Amino acids
Organic matter
Metabolism
Estuary
Netherlands coast

Acides aminés
Matière organique
Métabolisme
Estuaire
Côte des Pays-Bas

# Seasonal distribution of dissolved and particulate amino acids in the Ems-Dollart estuary

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ABSTRACT

The concentrations of dissolved and particulate amino acids (after hydrolysis) in the Ems-Dollart estuary were measured from March 1979 to August 1980.
The concentration of dissolved amino acids ranged from 0.19-3.61 mg. glycine. $l^{-1}$ (1.0-17.0% of dissolved organic carbon).
The concentration of particulate amino acids ranged from 0.1-14.1 mg. glycine. $l^{-1}$ (1.2-79.5% of particulate organic carbon). Highest values were measured in the outer part of the estuary and were correlated with the phytoplankton bloom.
It is suggested that the amino acids in the Ems-Dollart estuary are relatively more labile than other organic compounds.

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RÉSUMÉ

Distribution saisonnière des acides aminés dissous et particulaires dans l'estuaire de l'Ems-Dollart

Les concentrations en acides aminés dissous et particulaires (après hydrolyse) ont été mesurées dans l'estuaire de l'Ems-Dollart de mars 1979 à août 1980.
Les concentrations en acides aminés dissous varient de 0,19 à 3,61 mg. glycine. $l^{-1}$ (1-17% du carbone organique dissous).
La source principale des acides aminés dissous est liée à la production primaire dans l'estuaire.
Les concentrations en acides aminés particulaires varient de 0,1 à 14,1 mg. glycine. $l^{-1}$ (1,2 à 79,5% du carbone organique particulaire). Les plus fortes valeurs ont été rencontrées à l'extérieur de l'estuaire et sont corrélées avec des poussées de phytoplancton.
Ces résultats suggèrent que les acides aminés sont relativement plus labiles que les autres composés organiques dans l'estuaire de l'Ems-Dollart.

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## INTRODUCTION

In the Ems-Dollart estuary, the rivers Ems and Westerwoldse Aa discharge $36 \times 10^3$ and $24 \times 10^3$ tons organic carbon, respectively, into the estuary (van Es, Laane, 1982). The import of organic matter from the adjacent North Sea cannot yet be quantified properly (Postma, 1967; 1980; van Es, 1977). Primary production in the outer part of the estuary produces about $50 \times 10^3$ tons organic carbon per year (Colijn, 1978).
In earlier papers the concentration and distribution of dissolved organic carbon, lipids and polyphenolic substances have been described: the results for the dissolved constituents are summarized in Table 1.

Contribution No. 57 from the Biological Research Ems-Dollart Estuary.

Table 1
*Variation in the concentration (mgC.l$^{-1}$) of dissolved organic carbon, dissolved lipids, carbohydrates and polyphenolic substances and the proportion (%) of these dissolved compounds in dissolved organic carbon in the Ems-Dollart estuary* (1978-1981).

| | Concentration mgC.l$^{-1}$ | % DOC | References |
|---|---|---|---|
| Dissolved organic carbon (DOC) | 0.4 -15.6 | | Laane, 1980 *a* |
| Lipids | 0.045- 0.089 | 0.8- 1.5 | Laane, 1980 *b* |
| Carbohydrates | 0.04 - 4.08 | 7.4-51.3 | Laane, unpublished results |
| Polyphenolic substances | 0.2 - 5.4 | 16 -61 | Laane, 1981; 1982, Laane and Koole, 1982 |

The only sources of dissolved polyphenolic substances in the Ems-Dollart estuary are the river Ems and Westerwoldse Aa (Laane, 1981). There is no evidence that polyphenolic substances could be formed *in situ* (Laane, 1982).

The sources of carbohydrates and lipids in the Ems-Dollart estuary are the rivers and primary production (Laane, 1980 *b*; unpublished results). From decomposition experiments, van Es and Laane (1982) concluded that the potential utility of the organic matter from the rivers for heterotrophic organisms differs considerably, compared with the utility of the primary produced organic matter. The allochthonous organic matter has lost most of its easily utilizable organic matter, probably as a result of decomposition during its transport to the estuary. In consequence, the allochthonous organic matter has a low food value in the estuary, compared with the local primary production.

