University of Plymouth

PEARL **https://pearl.plymouth.ac.uk**

01 University of Plymouth Research Outputs University of Plymouth Research Outputs

The trophic level of Mytilicola intestinalis Steuer (Copepoda: Poecilostomatoida) in Mytilus edulis L., as determined from Stable Isotope Analysis

Gresty, K

http://hdl.handle.net/10026.1/14752

Bulletin of the Plankton Society of Japan
Plankton Society of Japan/Nihon Purankuton Gakkai

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

The Trophic Level of *Mytilicola intestinalis* Steuer (Copepoda: Poecilostomatoida) in *Mytilus edulis* L., as Determined from Stable Isotope Analysis

K. A. Gresty[1] and C. Quarmby[2]

[1] *Hatherly Laboratories, Exeter University*
Exeter, EX4 4PS, UK
[2] *Institute of Terrestrial Ecology, Merlewood Research Station*
Grange-over-Stands, Cumbria, UK

Abstract

Mytilicola intestinalis inhabits the digestive tract of the commercially important European blue mussel *Mytilus edulis*. Early researchers believed that *Mytilicola* damaged the host by feeding directly upon the epithelial lining of the host intestine, whilst more recent work indicated that it does not seriously affect the host and feeds upon excess food (phytoplankton and detritus) which passes through the mussel intestine. Comparative analysis of the stable isotope ^{15}N shows that it is 2.8‰ higher in whole *Mytilicola* than in mussel intestine, suggesting that *Mytilicola* occupies a higher trophic level than *Mytilus* and therefore is unlikely to use the same food source. Whilst the limitations and pitfalls of this method are recognised and discussed, it is concluded from these observations (and from evidence provided by other techniques) that *Mytilicola* utilizes mussel breakdown products such as mucus and sloughed-off cells as its major food source and thus feeds indirectly upon the host.

Introduction

The poecilostome copepod *Mytilicola intestinalis* Steuer 1902, has a long history of being regarded as a serious pest of *Mytilus edulis* L. in European waters where shellfish mariculture is of considerable commercial importance. The crash of the Dutch and German mussel fisheries in 1949 and 1950 were attributed to *Mytilicola* (Korringa 1951) and subsequent work by Meyer & Mann (1951), Cole and Savage (1951), Theisen (1966) and Williams (1969) confirmed its pest status. However, relatively recent studies have failed to detect any detrimental effects of *Mytilicola* upon its common host *Mytilus edulis* (Dethlefsen 1975; Gee et al. 1977; Davey et al. 1978; Dare 1985; Davey 1989). The gross discrepancies between these two conclusions have been attributed to poor scientific protocol and lack of rigorous statistical analysis by the earlier workers (Lauckner 1983; Davey & Gee 1988). However, there is still some

doubt over the status of *Mytilicola*—is it a harmful parasite or harmless commensal?

An understanding of the true mode of feeding and the diet of *Mytilicola* is an important facet of this argument and this has never been accurately determined. Guisti (1967) claimed that the copepod removes the microvillar border of the intestinal epithelium of the mussel, which could lead to serious damage of the commercial product and be a threat to the fishery. On the other hand, Moore et al. (1978), using histological and histochemical techniques suggested that *Mytilicola* was most probably a herbivore feeding on the phytoplankton material passing through the host gut; in which case it may only be a threat to the general mussel population in times of serious food limitation.

The determination of an animal's diet has traditionally been undertaken by critical analysis of the contents of its alimentary tract and faeces but this can prove to be difficult and/or unproductive when dealing with small organisms. Histological and tracer techniques have also been employed but recent work has indicated that the use of stable (non-radioactive) isotopes of carbon and nitrogen can be extremely useful in dietary analyses, particularly in determining the trophic level occupied by an organism.

