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Experiments to determine the relevance of dissolved amino acids for the nutrition of marine benthic animals

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Abstract

In vitro and *in situ* several macrobenthic species were found to show highest uptake rates of amino acids at low temperatures of 0° – 10° C. The rates decreased at a higher temperature of 15° C which favoured the activity and the development of microorganisms. However, the significance of competitive microorganisms was less striking in the *in situ* experiments than under laboratory conditions. Phenylalanine was most readily taken up although ''aufwuchs'' of *Fucus serratus* and *Gammarus* sp. was found to show primary uptake of aspartic acid. Arthropods were also able to take up dissolved amino acids but in much lower quantities than molluscs and polychaetes. Dissolved amino acids in the water column hardly penetrated the sediment if this was covered by a layer of detritus.

Introduction

The question of uptake of organic substances dissolved in seawater (sugars, amino acids, fatty acids, esters) by marine macrofauna was considered as early as 1909 by PÜTTER. Having been subsequently disregarded for many years, interest in this topic revived in the 1960's (see STEPHENS 1960, FERGUSON 1969), and more recently a number of experiments have been carried out (see BAMFORD and CAMPBELL 1976, SCHLICHTER 1978, SIEBERS 1980, and SOUTHWARD and SOUTHWARD 1970). Uptake rates and capacities could be established for various types of marine fauna under different environmental conditions. In the course of these experiments, however, it was found to be extremely difficult to provide an exact picture of the relevant parameters. The most important questions at issue include effectivity of uptake of available substances, concentrations of amino acids as limiting factors, the role of the sediment for sediment dwellers, and the importance of competition from heterotrophic microorganisms.

Most of these topics have been studied in laboratory experiments using physiological methods (e.g. SCHLICHTER 1978). Studying *Asterias rubens*, SIEBERS (1980) examined the role of heterotrophic microorganisms. With high concentrations of 10 $\mu mol \cdot l^{-1}$ he initiated mass development of microorganisms, reducing the quantities of the dissolved organic substances rapidly to minimum values so that the epidermal absorption of the animals was only poor. The importance of these substances for the animals is indicated by the fact that uptake could still be observed when very low concentrations were available. The laboratory and especially the *in situ* experiments reported in this study, where amino acids were offered in the same qualities and same small quantities as they appear in the biotope, should give an indication of where the next research should be concentrated.

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Material and methods

Several species of macrobenthic animals from the western Baltic (Kiel Bight) were used in the experiments (*Gammarus* sp., *Nereis succinea* and *Mytilus edulis*, *Littorina obtusata* from the phytal 0.5–2 m water depth and *Macoma balthica* from 5–6 m water depth from the sand bottom). All experiments were carried out with amino acids labelled with ^{14}C and ^{3}H.

Experimental runs were performed with

a. amino acid mixtures (labelled with ^{14}C (Amersham Batch 22 Code CFB 104), where two concentrations were offered:
   1. 17 nmol C·l^{-1} = 2 x 10^6 cpm·min^{-1} and
   2. 8 nmol C·l^{-1} = 1 x 10^6 cpm·min^{-1},

b. mixture of 1 μmol·l^{-1} containing 100 nmol ^{14}C labelled aspartic acid and 100 nmol ^{3}H labelled phenylalanine.

The amino acid solutions for the *in situ* experiments were prepared according to results of a previous seawater analysis (DAWSON and PRITCHARD 1978) (Instrument: Locarte Mark 5 NF), which showed the following amino acids to be common: aspartic acid 100 nmol·l^{-1}, glutamic acid 108 nmol·l^{-1}, serine 123 nmol·l^{-1}, glycine 199 nmol·l^{-1}, phenylalanine 100 nmol·l^{-1}, leucine 143 nmol·l^{-1}, tyrosine 65 nmol·l^{-1} and alanine 80 nmol·l^{-1}.

