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Re-evaluation of nutrient sources for deep-sea wood-boring bivalves using the isotopic composition of bulk C, N, S, and amino acid nitrogen

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ABSTRACT: Wood-boring bivalves (families Xylophagaidae and Teredinidae) are unique bivalves that are peculiarly adapted to feed on terrigenous woody materials, even though they inhabit the deep-sea floor far from land. Previous studies of their metabolic processes suggested the importance of symbiotic microbes that secrete cellulase to access woody carbon (carbohydrates), and supply organic nitrogen via nitrogen fixation. Since nitrogen is generally depleted in woody materials, dissolved dinitrogen in seawater has been proposed as a plausible nitrogen source for wood-boring bivalves. We evaluated the food ecology of wood-boring bivalves (genus: *Xyloredo*) obtained from the deep seafloor off the Ryukyu Islands by considering their bulk carbon, nitrogen, and sulfur isotope composition and potential dietary sources (i.e. logs, particulate organic matter, and surface sediments). We also investigated the trophic interactions between wood-boring bivalves and logs based on the amino acid nitrogen isotopic composition. The bulk isotope data revealed that in wood-boring bivalves these elements are derived mainly from the logs in which they live. These results were consistent with the trophic hierarchy calculated from the nitrogen isotopic composition of amino acids. Ecologically, wood-boring bivalves are one step higher than logs in terms of their trophic position. Based on these data, we propose that terrigenous woody materials are the major dietary sources for wood-boring bivalves and the same standard trophic interaction exists between diet and consumer species as in the grazing food web. The symbionts may aid the digestion of woody materials, but they do not supply nitrogen via nitrogen fixation in this case.

KEY WORDS: Wood-boring bivalves · *Xyloredo teramachii* · Compound-specific nitrogen isotope analysis · Amino acids · Nitrogen nutrition · Stable isotope composition

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INTRODUCTION

Primary production on land occasionally supports marine life, especially organisms that inhabit areas near the coast. Animals that rely on terrigenous organic matter utilize mainly fresh leaves, litter, and fruit, which are rich in easily digestible labile organic compounds such as sugars, fats, and proteins. One of the main components of wood is cellulose, which is a refractory macromolecule in animal diets. Some animals have been reported to have endogenous cellulase and xylanase genes and cellulase and hemicellulase activities (e.g. Sakamoto & Toyohara 2009). This suggests that they can directly use and digest

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cellulose. However, cellulase-synthesizing animals do not always use the cellulase they produce, but rather assimilate terrigenous organic matter indirectly through eating bacteria that colonize and degrade woody materials, including cellulose (Yamanaka et al. 2013). The source of nitrogen for these animals is likely to be more variable.

Two families of wood-boring bivalves are recognized: Xylophagaidae and Teredinidae. These wood-boring bivalves not only use wood as a shelter but also digest wood constituents as their nutrient source. Popham & Dickson (1973) reported that teredinid wood-boring bivalves harbor intracellular endosymbionts in their gills, a bacteriocytes-rich region, known as the 'gland of Deshayes', and Waterbury et al. (1983) demonstrated by cultivation *in vitro* that symbiotic bacteria are capable of cellulose digestion and atmospheric nitrogen fixation. In addition to the cultivation test using isolated symbiotic bacteria, multi-isotope imaging mass spectrometry has been applied to a teredinid wood-boring bivalve (*Lyrodus pedicellatus*), to visualize atmospheric nitrogen fixation by symbiotic bacteria and the transfer of fixed nitrogen to the host cells using ^{15}N labeled dinitrogen (Lechene et al. 2007).

Many studies have evaluated the importance of nitrogen fixation by endosymbionts (e.g. Waterbury et al. 1983, Nishimoto et al. 2009) due to the poor potential of woody materials as a nitrogen source for wood-dependent animals. Although the nitrogen content of woody materials varies depending on the species and circumstances, it is generally expected to be less than 0.5% on a weight basis (e.g. Santa Regina & Tarazona 2001, Mizota et al. 2011). This value is lower than that of marine plankton (ca. 3.8%; Bowen 1979); however, we suppose that the nitrogen in wood may still be useful for certain animals.

