Symbiosis, 17 (1994) 1–13
Balaban, Philadelphia/Rehovot

Endosymbiotic Bacterial Contribution in the Carbon Nutrition of *Loripes lucinalis* (Mollusca: Bivalvia)

MONIQUE JOHNSON[1,*], MARCEL DIOURIS[2] and MARCEL LE PENNEC[1]
[1] *Laboratoire de Biologie Marine, URA CNRS 1513, Institut d'Etudes Marines, Université de Bretagne Occidentale, 29287 Brest Cedex*
Tel. 98 01 62 68, Fax 98 01 63 11
[2] *Laboratoire d'Ecophysiologie et de Biochimie des Algues Marines, Institut d'Etudes Marines, Université de Bretagne Occidentale, 29287 Brest Cedex*
Tel. 98 01 64 86, Fax 98 01 63 11

Received June 13, 1994; Accepted July 27, 1994

Abstract

The carbon isotope composition ($\delta^{13}C$) as well as the Ribulosebisphosphate carboxylase (RuBCase) activity in the mollusc bivalve *L. lucinalis* were measured monthly over a one year period. This bivalve harbours symbiotic chemoautotrophic bacteria in its gill cells. The gills represent 35% of the total body fresh weight of the bivalve and there are approximately 2×10^{10} bacteria per gram of gill tissue. Gill colour varied from beige to very dark brown and colour did not seem to be seasonally dependent. No obvious relationship could be seen between gill colour and RuBCase or $\delta^{13}C$ values although very dark gills systematically had low RuBCase levels (mean 0.15 ± 0.04 units/g). The carbon isotope ratios were compared to values obtained from bivalves which do not harbour symbiotic sulphur oxidizing bacteria. The $\delta^{13}C$ of these control animals ranges from -18.1 to -20.1‰ which reflect a phytoplanktonic dietary carbon source. The tissues of *L. lucinalis* are substantially more depleted in ^{13}C, with an annual mean ranging from -29.7 ± 0.5‰ to -27.1 ± 0.5‰ for the gill and foot tissues respectively. $\delta^{13}C$ values obtained from the gill are positively correlated with those of the foot. It is estimated that, on the average, 63% of the host carbon is provided by the symbionts. In addition, it would seem that this contribution increases during the months of January to May. Mean RuBCase activity throughout the year was 0.54 + 0.24 units/g gill wet weight with a range from

* The author to whom correspondence should be sent

0334-5114/94 /$05.50

0.02 to 2.1 units/g. There was at times extreme variability in the RuBCase enzyme activity within a given month. Several factors capable of influencing host isotopic composition are discussed.

Keywords: chemoautotrophic bacteria, endosymbionts, Lucinacea, carbon isotope composition, Ribulosebisphosphate carboxylase activity

1. Introduction

Recent works have led to the discovery of chemoautotrophic endocellular bacteria in the gill tissues of several benthic bivalve species (Fisher and Hand, 1984; Dando et al., 1985; Dando and Southward, 1986; Dando et al., 1986; Southward, 1986; Distel and Felbeck, 1987; Bouvy et al., 1989; Cary et al., 1989; Herry et al., 1989). These bacteria are mostly sulfur-oxidizing prokaryotes which fix CO_2 autotrophically by means of the Calvin and Benson cycle. Several ultrastructural studies have already provided information on the physiology of this association (Giere, 1985; Vetter, 1985; Southward, 1986; Distel and Felbeck, 1987; Herry and Le Pennec, 1987; Le Pennec et al., 1988; Herry et al., 1989; Conway et al., 1992) and histoautoradiographic research has shown that there is indeed transfer of organic matter fixed by the bacteria to several organs of the host bivalve (Fisher and Childress, 1986; Bouvy et al., 1989; Herry et al., 1989). A trophic function for the bacteria in these associations has therefore already been confirmed. The use of histoautoradiography, however, can only provide qualitative information and necessitates the incubation of whole animals or of animal tissue in filtered seawater which is far from being a natural environment. To evaluate and quantify the importance of the symbiosis in the nutritional strategy of the host bivalve, measurement of the carbon isotope composition represents a more valid approach. Indeed, the carbon isotope composition ($\delta^{13}C$) provides an indication as to the origin of the nutritional carbon. During the enzymatic fixation of carbon dioxide by plants and bacteria, there is varying discrimination against ^{13}C and the organic matter formed is thus depleted in ^{13}C. Stable isotope ratios of species containing symbiotic bacteria investigated so far show unusually low $^{13}C/^{12}C$ ratios, suggesting bacterial carbon contributions to the host.

