

Ingestion of live filamentous diatoms by the Great Lakes amphipod, *Diporeia* sp.: a case study of the limited value of gut contents analysis

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Abstract

Individuals of the Great Lakes amphipod, *Diporeia* sp. (formerly named *Pontoporeia hoyi*) were collected from a 45-m deep station in southeastern Lake Michigan and isolated in small laboratory feeding vessels at 4 °C, after the animals had voided their guts over a 24-hour period. Over a 20-day period, following introduction of a single ration of live cells of the filamentous diatom, *Melosira varians*, 9 of 10 animals had ingested this material, and 7 of these 9 individuals had deposited fecal pellets. Subsequent examination of gut contents and fecal pellets showed that although animals had ingested whole algal cells/filaments, little of the material in gut contents or fecal pellets bore any identifiable structural similarity to cells/filaments prior to ingestion. The results suggest that earlier studies of pontoporeiid gut contents may seriously underestimate the importance of algal components in the amphipod's diet and imply that *Diporeia* sp. growth and production may be more closely linked to primary production than previously thought.

Introduction

Diporeia sp. (formerly named *Pontoporeia hoyi*, prior to a revision by Bousfield, 1989) is a prominent benthic macroinvertebrate of North America's Laurentian Great Lakes and a prey item that supports the food requirements of a wide variety of valuable commercial and sportfish species (Wells, 1980). In Lake Michigan, *Diporeia* sp. constitutes up to 60% of the resident macroinvertebrate biomass (Nalepa *et al.*, 1985). The amphipod commonly inhabits a thin (1–2 cm) surficial sediment layer (Nalepa & Robertson, 1981), where it presumably feeds on recently-

deposited detritus. To date, however, a definitive study and documentation of the nutritional sources supporting *Diporeia* sp. production has not been conducted. Laboratory sediment preference studies performed in the 1960's (Marzolf, 1965) indicated that when quantities of *Diporeia* sp. were introduced into aquaria containing a variety of sediment types, significantly more animals colonized fine, bacteria-rich sediments, compared to coarser or sterile substrates. Although subsequent gut analysis studies of both *Diporeia* sp. (formerly named *Pontoporeia affinis* (= *P. hoyi*) (Great Slave Lake, N.W. Territories, Canada; Moore, 1977) and *Monoporeia affinis* (formerly named *Pontoporeia affinis* prior to revision by Bousfield, 1989) (Baltic Sea; Ankar, 1977) indicated that particles found in the gut

were $\leq 10 \mu\text{m}$), the nutritional importance of microflora associated with particles in this size range is unknown. *Diporeia* sp. collected from Great Slave Lake contained algae, detritus, sand grains and oligochaete setae in their guts (Moore, 1977). While identifiable algae composed $\leq 1.5\%$ of gut contents, most species ingested were planktonic forms. In all ingested diatom species, intact chloroplasts were not observed, suggesting that cells were partially digested during passage through the gut, or that the cells were dead prior to ingestion. Green algae, (particularly *Scenedesmus quadricauda*) were seldom broken down and cell walls of most ingested species remained intact during gut passage. While ruptured chloroplasts indicated partial digestion and possible nutritional importance of algae, the relatively small fraction of algae in overall gut volume indicated that they constituted only a minor energy source.

Examination of guts of *Diporeia* sp. collected from a 97-m deep southern Lake Michigan site showed that 1% of the gut volume was composed of identifiable biological remains, including *Cyclotella* and *Melosira* fragments (Evans *et al.*, 1990).

Similarly, gut analysis of *M. affinis* populations in Lake Erken, Sweden indicated that algae composed only 2.3% of gut volume with detritus (97.6%) and animal remains (0.1%) constituting the remaining volume (Johnson, 1987). Although diatoms represented less than half of the algal component (and 1% of the total gut volume), highest *M. affinis* growth and production occurred soon after diatom blooms which provided a rich supply of organic matter to profundal sediments (Johnson, 1987). Though blooms of other algae (primarily *Cladophora*) occurred in October and November, corresponding *M. affinis* production was unusually low and implied that nutritional value of *Cladophora* and associated organic matter was minimal.

