What is the main food source of the shipworm (Teredo navalis)? A stable isotope approach

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Chapter 4

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Chapter 4

Abstract

Stable isotope analysis of soft bodies of the shipworm *Teredo navalis* demonstrated that this species is mainly feeding on seston by filter feeding in contrast to wood consumption. *Teredo navalis* showed similar stable isotope values (δ^{13}C, δ^{15}N) as *Mytilus edulis* and *Crassostrea gigas*, which species were attached to the wood instead of boring into.

Introduction

Boring behaviour protects bivalves against predation while they filter feed consuming seston. Within boring bivalves belonging to the Pholadacea two families are usually distinguished viz. Pholadidae (Angel Wings) which bore into hardened peat, wood, soft chalkstone, corals (and even ABS pipes e.g. Jenner et al., 2003) and Teredinidae (Shipworms) which are specialized to bore into wood (Turner, 1966). It is an ongoing debate if some species of the Teredinidae are able to feed on wood exclusively, or on symbiotic bacteria feeding on wood or also feed on seston (suspended organic matter: plankton and detritus) as additional or as main food source (e.g. Nair and Saraswathy, 1971, Pechenik et al., 1979 and literature therein).

Within specialized epithelial cells of several members of the Teredinidae, associations of symbiotic cellulolytic nitrogen fixing bacteria were found within the gills (Distel et al., 1991, Distel et al., 2002, Sipe et al., 2000, Betcher et al., 2012). Wood contains a very low level of nitrogen and has so a very high C/N ratio (Distel, 2003) and therefore the atmospheric nitrogen usable for some shipworm species fixed by endosymbionts (Lechene et al., 2007) may be the main source.

One of the most common teredinid species is *Teredo navalis*. Popham and Dickson (1973) demonstrated bacterial associations in the gills of *T. navalis* indicating that this species may be able to feed solely on wood. Mann and Gallager (1985) found no significant growth enhancement in the presence of a phytoplankton supplement (in addition to wood), which could suggest wood as the primary food resource. Paalvast and Van der Velde (2011) suggest that individuals that bore into hardwood need to acquire extra nourishment by filtration for at least their basal metabolism, and that this also accounts in highly infested wood when there is no more wood left to bore into.

To find out what *T. navalis* is mainly consuming can nowadays be tested by stable isotope analysis. Carbon isotope ratios ($\delta^{13}C$) in animal tissues closely resemble the food consumed over longer period of time, meaning you are (almost) what you eat. The enrichment in $\delta^{13}C$ in the consumer compared with its diet lies within 0 to 1‰ (DeNiro and Epstein, 1978, Rau et al., 1983, Fry et al., 1984, Gu et al., 1996, Vander Zanden et al., 1997, Vander Zanden et al., 1999). The nitrogen ratio ($\delta^{15}N$) increases by 3.4 ± 1.1‰ with each trophic level through the food web (DeNiro and Epstein, 1978, Minagawa and Wada, 1984, Wada et al., 1987). $\delta^{13}C$ values indicate the carbon sources, while $\delta^{15}N$ values indicate trophic position.

We hypothized that, when *T. navalis* feeds mainly on wood as a carbon source the $\delta^{13}C$ values of the shipworm would resemble the $\delta^{13}C$ values of the wood in

which they bore into. When *T. navalis* feeds on seston by filter feeding, than the $\delta^{13}C$ values of the shipworm resemble the $\delta^{13}C$ values of other bivalve filter feeders at the same location. We further hypothesized in the case of filter feeding on seston the $\delta^{15}N$ values of the shipworm are expected to be at least 3.4 ± 1.1‰ higher than the $\delta^{15}N$ of wood and should be similar to the $\delta^{15}N$ values of other bivalve filter feeders at the same location.

Material and methods

To test the above hypotheses the carbon and nitrogen stable isotope composition of the body tissue of the bivalves *T. navalis* (shipworm), *Mytilus edulis* (Blue mussel) and *Crassostrea gigas* (Pacific oyster) and the fir wood (*Picea abies*) where they lived in or on wood, was analysed.

