# Seawater flow into the digestive system of actinotroch larvae (Phoronida)

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## INTRODUCTION –

Actinotroch larva (Phoronida) concentrate and collect food particles during their planktonic existance. Reported mechanisms of particle capture include both ciliary reversal and direct interception along the larval tentacles, and suction of particle-containing seawater into the larval vestibule. These diverse mechanisms explain collection of particles, but not subsequent ingestion. Particle delivery into the digestive system is accomplished, at least in part, by the activity of the esophageal cilia. If and how the beat frequency of esophageal cilia is modified by the presence or absence of particulate or dissolved organic materials remains unknown for actinotroch larvae.

In the absence of large particulate foods (e.g., phytoplankton) the beating of esophageal cilia could deliver seawater into the larval digestive system. Dissolved and small (e.g., < 1 μm) particulate organic materials contained within this flow would be available for digestion and assimilation. The benefits of these alternative sources of nutrition depend upon (1) the delivery of seawater into the digestive system, (2) the abundance of these materials in seawater, and (3) the mode(s) of material digestion and assimilation exhibited by cells of digestive epithelia. This possibility was evaluated by exposing field-collected actinotroch larvae to the iron-containing protein ferritin and assessing the subsequent distribution of the label within the larval body.

## METHODS-

Actinotroch larvae (provisionally identified as *Phoronis architecta*, S. Santagata, pers. com) were collected using a 3/4 m diameter (202 μm mesh) plankton net deployed into the "Indian River" from the dock of the Smithsonian Marine Station, Fort Pierce, FL.

Actinotroch larvae were transferred to 0.2 μm (pore size) filtered seawater (FSW) and held at room temperature (27 ºC). For experiments, larvae (< 1 / mL) were incubated in a 0.5 mg / mL solution of the iron-containing protein ferritin suspended in 10 mL of FSW. After 4-h incubation, larvae were washed 3× in an isosmotic $MgCl_2$: FSW mixture to remove exogenous label and fixed a 2% paraformaldehyde in $HCO_3^-$-buffered saline. Identically treated control animals were not exposed to ferritin.

To detect the presence of iron atoms from ferritin, preserved larvae were incubated in a 2:3 mixture of 2% potassium ferrocyanide and 1% HCl for 1 h. In an acidic medium, the $Fe^{3+}$ ions complex with ferrocyanide ions to form a blue, insoluble reaction product. After the incubation in the reaction mixture, the larvae were dehydrated using an ascending ethyl alcohol series. For preparations of intact larvae, dehydrated individuals were cleared in xylene and whole mounts were prepared using Permount®. To confirm that the reaction product was located within cells, dehydrated larvae were embedded in plastic (Embed 812) and sectioned (1 μm). Sections were stained with 2.5% acid fuchsin.

## RESULTS-

- The blue reaction product was detected in actinotroch larvae not exposed to ferritin (controls), but the label abundance was low, the distribution diffuse, and it was not within the cells of the digestive system (Fig. 1A).

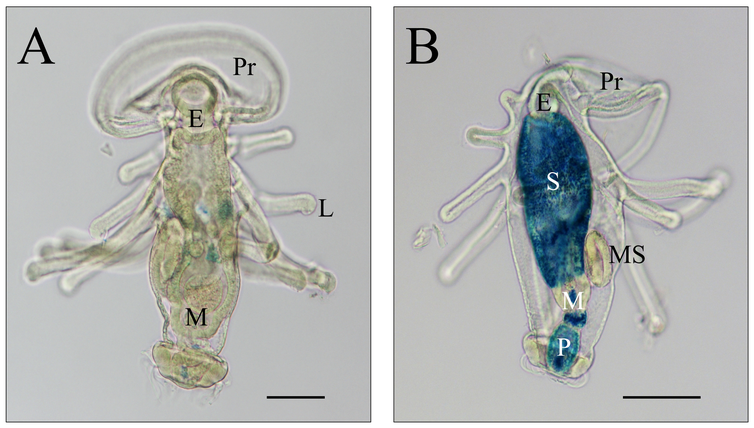


Figure 1: Light micrograph of larvae of *Phoronis architecta* not expose (A) and exposed (B) to 0.5 mg ferritin / mL in seawater for 4 hours. The blue color present in Fig. 1B represents the location of $Fe^{3+}$ (originally present in ferritin) within the digestive system and the bounding epithelia. Abbreviations: E = esophagus, L = larval tentacles, M = midgut, MS = metasomal sack, P = proctodeum, Pr = preoral lobe, S = stomach. Scale bars = 150 μm.

- All larvae exposed to ferritin (n = 21) contained an abundance of the blue reaction product. The label was present in the cells of the stomach, and proctodeum (Figs. 1B, 2B, 3B), but was not equivalently present in the esophagus (Fig. 2A) and midgut (Fig. 3A) epithelia.

- The blue reaction product was also present within the lumen of the digestive system (Fig. 3).

- There was no evidence of label translation from the sites of assimilation to other regions of the larval body.

