

The Development of a Natural Plankton Population in an Outdoor Tank with Nutrient-Poor Sea Water. II. Changes in Dissolved Carbohydrates and Amino Acids*

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ABSTRACT: Interrelations between plankton communities and dissolved carbohydrates and amino acids were investigated under near-natural conditions in sea water enclosed in plastic tanks. In summer 1972 the development of a natural plankton population was followed in a 3-m³ plastic tank for 28 d. In the course of this experiment, concentrations of dissolved neutral carbohydrates and free amino acids were determined. Results are in the range of published data for the open sea with respect to concentrations (0.2–2.5 $\mu\text{moles dm}^{-3}$ total sugar; 0.2–3.1 $\mu\text{moles dm}^{-3}$ total amino acids) and qualitative composition. A plankton succession was observed during the experiment; this was accompanied by distinct alterations in the concentrations of dissolved amino acids and carbohydrates. Glucose and lysine occurred in highest concentrations. Maximum rate of increase was 29 nmoles $\text{dm}^{-3} \text{h}^{-1}$ for glucose, and 25 nmoles $\text{dm}^{-3} \text{h}^{-1}$ for lysine. The rates of decrease are in the same range as bacterial uptake rates determined by various authors employing tracer methods. Numerous positive, highly significant correlations suggest heteropolysaccharides as one source of individual carbohydrates. Relations between certain species within the plankton succession and occurrence of dissolved organic substances were observed. Significant positive correlations existed between glucose and diatoms as well as between glucose, galactose, mannose, arabinose and ribose and phytoplankton biomass. There were also several significant positive correlations of amino acids, especially of valine, leucine and isoleucine with other biological parameters.

INTRODUCTION

The largest proportion of dissolved organic substances in sea water is presumed to be chemically resistant and of high molecular weight. Only a small fraction consists of chemically unstable substances, released by living organisms and subject to rapid biological and chemical transformation (Fogg, 1975; Bada and Lee, 1977). The latter group, which includes most of the amino acids and carbohydrates, may play an essential role in marine ecosystems in spite of low concentrations. These substances have a stabilizing effect, particularly in regeneration phases by extending the supply of nutrients (Wangersky, 1977). The processes

of production and utilization of dissolved organic matter are significant in a broad range of ecological interrelationships. Only a small percentage of dissolved organic matter (DOM) in the sea has been identified (Wangersky, 1972, 1978; Williams, 1975); its origin from plankton excretion or release is still a matter of discussion.

Phytoplankton is probably the most important source of DOM (Anderson and Zeutschel, 1970; Thomas, 1971; Haug and Myklestad, 1976; Duce and Duursma, 1977; Fogg, 1977; Myklestad, 1977; Sharp, 1977; Whittle, 1977). The release is dependent on the physiological state of the plankton cell. Carbohydrates are one of the main components of DOM released by phytoplankton, along with amino acids and fatty acids (Degens, 1970; Handa, 1970). It has been observed that, particularly when nitrate has become exhausted,

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the proportion of carbohydrates (and thus the C/N ratio) increases (McAllister et al., 1961; Antia et al., 1963; Myklestad, 1974; Brockmann et al., 1977b) mostly in form of fixed reserve carbohydrates (McAllister et al., 1961; Antia et al., 1963; Myklestad, 1974; Brockmann et al., 1977b). In the stationary phase, an increased amount of carbohydrates is often released (Myklestad, 1977; Brockmann et al., 1974a, unpublished); this varies in composition with the species concerned (Myklestad, 1974, 1977). The composition of extracellular monosaccharides is also species specific (Allan et al., 1972; Haug and Myklestad, 1976). Amino acids dissolved in sea water are released by phytoplankton (Hellebust, 1965; Gocke, 1970; see also Hellebust, 1974; Whittle, 1977), but very little is known about excretion or loss under natural conditions.

An intracellular amino acid pool has been observed in connection with uptake (North and Stephens, 1969; Liu and Hellebust, 1974; Smith and Wiebe, 1976). Thus, a direct release process would appear conceivable. Zooplankters also have a pool of free amino acids at their disposal (Cowey and Corner, 1963; Jeffries, 1969). Release of amino acids from zooplankters is therefore also a possibility to be considered (Cowey and Corner, 1963; Johannes and Webb, 1965, 1970; Webb and Johannes, 1967, 1969; Butler et al., 1970; see also Corner and Davies, 1971; Conover, 1978). During grazing, amino acids and proteins probably also go into solution along with carbohydrates (Corner and Davies, 1971; Wheeler et al., 1974, 1977; Antia et al., 1975; Williams, 1975; Sepers, 1977).