The shipworms, blue mussels and Pacific oysters were all collected from the last metre near the sea floor of 8 metre long fir beam that was attached to a mooring pole from 3 metres above the mean low water level (MLWL) till the bottom of the sea floor 5 m below MLWL in the polyhaline Scheurhaven of the port of Rotterdam, the Netherlands (51º 57' 43.33" N, 4º 8' 16.41" E). The fir beam was attached to the mooring pole in April 2009 and removed in April 2012. The last metre of the pole was cut off and transported to the laboratory of the Radboud University Nijmegen. Mussels and oysters that grew on the beam were removed, shipworms were retrieved by cutting and splitting the beam with an axe and samples of the wood were taken for stable isotope analysis.

In the laboratory, bivalves were rinsed first in tap water and then in distilled water. The soft bodies of 6-8 individuals were pooled for each sample and oven dried at 60 °C for 3-5 days. Shells of molluscs and pallets in the case of the shipworm were removed and the remaining body tissue dried for 48 h at $60^{o}C$, after which specimens were ground to a fine powder using a pestle and mortar and liquid nitrogen. Measurements were carried out for each individual using their powder stored in small new glass bottles with a plastic cap until weighing. Carbon and nitrogen stable isotopic compositions were measured with a Carlo Erba NA 1500 elemental analyzer coupled online via a Finnigan Conflo III interface with a ThermoFinnigan DeltaPlus mass spectrometer. Carbon and nitrogen isotope ratios are expressed in the standard delta notation ($\delta^{13}C$, $\delta^{15}N$) relative to Vienna PDB and atmospheric nitrogen. Average reproducibility based on replicate measurements of internal standards Sucrose (IAEA-CH-6) for $\delta^{13}C$ and Ammonium sulphate (IAEA-N-2) for $\delta^{15}N$ was ca. 0.15‰. Acetanilide was used as the laboratory reference.

Statistical analysis was carried out with SPSS 15.0 for Windows. Anova together with the Levene's test for equality of variance was conducted to compare the means of $\delta^{13}C$ and $\delta^{15}N$ of the species involved.
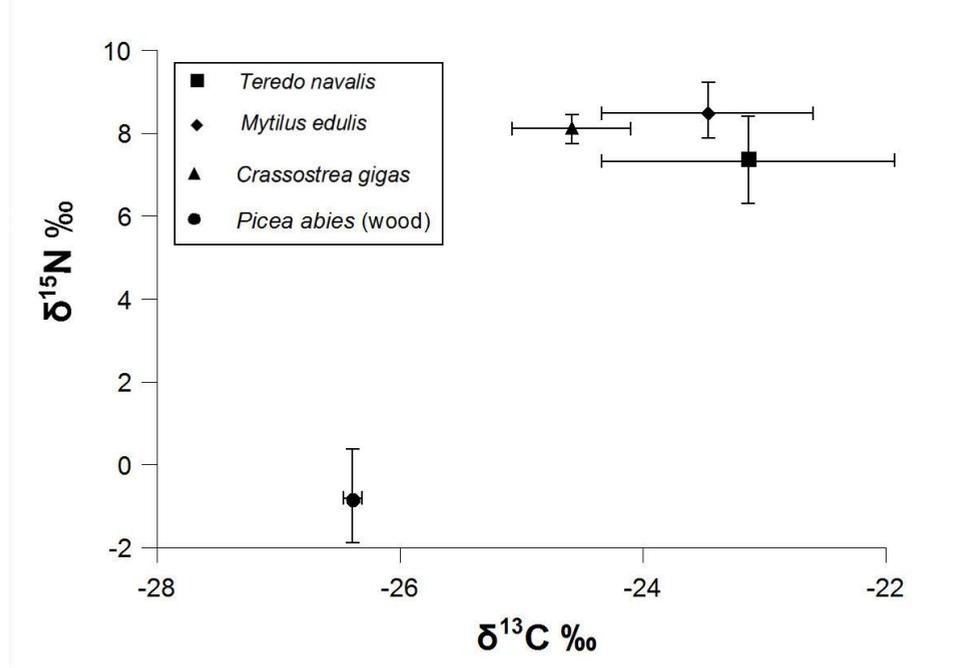
Figure 1 Stable isotope values for the shipworm (*Teredo navalis*), Blue mussel (*Mytilus edulis*), Pacific oyster (*Crassostrea gigas*) and wood (*Picea abies*).

Results

As fir wood contains very little nitrogen this led to overflow of 5 of the 9 samples during the stable isotope analyses for this element, but this was not the case for carbon. All other samples did not show any overflow.

