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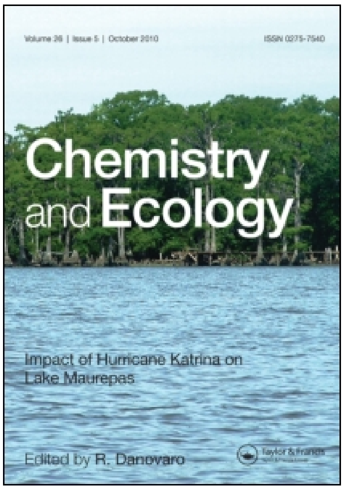

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Amino Acids in Sea Water

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Free amino acids in the waters of the English Channel have been measured over a twelve month period using a newly developed field technique. Samples have been taken over a wide area both from the surface and at depth. The results show that the levels of amino acids found in these studies are greater than those previously generally accepted. These findings are of considerable importance in productivity studies since amino nitrogen as a source of nutrient for phytoplankton has been largely neglected. This paper shows that the levels of amino acids in the upper layers of the sea are not insignificant and should not be disregarded as an alternative nitrogen source for phytoplankton.

INTRODUCTION

The role played by the dissolved nutrient nitrogen compounds in the growth of marine phytoplankton has always been difficult to quantify mainly through lack of information concerning the nature and concentrations of some nutrient constituents. Early studies were almost wholly concerned with nitrate due to the analytical difficulties of estimating other forms of dissolved nitrogen present. In the last twenty years routine reliable methods have been developed for the estimation of ammonia and urea. However when the measured concentrations of these are taken into account the amount of dissolved unidentified forms of nitrogen remaining in the upper layers of the sea is relatively large. (see figures 1 & 2))

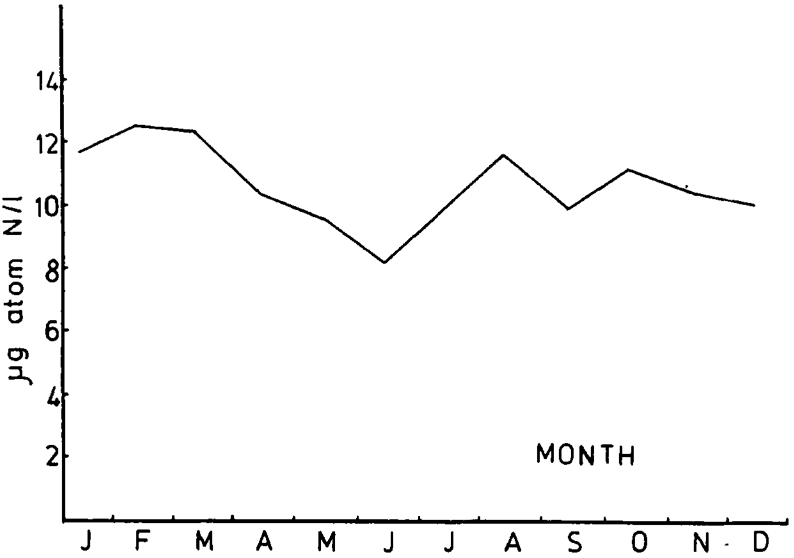

FIGURE 1 Total dissolved nitrogen compounds in the waters of the Western English Channel. Average of all the results obtained over 8 years. (Units μ g.at. N/l.). Butler *et al.* (1979).

It would be expected that amino acids would make some contribution to this unidentified fraction but estimation of these in sea water has always posed considerable analytical problems. Most published methods for measurements of individual amino acids are time consuming leading to losses and contamination. Total amino nitrogen measurements on sea water containing a mixed range of amino acids gave results which were difficult to interpret as the fluorescence techniques used depended upon unequal fluorescent intensity yields for the same amount of individual amino acids. Examples of reported levels of amino acids from various localities and using a variety of techniques are shown in Table I.