Compound-specific stable isotope analysis (CSIA) of amino acids potentially provides an accurate and precise estimation of the trophic level of organisms in a food web (e.g. McClelland & Montoya 2002, Popp et al. 2007, Chikaraishi et al. 2009, 2014). Chikaraishi et al. (2010) proposed that a comparison between the large ^{15}N-enrichment (+8.0 ± 1.1‰) in glutamic acid and the minor change (+0.4 ± 0.4‰) in phenylalanine with each increase in trophic level provides both an estimate of the trophic position (TP) of target organisms and the nitrogen isotopic ratio of phenylalanine from the primary producers in food webs. The TP value is calculated using the equation $TP = [(\delta^{15}N_{glutamic\ acid} - \delta^{15}N_{phenylalanine} + \beta)/7.6] + 1$ proposed by Chikaraishi et al. (2014). Here, the β represents the isotopic difference between glutamic acid ($\delta^{15}N_{glutamic\ acid}$) and phenylalanine ($\delta^{15}N_{phenylalanine}$) in primary producers; β = +8.4 for terrestrial C_3 plants and β = −3.4 for cyanobacteria and algae. If wood-boring bivalves assimilate nitrogen derived from the degradation of woody materials, it should appear at a low TP (i.e. TP = 2), and the $\delta^{15}N$ values of phenylalanine from wood-boring bivalves should be close to those of woody materials. However, if wood-boring bivalves assimilate fixed dinitrogen via endosymbionts, then $\delta^{15}N$ values in phenylalanine from wood-boring bivalves should be very different from $\delta^{15}N$ values in woody materials. Furthermore, in the former case, an adequate TP value is given using β = +8.4, while in the latter case, it is given using β = −3.4.

Sulfur is an essential element for life and is generally acquired from primary producers through the food web (Schiff & Fankhauser 1981). Common marine animals are thought to acquire their body sulfur from primary producers, which assimilate sulfate-sulfur as a primary sulfur source due to sulfate being an abundant anion in seawater (Trust & Fry 1992). This has been demonstrated using the sulfur isotopic signature as a food tracer, because seawater sulfate-sulfur is significantly enriched in ^{34}S, and is easily distinguishable from other sources (e.g. Yamanaka et al. 2013). The sulfur source of wood-boring bivalves has not been reported, and remains an interesting issue to investigate.

The present study investigated the sources of carbon, nitrogen, and sulfur for the wood-boring bivalve *Xyloredo teramachii* (Xylophagaidae) obtained from the deep seafloor off the Ryukyu Islands, where several logs (*Zelkova serrata*) cut from a single tree were placed at various depths (276, 500, and 1000 m). *Xyloredo teramachii* was originally described as one of the species of *Neoxylophaga* (Taki & Habe 1950); however, a recent study revealed that the species should be reallocated to the genus *Xyloredo* based on detailed morphological study (Haga & Kase 2008). Although molecular biological and taxonomic studies about endosymbionts of the family have not been reported, stable isotopic study using one of the species belonging to this family obtained around Japan suggests that the major food source of the bivalves is symbiotic bacteria (Nishimoto et al. 2009). We measured bulk carbon, nitrogen, and sulfur, and their isotopic composition, as well as the amino acid nitrogen isotopes in samples, to determine the primary nutrient sources and trophic interactions of the woodboring bivalves. The results will elucidate the fate of terrigenous organic matter and the biological cycles of nitrogen and sulfur.

MATERIALS AND METHODS

Study area

In June 2008, during the NT08-12 scientific cruise by the R/V 'Natsushima', the ROV 'HyperDolphin' was used to place *Zelkova serrata* logs at depths of 276, 500, and 1000 m off Miyako Island, southwestern Japan (Fig. 1, Table 1). The logs were ca. 30 cm thick, cut from one large tree (ca. 45 cm in diameter) in the Kanto district of central Japan. The study areas were on the landward slope of the Ryukyu Trench, where the average degree of incline was ca. 2°. The water temperature at the 3 sites was ca. 17°C at 276 m, ca. 11°C at 500 m, and ca. 4°C at 1000 m. Each site was covered with calcareous sandy silt, and a few benthic creatures were observed on the seafloor.

