DOES PHOTOSYNTHESIS TAKE PLACE IN THE GUT OF *PENTIDOTEA RESECATA?*

by

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Abstract

Pentidotea resecata is a species of isopod found along the western coast of the United States. Two separate color morphs are often reported, living and feeding on different substrates. A green color morph is found on the eelgrass *Zostera marina* in quiet, protected bays along the coast. The other color morph is brown and can be found on brown algae such as *Mactrocystis spp.* The brown color morph is more typical of the open coast. In addition to feeding on their primary substrate, *P. resecata* will also consume diatoms and other epiphytes found growing on the substrate surface. The purpose of this study was to determine whether chloroplasts within the plant and algal material consumed by the isopod were able to continue photosynthesis within the gut or possibly sequestered in gut-associated tissues following ingestion. In order to determine this, respirometry was done on living individuals of the green color morph in both light and dark following the removal of epiphytes from the surface of the isopods' cuticle. After sacrificing the isopods their gut was removed and respirometry was again run in both light and dark on the dead animals to determine the contribution of photosynthesis from any epiphytes remaining on the cuticle. Adult individuals between 45 and 54 mm in length were used in this study with no size-dependent variation in metabolic rate observed. Additionally, no oxyconformity was observed within the range of partial pressures of oxygen used. Isopods were placed in either a fed or a starved group. The fed isopods were provided with eelgrass ad libitum while the starved isopods were isolated from an eelgrass source. However, no significant difference was found between fed and starved isopods and they were analyzed together. Live

individuals showed consistently lower rates of oxygen consumption in the light than in the dark, indicating the contribution of oxygen to the system through photosynthetic processes. Analysis of isopod activity between light and dark environments showed that they were more active in the light and ruled out the possibility that the difference observed between light and dark oxygen consumption was due to increased activity in the dark. In dead isopods with their gut removed, no statistically significant difference was found in oxygen consumption between light and dark respirometry. This suggests that some ingested material within the gut of live individuals is indeed continuing to produce oxygen through active photosynthesis.

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INTRODUCTION

Symbiotic relationships occurring between algae and animals have been documented in a variety of organisms. A well-known example of this involves algal cells which live symbiotically within some marine organisms such as corals, anemones, and sponges (Venn et al., 2008). In cnidarians the symbionts live within vacuoles in the cytoplasm of the cells lining the blind gastrovascular cavity, contributing to the survival of the host in nutrient-poor waters (Davy et al., 2012; Muscatine & Porter, 1977). The algal symbiont receives a safe place to live and takes up nutrients from animal waste products, and in turn often supplies the animal with fixed carbon.

Symbiosis Within Mollusks

Such relationships are rare in more complex animals but some instances do exist. Some species of mollusks in particular participate in symbiotic relationships with algae. Giant clams within the family Tridacnidae have a complex system of algal symbiont-containing tubes which originate in the stomach and branch throughout the mantle (Norton et al., 1992).

Sea slugs within the order Sacoglossa are of particular interest as they sequester only the algal chloroplasts rather than the whole cells. These chloroplasts maintain functionality for extended periods of time. Over 20 years ago these sacoglossans were

described as the only known metazoans to retain functional chloroplasts as intracellular organelles (Clark, 1992), and no examples of other metazoans with this capability have been discovered in more recent years. Sacoglossans feed on siphonaceous algae, digesting most of the algal cell and sequestering only the plastids within the cells lining portions of their gut. This phenomenon of retaining only the chloroplasts has been termed kleptoplasty, as the plastids are being "stolen" from the algae which likely receive no benefit (Clark, 1992).

