Light and Photosynthesis in intertidal benthic diatoms[1]

W. Rowland Taylor

Department of Oceanography and The Chesapeake Bay Institute,
The Johns Hopkins University, Baltimore, U.S.A.

KURZFASSUNG: **Licht und Photosynthese bei im Gezeitenbereich lebenden benthonischen Diatomeen.** Diatomeen der Sandflächen des Barnstable Harbor (Massachusetts, USA) wurden eingesammelt und ihre Photosyntheserate bei verschiedenen Quantitäten natürlichen Sonnenlichts mit Hilfe der C^{14}-Methode ermittelt. Es zeigte sich, daß die Diatomeen dem Lichtfaktor gegenüber eine sehr große Toleranz besitzen. Die Intensität der Photosynthese erreichte Maximalwerte bei etwa 14 % des mittäglichen Mitte-Sommer-Sonnenlichts; aber selbst volles Sonnenlicht verursachte nur einen geringfügigen Leistungsabfall. Es wurden Messungen über das Durchdringungsvermögen der Sonnenstrahlung bis in die Sandschichten, in welchen die Diatomeen bei Niedrigwasser leben, durchgeführt. Diese Messungen zeigen, daß bei trockengefallenem Sand und an einem wolkenlosen Sonnentag gegen Nachmittag die Photosyntheserate die Atmungsintensität übertreffen kann, selbst wenn die Diatomeen 3,0 mm unter der Sandoberfläche leben. In 2,0 mm Sandtiefe bis zur Oberfläche kommt die Photosyntheserate unter den genannten Bedingungen bis auf 90 % oder näher an die Maximalleistung heran. Der hohe photosynthetische Nutzeffekt bei geringen Lichtintensitäten ermöglicht den Diatomeen, beachtliche Kohlenstoffmengen zu fixieren, selbst bei niedrigem Sonnenstand. Der Photosynthese-Mechanismus ist gut an die Wanderbewegungen der Diatomeen angepaßt.

INTRODUCTION

A unique feature of many intertidal areas is the concentration of benthic diatoms that appear on the surface as golden brown patches as the tide recedes. Many of these diatoms migrate to the surface as it becomes exposed. This phenomenon has been investigated by many biologists in different parts of the world. Aleem (1950) has reported on the diatom communities of the mud flats near Whitstable in England. Fauré-Frémiet (1951) studied the tidal rhythm of a diatom at Barnstable Harbor on the northeast coast of U.S.A. Callome & Debyser (1954) made extensive observations of the diatoms on the Bout-Blanc tidal flats near La Rochelle on the coast of Britany. Pomeroy (1959) has measured the photosynthetic productivity of diatom communities in the salt marshes of Georgia, U.S.A. Perkins (1960) has studied the diurnal rhythm of littoral diatoms in an estuary near St. Andrews, Scotland. The physiological mechanisms of benthic diatom migrations are not well understood. However, even casual observation shows that the organisms migrate into full sunlight.

[1] Contribution No. 72 Chesapeake Bay Institute, The Johns Hopkins University. Supported by the U. S. Atomic Energy Commission (Contract AT [30–1] 1477) and the Office of Naval Research (Contract Nonr 248 [54]).

The experiments of JENKIN (1937) and of RYTHER (1956) and others have shown that the photosynthetic activity of some planktonic species of diatoms are severely inhibited when exposed to full sunlight. Such data is apparently the basis for the suggestion that littoral diatoms migrate into an environment which may be inhibitory to their photosynthetic processes (POMEROY 1958, 1959). On the other hand, MOUL & MASON (1957) found diatoms several centimeters below the surface where it is difficult to imagine photosynthesis occurring.

PHOTOSYNTHESIS LIGHT RELATIONSHIP

A series of experiments were undertaken in an effort to determine the optimum light conditions for these migrating intertidal diatoms.