This difference will also be reflected in the chemical composition of the organic matter, especially in the concentration of amino acids, because amino acids are more readily utilized by heterotrophic organisms than are carbohydrates, lipids and polyphenolic substances (Maita, Yanada, 1978; van Es, Laane, 1982; Ittekkot, 1982).

In this paper the concentration and distribution of the amino acids in the Ems-Dollart estuary are described.

## MATERIALS AND METHODS

The sampling stations (at least 2 in each part of the estuary) were in the main tidal channel (Fig. 1).

On the basis of salinity (S), primary production (Colijn, 1978) and turbidity data, the estuary was divided into four parts: 1) the Dollart (S = 0-15‰); 2) the river Ems (S = 0-15‰); 3) the middle part of the estuary (S = 15-23‰); and 4) the outer part of the estuary (S = 23-31‰).

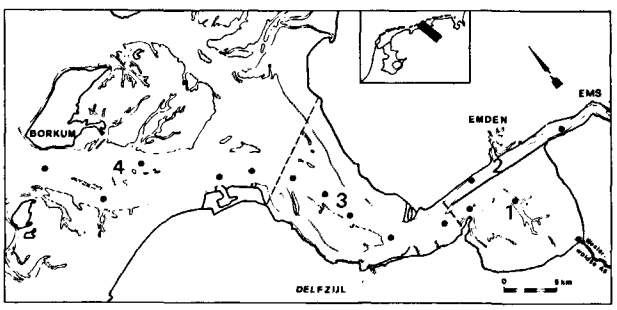


Figure 1
*Map of the Ems-Dollart estuary with sampling stations* (●).

Using a pumping system, water samples were collected from a depth of 1.5 m. For analysis of total organic carbon, nitrogen and total amino acids, about 150 ml unfiltered seawater from each sample was frozen to −20°C in serum bottles. Seawater from each sample was suction-filtered through Whatman GF/F filters (0.45 µm) and serum bottles were filled with 150 ml of the filtrate and frozen to −20°C. The filters and serum bottles were pre-combusted for 4.5 hours at 480°C.

For the determination of the concentration of amino acid, 2 ml samples were freeze-dried in a 10 ml glass ampoule (pre-combusted for 4.5 hours at 480°C). After the addition of 0.25 ml double-distilled 6 N HCl, the ampoule was sealed under nitrogen and heated to 120°C for 24 hours.

The hydrolysates were dried in vacuo over KOH pellets. After the addition of 4 ml aqua-bidest, the amino acids were detected with fluorescamine (North, 1975), and expressed in glycine equivalents (precision: 0.01 mgC.l$^{-1}$), which can be converted to organic carbon and nitrogen concentrations (1 mg glycine = 0.32 mgC or 0.187 mgN). Ammonia is excluded in this method (North, 1975).

Analyses for total nitrogen, nitrite and nitrate analyses were done according to Strickland and Parsons (1968). A modified phenol-hypochlorite method was used for determination of ammonia (Helder, de Vries, 1979).

Total and dissolved organic carbon were measured by wet oxidation (Menzel, Vaccaro, 1964). Total concentration of organic nitrogen was calculated as the difference between total nitrogen and inorganic nitrogen.

The particulate fractions of organic carbon and amino acids respectively were calculated by subtracting the appropriate dissolved fractions from the total fractions. These results were compared with those gathered by direct determination of particulate organic carbon and particulate amino acid carbon on the filter. No deviation could be found between these two detection methods within the analytical error.

## RESULTS AND DISCUSSION

### Dissolved amino acids

The concentration of dissolved amino acids varies between 0.19 (February 1980) and 3.61 mg. glycine.l$^{-1}$ (July 1979). Both concentrations were measured in the outer part of the estuary.