There is therefore some confusion as to the levels of amino acids normally present in sea water and the generally accepted ideas were summed up in the latest nitrogen review by McCarthy (1980). "The balance of the dissolved organic nitrogen is both large and virtually

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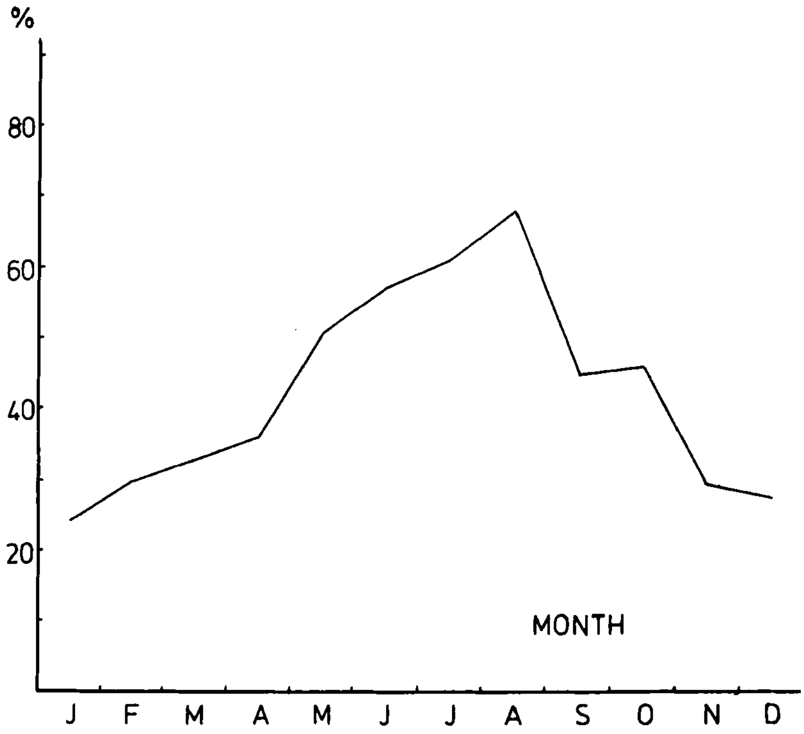

FIGURE 2 Unidentified fraction of the total dissolved nitrogen shown in Figure 1, after allowing for average concentrations of ammonia, nitrate and urea. (Expressed as a percentage of the total nitrogen.)

TABLE I

Examples of reported levels of dissolved free amino acids in European waters

	Site	μ moles/l
Chau & Riley (1966)	Irish sea	0.52–0.79
Starikova & Korzhikova (1969)	Black sea	0.15–0.46
Bohling (1970)	North sea	0.06–0.70
Riley & Segar (1970)	Irish sea	0.05–0.31
Andrews & Williams (1971)	English channel	0.20–0.80
Bohling (1972)	North sea	0.20–6.00
Daumas (1976)	Mediterranean sea	0.05–0.93
Garassi & Degens (1976)	North sea	0.20–1.80
Dawson & Pritchard (1977)	Baltic sea	0.05–0.85
Dawson & Goche (1978)	Baltic sea	0.13–0.39
Garrasi *et al.* (1979)	North sea	0.25–0.56

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unidentified. The amino acids constitute only a small component of the total and Thomas *et al.* (1971) demonstrated that the balance of this nitrogenous material was not suitable for oceanic phytoplankton in the eastern tropical Pacific. Hence it is uncertain as to whether any of the present unidentified dissolved organic nitrogen material should be considered as plant nutrient."

McCarthy goes on to discuss what hc calls "the something for nothing paradox" in the euphotic zone. Here all the indications are that the phytoplankton are doubling every 2–4 days and yet nitrogenous nutrients are apparently unavailable.

The development of High Performance Liquid Chromatography provided an opportunity to overcome the difficulty of amino acid estimation in sea water. In 1979 Lindroth & Mopper introduced an HPLC method for direct estimation of amino acids in sea water which involved no sample pretreatment. Initially this method was used in the studies described in this paper. During the course of the work certain disadvantages became apparent and the method was modified (Evens *et al.*, 1982). This modification has been used for two years for the estimation of amino acids both in fresh and saline waters and the results obtained are presented here.

APPARATUS AND METHODS

Samples were taken with standard reversing bottles, filtered and analysed as soon as possible after collection. No reliable method of sample preservation has been found although a number of techniques were tried. The speed with which significant changes occurred varied from sample to sample and the most reliable method was to take the instrument to sea and inject the sea water into the apparatus within a very short time of collection. The apparatus and method used were as per Evens *et al.* (1982) with the following slight refinement.

