# Tropical Macroalgae as Pollution Indicator Organisms[1]

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## INTRODUCTION

A great deal of emphasis has been placed on the role of phytoplankton as primary producers in marine biology. As our focus changes from oceanic to coastal processes, it becomes clear that benthic macrophytes emerge as dominant, if not predominant, in production of plant material. In two geographical regions is this particularly marked: the tropics and the very nearshore regions. Unfortunately, it is in these same regions that man's impact is rapidly occurring often resulting in irreversible destruction so that rapid and accurate information must be accumulated to control these impacts for optimum usage. Since a great portion of efforts in marine biology have been directed toward an understanding of the phytoplankton system, little data has been accumulated on the effect of various physical and chemical factors on macroalgae, especially tropical species.

The following is an attempt in the field and laboratory to accumulate a data base using tropical and subtropical macroalgae as indicator organisms. It is hoped that this would allow rational decision making as to discharges of thermal effluents from power plants as well as contribute to a fundamental understanding of the physiological ecology of tropical benthic macroalgae.

Temperature, exerting a fundamental influence on the rates of life processes, is one of the most important and universal environmental factors with which an organism must contend. This realization has prompted numerous investigators to amass quantities of temperature data for a wide variety of life processes. Excellent reviews have been presented by Precht (1949); Johnson, Eyring and Pollisar (1954); Gunter (1957); Marre (1962); Kinne (1963, 1964); Rose (1967); Prosser (1954) and Precht *et al.* (1973) in which thermal survival limits appear fundamental to evolution and distribution of organisms. Unfortunately, most survival limit studies of macroorganisms lack precise temperature control which has been utilized in studies of microorganisms. This is most glaring in the temperature studies on marine algae, an unfortunate situation since many consider temperature the most

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important factor in the marine environment, a major limiting factor for geographic and evolutionary coordinates (Gunter, 1957; Hedgepeth, 1957; Moore, 1958).

A review of marine macroalgae temperature limit studies clearly shows that we have a paucity of precise knowledge of tropical and Arctic species in particular (Oltmanns, 1905, 1923; Setchell, 1929; Blinks, 1951; Feldman, 1951; Doty, 1957; Gessner, 1959; Heim, 1959; Biebl, 1958, 1962; Marre, 1962; Abbott and North, 1972).

The marine tropics have been defined by temperature limits in biogeographical contexts from 25° to 30°C and 20° to 30°C and recently in a more physiological sense from 15° to 35°C (Scholander *et al.*, 1953). Unfortunately, most of the ecological work which substantiates these numbers has been based on animal studies and but a few measurements of algae temperature limits are available for comparison (Beibl, 1962; Jitts *et al.*, 1964; Abbott and North, 1972).

The tropics have been known to come close to their lethal limits during the summer since the time of Mayer (1914) who examined various animal lethal limits in the Dry Tortugas. "Tropics" itself being a term as the highest temperature regime in the marine environment affords an opportunity to explore the highest of the upper limits.

Temperature limit studies have been made on microorganisms where temperature control was as precise as $\pm 0.01$°C (Oppenheimer and Drost-Hansen, 1960; Miller *et al.*, 1966; Morita and Haight, 1964; Seiburth, 1964; Harden and Veldkamp, 1967). Judging from the above mentioned literature on macroalgae, the precision limits were one or two orders or less for macroalgae.

The laboratory section of this was an attempt to apply improved techniques of temperature control to determine the detailed temperature limits of tropical macroalgae. The major indicator organism studied was *Valonia*, which has long been used as a physiological tool for studying the green marine algae; however, the temperature limits have never been examined. It has the advantage of being a single cell and a whole organism. The improved techniques used in the microbial investigation were employed in hopes that a more precise limit of tropical algae could be found, as well as, giving a firmer basis on which to correlate the precise data obtained from biochemical studies and biophysical studies to the physiological and ecological data.

The ecological section was carried out at the site of a power plant complex (two fossil fuel and two nuclear plants) in Biscayne Bay, Florida, a subtropical estuary just at the edge of the tropics. The benthic macroalgal assemblage was tropical and not depauperate; however, the temperature regime and depauperate fish assemblage had been the basis on which the "subtropical" label had been given. Descriptions of the study area are found in Thorhaug *et al.* (1974).

## METHODS

### Field

#### FIELD PROCEDURES FOR GREEN ALGAE

Eight algal stations at Turkey Point and sixteen stations in Card Sound were

studied. The sampling procedures consisted of: a) The algae in a one square meter are counted via SCUBA, biweekly (during the summer of 1971 counting was done weekly). Each of the meter squares was subdivided into four equal sections with 1/8 inch polyethylene cord to facilitate counting. The number of each species present in each quadrant was recorded and can be used as replicate counts. The number of plants of each species which were in the reproductive phase were recorded. The number of juvenile, scenescent and dead and/or dying plants of each species was also recorded; b) Growth of algae was determined by measuring plants in one of the 1/4 $m^2$ quadrants on a biweekly basis. Measurements of *Penicillus, Halimeda, Rhipocephalus, Udotea* and *Avranvilla* were measured with vernier calipers according to the methods of Thorhaug (1965). c) Photographs were taken of the algal squares.

