Vol. 103: 285–289, 1994 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published January 10

Haemolymph nitrogen compounds and ammonia efflux rates under anoxia in the brackish water isopod *Saduria entomon*

Lars Hagerman[1], Anna Szaniawska[2]

[1]Marine Biological Laboratory, Copenhagen University, Strandpromenaden 5, DK-3000 Helsingør, Denmark
[2]Oceanographical Institute, Gdansk University, Al. Pilsudskiego 46, 81-378 Gdynia, Poland

ABSTRACT: Production and excretion of nitrogenous end products was studied in the benthic Baltic isopod *Saduria entomon* (L.) under normoxic and anoxic conditions. *S. entomon* is ammonotelic. Normoxic haemolymph ammonia concentrations were 176 ± 53 (SD) µmol NH_4^+ l^{-1} decreasing with time to 50 µmol NH_4^+ l^{-1} after 120 h anoxia. Normoxic ammonia efflux rate was 0.53 ± 0.07 (SD) µmol NH_4^+ g^{-1} h^{-1}, decreasing 90% after 96 h anoxia. Normoxic haemolymph urate amounted to 135 µmol l^{-1}, increasing after 120 h under anoxia to 741 ± 79 (SD) µmol l^{-1}. Haemolymph urate concentrations were relatively high because of the ammonia excretion and an undetectable urate excretion even under anoxia. Circulating free amino acids also increased progressively under anoxia, from the normoxic 28 to 165 mmol l^{-1} after 96 h anoxia. This increase was largely due to accumulation of alanine as a result of an anaerobic metabolism. Ammonia was calculated to be the quantitatively dominating nitrogenous end product in *S. entomon*. An effective efflux of ammonia prevents an excessive build-up of toxic ammonia in the haemolymph.

KEY WORDS: Ammonia · Amino acids · Urate · Total nitrogen · Blood · Excretion · Production

INTRODUCTION

The brackish water isopod *Saduria entomon* has been the subject of several ecological and physiological studies made in recent years (e.g. Leonardsson et al. 1987, Hagerman & Szaniawska 1988, 1990, 1991, 1992, Haahtela 1990, Vismann 1991). These studies have revealed *S. entomon* to be a most adaptable species with an exceptional tolerance of anoxia and hydrogen sulphide concentrations. The extent of its tolerance to periods of anoxia or to exposure of hydrogen sulphide whilst under anoxia is dependent on its activity pattern which determines whether the end product of the anaerobic metabolism will be lactate or alanine. Lactate accumulation relates to short-term survival whilst alanine accumulation leads to long-term survival under anoxia (Hagerman & Szaniawska 1990). In the presence of hydrogen sulphide, however, *S. entomon* invariably switches to anaerobiosis which also leads to lactate production – even if oxygen is present in amounts that normally permit the species to continue an aerobic metabolism (Hagerman & Vismann 1993).

In ammonotelic animals, ammonia dominates amongst the nitrogenous compounds which result from protein metabolism and which are excreted under normoxia. Most crustaceans are ammonotelic and also excrete lesser amounts of amino acids, urea and uric acid (Regnault 1987). As long as the respiratory mechanisms are maintained, the normal nitrogen metabolism is also maintained. It is only when the overall metabolic rate is impaired that nitrogen metabolism may be expected also to change and when environmental conditions worsen to severe hypoxia or anoxia, a change in the production and efflux of nitrogenous waste products may occur. It has been shown that increased production of uric acid (urate) occurs in *Carcinus maenas* and *Callinectes sapidus* when exposed to hypoxia (Lallier et al. 1987, DeFur et al. 1990). Furthermore, it has been shown that anaerobic metabolism

can lead to increased haemolymph levels of amino acids, with alanine being predominant, in *Saduria entomon* (Hagerman & Szaniawska 1990).

Although the respiratory responses of *Saduria entomon* have been studied intensively (Hagerman & Szaniawska 1988, 1990, 1991, 1992), the origin and fate of the nitrogenous end products of this species are not well known. The purpose of the present study was thus to investigate ammonia efflux rates and haemolymph nitrogen compounds under anoxia in *S. entomon*.

