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Dark Survival of Autotrophic, Planktonic Marine Diatoms

T. J. Smayda and B. Mitchell-Innes

Graduate School of Oceanography, University of Rhode Island; Kingston, Rhode Island, USA

Abstract

A general ecological problem is considered: how long can a photoautotrophic microalga, incapable of producing a resting spore (stage), retain its viability in the dark following removal from the euphotic zone? Nine coastal diatoms, including some capable of producing resting spores, were kept in the dark for 90 days at 15 °C, and their growth (viability) checked at periodic intervals upon reillumination. Seven of the 9 diatoms retained their viability for 90 days; generation time of illuminated cells then ranged from 2.5 to 10 days. *Skeletonema costatum* survived only 7 weeks of darkness. Based on the present and published observations, dark survival of this species is inversely related to temperature; it survives at least 24 weeks at 2 °C, and from 1 to 4 weeks at 20 °C. None of the species was observed to grow in the dark. The effects of temperature and light on dark survival, and of darkness on the chemical composition and photosynthesis following reillumination as reported in the scattered literature are evaluated. Together with the present observations, it is suggested that dark survival of photoautotrophic microalgae: (1) varies between species; (2) may be temperature dependent in some species, as in *S. costatum*; (3) may be prolonged by periodic illumination at subcompensation intensities for photosynthesis, as shown in *Dunaliella tertiolecta*. The potential ecological significance of these findings is also considered, should these *in vitro* results apply to natural populations.

Introduction

Many autotrophic species of marine phytoplankton undergo pronounced seasonal variations in abundance; some even disappear temporarily from the pelagic community. In some instances, this apparent disappearance is an artifact; the organisms are not detected by the techniques routinely used. Conceivably, some eurythermal species inhabiting colder waters and tropical species capable of active growth at very low nutrient concentrations maintain viable populations within the euphotic zone during their apparent absence, possibly as a component of the "hidden flora" (see Rahat and Dor, 1968). Other, less opportunistic species, however, may indeed disappear from the euphotic zone which would create for them a survival problem during periods of inimical growth, at least where autochthonous populations are concerned. Sea-ice and pelagic microalgae in polar regions must also survive long periods of winter darkness.

The production of resting spores by phytoplankton is commonly viewed as an adaptation allowing survival during such unfavorable periods. However, many species are not mero-planktonic (Smayda, 1958). For example, the ubiquitous, eurythermal diatoms *Skeletonema costatum* and *Asterionella japonica* are among those coastal species which apparently do not produce resting spores. The likelihood that these or more stenothermal species sink from the euphotic zone during unfavorable growth conditions (which might last for months) prompts the following question. How long can an autotrophic phytoplankter incapable of producing a resting spore (stage) retain its viability in the dark? Although many benthic diatoms are capable of facultative heterotrophy (Lewin and Lewin, 1960), the limited available evidence suggests that pelagic diatoms are not so endowed (Sloan and Strickland, 1966). The situation is more complex in diverse microflagellates (Yentsch and Reichert, 1963; Pintner and Provasoli, 1968).

Apart from the ecological consequences associated with this question of dark survival, it is relevant to such practical problems as the maintenance of microalgae in culture and their transfer interval. The latter prompted studies on dark survival by Antia and Cheng (1970) and Umebayashi (1972). Cursory observations on the dark survival of marine diatoms are also presented by Matsue (1954), Curl and McLeod (1961), Takano (1963), Ignatiades and Smayda (1970) and Bunt and Lee (1972). This study will report on such observations, using representative mero-planktonic and holo-planktonic coastal marine diatoms.

Materials and Methods

The 9 species of diatoms used in this study were isolated from Narragansett Bay into Guillard's (Guillard and Ryther, 1962) medium *f*/2. Unialgal cultures were maintained at 15 °C, under 1000 ft-c (footcandles) continuous illumination, in 125 ml Erlenmeyer flasks which contained 50 ml of medium (30‰S). For use in the experiments, cultures were grown at the above conditions for 1 week and, while still in exponential growth, then darkened by wrapping the flasks with aluminum foil. Caution was taken to eliminate light leaks. The darkened flasks were then incu-

bated for 90 days at 15 °C. The population density was enumerated prior to darkening using a Sedgwick-Rafter counting chamber.