FIELD PROCEDURES FOR RED ALGAE

Eight stations in Turkey point and sixteen stations in Card Sound were chosen from a grid. Aluminum frames were placed randomly at each station of one square meter. Each was subdivided into quadrants with 1/4 inch polyethylene cord. The percentage of each quadrant covered with red algal species was recorded as well as any unusual events such as epiphytes and gammete formation. Photographs were taken monthly of the algal squares.

## Laboratory

THE POLYTHERMOSTAT

The basic instrument used in the controlled temperature experiments was an aluminum bar bored to fit glass tubes, heated at one end and cooled at the other to provide the desired temperature. Selected organisms were placed in each tube and held at the observed temperature for the desired time and kept under nearly constant surveillance. Improvements over similar temperature control devices include the ability to fluctuate the temperature over short or long cycles, providing aeration for adequate oxygen for experiments and a constant accurate temperature readout for each tube.

Specifically, the polythermostat is a block of aluminum ($6' \times 3'' \times 9''$) precision bored to fit 24 sets of $19 \times 150$ mm glass cuvettes. The holes were spaced every 2.5 cm starting 24 cm from both ends of the bar. Twenty-four 3/32 inch thermocouple fittings were also bored in the block 0.5 cm from each tube (permitting temperature monitoring on each set of tubes); ten holes were bored for thermometers. One end of the block was heated with two strip heaters (750 and 400 watts) and the other end cooled by pumping a 50: 50 mixture of ethylene glycol and water at $-10°C$ through cooling fins cut into the aluminum bar. A 55-gallon drum containing the glycol-$H_2O$ mixture was cooled by a constant flow portable cooling unit. The mixture was pumped through 1/4 inch copper refrigeration tubing to the cold end of the temperature gradient bar. Both ends of the bar were temperature regulated to $\pm 0.05°C$ by two electric mercury thermoregulators inserted

directly into the bar, one at each end. These were, in turn, controlled by a special relay variable transformer circuit. Recording accuracy was better than $\pm 0.05$°C.

Insulation was found to be critical for maintaining the desired temperature gradient in the laboratory. Three inches of styrofoam sheeting was placed on the bottom and sides of the bar and the entire assembly mounted in a wooden casing. Strips of 1/4 inch styrofoam were fitted on the top of the bar. Laboratory air-conditioning was kept at 22°C for best results.

For fluctuating temperature experiments, a tripper switch was hooked into the circuit with the polythermostat. The switch could turn the machine on for a given length of time and then turn off, and the chart recorder would give the thermal history of each set of tubes during the specific time period. Also, the amount of heat produced could be varied and thus, the extremes of the fluctuating temperature regime by resetting the mercury thermoregulators. Fast and slow cycling could thus be accomplished.

Bubbling was supplied with an aquarium pump, with the air passing through an interconnected system of aquarium gang valves connected by plastic tubing to disposable Pasteur pipettes. The pipettes were inserted through corks and into the cuvettes containing the experimental organisms; penetration into the seawater was controlled. Under rates of bubbling ample to maintain the organisms, using this system, no temperature error or variability was observed.

Using two or three polythermostats at the same time permitted the fine discrimination over a large temperature range, for example one polythermostat could be set from 10–40°C; the other from 25–35°C also, one broad temperature range and one narrow, finely divided one could be observed. In short, many combinations of temperature ranges from 0–100°C could be selected; therefore, the system provided a way to set finely divided and accurate temperature gradients for the purpose of examining the effects of both fluctuating and constant temperatures on living processes.

### CULTURE METHODS

Single cell green marine alga, *Valonia*, has been grown for 70 years and its culture conditions are well known. The laboratory growth of the other green and red species was a continuation of earlier work; the methodological details are given in Thorhaug (1965).

### MORPHOLOGICAL CRITERIA OF DEATH

Despite common notions, it is often not too easy to determine when an organism is dead or dying; definitions are vague or non-existent. At times, the transition form the living to the dead is almost imperceptible, in other instances it proceeds slowly but with noticeable clarity and in some cases, as with the sporulation of *Valonia*, it is shockingly sudden. All species used in this investigation were observed over extended periods under a variety of conditions and the following morphological criteria have been developed for both field and laboratory.

*Halimeda*: (1) A color change from deep green to pastel green to pale yellow-green to white. All these may exist in small sections of a completely healthy speci-

men but when terminal segments are dramatically lighter than proximal ones death is indicated. (2) Individual segments crack easily. (3) Separation of segments on slight touching or shaking of tube. (4) Loss of turgor with a rubbery flexibility to branches, basal stalks, and the entire plant. (5) Care must be taken to note original condition of healthy plants which may be quite pale, with individual dead or damaged terminal segments, broken branches, etc., but with full turgor, and to individually observe changes from this point on.

*Penicillus*: (1) Color change from a healthy dark green to pale green to yellow green and then white, especially the filaments. (2) Loss of turgor of filaments. (3) Stalk becomes rubbery and then brittle. (4) Actual decay of plant with filaments decaying first, then interior of stipe.

*Acetabularia*: (1) Loss of color, change from green to white. (2) Sporulation and spores released from cap. (3) Breaking away of cap from stipe and decay of stipe.

*Valonia*: (1) Outright plasmolysis which is not reversible. (2) The formation of aplanospores. (3) Separation of plasma membrane from outer cellulose membrane forming a gap especially in medium and small cells. (4) The development of patchy grid-like reticulations on the cell wall. (5) Change from a dark green homogenous opaqueness or translucence to a spotty or complete transparency. (6) Loss of positive turgor; concavity may be introduced on the cell surface by slight pressure. The cells may not have plasmolyzed. (7) A loss of sheen to the cell wall.