MATERIAL AND METHODS

Experimental procedure. Specimens of *Saduria entomon* (L.) were collected in the Gulf of Gdansk, Poland (S 7‰) in 1991-92 and transported to the Marine Biological Laboratory (Helsingør, Denmark) where they were stored at ambient temperature and salinity (T 7 °C; S 7‰). Specimens (except those starved for experimental purposes) were fed twice weekly with shrimp or bivalve flesh. Anoxic conditions were maintained for up to 120 h by bubbling the medium with N_2 gas. Oxygen tension of the medium was controlled by a microprocessor-controlled regulator connected to a Radiometer PHM73 acid-base analyser supplied with a Radiometer E5046 oxygen electrode. The medium was considered to be anoxic when oxygen tensions were < 1 Torr. The aquaria were covered with small plastic spheres. This diminished contact with atmosphere and reduced the need for N_2 bubbling considerably. Change in water pH was thus reduced to an increase of only 0.4 pH units (from pH 8.1 to 8.5).

In experiments involving the assessment of excretion, *Saduria entomon* were kept individually in containers with 250 ml of the medium. These solutions were kept anoxic by bubbling N_2 gas through them as described above. Water samples were taken at timed intervals and either analysed immediately or kept frozen at -80 °C until needed for analysis.

Haemolymph samples were collected by inserting a hypodermic syringe (Terumo, 100 µl) into the heart region from a dorsoposterior direction. Each individual was sampled once only.

Analytical procedure. ***Total ammonia (NH_4^+):*** Ammonia concentrations were measured using a modified flow injection/gas diffusion technique (Clinch et al. 1988) as described by Hosie et al. (1991) and Hunter & Uglow (1993a). Water samples were injected directly into the NaOH carrier stream and blood samples (25 µl) were diluted 1:40 before injection. The peak heights of the chart outputs of the water and blood samples were compared with those of a series of fresh ammonium sulphate standards in the range 5 to 25 µmol NH_4^+ l^{-1} prepared daily with the medium or saline respectively.

Urate (uric acid) and urea: Haemolymph and water urate and urea were measured using Sigma Diagnostic Kits 685 and 635 respectively. To correct for haemocyanin interference at the wavelength 520 nm a blank absorbance measure was made on the same haemolymph volume as the test sample diluted in a buffer identical to the one in the Sigma Kit.

Amino acids: Amino acids in the haemolymph were measured by HPLC (Jasco 880 PU) connected to a Perkin Elmer LC 1000 fluorescence detector. The analytical method was pre-column (column: LiChrosorb RP18) derivatisation with orthophthalaldehyde (OPA) according to the description by Gardner & Miller (1980). Haemolymph (1 to 10 µl) was allowed to react with 5 times as much OPA 2 min before injection. Individual amino acid calibration was made with a Sigma amino acid calibration standard no. AA-S-18.

RESULTS

Under normoxic conditions, the blood ammonia concentrations in fed *Saduria entomon* are very variable but with a mean of 176 ± 53 (SD) µmol NH_4^+ l^{-1} (Fig. 1). The exoskeleton of *S. entomon* is relatively heavier than that of many other crustaceans and we may assume that the blood volume is correspondingly a smaller proportion of the fresh body weight. If 20% of the wet weight is taken to be a representative value for the blood volume (Lockwood 1968, Spaargaren 1972), then the weight-specific blood concentration is 0.04 to 0.05 µmol NH_4^+ g^{-1} wet wt. Initially there was a difference between the blood ammonia concentrations of the fed and starved groups with the latter having a mean of 100 to 120 µmol NH_4^+ l^{-1} (0.02 to 0.03 µmol NH_4^+ g^{-1} wet wt). These lower values in the starved specimens are possibly due to a decline in protein catabolism reducing

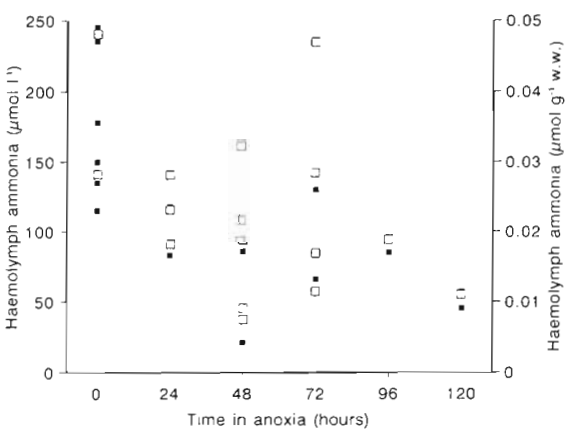

Fig. 1. *Saduria entomon*. Haemolymph ammonia concentrations in normoxia and as a function of time under anoxia. (■) µmol l^{-1} haemolymph; (□) µmol g^{-1} wet wt