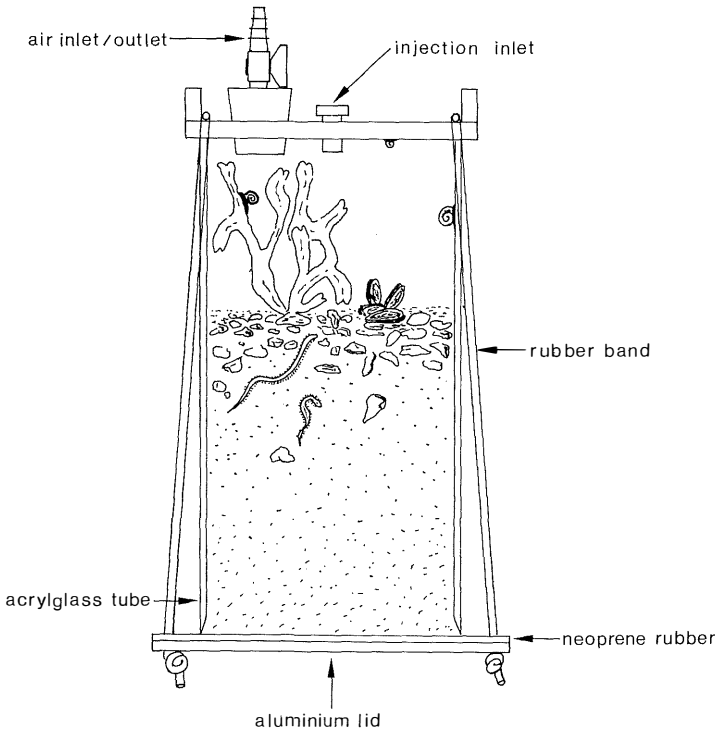

Figure 1

Special sediment corers made of acrylic glass were used for *in situ* experiments for incubation of small benthic microorganisms with labelled amino acids. (Length of the chamber: 25 cm; 15.2 cm ∅, Volume 1.5 l)

The experimental runs included laboratory tests under various conditions at different seasons and *in situ* experiments in summer. Laboratory tests were carried out with 100 ml aerated artificial seawater of 20 ‰ S at 0°, 5°, 10° and 15° C and in each case with 100 mg specimen weight (d. w.) in 100 ml seawater with heterotrophic microorganisms.

A water sample of 1 ml was taken at the beginning of all experiments in order to determine the initial radioactivity. At the end of the experiment 10 ml of the seawater used through the 6 h incubation period was passed through a membrane filter with 0.2 μm pore size (Biorad Nr. 3136059). 1 ml of this filtrate was used to determine terminal radioactivity of the water. The filter was also taken into scintillation fluid in order to measure the amino acid uptake of the microorganisms which had been filtered from the water.

At the end of the incubation period the samples were cleaned with seawater and distilled water to free them of attached amino acids. Then they were freeze-dried, weighed and reduced to ash in the ,,Packard sample oxidizer'', where the amount of ^{14}C and ^{3}H released during oxidation was chemically absorbed and measured in the scintillation counter.

A special type of sediment corer was devised for the *in situ* experiments. It is well suited for use in shallow and deep waters down to 20 m (Fig. 1). The corers were introduced into *Fucus* communities in 0.6 depth at 15° C, 17.6 ‰ S in Kiel Bight. The labelled amino acids in solution were administered to the system by way of a syringe. The system was allowed 6 h incubation. Three sediment corers were evaluated.

Results

Laboratory tests in filtered artificial seawater with heterotrophic microorganisms and an amino acid concentration of 17 nmol $C \cdot l^{-1}$ = 170 nmol $C \cdot 100\ ml^{-1}$ with *Macoma balthica* and *Mytilus edulis* reveal an optimal temperature range for the uptake of dissolved amino acids between 0° and 10° C. Uptake is reduced at 15° C (see. Fig. 2 and 3).

At 15° C the studied species made use of 10 % of the available amino acids from a concentration of 1 μCi = 17 nmol $C \cdot l^{-1}$ added to 100 ml seawater, and 6 % were taken up when a less high concentration of 0.5 μCi = 8 nmol $C \cdot l^{-1}$ in 100 ml was offered at the start of the experiment. At low temperatures (5° C or less) the uptake of the available amino acids was recorded at between 15 % and 40 %. Uptake by microorganisms accounted for only 1–2 % at these temperatures but increased to 15 % of the available amino acids at 15° C. Competition from microorganisms at warmer temperatures may account for the low uptake by the animal species studied. The untraceable quantity of dissolved amino acids at the end of the experiment rose from about 5 % at 0° C to 25 % at 15° C.