Loripes lucinalis is a littoral bivalve and is the most common Lucinacea to be found on the coast of Brittany (Herry, 1988). It lives in reduced sediments characterized by a blackish colour. Previous studies have already confirmed the existence of endocellular sulfur-oxidizing bacteria within the gill tissues (Herry et al., 1989; Diouris et al., 1988). The aim of the present study is to examine more closely the role of these chemoautotrophic bacteria in the diet of *Loripes lucinalis*. To this end, the carbon isotope composition as well as the

Ribulosebisphosphate carboxylase activity, a diagnostic enzyme of the Calvin-Benson cycle, of this lucinid was measured over a one year period. The $\delta^{13}C$ values for *Loripes* are compared with values obtained in control bivalves which do not harbour endosymbiotic bacteria. In addition, the $\delta^{13}C$ of the foot and gill tissues for each animal collected are compared and the contribution of the endosymbiotic bacteria in the carbon nutrition of *Loripes lucinalis* is estimated. The possible role of factors such as temperature and RuBCase activity in the isotopic fluctuations are then evaluated.

2. Materials and Methods

Loripes lucinalis specimens were sampled monthly from September 1991 to September 1992 from the Moulin Blanc Beach in the Bay of Brest at low tide (tidal coefficient > 90). The sediment was sifted through 1.5 mm mesh and the specimens recovered were placed in sea-water for their transport to the laboratory. Of the control bivalves not harbouring chemoautotrophic bacteria, only *Tapes decussatus* was sampled from the Moulin Blanc site. The other controls, *Abra tenuis* and *Tellina tenuis*, were sampled from neighbouring sites in the Bay of Brest, namely from Crozon and from a site in close proximity to the pleasure boat port.

The animals were dissected shortly following their arrival in the laboratory. The gills and the foot were removed with great care to prevent contamination of these organs by the digestive gland. The gill colour was evaluated on a scale from light beige to very dark brown and a number was assigned between 1 and 5 (5 representing a very dark brown colour). The tissues were then weighed and frozen (−80°C) until further analysis. To estimate $\delta^{13}C$ values, the samples were soaked in 1 M HCl to remove any residual carbonate and then lyophilized. The $^{13}C/^{12}C$ ratios were obtained using a mass-spectrometer coupled to a gas chromatograph and a combustion oven. The values were calculated in the following manner:

$$\delta^{13}C = \frac{^{13}C/^{12}C \text{ sample} - {}^{13}C/^{12}C \text{ standard}}{1000(‰)} \times {}^{13}C/^{12}C \text{ standard}$$

The classical Pee Dee Belemnite standard was used.

For Ribulosebisphosphate carboxylase (RuBCase) analysis, 200 μl of homogenizing buffer (0.3 M Tris-EDTA, pH 8) were added to the gills which were subsequently ground on ice for 45 sec with a hand-held grinder. Following grinding, the blade was rinsed with 100 μl of the homogenizing buffer. The mixture was then sonicated by 4 blasts of 15 sec and centrifuged for 10 min at 12 U/min at 0°C. RuBCase was then assayed in the manner described by

Diouris et al. (1988) with the exception that incubations were carried out at 25°C. Gills from control animals were assayed in the same manner.

In order to estimate the number of bacteria per gram of gill tissue, the gills of five individuals were weighed and subsequently ground in 10 ml of filtered sea-water (0.2 μm). A 0.5 ml volume of this mixture was diluted in filtered sea-water and incubated for one hour with the fluorescent dye Hoechst 33258 (Bisbenzimide, Sigma Chemical Corp., B-2883) at a final concentration of 20 μg/ml. Following incubation, the sample-dye mixture was vacuum filtered onto a 0.2 μm black Nuclepore filter. Filters were then mounted in immersion oil and examined with an Olympus BH-2 epifluorescence microscope equipped with an ocular micrometer. For all samples, at least 20 fields were counted spanning the entire diameter of the filter.

