Effects of Temperature on the Darkness Survival of Marine Microplanktonic Algae*

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Abstract. Thirty-seven species of marine microplanktonic algae from 10 taxonomic classes were tested for their viability in axenic culture after prolonged exposure to darkness at 2°, 10°, and 20°C. The darkness test periods were prolonged in weekly installments up to a maximum of 1 year, and viability retention (*survival*) was judged from the capability for resuming growth after replacement in light. The 2°C-tests showed 32% of the species reaching the limits of survival with 5–6 months of darkness exposure, but another similar percentage continued to tolerate darkness for double this period. These darkness toleration limits were considerably shorter at 20°C for the strains known to be isolated from cold marine regions, whereas the warm-water strains showed the reverse temperature effect in surviving significantly longer at 10°–20°C than at 2°C. Irrespective of temperature or algal class, the bulk of the more resistant survivors was formed by the strains qualifying as benthic types, about 70% of which tolerated 11–12 months and the rest at least 5–6 months of darkness. A few randomly chosen benthic strains extended this toleration to 3 years of darkness. It was concluded that phytoplankters retain darkness-endurance capacity determined by their ecological origin and with no obvious taxonomic correspondence. The concept of ecological races, characterized by temperature control of darkness survival, is discussed.

Introduction

The tenacious capacity of diverse marine phytoplankters for surviving long periods of continuous darkness is now well recognized from the results of culture studies reported by several laboratories [1, 6, 21]. Such physiological studies have served to provide a rational explanation for the ecological finding of pigmented microalgae in deep-water or polar, aphotic marine regions [8, 12, 18], where heterotrophic development appears unlikely [15]. According to this explanation, ungrazed phytoplankters (or those contained viable in fecal pellets) could sink below the euphotic zone (or become deprived of sunlight from polar

*The term *darkness-survival* is used throughout this report to denote the retention of cellular viability by an alga *without growth* (i.e., without significant increase in cellular mass or numbers) during exposure to darkness. Implicit in this definition is the denial of any known possibility of growth, either autotrophic requiring light or heterotrophic requiring organic-carbon, since both these agents are effectively absent in the survival cases considered here.

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winters and consequent ice-packs), whence after a period of darkness-survival they may be retransported by seasonal upward-pushing turbulence to the euphotic zone (or restored directly to sunlight from the return of polar summers) resulting in resumption of growth [6, 18]. It is expected from this interpretation that such phytoplankters would be exposed to a considerable temperature gradient during the process of sinking or during the seasonal cycle from spring–summer growth conditions to winter–darkness survival in temperate and polar latitudes. The anticipated effect of temperature on darkness-survival was deduced by Smayda and Mitchell-Innes [21] for cultured *Skeletonema costatum* by assembling results from various researchers, but in reality this question has never been directly investigated, neither for any one strain or group of microalgae nor with respect to the diversity of phytoplankters encountered in Nature. The present communication reports the results of such an investigation made at 3 temperatures on axenic cultures of 37 strains of marine microalgae belonging to 10 taxonomic classes and isolated from a wide assortment of marine areas.

Materials and Methods

Algal Strains

The 37 species (or undetermined strains) tested are listed in Table 1 according to their presently known taxonomic classification. Thirty-one of these strains are the same as those previously tested for darkness-survival at 20°C [1]; they have been regrouped into algal classes instead of the major phyletic divisions previously invoked, and the taxonomic amendments subsequently required for certain strains are incorporated in Table 1. It is pointed out that (a) the strains *Nannochloris oculata* and *Monallantus salina*, previously placed in the Chlorophyceae and Xanthophyceae, respectively, are now considered to belong to the Eustigmatophyceae [2]; (b) *Monochrysis lutheri*, previously placed in the Chrysophyceae, has been reidentified as *Pavlova lutheri* and transferred to the Haptophyceae [9]; (c) the identification of the listed cyanophyceaen strains is believed to be incorrect [22]; (d) in the first and third cases, nomenclatural revisions are known to be required and are being awaited.