Methods

The studies reported here were carried out at the Barnstable Harbor on the north shore of Cape Cod in Massachusetts, U. S. A. during the summer of 1963. There is a 2.7 meter tide in this area and at low tide vast expanses of the sand flats are exposed. The hydrography of this area has been described in detail by AYERS (1959). In order to obtain the maximum amount of sunlight so that light inhibition, if present, would be maximum, the experiments were carried out on cloudless days on which the low tide occurred near solar noon.

Diatoms were collected by the method of WILLIAMS (1963) in which two pieces of bolting cloth, having openings of approximately 200 microns, were spread over a diatom patch just as the tide receded. Diatoms migrated through both pieces of cloth and after 20 to 30 minutes the upper cloth was carefully lifted and rinsed into filtered sea water. Depending upon the time allowed for the collection, about 800 ml. of an algal suspension with a cell density of 3×10^4 to 7×10^4 cells/ml. was obtained. The distribution of littoral diatoms on the sand flats was heterogeneous and the species collected varied between collection sites. In the experiments described here only two species were used. One, an as yet unidentified large pennate diatom (*Tropidoneis sp.* ?) made up from 85 to 95 % of the cell suspension, while the remaining cells were *Hantzschia amphioxys, var. major* GRUN.

The suspension was dispensed into thirty milliliter clear plastic bottles and inoculated with 5 to 10 microcuries of carbon-14 labeled sodium carbonate depending upon the concentration of cells in the suspension. Using a series of plastic screens as light attenuators (MCALLISTER 1961), the bottles were incubated at a series of light levels varying from full mid-day sunlight to 1 % full sunlight. The temperature was maintained between 26.5° C and 27.5° C and was controlled by placing the bottles in a shallow pan which in turn was placed in a shallow tide pool just adjacent to the collection area. The surface temperature at the collection site was monitored and found to be within 0.5° C of the incubation temperature. Throughout the experiment, solar radiation was measured at regular intervals by use of a model 50 Eppley pyrheliometer.[2] Since the experiments were conducted near solar noon, the solar radiation varied less than 5 % throughout the incubation period and an average value was taken to calculate the incident radiation.

All bottles were run in duplicate or quadruplicate and dark bottles were included in each series so as to correct for nonphotosynthetic carbon uptake. This correction amounted to less than 0.5 % of maximum photosynthesis.

After one hour both photosynthesis and respiration were stopped by the addition of a drop of potassium iodide-iodine solution. On returning to the laboratory, the diatoms were collected on Millipore[3] AA filters (pore size 0.8 microns) washed with 5 ml. of 0.001 N HCl made up in 3 % NaCl, dried and assayed for radiocarbon content with a gas flow counter.

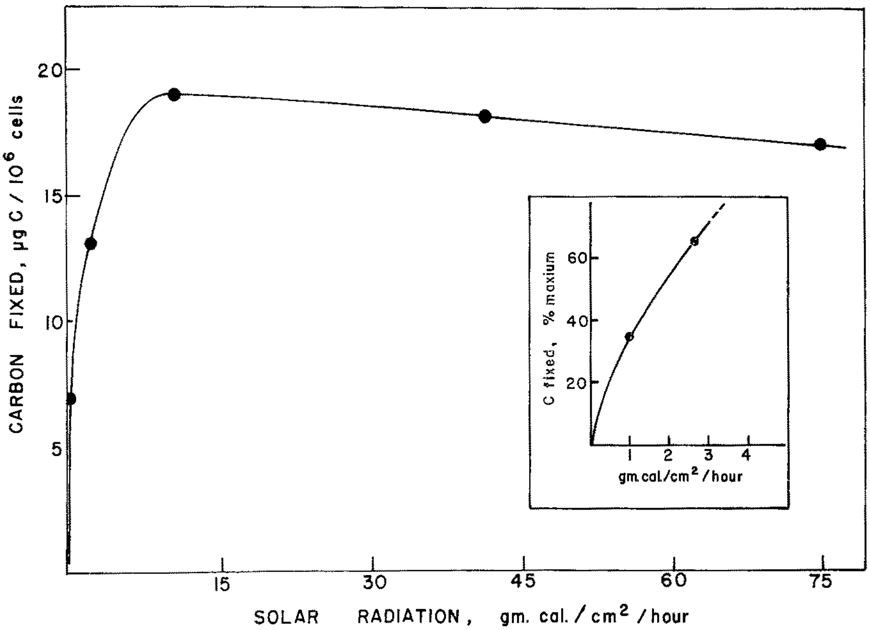

Fig. 1: Photosynthetic fixation of carbon in one hour by intertidal diatoms at varying intensities of natural sunlight. Insert: abcissa scale expanded to show rapid increase in photosynthesis at low light levels

