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Thermophilic Blue-Green Algae and the Thermal Environment

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INTRODUCTION

The blue-green algae (cyanophyta) are considered to be thermophilic (in this review) when part or all of their optimal growth temperature range is above 45 C. Different definitions are used for thermophily in bacteria, fungi, and animals. A maximal growth rate at temperatures over 45 C is mainly a characteristic of procaryotic organisms. Only a few species of eucaryotic protists or animals tolerate temperatures above this (Table 1). In the range between 50 and 60 C, there are a few fungi and the eucaryotic alga of acid waters, *Cyanidium caldarium*. Photosynthetic blue-green algae are known to grow at constant temperatures as high as 73 to 74 C (29), and nonphotosynthetic bacteria as high as 95 C (26, Table 1). Even many species of blue-green algae of nonthermal habitats have higher temperature optima than the eucaryotic algae of the same waters (79). Because of this, blue-green algae may be enriched for by incubating samples in light and nonselective mineral medium at 35 C (11).

Blue-green algae are becoming more conspicuous in this age of increasing environmental pollution. In nutrient-enriched waters, blooms of planktonic blue-green algae are more frequent, denser, and longer lasting (80). Similarly, thermal pollution from the water coolant of power plants (nuclear and conventional) has, as one of its most striking effects, the promotion of blue-green algal growth (164). It is probable that habitats suitable for thermophilic organisms are going to increase substantially.

Most thermal habitats are aquatic, and the source of heat is telluric for nearly all of these. Nevertheless, insolation can raise the temperature sufficiently in a few situations and the self-heating of organic materials (thermogenesis) may bring localized temperature to the point of ignition. Hot springs and their drainways provide the most abundant aquatic habitats for thermophilic blue-green algae. It will be these natural environments and these blue-green algae that are discussed through the remainder of this paper.

However, the other (rarer) thermal habitats require a brief description, even though little is known of their organisms in situ. Temperatures in excess of 50 C may be attained in a few truly aquatic situations solely from insolation. The monimolimnion (bottom waters) of a few small meromictic saline ponds and lakes may be heated (to >50 C) during the seasons of high light intensity and retain fairly high temperatures throughout the year, since circulation of the bottom water is completely lacking (15, 95). Considerably higher temperatures (90 to 95 C) were reached in artificial solar ponds at the Negev Institute in Israel (J. Schechter, *personal com-*

TABLE 1. *Organisms that live in hot springs at temperatures above 45 C*[a]

Organism	Temp
Filamentous and unicellular bacteria [mainly heterotrophic ? (26, 29, 32)]	95 C
Acidophilic thiobacilli (34)	about 60 C
Photosynthetic (purple sulfur) bacteria (*unpublished data*)	57 to 60 C
Photosynthetic blue-green algae (31, 38, 146)	74 C
Cyanidium [(acidophilic) (16, 34)]	56 to 57 C
Fungi [(?) in hot springs (58)]	60 C
Diatoms (120, 169; R. P. Sheridan, *personal communication*)	50 C
Green algae (120, 139, 169)	48 C
Ciliata (45)	50 C
Rotifera (45)	45 C
Ostracoda [crustacea (45; Castenholz, *unpublished data*)]	49 to 50 C
Acarina [arachnida (45, 46)]	50 to 51 C
Diptera [larvae (28, 45, 46, 175)]	50 C
Coleoptera [larvae (45)]	45 C

[a] Approximate highest constant temperatures at which they occur or at which growth has been demonstrated are indicated. Selected references on temperature limits are given for each group of organisms. Except for *Cyanidium* and certain bacteria and fungi, all of the organisms occur mainly in waters of *p*H above 6.

munication). In these, meromixis was established by artificially increasing the salt concentration towards the bottom. Small freshwater or saline pools in warm desert or tropical island environments sometimes exceed 40 C (B. Whitton, *personal communication*), although air temperature is also important in such circumstances. The origin of heat in the deep, hot brines of the Red Sea is telluric, and only bacterial life has been reported to date (174). Blue-green algae are the principal inhabitants of alternately moist and dry cliffs in most mountainous regions of the world. The conspicuous dark streaks of blue-green algae may be heated by sun and air to temperatures of over 40 C (109), but it is not known that growth occurs during such periods.

Hot springs and most solar-heated aquatic environments are composed of mineral waters, whereas the environment in self-heating piles of vegetation is richly organic. Thermophilic fungi, sporeforming bacteria, and actinomycetes appear to be the main agents of thermogenesis in accumulations of hay, compost, peat, and manure. A few of the fungi are active between 50 and 60 C, whereas some of the bacteria are active to about 70 C (5, 58, 77). Although self-heating vegetation may be one of the main habitats for the growth of thermophilic spore-forming bacteria, isolations of these organisms can easily be made from hot springs (134) and a variety of nonthermal habitats such as soil, sand, air, seawater, and snow (5).

Although the source waters of hot springs are usually quite low in dissolved organic compounds, there are obviously a number of heterotrophic bacteria associated with the photosynthetic blue-green algae on which they probably depend for carbon-energy sources. Some of these are filamentous flexibacteria; others are nonsporeforming filaments or rods of other types (42). However, in many hot springs in Yellowstone (26) and Oregon (*unpublished data*) there are bacteria growing above 90 C where no endogenous photosynthetic organisms are present to support heterotrophic growth. Either the rapid flow of spring water (containing a minute quantity of organic matter) is sufficient to sustain growth or these bacteria are autotrophs of some type.

Blue-green algae are particularly concentrated in hot-spring waters with a *p*H of over 6 where they form conspicuous and often unialgal matlike covers over submerged substrates. Since there is in many hot springs a surface effluent with a thermal gradient ranging from supraoptimal to ambient air temperature, specific differences in growth temperature optima may result in distinct species bands covering different portions of the gradient (Fig. 1). Since the component organisms differ in their amounts of chlorophyll-*a*, biliproteins, and carotenoid pigments, these bands may be quite different in color, but ranging from a dark brown to a yellow or rich green or blue-green. The orange or flesh color of many hot spring mats is often caused by compact masses of heterotrophic, carotenoid-containing, filamentous bacteria. Although these generally form gelatinous layers (sometimes a few centimeters thick) underneath a top cover of photosynthetic blue-green algae, they are sometimes exposed over wide areas (Fig. 1, 2). In some springs, pink to red layers of photosynthetic purple sulfur bacteria occur directly under the algal cover.

Because of the shallowness and the clarity of most thermal waters, and the exposure of many hot springs to high light intensities, various types of "sun adaptation" have occurred in many thermophilic organisms. Laboratory and field studies of this have added an interesting dimension to studies of adaptation to high temperature. In addition, the organisms of many hot springs have adapted to high salinity or to high concentrations of certain ions. Being partly of magmatic origin, most thermal waters differ considerably in their chemistry from surface waters of lakes and streams (Table 2; 127, 183). Most alkaline hot springs contain between 1,000 and 2,000

mg of total dissolved solids (TDS) per liter; some have salinities higher than that of seawater. Even those of lower salinity may be notably enriched in S (reduced), As, F, Mn, Fe, Al, Li, Si, or in several other elements (Table 2). Although the gross chemistry of a large number of hot springs is known and there is some information on the nutrition of thermophilic cyanophytes, few

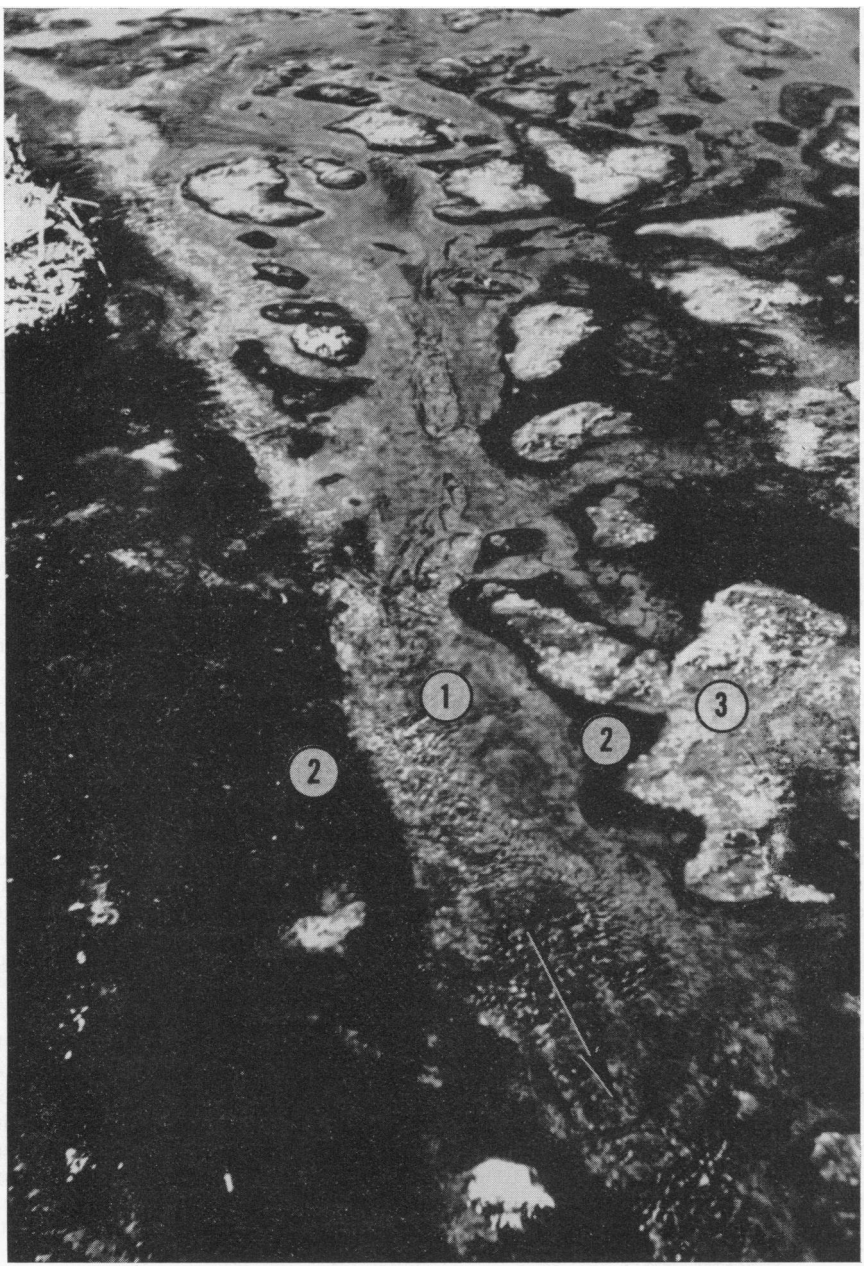

FIG. 1. *Hunter's Hot Springs, Lakeview, Ore.: a portion of the main thermal stream. The arrow indicates the direction of flow. The distance between the no. 1 and no. 3 markers is approximately 0.5 m. (1) Green-colored Synechococcus cover, >54 C. (2) Dark brown O. terebriformis cover, <54 C. (3) Pink-orange exposed undermat of filamentous bacteria, < about 45 C, plus several cyanophytes. The water depth ranges from a few millimeters to about 5 cm in midchannel. 30 March 1965.*

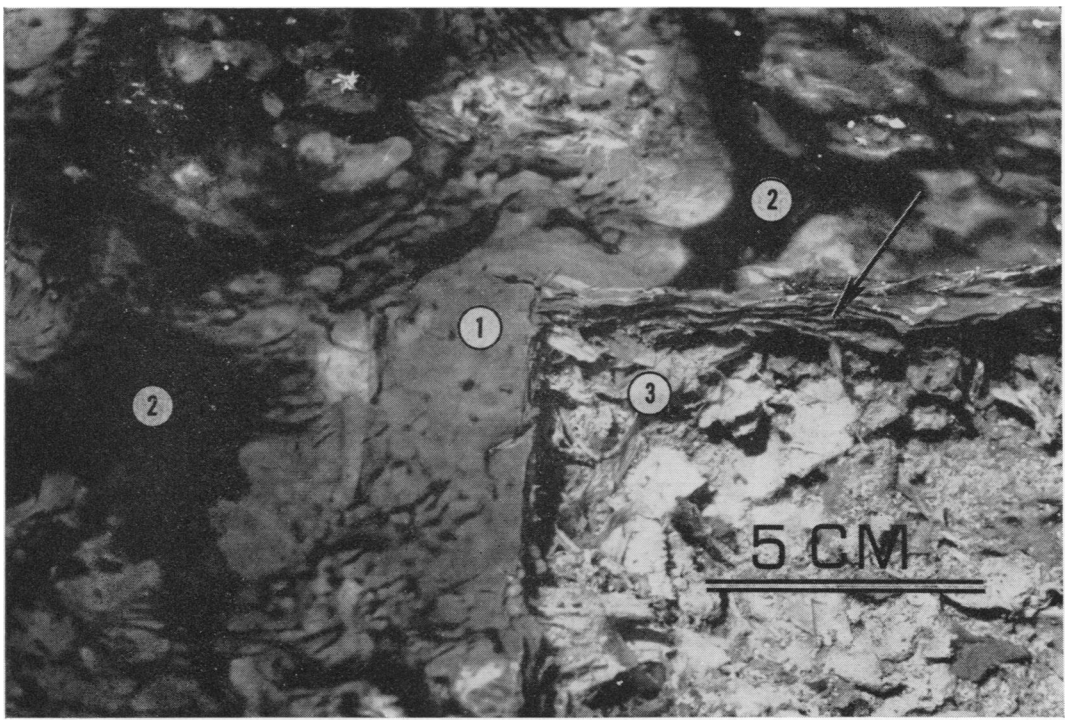

FIG. 2. *Hunter's Hot Springs: a closer view of a mat, with a portion cut away. (1) Green-colored top cover of Synechococcus. (2) Brown-colored O. terebriformis top cover in various contraction patterns and overriding the Synechococcus. (3) The undermat of pink-orange filamentous bacteria. Total mat thickness is about 2 cm. The arrow indicates the many separable layers of the undermat. The 5-cm scale marker is on the sandy substrate. Slow-moving water here was 1 cm or less deep over the top cover and about 53 C. 29 May 1967.*

attempts have been made to correlate field and laboratory data.