Elsewhere, the close association between diatom production in the water column and subsequent pontoporeiid production suggests that diatoms or fresh diatom-derived detritus may be far more nutritionally important than previous gut contents studies have revealed. In Lake Ontario,

Diporeia sp. instantaneous growth rates were highest in spring and closely followed spring peaks in phytoplankton productivity (Johnson & Brinkhurst, 1971). In the Baltic Sea, most of the annual growth of *M. affinis* and *Pontoporeia femorata* populations was restricted to spring, when peak phytoplankton production occurred (Hill & Elmgren, 1987). Although direct linkage between *Diporeia* sp. production and phytoplankton production has not been documented in Lake Michigan, there is evidence that the amphipod's reproduction is synchronized closely with peaks in phytoplankton productivity to ensure that the release of young occurs at times when phytoplankton-generated organic matter is most abundant (Lubner, 1979). In addition, the rapid increase in *Diporeia* sp. lipid content following spring diatom blooms (Gardner *et al.*, 1985) suggests that primary production in overlying water may subsequently provide a rich food source for the amphipod's growth and production (Gardner *et al.*, 1990). Lipid content of Lake Michigan *Diporeia* sp. may reach as much as 50% (dry weight) (Gardner *et al.*, 1985) and recent work (Quigley *et al.*, 1989) has indicated that most of this lipid fraction consists of storage lipids (triacylglycerols) which play a major energetic role throughout the animal's life history.

Recent gut fullness studies (Dermott & Corning, 1988; Quigley, 1988; Evans *et al.*, 1990) have demonstrated that *Diporeia* sp. feeds intermittently, unlike most amphipod species, which feed continuously (e.g. *Hyalella azteca*, Hargrave, 1970; *Gammarus pulex*, Welton *et al.*, 1983). Given the ability of *Diporeia* sp. to store large quantities of lipid, intermittent feeding may represent a strategy that allows the amphipod to fully exploit high-quality food during the brief period that it is available (i.e. following the deposition of organic matter from spring diatom blooms), and to feed sparingly at other times, while relying on lipid stores to support metabolic needs (Gauvin *et al.*, 1989). In fact, *Diporeia* sp. percent gut fullness data from Lake Michigan (Quigley, 1988; Evans *et al.*, 1990) and from Lake Ontario (Dermott & Corning, 1988) show that feeding is intensified following spring diatom

blooms. Thus, intensified feeding and associated increases in lipid storage at this time suggest that *Diporeia* sp. growth and production is closely linked with, and highly dependent on, inputs of organic matter derived from spring blooms. At present, little is known about the viability or decomposition stage of algal cells deposited at the sediment-water interface following spring blooms. However, silica flux measurements from Lake Michigan sediments (Quigley & Robbins, 1984; Conley & Schelske, 1989) have demonstrated that most of the silica released into overlying water is derived from a surficial floc layer of biogenic silica (primarily diatom frustules). Since mineralization of frustules can only proceed after disintegration of the outer diatom cell membrane, this floc layer should have considerable concentrations of senescent and dying diatoms. In fact, the introduction of moribund diatoms to the surface of estuarine Chesapeake Bay cores produced linear increases in the release of dissolved silica and implied that most of the upward flux of dissolved silica originated from a surficial floc layer that was rich in biogenic silica (primarily diatom frustules) (Yamada & D'Elia, 1984). Data on the seasonal concentration of photosynthetic pigments in surficial nearshore Lake Michigan sediments also demonstrated that pigment abundance is greatest in spring (Nalepa & Quigley, 1987). Senescent diatoms, in fact, might prove to be of particular nutritional value to *Diporeia* sp. since they may contain large amounts of lipids that are known to accumulate during low-growth periods (Parrish, 1986). The potentially high nutritional value of Baltic Sea surficial sediments, composed extensively of freshly-deposited phytoplankton, has also been cited in explaining high carbon assimilation efficiencies noted in *M. affinis* (~40%) and *P. femorata* (28%) (Lopez & Elmgren, 1989). Moreover, the sediment preference tests of Marzolf (1965) showed that *Diporeia* sp. selected sandy sediments overlain by a thin layer of fine detritus compared to sands into which this same detritus layer had been mixed. Although previous gut contents studies have tended to discount the direct ingestion of diatoms and other algal cells, such conclusions are limited