From pH, temperature, and salinity measurements on the filtered sea water used to make up the diatom suspensions, total inorganic carbon was calculated by the method of HARVEY (1960). Since the specific activity of the carbon-14 labeled sodium carbonate solution was known, the photosynthetic fixation of carbon could be calculated and is reported as micrograms of carbon assimilated by 10^6 cells in one hour.

[2] Eppley Laboratories, Inc. Newport, R. I., U. S. A.
[3] Millipore Filter Corp. Bedford, Mass., U. S. A.

Results

Data obtained from a typical experiment are shown in Figure 1. The diatoms were found to be very efficient at low light levels. This is more clearly shown in the insert to Figure 1 in which the abscissa scale is expanded. Diatoms receiving 0.75 gm. cals./cm.^{2}/hr. of solar radiation (1 % of incident radiation) fixed carbon at 35 % of their maximum photosynthetic capacity. At 1.88 gm. cals./cm.^{2}/hr. (2.5 % of incident radiation) they performed at 65 % of their maximum capacity. Maximum photosynthesis was reached at about 12 gm. cals./cm.^{2}/hr., which was 14 % of the full midday, mid-August sunlight.

At high light levels very little inhibition was found, there being approximately 10 % inhibition in full sunlight. Because this light response differed from that noted in plankton diatom species, the experiment was repeated with nearly identical results.

LIGHT PENETRATION THROUGH SAND

Since these diatoms are found a few millimeters below the surface, it was of interest to measure the penetration of solar radiation through the sand.

Methods

The apparatus used consisted of a square glass plate approximately 60 cm. on a side. It was mounted on a wooden frame supported on each corner by 30 cm. legs. The pyrheliometer was placed directly under the center of the glass plate and the solar radiation passing through the plate was measured. Surface sand from the top one to two millimeters was collected from the experimental area. It was passed through a 0.5 mm screen to remove the occasional small pelecypods *(Gemma gemma)* that were found. The sand was then evenly spread over the glass plate by means of a sliding guide bar. The distance between the bottom of the guide bar and the glass plate was adjustable and was set by the use of shims to one, two, or three millimeters. The technique was analogous, though more crude and on a larger scale, to the preparation of plates for thin layer chromotography. With practice quite uniform layers of sand were obtained and the solar energy penetrating the sand layers was determined.

Results

The sands inhabited by these diatoms are fine and well sorted. The results of a particle size analysis obtained by standard methods are given in Figure 2. Over 90 % of the sand grains were between 177 microns and 63 microns in diameter.

Sand from the surface millimeter of the diatom patches was washed thoroughly with filtered sea water, air dried, and then ignited in a muffle furnace. The organic content calculated from the weight loss averaged 0.30 % of dry weight. Unwashed sand had an organic content of 0.46 % of its dry weight.