Peterson et al. (1985), maintained that *Mytilus* should be classed as an opportunistic feeder with a wide range of dietary components. Traditionally, however, they have been regarded as herbivores with vegetable matter comprising the major portion of their diet (Widdows, pers. comm.) yet Galtsoff (1964) pointed out that the presence of an organism in the gut of bivalves is not necessarily proof that it is utilized or has nutritive value. Thus, zooplankton detritus, for example, may be found in the mussel intestine but it may pass through the mussel intestine untouched.

Mytilicola inhabits the alimentary canal of *Mytilus*, the early copepodites residing in the stomach and digestive tubules and the mature copepods in the intestine posterior to the style sac (Davey et al. 1978). At these sites there are four food categories potentially available to the copepod:-

1. Undigested vegetable matter such as phytoplankton (the major food source of the host).
2. The living tissue of the host gut epithelium.
3. Host breakdown products such as sloughed off cells from the gut lining and mucus secretions.
4. Zooplankton detritus.

The nitrogen-technique cannot differentiate between categories two and three because they are both derived from the host and therefore of the same trophic level. Similarly, if the zooplankton in category four had been feeding upon phytoplankton prior to being ingested by the mussel, they too would be at the same trophic level as the mussel and could not be distinguished from it. However, this technique can indicate whether animal or vegetable material is being consumed and therefore, whether the main food source of *Mytilicola* is the phytoplankton ingested by the mussel or other animal-derived products.

Background to Stable Isotope Technique

Nitrogen exists naturally as two stable isotopes ^{14}N (99.634% by atoms) and ^{15}N (0.366% by atoms). The measurement of the absolute abundance of ^{15}N and ^{14}N atoms is difficult and so determinations are generally made relative to a standard reference material of known composition. For nitrogen, the primary standard is taken to be atmospheric nitrogen (Mariotti 1983), although secondary standards which are more easily handled are more likely to be used in routine analysis. In this work, samples were measured against the secondary standard N-methyl glucamine, which had been previously calibrated against air.

The small differences which occur in natural abundances are described using the δ (delta) notation. The $\delta^{15}N$ value is expressed in ‰ and referred to as 'per mil'.

To calculate the $\delta^{15}N$ of a sample:

$$\delta^{15}N=\frac{R_{sample}-R_{standard}}{R_{standard}}\times 1000$$

Where R is either:

$$\frac{^{15}N}{^{14}N+^{15}N} \quad \text{or} \quad \frac{^{15}N}{^{14}N}$$

^{15}N and ^{14}N are the number of atoms of nitrogen in the sample or standard, of mass 15 and 14 respectively. The first form of the equation is more commonly used in biological work, the alternative form being found more often in geochemistry. At natural abundance levels however, the values of $\delta^{15}N$ calculated by the two forms of the equation are virtually identical, the difference being within experimental error (Shearer & Kohl 1986).

Work by Miyake and Wada (1967) initiated research into natural abundances of ^{15}N, by suggesting that there was a correlation between isotopic fractionation and links in the food chain in the marine environment. Isotopic fractionation occurs during the course of most biochemical reactions and arises when molecules of the same structure but with slightly different masses react at different rates, resulting in a different isotopic ratio in the product compared to the substrate (Peterson & Fry 1987; Montoya et al. 1990). Chemical bonds involving ^{14}N are more likely to break under a given set of conditions than equivalent bonds involving ^{15}N; catabolic reactions will therefore tend to lead to an increase of ^{15}N in an organism's tissues. Nitrogen excreted by fish (Minagawa & Wada 1984) and cows (Steele & Daniel 1978) is isotopically lighter than nitrogen in food, faeces and new tissues possibly due to fractionation during transamination (Macko et al. 1986). This is consistent with the stepwise increase in $\delta^{15}N$ with trophic level (Deniro & Epstein 1981; Minagawa & Wada 1984; Wada et al. 1987).