Test Methods

All algal stock cultures were ensured to be axenic prior to the tests. The same culture medium and test procedure were used as previously described ([1]). Sets of test flasks, inoculated with an alga (in stationary phase of growth), were incubated stationary in continuous darkness at each of the following temperatures (±2°C): 2°, 10°, and 20°C. The incubation periods were progressively prolonged by weekly installments until algal viability was completely lost or up to a maximum of 1 year if still viable. The tests at 10°C were effected only on a fraction of the total surveyed strains and were often incomplete in that, apart from showing a significant difference in survival value from the other 2 temperatures, these tests were not extended to the limits of viability retention nor to a maximum of 1 year. In all cases, viability retention was verified by replacing the darkened cultures in continuous light (200–300 ft-c illumination intensity) at 20 (±2)°C up to a maximum period of 8 weeks and visually noting resumption or failure of autotrophic growth during this period of light exposure.

Since the different temperature tests were started with inocula taken from a common stock culture grown at 20 (±2)°C and the receiving medium maintained at the same temperature level, care was taken to ensure that the subsequent cooling of the test-flask to the lower temperatures in darkness did not entail death from temperature shock. Cooling rate measurements (Fig. 1) indicated a hyperbolic drop in temperature from about 20° to 4°C requiring about 80 min, which rate was

Table 1

Summary of Maximum Periods of Darkness Survived by Marine Microalgae from Incubations in Culture Maintenance Medium at 3 Temperatures for Periods Tested up to (but not Exceeding) 52 Weeks.

Alga	Strain[a] (isolator; designation)	Isolation locale	Maximum survival (weeks)[b] at 2°C	10°C	20°C
CHLOROPHYCEAE:					
Brachiomonas submarina var. *pulsifera 7/2a*	M. Droop; Millport-44	Cumbrae, Scotland	40	nd	18
Dunaliella tertiolecta	——; Woods Hole-Dun	——	19	nd	12
PRASINOPHYCEAE:					
Prasinocladus marinus†	B.B.M.; Endoume	Gulf of Marseille	20-29[c]	nd	20-29[c]
Tetraselmis maculata†	T. Parsons; TMD	Departure Bay, Nanaimo, B.C.	52	nd	29
EUSTIGMATOPHYCEAE:					
Monallantus salina†	B.B.M.; Endoume	Gulf of Marseille	52	nd	52
Nannochloris oculata†	M. Droop; Millport-66	Cumbrae, Scotland	52	nd	36
HAPTOPHYCEAE:					
**Emiliania huxleyi* (=*Coccolithus huxleyi*)	R. Guillard; BT-6	Sargasso Sea (32°10′N, 64°30′W)	0.43	1	2
Isochrysis galbana†	M. Parke; Woods Hole-Iso	Port Erin, England	30	nd	20
Pavlova lutheri (=*Monochrysis lutheri*)	M. Droop; Millport-60	Cumbrae, Scotland	12	16	12
Prymnesium parvum	K. Reich; Israeli	Fish ponds, Israel	20	nd	12
XANTHOPHYCEAE:					
Heterothrix sp.†	B.B.M.; Endoume	Gulf of Marseille	52	nd	40-51[d]

Table 1 (continued)

Alga	Strain[a] (isolator; designation)	Isolation locale	Maximum survival (weeks)[b] at 2°C	10°C	20°C
CRYPTOPHYCEAE:					
Chroomonas salina	R. Guillard; 3C	Milford, Conn.	19	≥25[c]	14
Hemiselmis virescens	M. Droop; Millport-64	Cumbrae, Scotland	2	1	1
**Rhodomonas lens*	R. Lasker; Haskins	Gulf stream	0.57	1	4
undetermined cryptomonad	R. Guillard; Haskins-F5A (2)	Falmouth Great Pond, Mass.	22	nd	6
undetermined cryptomonad	L. Provasoli; Haskins-LIS (2)	Long Island Sound, N.Y.	20	nd	8
undetermined cryptomonad	E. Moul; Haskins-M3	Rutgers, N.J. (?)	24	nd	12
undetermined cryptomonad	L. Provasoli; Haskins-WH1	Woods Hole, Mass.	24	nd	8
BACILLARIOPHYCEAE:					
Centric diatoms					
**Bellerochea polymorpha*	R. Guillard; 675-d	Surinam coast (6°28′N, 54°59′W)	9	≥20	12
**Chaetoceros gracilis*	W. Thomas; TO-58-2	Gulf of Tehuantepec (14°40′N, 96°59′W)	16	≥30[c]	19
Cyclotella cryptica	R. Guillard; T-13L	Martha's Vineyard, Mass.	27	nd	15
**Fragilaria pinnata*	R. Guillard; 13-3	Sargasso Sea (33°11′N, 65°15′W)	40	nd	52
Melosira nummuloides†	R. Guillard; 0-8	Martha's Vineyard, Mass.	48	nd	15
Skeletonema costatum	R. Guillard; Skel	Long Island Sound, N.Y.	24	9	4
Thalassiosira fluviatilis	R. Guillard; Actin	Long Island Sound, N.Y.	20	nd	15
Thalassiosira pseudonana (=*Cyclotella nana*)	R. Guillard; 3H	Moriches Bay, Long Island, N.Y.	23	nd	15
Pennate diatoms					
Achnanthes brevipes†	S. Watson; 7	Falmouth (?) Great Pond, Mass.	52	nd	18