3. Results

L. lucinalis is a small bivalve with a shell that rarely exceeds 1.5 cm in length. The visceral mass is approximately 200 mg. The gill tissue accounts for, on average, $35 \pm 5.8\%$ of the total bivalve mass with a percentage that varies between 24 and 48%. There are approximately $2 \pm 0.1 \times 10^{10}$ bacteria per gram gill tissue. These bacteria are extremely pleomorphic. Some are spherical or oval, of small to medium size, measuring from 0.7 to 4.7 μm in diameter (mean 2.7 ± 0.85 μm). Others are rod-shaped and have an average length and width of 4.4 ± 1.6 and 2.5 ± 0.8 μm respectively. These rods can on occasion reach a length of 9 μm.

The evaluation of gill colour throughout the sampling year reveals that colour varied widely between individuals and did not seem to depend on the season. Similarly, no obvious correlation could be seen between gill colour and RuBCase or δ^{13}C values. It should be noted, however, that very dark gills systematically had low RuBCase levels (mean 0.15 ± 0.04 units/g, n=6).

The results obtained from δ^{13}C analysis of gill and foot tissues of *L. lucinalis* as well as values obtained for control bivalves which do not harbour chemoautotrophic bacteria are given in Table 1. The average $^{13}C/^{12}C$ values for the foot of control animals is $-18.7‰$. δ^{13}C values for *L. lucinalis* are more negative than control values, being $-29.7‰$ for the gill and $-27.1‰$ for the foot.

The δ^{13}C values of the foot and gill of each animal were compared to determine the isotopic differences between the gill tissue and the rest of the body. Figure 1 shows that there is a significant positive linear correlation between the gill δ^{13}C values and the organic isotopic signature of the rest of the body (r=0.84, p=0.05, n=34).

Table 1. Comparison of $\delta^{13}C$ values obtained for *Loripes lucinalis* and three bivalve species lacking chemoautotrophic bacteria

Species	$^{13}C(‰)$	
	Gill	Foot
Abra tenuis	−20.1	−19.4
Tellina tenuis	–	−18.8
Tapes decussatus	−20.0	−18.1
Loripes lucinalis	−29.7	−27.1

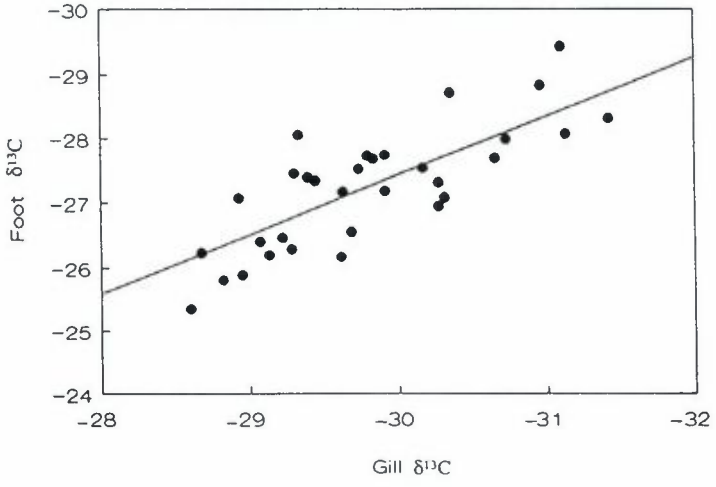

Figure 1. Relationship between foot and gill $\delta^{13}C$ values for *Loripes lucinalis*. The line drawn represents the equation $y = 0.92x + 0.14$.

Figure 2 represents the $\delta^{13}C$ values of the foot throughout the year-long sampling period. A slight increase in $\delta^{13}C$ values for the foot tissue is observed between January and May.

The contribution of bacterial autotrophically derived carbon to the diet of *L. lucinalis* was also estimated. A $\delta^{13}C$ value of −18.7‰, which represents the mean $\delta^{13}C$ foot value for control animals (Table 1), was used to represent the $\delta^{13}C$ value of an animal feeding heterotrophically on a phytoplankton derived carbon source. For the $\delta^{13}C$ value of the bacteria, a value of −31.42‰ was adopted, as this is the most negative value obtained for the gill tissue. Our calculations estimate that, on the average, 63% of the carbon nutrition of *L. lucinalis* is provided by the chemoautotrophic bacteria. Figure 2 also represents