since mechanical and digestive breakdown might greatly obscure the original identity and composition of the ingested material, and lead to an underestimate of the significance of algal components in the overall nutrition of *Diporeia* sp. If this was the case, we hypothesized that trituration and digestion of algal cells by the amphipod is extensive and that utilization of such food sources is far more important than previously believed.

To test this hypothesis, we introduced quantities of the diatom *Melosira varians* to individual *Diporeia* sp., and evaluated the degree of ingestion and breakdown of these live cells by the amphipod. *Melosira varians* is a large filamentous diatom that is typical in size and morphology among *Melosira* species of the Great Lakes. *Melosira* spp. are dominants of the spring bloom and are known to rapidly sink out of the water column at the onset of stratification (Holland & Beeton, 1972; Fahnenstiel & Scavia, 1987). Moreover, the large size and morphology of *M. varians* render it resistant to ingestion by some zooplankton during its pelagic existence, although *Diaptomus* spp. can ingest if after orienting the filaments normal to the long axis of the body (Vanderploeg *et al.*, 1988). As such, this diatom represented a particularly appropriate food item and a rigorous test of the digestive capabilities of *Diporeia* sp.

Materials and methods

Diporeia sp. obtained from a 45 m deep site in southeastern Lake Michigan were held in the laboratory at 4 °C. in sandy-silt sediments collected from the site. Prior to feeding tests, approximately 30 juveniles (5–6 mm total length) were isolated for 48 hours in lake water to allow the animals to void their guts. Following this isolation interval, 10 test animals with completely empty guts were individually isolated in feeding test vessels consisting of 20 ml high-density linear polyethylene liquid scintillation vials containing a 1 cm layer of 500–550 μ m ignited (550 °C. for 4 hours) sand overlain by filtered (2 μ m) lake water. The large particle size provided

a highly porous, well-oxygenated sediment environment and animals readily burrowed into the substrate following introduction. The coarse ignited sand also provided an inert substrate of particles that were too large to be ingested by *Diporeia* sp. and ensured that only introduced food could be potentially ingested. The substrate also permitted rapid location and retrieval of any fecal pellets produced during feeding experiments.

The tops of test vials consisted of open-ended polyethylene screw caps that were covered with 500 μm nylon screen. Test vials were held in an acrylic rack, submerged in a shallow tray, where filtered lake water was continuously aerated. The system also allowed individual vials to be periodically removed from the tray to inspect animals and sediments. This was accomplished by replacing the vial's screw cap with a double-width (5×7.5 cm) microscope slide and then placing the vial on the stage of a dissecting microscope where animals and sediments could be readily observed.

Following introduction to test vials, individual animals were held in the containers for 24 hours to adjust to conditions. Rations of laboratory-cultured *Melosira varians* (Vanderploeg *et al.*, 1988) were then introduced to individuals as follows: A 5 ml quantity of *Melosira* and culture water was drawn from the bottom of the culture flask and transferred to a 100 ml beaker where it was combined with 75 ml of filtered lake water. This suspension was then vigorously agitated by magnetic stirrer to ensure uniform distribution of *Melosira* filaments. Each test vial was then removed from the holding tray, opened, and 5 ml of the vial's water removed. A 2 ml volume of water from the *Melosira* suspension was then added and the cells allowed to settle to the sand substrate before the test vial was topped off with lake water and returned to the holding tray. To estimate amount of *Melosira* rations introduced to individuals, we also transferred 2 ml volumes of the suspension to pre-weighed aluminum dishes and these were dried for 24 hours at 60 °C and reweighed. Mean ration weight was 289 μg (dry weight) \pm (SE) 16 μg ($n = 5$). Following food introduction, all 10 test vials were incubated in

darkness at 4 °C. for a 20-day period during which vials were periodically removed from trays to examine conditions of test animals, the food ration, and to retrieve any recently-deposited fecal pellets. Any fecal pellets recovered were immediately preserved in 5% formalin solution.