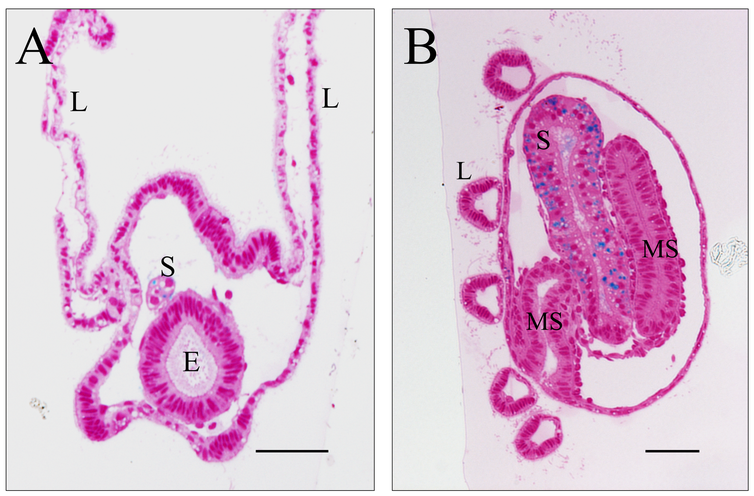


Figure 2: Light micrographs of 1-μm thick sections at the level of the esophagus (Fig. 2A) and the stomach (2B) of a larva of *Phoronis architecta* exposed to 0.5 mg ferritin / mL in seawater for 4 hours. The blue reaction product was not detected in the cells of the esophagus (Fig. 2A) and was abundant within the epithelium of the larval stomach (Fig. 2B). Abbreviations: E = esophagus, L = larval tentacles, MS = metasomal sack, S = stomach. Scale bars = 25 μm.

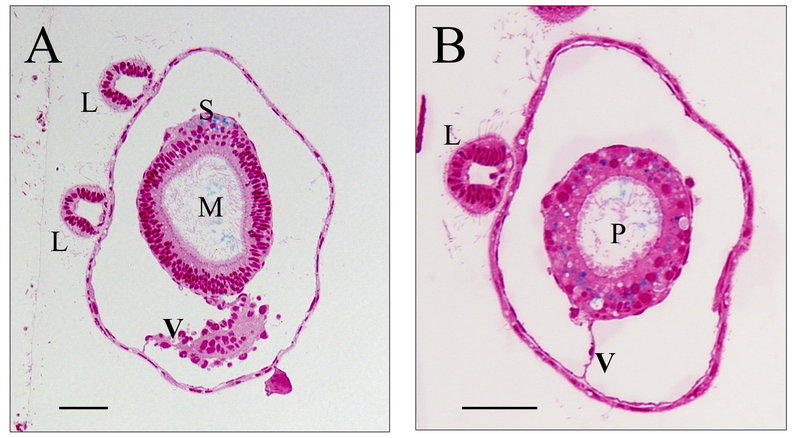


Figure 3: Light micrographs of 1-μm thick sections at the level of the midgut (Fig. 3A) and the proctodeum (Fig. 3B) of a larva of *Phoronis architecta* exposed to 0.5 mg ferritin / mL in seawater for 4 hours. The blue reaction product was not detected in the cells of the midgut (Fig. 3A) and was present within the proctodeal epithelium (Fig. 3B). The blue reaction product is present within the lumen of both regions of the digestive system. Abbreviations: L = larval tentacles, M = midgut, P = proctodeum, S = stomach, V = ventral mesentary. Scale bar = 25 μm.

## DISCUSSION-

Seawater passes into the digestive system of larvae of *Phoronis architecta*; the motive force for this fluid flow is presumed to be provided by the beating of the esophageal cilia with or without contributions from the esophageal musculature. Molecules of ferritin contained within this flow are readily assimilated by the epithelia of the larval stomach and proctodeum and not by the cells that line the esophagus and midgut. The existence of the blue reaction product as circular grains within cells suggests pinocytotic or endocytotic assimilation of ferritin and subsequent intracellular digestion of these molecules. There was no evidence of the movement of the iron label from the sites of assimilation to other regions of the larval body. However, this result does not preclude nor would the methods used here detect the distribution of unlabeled (without $Fe^{3+}$) organic materials.

The delivery of seawater into the digestive system in the absence of suspended particles suggests that "ingestion" is not coupled with the active removal of particles from suspension. This constitutive flow of seawater into the larval digestive system provides a plausible mechanism for the ingestion of particles (e.g., bacteria, colloids) too small to induce particle capture mechanisms, but may also contribute to larval nutrition. This seawater flow also makes available any dissolved organic material to the digestive epithelia.

The quantitative contribution to larval nutrition or energetics of organic materials (dissolved or particulate) that enters the digestive system of feeding larvae through "drinking" cannot be assessed here. These estimates, at a minimum, require knowledge of the abundance and compositional distribution of organic matter in seawater, the rate of seawater flow through the digestive system, and the assimilation efficiency of the digestive epithelium. Irrespective of these unknown quantities, the delivery of seawater into the digestive system of actinotroch larvae of *P. architecta* provides an alternate avenue for nutrient acquisition.