Typical data from light penetration measurements is shown in Figure 3. Ten per

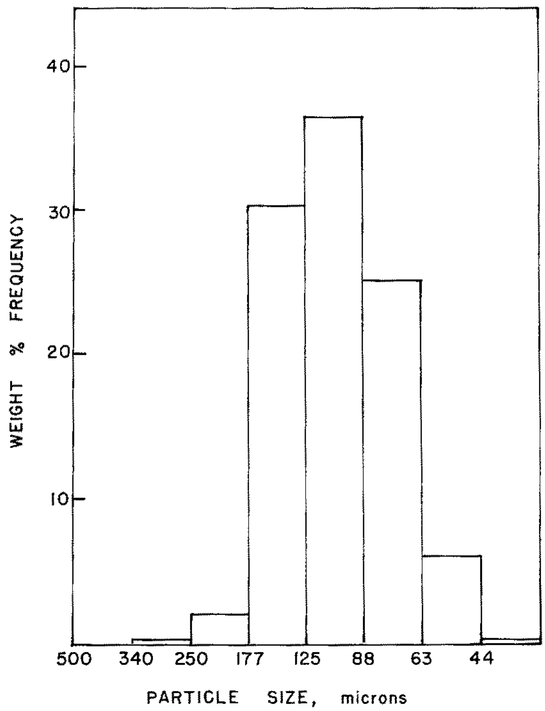

Fig. 2: Particle size distribution of Barnstable Harbor sand inhabited by migrating intertidal diatoms

cent of the solar radiation penetrated approximately 1.5 mm., while 1 % reached a depth of 3 mm. These measurements were taken at approximate solar noon but there was no significant difference in the slope curve obtained 2.5 hours after solar noon. The sand was applied to the glass plate in a moist condition but, when allowed to air dry, there was only a slight variation in the slope of the curve.

DISCUSSION

Since the attenuation of solar radiation through the sand was a logarithmic relationship, it can be described by the equation:

$$dI = -kIdx$$

where I is the radiation intensity, x is the thickness of sand and k is a constant. Integration of the equation and substituting values of I at different values of x results in an evaluation of the constant.

$$k = 0.67 \text{ mm.}^{-1}$$

While this constant might be considered analogous to the absorption coefficient describing the absorption of light by a solution, it is not used here in the same sense. It is merely a convenient means of describing the data and includes all processes involved

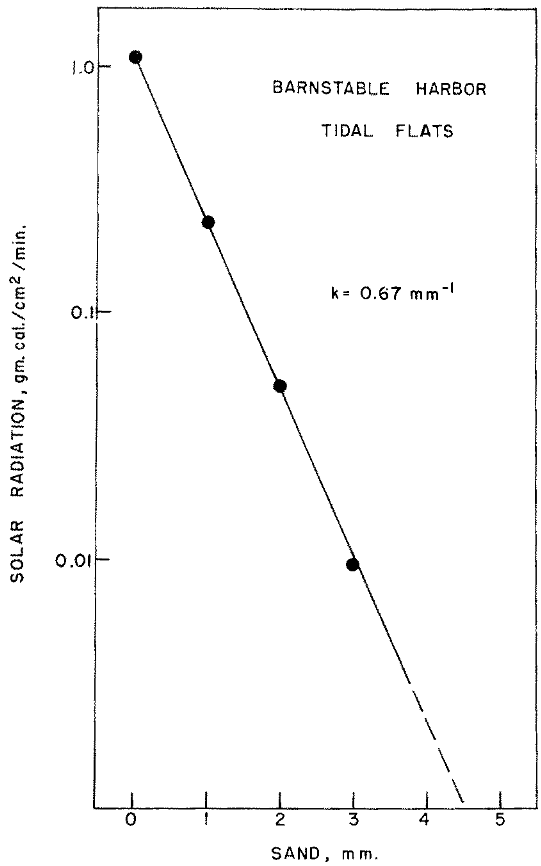

Fig. 3: Penetration of solar radiation through Barnstable Harbor sand inhabited by migrating intertidal diatoms

in reducing the solar radiation with increasing thickness of sand. These would include reflection, refraction, scattering and light absorbed by interstitial organisms and detritis. It includes all wave lengths of solar radiation that were detectable by the pyrheliometer. It is suggested that the term, *attenuation constant*, would be useful in describing such data.

