Oecologia (2021) 196:877–889
https://doi.org/10.1007/s00442-021-04973-0

ECOSYSTEM ECOLOGY – ORIGINAL RESEARCH

Autochthonous production contributes to the diet of wood-boring invertebrates in temperate shallow water

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Received: 17 June 2020 / Accepted: 13 June 2021 / Published online: 22 June 2021

Abstract

Marine wood-boring invertebrates rapidly fragment coarse woody debris in the sea. These wood borers have the ability to digest wood cellulose, but other potential food sources have been less investigated. To assess the contribution of each potential food source to the diet of wood borers, we traced seasonal and environmental changes in $\delta^{13}C$ of shipworms cultured under the same experimental conditions and related these changes to variations in $\delta^{13}C$ of potential food sources, i.e., wood log and particulate organic matter (POM) by using multiple linear regression models rather than the Bayesian mixing model. Based on the standardized partial regression coefficients in the model, it became clear that wood-derived organic carbon was the main carbon source for the teredinids, and POM also accounted for 37.9% of the teredinids' carbon source. Furthermore, we clarified variations in supplemental nitrogen sources for the teredinids: one species depended on both POM and wood log, whereas the other three species depended on either POM or wood log for their nitrogen source. $\delta^{13}C$ values of another wood-boring bivalve of *Martesia* (Pholadidae) increase as it grows, which suggests that the bivalve switches its feeding strategy from xylophagous to filter feeding as it grows. Wood borers are known to accelerate the transfer of organic materials derived from wood logs to marine ecosystems. However, this study suggests that autochthonous production strongly contribute to the diet of marine wood borers, helping them to decompose wood logs in temperate shallow water.

Keywords Wood decomposition · Wood fall · Shipworm · Stable isotope · Model selection

Introduction

Land–ocean linkage supports high productivity in coastal ecosystems, highlighting the importance of terrestrial input of both nutrients and particulate organic matter (POM) into the sea (Sholkovitz 1976; Matsunaga et al. 1998; Tsuda et al. 2003). Unlike the case with these forms of allochthonous input, not much attention has been paid to coarse organic matter, represented by vascular plant debris, as food sources for marine organisms. This is mainly because coarse plant debris has been regarded as resistant to biological decay. Nevertheless, coarse plant debris can harbor a dense and diverse assemblage of organisms in the sea (i.e., the sunken wood community) (in deep-sea floor: Bienhold et al. 2013; in mangrove swamp: Laurent et al. 2013), and wood-derived organic matter serves as one of the main carbon sources for the community, especially in the deep sea (Nishimoto et al. 2009).

In temperate shallow waters, coarse woody debris is fragmented by aggregation of wood borers, such as species of the molluscan bivalve families Teredinidae and Pholadidae

Communicated by Joel Trexler.

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and species of the crustacean isopod family Limnoriidae. Sedentary teredinid bivalves intensively fragment wood inside the logs, and the resulting honeycombed structure is collapsed from the surface by the attack of free-living limnoriid isopods (Nishimoto et al. 2015). These wood borers accelerate bacterial and fungal decomposition of the woody constituents in detritus (reviewed in Cragg et al. 2020) by increasing the surface/volume ratio of wood logs (Camilleri and Ribi 1986; Arnosti et al. 2018). When these wood borers are eaten by, for example, fish or predatory polychaetes (Russell 1997), organic matter derived from wood logs flows into higher trophic levels in the ecosystem as a result of predator–prey interactions. Thus, wood borers control the availability of food resources to members of the biological community and accelerate the assimilation of woody components into marine ecosystems.

A number of studies have addressed the ability of marine wood borers, especially members of the bivalve family Teredinidae, also known as shipworms, to digest wood logs. The teredinids harbor endosymbiotic cellulose-digesting and nitrogen-fixing bacteria in their gills (Distel et al. 2002). The bacteria-encoded enzymes are translocated from the gills to the gut and support the digestion of wood cellulose (O'Connor et al. 2014). Pechenik et al. (1979), however, reported the ability of a teredinid, *Lyrodus pedicellatus* Quatrefages, 1849, to assimilate phytoplankton in a feeding experiment using ^{14}C-labeled one, and some anatomical studies have supported this finding (Turner 1966; Lopes et al. 2000). POM, consisting of phytoplankton and detritus, has been considered a supplemental food source for teredinids, but the relative importance of wood log and POM in their diet is controversial (Turner 1966). For example, recent stable isotope techniques showed inconsistent results; a teredinid, *Teredo navalis* Linnaeus, 1758, mainly use POM as the carbon source (Paalvast and van der Velde 2013), whereas the main carbon source of another teredinid, *Bankia carinata* Gray, 1827, is wood log (Charles et al. 2018). The limnoriid isopods have the ability to digest lignocellulose without the help of symbiotic microbes (King et al. 2010), and their digestive tracts are always filled only with woody particles (e.g., Sleeter et al. 1978). However, their nitrogen source is still unknown, and it might serve as carbon source, too. In contrast, the obligate wood-boring pholadid bivalve, *Martesia striata*, Linnaeus, 1758, is believed to be a true filter feeder judging from its anatomical features (Purchon 1956; Turner and Johnson 1971; Haga and Kase 2011), but there have been no studies of its cellulase activity. Therefore, it remains a critical issue whether these marine wood borers depend on food sources in addition to wood during the process of fragmentation of wood logs.

Carbon and nitrogen stable isotope ratios (hereafter referred to as δ^{13}C and δ^{15}N, respectively) have often been used to estimate the diets of target species. Generally, the δ^{13}C value of a target species is slightly higher (around 0–1‰) than that of its prey (Hobson and Welch 1992), and a target species becomes enriched in δ^{15}N value relative to its prey by 3–4‰ (Peterson and Fry 1987). These differences are the so-called "trophic enrichment factors" (TEF). By using these TEF values and the δ^{13}C and δ^{15}N values of potential food sources, the simple linear stable isotope mixing model approach can uniquely determine the proportional contributions of up to three different food sources based on mass balance equations (Phillips 2001). However, wood logs are poor in nitrogen and provide little nitrogen to xylophages (wood eaters). Furthermore, symbiotic nitrogen-fixing microbes fix dissolved N_2 gas, and the resulting compounds are taken up by teredinid bivalves (Waterbury et al. 1983; Lechene et al. 2007). N_2 gas cannot be a carbon source for teredinid bivalves. Therefore, these two parameters should be used to trace carbon and nitrogen sources for wood borers independently. This use is sufficiently effective in the case with only two different food sources like carbon source of the teredinids, and provides useful information in other cases too.