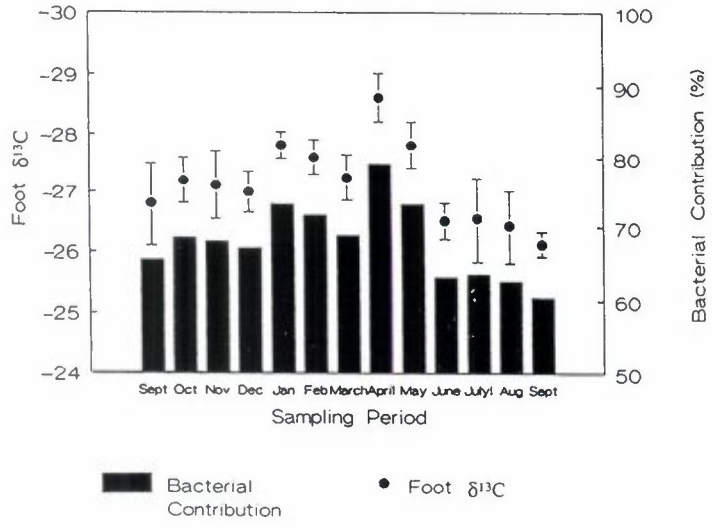

Figure 2. Mean $\delta^{13}C$ values (± standard deviation) for foot tissue as well as chemoautotrophic bacterial contribution in the carbon diet of *Loripes lucinalis* over a one year sampling period.

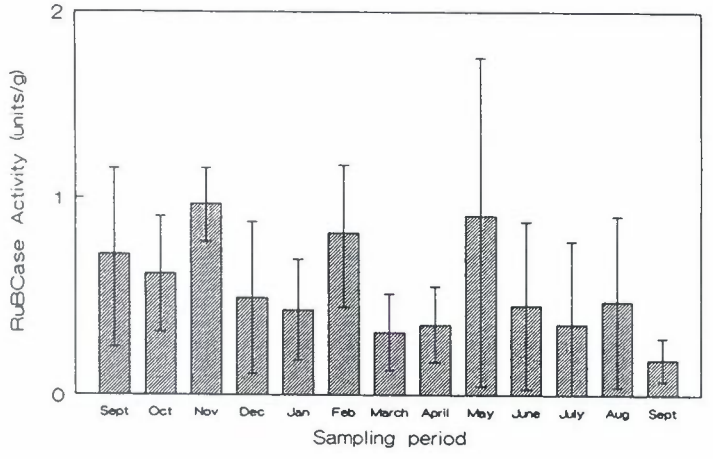

Figure 3. Mean Ribulosebisphosphate carboxylase (RuBCase) activity (± standard deviation) in the gill tissue of *Loripes lucinalis* over a one year sampling period where a unit of activity represents 1 μmole substrate converted to CO^2 min^{-1}.

the estimated bacterial contribution over a one year period. A slight increase in the bacterial contribution between the months of January and May can be observed, which reaches a maximum of 78% for the month of April.

Mean RuBCase activity throughout the year was 0.54 ± 0.24 units/g gill wet weight with a range from 0.02 to 2.1 units/g. Control animals showed zero RuBCase activity. Figure 3 shows that there was at times extreme variability in the RuBCase enzyme levels within any given month.

4. Discussion

In *L. lucinalis*, as in other littoral Lucinacea, the gill is adapted to harbour sulfur-oxidizing bacteria within specialized cells termed bacteriocytes. These bacteriocytes make up the majority of the gill epithelial cells (Herry et al., 1989; Diouris et al., 1988). The presence of endosymbiotic bacteria seems linked to an increase in gill size, which represents the largest organ in this bivalve. Indeed, the gill accounts for $35 \pm 6\%$ of the visceral mass. This is comparable to values found for other lucinids by Dando and Southward (1986) who observed gill contributions of $38.7 \pm 4.5\%$ and $30.6 \pm 5.6\%$ of the visceral masses of *Thyasira sarsi* and *Thyasira flexuosa* respectively. For *Lucinoma borealis*, a value of $28.06 \pm 4.39\%$ was reported (Dando et al., 1986) and for *Lucinoma aequizonata*, $35 \pm 3\%$ (Distel and Felbeck, 1987). These values are particularly indicative when compared to those found for *Mytilus edulis* where the gill accounts for only 10% of the bivalve's total mass (Distel and Felbeck, 1987).

Within the bacteriocytes, the envacuolated bacteria almost completely fill up the cell. Bacterial counts reveal that there are approximately $2 \pm 0.1 \times 10^{10}$ bacteria per gram gill tissue, which represents an important colonisation of the gill when compared to *Solemya velum* for which $1.2 \pm 0.4 \times 10^{9}$ bacteria per gram gill tissue have been reported (Cavanaugh, 1983).

