0630	occasional wetting	8.5°C	not sufficient light	100%			
0830	occasional wetting	8.5°C	no reading taken	100%			

Environmental parameters measured during the November *in situ* major fixation period.

TABLE 9

Time of Day	Number of Cells in Karyokinesis								Mitotic Index of Sections			Plant Mitotic Index
	Prophase			Prometa-Phase	Meta-Phase	Ana-Phase	Early-Telophase	Late-Telophase	Section 2	Section 3	Section 4	
	Early	Mid	Late									
1830	0	0	0	0	0	1	0	2	7.02%	1.60%	0.00	2.87%
2030	0	3	5	2	1	7	11	60	20.80%	26.40%	24.00%	23.73%
2230	0	0	0	1	0	4	0	29	14.28%	14.40%	2.40%	10.36%
0030	0	0	0	0	0	0	0	4	5.50%	0.80%	0.00	2.10%
0230	0	0	0	0	0	0	0	3	5.30%	0.00	0.00	1.77%
0430	0	0	0	0	0	0	0	4	3.20%	0.00	0.00	1.07%
0830	0	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00
1230	0	0	1	0	0	0	0	2	4.70%	2.40%	0.00	2.37%
1430	7	5	4	2	6	3	0	0	16.00%	5.60%	0.00	7.20%
1630	2	2	9	3	6	3	2	33	17.50%	33.33%	19.20%	23.08%

Numbers of nuclei in various stages of karyokinesis for three rectangles taken from the thallus per minor fixation time in January field material. Mitotic index per plant shown in final column, the plants all bore spores.

TABLE 10

me of Day	Plant Submerged or Emerged	Temp. of Plant Environment	Light Intensity Sea Cell	Relative Humidity	Ppt.	pH	Photo Pe Rise
0830	submerged (2.5 feet of water)	7.5°C	no reading taken	100%		7.8	8:03
1030	submerged (3 feet of water)	8.0°C	1065.88 f.c.	100%		7.8	
1230	submerged (2 feet of water)	7.75°C	1207.06 f.c.	100%		7.8	
1430	submerged (6 inches of water)	8.25°C	430.59 f.c.	100%		7.8	
1630	occasional wetting	9.0°C	dark	100%			
1830	occasional wetting	8.5°C	dark	100% (rain)			
2030	emerged	9.0°C	dark	100% (rain)	0.26"		
2230	emerged	9.0°C	dark	100% (rain)			
0030	occasional wetting	8.0°C	dark	100%			
0230	submerged (1 foot of water)	8.0°C	dark	100%			
0430	submerged (2 feet of water)	8.0°C	dark	100%			

Ppt. for January 12th - 0.26"

Ppt. for January 13th - 0.15"

Environmental parameter measured during the January *in situ* major fixation period.

TABLE 11

Numbers of nuclei in various stages of karyokinesis for three rectangles taken from the thalli of two plants per minor fixation time in January lab material submersed under constant light.

TABLE 11

Time of Day	Number of Cells in Karyokinesis: Prophase Early	Prophase Mid	Prophase Late	Prometa-Phase	Meta-Phase	Ana-Phase	Early-Telophase	Late-Telophase	Plant A or B	Mitotic Index of Sections: Section 2	Section 3	Section 4	Plant Mitotic Index	Minor Fixation Mitotic Index	Sex of Plant
0830	0	0	1	0	1	3	2	20	A	12.96%	4.00%	0.00	5.65%	4.24%	β
									B	2.50%	2.78%	3.20%	2.83%		β
1030	3	1	3	0	1	2	3	21	A	8.80%	6.40%	3.20%	6.13%	4.53%	β
									B	8.80%	0.00	0.00	2.93%		β
1230	0	0	3	2	2	1	0	19	A	17.58%	0.80%	0.00	6.13%	5.30%	β
									B	10.99%	0.80%	1.60%	4.46%		β
1430	0	1	6	1	2	2	0	11	A	25.68%	3.20%	0.00	9.63%	9.63%	β
									B	0.00	0.00	0.00	0.00		α
1630	4	2	19	9	4	5	4	30	A	14.40%	15.20%	0.00	9.87%	8.54%	—
									B	8.80%	10.40%	2.40%	7.20%		β
1830	0	0	0	0	0	0	1	4	A	2.40%	0.80%	0.00	1.08%	0.76%	—
									B	0.00	1.35%	0.00	0.45%		—
2030	0	0	1	0	1	1	1	31	A	37.50%	19.78%	15.74%	24.34%	24.34%	β
									B	0.00	0.00	0.00	0.00		—
2230	0	0	0	3	0	0	0	2	A	2.70%	0.00	0.00	0.90%	0.90%	β
									B	0.00	2.40%	0.00	0.80%		α
0030	0	0	0	0	0	0	0	0	A	0.00	0.00	0.00	0.00	0.00	β
									B	0.00	0.00	0.00	0.00		α
0230	0	0	0	0	0	0	0	16	A	0.00	14.86	4.00%	6.29%	6.29%	β
									B	0.00	0.00	0.00	0.00		α
0430	0	0	0	0	0	0	1	15	A	27.50%	12.16%	4.80%	14.82%	8.16%	β
									B	3.70%	0.80%	0.00	1.50%		β
0630	1	2	2	2	3	4	2	44	A	4.05%	15.00%	10.00%	9.68%	14.67%	α
									B	23.20%	13.60%	7.20%	14.67%		β
0830	0	0	0	0	1	2	0	4	A	6.48%	0.00	0.00	2.16%	2.16%	
									B	0.00	0.00	0.00	0.00		

TABLE 12

Minor Fixation Time	Mean Number of Cells in Prophase per Fixation Time	Significance Rating at 5% Level
1830	0	a
2230	0	a
0030	0	a
0230	0	a
0430	0	a
0830	0	a
0830	0.50	a
2030	0.50	a
1230	1.5	a
0630	2.5	a
1030	3.5	a
1430	3.5	a
1630	12.5	b *

Statistical data for the "early-stage" of karyokinesis in laboratory plants kept submersed under constant light.

* significant difference (5% level) at termination of each series of small letters.

TABLE 13

Minor Fixation Time	Mean Number of Cells in the "mid-stage" of Karyokinesis per Fixation Time	Significance Rating at 5% Level
0030	0	a
0230	0	a
0430	0.4	a
1830	1	a
2030	1.5	a
2230	1.5	a
0830	1.5	a
1230	2.5	a
1430	2.5	a
0830	3	a
1030	3	a
0630	5.5	a
1630	11	b *

Statistical data for the "mid-stage" of karyokinesis in laboratory plants kept submersed under constant light.

* significant difference (5% level) at termination of each series of small letters.

TABLE 14

Time of Day	Number of Cells in Karyokinesis: Prophase Early	Prophase Mid	Prophase Late	Prometa-Phase	Meta-Phase	Ana-Phase	Early-Telophase	Late-Telophase	Plant A or B	Mitotic Index of Rectangles: Rectangle	Rectangle	Rectangle	Plant Mitotic Index	Minor Fixation Mitotic Index	Sex of Plant
1830	0	0	0	0	0	0	0	31	A	0.00	10.19%	7.20%	5.80%	4.50%	β
									B	9.60%	0.00	0.00	3.20%		—
1930	0	0	2	2	4	0	1	20	A	25.27%	7.20%	0.80%	11.09%	11.09%	β
									B	0.00	0.00	0.00	0.00		—
2030	0	0	0	0	0	0	0	4	A	0.00	0.00	0.00	0.00	1.06%	β
									B	0.00	1.60%	1.60%	1.06%		—
2130	0	0	2	0	0	0	0	21	A	35.00%	6.48%	0.00	13.83%	10.91%	β
									B	17.58%	5.60%	0.80%	7.99%		β
2230	0	0	0	0	0	0	0	0	A	0.00	0.00	0.00	0.00	0.00	—
									B	0.00	0.00	0.00	0.00		—
2330	0	0	0	0	0	0	0	14	A	10.19%	4.05%	0.00	4.75%	4.75%	β
									B	0.00	0.00	0.00	0.00		—
0030	0	0	0	0	0	0	0	4	A	17.50%	0.80%	0.00	6.10%	6.10%	β
									B	0.00	0.00	2.40%	0.80%		—
0130	0	0	1	1	0	2	0	43	A	20.00%	5.26%	9.60%	11.62%	10.08%	β
									B	0.00	20.80%	4.80%	8.53%		β
0230	0	0	0	0	0	1	0	48	A	10.40%	10.40%	4.80%	8.53%	6.53%	β
									B	6.40%	7.20%	0.00	4.53%		β
0330	0	0	0	0	0	0	0	37	A	6.76%	12.04%	20.00%	12.93%	12.93%	β
									B	0.00	0.00	0.00	0.00		—
0430	2	2	2	2	6	2	0	3	A	0.00	0.00	0.00	0.00	4.80%	—
									B	4.80%	4.00%	5.60%	4.80%		—
0530	0	0	2	1	1	1	0	24	A	10.99%	4.80%	2.40%	6.06%	4.82%	β
									B	3.51%	4.00%	3.20%	3.57%		β

Numbers of nuclei in various stages of karyokinesis for three rectangles taken from the thalli of two plants per minor fixation time in January lab material emersed under constant light.

TABLE 15

Time of Day	Number of Cells in Karyokinesis: Prophase Early	Prophase Mid	Prophase Late	Prometa-Phase	Meta-Phase	Ana-Phase	Early-Telophase	Late-Telophase	Plant A or B	Mitotic Index of Sections: Section 2	Section 3	Section 4	Plant Mitotic Index	Sex of Plant
0830	0	0	3	0	5	12	4	3	A	0.00	20.80%	0.80%	7.20%	β
									B	0.00	0.00	0.00	0.00	α
1230	0	0	2	1	6	3	1	9	A	15.74%	0.00	0.00	5.25%	β
									B	0.00	0.00	4.00%	1.33%	α
1430	0	1	6	5	8	2	1	2	A	4.80%	6.40%	7.20%	6.13%	α
									B	1.60%	0.00	0.00	0.53%	α
1630	0	1	4	0	6	10	2	7	A	1.85%	3.20%	0.00	1.68%	β
									B	12.16%	12.00%	1.85%	8.67%	β
1830	0	0	3	0	8	4	8	44	A	14.04%	22.40%	15.20%	17.21%	β
									B	3.20%	2.40%	4.00%	3.20%	—
2030	0	0	3	2	3	7	0	52	A	24.32%	13.60%	7.20%	15.04%	β
									B	21.30%	0.00	0.00	7.10%	β
2230	0	2	8	4	11	8	0	11	A	0.00	5.60%	15.20%	6.93%	α
									B	10.19%	4.00%	1.60%	5.26%	β
0030	1	2	1	0	1	0	1	99	A	23.20%	20.00%	7.20%	16.80%	β
									B	0.00	27.78%	9.60%	12.46%	β
0230	0	0	0	0	1	0	0	14	A	8.80%	1.60%	1.60%	4.00%	β
									B	0.00	0.00	0.00	0.00	α
0430	0	0	0	0	0	6	0	17	A	1.35%	11.11%	2.40%	4.95%	β
									B	14.04%	4.00%	0.00	6.01%	β
0630	1	1	2	2	0	0	0	2	A	0.00	0.00	0.00	0.00	β
									B	6.48%	0.80%	0.00	2.43%	β
0830	0	0	0	0	3	3	1	2	A	35.00%	15.79%	6.59%	19.13%	β
									B	0.00	0.00	0.00	0.00	α

Numbers of nuclei in various stages of karyokinesis for three rectangles taken from the thalli of two plants per minor fixation time in January lab material submersed under

TABLE 16

Listing of previous workers and organisms investigated with regard to mitotic periodicity. This information is illustrated in figure 7.

Table 16

No.	Author	Species and Details of Mitotic Periodicity
1	Laughlin, Harry H., 1919	Roots of *Alluim cepa*. Peak of cells in various stages of mitosis at approximately noon at 30° C.
2	Jensen & Kavaljian, 1958	Roots of *Alluim cepa*. Two peaks of miotic activity: 1. 12:00 a.m. 2. 12:00 p.m.
3	Brown, 1951	Roots of pea seedlings. Photoperiod from 10:00 a.m. to 10:00 p.m. and peak of cell division occurred from 12:00 a.m. to 4:00 a.m.
4	Kellicott, 1904	Roots of *Allium*. Two maximum periods of cell division: 1. Primary at 12:00 a.m. 2. Secondary at 1:00 p.m.
5	Lance, 1952	Shoot apices of *Vicia faba*. Two periods of maximum mitotic activity: 1. 9:00 a.m. 2. 9:00 p.m.
6	Speese, 1939	Leaves of *Smilax*. Two periods of maximum mitotic activity: 1. Shortly after 12:00 a.m. 2. Shortly after 12:00 p.m.
7	Sweeney & Hastings, 1958	Populations of the dinoflagellate *Gonyaulax polyedra*. A circadian rhythm in cell division which takes place one hour before the beginning of the light period under natural conditions.
8-10	Sweeney, 1959	#8 *Gonyaulax sphaeriodea* - end of dark period #9 *Prorcentrum micans* -during light period #10 *Gymnodinium spiendens* - end of light period Photoperiod: 12 hours of light and 12 hours of dark.
11	Braarud & Pappas, 1951	Dinoflagellate *Peridinium triquetrum*. Mitotic activity during the dark period.
12	Jörgenson, 1911	Dinoflagellate *Ceratium* spp. Mitotic activity during the dark period.
13	Gough, 1905	A dinoflagellate species. Mitotic activity during the dark period.
14	Soroken, 1959	Cultures of *Chlorella pyrenoidosa*. Photoperiod of 9 hours light and 15 hours dark. Mitotic activity during the dark period.
15	Braun, 1851	Cell division takes place to a greater extent in *Spirogyra* sp. during the dark period.
16	Famintzin, 1867	Cell division in *Spirogyra* takes place at night.

17	Sachs, 1874	Nuclei of *Spirogyra, Vaucheria, Hydrodictyon* and *Ulothrix* divide during the dark period.
18	Strasburger, 1880	Cell division in *Spirogyra* takes place during the night.
19	Kurssanow, 1912	Cell division takes place during the dark period in *Zygnema* with a greater number of mitotic divisions occurring between 9:00 p.m. and midnight.
20	Gojdics, 1934	Cell division in *Euglena deses* takes place during the night. Cultured in 0.1% beef extract.
21	Leedale, 1959	*Hydrodictyon* sp., *Ulothrix* sp., *Mougeotia* sp., *Spirogyra* sp., *Zygnema* sp., *Closterium* sp., *Cosmarium* sp. and *Staurastrum* sp. have a periodicity in mitosis where nuclear division takes place almost entirely at night. The rhythm is exogenous. Some species of *Spirogyra* and *Zygnema* undergo mitosis when cultured under continuous light.
22	Leedale, 1959	16 species of Euglenineae were found to undergo cell division during the dark period. In all species the rhythm was exogenous. Mitosis began one to two hours after the onset of darkness.
23	Dangeard, 1902	In *Euglena* sp., *Phacus* sp. and *Trachelomonas* sp. cell division occurs during the dark period.
24	Baker, 1926	In *Euglena gracilis* cell division takes place during the dark.
25	Ratcliffe, 1927	In *Euglena spirogyra* cell division takes place during the dark, when cultured in modified Doflein's medium.
26	Hall, 1931	In *Euglena leuclops* cell division takes place during the dark period when a parasite in *Stenostomum*.
27	Johnson, 1934	In *Colacium vesiculosum* Ehrbg. Cell division takes place during the dark.
28	Chu, 1946	In *Euglena* sp. cell division takes place during the dark.
29	Rayns and Godward, 1965	*Eudorina elegans* cultured under conditions of 16 hours light and 8 hours of dark showed a primary peak in cell division two hours before the dark period and subsidiary peaks occurred to a greater extent during the dark period.
30	Iyengar & Balakrishnan, 1949	*Polysiphonia platycarpa* was fixed, both in the lab and in the field at twenty-four hour intervals; more nuclear division figures were observed in material fixed between 9:00 p.m. and 1:00 a.m.

31	Mullahy, 1952	*Lemanea australis* fixed over a period of a week may yield no division figures. The author states that cell division occurs mostly at night and sexual shoots fixed at 2:00 a.m. show the greatest number of mitotic figures.
32	Rao, 1956	*Polyides caprinus* fixed, when submerged, in both the lab and in the field yielded a greater abundance of mitotic division figures *in situ* between 12:30 p.m. and 2:30 p.m.
33	Yabu, 1964	The author implies that more karyokinetic figures are found at midnight in *Agarum cribrosum* Bory and *Alaria praelonga* Kjellman.
34	Yabu & Tokida, 1963	The authors imply that more mitotic division figures are found at midnight in *Arthrothamnus bifidus*.
35	Yabu & Kawamura, 1959	*Polysiphonia japonica* was fixed between 7:00 p.m. and 4:00 a.m.; active dividing nuclei were found between 9:00 p.m. and 3:00 a.m. with a peak in cell division between 11:00 p.m. and 2:00 a.m.
36	Yabu & Kawamura, 1959	The authors feel that karyokinesis peaks in the middle of the night in *Chondria crassicaulis*.
37	Løvlie, 1964	Cultures of a mutant strain of *Ulva mutabilis* divides synchronously during the dark period.
38	Krishnamurthy, V., 1959	*Porphyra umbilicalis* (L.) Kutz. var. *laciniata* (Lightf.) J. Ag. divides at any time during the day but light is not required as division digures were seen in material fixed in the lab at 10:00 p.m.
39	Wolfe, 1904	*Nemalion* when cultured in running seawater were observed to have a greater number of cells in karyokinesis during the night.

Fig. 1 Diagrams, to scale, of *P. lanceolata* plants to show positions from which rectangles (measuring 3/4 x 2 mm. in all cases) were removed for squashing and subsequent assessment of mitotic activity.

