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# Salinity Tolerance of Benthic Estuarine Diatoms as Tested with a Rapid Polarographic Measurement of Photosynthesis

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## Abstract

Measurements of net photosynthesis of benthic estuarine diatoms were made by polarographic registration of oxygen saturation. A measuring cell was constructed in which media with salinities of 2.0 to 100.7‰ were pumped over the algae between measurements. Diatoms from unialgal cultures and mixed populations from intertidal flats appeared to be highly tolerant of extreme salinities. During short-term exposures (20 min) the net photosynthesis of the algae did not drop below 70% of the initial values, within the salinity range 4.0 to 60.0‰. Prolonged exposure (up to 6 h) gave essentially the same results. Populations of benthic diatoms, sampled from field stations with mean salinities of about 30, 12, and below 5‰, showed only gradual differences in their tolerance of salinities between 2.0 and 33.7‰. Two species, *Navicula arenaria* and *Nitzschia sigma*, were cultured in media ranging in salinity from 8.0 to 45.0‰ and from 2.0 to 45‰, respectively. The tolerance to changing salinity was only slightly affected by the salinity of the medium in the preculture. The role of salinity in the production and distribution of intertidal diatoms is discussed.

## Introduction

Algae growing in estuarine areas are subjected to strongly changing salinities due to the varying degree of mixing of seawater and fresh water. Moreover, benthic algae living in the intertidal zone are exposed to rain and desiccation at low tide. The physiological consequences of an extreme salt regime have been extensively investigated for intertidal macro-algae (for review see Gessner and Schramm, 1971). However, little physiological information is available concerning the effect of salt concentration on marine benthic diatoms (Fischer, 1952, 1963, 1964; Nultsch, 1962; Williams, 1964). This scarcity of experimental data on the salinity tolerance of estuarine benthic diatoms contrasts strongly with the fact that some investigators in the field ascribe rather precise salinity preferences to the individual species (cf. van der Werff, 1960).

Most of the culture experiments with marine planktonic algae, devised to test the influence of salinity, pertain to constant levels of salinity and only a few to fluctuating salt concentrations (Nakanishi and Monsi, 1965; Vosjan and Siezen, 1968; Quasim *et al.*, 1972; Schobert, 1974; Schöne, 1974; Kirst, 1975).

The present investigation was carried out in the Eems-Dollard estuary (Fig. 2), a part of the Dutch-German Waddensea. A considerable part of the surface area consists of intertidal mud- and sandflats. The pennate diatoms constitute most of the benthic algal flora (Colijn, 1974). A previous investigation on the diatoms in the Eems-Dollard estuary (van der Werff, 1960) revealed, along the longitudinal axis of the estuary, a distinct distribution pattern which was thought to be caused by the salinity gradient.

Probably most of the primary production of the benthic diatoms in the area occurs at low tide when none of the light is being absorbed by the turbid water. Rain and desiccation at the surface layer of the sediment might have a negative effect on the rate of primary production. In this study, I investigated whether or not the postulated fluctuations in salinity in the field can have an inhibiting effect in the laboratory on the rate of photosynthesis of mixed populations of diatoms from the field and of unialgal cultures. Diatoms growing at various salinities in the estuary or in cultures were used.

## Materials and Methods

### *Measurements of Net Photosynthesis*

Photosynthesis was measured in a laboratory unit (Fig. 1). A Clark-type oxygen electrode and an oxygen monitor (Yellow Spring Institute 5300) were connected with a Perspex measuring cell. The oxygen-saturation values measured by the monitor were plotted by a recorder. The temperature was kept constant by a circulation waterbath. A strong water movement along the electrode and a gentle flow over the algae was achieved with a single magnetic stirring bar. The diatoms were kept in the main chamber of the measuring cell under a small perforated Perspex plate which moderated the water movement.