One of the most interesting ecological features of most hot springs is the great constancy of temperature and chemistry of the water at the source, generally unequaled by other aquatic systems. In practice, this means that a thermophilic alga has its optimal range of temperature available in the thermal gradient at any season. Similarly, any chemical limitations that exist exert their influence year round. This is quite unlike the situation in typical streams and lakes where great seasonal fluctuations of temperature and nutrient content may occur. In addition, the relatively few species of each trophic level greatly simplify studies of species interactions and of productivity.

It is generally assumed that blue-green algae evolved in the early Precambrian and were responsible for the first significant increase in atmospheric oxygen. Microfossils of cyanophyte-like filaments have recently been found in early, middle, and late Precambrian strata (171). Fossils of the late Precambrian (about 1 billion years ago) resemble living members of the blue-green algae (151). Most of the fossil-rich Precambrian material occurs as chert or as stromatolites which could have originated in aquatic thermal environments similar to those of today. In particular, the description by Schopf (151) of the probable conditions of deposition of the late Precambrian cyanophyte-rich material sounds very similar to the manner in which blue-green algae are embedded by siliceous sinter in contemporary hot springs of high or moderate salinity. The question of whether the adaptations of modern thermophiles to high temperature occurred in Precambrian or more recent times is unanswerable at present (*see* reference 5).

The physical-chemical bases of the stability of compounds and cellular structures at elevated temperature are still poorly understood. Most of the work has been with the thermophilic spore-forming bacteria, primarily *Bacillus stearothermophilus* (e.g., 3, 12, 18, 20, 25, 29, 34, 48, 50, 56, 72, 86). It may be fallacious to extrapolate these results in toto to the blue-green algae which are photosynthetic and from a different chemical

TABLE 2. *Chemical composition of selected hot springs and surface waters*[a]

Composition	Volcanic-Na, Cl-HCO_3 hot springs		Volcanic acid, SO_4 hot springs			Calcarous hot springs	Meteoric-Low salinity hot springs		Hot Na-Ca, Cl brines	Cold surface waters				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Na	352	156	243	22	7,100	129	74	4.8	7,030	2.0	1.5	3.2	11	21,400
K	24	15	61	7.1	926	69	1.9	1.6	901	0.2	0.8		3.1	1,120
Li	11	0.2	3.2	0.01	—	2.3	0.02	TR	9.9	—	0.014	TR	0.002	8.5
Ca	0.8	2.0	6.5	64	2,370	272	2.2	47	1,140	1.3	6	14	34	11
Mg	0.0	0.5	0.0	31	6,770	68	0	5.1	70	0.2	0.0	4.3	7.6	32
SiO_2	362	609	369	147	164	60	81	46	38	2.2	3.4	1.4	5.9	.0
HCO_3	0	112	0	0	0	667	79	168	192	1.0	12	65	101	26,430
CO_3	70	17	0	0	0	0	27	—	0	—	—	—	—	
SO_4	23	178	454	602[b]	2,170[c]	501	12	7.8	189	3.1	5	0.4	41	7,530
H_2S	2.6	2.5	—	35	28	2.6	<1	—	0.0	—	—	—	—	—
Cl	405	63	408	169	57,300	170	14	2.5	13,300	2.7	3.5	1.6	15	15,100
B	4.4	0.68	6.9	57	5.9	4.3	0.3	0.3	5.1	—	—	0.013	0.015	157
NH_4	0.0	0.1	3.4	3.1	17	1.0	—	0.04	5.4	—	—	—	—	—
NO_3	1.8	0.3	—	0.000	—	0	0.5	0.4	0.0	0.3	0.0	0.8	1.9	2.4
NO_2	0.00	0.03	—	0.000	—	0	—	0.002	0.00	—	—	—	—	—
PO_4	1.3	0.14	—	6.1	—	0.8	—	0.08	0.00	—	0.0	0.000	<0.184	38
Al	0.9	0.55	1.5	85	1,880	0.2	0.1	0.2	0.7	—	0.13	0.103	1.010	—
Fe	0.06	0.21	0.8	28	10,630	0.06	0.4		0.42	—		0.06	0.02	0.21
Mn	0.0	0.00	—	0.3	ca. 24	0.03	0.004	0.3	1.9	—	0.0054	0.0026	0.046	—
Sr	0.004	0.003	—	0.00	9.3	0.4	0.2	TR	28	—	0.015	0.037	0.061	96
Zn	0.00	0.00	—	0.14	—	—	—	—	0.00	—	<0.027	0.000	—	0
Cu	0.003	0.007	—	0.03	—	0.003	0.01	—	0.1	—	0.0014	0.0095	0.0090	0
As	1.5	0.5	—	1.9	5.7	0.5	<.1	0	0.00	—	—	—	—	—
F	25	3.3	—	0.4	806	2.4	11	0	3.2	—	0.0	0.0	0.1	23
Br	1.5	0.0	—	0.00	37	—	—	TR	8.2	—	—	—	—	—
I	0.3	0.0	—	0.000	5.6	0.1	—	TR	0.2	—	—	—	—	—
Pb	0.00	0.00	—	0.07	—	—	—	—	0.01	—	0.0018	0.0040	0.004	—
Mo	0.06	0.03	—	TR	—	—	—	—	—	—	0.000	0.000	0.000	—
Total	1,310	842	1,570	2,780	98,300	1,950	304	284	22,900	13	33	92	221	71,900
pH	9.6	8.7	2.47	1.5	Acid	6.6	9.1	—	7.3	—	—	—	—	9.7
Temp	94	90.5	87	69.9	Very hot	72	88	64	57	Cold	Cold	Cold	Cold	Cold

[a] Values are expressed a milligrams per liter. Data for 1–9 are from White et al. (183); data for 10–14 are from Livingstone (127). Locations: 1, Upper Geyser Basin; Yellowstone; 2, Iceland; 3, Norris Basin, Yellowstone; 4, Japan; 5, New Zealand, White Island; 6, Mammoth, Yellowstone; 7, Idaho; 8, Arkansas; 9, Utah; 10 Scotch tarn; 11, Glomma River, Norway; 12 Churchill River, Canada; 13, Mississippi River, Louisiana; 14, Mono Lake, California.

[b] *Also* 1,440 mg of HSO_4 + 2 mg of S_4O_6 per liter.

[c] *Also* 7,600 mg of HSO_4 + 157 mg of S_4O_6 + 9.3 mg of S_5O_6 per liter.

environment. It has been suggested (29) that the photosynthetic apparatus itself is the most temperature-sensitive component of the blue-green algae with the highest temperature tolerances (i.e., 73 to 74 C). On the basis of very little information, thermophilic blue-green algae appear to have the same functionally defined and structurally defined systems as other blue-green algae (8, 9, 69, 121, 132). Among thermophilic types, only *Synechococcus lividus* and *Mastigocladus laminosus* have been described at the ultrastructural level (69, 132), and even the gross aspects of photosynthesis, respiration, and other metabolic processes at high temperature are undescribed in most species (52, 133).

The isolation of thermophilic variants of mesophilic bacteria capable of growth at 55 C has been consistently successful with several spore-forming types (5). The ability to grow at 55 C has also been genetically transformed to nonthermophilic bacteria by mesophilic forms, indicating that the original thermolability may have resided in a single system or component (21, 136). In view of this, it seems possible at least that all cell systems of mesophilic procaryotes such as blue-green algae may be capable of operating at temperatures of about 55 C with only minor genetic alterations. Perhaps 55 to 60 C represents a borderline, above which cells are required to use very different and perhaps more cumbersome methods of assuring thermostability, methods which may prevent the operation of metabolic systems at lower temperatures (22).

DISTRIBUTION OF THERMAL WATERS

The water of hot springs may discharge at temperatures somewhat higher than the common boiling temperature, although superheated waters are uncommon except in volcanic regions (17). Superheated steam issuing from vents may have considerably higher temperatures (2, 17). A spring may discharge into a basin, thereby forming a pool or lake [limnotherm (179)]. Some of this type have no surface effluents. The pool itself (with or without an overflow) may be of a uniform temperature if the water is well mixed by a bottom source of gas or water. If less turbulent, water along the edges may cool slightly. Other pools have stream outlets. Whether thermal streams originate from pools or directly from the earth [rheotherm (179)], they will display an entire temperature gradient from source temperature to close to ambient atmospheric temperature unless truncated by the flow entering another body of water or disappearing into the ground. Although many springs have quite constant temperatures at their sources over many years, others vary considerably in the course of years, days, or hours (3, 17, 176). Flow rate may be less constant, particularly in geyser areas where the well-known surges of water cause great changes in drainway flow and temperature over short periods of time.

Springs in which the water discharges at a temperature of over 45 C occur in every continent except Antarctica (Fig. 3). Most of these spring areas have been mapped, listed, and referenced by Waring (181). Concentrated thermal activity is usually associated with Tertiary or Quaternary volcanism, where magma may still lie closer to the surface (Fig. 3). Many groups of springs, however, are associated solely with the deep penetration of water in fault zones. The greatest concentrations of thermal springs occur in the Yellowstone Plateau of North America, the North Island of New Zealand, Iceland, and Japan. Large numbers of hot springs are also widely scattered over most of the United States west of the Great Plains, the line of the Andes, Italy, Algeria-Tunisia, Greece, Turkey, portions of central Africa, India, central Asia, Indonesia, Melanesia, the Philippines, and Kamchatka (Fig. 3). Australia has few hot springs and the whole of northern Europe above 52° lat is devoid of thermal waters above 40 C. A few hot springs lie on the Greenland coast 300 to 400 miles to the west of Iceland, but all of North America east of the Rocky Mountains lacks thermal water, except for the few springs in Virginia, North Carolina, and Arkansas (181).

Although there is a great variety of chemical types, there seem to be thermal waters on every continent that are essentially identical to some on almost every other continent. Any area of intense thermal and volcanic activity usually has the greatest variety of spring types. The major areas of Yellowstone, Iceland, and New Zealand have probably been active continuously since the early Pleistocene. It is likely that throughout the biological history of the earth thermal water has existed, although not in the same areas. Terrestrial volcanic belts are not connected by underground water systems (13), although this has been suggested.

Exposure to extremely high light intensity is almost universally characteristic of hot spring environments. Most of the extensive thermal areas are devoid of higher vegetation, or nearly so. In addition, some of the best known areas are at high elevation or in regions of little overcast. Since the water of most thermal pools is clear and the effluent streams are usually shallow, there is little radiation extinction. As a result, a large number of thermophiles must adapt to high light-level situations or else physically avoid the light. In Yellowstone, (elevation 6,000 to 8,000 ft) exposures to 0.6 cal per cm^2 per min of radia-

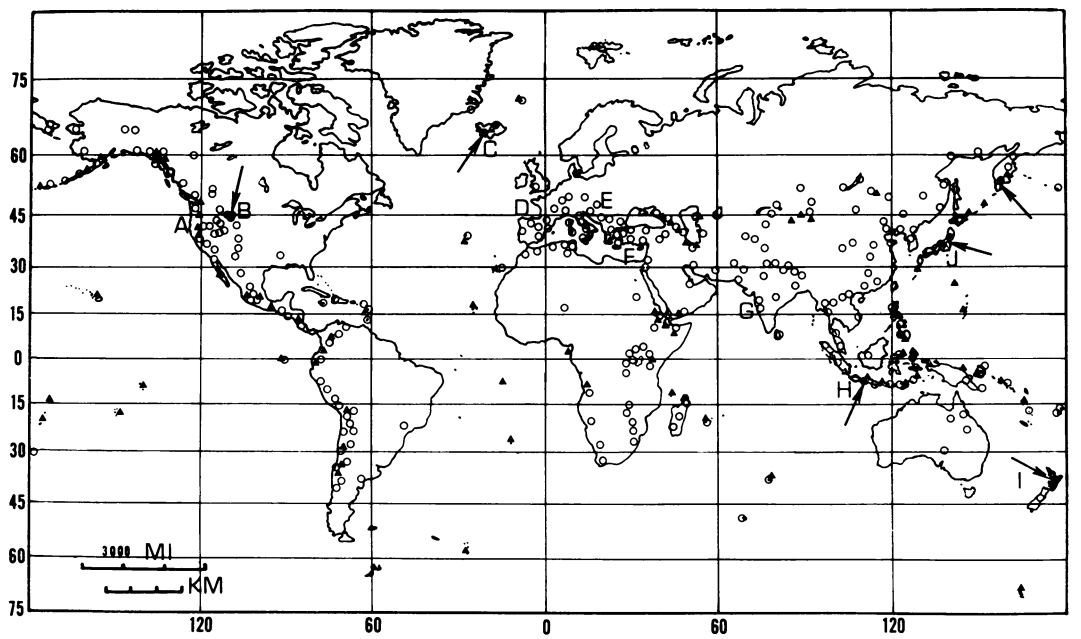

FIG. 3. *Global distribution of thermal areas with springs discharging at temperatures over 45 C (○) and of contemporary or Recent volcanism (▲). The regions with particularly high densities of hot springs are indicated by solid circles and arrows. Most of the information has been obtained from Waring (181). Capital letters refer to selected hot-spring areas where floristic studies of the component blue-green algae have been made. Major references are: A, Oregon (145); B, Yellowstone Park (59, 139); C, Iceland (23, 27, 54, 139, 153); D, France (73, 139); E, Hungary, Czechoslovakia, and Yugoslavia (57, 115, 116, 139); F, Greece and Israel (14, 62, 139); G, India (173); H, Indonesia (91); I, New Zealand (139); and J, Japan (71, 187). The reference to Nash (139) is included for several regions because of his comprehensive review of historical colections (before 1923) from European and North American hot springs.*

tion in the visible range (400 to 700 nm) are common at midday in summer, whereas similar intensities occur in the hot-spring areas of eastern Oregon (altitude 1,500 to 4,800 ft). Such high intensities, with spectral distributions similar to overcast or sun-plus-sky radiation, have been approached in the laboratory with quartz iodine vapor lamps and appropriate liquid filters (110).