Kleptoplasty may play a role in the survival of the sea slug host by providing nutrients through the process of photosynthesis, particularly in adverse feeding conditions. Chloroplast-containing sacoglossans which are starved in the light have less of a size decrease than those starved in the dark (Casalduero & Muniain, 2008), suggesting that the animals are indeed able to benefit from the photosynthesizing chloroplasts. The sacoglossan *Elysia chlorotica* is able to maintain its sequestered chloroplasts with most of their photosynthetic capability for 5 months, with some minimal function continuing for up to 9 months. Maintaining chloroplast functionality appears to be accomplished through horizontal gene transfer from the algae to the sacoglossan, allowing the animal to support the chloroplasts so that they can be functionally capable of transcription and translation without the presence of an algal nucleus (Green et al., 2000). The algal nuclear gene *prk* which encodes a protein in the Calvin cycle of the filamentous algae *Vaucheria litorea* has been found on one of the chromosomes of unhatched *E. chlorotica* embryos using FISH labeling (Schwartz et al., 2014). This finding confirms

horizontal gene transfer between the sacoglossan and their algal food source, and also suggests vertical transmission since the embryos have not yet begun to feed. Although different species of sacoglossan vary in the location of chloroplast storage as well as the duration of chloroplast viability, the majority of them gain some benefit from plastid photosynthesis (Pierce et al., 2015).

Symbiosis Within Crustaceans

Beyond the cited instances in mollusks, very few examples of symbiosis between complex animals and photosynthetic cells or chloroplasts are known. However, some evidence suggests that a few crustaceans may be capable of a similar relationship, at least to a limited extent. Some copepods have been shown to contain functional chloroplasts within their gut (Epp & Lewis, 1981, Jansen & Bathmann, 2007). Algae remaining in the guts of copepods were capable of photosynthesis for up to 24 h after ingestion (Epp & Lewis, 1981). In this instance it is likely that the whole algal cells are simply remaining within the gut for longer periods of time rather than having an uptake of viable chloroplasts by the animal tissues. Whether the animal obtains any benefits from this photosynthesis has not been determined. Microscopic examination of the gut of another small crustacean, *Daphnia obtusa*, however, has shown the presence of plastids within the endocytes of the midgut and cecae indicating chloroplast uptake (kleptoplasty) (Chang & Jenkins, 2000). Although no research has been done which

indicated plastid functionality, two types of plastids have been observed within the endocytes. Based on their internal structure, one type of plastid was interpreted as functional and the other type appeared to be senescent (Chang & Jenkins, 2000). Such an arrangement strongly implies active uptake of plastids by the animal and likely symbiotic benefit.

These examples suggest that plastids may be used symbiotically in some crustaceans, or at the very least are able to travel through the gut while maintaining functionality. With these examples in mind it seems possible that this could be occurring in other species of crustacean as well.

General Information on *Pentidotea resecata*

The crustacean *Pentidotea resecata* is a coastal marine isopod with two distinct color morphs reportedly associated with their primary diet and substrate (Lee & Gilchrist, 1972). The brown color morph is typically found on the kelp *Macrocystis pyrifera,* while the green morph typically lives on the eelgrass *Zostera marina*. Color morphs are strongly associated with their respective substrata and some literature claims that green *P. resecata* is never found on the brown substrate and brown individuals are never found on the green substrate (Lee & Gilchrist, 1972). Coloration is thought to come mainly from the body and cuticle color, with only the brown color morphs exhibiting expanded chromatophores. While chromatophores are also present

in the green color morph, they are punctate (Lee & Gilchrist, 1972). The maximum length which can be reached by this species is widely reported in the literature as 4 cm (Kozloff, 1983, Lamb & Hanby, 2005). The body is flattened dorsoventrally as seen in most species within suborder Valvifera; however the most diagnostic feature of *P. resecata* is their concave pleotelson (Kozloff, 1996).

The geographical range for this species extends from southeastern Alaska to Baja California with animals typically found in protected bays within the littoral or sublittoral zones. Large fluctuations in population density occur annually with the highest densities occurring between the months of May and September. During the rest of the year populations decrease dramatically and no *P. resecata* can be found during the fall and winter months (Lee & Gilchrist, 1972). While *P. resecata* is normally herbivorous, cannibalistic behavior has been observed in rare cases within this genus, particularly when high population density leads to extreme overcrowding (Jones, 1971, Lee & Gilchrist, 1972).

