Mar Biol (2009) 156:611–618
DOI 10.1007/s00227-008-1112-y

ORIGINAL PAPER

Stable isotopes changes in the adductor muscle of diseased bivalve *Ruditapes philippinarum*

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Received: 6 November 2008 / Accepted: 4 December 2008 / Published online: 24 December 2008

Abstract In this article, we show how a disease could bias stable isotope analyzes of trophic networks and propose a strategy in the choice of tissues to be analyzed. In the past few years, a new pathology (brown muscle disease or BMD) affecting the posterior adductor muscle of *Ruditapes philippinarum* has emerged in Arcachon Bay. BMD induces a necrosis of muscle tissues which become infused by conchiolin and hence calcified. As muscle of mollusks are often used for trophic food webs studies through stable isotopic analyzes, this work investigated the effect of BMD on carbon and nitrogen isotopic ratios of anterior and posterior adductor muscles of clams collected in February and August 2007. Infected clams displayed a lower condition index and a posterior adductor muscle $\delta^{13}C$ enrichment of 1.2‰ in February and 0.7‰ in August. $\delta^{15}N$ of posterior muscles was however not affected by the disease. Anterior muscle of diseased clams remained healthy and displayed the same isotopic signature as both posterior and anterior muscular tissues of healthy clam. Acidification significantly depleted $\delta^{13}C$ in posterior muscles of infected clams, suggesting calcification, contrary to anterior muscles of infected clam and to both muscles of healthy clams, where no effect was observed. An X-ray diffractometry analysis confirmed the presence of $CaCO_3$ (aragonite). Trophic food web studies relying on stable isotope ratios should utilize only healthy animals or anterior adductor muscles when expertise in mollusk pathology is lacking.

Communicated by U. Sommer.

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Introduction

The Manila clam *Ruditapes philippinarum* (Adams and Reeve 1850) originates from Indo–Pacific waters and nowadays contributes to more than half of global yields of clams. Since 1930, Manila clam has been introduced with Pacific oyster (*Crassostrea gigas*) seed into different parts of the world, e.g., from the United States to Canada and to the Hawaiian islands (Flassch and Leborgne 1992). In Europe, *R. philippinarum* was primarily introduced into France for culture purposes in 1972 and later to England, Spain and Italy (Flassch and Leborgne 1992). Within a few years, the species established natural populations in most southern European countries and particularly along the French Atlantic coast. In Arcachon Bay (SW France), *R. philippinarum* was first introduced in 1980 as a

commercially attractive species. It rapidly escaped from clam-growing areas and colonized intertidal sea-grass beds (*Zostera noltii*). Today, *R. philippinarum* undergoes intensive exploitation by local fishermen and has a relatively high economic value.

Since its introduction to France, *R. philippinarum* has endured several infectious pathologies such as the brown ring disease (BRD) caused by the bacterium *Vibrio tapetis* (Paillard 2004), perkinsosis induced by the protozoan *Perkinsus* sp. (Lassalle et al. 2007), and to a lesser extent, trematode infections (Lassalle et al. 2007). Recently, a presumptive viral pathology called the brown muscle disease (BMD) has occurred in Manila clam populations from localities of Arcachon Bay (Dang et al. 2008a; 2008b). The anterior adductor muscle never exhibits macroscopic signs of the disease (Dang et al. 2008a).

Stable isotope analyzes (SIA) are widely used to investigate dietary pattern and trophic relationships in the marine environment (Peterson and Fry 1987). δ^{13}C values help in identifying primary food sources assimilated by consumers (Fry and Sherr 1984) whereas δ^{15}N values allow determination of their trophic levels (DeNiro and Epstein 1981). SIA have also recently been used to characterize host-parasite systems and provide a means of exploring relationships between a host and its parasites (Deudero et al. 2002).

The objectives of this study were to investigate the effects of the BMD on the stable isotopic carbon and nitrogen ratios of adductor muscles, of Manila clams. Condition index and SIA are expected to provide complementary information on possible metabolic disturbances within muscles, as anterior and posterior adductor muscles of healthy and BMD-infected clams were comparatively analyzed by SIA. The idea was to compare stable isotopic signatures of infected muscle (posterior) with anterior muscle which never develops macroscopic signs of BMD in order to assess in what extent δ^{13}C and δ^{15}N values are affected by the disease and could lead to shortcomings in term of trophic relationships interpretations. The possibility that muscle was calcified by the disease was also investigated in order to test the possibility to acidify tissues prior to SIA.

