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## **Exploring feeding strategies and isotopic diversity in some Mediterranean marine wood-boring bivalves**

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HAL Authorization

Article title: Exploring feeding strategies and isotopic diversity in some Mediterranean marine wood-boring bivalves

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Highlights:

- Long-term experiment provides robust insights into the trophic ecology of wood borers
- *X. dorsalis*  $\delta^{15}\text{N}$  is minimally affected by access to suspended particles
- Comparative profiling of bacterial communities in co-occurring wood borers
- *X. dorsalis* likely acquires nitrogen from bacteria associated with digested wood

## Abstract

Wood serves as a resource for specialized marine organisms, such as wood-boring bivalves, which have evolved unique strategies to degrade and assimilate this highly refractory material. This study examines the feeding strategies of co-occurring wood-boring bivalves under controlled conditions by integrating isotopic analyses ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) with microbiota profiling. A comparison of one xylophagaid (*Xylophaga dorsalis*) and two teredinids (*Bankia carinata* and *Nototeredo norvagica*) reveals pronounced differences in their isotopic signatures, despite their shared trophic niche. While teredinids rely on diazotrophic symbioses for nitrogen acquisition, *X. dorsalis* exhibits elevated  $\delta^{15}\text{N}$  values, suggesting additional nitrogen inputs, possibly from bacteria associated with digested wood. Microbiota analyses confirm the presence of diazotrophic endosymbionts in *X. dorsalis*, whereas the nitrogen-fixing capacity of the dominant symbiont in *B. carinata* remains uncertain. These findings underscore the diversity of trophic mechanisms and symbiotic interactions among wood-boring bivalves, highlighting the complexity of nutrient assimilation strategies within this ecological guild.

## 1. INTRODUCTION

Although wood is scarce in the ocean, it serves as a principal food source for some specialized marine bivalves. These xylophages retain conventional filter-feeding structures (Morton, 1983) but have also evolved unique adaptations to exploit this highly refractory resource (Cragg et al., 2015). Their shells bear fine denticles for rasping wood, they possess a large blind stomach sac for storing wood chips (Turner, 1966), and they host endosymbiotic bacteria in their gills that contribute to wood digestion (Distel and Roberts, 1997; Popham and Dickson, 1973; Waterbury et al., 1983).

Although wood is rich in organic carbon, it presents two major challenges: it is composed of complex macromolecules, mainly cellulose embedded in lignin (Nishimura et al., 2018), requiring specialized enzymes for breakdown (Cragg et al., 2015), and it is extremely nitrogen-poor (Cowling and Merrill, 1966), limiting protein synthesis and growth in heterotrophic organisms. Consequently, xylophages must produce cellulolytic enzymes to degrade cellulose and also rely on alternative nitrogen sources to overcome wood's nutrient constraints.

Marine wood-boring bivalves belong to two families, Teredinidae and Xylophagidae, both of which have evolved to bore into and consume wood (Distel et al., 2011). While Teredinidae are better studied, both lineages descend from a common ancestor and harbor specialized gill endosymbionts thought to be essential for cellulolysis and nutrient acquisition (Distel and Roberts, 1997). In Teredinidae, these symbionts not only secrete cellulolytic enzymes but also fix atmospheric nitrogen, supplying their hosts with bioavailable N (Lechene et al., 2007; Waterbury et al., 1983). By comparison, the symbiotic partners of Xylophagidae remain poorly characterized (Distel and Roberts, 1997; Fagervold et al., 2014). The discovery of analogous endosymbionts in both families suggests a shared evolutionary strategy for exploiting wood, yet emerging isotopic evidence indicates that their modes of nitrogen acquisition may diverge (Voight et al., 2020).

Stable isotope analysis provides a powerful tool to unravel the feeding strategies of wood-boring bivalves by tracing the isotopic signatures of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in their tissues. Since an organism's isotopic composition reflects that of its diet (DeNiro and Epstein, 1981; DeNiro and Epstein, 1978), species consuming the same food sources would be expected to show similar isotopic signatures. However, interspecific comparisons are challenging due to the difficulty of collecting sufficient specimens from the same piece of wood and the incomplete knowledge of available food sources (Voight et al., 2020). Additionally, wood-boring bivalves may rely on both wood and suspended particles, complicating the precise quantification of each source's contribution to their diet (Charles et al., 2018; Nishimoto et al., 2021). Stable isotope analysis can help distinguish wood as a primary carbon source from suspended particulate organic matter (SPOM), which may supplement nutrient intake. This distinction is particularly clear when separating terrestrial from marine primary sources, as marine producers typically exhibit heavier  $\delta^{13}\text{C}$  values than terrestrial ones (Peterson and Fry, 1987).