A. November field material; rectangles removed from periphery of lower half of thallus.

B-F. Plants collected in January under field and laboratory conditions. Rectangles removed in sequence from extreme periphery (plants C-F), are referred to in text, as 1, 2, 3, 4, numbering from periphery inward.

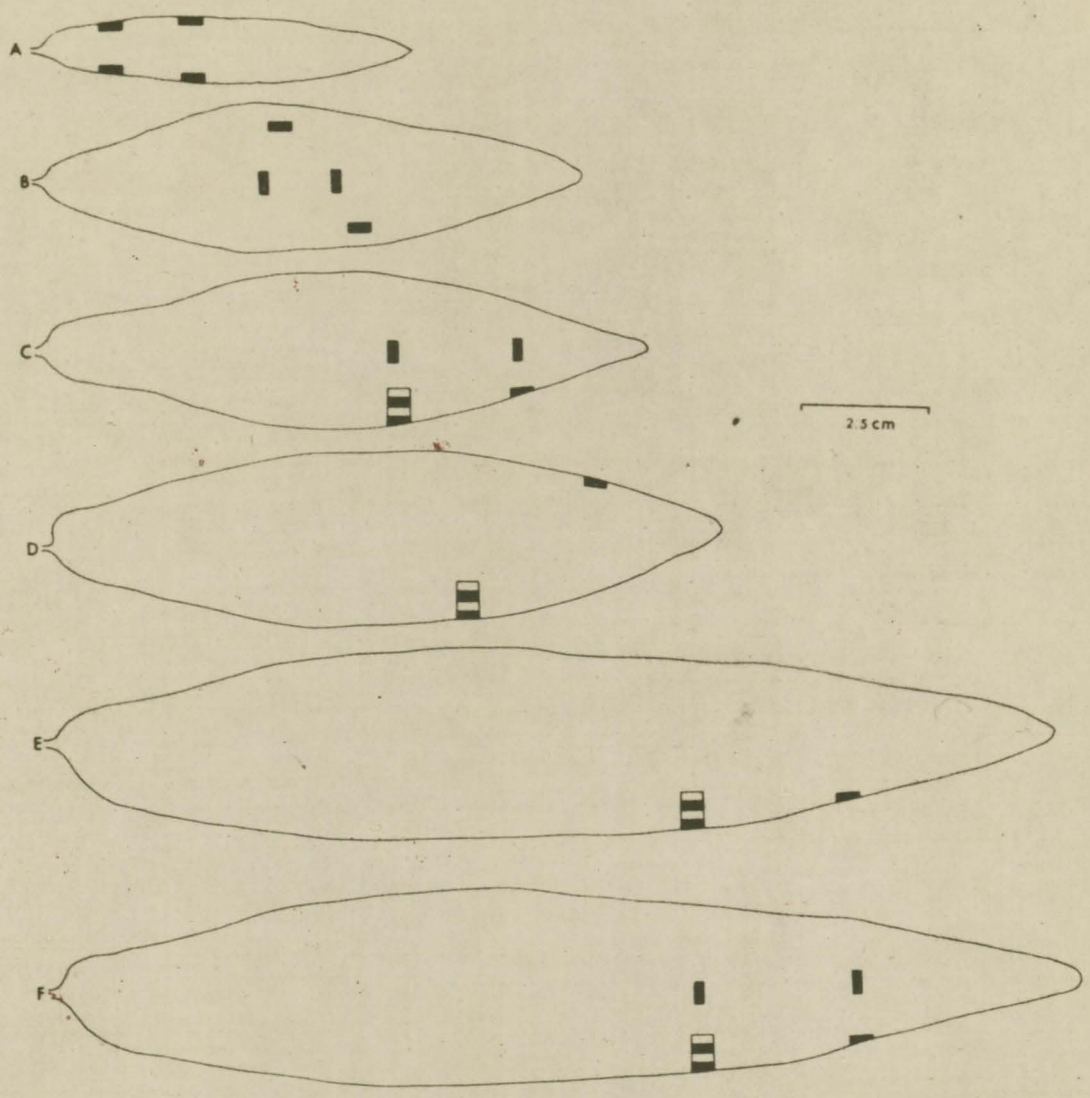
B. Field material: rectangles removed from just inside white reproductive strip (spores) and from the center of thallus.

C. Laboratory plants kept emersed under constant light: rectangles removed as shown from central locations and in a sequence inward from extreme periphery.

D. Field material: rectangles removed from peripheral locations as shown.

E. Laboratory material kept submersed under alternating light with rectangles removed as shown.

F. Laboratory material kept submersed under constant light with rectangles of tissue removed as shown.


A
B
C
D
E
F
2.5 cm

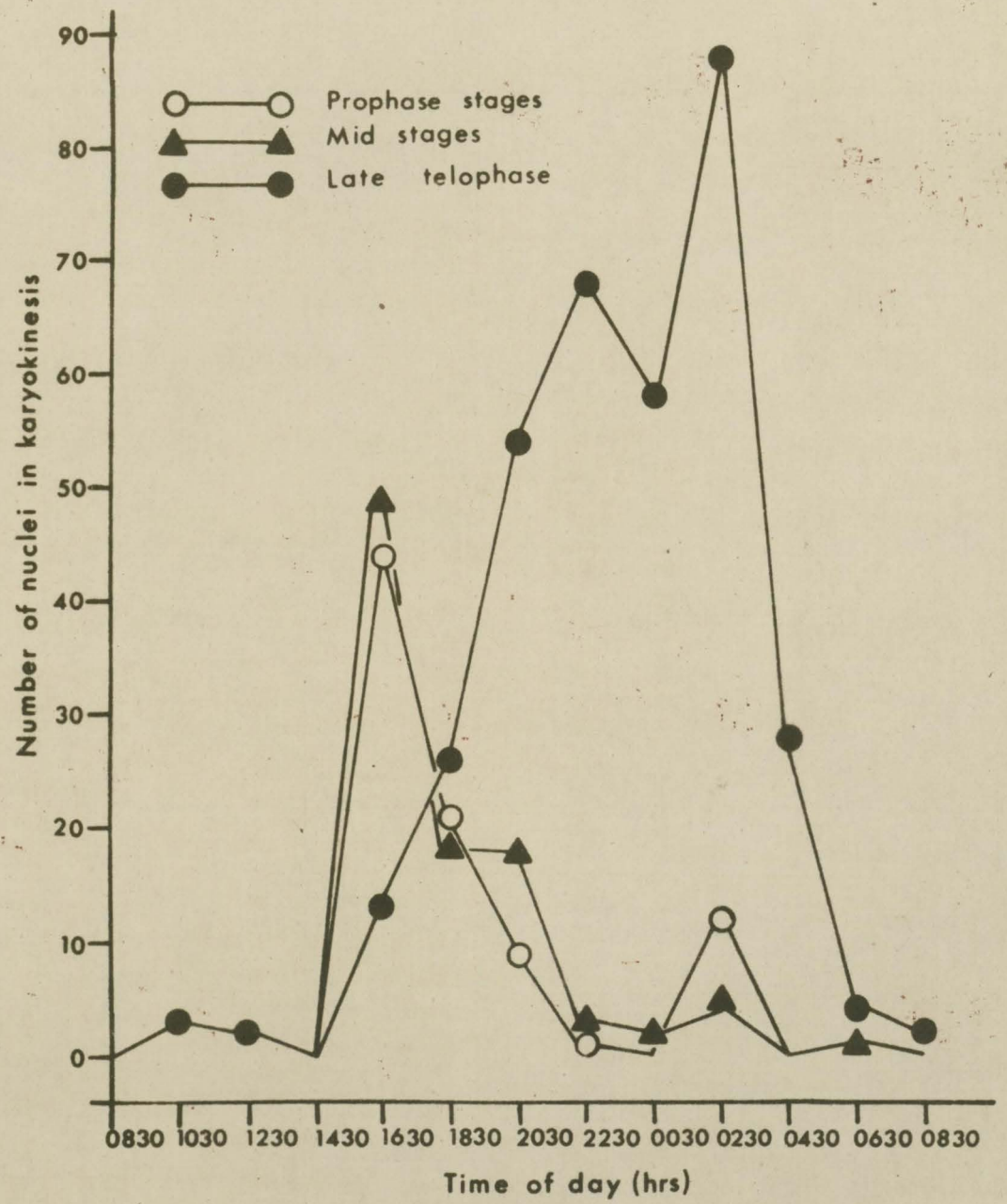
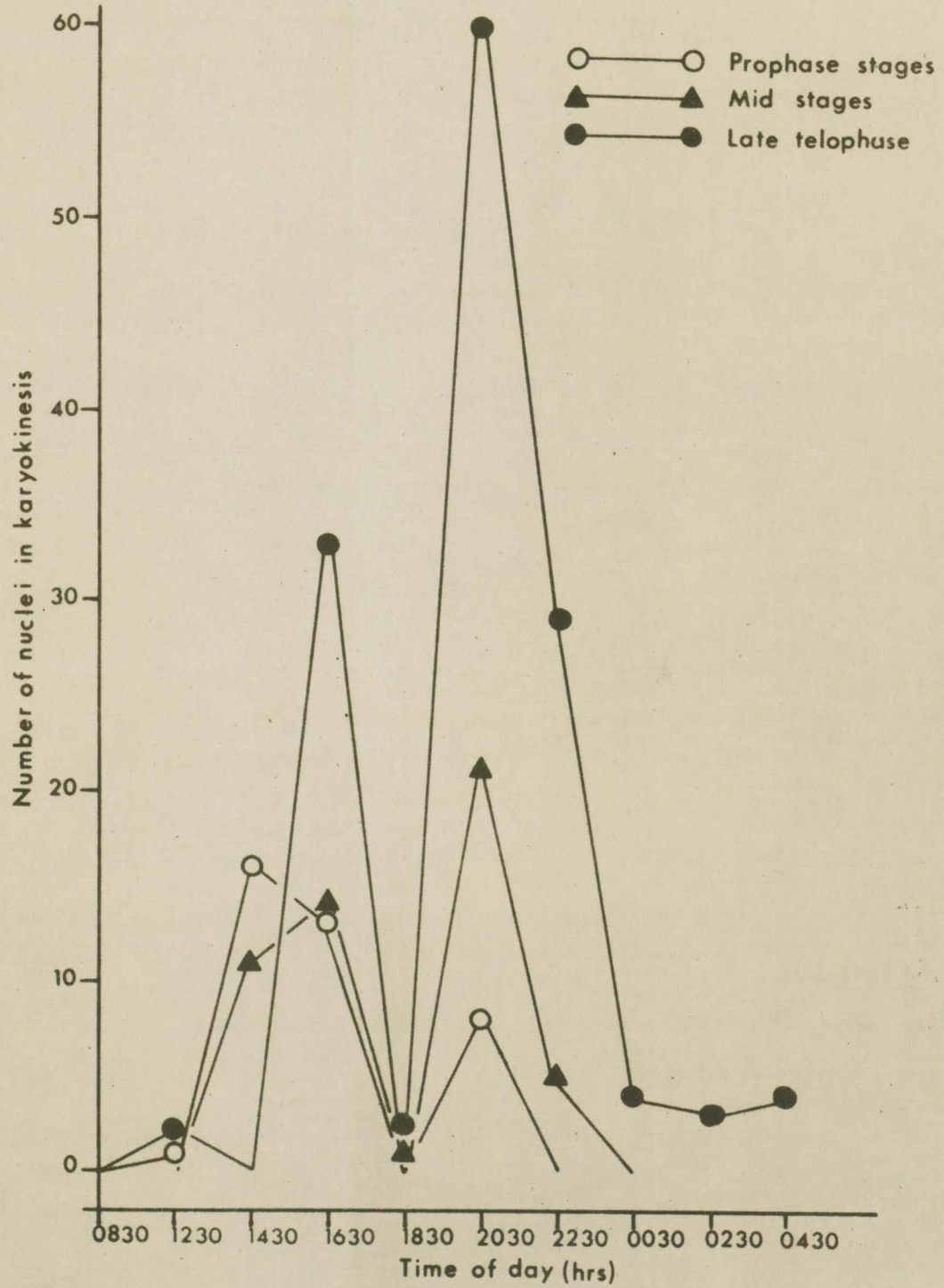


Fig. 2 Number of nuclei in karyokinesis, grouped into "early-" "mid-" and "late-" stages (see text), plotted against time in plants sampled from the field in January.

Fig. 3 Number of nuclei in karyokinesis, grouped into "early-", "mid-" and "late-" stages (see text), plotted against time in plants sampled from the field in January.


Prophase stages
Mid stages
Late telophase
Number of nuclei in karyokinesis
60
50
40
30
20
10
0
0830
1230
1430
1630
1830
2030
2230
0030
0230
0430
Time of day (hrs)

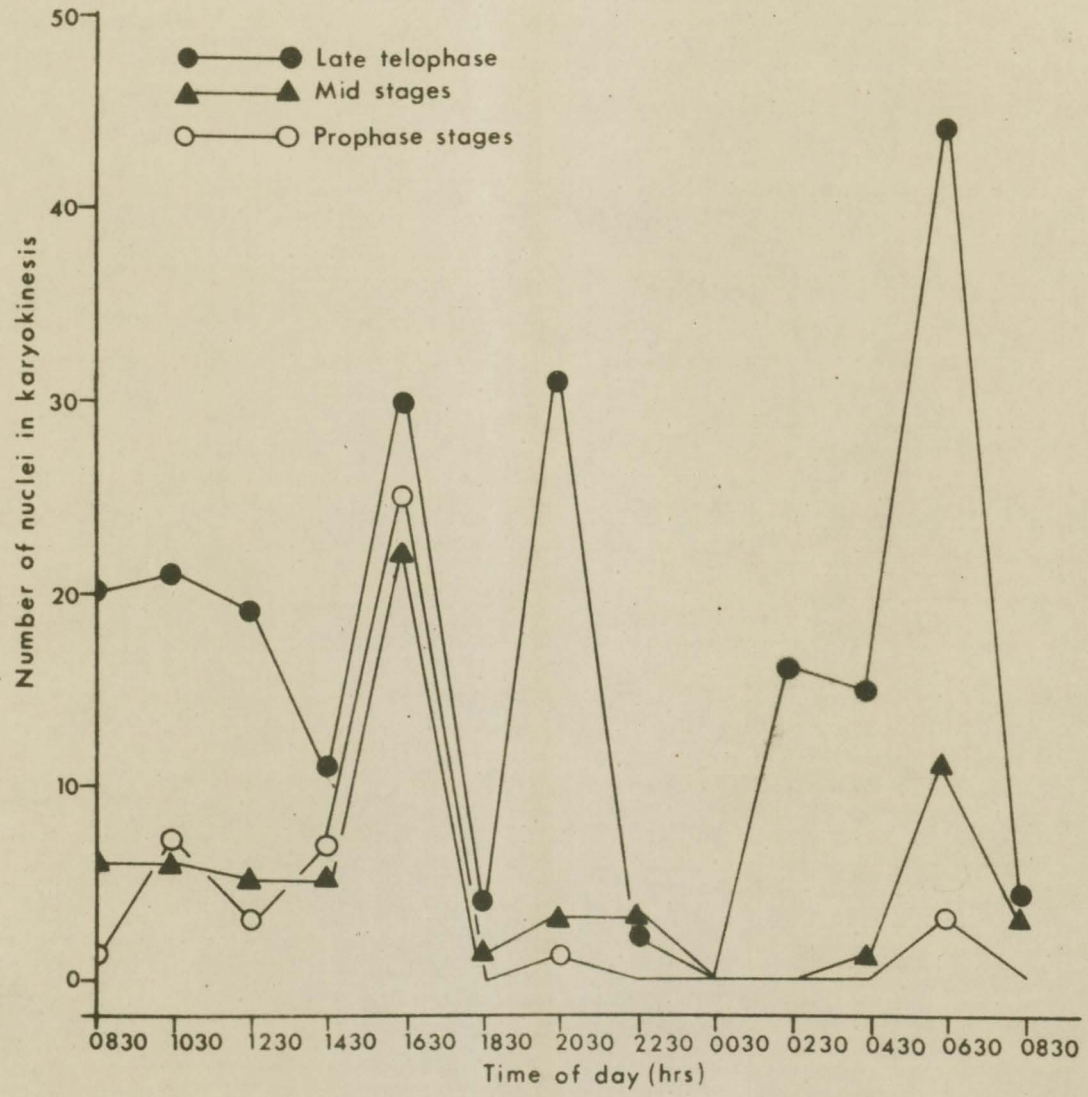


Fig. 4 Number of nuclei in karyokinesis, grouped into "early-", "mid-" and "late-" stages (see text), plotted against time in plants kept submersed under constant light in the lab.