The diatoms were put into the measuring cell on glass-fibre filters (Whatman GF/C). The algae were suspended, and 10 to 50 ml suspension was pipetted onto the glass-fibre filter under gentle suction (-50 cm water pressure). Detrimental effects were observed when higher pressures were used. No appreciable loss of the algae from the filter was observed over many hours, as long as the immersion of the filter was carried out very carefully to prevent the diatoms from becoming detached.

The rate of net photosynthesis of the diatoms, in mg $O_2$.mg chlorophyll $a^{-1}$.$h^{-1}$, was calculated from the following data: the slope of the oxygen saturation curve plotted by the recorder in %.$h^{-1}$; the oxygen saturation value at the particular salinity and temperature in mg $O_2$.$l^{-1}$; the volume of the measuring cell (0.038 l); and the chlorophyll *a* content of the diatoms on the filter in mg. All measurements of photosynthesis were started with air-saturated water samples. The measured saturation values were converted to absolute amounts of oxygen using the oxygen-saturation values of seawater given by Carpenter (1966). For salinities over 35‰ the solubility was measured by the Winkler method (45‰ S: 7.1 mg/l; 60‰ S: 6.6 mg/l; 78‰ S: 6.2 mg/l; 100.7‰ S: 5.7 mg/l). The chlorophyll *a* concentration of the diatoms on the filter was measured according to Lorenzen (1967). The measuring cell was illuminated with fluorescent tubes (Philips no. 34) at a quantum irradiance of 85 µE.$m^{-2}$.$sec^{-1}$ (4000 lux) at the surface of the cell.

### *Test Algae*

Mixed populations of micro-algae directly collected from the field and cultures of diatoms were used. Six species of common benthic diatoms from the Eems-Dollard estuary were grown in unialgal, but not bacteria-free cultures: *Navicula arenaria* Donkin; *Nitzschia* c.f. *dissipata* (Kützing) Grunow; *Nitzschia sigma* (Kützing) W. Smith; *Nitzschia closterium* (Ehrenberg) W. Smith; *Stauroneis constricta* (Ehrenberg?, W. Smith) Cleve; *Amphiprora* c.f. *paludosa* W. Smith. The diatoms were grown in Perspex boxes on analytical sand (Merck) immersed in 1 cm of enriched seawater medium. Only growing cultures were used in the experiments. The temperature in the climate rooms was 20°C, and extra cool white fluorescent light was supplied at a quantum irradiance of 85 µE .$m^{-2}$.$sec^{-1}$ (4000 lux) for 16 h per day.

Epipelic diatoms were obtained from field samples of sediment by the lens-tissue technique (Eaton and Moss, 1966). Three layers of tissue were spread over the sediment, and after one night in a climate room (12°C) the uppermost two layers were harvested. These tissues were shaken with artificial seawater and the suspension was filtered through a mesh (200 µ). The salinity of the artificial seawater was selected in accordance with the mean salinity at the sampling site. Field samples were obtained from three stations in the estuary (Fig. 2) from December, 1974 to March, 1975. The suspensions of the cultures and field samples, obtained with the lens-tissue technique, were prepared for the photosynthesis measurements as described above.

### *Test Media*

Two series of media were used; the artificial seawater media were used for the measurements of photosynthesis, and the enriched seawater media were used for the culturing of the diatom species. A basic artificial seawater of fairly natural ionic composition (Provasoli, 1957, code U) was prepared in concentrations of 2.0; 4.0; 8.0; 15.0; 33.7; 45.0; 60.0; 78.0; and 100.7 g total dry salts per litre. These solutions were supplied with the following additions: 0.2 g/l $NaHCO_3$; 0.5 g/l tris-buffer (pH 7.8); minor elements (Provasoli *et al.*, 1957, p. 414) and 26 mg/l $HBO_3$. Air was bubbled through the media at 20° and 12°C for one night to adjust the saturation value to 100%. The culture media of various salinities were prepared from North