Sample collection

The logs, together with the wood-boring bivalves (mainly *Xyloredo teramachii*) that bored into them, were subsequently recovered by the ROV 'HyperDolphin' from the 276 m deep site in July 2009 and from the other 2 sites in April 2010, during the NT09-10 and NT10-07 scientific cruises by the R/V 'Natsushima', respectively. At the 276 m deep site, some specimens belonging to genus *Coeloteredo* of the family Teredinidae were found with *X. teramachii*, and at the 500 m and 1000 m deep sites some specimens belonging to Teredinidae were also found. Degradation states of the recovered logs were quite similar based on macroscopic observation: on the upper surfaces of a cut end approximately 15–20 burrow holes (0.5–2 mm in diameter) per 1 cm^2 were observed and the burrows reached up to 5 cm depth (a few large burrows reached ~10 cm) from the surfaces. During the dive surveys, sediment cores and seawater were sampled from

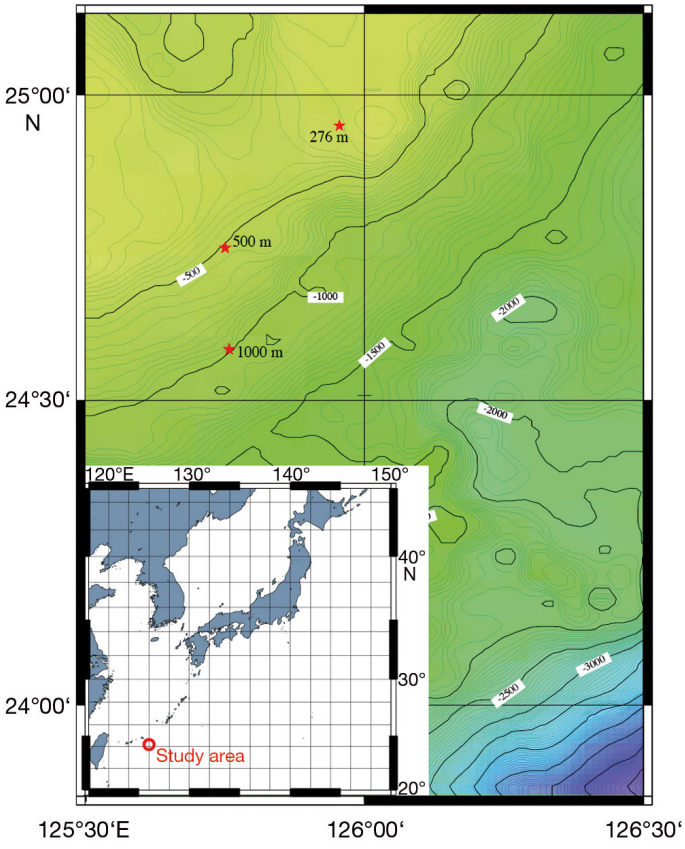

Fig. 1. Location and bathymetric map of the study sites (red stars) off Miyako Island, southwestern Japan

Table 1. Site information and description of samples. Dates in dd.mm.yyyy. POM: particulate organic matter

Depth (m)	Latitude (°N)	Longitude (°E)	Cruise	Dive no.	Date	Description
276	24° 57.19′	125° 57.29′	NT08-12	862	30.06.2008	Deployed logs, sampled control sediment
			NT09-10	1031	12.07.2009	Recovered logs with wood-boring bivalves, sampled sediment and POM
			NT10-07	1118	27.04.2010	Recovered logs with wood-boring bivalves, sampled sediment and POM
500	24° 45.00′	125° 44.99′	NT08-12	858	27.06.2008	Deployed logs
			NT10-07	1112	21.04.2010	Sampled sediment
			NT10-07	1114	22.04.2010	Recovered logs with wood-boring bivalves, sampled POM
			NT10-07	1119	28.04.2010	Sampled POM
1000	24° 35.01′	125° 45.51′	NT08-12	859	27.06.2008	Deployed logs, sampled control sediment
			NT10-07	1116	25.04.2010	Recovered logs with wood-boring bivalves, sampled sediment and POM

just below and above the logs using an MBARI-type push corer and a Niskin bottle operated by the ROV manipulators, respectively. Control sediments were collected just before placing the logs. Particulate organic matter (POM) was separated from the seawater samples by filtration using preheated glass-fiber filters (GC-50, Advantec) on board the research vessel. Table 1 provides details of the samples collected in this study.