A large range in the colour of the gills seems common in lucinids. Several authors have reported that there exists a distinct correlation between colour and sulfur content of the gills (Dando et al., 1985; Vetter, 1985; Cary et al., 1989) although such a correlation may not be systematically found for all lucinid species (Dando et al., 1986). Bivalves with yellow gills due to the presence of elemental sulfur are considered to be in good health while those with black gills are considered to be in poor condition (Cary et al., 1989). Elemental sulfur is assumed to be a form of energy storage for the bacteria and therefore indicates the "condition" of the animal/bacteria symbiosis (Dando et al., 1985). In the present study, gill colour varied between individuals and did not seem to depend on season, RuBCase or $\delta^{13}C$ values. Of interest is the fact that very

dark gills systematically had very low RuBCase activities. This would seem to indicate that these animals had a limited potential for bacterial chemoautotrophy. Cary et al. (1989) reported that black gills contain few symbionts. It is interesting to postulate that very dark gills indicate that the host bivalve is actively digesting its bacterial symbionts due to some endogenous metabolite requirement (e.g. gametogenesis) and that the resulting black colour is due to the increased presence of the dark residual bodies described in a great many symbioses and which are generally believed to represent lysed bacteria (Dando et al., 1986; Southward, 1986; Distel and Felbeck, 1987; Le Pennec et al., 1988). Extensive bacterial lysis would greatly affect RuBCase enzyme activity while not altering the $\delta^{13}C$ values which is consistent with the results of this study.

The $\delta^{13}C$ values obtained for the control bivalves which do not harbour chemoautotrophic bacteria can be considered to be representative of a phytoplankton-based diet. Indeed, planktonic photoautotrophy from middle latitudes produces $\delta^{13}C$ values between -18 and $-23‰$ (Spiro et al., 1986). The mean of $-18.7‰$ found in the present study is comparable to control values cited elsewhere (Dando and Spiro, 1993). The $\delta^{13}C$ values for *L. lucinalis* are substantially more depleted in ^{13}C than the control values which indicates a preferential incorporation of ^{13}C and, therefore, an important chemoautotrophic contribution to the carbon nutrition of the host. These values are comparable to those found for other Lucinacea (Table 2) and are always lower for the gill than for the rest of the body with a difference in $\delta^{13}C$ between gill and non-gill tissues which varies depending on the species. The almost identical values obtained from gill and body tissues for *Codakia orbicularis* (Berg and Alatalo, 1984) and *Myrtea spinifera* (Spiro et al., 1986) indicate qualitatively that most of the nutritional needs of the bivalves are provided by the chemoautotrophic endosymbionts. For *L. lucinalis*, the difference in $\delta^{13}C$ of 2.6‰ between the gill and the foot suggests the participation of an additional trophic resource in the carbon diet of *Loripes*. These results are not surprising in light of the mixotrophic diet attributed to lucinids which possess a functional, though reduced, digestive system (Allen, 1958). Indeed, particles of phytoplanktonic origin, including diatoms, are often present in the stomach lumen (Le Pennec et al, 1988).

A comparison of the $\delta^{13}C$ values for the different tissues from each animal allows us to evaluate the effectiveness with which chemoautotrophically derived carbon is transported from the gill to the remaining body parts. The significant positive correlation observed indicates that an increase in gill $\delta^{13}C$ leads to a proportional increase in the non-gill tissues. The close association between the gill harbouring the symbionts and the isotopic carbon signature of the rest of

Table 2. $\delta^{13}C$ values for *Loripes lucinalis* and published values for several other Lucinacea

Species	Gill tissue	Non-gill tissue	Difference
Loripes lucinalis	−29.7	−27.1	2.6
Lucinoma borealis (Spiro et al., 1986)	−28.5	−25.6	2.9
	−28.8	−24.1	4.7
Lucinoma borealis (Dando et al., 1986)	–	−28.1 to −29.0	–
Lucinoma aequizonata (Cary et al., 1989)	–	−29	–
Thyasira flexuosa (Dando and Southward, 1986)	–	−29.3	–
Thyasira sarsi (Dando and Spiro, 1993)	–	−25.4	–
Thyasira sarsi (Spiro et al., 1986)	−31	−28.2	2.8
Codakia orbicularis (Berg and Alatalo, 1984)	−23.9	−23.2 to −23.8	0.4
Myrtea spinifera (Spiro et al., 1986)	−24.2	−23.4	0.8

the body further emphasizes the trophic importance of the chemoautotrophic bacteria in this bacteria-bivalve association.