Table 1 (continued)

Alga	Strain[a] (isolator; designation)	Isolation locale	Maximum survival (weeks)[b] at 2°C	10°C	20°C
Amphiprora paludosa var. *duplex*†	R. Lewin; 73-M	Woods Hole, Mass.	27	nd	19
Cylindrotheca fusiformis†	S. Watson; 13	Woods Hole, Mass.	52	nd	52
Navicula incerta†	R. Lewin; 66-M	San Francisco, Calif.	52	nd	52
Nitzschia angularis var. *affinis*†	R. Lewin; 35-M	Herring Cove, Nova Scotia	52	nd	52
Phaeodactylum tricornutum†	R. Lewin; 74-M	Woods Hole, Mass.	52	nd	52
DINOPHYCEAE:					
Amphidinium carteri	R. Guillard; Amphi 1	Falmouth Great Pond, Mass.	2	4	3
RHODOPHYCEAE:					
Porphyridium cruentum†	W. Vischer; 107	Switzerland	52	nd	52
Rhodella maculata†	M. Droop; Millport-207	Southend-on-Sea, Essex, England	43	nd	14
CYANOPHYCEAE:					
**Agmenellum quadruplicatum*†	C. Van Baalen; PR-6	Magueyes Island, La Parguera, Puerto Rico	23	nd	35
Anacystis marina†	C. Van Baalen; 6	City Island, New York	52	nd	28

*Supposedly warm-water or tropical strains, as judged from the locale of their isolation.

†These strains appear to be benthic (i.e., meroplanktonic or tychopelagic) types, as judged from their known growth habits, life cycles and isolation locale.

[a]Abbreviations used: *B.B.M.*, research team of Drs. B.R. BERLAND, D. J. BONIN, and S. Y. MAESTRINI [5]; *Millport*, Marine Station at Millport, Scotland; *Woods Hole*, Oceanographic Institution at Woods Hole, Mass., U.S.A.; *Endoume*, Station Marine d'Endoume at Marseille, France; *Haskins* Laboratories (Dr. L. Provasoli) at New Haven, Conn., U.S.A.

[b]*nd*, not determined.

[c]Whereas definite evidence was obtained for survival from 20-weeks and complete loss of viability from 30-weeks of darkness, the intervening period (21–29 weeks) remained untested.

[d]The period between definite survival from 40 weeks and death from 52 weeks of darkness remained untested.

[e]In these cases, longer periods than the shown survivals remained untested.