SOLUTES OF THERMAL WATERS

The solute concentration of hot springs may vary greatly, even within a local area (2, 17, 61, 181, 183). Thermal water with less than 500 mg of TDS per liter is uncommon. The median concentration for the earth's thermal springs is probably only slightly less than 2,000 mg of TDS per liter. This is considerably higher than the typical value (less than 150 mg/liter) for surface waters of lakes and streams (127; Table 2). Hot subsurface waters are rich in solutes because of their greater leaching and carrying capacity. It is generally assumed that the mineral content of a hot spring remains quite constant. However, erratic or seasonal changes may occur, probably varying with the increase or decrease of precipitation in the watershed. Thus, the ratios of certain elements and the total salinity may change considerably in a short period (176). Nevertheless, the great majority of thermal springs appear to have remarkably constant chemical composition and temperature at the point of emergence.

Springs of somewhat lower temperatures (<80 C), in which the waters are wholly of recent meteoric origin, generally have lower salinities, often between 200 and 400 mg/liter (183; Table 2). Highly saline hot springs (>20,000 mg of TDS per liter) occur in widely scattered regions, associated with deep sediments or volcanism [e.g., Utah, Israel, Turkey, Greece, Japan, Philippines, Indonesia (181, 183; Table 2)]. In a highly saline spring on Reykjanes (SW Iceland), with a salinity of over 45,000 mg of TDS per liter, no algae were found (T. D. Brock, *personal communication*). The *p*H of this spring is below 7, and the water may contain poisonous concentrations of some elements (183). Blue-green algae are well known in highly saline lakes,

and many also thrive in certain saline hot springs. The Tiberias Hot Springs of Israel, containing over 30,000 mg of TDS per liter with a *p*H from 6.2 to 7.0 (183), have an abundant blue-green flora which includes about 35 species, according to Dor (62). In Greece, hot springs with over 15,000 mg of NaCl per liter also have several blue-green species (14).

It is difficult to estimate to what extent the water of thermal springs is of magmatic origin. In general, very hot waters with deep subterranean sources are partly derived from magmatic steam, although an ultimate meteoric source probably accounts for the major portion. Magmatic contributions are more significant in regions of Tertiary or Quaternary volcanism, where the magma is still relatively close to the surface. The water of a few springs, both thermal and nonthermal, is of a connate origin (i.e., trapped in sediment at the time of its deposition). Since the origins of thermal waters differ, so do the relative concentrations of numerous ions; there is no simple method of classification.

Somewhat condensing a general classification of subsurface waters used by White, Hem, and Waring (183), we may list the common types of thermal waters (based on origin and chemistry) as: (i) volcanic-Na, Cl-HCO_3; (ii) volcanic-acid, SO_4; (iii) calcareous, travertine-depositing; (iv) meteoric-low salinity; (v) brine—Na-Ca, Cl. Examples of the chemical compositions of these general categories are given in Table 2.

(i) Volcanic-Na, Cl-HCO_3 springs are neutral to highly alkaline waters which occur near Tertiary or more recent volcanic activity and are partly derived from magmatic steam. The principal solutes are sodium, chloride, and bicarbonate (or carbonate), and silicate (Table 2). The chloride may be largely magmatic. Salinities are generally between 1,000 and 3,000 mg/liter in geyser areas (i.e., high temperatures near the earth's surface), but may be higher elsewhere (183). Almost all the hot springs of the Yellowstone Plateau (>3,000) have salinities in this range (2). They usually also contain over 200 mg of silica per liter, in which case opaline (siliceous) sinter may be deposited. The sulfate concentration may be high but it varies with the spring (Table 2). This category of spring comprises the bulk of the alkaline hot springs of the earth.

(ii) Volcanic-acid SO_4 waters are closely associated with volcanic activity and surface fumaroles (solfataras). Sulfuric acid, resulting in *p*H values between 1 and 4, originated mainly from the oxidation of the abundant sulfides in the subterranean water. Hydrochloric acid (magmatic chloride) may contribute to the acidity in some waters (Table 2). Silica is generally quite high (>120 mg/liter). Most volcanic acid springs (e.g., North America, Japan, Indonesia, New Zealand, Italy, Iceland) are inhabited by the photoautotrophic eucaryote, *Cyanidium caldarium* in the thermal range below 56 to 60 C (34, 54). Highly saline acid springs are also common, but I am not aware of any reports of photoautotrophs in such waters (Table 2). Salinities of from 35,000 to 100,000 mg/liter are known in western parts of the Pacific circle of contemporary volcanism (183).

(iii) Calcareous, travertine-depositing springs are widely scattered, and are probably in contact with limestone deposits (183). They are generally near neutrality and have higher concentrations of Ca, Mg, and HCO_3 (relative to sodium and chloride) than neutral or alkaline springs of the volcanic type (Table 2). Hydrogen sulfide may be high in some springs of this type. They precipitate large amounts of calcite or aragonite travertine. This material is also deposited by the common volcanic-Na, Cl-HCO_3 springs, but not in such large amounts. The water is mostly, if not entirely, of meteoric origin. All travertine-depositing springs are supersaturated with CO_2. As a result of the decrease in pressure upon surfacing, CO_2 is evolved and $CaCO_3$ is precipitated.

(iv) Meteoric, low-salinity (<500 mg of TDS per liter) thermal waters are characterized by their association with diastrophism rather than volcanism. Nitrogen is usually the major gas. Sodium or Ca is the dominant cation and Cl is usually low compared to HCO_3 or even SO_4 (Table 2). Temperatures are often lower than those of volcanic springs, although the circulation depth may reach thousands of feet (183).

(v) Thermal brines (Na-Ca, Cl), resembling oil-field brines, are not common but occur in many geographical regions. Salinites range from below to well above that of sea water, and the *p*H is usually between 6 and 7 (Table 2). These waters are thought to be connate (183). High concentrations of methane are common.

Hot acid sulfur springs and acid brines provide such restrictive conditions that blue-green algae are either absent or very rare. However, most hot springs, including the common neutral-alkaline types of the volcanic areas, are greatly enriched with many ions rare in the surface waters of lakes and streams (Table 2). Some of these elements are micronutrients, but are present at concentrations that are probably toxic to many microorganisms. Some are lost from solution soon after surfacing, but this may depend on the temperature, *p*H, and oxygen tension. Uzamasa (176) reported the following concentrations as extremes from a variety of volcanic hot springs

in Japan (mg/liter): Al, 1,000; As, 5.1; Co, 2.19; Cu, 68; F, 16; Fe, 1,000; Pb, 2.6; Ni, 9.38; Mn, 278; and Zn, 2.0. The greatest enrichment of the metallic ions is in the more acid waters (Table 2). The mean copper level was about 0.098 mg/liter in the acid springs of Hokkaido, whereas concentrations between 0.020 and 0.030 mg/liter occurred in the neutral-alkaline springs (176). Manganese is also higher in the acid springs, but waters with a *p*H of up to 8.0 may still contain large quantities. The mean for Mn-containing springs in Japan is 2.3 mg/liter, an extremely high level compared to lake or stream water. Dark oxides of Mn and Fe are characteristic deposits in the drainways of many *Na, Cl-HCO*$_3$ springs. Aluminum and Fe are commonly at concentrations over 10 mg/liter in volcanic-acid waters, and extremes of over 1,000 and 10,000 mg/liter, respectively, have been reported (183; Table 2). Arsenic may be high in acid or alkaline springs, and values over 2.0 mg/liter are common in Yellowstone (2) and in other alkaline hot springs (183). In 174 neutral and alkaline springs in Japan, the mean concentration was 0.28 mg/liter. About twice as much occurred in 16 acid springs (176). Fluoride is generally higher in mildly alkaline waters. For Japanese springs, the concentration was generally between 1.0 and 2.0 mg/liter. Yellowstone springs are noted for high fluoride concentrations, commonly between 15.0 and 20.0 mg/liter (2; Table 2). Bromide concentrations of over 1.0 mg/liter are most common in more saline springs of either the volcanic or connate types (Table 2). Sulfides may be common in springs of diverse types.

Tolerances of blue-green algae to most of the above solutes have not been established (*see* reference 94). The high tolerances of some blue-green algae are evident from their growth in hot springs containing large amounts of some of these elements. Some of the obvious differences in flora and productivity among hot springs may result in part from the different concentrations of "minor" elements. Perhaps the frequently conspicuous absence of eucaryotic algae below about 25 to 30 C in drainways of many hot springs (particularly in volcanic types) is explicable by their relatively high sensitivity to certain ions.

Practically all analyses of thermal springs have been made by geochemists or hydrologists. Consequently, analyses of biologically important elements have been incomplete. The elements known to be micronutrients for blue-green algae (e.g., Cu, Co, Zn, Mn, Fe, B, Mo) are often omitted from the analyses unless their presence in large quantities is suspected. Except possibly for Mo and Co, these elements are usually more abundant than in nonthermal surface waters (176)

The macronutrients which are most often limiting in lake waters are inorganic phosphate and combined nitrogen. Thermal waters, although of comparatively high salinity, did not acquire their salts in the same evaporative manner as surface waters of closed basins which are usually enriched in both, but particularly in PO_4. From the relatively few analyses available, it appears that NO_3-N may be very low or lacking entirely in many hot spring sources (30, 52, 183). However, even low levels may not limit microbial growth if the flow rate is sufficiently high. Springs which do not have detectable amounts of NO_3 at their sources may acquire higher levels downstream, either from surface drainage or as a result of N-fixation. In Hunter's Hot Springs of eastern Oregon, NO_3 was undetectable at one source, but ranged from 0.042 to 0.142 mg of NO_3-N per liter 20 m downstream (52). Brock also found an increase in nitrate and ammonia in a downstream direction (30). NO_3 ion was found in 15% of the Japanese springs, presumably at the sources (176). In those, the mean level was about 0.2 mg/liter. Much higher values may occur in more saline thermal waters (183). Among the blue-green algae there is increasing evidence that N-fixation is restricted to heterocyst-containing species, possibly to the heterocyst itself (76), but apparently exceptions may occur (186). Among blue-green algae which grow above 54 C, only *M. laminosus* has heterocysts, and N-fixation has been demonstrated in axenic cultures and cell-free preparations (78, 152). Steward has also shown in situ N-fixation in hot spring drainways dominated by *Mastigocladus* (168).

Ammonium-N is generally more abundant than NO_3 or NO_2, at least at the spring source, in both alkaline and acid types, but particularly in the latter (183; Table 2). Ammonium ion was found in less than one-half of 860 hot springs analyzed in Japan; in these it averaged about 1.6 mg/liter—considerably higher than NO_3 (176). The depressed upper temperature boundary for *Synechococcus* in some springs might be attributable to source waters very low in all forms of combined nitrogen, although such correlations have not been made.

Inorganic phosphate analyses of thermal water sources often show values much higher than those found in surface fresh waters (30, 127, 170, 183; Table 2). Nevertheless, PO_4 was detectable in only 37% of the Japanese springs; in these it averaged about 6.5 mg/liter—a very high value when compared to surface waters (176). Mushroom Spring (Yellowstone), Brock's main re-

search spring, contained 2.7 mg of PO_4/liter—also high (30). In some springs, phosphate values may increase downstream if drainage or seepage from the shore occurs. In Hunter's Hot Springs, a concentration of <0.040 mg of PO_4-P/liter at one source increased to over 0.100 mg of PO_4-P/liter (i.e., 0.300 mg of PO_4 ion/liter) 20 m downstream (52).

It is probably safe to state that most hot springs have the high nutrient characteristics of eutrophic waters, but these are often countered by poisonous concentrations of certain elements, low *p*H, or extremely high salinity. It is probable that many thermal streams cause eutrophication in the bodies of water into which they drain. Harrington and Wright (96) have measured a considerably higher standing crop and primary productivity in the Firehole River below the thermal tributaries from the Upper Geyser Basin (Yellowstone) than above. Similarly, the chemistry or temperature of hot spring effluents apparently causes increases in insect biomass in the Gibbon River of Yellowstone (178). If poisonous concentrations of some elements were present in the thermal streams, these would be diluted to subtoxic levels in major freshwater streams.