At periodic intervals, an aliquot of 0.3 to 0.4 ml was transferred from the darkened flasks into 20 × 150 mm test tubes containing 10 ml of fresh media. Only the aluminum foil enclosing the screw cap of the darkened flask was removed during this step — carried out in a transfer hood. The darkened population was probably then exposed to low light intensity for about 30 sec. Following inoculation, the test tubes were incubated under continuous illumination (1000 ft-c) at 15 °C, and the population enumerated usually after 2 days initially, and at somewhat longer intervals thereafter when growth was less vigorous and/or delayed. Observations on the general condition of the cells were also made.

The inoculum from the darkened parent cultures ranged from 100 to 1800 cells ml^{-1}, depending on the species. From this and the population densities produced upon reillumination, the mean daily division rate ($\overline{K}$) was calculated from

$$K = \ln \frac{C_t}{C_o} \left(\frac{1}{t \ln 2} \right),$$

where C_t and C_o are cell concentrations at times t and o, and t is time in days. It was impossible to determine with confidence the abundance of viable cells remaining in the darkened flasks with time. Recognition of viable cells became difficult and unreliable due to cellular abnormalities, general deterioration, and an increasing amount of particulate material. Thus, the number of viable cells in the inoculum (100 to 1800 cells ml^{-1}) established initially for a species was considered to remain constant throughout the experiment. This assumption is obviously incorrect, and it is likely that the growth rates were underestimated for populations reilluminated after the first week of dark incubation. This is partially demonstrated by the following results. After 6 days of dark incubation, the number of viable cells of *Chaetoceros curvisetus* recognizable decreased from 760 to 240 cells per ml. The effect of using these different inoculum levels in computing its growth rate is evident (Fig. 2). Despite this shortcoming, the relative differences in growth with time and between species are presented only to provide some indication of their potential survival characteristics in darkness, particularly duration.

The potential influence of inorganic nutrient and vitamin enrichment on the dark survival of *Ditylum brightwelli* and *Skeletonema costatum* was also examined. Each species was grown at the environmental conditions stated previously in 250 ml Erlenmeyer flasks containing *f*/2 media. After 7 days, the contents were sub-divided into two equal portions (50 ml each) in 125 ml Erlenmeyer flasks; one flask was enriched to yield Guillard's medium *f* levels, while the other was not modified. Both flasks were then darkened by wrapping with aluminum foil, incubated and monitored, as described previously. Periodically, an inoculum from the darkened flasks was added to paired culture tubes containing medium *f* and *f*/10. One set was incubated at 1000 ft-c of continuous illumination and the other at 200 ft-c. This experiment was designed to examine whether dark survival was favored by enrichment, and to examine generally the effect of enrichment and light intensity on growth, following transfer from the dark.

Results

Three general types of dark survival patterns are suggested by the experiments (Figs. 1, 2):

Type 1. Following maximum growth ($\overline{K}$ max) capability during the first week in darkness, a progressive, curvilinear decrease in $\overline{K}$ occurs upon reillumination; $\frac{1}{2}\,\overline{K}$ max was reached after 2 to 3 weeks: *Asterionella japonica, Lithodesmium undulatum, Skeletonema costatum,* possibly *Thalassiosira* sp. (Clone No. 28) (Figs. 1, 2).

Type 2. A high, constant $\overline{K}$ capability occurred during the first 2 weeks in darkness, followed by a precipitous, almost overnight, decrease in $\overline{K}$ to more or less constant levels thereafter: *Ditylum brightwelli* (Fig. 1B).

Type 3. Intermediate between Types 1 and 2. A high growth capability and a less pronounced curvilinear decline than in Type 1 during the first 3 to 4 weeks in darkness is followed by a precipitous decrease to a low, constant growth potential thereafter: *Chaetoceros didymus, Chaetoceros curvisetus, Thalassiosira gravida* (Figs. 1B, C; 2).

The poor response of *Stephanopyxis turris* does not fit into any of these categories.