The results of the *in situ* experiment are summarized in Fig. 4. At the end of the 6 h incubation period macro-algae with aufwuchs and macrofauna had taken up about 8 % of the two labelled amino acids. The macrofauna alone accounted for 4.5 %. A detailed uptake pattern of the dominant species of the *Fucus* region in these experiments can be seen in Fig. 5.

It should be noted that in contrast to the results of the laboratory experiment at 15° C, microorganisms *in situ* were found to take up less than 1 % of the available amino acids within 6 hours and that roughly equal quantities of both labelled amino acids were taken up, while *Fucus* with aufwuchs showed a marked preference for aspartic acid (Fig. 5). The macrofauna, with the exception of gammarids, preferred phenylalanine.

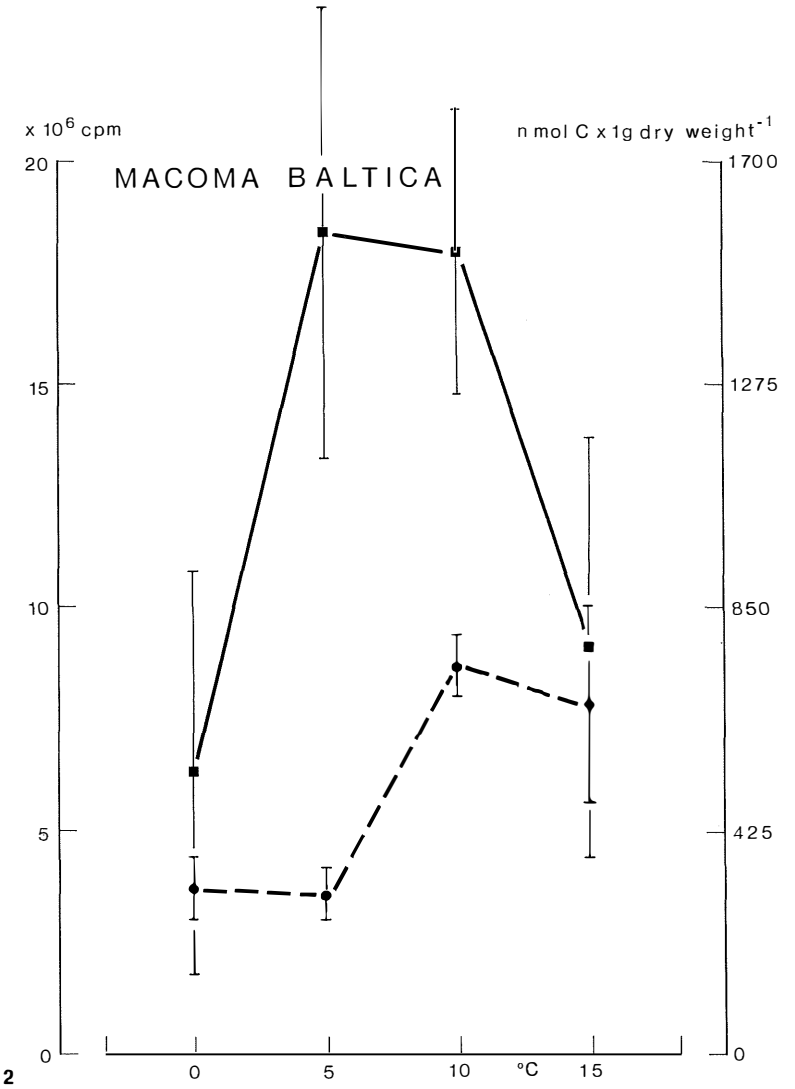

Figure 2

Macoma balthica uptake of dissolved amino acids during 6 h exposure. Experimental conditions:
——— 1 μCi = 17 nmol C·l^{-1}
– – – 0.5 μCi = 8 nmol C·l^{-1}
amino acid mixture + 100 ml filtered artifical seawater ( ), 20 ‰ S, various temperatures.