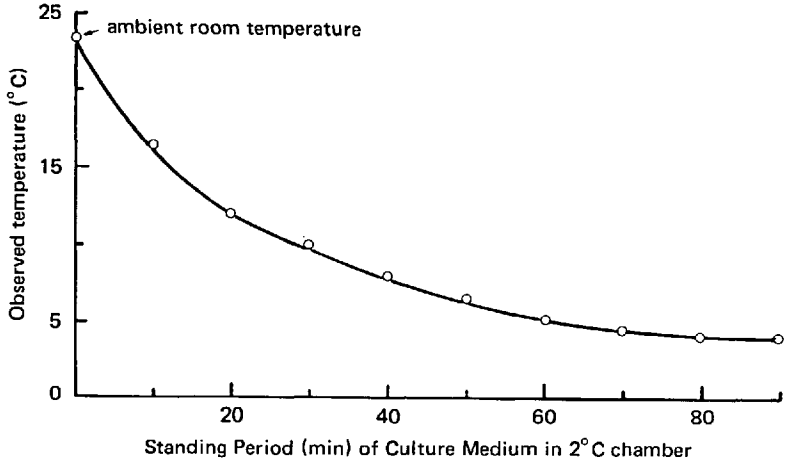

Fig. 1. Cooling rate of test-cultures from ambient room temperature to that of a refrigerated chamber at 2(±2)°C. A test-flask, containing the culture medium, was transferred from the laboratory, at temperatures ambient between 20°–25°C, to the refrigerated chamber, and subsequent changes in the medium-temperature were periodically recorded at 10-min intervals as shown.

inferred to be nonlethal even for the algal strains most sensitive to cold-cum-darkness (see Results) in that these strains resumed growth after 3–4 days of placement at 2°C in darkness.

Great care was taken to ensure complete exclusion of microbial or cross-contamination during the entire investigation by using standard aseptic techniques and periodically checking for sterility against bacteria and moulds.

Results

The individual results for the various algal strains are listed in Table 1. About 60% of the strains remained viable after 24 weeks or longer darkness at 2°C, and 32% continued to show this property up to 48–52 weeks without light. The 20°C-tests gave results substantially similar to those previously reported [1], although in several cases significantly longer survivals were obtained presumably on account of the stricter temperature control used in the present study. Nevertheless, the proportions of strains showing maximum durability at 2°C were markedly reduced at 20°C, with 35% of the strains remaining durable for 24 weeks or longer and 19% for 48–52 weeks darkness. weeks darkness.

An explanation was sought for these temperature-related survival statistics by tracing the native habitats whence the surveyed strains were isolated (see Table 1). It was indeed found that the bulk of the strains originated from marine regions facing *cold* winters or periods of darkness, with the consequence that the survey reflected a bias in favor of such strains, i.e., longer survival at 2°C relative to 20°C. This beneficial effect of lower temperature on darkness-

resistance of the *cold-water* strains was most strikingly revealed from the graphical plots shown in Fig. 2. There is no doubt that *Skeletonema costatum*, cryptomonad *F5A(2)*, *Tetraselmis maculata*, and *Anacystis marina* are strongly favored by the lowest temperature tested. On the other hand, a reverse tempera-