The dissolved organic compounds present in thermal streams have seldom been measured. Brock (30) found 2.4 mg of organic carbon/liter both in the source pool and at the foot of the drainway of his main research spring. The concentration of organic solutes and their identities should be of considerable interest, since little is known about the natural energy or carbon sources for bacteria that grow at very high temperatures [75 to 95 C (26)]. Brock and Freeze

TABLE 3. *Blue green algae of thermal springs*[a]

Order: *Chroococcales* Family: *Chroococcaceae*	
Synechococcus lividus Copeland var. (31, 38, 146) A, B, (I?), J	74 C
"Type IV" (146)	72 C
"Type III" (146)	67 C
"Type II" (146)	62 C
"Type I" (146)	58 C
Synechocystis elongatus Naeg. var. (14, 91) A, B, E, F, G, H, (I?), J	(66–70 C)
S. minervae Copeland var. (14, 145) A, B, F, (I?), J	(60 C)
S. aquatilis Sauvageau (62) (B?), E, F, G, H, J	(45–50 C)
Aphanocapsa thermalis (Kütz.) Brügger (14, 145) A, B, D, E, F, G, I, J	(55+ C)
Order: *Chamaesiphonales* Family: *Pleurocapsaceae*	
Pleurocapsa [? *minor* Hansg. (145)] A, G, (H?), J	(52–54 C)
Order: *Oscillatoriales* Family: *Oscillatoriaceae*	
Oscillatoria terebriformis Ag. "thermal-red" (53) A, (J?) (B, D, E, F, G, and I—other forms ?)	53 C
O. animalis Ag. (73) A, D, E, F, G, J	(?55 C)
O. amphibia Ag. (62) B, D, E, F, G, J	(57 C)
O. geminata Menegh. (62, 145) A, B, D, E, F, G, H, I, J	(55 C)
O okenii Ag. (116) (B?), E, F, H, J	(60+ C)
O. tenuis Ag. (*unpublished data*) B, C, D, E, F, G, H, I, J	45–47 C
Spirulina sp. [incl. *S. labyrinthiformis* Gom. (*unpublished data*)] A, B, F, H, J	(55–60 C)
Phormidium laminosum (Ag.)Gom. (54) [? incl. *P. tenue* (menegh.) Gom. and *P. valderianum* (Delp.) Gom.] A, B, C, D, E, F, G, H, I, J	57–(60) C
P. purpurascens (Kütz.) Gom. (*unpublished data*) B, C, D, I, J	46–47 C
Symploca thermalis Kütz. var. (*unpublished data*) (A?), C, D, F, J	45–47 C or (?>50 C)
Order: *Nostocales* Family: *Rivulariaceae*	
Calothrix sp.[incl. *C. thermalis* (Schwabe) Hansg. (145)] A, B, C, E, J	(52–54 C)
Order: *Stigonematales* Family: *Stigonemataceae*	
Mastigocladus laminosus (Ag.) Cohn (54, 154) Second type (54) A, B, C, D, E, F, G, H, I, J	63–64 C 57 C

[a] Selected list of species that are common in at least some thermal areas. The upper limit without parentheses refers to the maximum constant temperature at which growth has been demonstrated (in the field or in culture); the upper limit with parentheses is based on certain observations of occurrence only. The references apply mainly to temperature limits. The distribution of species with respect to some of the thermal areas of the world is indicated by the letters in capitals. The *locations* of these are shown in Fig. 3, and relevant references are given there.

(42) have recently described a new aerobic, nonsporulating bacterium (*Thermus aquaticus*) isolated by high temperature (70 to 75 C) enrichment from many hot springs. This may represent one of the important heterotrophs of hot-spring mats.

The undermat of carotenoid-containing filamentous bacteria (presumably heterotrophs) must also depend on organic solutes either from the spring source or from excretions or lysates of the photoautotrophs of the upper layers. In some springs (e.g., Kah-nee-ta, Oregon) thick top mats of orange filaments (about 1.5 μm wide) predominate in summer over large areas at temperatures ranging from about 55 to 40 C. The specific energy and carbon sources for growth are unknown, but chlorophyll-*a* is lacking.

DISTRIBUTION OF SPECIES

Upper and Lower Temperature Limits

The maximum *constant* temperature that will sustain growth under natural conditions is still unresolved for most cyanophytes, but 73 to 74 C may be the upper limit for the highest temperature type [*Synechococcus* (Table 3)]. Brock (31) has demonstrated photoincorporation of ^{14}C-carbonate in *Synechecococcus* populations at temperatures as high as 73 C in a Yellowstone hot spring. In complex hot-spring medium, *S. lividus* has a generation time of less than 24 hr at 70 C (146). There are numerous reports of blue-green algae occurring at higher temperatures (*see* 27, 52, 59, 145, 179, 187). Copeland (59) listed five species above 75 C in Yellowstone, one as high as 85 C. Mann and Schlicting (131), more recently, reported similarly high temperature ranges in Yellowstone. Brock (29, 32) and I (52) have examined some of the same springs and have concluded that the organisms in question are probably flexibacteria or other nonchlorophyllous filamentous procaryotes, not photosynthetic cyanophytes. Although fluorescence microscopy may not be capable of resolving very small quantities of pigment, Brock did not find any indication of chlorophyll-*a* in the material collected (32). Organisms of these higher temperatures are sometimes brightly colored, presumably because of carotenoid pigments. So far, there has been no demonstration of photosynthesis or growth of photoautotrophs over 73 C. Most of the reports of higher tolerances (particularly numerous in the older literature) are based simply on presence; and the temperature, even if measured at the precise site of the specimen, was probably taken with a bulb thermometer in water, which may often stratify to the extent of a 10-degree difference in a vertical centimeter. On the other hand, Setchell (158) and Nash (139) reported blue-green algae in Yellowstone at temperatures no higher than about 73 and 75 C, respectively, which agrees essentially with the observations by Brock and myself. Kempner (113) measured ^{32}P incorporation into nucleic acids in a few adjacent springs in Yellowstone and found none above 73 C; he concluded that this temperature represented the upper limit of life. Probably he was working with waters that contained *Synechococcus* but not the bacteria which grow at considerably higher temperatures (>90 C) in nearby springs (26, 29).

Some of the discrepancies referred to might be resolved if the duration of exposure to the temperature in question were known. Some of the reports of blue-green algae above probable maximum *constant* growth temperatures may represent short-term exposures to the higher temperature. For example, *M. laminosus* was found in Iceland in a particular spring that varied from about 60 to 70 C approximately every 20 min (54). What is thought to be the same organism was isolated from the spring, cultured, and cloned. In the laboratory, its maximum constant growth temperature was 63 to 64 C. However, it could withstand about 9 hr of exposure to 70 C without noticeable mortality (54).

In North American springs, 74 C is about the maximum constant temperature at which one or more species of *Synechococcus* occurs (Table 3). Where these species do not occur or where chemical conditions may be inhibitory, the upper temperature boundary for photoautotrophs is lower. In hot springs of the Cascade Range in Oregon, 64 to 68 C is approximately the upper limit; unicellular *Synechococcus* is usually the only cyanophyte above 58 C. Farther to the east in Oregon, and in Yellowstone, 73 to 74 C is the apparent boundary in alkaline springs. In acid-sulfur springs below *p*H 4 in Yellowstone, no cyanophytes occur; *C. caldarium* is the only photoautotroph over 45 C and it extends to about 57 C (16, 34). In Iceland, the photosynthetic upper limit in alkaline springs is about 63 C. This is a reflection of the absence of all species of *Synechococcus* and the presence of *M. laminosus* which tolerates this temperature in many alkaline springs throughout the world (Table 3; 54, 154). Similar reasons for depressed upper-temperature borders probably apply to New Zealand (139) and many other thermal areas lacking high-temperature races of *Synechococcus*.

The lower temperature limits for growth are very poorly defined. Most of the thermophilic cyanophytes which have been examined in this respect grow poorly or not at all below 30 to 35 C. Certain races of *Synechococcus* will not grow

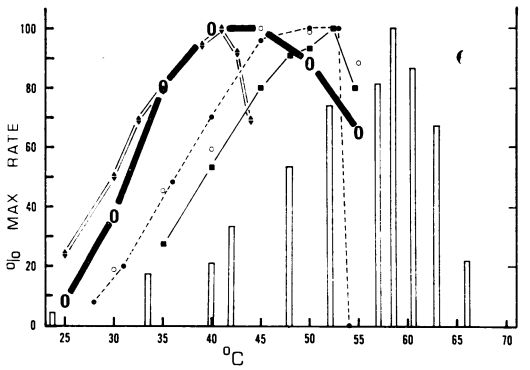

FIG. 4. *Growth and photosynthetic rates of blue-green algae at different temperatures, as percentage of the maximum rate. Symbols:* ▲▼, *A. nidulans (117)—growth rate with 0.5% CO_2 and 450 ft-c light (saturating)—maximum rate was about 11 doublings per day; elongated circle, M. laminosus (105)—growth rate with line air and about 1,000 ft-c—maximum rate was less than 1.5 doublings per day;* ●, *O. terebriformis—growth rate with air and saturating light intensities—maximum rate was about 4.8 doublings per day;* ○, *S. lividus (145)—average growth rates of three clones, probably representing one race, no aeration, and about 1,000 ft-c—maximum rate about 8.3 doublings per day;* ■, *S. lividus (66)—growth rate with 2% CO_2 and 400 ft-c (below 45 C) and 1,500 ft-c at 45 to 55 C—maximum rate was 6.6 doublings per day; vertical bars represent photosynthetic rates ($^{14}CO_2$ incorporation) of a field population of Synechococcus collected at 58.5 C (31).*

below 55 to 50 C; others ceased growth between 50 and 45 C, some between 40 and 35 and others below 30 C (146). High-temperature clones of *M. laminosus* from Iceland, however, are able to grow over the range of 62 to 29 C and perhaps lower (54). There are great differences among cyanophytes, and unlike the upper temperature limit the real lower boundary is much harder to define in field populations, since growth rates taper off gradually below the optimum (Fig. 4). Upper temperature limits, on the other hand, are often a few degrees or less above the optimum. The lower limit is flexible, depending on previous conditioning, light intensity, and other factors. Field populations taper off gradually in some cases, but competition with species better adapted to lower temperatures may result in an abrupt lower boundary in field population, which is often several degrees higher than the real minimum temperature for growth.

Classification and Geographical Distribution

Individual species of thermophiles appear generally to have very wide geographical distributions. However, it is difficult to make categorical statements about this, primarily because the specific identification of blue-green algae is so uncertain at present and few thermal areas have been examined exhaustively. Nevertheless, I have chosen several of the species of blue-green algae that are most frequently reported from hot springs above 45 C. These are included in Table 3 together with some general information on their global distribution. On the world map (Fig. 3) the few hot-spring areas where reasonably complete floristic studies have been made are spotted with capital letters, and references are included in the figure legend. The reader should be warned that the distribution patterns implied by the information provided must be considered tentative. My choice of binomials used in Table 3 has been difficult because of conflicting systems of classification. Gross morphology has been used as the basis of all systems, yet blue-green algae present relatively few morphological characters to the observer under the light microscope. After examining the classical compilation of Geitler (90), one has the impression that different names were frequently given to specimens that are only variants of the same genome. Drouet (64, 65) has simplified the matter with respect to the unicellular types and the oscillatoriaceae by reducing large numbers of species to synonomy and by considering many morphological types as ecophenes (nongenetic variants) of a few species with high degrees of plasticity. However, Drouet's decisions were not based on observed variations in clonal cultures, and, unless the simplicity which he implies has a genetic basis, the new system has no advantage over the old. The reader should be warned that the use of the Drouet system exclusively might lead to considerable confusion. For example, the organism that I call *S. lividus* Copeland (old system) is apparently the same organism that Stockner (169) called *Schizothrix calcicola* (Ag.) Gom. We are both referring to the unicellular rodlike organism that divides in a single plane transverse to the long axis and which grows at high temperatures in hot springs of Oregon (146) and Yellowstone (29). In addition, the filamentous blue-green alga which is primarily responsible for building calcareous stromatolites in marine waters of Bermuda is called *S. calcicola* (159, 160). Drouet considers all of these and many other morphological forms as ecophenes of *S. calcicola*, normally a filamentous nonthermophilic organism (63, 64). This apparent problem cannot be resolved at present because neither Drouet nor I knows the degree of genetic homology between the obligate thermophile of the hot springs and the marine type. However, information of a genetic type, based on deoxyribonucleic acid

hybridization studies, base composition, and other characteristics may soon revolutionize the classification of blue-green algae (60, 68, 130). At present, to avoid the confusion of organisms by oversimplification, it is important that the more narrowly descriptive names be used, with Drouet's name in addition, or vice versa.

Besides being the most ubiquitous and cosmopolitan thermophile, *M. laminosus* (Ag.) Cohn and its several "forms" is one of the easiest species of blue-green algae to recognize because of its unusually high degree of morphological complexity (27, 85, 154). Some of the morphological forms may simply be developmental phases or temperature-controlled manifestations of the same organism. Preliminary experiments have indicated, however, that there are at least two genetic "races" with unique morphologies and temperature tolerances (54). Although the literature has not been exhaustively searched, it is evident that *M. laminosus* has been reported in thermal waters (*p*H 5 to 9+) from almost all parts of the earth (154), with the possible exception of some midoceanic islands [e.g., the Azores (37)] or recently deglaciated areas [e.g., west coast of Greenland (27)]. In regions of many hot springs, such as Yellowstone Park, some springs are dominated by *Mastigocladus*, whereas others (superficially similar chemically) are almost completely dominated by *Synechococcus*. Throughout the world a "*Mastigocladus* Spring" appears to be a recognizable entity. No one, however, has fixed the reasons for this discrimination by habitat. From the little that is known about *Mastigocladus*, other thermophiles, and hot spring chemistry, it is tempting to suggest that spring sources deficient in combined nitrogen will allow the N-fixing *Mastigocladus* to dominate from about 60 to about 50 C, since neither *Synechococcus* nor other thermophiles of this temperature range are apparently capable of nitrogen fixation (168). On the other hand, nitrate- or ammonium-rich springs may allow other thermophiles to compete successfully against *Mastigocladus*, perhaps through more rapid growth rates. There are also reports that *Mastigocladus* is less tolerant of hydrogen sulfide than other species in hot springs (*see* reference 154). Other chemical constituents could also be involved in the exclusion of *Mastigocladus* from some springs.

Most remote thermal areas have not been investigated with regard to the species present. *Phormidium laminosum* is alleged to be a common companion of the cosmopolitan *Mastigocladus*. However, specific differentiations in the narrow-trichome (<3 μm) members of the genus *Phormidium* are so uncertain that one cannot be confident that the same species occurs in all these localities.

Iceland probably has only about six or seven blue-green algae capable of growing above 45 C, whereas hot waters of Europe, Asia, and North America generally support at least twice this number of species (54). Iceland has been active thermally throughout the last glaciation; but hot waters were probably covered by the ice until about 9,000 years ago. Relative youth and the rarity of dispersal from other thermal areas are probably major factors in accounting for the impoverished condition of the Iceland flora (54). The range in chemistry of thermal waters in Iceland is probably great enough to support any of the earth's thermophilic blue-green species (17, 174, 181; Table 2). The species in Iceland all appear to be cosmopolitan.