According to the World Register of Marine Species (WoRMS, <https://www.marinespecies.org> accessed 2025-10-03. doi:10.14284/170), eight species of Teredinidae and four species of Xylophagidae have been documented in the Mediterranean. Co-occurrence of multiple species on the same piece of wood is plausible (Voight, 2008; Voight et al., 2020), offering an opportunity to investigate whether wood-boring bivalves share similar trophic niches. To explore this, we conducted an experiment using beech wood previously exposed to

shallow coastal waters to allow natural infestation by xylophagous bivalves. The infested logs were then incubated under filtered and unfiltered seawater to assess the impact of SPOM availability on isotopic composition and to determine whether coexisting species exhibit similar feeding strategies. Finally, to better understand the processes underlying isotopic variation, we characterized the bacterial communities associated with bivalve tissues and examined whether differences in microbial composition aligned with distinct feeding strategies or nutrient acquisition mechanisms.

## 2. MATERIAL AND METHODS

### 2.1 Experimental setup

Beech wood (*Fagus sylvatica*) was chosen as a regionally appropriate substrate, given the proximity of the Albères massif, which hosts one of Europe's southernmost beech forests (Charles et al., 2022). Two bundles, each from the same tree branch, were prepared, containing five dried sections (20 cm × 2–4 cm) with bark intact.

Bundles were submerged at 27 m depth at the "SOLA" coastal observation station (Banyuls-sur-Mer, 42°29'300 N, 03°08'700 E; <https://www.somlit.fr/en/>) in late June 2021 for a two-month colonization by wood-boring bivalve propagules.

After colonization, the bundles were transferred to two 60 L tanks for an 8-month experiment. Both tanks received continuous seawater flow from the bay, with distinct particle treatments: one tank contained unfiltered seawater representing ambient coastal particles, and the other contained particle-depleted seawater, pre-filtered sequentially through 60 µm nylon, 20 µm polypropylene, and 5 µm polypropylene cartridges (Diproclean). Tanks were kept in the dark at 14 °C, matching the site's average annual temperature ( $14.4 \pm 1.7$  °C).

Water conditions were monitored every two months by measuring chlorophyll a and phaeopigment concentrations fluorometrically (Holm-Hansen et al., 1965; Strickland and Parsons, 1972; Yentsch and Menzel, 1963). One-liter samples were filtered through GF/F glass fiber filters, pigments were extracted in 90% acetone overnight, and fluorescence was recorded at 432 nm and 664 nm before and after acidification using a Turner Design 10-AU fluorometer.

### 2.2 Sample collection and preparation for stable isotope analyses

Stable isotope analyses (SIA) of suspended particulate matter was performed at the same intervals as pigment measurements. Two 60 mL water samples per tank were filtered through pre-combusted (450 °C, 4 h) 13 mm GF/F filters, dried at 50 °C overnight, and stored in a desiccator. Filters were halved: one half was acidified with HCl fumes for 4 h to remove inorganic carbon (Søreide et al., 2006) for  $\delta^{13}\text{C}$  measurements, and the other half was used directly for  $\delta^{15}\text{N}$  (Bunn et al., 1995; Kennedy et al., 2005).

After 8 months, wood sections were carefully opened with chisels, and bivalves were extracted intact. Species were identified morphologically (Turner, 1971, 1966) and Romano et al. (2014). Animals were cleared of shells (and pallets for Teredinidae), which were preserved as vouchers at the Observatoire Océanologique de Banyuls-sur-Mer. The caecum and digestive tract were purged gently, then samples were frozen, freeze-dried, and finely ground for analysis.

Wood samples were meticulously cleaned under a binocular microscope to remove bark, limestone, and debris, then frozen, freeze-dried, and homogenized using a TissueLyser II (Qiagen).

### 2.3 Determination of isotopic compositions

All samples were packed into tin capsules, with masses ranging from 0.3 to 0.4 mg for animal tissues and for analysis of wood carbon isotopic composition, and masses of 1.4 to 8.7 mg for analysis of wood nitrogen isotopic composition.

