

A NEW THERMAL GRADIENT DEVICE FOR CULTURING ALGAE

By PETER ALLAN SIVER

Department of Biology, Western Connecticut State University, Danbury,
Connecticut 06810, USA

A temperature gradient plate was constructed for use in culturing marine and freshwater microalgae. The apparatus can be used to study the effect of temperature and its interaction with other variables on the growth, reproduction and physiology of many aquatic organisms. In the present apparatus a constant temperature gradient from 0–40°C was produced along the length of a 1 m × 1 m × 1.25 cm aluminum plate by heating one end of the plate with a heating element and cooling the opposite end with a standard refrigeration evaporator. Variations from mean temperatures along a transect at right angles to the gradient were less than 1.5°C. The temperature gradient device discussed here is easy to build, has a low cost compared to devices previously described and requires little maintenance.

Temperature, light and nutritional status are three of the major parameters that regulate the growth and development of algae. The effects of the interactions between these parameters have become increasingly important to our understanding of algal physiology and ecology. Analysis of the interactions between any two of the parameters can be quite tedious and time consuming, especially if separate temperature control incubators are used. The use of an apparatus constructed to yield a cultivation surface with a temperature gradient at right angles to either a light or nutrition gradient has been most beneficial to such investigations (Yarish, Lee & Edwards, 1979).

An apparatus using a temperature/light cross gradient was first developed by Halldal & French (1956) to study the growth of microalgae on agar. In their design a thermal gradient was established by pumping hot and cold water through holes bored down opposing sides of an aluminium plate. The result was a linear thermal gradient across the plate on which the algae were grown. The light gradient was established using overhead lamps at one end of the plate in conjunction with a translucent light weakening paper comb (Halldal & French, 1956). A similar apparatus, also using heated and cooled water to produce a temperature gradient on an aluminium plate, was designed (Edwards & Van Baalen, 1970) and later modified (Yarish, Lee & Edwards, 1979) for the culture of benthic marine algae.

This paper describes the design of a new thermal gradient device constructed in the author's laboratory for culturing microalgae. The device is also easily adapted for macroalgal culture as well as other organisms. This apparatus incorporates the cross gradient principle but uses different modes of heating and cooling the growth surface than previously described. The device yields a con-

stant thermal gradient, is inexpensive and is easily constructed. Comparisons are made with previously described devices.

DESCRIPTION OF THE APPARATUS

The culture apparatus consists of a 1.25 cm × 1 m × 1 m aluminium plate resting on metal spacers that suspend it 4 cm from a supporting table top (Fig. 1). One side of the plate is cooled using a standard refrigeration evaporator while the opposite side of the plate is warmed with a heating element. The result of cooling and heating opposing sides of the plate is the formation of a linear temperature gradient across the plate. The temperature range is controlled by adjusting heating and cooling thermostats. Two moveable thermocouples, connected to the cooling and heating thermostats, are clamped on to the plate near the cooling evaporator and heating element respectively. If only high or low temperatures are of interest, the cooling or heating units are turned off.

The cooling unit is a standard compressor-condenser-evaporator refrigeration unit utilizing Freon 12 as the coolant. The evaporator consists of a copper pipe (OD of 1.3 cm) anchored by metal strips in a 0.75 cm deep groove on the base of the plate. The groove runs the width of the plate four times (note that for convenience only one turn in the groove is illustrated in Fig. 1). The exposed portions of the evaporator are covered with refrigerant insulating tape. The groove configuration, which is confined to the end 10 cm of the plate, provides more than adequate surface area contact between the evaporator and the plate to lower the plate temperature to 0°C. On the opposite end, a piece of sheet metal anchors the heating element (440 W) in a U-shaped groove (0.75 cm deep) that runs the width of the plate twice and protects the table top from excessive heat. The heating element, which also uses a 10 cm wide portion of the plate, provides stable temperatures up to 40°C. Each temperature control unit has its own master switch and fuse box (Fig. 1).

Lighting is provided by five 10-W cool-white fluorescent lights suspended above the growing area by chains. If a temperature-nutrition cross gradient is to be utilized for experimentation, the lights are suspended at the same height, providing similar light intensities across the surface of the growing area. The light intensity is controlled by raising and lowering the fixtures. A light intensity gradient is achieved in one of two ways: (a) Several fixtures are suspended above only one end of the growing area parallel to the thermal gradient or (b) one end of each light is lowered closer to the plate relative to the other (illustrated in Fig. 1). The lights are connected in series to an interval timer for regulation of the photoperiod.

