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The effect of bacterivorous nematodes on detritus incorporation by macrofaunal detritivores: A study using stable isotope and fatty acid analyses

D. Leduc *, P.K. Probert

Department of Marine Science, University of Otago, P.O. Box 56, Dunedin, New Zealand

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Several studies have investigated the effect of nematodes on microbial degradation of macrophyte detritus, but little is known about the potential effect of bacterivorous nematodes on productivity of macrofaunal detritivores. We investigated the influence of the bacterivorous nematode *Rhabditis* (*Pellioditis*) *mediterranea* on the incorporation rate of isotopically-labelled macroalgal detritus by the amphipod *Paramoera chevreuxi* in a laboratory microcosm. The fatty acid composition of amphipods, nematodes, and macroalgal detritus was characterized to help determine the pathway of detritus incorporation by amphipods. The potential importance of *R. mediterranea* as a source of highly unsaturated fatty acids (HUFAs) to higher trophic levels was also investigated.

We found no clear evidence for an effect of nematodes on the incorporation rate of fresh macroalgal detritus by amphipods, although there was some indication that the type of detritus (i.e. the green *Chaetomorpha* sp. vs the red *Polysiphonia* sp.) is important in determining the nature and extent of the relationship between nematodes and macrofaunal detritivores. Fatty acid data indicated that nematodes did not contribute significantly to the diet of amphipods when detritus was present, and there was no evidence that nematodes affected the pathway of detritus incorporation by amphipods. Amphipods incorporated *Chaetomorpha* sp. detritus about 10 times faster than *Polysiphonia* sp. detritus despite the higher C/N ratio and lower HUFA content of the former. This suggests that other factors, such as the presence of grazer deterrent compounds, are important in determining the palatability of macroalgal detritus. Amphipods fed exclusively on nematodes retained high HUFA levels but suffered high mortality. The burrowing behaviour of nematodes is suggested as the most likely factor limiting their availability to predators.

Results suggest a limited interaction between amphipods and bacterivorous nematodes in detrital food webs. Further experiments are needed to test the wider applicability of these findings to different nematode and macrofaunal taxa, and for different types of detritus.

1. Introduction

1.1. Interactions between nematodes and macrofaunal detritivores

Nematodes are amongst the most abundant animals living in marine sediments worldwide, and play a significant role in the ecology of benthic communities (see reviews by Platt & Warwick 1980; Heip et al., 1985; Coull, 1999). The positive effect of nematodes on bacterial growth (e.g. Ingham et al., 1985; Griffiths, 1994; Riemann and Helmke, 2002) and detritus mineralization rates (e.g. Findlay and Tenore, 1982; Alkemade et al., 1992; Lillebo et al., 2007), in particular, is well documented. How nematodes may affect macrofaunal productivity of detrital-based ecosystems is less clear. The presence of nematodes could decrease the availability of detritus to macrofauna due to increased mineralization rates (Anderson et al., 1983) and/or direct competition for food (McIntyre, 1969; Alongi and Te nore, 1985). Experimental evidence, on the other hand, suggests that meiofauna (consisting mostly of nematodes) has a positive effect on the assimilation of aged seagrass detritus by the polychaete *Nephtys incisa* (Tenore et al., 1977). A relationship between nematodes and macrofaunal detritivores could be ecologically significant given the wide distribution of nematodes and the importance of detritus in the energetics of coastal communities (e.g. Mann, 1988; Kirkman and Kendrick, 1997).