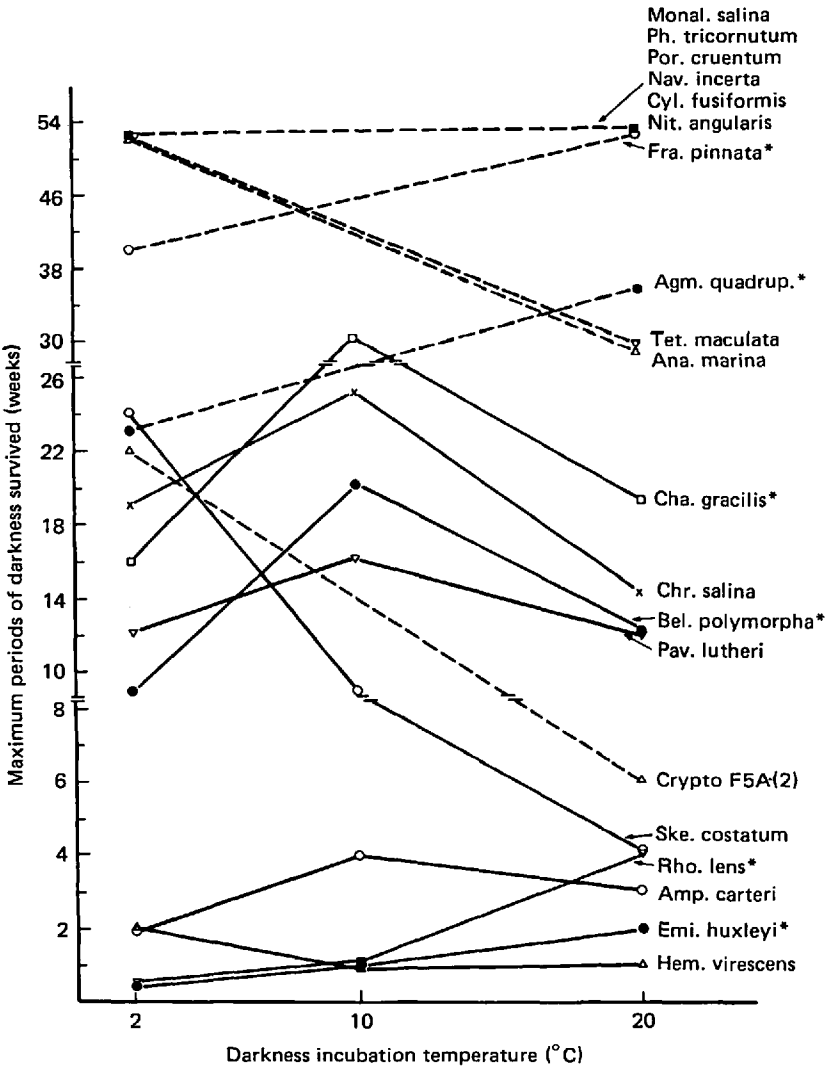

Fig. 2. Influence of temperature on the darkness durability of some marine microalgae. The warm-water strains are denoted by asterisks. The solid and broken lines are used to differentiate between the cases for which 10°C-values are known or unknown, respectively. It is pointed out that the 10°C-values may be greater for certain strains (see Table 1) than those shown.

ture effect was generally revealed for those strains known to have been isolated from *warm-water* or tropical regions (e.g., *Fragilaria pinnata, Agmenellum quadruplicatum*). In several cases (e.g. *Chaetoceros gracilis, Bellerochea polymorpha, Pavlova lutheri, Chroomonas salina*) showing relatively small or insignificant difference between the 2°- and 20°C-tests, the temperature optimum for darkness-survival was achieved at 10°C (see Fig. 2), suggesting that these species (although tolerant of 2°–20°C) may have been best habituated to *cool waters* (intermediate between warm and cold) for darkness survival. Unfortunately, not enough 10°C-tests were carried out to establish this possibility for other strains. *Emiliania huxleyi, Hemiselmis virescens, Rhodomonas lens,* and *Amphidinium carteri* showed the poorest potential (maximum of 2–4 weeks) for darkness-survival in this survey, but even at this level significant temperature effects could be correlated with their origins (see Fig. 2).

There were several strains (Fig. 2) which survived 1 year of darkness without temperature effect from 2° to 20°C. Although these strains ranged from various classes of algae, it was noticed that they share the ecological characteristic of being "benthic-type" organisms, with either meroplanktonic or tychopelagic habits as defined by Hendey [14]. The possibility exists however that these strains may show a temperature effect in tests extended beyond 1 year, since other benthic-type species (*T. maculata, A. marina, Nannochloris oculata*) survived 1 year of darkness at 2°C but considerably less at 20°C. A single experiment was carried out to examine the durability of both types in excess of 1 year, when 4 out of 6 randomly chosen strains showed viability retention up to 3 years of darkness, the maximum period hitherto tested (see Table 2); unfortunately the effects of temperature were not tested at the same time. It must be pointed out that (a) in many cases, precise information on the natural habitat of the strains, considered benthic in Table 1, was not available from the respective suppliers or isolators of these strains; (b) the "benthic" nature of such strains was deduced by the present author on the basis of their

Table 2
Capacity of Certain Algal[a] *Strains for 1–3 Years' Darkness Survival at 2°C*

Alga	Retention (+) or loss (−) of viability after darkness exposure for	
	1 Year	3 Years
Porphyridium cruentum	+	+
Navicula incerta	+	+
Nitzschia angularis	+	+
Nannochloris oculata	+	+
Cylindrotheca fusiformis	+	−
Tetraselmis maculata	+	−

[a]The algal strains shown were randomly chosen from those known to possess extreme darkness durability (see Table 1).

known life-histories and growth habits in culture, particularly the capacity to grow on agar–gel surfaces. Furthermore, the term "benthic" is used to denote not only "bottom-dwelling" but in the wider sense of Round [20] covering close (but nonparasitic) association with all solid surfaces (animal, plant, soil, sand, rocks, etc.) in contact with seawater; this surface association may be periodic (e.g., meroplanktonic behavior) or virtually permanent (e.g., tychopelagic behavior).