The mean concentration in each part of the estuary was calculated using the results from at least two stations in that part. The mean concentration of dissolved amino acids in the river Ems varies between 0.06 and

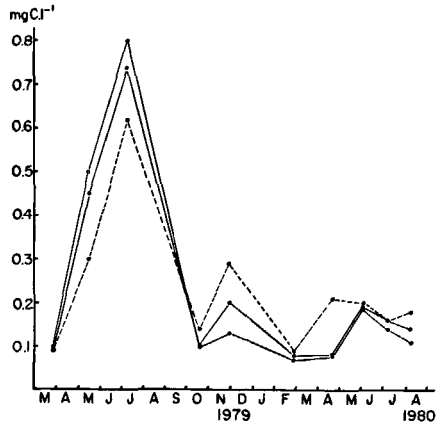


Figure 2

*Seasonal variation in the mean concentration of dissolved amino acids (mgC.l$^{-1}$) in the Dollart (●- -●) and in the middle (●——●) and outer (○——○) parts of the Ems-Dollart estuary.*

0.13 mgC.l$^{-1}$. Figure 2 shows the seasonal variation in the mean concentration of dissolved amino acids for the other three parts of the estuary.

The concentrations of dissolved amino acids in the Ems-Dollart estuary are comparable with those summarized in the literature, ranging from 6.6-1 530 µg.l$^{-1}$ (Dawson, Pritchard, 1978). However, the concentration of 3.61 mg. glycine.l$^{-1}$ found in the outer part of the estuary is rather high.

Figure 3 presents the percentage of the mean concentration of dissolved amino acids (mgC.l$^{-1}$) in the mean concentration of dissolved organic carbon for three parts of the estuary. These range from 0.1 to 17.0%. In the river Ems this percentage varied between 1.1 and 5.7%.

These results agree with Ittekkot's (1982) observations. He found that the percentage of dissolved amino acids in dissolved organic carbon during a plankton bloom on the Northern North Sea, ranged from 1-15%.

During the winter months the highest concentrations were measured in the Dollart. Water discharged from the Westerwoldse Aa into the Dollart in the period October-December, contains enormous quantities of waste from potato flour mills (de Wolf, 1977; van Es, 1977).

In the months October 1978 to January 1979, integrated water samples were taken daily during the sluicing. The monthly means of the amount of amino acids sluiced into the estuary during the potato flour processing are given in Table 2.

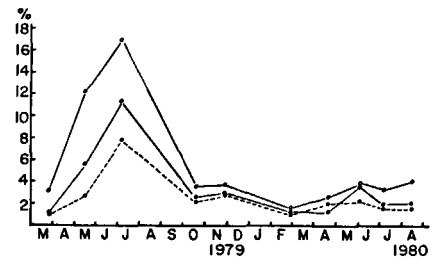


Figure 3

*Proportion (%) of the mean concentration of dissolved amino acids (expressed as glycine carbon) in the mean concentration of dissolved organic carbon for the Dollart (●- -●) and the middle (●——●) and outer (○——○) parts of the Ems-Dollart estuary.*

Table 2

*Amount of total amino acids discharged by the Westerwoldse Aa into the Dollart in the period October 1978 to January 1979 and the concentration of amino acids as a percentage of total organic carbon (% TOC).*

| Date | $10^3$ kg glycine day$^{-1}$ | % TOC |
|---|---|---|
| 23-31 October 1978 | 15.3 | 2.5 |
| 1-30 November 1978 | 11.6 | 2.6 |
| 1-31 December 1978 | 16.9 | 2.4 |
| 1-10 January 1979 | 5.6 | 5.9 |

A possible explanation for the increase in the dissolved amino acids during the summer months may be the primary production in the estuary and the adjacent North Sea.

Primary production produces particulate organic matter, approximately 50% of which consists of amino acids (Parsons *et al.*, 1961). Any increase in primary production during the summer months will also be reflected in the concentration of particulate amino acids.

## Particulate amino acids

The concentration of particulate amino acids ranged from 0.1 mg. glycine.l$^{-1}$ (February 1980) to 14.1 mg. glycine.l$^{-1}$ (July 1979); both measured in the outer part of the estuary.

The mean concentration of particulate amino acids in the river Ems during 1979 and 1980 was rather uniform and ranged between 0.05 and 0.18 mgC.l$^{-1}$.

The mean concentration of particulate amino acids in the other parts of the estuary are given in Figure 4. The concentrations of particulate amino acids observed in the Ems-Dollart estuary were higher than those mentioned in the literature; they ranged from 2.0 to 1 157 µg.l$^{-1}$ (Maita, Yanada, 1978; Siezen, Mague, 1978; Garfield *et al.*, 1979; Hollibaugh *et al.*, 1980).