Irrespective of their response, after 1 week in darkness all species exhibited very high division rates upon reillumination ($\overline{K} = 1.8$ to 2.8); the maximum growth potential of *Chaetoceros didymus* ($\overline{K} = 2.1$) and *C. curvisetus* ($\overline{K} = 4.2$) occurred after 2 weeks of dark incubation. Following the period of $\overline{K}$ max, an increasing loss in viability accompanied prolonged exposure to darkness which reduced the potential growth rate to about $\overline{K} = 0.1$ after 35 to 90 days in the dark, depending on the species. Nonetheless, all species remained viable after 90 days in the dark, except *Skeletonema costatum* which did not survive beyond 7 weeks (Fig. 1A). The growth potential of *Thalassiosira* sp. (Clone 28) reached a minimum after 90 days. The slight increase in $\overline{K}$ apparent after 1 to 2 months of continuous darkness found for the other species which survived 90 days of darkness (Figs. 1, 2) is probably an artifact, since a constant incubation period was not used throughout the experiment. (During this period, 14 days elapsed before the cultures could be examined, in contrast to the normal period of 7 days subsequently.) However, the calculated generation time

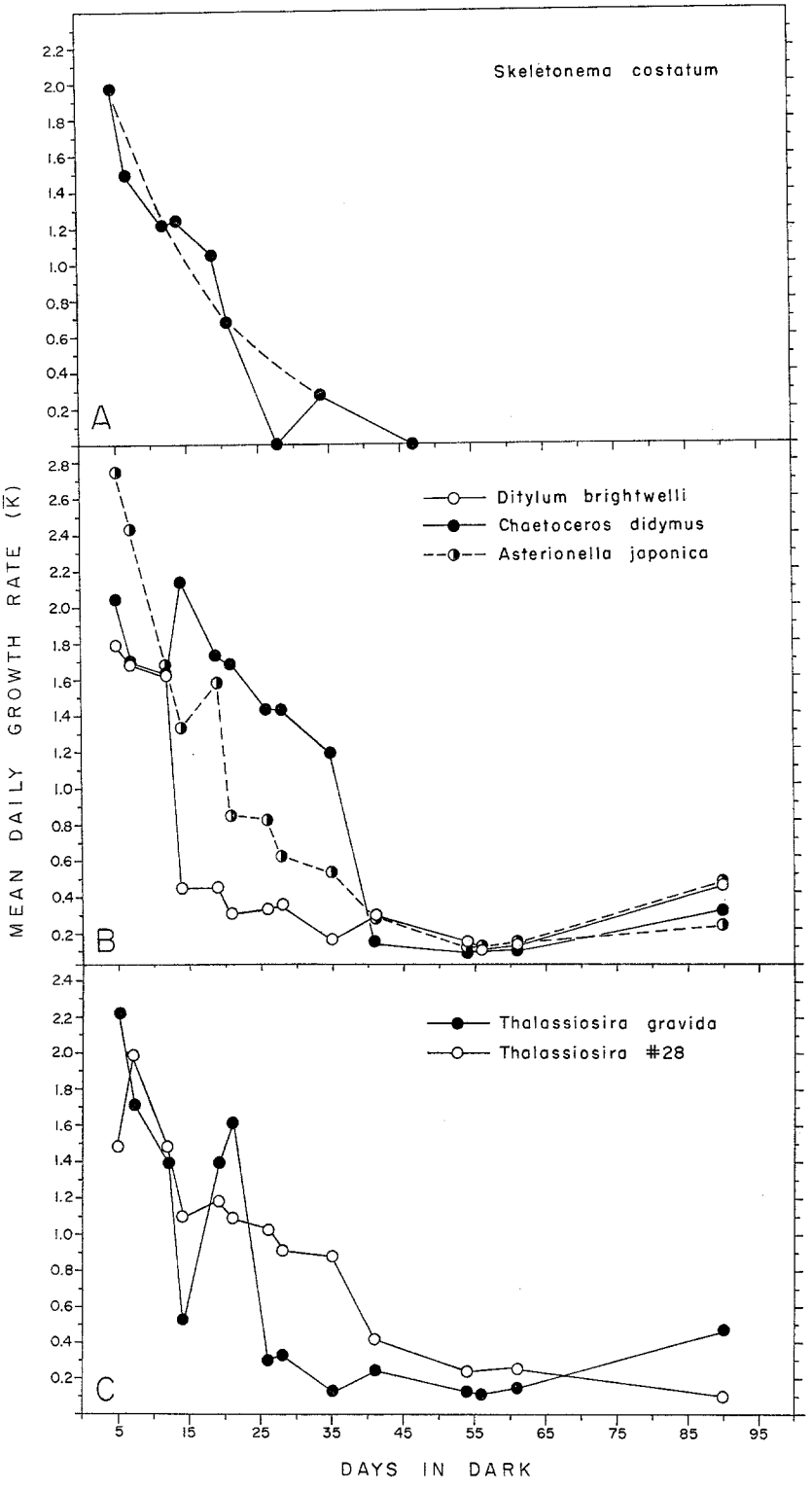

Fig. 1. Mean growth rates ($\bar{K}$) of 6 diatom species incubated at 15 °C, 1000 ft-c continuous illumination, and in *f*/2 medium, following inoculation from cultures stored for up to 90 days in darkness at 15 °C. Broken line for *Skeletonema costatum* response (A) represents an idealized response