Some thermophilic species and varieties are broadly endemic, but are absent from a few geographical regions (*see* Fig. 3). For example, the narrowly elongate species of *Synechococcus* (e.g., *S. elongatus*) which inhabit waters to about 70 C in North America, Japan, and the eastern Mediterranean are absent from parts of western Europe, Iceland, and possibly New Zealand. *S. lividus*, as such, has only been reported from North America and Japan (71, 187). Narrower distribution patterns, such as that of *Oscillatoria terebriformis*, probably occur, but may represent the distribution of acceptable habitats rather than actual momentary stages of geographical spread. *O. terebriformis* apparently occurs only in restricted hot-spring regions of western North America. The same red-brown thermophilic type may also occur in Japan (155), but doubtfully in other continents, although a number of different genetic entities fit the broad description (Agardh ex Gomont) bearing this binomial. "Thermal-red" *O. terebriformis* seems to occur in almost all alkaline springs of western and eastern Oregon, northern California, and Nevada (53). However, it has not been found by me or Brock in the diverse springs of Yellowstone National Park, which are merely 600 miles from abundant populations in Oregon. The *O. terebriformis* referred to by Stockner (169) in tepid springs at Mt. Rainier is nonthermophilic, green, and with different cell dimensions in culture (*unpublished data*).

Problems of Survival and Transport

The capacity of thermophilic cyanophytes to survive outside the normal habitat has been little studied. None of the extreme thermophiles produce typical resting spores. Certain species, such as *M. laminosus* and *P. laminosum*, are very tolerant of freezing and drying, whereas *S. lividus*

is less so and *O. terebriformis* is very labile. The transport of thermophiles over long distances would probably require drying or freezing, or both, during the journey if the cells were located on the outside of the carrier. Proctor et al. (149) demonstrated that freshwater algae, other cryptogams, and small invertebrates are transported internally by various water birds, but few blue-green algae were found (148).

In hot springs of equatorial or temperate latitudes, a dormancy period is not required for winter survival. In Yellowstone and Oregon hot springs, midday light intensities in winter often exceed 0.2 cal per cm^2 per min [400 to 700 nm (88)], and blue-green algal populations similar to those of summer are maintained in the steepened thermal gradient (39, 41, 52, 53, 145, 169). In Iceland, however, the 2 months of near total darkness in winter implies a dormant phase for the cyanophytes, since they are probably obligate photoautotrophs. The hot-spring mats disintegrate and wash out (54, 153, 175). The stress of prolonged darkness may also limit the number of species to some extent, although species unable to withstand these conditions probably would not easily survive transport to Iceland initially (54). *O. terebriformis* ("thermal-red") of North America may be such a labile species.

One of the interesting questions is whether, in the dispersal of thermophilic blue-green algae, the carrier must transport the inoculum from one hot spring to another or whether sources of inoculum are available elsewhere. Thermophilic spore-forming bacteria (e.g., *B. stearothermophilus*) can be isolated from common soils as well as from hot springs (31). Viable cells of obligate blue-green algal thermophiles (at least extreme types) may not be found far from thermal areas, but no one has really looked. I have occasionally grown blue-green algae out of samples of common soil in the Willamette Valley (Oregon), with incubation at 45 C, but I have never had positive results at 55 C.

STUDIES OF NATURAL POPULATIONS

Mat Formation and Stability

In the thermal range (above 45 C) of nonacid hot springs, there occur gelatinous or calcareous mats of various colors, sometimes several centimeters in thickness (Fig. 1, 2). The topmost layer is generally composed of photosynthetic blue-green algae. A variety of mat structures has been described by Schwabe (155, 156), Brock (33), Stockner (170), and a few others. One of the striking aspects of these mats is the variety of bright green, brown, red, orange, and yellow colors. These large mats accumulate without apparent deterioration at high temperatures. These mats are largely composed of living microorganisms, held together by abundant extracellular gelatinous materials. The cyanophytes usually occupy only the first few millimeters or less, and orange, yellow, red, or flesh-colored filamentous bacteria make up the rest. Although unicellular bacteria are also present, and become abundant in organic enrichments (31), carotenoid-containing flexibacteria may be the principal heterotrophs in many hot springs. The gliding movements of these bacteria (and flexional movements of some) together with slight similarities in carotenoid pigments (1, 84) indicate that they may be nonchlorophyllous homologues of blue-green algae. The leakage or lytic products of the primary producers are probably the main energy sources available to the bacteria, although the source waters of some hot springs may contain some organic solutes derived ultimately from surface waters. The bacterial undermat is essentially the closed end of energy flow. This material does not readily decompose except when washed downstream into nonthermal waters.

It was suggested earlier by Schwabe (157) and by Brock (30) that the organic accumulation in thermal waters is due primarily to the general absence of herbivores above 43 to 48 C. This may be difficult to demonstrate directly, but what would appear to be the effect of animal grazing is quite apparent below certain temperatures in numerous hot springs. In many cases (Oregon, Iceland, and Yellowstone) the mat is corrosively dissected by burrowing ephydrid (diptera) larvae which may reach large numbers below above 45 C, although specific predators such as dolichopodid flies are also present at some springs (28, 175). In some Oregon hot springs, a thermophilic ostracod forms very dense populations up to about 47 to 48 C without any apparent competitors or predators present. In such situations, there is an abrupt change both in the algal species and total mass below the maximal temperature of these animals. There is generally no mat accumulation at all. Instead, there may be leathery nodules composed of the cyanophytes *Pleurocapsa* sp. and *Calothrix* sp. These nodules are able to withstand the apparent grazing pressure in flowing waters from about 47 to 35 C. Brock et al. (28) have demonstrated by direct means, using ^{14}C-labeled blue-green algae and bacteria, that ephydrid larvae and adults feed on the microorganisms of hot springs. I have found that the thermophilic ostracods of Hunter's Hot Springs (Oregon) can rapidly decimate a population of *O. terebriformis*. In these springs, the lower temperature edge of *O. terebriformis* mats (46 to 48 C) is probably determined by the

feeding range of this herbivore rather than by a greatly decreased growth rate of the algae at that temperature (*see* Fig. 4).

The grazers of the mats at lower temperatures probably aid in decomposition by ingesting microorganisms and depositing large amounts of partly changed fecal matter which may then be further decomposed by the microorganisms of lower temperatures. In areas worked over by dipteran larvae, ostracods, amphipods, and other animals, large amounts of characteristically flocculent fecal matter with associated microorganisms may accumulate in pockets of quiet water. This is probably a reliable indicator of heavy grazing activity.

In most alkaline hot springs, the blue-green algal cells are concentrated in the uppermost few millimeters of the mat, although filamentous bacteria may also be abundant there. Brock (33, 41) has demonstrated by $^{14}CO_2$ incorporation that photosynthesis is limited to the upper portion of the mat. For example, in a mat 1 to 2 cm thick essentially all activity was restricted to the top 2 to 5 mm. Chlorophyll measurements made by spectrophotometry or fluorescence microscopy corresponded with the incorporation results. In the top 5 mm of a typical core from 57 C in a Yellowstone spring, unicellular *Synechococcus* was essentially the only photoautotroph (41). This upper portion was subdivided into four separate layers for autoradiographic analyses after photosynthetic incubation. In the uppermost 1.2 to 1.3 mm, 95% of the cells were labeled, whereas only 35% (of fewer cells) in the lowest sublayer were labeled. Furthermore, the top cells averaged 4.8 silver g per cell and the lowest only 1.3 per cell, indicating the obvious light limitations at depth. *O. terebriformis* ("thermal red") is ubiquitous in Oregon springs below about 54 C (Fig. 1, 2). It forms deep red-brown mats 2 mm in thickness and so dense that commonly only about 1% of the incident light is transmitted (53). Since these mats are motile and tend to override immotile *Synechococcus* surface covers, the latter become severely shaded. Eventually the *Synechococcus* may disappear (53). In North America, the photosynthetic layer above about 60 C is dominated by narrow-cell forms of *Synechococcus* resembling *S. lividus* Copeland. In Yellowstone, Oregon, and other hot springs there are great variations in photosynthetic covers below this temperature. The photosynthetic top layer may consist of densely bound or unbound populations of unicells (e.g., *S. lividus*, *S. minervae*), compact masses of gel-bound or free filaments (*Phormidium* sp., *Oscillatoria* sp.), or tufts of branching filaments (e.g., *M. laminosus*). There are also situations (e.g., Kah-nee-ta, Oregon) where a gel-like translucent orange layer a few millimeters thick (composed of filamentous "bacteria") lies over a dark green layer of *Synechococcus*.

Under nearly all types of photosynthetic covers in nonacid springs lies the orange-to-flesh colored, heterotrophic undermat. In an early statement, Brock (30) suggested that the many layers of typical hot-spring undermats represented the accumulation of several years. Later, he realized that accumulation is considerably more rapid and that the distinct layering cannot be explained easily (39). Stockner (170), studying mainly tepid calcareous springs at Mt. Rainier, came to a similar realization.

Brock has examined the manner in which mats are formed (33). He replaced a portion of the hot-spring drainway (about 55 C) with a wooden channel 6 inches wide and 7 ft long. An orange cover of undermat formed first and was about 2 mm thick in 3 weeks. This was followed by a top cover of green *Synechococcus* which first appeared as small patches, eventually making a complete cover in an additional 4 weeks. In hot springs of eastern Oregon, I found that the blue-green alga was the first *apparent* colonizer on natural and plexiglass substrates. It is possible that in some stretches of thermal streams organic solutes are at such low concentrations that bacterial growth depends on intimate contact with photoautotrophs. During optimal periods, a new covering of *Synechococcus* will coat plates (15 by 15 cm) in a few days, and a typical orange undermat will be visible a few days later. The orange undermat increases in thickness by growth and not by immigration.

In eastern Oregon hot springs, new mat material on denuded substrates and submerged plexiglass plates increased in thickness at a rate of 1 to 2 mm per week. Commonly, this corresponded to values of about 1 to 2 g of organic matter (ash-free dry weight) per m^2 per day and 5 to 10 mg of chlorophyll-*a* per m^2 per day. These are not true growth rates since they exclude losses from decomposition, grazing, and washout and include the possible addition of cells recruited from upstream. Cells of *S. lividus* are almost bacterial in size (5 by 1.5 μm) and settle out very slowly even in unshaken flasks. The ability of a dislodged cell to successfully recolonize will surely diminish with increased flow rate or steepness of the thermal gradient. In a few cases, however, whole masses of detached mat from upstream became lodged on new substrate and obviously are added to the yield. The cause of the very distinct layering of most mats is not understood (Fig. 2). It occurs in mats of flowing streams and nonflowing thermal pools. If the specific organ-

isms of the various layers of undermat were known, the reasons for layering might be more obvious. The steepness of the vertical oxygen gradient in the mat should also have an important influence on the type and growth rate of these. In the Oregon springs, there is often a vivid pink-red layer of *Chromatium* (thiorhodaceae) immediately under *O. terebriformis* at temperatures below about 57 to 60 C. If the *Chromatium* were active photosynthetically, the local conditions should be nearly anaerobic and at greater depths in the orange undermat also, at least above the temperature of extensive burrowing by dipteran larvae (about 45 C). Species of *Saprospira* isolated from hot springs by Lewin (126) showed aerobic tendencies, but it is not known whether these were important components of undermats. Similarly, the nonsporulating bacterium *T. aquaticus*, isolated from Yellowstone springs by incubation at 70 to 75 C, is an obligate aerobe (44).

Movements of Filaments and Mats

In most hot springs in Oregon [and apparently in Japan also (155)], top layers of highly motile *O. terebriformis* are common (Fig. 1, 2, 53). *O. terebriformis* forms dense mats, commonly over 2 mm in thickness, which consist of free-moving but intricately interwoven trichomes with little or no gel binding them. The integrity of the mat is maintained by the intricacy of the trichome entanglement, and the position of the whole mat in flowing water is probably maintained by the penetration of free trichome ends into the substrate. Almost all other cyanophyte covers in hot springs are held together and kept in place by the gelatinous material formed by the blue-green algae, the filamentous bacteria, or both. *O. terebriformis* mats, where found, dominate the thermal gradient from about 54 C down to about 48 to 35 C, the lower edge apparently depending on the abundance of the herbivorous ostracod described earlier.

During midday periods of high light intensity, *O. terebriformis* mats may contract to only a small fraction of their area under low light or darkness, exposing covers of *Synechococcus* or the orange undermat of filamentous bacteria. Mat-edge retraction rates may be as rapid as 100 cm/hr. Trichomes may migrate downward into some substrates en masse (53). With light reduction they will rapidly return to the surface and spread out by gliding. Mass aggregations and contractions were described in *Oscillatoria* (51, 53), and a somewhat similar phenomenon in a nonthermophilic *Anabaena* (180). Contraction of the mat edge in *O. terebriformis* in response to supraoptimal temperatures has also been noted (53). In addition, the upper edge of the *Oscillatoria* mat will advance upstream (by mass gliding of trichomes), keeping pace with the 54 C point when the temperature drops because of wind or decreasing air temperature. Most of the movements of *Oscillatoria* described are probably adaptive in that they enable the cells to maintain themselves close to optimal light and temperature conditions which change diurnally and seasonally. The mat of moving trichomes also allows little competition within its optimal temperature range, since it is always able to form the topmost layer. The common, more sedentary cyanophyte covers of hot springs have no adequate way to cope with diurnal fluctuations in temperature and light except by physiological tolerance. In many cases, these types are not able to colonize or grow rapidly enough in an upstream direction to keep pace with the steepening of the temperature gradient with on-coming cold weather. As a consequence, many thermal mats are left below the growth temperature range in winter when they bleach, disintegrate, and wash downstream (53). Over wintering is probably by vegetative or metabolically inactive cells along the upstream edges of the springs.