The schematic representation of the whole system (Fig. 6) shows the distribution of the amino acids in the system and indicates that only very little of the dissolved amino acids in seawater penetrates the sediment after 6 h. The slight activity that was nevertheless found in the top sediment layer down to 20 mm depth may be attributed to bioturbation due to the nereids present in the system. From the results of laboratory experiments (Fig. 7) it appears that a thick layer of detritus over the sediment prevents the exchange of dissolved substance. However, labelled amino acids were found down to a depth of 100 mm in the sediment at the end of 6 hours, if no visible detritus layer was present.

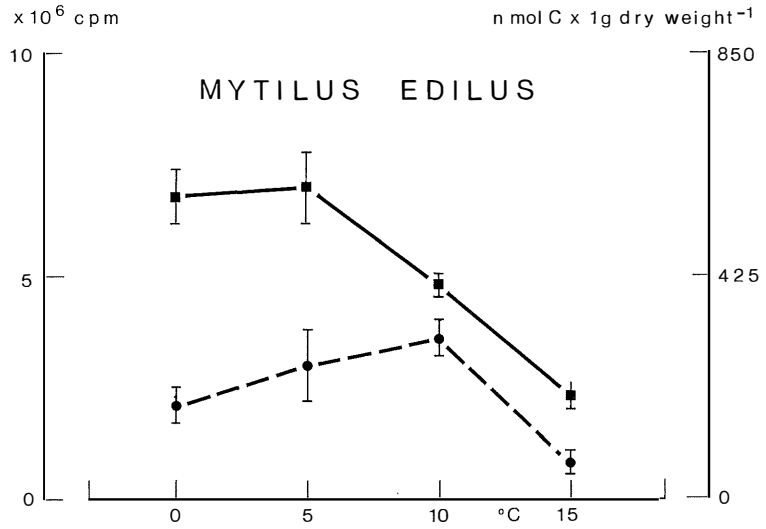

Figure 3

Mytilus edulis uptake of dissolved amino acids during 6 h exposure. Experimental conditions:

——— 1 μCi = 17 nmol C·l^{-1}
– – – 0.5 μCi = 8 nmol C·l^{-1}

amino acid mixture + 100 ml filtered artificial seawater (incubated with heterotroph microorganisms), 20 ‰ S, various temperatures.

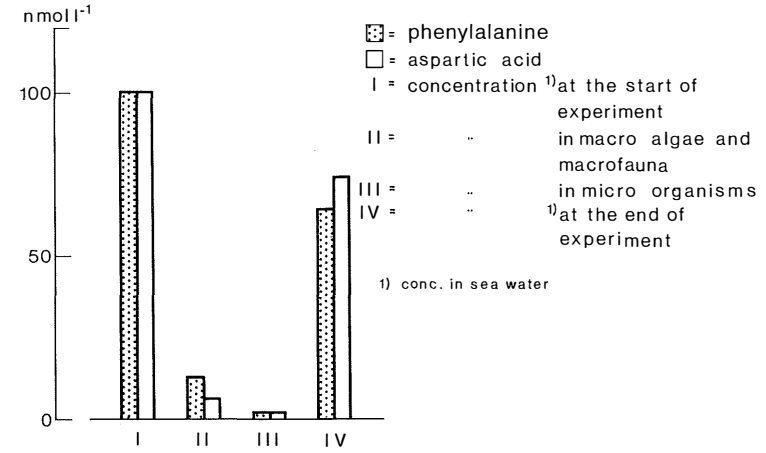

Figure 4

In situ experiment: Uptake of an amino acid mixture of 1 μmol·l^{-1} with 100 nmol·l^{-1} phenylalanine ^{3}H and 100 nmol·l^{-1} aspartic acid ^{14}C by organisms of a *Fucus* community in Kiel Fjord. June 1980; 15° C, 17.6 ‰ S; 0.5 m depth;