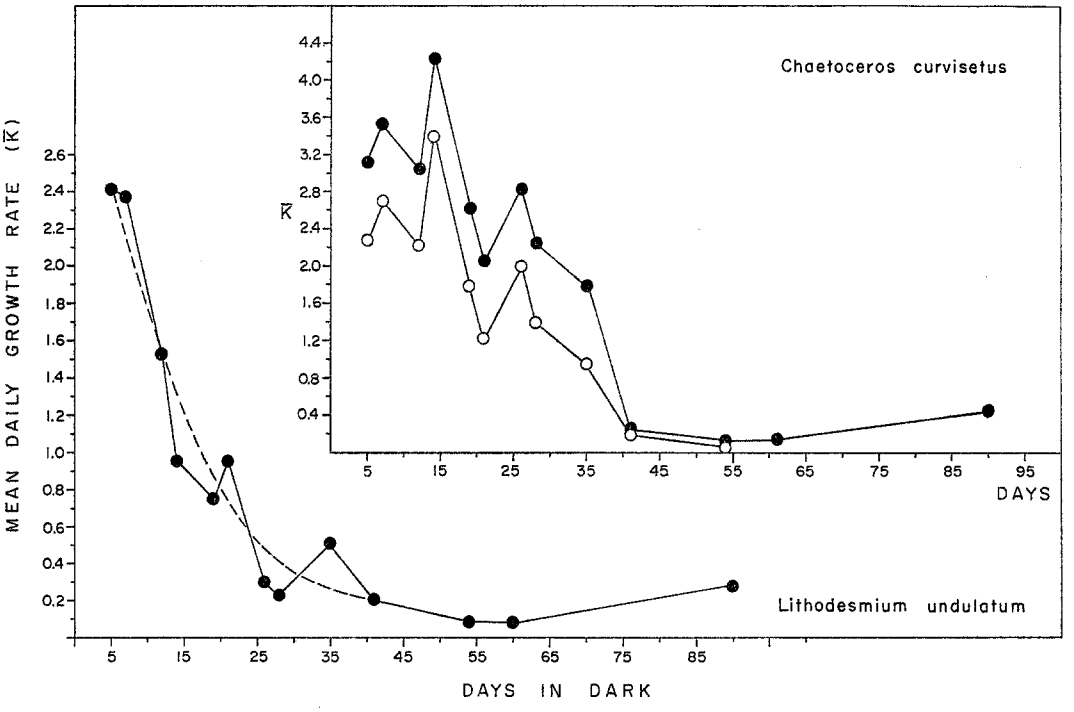

Fig. 2. Mean growth rates ($\overline{K}$) of *Chaetoceros curvisetus* and *Lithodesmium undulatum*. For further details see legend to Fig. 1. Dual response illustrated for *C. curvisetus* is based on inoculum level of 760 cells ml^{-1} (filled circles) and 240 cells ml^{-1} (open circles)

upon reillumination of 2.2 to 5 days is probably more representative of their growth capacity then than the 10 days based on $\overline{K}$ calculated over 14 days.

In addition to the decrease in viable cells accompanying prolonged exposure to darkness, morphological changes occurred. *Skeletonema costatum* began to lyse after 5 weeks; it then became especially difficult to distinguish living cells from particulate debris. The culture virtually disintegrated after 6 weeks. *Asterionella japonica* became increasingly chlorotic, and after 25 days the chains broke up into individual cells in which the "Kopfpol" end was lost or became shorter and curved. The species of *Thalassiosira* likewise became chlorotic; elongated cells resulting from uncompleted cell divisions were commonplace. The species of *Chaetoceros* formed resting spores, and the cellular contents of *Ditylum brightwelli* and *Lithodesmium undulatum* rounded off soon after placement in the dark. After about 1 month in the dark, the unialgal species became encrusted with particulate matter.