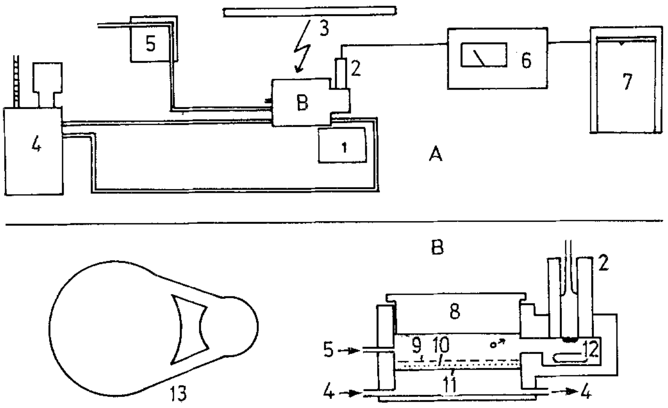

Fig. 1. Diagram of unit used for oxygen measurements (A) and the measuring cell (B). 1: Magnetic stirring motor; 2: Clark electrode; 3: fluorescent tubes; 4: waterbath; 5: high-capacity peristaltic pump; 6: oxygen monitor; 7: recorder; 8: Perspex stopper; 9: Perspex grid; 10: glass-fibre filter with diatoms; 11: stainless-steel plate; 12: stirring bar; 13: chambers of the measuring cell seen from above

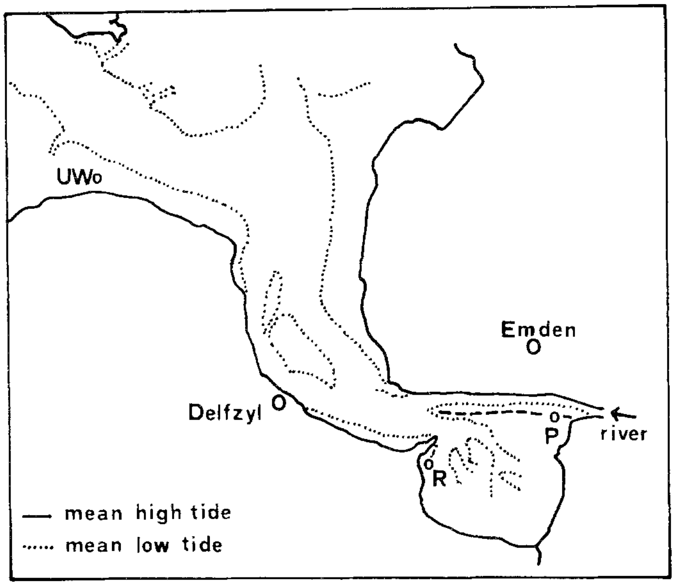

Fig. 2. Three field stations in Eems-Dollard estuary. Mean salinity at UW is about 30‰; at R about 12‰; and at P below 5‰

Sea water (30‰ S), diluted with demineralized water (to 15.0; 8.0; 4.0; and 2.0‰ S), or concentrated by addition of 100.7‰ S artificial seawater (to 45.0‰ S). These basic media were used to prepare enriched seawater media based on the "f" medium of Guillard and Ryther (1962). The additions were used in half the concentrations given by Guillard and Ryther; tris-buffer (0.5 g/l, pH about 7.8) and NH$_4$Cl (26.5 mg/l) were added (cf. Admiraal, 1976).

### *Experimental Procedure*

Two kinds of experiments were devised, differing in the time of exposure to the various salinities.

*Short-Term Exposure.* The test algae were kept for 20 min in a medium of one given salinity, and the photosynthesis was measured. Subsequently, a quantity of medium of another salinity, equalling 7 volumes of the measuring cell was pumped through the measuring cell within a few minutes. The sequence of tested salinities was always from medium to extreme ones. For example, a culture grown at 30‰ S was exposed to different salinities in the following sequence: 33.7; 15.0; 8.0; 4.0; 2.0‰ S, and another sample of the same culture to 33.7; 45.0; 60.0; 78.0; 100.7‰ S.