The fact that so few investigations have been carried out on the relationship between DOM and plankton development (Morris and Foster, 1971; Storch and Saunders, 1978) can be explained by the difficulties arising from hydrodynamic influences leading to inhomogeneous plankton and substrate distributions in the field, thus complicating the analysis of biochemical interactions. In addition, laboratory results cannot be directly applied to the open sea. This is particularly the case for DOM release rates and factors influencing these rates (Nalewajko, 1966; Smith et al., 1977).

In water bodies separated from horizontal water exchange by physiologically inert plastic bags (Brockmann et al., 1974b), concentration changes of DOM and plankton development may be observed under near-natural conditions. In 1972, from July 8 until August 4, we investigated a natural plankton population contained in 3 m³ sea water collected 1 nautical mile south of Helgoland (Southern North Sea). The unfiltered population within the untreated, relatively nutrient-poor sea water was kept in a flexible plastic tank of 4 m depth in the outer harbour of Helgoland. Within a few days, there were alternations of anabolic and catabolic phases in the regeneration system. This

led to a succession of phytoplankters with 3 biomass maxima (diatoms, dinoflagellates and again diatoms) (Brockmann et al., 1977a) as well as a succession of zooplankters (ciliates and different small copepods with up to 3 cohorts (Brockmann et al., in preparation). Concentration changes of dissolved neutral carbohydrates and free amino acids were observed which paralleled the development of the natural phytoplankton population. The objectives of our research included assessment of the degree to which the composition and concentration of DOM in the tank corresponded to that in the open sea, as well as of time scales for qualitative and quantitative DOM changes compared with the plankton development in the tank.

METHODS

Experimental procedures and statistical evaluation methods for the tank experiment in 1972 have been described previously (Brockmann et al., 1974b; Brockmann et al., 1977a). Samples of 250 ml were collected at 0.2, 1.0, 1.8 and 2.6 m depth in the tank and were then combined.

Analysis of Dissolved Neutral Carbohydrates

Immediately after collection, 1 dm³ samples were fixed by addition of 3 cm³ of 3.5% (wt/vol) HgCl₂ solution. After 2 h at most they were filtered through 0.45- μ m membrane filters (cellulose nitrate; Sartorius) and stored at 4 °C. Desalination was performed by means of electro dialysis with ion exchange membranes as described by Josefsson (1970), using Nepton membranes (Serva, C 61 AZL 183 and A 111 BZL 183). A constant electrical current of 1 A was maintained by a regulated D. C. power supply (PE 1213, Philips), adjusted to a maximum potential of 250 V. After reaching the crossover point, this instrument operated in constant voltage service. In order to achieve complete desalination, tap water for cooling and degassing was led into the electrode compartments of the desalinators under constant pressure. After 36 h the desalted samples were removed and evaporated to 1 cm³. Then 1 cm³ of 2 N HCL was added and the mixture heated in a screw-capped tube fitted with a Teflon liner (Sovirel, Levallois's-Perret, France) for 2 h at 100 °C. The hydrolysates were neutralized with Dowex 1 (HCO₃⁻) (Niedermeier, 1971) and purified by hexane extraction. The monosaccharides were extracted from the aqueous phase after reducing the volume with 85% ethanol. After vacuum desiccation over P₂O₅ they were converted to trimethylsilyl ethers (Vance and Sweeley, 1967). The derivatives were evaporated to dryness and then

redissolved in *n*-hexane. Finally, a defined amount of trimethylsilyl-mannitol was added as internal standard (Clamp et al., 1967; Vance and Sweeley, 1967; Bhakti et al., 1970). For gas chromatography of the carbohydrate derivatives at different temperature programs see Siebers et al. (1972).

For method intercalibration some samples were additionally analysed with a carbohydrate analyzer (borate buffer) ZA 5100 (Biotronik, Munich).