Bulk carbon, nitrogen, and sulfur isotopes and elemental analyses

Soft parts of the wood-boring bivalve *X. teramachii* were carefully picked from the recovered logs. The gut and gills were removed under a binocular microscope, and then the remaining tissues were provided for the following analyses. Log samples were carefully cleaned and chipped with a knife. The remaining bivalve organs and log chips were centrifugally washed repeatedly in a 0.1 M LiCl solution and MilliQ water, respectively, to eliminate seawater sulfates, treated with 1 N HCl to remove carbonate, and then freeze-dried and pulverized. Sediment samples were also centrifugally washed repeatedly in MilliQ water to eliminate pore-water sulfates, treated with 1 N HCl to remove carbonate, and then freeze-dried and pulverized. Bulk carbon and nitrogen isotopic compositions of those samples were determined using the pulverized samples. The measurement of bulk sulfur content and isotopic composition followed the procedures described in previous studies (Mizota et al. 1999, Yamanaka et al. 2000a, 2000b). The pulverized samples were placed in a Parr bomb 1108, a stainless steel vessel filled with oxygen gas under high pressure (30 kg cm^{-2}) and a few milliliters of distilled water. Only sediment samples not treated with HCl were used for sulfur measurement to avoid loss due to sulfide vaporization. After combustion, the samples were converted completely into gas, and all sulfur compounds were trapped as sulfates in distilled water in the vessel. The resulting sulfates were recovered as a $BaSO_4$ precipitate, which was weighed to calculate the sulfur content and used for isotopic ratio measurement. The sulfur content of individual wood-boring bivalves was insufficient to measure the sulfur isotopic ratio, so composite precipitates were used. Carbon, nitrogen, and sulfur isotopic compositions were measured by elemental analysis/isotope ratio mass spectrometry (EA/IRMS) (IsoPrime coupled with Euro Vector EA3000, GV Instruments). The carbon and nitrogen contents of the logs were calculated using a thermal conductivity detector signal output in EA/IRMS.

Sediment samples were dialyzed against distilled water to eliminate pore-water sulfates, and then freeze-dried and pulverized. Total organic carbon (TOC) and total nitrogen (TN) concentrations in the surface core sediments and POM were measured in pulverized dry samples, after treatment with 1 N HCl to remove carbonate, by the dry combustion method using an elemental analyzer (EA3000, GV Instruments). The carbon and nitrogen isotopic compositions of TOC and TN were then measured by EA/IRMS.

All isotopic compositions were expressed using the δ notation, with a per mille deviation (‰) from international reference materials (Vienna Pee Dee Belemnite [VPDB] for $\delta^{13}C$, atmospheric N_2 for $\delta^{15}N$, and Cañon Diablo Troilite [CDT] for $\delta^{34}S$). The analytical errors associated with the overall process of these determinations were less than 0.2, 0.3, and 0.3‰, for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively.

CSIA of amino acids in wood-boring bivalves and logs

Four wood-boring bivalve and 3 log samples obtained at a depth of 276 m during the HyperDolphin no. 1031 dive were prepared for the CSIA of amino acids with HCl hydrolysis followed by *N*-pivaloyl/isopropyl (Pv/iPr) derivatization, according to the methods described by Chikaraishi et al. (2014). In brief, samples were hydrolyzed using 12 M HCl at 110°C. The hydrolysate was washed with *n*-hexane/dichloromethane (3:2, v/v) to remove hydrophobic constituents (e.g. lipids). Then, derivatizations were performed sequentially with thionyl chloride/2-propanol (1:4, v/v) and pivaloyl chloride/dichloromethane (1:4, v/v). The Pv/iPr derivatives of the amino acids were extracted with *n*-hexane/dichloromethane (6:5, v/v). The nitrogen isotopic composition of individual amino acids was determined using gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) (Agilent Technologies 6890N GC coupled to DeltaplusXP IRMS with a GC-C/TC III interface, Thermo Fisher Scientific). The nitrogen isotopic composition ($\delta^{15}N$) was determined for 8 amino acids in samples (alanine, glycine, valine, leucine, isoleucine, proline, glutamic acid, and phenylalanine), based on a S/N ratio of ≥20, with baseline separation on the chromatogram. The analytical error associated with the isotope measurements of amino acids was approximately ±0.5‰.