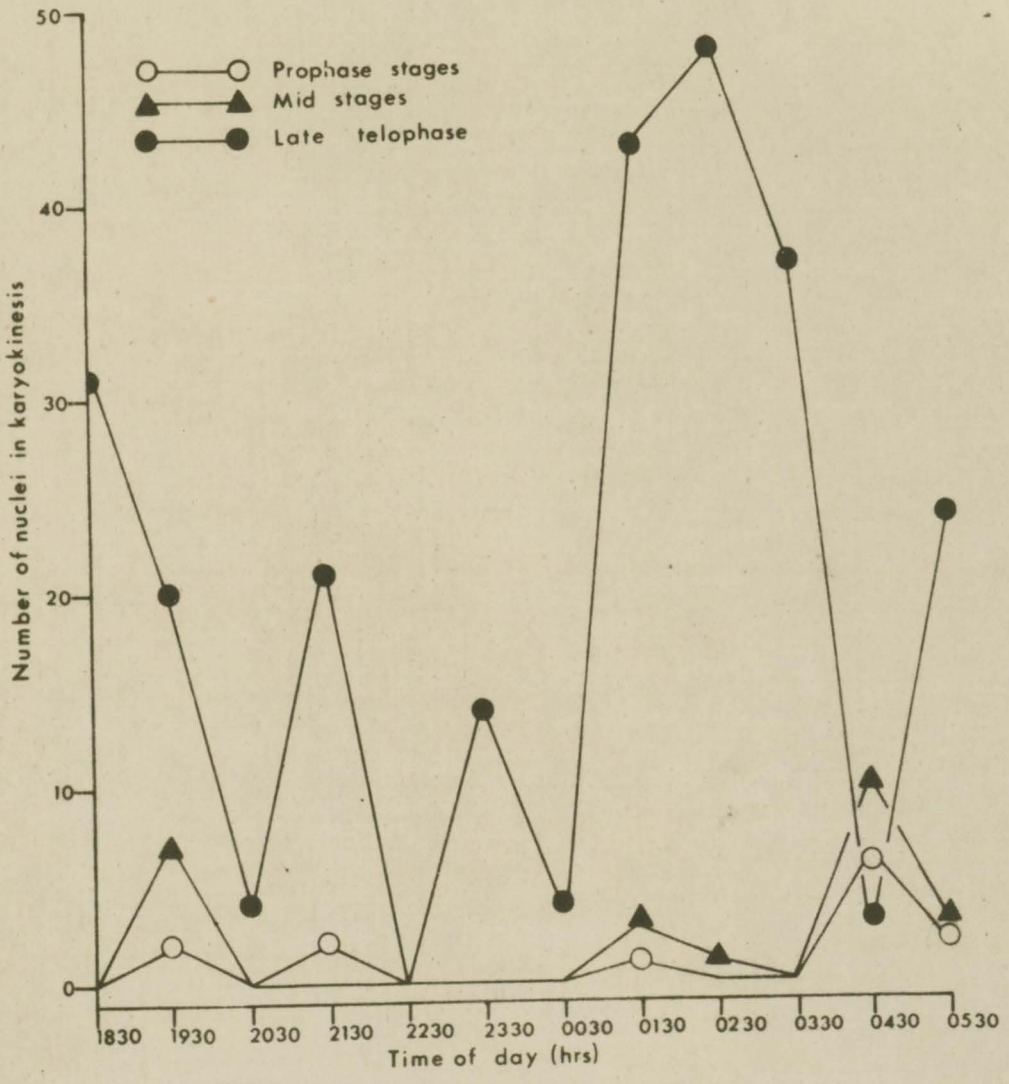


Fig. 5 Number of nuclei in karyokinesis, grouped into "early-", "mid-" and "late-" stages (see text), plotted against time in plants kept emersed under constant light in the lab.

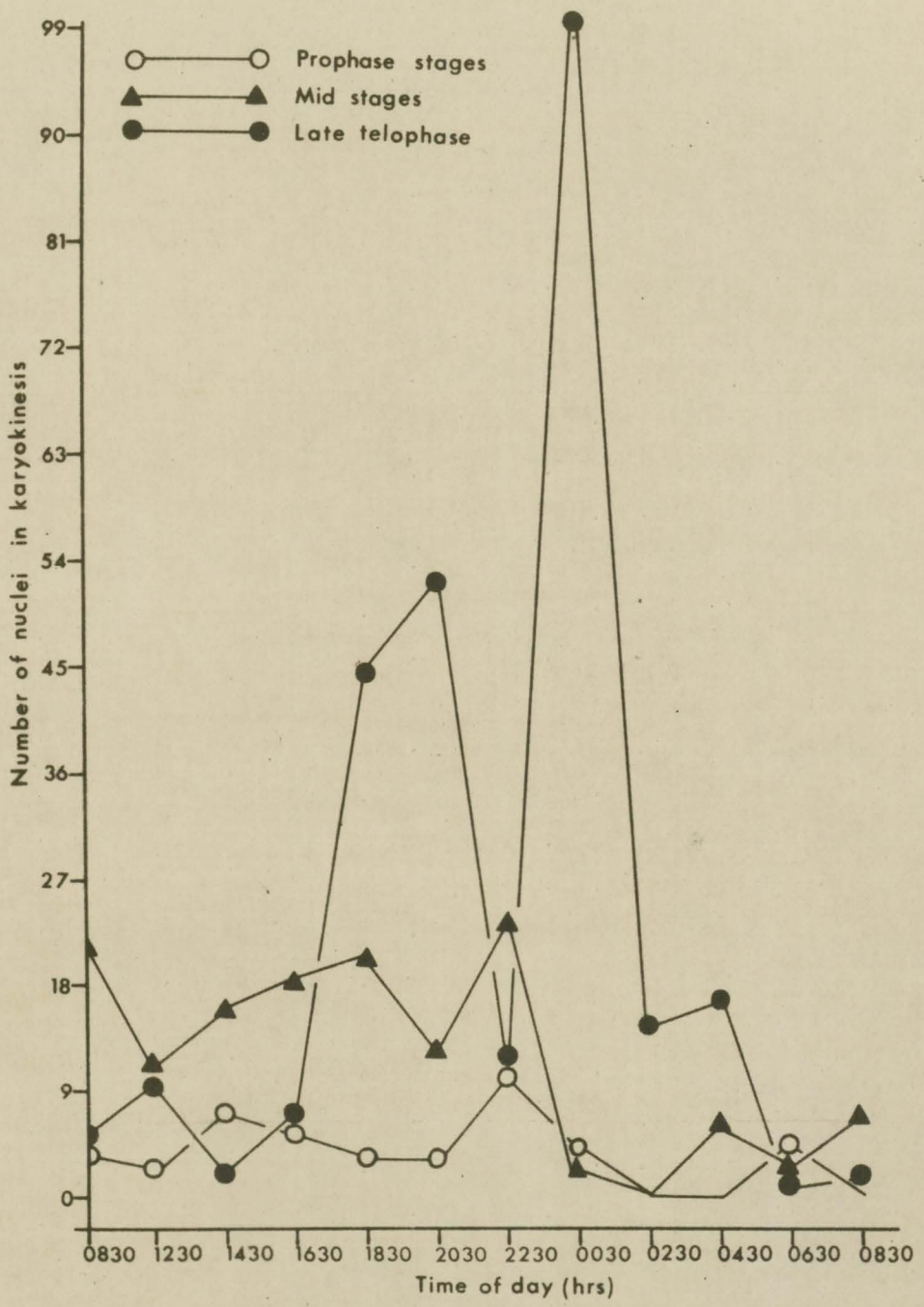


Fig. 6 Number of nuclei in karyokinesis, grouped into "early-", "mid-" and "late-" stages (see text), plotted against time in plants kept submersed under alternating light in the lab.

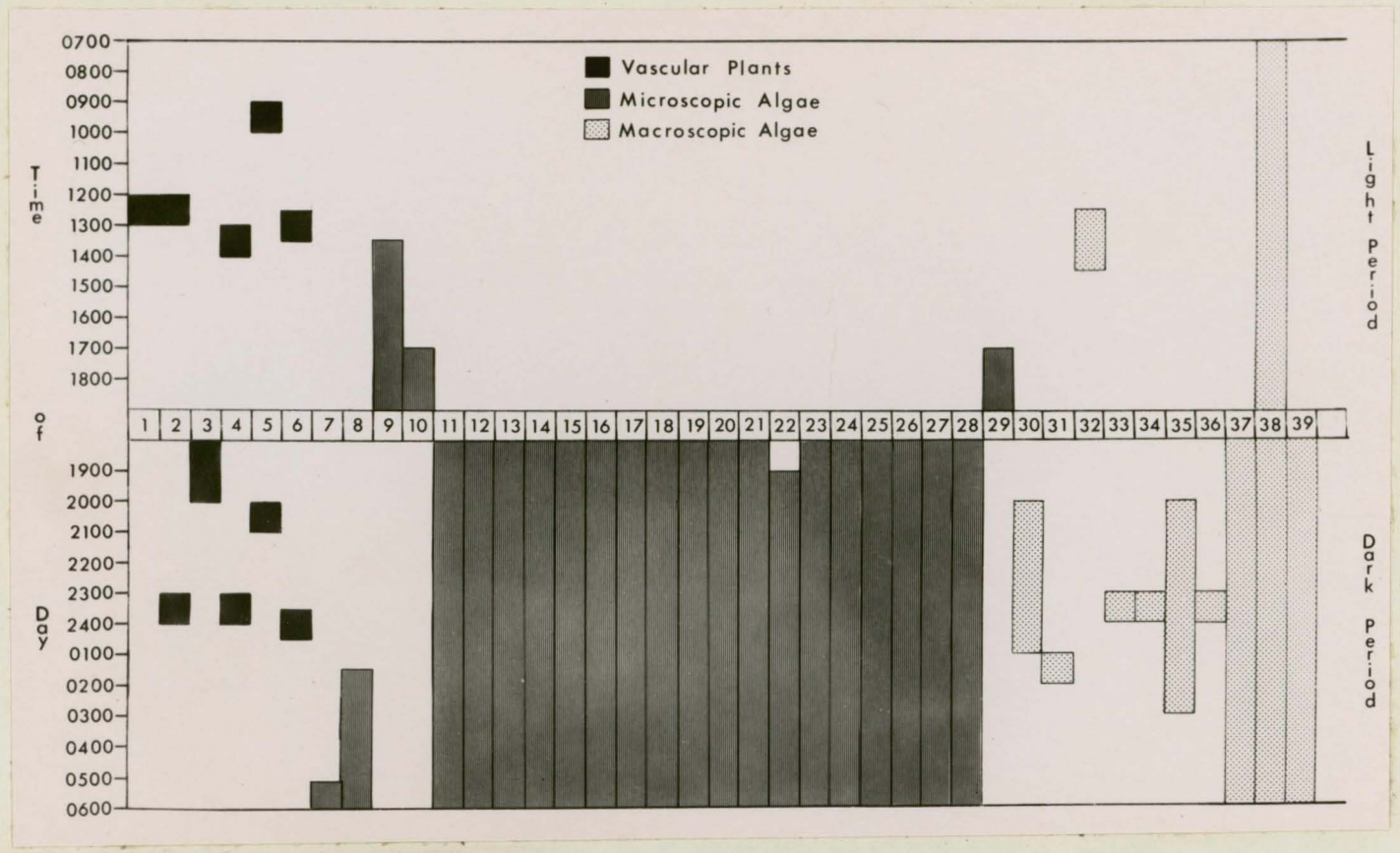


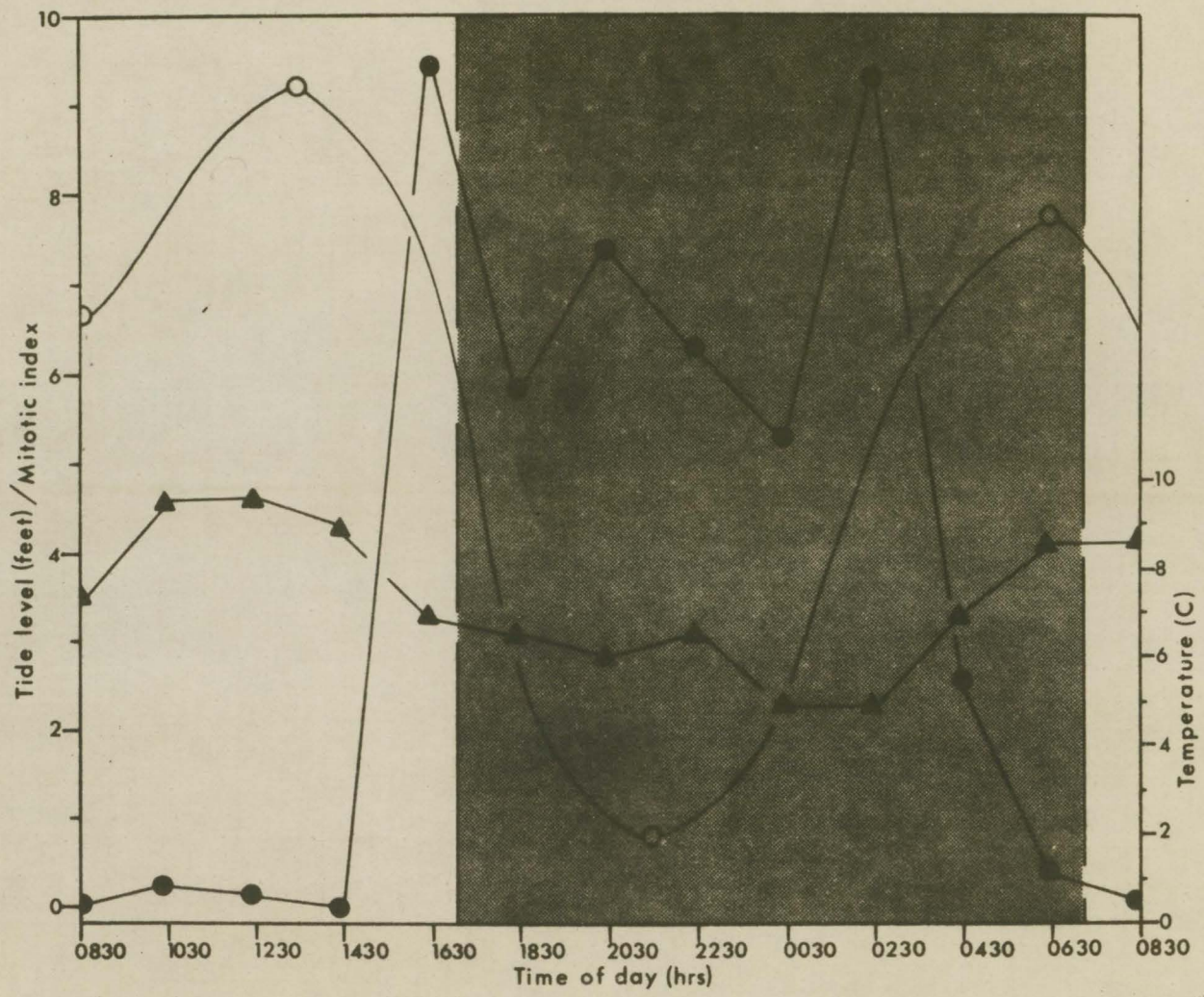
Fig. 7. A diagrammatic representation, arbitrarily compiled from the literature, of the diurnal distribution of mitotic phenomena in a variety of plants grouped as shown. The numbers 1-39 in this figure represent authors and the plants they worked on and these are listed in some detail in Table 16 which should be used in conjunction with this figure.

Fig. 8 Distribution of mitotic index, tide levels natural photoperiod and temperature throughout the 24 hour major fixation sampling period in November.

Black circles = mitotic index

White circles = tide levels

Triangles = temperature.


Tide level (feet)/Mitotic index
Temperature (C)
Time of day (hrs)
0830
1030
1230
1430
1630
1830
2030
2230
0030
0230
0430
0630
0830

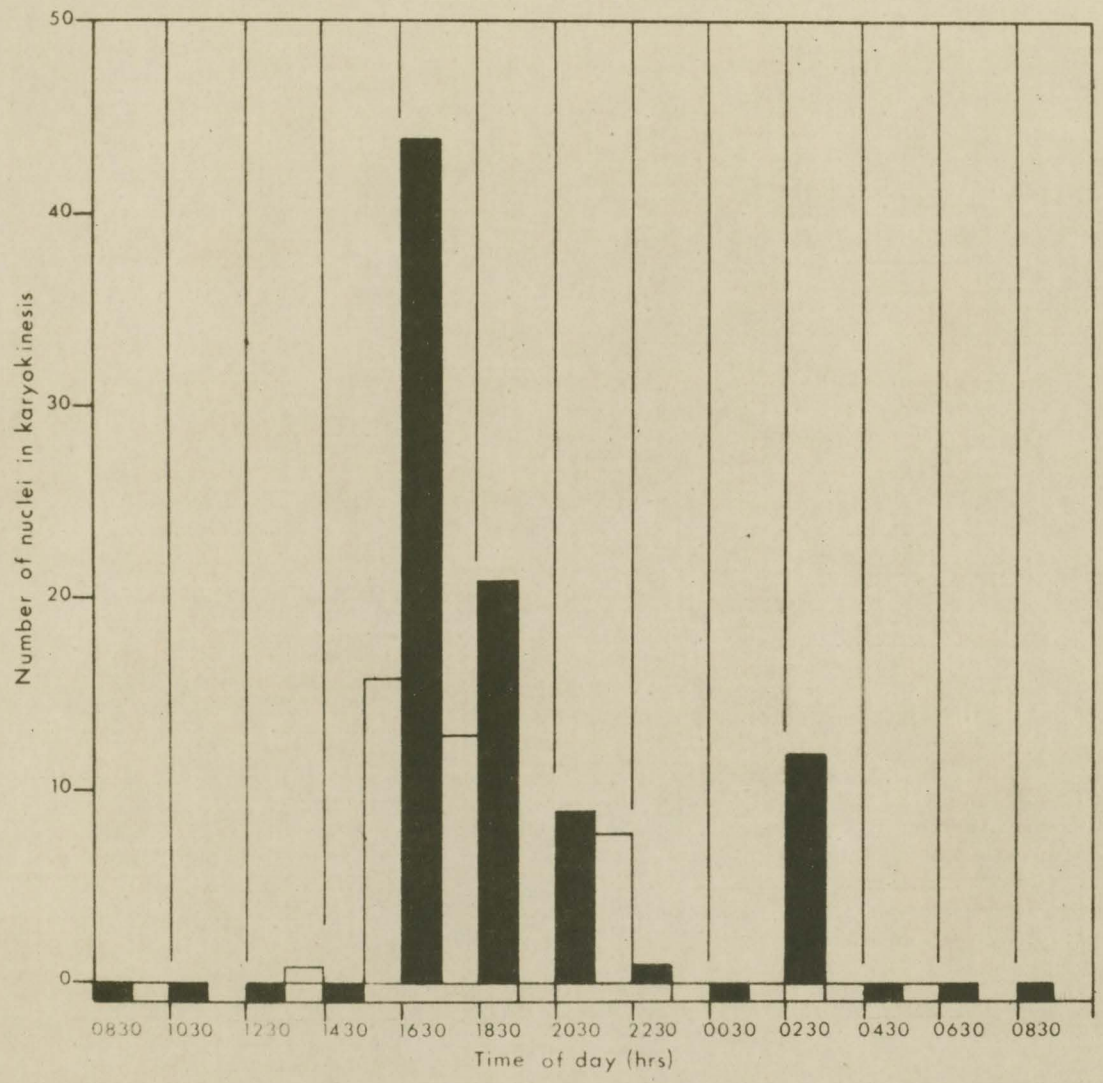


Fig. 9 Comparison of the distribution of the number of nuclei in karyokinesis for both the November and January field sampled plants. Where zero line incomplete no plants were sampled, otherwise plants were sampled and no activity recorded.