Materials and methods

Sampling strategy and clams treatment

Specimens of *R. philippinarum* were collected at Lanton, an intertidal area of Arcachon Bay, which is located on the southwestern Atlantic coast of France. Manila clams were then selected at different macroscopic stages of the BMD: healthy (Fig. 1a), intermediate and advanced (Fig. 1b)

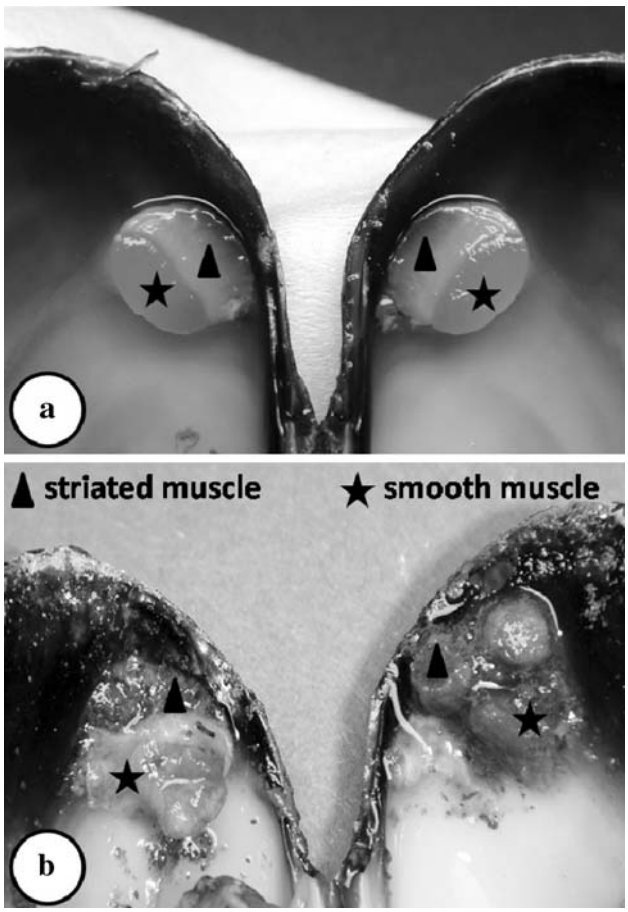

Fig. 1 Naked-eye observations of healthy (FDI = 0) (**a**) and diseased (FDI = d) (**b**) posterior adductor muscle (*striated* and *smooth muscles*) of *Ruditapes philippinarum*

(Dang et al. 2008a). At the intermediate stage, the striated part of the posterior adductor muscle was more severely infected than the smooth part. The adductor muscle attachment was reduced by the presence of brown spots which enlarged (initial calcification). The advanced stage of BMD displays a quasi-complete brown calcification of the striated muscle like the major part of the smooth muscle.

Ten healthy and ten diseased clams (30–36 mm shell length) were sampled in February 2007. Diseased clams were only collected in the intermediate stage of BMD. Posterior adductor muscles were dissected and freeze-dried for 48 h. According to the preliminary results obtained in February, ten healthy and ten diseased clams of the same shell length were sampled at the same location in August 2007 and both anterior and posterior adductor muscles were analyzed. Infection was quantified in clams in advanced stages of BMD in August by the final disease index (FDI) proposed by Dang et al. (2008a) from 0 (no infection observed) to 16 (stage d, fully infected). Clams were opened, their FDI evaluated and their posterior and

anterior muscles dissected, freeze-dried and weighed. Dry weights (mg) of the remaining tissues together with dry weights of their shells (g) were utilized for the calculation of the condition index (CI). The condition index is reported as CI = dry flesh weight (mg)/dry shell weight (g).

Isotopic and elemental analysis

Samples of clam muscle tissues were ground to a homogeneous fine powder and 1 mg was folded into tin cups (9-mm height, 5-mm diameter). Stable-isotope ratios measurements were performed on a Carlo Erba 2500 elemental analyzer in line with a VG Isoprime. The analytical precision was 0.2‰ for both δ^{13}C and δ^{15}N, estimated from several calibrated laboratory standards analyzed along with the samples. Replicates of February samples were performed to confirm the analytical reproducibility that was within 0.2‰. Stable isotopic ratios are reported as

$$\delta^{A}\mathrm{X} = [(R_{\mathrm{sample}}/R_{\mathrm{ref}}) - 1] \times 1,000$$
$$w\delta\mathrm{X} = (R_{\mathrm{sample}}/ - 1) \times 1,000$$

where A is the atomic mass of the heavy stable isotope of the element X, and R_{sample} and R_{ref} are the ratios of heavy to light isotope of sample and reference, respectively. References are atmospheric nitrogen for δ^{15}N and Vienna Pee Dee Belemnita (PDB) for δ^{13}C. Organic carbon and nitrogen contents were measured simultaneously with the stable isotopic ratios by integrating the voltage of the main ion beam (IRMS).