The diet of *P. resecata* consists mainly of their host substrate (eelgrass or kelp), or algal material such as diatoms scraped from their host. Eelgrass and diatoms are found within the isopod gut, confirming ingestion (Lee & Gilchrist, 1972). After ingestion the food passes through the stomach into the blind-ended hepatopancreas and the hindgut (Figure 1). The majority of digestion and absorption occurs within the hepatopancreas, with waste material being moved back into the stomach and down into

the hindgut for elimination. The hindgut has very little if any function in nutrient absorption within herbivorous isopods (Guarino et al., 1994). Other than the transport of fecal material out of the body, no definite function has been assigned to the hindgut; however, undigested plant material is often found within the feces (Lee & Gilchrist, 1972). In other species of herbivorous isopods, algal material can stay intact within the hindgut for extended periods of time during starvation (Holdrich & Ratcliffe, 1970).

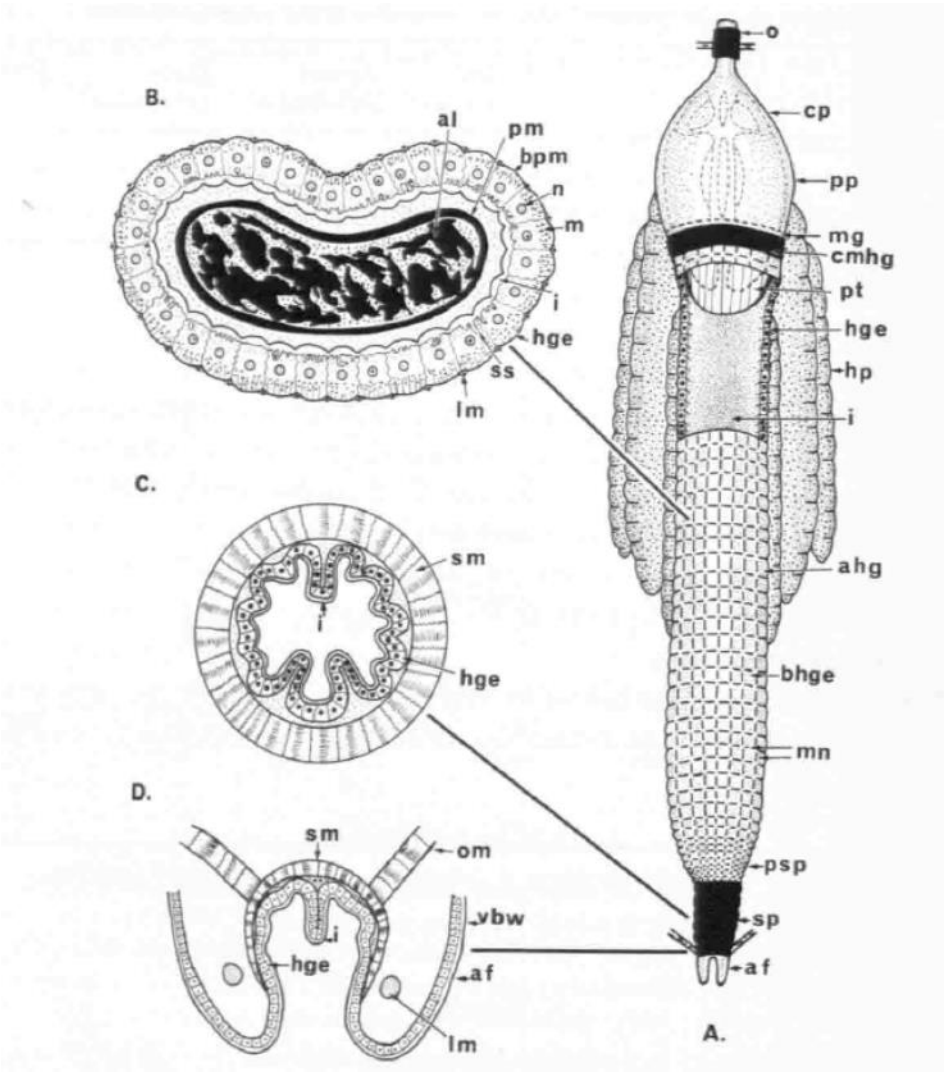

Figure 1. Diagram of the gut of the herbivorous isopod *Dynamene bidentatus*. The whole gut is shown in (A) with the six blind-ended hepatopancreas (hp) tubules lying on either side of the hindgut. B-D show cross sections through the hindgut, sphincter, and anal flaps respectively (Holdrich & Ratcliffe, 1970).

Preliminary Investigation

Preliminary investigations with the green color morph of *P. resecata* using spectrophotometry indicated the presence of chlorophyll within whole animal tissue (Cowles et al., 2011). Initial results from respirometry also suggested that photosynthesis was taking place in or on the isopods while they were held in light conditions. In the light the isopods had a significantly lower apparent oxygen consumption than they did in the dark, suggesting that photosynthesis may be occurring and contributing oxygen to the system when they are exposed to light conditions (Cowles et al., 2011). While these results could be explained by photosynthesis taking place within the gut of *P. resecata,* it is also possible that both the presence of chlorophyll and the evidence of photosynthesis are due to algae clinging to the cuticle of the isopod and are not from the gut at all.

Current Research

My goals for this research project were to ascertain the source and persistence of photosynthesis within the green color morph of *P. resecata.* Respirometry was used to measure changes in oxygen levels, an indicator of respiration or photosynthesis, in the light and in the dark. In addition, the isopods were sacrificed and their gut removed to determine whether photosynthesis was coming mainly from diatoms on the cuticle or from within the gut. Another goal was to use respirometry to track changes in the level

