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# Uptake and Assimilation of Dissolved Organic Matter by *Bosmina longirostris (Crustacea: Cladocera)*

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# Uptake and Assimilation of Dissolved Organic Matter by *Bosmina longirostris* (Crustacea: Cladocera)

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## Abstract

A considerable amount of dissolved organic matter (DOM) exists in freshwater environments (5-40 µg/mL). However, freshwater invertebrates, including crustaceans, are viewed as incapable of taking up and metabolically utilizing DOM. We evaluated this hypothesis by exposing the freshwater crustacean *Bosmina longirostris* to labeled proteins and polysaccharides. Individuals readily ingested these materials as evidenced by the presence of molecule-specific labels in the digestive system. Histological examination of specimens exposed to the protein ferritin revealed that the label was in the lumen of the digestive tract but not in the surrounding cells. Digestion in crustaceans is extracellular, and the absence of a label within cells is not necessarily unexpected. *Bosmina longiristris* is capable of supplementing its diet of particulate foods through the acquisition and assimilation of DOM from its environment.

## Introduction

Dissolved organic matter (DOM) is potentially an important nutritive source for marine and freshwater invertebrates. Components of DOM include dissolved organic nitrogen, free and combined amino acids, and carbohydrates. Beginning in 1907, studies reported DOM uptake by marine invertebrates. These earlier reports have been confirmed by other studies using labeling technologies (Legrand and Carlsson 1998 and Perls 1867). In contrast, DOM uptake by freshwater invertebrates has been considered either low or completely absent (Stephens 1964). However, more recent studies have shown that freshwater invertebrates, such as oligochaetes, snails, and bivalves, are capable of DOM uptake from freshwater (e.g. Thomas 1997). One group of invertebrates, the crustaceans, were thought to be incapable of DOM uptake due to their exoskeleton. However, Gellis and Clarke (1935) discovered that the crustacean *Daphnia magna* (Arthropoda: Crustacea) was able to utilize filter-passing material as food. Although this paper doesn't directly discuss where assimilation occurs, Quaglia *et. al* (1970) examined copepod alimentary tracts and discovered that the midgut is the best equipped place for assimilation of materials.

The freshwater cladoceran *Bosmina longirostris* (Arthropoda: Crustacea) is highly abundant in North American lakes and ponds. *B. longirostris* is known to feed on particulate matter in water (Tóth and Kato 1997), but feeding on DOM has not yet been addressed. We tested the hypothesis that *B. longirostris* can take in and assimilate DOM in freshwater. We hypothesized that the tissues surrounding the gut lumen of *B. longirostris* would assimilate DOM based on past evidence on the uptake of DOM by other freshwater cladoceran such as *Daphnia magna* (Gellis and Clarke 1935).

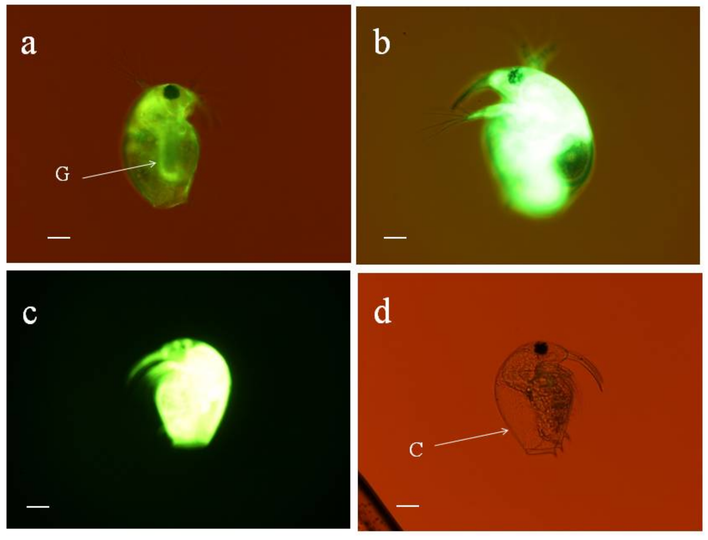


Figure 1: Fluorescence micrograph of *B. longirostris* incubated in FLW with 1 mg/mL BSA-FITC for a) 2 hours b) 3 hours c) 4 hours. d) Fluorescence micrograph of *B. longirostris* control incubated in FLW. Scale bars= 100 µm. G= gut, C= carapace. Viewed using fluorescence microscopy and transmitted light microscopy at the same exposure time and digital gain.

## Materials and Methods

*Bosmina longirostris* were collected from the surface waters of Angler's Lake in Bloomington, IL.

Experiments

Experimental animals were allowed to clear their digestive tracts for 30 minutes in 0.2 µm (pore size) filtered lake water (FLW) and were then tested for uptake and assimilation by transferring them to one of the following three solutions:

- 1.0 mg / mL of the fluorescein-labeled protein, bovine serum albumen (BSA, mw= 68 kDa) in FLW
- 1.0 mg / mL of the protein ferritin (mw= 440 kDa) in FLW
- 0.5 mg / mL iron dextran (mw=73 kDa) in FLW

Detection of Labels in *B. longirostris*

At known time intervals (2-4 hours) specimens were removed from the medium, washed and immobilized with carbonated FLW, and examined using one of two techniques:

- Individuals exposed to BSA were immediately examined using a Nikon E600 compound microscope equipped for fluorescent microscopy.
- Individuals exposed to ferritin and iron dextran were fixed with a 2.5% paraformaldehyde, and then the presence of the iron label was detected using the "Prussian Blue" reaction. Some specimens were examined as whole mounts, others were sectioned (1µm), and all were viewed using a Nikon E600 microscope with transmitted light.