The analytical column temperature was maintained at 30°C as this improves the resolution of tryptophan and phenylalanine. At this temperature the life of the stationary phase is prolonged if the pH of the phosphate buffer is reduced from 7.5 to 7.0. This causes no loss of amino acid resolution.

Standardisation

Prior to starting sample analysis the normal practice of calibrating the instrument was carried out using a standard solution containing 20 amino acids. To guard against the consequences of any instrumental malfunction during the subsequent run of analyses an internal standard was added to each sample. The internal standard used was α-amino adipic acid which has not been found in sea water. This acid was chosen following experimental work by M. Righton which will be reported elsewhere.

Analysis at sea

Because of the need for speedy analysis, due to the labile nature of amino acids in water, the method was originally designed for fieldwork. In the last two years the apparatus has proved to be robust, able to operate satisfactorily either aboard ship in difficult weather conditions including a force 8 gale or on a land based vehicle. Given a smooth electrical supply it is quite suitable for general environmental use in the field.

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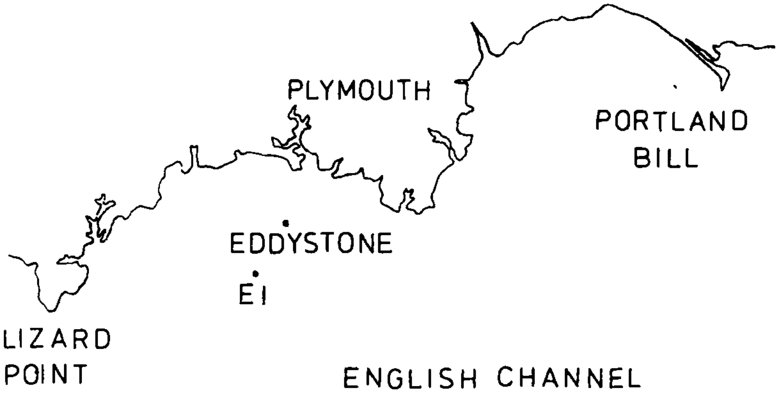

FIGURE 3 Area of sampling.

Sample area

Samples are from the area shown in the chart (Figure 3.). The average depth of the water in the area is about 50 meters. Depth profiles are from International Hydrographic Station E 1. Over most of the area a thermocline is present during the summer months. The salinity range is from 34.2 to 35.4 parts per thousand.

RESULTS

Table II shows the average of all the results taken during a twelve month period. The total amino acid concentration shows a seasonal variation; the lowest values being found in the summer months. The amino acid nitrogen makes up about 30% of the total dissolved nitrogen during the early part of the year but this falls to 13% in August. It accounts for a considerable part of the previously unidentified fraction.

TABLE II

Average monthly levels of total amino acid nitrogen (units μ moles/l)

Month	Ave. NH_2—N	No. Samples	Std. Dev.	Coeff. Var.	%NH_2 of TN	%NH_2 of DON
JAN	3.7	7	1.98	54%	32%	78%
FEB	3.6	1	0	0%	29%	65%
MAR	3.6	2	1.27	36%	29%	61%
APR	2.2	20	0.85	39%	21%	36%
MAY	2.6	25	1.46	57%	27%	35%
JUN	2.1	6	1.20	58%	26%	27%
JUL	1.4	18	0.95	68%	14%	16%
AUG	1.6	15	0.63	40%	13%	15%
SEP	2.7	33	2.57	96%	26%	39%
OCT	2.2	22	1.09	49%	20%	33%
NOV	2.0	2	0.04	2%	19%	37%
DEC	1.9	4	2.11	110%	19%	42%

Table III shows the variation in % amino acid composition of the sea water samples used for Table II. The main amino acids found are serine, glycine, tyrosine, alanine and aspartic acid.

The variability of both the total amino acid concentrations and the percentage composition of individual acids is shown in Tables IV, V, & VI.

Table IV gives the results from a series of surface samples taken at equally spread intervals during a two hour period over a distance of 10 miles and Table V presents the composition of this amino nitrogen.

Table VI shows a depth profile in an unstratified water at international hydrographic station E1.

DISCUSSION

The results from these studies show that amino acids are often present in sea water at higher levels than was previously generally accepted.