Isotopic compositions were determined at the stable isotope facility of the joint research unit LIENSs (CNRS - La Rochelle University), France, using an elemental analyzer (EA Flash 2000, Thermo Scientific, Milan, Italy) equipped with an autosampler (Zero Blank, Costech, Valencia, CA, United States) coupled to a continuous flow isotope-ratio mass spectrometer (Delta V Plus with a ConFlo IV interface, Thermo Scientific, Bremen, Germany) equipped with the Smart EA option. Data are expressed using the  $\delta$  notation (in ‰) and were normalized using reference materials, i.e., Vienna Pee Dee Belemnite for  $\delta^{13}\text{C}$  values and atmospheric nitrogen (Air- $\text{N}_2$ ) for  $\delta^{15}\text{N}$  values, following the formula:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \times 1000$ , where R is  $^{13}\text{C}/^{12}\text{C}$  for carbon and  $^{15}\text{N}/^{14}\text{N}$  for nitrogen, respectively. Normalization was done using USGS61 and USGS63 (US Geological Survey, Reston, VA, USA) based on their nitrogen isotope-delta values and standard uncertainties (i.e.,  $-2.87 \pm 0.04\text{‰}$  and  $+37.83 \pm 0.06\text{‰}$  respectively). Uncertainty was  $<0.10\text{‰}$  for both carbon and nitrogen.

### 2.4 Bivalve microbiome characterization using 16S rRNA barcoding

To investigate the microbiota associated with wood-boring bivalves, additional individuals of the same species were sampled from the same experimental setup at the end of the incubation period, concurrently with those used for stable isotope analysis. For each specimen, the gills were separated from the rest of the body tissues for distinct analyses. Dissections were performed in RNA Later solution to preserve sample integrity, ensuring high-quality sequencing data. Cells were lysed with microbeads from the Fecal/Soil MicroPrep kit (Zymo) in 600 $\mu\text{l}$  RLT buffer from the AllPrep DNA/RNA mini kit from Qiagen, in a FastPrep apparatus (MP Bioscience, 2 times 5s at 5m/s). DNA was then purified with the AllPrep DNA/RNA mini kit (Qiagen) following the manufacturer's instructions and RNase treated. The V3V5 region of 16SrDNA gene was amplified for Bacteria using two universal primers : 357F (Schuurman et al., 2004) and 926R (Walters et al., 2016). High-throughput sequencing was performing using MiSeq 300 bp technology (Novogene). Raw sequences were processed using the FROGS pipeline (Escudié et al., 2018) on the Galaxy platform. After quality filtering, dereplication, and chimera removal, sequences were clustered into amplicon sequence variants (ASVs), defined as unique, high-resolution sequence groups produced by Swarm clustering without a fixed similarity threshold. Only ASVs present in at least four samples and accounting for a minimum of 0.05% of the total read count were retained for downstream analyses. Sequences were assigned to the species level by comparison of the 373 bp 16S rRNA gene fragment with the SILVA 138.1 database (Quast et al., 2012), using a 97% identity cutoff over at least 95% of the sequence length. While this fragment length provides moderate taxonomic resolution, it may not fully resolve closely related species. The 97% identity threshold is a classical standard for near full-length 16S sequences (Stackebrandt and Goebel, 1994), but its application to shorter amplicons such as the V3–V5 region is debated, with some authors recommending higher

thresholds (e.g., 98.5–99%) for species-level discrimination (Kim et al., 2014; Yarza et al., 2014). We chose the 97% cutoff as a practical compromise, consistent with previous studies and current database limitations. Overall, we analyzed 1,264,373 reads, which were normalized to 63,989 reads per sample. The DNA sequences have been submitted to the European Nucleotide Archive (ENA) at EMBL-EBI and are publicly available under accession number PRJEB88332.

## 2.5 Data analysis and statistical treatment

Due to the small sample sizes, non-parametric methods were employed to analyze the stable isotope data. Specifically, Kruskal-Wallis tests were first applied to assess overall differences in isotopic compositions among species under each experimental condition. Pairwise comparisons were then conducted using Wilcoxon rank sum tests to evaluate the influence of SPOM presence on the isotopic composition of each species. All statistical analyses were performed at a significance level of  $\alpha = 0.05$  using R version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Additionally, the PMCMRplus package (version 1.9.10) in R was used for post-hoc multiple comparison tests (Pohlert, 2023).