Black = November

White = January

(a) "Early-stages" of karyokinesis.

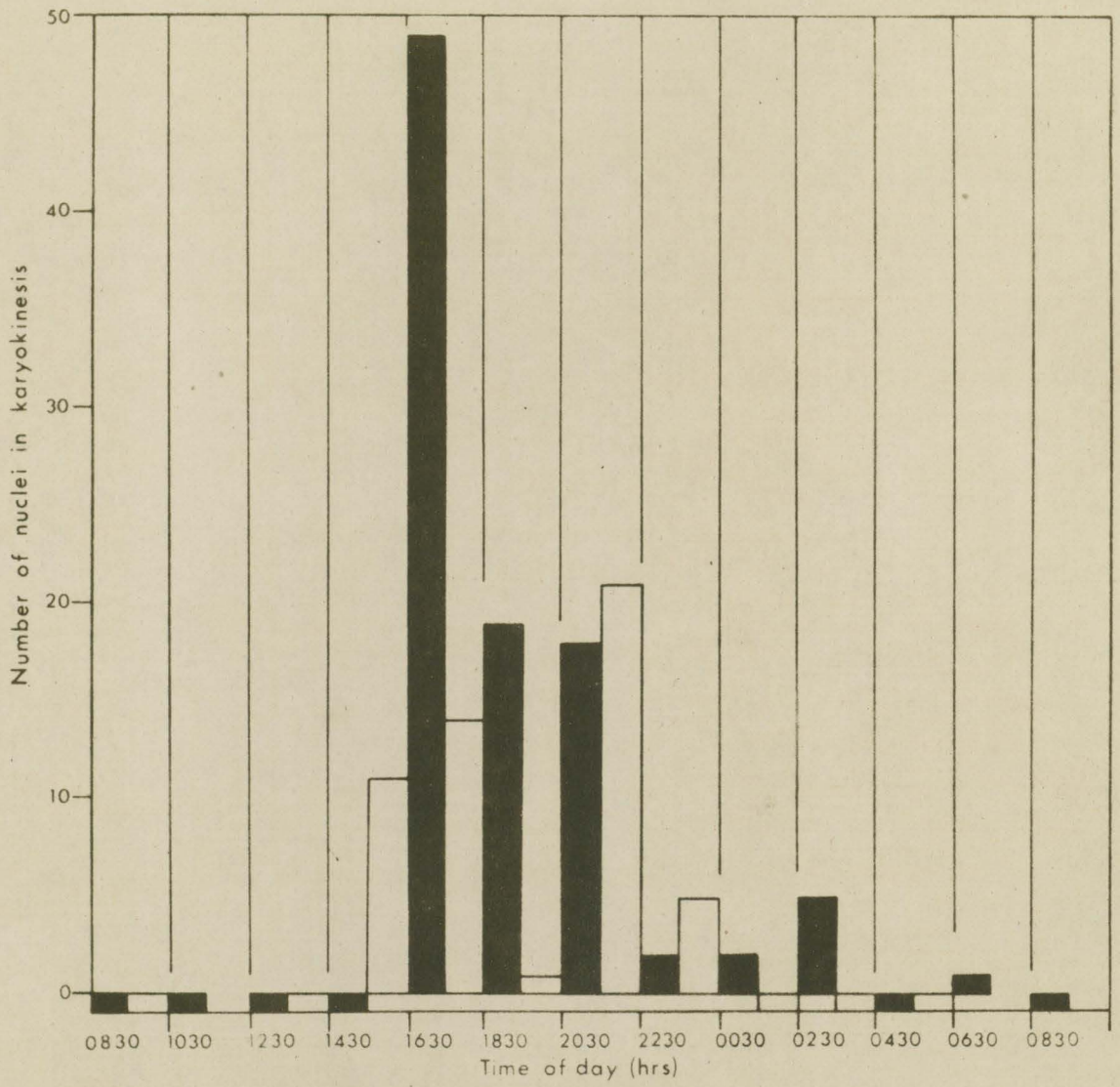


Fig. 9 (b) "Mid-stages" of karyokinesis.

Fig. 10 Distribution of mitotic index, tide levels and temperature throughout the 24 hour major fixation sampling period in January.

Black circles = mitotic index

White circles = tide levels

Triangles = temperature.

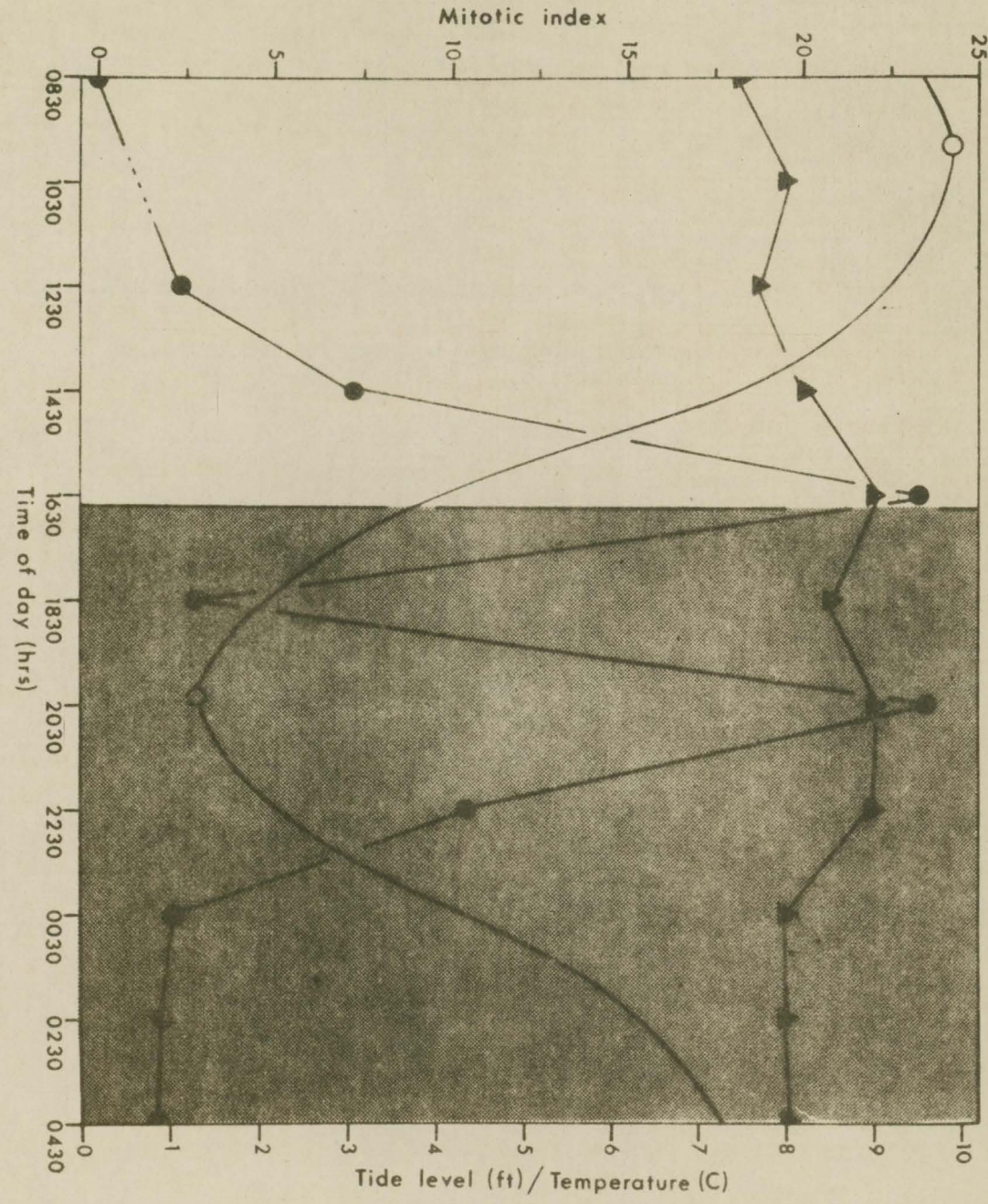

Mitotic index
0
5
10
15
20
25
0830
1030
1230
1430
1630
1830
2030
2230
0030
0230
0430
Time of day (hrs)
0
1
2
3
4
5
6
7
8
9
10
Tide level (ft) / Temperature (C)

Fig. 11 Comparisons of number of nuclei in karyokinesis in plants sampled from the field and from two laboratory situations.

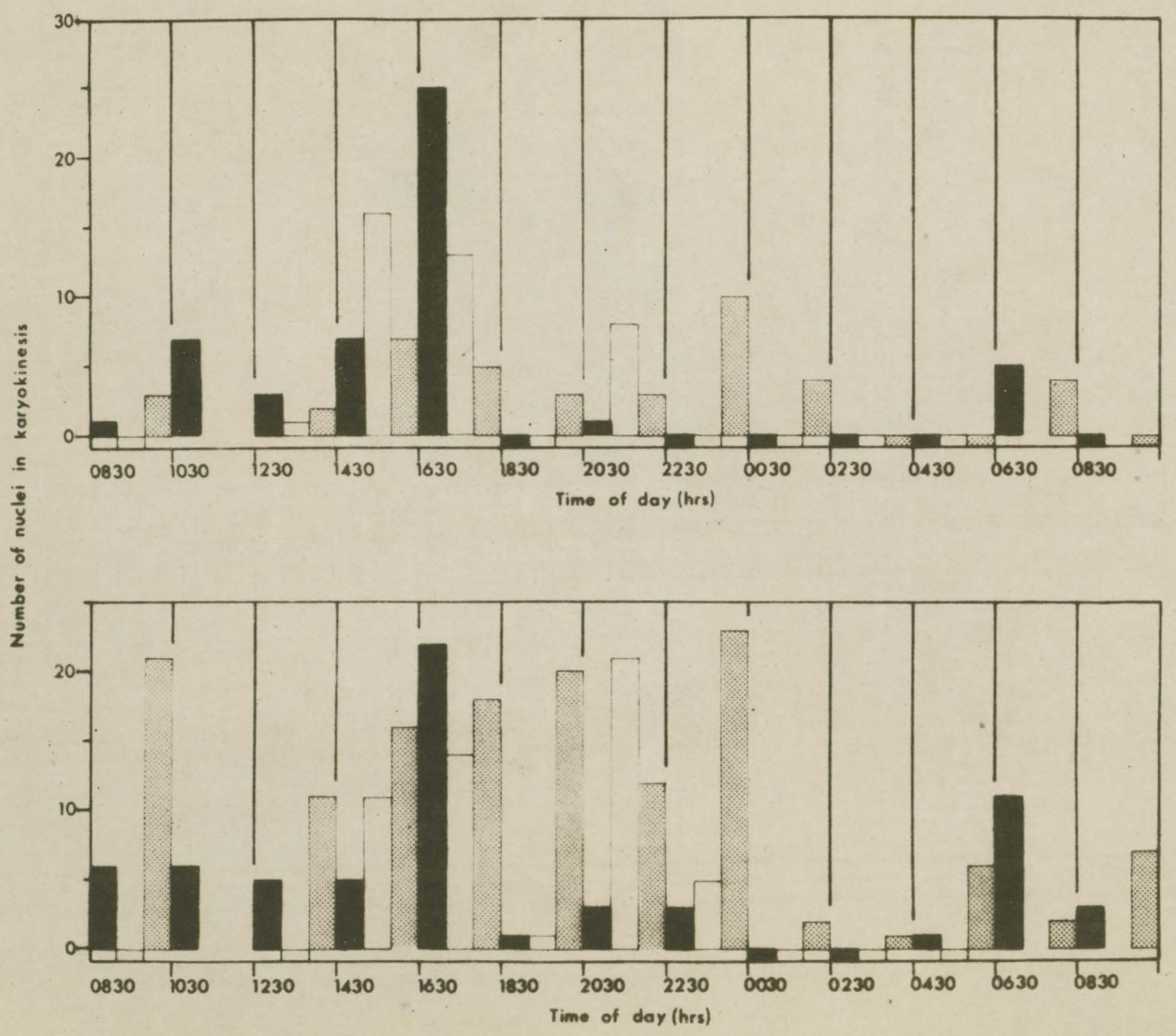
White = field

Stippled = alternating light submerged in lab.

Black = constant light submerged in lab.

upper figure - "early-stages" of karyokinesis

lower figure - "mid-stages" of karyokinesis.


Number of nuclei in karyokinesis
30
20
10
0
0830
1030
1230
1430
1630
1830
2030
2230
0030
0230
0430
0630
0830
Time of day (hrs)
20
10
0
0830
1030
1230
1430
1630
1830
2030
2230
0030
0230
0430
0630
0830
Time of day (hrs)

PART III

ASPECTS OF THE CYTOLOGY OF *PORPHYRA LANCEOLATA*

INTRODUCTION

Porphyra is a widely distributed seaweed which is used in some parts of the world as a supplementary source of food. Japan has been cultivating the genus for 300 years and by 1955 had 500 million square meters under cultivation (Tseng and Chang 1955). Many workers (Berthold 1882; Ishikaw 1921; Dangeard 1927; Kunieda 1939) had studied various aspects of the biology of Porphyra, but it was not until 1949 that all the then apparent stages in the life history of the genus had been demonstrated (Drew 1949), and even today relatively little literature is available on the cytological status of the various stages and their respective reproductive cells.

The most economically important and ecologically dominant phase of Porphyra consists of a leaf-like mono- or di-strommatic thallus that liberates various types of reproductive cells whose function had only been surmised and nuclear cytology and chromosome complements ignored in the majority of species. One of the types of reproductive cells is formed as packets of 64-128 cells which, when mature, constitute a white edge around the plant and have been described for most species of Porphyra. These cells have been termed spermatia (Ishikawa 1921; Kunidea 1939; Drew 1956), although they have not been directly linked with fertilization. Similarly, carpospores have been described for species of Porphyra (Berthold 1882; Kunieda 1939; Ishikawa 1921) although little evidence existed that fertilization had taken place, nor was the chromosome complement given. Other problems centered around the phenomenon of fertilization, where and how it takes place and where reduction division occurs are,

as yet, unsolved for Porphyra or for the Bangioideae. Fertilization was first reported as occurring in Porphyra by Berthold (1882), and later by Joffe (1896), but neither author actually observed the process. Later Knox (1926), Dangeard (1927), Magne (1952), and Tseng and Chang (1955), gave evidence that fertilization occurred in certain species of Porphyra and other authors (Ishikawa 1921; Kunieda 1939), stated that it takes place in P. tenera, but give little evidence of having observed it. Those authors that have described the fertilization process in Porphyra vary in their description of how and where it takes place. Berthold (1882), and Dangeard (1927), both felt that it takes place within the female thallus but Knox (1926) described the liberation of gametes into the culture media and observed fertilization taking place some distance from the thallus. It is unlikely that two totally different methods of fertilization exist in one genus, although Flint (1953) considers that two types of sexual reproduction occur in a related genus, Kyliniella, but his report is not well documented. Hus (1902), working on the taxonomy of a number of species of Porphyra from the eastern Pacific stated that no evidence of fertilization was procured in any one of these species. Krishnamurthy (1959) carried out the only complete cytological study on a species of Porphyra but the process of fertilization was not observed nor did he find any evidence that sexual reproduction takes place at all in this species. Due to the lack of definite evidence that sexual reproduction takes place in Porphyra, Conway (1964 b) reclassified the supposed sexual reproductive cells removing the implication they are sexually oriented. The new classification includes the terms α spores for the carpospores and β spores for the

spermatia. These α and β spores are a further breakdown of the Type III spores of Drew (1956), who had previously classified the spores of the Bangiophycidae on the basis of their formation and not their function.

However, in addition to the reports of fertilization by the older workers (Berthold 1882; Joffe 1896; Dangeard 1927), recent workers suggest that fertilization may occur in certain species. Magne (1952), working on P. linearis, describes a prophase nucleus in a fine tube which connects a structure on the surface of the thallus with a cell inside the thallus. Furthermore, he found the resulting carpospores to be diploid. Kito (1966), studying the cytology of a species of Porphyra epiphytic on Grateloupia, observed four chromosomes in the vegetative and antheridial cells and eight in the mitotically active carpospores. Thus, in the latter plants, either fertilization or polyploidy had taken place.

Assuming fertilization, certain workers (Berthold 1882; Ishikawa 1921) felt that meiosis occurred in the zygote immediately following fertilization, but no evidence in the form of illustrations was presented. Magne (1952) and Kito (1966) both state that meiosis does not take place previous to the production of carpospores. The latter authors at least give some evidence to back up their statements, as they observed cell division after fertilization and preceding carpospore formation and found it to be mitotic and the chromosome complement to be diploid.

Other aspects of cytology in Porphyra have not, or very

rarely, been demonstrated. Qualitative and quantitative data on stages of mitosis, stages of meiosis, nuclear size and cell size are almost totally lacking in the literature. Krishnamurthy (1959), whilst presenting drawings of the various stages of mitosis, did not give complete photographic evidence and Japanese workers (Kito 1966; Yabu and Tokida 1963) describe the various stages of mitosis but illustrate these stages with inadequate photographs.