To exclude external light black fabric is suspended from the upper frame supporting the light fixtures, enclosing a growing chamber. To avoid excessive heat build up within the growth chamber, the ballasts were removed from the light fixtures and remounted on a lower shelf. All equipment and operating controls are mounted on two shelves directly below the plate. The frame supporting the light fixtures and black fabric consists of aluminium poles and is anchored to the sides of the table. The entire apparatus (frame, table-top and shelves) rests on rollers for ease in relocation.

In initial experiments 50 ml Erlenmeyer flasks were used to culture 10–15 ml

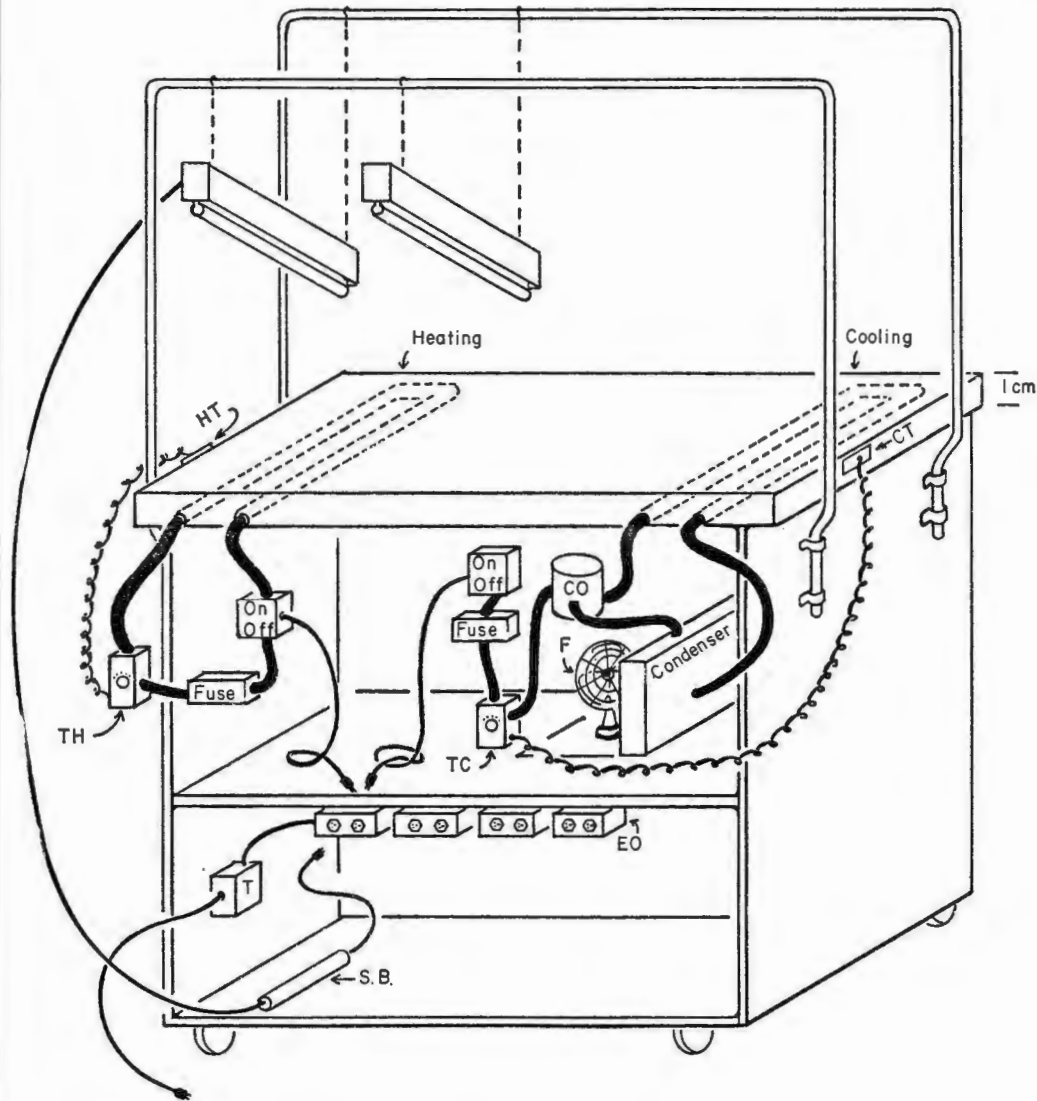


FIG. 1. Diagram of the thermal gradient plate constructed in the author's laboratory. The growing surface consists of an aluminium plate that has one end cooled by a standard refrigeration evaporator and the opposite end heated using a heating element. The result is the formation of a linear temperature gradient down the length of the plate. Fluorescent lights are suspended above the growing surface and the entire apparatus is on rollers for ease in relocation. Key: TH, heating thermostat; HT, heating thermocouple; TC, cooling thermostat; CT, cooling thermocouple; CO, compressor; T, interval timer; SB, starter ballast; F, fan; EO, electrical outlet.

TABLE I. The temperatures of 10 ml of culture medium in 50 ml Erlenmeyer flasks over a 5-day period on the temperature gradient plate

Day	Light or dark	Front or back	Temperature (°C)					
			A	B	C	D	E	F
1	L	F	15	17	22	27	30	35
		B	15.5	17	22	27.5	31	35
2	L	F	14.5	17	23	28	32	35
		B	15	16.5	22	29	31	34
3	L	F	15	18	23	28.5	32.5	36
		B	15.5	17	21	29	30	35.5
3	D	F	15	18.5	22	27.5	31	35.5
		B	15	17	22	27	29	34
3	D	F	14.5	17	22.5	27.5	31	34
		B	15	16	20.5	27	30	34
4	L	F	15	17	22	27	31	36
		B	15	16	21	27	29.5	34
4	D	F	14.5	17.5	22	28	29.5	33
		B	15	17	20	27	29	33
5	L	F	15	17	21	27.5	30	35
		B	15	17	20	27	30	34
Mean:			15	17	21.6	27.6	30.4	34.6

The temperature was monitored along six parallel transects, each perpendicular to the gradient, either during the light (L) or dark (D) period. Temperatures reported were measured in similar flasks at the ends of each transect (front, F, and back, B).

volumes of microalgae. Ten flasks were arranged along six transects, each perpendicular to the gradient, and temperatures monitored in 50 ml flasks placed on the ends of the transects. Over a 5-day period the mean temperature along the transects ranged from 15–34.6°C (Table I). Temperature variations from the means were less than or equal to 1.5°C. Essentially, there was no difference between the temperatures on the ends of each transect nor between light and dark periods (Table I). Such temperature stability was maintained indefinitely. It should be noted that this temperature stability was achieved in a room where the air temperature was not controlled and thus probably caused some of the variation.