of photosynthesis which occurred during a period in which the animal did not have access to fresh plant cells with functional chloroplasts. I hypothesized that photosynthesis is taking place in the gut of *Pentidotea resecata*, and that intact chloroplasts are preserved in some way to such an extent that photosynthesis would still occur within the gut after 3 weeks of starvation.

MATERIALS AND METHODS

GENERAL

Capture and Transportation

Thirty-six *P. resecata* were collected from the eelgrass beds of *Zostera marina* located near March Point on Padilla Bay, Washington (Figure 2) between June and August of 2014. A seine net approximately 2 m wide was pulled over the surface of the eelgrass. This disturbed the isopods and caused them to swim to the surface where they were captured by hand. Only isopods with a total length of 45 mm or above were collected. Smaller individuals were released to limit differences due to size variation and to avoid the effects of variability on metabolism due to juvenile or sub-adult growth. Total length was defined as the length from the tip of the pleotelson to the base of the antennae. All individuals collected were between 45 and 54 mm in length and ranged in mass from 0.72 g to 1.45 g $\bar{x}$=0.92 ± 0.13 g. Mass was determined using a tabletop balance accurate to 0.01g. After collection the isopods were transported to the Rosario Beach Marine Laboratory in an insulated container containing saltwater from Padilla Bay to maintain temperature and a bubbler to maintain oxygen levels.

Figure 2. The location of isopod collection at March Point, Skagit County WA, coordinates 48.496541, -122.555333 (indicated by white mark). Image taken from Google maps. Numbers mark longitude and latitude coordinates.

Holding Conditions

Each individual isopod was initially measured for mass and length before being placed in individual housing containers. Containers were clear plastic jars with screw lids and ranged in size from 230 to 475 mL. A series of holes were drilled around the walls and bottom of the container to allow free water circulation while preventing escape of the isopod. Containers with live isopods were placed upside down in an outdoor circulating seawater tank measuring 1.1 m by 2.3 m with running seawater at a depth of approximately 0.16 m. A rock was fastened to the inside of each lid to add extra weight and anchor the containers, preventing them from being moved around the tank by water currents. Adherence of rocks to the inside of the lid was initially accomplished using a latex based calking, however this disintegrated over time and was replaced by aquarium silicone sealant. The bottom and lid of each container was labeled with an ID to keep track of individual isopods.

