Gut Content and Pigment Analysis in the Marine Isopod *Pentidotea resecata*

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Abstract

The green morph of the marine isopod *Pentidotea resecata* lives and feeds on eelgrass, while the brown morph lives on kelp. The coloration of the green morph closely matches that of its substrate. The goal of this project was to determine whether the green isopod's coloration is due to the presence of chloroplasts and/or chlorophyll. Using spectrophotometry, we analyzed isopod tissue extracts for the presence of chlorophyll *a*. The extracts did exhibit peaks near 430 and 664 nm, as expected for chlorophyll *a*. However, acidification of the extracts produced little change in the extracts' absorption spectra, indicating that they contained mostly pheophytin *a*, a degradation product of chlorophyll *a*. The digestive system of *P. resecata* consists of a mouth, esophagus, stomach, hepatopancreas, hindgut, and anus, which exits ventrally into the valve formed by the uropods. The stomach contains separate openings into both the hepatopancreas and the hindgut, which are the locations where most of the materials being digested were found on dissection. The hepatopancreas of this species consists of several tan-colored midgut glands that surround the stomach and hindgut. The hindgut contents include chunks of whole eelgrass cells and a variety of diatoms along with other debris. Although fluorescence suggests that some chlorophyll in the material within the hindgut lumen may still retain functionality, we did not find any indication of plant cells or chloroplasts within the isopod tissue itself. While it is likely that *P. resecata* derives its green pigmentation from its eelgrass diet, these animals do not appear to contain functional chlorophyll *a* within their tissues.

Introduction

The marine isopod *Pentidotea resecata* can be found in beds of eelgrass (*Zostera marina*) or brown kelp such as *Macrocystis* along the west coast of North America. Isopods found on kelp are brown in color, whereas those found on eelgrass have green pigmentation. These two color varieties appear to exist as separate populations that do not intermingle (Lee & Gilchrist 1972).

Some marine invertebrates are known to harbor photosynthetic organisms. These include corals and their zooxanthellae symbionts, some sacoglossan sea slugs, and even some crustaceans (Muscatine & Porter 1977, Clark 1992, Epp & Lewis 1981, Chang & Jenkins 2000).

The goal of this project was to establish whether the green color of *P. resecata* found in eelgrass beds was due to the presence of photosynthetic material within the isopod, specifically intact chloroplasts and chlorophyll *a*. Since these photosynthetic elements would presumably be obtained via the isopod's eelgrass diet, we examined the animal's digestive system for the presence of whole plant cells and intact chloroplasts. The isopod digestive system consists of a mouth, esophagus, stomach, hepatopancreas, hindgut, and anus, all of which are lined by cuticle with the exception of the hepatopancreas (Storch et al. 2002). We used spectrophotometry to check for the presence of functional chlorophyll within the whole isopod, including its cuticle, and compared the optical density of isopod extracts with that of eelgrass extracts.

Methods: Sample Collection

Green eelgrass isopods (*Pentidotea resecata*) were collected from Padilla Bay, WA along with samples of their eelgrass (*Zostera marina*) substrate. Samples were transported to Rosario Beach Marine Laboratory where they were maintained in outdoor and indoor seawater tanks. Isopods were anesthetized with carbonated saltwater prior to dissection.

Methods: Spectrophotometry

- Eelgrass and isopod samples were ground in a tissue grinder with a 90% acetone-magnesium carbonate solution according to a standard protocol for chlorophyll extraction (Rice et al. 2012).
- Pigment extracts were analyzed using a Beckman DU520 UV/Vis spectrophotometer.
- We tested for chlorophyll *a* following a standard acidification protocol (Rice et al. 2012).
  - We recorded the absorbance of the extract at 664 nm before adding 0.1M HCl and at 665 nm after the addition of acid.
  - The optical densities were corrected based on absorbance readings at 750 nm.
  - The corrected optical densities were used to calculate an acidification ratio.
  - Pure chlorophyll *a* was represented by a ratio of 1.7, whereas a ratio of 1.0 indicated the presence of pheophytin *a,* a degradation product of chlorophyll *a*.

Figure 1: (A) Pre and post-acidification eelgrass extracts. (B) Isopod digestive system *in situ*. (C) Anterior isopod digestive system at 6.5x.