ammonia production or to starvation-induced tissue shrinkage causing an increased blood volume. Blood ammonia levels decrease with time under anoxia and in fed isopods drop quickly over the first 24 h to values similar to those of the starved specimens. In both fed and starved groups, the mean blood ammonia values after 120 h under anoxia had dropped to a mean of 50 µmol NH_4^+ l^{-1} (0.011 µmol NH_4^+ g^{-1} wet wt) – a >4-fold change from normoxic values.

The mean normoxic weight-specific ammonia efflux rate was 0.53 ± 0.07 (SD) µmol NH_4^+ g^{-1} h^{-1} (Fig. 2). Efflux rates showed a wide variability and no difference between the means of the starved and fed groups was found. The efflux rates decreased steadily after 24 h under anoxia and after 96 h reached 0.052 ± 0.017 (SD) µmol ammonia g^{-1} h^{-1}. This represents a 90% drop from original rates, corresponding to a decrease of 4.7 nmol h^{-1} or 0.9% h^{-1} of the normoxic efflux rate.

The weight-specific blood ammonia concentration and excretion rate can be used to derive a turnover ratio – the time required to effect a complete replacement of the blood ammonia. Under steady state, weight-specific blood ammonia production equals excretion. Under normoxia, the ammonia replacement time for *Saduria entomon* under the same salinity/temperature conditions used here is 6.9 min. This represents a tissue ammonia production of 0.55 µmol NH_4^+ g^{-1} wet wt h^{-1}. After 96 h under anoxia, the replacement rate became 20.7 min which equates to a tissue production rate of 0.1 µmol NH_4^+ g^{-1} wet wt h^{-1}. Data obtained on ammonia production and replacement rates during the experiments are given in Table 1. These data show a lower or no initial decrease in production (= excretion) rates which probably reflect the stress-induced elevation of metabolic rates which accompanied the transfer of the isopods to the experimental conditions.

Table 1. *Saduria entomon*. Haemolymph ammonia production and haemolymph ammonia replacement times

	Production (µmol g^{-1} wet wt h^{-1})	Replacement time (min)
Normoxia	0.54	6.9
24 h anoxia	0.48	3.8
48 h anoxia	0.37	3.2
72 h anoxia	0.18	5.5
96 h anoxia	0.1	20.7

No urea was detected in any of the water or blood samples collected under normoxia or whilst under anoxia. Small quantities of urate were present in the haemolymph samples collected under normoxia and these amounted to 135 ± 21 (SD) µmol urate l^{-1} haemolymph (Fig. 3). The urate concentrations increased progressively during the anoxic period and reached 741 ± 79 µmol urate l^{-1} haemolymph (= 0.15 µmol urate g^{-1} wet wt) after 120 h. Excretion of urate was not noticed; urate water concentration was below the detection limit.

The total concentration of circulating free amino acids under normoxia was 28.1 mmol l^{-1} haemolymph, increasing steadily under anoxia to reach 165.1 mmol l^{-1} after 96 h. This large increase was due mainly to an accumulation of alanine which, as a result of anaerobic metabolism, increased from the normoxic 4.7 to 100 mmol l^{-1} by the end of the anoxic period.

Data relating to the haemolymph concentrations of the measured variables are given in Table 2 which shows the anoxia-induced accumulation of urate and amino acids. This resulted in an increased ratio of the urate-N : ammonia-N in the haemolymph over the anoxic period, urate-N is 57 times higher than ammonia-N after 120 h anoxia. This is likely caused

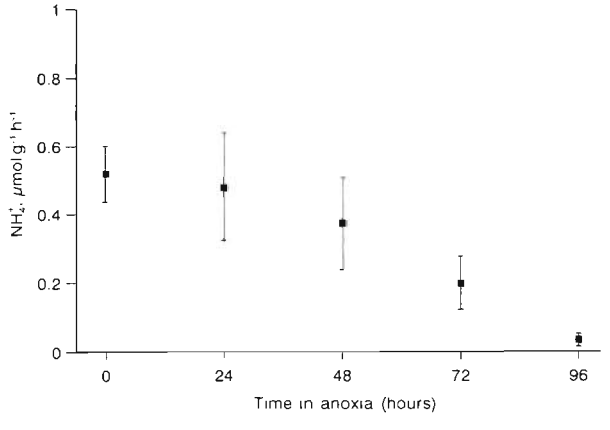

Fig. 2. *Saduria entomon*. Ammonia excretion (µmol g^{-1} wet wt h^{-1}; mean ± SD) under normoxia and as a function of time under anoxia