The outdoor seawater tank was exposed to normal day-night cycles and the temperature remained between 12 and 13 °C, which is within the range of temperatures in Padilla Bay during the summer. This was determined by examining data gathered from Padilla Bay by the National Estuarine Research Reserve System, a department of NOAA. The average temperature from May 1st through July 20, 2014 was 14.6° C with a range from 9° to 27° C (National Estuarine Research Reserve, 2014).

Maintenance

Throughout the holding period the growth of diatoms within the outdoor tank and on the isopods themselves was problematic. Although not identified to species, the brown filamentous algae growing on the inside of the tank was examined under magnification and was consistent with the appearance of filamentous diatoms. The filamentous algae growing on the cuticle surface of the isopods themselves appeared to be the same as that growing in the tank and were also assumed to be diatoms (McLarty, personal communication. April 21, 2015). To limit diatom growth each isopod was removed from its container every other day and gently brushed with a small soft-bristle toothbrush to remove diatom buildup. Diatoms were removed from both dorsal and ventral surfaces in this manner. Buildup of diatoms was also observed underneath the third pleopod inside the branchial chamber of some animals; however these could not be removed without injuring the animal. Additionally, diatoms clinging to the legs and antennae were difficult to remove. Green filamentous algae were observed growing on the dorsal surface or appendages of a few isopods, and these algae were removed using forceps. The containers as well as the sides and bottom of the seawater tank were brushed down to remove diatoms nearly every day. On a bi-weekly basis the isopods were temporarily moved and the outdoor tank was drained and cleaned with a power washer to remove diatom buildup.

Activity Level

The activity of *P. resecata* was quantified in both light and dark environments to determine if there were differences in activity that would have an effect on O_2 consumption. The experiment was conducted in an acrylic tank (40x20 cm) with circulating seawater. The bottom of the tank was covered with a plastic, white grid with cells of 1.75 cm^2. A larger blue grid of 3.5 cm^2 (the equivalent of 2x2 white squares) was painted on the white, plastic grid using blue nail polish which was visible under infrared lighting (Figure 3). During the light intervals two 60W LED bulbs were shone directly on the tank in addition to the artificial light provided by the room's fluorescent lights, which created a light intensity of 90-100 μmol photons m^{-2} sec^{-1} inside the tank. The windows were covered to standardize light levels across all trials. Dark conditions were maintained by turning off all the lights, with the exception of an infrared light, which was turned on and shone into the tank from the side. A Sony HandyCam (DCR-SR300) was mounted above the tank and used to track isopod activity under both light and dark conditions. All of the trials were conducted during daylight hours to eliminate any possible circadian effect.

In each of six trials, four isopods were placed in a 12-by-20 cm section blocked off in the center of the tank with ample seawater circulation and allowed 15 min to acclimatize to the new environment (Figure 3). Their activity, as indicated by the number of blue grid lines crossed by the cephalon of each individual, was recorded for a

10 min interval using the camcorder mounted above the tank. The lights were turned off and the isopods allowed another 15 min period to acclimatize to the dark conditions before their activity was recorded for 10 min. This was done with a total of 24 animals. Since the experiment was conducted during the summer which is within *P. resecata's* breeding season, only male or non-brooding female isopods were used to control for any potential differences in activity due to brooding. Brooding females were determined by the presence of eggs or young within the marsupium.