Table 1 shows the effect of nutrients (equivalent to medium *f* enrichment) on the dark survival of *Skeletonema costatum* and *Ditylum brightwelli* and on their growth when reilluminated after 5 days at 200 and 1000 ft-c. Growth was also checked after 2 days, but it was usually not evident in *S. costatum*, unlike *D. brightwelli*. After 4 days in the dark, *S. costatum* grew better at 200 ft-c ($\overline{K} = 0.79$ to 0.92) than at 1000 ft-c ($\overline{K} = 0$ to 0.64), independent of the pre- and post-enrichment treatments (Table 1). When darkened for 18 days, growth of the unenriched population was not detected in *f*/10 at either light intensity, unlike in the other treatments. The darkened population looked moribund, and was barely distinguishable from debris. The growing, illuminated cells also appeared unhealthy. Cells darkened for 32 and 46 days did not grow in any treatment.

Enriched, darkened cells looked more robust initially than those unenriched, but enrichment did not prolong *Skeletonema costatum*'s survival in these experiments.

Darkened cells of *Ditylum brightwelli* appeared healthy after 4 days in the enriched treatment, with evidence of recent cell division, whereas dysplastic cells (Boleyn, 1972) were common in the unenriched treatments. Enriched cells darkened for 18, 32, and 46 days also appeared to be more robust, although lysis was evident in both treatments after 46 days. The mean daily division rate after 2 days exceeded the 5-day mean at all treatments, except for the enriched *f*/10 and 200 ft-c treatment (Table 1). This contrasts with the poorer response of *Skeletonema costatum* initially, i.e., a longer lag preceded its active growth. *D. brightwelli*, unlike *S. costatum*, retained its viability after 46 days in the dark, i.e., it was evident within 5 days of transfer into light. However, similar to *S.*

Table 1. *Skeletonema costatum and Ditylum brightwelli. Mean daily division rates after incubation for 5 days at 200 and 1000 ft-c in f and f/10 media following periodic inoculation from cultures stored in the dark for up to 46 days, and which were unenriched (U) and enriched (E) with medium f; —: no observed growth*

Days in dark	Light intensity (ft-c)							
	200				1000			
	Enrichment							
	U (*f*/10)	E (*f*/10)	U (*f*)	E (*f*)	U (*f*/10)	E (*f*/10)	U (*f*)	E (*f*)
				Skeletonema costatum				
4	0.79	0.83	0.87	0.92	0.10	—	0.60	0.64
18	—	0.57	0.45	0.43	—	0.39	0.53	0.70
32	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—
				Ditylum brightwelli				
4	0.25	0.55	0.51	0.64	0.33	0.49	0.33	0.46
18	—	—	0.19	0.09	—	0.05	—	—
32	—	—	—	0.09	—	—	—	0.05
46	—	—	—	—	—	0.13	0.05	0.05

costatum, there is no clear indication that the enrichments used promoted dark survival.

Discussion

The 3 types of response characterizing the patterns of decrease in the mean daily division rates of the 9 diatoms studied here accompanying prolonged incubation in the dark are offered only as qualitative expressions of their varying survival potential. The experiments were designed only to explore this problem and its potential ecological importance; the data indicate that detailed studies are now warranted, particularly under an experimental matrix of differing temperatures, photoperiods and light intensity. The only important difference between species in their ability to survive darkness may ultimately prove to be survival time; the morphological and physiological changes accompanying dark stress of photoautotrophic microalgae may be similar although, as will be demonstrated, the limited data challenge this notion. Nonetheless, and despite the possibility that experimental artifact accounts for the trends established, continuous dark incubation progressively increases the time that it takes an inoculum of an autotrophic species to reach a constant number of cells upon reillumination (see Umebayashi, 1972). Presumably this reflects a progressive loss of viability and a reduced growth rate of the survivors preliminary to their eventual lysis and death. The approximate curvilinear decrease in growth potential upon reillumination of increasingly dark-stressed cells noted here (Figs. 1, 2) might, thus, be anticipated.

Seven of the 9 diatoms survived 90 days of darkness at 15 °C, the duration of the experiments. Bunt and Lee (1972) reported that 2 diatoms and a chlamydomonad (of 4 species examined) isolated from Antarctic sea-ice also survived 90 days (duration of the experiment) of darkness at —1.8 °C. Antia and Cheng (1970) found that the survival potential of 31 species of marine microalgae kept in continuous darkness at 20 °C ranged from 1 week to 6 months (maximum tested). These authors could not relate the considerable differences in survival potential observed between species to algal class or to their subdivisions. Such attempts may be premature, however, until additional observations are available and some conflicting data reconciled. For example, the limited survival (4 to 5 days) of *Dunaliella tertiolecta* in darkness (Yentsch and Reichert, 1963; Hellebust and Terborgh, 1967) contrasts with the 7-week survival period for this species at the same temperature reported by Antia and Cheng. (Note, however, that cell division was the response measured by the latter and photosynthesis by the former investigators.) Other microflagellates survived up to 14 weeks at 20 °C, but *Coccolithus huxleyi* and *Amphidinium carterae* only 1 and 3 weeks, respectively. Using techniques similar to ours, Ignatiades and Smayda (1970) found that *Rhizosolenia fragilissima* survived only 1 month in the dark at 18 °C, and exhibited a Type 1 response. This survival potential is even lower than that of *Skeletonema costatum*, which survived only 7 weeks in the present study, and whose response will be re-examined shortly.