According to the extreme hardness of diseased posterior adductor muscles and following preliminary positive tests with HCl, the presence of calcification and therefore inorganic carbon was expected to occur. In many ecological studies, it has been common practice to acidify samples to remove inorganic carbonates which are less negative in δ^{13}C than other fractions (DeNiro and Epstein 1978).

To confirm this hypothesis and to exclusively evaluate the δ^{13}C of organic carbon in muscle tissue, all February and August samples were decarbonated by acidification for a second carbon isotopic analysis: 1.3 mg of powder was deposited into silver cups and acidified by adding 1 M HCl drop by drop until the cessation of bubbling. At last, 140 µL was added and samples were left for 4 h in acid. Then, they were again dehydrated and freeze-dried for 48 h. Both carbon isotope ratios and carbon contents were measured, as previously described.

Powder X-ray diffractometry (XRD)

Powder XRD is a convenient, non-destructive tool used to differentiate between multiple phases of materials, owing to the unique diffraction patterns produced from the crystallographic structures of each polymorph. Since the diffraction pattern of each crystalline form of a compound is unique, XRD is particularly suited for analyzes of solid mixtures. Moreover, the intensities of the peaks are unique to each phase and enable qualitative analyzes. The analysis is then expected to confirm calcification of the diseased muscle and to determine which crystal is present. Powder remaining from the isotopic analyzes of August posterior muscles was used. The ten healthy and the ten diseased samples were pooled in order to get enough healthy and diseased powder for the XRD analysis. Then, powder were placed in aluminium holders, pressed with a glass blade and exposed to CuKα radiation (Kα = 1.5418 Å) in a wide-angle X-ray powder diffractometer (Model, PANalytical X'pert MPD, Bragg–Brentano geometry). The angular range was 8.020°–79.980° and the angular step size was 0.02°. Eventually, diffraction patterns of samples were elaborated and compared to diffraction patterns of known crystals using DIFFRACplus software in order to determinate which crystals, if any, were present in the samples.

Statistical analyzes

Differences between diseased and healthy muscles were tested with analyzes of variance and/or non-parametric test. Maximum type I error rates were set at $\alpha = 0.05$. Homogeneity of variance was checked using Cochran test. Significant ANOVA results were followed by multiple comparisons using the conservative Tukey's HSD post hoc test (Sokal and Rohlf 1981). When variances were not homogeneous, the non-parametric Mann–Whitney U test and Kruskal–Wallis test were used to assess differences between two and several groups of samples, respectively. Correlation between variables non-normally distributed was tested by the Spearman's rank correlation method. Statistical analysis was performed using Statistica software 7.1.

Results

Condition index and final disease index

Healthy and diseased adductor muscles can be easily distinguished (Fig. 1). Healthy clams displayed a significant higher condition index than infected clams ($P = 0.007$) with average values of 54.4 ± 4.8 (± SE) and 34.4 ± 4.5, respectively. FDI was calculated for each clam processed in August and FDI ranged between 8 and 16 for diseased clams. A significant negative correlation was found between FDI and the condition index ($r = -0.54$, $P = 0.013$, $n = 20$).

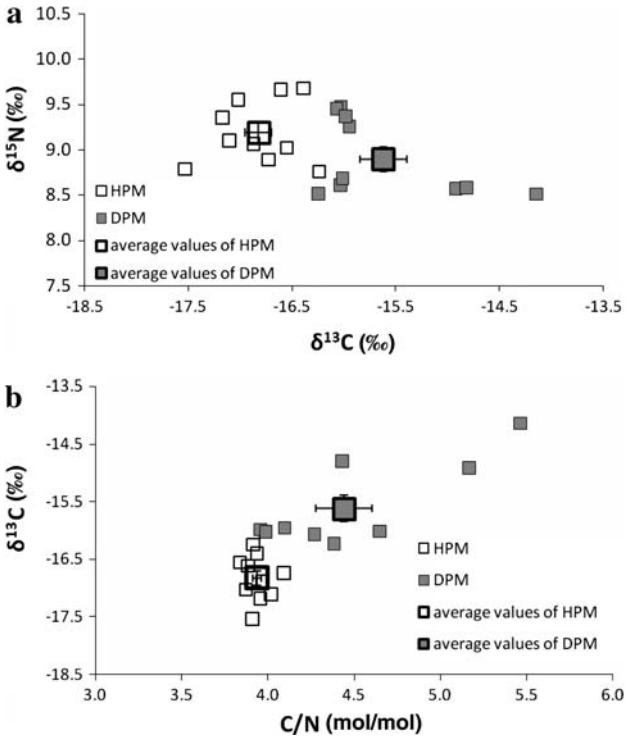

Fig. 2 Dual plots of nitrogen versus carbon stable isotope ratios (**a**) and carbon stable isotope ratio versus C/N ratio (**b**) in posterior adductor muscles of *Ruditapes philippinarum* in February 2007. *HPM* healthy posterior muscle. *DPM* diseased posterior muscle. Averages are represented ± standard error