*Long-Term Exposure.* The rate of photosynthesis of a diatom sample was measured at regular intervals for about 6 h in medium of the same salinity. Medium was pumped through the measuring cell at a rate of about 7 ml/min between measurements. When the test salinity was not the same as that used during the growth of the algae, they were transferred via intermediate salinities within 30 min. The measuring unit was used in duplicate to make two exposures each time.

The experiments with cultures were carried out at 20°C; the experiments with field samples at 12° or 20°C.

## Results

The net photosynthesis as measured by the oxygen method equals gross photosynthesis minus respiration. Samples from the field and cultures had respiration rates of about 10% of the oxygen production in the light when random test measurements were made. Photosynthesis at the quantum irradiance used (85 $\mu E.m^{-2}.sec^{-1}$) was probably not fully saturated. Yet fairly high rates were measured: 2.7-5.7 and 4.5-5.9 mg O$_2$.mg chlorophyll a$^{-1}$.h$^{-1}$ in cultures and field samples, respectively. Comparable values have been reported for the maximum rate of photosynthesis by phytoplankton (Parsons and Takahashi, 1973).

### *Short-Term Exposures of Cultures*

Cultures of 6 diatom species were exposed to step-wise decreasing and increasing salinity. Most of the cultures were tested in two independent experiments. Fig. 3 shows that the photosynthesis of these benthic diatoms hardly depends on strong fluctuations in salinity. A constant rate of oxygen production was observed after a few minutes incubation in the new medium, except at the most extreme salinities. The rate of net photosynthesis did not drop below 70% of the initial value within the salinity range 4.0 to 60.0‰. Specific differences among the species were small. In *Nitzschia sigma* and *N.* c.f. *dissipata* photosynthesis was distinctly increased at low salinity.

### *Short-Term Exposures of Field Samples*

Mixed populations of two field stations (Fig. 2, UW and R) were collected on two occasions; the second sample from Station R was taken during rain in pools having a salinity of 7‰. The rate of photosynthesis of the field samples of Station UW showed a relation with the salinity of the medium which was quite similar to that of the unialgal cultures (Fig. 4). The populations from Station R displayed a different response since they tolerated 2.0‰ S very well. These algae grew in the field at a mean salinity less than half that at Station UW.

### *Long-Term Exposures of Cultures*

The net photosynthesis of two diatom species was registered for about 6 h in media with salinities of 4.0, 33.7, and 60.0‰. The rate of net photosynthesis did not appear to be strictly constant during the experiment (Fig. 5). This experiment suggests that the observed tolerance of the photosynthesis of the diatoms to extreme salinities is not restricted to the first 20 min after transfer, but may extend over many hours.

### *Long-Term Exposures of Field Samples*

Mixed populations of diatoms from three field stations in the Eems-Dollard estuary were used for this experiment. The stations UW, R, and P (Fig. 2) differ in