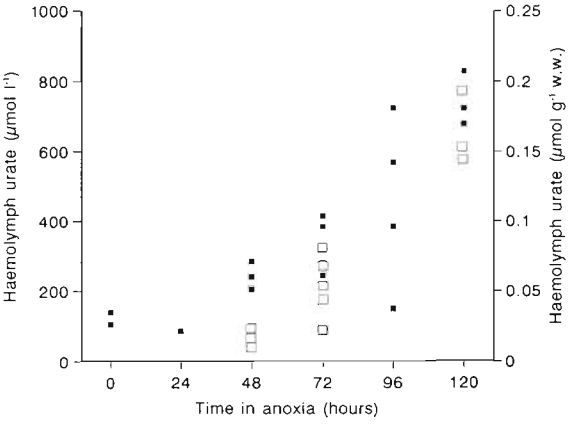

Fig. 3. *Saduria entomon*. Haemolymph urate concentrations in normoxia and as a function of time under anoxia. (■) µmol l^{-1} haemolymph; (□) µmol g^{-1} wet wt

Table 2. *Saduria entomon*. Haemolymph concentrations (µmol l^{-1}) of nitrogenous compounds in normoxia and after 120 h anoxia (96 h for FAA)

	NH_4^+	Urate	Urea	Amino acids (FAA)	Alanine (% FAA)
Normoxia	176 ± 53	135 ± 21	0	28 100	16.7
Anoxia 120 h	50 ± 5	741 ± 79	0	165 100	60.5

entirely by an efficient ammonia excretion and a negligible urate excretion. Assuming no normoxic ammonia excretion (normally ca 0.5 µmol NH_4^+ g^{-1} h^{-1}) during a 24 h period and an initial haemolymph concentration of 0.04 µmol NH_4^+ g^{-1}, the accumulated blood ammonia would be 12.4 µmol NH_4^+ g^{-1} after 24 h. This should be compared to an accumulated urate of ca 0.02 µmol g^{-1} or a production of 0.1 nmol g^{-1} h^{-1}, a value practically negligible compared to the ammonia production of up to 0.54 µmol g^{-1} h^{-1}. When using anoxic excretion and blood ammonia values, the corresponding accumulated blood ammonia would be 3.6 µmol g^{-1}, still accentuating the importance of the ammonia excretion in preventing excessive build-up of nitrogenous by-products in the haemolymph.

DISCUSSION

The ammonia efflux rate in crustaceans is size-dependent (Regnault 1987) and the normoxic weight specific ammonia efflux rates found here for *Saduria entomon* (mean = 0.5 µmol NH_4^+ g^{-1} h^{-1}) are in the normal range found for crustaceans (0.2 to 1.6 µmol g^{-1} h^{-1}; Regnault 1986). The individual variation of efflux rates probably reflects individual differences in metabolic rate due to activity and nutritional differences. A reduced rate of efflux under severe hypoxia has been observed to occur in some species and may be a general trend amongst crustaceans (Regnault & Aldrich 1988). The decrease in efflux rate found to occur here during anoxia seems to follow this trend.

The normoxic haemolymph ammonia concentrations found here for *Saduria entomon* ranged from 115 to 250 µmol NH_4^+ l^{-1} and also compare well with what is known for other (mainly decapod) crustaceans (Hagerman et al. 1990). The concentrations are however much lower when expressed as weight specific (mean 0.04 µmol ammonia g^{-1} wet wt). Assuming that the blood volume of *S. entomon* is approximately 20% (which is slightly less than values quoted for decapod species, e.g. Spaargaren 1972, because of the relatively greater exoskeleton weight in these isopods), then normoxic ammonia replacement times of 6.9 min were calculated. This time is short compared with that for *Nephrops norvegicus* (Hosie et al. 1991) but similar to that of *Crangon crangon* (Hunter & Uglow 1993b) which probably reflects the activity/metabolic level of the species. The combination of the lowered haemolymph ammonia levels and the decreased efflux rate found after 96 h anoxia indicates ammonia production inhibition and parallels the decrease in metabolic levels found in *S. entomon* under such anoxia (Hagerman & Szaniawska 1990).