Pre-acidification stable isotopes analyzes

In February, δ^{13}C and δ^{15}N values of the posterior adductor muscle of *R. philippinarum* ranged from −17.5 to −16.6‰, and 8.8 to 9.7‰, respectively for healthy clams whereas these ratios varied from −16.2 to −14.1‰, and 8.5 to 9.5‰, respectively for diseased clams (Fig. 2a). δ^{13}C values of infected clams exhibited a higher range than those of healthy clams (2.1 vs. 0.9‰). Muscles of diseased clams were significantly enriched in ^{13}C ($P = 0.0002$) by 1.2‰ on average (−15.6 vs. −16.8‰) compared to healthy clams (Fig. 2a; Table 1). No significant difference was noted in the δ^{15}N values between healthy and diseased clams (8.9 vs. 9.2‰) ($P = 0.111$). C/N ratios in healthy animals ranged from 3.8 to 4.1 with a mean value of 3.9 ± 0.02 (Fig. 2b; Table 1). Conversely, diseased clams exhibited scattered values between 3.9 and 5.5 (mean = 4.4 ± 0.16) (Fig. 2b). Posterior muscles of BMD infected clams had significantly higher C/N ratios than healthy bivalves ($P = 0.001$).

In August, both anterior and posterior muscles were analyzed. Due to seasonal variations, posterior adductor muscles of healthy clams in August were depleted in ^{13}C compared to February (−17.6 vs. −16.8‰) (Figs. 2a, 3a; Table 1). The dual plot of δ^{13}C and δ^{15}N in August exhibited the same trends as in February with a higher range in δ^{13}C for posterior muscles of diseased clams compared to those of healthy clams (2.4 vs. 0.6‰) combined with a ^{13}C enrichment (0.7‰) in diseased clams. δ^{13}C and δ^{15}N values varied from −17.9 to −17.2‰, and 7.9 to 9‰, respectively, for healthy clams and from −17.9 to −15.6‰, and 7.8 to 8.4‰, respectively for infected clams (Fig. 3a). Mean values of carbon and nitrogen stable isotope signatures were −17.6 ± 0.05‰ and 8.6 ± 0.05‰, respectively for healthy bivalves, −16.8 ± 0.25‰ and 8.1 ± 0.07‰, respectively for diseased clams.

For anterior muscles, δ^{13}C and δ^{15}N values ranged from −17.8 to −17‰, and 8 to 9.7‰ with mean values averaging −17.5 ± 0.05 ‰ and 8.9 ± 0.11 ‰, respectively for healthy clam, and ranged from −17.8 to −17.1‰, and 8.7 to 9.9‰ with mean values of −17.6 ± 0.09‰ and 9.1 ± 0.13‰, respectively for diseased clams. There was no significant difference in both δ^{13}C and δ^{15}N values of anterior muscles between healthy and diseased clams ($P = 0.22$, $P = 0.39$, respectively). δ^{13}C range was low and averaged 0.7‰ for both infected and no infected animals (Fig. 3a).

Posterior muscles of infected clams were significantly enriched in ^{13}C compared to anterior muscles of both healthy and infected clams and to posterior muscles of healthy bivalves ($P = 0.001$, Tukey Test, Figs. 3a, 4). δ^{15}N values of both infected and healthy clams anterior muscles were significantly different from those of both posterior muscles ($P < 0.001$, Tukey Test, Fig. 4). δ^{15}N

Table 1 Means (± SE, $n = 10$) of carbon and nitrogen stable isotope ratios (‰) and C/N content (mol mol^{-1}) of *Ruditapes philippinarum* adductor muscles before and after acidification

Date	Status	Adductor muscle	Before acidification			After acidification
			δ^{15}N	δ^{13}C	C/N	δ^{13}C
February	H	P	9.2 (± 0.11)	−16.82 (± 0.13)	3.94 (± 0.02)	−16.85 (± 0.13)
	D	P	8.9 (± 0.13)	−15.62 (± 0.23)	4.44 (± 0.16)	−16.43 (± 0.12)
August	H	A	8.9 (± 0.15)	−17.49 (± 0.07)	3.68 (± 0.02)	−17.60 (± 0.08)
		P	8.55 (± 0.06)	−17.57 (± 0.06)	3.70 (± 0.02)	−17.53 (± 0.07)
	D	A	9.10 (± 0.14)	−17.60 (± 0.09)	3.85 (± 0.02)	−17.40 (± 0.09)
		P	8.13 (± 0.07)	−16.80 (± 0.25)	4.59 (± 0.07)	−17.89 (± 0.11)