TEF values are often quoted from past studies dealing with similar relationships, because it is not realistic to calculate TEF values for every prey–predator relationship. The quoted TEF values are helpful for rough estimation of the proportional contribution of each potential food source in complex food web structures. In xylophages, however, TEF values are hard to determine, because their potential food sources are mixtures of labile and refractory organic matter with different stable isotope ratios. For example, teredinid bivalves selectively assimilate the labile components from wood logs (i.e., cellulose and hemicellulose). Among them, teredinid bivalves mainly utilize cellulose (Sabbadin et al 2018), which has a δ^{13}C value approximately 3‰ higher than that of lignin and 1–2‰ higher than that of wood as a whole (Loader et al. 2003, in one of the oak woods, *Quercus robur* L.). In previous studies, the δ^{13}C of whole wood was generally used as one of the end members in the mixing model without considering the gaps in δ^{13}C values between wood logs and wood cellulose. In addition, the gaps are not necessarily 1–2‰ because of the differences in lignin contents among tree species. These discrepancies might lead to misinterpretation of the proportional contribution of each potential food source for xylophages in the use of the stable isotope mixing model.

The δ^{13}C value of wood cellulose is known to fluctuate, together with that of wood as a whole (Loader et al. 2003), and, therefore, the variation in δ^{13}C value of wood as a whole can be regarded as that of cellulose. POM, another independent carbon source of teredinid bivalves, is mainly consisted with labile marine phytoplankton and unidentifiable detrital particle. The δ^{13}C value of POM is also known to fluctuate seasonally (e.g., Vizzini and Mazzola 2003).

This variation is attributed to the seasonal changes in growth speed of marine phytoplankton (Laws et al. 1995) and/or in intensity of riverine organic inputs. In the area far from large river mouth, the fluctuation in $\delta^{13}C$ values of POM can be regarded as the fluctuation in $\delta^{13}C$ value of marine phytoplankton. Indeed, $\delta^{13}C$ value of POM in this study site was the highest in summer, supporting this assumption. Therefore, as an alternative approach to the mixing model, we propose a basic multiple linear regression model in which the $\delta^{13}C$ values of wood logs and POM themselves are adopted as parameters. We traced the seasonal and environmental changes in $\delta^{13}C$ values of teredinid bivalves cultured under the same experimental conditions and related these changes to the variations in $\delta^{13}C$ values of potential food sources by using a multiple linear regression model. This approach is advantageous in that it can estimate the proportional contribution of each potential carbon sources without the use of uncertain TEF values.

Materials and methods

Sampling design

In September 2008, we placed pieces of logs (ca. 10 cm in diameter and ca. 20 cm in length) of Japanese cedar, *Cryptomeria japonica* (Thunb. ex L.f.) D. Don, at ca. 2 m depth on the muddy bottom of Tanabe Bay, Kii Peninsula, on the Pacific coast of central Honshu, Japan (33°40′46.80″ N, 135°21′46.00″ E). The logs were fixed to the top of a polypropylene net that had been anchored to the sea floor. We randomly collected three log samples every 2 months for the first 16 months, and every 4 months thereafter, for a total of 48 months. At each sampling month, we also sampled 1 L of seawater from the sea bottom. Details of the experimental settings are given in Nishimoto et al. (2015).

After collecting the organisms attached to the wood surface, we manually broke the logs into small pieces and collected the wood-boring invertebrates. After measuring the shell length of the wood-boring bivalves, we analyzed the stable isotope ratios in tissues of five teredinid species, *Teredo navalis* Linnaeus, 1758, *Teredo bartschi* Clapp, 1923, *Teredo clappi* Bartsch, 1923, *Lyrodus pedicellatus*, and *Nototeredo edax*, Hedley, 1895, which was once misidentified as *Psiloteredo megotara*, Hanley in Forbes & Hanley, 1848 in Nishimoto et al. (2015), one pholadid bivalve, *Martesia striata*, and two limnoriid isopods, *Limnoria tuberculata* Sowinsky, 1884 and *Limnoria saseboensis* Menzies, 1957. Four epibenthic bivalve species, *Brachidontes mutabilis*, Gould, 1861, *Striarca symmetrica*, Reeve, 1844, *Barbatia virescens*, Reeve, 1844 and *Crassostrea gigas*, Thunberg, 1793 were also analyzed as examples of non-wood-boring bivalves.

Preparation of samples for stable isotope analysis

Animal samples collected during 2008–2012 were kept at − 20 °C until 2012 and were thereafter preserved in 99.5% ethanol at room temperature until further processing in 2016–2017 for stable isotope analysis. Freezing is the most common method to preserve samples for stable isotope analysis, because exposure to storage solution such as ethanol and formalin results in the changes in stable isotope values. The changes in $\delta^{13}C$ values caused by ethanol-preservation are mainly due to the loss of lipid contents (Post et al. 2007), whereas organic solvents, such as chloroform–methanol solutions (Bligh and Dyer 1959), are generally used for the degreasing protocol in stable isotope analysis (Elliot et al. 2017). This is because $\delta^{13}C$ is depleted in lipids compared to proteins, and there are variations in lipid content among specimens depending on the body conditions (Elliot et al. 2017). This degreasing procedure is, therefore, essential to compare food sources between specimens in different body conditions. Besides this degreasing effect, the loss or alteration of proteins also might affect stable isotope values. However, the shifts in $\delta^{13}C$ and $\delta^{15}N$ values after ethanol preservation were less than 0.5 ‰ for both the muscle tissues of marine fishes (average + 0.34 ‰ for $\delta^{13}C$ and + 0.45 ‰ for $\delta^{15}N$ in Durante et al. 2020, based on the review of past studies) and the foot tissues of freshwater bivalves (Syväranta et al. 2010). Furthermore, the potential changes in $\delta^{13}C$ and $\delta^{15}N$ values were suppressed in the multiple linear regression model. This is because the multiple linear regression model deals with the variation in stable isotope ratios, not the values themselves. The variations would be maintained among muscle tissue under the same preanalytical procedures (Arrington and Winemiller 2002), i.e., sample storage methods (presence or absence of fixation, type of fixative solution, and length of treatment) and tissue excision methods.

In molluscan specimens, $\delta^{13}C$ and $\delta^{15}N$ values differ among tissues, such as muscle, gonad, gill, digestive diverticula, and shell (McConnaughey and Gillikin 2008). In this study, adductor muscles were selectively used for stable isotope analysis because $\delta^{13}C$ values in muscle tissues are expected to be less affected by ethanol preservation as mentioned above. To obtain sufficient volume for stable isotope analysis, larger specimens were selected from the sample. If there were no large specimens, siphons and/or mantles were used for the analysis in addition to adductor muscles. In the smaller pholadid bivalve and the limnoriid isopod specimens, the whole tissues of several specimens, from which the digestive tracts were thoroughly removed, were mixed. Using large pholadid specimens, we also investigated the variations in $\delta^{13}C$ and $\delta^{15}N$ values among body tissues, i.e., adductor muscle, gill, siphon, and other nondigestive tissues.

The animal tissues were vacuum freeze-dried at − 20 °C for more than 3 days, then powdered. These samples were used for stable isotope analysis without further degreasing with organic solvent, because they already had been degreased during long-term ethanol preservation (Syväranta et al. 2008). The limnoriid isopod specimens were acidified with 1 N HCl to remove calcium carbonate from the exoskeleton, because the value of $\delta^{13}C$ in calcium carbonate is considerably higher than that in muscle.

