

Short Communication

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Grazing of free-living *Pylaiella littoralis* by the amphipod *Gammarus tigrinus*

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Abstract: *Pylaiella littoralis* is a brown alga with a cosmopolitan distribution. A free-living form of the alga fouls the beaches of Nahant, Massachusetts (USA). The amphipod *Gammarus tigrinus* is found within the floating drifts of algae. Gut contents confirmed that *G. tigrinus* consumed *P. littoralis*. Significantly, culture studies revealed that ingested *P. littoralis* survives and grows from fecal pellets produced by *G. tigrinus*. Also, inefficient grazing produced vegetative fragments that grew and survived. $\delta^{13}\text{C}$ values for the amphipods and *P. littoralis* averaged -17.4‰ and -17.7‰ , respectively, suggesting that *G. tigrinus* acquires most of its carbon from *P. littoralis*.

Keywords: *Pylaiella littoralis*; *Gammarus tigrinus*; grazing; digestion resistance; carbon isotope

The effects of grazers on the abundance, distribution, and morphology of seaweeds are well documented (Gaines and Lubchenco 1982; Hawkins and Hartnoll 1983; Lubchenco and Gaines 1981; Slocum 1980; Underwood 1979). Herbivores may also facilitate the reproduction and dispersal of seaweeds when grazed filaments are released as drift or when seaweeds survive ingestion and germinate from fecal pellets (Breeman and Hoeksema 1987; Santelices and Correa 1985; Santelices and Ugarte 1987; Santelices et al. 1983). In freshwater, changes in community structure can result from the differential ability of freshwater algae to survive ingestion (Gibor 1956; Nicotri 1977; Porter 1973). A wide variety of seaweeds survive ingestion by sea urchins, many gastropods, and a chiton (Breeman and Hoeksema 1987;

Santelices and Correa 1985; Santelices and Ugarte 1987; Santelices et al. 1983).

We investigated the relationship between the filamentous brown seaweed *Pylaiella littoralis* (L.) Kjellman and the amphipod, *Gammarus tigrinus* Sexton, in Nahant, Massachusetts, USA (Figure 1), where *P. littoralis* occurs as a free-living perennial population (Miller 1988; Wilce et al. 1982). The alga reproduces solely by vegetative fragmentation. Reproductive unilocular and plurilocular organs are rarely present and do not contribute significantly to population growth (Wilce et al. 1982). Nuisance accumulations of *P. littoralis* occur in the surf zone of beaches fringing Nahant and adjacent bays. The amphipods are present year-round and account for at least 15 % of the dry weight of the free-living *P. littoralis* community during summer months (T. Briggs, pers. comm.).

To evaluate the effects of *G. tigrinus* grazing on the free-living *P. littoralis* population in Nahant, we conducted gut analyses to establish if *G. tigrinus* consumes *P. littoralis* *in situ*. After we serendipitously observed algal filaments growing out of amphipod fecal pellets in culture, we conducted laboratory tests to describe and quantify the phenomenon. After we confirmed that gut contents of *G. tigrinus* collected *in situ* mainly contained *P. littoralis*, we performed stable carbon isotope analyses to evaluate if *P. littoralis* was a primary dietary carbon source for *G. tigrinus*.

In 1987 we collected *G. tigrinus* from *in situ* populations of free-living *P. littoralis*. We used a compound microscope to identify gut contents. In a germination test, 80 fecal pellets were rinsed once in sterile seawater and then set aside for incubation at 10 °C and $30\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ under long days (16:8 h light:dark cycle). As a control, seawater used to rinse the pellets was checked for subsequent algal development. After one week, pellets were transferred to either 10 ml of sterile F/4 culture medium (Guillard and Ryther 1962) in a plastic petri dish or 200 ml of F/4 medium in a glass storage dish. Cultures were transferred to new media weekly and were monitored for 30 days to check for the development of algal filaments from fecal pellets. Fecal pellets were also collected from amphipods placed in unialgal cultures of free-living *P. littoralis*.