H healthy, *D* diseased, *P* posterior, *A* anterior

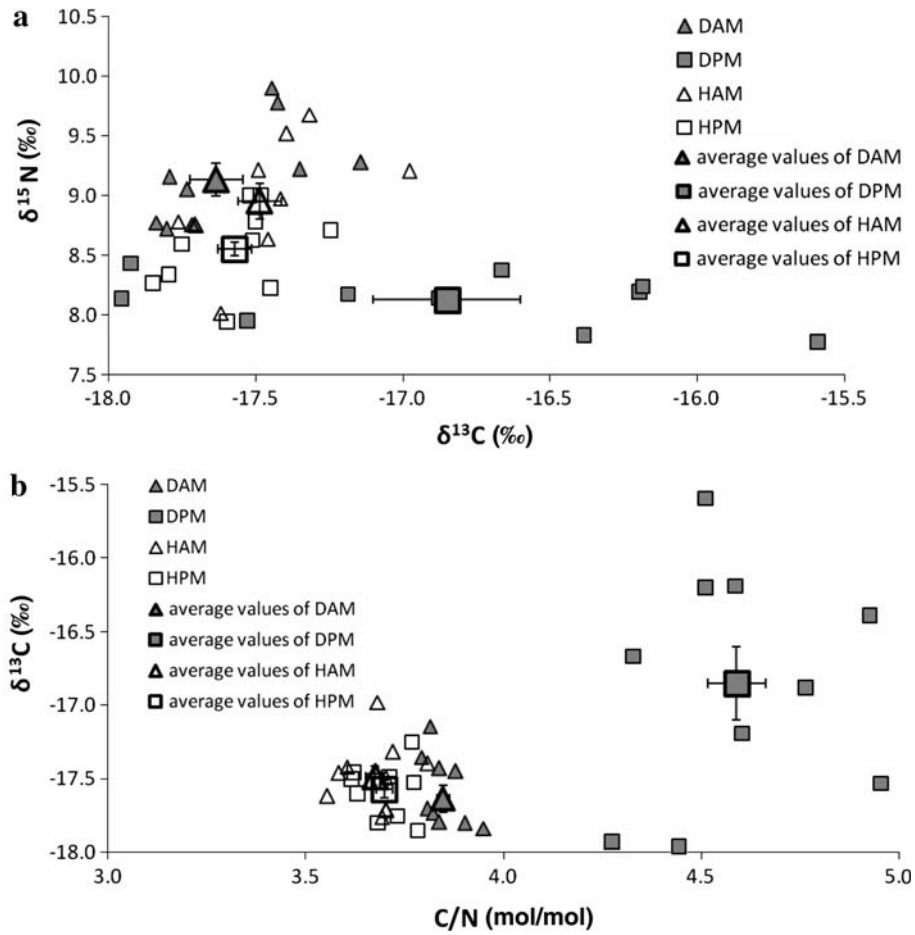

Fig. 3 Dual plots of nitrogen versus carbon stable isotope ratios (**a**) and carbon stable isotope ratio versus C/N ratio (**b**) in anterior and posterior adductor muscles of *Ruditapes philippinarum* gathered in August 2007. *HPM* healthy posterior muscle. *DPM* diseased posterior muscle. *HAM* healthy anterior muscle. *DAM* diseased anterior muscle. Averages are represented ± standard error

values of posterior muscles were not significantly different from those of anterior muscles in healthy clams in contrast to diseased clams ($P < 0.001$, Tukey Test, Fig. 4).

C/N ratios ranged from 4.3 to 4.9 and averaged 4.6 ± 0.07 for posterior infected muscles and ranged from 3.6 to 3.9 for anterior diseased muscles and both healthy muscles (Fig. 3b). C/N ratios were significantly higher for posterior muscles in infected clams compared to anterior muscles in infected clams and both muscles in healthy clams ($P < 0.001$).