Once manually broken into small pieces, the wood samples were immediately dried for about a week at 80 °C. Then, were put into polyvinyl bags and kept at room temperature for 4 to 9 years. After this long-term storage, no visible mold was found from the wood samples. Before stable isotope analysis in 2016–2017, the sapwood sections where were not heavily attacked by limnoriids were sub-sampled and dried again for 2 days at 80 °C. The dried sub-samples were minced with scissors for stable isotope analysis. The seawater samples were filtered on precombusted (485 °C, 4 h) GF/F filters on the day of sampling and kept at − 20 °C until further processing in 2016. After drying for 2 days at 60 °C, the filtered POM samples were put in a desiccator for 3 h with 12 N HCl fumes to remove inorganic calcium carbonate and dried overnight at 60 °C.

Stable isotope analysis

Stable isotope analyses were conducted using a mass spectrometer IsoPrime 100 (Elementar UK Ltd., Cheadle, UK) coupled with an elemental analyzer vario MICRO cube (Elementar Analysensysteme, Lagenselbold, Germany). The $\delta^{13}C$ and $\delta^{15}N$ values of all animal specimens were measured using samples ranging from 0.2 to 1.2 mg dry mass. Because of the small nitrogen content, the $\delta^{13}C$ and $\delta^{15}N$ values of the wood logs were measured using subsamples of about 1 and 40 mg dry mass, respectively. To ensure data quality, we omitted animal samples with high C/N ratios (> 4), because these samples were suspected of being contaminated by lipids or woody particles or being altered in long-term preservation in ethanol. Considering measurement accuracy, the stable isotope ratios were rounded to the nearest decimal place.

Data analysis

Differences in values of $\delta^{13}C$ and $\delta^{15}N$ among bivalve species and in shell length among teredinid bivalves were statistically evaluated by one-way analysis of variance (ANOVA), and post hoc Tukey–Kramer multiple comparison tests were used to determine which pairwise comparisons of species were significant. The differences in values of $\delta^{13}C$ among body tissues of the pholadids were tested by a paired *t* test.

To clarify the relative importance of potential carbon sources, i.e., wood logs and POM, for the teredinids, we applied a multiple linear regression model to the $\delta^{13}C$ dataset. Seasonal variations in the $\delta^{13}C$ values of POM might be reflected in the $\delta^{13}C$ values of the teredinids after several months of delay. The full model was therefore assumed to be "Wood + $POM_{X\text{ months before}}$ ($X = 0$, 2, 4, mean 0 and 2 or mean 2 and 4)." In this model, $POM_{\text{mean 0 and 2 months before}}$ represented the mean value of the stable isotope ratios of POM collected during the sampling month of the teredinids and 2 months before. If we needed $\delta^{13}C$ values of uncollected POM to run the model, we replaced the value with the mean $\delta^{13}C$ value of POM collected in the same month in other years. The best model was identified through forward stepwise variable selection based on the Akaike information criteria (AIC) procedure. After checking for multicollinearity between explanatory variables based on the variation inflation factor (VIF), we compared the effect of each potential carbon source using the standardized partial regression coefficient of the best model.

For nitrogen, the teredinids have another potential source, i.e., nitrogen fixation by symbiotic microbes. Its $\delta^{15}N$ value is incorporated into the intercept of the model, because the $\delta^{15}N$ value of dissolved dinitrogen in seawater is constant at around 0.5‰ in coastal areas (Naqvi et al. 1998). This makes it impossible to quantify the contribution of each potential nitrogen source to the diet of the teredinid bivalves. The $\delta^{15}N$ dataset was therefore tested under the same procedure to check whether wood logs and/or POM contribute to their nitrogen sources.

We also applied our dataset to a widely used Bayesian stable isotope mixing model, in which the TEF values were quoted from Charles et al. (2018). We then compared the resulting proportional contributions with the values obtained in the multiple linear regression model. These statistical tests were conducted in R ver. 3.5.1 (R Core Team 2018), using packages such as "multcomp" for ANOVA and the Tukey–Kramer test and "siar" for the Bayesian stable isotope mixing model approach.

Results

$\delta^{13}C$ and $\delta^{15}N$ values of wood borers and non-wood-boring bivalves

The mean $\delta^{13}C$ and $\delta^{15}N$ values of the teredinid bivalves were lower than those of POM and higher than those of the wood logs (Fig. 1). In contrast, the non-wood-boring bivalves had higher mean $\delta^{13}C$ and $\delta^{15}N$ values than POM. The $\delta^{13}C$ and $\delta^{15}N$ values of each teredinid species were significantly different from those of non-wood-boring bivalves (Table 1) (one-way ANOVA, $P < 2e\text{-}16$, $F = 49.76$ for $\delta^{13}C$

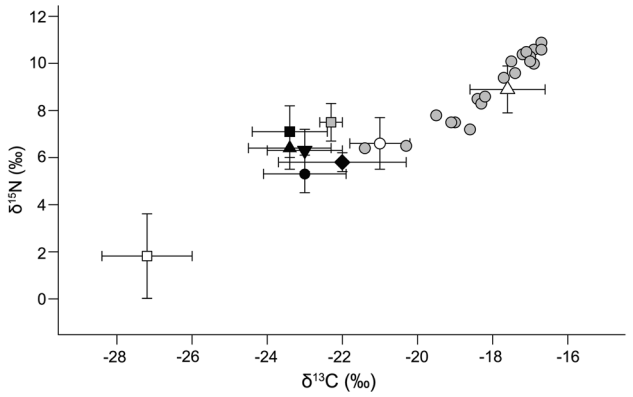

Fig. 1 Carbon and nitrogen stable isotope ratios of marine wood-boring invertebrates and non-wood-boring bivalves. Error bars: standard deviations: *Teredo navalis* ($N=30$: closed circle), *Teredo bartschi* ($N=30$: closed square), *Teredo clappi* ($N=30$: closed triangle), *Lyrodus pedicellatus* ($N=30$: closed inverted triangle), *Nototeredo edax* ($N=3$: closed diamond), *Martesia striata* ($N=20$: gray circle), Limnoriids ($N=5$: gray square), non-wood-boring bivalves ($N=16$: open triangle), Particulate organic matter ($N=26$: open circle), and *Cryptomeria japonica* ($N=45$ for carbon and 33 for nitrogen: open square). For *Martesia striata*, gray circles denote individuals

and 21.86 for $\delta^{15}N$, and Tukey–Kramer, $P<0.001$), except for the relationships between *T. bartschi* and the two non-wood-boring bivalves, *B. mutabilis* and *C. gigas*, in terms of $\delta^{15}N$ (Tukey–Kramer, $P=0.32$ and 0.10, respectively). $\delta^{13}C$ values of *M. striata* was characterized by a wide variation, including both values characteristic of the non-wood-boring bivalves and values characteristic of the teredinid bivalves (Fig. 1), and its $\delta^{15}N$ value was positively correlated with the $\delta^{13}C$ value (Fig. 1). The mean $\delta^{13}C$ value of limnoriids, including both *L. tuberculata* and *L. saseboensis*, was significantly lower than that of non-wood-boring bivalves (*t* test, $P=1.75e\text{-}09$) and slightly higher than that of teredinid species (Fig. 1). The mean C/N ratio in the limnoriids was significantly different from (*t* test, $P=7.73e\text{-}14$) and higher than that in all bivalve species (Table 1).