It is customary to assume that the rate of respiration of diatoms is approximately 10 % of their maximum photosynthetic rate. Direct measurements of the respiratory rate of these diatoms are not yet available but, if the above assumption is adapted, the light level at which photosynthesis and respiration are equal is about 0.3 % of mid-day summer sunlight. This is best shown in the insert to Figure 1. This light level is considerably less than the usual one per cent of incident radiation that is used for "average" plankton species and more closely resembles the light response curves for "shade" forms described by Ryther & Menzel (1959). However, no shade adaption could have taken place since the method of collection depended upon the diatoms' migration into bright sunlight. This photosynthetic efficiency at very low light levels would mean that these species of diatoms existing as deep as 3 mm. in the sand would be above their compensation point. It would also mean that diatoms can photosynthesize near their maximum rate even if the sand flats are exposed at a time considerably different from solar noon. For example, using RYTHER's data (1956) for incident radiation in mid-June, they would photosynthesize at 90 % or better of their maximum capacity at any time that the tide was out between 0530 and 1830.

The lack of inhibition of photosynthesis of these species by full summer sunlight, on the other hand, fits in well with their migratory behavior. Diatoms which are anywhere from the surface to 2 mm. deep in the sand can, on such a day, photosynthesize at 90 % or better of their maximum capacity. At high levels then these diatoms resemble the "sun" forms of plankton organisms noted by RYTHER & MENZEL (1959).

The light penetration measurements are subject to several reservations. The instrument used to detect solar radiation is equally sensitive to all wave lengths reaching the earth's surface through a range of 380 to 2900 mμ (MACDONALD 1951). EDMONDSON (1956) suggests that about 45 % of the solar energy detected by this instrument is useful for photosynthetic purposes. Since no filters were used, the possibility exists that this long wave length energy may be attenuated by the sand at a different rate than the photosynthetically active light. The results obtained by the present techniques may therefore be in error and may not represent the penetration of solar radiation that is actually useful to the plants.

The sand used for these measurements was collected from the top 2 mm. and the attenuation data apply only to it. At approximately 3 mm. depth the sand is black due to bacterial sulfide formation and such anaerobic sand probably attenuates light more strongly than surface sand. The system is further complicated in that often a layer of purple sulfur bacteria is found between the anaerobic and aerobic layers of sand. Thus the diatoms may not be able to photosynthesize at depths below the aerobic zone as successfully as this data would indicate. POMEROY (1959) by using frozen sections has found most diatoms to be at or very near the surface in the mud flats of the Georgia salt marsh and suggested that vertical migration was limited to a few microns. Preliminary observations of the Barnstable sand flat populations show considerable numbers of these large migrating, pennate diatoms at 2 to 3 mm., while fewer organisms were deeper than this.

As used, the pyrheliometer measured the brightness of a layer of sand above it. Diatoms existing a few millimeters below the surface, while receiving most of their light from above, may because of scattering and reflection obtain light from all angles, even below. Thus the algae may be able to successfully photosynthesize at depths even greater than indicated by these data. The quantitative aspects of this possibility are unknown.

Future measurements will be made employing an infra-red cutoff filter, collimated light and other refinements which should help to resolve some of these experimental deficiencies.

SUMMARY

1. The rate of photosynthesis at varying amounts of natural sunlight has been measured for large pennate littoral diatoms which inhabit the sand flats of Barnstable Harbor, Massachusetts, and which exhibit vertical migration to the surface at low tide.
2. The algae require only 12 gm. cal./cm.^{2}/hr. to reach their maximum photosynthetic capacity.
3. Full mid-day summer sunlight (75 gm. cal./cm.^{2}/hr.) results in only a 10 % inhibition of maximum photosynthesis.
4. Measurements of light penetration through the sand in which the diatoms are found indicated 10 % of solar radiation reaches 1.5 mm.
5. At noon on a cloudless mid-summer day these species existing as deep as 3.0 mm. are probably above their compensation level, while those from 2.0 mm. to the surface are able to photosynthesize at 90 % or better of their maximum rate.
6. The photosynthetic efficiency at low light levels enables these diatoms to fix considerable carbon even when the flats are exposed when the sun angle is low.
7. The migration pattern of these diatoms does not result in significant inhibition of photosynthesis but rather their photosynthetic apparatus is well adapted to such migratory activity.