One of the criticized aspects of this technique in the estimation of nutritive carbon is that dissolved organic matter may contribute to the isotopic signature of the host. Indeed, dissolved amino acids of chemosynthetic origin can be liberated from sediment and contribute to bivalve nutrition (Degens, 1969). It has also been suggested that the assimilation of organic particles of photoautotrophic origin may take place within the digestive gland. Although the utilization of these trophic resources cannot be excluded, the close link between the $\delta^{13}C$ of the gill and the foot suggests that the transfer of molecules elaborated by the gill bacteria represent the most important factor determining the isotopic signature of *L. lucinalis*.

An estimation of the bacterial contribution to the host's carbon nutrition was made. For the bacterial $\delta^{13}C$, a value of −31.42‰ was adopted, this value corresponding to the most negative value obtained for the gill. This value, obtained from a gill tissue/bacteria mixture, is therefore a maximum value but remains comparable to the values reported in other studies: from −31.7 to

−33.6‰ for the isolated symbionts of *Solemya velum* (Conway et al., 1989), and −34±0.8‰ for *Lucinoma aequizonata* (Cary et al., 1989). For this last species, the authors estimated that at least 25% of the bivalve's organic matter did not come from the symbionts. More recently, Dando and Spiro (1993) used a $\delta^{13}C$ value of −32.8‰ to estimate that a population of *Thyasira sarsi* obtained between 26 and 76% of its organic input via their chemoautotrophic bacteria. According to our calculations, approximately 63% of the carbon nutrition of *L. lucinalis* is provided by the endosymbiotic bacteria.

If we consider the variations in $\delta^{13}C$ throughout the year, we observe a slight increase in the bacterial contribution between January and May, which reaches a maximum of 78% in April. It is during this same period, from February to May, that important gonad development is observed. A complete spawn was recorded in May (Johnson and Le Pennec, 1994).

It is difficult to determine the reasons behind this spring increase in $\delta^{13}C$, as several factors can affect the observed organic composition of an animal. These factors include bacterial metabolism, carbon source, environmental temperature, and the level of substrate availability (Kennicutt et al., 1992). Temperature could play a role in the present study, as bacteria will preferentially utilize ^{13}C at lower temperatures, as has been shown for phytoplanktonic species (Sackett et al., 1965). As previously described (Johnson and Le Pennec, 1994), it is between December and May that sediment temperatures at the Moulin Blanc Beach are the lowest.

To evaluate bacterial metabolism, the activity of RuBCase, a key enzyme in the Calvin-Benson cycle of CO_2 fixation, was measured. Fisher and Childress (1986) observed that specimens of *Solemya reidi* had an average RuBCase activity of 0.68 μmole min^{-1} g^{-1} wet weight. This activity dropped by over 90% when animals were kept under adverse conditions for an extended period of time. It would seem logical that similar decreases in enzyme activities would occur *in situ* due to changing environmental conditions and physiological status of the animal. Mean RuBCase activity throughout the sampling period of the present study was 0.54 units g^{-1} gill wet weight (units=μ mole substrate converted to CO^2 min^{-1}) which is similar to values found elsewhere. For example, Bouvy et al. (1989) found RuBCase activity to be, on average, 0.38 μmoles g^{-1} min^{-1}. The values found for a hydrothermal vent gastropod were reported to be 0.2 ± 0.02 μmole min^{-1} g^{-1} wet weight (Stein et al., 1988). Other reported values, however, are lower than average value of the present study. For example, *Myrtea spinifera* was observed to have an average RuBCase activity of 0.037 μmole g^{-1} min^{-1} (Dando et al., 1985). In addition, a previous study involving *Loripes lucinalis* estimated RuBCase activity to be only 0.046 μmole min^{-1} g^{-1} wet weight (Diouris, 1988). These differences could result from the

different incubation temperatures in addition to interspecies variations but could also stem from the fact that enzyme activity levels vary widely from one individual to the next. Indeed, the RuBCase activity range of the present study was from 0.02 to 2.1 units g^{-1} wet weight, this latter value being one of the highest RuBCase activity levels recorded for a bacterial/bivalve symbiosis to date. Although the RuBCase activity fluctuated from month to month, it did not seem to be correlated with the seasons, and no obvious relationship was seen between these activity levels and the $\delta^{13}C$ values.