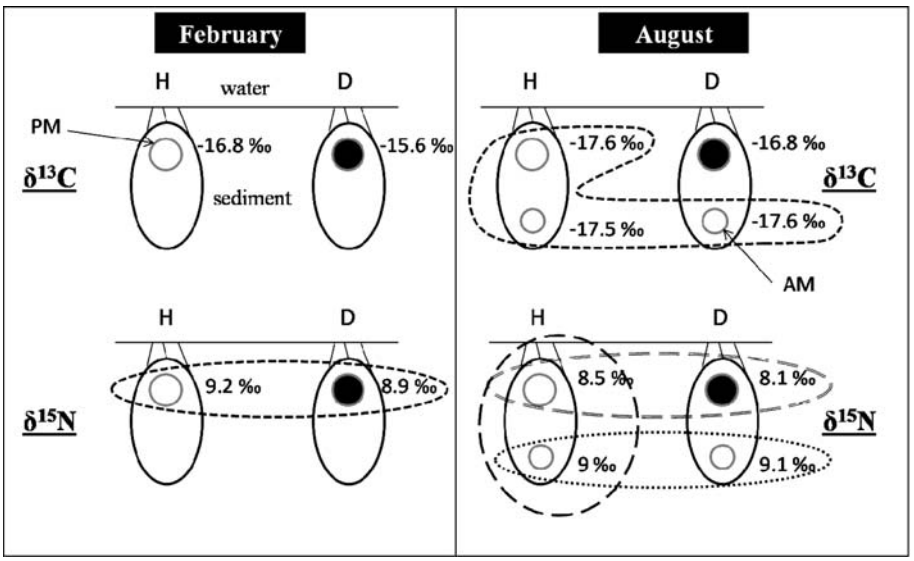

Fig. 4 $\delta^{13}C$ and $\delta^{15}N$ averages of healthy (*H*) and diseased (*D*) clams in February and August 2007. *Dotted lines* gather values that were not significantly different (Anova, Tukey test $P > 0.05$). *AM* anterior muscle, *PM* posterior muscle

FDI calculated for each clam processed in August varied between 8 and 16 with a high proportion of clams in class 16. Significant positive rank correlations were found between FDI and both δ^{15}N and C/N ratios ($r = -0.5$, $P = 0.01$ and $r = 0.9$, $P < 0.001$, respectively, $n = 20$). No significant correlation was observed between FDI and δ^{13}C ($r = 0.3$, $P = 0.19$, $n = 20$).

Post-acidification stable isotopes analyzes

All clam samples from both February and August samples were analyzed for stable isotopes after acidification to remove inorganic carbon. No significant effect of the acidification process was noted for both muscles of healthy clams and for the anterior muscle of diseased clams ($P > 0.05$). In contrast, significant ^{13}C depletions of 0.8‰ in February and 1.1‰ in August were observed after acidification of the posterior muscle of diseased clams ($P = 0.005$) (Table 1). δ^{13}C values of infected posterior muscles were −15.6 and −16.8‰ before acidification and became −16.4 and −17.9‰ in February and August, respectively after acidification (Table 1). Acidification reduced the range in δ^{13}C values of the posterior infected muscle, which became similar to the low variability observed in δ^{13}C values of healthy clams (Table 1). However, the posterior adductor muscle after acidification remained significantly different ($P < 0.05$) from both healthy muscles and anterior muscle of diseased clams.

X-ray diffractometry

Apart from the occurrence of four peaks corresponding to aluminum of the holder, the XRD pattern of posterior adductor muscle of diseased and healthy clams greatly differed (Fig. 5). No mineral was detected within the healthy muscle sample. However, a bulge was noticed around 20° angle (2θ) and was assumed as the sign of amorphous organic material. This bulge was not found in the XRD pattern of diseased clams, which contained numerous peaks of crystalline compounds such as halite (NaCl) and aragonite ($CaCo_3$). Aragonite has an orthorhombic crystalline structure and was found in high concentration within the infected posterior muscles.

Discussion

BMD significantly modifies the δ^{13}C values of the posterior adductor muscle. Compared to healthy clams, the observed ^{13}C enrichment, i.e., 1.2‰ in February and 0.7‰ in August, is mainly but not exclusively due to the calcification by

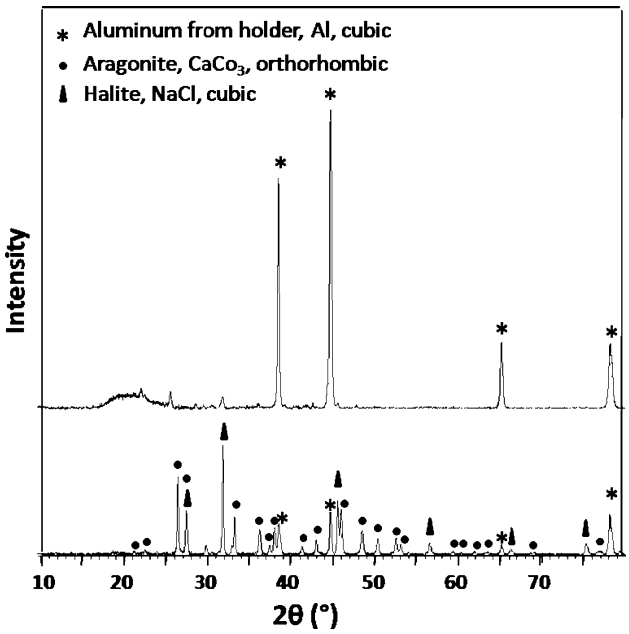

Fig. 5 X-ray diffraction patterns of healthy (*above*) and diseased (*below*) muscles

aragonite ($CaCo_3$). Indeed, after acidification, δ^{13}C of diseased posterior muscles is closer, but remains slightly different from δ^{13}C values of healthy muscles. Conversely, neither δ^{13}C of anterior muscles, nor δ^{15} N of both anterior and posterior muscles are modified by BMD.