ACKNOWLEDGMENTS

The initial stages of this investigation were greatly aided by advice from Dr. John D. PALMER whose interest in the migratory rhythms of these algae has resulted in extensive knowledge of the tidal flats of Barnstable Harbor. A travel grant (GB-1637) from the National Science Foundation made possible the presentation of this paper at the *Fourth Marine Biological Symposium*, Biologische Anstalt Helgoland, Hamburg, and is gratefully acknowledged.

LITERATURE CITED

ALEEM, A. A., 1950. The diatom community inhabiting the mud flats at Whitstable, Kent. *New Physiol.* **49**, 179–188.

AYERS, J. C., 1959. The hydrography of Barnstable Harbor, Massachusetts. *Limnol. Oceanogr.* **4**, 448–462.

CALLOME, B. & DEBYSER, J., 1954. Observations sur les mouvements des diatomees a la surface des sediments marins de la zone intercotidale. *Vie et Milieu* **5**, 242–249.

EDMONDSON, W. T., 1956. The relation of photosynthesis by phytoplankton to light in lakes. *Ecology*, **37**, 161–173.

FAURÉ-FRÉMIET, E., 1951. The tidal rhythm of the diatom, *Hantzschia amphioxys. Biol. Bull., Woods Hole* **100**, 173–177.

HARVEY, H. H., 1960. The chemistry and fertility of sea waters. 2nd ed. Cambridge Univ. pr., London, 161–174.

JENKIN, P. M., 1937. Oxygen production by the diatom, *Coscinodiscus excentricus*, EHR. in relation to submarine illumination in the English Channel. *J. Mar. biol. Assoc. U. K.* **22**, 301–343.

McALLISTER, C. D. & STRICKLAND, J. D. H., 1961. Light attenuators for use in phytoplankton photosynthesis studies. *Limnol. Oceanogr.* **6**, 226–227.

MACDONALD, T. H., 1951. Some characteristics of the Eppley pyrheliometer. *Mon. Weath. Rev., Wash.* **79**, 153–159.

MOUL, E. T. & MASON, D., 1957. Study of diatom populations on sand and mud flats in the Woods Hole area. *Biol. Bull., Woods Hole* **113**, 351.

PERKINS, E. J., 1960. The diurnal rhythm of the littoral diatoms of the River Eden estuary, Fife. *J. Ecol.* **48**, 725–728.

POMEROY, L. R., 1958. Algal productivity in salt marshes. *Proc. Salt Marsh Conference, The Mar. Inst. Univ. Georgia, Sapelo Island, Georgia, March 25–28, 1958*, 88–95.

— 1959. Algal productivity in salt marshes of Georgia. *Limnol. Oceanogr.* **4**, 386–398.

RYTHER, J. H., 1956. Photosynthesis in the ocean as a function of light intensity. *Limnol. Oceanogr.* **1**, 61–70.

— & MENZEL, D. W., 1959. Light adaption by marine phytoplankton. *Limnol. Oceanogr.* **4**, 492–497.

WILLIAMS, R. B., 1963. Use of netting to collect motile benthic algae. *Limnol. Oceanogr.* **8**, 360–361.

Discussion following the paper by TAYLOR

WELLS: Is it known whether or not intertidal benthic diatoms produce ectocrine substances which would make the mud unpalatable or even poisonous to animals which live by swallowing it?

TAYLOR: Many of these diatoms obviously produce some sort of gelatinous material, but I know of no work concerning the effects of this material on other organisms. The diatoms with which I have been concerned are two very large pennate diatoms that actively migrate into bright sunlight. These are by no means all of the diatoms in these golden patches I have shown on my slides. Many of the diatoms present do not migrate. Furthermore, there are many blue-green algae and sessile chrysomonads present. There is a great deal of species diversity between these golden patches, even those only a meter apart.

OVERBECK: Beeinflußt Schwefelwasserstoff die Assimilation?

TAYLOR: If you recall I mentioned that often between the aerobic and anaerobic layers of sand are found purple sulfur bacteria. These organisms, of course, metabolically convert sulfide to sulfate. On the average, the top 2 mm of sand are aerobic and free of sulfide. Our studies were not involved with organisms which were found to any extent below this aerobic zone.