*Laurencia*: (1) The foremost criteria is the condition of the vegetative buds on the tips, including color, shape, and degree of translucency when viewed under low power of a dissecting scope. Death caused the buds to become opaque and lighter in color, and to swell. (2) Secondary indications are color changes on the stem and slightly bloated appearance. When 50% of the buds were dead the entire tip was called dead. Biebl (1962) used staining techniques and noted that the cells swelled upon thermal death. This is interesting because *Valonia* cells among others shrink upon thermal death.

## RESULTS

### Halimeda incrassata

An earlier investigation (Thorhaug, 1965) showed that *Halimeda incrassata* could be successfully grown under laboratory conditions with rates of growth close to those in the field. In view of this and the fact that this ubiquitous algae is very abundant in Biscayne Bay and Card Sound, it made an excellent experimental plant. Specimens were obtained from the field and gently cleaned to remove epiphytes and debris. The results of three experiments indicated that exposure of eight days at temperatures from 32.9° to 34.8°C caused death (See Fig. 1). Field studies indicated that those stations at which the temperature rose above average daily temperatures of 33°C or a measured mid-day temperature of 32.6°C produced no young *Halimeda* (Tables 1 and 2) and the general condition of the algae began to deterio-

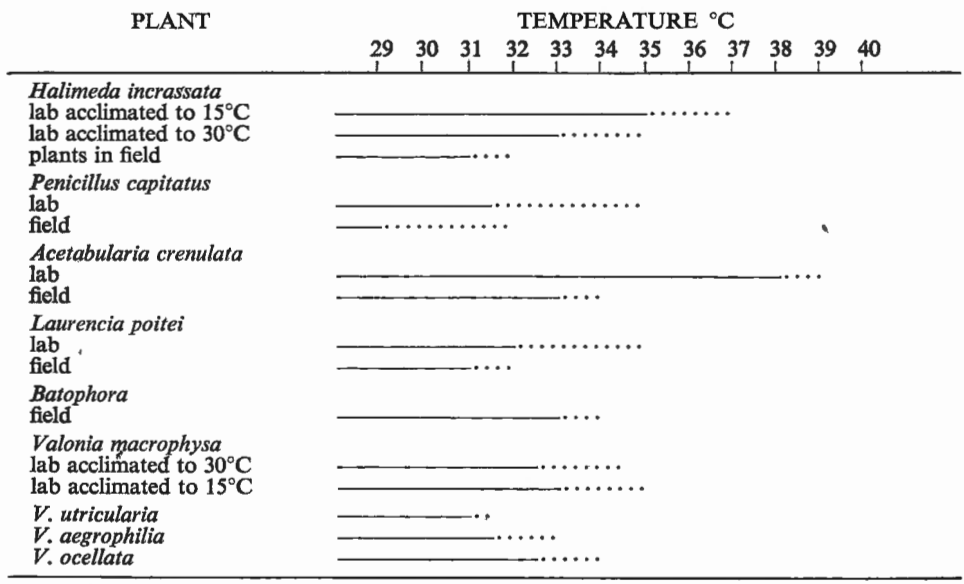


Fig. 1. Comparison of laboratory and field measurements of upper temperature tolerances of various marine tropical macroalgae. The solid line for the lab data indicates 100% survival and the dotted line indicates survival between 100% and 0%. For the field data, the solid line indicates normal abundance of this species and the dotted line indicates a marked decline in the population, but there are still some specimens left. Field data obtained from Thorhaug 1971 and 1974, laboratory data from Thorhaug, 1974.

Table 1. The distribution of standing crop at Card Sound, Florida with ambient temperature averaged over the previous two weeks for important macroalgae. At the time these readings were taken, no thermal effluent had been released.

| Temperature | *Penicillus capitatus* | | | *Halimeda incrassata* | | | *Acetabularia crenulata* | | | *Laurencia poitei* |
|---|---|---|---|---|---|---|---|---|---|---|
| °C | Total | %U* | %J* | Total | %U | %J | Total | %U | %J | % Cover |
| 16.1–17.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17.1–18.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18.1–19.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19.1–20.0 | 245 | 33 | 16 | 181 | 29 | 3 | 301 | 2 | 0 | 35 |
| 20.1–21.0 | — | — | — | — | — | — | — | — | — | — |
| 21.1–22.0 | 170 | 32 | 16 | 171 | 15 | 14 | 363 | 0 | 19 | 81 |
| 22.1–23.0 | 400 | 37 | 29 | 257 | 20 | 12 | 459 | 18 | 17 | 224 |
| 23.1–24.0 | | | | | | | | | | |
| 24.1–25.0 | These temperatures were not encountered. | | | | | | | | | |
| 25.1–26.0 | | | | | | | | | | |
| 26.1–27.0 | 557 | 34 | 21 | 413 | 11 | 11 | 804 | 1 | 16 | 403 |
| 27.1–28.0 | 160 | 49 | 15 | 102 | 13 | 25 | 108 | 21 | 6 | 128 |
| 28.1–29.0 | 252 | 50 | 19 | 134 | 21 | 11 | 118 | 17 | 10 | 180 |
| 29.1–30.0 | 847 | 42 | 17 | 571 | 22 | 16 | 547 | 19 | 26 | 405 |
| 30.1–31.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31.1–32.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32.1–33.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33.1–34.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* %U = % unhealthy, %J = % juvenile.