Analysis of Dissolved Amino Acids

Samples of 1 dm³ were fixed with 10 ml chloroform. Within 2 h they were filtered through 0.45 µm membrane filters (cellulose nitrate, Sartorius) and stored at 4 °C. Before desalination the samples were adjusted to a pH of 2.0 with HCl and purified by threefold extraction with ethyl acetate. After the remaining ester had been removed by rotation evaporation, the aqueous phases were desalinated by ion exchange (Siegel and Degens, 1966; Palmork, 1969; Andrews and Williams, 1971). After adjustment of the pH to 9.5 with NaOH the samples were desalinated on a Chelex 100 column (Cu²⁺-form, 200–400 mesh, Biorad), followed by elution of the sea water and rinsing with aqua bi-dist. Thereafter the amino acids were eluted from the column with 3 N NH₃. Copper ions were removed with Chelex 100 (NH₄⁺-form). The elutes were evaporated and analysed on a Unichrom-amino acid analyzer (Beckman).

RESULTS

Neutral Carbohydrates

The total concentration of dissolved neutral monosaccharides in the tank reached values of about 2.5 µmoles dm⁻³. For comparison, the data for carbohydrates, phytoplankton and zooplankton are combined in Figure 1. All three components are of the same order of magnitude, between 20 and 200 µg C dm⁻³. The carbohydrate carbon temporarily reached values several times higher than those of the phyto- or zooplankton. The concentration of particulate carbon during the entire experiment remained between 0.5 and 1 mg dm⁻³ (Brookmann et al., 1977a). Glucose, galactose, mannose, fucose, rhamnose, xylose, arabinose and ribose were detected, along with some unidentified substances (Fig. 2). Glucose was the major component of the analysed carbohydrates with up to 74 mol %. It always accounted for at least 50 % of the carbohydrate fraction. The concentration of the unidentified components x₂, x₃, x₄, probably hexoses, was related to mannitol as internal standard. The retention times of these compo-

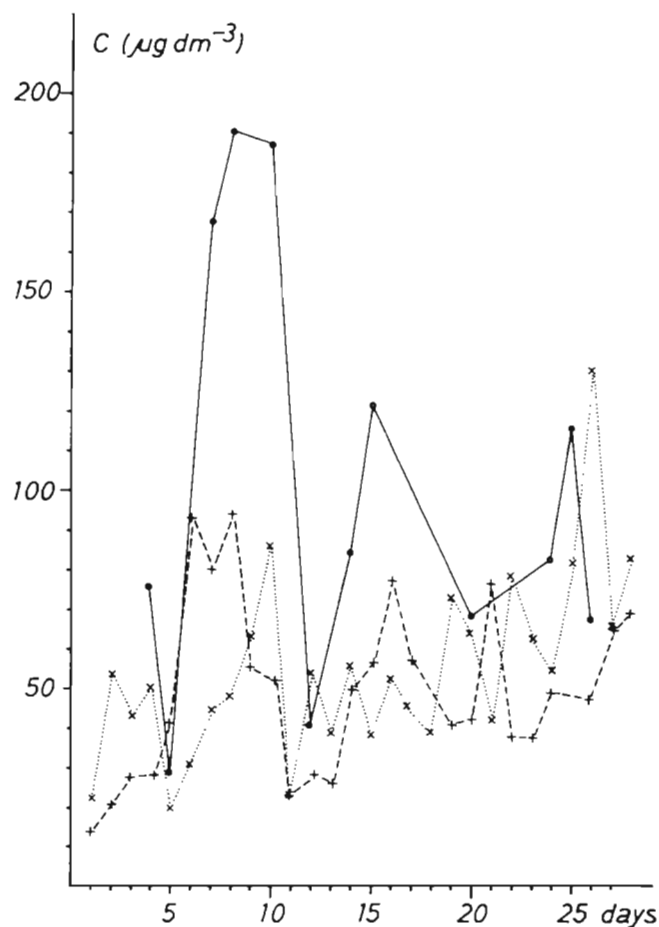


Fig. 1. Concentrations of dissolved carbohydrates (—), phytoplankton (---) and zooplankton (...) in µg C dm⁻³ in a 3 m³ large foil tank from July 8 until August 4, 1972

nents, relative to α -glucose, were 0.58, 0.61 and 0.77, respectively.

Many of the sugars exhibited distinct maxima at various times between the 7th and 10th day. The maximum for glucose extended over a longer period including the maxima of all other sugars. The maximum rates of increase and decrease of glucose were 0.7 µmoles dm⁻³ d⁻¹. Relative maxima also occurred later in the experiment, around the 15th and 25th day.

The individual carbohydrates have numerous significant, positive rank correlations with each other (Fig. 3). Particularly mannose, xylose and rhamnose are related to each other, by highly significant correlations. Fucose is only correlated significantly with mannose and rhamnose.