There was a significant difference in the means of $\delta^{13}C$ values between the species (one-way ANOVA, F(3,52)= 31.1, p< 0.001) (Fig. 1). Wood (N = 9, M ($\delta^{13}C$) = -26.41 ‰, SE = 0.023) compared with the bivalves had a significant (Tamhane at the 0.05 level) lower $\delta^{13}C$ value ranging from -1.8‰ for the Pacific oyster (N = 8, M ($\delta^{13}C$) = -24.58 ‰, SE = 0.213), -3.0‰ for the Blue mussel (N = 23, M ($\delta^{13}C$) = -23.46 ‰, SE = 0.181) to -3.3‰ for the shipworm (N = 16, M ($\delta^{13}C$) = -23.13 ‰, SE = 0.302). Furthermore the Pacific oyster's $\delta^{13}C$ was significantly lower (Tamhane at the 0.05 level) than those of shipworm and Blue mussel.

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There was also a significant difference in the means of $\delta^{15}N$ values between the species (one-way ANOVA, $F(3,47)= 170.6$, $p< 0.001$) (Fig. 1). Wood (N = 4, M ($\delta^{15}N$) = -0.86 ‰, SE = 0.538) compared with the bivalves had a significant (Tamhane at the 0.05 level) lower $\delta^{15}N$ value ranging from -8.2‰ for the shipworm(N = 16, M ($\delta^{15}N$) = 7.38 ‰, SE = 0.251), -9.0‰ for the Pacific oyster(N = 8, M ($\delta^{15}N$) = 8.14, SE = 0.120) to -9.3‰ for the Blue mussel (N = 23, M ($\delta^{15}N$) = 8.48 ‰, SE = 0.130). Between the bivalves there was only a significant difference between the mean $\delta^{15}N$ values of the shipworm and the Blue mussel.

Discussion

Several species of shipworms including *Teredo navalis* seem to be able to grow in the absence of food (apart from wood) as demonstrated under laboratory conditions (Gallager et al., 1981, Man and Gallager, 1985). The presence of phytoplankton supplement enhanced growth in the shipworm, *Bankia gouldi*, but was progressively less significant in the shipworms *T. navalis* and *Lyrodus pedicellatus* (Man and Gallager, 1985). Becker (1959, in Nair and Sarawathy) managed to rear *L. pedicellatus* through four generations in artificial sea water without any additional food. He was unsuccessful with *T. navalis* that failed to breed probably on account of inadequate nutrition, owing to the absence of protein rich plankton. Mann and Gallager (1985) observed well developed gonads with sperm and ova present for *Bankia gouldi* in the absence of planktonic food but did not mention this for *T. navalis* as gonad development was not part of their study. Turner (1966) suggested that the adults of some species require planktonic food during the breeding period, while others may be capable of surviving on plankton only as do many other bivalves.

The $\delta^{13}C$ values of the shipworm *T. navalis* were on average 3.3‰ higher than that of the wood it lived in. This is far above the range of 0 to 1‰ if wood would have been the main source of carbon. The shipworm's $\delta^{13}C$ values closely resembled the $\delta^{13}C$ values of the Blue mussel and were higher than the $\delta^{13}C$ values of the Pacific oyster. This means that not the wood it bores into but food obtained by filter feeding via the siphons was the main source of carbon, despite the presence of possible symbiotic cellulolytic nitrogen fixing bacteria in the gills of this species of shipworm. It further indicates that under natural conditions drilling in wood by the shipworm, *T. navalis*, is more important for shelter than nutrition.

The positive difference between the $\delta^{15}N$ of the bivalves compared with the $\delta^{15}N$ of wood was more than twice the 3.4 ± 1.1‰ value for each consecutive trophic level through the food web (DeNiro and Epstein, 1978, Minagawa and Wada,

1984, Wada et al., 1987), further the $\delta^{15}N$ of the shipworm was very close to the $\delta^{15}N$ of the Blue mussel and Pacific oyster. This excludes wood as the main source for nitrogen, and that food must have been acquired by filter feeding of seston. It would be very illogical that food present in the inhaled water would not be utilized as a carbon and nitrogen source.

This means that under natural conditions symbiosis between shipworm, *T. navalis*, and endosymbionts is not a case of mutualism but rather of commensalism from the latter.

Acknowledgements

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