Carbon sources for teredinid bivalves

The $\delta^{13}C$ values did not significantly differ among the teredinid species (one-way ANOVA, $F=1.677$, $P=0.16$), and therefore in the following statistical analysis we tested the carbon source not for each teredinid species but for the teredinids altogether. The $\delta^{13}C$ values of the wood logs ranged from -28.8 to -24.5‰ (Table 1), and there was also wide seasonal variation in the $\delta^{13}C$ values of POM, ranging from -21.9 to -19.2‰. The $\delta^{13}C$ values of both potential carbon sources were significantly and positively related to those of the teredinids (minimum -25.2‰, maximum -20.2‰) ($P<2.2e\text{-}16$ for wood, and $P<3.614e\text{-}11$ for POM, respectively) (Fig. 2). Based on AIC, the best model of the carbon source for teredinids was identified as "Wood + POM $_{0\text{ month before}}$" (Table S1), and the $\delta^{13}C$ of the teredinid bivalves was best explained by the following multiple linear regression model formula (1), in which all explanatory variables were statistically significant ($P<0.05$) (Table S1):

$$\delta^{13}C_{\text{Teredinids}} = 0.69 \times \delta^{13}C_{\text{Wood}} + 0.38 \times \delta^{13}C_{\text{POM 0 month before}} + 3.70 \quad (1)$$

The VIF value between $\delta^{13}C_{\text{wood}}$ and $\delta^{13}C_{\text{POM 0 month before}}$ was only 1.07, which resulted in rejection of the multicollinearity between them. Using partial regression coefficients (0.69 for wood logs and 0.38 for POM), we calculated the standardized partial regression coefficients. The standardized partial regression coefficient of wood logs (0.64) was larger than that of POM (0.39). To determine the contribution of POM to their carbon source, we canceled the effect caused by the variation in $\delta^{13}C$ values of wood logs by using the following correction formula (2) based on the regression line in Fig. 2a:

$$\delta^{13}C^{*}_{\text{Teredinids}} = \delta^{13}C_{\text{Teredinids}} - \left(\delta^{13}C_{\text{Wood}} \times 0.802 - 1.092\right) + \text{mean}\,\delta^{13}C_{\text{Teredinids}} \quad (2)$$

The corrected $\delta^{13}C$ values (hereafter referred to as $\delta^{13}C^{*}$) of the teredinids were high from July (early summer) to September (late summer) and fluctuated seasonally, synchronously with the $\delta^{13}C$ values of POM (Fig. 3). When we compared the $\delta^{13}C^{*}$ values among the teredinid species, that of *N. edax* was significantly different from (one-way ANOVA, $P=0.0089$, $F=3.56$, Tukey–Kramer, $P<0.05$) and higher than those of the other teredinid species (Fig. 4a).

We also investigated the size-related $\delta^{13}C^{*}$ values of each teredinid species. Small individuals had wide variation in $\delta^{13}C^{*}$ values, and their $\delta^{13}C^{*}$ values converged to relatively high values as they grew (Supplementary Fig. 1). Among the four teredinid bivalves except *N. edax*, of which sample size was small, *T. bartschi* [shell length (SL) 5.7 ± 1.4 mm (mean ± standard deviation)] was the largest species in shell length, and its shell length was significantly different from that of the smallest teredinid species, *T. clappi* (SL 4.2 ± 0.7 mm) (one-way ANOVA, $P=0.00018$, $F=7.22$, Tukey–Kramer, $P<0.001$). The remaining two species, *T. navalis* and *L. pedicellatus*, had medium shell lengths of 4.9 ± 1.6 and 4.9 ± 1.5 mm, respectively.

Nitrogen sources for teredinid bivalves

$\delta^{15}N$ values differed significantly among the teredinid species (one-way ANOVA, $P=5.6e\text{-}10$, $F=15.07$). The lowest $\delta^{15}N$ value [5.3 ± 0.8‰ (mean ± standard deviation)]

Table 1 Results of stable isotope analysis of animals and potential food source

	$\delta^{13}C$ (‰) Mean ± SD (min/max)	$\delta^{15}N$ (‰) Mean ± SD (min/max)	C/N	*N*
Potential food sources				
Wood log				
Cryptomeria japonica	− 27.2 ± 1.2 (− 28.8/− 24.5)	1.8 ± 1.8 (− 3.3/5.1)	460 ± 91	45
				33
Particulate organic matter (POM)	− 20.9 ± 0.8 (− 21.9/− 19.2)	6.6 ± 1.1 (4.0/8.4)	5.63 ± 0.50	26
Wood borers				
Teredinidae	− 23.2 ± 1.1	6.3 ± 1.1	3.41 ± 0.14	123
Teredo navalis	− 23.0 ± 1.1 (− 24.7/− 20.7)	5.3 ± 0.8 (3.9/6.7)	3.37 ± 0.12	30
Teredo bartschi	− 23.4 ± 1.0 (− 25.0/− 21.3)	7.1 ± 1.1 (4.6/8.8)	3.45 ± 0.17	30
Teredo clappi	− 23.4 ± 1.1 (− 25.2/− 21.1)	6.4 ± 0.9 (4.3/8.7)	3.42 ± 0.13	30
Lyrodus pedicellatus	− 23.0 ± 1.0 (− 24.8/− 20.9)	6.3 ± 0.9 (4.8/9.5)	3.40 ± 0.15	30
Nototeredo edax	− 22.0 ± 1.7 (− 23.6/− 20.2)	5.8 ± 0.4 (5.5/6.2)	3.45 ± 0.08	3
Pholadidae				
Martesia striata	− 18.0 ± 1.3	9.0 ± 1.5	3.43 ± 0.17	20
Shell length: ~ 5 mm	− 19.2 ± 1.2 (− 21.4/− 18.2)	7.6 ± 0.9 (6.4/8.6)	3.51 ± 0.18	7
Shell length: 5 mm ~	− 17.4 ± 0.9 (− 19.5/− 16.7)	9.8 ± 1.1 (7.5/10.9)	3.38 ± 0.16	13
Adductor muscle	− 17.0 ± 0.1 (− 17.0/− 16.9)	10.1 ± 0.2 (10.0/10.3)	3.43 ± 0.13	3
Gill	− 17.0 ± 0.2 (− 17.2/− 16.9)	9.9 ± 0.5 (9.3/10.2)	3.48 ± 0.09	3
Siphon	− 16.6 ± 0.4 (− 17.1/− 16.2)	9.6 ± 0.6 (8.9/10.1)	3.33 ± 0.10	3
The rest of non-intestinal tissue	− 17.7 ± 0.3 (− 18.0/− 17.4)	8.8 ± 0.6 (8.2/9.4)	3.74 ± 0.15	3
Limnoriidae	− 22.3 ± 0.3	7.5 ± 0.8	3.96 ± 0.03	5
Limnoria saseboensis	− 22.5 ± 0.3 (− 22.7/− 22.3)	6.7 ± 0.8 (6.2/7.2)	3.96 ± 0.04	2
Limnoria tuberculata	− 22.2 ± 0.2 (− 22.4/− 22.0)	7.9 ± 0.1 (7.8/8.1)	3.97 ± 0.03	3
Non-wood-boring bivalves	− 17.6 ± 1.0	8.9 ± 1.0	3.20 ± 0.08	16
Brachidontes mutabilis	− 18.1 ± 0.3 (− 18.3/− 17.7)	8.5 ± 0.6 (7.8/8.9)	3.19 ± 0.10	3
Striarca symmetrica	− 17.1 ± 0.2 (− 17.4/− 16.9)	9.4 ± 0.2 (9.1/9.6)	3.24 ± 0.10	4
Barbatia virescens	− 17.3 ± 0.9 (− 18.4/− 16.8)	9.6 ± 1.0 (8.4/10.3)	3.13 ± 0.05	3
Crassostrea gigas	− 17.8 ± 1.4 (− 19.2/− 16.0)	8.3 ± 1.2 (6.8/9.6)	3.21 ± 0.07	6