Miyake & Wada (1967) showed that, amongst marine samples, ^{15}N is enriched in organisms in the following order: inorganic nitrogen; phytoplankton; zooplankton; fish. They suggested that a ^{15}N enrichment is correlated with an increase in trophic level in shallow water communities and this concept was further investigated by Wada & Hattori (1976), Hattori (1976), Rau (1982), Minagawa & Wada (1984) and Van Dover et al. (1988).

Research by Minagawa & Wada (1984) has indicated that approx. a 3‰ shift in the ^{15}N isotope indicates a possible single trophic step up the food-chain. Thus, if the mussel and the parasite have similar values of ^{15}N then they are on the same trophic level. However, if the parasite has a $\delta^{15}N$ of 3‰ higher than the mussel, then it is a trophic level higher. Using the ^{15}N results from the mass spectrometer, it should be possible to determine the nature of the diet of *Mytilicola intestinalis* by eliminating certain elements from the potential diet and examining the evidence for the remaining food-sources.

Materials and Methods

In order to assess accurately the ratio of nitrogen isotopes in the materials under investigation, at least 20–30 mg dry weight of each tissue is required and this took three months to collect. Mussels infected with *Mytilicola* were obtained from the Bull Hill mussel bed in the Exe estuary, Devon, and kept in the laboratory in a re-circulating seawater system. The water in this system was replenished from the estuary once a month and the diatom *Phaeodactylum tricornutum* added as food for the mussels.

Different organs and tissues of *Mytilus* can fractionate ^{15}N to different extents, so that the ^{15}N content of one tissue might be very different to that of the whole animals. As *Mytilicola* lives in the intestine of *Mytilus,* only that tissue was collected. The posterior section of intestine was dissected out of individual mussels within 2–3 weeks of collection; squeezed with forceps to evacuate its contents; stripped to remove any attached debris; washed in filtered seawater, blotted dry, washed and dried again. Pieces of intestine were placed in pre-weighed glass tubes, spun for 15 minutes in a freeze-drier until frozen and then left overnight to ensure complete evaporation of any remaining water. The tubes were then labelled, weighed, sealed and stored in a dessicator until required for analysis.

Because of their small size, ^{15}N analysis was carried out on whole *Mytilicola.* These were removed from the host intestine, carefully cleaned of any attached debris, washed in filtered seawater to remove any enveloping mucus, freeze-dried and stored as detailed above.

A stock culture of *Phaeodactylum tricornutum* was obtained from the Plymouth Marine Laboratory and was grown under sterile conditions in artificial seawater to which sodium nitrate was added as a nutrient. 500 ml aliquots of the diatom culture were taken at intervals until sufficient material had been obtained. The diatom cells were separated from the culture medium by centrifugation at 1500 rpm for 10 minutes, resuspended in distilled water to remove excess sodium chloride and quickly re-centrifuged. The resulting pellet was pipetted into a pre-weighed glass tube, freeze dried and stored as detailed above.

The suspended matter in the seawater was collected by means of a filter and then treated as the diatom culture. The artificial seawater and sodium nitrate were treated in the same manner as the other tissues for freeze-drying and storage.

The freeze-dried samples were analysed at the Institute of Terrestrial Ecology, Cumbria using the Natural Environment Research Council stable isotope facilities. Samples were ground into a fine powder in a freezer mill (Spex Industries Inc. USA)

which runs at liquid nitrogen temperatures and 10 replicate 3 mg sub-samples were weighed out into small tin containers (6 mm by 4 mm) and then processed. The analytical system consisted of a 'Roboprep' analyser (a Dumas-type continuous flow CHN preparative analyser) coupled to a 'Tracermass' isotope ratio mass spectrometer, which is a magnetic sector, single inlet, triple collector instrument. Both instruments were made by Europa Scientific Ltd, Crewe, UK. A dedicated computer calculated the ^{15}N values in each tissue from the isotope ratios.

Full details of the principle of the method and of the operating conditions employed, have been given by Barrie & Lemley (1989), Harris & Paul (1989) and by Schepers et al. (1989).