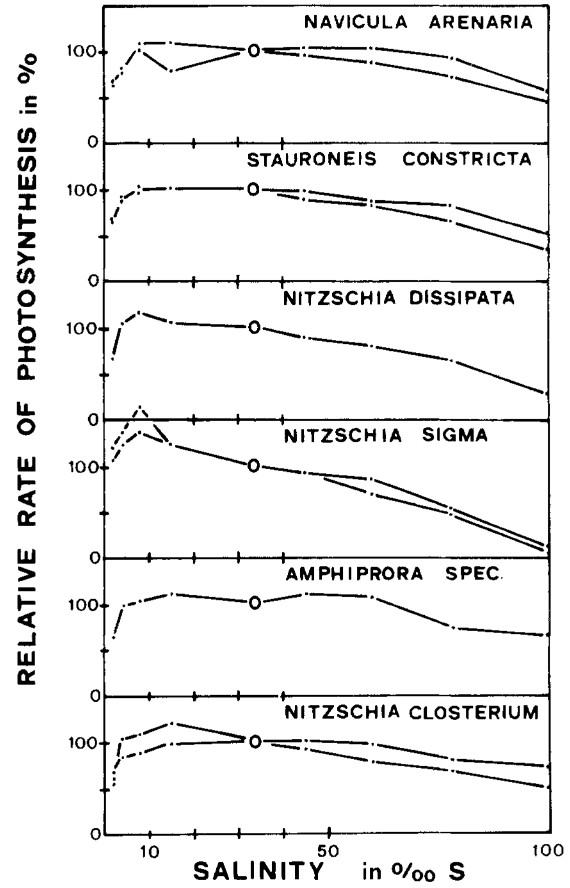

Fig. 3. Photosynthesis during short-term exposure of unialgal diatom cultures to step-wise variation in salinity. Open circles: initial values

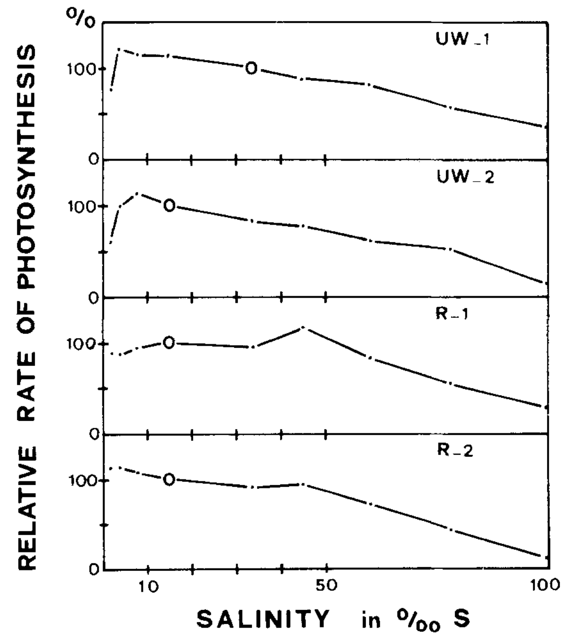

Fig. 4. Photosynthesis during short-term exposures of mixed diatom populations collected from the field to step-wise variation in salinity. UW and R refer to field stations in Fig. 2. Temperature: 20°C. Open circles: initial values

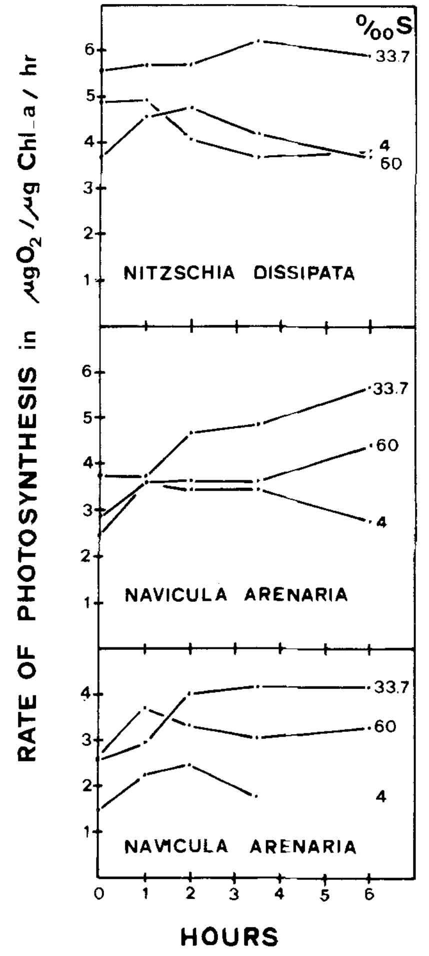

Fig. 5. Photosynthesis of two diatom species, precultured at 30‰ S, after transfer to media of extreme salinities. Temperature: 20°C

