OPTIMUM LIGHT AND TEMPERATURE REQUIREMENTS FOR *GYMNODINIUM SPLENDENS*, A LARVAL FISH FOOD ORGANISM[1,2]

In recent years, it has been possible to substitute the marine dinoflagellate *Gymnodinium splendens* for wild natural plankton as a food source for first-feeding larval anchovies, *Engraulis mordax* (Lasker et al., 1970). *G. splendens* is ideal for this larval marine fish at this developmental stage because the alga's size (approximately 53 μm diameter) is similar to the size range of natural food particles. Reasonably lengthy larval survival (up to 19 days) can be achieved in the laboratory by first using this dinoflagellate and later supplementing with veligers (Lasker et al., 1970). The alga has also been used in combination with the rotifer *Brachionus plicatilis* as a larval food source (Theilacker and McMaster, 1971).

These developments have made possible new studies of anchovy larval behavior (Hunter, 1972) and nutrition (Conklin, unpublished data). Furthermore, the use of *G. splendens* may be of help in rearing other larval fish.

G. splendens is easily grown in an enriched seawater medium that was developed by Sweeney (1951, 1954) and Sweeney and Hastings (1957). The organism was first cultured with soil extract in the medium (Sweeney, 1951) which was later replaced with vitamin B_{12} (Sweeney, 1954). However, for routine culturing soil extract suffices and also provides trace metals and unknown organic factors.

The dinoflagellate has been cultured at 16°C and a light intensity of 60-70 footcandles (Sweeney, 1951); at 20°C and 200-400 footcandles (Sweeney, 1954) and at 20°C and 500 footcandles (Lasker et al., 1970), but optimum physical conditions for growth were not determined. Such information would be of value in setting up continuous cultures of *G. splendens* or in further improving conditions for mass cultures. The purpose of this paper is to establish the optimum conditions of light and temperature for *G. splendens* culture—conditions that result in a maximum growth rate.

Materials and Methods

Our culture of *G. splendens* was isolated from a water sample taken from the Scripps Institution pier in March 1969. It is unialgal but not bacteria-free. *Gymnodinium* is maintained in the enriched seawater medium of Sweeney and Hastings (1957) plus the Fe solution of Rodhe (1948). One liter of medium contains 750 ml seawater, 10 ml 0.1% EDTA (disodium ethylenedinitrilo tetra-acetate), 2 ml 1 M KNO_3, 2 ml 0.1 M K_2HPO_4, 20 ml of soil extract (prepared by autoclaving equal weights of untilled loam and glass distilled water followed by filtration through Whatman[3] No. 4 paper), 0.25 ml Rodhe Fe solution (prepared by dissolving 1.33 g ferric citrate and 1.33 g citric acid in 100 ml of double distilled water) and 216 ml (glass) distilled water. Autoclaving the medium results in a precipitate, which can be avoided by sterile filtration. Stock cultures of *G. splendens* were maintained at 500 footcandles (5,000 lux) and 21°C.

Experimental cultures were grown in this same medium. Cells were concentrated from exponentially growing stock cultures by gentle reverse filtration (Dodson and Thomas, 1964) and inoculated into 65-ml bottles containing 50 ml of medium to give an initial cell count of 200-500 cells/ml.

The culture bottles were then placed in temperature gradient block A of Thomas, Scotten, and Bradshaw (1963). This apparatus consisted of an aluminum block containing 30 holes. Prior to starting the experiment, thermostatted cold water was pumped through one end of the block and warm water was pumped through the other end. This set up a thermal gradient so that there were six rows

[1] Contribution from the Scripps Institution of Oceanography.

[2] This work was part of the STOR (Scripps Tuna Oceanography Research) Program. It was supported by the Oceanography Section, National Science Foundation Grant No. GA-32529X and by Marine Research Committee (California Department of Fish and Game) Grant No. M-13 to Reuben Lasker. The work was performed in the Southwest Fishery Center, National Marine Fisheries Service, NOAA.