Stable carbon isotope analyses ($\delta^{13}\text{C}$) were performed on *G. tigrinus* collected *in situ* and starved for one week to

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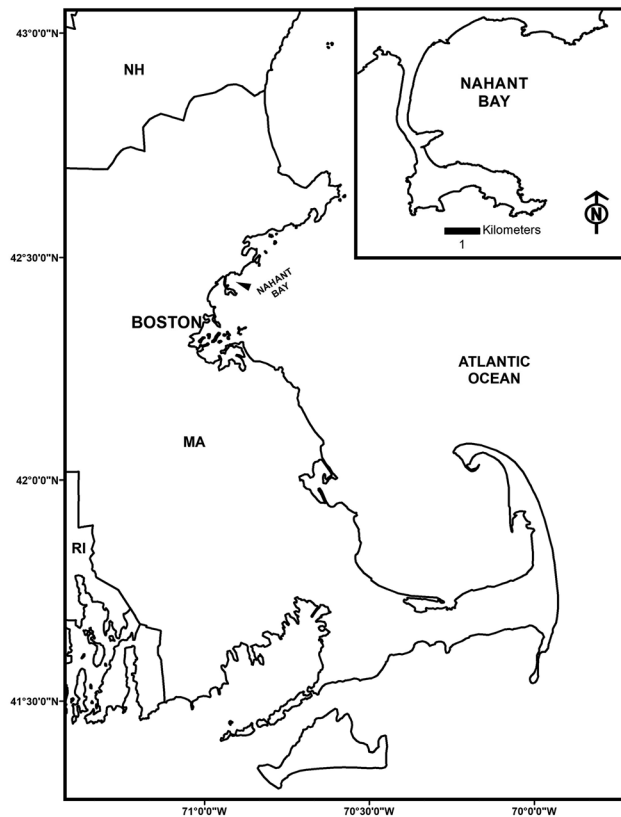


Figure 1: Map of northeast United States with Nahant Bay (insert). NH, New Hampshire; MA, Massachusetts; RI, Rhode Island.

evacuate their digestive tract. In addition, the $\delta^{13}\text{C}$ value of free-living *P. littoralis* was determined from material collected with the amphipods and meticulously picked clean of other algae and invertebrates (mostly amphipods and copepods). In addition, we obtained stable carbon isotope values for the free-living *P. littoralis* community, including unsorted algae, animals and debris. We tested the null hypothesis that no difference in carbon isotope ratios exists between free-living *P. littoralis* and *G. tigrinus*. Rejection of the null hypothesis (t -test $p \leq 0.05$) implies that the amphipods assimilate considerable carbon from a source other than *P. littoralis*.

Samples were prepared for stable carbon isotope analysis following Dunton and Schell (1986). Isotope measurements were performed on a Micromass 602E dual inlet, double collector isotope-ratio mass spectrometer. Results were expressed as $\delta^{13}\text{C}$ values relative to the limestone standard Pee Dee Belemnite (Craig 1957), where:

$$\delta^{13}\text{C} = \left| \left[\left(\frac{{}^{13}\text{C}:{}^{12}\text{C}_{\text{sample}}}{({}^{13}\text{C}:{}^{12}\text{C}_{\text{standard}})} \right) - 1 \right] \times 10^3 \right|$$

Secondary standards were run daily as cross-calibration checks of the AER oil working standard, combusted daily. NBS-22 ($\delta^{13}\text{C} = -29.8\text{‰}$) was used as a common secondary standard.