The present work is a segment of a larger study on the periodicity of cell division in various species of eastern Pacific intertidal red algae (Austin and Pringle 1968) Porphyra lanceolata (Setchell and Hus) G.M. Smith, was chosen as a high shore species and quantitative data on mitosis throughout 24 hour periods were obtained (Pringle and Austin unpublished data). In conjunction with this work, aspects of the cytology and life history of P. lanceolata were studied and are presented below.

MATERIALS AND METHODS

P. lanceolata was collected at the Ogden Point Breakwater in Victoria, B.C. on November 22-23, 1967 and January 12-13, 1968 and the plants placed in Carnoy's fixative every two hours for each of the two 24 hour periods. Further details on the methodology of sampling the plants throughout all tidal phases is presented in another paper on mitotic periodicity of the species (Pringle and Austin unpublished data). After 24 hours the plants were removed from the fixing solution and placed in 100% alcohol for 15 minutes after which they were put in 70% alcohol and stored in a refrigerator.

The aceto-carmine squash technique of Austin (1959), with minor modifications, was employed in preparing the material for cytological studies. Observations on the squashed and stained material was accomplished using a Zeiss WL microscope employing a green filter and a 100X Zeiss Planapochromat oil immersion objective. Photomicrographs were taken with a Reichert VbX plate camera.

Measurements of cytological components were carried out using an occular micrometer and are given for both vegetative and reproductive structures. Most structural sizes given were obtained by acquiring an average from a varying number of plants, this latter number depending on the structure being measured.

The intact plants were scanned under an Olympus tri-nocular dissecting microscope and each plant sampled was sketched and its linear measurements recorded.

OBSERVATIONS AND DISCUSSION

P. lanceolata occurs 7.5 feet above zero tide at the Ogden Point Breakwater and elsewhere on adjacent coastlines in semi-exposed and exposed locations. The morphology of the plants, their size and reproductive structures varied between November and January.

November Plants - reproductive bodies present.

In November 39 plants were processed which ranged in size from 3.5 cm. to 12.0 cm. with an average size of 7.23 cm. x 1.13 cm. (Table 1). Rectangles of thallus were removed from various locations on a number of randomly selected plants in an attempt to obtain vegetative division. At this time of year the most mitotically active groups of cells occurred mid-way along the length of the plants in a peripheral location.

Certain reproductive structures were evident in November and most peripheral locations on the majority of the plants revealed "globular cells" (Fig. 3), which were in contrast to the oval shaped vegetative cells (Fig. 4 and 5). Two types of globular cells were found and both may exist in one plant. The two types are very similar in shape and size, but they differ in formation.

Type I Spores? (Drew 1956)

The most abundant type of globular cell has the following characteristics; it is thin walled, stains lightly, averages 24 x 20 microns in size and is formed by the division of a mother cell. These particular cells are found adjacent to large oval cells which

are approximately 37 x 18 microns. These latter cells are surrounded by thick gelatinous material and divide longitudinally to form two oval cells (Fig. 1) each of which divides transversely to form two globular cells (Fig. 2).

These round cells relate to reproductive cells found in other species in that they closely resemble the illustration of carpospores for P. tenera given by Kunieda (1939), but this author gives little detail of their formation except that they are thought to follow fertilization and result in batches of 8-16 cells. There is no evidence that these round cells of P. lanceolata are carpospores and only two are formed from each mother cell. They also resemble α spores found in P. umbilicalis, but the formation of the latter is quite different (Conway 1964 b). It is plausible that carpospores, as described for P. tenera (Kunieda 1939), are α spores. Drew (1956), in her classification of Bangiophycidae spores, describes the cytokinesis of a mother cell resulting in the formation of two daughter cells of unequal size, one of which becomes a sporangium which liberates its entire contents as in monospore formation. Drew classifies this type of spore production as Type I. Although this type of spore formation has not been recorded previously for species of Porphyra, it is very similar to that described above P. lanceolata, a difference being that in P. lanceolata both cells become a "sporangium" (Fig. 2 and 3). These Type I spores, as described here for P. lanceolata, are easily distinguished from the vegetative cells; the former are globular in shape as opposed to the latter's oval shape, during formation they are embedded in a thick gelatinous

material and the spore mother cell divides in a transverse fashion (Fig. 2), which is not the case with vegetative cells which divide longitudinally.

Monospores

The less common type of globular cells (Fig. 8 and 9), found in P. lanceolata in November, differ from the Type I described above in appearance and manner of formation. They are not thin walled and embedded in a loose matrix of gelatinous material but are cells with extremely thick walls (Fig. 8), whose cytoplasm stains very densely. The average size of these cells is 26 x 21 microns and they are found on the extreme edge of the thallus in only one or two rows which is similar to the location of monospores on thalli of P. tenera (Kunieda 1939). The main distinction between these round cells and those previously described here as Type I spores is their formation (Fig. 10-14). The former develops by the rounding up of typical vegetative cells, a process easily followed in a section of thallus, for there is a gradation in cell morphology between the thick walled round cells and the nearest vegetative cell. Initially, the vegetative cell becomes more spherical in shape (Fig. 11), then decreases in size (Fig. 12 and 13) and a thick cell wall is laid down (Fig. 14). The cytoplasm of the spore increases in its capacity for absorbing stain as each of these steps progresses. Infrequently these cells may undergo division (Fig. 9) to give rise to two similar thick walled cells. Drew (1956) classified spores of this derivation as Type II spores or monospores. Similarly other workers (Tseng and Chang 1955; Hollenberg 1958; Conway 1964 b) term this type

of spore a monospore. They are spores that are derived from a mother cell without the incidence of cytokinesis and germinate in a bipolar fashion which ultimately gives rise to a plant similar to the parent plant. Although culture studies were not carried out on this type of spore described here for P. lanceolata, it can be inferred that they are monospores due to their morphology, size and formation.

In this study monospores were relatively abundant on most plants collected in November but in January only a few of the smaller plants possessed them. Thus there is the possibility only young plants form monospores and that the smaller plants of January, which possess monospores, were initiated from either monospores or the Type I spores shed in late October or November. Similarly Hollenberg (1958), culturing P. perforata collected in California, suggests that monospores may not be liberated throughout a plant's life span.

Type III Spores (Drew 1956)

The third type of spore formed by P. lanceolata in November is liberated from a packet of 16 cells. These packets of cells result from the repeated division of a pale spore mother cell, which is quite distinct from darker, typical vegetative cells (Fig. 15-26). The spore mother cell firstly undergoes a transverse division (Fig. 16) after typical mitosis which resembles that of vegetative cells. The two daughter cells increase in size longitudinally becoming wedge shaped, and eventually lie side by side (Fig. 17 and 18). The second division results, not in wedge-shaped growth and parallel arrangement, but is somewhat different

in that each daughter cell grows evenly at each pole increasing the size of the packet of four cells by almost half (Fig. 20). The third division (Fig. 21) produces a packet of eight cells (Fig. 22) and this time, as in the very first division, the daughter cells extend towards each other again forming the characteristic wedge shape with oblique walls (Fig. 23), and eventually lie in a parallel fashion (Fig. 24). Finally a fourth division occurs (Fig. 25) in all the cells producing sixteen cells (Fig. 26), the ultimate number of spores in the complete packet. The cytoplasm of the individual cells and the gelatinous matrix, which surrounds the packet of cells subsequent to the second division, do not stain darkly and thus the haploid number of chromosomes are easily seen throughout spore formation. The packet of cells was observed to increase in size with each cell division and is eventually approximately 1/3 larger than its original size (Fig. 15 and 26). When liberated the spores are similar to the asexual spores described by Conway (1964 b) for P. umbilicalis. According to Drew (1956), these spores would be classified as Type III, although in her description of asexual Type III spores there is very little change in total size between the original mother cell and that of the ultimate packet of 16 cells.

The size and shape of the cells found in P. lanceolata vary depending on their location in the thallus and presumably their function. In November, the extreme peripheral edge of the thallus is made up of Type I spores adjacent to large oval cells which have an average size of 36 x 18 microns. These cells typically have a large central pyrenoid which averages 8 x 10 microns in size and a nucleus

located in a mid-parietal location having an average size of 7.0 x 2.1 microns. During cytokinesis these cells divide longitudinally giving rise to the spore mother cells of the Type I spores.

Vegetative Cells and Mitosis

A rectangular section of material, ¼ mm x 2 mm, removed from the periphery of the plant typically contains, in addition to the Type I spores and the large oval cells, small oval cells which average 26 x 19 microns in size which are the typical vegetative cells of the November plants (Fig. 4 and 5). The pyrenoid is centrally located, averages microns in diameter and its appearance fluctuates markedly throughout a 24 hour period. In stained material, at certain times of day, it is very dark and granular and resembles a nucleus whilst at other times it is a pale yellow or white in color. The nucleus is located in a mid-parietal location adjacent to the pyrenoid (Fig. 4 and 5). It is from these vegetative cells that quantitative data on mitotic periodicity was acquired.

Karyokinesis has been observed in the vegetative cells of P. lanceolata and eight distinct stages have been discerned (Fig. 28 -35). The interphase nucleus (Fig. 27) is characterized by a large, solid, dark nucleolus which is surrounded by a narrow distinct ring of light-staining nucleoplasm which delimits the granular appearing cytoplasm. The nucleolus gradually becomes fainter as prophase proceeds and by prometaphase it is no longer in evidence. Prophase consists of three distinct stages, the first of which is early-prophase where three to five irregular strands of chromatin of different lengths appear (Fig. 28) and by mid-prophase these have shortened, become more regular in appearance and unite, forming three distinct chromosomes

(Fig. 29). The chromosomes continue to decrease in length and increase in width and by late-prophase appear as stubby rods and frequently a bifurcation in the chromosomes is evident (Fig. 30). In the interval between mid-prophase and metaphase, the chromosomes gradually move toward the mid-point of the nuclear area where they line up adjacent to one another and form a regular configuration (Fig. 31) which has been designated prometaphase. At this stage the chromosomes lie parallel to the long axis of the cell. They continue to shorten until at metaphase (Fig. 32) they have reached their smallest size and, due to their close clumping, the discernment of individual chromosomes is rarely possible. Anaphase (Fig. 33) is first evident when a split is observed in the metaphase configuration. The division of the chromosomes into two chromatids appears to occur simultaneously as the two groups of chromatids, moving in opposite directions, are parallel to one another. But this parallelity does not persist, for as the groups move farther apart they each typically take on a crescent shape and at this point the individual chromatids can frequently be seen. Each group moves rapidly toward opposite poles in a direction parallel to the long axis of the cell. As they move toward their respective poles, the chromatids coalesce to form a solidly staining structure (Fig. 34) which is typical of the early telophase figure. The groups increase in size and move to a mid-parietal location on opposite sides of the pyrenoid (Fig. 35). The cell and nuclear material remain in this stage, late telophase, for an extended period of time; finally the pyrenoid splits and cytokinesis is initiated. The cell divides longitudinally with the initial cleavage furrows appearing at both ends of the rectangular cell.

Under the environmental conditions of November 1967, P. lanceolata exhibited a definite, statistically significant periodicity in cell division. Details on the periodicity are given in another paper (Pringle and Austin unpublished data). Further data on mitotic index, mitotic rate and stage index will also be presented elsewhere.

January Plants - reproductive bodies present.

The plants collected in January were significantly different morphologically (Fig. 46 and 47) from those collected in November (Fig. 45). Furthermore, the average length and width of the plants had increased to twice what it was in November (Table I). In January three distinct types of plants were observed:

1. plants oblanceolate in shape and similar in size to those plants collected in November,
2. larger oblanceolate plants with an entire outline which has a regular, pale white margin, from 1-4 mm wide, on the sides and distal end of the plant (Fig. 46) and
3. large oblanceolate to spatulate plants with an irregular sinuate margin (Fig. 47).

The latter two types of plants were found in approximately equal numbers.

The objective of the original work on Porphyra was to accumulate quantitative data on the occurrence of karyokinesis in vegetative cells but the majority of plants in January had formed reproductive structures, dissimilar to the spores produced on plants in November, and these structures were located along the edge of the

thallus where the plants were sampled for vegetative growth in November. Thus, in search of maximum vegetative division, pieces of thalli were removed from various areas on the plant other than where the reproductive structures were found. Most of the samples were removed approximately 3-4 mm inwards from the edge of the thallus on both sides of the plant. In 39 plants sampled (three plants from each of 13 collections taken every two hours for 26 consecutive hours), no vegetative cell division was found. This supported the authors' observation that vegetative growth was less than in November and it was decided to sample reproductive areas in an attempt to locate cells undergoing karyokinesis.

β Spores? (Conway 1964 b)

The white margins (Fig. 48) which occurred on certain of the Porphyra plants were found to be made up of packets of cells which were thought to be spermatia by certain previous workers (Hus 1902; Kunieda 1939; Drew 1956). Recently Conway (1964 b) reclassified these cells and termed them β spores as little evidence was available that they participated in a sexual process. A preliminary series of squashes of material removed from the extreme periphery of the white edge of P. lanceolata revealed individual packets (Fig. 6) of 64-128 cells which ultimately formed haploid β spores (Fig. 7) 2-4 microns in size. Adjacent material was then removed from the edge inward toward the middle of the thallus. The packets of cells were still evident but there was a gradation in the number of cells per packet from approximately 130 cells in the extreme outer material to 2-4 cells in material 2-3 mm in from the edge. Neighboring these smaller groups

of cells were large individual cells, 50 x 20 microns in size (Fig. 36), which were much larger than the average vegetative cell and are termed here β spore mother cells. The β spore mother cells of P. umbilicalis were also found to be much larger than the vegetative cells (Krishnamurthy 1959).

Although the β spores resemble in appearance one of the types of asexual spores (Fig. 15-26) formed in November, their formation is dissimilar (Fig. 36-40). The asexual spores of November are formed in a regular pattern but β spore formation is more irregular. There is disagreement among workers as to the direction in which the first wall is laid down after karyokinesis in the β spore mother cell (Drew 1956). Dangeard (1927), Grubb (1924) and Ishikawa (1921) describe the primary wall as bisecting the mother cell in a transverse fashion. Conversely, Hus (1902) found, for 12 species of Porphyra located on the west coast of North America, that the first wall laid down is longitudinal. Similarly the first wall is longitudinal in the material described here for P. lanceolata (Fig. 37 and 38) and the second wall is perpendicular to it (Fig. 39). The following divisions occur in a random fashion (Fig. 40), which ultimately results in a packet consisting of approximately 130 cells. Mitotic division in β spore formation is similar to that described for vegetative cells and is very easily followed as the cytoplasm is lightly stained and the chromosomes are dark. The last nuclear division is not completed and terminates at late prophase (Fig. 7), which is similar to P. tenera (Ishikawa 1921; Yabu and Tokida 1963). The β spores, when liberated from their packet, expand in size to approximately 4 microns and the chromosomes become

more prominent, transforming from a circular shape to square configuration which may represent chromatid formation.

Krishnamurthy (1959) found it difficult to locate the initial division of the β spore mother cell in P. umbilicalis as the chromosomes stained poorly and very few mother cells were in evidence. A further explanation for this may be that the mitotic periodicity found in P. lanceolata (Pringle and Austin unpublished data) may prevail in P. umbilicalis; thus if the latter species were not fixed at the time of day when karyokinesis was initiated it would be difficult to locate β spore mother cells with dividing nuclei. The mitotic perioicity of the vegetative cells of P. lanceolata is maintained in the initial cell division of all types of mother cells, but is gradually lost as the number of cells per packet increases.

α Spores? (Conway 1964 b)

Plants of Type 3 (Fig. 47) were sectioned at similar locations to the plants that produce β spores; thus four adjacent pieces of thalli were removed. There was no uniformity in cell structure and size in this region and each squash exhibited a wide range of cell types. This diversity in cell types is in complete contrast to the same regions of the plants which bear β spores where there is a complete homogeniety of cells. Some of the cell types contained six chromosomes, the diploid number, and thus the possibility existed that fertilization takes place in this species of Porphyra. A review of the literature revealed that very little is known about the reproductive structures in P. lanceolata but recent workers (Conway 1964 a, b;

Krishnamurthy 1959) suggest that fertilization may not occur in all species of Porphyra. The few illustrations presented in the literature of the structures involved in fertilization for Porphyra were not similar to those structures contiguous to the cells which contained six chromosomes in material of the present work. Hence, representative cell types were drawn and their respective measurements recorded in an attempt to identify the carpogonium and describe the fertilization process for this species.

The most abundant structures in these latter types of plants are distinct groups of dark staining, wedge shaped cells (Fig. 43 and 44) which are present on most plants collected in January which do not contain βspores. These "groups" of cells are, at times, located adjacent to the cells with the diploid number of chromosomes but there does not appear to be a direct connection between them. The mother cells, which give rise to these groups of cells, are large, dark and are about 45 x 30 microns in size. The nucleus goes through the various stages of karyokinesis and cytokinesis resulting in the first wall being layed down parallel with the longitudinal axis of the cell (Fig. 41), giving rise to two daughter cells 45 x 10 microns in size. The nucleus of each daughter cell divides again and cytokinesis takes place in a transverse direction (Fig. 42). Cell division then occurs in a random fashion until approximately sixteen cells have been formed with oblique cell walls (Fig. 43 and 44) and remain as such for a considerable period of time. As each cell division takes place the cytoplasm stains more darkly and the cells remain in their individual groups although there is no visible enveloping material.

These groups of cells do not give rise immediately to spores at this time of year, if indeed they do at any time, but remain in groups for a period of time; in this respect they may resemble what Kunieda (1939) termed as carpospore formation in P. tenera. The latter author observed that pieces of thalli containing groups of carpospore mother cells would drop away from the thallus before carpospores were formed and, to find mature spores, the latter author suggested picking up drifting chunks of thalli.