Several factors, such as available caloric content, nitrogen content, and presence of essential nutrients, determine the quality of detritus as a food source for detritivores (Tenore, 1983; Phillips, 1984; Tenore et al., 1984). Highly unsaturated fatty acids (HUFAs, defined as fatty acids having carbon chain length of $>C_{20}$ and with >3 double bonds), for example, cannot be biosynthesized by most metazoans, but are essential for the normal functioning of cell membranes and membrane-bound enzyme systems (Bishop, 1976; Wantanabe et al., 1978; Phillips, 1984). HUFA content has been shown to be a good indicator of food quality (e.g. Langdon and Waldock, 1981; Read, 1981; Wantanabe et al., 1983) and an important determinant of trophic transfer efficiency

* Corresponding author. Tel.: +64 3 479 5821; fax: +64 3 479 8336.
E-mail address: daniel.leduc@gmail.com (D. Leduc).

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in freshwater systems (Muller-Navarra et al., 2004; Persson et al., 2007). The amount of HUFAs in macrophyte detritus, however, is often low and can decrease rapidly during decomposition (Nichols et al., 1982; Tenore et al., 1984; Kharlamenko et al., 2001), which could lower its palatability to detritivores.

Unlike most metazoans, nematodes can biosynthesize HUFAs from acetate (Rothstein and Gotz, 1968; Bolla, 1980). Although little is known about the fatty acid (FA) composition of marine nematodes, recent data on *Oncholaimus moanae* from intertidal sediments suggest that marine nematodes are rich in HUFAs (Leduc, in press). The biochemical upgrading of HUFA-poor microalgae by HUFA-producing protists has been documented in pelagic food webs (e.g. Breteler et al., 1999; Tang and Taal, 2005; Veloza et al., 2006). The presence of nematodes could lead to greater trophic transfer efficiency in detrital-based food webs by alleviating HUFA limitation, but the potential importance of this process has not yet been investigated (Phillips, 1984; Moens et al., 2006).

1.2. Nematodes as prey for higher trophic levels

Nematodes may be ingested passively by non-selective deposit feeders (e.g. holothurians, polychaetes) and surface grazers (e.g. gastropods), or actively sought by small predators (e.g. juvenile flatfish, shrimps, amphipods) (see Coull, 1990, 1999 for reviews). Whilst nematodes may contribute to the diet of non-selective deposit feeders, how much they contribute to the nutrition of this feeding guild remains unclear. The potential importance of nematodes to the energy requirements of non-selective deposit feeders was highlighted by Sikora et al. (1977), who found that nematodes represented 78–92% of the biomass (measured as ATP content) of muddy sediments in North Inlet Estuary, South Carolina. Small predators (or selective deposit feeders) may also benefit from actively targeting nematodes. The "packaging model" (Lackey, 1936, cited in Giere, 1993) suggests that energetically it is more advantageous for a consumer to eat one nematode than numerous smaller food particles of the same energetic value. Similarly, Sikora et al. (1977) described the role of nematodes as converting "a diffuse substrate into a discrete, particulate, high-energy food source for the next trophic level". Relatively few studies, however, have reported nematodes as important prey items (e.g. Fitzhugh and Fleeger, 1985; Marinelli and Coull, 1987; Yu et al., 2003). It has been suggested that fish may select harpacticoid copepods over nematodes due to the epibenthic lifestyle and greater visibility of the former (Coull, 1990). The greater digestibility of nematodes compared to harpacticoid copepod exoskeletons (Coull, 1990; Feller and Coull, 1995), however, could make nematodes harder to detect in gut contents.

1.3. Tracing organic matter flow using biomarkers

Stable isotopes have been used extensively to trace the flow of organic matter in marine food webs (e.g. Lepoint et al., 2004; Fry, 2006; Mateo et al., 2006). Stable isotope enrichment studies are particularly useful in short term (i.e. days or weeks) feeding experiments (e.g. Herman et al., 2000; Moens et al., 2002; Rossi, 2007). Additional biomarkers are needed, however, to understand the pathways through which food sources are assimilated (e.g. is detritus assimilated directly, or indirectly through the ingestion of associated microbiota and/or nematodes?). FAs can prove useful for tracing the flow of detritus by providing biomarkers for macrophytes (e.g. 18:2n6, Meziane and Tsuchiya, 2000; Kharlamenko et al., 2001) and bacteria (e.g. 15:0, 17:0, and 18:1n7, Volkman et al., 1980; Findlay et al., 1990). FAs have also been used to investigate the role of terrestrial nematodes in the diet of collembolans (Chamberlain et al., 2005; Ruess et al., 2005; Chamberlain et al., 2006). Combining stable isotope and FA biomarker approaches is likely to provide a powerful method to study the effect that nematodes may have on the assimilation of detritus by detritivores.