Table 1. Continued

| Temperature | *Udotea flabellum* | | | *Rhipocephalus phoenix* | | | *Batophora oestedii* | |
|---|---|---|---|---|---|---|---|---|
| °C | Total | %U* | %J* | Total | %U | %J | Total | %U |
| 16.1–17.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17.1–18.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18.1–19.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19.1–20.0 | 49 | 31 | 6 | 86 | 9 | 0 | 2695 | 0 |
| 20.1–21.0 | — | — | — | — | — | — | — | 0 |
| 21.1–22.0 | 50 | 30 | 0 | 64 | 45 | 0 | 1956 | 0 |
| 22.1–23.0 | 77 | 30 | 12 | 104 | 28 | 27 | 3609 | 0 |
| 23.1–24.0 | These temperatures were not encountered. | | | | | | | |
| 24.1–25.0 | | | | | | | | |
| 25.1–26.0 | | | | | | | | |
| 26.1–27.0 | 137 | 26 | 9 | 175 | 25 | 17 | 4622 | 0 |
| 27.1–28.0 | 14 | 29 | 36 | 27 | 48 | 15 | 1100 | 0 |
| 28.1–29.0 | 46 | 50 | 4 | 65 | 29 | 9 | 1647 | 0 |
| 29.1–30.0 | 145 | 38 | 10 | 380 | 13 | 4 | 10259 | 0 |
| 30.1–31.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31.1–32.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32.1–33.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33.1–34.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34.1–35.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 2. The distribution of standing crop at Turkey Point, Biscayne Bay, Florida with ambient temperature averaged over the previous two weeks for important macroalgae. Many of the stations examined were in a thermal effluent from a power plant.

| Temperature | *Penicillus capitatus* | | | *Halimeda incrassata* | | | *Acetabularia crenulata* | | | *Laurencia poitei* |
|---|---|---|---|---|---|---|---|---|---|---|
| °C | Total | %U* | %J* | Total | U* | %J | Total | %U | %J | % Cover |
| 16.1–17.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17.1–18.0 | 2 | 0 | 0 | 35 | 86 | 9 | 0 | 0 | 0 | 88 |
| 18.1–19.0 | 227 | 37 | 24 | 51 | 4 | 2 | 9 | 0 | 0 | 16 |
| 19.1–20.0 | 140 | 31 | 14 | 66 | 3 | 24 | 0 | 0 | 0 | 0 |
| 20.1–21.0 | 172 | 45 | 2 | 122 | 3 | 8 | 39 | 0 | 3 | 7 |
| 21.1–22.0 | — | — | — | — | — | — | — | — | — | 0 |
| 22.1–23.0 | 292 | 70 | 17 | 101 | 10 | 18 | 8 | 0 | 25 | 99 |
| 23.1–24.0 | 139 | 75 | 12 | 19 | 58 | 21 | — | — | — | — |
| 24.1–25.0 | 323 | 63 | 7 | 211 | 9 | 15 | 47 | 34 | 0 | 89 |
| 25.1–26.0 | 481 | 50 | 18 | 589 | 26 | 29 | 133 | 1 | 20 | 3 |
| 26.1–27.0 | 245 | 44 | 25 | 195 | 17 | 4 | 6 | 17 | 0 | 84 |
| 27.1–28.0 | 100 | 73 | 13 | 226 | 10 | 6 | 29 | 52 | — | — |
| 28.1–29.0 | 755 | 39 | 12 | 371 | 2 | 14 | 133 | 5 | 34 | 67 |
| 29.1–30.0 | 1710 | 44 | 16 | 160 | 7 | 9 | 335 | 21 | 20 | 10 |
| 30.1–31.0 | 1701 | 41 | 13 | 898 | 7 | 11 | 720 | 18 | 25 | 114 |
| 31.1–32.0 | 1016 | 49 | 15 | 1385 | 9 | 6 | 641 | 16 | 22 | 41 |
| 32.1–33.0 | 262 | 46 | 16 | 292 | 8 | 5 | 147 | 21 | 27 | 0 |
| 33.1–34.0 | 376 | 42 | 14 | 273 | 11 | 3 | 620 | 5 | 28 | 0 |
| 34.1–35.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* %U = % unhealthy, %J = % juvenile.