Methods: Isopod Dissection and Light Microscopy

Isopods were dissected by removing the dorsal exoskeleton from the head to the telson.

- A Jenco GL7-280 dissecting microscope was used to photograph the isopod digestive system.
- A Nikon Eclipse E200 compound microscope at 40-1000x was used to check for green plant cells and chloroplasts within each section of the digestive system (stomach, midgut, hindgut).
- Images were captured using a Nikon D70 digital camera.

Methods: Fluorescence Microscopy

We looked for chlorophyll fluorescence in eelgrass and isopod samples using two different dichroic edge filters.

- A blue short-pass filter (<550 nm) was secured to the light source.
- A red long-pass filter (>600 nm) was secured to the microscope objective lens. Maximum chlorophyll fluorescence has been observed at emission spectra of 685 nm (Gitelson et al. 1999).
- Photographs were taken with a Nikon D70 digital camera.

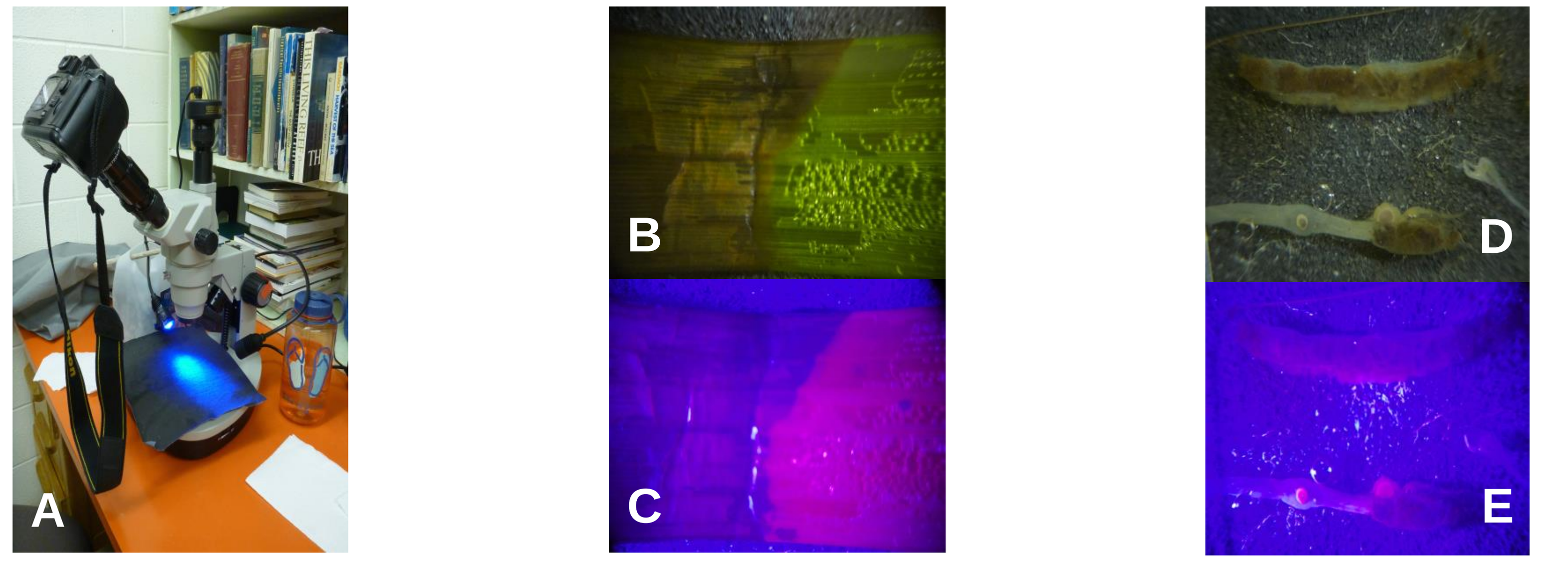

Figure 2: (A) Dissection scope with camera attached and blue filter over light source. (B) Eelgrass under white light. (C) Eelgrass under filtered light. (D) Isopod hindgut under white light. (E) Isopod hindgut under filtered light conditions.

Results: Spectrophotometry

- Eelgrass extracts exhibited absorption spectra characteristic of chlorophyll *a* with two strong peaks, one near 430 nm, and the other at 663-664 nm (Figure 3).
- Acidification of eelgrass extracts resulted in absorption ratios between 1.1 and 1.7.
- Isopod extracts exhibited rounded peaks near 430 nm and at 665 nm (Figure 3).
- Acidification of isopod extracts resulted in absorption ratios of 1.0-1.1.