[3] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

of five holes each at a given temperature. The bottles were illuminated from below through plastic windows in each hole. The continuous fluorescent light was attenuated with fogged photographic film filters placed below each window. Thus, at each of six temperatures, five light intensities were achieved. The light intensity in each hole was measured by an International Rectifier I2BM silicon photocell which was calibrated against a Weston Model 756 footcandle meter and an Eppley radiometer using the same fluorescent light source.

Counts of cells in each culture were made at daily intervals by taking 1-ml aliquots and counting all the cells in a 0.1-ml portion using a Palmer-Maloney counting chamber. Cell numbers were plotted against time in days on semilogarithmic paper. Growth rates during the exponential phase of growth were calculated by the equation:

$$\mu_2 = \frac{\log_2 N_2 - \log_2 N_1}{t_2 - t_1}$$

where N_1 and N_2 are cell counts at times t_1 and t_2. μ_2 is expressed as doublings of cell numbers per day.

Results and Discussion

Three experiments were performed. The first two were terminated after only 4 days of growth because of failure of the temperature-controlling equipment and because of contamination of some of the cultures with a small, colorless flagellate. Nevertheless, these preliminary experiments served to establish that growth of *G. splendens* was optimum at moderate temperatures and light intensities.

This result was confirmed in the third experiment in which growth was followed for 8 days. Figure 1 shows the growth rates that were achieved during the exponential phase of growth at different temperatures and intensities in this experiment. No growth occurred at 10° and 30°C and was suboptimal at 15°C. Growth was maximal at 20-27°C and at a light intensity of 2-9 kilolux. These are rather wide ranges of

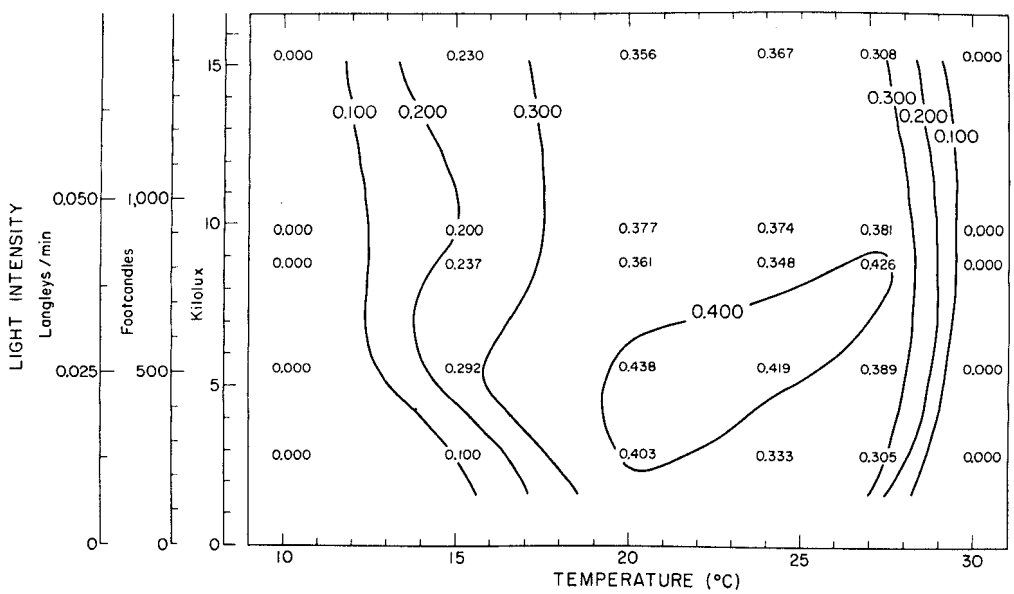

FIGURE 1.—Growth rates of *Gymnodinium splendens* at various light intensities and temperatures. The numbers are rates in doublings per day at each intensity and temperature and the data are also contoured to show similar rates.

TABLE 1.—Optimum temperatures and light intensities for growth of marine dinoflagellates.