Results and discussion

From the very outset of the feeding experiment, it was apparent that *Diporeia* sp. would readily ingest introduced *Melosira* cells/filaments. In one instance during the initial introduction of the *Melosira* ration, we observed one animal which emerged from its partially-buried position, to move directly to a large clump of *Melosira* filaments. While lying on its side at the sediment surface, the animal could be clearly observed guiding tangled masses of *Melosira* to its mouth where ingestion occurred.

Over the first 10 days of the test period, 6 of 10 test animals had partially or totally full guts of *Melosira* and 4 of these 6 animals had deposited fecal pellets. By 20 days, 9 animals had *Melosira* gut contents and 7 of these 9 had produced from 1 to 15 fecal pellets.

Fecal pellets produced by test animals fed *Melosira* were visibly different than *Diporeia* sp. fecal pellets we have previously collected from Lake Michigan sediments, and from sediment-fed animals in the laboratory. These fecal pellets typically consisted of cylindrically-shaped, highly compacted fine material that was tightly enclosed within a durable peritrophic membrane. By contrast, *Melosira*-fed *Diporeia* sp. released fecal pellets that were more amorphous, had less compacted material and more loosely fitting peritrophic membranes. Gut turnover rates (as defined by fecal pellet (F.P.) output per unit time) of *Melosira*-fed animals were also far lower than rates we have previously observed in sediment-fed *Diporeia* sp. (Quigley, unpublished data). The maximum of 15 fecal pellets produced over 20 days by *Melosira*-fed animals (0.75 F.P. d^{-1}) was far lower than the 100 or more fecal pellets we have observed produced by sediment-fed animals

over 10 days (10 F.P. d^{-1} or a daily gut turnover rate of 1.5–2.0). Similar gut turnover rates have also been noted for sediment-fed Lake Ontario *Diporeia* sp. (Dermott & Corning, 1988) which took a mean of 6.7 hours to completely fill guts at 5 °C. Since the animals would require some time to void their guts, the expected daily gut turnover rate will be less than a maximum of 3.6 (assumes instantaneous emptying of gut) and is probably closer to the range of 1.5–2, we observed in Lake Michigan *Diporeia* sp.

The markedly lower gut turnover rate of *Melosira*-fed animals might, in part, be due to the relatively higher nutritive quality of this food item and a corresponding response by *Diporeia* sp. that extended the residence time of this material in the gut, thereby allowing more time for the full assimilation of this food. Alternatively, the relatively long gut passage time of ingested *Melosira* might represent an inability of *Diporeia* sp. to efficiently process the ration in the complete absence of fine

sediment particles which may be required for rapid, efficient passage of gut contents.

Inspection of both gut contents and fecal pellets (under a compound microscope, Figs. 1 and 2) revealed that *Diporeia* sp. was extremely efficient in breaking down live *Melosira* cells. Whole intact cells were rarely observed in the gut contents or in fecal pellets. While some cell wall structures were occasionally observed, most of the remaining gut and fecal pellet material bore no identifiable similarity to the *Melosira* cells that had been originally ingested.