Fucus serratus + aufwuchs:	1291 mg
Mytilus edulis:	632.3 mg
Nereis succinea:	304.3 mg
Gammarus sp.:	17.0 mg
Littorina obtusata:	10.2 mg
Microorganisms:	1,500 cm³

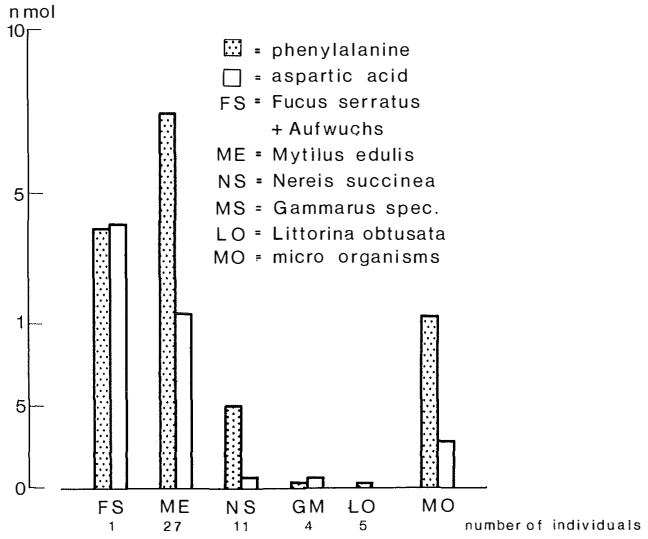

Figure 5
Uptake balance of phenylalanine and aspartic acid in the *in situ* experiment

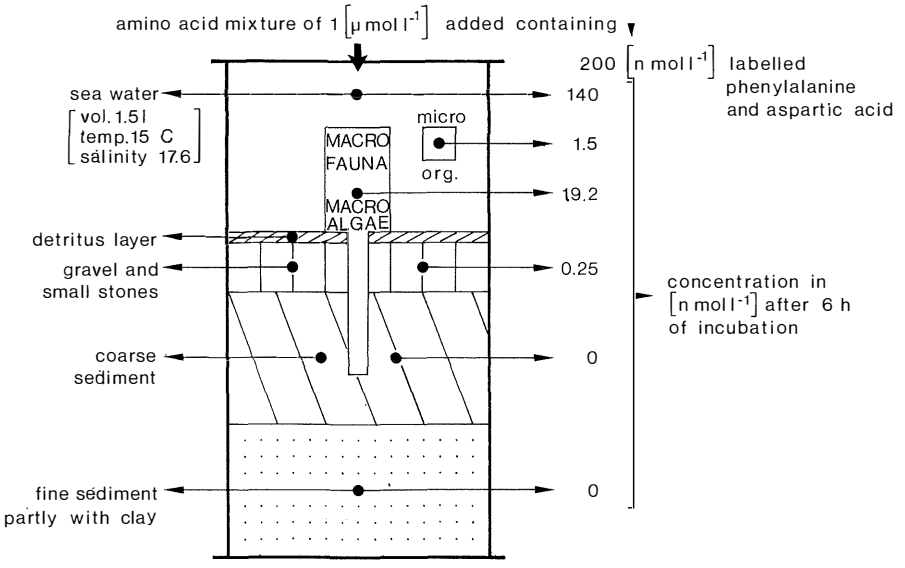

Figure 6
Schematic representation of the allocation of the amino acids in the closed ecosystem under *in situ* conditions after 6 h incubation