RESULTS

The bulk isotope compositions of wood-boring bivalves and their associated logs, POM, and surface sediments are summarized in Table 2 and Fig. 2. The $\delta^{13}C$ values of the samples without surface sediments, obtained from just below the logs, were lower than −26‰, whereas the values in surface sediment, including the control samples, were distinguishably higher than −22‰. The $\delta^{15}N$ values of the POM and

Table 2. Bulk isotopic ratios of wood-boring bivalve and log samples. NM: not measured. Surface sediment samples were taken 0–5 cm below the seafloor

Sample	Sampling site depth (m)	Isotopic ratio $\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	No. of analyses	HyperDolphin dive no.
Bivalve *Xyloredo teramachii*	276	−27.5 ± 0.2	+3.2 ± 1.6	−1.9	4	1031
	276	−26.6 ± 0.8	+3.8 ± 1.1	+4.6	3	1118
	500	−25.8 ± 0.8	+2.3 ± 0.8	NM	3	1114
	1000	−25.7 ± 1.1	+0.8 ± 1.0	+7.0	4	1116
Tree log *Zelkova serrata*	Original	−29.4 ± 0.1	+0.8 ± 0.1	+6.3	2	–
	276	−27.4 ± 0.3	−0.2 ± 1.2	+4.6	3	1031
	276	−27.3 ± 0.3	−0.3 ± 0.4	NM	3	1118
	500	−28.0 ± 1.2	+0.8 ± 1.6	+8.8	3	1114
	1000	−28.3 ± 0.7	−1.1 ± 1.7	+10.8	3	1116
Particulate organic matter	276	−23.3	+7.4	NM	1	1031
	276	−25.9 ± 0.2	+5.4 ± 2.1	NM	2	1118
	500	−25.0 ± 0.6	+6.8 ± 2.1	NM	2	1114
	500	−25.9 ± 0.5	+4.0 ± 2.1	NM	2	1119
	1000	−26.0 ± 0.4	+4.6 ± 1.5	NM	2	1116
Surface sediment: below tree log	276	−20.0	+7.2	NM	2	1031
	276	−20.6	+6.1	NM	2	1118
	500	−19.2	+5.4	NM	2	1112
	1000	−21.4	+3.8	NM	2	1116
Surface sediment: control	275	−19.3	+7.0	NM	1	862
	1000	−21.9	+5.4	NM	1	859