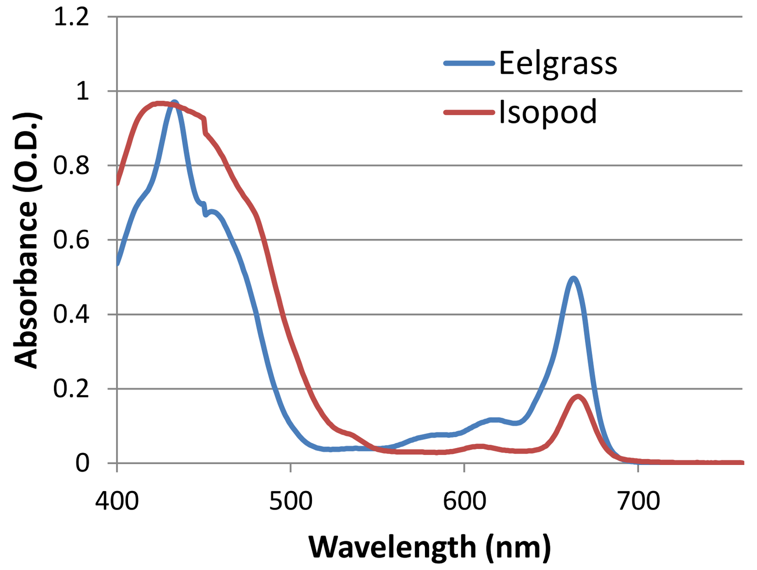

Figure 3: Absorption spectrum of eelgrass and isopod extracts.

- A t-test showed that the acidification ratios from the eelgrass and isopod samples were significantly different from each other (p=6.65E-06).

Results: Isopod Gut Anatomy

- The isopod digestive system consists of a mouth, esophagus, stomach, hepatopancreas, hindgut, and anus.
- The stomach contains a filter-apparatus that directs the flow of ingested material to either the hepatopancreas or hindgut.
- The hepatopancreas consists of three pairs of long midgut glands, which bulge with brownish fluid when full (Figure 4).
- The hindgut forms a long tube and has a striated appearance under the light microscope (Figure 5).

Figure 4: Two midgut glands from the same animal (100x).

Figure 5: A comparison of the hindgut with a midgut gland (40x).

Results: Hindgut Contents

- The hindgut frequently contained large fragments of eelgrass blades with whole plant cells containing chloroplasts (Figure 6).
- There were also a variety of diatoms within the isopod gut.

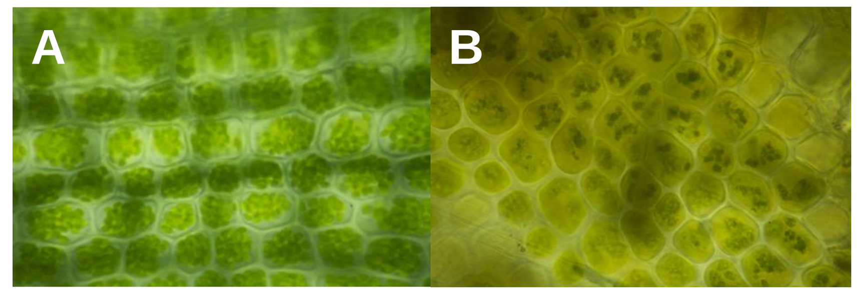

Figure 6: Eelgrass cells with chloroplasts in (A) a blade of eelgrass and (B) the hindgut of an isopod. Photographed at 1000x.

Summary

- The isopod *Pentidotea resecata* lives and feeds on eelgrass (*Zostera marina*).
- Chlorophyll *a*, which can be easily extracted from eelgrass blades, appeared to be rapidly degraded to pheophytin *a* inside the isopod.
- Some isopod tissue appeared red under conditions that should detect red chlorophyll fluorescence.
- Chunks of whole eelgrass cells containing chloroplasts could be found within the lumen of the isopod's hindgut, but not inside the midgut or within isopod cells.
- The eelgrass isopod's green coloration did not appear to be due to the presence of photosynthetically active material within its tissue.

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