The data are expressed as means ± standard deviation with minimum and maximum values

N indicates the total number of analyses for each species

characterized *T. navalis*, and *T. bartschi* had the highest $\delta^{15}N$ value (7.1 ± 1.1‰) (Fig. 4b). The $\delta^{15}N$ values of *T. clappi* and *L. pedicellatus* were intermediate (6.4 ± 0.9‰ and 6.3 ± 0.9‰, respectively). The $\delta^{15}N$ values of wood logs and POM were widely variable, ranging from − 3.3 to 5.1‰ and from 4.0 to 8.4‰, respectively (Table 1). Using these variations in potential nitrogen sources, we modeled changes in $\delta^{15}N$ of each teredinid species except *N. edax*, of which sample size was small, according to the following multiple linear regression model formulas (3–7):

Teredo navalis:

$$\delta^{15}N = 0.40 \times \delta^{15}N_{\text{POM mean 2 and 4 month before}} - 0.12 \times \delta^{15}N_{\text{Wood}} + 3.11 \quad (3)$$

$$\delta^{15}N = 0.47 \times \delta^{15}N_{\text{POM mean 2 and 4 month before}} + 2.44 \quad (4)$$

Teredo bartschi:

$$\delta^{15}N = 0.58 \times \delta^{15}N_{\text{Wood}} + 0.45 \times \delta^{15}N_{\text{POM 2 and 4 month before}} + 3.18 \quad (5)$$

Teredo clappi:

$$\delta^{15}N = 0.70 \times \delta^{15}N_{\text{Wood}} + 0.19 \times \delta^{15}N_{\text{POM 4 months before}} + 3.11 \quad (6)$$

Lyrodus pedicellatus:

$$\delta^{15}N = 0.41 \times \delta^{15}N_{\text{POM mean 2 and 4 month before}} + 3.76 \quad (7)$$

If the consumer assimilates nitrogen compounds from the diet, its coefficient never becomes negative. The relationship between *T. navalis* and wood logs in formula (3), therefore, could be regarded as a false correlation,

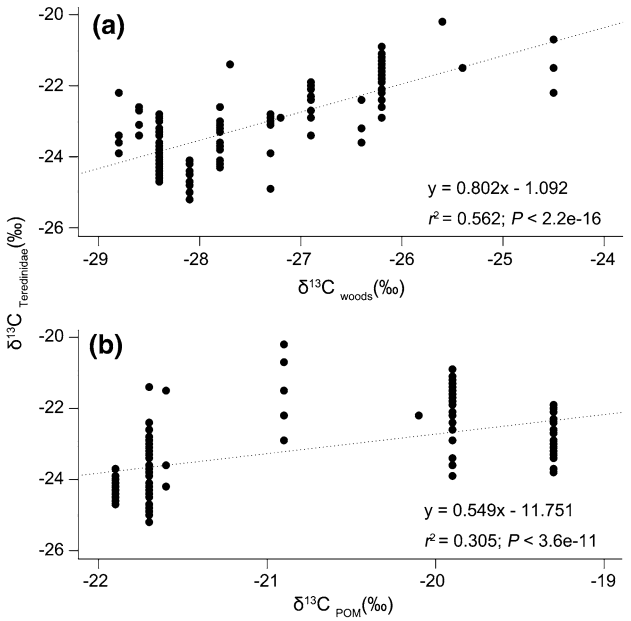

Fig. 2 Correlation between carbon stable isotope ratios of the teredinid bivalves ($N = 123$) and their potential food sources in **a** woods and **b** particulate organic matter (POM)

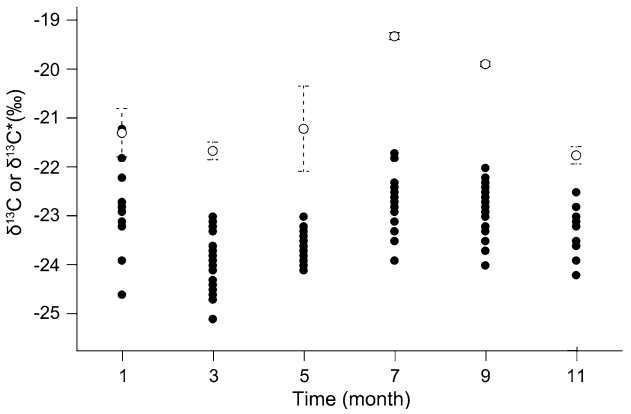

Fig. 3 Monthly changes in carbon stable isotope ratios of the teredinid bivalves ($N = 123$: closed circle) and particulate organic matter (POM) (open circle). Error bars: standard deviations: $\delta^{13}C^*$ refers to corrected $\delta^{13}C$ values. Further details are given in the text

and we also show the second best-fitting model formula (4). In these multiple linear regression models, $\delta^{15}N_{\text{POM mean 2\&4 months before}}$ was statistically significant for three teredinid species ($P = 0.014$ for *T. navalis*, $P = 0.010$ for *T. bartschi*, and $P = 0.038$ for *L. pedicellatus*) (Table S2). Among them, the $\delta^{15}N$ value of wood logs was also selected in the best model (formula 5) and was positively correlated with that of *T. bartschi* ($P = 9.6e\text{-}07$, Table S2). For another teredinid species, *T. clappi*, $\delta^{15}N_{\text{POM 4 months before}}$ was selected as well as that of wood logs

