Helgoländer wiss. Meeresunters. 20, 104–110 (1970)

Osmotic and ionic requirements of the marine centric diatom *Cyclotella nana**

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KURZFASSUNG: **Osmotische und ionische Ansprüche der marinen, zentrischen Diatomee *Cyclotella nana*.** Die osmotischen und ionischen Ansprüche der Diatomee *Cyclotella nana* HUSTEDT (Klon 13–1) wurden untersucht. Osmotische Reaktionen wurden bei relativ hohem osmotischem Druck nachgewiesen. *C. nana* vermehrt sich in Salinitäten von 15 bis 50 ‰. Wird der osmotische Druck des Mediums mit Saccharose ausgeglichen, so konnte eine Verdünnung bis zu einem Anteil von 1/6 Seewasser erfolgen, ohne die Vermehrungsvorgänge der Diatomee nachhaltig zu beeinträchtigen. Der Bedarf an Na, K, Ca und Mg sowie die Wirkungen von Konzentrationsveränderungen dieser Kationen werden erörtert.

INTRODUCTION

It is well established that many marine planktonic algae die or grow poorly when sea water is diluted below 50 % (BRAARUD 1961, PINTNER & PROVASOLI 1963, LEWIN & GUILLARD 1963). This is the case for *Cyclotella nana* clone 13–1, which was isolated from the Sargasso Sea (GUILLARD & RYTHER 1962). There is very little information, however, on the reason. DROOP (1958) has suggested from study of the neritic diatom *Skeletonema costatum* that the salinity response of this alga is due mainly to its sodium requirement. MCLACHLAN (1960) found that *Dunaliella tertiolecta* could tolerate an osmotic pressure as low as the equivalent of 1/10 sea water and a sodium concentration not lower than that equivalent to 1/50 sea water. None of the other monovalent cations examined were able to replace the minimum sodium requirement. The present work is an attempt to investigate the ecologically important problem of the growth response of marine phytoplankton to salinity by means of growth studies of *Cyclotella nana* HUSTEDT clone 13–1.

* Contribution No. 2387 from the Woods Hole Oceanographic Institution. This study was supported in part by National Science Grant GB 7682.

MATERIALS AND METHODS

Bacteria free cultures of *C. nana* (clone 13–1) were used. Data of isolation etc. have been given previously (GUILLARD & RYTHER 1962). The culture was maintained in medium f/2, which is the standard enrichment at half strength (GUILLARD & RYTHER 1962). In most experiments, f/2 using Sargasso Sea water was the basic medium. When the sea water was diluted, the dilution was made with distilled water containing the f/2 enrichment. In some experiments, a modified synthetic LEWINS' medium (LEWIN & LEWIN 1967) consisting of vitamins and microminerals as in medium f/2 was used. Medium containing sucrose or glucose was sterilized by membrane filtration.

The alga was cultured in 15 ml of medium in 50 ml conical flasks under a light intensity of 4–5000 lux and with a day/night cycle of 14/10 hours. The temperature was kept at about 20^{0} C. The growth was followed by counting cells in a haemocytometer. Standard inoculum was about 10^4 cells/ml. The results are given in divisions per day (d) calculated from the formula given in GUILLARD & RYTHER (1962).

RESULTS AND DISCUSSION

Figure 1 shows the effect of dilution of sea water on the growth rate. Total solids in sea water are given as g/l. Water with a salinity of 35 $^{0}/_{00}$ has a total salt content of about 36.2 g/l, with osmotic pressure of ca. 23.1 atmospheres (970 milliosmoles). Solutions of sucrose, 0.9 M, or NaCl, 0.53 M, have about this osmotic pressure (BARNES 1954, HARVEY 1966). The curve to the right in Figure 1 represents dilution of sea water with correspondingly different osmotic pressures, while the curves to the