The extensive breakdown of *Melosira* by *Diporeia* sp. reflects an ability to crush and grind ingested material and is similar to the behavior exhibited by *M. affinis* in the Baltic Sea, where this species is capable of crushing the 220–250 μm shells of the spat of the bivalve *Macoma balthica*, presumably ingesting the remains (Elmgren *et al.*, 1986). We examined *Diporeia* sp. mandibles and found that they con-

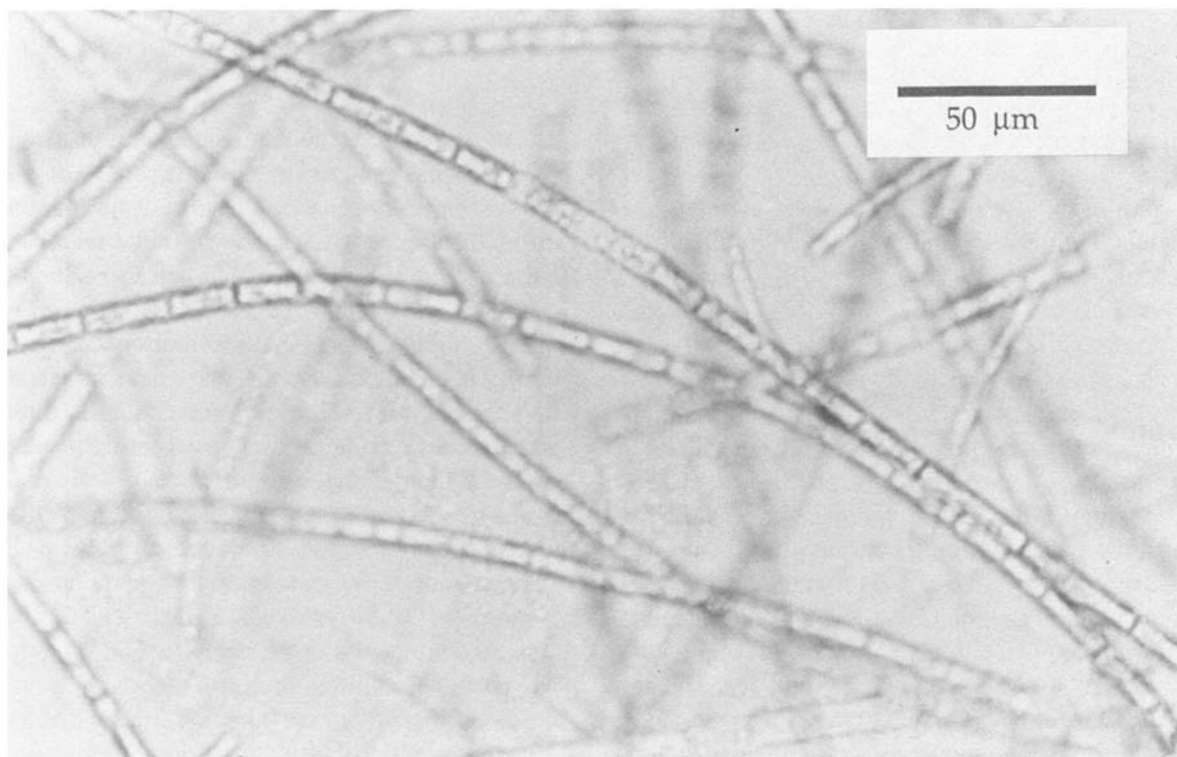


Fig. 1. Live *Melosira varians* cells/filaments prior to ingestion by *Diporeia*. Black bar (upper right) denotes scale (50 μm).