The usefulness of FA biomarkers in food web studies can be established only by feeding experiments and through thorough sampling of food sources in the field (Chamberlain et al., 2005). Feeding experiments, for example, led Hall et al. (2006) to question the usefulness of long chain FAs (LCFAs) as biomarkers of mangrove assimilation by invertebrates (e.g. Meziane and Tsuchiya, 2000; Meziane et al., 2006). The FA composition of different species fed the same diet can also differ significantly (Ruess et al., 2002). FA profiles can be affected by physiological (e.g. starvation, Wen et al., 2006) and environmental factors (e.g. temperature, Pernet et al., 2007). The influence of diet on the abundance of particular FAs should, therefore, be determined in controlled conditions prior to the interpretation of food web relationships based on data collected from the field.

1.4. Amphipods as study organisms

Amphipods are common in most benthic communities and are important prey for various larger organisms (Kline and Wood, 1996; Beare and Moore, 1997; Macneil et al., 1999). Amphipods span a variety of feeding guilds including grazers, detritivores, and carnivores, with individual species often showing considerable flexibility in their feeding habits (MacNeil et al., 1997; Blankenship and Levin, 2007). Due to their small size, amphipods have the ability to select meiofaunal prey (Yu et al., 2003), and may have the ability to seek out nematodes when high quality food sources are absent. A trophic preference for nematodes (high in HUFAs) over fungi (low in HUFAs), for example, has been shown for collembolans in a study using stable carbon isotopes and FA biomarkers (Chamberlain et al., 2006).

Many authors have studied the diet of amphipods using FA biomarkers (e.g. Nelson et al., 2001; Auel et al., 2002; Guerra-Garcia et al., 2004; Nyssen et al., 2005; Biandolino and Prato, 2006). However, to our knowledge, no studies have evaluated the validity of FA biomarkers in amphipods under controlled conditions, which contrasts with the numerous experimental studies carried out on other organisms such as calanoid copepods (e.g. Graeve et al., 1994, 2005; Veloza et al., 2006) and crabs (e.g. Hall et al., 2006; Wen et al., 2006; Alava et al., 2007). Feeding experiments may, in addition to providing an assessment of the usefulness of common macrophyte biomarkers, help uncover potential biomarkers of nematode predation in amphipods (Ruess et al., 2004).

1.5. Study objectives

The main objectives of the present study were 1) to determine the effect of the bacterivorous marine nematode *Rhabditis* (*Pellioditis*) *mediterranea* on the incorporation rate of ^{13}C-labelled macroalgal detritus of high and low HUFA content by the gammarid amphipod *Paramoera chevreuxi;* and 2) identify the pathways through which detritus is assimilated (i.e. directly or indirectly through the assimilation of microbiota and/or nematodes) by comparing the FA profile of potential food sources and amphipods before and after the experiment. The assimilation of *R. mediterranea* by amphipods was also quantified using ^{15}N-labelled nematodes. Information gathered during this experiment was also used to assess the ability of *R. mediterranea* to biosynthesize HUFAs and to determine the usefulness of different FAs as biomarkers of macrophyte and nematode assimilation in amphipods.

2. Methods

2.1. Labelling of macroalgal food sources

Green macroalgae are usually characterized by low levels of HUFAs, whereas red macroalgae often contain high levels of the HUFAs 20:5n3 and 20:4n6 (e.g. Graeve et al., 2002). The green macroalga