Measurements of Photosynthesis and Growth

In 1966, Thomas D. and M. Louise Brock determined the standing crop of algae and other material along a thermal gradient in alkaline hot springs in Yellowstone and Iceland (35) by measurements of chlorophyll, ribonucleic acid, and protein (36). In the Yellowstone drainway all three materials were produced at maximal levels at a temperature between 51 and 56 C. This was also the region in the gradient with the greatest mat accumulation as determined visually. Common values for chlorophyll were 700 to 800 mg/m^2 (35). In the single Icelandic spring examined, all three materials were maximal at a temperature of about 48 C. It has already been mentioned that the species may be different in the two regions, and that the upper temperature limit of photoautotrophs in Iceland is only about 63 C. In both cases, however, the standing-crop maxima were above the temperature range of herbivores or any other animals in the springs. Positions of maximal mat accumulation do not necessarily fix the locations of the maximal photosynthetic or growth rates of blue-green algae or the heterotrophs. Since the first paper, the Brocks have published several additional reports, most of which are concerned with natural rates of photosynthesis in Yellowstone hot springs (30–33, 38–41). Their principal experimental stream was Mushroom Spring in the White Creek drainage

of the Lower Geyser Basin, a few hundred meters from Great Fountain Geyser. The temperature for the maximal crop of chlorophyll was again between 50 and 60 C (30). The incorporation of $^{14}CO_2$ per *unit area* was also highest in the general area of maximal algal standing crop, as expected. Incorporation studies were performed with mat cores set upright in horizontally positioned vials. These cores were generally incubated with $NaH^{14}CO_3$ in spring water at original temperatures for 1 hr. Although objections might be raised about possible stress conditions in the vial (170), the time is short, and there has been no indication yet that thermophilic blue-green algae have a requirement of high-water velocity which has been attributed to some algae of streams (184).

Brock (30) also plotted the photosynthetic rates on a per *chlorophyll* basis to determine photosynthetic efficiency. The greatest efficiency occurred at temperatures between 40 and 50 C instead of in the region of the greatest chlorophyll. It is often useful to express photosynthesis on some basis other than area, but in this case, the use of pigment as a basis must be distinguished from the same expression in laboratory studies (*see* reference 167). Since intact cores of the algal mat were used in the experiments, all photosynthetic cells were compacted in the upper few millimeters. Nevertheless, even in this short vertical distance there is probably a steep drop in light intensity along with an increase in individual cell chlorophyll (*see* references 44, 137). The photosynthetic rate at the top may have been light saturated (or even inhibited) at the same time that cells at greater depth were severely limited by dim light. Thus, the expression of photosynthetic rate per unit of chlorophyll in the case of the field experiments differs in meaning from the same expression in laboratory studies where it would be assumed that all of the cells are in suspension, equally exposed to light, and contain the same amounts of chlorophyll. In the field, it should be expected that photosynthetic efficiency (of intact mats) will be less in regions of thickest algal mat (highest chlorophyll values) because of the greater degree of light extinction.

The photosynthetic characteristics of natural algal mats have been illustrated in several studies by the Brocks. In one experiment, Brock showed that the photosynthetic rate per unit of chlorophyll was dependent on light intensity throughout the course of a single day in summer (30). This can be best explained by assuming that light attenuates greatly within the algal layer, leaving many cells photosynthetically unsaturated; the percentage of cells saturated would depend on the external light intensity. In a later study, the Brocks found that there was seldom any indication of photosynthetic inhibition of algal mats even by the highest light intensities [about 0.56 cal per cm^2 per min, corrected for 400 to 700 nm by a factor of 0.4 (*see* reference 172)] unless the populations had been adapted to reduced light intensities previously (41). Photosynthetic light saturation, however, occurred in some cases at values of about 0.3 cal per cm^2 per min (my estimate for station I, 71 C, corrected for 400 to 700 nm) which was about 50% of full midday summer insolation. Brock (in Yellowstone) and I (in Oregon) have noted that mats of *Synechococcus* at the higher temperatures (i.e., 68 to 74 C) appear thinner than at lower temperatures. Thus, it is probable that a greater percentage of the cells in these thinner mats become light saturated, resulting in a relatively high photosynthetic efficiency expression. An increase in apparent efficiency near the upper temperature limit was indeed found by Brock in one study (30). However, the *Synechococcus* mats at these high temperatures may be thinner because of poorer photosynthetic and growth rates for individual light-saturated cells than at somewhat lower temperatures. If this is true, the higher efficiency expression would be rather misleading in terms of physiology.

Additional information has come from two experiments in Oregon hot springs (*unpublished data*). In these springs, *Synechococcus* cells can be easily removed by suction from the mat surface since they are not bound in place by mucilage as in many Yellowstone springs. Thus, dilute cell suspensions with a minimal degree of self-shading can be prepared for photosynthetic studies, although the cells used may still have quite mixed light histories. Using methods of ^{14}C incorporation and analysis similar to those of Brock, I measured the photosynthetic rates of replicate suspensions of *Synechococcus* for 30 min in two Oregon springs under different light intensities, simultaneously. In both experiments, the cells were collected from a temperature of 60 C and incubated at the same temperature. The results showed that photosynthetic saturation for these cells occurred at about 0.07 to 0.08 cal per cm^2 per min (400 to 700 nm), approximately 1,500 to 2,000 ft-c. Intensities of about 0.2 cal per cm^2 per min were still in the saturation range. However, an intensity of about 0.4 depressed photosynthesis to about 50% of the maximal rate.

Although the conditions and the species strains used might be different in Oregon and Yellowstone, comparisons are of interest. Brock's finding (41) of photosynthetic saturation at about 0.3 cal per cm^2 per min in the thinner mat at 71 C

would indicate that mat thickness is still a factor at intensities lower than this, if we accept the evidence that indicates individual cells are saturated at intensities as low as 0.07 cal per cm^2 per min. Thicker mats at lower temperatures should probably not show photosynthetic saturation even under the brightest natural light.

The Brocks (38) measured steady-state growth rates of natural populations of *Synechococcus* by novel methods. On a hard-bottom drainway of siliceous sinter in shallow thermal streams, the complete darkening of a *Synechococcus* mat apparently stops growth completely, as would be expected in the case of obligate photoautotrophs. In the dark, washout of cells appears to have continued at a normal rate until eventually the sinter was bare. The loss rate was exponential. The time when the population was 50% of the original size (as determined by cell count) was equated with the generation time required to maintain the observed steady-state population under photic conditions. The estimates of generation time were 40 hr at the 70 C station and 22 hr at the 72 C station. The rate of increase in cell numbers can generally be equated safely with the rate of increase in mass over a 24-hr period, in the case of unicellular organisms. Assuming that mass increase is possible only during the 15-hr light period, I have shortened the Brock values for generation times to 25 and 13.7 hr, respectively. This means that during the light period the rate would be 1.0 and 1.7 mass doublings per day. These rates are very close to the maximal rate (about 2.0 mass doublings per day) obtained for a high temperature strain of *Synechococcus* in my laboratory at 65 C, under continuous light and a variety of intensities, gas mixtures, and agitation rates (J. Meeks, *unpublished data*). Maximal rates at 70 C are consistently somewhat lower and even more comparable with the Brock field values. The field method described should be very useful in springs or streams where flow rates are constant and where unicellular algae predominate. Difficulties would arise if components of the algal mat were facultative dark heterotrophs, if darkness affected the tenacity of cell attachment to the substrate, or if washout was erratic and involved chunks or tufts of mat rather than individual cells.

Stockner (170) has estimated primary production by measuring diurnal changes in dissolved oxygen and also increases in organic matter on denuded substrates in the drainways of tepid (37 C) springs at Mt. Rainier, Wash. He found a correlation between blue-green algal production and monthly light intensity—light duration means (gram calories per square centimeter per day) throughout most of the year, even summer. This may be expected, however, with or without self-shading, since the number of productive hours changes with day length, even though photosynthesis may be saturated during many of the daylight hours.

CULTIVATION OF THERMOPHILIC CYANOPHYTES

There is little information on the cultivation of thermophilic blue-green algae. The following section will summarize primarily the methods which have been used in my laboratory. Most media were originally designed for nonthermophilic cyanophytes (7, 108) and later modified and used with thermophiles (67, 69, 105). Media and methods for unicellular types, including the semithermophilic *Anacystis nidulans*, have been developed by Van Baalen (177) and Allen (10). A more detailed description of the culture techniques and of the culture collection of thermophilic blue-green algae at the University of Oregon will be published in Schweizerische Zeitschrift für Hydrologie during 1970 (55).

Medium and Nutrition

Many of the predominant photosynthetic blue-green algae of hot springs grow vigorously in mineral medium. Species from hot springs of many types and a variety of geographical regions are grown on defined medium *D*, with minor modifications (Table 4). Complex media with appropriate hot-spring waters have also been used extensively for some species (Table 4; 146). In all cases, medium *D* has worked as well or better. The only organic compound in the medium is the metal chelator, nitrilotriacetic acid (NTA). Although NTA may be used as a C and N source by *Escherichia coli* (83), it is probably not metabolized by cyanophytes. The medium contains all of the nutrients known as requirements for blue-green algae (92, 104, 142). Nitrate is probably the sole source of combined nitrogen in the unmodified medium, although NTA may conceivably provide nitrogen to some organisms. *M. laminosus* and *Calothrix* sp. are the only thermophilic blue-green algae for which nitrogen fixation has been reported (78, 152, 168). Auxotrophy is known in relatively few cyanophytes (104) and not at all in thermophilic species. Although photoassimilation of organic compounds, such as acetate, may be common in cyanophytes (101, 143), only a few blue-green algae will grow heterotrophically in the dark (4, 75, 104, 114, 122, 162). Among photosynthetic thermophiles, only the *eucaryotic* alga *C. caldarium* is known to be a heterotroph (6, 16).

The salinity of the great majority of neutral-alkaline hot springs is much higher than that of

TABLE 4. *Composition of media and solutions*

Medium D[a]	
Double distilled water	1,000 ml
Nitrilotriacetic acid	0.1 g
Miconutrient solution	0.5 ml
$FeCl_3$ solution[b]	1.0 ml
$CaSO_4 \cdot 2\ H_2O$	0.06 g
$MgSO_4 \cdot 7\ H_2O$	0.10 g
NaCl	0.008 g
KNO_3	0.103 g
$NaNO_3$	0.689 g
Na_2HPO_4	0.111 g
Micronutrient Solution	
Distilled water	1,000 ml
H_2SO_4 (concd)	0.5 ml
$MnSO_4 \cdot H_2O$	2.28 g
$ZnSO_4 \cdot 7H_2O$	0.50 g
H_3BO_3	0.50 g
$CuSO_4 \cdot 5H_2O$	0.025 g
$Na_2MoO_4 \cdot 2H_2O$	0.025 g
$CoCl_2 \cdot 6H_2O$	0.045 g
Complex hot spring water medium	
Hot spring water	1,000 ml
Soil extract[c]	50 ml
Ethylenediaminetetraacetate-Fe (13% Fe)	0.005 g
$MgSO_4 \cdot 7H_2O$	0.100 g
KNO_3	0.260 g
K_2HPO_4	0.100 g

[a] Designed by R. P. Sheridan, medium *D* is prepared as a 10-fold concentrated stock and stored at 4 C unautoclaved. The *p*H is adjusted after dilution (double-distilled water) to 8.2 with 1 M NaOH. The final *p*H is 7.5 to 7.6 after the autoclaved medium has cooled and cleared completely.

[b] $FeCl_3$ solution consists of 1,000 ml of distilled water and 0.2905 g of $FeCl_3$.

[c] Approximately 400 g of brown loam is autoclaved in 1,000 ml of water for 40 min. Mixed slurry is filtered through triple-layered Whatman no. 1 paper while warm. Clear amber liquid results.

most surface waters. Nevertheless, some essential nutrients such as combined nitrogen or phosphate may be very low. However, none of the thermophiles in culture seems to be sensitive to high levels of these elements, unlike obligate oligotrophs (*see* reference 82). Nevertheless, there are some very common thermophilic blue-greens that have not responded to my isolation techniques. These include the varieties of *S. minervae* and species of *Spirulina*.

Medium *D*, with the addition of 0.1% tryptone and 0.1% yeast extract, was also used successfully (46 to 79 C) for the culture of the nonsporulating bacterium *T. aquaticus*, obtained from hot springs and hot tap water (42). Sporulating, thermophilic bacteria (e.g., *B. stearothermophilus*) from hot springs and many other sources have been grown in a variety of complex and defined organic culture media (5), as well as in relatively simple medium composed of distilled water containing 0.08% $MgSO_4 \cdot 7\ H_2O$, 0.018% $CaCl_2 \cdot 2\ H_2O$, 0.05% KNO_3, 0.28% NH_4Cl, 4% glycerol, 0.6% sodium glycerophosphate, and 1% glucose monohydrate, which was adjusted to *p*H 6.8 and sterilized by filtration through a membrane filter (47).

Isolation and Maintenance

Algal material collected from hot springs should be *quite* dilute in sample vials containing spring water. Most keep very well, often for several weeks or months, in the dark at a temperature between 10 and 20 C. A temperature below 5 C is quite detrimental to some. However, even 20 to 30 C in darkness will generally not harm samples for several days if kept dilute. Therefore, no special precautions are required for shipment except padding and darkness.

Normal *D* medium (Table 4) with a *p*H of 7.5 after autoclaving is used as almost a universal medium for the isolation and growth of cyanophytes from hot springs which range in *p*H from 5 to over 9. Most thermophilic blue-green algae, as well as the great majority of mesophilic types, prefer an alkaline environment (104). The *p*H of *D* medium rises as a result of algal growth, as it is essentially unbuffered. Medium *D* of *p*H 4 has been used to isolate and cultivate the thermophilic eucaryote *Cyanidium caldarium* from acid-sulfate hot springs.