Discussion

Table 3 attempts to statistically evaluate the darkness durability of the total and different types of microalgal assemblages surveyed, with temperature effects considered both separately and in entirety. Clearly, the benthic and cold-water types are predominant in lasting capacity. Apart from this feature, they also appear to show a seasonal pattern with prominent clusters at 3–4, 5–6, and 11–12 months of darkness. The question is raised whether these clusters represent natural periodic (or seasonal) rhythms in bulk for these algae obliged to spend quarter- or half- or one-yearly cycles of displacement from the euphotic to aphotic zones in the marine environment. On the other hand, the warm-water group shows no definable seasonal pattern, but this may be due to insufficient species surveyed.

Previous workers have reported the finding of ecological races of phytoplankters whose physiological behavior in culture may be predetermined by various conditions normally prevalent in their natural habitat. When ecologically different clones of the same diatom species were compared in culture, the warm-water open-ocean clone failed to grow below 15°C or 17.5‰ and showed distinct preference for higher temperatures and salinities to support good growth, in contrast to the wide toleration displayed by the cold-water coastal and estuarine clones capable of growing not only at far lower temperatures and salinities but also at the higher ones appreciated by the former clone [10]. However, another cold-water coastal diatom could not grow at temperatures above 15°C while tolerating salinities up to a lower limit of 8‰ [10]. Unlike the cold-water tolerant *Skeletonema costatum* found ubiquitous in tropical and far-northern latitudes, the distribution of *Skeletonema tropicum* was reported to be limited to warm-water (south of 30°N) latitudes in the western Atlantic Ocean on account of its inability to live at temperatures less than 13°C [16]; another *Skeletonema* species from the Sargasso Sea showed a similar cold-water inaptitude [11]. An unexpected effect of light intensity was noted for the cold-water diatom *Thalassiosira nordenskiöldi*, which showed growth capability over a wide temperature range (2°–18°C) at low light intensity but could not tolerate the lower temperatures at high light intensity [17]. This complication was furthered by a recent report that there may be a considerable overlap of temperature ranges permitting light-mediated growth of certain cold-water, warm-water, and presumably intermediate cool-water strains [13]. Another physiological parameter invoked to distinguish ecological races of phytoplankters is their inherent

Table 3

Estimated Toleration Limits of Various Algal Assemblages Obliged to Survive Continuous Prolongation of Darkness

Algal assemblage represented from present survey	Total no. of strains considered	Darkness exposure temperature (°C)	Percentage strains of assemblage reaching viability limits from the following periods (months) of darkness						
			<1	1–2	3–4	5–6	7–8	9–10	11–12
Total of all types[a]	37	2	11	3	5	32	8	8	32
		20	8	14	35	11	8	5	19
		2–20[b]	8	3	5	32	11	5	35
Benthic types	17	2	0	0	0	12	12	6	71
		20	0	0	12	24	24	6	35
		2–20[b]	0	0	0	6	18	6	71
Cold-water types	26	2	8	0	4	38	4	8	38
		20	11	12	39	8	11	0	19
		2–20[b]	8	0	4	38	4	8	38
Warm-water types	6	2	33	17	17	17	0	17	0
		20	17	17	17	17	0	17	17
		2–20[b]	17	17	17	17	0	17	17

[a]Entire list of strains shown in Table 1.

[b]In considering this whole range, that temperature (2°C or 20°C) was chosen which supported the longer survival of each strain; the 10°C-results were ignored because of their incompleteness.

difference in uptake capacity for essential (but scarce) nutrients for growth, reflecting ecological adaptation to high- or low-nutrient regions. Thus, diatom clones from oceanic low-nutrient areas showed considerably lower half-saturation constants for nitrate uptake than those isolated from estuarine regions, with intermediate values observed for clones from continental-shelf areas [7]. The results from the present investigation suggest that the temperature optimum of darkness-durability may serve as an additional useful criterion for distinguishing ecological races from cold, warm, or intermediate cool-water regions, with appropriate redefinitions of these regions to cover the underlying aphotic zones. This parameter has the added advantage of being independent of complications from the effects of light and presumably nutrients.