The various observations on the dark survival potential of photoautotrophic centric diatoms, including our results, provide no evidence that growth occurred in the dark (Takano, 1963; Antia and Cheng, 1970; Umebayashi, 1972), even when darkened species were provided various organic substrates (Bunt and

Lee, 1972). This is consistent with the limited data available on heterotrophy of pelagic species (Sloan and Strickland, 1966). Simple enrichment with inorganic salts and vitamins were likewise without effect on the dark survival potential of *Skeletonema costatum* and *Ditylum brightwelli* in our preliminary experiments (Table 1).

The changes occurring in dark-stressed cells are poorly understood, but morphological, physiological, and biochemical modifications are evident. Morphological changes observed in the present experiments included the formation of resting spores (*Chaetoceros* spp.), formation of dysplastic cells (*Ditylum brightwelli*), break-up of chains (especially in *Asterionella japonica*) and, in all cultures, cell lysis.

Iizuka (1963) established that the chloroplast appearance and the absorption spectra of pigment extracts of wild phytoplankton species became abnormal following prolonged exposure to darkness. After 30 days in the dark, normal chloroplasts developed upon reillumination. However, Bunt and Lee (1972) found no differences in the ratios of carbon: chlorophyll *a* and carotenoids: chlorophyll *a* between illuminated and darkened cultures. The protein content of *Phaeodactylum tricornutum* cells did not change significantly when darkened up to 16 days (Griffiths, 1973). However, when *Skeletonema costatum* is darkened following cultivation under continuous illumination, its chlorophyll *a*, carbohydrate, protein, and lipid contents decrease (Handa, 1969). Handa suggested that these changes explained the decreased photosynthetic activity observed when darkened *S. costatum* cells were reilluminated.

Thus, severe cyto-chemical modifications of dark-stressed cells may accompany the observed morphological changes. Species differences in dark survival potential may be linked to their relative ability to minimize cellular damage which ultimately results in a loss of viability, beyond any potential uptake of organic material to satisfy respiratory needs. The potential influence of external physical factors on dark survival is, therefore, of interest.

Skeletonema costatum survived about 7 weeks of darkness at 15 °C (Fig. 1); the lessened, apparent survival in the enrichment experiments (Table 1) probably reflects the short incubation period (5 days) upon reillumination prior to monitoring its growth, probably preceded by a considerable lag. The 7 weeks' survival agrees with previous observations on *S. costatum* (Matsue, 1954; Curl and McLeod, 1961; Takano, 1963). However, this survival period is substantially higher than that reported by Antia and Cheng (1970), and lower than found by Umebayashi (1972) and in recent observations by Antia at 2 °C (personal communication). We have plotted these observations against the temperature (during) dark incubation reported by these authors (Fig. 3). It is clearly seen that the dark survival potential of *S. costatum* is inversely related to the temperature during dark incubation (Fig. 3). *S. costatum* survived 24 weeks in the dark at 2 °C and from 1 to 4 weeks at 20 °C. Should *S. costatum*'s response be either representative generally or applicable to natural populations, then the ecological implications are readily apparent. For example, *S. costatum* [or other eurytherms also incapable of forming resting spores (stages)] might have to compete for nutrients within the euphotic zone more continuously in the sub-tropical portion of its range