The formation of these groups of cells with oblique walls (Fig. 43 and 44) in P. lanceolata resembles the description given for α spore formation by Conway (1964 b). The latter author describes globular spores found in large proportions on the thallus of P. umbilicalis. These spores are formed from a mother cell which undergoes repeated division with the initial division, in most cases, taking place longitudinally; the following divisions taking place in a random fashion forming a packet of cells of no more than 16 in number, each of which when liberated are 10-12 microns in diameter.

The groups of cells described lastly for P. lanceolata could conceivably give rise to spores similar to α spores; thus, in this species, the formation of α spores is inferred.

Sexual Reproduction

Male and Female Cells

Structures, quite distinct from the groups of α spore mother cells, occur abundantly in the peripheral three millimetres of the plants.

One of these consists of large, lightly staining cells, 50 x 25 microns in size (Fig. 58), which at times contain the diploid number of chromosomes. It was felt that these cells may be carpogonia, thus agreeing with the description given for carpogonia in P. leucosticta (Berthold 1882). Cells containing from one to ten densely staining structures are found in close association with the carpogonia and, although similar structures have not been related to fertilization in Porphyra, nor have they been described, they will be referred to here as male "gametangiophores" (Fig. 50, 51 and 52).

Fertilization

The "gametangiophore" is initially observed containing small male gametangia whose nuclei divide repeatedly (Fig. 50 and 51). Nuclear division continues until the gametangia have expanded and are of sufficient size to fill the somewhat expanded gametangiophore (Fig. 52). In some instances what may be a fertilization tube develops from the gametangium which penetrates the wall both of the gametangiophore and also an adjacent carpogonium (Fig. 53 and 54). The male gametes are seen in the "fertilization tube" and may be discharged into the carpogonium (Fig. 54) where, after discharge, one male nucleus increases in size and is contained by a distinct nuclear membrane (Fig. 54). The latter characteristic distinctly separates it from typical vegetative nuclei which have indistinct nuclear membranes.

Other structures were observed (Fig. 55-57), which may be another form of the "male sexual complex" of P. lanceolata and are more numerous than the above described gametangiophores which bear

gametangia with "fertilization tubes". This "other type" of gametangium does not increase in size inside the gametangiophore as described above, but continues to divide until the gametangiophore is filled with many small gametangia (Fig. 55). These gametangia are then liberated from the gemetangiophore (Fig. 55) to an inter-cellular position, where they increase in dimension (Fig. 56) and become funnel shaped as the nuclei within multiply in number (Fig. 57). At this stage the gametes are intensely stained and resemble small nuclei.

It is not clear as yet why the male reproductive complex exists in two forms, but it could be that if the gametangiophore develops adjacent to a carpogonium, then the gametangia mature intra-cellularly, due to female hormonal influence, which may also induce the proliferation of the fertilization tube. Otherwise the gametangia are liberated, develop inter-cellularly and take on a funnel shape when in contact with a carpogonium or a hormone derived from such.

Carpospore Development

The post-fertilization process, as observed here, does not include meiosis, which agrees with the findings of recent workers (Magne 1952; Kito 1966), although older workers (Berthold 1882; Dangeard 1927) claimed that reduction division takes place in the zygote. Many carpogonia were observed to contain a single, globular, dark staining structure (Fig. 58), which has been interpreted here as the fusion nucleus. If the latter interpretation is correct, then it is a prolonged state, as many carpogonia were found to contain this structure. Mitosis then proceeds in the diploid cells (Fig. 65-73), as

described for the haploid vegetative cells, except that the resulting daughter groups of chromatin remain at the poles of cells (Fig. 72) and cytokinesis takes place in a transverse fashion. The carpogonium is then divided into two equal daughter cells (Fig. 59 and 60) and cell division may proceed in either of the two halves or both of them. The second nuclear division results in the cell wall being laid down parallel to the longitudinal axis of the cell (Fig. 61) and transverse divisions then ensue to form the ultimate carpospore-containing structure (Fig. 62). Carpospores, 8-14 microns in size, are liberated (Fig. 63) with a few germinating while still in the thallus (Fig. 64). Conway (1964 b) also observed carpospores germinating in the thallus of P. umbilicalis, as they had become enmeshed in the gelatinous investment which surrounds the cells.

The "fertilization" process, as described here, differs significantly from that previously described for the genus Porphyra. Certain previous workers (Berthold 1882; Dangeard 1927; Ishikawa 1921; Magne 1952) maintained that the spermatia are the cells that are now termed β spores (Conway 1964 b). Berthold (1882), in first describing fertilization in Porphyra, observed a number of spermatia adhering to to thalli of P. leucosticta and below them canals through the mucilage were observed which terminated at what he termed carpogonia. These canals were thought to function as fertilization tubes. Without having observed a gamete pass down the canal, Berthold felt that this represented the male reproductive complex of Porphyra. Other authors (Ishikawa 1921; Kunieda 1939; Magne 1952; Krishnamurthy 1959) had observed the round structures attached to the surface of the thallus but very few of these authors presented convincing evidence of

fertilization. However, Magne (1952) illustrates a prophase nucleus in a fine tube which connects a structure on the surface with a cell in the thallus. Conversely Hus (1902) did not find any evidence fertilization took place in 13 species of Porphyra although he did observe structures on the surface of the thallus and canals projecting from them through the mucilage but stated that these structures were fungal in origin. Similarly, Kunieda (1939) described similar structures on P. tenera and felt they were the result of an infection by oomycetes. The latter author recorded other structures on the surface of the thallus which he felt were spermatia, and suggests yet another type of fertilization for Porphyra. He claims the carpogonia extend a primitive trichogyne through the surface of the thallus which engulfs the spermatia, and mentions that a more complete description of the fertilization process would be forthcoming at a later date; as far as the present authors are aware, it was never published. In contrast, Ishikawa (1921) agreed with the interpretation of Berthold regarding fertilization in P. tenera as he saw many spermatia on the surface of the thallus with a few of them attached to a primitive trichogyne. Again, fertilization was not observed, but subsequent work was promised which, as far as the present authors are aware, never appeared. Krishnamurthy (1959) sampled 50 plants of P. umbilicalis in an attempt to locate the fertilization process. Infrequently, he observed plants with spermatia on their surface but further evidence of fertilization was not procured and a comprehensive cytological study showed a complete lack of diploid cells.

Plants of P. lanceolata, as described here, form βspores, or what have been termed spermatia but at no time were these spores observed in connection with the plants that bear carpospores.

A possible reason for the lack of evidence in the literature regarding sexual reproduction in Porphyra may be due to the existence in the genus of marked periodic rhythms in its developmental process which may well be linked to environmental conditions. A statistically significant periodicity of mitosis (Pringle and Austin unpublished data), exists in P. lanceolata and the rhythm is such that karyokinesis is initiated only between the hours of 1430-1630 in both haploid and diploid cells. Thus, if these plants were fixed at an hour of the day when cells were not undergoing the early stages of mitosis, the diploid cells would not be seen and therefore, assumed to be lacking.

The marked periodicity evident for certain cytological aspects of P. lanceolata suggests that other activities, such as the initiation of sexual reproductive structures, might be a periodic phenomenon controlled by an exogenous factor. Such is the case for Prasiola (Friedman 1963) and Halicystis (Page and Kingsbury 1968). If the initiation of reproductive structures is controlled by an exogenous factor, there is then the possibility that all plants of that species in a particular geographical area, or even in an association, may not have been subjected to the condition required to initiate the response. For instance, meiosis in a mutant of Ulva mutabilis (Thiadens and Zeuthen 1967) is initiated by a change in culture media and a similar phenomenon controls the occurrence of meiosis in Prasiola (Friedman 1963), when under in situ conditions. Similarly, meiosis in Porphyra could be influenced by environmental factors which could

explain the finding of meiosis at the zygote stage by some workers (Berthold 1882; Dangeard 1927) and not by others (Magne 1952; Kito 1966). Because meiosis was not found in carpospore formation in January for material of P. lanceolata described here, does not imply that it may not occur at this location in February or March. It is not novel to suggest that it may not be solely an endogenous factor which initiates the cell to undergo structural change nor a structure to undergo a phenomenon such as meiosis, but that there may be an interaction between the physiology of the cell and environmental factors. In a plant such as Porphyra, where a number of types of reproductive structures are formed consistently it is possible that their formation may be at least partly governed by the capricious nature of the environment.

SUMMARY

P. perforata, when collected in November, was found to average 7.0 cm. x 1.1 cm. in size and most plants had an external morphology which could be described as oblanceolate. Three types of spores were produced which resembled those described by previous authors who have stated that they are asexual. No evidence existed that fertilization had occurred prior to the collection of plants in November as all cells studied contained three chromosomes, the haploid number.

Plants collected in January could be classified into three distinct groups on the basis of their morphology, size and spore production. The average size of the plants was double that of those collected in November. Certain large oblanceolate plants had a pale yellow peripheral edge which extended from the distal end of the plant toward the holdfast. The pale yellow areas consisted of packets of haploid cells which ultimately produced approximately 130 β spores. Other plants were spatulate to oblanceolate and had an irregular sinuate edge which was heterogenous as regards cell type, and also contained groups or packets of cells. Most frequent amongst these were packets of approximately 16 dark, wedge shaped cells which are thought to give rise to α spores. Intermingled amongst these groups of cells were large, pale carpogonia, some of which contained the diploid number of chromosomes. Following what might be interpreted as fertilization, cytokinesis takes place in the carpogonia a number of times, resulting in the formation of carpospores. Fertilization is thought to be effected by "male gamete cells" (spermatia), which are formed

in large numbers within gametangia. The latter were either liberated from a gametangiophore or they matured within the gametangiophore. In both cases, fertilization tubes are formed which liberate a gamete after penetrating the carpogonium. Mitosis, during which the diploid number of chromosomes was observed, was found to be similar to that of the vegetative haploid cells. Mitosis in P. lanceolata was not as Ishikawa (1921) described it for P. tenera, where he felt it resembled in part mitosis and in part amitosis, but similar to that of Rhodomela larix (Austin and Pringle 1968) and not unlike that described for higher plants.

The small oblanceolate plants collected in January resembled the plants collected in November and may result from the germination of asexual spores produced in the Fall. These plants were not observed to give rise to α or β spores but did produce asexual spores which were similar in size and morphology to the asexual spores produced in November.

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TABLE I

Month Plants Collected	Experiments in January	Number of Plants Measured	Average Plant Length for each Experiment	Average Plant Width for each Experiment
	Experiment #1	24	19.52 cm	3.72 cm
	Experiment #2	36	10.5 cm	2.76 cm
January	Experiment #3	11	13.10 cm	3.42 cm
	Experiment #4	24	11.71 cm	2.78 cm
	Experiment #5	24	19.73 cm	3.65 cm
January Total		119	74.56 cm	16.33 cm
January Average			14.91 cm	3.27 cm
November		39	7.23 cm	1.13 cm

Fig. 1-3 Type one spore formation (X900)

Fig. 1 spore mother cells.

Fig. 2 transverse division of mother cell.

Fig. 3 three spores surrounded by gelatinous-like material.

Fig. 4 Vegetative cells in "early-" and "mid-" stages of karyokinesis (X600)

Fig. 5 Field of vegetative cells.
Binucleate cells are in the late telophase of karyokinesis (X240)

Fig. 6 Broken packets of β spores. (X660)

Fig. 7 Liberated β spores. (X2660)

Fig. 8-9 Typical monospores. (X450)

Fig. 8 Thick walled monspores.

Fig. 9 Round and oval shaped monospores.
The oval shaped spores divide to give rise to two monospores.

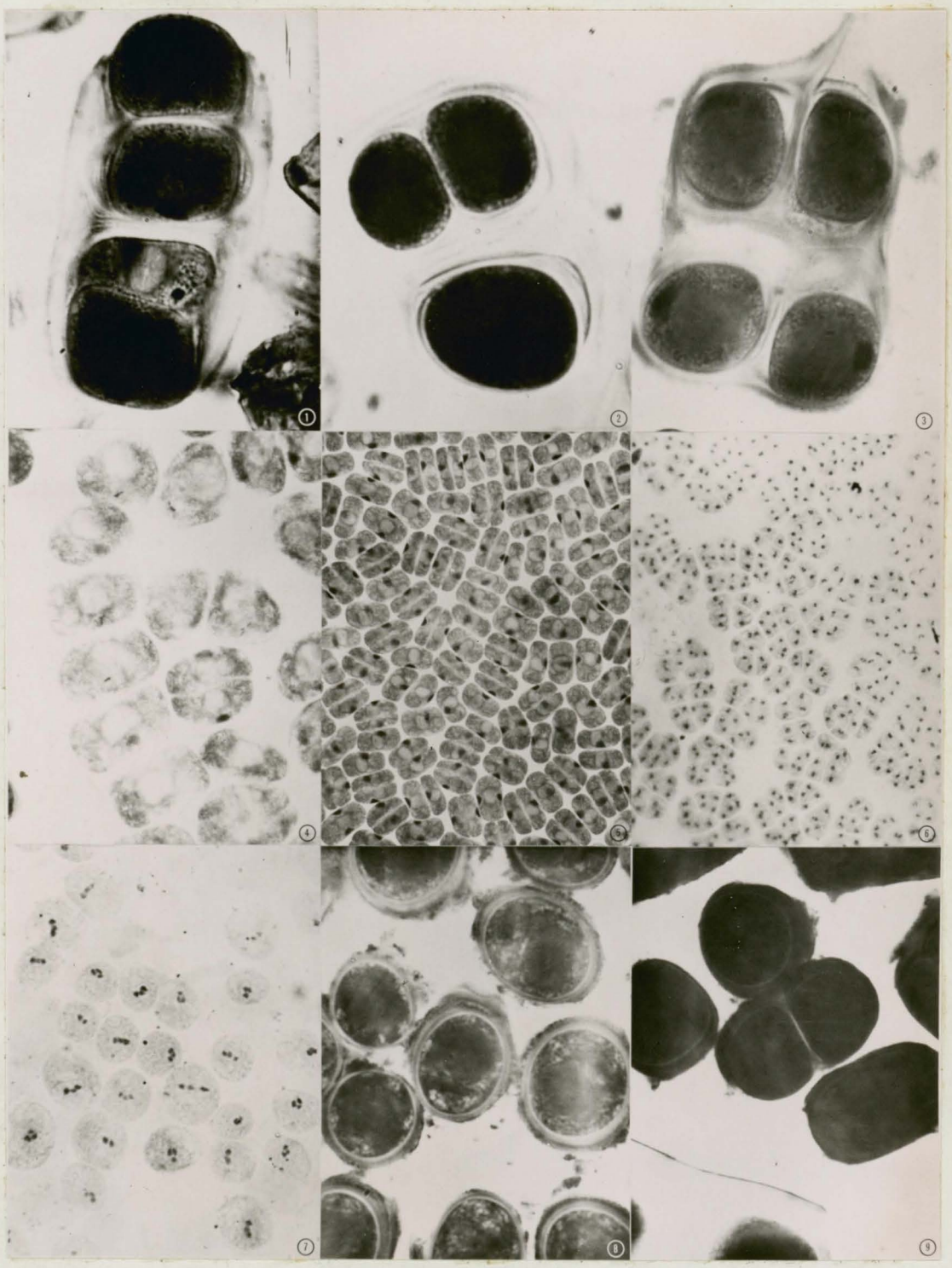
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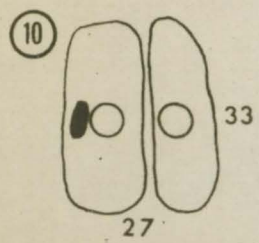
Fig. 10-14 Monospore formation.