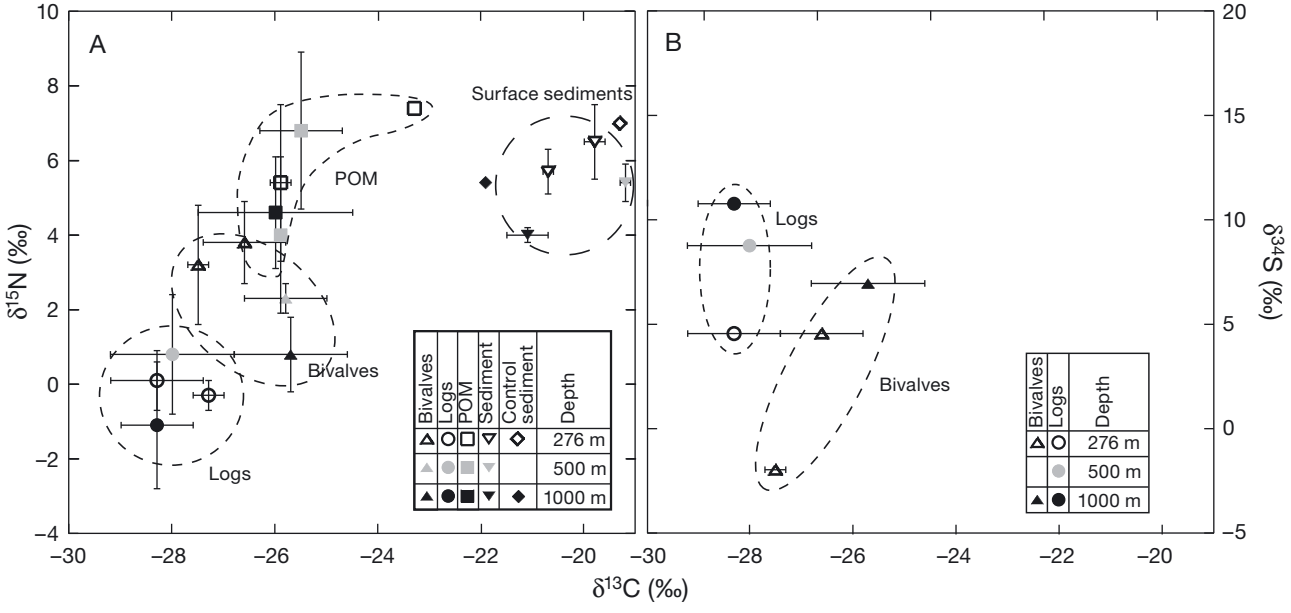

Fig. 2. Bulk isotope compositions of the wood-boring bivalves, logs, particulate organic matter (POM), and surface sediment samples. (A) $\delta^{13}C$ versus $\delta^{15}N$; (B) $\delta^{13}C$ versus $\delta^{34}S$

surface sediments were similar and higher than +4‰, while the wood-boring bivalves and logs had distinguishably lower values, as shown in Fig. 2A. The $\delta^{15}N$ values of the surface sediments sampled from beneath the logs were slightly lower than the values in the control samples obtained before the logs were placed at each site. In addition, the $\delta^{34}S$ values of the wood-boring bivalves and logs ranged from −1.9 to +10.8‰ (Fig. 2B), significantly lower than those of common marine animals (+15–20‰; Kaplan et al. 1963, Mekhtiyeva et al. 1976). These results suggest that organic matter in surface sediments and POM were not a plausible food source for the wood-boring bivalves.

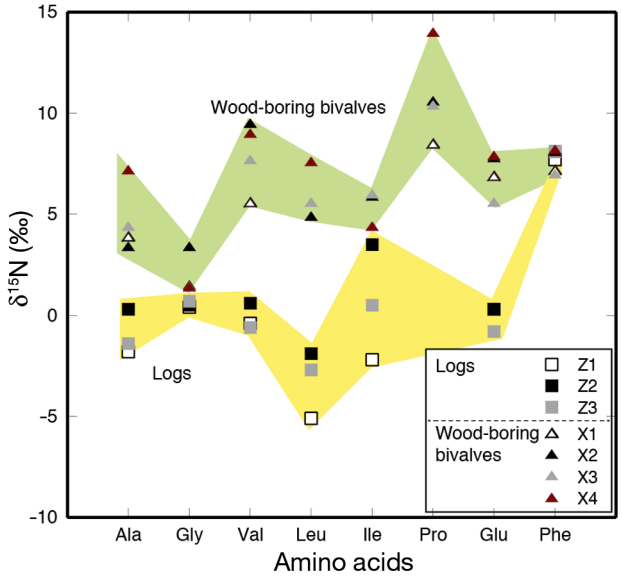

Fig. 3. Compound-specific nitrogen isotope compositions of amino acids extracted from selected wood-boring bivalve and log samples. Triangles and squares indicate woodboring bivalves and logs, respectively. Values are shown in Table 3. Ala: alanine; Gly: glycine; Val: valine; Leu: leucine; Ile: isoleucine; Pro: proline; Glu: glutamic acid; Phe: phenylalanine

The $\delta^{15}N$ values of individual amino acids extracted from the selected bivalve and log samples are shown in Table 3 and Fig. 3. The patterns of $\delta^{15}N$ values of amino acids were similar for the 4 bivalves and within the 3 logs. Although there was a negligible difference in the $\delta^{15}N$ values of phenylalanine between wood-boring bivalves and logs, there was a difference of ~7‰ in the $\delta^{15}N$ values of glutamic acid. The TP of the wood-boring bivalves and logs shown in Table 3 was calculated according to Chikaraishi et al. (2014). The TP values calculated using these data were ca. 1 and ca. 2 for logs and wood-boring bivalves, respectively, when β = +8.4 was applied (Table 3).

Table 4 shows the elemental composition of the logs and surface sediments. The organic carbon contents were ca. 50% for logs and 0.08–0.17% for surface sediments. The nitrogen contents were 0.09–0.20% for logs and 0.02–0.05% for surface sediments. The sulfur content of logs was 0.06–0.19%, but sulfur was not detected in the surface sediments. The TOC content of the control sediments and the sediment beneath the logs was within the same range, as shown in Table 4, while the TN content of the sediment beneath the logs was slightly higher than in the control sediments.