For microbiome data, alpha diversity metrics (including the Shannon index) and rarefaction curves were computed using the tools integrated in the FROGS pipeline on the Galaxy platform (Escudié et al., 2018). Beta diversity analyses were based on Bray-Curtis dissimilarities calculated from relative abundance data, and hierarchical clustering was performed within FROGS using default parameters to explore community similarities across host species and tissue types.

## 3. RESULTS

### 3.1 Water quality in tanks

Chlorophyll pigment analyses revealed natural fluctuations in the tank supplied with unfiltered seawater, reflecting variations in phytoplankton biomass, though changes were relatively moderate within the tank (Figure S1). In contrast, pigment concentrations in the filtered seawater tank remained below the detection limit, confirming that filtration effectively removed most particles. No filter-feeding propagules (e.g., bivalves, serpulids, or ascidians) were observed in either tank, indicating that particle reduction prevented propagule development.

### 3.2 Isotopic compositions

In the filtered seawater tank, SPOM loads were below detection limits, confirming that wood was the sole available food source. Beech wood  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were  $-28.3\text{‰}$  and  $-2.1\text{‰}$ , respectively, and remained consistent in the unfiltered seawater condition. In the latter, SPOM was detected with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  $-25.1 \pm 0.8\text{‰}$  and  $3.7 \pm 0.8\text{‰}$ , representing differences of  $\sim 3\text{‰}$  in  $\delta^{13}\text{C}$  and  $\sim 5.9\text{‰}$  in  $\delta^{15}\text{N}$  compared to beech wood (Figure 1).

At the end of the experiment, three wood-boring bivalve species, *Bankia carinata*, *Nototeredo norvegica*, and *Xylophaga dorsalis*, were collected from both treatments. Pronounced differences in  $\delta^{15}\text{N}$  were observed among species: *X. dorsalis* consistently exhibited higher  $\delta^{15}\text{N}$  ( $\sim 5\text{‰}$ ) than the two shipworms (*B. carinata* and *N.*

*norvagica*, ~1‰). This difference was statistically significant under both conditions (Kruskal-Wallis tests:  $p = 0.008$  with SPOM;  $p = 0.003$  in filtered seawater). Exposure to SPOM caused a slight but consistent  $\delta^{15}\text{N}$  increase across all species (Wilcoxon tests,  $p < 0.011$ ).

For  $\delta^{13}\text{C}$ , only *N. norvagica* showed a small but significant enrichment under SPOM (−26.5 to −25.5‰ in filtered vs. −25.2 to −24.5‰ in unfiltered; Wilcoxon test,  $p = 0.011$ ). No significant  $\delta^{13}\text{C}$  changes were observed in the other two species.

### 3.3 Bivalve microbiome

Microbiome analyses were performed on bivalves from the filtered seawater tank, the only condition that provided sufficient *X. dorsalis* specimens (>5 individuals). Three individuals of *X. dorsalis*, two of *N. norvagica*, and two of *B. carinata* were analyzed. To separate gill-associated bacteria from the rest of the body, animals were dissected accordingly. Rarefaction curves plateaued for all samples, indicating sufficient sequencing depth.

Bacterial diversity (Shannon index) was low in *X. dorsalis* ( $0.86 \pm 0.51$ ) and *B. carinata* ( $0.67 \pm 0.29$ ) but higher in *N. norvagica* ( $2.28 \pm 0.2$ ). Hierarchical clustering (Bray-Curtis distance) grouped microbiota by host species: *X. dorsalis* and *N. norvagica* were more similar, whereas *B. carinata* hosted a distinct community (Figure 2A). Within each species, gill and body microbiota were comparable, so subsequent analyses focused on branchial tissues (Figure 2B).

Across all samples, communities were dominated by one or two clusters of gamma-proteobacteria, representing 66–98% of total reads. In branchial tissues, sequences related to known shipworm symbionts were detected in *N. norvagica* and *X. dorsalis*. In *X. dorsalis*, 87–98% of sequences were identical to the symbiont LP1 of *Lyrodus pedicellatus* (16S rRNA, AY150183.1). In *N. norvagica*, 66–75% of sequences were potential symbionts: half matching LP1 (100% identity) and half matching *Teredinibacter turnerae* T7901 (99.73% identity, CP001614.2). In contrast, sequences from *B. carinata* showed low similarity to known shipworm symbionts (4–18%), with the dominant sequence closely related (99.7%) to an uncultured marine gamma-proteobacterium clone Sp02sw-4 (HQ241799.1).