Standards errors (SE) of carbon isotopic ratios of posterior adductor muscle of *R. philippinarum* were 0.13 and 0.06‰ in February and August, respectively (Table 1). This variability was similar to that observed in other bivalve species. In general, SE were lower than 0.15 with δ^{13}C ± SE of −22.6 ± 0.06‰ in *Crassostrea gigas* (Malet et al. 2007), −15.2 ± 0.03‰ in *Pecten maximus* (Lorrain et al. 2002), −16.4 ± 0.06‰ in *Mytilus galloprovinciallis* (Machás et al. 2003), −16.6 ± 0.08‰ in *Ruditapes decussatus* (Machás et al. 2003) and −14.3 ± 0.13 in *Cerastoderma edule* (Page and Lastra 2003). Conversely, diseased posterior muscle of Manila clam exhibited higher variability with δ^{13}C ± SE values of −15.62 ± 0.23 and −16.80 ± 0.25‰ in February and August, respectively (Table 1), according to various level of calcification within muscles.

The inter-individual variability in δ^{13}C in the present study was lower than 1‰ for posterior and anterior muscles of healthy clams and for anterior muscles of diseased clams. In contrast, this variability rose above 2‰ for diseased posterior muscles. This variability is very large compared to the usual trophic enrichment of 0.5 − 1‰ that occurs with trophic level increase in food webs (DeNiro and Epstein 1978; McCutchan et al. 2003). Consequently, BMD could bias the interpretation of isotopic data in the

context of trophic studies if BMD were not recognized prior tissue analyzes. Indeed, the adductor muscle is commonly used in trophic studies of clams because of its long turnover rates compared to other tissues with fast turnover such as digestive glands and gonads (Kasai et al. 2004; Kanaya et al. 2005).

Even if BMD did not affect the isotopic signature of the anterior adductor muscle, the lower condition index of diseased clams compared to healthy clams suggests a general weakening of the animal. At this period of the year, the condition index of affected clams should be one and a half higher, like unaffected clams (Laruelle et al. 1994). The decrease of the condition index was in minor part due to the dry weight of muscles and in major part due to a weight loss of the whole animal.

BMD induces a calcification of the adductor posterior muscle as revealed by X-ray diffractometry. The presence of aragonite and not calcite within the muscle in clams infected by BMD appears normal because of its inner position within the clam. It may indicate that the calcification is controlled by biogenic processes, i.e., protein matrix (Goulletquer et al. 1989), and immune response through enzyme activity (Jing et al. 2007).

The isotopic signature measured in infected clams corresponded with the cellular response of clam in respect to an infectious agent and not to the infectious agent, like in many other studies where the parasite could be analyzed separately from the tissue (Deudero et al. 2002). Thus, the observed shift in $\delta^{13}C$ values actually reflects the metabolic consequences of the disease on the clam tissues and is thus a pathologic shift. To our knowledge, such pathologic shift in mollusk bivalve tissues has never been reported before.

Our results on SIA evidenced that precaution must be taken if Manila clams are planned to be included in trophic food web studies. Only healthy individuals should be considered after a closer examination of their posterior muscles or, as a conservative alternative, only anterior adductor muscles should be sampled, when expertise in mollusk pathology is lacking. Finally, this work displayed the importance to acidify Manila clam tissues to remove inorganic carbonates in trophic web studies.

Acknowledgments We are particularly grateful to E. Lebraud from Institut de Chimie et de la Matière Condensée de Bordeaux to permit us to realize diffractometry analyzes; to P. Lebleu, F. Prince for their assistance in the field; I. Billy, K. Charlier for technical assistance with elemental analyzer. The study was partly financed by ANR (Multistress project). C. Dang was supported by a grant from Fonds Commun de Coopération Aquitaine-Euskadi and Conseil Général de la Gironde. Thanks to R. B. Carnegie for discussion and English editing. The experiments comply with the current laws of the country in which the experiments were performed.