Table 3. Compound-specific nitrogen isotope compositions ($\delta^{15}N$ [‰]) of amino acids extracted from the selected log samples (Z1–Z3) and wood-boring bivalves (X1–X4) obtained from a depth of 276 m during HyperDolphin dive no. 1031. Calculated trophic positions were estimated using the following formula: TP = [($\delta^{15}N_{glutamic\ acid}$ − $\delta^{15}N_{phenylalanine}$ + β)/7.6] + 1; β = −3.4 for coastal marine samples and β = +8.4 for terrigenous samples (Chikaraishi et al. 2014)

	Tree logs (n = 3)				Wood-boring bivalves (n = 4)				
Sample ID	Z1	Z2	Z3	SD	X1	X2	X3	X4	SD
Amino acid									
Alanine	−1.8	+0.3	−1.4		+3.9	+3.4	+4.4	+7.2	
Glycine	+0.4	+0.5	+0.7		+1.5	+3.4	+0.8	+1.4	
Valine	−0.4	+0.6	−0.6		+5.6	+9.5	+7.7	+9.0	
Leucine	−5.1	−1.9	−2.7		–	+4.9	+5.6	+7.6	
Isoleucine	−2.2	+3.5	+0.5		–	+5.9	+6.0	+4.4	
Proline	–	–	–		+8.5	+10.6	+10.4	+14.0	
Glutamic acid	+0.3	+0.3	−0.8		+6.9	+7.8	+5.6	+7.9	
Phenylalanine	+7.7	+8.1	+8.1		+7.2	+8.1	+7.0	+8.2	
Calculated trophic position									
Coastal marine	−0.4	−0.5	−0.6	0.0	0.5	0.5	0.4	0.5	0.1
Terrigenous	1.1	1.1	0.9	0.0	2.1	2.1	1.9	2.1	0.1

Table 4. Elementary composition of tree-log and surface sediment samples. ND: not detected. Surface sediment samples were taken 0–5 cm below the seafloor

Sample		Depth (m)	Elemental composition (wt. %) Organic carbon	Nitrogen	Sulfur	Atomic ratio C/N	C/S	No. of analyses	HyperDolphin dive no.
Tree log	*Zelkova serrata*	Original	49.32 ± 2.29	0.20 ± 0.01	0.06 ± 0.01	291.9	2364.0	2	–
		276	52.08 ± 1.07	0.11 ± 0.01	0.13 ± 0.02	554.3	1055.0	3	1118
		500	48.63 ± 1.87	0.11 ± 0.00	0.14 ± 0.05	509.1	942.2	3	1114
		1000	50.43 ± 0.28	0.09 ± 0.00	0.19 ± 0.11	685.6	716.7	3	1116
Surface sediment	Beneath tree logs	276	0.15 ± 0.02	0.03 ± 0.02	ND	5.3	–	2	1031
		276	0.09 ± 0.01	0.03 ± 0.00	ND	3.0	–	2	1118
		500	0.16 ± 0.02	0.04 ± 0.01	ND	4.4	–	2	1112
		1000	0.16 ± 0.01	0.04 ± 0.00	ND	5.0	–	2	1116
	Control	275	0.17	0.02	ND	8.5	–	1	862
		1000	0.18	0.02	ND	8.3	–	1	859

DISCUSSION

Nutrient source of wood-boring bivalves

The carbon isotope composition of wood-boring bivalves was more enriched than that of the logs. Such differences have frequently been observed between food and consumer species in grazing food webs. If the main carbon source for wood-boring bivalves is limited to cellulose, as reported previously (Waterbury at al. 1983, Nishimoto et al. 2009), the difference may be due to the enrichment of ^{13}C in cellulose relative to the whole wood (2–3‰ higher; Loader et al. 2003). The nitrogen isotopic ratios also support this conclusion, because the $\delta^{15}N$ values of wood-boring bivalves were slightly higher than those of the logs; however, another possible food source, POM, was enriched in ^{15}N relative to the bivalves. The isotopic compositions of POM are considered to represent the planktonic material in the bottom seawater around the logs. Furthermore, the sulfur isotopic ratios of wood-boring bivalves were significantly lower than those of common marine animals ($\delta^{34}S$ = +15–20‰; Kaplan et al. 1963, Mekhtiyeva et al. 1976), and were almost comparable with the ratios of the associated logs as shown in Table 2. These results suggest that the associated logs were the most likely food source of the wood-boring bivalves.