Results

The values obtained from the stable isotope analysis are shown in Table 1. A oneway analysis of variance indicates that the $\delta^{15}N$ values are all statistically significant ($F_{4,45}=565.8$, $p<0.001$). The artificial seawater mix contained a negligible amount of nitrogen and the total % nitrogen for the other samples was lowest in the suspended matter and highest in the *Mytilus* gut. The total percentage nitrogen content in the tissue samples did not show a correlation between organism and trophic level.

The value obtained for *Mytilicola* was 7.3, 2.8‰ higher than that for the *Mytilus* intestine which was 4.5. However, the value for the diatom culture (9.9) was very much higher than for *Mytilus* tissue, when the trophic chain model predicts that it should be lower. This anomolous result is discussed below.

Discussion

The $\delta^{15}N$ results obtained in this study are relative values and should not be compared directly with those obtained by other workers. However, the actual differences between the ^{15}N values of the sample materials do correlate with the findings of other studies, showing the possibility of a rise in trophic level with an increased value of $\delta^{15}N$. The ^{15}N value for *Mytilicola intestinalis* was 7.3‰, almost 3‰ higher than the value for the *Mytilus* gut. If the two organisms were consuming the same kind of food substance, then the $\delta^{15}N$ values would be expected to be the same because they would both be primary consumers on the same trophic level. However, as a 3‰ in $\delta^{15}N$ enrichment is correlated with an increase in trophic level (Miyake & Wada 1967; Rau 1982; Minagawa & Wada 1984; Montoya et al. 1990; Van Dover, pers. comm.)

Table 1. The Mean values (±1 SD) for relative $\delta^{15}N$ and % nitrogen in 10 replicate samples of the 5 tissue types analysed.

Tissue type	Relative $\delta^{15}N$	% N. freeze dried
Mytilus gut	4.5±0.2	8.9±0.1
Mytilicola	7.3±0.2	7.9±0.2
Phaeodactylum	9.9±0.2	5.7±0.1
Suspended matter	11.8±1.0	0.4±0.01
Sodium nitrate	2.7±0.4	—

the present results indicate that *Mytilicola intestinalis* is either feeding directly upon the gut tissue or sloughed-off cells and mucus of *Mytilus* and/or consuming particles of zooplankton detritus present in the intestine. This finding is supported by other more traditional techniques which have been used to study the food and feeding biology of *Mytilicola* (Gresty 1990). These studies included scintillation assay of labelled diatoms through the mussel gut, copepod gut content analysis by histology and also general alimentary canal observations of *Mytilicola,* which all indicated that plant matter was not the major dietary constituent of *Mytilicola.* Examination of the mouthparts of *Mytilicola* revealed that this copepod lacked a mandible, the gnathobase of which is the major cutting or grinding tool and it is therefore unlikely to be grazing directly upon the intestinal epithelium of the mussel, as Guisti (1967) proposed. Transmission electron microscopy has shown cellular components and mucus to be present in the copepod's gut, suggesting that *Mytilicola* is scavenging upon detrital matter and mussel break-down components. The actual percentage of zooplankton detritus present in the diet of *Mytilus* is unknown because the estuary is a constantly changing environment and the mussel can only feed upon the available foodsources. However, providing the detritus particles are small enough to pass through the selective feeding mechanism of *Mytilus,* they will eventually be made available to *Mytilicola* along with sloughed-off *Mytilus* cells in a mucus envelope. Such feeding activity by *Mytilicola* suggests that it occupies a higher trophic level than *Mytilus.*