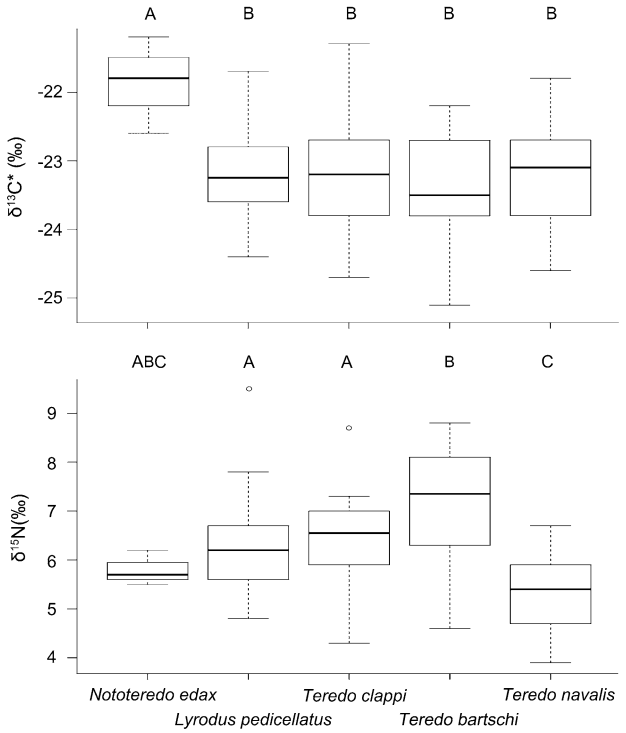

Fig. 4 **a** Carbon and **b** nitrogen stable isotope ratios among the teredinid bivalves. Different letters (A–C) indicate significant differences by the Tukey–Kramer test ($P < 0.05$). Horizontal lines in each box plot represent third quartile, median and first quartile. The whiskers extend to 1.5 × interquartile range. Dots represent outliers

(formula 6), but only the latter was statistically significant ($P = 2.3e\text{-}05$) (Table S2). The intercept was always statistically significant ($P < 0.01$) for every teredinid species, whereas the coefficients differed among the teredinid species, ranging from 2.44 for *T. navalis* to 3.76 for *L. pedicellatus* (Table S2).

Carbon sources for pholadid bivalves

The $\delta^{13}C$ values of the pholadids ranged from −21.4 to −16.7‰ (Table 1), and the $\delta^{13}C$ values increased as the bivalves grew (Fig. 5a). The mean $\delta^{13}C$ value of whole body of small pholadid individuals (SL < 5 mm; −19.2 ± 1.2‰) was low and was significantly different from that of adductor muscles of large individuals (SL > 5 mm; −17.4 ± 0.9‰) (Table 1) (*t* test, $P = 0.015$). Among the soft body tissues of large, full-grown specimens, the non-intestinal tissues had the lowest mean $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 5b), which were significantly different from those of the adductor muscles (paired *t* test, $P = 0.013$ for $\delta^{13}C$, and 0.021 for $\delta^{15}N$). The gaps between them were only 0.7‰ for $\delta^{13}C$ and 1.3‰ for $\delta^{15}N$ (Table 1).

We also performed fine dissection ($N = 10$) and scanning electron microscopy observations ($N = 4$) preliminary over

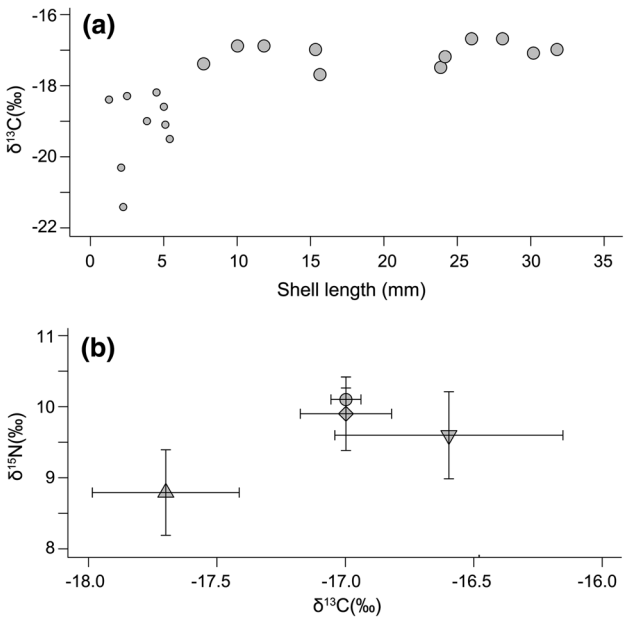

Fig. 5 **a** Shell length-related variation in carbon stable isotope ratios of the pholadid bivalve, *Martesia striata* ($N=20$). Note that whole body without intestines (gray small circle) was used for small individuals. **b** Carbon and nitrogen stable isotope ratios of *M. striata* tissues, i.e., muscle ($N=3$: gray circle), gill ($N=3$: gray diamond), siphon ($N=3$: gray inverted triangle), and other tissues without intestine ($N=3$: gray triangle). Error bars: standard deviations

the alimentary system of ten preserved specimens ranging from 5.6 to 23.5 mm in shell length, with and without gonad/callum development, to obtain morphological evidence. Details will be reported separately elsewhere, but tiny but distinct reniform or sac-like cecum was found on the right wall of the stomach in smaller immature individuals (< 15 mm in shell length, $N=7$), and its stomach, digestive tracts and the cecum were filled with ingested fragments of wood and filter-fed particles such as fragments of diatom frustules (Supplementary Fig. 2c, d). Wood debris were the predominant contents (Supplementary Fig. 2d, e) and were readily recognized through the tracts in having brownish color [Supplementary Fig. 2b: (gc) and (sc)]. On the other hand, the cecum was rudimentary or even absent in full-grown mature individuals that had developed callum (9.5–23.5 mm in shell length, $N=3$), and there were few wood fragments in their tracts.

Discussion

POM contributes to the diet of marine wood borers in temperate shallow water

The teredinids have long been believed to be obligate xylophages, whereas a feeding experiment using ^{14}C-labelled phytoplankton (Pechenik et al. 1979) and some anatomical studies of the alimentary systems and gill structures (Turner 1966; Lopes et al. 2000) suggested that they used POM as a part of their food resources. The present study supports the conclusion that wood-derived organic carbon is the main carbon source for the teredinids, by comparing the standardized partial regression coefficients of wood logs and POM in the best carbon source model of multiple linear regression analysis. However, seasonal synchronicity between the $\delta^{13}C^*$ value of the teredinids and the $\delta^{13}C$ value of POM strongly supports the non-negligible contribution of POM to the carbon sources of the teredinid bivalves. A low VIF value guaranteed the independence of explanatory variables so we regard their standardized partial regression coefficients as indicators of their relative importance as carbon source for the teredinids. The contribution is calculated to be 62.1% for wood and 37.9% for POM based on the following formulas: wood contribution (%) $= 0.64/(0.64+0.39) \times 100$ and POM contribution (%) $= 0.39/(0.64+0.39) \times 100$. Thus, autochthonous production of POM strongly contributes to the diet of wood-boring invertebrates, helping them to decompose wood logs in temperate shallow water, particularly in its initial phase, where the teredinids are known to play an important role (Nishimoto et al. 2015).