As a result of the inland transport of suspended matter (Postma, 1967; 1980) there is a relatively high

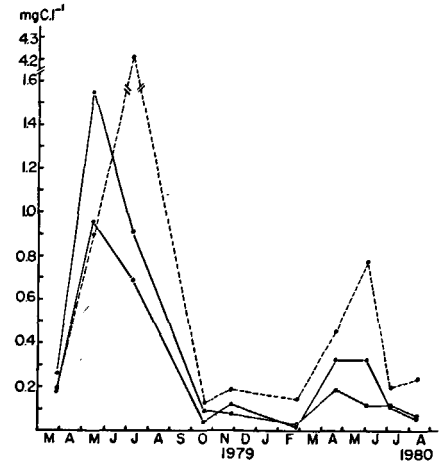


Figure 4

*Seasonal variation in the mean concentration of particulate amino acids (mgC.l$^{-1}$) in the Dollart (●- -●) and the middle (●——●) and outer (○——○) parts of the Ems-Dollart estuary.*

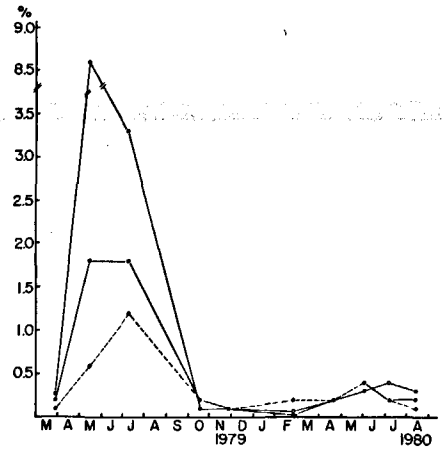


Figure 5
*Seasonal variation in the proportion (%) of mean particulate amino acid in mean concentration of suspended matter in the Dollart (●- - ●) and the middle (●——●) and the outer (○——○) parts of the Ems-Dollart estuary.*

concentration of suspended matter in the Dollart. An increase in particulate amino acid concentration could be caused by primary production and/or an increase in the concentration of suspended matter. Related for the amount of suspended matter, the highest percentages of amino acids in the suspended matter were found in the outer part of the estuary (Fig. 5).

The percentages of mean particulate amino acid carbon in mean total particulate organic carbon (Laane, unpublished results) are given in Figure 6 for three parts of the estuary.

In the river Ems this fraction varied between 1.1 and 12.2% during 1979 and 1980. These results are comparable with the observations of Holm-Hansen (1968), Maita and Yanada (1978) and Siezen and Mague (1978). They found that during one year this fraction ranged from 9.2 to 63.5%. The highest values they measured were in coastal waters, where phytoplankton growth was abundant.

In 1979 the highest concentration of chlorophyll-*a* ($38.7\,\mu g.l^{-1}$) was measured during the cruise in May in the outer part of the estuary (Colijn, unpublished results). This maximum corresponds with the maximum in the concentration of particulate amino acids (Fig. 4). The proportions of particulate amino acids in the suspended matter and in particulate organic carbon were also highest in that month (Fig. 5 and 6).

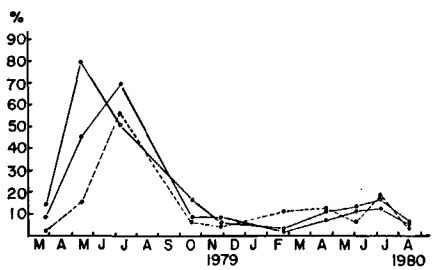


Figure 6
*Seasonal variation in the proportion (%) of mean particulate amino acid carbon in mean total particulate organic carbon in the Dollart (●- - ●) and the middle (●——●) and outer (○——○) parts of the Ems-Dollart estuary.*

From these results it is inferred that the main source of particulate amino acids is the phytoplankton species in the estuary. The relatively high concentrations of particulate amino acids (compared with the concentrations quoted in the literature) in the outer part of the estuary during the summer months probably result from the high primary production in that part of the estuary, up to $400\,gC.m^{-2}.yr^{-1}$ (Colijn, 1978).

Maita and Yanada (1978) concluded that particulate amino acids in coastal waters decompose rapidly by comparison with other dissolved compounds.