Variations (of ^{15}N) in the diet of individuals of the same species can affect profoundly the organism's nitrogen content and so make general trophic level determinations difficult to interpret. Peterson et al. (1985) found that a single population of mussels showed a gradient of ^{15}N content along an estuary, resulting from the fact that mussels near the mouth of the estuary were primarily feeding upon phytoplankton whilst those further up the river were feeding mainly on *Spartina* detritus. Therefore it is very important to know the exact diet of an organism under field conditions if its position in the food chain is to be interpreted correctly, using ^{15}N analysis. However, in the case of the relationship between *Mytilicola intestinalis* and *Mytilus edulis,* the situation is simplified somewhat. In the life-cycle of *Mytilicola,* only the naupliar stages are free-living and these are non-feeding; the remaining developmental stages and the adult can only be found inside a single host and there is no transfer of parasites from one host to another. In this time, the copepods have no access to food other than that collected or produced by the mussel. Consequently, it is immaterial how the nitrogen isotope ratios vary in the mussel's diet as, ultimately, they will be reflected in the parasite's isotopic composition.

The problem of pinpointing the foodsource of the mussels is particularly highlighted by the relative values of ^{15}N in mussels and the diatom culture in this investigation. If *Mytilus* had been feeding on the *Phaeodactylum* culture, then it would be expected that there would be a 3‰ increase in the $\delta^{15}N$ value of mussel gut over *Phaeodactylum.* However, the mussel gut ^{15}N value (4.5) is very much lower than that for the diatom culuture (9.9), which apparently rules out *Phaeodactylum* as a food source.

A possible, alternative, foodsource of the mussels was the suspended matter in the

aquarium seawater. The mussels were kept for a short while in the laboratory in seawater that had come from the mussel sample site in the Exe estuary, Exmouth. This seawater was not filtered and so contained particulate and suspended matter which could have been used as a food source. However, the $\delta^{15}N$ value for this material (11.8) was higher than that of the diatom culture, which would appear to rule this out as a possible foodsource.

However, it should be borne in mind that it takes time for the isotopic composition of a mussel to change. When the mussels were collected from the estuary, large specimens were chosen because they usually contain the greatest number of adult *Mytilicola,* thereby speeding up the collection of copepod material. The nitrogen composition of the mussels would have reflected their diet in the estuary and it may take a long time for a new isotopic composition, following a new diet, to come to equilibrium. This is particularly true for older mussels which have a reduced growth rate and a longer turn-over time for tissue growth and cell renewal.

The result of this work strongly suggests that the parasite *Mytilicola* is feeding almost exclusively upon cellular detritus and mucus derived from the mussel host but also indicates that the food source for the mussel has not been indentified with certainty. However, there is a possible alternative explanation for the higher ^{15}N values found in *Mytilicola* than in *Mytilus,* which is nothing to do with differences in the trophic level of these two animals. If, when brought into the aquarium and presented with food (diatoms and/or suspended matter) with considerably higher ^{15}N content than the food utilized in the estuary, *Mytilicola* might incorporate the added ^{15}N more quickly than the mussel. Chance timing of specimen collection and analysis, may then have suggested that the average ^{15}N content of the parasite was approx. 3‰ higher than the average mussel concentration, just the right amount to indicate a difference in trophic level, whereas in fact they are merely random figures in passing from one equilibrium state to another. This explanation, which might be termed a differential isotopic memory effect, relies heavily upon coincidence but cannot be ruled out without a more careful monitoring of *Mytilicola/Mytilus* over a period of time, or alternatively, identifying with certainty the mussel's food source.

Finally, a comment on the unexpectedly high value of ^{15}N found in the cultured diatoms. The artificial seawater medium contained a negligible amount of ^{15}N and the sodium nitrate that was added as a nutrient had a $\delta^{15}N$ value of 2.7‰. It was not possible therefore, for this to be responsible for the high phytoplankton value (9.9‰). However, it was subsequently discovered that during the log-phase of the diatom culture's growth in Plymouth, a solution of Fison's Liquinure (a garden-type fertilizer) was added periodically to encourage the rapid growth of the culture. This extra nitrogenous compound is the only unaccounted for source of ^{15}N and so is most likely to be responsible for the greater than expected value of the phytoplankton. In the natural field environment, phytoplankton have been found to have $\delta^{15}N$ much lower than zooplankton and bivalves (Miyake & Wada 1967; Minagawa & Wada 1984; Checkley & Miller 1989; Montoya et al. 1990).