DISCUSSION

The use of two-way parameter gradients has been most beneficial to our understanding of algal growth (Halldal, Huang & French, 1956; Edwards, 1971; Yarish, 1976; Kapraun, 1977), physiology (Halldal, 1956; Halldal & French, 1958; Siver & Trainor, 1981) and ecology (Edwards, 1970; Rhyne, 1973). A thermal gradient apparatus such as described here greatly reduces the time and space necessary to obtain growth responses at many combinations of temperature with nutrition, pH, light intensity and photoperiod.

As with the thermal gradient plate described by Yarish, Lee & Edwards (1979) the present device yields a large temperature range (0–40°C) with discrete areas on the plate where the temperature remains constant (variations from the mean temperatures are less than 1.5°C). It is believed that if the gradient plate was in a temperature controlled room the temperature variation from the mean would be even smaller. With the ballasts removed from the fluorescent lights there are no differences in the temperatures of the cultures between light and dark periods.

In the present design, if temperatures greater than 40°C were required, a heating element with greater wattage and thus a higher temperature range could be used.

One of the advantages when compared to previously described units (Halldal & French, 1956; Edwards & Van Baalen, 1970; Yarish, Lee & Edwards, 1979) is the lower cost of equipment and parts needed to heat and cool the plate. Assuming equal cost for the aluminium plate and lighting fixtures, a common compressor–condenser–evaporator unit and a standard heating element can be purchased at a fraction of the cost of a heating or cooling circulator. The refrigeration unit of an old refrigerator was used to cool the plate and thus further cut the cost of the apparatus.

In previously described gradient plates cooled and heated by water (Halldal & French, 1956; Edwards & Van Baalen, 1970; Yarish, Lee & Edwards, 1979) a thick aluminium plate was needed to provide the necessary borings through which the water was channelled. Although the gradient plate described here uses a 1.25 cm thick aluminium plate, a much thinner plate (0.25 cm) could be used to provide similar results. In this case the cooling evaporator and heating elements would be clamped to the basal portion of the plate. With the thinner aluminium plate, a smaller surface area contact between the plate and the evaporator and heating element would be required to achieve a working temperature gradient.