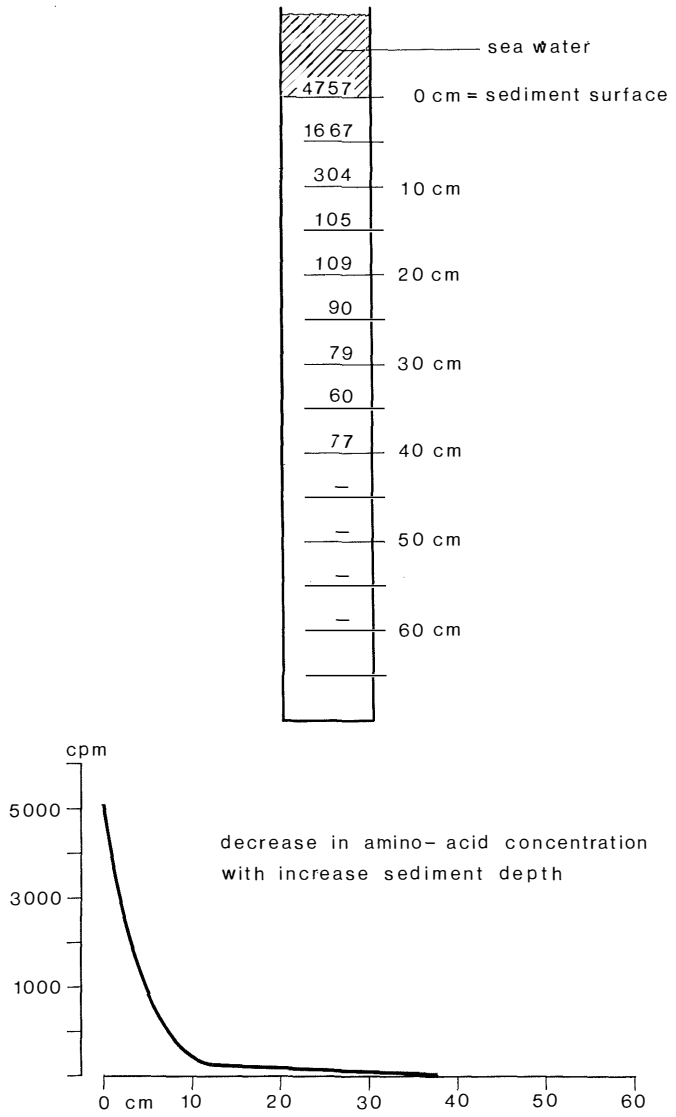

Figure 7

Amino acid transport in sandy sediment with no detritus layer after 6 h (activity at the start of experiment 2.5 μCi)

In situ experiment: uptake values for 24 hours

Uptake/d/g_{dw}	Biomass $g_{dw}m^{-2}$	Organisms
38 nmol	6	Macrofauna
27 nmol	14	Macroalgae + aufwuchs
8 nmol $(0.2\ m^3)^{-1}$	—	Microorganisms

Discussion

When bacteria are inactivated by means of antibiotics, uptake rates for macrofauna increase with increased temperature, reaching a maximum at temperatures over 20° C (GILBERTSON and JONES 1972, SIEBERS 1976, TEMPEL and WESTHEIDE 1980).

The experiments discussed here (where no antibiotics were used) produced other results. Maximal uptake rates for the macrofauna were found to lie in the lower temperature range (0° – 10° C). This may be due to specific seasonal adaption of the animals as TEMPEL and WESTHEIDE (1980) suggest or to reduced competition by microorganisms under these conditions.

Previous results have led to the assumption that a concentration of 5 – 30 $\mu mol \cdot l^{-1}$ of amino acids is necessary for the uptake of dissolved organic substances, showing a tolerable energic balance for the organisms (JØRGENSEN 1979, SIEBERS 1980), but the values found here lay well below this level. The high level of utilization of up to 40 % of the available amino acid concentration of 170 nmol $C \cdot l^{-1}$ seems to indicate the significance of amino acids for the animals.

Seasonal analysis of the seawater has shown that in the area of the Kiel and Eckernförde Bight concentration of amino acids in the water above the sediment is rarely higher than 1–5 $\mu mol \cdot l^{-1}$ in summer and 0.1–1 $\mu mol \cdot l^{-1}$ in winter (DAWSON and PRITCHARD 1978). This means that in this natural habitat very low concentrations are available that may only be utilized at considerable energy expenditure.

Under good assimilation conditions, however, algal exudation can offer special short-term peaks of dissolved organic substances. The macrofauna could profit from this before heterotrophic microorganisms can develop and, by their own uptake, reduce the available substances to minimum concentrations (SIEBERS 1980). Within the 6 h incubation time of the field experiments done here the microorganisms did not compete very much. Their uptake was less than 1 % of the offered amino acids, in comparison to the laboratory experiments where utilization of 6–15 % was observed depending on the offered concentrations. This high percentage may be due to the good growth conditions afforded to microorganisms in artificial, sterile seawater with a diet of amino acids, leading to their explosive development.