Acknowledgments

Grateful thanks are due to many: Phil and Jan Shears for help with specimen collection and dissection; Plymouth Marine Laboratory for diatom cultures, Dr. T. Harris and Prof. C. R. Kennedy for discussions and proof-reading, Brad Bagilhole for demystifying the intricacies of the TEX typesetting package, Dr. C. L. Van Dover for the initial impetus and finally, Dr. M. J. Attrill for inspired comments and support. This study was carried out with the aid of a N. E. R. C research studentship No. GT4/87/ALS/9 (to K. A. G).

Literature Cited

Barrie, A. & M. Lemley, 1989. Automated $^{15}N/^{13}C$ analysis of biological materials. *Int. Lab.*, 19:82-91.

Checkley, D. M. & C. A. Miller, 1989. Nitrogen isotope fractionation by oceanographic zooplankton. *Deep-Sea Res.*, 36:1449-1456.

Cole, H. A. & R. E. Savage, 1951. The effect of the parasitic copepod *Mytilicola intestinalis* (Steuer) upon the condition of mussels. *Parasitology*, 41:156-161.

Dare, P. J., 1985. The status of *Mytilicola intestinalis* Steuer as a pest of mussel fisheries: Harmful parasite or benign commensal? *Int. Coun. Exp. Sea*, CM. 1985/K:39, Copenhagen.

Davey, J. T., 1989. *Mytilicola intestinalis* (Copepoda, cyclopoida): A ten year survey of infested mussels in a Cornish estuary, 1978-1988. *J. mar. biol. Ass. U. K.*, 69:823-836.

Davey, J. T. & J. M. Gee, 1988. *Mytilicola intestinalis*, a copepod parasite of blue mussels, pp. 64-73. In *Disease Processes in Marine Bivalve Molluscs* (ed. Fisher, W. S.). Am. Fish. Soc., Spec. Pub. 18.

Davey, J. T., J. M. Gee & S. L. Moore, 1978. Population dynamics of *Mytilicola intestinalis* in *Mytilus edulis* in South-West England. *Mar. Biol.*, 45:319-327.

Deniro, M. J. & S. Epstein, 1981. Influence of diet on distribution of nitrogen isotopes, in animals. *Geoch. Cosmoch. Acta*, 45(3):341-351.

Dethlefsen, V., 1975. The influence of *Mytilicola intestinalis* (Steuer) on the meat content of the mussel *Mytilus edulis* (L.) *Aquaculture*, 6:83-97.

Galtsoff, P. S., 1964. *The American Oyster Crassostrea virginica Gmelin.* Fish and Wildlife Service Fishery Bulletin 64:(Chapter 10). Washington, D. C.

Gee, J. M., L. Maddock & J. T. Davey, 1977. The relationship between infestation by *Mytilicola intestinalis* Steuer (Copepoda, cyclopoida) and the condition index of *Mytilus edulis* in South-West England. *J. Cons. int. Explor. Mer*, 37:300-308.

Gresty, K. A., 1990. *The Feeding Biology of Mytilicola intestinalis Steuer, in the Mussel Host Mytilus edulis L., and Other Host-Parasite Relationships.* Ph. D Thesis, University of Exeter. England, 200pp.

Guisti, F., 1967. The action of *Mytilicola intestinalis* Steuer on *Mytilus galloprovincialis* Lam. of the Tuscan coast. *Rev. Parassitol.*, 28:17-26.

Harris, A. F. & E. A. Paul., 1989. Automated analysis of ^{15}N and ^{14}C in biological samples. *Comm. Soil Sci. Plant Anal.*, 20:935-947.

Korringa, P., 1951. Le *Mytilicola intestinalis* Steuer (Copepoda parasitica) menace l'industrie moulier en Zelande. *Rev. Trav. Inst. Peches Mar.*, 17:9-13.