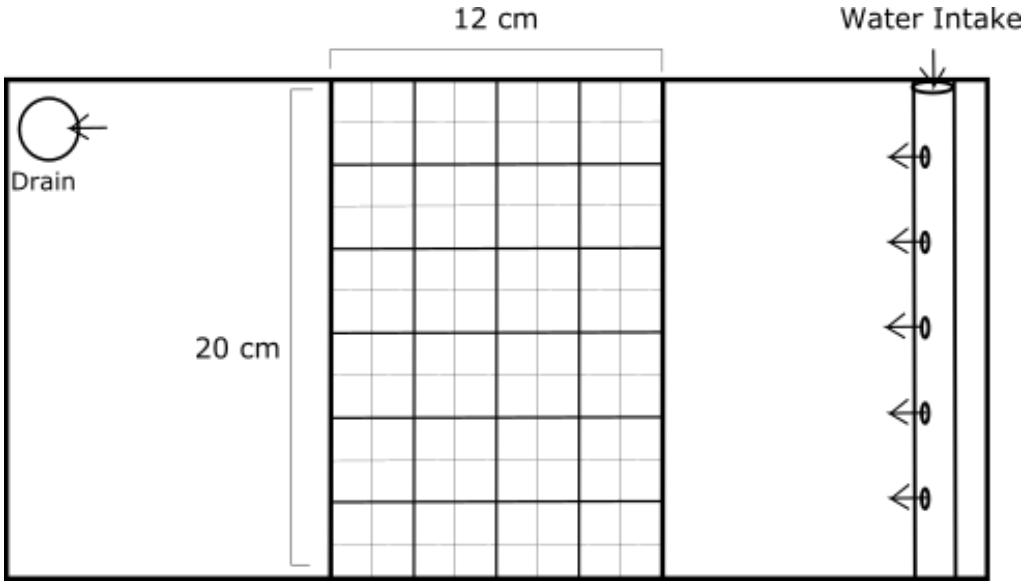

Figure 3. Tank with grid-covered arena used in quantifying isopod activity level. The 12 by 20 cm arena in the middle of the tank was blocked off from the rest of the tank while still maintaining adequate flow of water. Each of the small squares was 1.75 cm^2, the larger squares 3.5 cm^2. After placing four isopods into the gridded area of the arena, their activity was quantified by counting the mean number of times the isopod's cephalon crossed one of the darker lines marking the larger squares on the grid in a 10 minute period. Arrows indicate the direction of water flow.

RESPIROMETRY

Equipment and Settings

Respirometry was conducted using a Hach HQ40d® meter with Hach LDO10103® oxygen optodes within respirometry chambers under both light and dark conditions. Each of the two respirometry chambers consisted of a 189 mL glass chamber with a stir bar enclosed in a perforated pipe and covered by mesh to ensure proper water circulation without interfering with the animal. A water jacket circulating water around the internal chamber was maintained at a temperature of 15 ° C (the average summer temperature in Padilla Bay) throughout all experiments (Figure 4). A wide-spectrum LED grow light was used as the light source to standardize the amount of light used for photosynthesis while eliminating issues with heat dissipation. The light was manufactured by HydroGrow® and had an emission spectrum between 440 and 740 nm, which spans most of the effective range for both photosynthesis and vision (Falkowski & Raven, 2013). Light intensity was set at 100 µmol photons m^{-2} s^{-1}, an intensity at which photosynthesis is functioning robustly in eelgrass (Hylarides, 2015). A higher intensity was avoided to limit the risk of photo-oxidative damage.