References

Dang C, de Montaudouin X, Gonzalez P, Mesmer-Dudons N, Caill-Milly N (2008a) Brown muscle disease (BMD), an emergent pathology affecting Manila clam *Ruditapes philippinarum* in Arcachon Bay (SW France). Dis Aquat Organ 80:219–228. doi:10.3354/dao01941

Dang C, Gonzalez P, Mesmer-Dudons N, Caill-Milly N, Bonami JR, De Montaudouin X (2008b) Virus-like particles associated with brown muscle disease in Manila clam (*Ruditapes philippinarum*) in Arcachon Bay (France). J Fish Dis (in press)

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506. doi:10.1016/0016-7037(78)90199-0

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351. doi:10.1016/0016-7037(81)90244-1

Deudero S, Pinnegar JK, Polunin NVC (2002) Insights into fish host-parasite trophic relationships revealed by stable isotope analysis. Dis Aquat Organ 52:77–86. doi:10.3354/dao052077

Flassch JP, Leborgne Y (1992) Introduction in Europe, from 1972 to 1980, of the Japanese Manila clam (*Tapes philippinarum*) and the effects on aquaculture production and natural settlement. ICES Mar Sci Symp 194:92–96

Fry B, Sherr EB (1984) $d^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib Mar Sci 27:13–47

Goulletquer P, Héral M, Béchemin C, Richard P (1989) Anomalies de calcification chez la palourde japonaise *Ruditapes philippinarum*: caractérisation et comparaison des compositions en acides aminés de différentes parties de la coquille analysées par HPLC. Aquaculture 81:169–183. doi:10.1016/0044-8486(89)90243-3

Jing G, Yan Z, Li Y, Xie L, Zhang R (2007) Immunolocalization of an acid phosphatase from pearl oyster (*Pinctada fucata*) and its in vitro effects on calcium carbonate crystal formation. Mar Biotechnol 9:650–659. doi:10.1007/s10126-007-9018-0

Kanaya G, Nobata E, Toya T, Kikuchi E (2005) Effects of different feeding habits of three bivalve species on sediment characteristics and benthic diatom abundance. Mar Ecol Prog Ser 299:67–78. doi:10.3354/meps299067

Kasai A, Horie H, Sakamoto W (2004) Selection food sources by *Ruditapes philippinarum* and *Mactra veneriformis* (Bivalva: Mollusca) determined from stable isotope analysis. Fish Sci 70:11–20. doi:10.1111/j.1444-2906.2003.00764.x

Laruelle F, Guillou J, Paulet YM (1994) Reproductive pattern of the clams, *Ruditapes decussatus* and *R. philippinarum* on intertidal flats in Brittany. J Mar Biol Assoc UK 74:351–366

Lassalle G, de Montaudouin X, Soudant P, Paillard C (2007) Parasite co-infection of two sympatric bivalves, the Manila clam (*Ruditapes philippinarum*) and the cockle (*Cerastoderma edule*) along a latitudinal gradient. Aquat Living Resour 20:33–42. doi:10.1051/alr:2007013

Lorrain A, Paulet Y-M, Chauvaud L, Savoye N, Donval A, Saout C (2002) Differential $\delta^{13}C$ and $\delta^{15}N$ signatures among scallop tissues: implications for ecology and physiology. J Exp Mar Biol Ecol 275:47–61. doi:10.1016/S0022-0981(02)00220-4

Machás R, Santos R, Peterson B (2003) Tracing the flow of organic matter from primary producers to filter feeders in Ria Formosa lagoon, southern Portugal. Estuaries 26:846–856. doi:10.1007/BF02803343

Malet N, Sauriau P-G, Faury N, Soletchnik P, Guillou G (2007) Effect of seasonal variation in trophic conditions and the gametogenetic cycle on $\delta^{13}C$ and $\delta^{15}N$ levels of diploid and triploid Pacific oysters *Crassostrea gigas*. Mar Ecol Prog Ser 346:203–217. doi:10.3354/meps06953

McCutchan JHJ, Lewis WMJ, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390. doi:10.1034/j.1600-0706.2003.12098.x

Page HM, Lastra M (2003) Diet of intertidal bivalves in the Ría de Arosa (NW Spain): evidence from stable C and N isotope analysis. Mar Biol (Berl) 143:519–532. doi:10.1007/s00227-003-1102-z

Paillard C (2004) A short review of brown ring disease, a vibriosis affecting clams, *Ruditapes philippinarum* and *Ruditapes decussatus*. Aquat Living Resour 17:467–475. doi:10.1051/alr:2004053

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320. doi:10.1146/annurev.es.18.110187.001453

Sokal RR, Rohlf FJ (1981) Biometry. The principles and practice of statistics in biological research. Freeman W.H. and Co., NJ, pp 1–859