With an utilization rate of 4.5 % of the macrofauna and 3.5 % of the macroalgae + aufwuchs, the uptake rate of the *in situ* experiment was close to the lower values of the *in vitro* experiments. It should, however, be noted that only two amino acids of the amino acid mixture were measured in the *in situ* experiments. The values of the 24 h turnover of 38 $nmol \cdot d^{-1} \cdot g.d.w.^{-1}$ of the macrofauna and 27 $nmol \cdot d^{-1} \cdot g.d.w.^{-1}$ of *Fucus serratus* + aufwuchs correspond to the lower values found by WILLIAMS et al. (1976) for heterotrophic microorganisms on the Californian coast. Further investigations are necessary to see whether this uptake is due only to the aufwuchs, or whether *Fucus* itself participates in the uptake.

Where there is no detritus layer, sediment dwellers would not appear to have difficulty in taking up amino acids from the seawater above the sediment due to the water fluctuations in their systems, and need not rely wholly on interstitial water. However, where there is a thick detritus layer over the sediment, substances in the water above the layer only seem to be available to those macrobenthic sediment dwellers with a high degree of bioturbation by means of a tube system. On the other hand, several organisms profit from the detritus layer as the centre of microbial decomposition. The relative importance of availability of different components of the detritus layer needs further investigation. High quantities of amino acids seem to be made available here (100 to 1000 $\mu mol \cdot l^{-1}$) during short-term peaks of decomposition.

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References

BAMFORD, D. R. and E. CAMPBELL 1976. The effect of environmental factors on the absorption of L-Phenylalanine by the gill of *Mytilus edulis*. Comp. Biochem. Physiol. **53 A**, 295–299.

DAWSON, R. and R. G. PRITCHARD, 1978. The determination of α – amino acids in seawater using a fluorimetric analyser. Mar. Chem. **6**, 27–40.

FERGUSON, J. C., 1969. Feeding activity in Echinaster and its induction with dissolved nutrients. Biol. Bull. **136**, 374–384.

GILBERTSON, D. E. and K. G. JONES, 1972. Uptake and assimilation of amino acids by *Biomphalaria glabrata* (*Gastropoda: Planorbidae*). Comp. Biochem. Physiol. **42 A**, 621–626.

JØRGENSEN, N. O. G., 1979. Uptake of L-Valine and other amino acids by the polychaete *Nereis virens*. Mar. Biol. **52**, 45–52.

PÜTTER, A., 1909. Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer. Jena, Fischer: 168 pp.

SCHLICHTER, D., 1978. On the ability of *Anemonia sulcata* (*Coelenterata: Anthozoa*) to absorb charged and neutral amino acids simultaneously. Mar. Biol. **45**, 97–104.

SIEBERS, D., 1976. Absorption of neutral and basis amino acids across the body surface of two annelid species. Helgol. Wiss. Meeresunters. **28**, 456–466.

SIEBERS, D., 1980. Can sea stars feed themselves by means of absoprtion of organic substances dissolvec in seawater? Verh. Dtsch. Zool. Ges. 1980, 314.

SOUTHWARD, A. J. and C. SOUTHWARD, 1970. Observations on the role of dissolved organic compounds in the nutrition of benthic invertebrates (Exp. on three species of Pognophora). Sarsia **45**, 69–96.

STEPHENS, G. C., 1960. Uptake of glucose from solution by the solitary coral, Fungia. Science (Wash.) **131**, 1532.

TEMPEL, D. and W. WESTHEIDE, 1980. Uptake and incoporation of dissolved amino acids by interstitial *Turbellaria* and *Polychaeta* and their dependence on temperature and salinity. Mar. Ecol. Prog. Ser. **3**, 41–50.

WILLIAMS, P. J. LeB., T. BERMAN and O. HOLM-HANSEN, 1976. Amino acid uptake and respiration by marine heterotrophs. Mar. Biol. **35**, 41–47.