The levels of available sulfur in the sediment should also be considered as they will directly affect the bacterial endosymbionts and, therefore, the hosts as well. The results of a recent 5 year study show that substantial variations in $\delta^{13}C$ can be observed and, in extreme cases, the depletion of sediment sulfur levels can cause the crash of Thyasiridae populations (Dando and Spiro, 1993). The results of the present study show that feeble variations in the $^{13}C/^{12}C$ ratio do occur and these could be linked to the dependence of the symbiotic bacteria to sediment sulfur levels. A lack of sulfur could lead to an increased dependence on heterotrophic nutrition which would have repercussions on the isotopic signature of the host.

In addition, although there are a multitude of papers dealing with the symbiotic bacteria/bivalve interactions, data on the metabolism of the bacteria themselves are fragmentary due mainly to the limited success in culturing the symbionts (Wood and Kelly, 1989a, Wood and Kelly, 1989b). It is evident that increased knowledge of the symbiont's metabolic capabilities is necessary if we are to fully understand the isotopic fluctuations of the host bivalve tissues.

Acknowledgements

We would like to thank N. Casse, A. Marhic, S. Plana, A. Johnson and A. Talarmin for their help in sample collection. We are also indebted to Mrs. Pachiaudi for her technical assistance in $\delta^{13}C$ analysis.

REFERENCES

Allen, J.A. 1958. On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). *Phil. Trans. R. Soc. Lond. B.* **241:** 421–484.

Berg, C.J. and Alatalo, P. 1984. Potential of chemosynthesis in molluscan mariculture. *Aquaculture* **39:** 165–179.

Bouvy, M., Soyer, J., Cahet, G., Descolas-Gros, C., Thiriot-Quiévreux, C., and Soyer-Gobillard, M.O. 1989. Chemoautotrophic metabolism of intracellular gill bacteria in the marine bivalve *Spisula subtruncata* (Da Costa). *Neth. J. Sea Res.* **23(1):** 29–34.

Cary, S.C., Vetter, R.D., and Felbeck, H. 1989. Habitat characterization and nutritional strategies of the endosymbiont-bearing bivalve *Lucinoma aequizonata*. *Mar. Ecol. Prog. Ser.* **55:** 31–45.

Cavanaugh, C.M. 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphide-rich habitats. *Nature* **302(5903):** 58–61.

Conway, N.M., Howes, B.L., McDowell Capuzzo, J., Turner, R.D., and Cavanaugh, C.M. 1992. Characterization and site description of *Solemya borealis* (Bivalvia; Solemyidae), another bivalve-bacteria symbiosis. *Mar. Biol.* **112:** 601–613.

Conway, N.M., McDowell Capuzzo, J., and Fry, B. 1989. The role of endosymbiotic bacteria in the nutrition of *Solemya velum:* Evidence from a stable isotope analysis of endosymbionts and host. *Limnol. Oceanogr.* **34:** 249–255.

Dando, P.R. and Southward, A.J. 1986. Chemoautotrophy in bivalve molluscs of the genus *Thyasira*. *J. Mar. Biol. Ass. U.K.* **66:** 915–929.

Dando, P.R., Southward, A.J., and Southward, E.C. 1986. Chemoautotrophic symbionts in the gills of the bivalve mollusc *Lucinoma borealis* and the sediment chemistry of its habitat. *Proc. R. Soc. Lond. B.* **227:** 227–247.

Dando, P.R., Southward, A.J., Southward, E.C., Terwilliger, N.B., and Terwilliger, R.C. 1985. Sulphur-oxidizing bacteria and haemoglobin in gills of the bivalve mollusc *Myrtea spinifera*. *Mar. Ecol. Prog. Ser.* **23:** 85–98.

Dando, P.R. and Spiro, B. 1993. Varying nutritional dependence of the thyasirid bivalves *Thyasira sarsi* and *Thyasira equalis* on chemoautotrophic symbiotic bacteria, demonstrated by isotope ratios of tissue carbon and shell carbonate. *Mar. Ecol. Prog. Ser.* **92:** 151–158.

Degens, E.T. 1969. Biogeochemistry of stable isotopes. In: *Organic Geochemistry Methods and Results*. G. Eglington and M.T.G. Murphy, eds. Springer, Berlin, pp. 304–329.