However, bulk nitrogen isotopic compositions can be misleading in the evaluation of nitrogen sources with respect to the relationship between prey and predator species, mainly due to temporal and spatial variations in the isotopic composition of prey species (Chikaraishi et al. 2014). To overcome this concern, CSIA of amino acids provides a more reliable estimation of TP (McClelland & Montoya 2002, Popp et al. 2007, Chikaraishi et al. 2009). The $\delta^{15}N$ values in phenylalanine from the wood-boring bivalves and logs were similar, whereas glutamic acid $\delta^{15}N$ values were higher in the bivalves than in the logs. The calculated TP values for wood-boring bivalves and logs based on the analytical data are shown in Table 3. To obtain adequate TP values, a β value of +8.4 was used in the calculation, and the resulting TP values for wood-boring bivalves and logs were ca. 2 and ca. 1, respectively. If nitrogen fixation occurred by the endosymbionts in the bivalve gills, adequate TP values will be given using a β value of −3.4, because the TP value of the nitrogen-fixing cyanobacteria is calculated to be 1, using a β value of −3.4 (Chikaraishi et al. 2009). In the same way, if the bivalves feed on planktonic material, the TP values of bivalves should be calculated using a β value of −3.4. This means that the TP values of bivalves should be calculated using a β value of −3.4. The small difference (±0.1) in calculated TP values of the bivalves (1.9–2.1 using a beta value of +8.4) as shown in Table 3 suggests a minor contribution of fixed nitrogen and/or planktonic material. This is consistent with the primary food source for the wood-boring bivalves being logs, with a terrigenous origin suggested by the bulk isotope analyses.

Elemental composition changes of the logs after placement on the seafloor

Compared to the original elemental composition of the logs, the nitrogen contents of recovered log samples were almost half of original value (Table 4). This result suggests preferential loss of nitrogen from the log material. The lost nitrogen from log materials may be assimilated by the wood-boring bivalves.

The sulfur content of the recovered logs (0.13–0.19%) was higher than that of the original log (0.06%; Table 4). The log samples recovered at depths of 500 and 1000 m also had higher $\delta^{34}S$ values relative to the original log (Tables 2 & 4). Therefore, the enhancement of the sulfur content and $\delta^{34}S$ values in the recovered log samples suggests that seawater sulfate-sulfur, which has a high $\delta^{34}S$ value (ca. +20‰), is increasingly incorporated into the log as water depth increases. However the mechanism for the incorporation of ambient sulfate-sulfur remains unknown.

CONCLUSION

Wood-boring bivalves *Xyloredo teramachii* that bored into logs placed on the seafloor at depths of 276, 500, and 1000 m were analyzed to determine their nutrient sources. The bulk carbon, nitrogen, and sulfur isotopic compositions of the wood-boring bivalves and logs suggested that the primary nutrient source of the wood-boring bivalves was the associated logs. This conclusion is consistent with the results of the CSIA of amino acids, which indicated similar $\delta^{15}N$ values in phenylalanine in the wood-boring bivalves and logs, and enabled TP values to be estimated (TP $\simeq$ 1 for the logs and TP $\simeq$ 2 for the wood-boring bivalves). This TP estimation was applied to the equation for a terrigenous C_3 food web, suggesting that the relationship between the wood-boring bivalves and logs is an analogue of a terrigenous food web.

Wood-boring bivalves can harbor symbiotic bacteria that secrete cellulase and have the ability to fix nitrogen (Waterbury et al. 1983). Therefore, primary nitrogen nutrition is thought to use dissolved dinitrogen in seawater. However, if a plentiful supply of nitrogen from tree constituents is available, wood-boring bivalves may not require nitrogen nutrition via symbiotic bacteria. As a result, nitrogen contents in the logs were preferentially decreasing relative to carbon. Although seawater sulfate-sulfur is expected to be incorporated into the log, the sulfur nutrition of wood-boring bivalves is provided mainly by constituents of the tree itself. Wood-boring bivalves rely almost entirely on terrigenous primary production even though they inhabit the deep-sea floor.

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