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TABLE III

% Composition of total amino nitrogen shown in Table II

	Jan	Feb	Mar	Apr	May	Jun	Jly	Aug	Sep	Oct	Nov	Dec	Average for Year
ASP	7	6	7	5	7	6	7	5	6	5	5	9	6
GLU	5	4	6	2	4	4	6	3	3	3	3	3	4
ASN	2	1	2	1	1	0	0	1	1	0	1	0	1
SER	6	12	15	12	14	11	12	11	15	7	11	12	12
HIS	4	7	4	3	4	7	3	2	2	4	8	3	4
GLY	8	16	12	10	12	13	9	10	14	7	14	13	11
THR	5	4	4	3	5	4	3	2	3	7	5	5	4
ARG	1	0	0	0	0	2	0	0	0	1	1	0	0
ALA	6	7	7	6	8	8	7	7	7	13	6	9	8
TYR	24	10	14	28	13	6	15	16	9	6	4	10	13
ABU	4	2	3	2	3	3	2	2	3	3	3	2	3
GAB	0	0	2	2	0	0	0	0	0	6	0	10	2
VAL	5	4	4	4	4	5	4	4	2	3	5	4	4
MET	0	0	0	0	0	0	0	0	0	0	0	0	0
TRY	2	0	0	0	0	0	0	0	1	0	1	0	0
PHE	4	3	2	3	4	6	2	3	1	13	3	4	4
ILE	4	3	1	4	4	4	1	7	2	7	4	4	4
LEU	6	5	3	4	7	5	4	6	14	8	3	4	6
ORN	6	12	7	9	7	13	19	16	13	6	12	8	11
LYS	2	4	5	1	2	4	4	3	3	1	9	1	3

TABLE IV

Results of surface samples taken along a line from the Eddystone to Plymouth breakwater at equal intervals (units μ moles/1)

SAMPLE	1	2	3	4	5	6	7	8
ASP	0.03	0.18	0.07	0.06	0.06	0.07	0.15	0.03
GLU	0.03	0.15	0.08	0.06	0.01	0.03	0.08	0.02
ASN	0.00	0.07	0.06	0.00	0.02	0.02	0.04	0.04
SER	0.08	0.34	0.14	0.11	0.11	0.11	0.31	0.08
HIS	0.00	0.08	0.00	0.00	0.03	0.06	0.08	0.01
GLY	0.09	0.16	0.17	0.13	0.09	0.03	0.29	0.07
THR	0.00	0.14	0.00	0.00	0.01	0.01	0.12	0.02
ARG	0.00	0.02	0.00	0.00	0.02	0.03	0.02	0.00
ALA	0.06	0.24	0.10	0.09	0.07	0.09	0.22	0.05
TYR	0.17	0.22	0.20	0.26	0.23	0.18	0.21	0.19
ABU	0.02	0.06	0.03	0.04	0.03	0.02	0.06	0.02
GAB	0.00	0.04	0.00	0.00	0.00	0.00	0.02	0.00
VAL	0.00	0.16	0.08	0.07	0.06	0.04	0.14	0.04
MET	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
TRY	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
PHE	0.00	0.18	0.00	0.00	0.03	0.04	0.06	0.01
ILE	0.00	0.10	0.00	0.00	0.10	0.01	0.10	0.04
LEU	0.00	0.29	0.17	0.18	0.12	0.05	0.21	0.16
ORN	0.15	0.31	0.19	0.15	0.18	0.12	0.28	0.14
LYS	0.00	0.16	0.14	0.00	0.13	0.05	0.15	0.00
TOTAL	0.63	2.92	1.43	1.15	1.30	0.96	2.54	0.93

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TABLE V

% Composition of the total amino nitrogen shown in Table IV

SAMPLE	1	2	3	4	5	6	7	8
ASP	5	6	5	5	5	7	6	3
GLU	5	5	6	5	1	3	3	2
ASN	0	2	4	0	2	2	2	4
SER	13	12	10	10	8	11	12	7
HIS	0	3	0	0	2	6	3	1
GLY	14	5	12	11	7	3	11	8
THR	0	5	0	0	1	1	5	2
ARG	0	1	0	0	2	3	1	0
ALA	10	8	7	8	5	9	9	5
TYR	27	8	14	23	18	19	8	20
ABU	3	2	2	3	2	2	2	2
GAB	0	1	0	0	0	0	1	0
VAL	0	5	6	6	5	4	6	4
MET	0	0	0	0	0	0	0	1
TRY	0	1	0	0	0	0	0	0
PHE	0	6	0	0	2	4	2	1
ILE	0	3	0	0	8	1	4	4
LEU	0	10	12	16	9	5	8	17
ORN	24	11	13	13	14	13	11	15
LYS	0	5	10	0	10	5	6	0