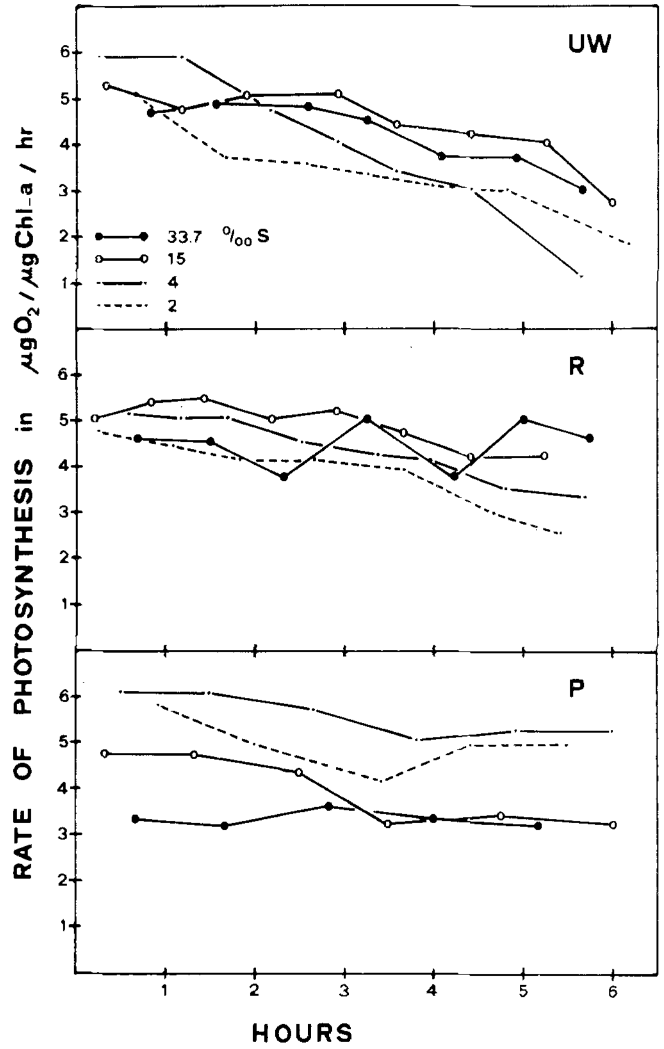

Fig. 6. Photosynthesis of mixed diatom populations, collected from 3 field stations, exposed to media of various salinities. UW, R, and P refer to field stations in Fig. 2. Temperature: 12°C

their mean salinity (about 30, 15, and below 5‰ S, respectively). The diatoms were exposed to 4 media with salinities from 2.0 to 33.7‰ for 6 h, and their photosynthesis was intermittently measured. It was evident that the three populations of diatoms were able to photosynthesize in all the test solutions (Fig. 6). The diatom population that grew in the field at the highest salinity (Station UW) showed, however, a somewhat higher activity in the most saline media. The reverse was true for the sample collected at the field station with the lowest salinity (Station P).

*Culturing at Various Salinities*

*Nitzschia sigma* grew well in all the culture media with salinities of 2.0 to 45.0‰, whereas *Navicula arenaria* was not able to grow in media of salinity below 8.0‰, and seemed somewhat retarded at 8.0‰. The two species were used in an experiment to test their ability to photosynthesize under short-term exposures to extreme salinities after preculturing in media of various salinities. The preculturing of *Navicula arenaria* at 15.0, 30, and 45‰ S did not seem to affect its relative inability to photosynthesize at the lowest salinities (Fig. 7). Similarly, *Nitzschia sigma* did not need low-salinity preculturing for it to be able to photosynthesis fully at low salinity (Fig. 8). Only when grown at 45‰ S was the photosynthesis of *N. sigma* inhibited at salinities below 8.0‰ S. The preculturing of the two species at high salinity increased their ability to photosynthesize at extremely high salini-