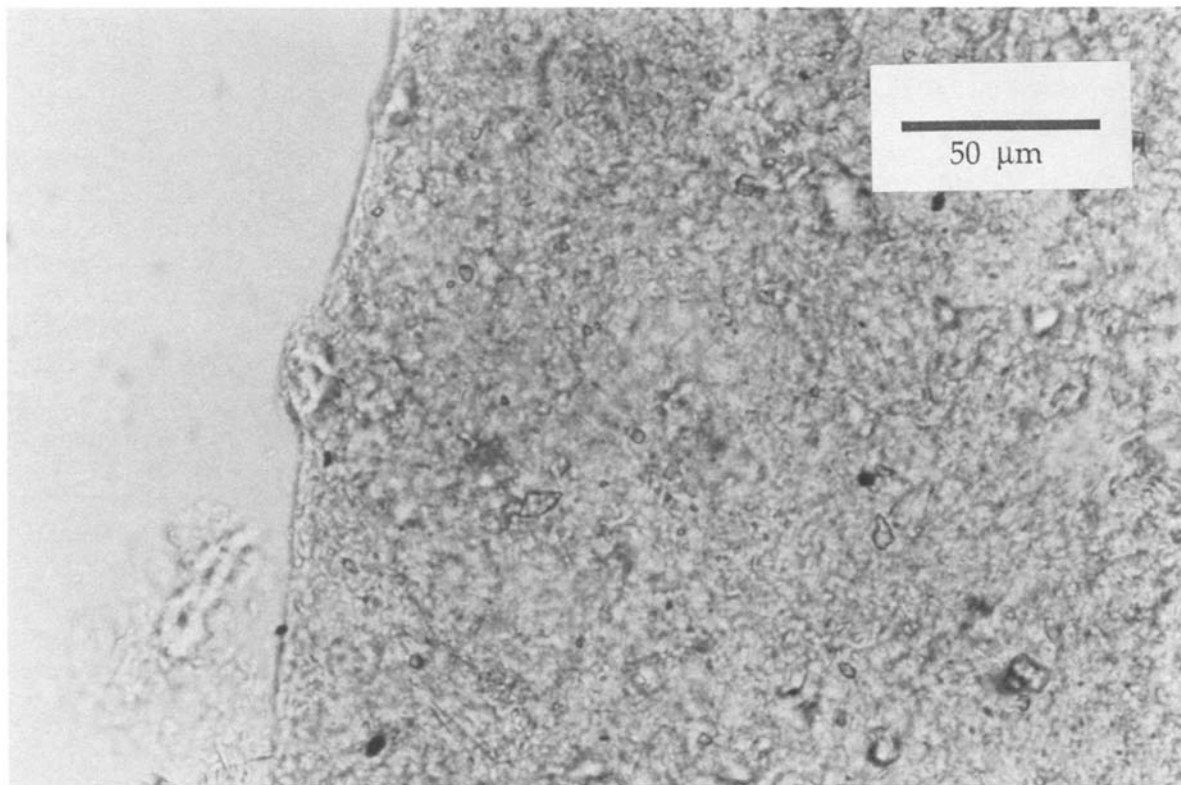


Fig. 2. Portion of fecal pellet collected from a *Melosira*-fed *Diporeia*. Note peritrophic membrane (left) and absence of any discernible remaining *Melosira* cellular structure. Black bar (upper right) denotes scale (50 μm).

sisted of well-developed multi-toothed incisors and strong triturative molar processes. These morphological traits are typical of the biting-rasping mandible commonly found in gammaridean amphipods (Bousfield, 1973; Barnard, 1969). Further mechanical breakdown may also occur in the foregut if *Diporeia* sp. possesses a cardiac stomach (and gastric mill) similar to those noted in *Gammarus lacustris* (Schmitz, 1967) and *Hyalella azteca* (Schmitz & Scherrey, 1983). At present, there is little detailed knowledge of the morphology of the gut of *Diporeia* sp. However, Bousfield (pers. comm.) believes that its gut is structurally typical of most gammarids and should include a cardiac stomach and gastric mill. In summary, given the demonstrated ability of *Diporeia* sp. to break down and digest *Melosira* to the extent that few recognizable features remain, it appears that previous gut content analysis of field-sampled animals may grossly underestimate

the overall importance of algal material in supporting the amphipod's growth and production. Thus, *Diporeia* sp. production may be far more intimately linked with primary production in overlying water than previously thought.

Finally, we believe that our results illustrate the often limited value of extrapolating gut contents observations to draw inferences about an aquatic invertebrate's overall diet. Peters (1984) emphasized the use of caution in interpreting gut contents data and provided a useful review of factors that often confound or limit such studies. Our observations of *Diporeia* sp. feeding reaffirm that such caution is fully warranted.

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