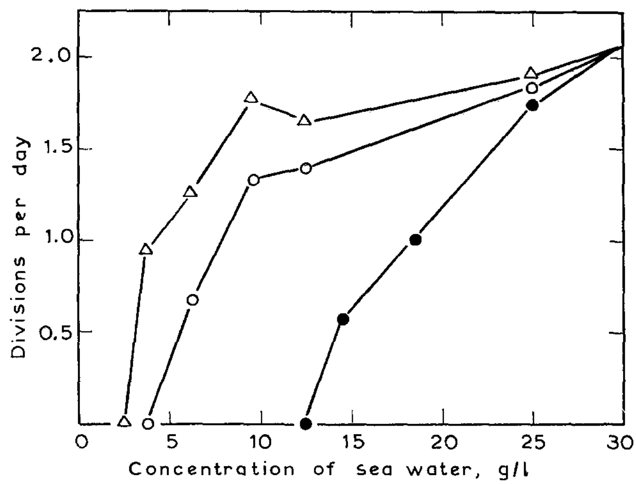

Fig. 1: Influence of dilution of sea water on the growth rate. ● = Sea water dilution, variable osmotic pressure. △ = NaCl added to 30 g/l equivalent osmotic pressure. ○ = Sucrose added to 30 g/l equivalent osmotic pressure

left represent experiments in which the osmotic pressure was kept constant and equivalent to sea water with a total salt content of 30 g/l by addition of sucrose or sodium chloride respectively. The figure shows that clone 13–1 grows in dilutions of sea water down to a total solid content of about 15 g/l in accordance with previous results (Guillard & Ryther 1962). The two other curves show that, when the osmotic pressure of the medium was adjusted with sucrose of NaCl, it was possible to dilute sea water further down to 4–6 g/l and still retain some growth. Note: the growth rate was a little higher when NaCl was used as the osmoticum, compared with sucrose.

Figure 2 shows growth rate as a function of sea water concentration at osmotic pressure equivalent to 20 g/l total solids obtained in three different ways: by adding four salts (Na-, K-, Ca-, Mg-chloride), one salt (NaCl) and sucrose. Dilution of sea water from 20 g/l to 2 g/l and adding the four major cations in the same proportion

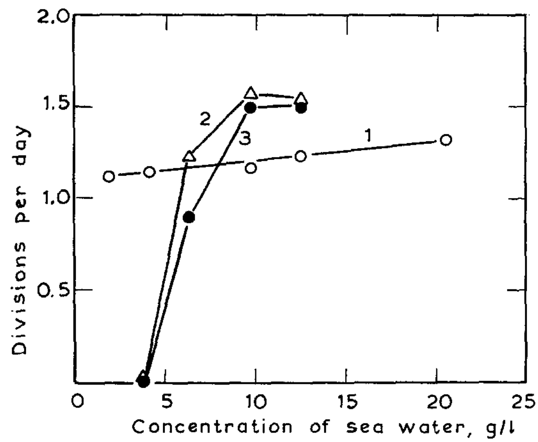

Fig. 2: Growth rate versus concentration of sea water at osmotic pressure equivalent to 20 g/l by addition of: (1) 4 salts (Na, K, Ca, Mg), (2) 1 salt (NaCl), (3) sucrose

as in sea water, gave practically the same growth rate as sea water (this indicates that minor elements are not involved). On the other hand, a marked decrease in growth rate was observed by diluting further than 9 g/l when the osmotic pressure was adjusted with NaCl or sucrose. Figure 2 indicates that addition of sodium is insufficient for growth at low dilutions of sea water. The difference between the sucrose and NaCl curve is not very pronounced.

In some experiments, the effect of added potassium on the growth rate in a modified synthetic Lewins' medium with a low sodium concentration (230 mM NaCl) was investigated. Three levels of potassium were used: 30, 70 and 210 mM KCl. Somewhat surprisingly the growth was good at 30 mM of KCl but by 70 mM (and 210 mM) of KCl there were toxic effects and growth ceased. The results confirm that the reason this alga stops growing upon continued dilution of sea water is not because of a specific sodium requirement. Potassium and sodium can, however, be interchanged to a very limited extent only.