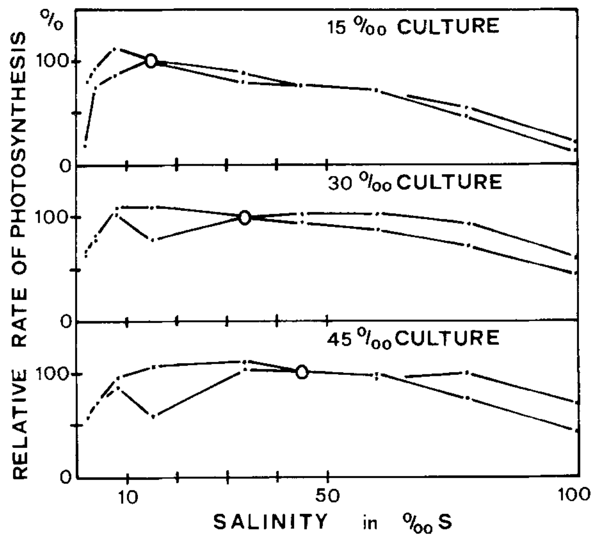

Fig. 7. *Navicula arenaria*. Photosynthesis of diatoms precultured at 15, 30, and 45‰ S, during short-term exposure to step-wise variation in salinity. Temperature: 20°C

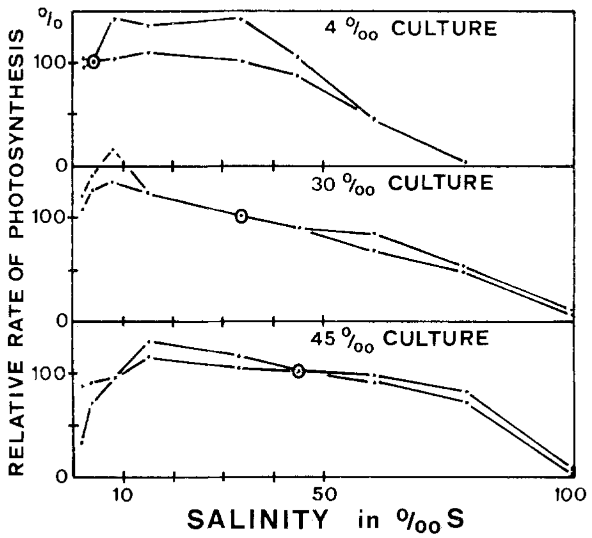

Fig. 8. *Nitzschia sigma*. Photosynthesis of diatoms precultured at 4, 30, and 45‰ S, during short-term exposure to step-wise variation in salinity. Temperature: 20°C

ties (Figs. 7 and 8). Yet *N. sigma*, precultured at 4‰ S, still showed a high rate of photosynthesis even at a salinity of 45.0‰.

## Discussion and Conclusions

The photosynthesis rate of the benthic diatoms, measured as oxygen production, was hardly affected by a wide range of salinities. The tolerance of benthic diatoms to strong fluctuations in salt concentration contrasted sharply with the sensitivity of the marine phytoplankton species *Thalassiosira rotula* (Schöne, 1974), and *Asterionella japonica* (Kain and Fogg, 1958), and was clearly higher than that of estuarine phytoplankton (Nakanishi and Monsi, 1965; Quasim *et al.*, 1972; Schobert, 1974). A comparable tolerance to extreme salinities was found by Vosjan and Siezen (1968) for *Chlamydomonas uva-maris* and by Höfler (1956, cited by Gessner and Schramm, 1971) for *Amphidinium höflerii*. The present results agree with the microscopical observations of Fischer (1952, 1963, 1964) and Nultsch (1962) on salt-marsh diatoms in hypotonic and hypertonic solutions. Williams (1964) cultured 14 species of benthic diatoms from a salt-marsh in a wide range of salinities; high growth rates occurred from 1.5 or 7‰ to about 40‰ S in most species. This was a very similar salinity tolerance range to that found in the present investigation for all short-term exposures and culture experiments on two species. Williams (1964) found, in the uppermost 0.5 to 1.0 mm of sediment of an emerged salt-marsh, salt concentrations of 2 or 3‰ S after rain and up to 45‰ S after several hours exposure to sunlight and wind. The present study suggests that these salinities permit a relatively high rate of photosynthesis in benthic diatoms. The rate of photosynthesis of samples of mixed diatom populations from the Eems-Dollard estuary was highest in media with salinities corresponding to the mean salinity at the field stations. Salinities markedly different from the mean salinity at the sampling site caused some decrease in the rate of photosynthesis (Fig. 5). This may have been caused partly by the species composition of the populations and partly by the physiological adaptation of the diatoms to the salinities prevailing in the field. Slight differences in the lower limit of tolerance occurred among the species investigated (Fig. 3). Adaptation to very high salinities, but not to low salinities, was demonstrated in two species (Figs. 7 and 8). Williams (1964) found no adaptation in long-term growth experiments, except for one species (*Rhopalodia musculus*) that was favoured by adaptation at very high salinities. In contrast, Kirst (1975) found in cultures of *Platymonas subcordiformis* that the decline of photosynthesis in low-salinity solutions was dependent on the NaCl concentration of the culture medium.