Diouris, M., Moraga, D., Le Pennec, M., Herry, A., and Donval, A. 1988. Chimioautotrophie et nutrition chez les Lucinacea, bivalves littoraux de milieux réducteurs. I. Activités enzymatiques des bactéries chimioautotrophes associées aux branchies. *Haliotis* **18:** 195–205.

Distel, D.L. and Felbeck, H. 1987. Endosymbiosis in the lucinid clams *Lucinoma aequizonata*, *Lucinoma annulata* and *Lucina floridana:* a reexamination of the functional morphology of the gills as bacteria-bearing organs. *Mar. Biol.* **96:** 79–86.

Fisher, M.R. and Hand, S.C. 1984. Chemoautotrophic symbionts in the bivalve *Lucina floridana* from seagrass beds. *Biol. Bull.* **167:** 445–459.

Fisher, C.R. and Childress, J.J. 1986. Translocation of fixed carbon from symbiotic bacteria to host tissue in the gutless bivalve *Solemya reidi*. *Mar. Biol.* **93:** 59–68.

Giere, O. 1985. Structure and position of bacterial endosymbionts in the gill filaments of Lucinidae from Bermuda (Mollusca, Bivalvia). *Zoomorphology* **105:** 296–301.

Herry, A. 1988. Chimioautotrophie bactérienne dans la branchie de quarte espèces de Lucinacea (Bivalvia). Thèse 3e cycle, U.B.O., Brest: 10.

Herry, A., Diouris, M., and Le Pennec, M. 1989. Chemoautotrophic symbionts and translocation of fixed carbon from bacteria to host tissues in the littoral bivalve *Loripes lucinalis* (Lucinidae). *Mar. Biol.* **101:** 305–312.

Herry, A. and Le Pennec, M. 1987. Ultrastructure of the gonad of a deep hydrothermal vent mytilid from the East Pacific Rise. *Haliotis* **16:** 295–307.

Johnson, M.A. and Le Pennec, M. 1994. The development of the female gamete in the endosymbiont-bearing bivalve *Loripes lucinalis. J. Mar. Biol. Ass. U.K.* **74:** 233–242.

Kennicutt II, M.C., Burke, R.A., MacDonald, I.R., Brooks, J.M., Denoux, G.J., and Macko, S.A. 1992. Stable isotope partitioning in seep and vent organisms: chemical and ecological significance. *Chem. Geol. (Isot. Geosci. Sect.)* **101:** 293–310.

Le Pennec, M., Herry, A., Diouris, M., Moraga, D., and Donval, A. 1988. Chimioautotrophie et nutrition chez les Lucinacea, bivalves littoraux de milieux réducteurs. II. Caractéristiques morphologiques des bactéries symbiotiques et modifications structurales adaptatives des branchies de l'hôte. *Haliotis* **18:** 207–217.

Sackett, J.H., Eckelmann, W.R., Bender, M.L., and Bé, A.H.W. 1965. Temperature dependence of carbon isotope composition in marine plankton and sediments. *Science* **148:** 235–237.

Southward, E.C. 1986. Gill symbionts in Thyasirids and other bivalve molluscs. *J. Mar. Biol. Ass. U.K.* **66:** 889–914.

Stein, J.L., Craig Cary, S., Hessler, R.R., Ohta, S., Vetter, R.D., Childress, J.J., and Felbeck, H. 1988. Chemoautotrophic symbiosis in a hydrothermal vent gastropod. *Biol. Bull.* **174:** 373–378.

Spiro, B., Greenwood, P.B., Southward, A.J., and Dando, P.R. 1986. $^{13}C/^{12}C$ ratios in marine invertebrates from reducing sediments: confirmation of nutritional importance of chemoautotrophic endosymbiotic bacteria. *Mar. Ecol. Prog. Ser.* **28:** 233–240.

Vetter, R.D. 1985. Elemental sulfur in the gills of three species of clams containing chemoautotrophic symbiotic bacteria: a possible inorganic energy storage compound. *Mar. Biol.* **88:** 33–42.

Wood, A.P. and Kelly, D.P. 1989a. Isolation and physiological characterisation of *Thiobacillus thyasiris* sp. nov., a novel marine facultative autotroph and the putative symbiont of *Thyasira flexuosa. Arch. Microbiol.* **152:** 160–166.

Wood, A.P. and Kelly, D.P. 1989b. Methylotrophic and autotrophic bacteria isolated from lucinid and thyasirid bivalves containing symbiotic bacteria in their gills. *J. Mar. Biol. Ass. U.K.* **69:** 165–179.