Uncircled figures represent size of cell in microns.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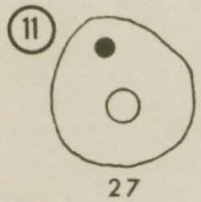
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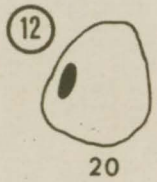
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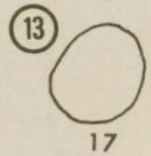
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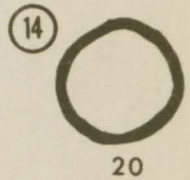
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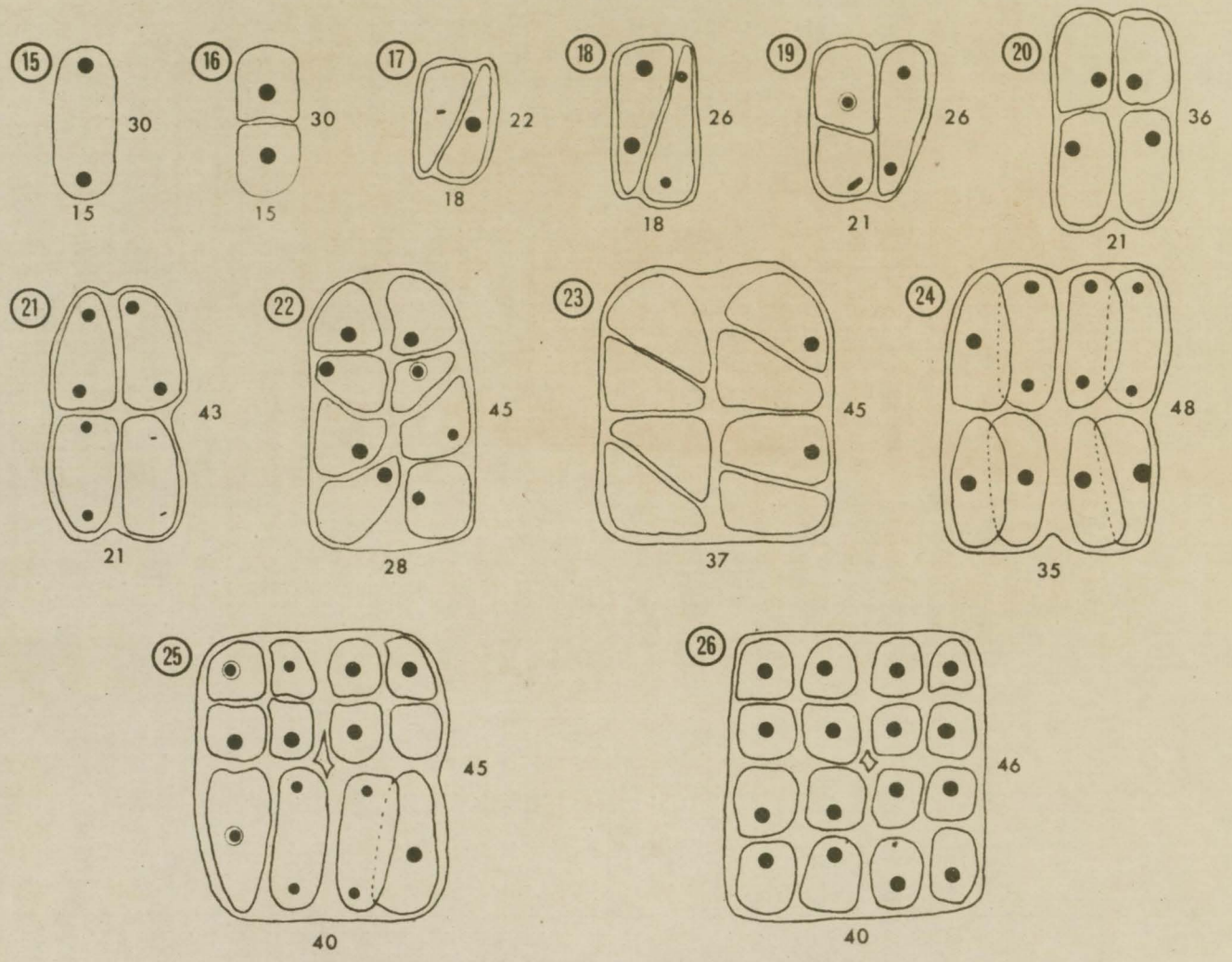
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Fig 15-26 Formation of Type III spores.

Uncircled numbers represent the size of the structure in microns.

Fig. 27 Vegetative cell with a granular pyrenoid (P) and an interphase nucleus (N). (X1400)

Fig. 28-35 Haploid phases of Karyokinesis.

Fig. 28 Early prophase. (X4000)

Fig. 29 Mid prophase. (X4000)

Fig. 30 Late prophase. (X4000)

Fig. 31 Prometaphase. (X4000)

Fig. 32 Metaphase (X4000)

Fig. 33 Early anaphase (X4000)

Fig. 34 Early telophase (X2000)
(N) nucleus (P) white pyrenoid

Fig. 35 Late telophase (X2000)

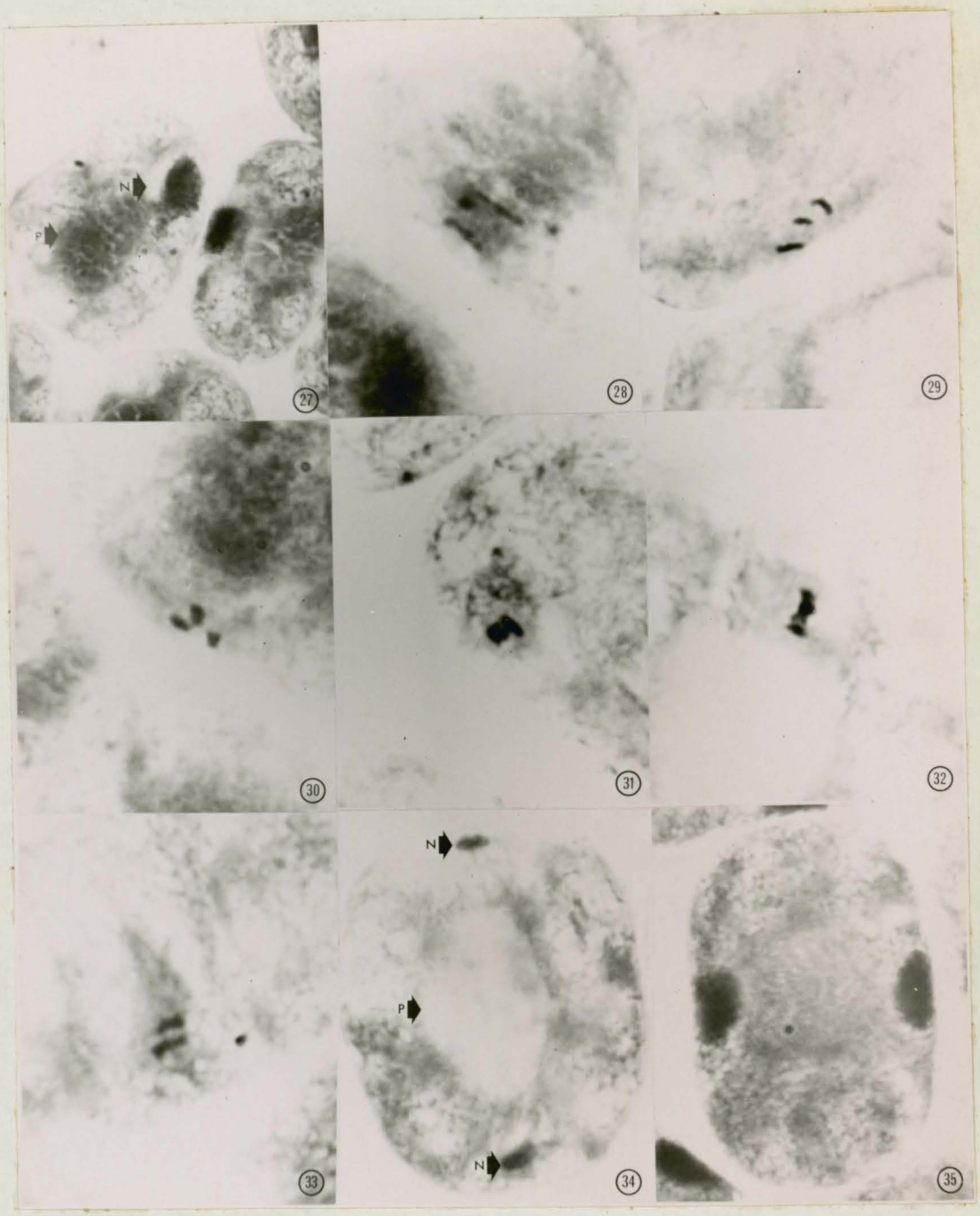
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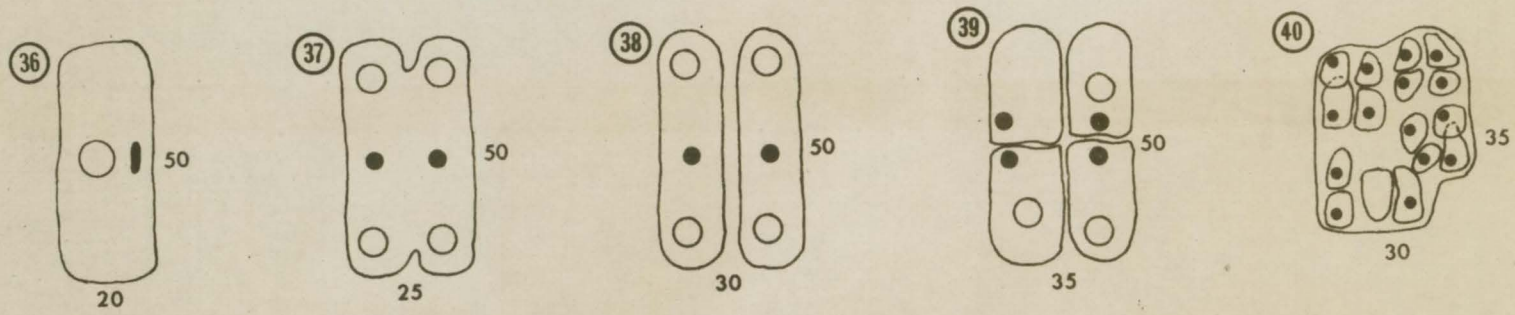
Fig. 36-40 β spore formation. Uncircled numbers are size of structure. Black circles = nucleus
White circles = pyrenoid

Fig. 36 β spore mother cell.

Fig. 37 Longitudinal division of the β spore mother cell.

Fig. 39 Transverse division.

Fig. 40 Packet of 16 cells in typical irregular appearance.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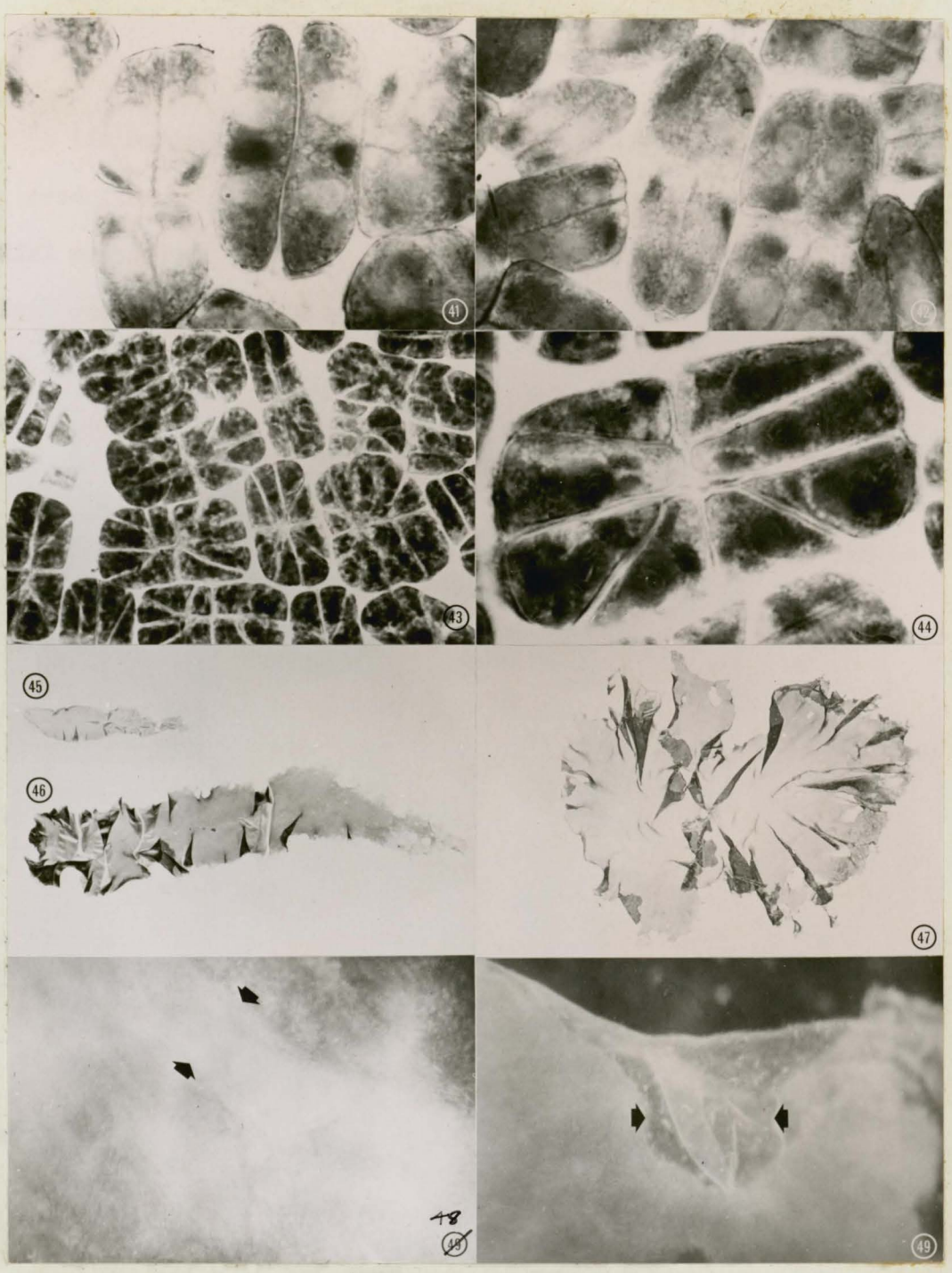
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Fig. 41-44 spore formation.

Fig. 41 Large mother cell following longitudinal cell division. Pyrenoids of each cell have split which preceeds the second division (X1000).

Fig. 42 Group of four cells following transverse division. Third mitotic division proceeding (X1000).

Fig. 43 Packets of cells which may give rise to spores (X500).

Fig. 44 Packet of cells (X1500).

Fig. 45 Typical November plant. (X1/3)

Fig. 46 Typical January plant which give rise to β spores. (X1/3)

Fig. 47 Typical January plant which bears sexual reproductive structures and α spores. (X1/3)

Fig. 48 Peripheral white edge (between arrows) of January plant which bears β spores. (X4)

Fig. 49 Transparent peripheral areas (between arrows) of the thallus of plants which give rise to α spores in January. (X4)

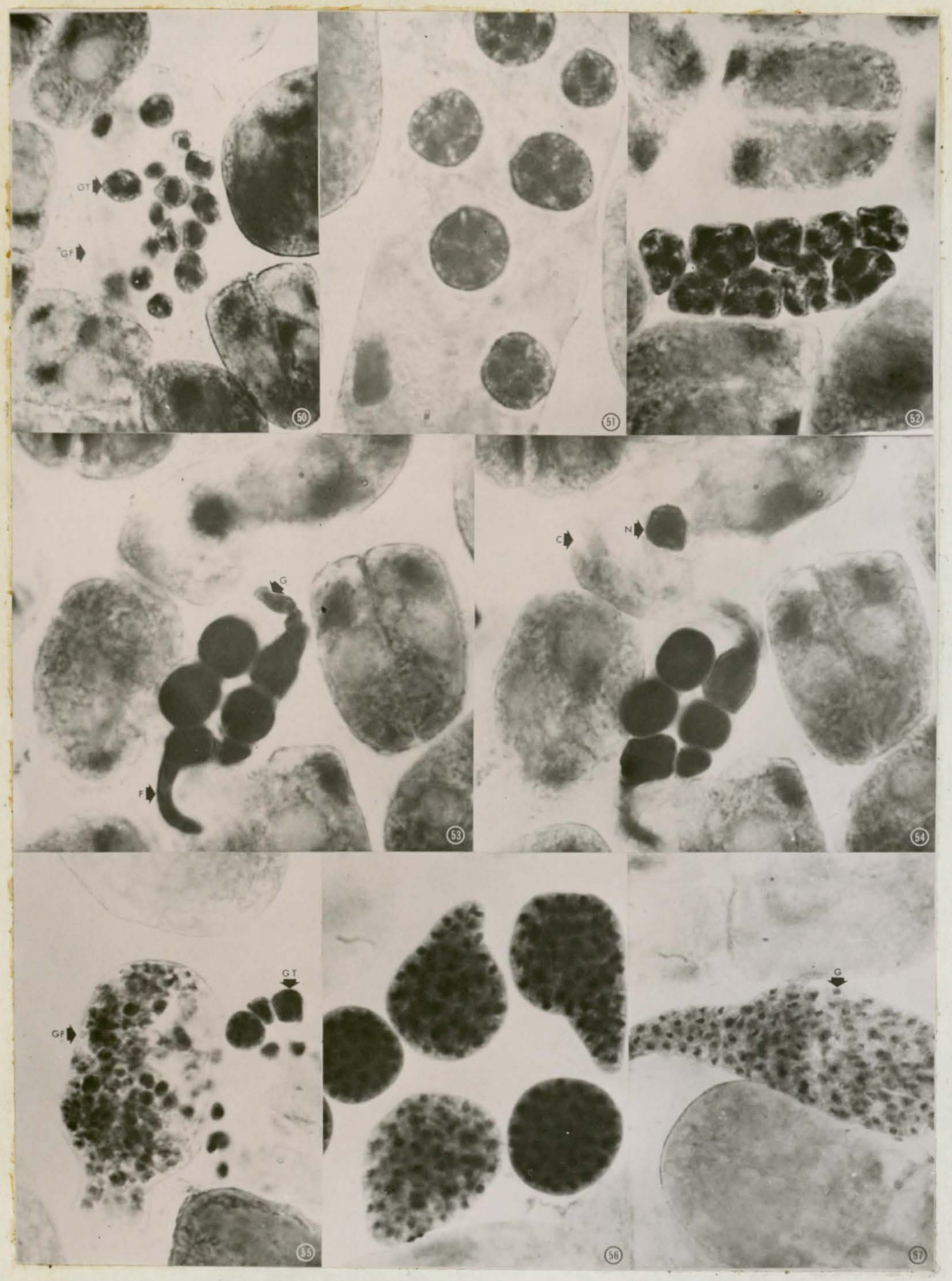
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Fig. 50-57 Formation of male reproductive structures.