Lauckner, G., 1983. Diseases of Mollusca: Bivalvia, pp. 477-961. In *Diseases of Marine Animals*, Marine Ecology Vol. 2 (ed. Kinne. O) Biologische Anstalt Helgoland, Hamburg.

Macko, S. J., M. L. F. Estep, M. H. Engel & P. E. Hare, 1986. Kinetic fractionation of stable nitrogen isotopes during amino-acid transamination. *Geoch. Cosmochi. Acta*, 50:2143-2146.

Mariotti, A., 1983. Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. *Nature*, 303:685-687.

Meyer, P. F. & H. Mann, 1951. Recherches allemandes relatives au *Mytilicola*, copepode parasite de la moule, existant dans les Watten Allemandes 1950-51. *Rev. Trav. Inst. Peches Mar.*, 17:63-74.

Minagawa, M. & E. Wada, 1984. Stepwise enrichment of ^{15}N and animal age. *Geochi. Cosmochi. Acta,* 48: 1135-1140.

Miyake, Y. & E. Wada, 1967. The abundance ratio of $^{15}N/^{14}N$ in marine environments. *Rec. oceanog. Works Japan,* 9:37-53.

Montoya, J. P., S. G. Horrigan & J. J. McCarthy, 1990. Natural abundance of ^{15}N in particulate nitrogen and zooplankton in the Chesapeake Bay. *Mar. Ecol. Prog. Ser.,* 65:35-61.

Moore, M. N., D. M. Lowe & J. M. Gee, 1978. Histopathological effects induced in *Mytilus edulis* by *Mytilicola intestinalis* and the histochemistry of the copepod intestinal cells. *J. Cons. int. Explor. Mer,* 38:6-11.

Peterson, B. J. & B. Fry, 1987. Stable isotopes in ecosystem studies. *An. Rev. Ecol. Systems,* 18:293-320.

Peterson, B. J., R. W. Howarth & R. H. Garritt, 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food web. *Science,* 227:1361-1363.

Rau, G. H., 1982. The relationship between trophic level and stable isotopes of carbon and nitrogen, pp. 143-148. In *Coastal Water Research Project, Biennial Report 1981-1982* (ed. Bascom, W) Southern California Coastal Water Research Project. Long Beach, California.

Schepers, J. S., D. D. Francis & M. T. Thompson, 1989. Simultaneous determination of total carbon, total nitrogen and ^{15}N on soil and plant material. *Comm. Soil Sci. Plant Anal.,* 20:949-959.

Shearer, G. & D. H. Kohl, 1986. N_2-fixation in field settings: estimation based on natural ^{15}N abundance. *Aust. J. Plant Physiol.,* 13:699-756.

Steele, K. W. & R. M. Daniel, 1978. Fractionation of nitrogen isotopes by animals: A further complication to the use of variation in the natural abundance of ^{15}N for tracer studies. *J. Ag. Sci.,* 90:7-9.

Theisen, B. F., 1966. *Mytilicola intestinalis* Steuer in Danish Waters. 1964-65. *Meddeleser fra Danmarks Fiskeri- og Havundersogelser,* 4:327-337.

Wada, E. & A. Hattori, 1976. Natural abundance of ^{15}N in particulate matter in the North Pacific ocean. *Geoch. Cosmoch. Acta,* 40:249-251.

Wada, E., M. Terazaki, Y. Kabaya & T. Nemoto, 1987. ^{15}N and ^{13}C abundances in the Antarctic ocean with emphasis on the biogeochemical structure of the food web. *Deep-Sea Res.,* 34:829-841.

Williams, C. S., 1969. The effect of *Mytilicola intestinalis* on the biochemical composition of mussels. *J. mar. biol. Ass., U. K.,* 49:161-173.

Van Dover, C. L., B. Fry., J. F. Grassle., S. Humphris & P. A. Rona, 1988. Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the mid-Atlantic ridge. *Mar. Biol.,* 98:209-216.