Organism	Optimum temperature range (°C)	Optimum light intensities (kilolux)	(ly/min)	Reference
Peridinium sp.	18-20	—	>0.023	Barker (1935)
Prorocentrum micans	25	—	>0.023	Barker (1935)
Prorocentrum micans	—	>6	>0.03	Kain and Fogg (1960)
Prorocentrum gracile	18	—	>0.023	Barker (1935)
Amphidinium carteri	20-30	—	0.1-0.4	Jitts et al. (1964)
Gonyaulax polyedra	25	5-10	—	Hastings and Sweeney (1964) Sweeney (unpublished data)
Gymnodinium simplex	23-28	6-20	—	Thomas (1966)
Gymnodinium splendens	20-27	2-9	0.01-0.04	This paper

temperature and light, but it was surprising that rates were reduced slightly at higher light intensities.

Optimum temperatures and intensities for some other marine dinoflagellates are given in Table 1. *G. splendens* has temperature optima that are close to those of other dinoflagellates, but its light intensity requirements are somewhat lower.

These results are useful in providing proper conditions for mass cultures. Normal room temperatures are proper and optimum light intensities (about 5 kilolux) can be provided with banks of 40-W fluorescent lights placed about 5-10 cm away from culture flasks. At a growth rate of 0.4 doublings per day, a culture inoculated with a few hundred cells per milliliter will increase 10 fold in about 8 days.

Literature Cited

BARKER, H. A.
1935. The culture and physiology of the marine dinoflagellates. Arch. Mikrobiol. 6:157-181.

DODSON, A. N., AND W. H. THOMAS.
1964. Concentrating plankton in a gentle fashion. Limnol. Oceanogr. 9:455-456.

HASTINGS, J. W., AND B. M. SWEENEY.
1964. Phased cell division in the marine dinoflagellates. *In* E. Zeuthen (editor), Synchrony in cell division and growth, p. 307-321. John Wiley, N.Y.

HUNTER, J. R.
1972. Swimming and feeding behavior of larval anchovy, *Engraulis mordax*. Fish. Bull., U.S. 70:821-838.

JITTS, H. R., C. D. MCALLISTER, K. STEPHENS, AND J. D. H. STRICKLAND.
1964. The cell division rates of some marine phytoplankters as a function of light and temperature. J. Fish. Res. Board Can. 21:139-157.

KAIN, J. M., AND G. E. FOGG.
1960. Studies on the growth of marine phytoplankton. III. *Prorocentrum micans* Ehrenberg. J. Mar. Biol. Assoc. U.K. 39:33-50.

LASKER, R., H. M. FEDER, G. H. THEILACKER, AND R. C. MAY.
1970. Feeding, growth, and survival of *Engraulis mordax* larvae reared in the laboratory. Mar. Biol. (Berl.) 5:345-353.

RODHE, W.
1948. Environmental requirements of freshwater plankton algae. Symb. Bot. Ups. 10:1-149.

SWEENEY, B. M.
1951. Culture of the dinoflagellate Gymnodinium with soil extract. Am. J. Bot. 38(a):669-677.
1954. Gymnodinium splendens, a marine dinoflagellate requiring vitamin B_{12}. Am. J. Bot. 41:821-824.

SWEENEY, B. M., AND J. W. HASTINGS.
1957. Characteristics of the diurnal rhythm of luminescence in Gonyaulax polyedra. J. Cell. Comp. Physiol. 49:115-128.

THEILACKER, G. H., AND M. F. MCMASTER.
1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Mar. Biol. (Berl.) 10:183-188.

THOMAS, W. H.
1966. Effects of temperature and illuminance on cell division rates of three species of tropical oceanic phytoplankton. J. Phycol. 2:17-22.

THOMAS, W. H., H. L. SCOTTEN, AND J. S. BRADSHAW.
1963. Thermal gradient incubators for small aquatic organisms. Limnol. Oceanogr. 8:357-360.

WILLIAM H. THOMAS
ANNE N. DODSON
CAROL A. LINDEN

Institute of Marine Resources
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA 92037