Fig. 50 Male gametangiophore (GF) bearing black circular structures which are gametangia (GT). (X1100)

Fig. 51 Gametangiophore bearing circular gametangia. (X2000)

Fig. 52 Gametangiophore bearing nine gametangia. Each gametangia contains from 3-5 gametes. (X1100)

Fig. 53 Gametangiophore containing six gametangia each one bearing a number of gametes. Two of the gametangia have produced fertilization tubes (F) the upper one having penetrated a carpogonium. A gamete (G) is visible in it. (X1100)

Fig. 54 A possible male or fusion nucleus (N) inside the carpogonium (C). (X1100)

Fig. 55 A male gametangiophore (GF) liberating gametangia (GT) from an oepning on the lower end of the structure. (X1500)

Fig. 56 Expanded male gametangia in an intercellular location. Three have taken on a funnel shape. Each gametangium bears a number of gametes. (X2000)

Fig. 57 A large funnel shaped gametangium containing a number of easily visible gametes (G). (X2000)

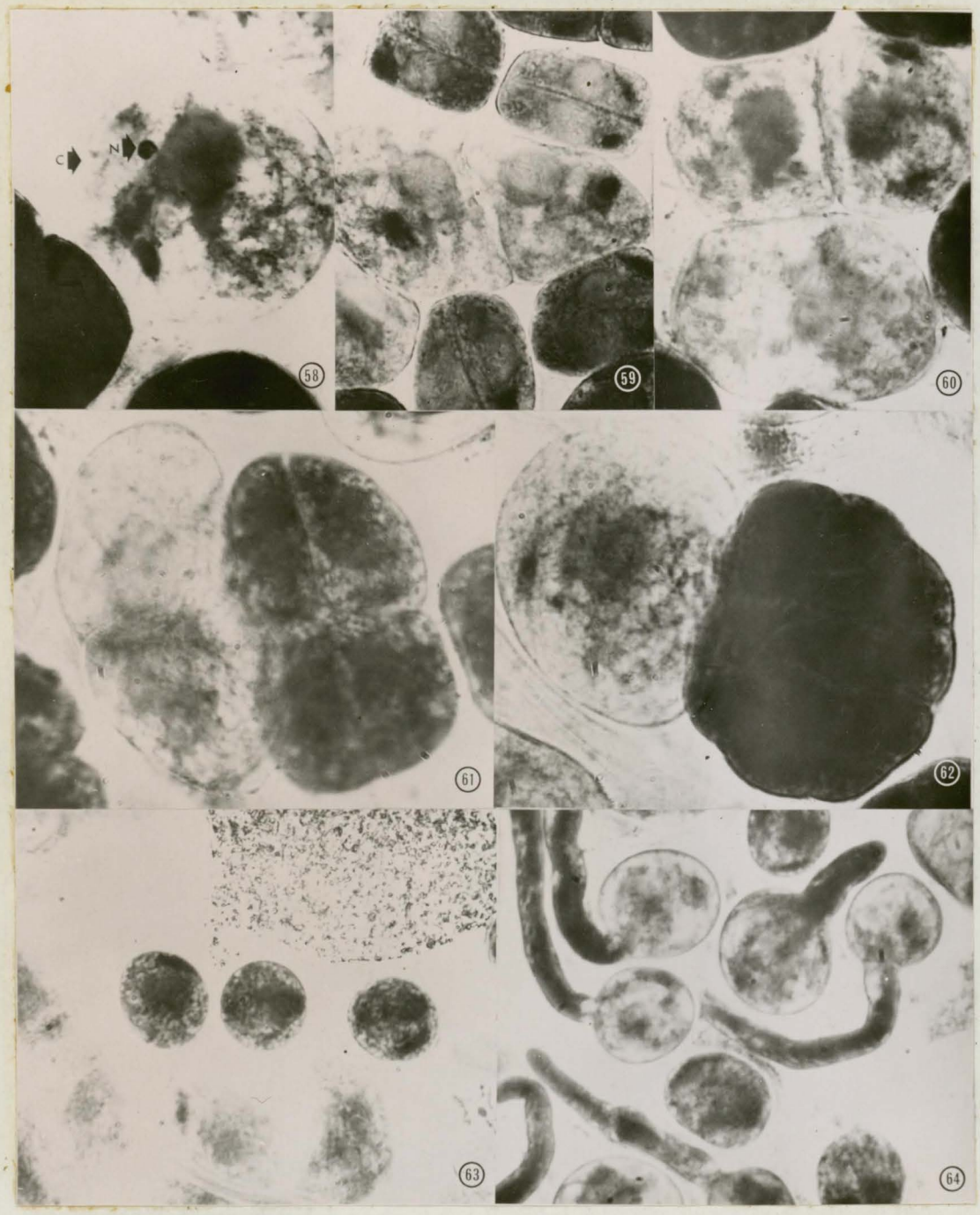
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Fig. 58-62 The post-fertilization process.

Fig. 58 A light-staining circular carpogonium (C) which bears a black nucleus (N) which may either be the male or fusion-nucleus (X1000).

Fig. 59 First division (transverse) of a carpogonium (X1000).

Fig. 60 Two lightly-staining carpogonia one of which had undergone cell division (X1000).

Fig. 61 Two carpogonia one of which has undergone two cell divisions (X1200).

Fig. 62 A carpogonium which continued division on only one side, the dark side (X1200).

Fig. 63 Possible carpospores (X1500).

Fig. 64 Carpospores which have germinated in the thallus (X1500).

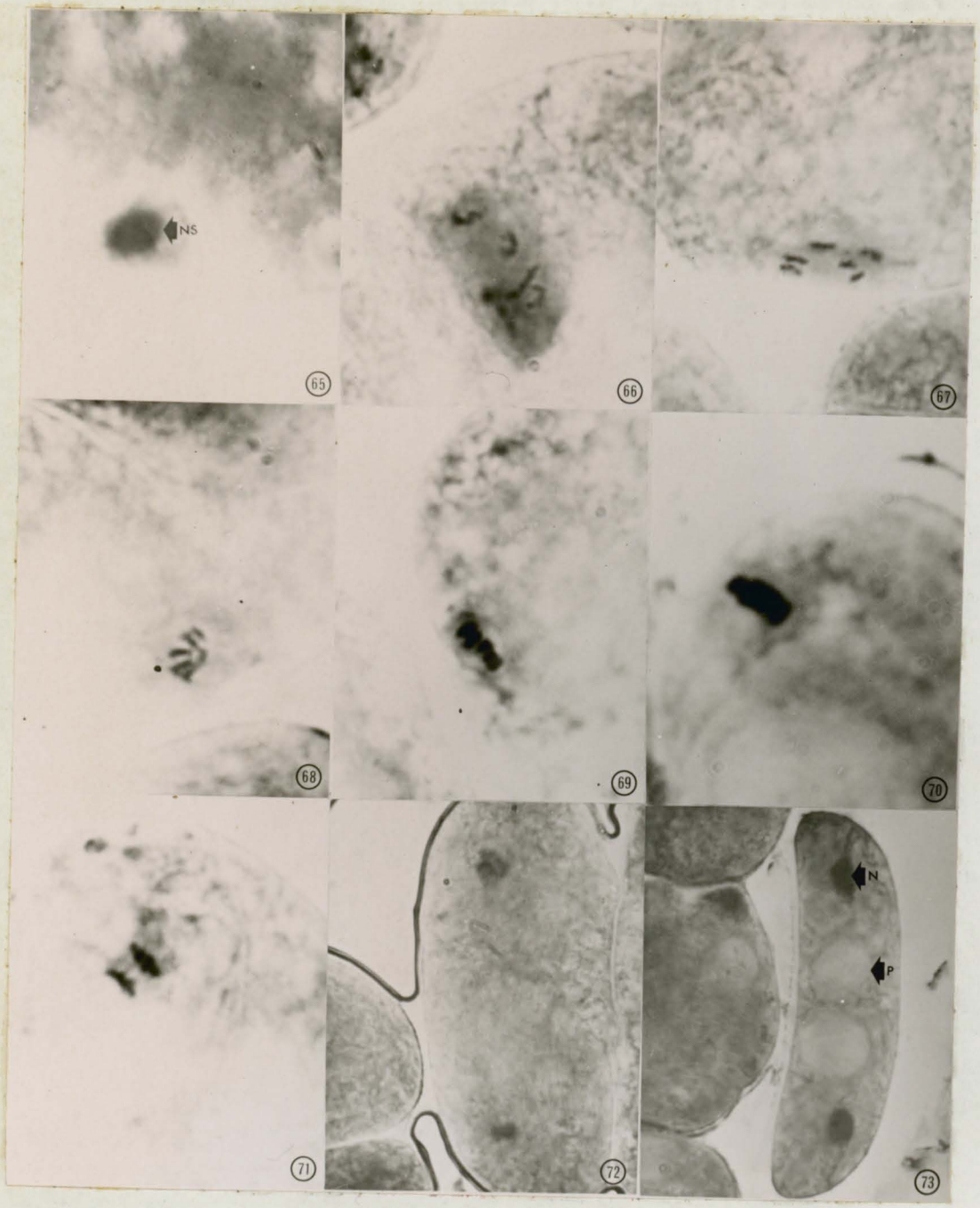
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Fig. 66-73 The stages of diploid karyokinesis.

Fig. 65 Interphase nucleus with a prominent nucleolus (NS) (X4000)

Fig. 66 Early prophase (X4000).

Fig. 67 Mid prophase (X4000).

Fig. 68 Late prophase (X4000).

Fig. 69 Prometaphase (X4000).

Fig. 70 Metaphase (X4000).

Fig. 71 Anaphase (X4000).

Fig. 72 Telophase (X1300).

Fig. 73 Telophase with distally located nuclei (N) and lightly staining pyrenoids (P). The latter having just divided prior to cytokinesis (X1200).

APPENDIX

A PERIODIC DISTRIBUTION OF MITOTIC INDEX IN *PORPHYRA LANCEOLATA* AND *RHODOMELA LARIX*

Over the years most nuclear cytologists working on intertidal marine algae, have collected their plant material at low tide when the plants become readily available. Other workers have stated that environmental conditions affect the temporal occurrence of mitosis and collect their plants at specific times of day or night and at various combinations of tidal level. However to the authors' knowledge no attempt has been made to acquire quantitative data on the distribution of karyokinetic figures over a 24-hour period either in the lab or in situ. Evans (1), working on three species of Fucus, apparently fixed his material throughout all seasons, at all times of day and night during which times the plants were subject to varying environmental conditions. No periodicity in the occurrence of mitosis was evident although the author did not use quantitative methods.

However, quantitative data on diurnal mitotic patterns have been obtained for various higher plant apical and root meristems (2). Certain of these plants showed peaks in karyokinesis during a 24-hour period, but most stages of mitosis were observed at all hours of the day and night; a synchrony in mitosis was thus not evident.

Daily patterns in the incidence of mitotic figures have been studied extensively in unicellular algae (3), and the cells of most species maintain a natural periodicity which can be synchronized (3) under rigid culture conditions. The dinoflagellate Gonyaulax polyedra (4), exhibits an endogenous rhythm in cell division, but each cell in the population does not divide every day. This partial

synchrony of cell division is referred to as phased cell division.

This short note reports the initial findings in a programme involving the sequential sampling and fixation, over 24-hour periods, of selected marine algae from various levels of the littoral and sub-littoral, using aqua lung techniques, together with the monitoring of gross environmental factors. Reported here are preliminary data on Porphyra lanceolata (Setchell & Hus) G.M. Smith, a high-intertidal plant and Rhodomela larix (Turner) C. Agardh, a mid-intertidal plant. The plants were fixed and stained according to the procedure of Austin (5).

P. lanceolata was collected in situ every two hours for a 24-hour period on November 2-3, 1967. Quantitative data on the occurrence of mitosis in these plants was obtained from three, $\frac{3}{4}$ mm x 2 mm, squashed and stained rectangles of material removed from the peripheral edge of each plant. The cells from each individual squash were sampled using a Zeiss Integrating Eyepiece which randomly designated 25 cells per high power microscope field. The nucleus of each of these cells was categorized as either interphase or as one of the stages of karyokinesis and since five fields per squash were sampled, 125 cells per squash or 375 cells per plant were scored. Three plants per fixation hour were sampled in this manner in an attempt to acquire statistically significant data. A plant mitotic index was calculated and combined for the three-plants taken per fixation-hour, yielding one mitotic index per fixation hour.

The vegetative cells of P. lanceolata exhibit a distinct periodicity in cell division (Fig. 2, Part II), and the peaks are statistically significant at the 5% level (Table 1). The rhythm is not of the type found in higher plants, as all stages of mitosis are not found throughout the 24-hour period, however it does resemble the phased cell division rhythm found in Gonyaulax polyedra (4). Karyokinesis was initiated somewhat later than 1430 and at 1630 the prophase stages of mitosis together with prometaphase, metaphase, anaphase and early telophase were observed in far greater numbers than at any other time during the entire 24-hour period, (Fig. 2, Part II, Tables 2 and 3). The cells observed in the "early-" and "mid-" stages of karyokinesis rapidly decreased in number in the fixation hours immediately following 1630 until by 2030 there are virtually none in evidence. The interval between the completion of karyokinesis and the initiation of cytokinesis is prolonged in this particular material, thus there were far more cells observed in the late telophase stage (Fig. 2, Part II), than in the other phases. The late telophase stage reached a maximum at 0230 and then decreased rapidly and at 1430 not one single cell was observed in any one of the stages of karyokinesis.

R. larix was collected in situ on July 27-28, 1966 and after a preliminary cytological exploration young ramuli at the apex of the plant were chosen for mitotic investigation throughout the study. These small, apical ramuli, when compared to the larger ramuli found throughout the rest of the plant, contained a far greater number of cells with nuclei in karyokinesis. There was no gradient in mitotic index throughout the first millimeter of these ramuli but the distal

250 microns of each chosen ramulus was removed and discarded due to the small cell size and the adjacent 500 microns was squashed, stained and quantitatively assessed for mitotic index. Squashes were made from three ramuli per plant, and three plants per fixation hour were sampled for cells with nuclei in karyokinesis. The sampling of individual squashes and the number of cells counted were as described for Porphyra.

A periodicity in mitosis is evident for R. larix (Fig. 2, Part I) which is statistically significant at the 5% level (Table 4), and resembles the mitotic rhythms found in higher plants, thus it is asynchronous and not a phased type of rhythm. Cells are found at each fixation period with nuclei in all stages of mitosis, but at certain times of day there is a greater number of nuclei actively engaged in karyokinesis.

Further studies from collections made both in situ and under laboratory conditions are under way in an attempt to investigate the nature of the rhythms. Various environmental parameters were monitored during the work and data correlating the appearance of mitosis with certain gross environmental conditions will be reported elsewhere.

1. Evans, L., Ann. Bot., 26, 345 (1962)

2. Erickson, R.O., In Synchrony in Cell Division, Interscience 1 (1964).

3. Tamiya, H., Ann. Rev. Plant Physiol., 17, 1 (1966).

4. Sweeney, B.M., and Hastings, J.W., J. Protozool., 5, 217 (1958).

5. Austin, A.P., Stain Tech., 34, 69 (1959).

Table 1.

P. lanceolata fixation data, showing the mean values for three plants per fixation time and its rank in relation to other fixation times. Statistical significance between means (Duncan's test) at the 5% level is shown.

Minor Fixation Time	Mitotic Index per Minor Fixation Hour	Rank		
0830	0.0	a		
1430	0.0	a		
0830	0.17	a		
1230	0.18	a		
1030	0.27	a		
0630	0.35	a		
0430	2.58	a	b	
0030	5.38		b	c
1890	5.87		b	c
2230	6.31		b	c
2030	7.20		b	c
0230	9.33			c
1630	9.42			c *

* significant difference (5% level) at termination of each series of letters

Table 2.

The rank of the treatement means for the number of cells with nuclei in a stage of prophase observed per minor fixation time. Statistical significance between means (Duncan's test) at the 5% level is shown.

Minor Fixation Time	Treatment Means ranked in size	Significance Rating at 5% level		
0830	0	a		
1030	0	a		
1230	0	a		
1430	0	a		
0030	0	a		
0430	0	a		
0630	0	a		
0830	0	a		
2230	0.333	a		
2030	3.00	a	b	
0230	4.00	a	b	
1830	7.00		b	
1630	14.67			c *

* significant difference (5% level) at termination of each series of letters

Table 3.

The rank of the treatment means for the combined totals of cells in prometaphase, metaphase, anaphase and early telophase stages (mid-mitotic stages) observed per minor fixation time. Statistical significance between means (Duncan's test) at the 5% level is shown.

Minor Fixation Time	Treatment Means ranked in size	Significance Rating at 5% level
0830	0	a
1030	0	a
1230	0	a
1430	0	a
0430	0	a
0830	0	a
0630	0.333	a
2230	0.666	a
0030	0.666	a
0230	1.666	a
2030	6.00	b
1830	6.33	b
1630	16.33	c *

* significant difference (5% level) at termination of each series of letters

Table 4.

R. larix fixation data, showing the mean mitotic index values for three plants per fixation time and its rank in relation to other fixation times. Statistical significance between means (Duncan's test) at the 5% level is shown.

Time of day	Mitotic Index per Fixation Time (three plant mean)			
1100 hrs.	42.56%	a		
0100 hrs.	51.42%	a		
0500 hrs.	68.16%		b	c
1445 hrs.	68.75%		b	c
2045	82.67%			c *

* significant difference (5% level) at termination of each series of letters

VITA

Surname: PRINGLE Given Names: JOHN DOUGLAS

Place of Birth: VANCOUVER, B. C. Date of Birth: October 22, 1940

Educational Institutions Attended, with Dates of Entering and Leaving:

UNIVERSITY OF BRITISH COLUMBIA	1962	to	1963
UNIVERSITY OF VICTORIA	1964	to	1968

Degrees, Diplomas, Etc. Awarded, with Dates and Names of Institutions:

B.Sc.	1966	University of Victoria

Honors and Awards:

University of Victoria Bursary, 1966/67

University of Victoria Fellowship, 1967/68

N. R. C. Fellowship, 1968/69

Publications:

Mitotic Index in Selected Red Algae in situ

I. Preliminary Survey.

2758 Dunlevy,
Victoria,
British Columbia,
Canada.

August 19th 1968.

To the Supervisory Committee of John D Pringle's
M.Sc., Programme.

I have read the material being submitted by John D. Pringle for the M.Sc., at the University of Victoria and find it well presented and fully adequate for the degree for which it is submitted.

signed..... 

L. G. Saunders

Professor Emeritus, University of
Saskatchewan, Saskatoon.