A few significant correlations with several phytoplankton groups were found. For the entire period of investigation significant (95 % confidence interval) positive correlations existed between glucose and the diatoms as well as between glucose, galactose, mannose, arabinose and ribose and the phytoplankton

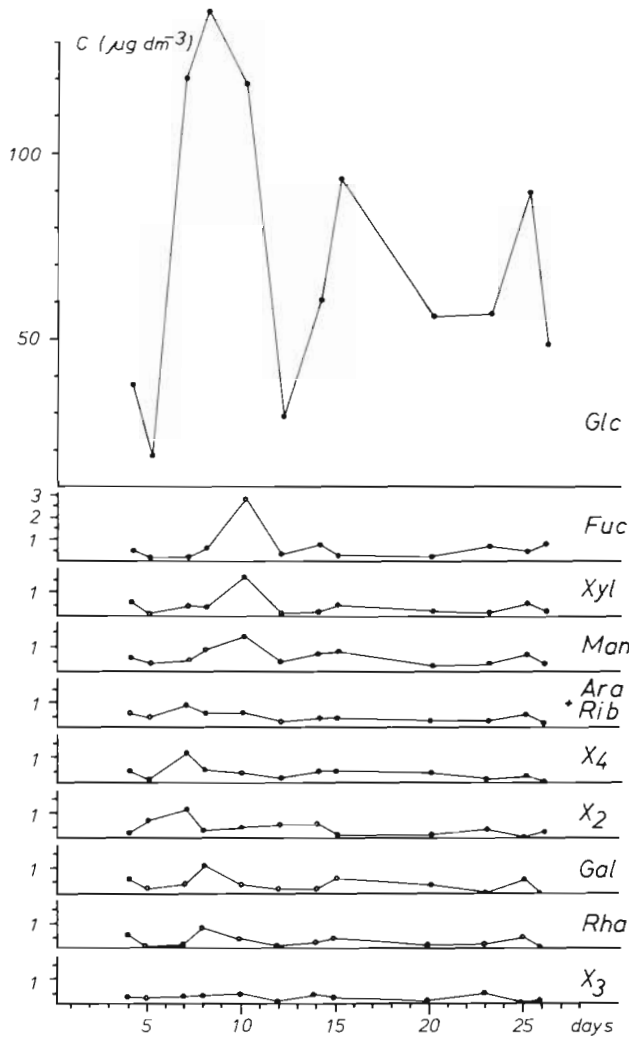


Fig. 2. Concentrations of individual carbohydrates after hydrolysis. ara: arabinose, fuc: fucose, gal: galactose, glc: glucose, man: mannose, rha: rhamnose, rib: ribose, xyl: xylose. x₂, x₃ and x₄: unknown components (see text)

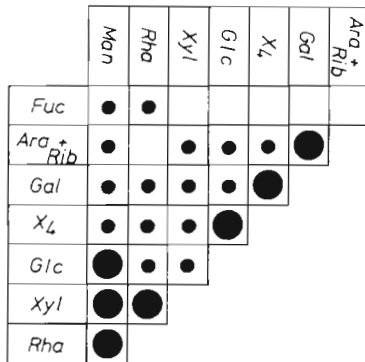


Fig. 3. Rank-correlations of individual carbohydrates. There are only positive significant correlations (filled circles.) Small points: statistical probability of 95,0 to 99,0 %; large points: 99,0 to 99,9 %. (x₄ see legend to Fig. 2 and text)

biomass. The sum of the phyto- and zooplankton biomass was also significantly correlated with glucose, as well as with other carbohydrates.

Free Amino Acids

In the course of the experiment the dissolved free amino acids reached a total concentration of 0.2-3.1 µmoles dm⁻³. This value corresponds to about 200 µg C dm⁻³, comparable to the value for dissolved carbohydrates and thus in the same range as the phyto- and zooplankton carbon.

In terms of nitrogen, the dissolved amino acids, with 5 µg at N dm⁻³, reached the same concentrations as nitrogen and ammonia, and half about the maximum N-concentration of the particulate matter (Fig. 4).

The predominating amino acids were lysine and glycine (Fig. 5), followed by glutamic acid, serine and alanine. In addition to the amino acids shown in Figure 5, phenylalanine, proline and arginine were found in traces.