Table 2. Continued

| Temperature °C | *Udotea flabellum* Total | *Udotea flabellum* %U* | *Udotea flabellum* %J* | *Rhipocephalus phoenix* Total | *Rhipocephalus phoenix* %U | *Rhipocephalus phoenix* %J | *Batophora oestedii* Total | *Batophora oestedii* %U |
|---|---|---|---|---|---|---|---|---|
| 16.1–17.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17.1–18.0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 18.1–19.0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 |
| 19.1–20.0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 |
| 20.1–21.0 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 0 |
| 21.1–22.0 | — | — | — | — | — | — | — | — |
| 22.1–23.0 | 5 | 20 | 0 | 1 | 0 | 0 | 25 | 0 |
| 23.1–24.0 | — | — | 0 | 5 | 20 | 80 | 26 | 0 |
| 24.1–25.0 | 24 | 42 | 0 | 7 | 0 | 29 | 53 | 0 |
| 25.1–26.0 | — | — | 0 | 5 | 40 | 20 | 561 | 0 |
| 26.1–27.0 | 11 | 36 | 0 | 2 | 50 | 0 | 113 | 0 |
| 27.1–28.0 | — | — | 0 | — | — | 0 | 12 | 0 |
| 28.1–29.0 | 12 | 33 | 0 | 15 | 20 | 0 | 18 | 0 |
| 29.1–30.0 | — | — | 0 | — | — | 0 | 35 | 0 |
| 30.1–31.0 | 54 | 39 | 4 | 24 | 33 | 4 | 3585 | 0 |
| 31.1–32.0 | 14 | 21 | 14 | 6 | 33 | 0 | 310 | 0 |
| 32.1–33.0 | 0 | 0 | 0 | 0 | 0 | 0 | 41 | 0 |
| 33.1–34.0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 100 |
| 34.1–35.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 3. The standing crop summary of major macroalgae as a function of temperature at Turkey Point, Florida. Many of these data were examined under the influence of a thermal effluent.

| Temperature °C | Total for 7 major species | % Unhealthy | % Juvenile | Total for 16 species |
|---|---|---|---|---|
| 15.1–16.0 | 0 | 0 | 0 | 0 |
| 16.1–17.0 | 0 | 0 | 0 | 0 |
| 17.1–18.0 | 18 | 12 | 1 | 5 |
| 18.1–19.0 | 46 | 5 | 3 | 0 |
| 19.1–20.0 | 32 | 4 | 5 | 0 |
| 20.1–21.0 | 52 | 6 | 1 | 44 |
| 21.1–22.0 | — | — | — | — |
| 22.1–23.0 | 75 | 14 | 8 | 27 |
| 23.1–24.0 | 27 | 21 | 16 | 0 |
| 24.1–25.0 | 107 | 34 | 7 | 15 |
| 25.1–26.0 | 253 | 16 | 12 | 58 |
| 26.1–27.0 | 93 | 23 | 4 | 10 |
| 27.1–28.0 | 52 | 19 | 2 | 21 |
| 28.1–29.0 | 195 | 14 | 8 | 22 |
| 29.1–30.0 | 321 | 10 | 6 | 0 |
| 30.1–31.0 | 1013 | 19 | 8 | 104 |
| 31.1–32.0 | 487 | 18 | 8 | 259 |
| 32.1–33.0 | 106 | 10 | 17 | 24 |
| 33.1–34.0 | 189 | 22 | 6 | 10 |
| 34.1–35.0 | 0 | 0 | 0 | 0 |
| 35.1–36.0 | 0 | 0 | 0 | 0 |

rate. Above 32°C the standing crop decreased markedly. Temperatures rose to this level in late May and early June, 1971. When the temperature dropped below 30°C, *Halimeda* began to recolonize in the fall. Under normal conditions summer was the time of highest standing crop (Table 3).

Acclimation studies were attempted by holding *Halimeda* in a controlled environment for two weeks. One group of plants held at 15°C had upper lethal temperature limits between 33.2° to 34.7°C. A group held at 30°C had upper limits between 32.6° to 34.2°C. Obviously, before valid statements on acclimation can be made, one must investigate various acclimation periods ranging from days to several generations. However, these preliminary results, coupled with experimental data on *Valonia macrophysa* (presented below) suggest that little if any acclimation occurs with some tropical algae; if anything, those plants held for extended periods close to their upper thermal limits have a lower lethal limit than those held at lower temperatures. This is different than what was seen in some fishes (Brett, 1956).

## Penicillus capitatus

The *Thalassia* community contained an abundance of *Penicillus*. Specimens of *P. capitatus* from the Florida Keys were used in five temperature tolerance experiments that ranged from 3 to 12 days duration (see Fig. 1). As an additional control, *Penicillus* plants were held in the polythermostat at 24°C for 8 weeks; they continued to be in excellent health. Previous laboratory studies demonstrated that specimens and their clones could be held for a year or more (Thorhaug, 1965). The temperature tolerance experiments showed that after 8 days *Penicillus* kept at temperatures below 31.5°C were all alive while those held above 34.7°C were dead. This compared well with the field studies at Turkey Point (Table 2) where *Penicillus* was stressed or non-healthy when the temperature in May and early June rose to 32°C. Maximum standing crops appeared between 29° and 31°C. At 31° to 32°C percentage of unhealthly plants increased while standing crop dropped nearly 50%. From 32° to 34°C the decisively lower standing crop was composed chiefly of dead and/or dying specimens. Above 34°C, no specimens were found. There was growth renewal only after the temperatures fell below 31°C in the fall of 1970; however, some stations did not attain the previous abundance until the thermal effluent was removed. *Penicillus* was a major recolonizer. This observation was in agreement with laboratory experiments which showed *H. incrassata* withstand temperatures slightly higher than did *Penicillus capitatus*.