## 4. DISCUSSION

The controlled, long-term experiment with well-defined food sources allows us to interpret the observed interspecific differences as biologically meaningful rather than artefacts of prior diet or short-term variability. Because all individuals were maintained under identical conditions and fed wood, either alone or with SPOM, the differences in isotopic composition likely reflect intrinsic species traits. In this context, the observed trophic fractionation factors deviate from commonly accepted values (ca. 0–1‰ for  $\delta^{13}\text{C}$  and 3–4‰ for  $\delta^{15}\text{N}$ ; DeNiro and Epstein, 1978, 1981), which is consistent with expectations if wood were the sole dietary source. Despite their shared wood-boring lifestyle and functional traits, species differed markedly. *Xylophaga dorsalis*, although hosting diazotrophic endosymbionts related to those of teredinids, showed consistently higher  $\delta^{15}\text{N}$  values than *Bankia carinata* and *Nototeredo norvagica*. These differences point to distinct nitrogen sources or metabolic pathways.

#### 4.1 Isotopic composition

##### Carbon assimilation

SPOM was significantly enriched in  $^{13}\text{C}$  and  $^{15}\text{N}$  relative to wood. Nevertheless, bivalve tissue  $\delta^{13}\text{C}$  did not differ between filtered and unfiltered treatments, suggesting a limited contribution of SPOM to carbon assimilation. This inference is, however, complicated by selective processing of wood components: these bivalves preferentially metabolize carbohydrate fractions (Boynton and Miller, 1927; Dore and Miller, 1923; Sabbadin et al., 2018) rather than the whole lignocellulosic matrix, and cellulose is naturally  $^{13}\text{C}$ -enriched relative to lignin (Benner et al., 1987; Loader et al., 2003). Consequently, isotopic overlap between cellulose-derived carbon and SPOM can mask any minor SPOM-derived signal, especially in *B. carinata* and *X. dorsalis*. *X. dorsalis* showed a broader range of  $\delta^{13}\text{C}$  values independent of treatment, a pattern that may reflect greater metabolic flexibility or variability in carbon-source processing and in the coupling between carbon and nitrogen assimilation.

##### Nitrogen assimilation

The very high C:N ratio of beech wood ( $\sim 370$ ) imposes strong nitrogen limitation. The low  $\delta^{15}\text{N}$  values ( $\sim +1\text{‰}$ ) observed in *B. carinata* and *N. norvegica* are consistent with provisioning by diazotrophic gill symbionts, which fix atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N} \approx 0\text{‰}$ ) and supply ammonia with minimal fractionation (Hoering and Ford, 1960; Lechene et al., 2007). These results align with previous studies (Charles et al., 2020, 2018) and indicate a stable reliance on symbiotic nitrogen fixation in these tereidids. By contrast, *X. dorsalis*—despite hosting endosymbionts whose 16S rRNA sequences match the LP1 diazotrophic symbiont of *Lyrodus pedicellatus*—exhibited higher  $\delta^{15}\text{N}$  values (+4 to +5‰). High  $\delta^{15}\text{N}$  persisted even in the filtered-treatment where SPOM was undetectable, implying that particulate inputs alone cannot account for the enrichment.