The concentration development of individual amino acids was subject to relatively large fluctuations in the

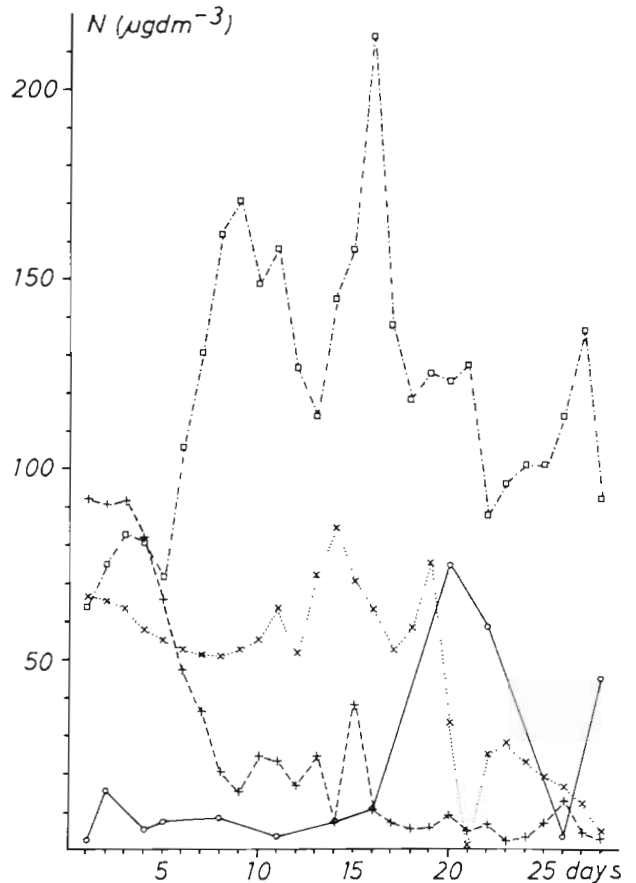


Fig. 4. Dissolved free amino acids (—), ammonia (--), nitrate (...) and particulate nitrogen (-.-) in µg N dm⁻³

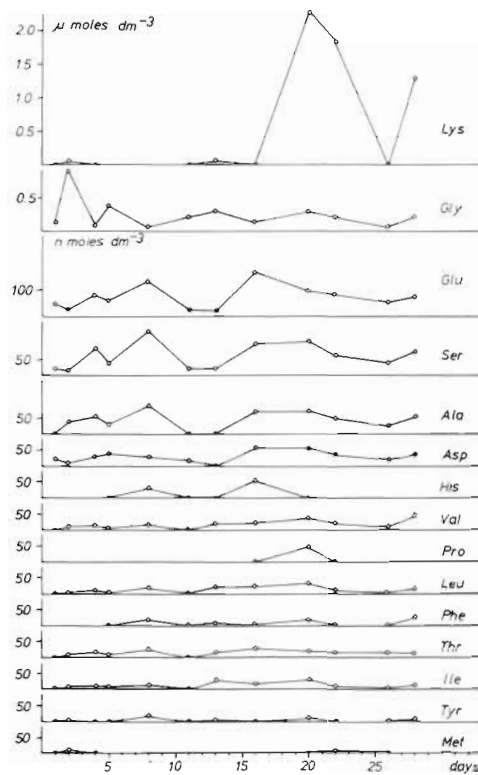


Fig. 5. Individual dissolved free amino acids. ala: alanine, asp: aspartic acid, glu: glutamic acid, gly: glycine, his: histidine, ile: iso-leucine, leu: leucine, lys: lysine, met: methionine, phe: phenylalanine, pro: proline, ser: serine, thr: threonine, tyr: tyrosine, val: valine

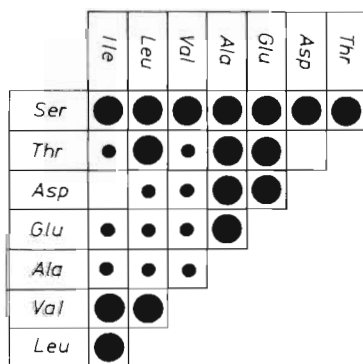


Fig. 6. Rank-correlations of individual free dissolved amino acids. Only positive significant correlations (see legend to Fig. 3)

12 samples analysed. This was especially the case for lysine, with maximum increase and decrease rates of 0.6 and 0.5 $\mu\text{moles dm}^{-3} \text{d}^{-1}$, respectively. In the first half of the experiment glycine accounted for 80 molecular percent of the dissolved amino acids. Lysine made up the same proportion in the second experimental period. Glutamic acid, serine and alanine had relative concentrations reaching 20 molecular percent

while all other components remained under 10 %. Several positive, significant rank correlations were found among the amino acids. Figure 6 shows the compounds with the most frequent significant correlations.