## Results

When exposed to 1.0 mg/mL FITC-BSA for two hours, the fluorescence initially associated with FITC-BSA was found in the gut lumen (Figure 1a). With continued exposure, the entire animal filled with the fluorescent label (Figures 1b and 1c), while the control individual showed no fluorescence (Figure 1d).

In whole mounts of specimens exposed to 1.0 mg/mL ferritin for one hour, the blue reaction product was seen inside the lumen of the gut and into the tissues immediately surrounding the gut lumen (Figure 2a). In the 3 and 4 hour incubations, ferritin could again be detected in the tissues surrounding the gut lumen (Figures 2b and 2c), while the control specimens contained no indication of the reaction product (Figure 2d).

Specimens incubated with iron dextran revealed the presence of the blue reaction product in the midgut and surrounding tissues in as little as two hours (Figure 3a). As time progressed, the distribution of the reaction product expanded nearly throughout the entire body after three and four hours of incubation (Figures 3b and 3c). The control specimens again showed no indication of reaction product (Figure 3d).

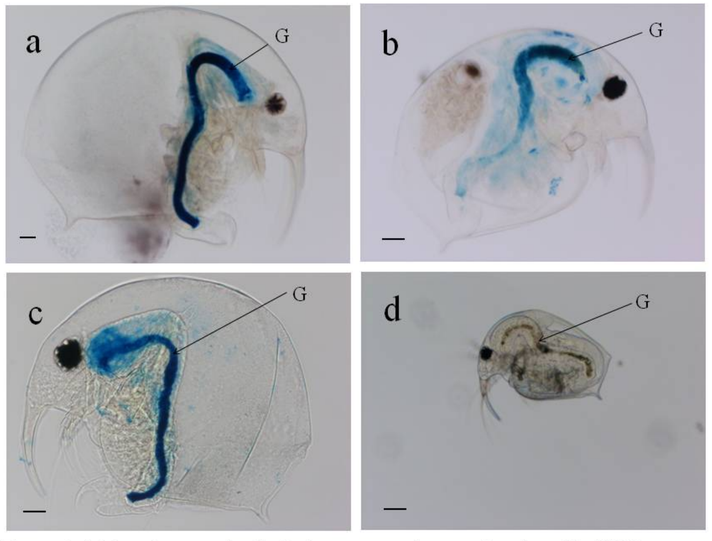


Figure 2: Light micrograph of a *B. longirostris* that was incubated in FLW with 1.0 mg/mL ferritin for a) 1 hour b) 3 hours, and c) 4 hours. d) Light micrograph of a *B. longirostris* that was incubated in FLW for 3 hours. Scale bars= 100 µm. G= gut. Viewed using light microscopy with same exposure time and digital gain.

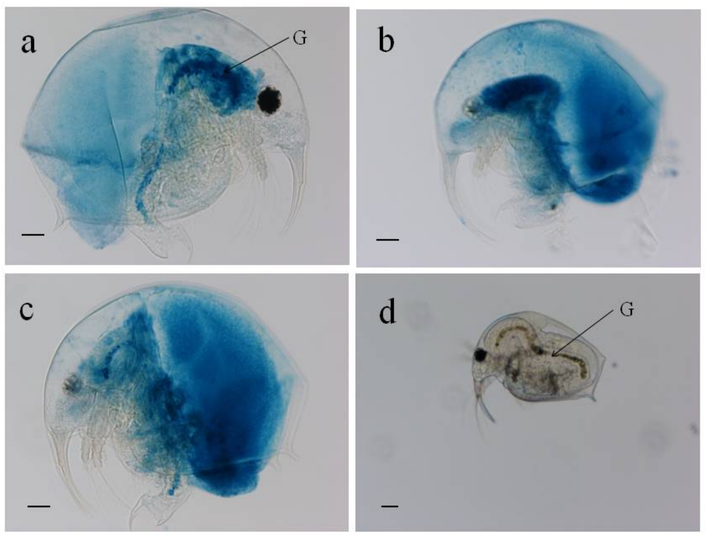


Figure 3: Light micrograph of a *B. longirostris* that was incubated in FLW with 1.0 mg/mL iron dextran for a) 2 hours b) 3 hours c) 4 hours. d) Light micrograph of a *B. longirostris* that was incubated in FLW for 3 hours. Scale bars= 100 µm. G= gut. Viewed using light microscopy with same exposure time and digital gain.

## Conclusions

The freshwater cladoceran, *Bosmina longirostris* is capable of ingesting DOM from the environment. Digestion of these materials occurs in the alimentary canal, and incorporation of the materials occurs in the tissues surrounding the gut. These results support our hypothesis that DOM is digested by *B. longirostris* and that the cells surrounding the gut lumen can assimilate materials that pass through the digestive tract. Our results suggest that this form of nutrient acquisition can occur at DOM concentrations equivalent to those of natural freshwaters. We saw evidence of uptake and assimilation by the tissues of B. longirostris in concentrations as low as 10 µg/mL, which falls within the concentration range (5-40 µ/mL) of freshwater lakes (Thomas 1997). Our conclusion is similar to previous studies on freshwater invertebrates, especially the work done with *Daphnia magna* (Gellis and Clarke 1935), and our assumptions on location of this assimilation are consistent with previous reports as well (Quaglia *et. al* 1970).

## Acknowledgements

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