A plausible explanation for the elevated  $\delta^{15}\text{N}$  values in *X. dorsalis* is the use of multiple nitrogen sources. Its short exhalant siphon opens into the burrow, where accumulations of digested wood (Figure 3) likely create organic-rich, micro-anaerobic niches that favor nitrogen-cycling bacteria (Maier, 2015). Under such conditions, dissimilatory nitrate reduction to ammonium (DNRA) can occur, converting  $\text{NO}_3^-$  into  $\text{NH}_4^+$ , a bioavailable nitrogen form for assimilation (Girguis et al., 2000; Maier, 2015; Minic and Hervé, 2004; Rau, 1981; Rütting et al., 2011). DNRA is promoted in saline, sulfidic, and organic-rich environments (Nie et al., 2021), and taxa such as Desulfobacterales, some of which perform DNRA, have been detected in submerged beech wood under comparable conditions (Charles, Paoletti & Agogué, in prep.). Coupled nitrification–DNRA processes at transient oxic–anoxic interfaces may further regenerate nitrate for immediate reduction (Marchant et al., 2016). DNRA typically produces isotopically enriched nitrogen (+5‰ to +10‰; Chen et al., 2024; Granger et al., 2010), consistent with the elevated  $\delta^{15}\text{N}$  values observed in *X. dorsalis*. These values therefore likely reflect a mixed nitrogen supply: near-0‰ input from diazotrophic symbionts and enriched compounds generated by DNRA in the burrow. Whether *X. dorsalis* acquires this nitrogen by ingesting DNRA-performing bacteria or by directly assimilating ammonium remains unresolved. Reduced  $^{15}\text{N}$  excretion (Vanderklift and Ponsard, 2003) may provide an additional explanation for the observed enrichment.

#### 4.2 Bacterial community associated with the wood-borers

Analysis of bacterial community composition based on 16S rRNA gene sequences (373 bp) revealed a dominant bacterial phylotype shared between *X. dorsalis* and *N. norvegica*, consistently observed across the individuals and tissues examined (Figure 2). This phylotype matched the symbiont LP1 of *Lyrodus pedicellatus*, previously identified in shipworm gill tissues. The overwhelming prevalence of this single ASV in *X. dorsalis* strongly supports the functional importance of gill-associated symbionts in this genus (Distel and Roberts, 1997), extending earlier observations from deep Mediterranean *Xylophaga* (Fagervold et al., 2014). While short sequences limit fine-scale taxonomic resolution, the dominance of a single ASV across all samples highlights a highly constrained and specialized symbiotic association in *X. dorsalis*. These findings emphasize clear functional differentiation among host species, particularly regarding nitrogen acquisition strategies, as reflected in the contrasting isotopic signatures.

The bacterial community associated with *N. norvegica* displayed the highest diversity among the three species, including symbionts from the genus *Teredinibacter* as well as bacteria related to *Pseudomonadales* and *Alteromonas*, the latter recognized for its role in lignocellulose degradation (Goodell et al., 2024). In contrast, *B. carinata* exhibited a microbiota dominated by a single cluster corresponding to clone Sp02sw-4, an uncultured marine bacterium (GenBank accession number HQ241799) previously isolated from a sponge (Walmsley et al., 2012). Additional amplicons detected in *B. carinata* included LP1 of *Lyrodus pedicellatus*, *Teredinibacter* sp., and *Teredinibacter turnerae*, all previously reported in shipworms. The functional role of clone Sp02sw-4 remains to be clarified, but its dominance underscores the existence of host-specific microbial partnerships.

Overall, these results reveal the remarkable complexity and specialization of wood-borer microbiota, highlighting distinct species-specific assemblages that likely reflect adaptation to differing ecological and nutritional niches. The diversity of bacterial strains documented to date (Betcher et al., 2012; Distel et al., 2002, 1991; Goodell et al., 2024; Waterbury et al., 1983), together with ongoing discoveries regarding lignocellulose digestion by wood-boring bivalves (Goodell et al., 2024), underscores that these symbiotic relationships are still far from fully characterized.

#### 5. Conclusion

The coherence of isotopic and microbiome profiles provides biologically relevant information. *Xylophaga dorsalis* exhibits greater ecological flexibility, occupying a broader bathymetric range and consistently displaying elevated  $\delta^{15}\text{N}$  values compared to teredinids. This pattern suggests reliance on multiple nitrogen sources: teredinids appear to depend primarily on diazotrophic symbionts, whereas *X. dorsalis* likely acquires nitrogen from both symbiotic fixation and microbial processing within its burrow. Nitrogen conservation within host tissues (Wang and Douglas, 1998) may also represent an important but understudied mechanism for optimizing nitrogen use. While further integrative studies are needed to fully disentangle the relative contributions of multiple nitrogen sources and conservation pathways, this controlled-feeding study of co-occurring wood-boring bivalves reveals species-specific strategies for nitrogen acquisition and microbial contributions.