According to Regnault (1987) some 1 to 5% of the end products of nitrogen metabolism could be urea. Urea was, however, not detected in the haemolymph of *Saduria entomon*, either under normoxia or anoxia but a conspicuous production of urate was found under anoxia. The final urate concentrations in the haemolymph were high relative to those of blood ammonia (Table 2), mainly because of the efficiency of the ammonia excretion even under anoxia.

Lallier et al. (1987) found a significant increase in *Carcinus maenas* haemolymph urate after 24 h under hypoxia (20 Torr). Urate has been found also in other species when subjected to hypoxia, e.g. *Penaeus japonicus* (Lallier & Truchot 1989), *Homarus vulgaris* (Nies et al. 1992) and *Nephrops norvegicus* (H. Schlütter & L. Hagerman unpubl.). It may thus be that anaerobiosis-induced haemolymph urate accumulation is a widespread phenomenon in benthic crustaceans.

The normoxic levels of haemolymph free amino acids (FAA) found in *Saduria entomon* lie within the range found for higher crustaceans. Free-living marine (brackish water) isopods seem to have haemolymph FAA concentrations higher than those of most decapods (Charmantier et al. 1976) and this also appears to be the case for *S. entomon*. The normoxic amino acids (especially glycine, proline and alanine) help to maintain the cellular osmotic pressure (Weber & van Marrewijk 1972) and haemolymph amino acids are also the products of protein turnover (Claybrook 1983). The anoxia-induced increase in haemolymph FAA is probably due to protein catabolism as anoxia is invariably associated with starvation and an accumulation of alanine as an anaerobic end product (Hagerman & Szaniawska 1990). Although haemocyanin is also broken down under anoxia it is unlikely to be a major contributor to the FAA build-up because it is not particularly rich in proline, glycine and alanine (Boone & Schoffeniels 1979).

The present study confirms that *Saduria entomon* can tolerate a considerable time under anoxia and this presumably has considerable survival value to a mud-dwelling organism. During anoxia there appears to be

an enhanced build-up of urate and certain free amino acids in the haemolymph and a reduced but still effective efflux of ammonia. Presumably, this combination of strategies effects a removal of nitrogenous waste and minimises the attendant problems of an excessive build-up of potentially toxic ammonia in the haemolymph.

Acknowledgement. The study was supported by the Danish Science Research Council grant no. 11-8391.

LITERATURE CITED

Boone, W. R., Schoffeniels, E. (1979). Hemocyanin synthesis during hypo-osmotic stress in the shore crab *Carcinus maenas* (L.). Comp. Biochem. Physiol. 63B: 207–214

Charmantier, G., Charmantier, M., Voss-Foucart, M.-F., Jeuniaux, C. (1976). Les acides aminés libres de l'hémolymphe des isopodes marins *Sphaeroma hookeri, Sphaeroma serratum* (Flabellifera) et *Idotea baltica* (Valvifera). Arch. int. Physiol. Biochim. 84: 989–996

Claybrook, D. L. (1983). Nitrogen metabolism. In: Mantel, L. (ed.) The biology of Crustacea, Vol. 5, Internal anatomy and physiological regulation. Academic Press, New York, p. 163–213

Clinch, J. R., Worsfold, P. J., Sweeting, F. W. (1988). An automated spectrophotometric field monitor for water quality parameters. Determination of ammonia. Analyt. chim. Acta 214: 401–407

DeFur, P. L., Mangum, C. P., Reese, J. E. (1990). Respiratory responses of the blue crab *Callinectes sapidus* to long-term hypoxia. Biol. Bull. 178: 46–54

Gardner, W. S., Miller, W. H. (1980). Reverse-phase liquid chromatographic analysis of amino acids after reaction with o-phthalaldehyde. Analyt. Biochem. 101: 61–65

Haahtela, I. (1990). What do Baltic studies tell us about the isopod *Saduria entomon*? Acta zool. fenn. 27: 269–278

Hagerman, L., Sondergaard, T., Weile, K., Hosie, D., Uglow, R. F. (1990). Aspects of blood physiology and ammonia excretion in *Nephrops norvegicus*. Comp. Biochem. Physiol. 97A: 51–55