Vosjan and Siezen (1968) found a discrepancy in the photosynthesis of algae measured with the $O_2$ and the $^{14}C$ methods.

Cultures of *Chlamydomonas uva-maris* and *Scenedesmus obliquus,* incubated at very high salinities, showed a relative low rate of $^{14}C$ incorporation compared with the rate of oxygen production. The cause of this phenomenon was not clear, but it was possible that the $^{14}C$ incorporation in the algal cells was counterbalanced by excretion of labeled photosynthetic products. However, the oxygen production provided a direct measure for the quantity of carbon reduced and energy fixed.

The species investigated have been described, as to their salt preference, in several primarily taxonomic publications on diatoms. *Nitzschia sigma* was referred to as a brackish-freshwater species by Hendey (1964) and van der Werff (1960) and as a "mesohalob" and "euryhaline" species (Hustedt, 1939). *Navicula arenaria* was referred to as a marine littoral species, capable of withstanding considerable ranges of salinity by Hendey (1964), and as a "euhalob" and "euryhaline" species by Hustedt (1939). *Nitzschia dissipata* is a common freshwater species (Hustedt, 1930), but has been described from fresh and brackish waters also (van Heurck, 1896). *Amphiprora paludosa* occurs in brackish water or in water with a high mineral content according to van Heurck (1896), and has been described as a "mesohalob" and "euryhaline" species by Hustedt (1939). *Nitzschia closterium* f. *minutissima* has been found in brackish waters (Hendey, 1964). The present results do not contradict these indications of the salt preferences of the investigated estuarine benthic diatoms, but suggest that most species have rather broad ranges of salinity tolerance. The data on two species investigated might explain their occurrence in the field. *Navicula arenaria* was isolated from Field Station UW (Fig. 2), having a mean salinity of about 30‰, and *Nitzschia sigma* was isolated from the inner part of the estuary (between Stations R and P, Fig. 2, mean salinity about 10‰). *Navicula arenaria* showed retarded growth in media with a salinity of 8‰; no growth was observed in media with 4‰ S, whereas *Nitzschia sigma* grew well in the most dilute medium (2‰ S). The growth responses of these two species to low salinities are compatible with their occurrence in their original habitat.

The present investigation and those of Fischer (1952, 1963, 1964), Nultsch (1962), and Williams (1964) all indicate that estuarine benthic diatoms grow well under highly divergent salt concentrations. This contradicts the theory (van der Werff, 1960) that salinity level is the main factor governing the distribution of benthic diatoms within an estuary. Martin (1970) carried out culture experiments designed to relate salt requirements of benthic diatoms to their distribution in an estuary, but did not establish a positive correlation. Bakker and de Pauw (1974) postulated that in tidal brackish waters (Westerschelde estuary), turbulence acts as an ecological master-factor in relation to phytoplankton development, dominating salinity in importance within the chosen salinity ranges. Thus, it seems necessary to explore more ecological factors, other than salinity, in order to explain the distribution of benthic diatoms in estuaries.

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