A number of amino acids, particularly valine, leucine and isoleucine, are significantly correlated with plankton groups and individual plankton species such as *Nitzschia longissima*, *Thalassiosira rotula*, *Prorocentrum micans*, *Ceratium spec.* and the adult copepods (Fig. 7). In contrast to the diatoms, the dinoflagellates taken collectively only have positive significant correlations with threonine. The total concentration of dissolved amino acids and several individual amino acids are significantly positively correlated with the chlorophyll concentration. There are several negative correlations with nitrate and, especially, with ammonia.

Phyta-and Zooplankton	●	●	●	●	●	●	●	●
Phytoplankton	●	●	●	●	●	●	●	●
Diatoms	●	●	●	●	●	●	●	●
<i>Nitzschia longissima</i>			●	●	●			
<i>Thalassiosira rotula</i>			●	●	●			
Dinoflagellates	●							
<i>Prorocentrum micans</i>	●	●		●	●			
<i>Ceratium sp.</i>				●	●			
Zooplankton	●		●					
Copepods (adult)			●	●				
Chlorophyll	●	●	●	●	●	●	●	●
NO_3^-						○	○	○
NH_4^+	○			○	○	○	○	○
	TAA	Thr	Ala	Val	Leu	Ile	Ser	Asp
								Glc

Fig. 7. Rank-correlations of amino acids with some ecosystem-factors. Open symbols: negative correlations. For statistical probability see legend to Figure 3. TAA: total amino acids

DISCUSSION

The well-defined plankton succession observed in the tank over a period of 28 d (Brookmann et al., 1977a and in preparation) appears to have been responsible for distinct variations in the concentrations of dissolved carbohydrates and, especially, dissolved amino acids.

Neutral Carbohydrates

The concentration range of dissolved neutral carbohydrates of 0.05–0.50 mg dm^{-3} shows good agreement

with the results of Walsh and Douglas (1966), Kawahara et al. (1967), Okaichi (1967), Handa (1970) and Burney and Sieburth (1977) from various marine areas.

The individual monosaccharides, with the exception of fucose, all have been previously detected in sea water samples by Wangersky (1952), Lewin (1956), Degens et al. (1964), Handa and Yanagi (1969), Handa (1970) and Josefsson (1970). Nevertheless, fucose, among other carbohydrates, has been found frequently in marine diatoms and their release products (Lewin et al., 1958; Parsons et al., 1961; Coombs and Volcani, 1968; Hecky et al., 1973; Haug and Myklesstad, 1976). The ratio hexoses to pentoses in the tank of about 12 corresponds to that found by Hirayama (1974) off the Japanese coast.

Glucose was present in concentrations ($0.2\text{--}1.9 \mu\text{moles dm}^{-3}$) similar to those found by Vaccaro et al. (1968) along a section in the Atlantic Ocean ($0.3\text{--}0.8 \mu\text{moles dm}^{-3}$), as well as to our values from summer of 1973 near Helgoland ($0.2 \mu\text{moles dm}^{-3}$) (Brockmann et al., unpublished). Andrews and Williams (1971), however, detected only $0.002\text{--}0.03 \mu\text{moles dm}^{-3}$ of glucose in the English Channel.

Glucose, always accounting for at least 50 % of dissolved monosaccharides in our tank, has often been observed to be the major carbohydrate component, particularly in the euphotic zone and in the phytoplankton (Parsons et al., 1961; Handa, 1969; Handa and Tominaga, 1969; Handa and Yanagi, 1969; Handa, 1970; Myklesstad et al., 1972; Hecky et al., 1973; Haug and Myklesstad, 1976). Highly positive correlations among monosaccharides implies that they have originated from hydrolysis of heteropolysaccharides. The concentration increase of glucose in the first third of the experimental period is probably related to the occurrence of *Lauderia borealis*. The maximum concentration of dissolved carbohydrates was observed in the stationary phase of *Lauderia borealis*; this is in agreement with the findings of Myklesstad (1977) and our own studies of monocultures of *Thalassiosira rotula* (Brockmann et al., unpublished).