Unlike the sedentary teredinid bivalves, free-living limnoriid isopods, serving as one of the keystone members in decomposing wood logs in the sea, do not live in one permanent shelter. Such a mode of life eventually causes the collapse of the honeycombed wood logs (Nishimoto et al. 2015). These limnoriid isopods have endogenous lignocellulase activity (King et al. 2010), and their digestive tracts are always filled only with woody particles (e.g., Sleeter et al. 1978). Therefore, they have long been believed to depend exclusively on woody debris as their carbon source. In the present study, the mean $\delta^{13}C$ value of the limnoriids is slightly higher than that of the teredinids, in which 37.9% of carbon is derived from POM. It seems to be reasonable to conclude that the limnoriid isopods are not obligate xylophagous animals and partially depend on other food sources. For example, free-living xylophagous microorganisms, such as fungi and bacteria may also provide a supplemental nitrogen to the limnoriids. In addition, their exoskeletons were often covered with ciliates, fungal hyphae, bacteria, and microphyte remains, and the limnoriid isopods might use these organisms as carbon and nitrogen source by grooming their exoskeleton (Charles et al. 2019). These organic matters on the exoskeleton may also affect $\delta^{13}C$ and $\delta^{15}N$ values of the limnoriids specimens themselves. Indeed, most limnoriids samples showed high C/N ratios over 4, and then removed from this study to maintain data quality. In the present, it is technically difficult to measure the stable isotope ratios of these potential food sources due to their insufficient weight.

Future studies should also avoid the contamination of these organic matters, for example, by using the limnoriid specimens immediately after molting.

Martesia striata changes its feeding strategy from xylophagous to filter feeding as it grows

Unlike the teredinids, a wood-boring pholadid bivalve, *Martesia striata*, has been thought to be a filter feeder judging from morphological features, such as the lack of a wood-storing cecum in the stomach (Purchon 1956; Turner and Johnson 1971). In the present study, however, the smaller young individuals of *M. striata* had relatively low $\delta^{13}C$ and $\delta^{15}N$ values. The low $\delta^{13}C$ and $\delta^{15}N$ values are not explained by the small variation among body tissues nor by possible contamination of the tissue samples by woody pellets with low nitrogen content, respectively.

Our preliminary anatomical observations first revealed the presence of a tiny but distinct reniform cecum in smaller immature specimens, whose contents of the cecum, stomach and digestive tracts were dominated by fragments of wood, as in the exclusive wood-eating teredinids and xylophagaids. The cecum present in juvenile *Martesia* appears to have been overlooked previously because of its tiny size and fragility, or because samples of small young individuals have not been considered (Purchon 1956; Turner and Johnson 1971). This finding in this study indicates that wood logs provide young *M. striata* with the carbon that is necessary for their initial growth. The $\delta^{13}C$ values of large, full-grown individuals were relatively high, suggesting that the importance of POM as their food source increased as they grew. After they terminate boring activity, the callum covers their foot and they become true filter feeders as previously believed (Turner 1955; Turner and Johnson 1971).

Species-specific characteristics of feeding strategies among shipworms

Teredinid species have been thought to switch their feeding strategy from xylophagous to filter feeding under crowding or other environmental circumstances (Turner 1966; Lopes et al. 2000). This study provides another aspect of the diversity in feeding strategies in the teredinid species.

Specimens of *N. edax* have obviously higher $\delta^{13}C^*$ values than the other four teredinid species, indicating that *N. edax* depends on POM for its carbon sources more than other teredinid species. *Nototeredo edax* is the only free-spawning species at this study site, and it tends to occur in open waters (Scheltema 1971; MacIntosh et al. 2012). Especially in open waters, with sparse distribution of driftwood, it is advantageous for the species to increase their fecundity (Scheltema 1971; MacIntosh et al. 2014). We think it is reasonable to assume that spawning species rely more heavily on POM than brooding species, which helps them to maintain their large body size and consequent fecundity. Future studies need to investigate the anatomical relevance of interspecific differences in stable isotope approaches.

Because of the low nitrogen content of woody tissues, it has been widely believed that endosymbiotic nitrogen-fixing bacteria are the major nitrogen source for the teredinids (Gallager et al. 1981; Waterbury et al. 1983; Lechene et al. 2007). If nitrogen fixation was the only source of nitrogen for the teredinids, their $\delta^{15}N$ values would be similar among the teredinid species. This study, however, has shown that $\delta^{15}N$ values are significantly different among teredinid species, which suggest that nitrogen source is different between the teredinid species collected from a single location. The multiple linear regression models indicate that the supplemental nitrogen sources of four teredinid bivalves are diverse.

$\delta^{15}N_{\text{POM mean 2 and 4 months before}}$ values were positively correlated with $\delta^{15}N$ values in three teredinid bivalves, suggesting that some nitrogen is assimilated from POM following a 2- to 4-month delay. Nitrogen is more valuable than carbon for xylophages, so it seems to take longer for turnover than carbon. For *T. navalis* and *L. pedicellatus*, $\text{POM}_{\text{mean 2 and 4 months before}}$ is the only parameter adopted in the best model, but the intercepts are considerably different between them, being high for *L. pedicellatus* and low for *T. navalis*. The low $\delta^{15}N$ value is attributed to the high contribution of nitrogen fixation, in which $\delta^{15}N$ value is apparently lower than that of POM (Naqvi et al. 1998). Therefore, *T. navalis* depend on nitrogen fixation for its nitrogen more than *L. pedicellatus*. The former species is the only short-term brooder collected at this study site and has pelagic larval stage for 10–15 days (Calloway and Turner 1988). Compared to long-term brooder with larval stage from a few hours to days, this species is not capable of local retention. Suppressing the relative importance of wood logs and POM as nitrogen sources, *T. navalis* may be able to bore into wood logs, even if the conditions in the recruitment area are somewhat unfavourable.

Unlike these two teredinid bivalves, the $\delta^{15}N$ value of *T. clappi* is positively correlated only with that of wood logs. Wood logs have been ignored as the nitrogen source for xylophages; however, they serve as a supplemental nitrogen source for *T. clappi*, despite their low nitrogen content. Yamanaka et al. (2015) found that wood-derived nitrogen also served as the main nitrogen source in a deep-sea xylophage of the Xylophagaidae, *Xyloredo teremachii* (Iw. Taki & Habe, 1950). A feeding strategy that does not rely on POM as a nitrogen source probably have resulted in *T. clappi* having the smallest shell length among the four teredinid species, but this feeding strategy seems to be adapted to waters with low productivity. Contrary to other three teredinid species, the $\delta^{15}N$ value of *T. bartschi*, the largest

teredinid species at this study site, is positively correlated with that of both wood logs and POM. By using several nitrogen sources in addition to nitrogen fixation, *T. bartschi* appears to be the most highly adapted to this experimental condition among the four teredinids species.