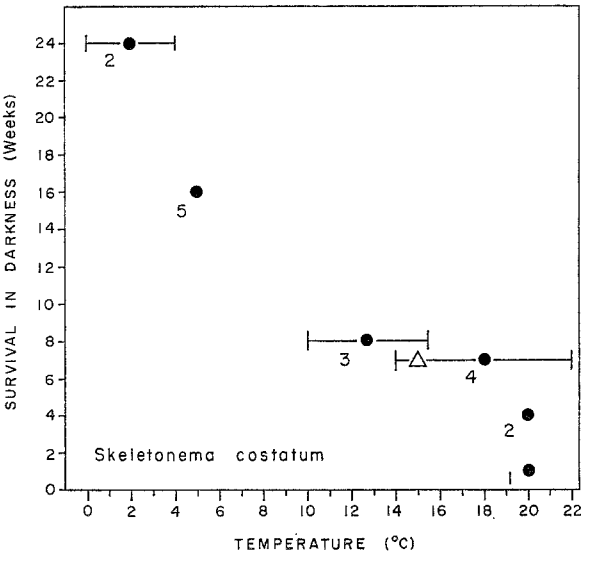

Fig. 3. *Skeletonema costatum*. Influence of temperature on survival in darkness without loss of viability. Data sources: 1: Antia and Cheng (1970); 2: Antia (unpublished); 3: Matsue (1954); 4: Takano (1963); 5: Umebayashi (1972); triangle: present data. Range bars represent temperature range reported during dark survival experiments, data points are positioned at the median temperature. In Umebayashi's experiment the viability test was made at 15 °C; in the others the dark storage and viability-test temperatures were similar

than in colder regions (where dark survival is favored) to ensure autochthonous populations. If this were generally true, then polar and tropical species (considering the extremes) may have the same constraints.

However, a direct relationship between temperature and dark survival may also occur, as suggested by the only other pertinent observations on diatoms known to the authors. *Phaeodactylum tricornutum* survived 3 months at 5 °C (Umebayashi, 1972), but at 20 °C, a different clone (Antia and Cheng, 1970) survived 6 months. Unlike for darkened cells, high temperatures (28 °C) have a pronounced deleterious effect on the photosynthetic capacity of illuminated cells of this species (Griffiths, 1973).

The influence of temperature on the dark survival of photoautotrophic microalgae may, *inter alia*, be

through its potential effect on respiration, photosynthesis and, perhaps, other vital processes, which will be considered shortly. Of other environmental factors, light is also potentially important. Umebayashi's (1972) findings on the survival of 5 diatoms kept in complete darkness relative to that in a darkened refrigerator (both at 5 °C) which was opened "several times" daily are provocative. The survival of *Skeletonema costatum*, *Cyclotella nana* and *Chaetoceros calcitrans* forma *pumilus* increased from 4 to 9 months in the opened refrigerator, while survival of *Nitzschia closterium* increased from 3 to 25 months and *Phaeodactylum tricornutum* from 6 to 34 months! Conceivably, such an effect contributed to the dark survival of 7 of the 9 diatoms for 90 days in the present experiments. The screw caps of the darkened culture flasks (wrapped in aluminum foil) were removed periodically for very short periods (less than 30 sec) in the dimly lit transfer room to carry out the experiments. Perhaps any illumination received by the populations was sufficient to prolong their dark survival.

Hellebust and Terborgh's (1967) study with *Dunaliella tertiolecta* is pertinent to Umebayashi's observations. This flagellate retained more than 80% of its photosynthetic capacity at 18 °C when illuminated for 5 days with blue, blue-green, and red light at intensities considerably below the compensation intensity for photosynthesis. In contrast, cells kept in total darkness for the same period lost completely their ability to photosynthesize.

It seems unlikely that the *Dunaliella tertiolecta* response explains the observed effect of temperature on the dark survival of *Skeletonema costatum* (Fig. 3). However, it possibly explains Umebayashi's startling results (1972), and may even be relevant to the apparent heterotrophy in subdued light reported for other micro-flagellates (Pintner and Provasoli, 1968).

The potential ecological significance of the *Dunaliella tertiolecta* type of response is clear. The dark survival of cells which have sunk below the euphotic zone might be prolonged by periodic exposure to light levels which are below the compensation intensity, even though this intensity cannot support growth and cell division. (Such illumination, however, may possibly minimize the secretion or modification of reserve products useful in respiration, among other positive effects.) Mixing of the cells upward in the water column or a deepening of the euphotic zone are possible mechanisms allowing periodic, low-level illumination. Should such a means of dark survival occur *in situ*, then its potential significance to cells sinking in shallow waters or those near the base of the euphotic zone in deeper waters is evident. Populations growing in deeper waters have a greater risk of sinking much deeper into the aphotic zone which would be detrimental to periodic natural illumination. Their survival problem (even if capable of producing a resting stage) is thus compounded by that of potentially sinking to great depths. At present, mixing processes must be considered the essential mechanism permitting the retention of a minimal, viable population in, or their periodic return to, the euphotic zone in such areas. Quantitative data demonstrate that such a mechanism can adequately account for phytoplankton suspension generally (Smayda, 1970) and, given the organisms involved, should be applicable here as well.