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## Author contributions: CRediT

Conceptualization: F.C, P.R., B.L.; Data curation: F.C, B.L., M.P., F.L.; Formal analysis: F.C., M.P., H.A.; Funding acquisition: F.C. and any other; Investigation: F.C., E.P.; Methodology: G.G., F.A., M.P.; Project administration: F.C.; Validation: B.L., H.A., M.P.; Writing – original draft: F.C.; Writing – review and editing: P.R., B.L., F.L., M.P., H.A., F.C.

## Figures captions

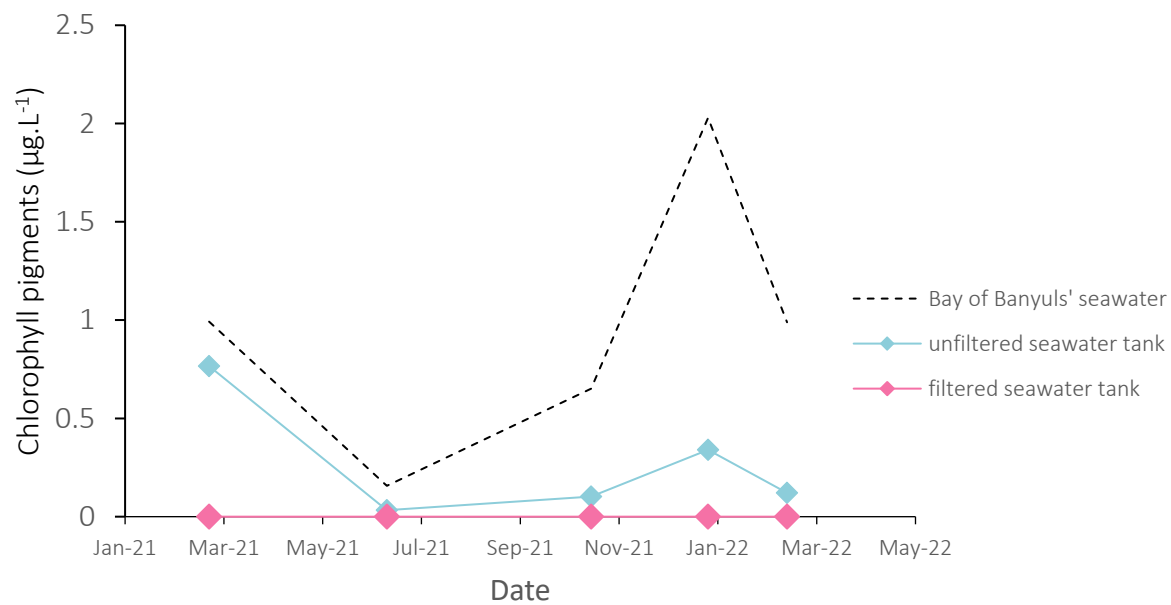
Figure S1. Comparison of chlorophyll pigments concentration between the Bay of Banyuls-sur-Mer and the experimental tanks, providing insight into phytoplankton availability as a potential food source for wood-boring bivalves in the two experimental setups.

Figure 1. Carbon and nitrogen isotopic compositions of wood-boring bivalves relative to potential food sources. Symbol color indicates seawater particle load (unfiltered vs. filtered); symbol shape denotes wood-boring bivalve species.