Graphs of growth rate as a function of osmotic pressure are shown in Figure 3. The dotted curve is the same as the curve to the left in Figure 1 (dilution of sea water). The other curves show the growth response of the alga in media made by

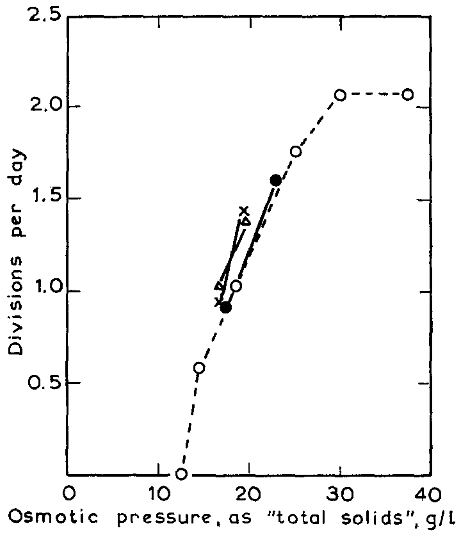

Fig. 3: Growth as a function of osmotic pressure, sea water concentration 14.4 g/l. Sea water (o); NaCl (●), sucrose (△), mannitol (×) added to 14.4 g/l of sea water

diluting small batches of sea water to 14.4 g/l and increasing the osmotic pressure by addition of two different amounts of NaCl, by sucrose, or by mannitol, respectively. The four curves all indicate the same tendency; a very marked increase in growth rate with increasing osmotic pressure. The range of osmotic pressure investigated is

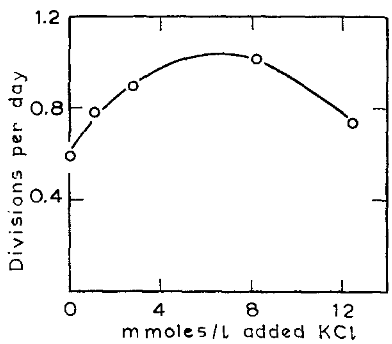

Fig. 4: Effect of added K. Basal medium has sea water diluted to 14.4 g/l of "total solids" (ca. 4 mM K)

equivalent to the range of salinity where dilution has previously been shown to give a marked decrease in growth rate (Guillard & Ryther 1962). It seems, therefore, safe to conclude that when growing *C. nana*, clone 13–1, in sea water, the first deficiency to be detected by dilution is caused by osmotic stress.

That a small change in ionic composition can markedly influence the rate of growth in the region of osmotic stress has been demonstrated by addition of potassium chloride to a sea water medium diluted to 14.4 g/l total solids. The results, given in Figure 4, show that an increase in potassium salt concentration from 4 mM to 12 mM increased the growth rate from 0.6 to 1.0 divisions per day. The increase of osmotic pressure by this addition is negligible.

Figure 5 shows the growth rate over a wide range of osmotic pressure, which was adjusted by adding NaCl to samples of different dilutions of sea water. The curves have a maximum at about an osmotic pressure equivalent to that of sea water.

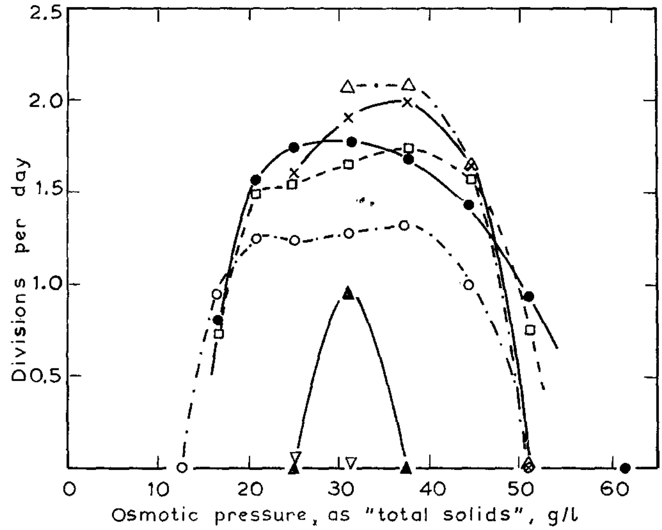

Fig. 5: Influence of osmotic pressure (adjusted with NaCl) on the growth rate at different sea water dilutions: △ = 31.1; × = 24.9; □ = 12.41; ● = 9.35; ○ = 6.26; ▲ 3.74; ▽ = 2.5 (g/l)

The curve for the dilution 9.35 g/l "total solids" is somewhat exceptional, having a maximum in growth at a little lower osmotic value. Below an osmotic pressure corresponding to 20 g/l the growth rate decreased very markedly for dilutions corresponding to 6.26–12.41 g/l. The most likely reason is an osmotic deficiency of the medium, as discussed above. The decrease in growth rate at high osmotic pressure (ca. 50 g/l "total solids") is also very pronounced. It is unlikely that this is a toxic effect of sodium. Experiments with added glucose or sucrose to sea water, and with sea water concentrates, showed that, in any case, the alga could not survive above an osmotic pressure of about 50 g/l "total solids". The decrease in growth rate at high concentrations, therefore, can be attributed mostly to excess osmotic pressure.