## Acetabularia crenulata

Specimens were taken from the field attached to small rocks. The rhizoids were carefully detached with needles and held for several days before use in order to insure that the alga was not damaged in handling. When kept under carefully

controlled conditions the plants reacted favorably to transplanting and detaching. Thirty replicate tubes each containing five specimens of *Acetabularia crenulata* were held at temperatures between 10° and 45°C. Between 38.1° and 39.1°C the specimens were no longer able to survive. One might well expect the lethal temperature of *Acetabularia* to be higher than that seen for any of the other algae since it is an intertidal form. Such plants and animals are well known to be very resistant to many physical stresses including temperature. These data are summarized in Table 1. Field data (Table 2) indicated that larger populations of *Acetabularia* at 33° to 34°C than any other major macrophyte. This alga was one of the first recolonizers in the fall, when temperatures began to decrease as well as when effluents were no longer released.

## Valonia

Since 1891 when Meyer performed the first physiological experiments with *Valonia*, it has been used as an indicator of marine algal physiological properties by many investigators. This plant is a large single-celled, tropical benthic green algae found only in the marine environment and can attain a diameter of more than 10 cm. Because of its large size, morphological observations which indicate cell death are relatively easy. *Valonia* grows in Biscayne Bay, Card Sound and in the waters of the Florida Keys as a part of the abundant green algal community. In addition, *Valonia* is a member of the Order Siphonales which includes the important estuarine algal families of Caulerpaceae, Codiaceae and Vaucheraceae. These families include most of the major macroalgae in Biscayne Bay and Card Sound (*Caulerpa, Avainvillea, Halimeda, Penicillus, Udotea, Rhipocephalus, Chamaedemis* and *Dictosphaeria*), hence *Valonia* may provide useful extrapolation.

For these above reasons, it was decided to use *Valonia* as the tool to study many of the details of thermal stress. It was most thoroughly investigated during this study and many of the findings are applicable to *Penicillus, Halimeda, Acetabularia, Laurencia* and even *Thalassia.* The understanding of the gradual process of heat death by observing these giant cells was invaluable for comprehending the events in this field.

One very important consideration in thermal stress studies is the ability of, and ease, to which an organism can acclimate to changing conditions. In order to investigate this, five species of *Valonia* from eight locations were used. The organisms were: *V. macrophysa, V. ventricosa, V. utricularis, V. ocellata* and *V. aegrophilia.* The cells were collected from Biscayne Bay the Florida Keys, the Dry Tortugas in the moat at Fort Jefferson, Puerto Rico (La Parreguera), Jamaica (Port Royal), Curacao (Pescadera Baai), Bermuda (St. Georges) and Venezuela (Cumana). They were flown directly to Miami and immediately used in the experiments. Other algae collected locally were maintained in the laboratory under culture conditions resembling the natural habitat in an aquaria outside the laboratory that had continuously running seawater percolating up through the sand and rock on the bottom (Thorhaug, 1965).

1. *Valonia macrophysa*: A number of experiments, including all the acclimation studies, were conducted using this species. A summary of the results is given in Fig. 1.

In one set of experiments different sized cells of each of three species of *Valonia* (*macrophysa, ventricosa* and *utricularis*) were compared to test if there were differences in temperature tolerances between different sizes (age) of cells within a species. We concluded that temperature tolerance was not dependent on cell size in any of the three species. Naturally, as in all these experiments, encrusting growth was removed from the plants and only healthy cells were selected. The cells in the polythermostat were observed at appropriate intervals, the light was kept at less than one foot candle and the light-dark periods were 14 hours and 10 hours, respectively.

Two experiments were conducted using cells from Biscayne Bay and the Florida Keys. The first consisted of two replicates of 19 sets of six cells held at temperatures ranging from 7.0° to 36.6°C for a period of three days, at a salinity of 32‰. The cells maintained a healthy condition between 15° and 31.5°C. Irreversible plasmolysis occurred abruptly below 14°C and above 33.5°C. Death began at 15° and 31.5°C. A similar experiment conducted at a lowered salinity (25‰) gave the same thermal tolerance limits.

Another experiment used 16 cells held at 30 different temperatures ranging from 8.0° to 38.2°C for a period of five days. The temperature interval was 1.1°C, a more closely-spaced interval than used in the previous experiment. During the first 24 hours all cells remained healthy; after 48 hours of exposure above 31.5°C they began to show distress. On the third day, complete irreversible plasmolysis occurred below 15°C and above 33.5°C, partial mortality took place at 13.3° and 31.5°C. No change occurred over a two week period.

To determine if acclimation due to long-term growth at various temperature regimes in the tropics would cause the thermal limits to change, experiments were conducted utilizing *V. macrophysa* from Puerto Rico where the mean water temperature was 28.5°C. Nineteen sets of 24 cells each were held at temperatures ranging from 7.9° to 38.1°C for 72 hours. Almost all the cells had undergone irreversible plasmolysis at temperatures below 15.6°C and above 29.7°C. Partial mortality was observed between 14.6° and 15.6°C and between 29.7°C and 30.7°C. All cells appeared healthy at the intermediate temperatures.

Since acclimation at the warmer temperatures of Puerto Rico did not affect the lethal limits of *Valonia* the effects of acclimation on cells living in a cooler area were tested using Bermuda specimens where the mean annual temperature was 22.6°C. In one polythermostat, sets of 16 cells each were held at temperatures ranging from 8.0° to 38.0°C at intervals of 1.5°C. The results showed that below 13.9°C and above 33.6°C, all the cells died after three days. In the second polythermostat 30 sets of 13 cells each were placed at 0.33°C intervals between 24° and 34°C. The results show that between 32.0° and 32.6°C more than 50% of the cells died after three days.