Analyses of *G. tigrinus* gut contents revealed that *P. littoralis* comprised most of the material ingested by these animals, though unidentified digested material that resembled *P. littoralis* was common. The condition of the material in the guts of *G. tigrinus* varied from unidentifiable debris to intact pieces (Figure 2). Discoid plastids of *P. littoralis* could sometimes be discerned, even from material collected in the lower third of the amphipod gut. Cell wall ghosts with cellular dimensions similar to *P. littoralis* could frequently be discerned among the debris. The survivorship of ingested filaments by amphipods collected from floating drifts of algae at Nahant was low. Three pellets out of 80 produced live algal filaments. *Pylaiella littoralis* was the only alga that grew vigorously from the fecal pellets, though two other algae were observed, including *Ulva* sp. and an unidentified brown alga. Filaments of *P. littoralis* also grew from fecal pellets produced by amphipods fed unialgal *P. littoralis* (Figure 3).

The $\delta^{13}\text{C}$ values for free-living *P. littoralis* and *G. tigrinus* overlapped in their ranges and averaged -17.7‰ and -17.4‰ ,

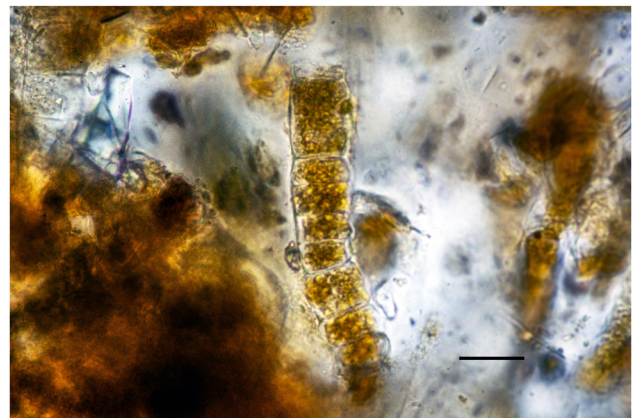


Figure 2: Gut contents of *Gammarus tigrinus* collected among free-living *Pylaiella littoralis*, with intact filament resembling *P. littoralis*. Scale bar = 25 μm .

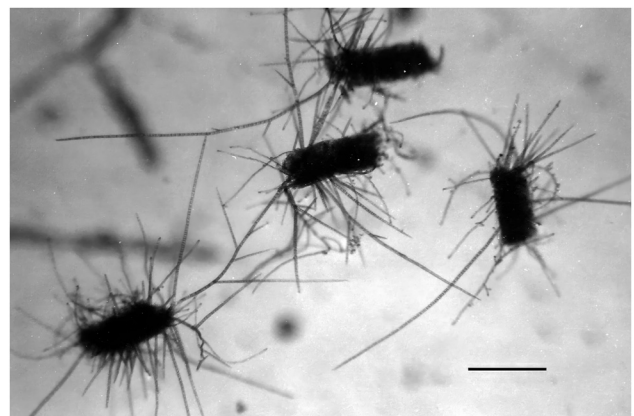


Figure 3: Fecal pellets produced by *Gammarus tigrinus* in culture with unialgal free-living *Pylaiella littoralis* (top). Scale bar = 1 mm.

respectively. The isotope value of material collected from the *P. littoralis* community, but not cleaned of associated animals and other algae, was -17.6‰ . These values are not significantly different ($t = 1.53$, $df = 5$).

Results of this study document that *P. littoralis* can survive ingestion by the amphipod *G. tigrinus*. New filaments grew from fecal pellets produced by amphipods collected in laboratory culture and from the free-living algal community in Nahant Bay. Before these observations, fragmentation because of physical disturbance from wave action or by filament breakage following infection by the holocarpic chytrid *Eurychasma dicksonii* (Wright) Magnus were the primary mechanisms proposed to increase population numbers (Wilce et al. 1982). Survivorship after ingestion adds another means of reproduction. However, the relative contribution of the different fragmentation modes to population increases or maintenance is unknown.