The maximum rate of increase for glucose of $0.7 \mu\text{moles dm}^{-3} \text{d}^{-1}$ or $50 \mu\text{g C dm}^{-3} \text{d}^{-1}$ was accompanied by an increase in particulate carbon ($175 \mu\text{g C dm}^{-3} \text{d}^{-1}$) and diatoms ($40 \mu\text{g C dm}^{-3} \text{d}^{-1}$ between the 5th and 7th day). Thus, at this time the dissolved carbohydrates increased at a rate greater than the development of the phytoplankton biomass. The maximum carbohydrate concentration ($190 \mu\text{g C dm}^{-3}$) was also greater than that of the phytoplankton biomass ($67 \mu\text{g C dm}^{-3}$) (Brockmann et al., 1977a).

Since carbohydrates, and glucose polymers in particular, are stored within an intracellular pool, it is probable that they seeped out of the diatoms during grazing (Whittle, 1977; Wangersky, 1978). The sig-

nificant positive correlations between various plankton groups and individual carbohydrates support this hypothesis. In this context especially glucose has been found in high concentrations in *Chaetoceros* sp. and *Skeletonema costatum*, particularly in the water-soluble fraction (Handa, 1969; Handa and Yanagi, 1969).

The desoxyhexoses fucose and rhamnose have been detected as extracellular carbohydrates along with galactose in *Chaetoceros* cultures (Haug and Myklesstad, 1976). Rhamnose and fucose, along with galactose and mannose have been reported as extracellular products in other diatoms (Allan et al., 1972).

The rapid decrease of extracellular carbohydrates results from heterotrophic utilization (Gocke, 1975, 1977; Williams and Yentsch, 1976). We found a maximum rate of decrease of $30 \text{ nmoles glucose dm}^{-3} \text{h}^{-1}$, which is in the same range as bacterial uptake (Crawford et al., 1974; Gocke, 1975, 1977; Seki et al., 1975).

Amino Acids

We found total concentrations in the same range as most of the authors reviewed by Dawson and Pritchard (1978).

Amino acids spectra (Fig. 5) agree with data published by Bohling (1970, 1972) with respect to concentrations, parallel development, investigation area and time of year.

The findings of Pocklington (1971), Williams (1975), Whittle (1977), and others, that phytoplankton is a significant source of amino acids dissolved in sea water are confirmed by positive, significant correlations (Fig. 7). The monomeric compounds are most probably released by diffusion (Hellebust, 1974). The amino acids asparaginic acid, glutamic acid, threonine, isoleucine, glycine, serine, alanine, valine and leucine are synthesized *de novo* within seconds by unicellular algae in the course of primary production (Beardall et al., 1976). It might be conceivable that some of them are released from intracellular pools in the course of biosynthesis. In this context, significant positive interrelationships between individual amino acids are of particular interest: phenylalanine and tyrosine; threonine, isoleucine, asparaginic acid; and alanine, valine and leucine (Fig. 6).

Zooplankton biomass (Brockmann et al., in preparation) is significantly correlated to total dissolved amino acids indicating another amino acid source from release by zooplankton (cf. Corner and Davies, 1971) or the process of grazing (Wheeler et al., 1974, 1977; Sepers, 1977). Adult copepods appear to be of importance in this respect (Fig. 7).

The decrease in inorganic nitrogen is accompanied by an increase in organic nitrogen compounds (Fig. 4),

as confirmed by significant, negative correlations, particularly in the case of ammonia and individual amino acids (Fig. 7). Diatoms appear to play a key role here, as reflected by the negative correlations with ammonia and nitrate (Brockmann et al., 1977a), as well as positive correlations with amino acids, which may thus have a stabilizing effect by nutrient conservation (Wangersky, 1977).

It is assumed that uptake of dissolved amino acids by heterotrophic bacteria is considerably more important in the open water than uptake by phytoplankton (Hollibaugh, 1976; Hoppe, 1976). The maximum rate of decrease of lysine amounting to 21 nmoles $\text{dm}^{-3} \text{h}^{-1}$ corresponds to bacterial uptake rates (Crawford et al., 1974; Seki et al., 1975; Gocke, 1977).

In summary, plastic bags have been shown as a useful experimental tool for the detection of ecological processes. They represent the necessary link between small-scale laboratory experiments and sampling in the open sea. They have enabled the comprehension of interrelationships between plankton organisms and DOM, by excluding hydrodynamic factors. In this way hitherto unknown rapid concentration changes of DOM could be detected during a plankton succession.

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