Respiration in *Skeletonema costatum* decreases with temperature (Ryther and Guillard, 1962), but it is unknown how important this is in prolonging its dark survival at lower temperatures (Fig. 3). Clearly, growth upon reillumination requires that photosynthesis occurs and, hence, a temperature-dependent impairment of the potential photosynthetic capacity of darkened cells must be expected. *Skeletonema costatum*'s dark survival curve suggests that the time required for irreversible damage of the photosynthetic mechanism in the dark progressively decreases as temperature increases. In this regard, the lessened impairment of the photosynthetic mechanism and of the activities of enzymes involved in photosynthetic dark reactions in *Dunaliella tertiolecta* at 5 °C relative to higher temperatures is of interest (Hellebust and Terborgh, 1967).

The capability of darkened cells to photosynthesize upon reillumination is also modified, and is especially influenced by the light conditions preceding darkening. This effect is also relevant to natural populations, particularly since upon sinking below the euphotic zone the chances of periodic natural illumination become lessened. An accelerated rate of photosynthesis upon temporary reillumination would be beneficial to the retention of viability. Thus, Yentsch and Reichert (1963) reported that photosynthesis was enhanced in axenic *Dunaliella tertiolecta* (not *euchlora*, *vide* Hellebust and Terborgh, 1967) when reilluminated at I_{sat} (saturation intensity), after 12 h of darkness following cultivation under continuous illumination. The photosynthetic capacity exceeded by 5-fold that in cells grown under continuous illumination. After 24 h darkness, this enhancement was lost, as was viability after 100 h. Hellebust and Terborgh (1967) confirmed this effect, but demonstrated its dependence on photoperiod. An initial, temporary enhancement of photosynthesis upon reillumination did not occur when *D. tertiolecta* was grown on a 12 h light:12 h dark cycle prior to dark incubation.

A similar initial, temporary enhancement of photosynthesis accompanies the reillumination of darkened diatom cells (Handa, 1969; Griffiths, 1973). However, here enhancement occurs irrespective of the photoperiod under which the cells are grown prior to dark incubation. For *Skeletonema costatum*, this was continuous illumination (Handa, 1969), and for *Phaeodactylum tricornutum* it was a 16 h light:8 h dark photoperiod (Griffiths, 1973). Griffiths detected a possible harmful effect on the photosynthetic capacity

of *P. tricornutum* if grown for a prolonged period under alternating light and dark cycles. He suggested that an occasional period of prolonged darkness might be beneficial to this diatom. Similarly, Hellebust and Terborgh (1967) felt that *Dunaliella tertiolecta* when grown under continuous light for a long period needed a period of darkness to develop a high photosynthetic capacity.

In summary, the present experimental results, together with scattered observations, suggest that the dark survival potential of photoautotrophic microalgae when displaced from the euphotic zone by vertical mixing or sinking: (1) will vary between species; (2) may be dependent on temperature, as demonstrated for *Skeletonema costatum*; (3) may be prolonged by periodic illumination at sub-compensation intensities, as shown in *Dunaliella tertiolecta*. Changes in cellular chemical composition also occur during dark incubation, and the photosynthesis of darkened cells may be temporarily enhanced following reillumination. This important problem is worthy of serious study, with emphasis on the physiological aspects governing dark survival.

Summary

1. The dark survival of 9 coastal diatoms, including some incapable of forming resting spores, was followed for 90 days at 15 °C.

2. Seven diatoms survived 90 days of darkness; *Skeletonema costatum* survived 7 weeks in the dark; 3 general types of dark survival patterns are tentatively recognized. Growth in the dark was not observed.

3. The retention of viability in darkened *S. costatum* cells is inversely related to the temperature during dark incubation; at 2 °C it survives for at least 24 weeks, but only 1 to 4 weeks at 20 °C.

4. The effects of temperature and light on dark survival, and of darkness on chemical composition and photosynthesis following reillumination noted by other investigators for certain species are evaluated and, together with the present observations, used to consider this general ecological problem namely, the dark survival of photoautotrophic microalgae.

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First author's address: Dr. T. J. Smayda
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881
USA

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