Collected material may be handled in several ways in the laboratory. Filamentous types which are motile or have motile hormogonia (e.g., *Oscillatoria*, *Phormidium*, *Lyngbya*, *Symploca*, *Calothrix*, *Mastigocladus*) will generally isolate themselves by gliding away from the inoculum particle in a few to several hours on *D* medium or Cg-10 medium (177) solidified with 1.0 to 1.5% agar in plates and incubated at an appropriate temperature. Blocks of agar with single trichomes may then be picked off for inoculation into liquid or agar medium, in one step establishing clones which may also be axenic. Some thicker trichomes (e.g., *O. princeps*) penetrate into and glide well *through* agar medium, and there is an even greater likelihood in such cases of freeing the trichomes of bacteria. With some slow-moving species the original inoculation on agar medium may be incubated for a few to several days. This will eventually establish a peripheral mass of trichomes for subsequent transfer to new agar.

A gross inoculation on agar medium may also be used for nonmotile cyanophytes, but not if there is an abundance of motile types in the sample. The almost ubiquitous *M. laminosus* and

P. laminosum have often been separated by dilution streaking. Unicellular forms, such as *Synechococcus* sp., may also be isolated in this manner, especially on the Cg-10 agar of Van Baalen (177), and probably on the unicellular medium of Allen as well (10). *Mastigocladus*, however, is somewhat unique among thermophilic types in its ability to come up in agar stabs or in agar shake cultures. In the latter method the inoculum is washed, blended, and diluted before dispersing in the unsolidified medium at approximately 45 C. Axenic cultures of *Mastigocladus* have also been obtained in this way. For the most common unicellular thermophiles of North American springs, *Synechococcus* sp., isolations are very simple for the narrow-form rod types (resembling *S. lividus*) which are described as several species by Copeland (59). In springs in which *Synechococcus* is present, the bulk inoculation of collected material into liquid *D* medium usually results in the growth of several blue-green algae. However, with time, narrow-form types of *Synechococcus* almost always outgrow the others and often form a dense suspension bloom. One or more transfers of suspended cells generally results in an unialgal culture. Clones of these rods (1.2 to 2.2 μm wide) may be established in different ways. For incubation temperatures of 50 C or less, *Synechococcus* suspensions may be diluted by streaking on the Cg-10 agar medium developed by Van Baalen (177) for single cells of *A. nidulans*. In my laboratory this medium is modified only slightly: the glycylglycine buffer is reduced to 0.5 g/liter, 1% agar is used, and the *p*H is adjusted to 8.2 with 1 M NaOH before autoclaving. Colonies from single cells of *Synechococcus* develop on this medium but generally not on *D* agar. Axenic cultures of *Synechococcus* may be obtained in this way. Difficulties arise, however, with the high temperature "races" of *Synechococcus* which will not grow below 50 to 55 C (146). These may be easily enriched for by incubating the sample in liquid *D* medium at higher temperatures (e.g., 60 to 70 C). Material collected above 65 C in most hot springs will contain narrow-form *Synechococcus* as the only viable photosynthetic organism. However, syneresis of agar or silica gel occurs and the surface dries rapidly at temperatures above 55 C; streak and plating isolation methods were unsuccessful. A tedious, but sometimes effective, method of cloning and purification by manual isolation has been used (124). It involves the spotting of minute drops of a dilute cell suspension in a film of mineral oil spread on a glass plate or slide. After microscopic examinations, drops with single cells are sucked up with a capillary pipette and inoculated into liquid medium. This has not worked well with high-temperature *Synechococcus*, however, since cells may not generally survive either the pick-up by pipette or the transfer to fresh or conditioned medium. The picking up of agar blocks with single cells that have been sprayed onto the surface of a plate may prove less damaging. We have had some success by inoculating liquid *D* medium with a small amount of a very dilute cell suspension. In new medium, the cells will settle out and small clonal colonies will appear on the flat bottom, but the flask must not be agitated at all during the incubation. Another method has been to pull a very dilute cell suspension on to a Whatman GF/C glass-fiber filter, then to place the whole disc in liquid *D* medium. Since these filters become quite fuzzy when submerged, small colonies may be removed by picking off a submerged tuft with a forceps.

Many larger cells or filaments (e.g., *Mastigocladus*) may be cloned and also purified by a series of dilution washes when manually carried through a series of depression wells, each with sterile medium. A pulled glass capillary tube, point, or hook may be used under a dissecting microscope for the transfers. Agar plates may also be conveniently used as a working surface for dragging out and isolating single cells or filaments from mixed samples (*see* reference 125).

Axenic cultures of some cyanophytes may be established by treatment with ultraviolet radiation (104). With some of thermophilic types, however, it appears that the several orange-colored bacteria often present are less sensitive to ultraviolet treatment than the cyanophyte.

Stock cultures here are usually maintained in 125-ml Erlenmeyer flasks with 80 ml of liquid *D* medium or in test tubes with 15 to 20 ml of medium. Incubation temperatures vary with the species. However, the bulk of the cultures are kept at 45 C in constant-temperature water baths. The flasks are usually stoppered with nonabsorbent cotton, but at higher temperatures (>55 C) Morton metal closures with "fingers" are used with Bellco (Bellco Glass, Inc., Vineland, N.J.) flasks to slow evaporation. Growth is considerably slower in the vessels with closures; in some cases this is preferred. The light intensity at the flask level is kept at about 200 to 500 ft-c (continuous light from Coolwhite fluorescent lamps). The intensity at the culture level in the tubes is often only 50 to 100 ft-c. Both flask and tube cultures, particularly when aged, should never be exposed to high intensities (>600 ft-c) for more than a couple of hours; death of all cells may occur. The flask cultures require transferring about every 4 to 5 weeks, whereas the slower growing tube cultures are generally transferred every 8 to 9 weeks. Agar surfaces are used in flasks

for maintaining axenic cultures, but agar slants in tubes dry too quickly to be used at these temperatures. Cotton plugs can best be protected from dust contamination by capping these with a soft porous tissue (e.g., Kimwipes) held with a rubber band. Nonporous caps cause wetting of the cotton plugs at these temperatures. Air-circulated incubators at 45 C and above result in excessive evaporation; consequently, water baths are preferred.

Many thermophilic cyanophytes will tolerate freezing or drying for a long time. Slow freezing (5 to 10 min) to −20 C works with several common thermophiles. Holm-Hansen (102) found this method somewhat preferable to rapid freezing, although the blue-greens used were nonthermophilic types from either polar or temperate regions. All culture strains of the ubiquitous *M. laminosus* and *P. laminosum* tolerated slow freezing and storage at −20 C in my laboratory without any apparent cell death, although only *Mastigocladus* was stored as long as 3 months. During some trials, various strains of *S. lividus* tolerated freezing, and some cells were viable after 1 to 2 years of storage, but this method has not worked in every case. *O. terebriformis*, a common thermophile in Oregon, will not tolerate slow or rapid freezing. Freeze-drying may be effective with the more labile types, but it has not yet been tried with these thermophiles (*see* reference 103).

Dimethyl sulfoxide, glycerol, or other cryoprotective agents might also enhance survival of cells at low temperatures, but they have not been tested with thermophilic blue-green algae.

Dilute cultures and samples of several species in liquid medium or spring water have been stored in complete darkness at about 15 C and have survived well for over 4 months. Such a simple method deserves more intensive trials.

M. laminosus will also tolerate dryness at about 25 C (with or without a desiccant) for at least a few months with little loss of viability. Narrow-form *Synechococcus* sp. survived, at most, a few days of drying, and *O. terebriformis* does not survive at all.

Rates of Growth, Photosynthesis, and Respiration in Culture

The remark by Marrè (133) that thermal algae generally grow slowly was based on the few studies available at that time. Since 1961, however, there have been enough additional reports of thermal growth rates to indicate that such a generalization is incorrect. A short generation time (about 2 hr) was known in the case of *A. nidulans*, which has a maximal growth rate at 41 C but tolerated a temperature no higher than 45 C (117). Dyer and Gafford (66, 67) reported nine doublings per day for *S. lividus* at 52 C, which is almost comparable to *A. nidulans*, *Chlorella pyrenoidosa* TX 7-11-05 at 39 C (166), and *Chlamydomonas mundana* at 33 C (129). Similarly, several isolates of *S. lividus* from eastern Oregon springs grew at rates ranging from 8 to 10 doublings per day from 45 to 55 C (146). Both *A. nidulans* and *S. lividus* are small unicellular rods ranging in cell width from about 1.2 to 2.2 μm. *Chlorella* and *Chlamydomonas* are also small, but most species or strains do not exceed 3 to 4 doublings per day (106). Over a fairly wide range of phylogenetic groups there appears to be an inverse correlation between cell size and growth rate (*see* reference 185), but no constant relationship between high growth rates and high temperature, although rates of over 3.0 doublings per day appear to be confined to temperatures over 20 C (106). Brock (29) cites somewhat shorter generation times for higher temperature species of bacteria than for lower temperature types. In thermophilic *Synechococcus*, the highest temperature races have reduced growth rates (146). Whether the observed limit is genetically controlled or whether other problems, such as nutrient or CO_2 availability, are more manifest at higher temperatures in still unresolved. Current studies at 60, 65, and 70 C indicate that the maximal growth rate under a variety of aerated conditions is only about 2.0 doublings per day (J. Meeks, *unpublished data*). Estimates of growth rates of *Synechococcus* near 70 C in natural populations gave comparable values (38; *see* earlier section on field results).

Holton (105) estimated a growth rate of about 1.5 doublings per day for *M. laminosus* and considered it quite low. It is lower than many blue-green algae but is nevertheless comparable to a large number of eucaryotes (106). Nonthermophilic filamentous cyanophytes such as *Schizothrix calcicola*, *Anabaena variabilis*, *Nostoc muscorum*, and *Tolypothrix tenuis* reached maximal growth rates (3.0 to 4.0 doublings per day) in culture above 30 C (106). The filamentous but undifferentiated *O. terebriformis* (trichome width, 4 to 6 μm) approached 5 doublings per day under optimal conditions from 45 to 53 C (Fig. 4). *O. princeps*, with the great trichome width of 20 to 30+ μm, has not surpassed 0.5 doublings per day in culture at its 40 C optimum (L. Halfen, *unpublished data*). Even though information on growth rates of the blue-green algae is still scanty, it seems that the variation among species is as great in thermophiles as in mesophiles and that no clear trends are evident.

Photosynthetic rates in thermophiles, as measured by $H^{14}CO_3$ or $^{14}CO_3$ incorporation,

have been measured mainly in field populations. However, neither these nor the few laboratory measurements have distinguished thermophilic cyanophytes from the mesophiles or from the bulk of the eucaryotic algae (49, 67, 118, 137, 147, 161).

Cyanophytes respire aerobically in the dark, but sometimes at rates lower than those of eucaryotic algae (24, 43, 104, 105, 118, 182). In the thermophilic *M. laminosus*, Holton (105) found a dark endogenous respiratory rate [μliters of O_2 uptake per mg (dry weight) per hr] of 8.0 for actively growing material at 45 C, as compared to 7.5 for active cells of *A. nidulans* at 39 C (118). Biggins (24) found somewhat lower rates (4 to 6 μliters of O_2 per mg per hr) for *A. nidulans* at 25 C (a suboptimal temperature). The existence of peculiarities in respiration, oxidative phosphorylation, and other aspects of "dark metabolism" in blue-green algae has been suggested and argued recently by several persons (19, 24, 104, 107, 123, 144, 165).

RESPONSES TO TEMPERATURE AND LIGHT INTENSITY

Optimal Temperature and Light Intensity

The optimal conditions for growth may seldom be described in terms of a single factor. Nevertheless, this has been attempted, using temperature alone, nutrient concentration alone, light alone, etc. Most of these factors influence the effect of one or more of the others. Thus, experimental procedure can become very complex when one attempts to consider several factors simultaneously. Few workers have done so. The temperature optimum for growth or photosynthesis of microalgae may be dramatically or subtly influenced by light intensity, day length, nutrient concentration and availability, CO_2 concentration, *p*H, constancy of the several factors, and preconditioning. In a series of field experiments, Brock (31) found that the maximal rate of ^{14}C photoincorporation in natural populations of *Synechococcus* (mainly) taken from several different temperature regimes was very close to the temperature from which they had been collected (Fig. 4). For example, sample cores were collected at 58.5 C in the thermal stream. These were equilibrated for about 5 min in as many as 10 new temperatures (about 23 to 73 C) in glass vials, followed by a 1-hr incubation with the isotope added. The photosynthetic population at 58.5 C in this Yellowstone spring consisted almost entirely of *Synechococcus* sp. Thus, although genetic adaptation was avoided by the short time involved, not enough time for physiological acclimation was allowed. Maximal ^{14}C assimilation occurred at the temperature of collection only; rates decreased with temperature below and above 58.5 C (Fig. 4). In the few thermophilic cyanophytes that have been studied in the laboratory, maximal growth or photosynthetic rates occurred over a considerably greater temperature range (Fig. 4). For this reason, I suspect that the sharpness of Brock's optimum temperature peaks is not real and that the species involved have the potential to photosynthesize and grow at maximal rates over a considerably wider range, given time for acclimation. Little is known about the resilience of thermophilic algae subjected to temperature changes. Several strains or races of *S. lividus* were able to tolerate abrupt changes within their growth range or below it without apparent damage (145, 146). Lag periods in the growth of some clones usually varied from a few to several hours, the time being somewhat proportional to the temperature difference involved. A displacement from a suboptimal temperature (e.g., 30 to 40 C in *clone 53*) to a higher temperature in the optimal range (45 to 55 C) generally resulted in a shorter lag ($<$10 hr) than when the direction was reversed (15 to 72 hr). The ability to withstand considerable changes in temperature is also known in obligate thermophilic bacteria, but rapid displacements to higher temperatures are often fatal to facultative thermophiles (*see* reference 21). To have a wide range in growth temperature would have considerable adaptive value in a thermal stream.