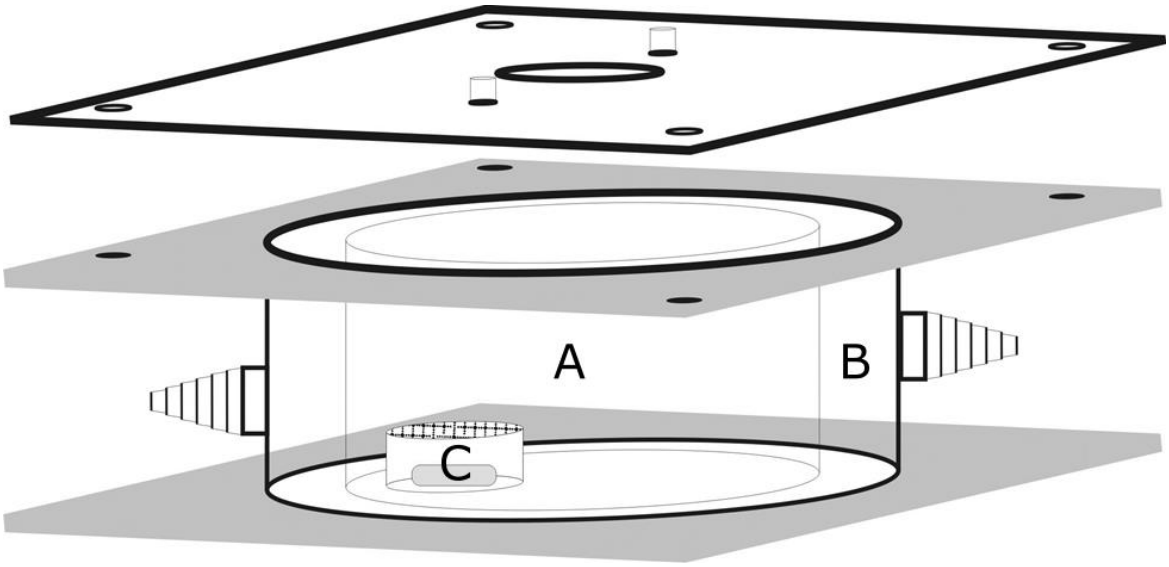

Figure 4. Respirometry chamber (A) with recirculating water jacket (B) for maintaining temperature and enclosed stir bar (C) for adequate water circulation. The Hach LDO10103® oxygen optode was inserted through the hole in the center of the lid. The holes at the corners of the lid enabled it to be screwed down once the isopod was in place.

Experimental Groups

Respirometry was conducted on four groups of isopods during a three week period. One group of six freshly caught individuals was used for initial respirometry. The three remaining groups were used for respirometry at the end of each of the following three weeks, one group for each week. Of the 10 individuals in each of these groups, 5 (the 'fed' subgroup) were provided with eelgrass ad libitum for the duration of their time in captivity while the other 5 (the 'starved' subgroup) did not have access to eelgrass.

Respirometry

The isopods were blot dried and weighed before being placed back into their container for at least 1 h prior to respirometry. The filtered seawater in the respirometry chamber was brought as close to oxygen saturation as possible (155 mm Hg) by blasting it through a 50 mL syringe 10 times. Once placed in the respirometry chamber the live isopod remained undisturbed for 10 min with the oxygen optodes running before respirometry data were gathered. This time allowed the animal to acclimate in the case of live runs and the optode to fully stabilize in the case of all runs.

Respirometry experiments were conducted in the light with the lab door closed and the fluorescent room lights and both LED lights on in order to standardize light

exposure. For respirometry measurements in the dark the door was closed and all lights turned out. In addition, a thick black cloth was wrapped around each respirometry chamber and another black cloth was draped over the top. The windows were covered to standardize light levels in both the light and dark conditions. All runs were done over a period of at least 1 h. Since light respirometry would include changes in oxygen due to both photosynthesis and respiration, this was referred to as “apparent rate”.

Respirometry on dead individuals with their gut removed was conducted in much the same way; however, the chambers were rinsed between runs of dead individuals to eliminate bacterial growth that may occur if decay ensues after death. For the freshly caught isopods and the 3 week group the chambers were rinsed twice with freshwater and a third time with filtered seawater between runs. For the week 1 and week 2 groups the chamber was rinsed once with 10-20 mL of hydrogen peroxide and then rinsed twice with freshwater and a fourth time with filtered seawater.

Figure 5 shows the treatment and respirometry for each of the four groups.