They calculated a turnover time of 7-11 days for particulate amino acids in the upper layer during the summer. Compared with the concentrations of particulate amino acids the concentration of dissolved amino acids is very low. Total organic matter normally comprises 60-80% of dissolved organic matter and 20-40% of particulate organic matter. This proportion is not reflected in the distribution of the amino acids over the dissolved and particulate phases (*see* Fig. 2 and 4). From these results it is presumed that the turnover rate of the dissolved amino acids is high compared with other dissolved constituents in the Ems-Dollart estuary.

In decomposition experiments, van Es and Laane (1982) concluded that during the summer 70-90% of the concentration of particulate amino acids in the outer part of the estuary was degradable within 30 days of incubation.

This fraction diminished up the estuary. From these experiments they also concluded that during the summer months the dissolved amino acids decomposed more quickly than dissolved organic matter as a whole.

For heterotrophic organisms amino acids are as a food source besides a carbon pool also a nitrogen pool.

Tuschall and Brezonik (1980) isolated dissolved amino acids and found that the amino acid nitrogen accounted for 14-34% of the total dissolved organic nitrogen. In the literature they cited this fraction varied between 16-60%. Siezen and Mague (1978) calculated that the nitrogen content of the particulate amino acid pool was sufficient to account for all the particulate organic nitrogen.

During 4 surveys in 1978 the contribution of total (dissolved and particulate) amino acid nitrogen to total organic nitrogen was calculated for the different parts of the estuary (Table 3).

The relatively low proportion of amino acid nitrogen in organic nitrogen in the Dollart and Ems, for all dates, indicates that the freshwater sources discharge organic nitrogen compounds into the estuary that are not amino acids. Most of this is probably urea (Berman, 1974; Mitamura, Saijo, 1980).

Table 3
*Percentage distribution of mean total amino acid nitrogen in mean total organic nitrogen in the Ems-Dollart estuary, for different dates in* 1978.

| Area | 6 June | 17 July | 5 Sept. | 23 Oct. |
|---|---|---|---|---|
| Dollart | 8.6 | – | 6.1 | 10.7 |
| Ems | 5.0 | 1.4 | 2.6 | 2.3 |
| Middle part | 85.3 | 65.0 | 43.0 | 36.5 |
| Outer part | 78.2 | 79.9 | 27.0 | 28.0 |

During the summer months, the calculated concentration of amino acid nitrogen is sufficient, in many cases, to account for nearly all of the organic nitrogen present in the middle and outer parts of the estuary.

These results are in agreement with those of Tuschall and Brezonik (1980) and Siezen and Mague (1978). The high fraction of amino acid nitrogen in total organic nitrogen in the outer part of the estuary, suggests that the terrestrial organic nitrogen compounds, not being amino acids, are decomposed during their transport through the estuary. This possibility is confirmed by data on the behaviour of inorganic nitrogen compounds in the Ems-Dollart estuary (Helder *et al.*, 1982). They found that nitrate, nitrite deviate positively from conservative behaviour.

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REFERENCES

**Berman T.**, 1974. Urea in the waters of Lake Kinneret (Sea of Galilee), *Limnol. Oceanogr.*, **19**, 977-980.

**Colijn F.**, 1978. *Primary production measurements in the Ems-Dollart estuary during* 1975 *and* 1976, BOEDE Publ. en Versl., 1978-1, 1-15.

**Dawson R., Pritchard R. G.**, 1978. The determination of amino acids in seawater using a fluorometric analyser, *Mar. Chem.*, **6**, 27-40.

**Es F. B. van**, 1977. A preliminary carbon budget for a part of the Ems estuary: the Dollart, *Helgol. Wiss. Meeresunters.*, **30**, 283-294.

**Es F. B. van, Laane R. W. P. M.**, 1982. The potential utility of organic matter in the Ems-Dollart estuary, *Neth. J. Sea Res.* (to be published).

**Garfield P. C., T. T. Packard, Codispoti L. A.**, 1979. Particulate protein in the Peru upwelling system, *Deep-Sea Res.*, **26**, 623-639.