TABLE VI

Samples taken at 7 depths from E1 showing amino acid levels in unstratified waters and relationship to other parameters

MAY 1983

DEPTH	TEMP	SAL	TN	NO_3^-	NH_4^+	$-NH_2$	REMAINDER	
								%
0M	10.59	35.07	11.45	0.16	1.71	2.76	6.82	60.0
5M	10.50	35.07	7.85	0.32	0.76	1.94	4.83	61.5
10M	10.44	35.09	7.60	0.27	1.23	4.35	1.75	23.0
20M	10.43	35.09	7.96	0.09	1.32	2.33	4.22	53.0
35M	10.39	35.09	6.49	0.36	1.15	1.35	3.63	55.9
50M	10.15	35.13	12.24	1.70	1.26	2.25	7.03	57.4
70M	10.12	35.14	9.59	1.83	1.91	1.65	4.20	43.8

units: temperature(TEMP) °C; salinity(SAL) 0/00; total nitrogen(TN) μ g.ats.N/l; nitrate(NO_3) μ g.atsN/l; ammonia(NH_4) μ g.ats.N/l; amino acid($-NH_2$) μ moles/l.

(compare Table I & II). Moreover the fraction of the dissolved organic nitrogen identified as amino acids cannot really be considered as a small component.

Tables II & III show a clear variation in both the amount of amino acid nitrogen present in the sea and the amino acid composition of sea water. The total amino acid concentration is lowest in the summer

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months and 30% of the dissolved total nitrogen is accounted for by the amino acids in the early part of the year falling to 13% in August. Amino acids therefore account for a considerable part of the previously unidentified fraction. This is of considerable importance in productivity studies if this amino nitrogen is directly utilised by phytoplankton. There is now considerable evidence that as well as marine bacteria phytoplankton can use amino acids for their nitrogen requirements. This evidence has been summed up by Dring (1982) who writes "Recent studies have shown amino acids to support the growth of most marine algae tested. The marine algae used in these experiments included representatives from all the algal divisions except the Phaeophyta." As early productivity studies did not take into account the presence of significant quantities of amino nitrogen, this may well be one of the factors involved in "the something for nothing paradox" described earlier in this paper. These early studies by analogy with agriculture tended to concentrate on establishing the relationship between nitrate and total productivity. The object of the agricultural system however is to produce the greatest yield of a single crop relative to the amount of nitrate fertilizer added, and to achieve this every possible constraint such as pesticides and weedkillers are applied to the system. Thus the system cannot be regarded as natural but more akin to a unialgal culture grown under laboratory conditions. The marine ecosystem on the other hand is subject to gradual changes throughout the year so that a succession of phytoplankton species flourish as these changing conditions produce optimum conditions of growth for particular species. With regard to essential nutrients such as nitrogen, the nature of the nitrogen will be one factor in the optimum growth conditions as some phytoplankton species preferentially utilise specific forms such as ammonia, urea and amino acids.

It is interesting to note that the most predominant amino acids found in sea water do not include those which are essential for most vertebrates, insects and some protozoa. (Ile, Leu, Lys, Met, Phe, Thr, Try, Val).

The results given in Tables IV–VI illustrate the patchiness of the total amino acid concentrations and the variability of the composition. This patchiness is typical of all the nutrients in the upper layers of the sea during periods of high biological activity. These results are part of a wider study which includes some experiments designed to measure the flux of amino acids in the sea. The results from this wider study will be reported elsewhere together with some biological observations.

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The main object of the present paper is to show that the levels of amino acids in the upper layers of the sea are not insignificant and cannot be disregarded in productivity studies.

We gratefully acknowledge the assistance of the captain and crews of the Marine Biological Association research vessels and of the NERC research vessel Frederick Russell for the collection of samples. We also extend our gratitude to Mr M. I. Liddicoat for analytical assistance in providing some of the data shown in Table VI.

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