The implications of resistance to digestion for *P. littoralis* are magnified if one considers that other animals (isopods, small decapod crustaceans, and fishes) could similarly increase the rate of vegetative reproduction of free-living *P. littoralis*. Although the production of algal filaments from fecal pellets collected from field amphipods was low (less than 4%), this does not diminish the potential significance of the phenomenon since the amphipod population produces large numbers of pellets. Unlike some plant seeds that appear to be pre-adapted to survive ingestion with impenetrable outer coverings, how *P. littoralis* survives ingestion by *G. tigrinus* is unknown. Rapid transit through the amphipod gut, inefficient digestion or both are likely explanations. The phenomenon may be widespread as *P. littoralis* also survives ingestion by two limpets (Santelices and Correa 1985). A closely related algal genus, *Ectocarpus* sp., survived ingestion by seven herbivores (Santelices and Correa 1985; Santelices and Ugarte 1987). Other macroalgae that survive digestion include red algal species by herbivorous fish (Vermeij et al. 2012) and 14 macroalgae among 47 species found in the gut contents of three sea urchin species (Cabral de Oliveira 1991). Kelp zoospores also survive ingestion by a fish in Chile (Ruz et al. 2018).

Two additional observations are noteworthy. First, we did not observe fecal pellets in collections of free-living *P. littoralis*, but the pellets are negatively buoyant and could easily fall to the bottom. Second, cultures of unialgal free-living *P. littoralis* with *G. tigrinus* produced abundant algal filaments less than 0.5 mm long. Notably, the size and number of filaments are not seen in unialgal cultures without amphipods. Regeneration of entire individuals from filaments of this size occurred frequently in cultures. Therefore, filament fragmentation by amphipod grazing should be considered another means of vegetative reproduction.

Interpreting our stable isotope results is straightforward because *P. littoralis* was the main source of primary production in the algal drifts, which presented an ideal opportunity to determine if the algal carbon was assimilated by the amphipods (Dunton and Schell 1986; Kitting et al. 1984; Simenstad and Wissmar 1985; Suchanek et al. 1985). Our isotopic data show that the amphipod *G. tigrinus* derives most of its dietary carbon from *P. littoralis*. Although other algae likely possess an isotopic signature similar to *P. littoralis*, their biomass in the study area was small compared to the extensive drifts of floating *P. littoralis*. The carbon isotope values of *P. littoralis* are similar to that of other macroalgae, between 17 and 20 ‰ (Fry and Sherr 1989) and 14–29 ‰ (Stephenson et al. 1984). We could strengthen our interpretation that *Gammarus tigrinus* acquires most of its carbon from *P. littoralis* by conducting $\delta^{13}\text{C}$ analyses on amphipods collected from mud flats and marshes where other food sources dominate. For example, a broad survey throughout multiple habitat types in Plum Island Sound, Massachusetts, USA, found $\delta^{13}\text{C}$ values from -24.3 to -16.2‰ (Deegan and Garritt 1997). The heavier values indicated that the amphipods used more marsh organic matter than zooplankton as a dietary source.

The survival of ingested material and fragments generated by grazing makes the algal-amphipod relationship a two-edged sword. Amphipods reduce biomass through feeding, but inefficient digestion of filaments and the production of fragments by grazing can increase the number of individuals within the population.

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Research ethics: Not applicable.

Author contributions: SLM conceived and conducted the field and culture studies. RTW provided research facilities and supervised the project and writing by SLM for his doctoral thesis.

Competing interests: The authors state no conflict of interest.

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Data availability: Not applicable.

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Bionotes



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Steven L. Miller's current research focuses on coral reef monitoring to assess changes in benthic community structure throughout the Florida Keys. The program includes multiple habitat types and no-take marine reserves in its design. He was recording secretary for the International Coral Reef Society and previously served on the board of directors of the Ocean Conservancy and Coral Restoration Foundation. He is a Fellow of the American Association for the Advancement of Science.



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Robert T. Wilce (1924–2022) used scuba diving starting in the 1960s to study algae, often under ice. He pioneered arctic phycology and contributed dozens of taxonomic publications (Küpper 2022). He was an expert with a *camera lucida* and loved, in his words, “microscoping.” His world-class collection of seaweeds resides at the University of Massachusetts, Amherst, where he retired as a professor. He was a founding member of the Northeast Algal Society and received numerous awards during his long career.