Several items should be considered when dealing with light gradients on the plate. Firstly, because of lower light intensities at the ends of the fluorescent lights, only the inner 0.9 × 0.9 m area of the plate was used to culture microalgae. Secondly, the quantity and quality of light will change as it filters through the cultures. Thus, when a temperature–light cross gradient was utilized for experimentation the photosynthetic active radiation (PAR) was measured with a quantum sensor and culture flasks were moved accordingly to attain as true a light intensity gradient as possible.

The plate constructed by Halldal & French (1956) had a growth chamber through which CO₂ was pumped, however, their design was for growth of algae on agar plates and not liquid culture. If CO₂ becomes limited sodium bicarbonate could be added or CO₂ bubbled into the cultures.

When culturing macroalgae in Petri dishes, Edwards & Van Baalen (1970) reported the formation of condensation droplets on the lids. They placed a heated glass plate on the Petri dishes and this alleviated the condensation problem. In experiments using 50 ml Erlenmeyer flasks as culture vessels, I have noted little condensation on the insides of the flasks. Generally, if a condensation droplet formed, it ran down the flask side to the culture medium.

In summary, the apparatus discussed here, as with previously described gradient plates, allows for the culture of organisms under several gradients and yields a large amount of data over a short time period. Advantages of the present apparatus include lower cost and maintenance.

ACKNOWLEDGEMENTS

I would like to extend special thanks to Keith Harry and Henry Malonowski for aid in the construction of the apparatus and Dr Louis Rigley, and Henry Steuben for technical assistance and advice. I would also like to thank Dr Les Turoczi for helping to procure the aluminium plate, Mary Jane Spring who drew the illustration and Bernadette Nardone and Angela Foti for typing the manuscript.

REFERENCES

- EDWARDS, P., 1970. Field and cultural observations on the growth and reproduction of *Polysiphonia denudata* from Texas. *Br. phycol. J.*, **5**: 145-153.
- EDWARDS, P., 1971. Effects of light intensity, day-length, and temperature on growth and reproduction of *Callithamnion byssoides*. In *Contributions in Phycology* (Parker, B. C. and Brown, R. M. Jr., editors), 163-174. Allen Press, Lawrence, Kansas.
- EDWARDS, P. & VAN BAALEN, C., 1970. An apparatus for the culture of benthic marine algae under varying regimes of temperature and light intensity. *Botanica mar.*, **13**: 42-43.
- HALLDAL, P., 1956. *Effect of light and temperature on pigment ratios in bluegreen algae*. Carnegie Institution of Washington Year Book No. **56**: 272-275.
- HALLDAL, P. & FRENCH, C., 1956. *The growth of algae in crossed gradients of light intensity and temperature*. Carnegie Institution of Washington Year Book No. **55**: 261-265.
- HALLDAL, P. & FRENCH, C., 1958. Algal growth in crossed gradients of light intensity and temperature. *Pl. Physiol., Lancaster*, **33**: 249-252.
- HALLDAL, P., HUANG, H. & FRENCH, C., 1956. *Growth patterns of various algae in crossed gradients of light intensity and temperatures*. Carnegie Institution of Washington Year Book No. **56**: 275-278.
- KAPRUAN, D., 1977. Asexual propagules in the life history of *Polysiphonia ferulacea* (Rhodophyta, Ceramiales). *Phycologia*, **16**: 417-426.
- RHYNE, C., 1973. Field and experimental studies on the systematics and ecology of *Ulva curvata* and *Ulva rotundata*. *Sea Grant Publ. UNC-SG-73-09*, 123 pp.
- SIVER, P. & TRAINOR, F. R., 1981. Morphological control and physiology of *Scenedesmus* strain 170. *Phycologia*, **20**: 1-11.
- YARISH, C., 1976. Polymorphism of selected marine Chaetophoraceae (Chlorophyta). *Br. phycol. J.*, **11**: 29-38.
- YARISH, C., LEE, K. & EDWARDS, P., 1979. An improved apparatus for the culture of algae under varying regimes of temperature and light intensity. *Botanica mar.*, **22**: 395-397.

(Accepted 21 December 1982)