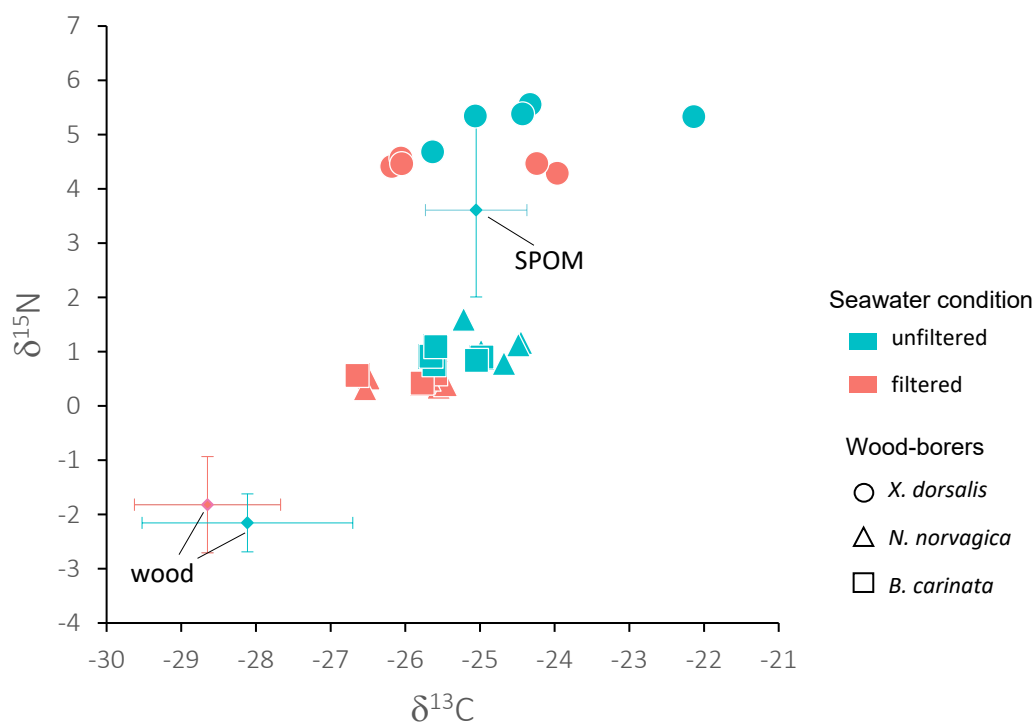
Figure 2. 16S rDNA barcoding analysis of microbiome from xylophagous bivalve species. Samples are labeled by host species (Xd = *X. dorsalis*, Nn = *N. norvegica*, Bc = *B. carinata*). Gill samples are highlighted in grey, while body samples are not highlighted. (A) Bacterial community clustering based on a Bray-Curtis distance matrix. (B) Bacterial species composition specific to gill tissues.

Figure 3. Interior of a *Xylophaga dorsalis* burrow, showing a mat of digested wood near the entrance, and a smaller but more complete fecal chimney on the right side

**Figure S1.** Comparison of chlorophyll pigments concentration between the Bay of Banyuls-sur-Mer and the experimental tanks, providing insight into phytoplankton availability as a potential food source for wood-boring bivalves in the two experimental setups.

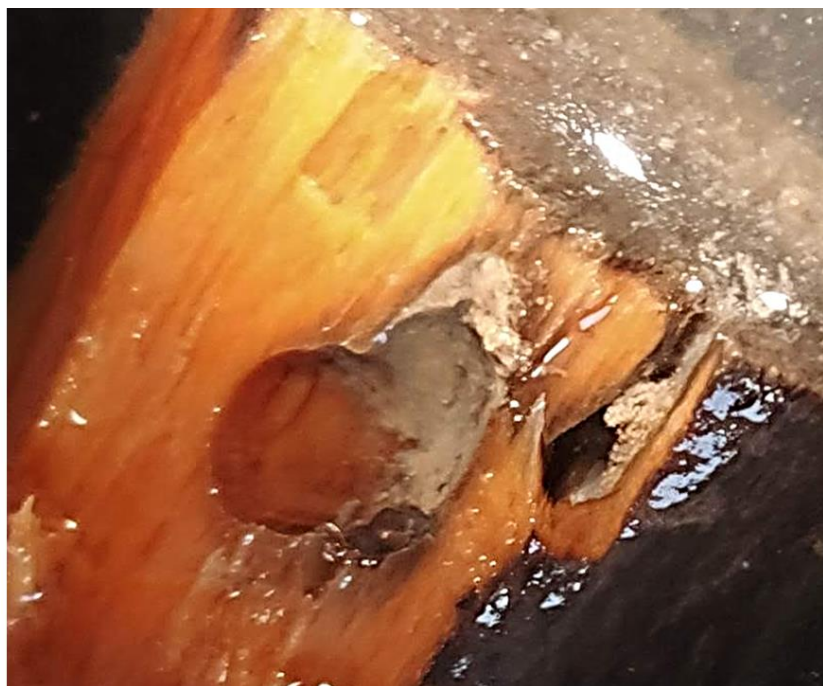


**Figure 1.** Carbon and nitrogen isotopic compositions of wood-boring bivalves relative to potential food sources. Symbol color indicates seawater particle load (unfiltered vs. filtered); symbol shape denotes wood-boring bivalve species.





**Figure 3.** Interior of a *Xylophaga dorsalis* burrow, showing a mat of digested wood near the entrance, and a smaller but more complete fecal chimney on the right side



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