Cells from the Dry Tortugas, located at the tip of the Florida Keys and close to Yucutan, where the mean annual temperature is 27.0°C, were examined. In one polythermostat 19 sets of 12 cells each were held between 9.8° and 36.8°C. After five days death occurred in all cells held below 12.3°C and above 32.8°C. In a second trial, four sets of 10 cells were tested in the range of 29.3° to 32.8°C. Between 31.3 and 31.6°C more than 50% of the cells died after five days.

Two final acclimation experiments were run using specimens from Biscayne Bay. Over 500 cells were held for 10 and 14 days at 30° and 15°C. Subsequently, they were placed in a polythermostat and held for 160 hours. One cell of each sample survived at 33.4°C; all cells died above this temperature. At lower temperatures 14% or less mortality occurred in the 30°C acclimated cells with 1% or less occurring in the cells, acclimated at 15°C. The critical interval was 32.3° to 33.4°C for those acclimated at 30°C and 33.4° to 34.5°C for those at 15°C. It should be observed that those cells held for extended periods (acclimated) at the higher temperature not only had a lower upper thermal limit but also had a much higher mortality at "normal" or "optimal" temperatures. This observation matched that found with the *Valonia* from Puerto Rico where the mean annual temperature was 28.5°C, the highest for all specimens examined. These results were remarkably close to those using "non-acclimated cells" and suggested that the algal thermal limit was very closely defined with little possibility for acclimation. This, of course, is in variance with what is known about bacteria and fishes. In addition, it strongly indicated that although the thermal limit appeared abrupt, the organisms were under severe thermal stress at temperatures below the death point and that exposure to slightly higher temperatures for short periods will prove fatal.

2. *Valonia utricularis*: Comparative experiments were conducted utilizing *V. utricularis* specimens from two locations, Bermuda and the Florida Keys near Miami. Two sets of 13 cells from Bermuda were held at 30 different temperatures ranging from 8.6° to 37.1°C for five days. The results showed that those cells exposed to temperatures below 13°C and above 31.0°C died within three days. For the Florida Keys specimens, 30 sets of 10 cells each were held at temperatures ranging from 26.6° to 32.7°C. Within the range of 31.0° to 31.4°C there was over 50% mortality after 5 days. The similarity of temperature tolerance for cells from the two areas is obvious; there is also good agreement with the thermal tolerance of *V. macrophysa.*

3. *Valonia ventricosa*: Specimens of this third species from the Florida Keys, Curacao and Jamaica were examined; the results are shown in Figure XI-4 and Table XI-1. For the Florida Keys specimens, 19 sets of six cells each were held at temperature intervals between 7.7° and 38.9°C. After three days of exposure, over 50% of the cells underwent irreversible plasmolysis below 14.3°C and above 33.0°C. Cells from Curacao, where the mean annual temperature is 24.5°C, were held between 9.7° and 36.9°C in 19 groups of six each. The cells were unable to survive a six day exposure below 12.1°C and above 31.5°C. Three additional trials using Curacao cells showed that this species had a lower tolerance limit of 14.5°C

and an upper limit of 33.0°C with death beginning at 31.5°C. Cells from Cumana, Venezuela had a 100% mortality below 15.5°C after five days. The upper critical limit was between 29.1° and 31.9°C.

Nineteen sets of 17 cells each collected in Jamaican waters, where the mean annual temperature is 27.4°C, were held between 9.7° and 37.0°C. The results showed that below 12.2°C and above 31.5°C more than 50% of the cells were unable to survive after five days. Irreversible plasmolysis began to take place at 13.8° and 29.9°C; cells held between 23° and 26°C for a period of three weeks remained healthy. These limits are very similar to those found for *V. ventricosa* for Curacao and only tenths of a degree from the Florida Keys specimens. The striking similarity of the upper death limits of the *V. macrophysa, V. ventricosa* and *V. utricularis* is also obvious.

4. *Valonia ocellata*: Cells from the Florida Keys were tested over the temperature range of 8.1° to 40°C. After a three day exposure to temperature below 14.7° or above 34.0°C all cells died; those from Curacao had a very similar limit of 34.6°C. Temperature intolerance began at 32.8°C.

5. *Valonia aegrophilia*: This is a very small, relatively rare species collected from the Dry Tortugas. Nineteen sets of 32 cells each were held between 9.5° and 37.0°C. After three days cells ceased to survive below 10.5° and above 33.0°C. The cells began to die at 12.0° and 31.4°C.

## Laurencia poitei

The red algae, *Laurencia poitei*, is found in many tropical and subtropical waters and is a dominant species in Biscayne Bay and Card Sound. It exists in non-attached clumps of single strands and masses which move freely with the tide and current except when caught on projections of the bottom. It is not known whether herbivores use it directly as food but it does form a significant portion of the biomass and this is a major contributor to the bottom detritus. In addition, it provides a substrate for many algae and sessile animals as well as shelter for small fish, polychaetes, molluscs and crustaceans as suggested by Thorhaug *et al.* (1974). The color of the plant ranges from a light yellow to a dark purple-red; in summer it tends towards lighter colors. Fall is the time of dominant growth.

The algae were collected by hand from Card Sound and brought back in large plastic containers equipped with aeration systems. Debris, animals, and other foreign matter were gently removed by mechanical cleaning in running seawater. The plants were held in 5 gallon glass tanks, the water was changed every two days and the salinity, pH, and temperature recorded.