Increasing dilution of the sea water at constant osmotic pressure led, with few exceptions, to a decrease in growth rate. As previously shown for one particular osmotic pressure in Figure 2 one tenth sea water (3.74 g/l) was the highest dilution giving growth. However, many cells were long and thin and often connected with each other.

Thus far, the sodium requirement of clone 13–1 has not been determined. However, the greatest dilution of sea water in which growth was possible (using sucrose as osmoticum) was $^{1}/_{6}$ sea water or about 83 mM sodium (Fig. 1). If one again considers Figures 1 and 2, it seems clear that the alga prefers NaCl to sucrose, especially at higher concentrations. This may reflect a sodium deficiency at dilutions below $^{1}/_{4}$ sea water. Droop (1958) found a lower sodium limit for *S. costatum* at 3000 mg Na/l or about 130 mM NaCl. McLachlan (1964) found reduced growth of *Skeletonema* at and below 200 mM sodium, and reduced growth of *Amphidinium* and *Olisthodiscus* below 200 mM. However, their experiments were not designed to separate the effects of lowered osmotic pressure from the other effects of reduced sodium concentration.

Figure 5 shows that the $^{1}/_{10}$ sea water dilution led to poor growth, this cannot be due to a sodium deficiency because NaCl was used as osmoticum. Growth experiments with synthetic media indicated that the alga could survive at a magnesium concentration of 0.85 mM, a calcium concentration of 1 mM and a potassium concentration of 1 mM (unpublished results). $^{1}/_{10}$ sea water is equivalent to concentrations of potassium and calcium salts of about 1 mM. It is, therefore, likely that the poor growth in $^{1}/_{10}$ sea water (Fig. 5) is caused by too low concentrations of both potassium and calcium – or, at least, one of them. Relatively high calcium requirements have been reported for other algae (Droop 1958, McLachlan 1964, Vollenweider 1950). The potassium requirement of planktonic algae, however, seems to have received relatively little attention. McLachlan (1964) found that reducing the potassium content of artificial sea water to $^{1}/_{10}$ that of natural sea water reduced growth of *Skeletonema, Amphidinium* and *Olisthodiscus*. In the case of clone 13–1, potassium can also have an effect on growth at concentrations above the estimated minimum level (ca. 1 mM). This is shown in Figure 4. In the region of osmotic stress, an increase in potassium concentration from 4 mM to 12 mM increased the growth rate significantly. A marine fungus also has a relatively high potassium requirement (Vischniac 1955).

Guillard (1960) showed that a mutant strain of *Chlamydomonas moewusii* survived and grew only if the osmotic pressure of the medium exeeded 1.5 atm (salinity about 2.3 $^{0}/_{00}$). Compared with this alga and *Dunaliella tertiolecta* with a lower tolerance limit of osmotic pressure at about a salinity of 3.5 $^{0}/_{00}$, *C. nana* clone 13–1 shows an osmotic response at a much higher salinity – at about half strength sea water. It would not be surprising if other algae, which grow poorly in sea water diluted to 50 $^{0}/_{0}$, will show a similar osmotic response.

SUMMARY

1. *Cyclotella nana* Hustedt (clone 13–1) was found to grow in sea water at salinities ranging from about 15 to 50 parts per thousand.
2. Investigation of the osmotic requirement in the region of poor growth at the lower salinities, revealed that this alga gives an osmotic response at a relatively high osmotic pressure.

3. The osmotic and ionic requirements can be clearly distinguished and it was shown that this oceanic diatom can survive at sea water dilutions as low as 1/6 of full strength of water from the Sargasso Sea when the osmotic pressure is adjusted with sucrose.
4. The requirements of the four main cations (Na, K, Ca, Mg) are discussed.

Acknowledgement. One of us (S. MYKLESTAD) wishes to thank the Norwegian Council of Scientific and Industrial Research for the award of a research fellowship which made possible his contribution to the work.

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