The loss of the ability to grow over the original full temperature range occurs commonly in some bacteria (74, 141). This is often a loss of low-temperature ability rather than a change in upper limit. Allen (6), using *C. caldarium*, and Löwenstein (128), using *Mastigocladus*, reported that the maintenance of cultures for long periods below the optimal growth temperature resulted in a lowering of the upper tolerance limit and of the optimum. Allen had mistakenly assumed that the original temperature maximum for *Cyanidium* was well above 55 C. Subsequent studies have shown that 55 C is very close to the upper limit for this organism in the laboratory (16) or field (34). In the case of *Mastigocladus*, the "maintenance" consisted of cold storage at a temperature of 5 to 8 C, well below the growth minimum of 25 C (128). Holton (105), while "cleaning up" his crude material from Laird Hot Springs, B.C., which contained *M. laminosus*, found there was a loss in tolerance to high temperature. In isolations from Iceland hot springs in 1968, I found that by first enriching for the high temperature race in culture medium at over 60 C followed by cloning with agar shake or streak methods at 45 C, no cultures were produced that were sensitive to the

highest temperatures normally attained by this organism [about 62 to 63 C (54)].

Peary (145) found no deadaptation in one clone of *S. lividus* after 8 months of growth at 30 C, a distinctly sub-optimal temperature. At that time it responded with the same growth rates from 55 to 30 C as the normal culture which had been maintained at 50 C. There were also no differences in lag periods, except at 30 C where the 30 C-grown material reached exponential phase first. The maintenance of the same clone of *O. terebriformis* at 29 to 30 and 44 to 45 C for 3 years has resulted in initially better growth rates at 30 C for the 30 C stocks than for the 45 C stocks (*unpublished data*). However, the 30 C stock was still able to grow well at 50 C. Similarly, Bünning and Herdtle (49) reported higher photosynthetic rates at 20 to 30 C in *O. geminata* that had been grown in that range than for those grown at the optimum of 40 C. The photosynthetic rates at 40 C were similar for both cultures. *M. laminosus* was collected (by T. D. Brock) from 58 C water in Iceland in 1966 but was isolated and cultivated in my laboratory at 45 C for 2.5 years. Upon raising the temperature to 62 C, the culture continued to grow and appeared similar to others cultured at 62 C (54). Another strain which had a lower initial maximum (about 57 C) retained this characteristic. Therefore, although loss of growth potential at the upper or lower end of the temperature range may be real in some cases, it is not general.

In some thermophilic blue-greens, the growth temperature optimum is a broad plateau, but it is usually skewed toward the upper end of the full temperature range (Fig. 4). In the case of *O. terebriformis*, the maximal growth rate was retained to about one degree from the normal lethal temperature of 54 C. The curve for *Mastigocladus* growth, which is broad and well graded at both ends, was sharpened at the upper end with CO_2 increase (105).

In thermophilic blue-green algae, the growth temperature optimum or range may be modified by other external factors; light intensity has one of the most profound effects. Most of the curves in Fig. 4 reflect changes in temperature under the same light intensity. In *O. terebriformis*, however, growth rates were measured after full acclimation at each temperature under a light intensity saturating for that temperature. An air (CO_2) supply sufficient to saturate growth for each light intensity and temperature was also used. In this species, the growth-saturating light intensity varied from about 1,200 ft-c (Coolwhite fluorescence) at 45 C to about 350 ft-c at 31 C, and inhibitory effects of bright light were felt at about 5,000 and 1,500 ft-c, respectively. The maximum growth rate at 31 C was only about 20% of the 45 to 53 C value (Fig. 4). Therefore, although acclimation periods and lags differed, maximum sustained growth rates could be attained at about 1,200 ft-c for the entire temperature range mentioned. Photosynthetic saturation occurred at about 800 ft-c in one Yellowstone strain of *Synechoccus lividus* at 45 C, when the cells were pregrown at that intensity (161). Similar values often apply to *Chlorella*, but the preceding light intensity for growth has a great influence on the saturation intensity for photosynthesis (167). *A. nidulans* appears to have a similarly high saturation intensity even when pregrown at much lower levels (118, 137). In natural hot spring mats, evidence has already been presented that suggests that many cells making up the algal layer might not become light saturated even at very high midday intensities because of self-shading.

The high light intensities reaching the algal surface in many thermal waters should result in "sun-adapted" forms. Typical manifestations of "sun adaptation" are the lack of inhibition by bright light and saturation of photosynthesis and growth by a relatively high intensity (*see* references 44, 167). Some of Brock's field results indicate that there is little or no inhibition of photosynthesis in the compact mats of *Synechococcus* at the highest natural light intensities. My results with suspensions of *Synechococcus* cells demonstrate that inhibition at the level of an individual cell does occur, but probably only above about 5,000 ft-c [about 0.2 cal per cm^2 per min (400 to 700 nm)]. With the high intensity values for saturation and inhibition, *S. lividus* qualifies well as a sun-adapted organism. In contrast, some blue-green algae are inhibited by light intensities lower than 500 ft-c (44).

Effects of Light and Temperature on Pigmentation

Except for thermophilic organisms that may avoid bright light by growing at some depth in the microbial mat or by responding phototactically and gliding away from it (53), most thermophilic microorganisms are probably able to tolerate exposures by making physiological adjustments which usually involve changes in chlorophyll and carotenoid pigments.

Some cyanophytes, at least, seem to contain carotenoids which are not efficiently coupled to photosynthesis (70, 93, 111, 140). Goedheer (93) has evidence to suggest that energy transfer from β-carotene to chlorophyll occurs in photosystem I in blue-green algae and that light energy absorbed by xanthophylls is not transferred to chlorophyll. Some of the carotenoids in most (or all) microorganisms function in protecting living

cell constituents from photooxidations sensitized by chlorophyll and other pigments (81, 119). This has been demonstrated in photosynthetic bacteria (163), heterotrophic bacteria (135), and eucaryotic photoautotrophs (*see* references 119, 138). However, the physicochemical nature of the molecular interactions which constitute protection are still poorly understood (81).

At this point, there is no reason to suggest that there is any consistent difference in the pigments of thermophilic and nonthermophilic blue-green algae (however, *see* reference 22). Phycoerythrin is absent from *S. lividus*, *M. laminosus*, and apparently all blue-green algae which grow at temperatures above 60 C. It is also lacking in the mesophiles *A. nidulans*, *Gloeocapsa alpicola*, and *P. luridum* (44). Phycoerythrin occurs in addition to phycocyanin in thermophilic *S. minervae* (maximum, 60 C), *O. terebriformis* "thermal-red" (maximum, 53 C), and in numerous mesophiles (*unpublished data*). The identity of some of the carotenoids in blue-green algae is still uncertain and there is variation among species (97–100). Myxoxanthophyll, echinenone, and β-carotene, however, appear to be almost universally present. It has been known for many years that the amount of chlorophyll decreases and that the carotenoid content increases (at least relative to chlorophyll) when blue-green algae are grown under bright light. Complementary chromatic adaptations (87, 112) have also been described in blue-green algae, but these are probably over ridden by high intensity responses in most hot-spring environments.

Sargent (150) found that high intensity white light or light of various broad spectral bands caused a change in *Gloeocapsa montana* from dark blue-green to yellow, and that lowering the intensity reversed the process. The color changes could be explained solely by a chlorophyll decrease over a few cell generations. *A. nidulans* (137) and *Phormidium persicinum* (44) responded similarly. In *Anacystis*, phycocyanin paralleled chlorophyll in its decline with increasing light intensity (no phycoerythrin), and in *P. persicinum* phycoerythrin was even more sensitive to bright light than chlorophyll (no phycocyanin present). In the field, the greening and yellowing of thermal *Synechococcus* populations is controlled by the amount of shading, at least in summer (41, 52). In *S. lividus* (41) and *A. nidulans* (8) grown at high light intensities, the decrease in chlorophyll was apparently accompanied by a decrease in the photosynthetic thylakoids. In the light intensity range for saturation, photosynthesis is no longer limited by the amount of light received but by the maximum rate of the "dark" reactions. A continuing decline in the bulk, light-harvesting pigments as light intensities are increased should be expected on the grounds that the excess pigment would not be required. A decrease in the amount of photochemical machinery during bright-light exposure and the maintenance of relatively large amounts of carotenoids at the thylakoids should be of value in reducing photodynamic lesions.

At a constant temperature and light intensity it was characteristic of the chlorophyll and carotenoid content of cells (pigment per unit of dry weight) to remain fairly constant during the exponential phase of growth in *O. terebriformis*, with self-shading kept to a minimum (*unpublished data*). At 45 C, the mean chlorophyll and carotenoid values for the exponential growth phase varied inversely with the light intensity. From 200 to 4,000 ft-c, there was approximately an eightfold decrease in the characteristic amounts of chlorophyll per cell during exponential growth, but the decrease in total carotenoids was only about twofold. The ratio of carotenoid to chlorophyll (optical density at 472 nm to optical density at 665 nm, in methanol) was about 0.5 to 0.7 when grown in dim light in contrast to about 2.5 when grown at 4,000 ft-c and higher. The decrease in chlorophyll with ascending light intensities was accompanied by a large decrease in phycoerythrin. Thus, *O. terebriformis* t-r (thermal-red) was a deep red-brown color at low light intensity, due primarily to the large amounts of phycoerythrin, whereas at very high intensity it was a pale ochre-yellow with carotenoids predominant. Growth of *O. terebriformis* in turbulent aerated cultures at high light intensities resulted in approximately 100 times more myxoxanthophyll than at the low intensities, although the other carotenoids were reduced.

At optimal temperatures (45 to 53 C), abrupt upward or downward shifts in light intensity (involving a range of up to 4,000 ft-c) caused no distress in the cultures. An upward shift to bright light resulted in an immediate increase in growth rate (dry weight increase). Carotenoid and chlorophyll pigments adjusted to new levels and new ratios in about 12 to 24 hr after the shift. Thereafter they generally increased at the same rate as total dry weight (*unpublished data*).

Growth and Survival at Suboptimal Temperatures

When *O. terebriformis* cultures were shifted from an optimal to a suboptimal temperature, the initial pigment content appeared to be a critical quantity (*unpublished data*). If the cultures were preconditioned by growth at 45 C at a high light intensity (e.g., >2,000 ft-c) there was no lag in growth when the culture was shifted to 31 C

at 350, 860, or 1,500 ft-c, but the rate dropped to that typical of light saturation at 31 C (Fig. 4). However, if cells were preconditioned at a low light intensity (350 ft-c) at 45 C and then shifted to 800 or 1,500 ft-c at 31 C, a lag of 6 to 7 days occurred, followed by a gradual attainment of exponential growth. The preconditioning under dim light at 45 C resulted in low carotenoid-chlorophyll ratios (about 0.5); in bright light, values of 1.0–2.5 were obtained. In the lagging cultures, exponential decreases in chlorophyll occurred for the period of the lag. The carotenoid drop was not as great proportionately. Only after high pigment ratios were finally attained at the lower temperature did exponential growth begin. A shift to 28 C and 300 ft-c (after growing at 45 C and 200 ft-c) resulted in an initial increase in dry weight for a period of about 48 hr while chlorophyll was simultaneously declining. This was followed by a decrease in dry weight until about the sixth day when almost all of the cells had lysed. An initial increase as described was not uncommon in downward shifts of temperature, even to temperatures below the growth minimum. Cultures grown in bright light (about 5,000 ft-c), however, withstood the shift to 28 C without a lag and grew at slow exponential rates for several days. Even with properly preconditioned cells, 28 C seemed to be about the lowest temperature at which aerated cultures could be maintained. Cells grown at 45 C and 5,000 ft-c were also shifted to 27 and 25 C. These grew for a limited time at very low rates (0.2 to 0.4 doublings per day at 27 C), but eventually diminished to zero. The highest growth rates of separate cultures differed from each other under identical conditions at 27 or 25 C, apparently reflecting differences in initial pigment content.

The probable protection provided by carotenoids against detrimental lesions was also demonstrated in light at much lower temperatures with *O. terebriformis*. At 13 or 18 C, the death rate may be reduced considerably if cells have an initially high carotenoid to chlorophyll ratio. At 700 ft-c, the lysis of almost the entire cell population occurred in 24 hr or less at 13 or 18 C when the initial pigment ratios were below 1.0. With higher ratios (about 2.5), no significant lysis occurred for about 3 to 4 days, and during the first 24 hr there was even a slight increase in dry weight. The death rate in darkness at similar temperatures was independent of pigment content, and there were large populations of viable cells after 2 weeks at temperatures ranging from 10 to 25 C.

These results indicate that the most deleterious effect of exposure to suboptimal or subminimal temperatures in the light may be the photooxidation of essential cellular (thylakoidal?) components which include chlorophyll, the sensitizing pigment. In the absence of syntheses, or with very slow synthetic rates, these photodynamic lesions would eventually cause the death of the cell and its subsequent lysis. Even when initially high carotenoid levels apparently afford complete photoprotection at first, eventually disproportionate changes in pigments will occur at temperatures where synthetic rates are too slow to maintain balances.

In nature, it would seem that *O. terebriformis* (and perhaps other blue-green algae, too) would lack the resilience to withstand abrupt increases in light intensity when stranded at suboptimal temperatures, unless pigment balances were favorable when the temperature change occurred. Analyses of the pigments of natural populations of *O. terebriformis* at various seasons have shown, however, that carotenoid-chlorophyll ratios are very much lower than for comparable light intensity regimes in the laboratory (*unpublished data*). Nevertheless, this should be expected, since this and other species of blue-green algae in the springs occur as dense mats with much self-shading. In the case of the *Oscillatoria* mats, the trichomes are so motile that no individual is likely to maintain an exposed position for very long. Within an optimal temperature range, great increases in light intensity should be tolerated even by the most exposed trichomes. However, it is probable that this resilience would be lost if a trichome were washed downstream to lower temperatures or if the thermal gradient steepened suddenly. Continued growth or survival would probably depend in large part on the pigment content of the cells at the time.

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