A plant tip (6 to 10 cm) was placed in each of 48 cuvettes containing 20 ml of filtered sea water; the salinity, pH, and appearance of the tip were noted. The temperature gradient used was from 6° to 45°C for a period of 10 days. The tips were examined daily and the water replaced with water of the same temperature. Three trials showed that at the end of 10 days more than 80% of the cells held below

30.1°C were healthy; even those held at 6.3°C were alive. At temperatures from 31.7° to 33.3°C less than 40% of the tips were living and above 34.9°C all were dead. Due to the difficulty in establishing indications of the morphological death point, the upper tolerance can only be expressed as a range of 31.7° to 34.9°C. This information is presented in Table 1 and agrees with field data which indicates that no healthy *Laurencia* occurred above a temperature averaging 32°C for 10 or more days and that there was a significant drop in abundance from 30° to 31°C and above. The benthic biology studies show that the animal populations closely associated with *Laurencia* became less abundant after sustained periods with average daily temperatures in excess of 33°C. In addition, these values agree with Biebl (1962) for *Laurencia poitei* held at 32° to 35°C for 12 hours.

### Other Macrophytes

*Udotea flabellum* and *Rhipocephalus phoenix* have very similar upper thermal limits in the field. Summer is their time of peak abundance in control conditions (Table 5). Between 30.1° to 31°C to 31.5° to 32°C standing crop fell significantly. Above 32.1°C none were encountered. On the other hand, *Batophora oestedii*, an intertidal green, did show fairly high standing crops from 31.1° to 32.0°C and specimens were noted up to 34.0°C. It is not surprising that this intertidal species like *Acetabularia* has higher thermal limits than the sublittoral greens. It should be noted that even a large standing crop of *Batophora* does not significantly contribute to the biomass.

The sixteen most abundant species which include *Penicillus capitatus, Halimeda incrassata, Acetabularia crenulata, Anadyomene stellata, Digenia simplex, Laurencia poitei, Rhipocephalus phoenix, Udotea conglutinata, Avranvillia nigricans, Batophora oerstedii, Cladophora fuliginosa, Sargassum pteropleuron, Halimeda opuntia, Caulerpa cuppressoides, Caulerpa paspaloides, Caulerpa sertularoides* have the highest standing crop between 31° and 32°C.

## DISCUSSION

The surprising result of a long term upper thermal limit being within a degree or two of mean summer ambient temperatures should cause us to reappraise a false set of logic to which we have grown accustomed in marine ecology. We often reason backwards from principles formulated chiefly from temperature land systems to tropical terrestrial, the marine temperate, and then, the marine tropics. This may prove that we are reasoning from the most complex to the simplest, which has often been demonstrated to be fallacious. In particular, from what we can ascertain at present, life evolved in the nearshore marine tropics, then evolved outward eventually forming the temperate land communities. The tropical benthic macroalgae, in particular some of the calcareous Siphonales such as *Penicillus* and *Halimeda* are found in very early fossil records (Johnson, 1966). These have

evidently always existed within the narrow temperature regime which defines the sublittoral marine tropics and have never had to evolve any complex mechanisms for dealing with a wide range of temperatures or rapidly changing temperatures. Thus, to predict temperature tolerances that these more primitive algae can withstand, it is not logical to ascertain thermal limits of freshwater, terrestial or even marine temperate plants above the mean annual high temperature and then apply these to the marine sublittoral tropicals. This was done by some very competent biologists at the onset of the "thermal pollution" problems. Clearly the marine tropical organisms must be tested for their own tolerances to each resulting pollutant rather than reasoning from the more complex responses of temperate (freshwater or marine) organisms. It is the thesis of this discussion that the first link in the food web, the sublittoral benthic macroplants may prove to be the most sensitive link to a whole range of other substances as they have to temperature and therefore prove highly valuable indicator organisms.

The abrupt upper thermal limit of these macroalgae add further substantiation to the hypothesis of Moore (1972) that the tropics are far from the "happy, stable" environment that many investigators have categorized them, but rather are "unhappy" and on the brink of diaster. Certainly, a small temperature increase pushed the system previously described to the brink of diaster whereas larger temperature changes were necessary for such an effort in temperate waters. Moore's hypothesis includes the physiological principle that an organism which is stressed for one factor is more vulnerable to a second stress factor than if it encountered the second factor alone. As a corallary, one may reason that since the tropical organisms are already near their upper temperature limit, that their vulnerability to other factors (or in the case of man's activities-pollutants) may be greater than that of temperate species.

One may also note that the optimum growth conditions for these algae as indicated by the large seasonal increase in standing crop in the summer are very close to the lethal and sublethal temperatures.

The extension of these concepts into one of the central questions of the tropics, i.e. why the tropics has more species than temperate or arctic, poses a fascinating question for further investigation. Does the continual existence of the assemblage of tropical marine organisms in a temperature regime close to and/or within their sublethal range cause more genetic mutation or change than organisms which live more of their lives at a more central portion of their temperature range. This is particularly interesting where one notes that late spring and summer, when temperature is the highest, is often the time for reproduction among tropical species. Voluminous data exists from genetic studies which indicate gene breakage and cross-over occurs in sublethal temperature ranges.

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