This study attributes all the variation in C and N source utilization to host phylogeny without considering other sources of variability. This is because all teredinid specimens, regardless of species, were collected from a limited number of wood logs deployed at the same site, and it is unlikely that there are significant differences in environmental conditions between the teredinid species. However, it is important to bear in mind that these results may vary depending on environmental conditions. In the Xylophagaidae, a deep-water sister taxon of the teredinid bivalves, all members have the reduced labial palps and gills, indicative of the low filter-feeding function (e.g., Turner and Johnson 1971). However, their $\delta^{15}N$ values tend to be higher in the specimens collected from shallower sites, which indicates the contribution of other nitrogen sources, such as POM (Voight et al. 2020). That is, the feeding value of each potential food source for xylophagous animals is not universal, and change depending on the environmental conditions. In this study, we estimated that 37.9% of carbon is derived from POM in temperate shallow water, but the degree of contribution also might change depending on the conditions, such as in-situ productivity and symbiotic community composition.

Sampling and pretreatment for appropriate estimation of food sources

Paalvast and van der Velde (2013) concluded that *T. navalis* collected from the polyhaline zone in the Netherlands was a filter feeder, based on their stable isotope analysis. The $\delta^{13}C$ value of *T. navalis* (−23.13‰) was considerably higher than that of wood logs (−26.41‰) and was comparable to or slightly higher than those of known filter-feeding bivalves collected from the same site (−23.46‰ for *Mytilus edulis* Linnaeus, 1758 and −24.58‰ for *C. gigas*). In brackish waters, however, the $\delta^{13}C$ value of POM is relatively low owing both to the high concentration of terrestrial input and to autochthonously produced phytoplankton with low $\delta^{13}C$ values (Chanton and Lewis 2002; Fry 2002), which reduce the gaps in $\delta^{13}C$ values between wood logs and POM. The small gaps in $\delta^{13}C$ of potential food sources make it difficult to correctly estimate the contribution of each food source.

Various protocols are available for pretreatment of samples for stable isotope measurements (Caut et al. 2009). Some studies use only muscles, whereas others use the whole body. Additionally, some studies carried out premeasurement degreasing and/or acidification protocols. These different pretreatment protocols make it difficult to compare the stable isotope ratios among studies (e.g., Post et al. 2007). Here, we consider that the C/N ratio of target species and its standard deviation are important. Post et al. (2007) argued that C/N ratios could be lower than 3.5 when lipid content is consistently low in aquatic animals. Moreover, when there are large variations in C/N ratios among specimens, the samples are heterogeneous in chemical composition, and it becomes unclear what the $\delta^{13}C$ and $\delta^{15}N$ values represent. Charles et al. (2018), however, estimated the food resources in *B. carinata* using specimens with high C/N ratios and high SD values (8.3 ± 2.5). The $\delta^{13}C$ value of *B. carinata* (-27.5 ± 0.6‰) might be underestimated, resulting in further misinterpretation of the proportional contributions of carbon sources in the mixing model approach. Pretreatment that reduces the C/N ratio and its standard deviation in target species is therefore indispensable for comparable and repeatable studies. In the present study, we measured $\delta^{13}C$ and $\delta^{15}N$ values using ethanol-preserved animal muscles, and the C/N ratios were often lower than 3.5, with small SD values, except for the limnoriids.

Risk of the use of uncertain TEF values in the mixing model approach

Recent stable isotope mixing model approaches in Bayesian frameworks, such as the SIAR algorithm (Parnell et al. 2010), can yield probabilistic estimates for the proportional contributions of each food source to the target species, even if the number of potential food sources exceeds $n+1$ (where n is the number of isotopes employed). These approaches are widely accepted for estimation of animal food sources. However, they are often based on many assumptions in setting the parameters. Charles et al. (2018) reported that a fully marine teredinid, *B. carinata*, derived 26% of its carbon from POM, based on a Bayesian stable isotope mixing model. In their model, the $\delta^{13}C$ and $\delta^{15}N$ values of both potential food sources (i.e., wood logs and POM) were used as end members, and the TEF values were quoted from previous studies dealing with the whole bodies of invertebrates, i.e., 0.30 ± 0.21‰ for $\delta^{13}C$ and 2.5 ± 0.25‰ for $\delta^{15}N$ (Caut et al. 2009). Concerning the assimilation of dissolved N_2 by bivalves with the help of symbiotic bacteria, they assumed that the TEF was −1‰ between dissolved N_2 and fixed N and there was no fractionation between the endosymbionts and their host. When we carried out the Bayesian stable isotope mixing model using our dataset under the same settings, POM accounted for 59.2% of the teredinids carbon source (Supplementary Figs. 3 and 4). This ratio is considerably different from that of *B. carinata* in Charles et al. (2018) and the output from the multiple linear regression model approach in this study. These facts clearly indicate that the TEF values used in Charles et al. (2018) are not applicable for estimating the food resources in marine xylophagous animals. Thus, the mixing model should be used with care

only when the TEF values and stable isotope ratios of the end members are correctly assigned (Ben-David and Schell 2001). Contrary to the mixing model approach, for carbon source analysis in the teredinids bivalves, the multiple linear regression model approach is advantageous in that it can estimate the proportional contribution of each potential carbon sources without use of the uncertain TEF values.

Speculations

This study highlights that marine wood borers depend on autochthonous production as their source of carbon and/or nitrogen in a coast of Tanabe Bay, central Japan. We assume that the same setting occurs in temperate shallow waters around the world, and offer the novel hypothesis that the transfer of organic carbon from coarse woody materials into marine ecosystems is controlled by the productivity of the ocean. This hypothesis also implies that the importance of wood logs as a food source increases in low-productivity conditions such as the deep-sea floor. By comparing wood-boring activities and autochthonous productivity among sites, we can elucidate the ecological role of terrestrial input in the form of coarse plant debris into marine ecosystems.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-021-04973-0.

Acknowledgements We thank Keita Harada (Seto Marine Biological Laboratory, Kyoto University), Go Suzuki (Seikai National Fisheries Research Institute, Fisheries Research Agency, Japan) and Hiroshi Fukuda (Faculty of Agriculture, Okayama University) for their assistance with the field sampling, Michitaka Shimomura (Seto Marine Biological Laboratory, Kyoto University) for his identification of limnoriid isopods, Keiko Yamada (National Research Institute of Fisheries Science, Fisheries Research Agency, Japan) for her help in running the elemental analyzer and mass spectrometry. The authors wish to thank three anonymous reviewers for valuable comments on earlier version of the manuscript.

Author contribution statement AN drafted a research plan, designed the methodology and collected the samples; TH identified the bivalves and produced Suppl. Fig. 2; AN produced other figures and tables; AN analyzed the stable isotope ratios; TH, AA and YS guided the work; AN drafted the manuscript and TH and AA contributed critically to the manuscript writing; all the authors gave final approval for submission.

Funding This study was partially funded by a Grant-in-Aid for Japan Society for the Promotion of Science fellows to AN (222580) and to TH (237855). Stable isotope analysis was supported by National Research Institute of Fisheries Science, Fisheries Research Agency, Japan.

Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability R packages are listed in "Materials and methods".

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional guidelines for the care and use of animals were followed.

Consent to participate Not applicable.

Consent for publication Not applicable.

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