It is interesting to compare the darkness survival-temperature optima of two supposedly cold-region species with the growth-temperature optima previously reported for the same strains (of identical origin as used in the present work). Jitts *et al.* [17] reported growth optima of *Skeletonema costatum* and *Pavlova* (*Monochrysis*) *lutheri* at 19°–20°C in culture, which temperatures contrast with their darkness-survival optima at 2° and 10°C, respectively. Ecologically, these temperature differences between growth and darkness-survival reflect differences between summer-growth conditions and winter-survival in both cases, and the identical growth optima suggest comparable seasonal growth conditions for the two species. However, it appears that *S. costatum* has developed better potential to survive near-freezing winter conditions than *P. lutheri*. This potential is reflected not only by the temperature optimum but also by the time-magnitude of survival. Whereas *S. costatum* can survive 24 weeks at 2°C and 8–9 weeks at 10°C, *P. lutheri* shows only half this survival magnitude at 2°C but almost double that at 10°C. Ecologically interpreted, these magnitude differences indicate that, in the native habitat, *S. costatum* may be normally exposed to long near-freezing aphotic winter conditions, whereas *P. lutheri* is equipped to weather shorter higher-temperature (albeit still cold) winters. On the other hand, it appears that *P. lutheri* is better equipped to tolerate temperature-fluctuation stresses during darkness-survival than *S. costatum*. The former shows equal survival magnitude at 2° and 20°C, with about 35% greater value at the optimum of 10°C, whereas the latter survives 5-fold longer at 2°C and 1-fold longer at 10° than at 20°C. These darkness-survival considerations indicate finer temperature-governed ecological differences between the 2 species than was shown by their growth-temperature optima, and suggest that (a) the northeast American coastal *S. costatum* is a colder-water strain than the west Scottish coastal *P. lutheri*; (b) the former is habituated to *uniform* seasonal cooling to near-freezing temperatures with deprivation of light, while the latter may be accustomed to weathering periodic temperature *irregularities* in darkness.

The poor potential for darkness-survival shown by a dinoflagellate (*A. carteri*), a coccolithophorid (*E. huxleyi*), and two cryptomonads (*R. lens, H. virescens*), at all the temperatures tested in this survey, poses a baffling ecological question. Are these strains never exposed to total darkness longer

than 3–4 weeks in the natural environment? Perhaps the test-medium used here was inadequate to reveal their true ecological potential, or maybe these strains require brief intermittent exposures to dim light for continuation of existence under nongrowing seasonal conditions. Umebayashi [23] and Smayda and Mitchell-Innes [21] have collected some evidence suggesting that the latter treatment of certain phytoplankters in culture can considerably prolong their darkness-survival. This consideration leads to more fundamental questions on the basic mechanisms underlying the darkness survival of all photoautotrophic microalgae in the absence of exogenous energy sources. It seems reasonable to link the darkness-durability of many species from the present survey to their known capacity for producing some form of resting stage in their life-cycle. Such resting stages, variously termed endospores or endocysts, asexual or resting cysts, aplanospores, hypnospores, etc., have been reported for some diatoms [14], prasinophytes [4], dinoflagellates [24], haptophytes [19], and chlorophytes [3]. But there are several members of these and other groups for which no clearly discernible resting stages have been documented; this is particularly true of the chlorophytes, eustigmatophytes, cryptophytes, rhodophytes, cyanophytes, most centric, and some pennate diatoms included in the present survey. It is possible that their ultraminute size may have defeated previous light-microscopic attempts to define the resting stages or even possible life-cycles of these microalgae. On the other hand, as discussed by Smayda and Mitchell-Innes [21], some holoplanktonic strains (such as *S. costatum*) may lack the cytological machinery for differentiation of a morphologically distinct resting-stage, but they may invoke physiological–biochemical mechanisms for darkness survival by controlling energy expenditure from endogenous metabolism (or respiration) to the bare minimum required for maintaining viability; in other words, they may resort to biochemically "quiescent" phases believed to occur during dormancy or hibernation of plants and animals. Both electron-microscopic and physiological studies are required to distinguish these alternatives, and it is not unlikely that the ultrastructural and biochemical insights, so obtained, may reveal a whole spectrum of survival mechanisms operating in the diverse microalgae encompassed by this survey.

Acknowledgments

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