**Helder W., de Vries R. T. P.**, 1979. An automatic phenol-hypochlorite method for the determination of ammonia in sea- and brackish waters, *Neth. J. Sea Res.*, **13**, 154-160.

**Helder W., de Vries R. T. P., Rutgers van der Loeff M. M.**, 1982. Observations on behaviour of nitrogen nutrients and silica in the Ems-Dollart estuary, *Can. J. Fish. Aquat. Sci.* (to be published).

**Hollibaugh J. T., Carruthers A. B., Fuhrman J. A., Azam F.**, 1980. Cycling of organic nitrogen in marine plankton communities studied in enclosed watercolumns, *Mar. Biol.*, **59**, 15-21.

**Holm-Hansen O.**, 1968. The distribution and chemical composition of particulate material in marine and fresh waters, *Mem. Ist. Ital. Idrobiol.*, **29** (suppl.), 37-51.

**Holmes R. W., Williams P. M., Eppley R. W.**, 1967. Red water in La Jolla Bay, 1964-1966, *Limnol. Oceanogr.*, **12**, 503-512.

**Ittekkot V.**, 1982. Variations of dissolved organic matter during a plankton bloom: qualitative aspects, based on sugar and amino acid analyses, *Mar. Chem.*, **11**, 143-158.

**Laane R. W. P. M.**, 1980*a*. Conservative behaviour of dissolved organic carbon in the Ems-Dollart estuary and the Western Wadden Sea, *Neth. J. Sea Res.*, **14**, 192-199.

**Laane R. W. P. M.**, 1980*b*. Some observations on the lipid concentration in the Ems-Dollart estuary and the Western Wadden Sea, *Estuarine Coastal Mar. Sci.*, **10**, 589-596.

**Laane R. W. P. M.**, 1981. Composition and distribution of dissolved fluorescent substances in the Ems-Dollart estuary, *Neth. J. Sea Res.*, **15**, 88-89.

**Laane R. W. P. M.**, 1982. Influence of pH on the fluorescence of dissolved organic matter in estuaries, *Mar. Chem.*, **11**, 395-401.

**Laane R. W. P. M., Koole L.**, 1982. The relation between fluorescence and dissolved organic carbon in the Ems-Dollart estuary and the Western Wadden Sea, *Neth. J. Sea Res.*, **15**, 217-227.

**Maita Y., Yanada M.**, 1978. Particulate protein in coastal waters with special reference to seasonal variation, *Mar. Biol.*, **44**, 329-336.

**Menzel D. W., Vaccaro R. F.**, 1964. The measurement of dissolved organic and particulate carbon in seawater, *Limnol. Oceanogr.*, **9**, 138-142.

**Mitamura O., Saijo Y.**, 1980. *In situ* measurement of the urea decomposition rate and its turnover rate in the Pacific Ocean, *Mar. Biol.*, **58**, 147-152.

**North B. B.**, 1975. Primary amines in California coastal waters: utilization by phytoplankton, *Limnol. Oceanogr.*, **20**, 20-27.

**Parsons T. R., Stephens K., Strickland J. D. H.**, 1961. On the chemical composition of eleven species of marine phytoplankters, *J. Fish. Res. Board Can.*, **18**, 1001-1016.

**Postma H.**, 1967. Sediment transport and sedimentation in the marine environment, in: *Estuaries*, edited by G. H. Lauff, Publ. No. 83 A.A.A.S., 158-179.

**Postma H.**, 1980. Sediment transport and sedimentation, in: *Chemistry and biogeochemistry of estuaries*, edited by E. Olausson and I. Cato, John Wiley and Sons, 153-186.

**Siezen R. J., Mague T. H.**, 1978. Amino acids in suspended particulate matter from oceanic and coastal waters of the Pacific, *Mar. Chem.*, **6**, 215-231.

**Strickland J. D. H., Parsons T. R.**, 1968. A practical handbook of seawater analysis, *Bull. Fish. Res. Board Can.*, **167**.

**Tuschall J. R. Jr., Brezonik P. L.**, 1980. Characterization of organic nitrogen in natural waters: its molecular size, protein content and interactions with heavy metals, *Limnol. Oceanogr.*, **25**, 495-504.

**Wolf P. de**, 1977. An introduction and a review of a waste-water research project, *Hydrobiol. Bull.*, **11**, 7.