Hagerman, L., Szaniawska, A. (1988). Respiration, ventilation and circulation under hypoxia in the glacial relict *Saduna (Mesidotea) entomon*. Mar. Ecol. Prog. Ser. 47: 55–63

Hagerman, L., Szaniawska, A. (1990). Anaerobic metabolic strategy of the glacial relict isopod *Saduria (Mesidotea) entomon*. Mar. Ecol. Prog. Ser. 59: 91–96

Hagerman, L., Szaniawska, A. (1991). Ion regulation under anoxia in the brackish water isopod *Saduria (Mesidotea) entomon*. Ophelia 33: 97–104

Hagerman, L., Szaniawska, A. (1992). *Saduria entomon*: ecophysiological adaptations for survival in the Baltic. In: Bjørnestad, E., Hagerman, L., Jensen, K. (eds.) Proc. 12th Baltic Marine Biologists Symp. Olsen & Olsen, Fredensborg, p. 71–76

Hagerman, L., Vismann, B. (1993). Anaerobic metabolism, hypoxia and hydrogen sulphide in the brackish water isopod *Saduria entomon* (L.). Ophelia 38: 1–11

Hosie, D. A., Uglow, R. F., Hagerman, L., Sondergaard, T., Weile, K. (1991). Some effects of hypoxia and medium ammonia enrichment on efflux rates and circulating levels of ammonia in *Nephrops norvegicus*. Mar. Biol. 110: 273–279

Hunter, D., Uglow, R. F. (1993a). A technique for the measurement of total ammonia in small volumes of seawater and haemolymph. Ophelia 37: 31–40

Hunter, D., Uglow, R. F. (1993b). Moult stage-dependent variability of haemolymph ammonia and total protein levels of *Crangon crangon*. Ophelia 37: 41–50

Lallier, F., Boitel, F., Truchot, J P. (1987). The effect of ambient oxygen and temperature on haemolymph l-lactate and urate concentrations in the shore crab *Carcinus maenas*. Comp. Biochem. Physiol. 86A: 255–260

Lallier, F., Truchot, J. P. (1989). Modulation of haemocyanin oxygen-affinity by l-lactate and urate in the prawn *Penaeus japonicus*. J. exp. Biol. 147: 133–146

Leonardsson, K., Bengtsson, Å., Linner, J. (1987). Size selective predation by fourhorn sculpin, *Myoxocephalus quadricornis* (L.) (Pisces) on *Mesidotea entomon* (L.) (Crustacea, Isopoda). Hydrobiologia 164: 213–220

Lockwood, A. P. M. (1968). Aspects of the physiology of Crustacea. Oliver & Boyd, Edinburgh

Nies, A., Zeis, B., Bridges, C. R., Grieshaber, M. K. (1992). Allosteric modulation of haemocyanin oxygen affinity by l-lactate and urate in the lobster *Homarus vulgaris*. II. Characterisation of specific effector binding sites. J. exp. Biol. 168: 111–124

Regnault, M. (1986). Production d'NH_4^+ par la crevette *Crangon crangon* L. dans deux écosystèmes côtiers. Approche expérimentale et étude de l'influence du sédiment sur le taux d'excrétion. J. exp. mar. Biol. Ecol. 100: 113–126

Regnault, M. (1987). Nitrogen excretion in marine and freshwater crustacea. Biol. Rev. 62: 1–24

Regnault, M., Aldrich, J. C. (1988). Short-term effect of hypoxia on ammonia excretion and respiration rates in the crab *Carcinus maenas*. Mar Behav. Physiol. 6: 257–271

Spaargaren, D. H. (1972). The determination of the extra cellular volume in the shrimp *Crangon crangon* (L.). Comp. Biochem. Physiol. 43A: 843–848

Vismann, B. (1991). The physiology of sulfide detoxification in the isopod *Saduria (Mesidotea) entomon* (L.). Mar. Ecol. Prog. Ser. 76: 283–292

Weber, R. E., van Marrewijk, W. J. A. (1972). Free amino acids in the shrimp *Crangon crangon* and their osmoregulatory significance. Neth. J. Sea Res. 5: 391–415

This article was submitted to the editor

Manuscript first received: March 22, 1993
Revised version accepted: October 13, 1993