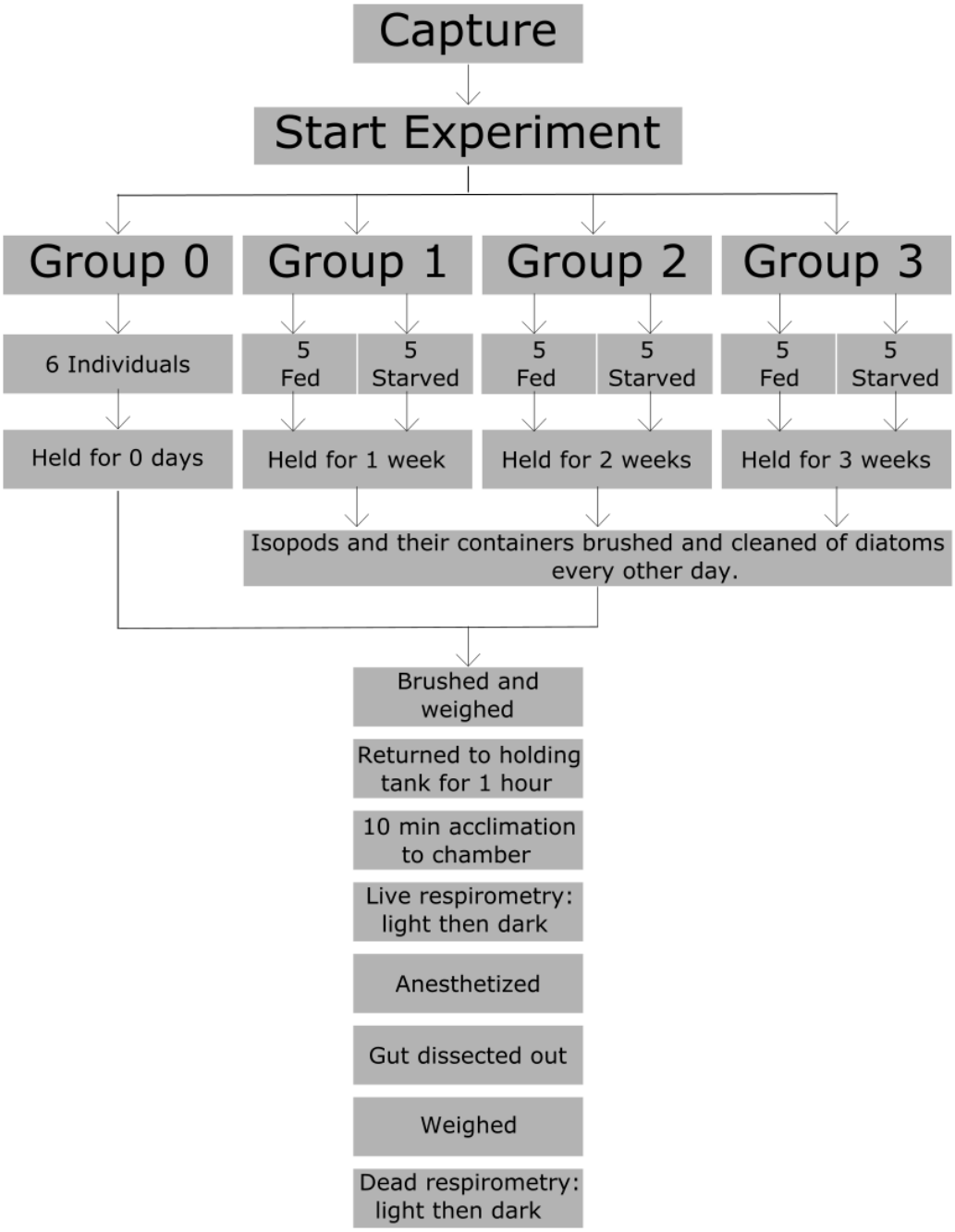

Figure 5. Flow chart outlining the conditions and treatments for the isopods used in respirometry for each of the groups. Each isopod was held for a specific length of time (0, 1, 2, or 3 weeks) under experimental conditions, during which their cuticle was brushed every other day. Following this period of time, the oxygen consumption of each live isopod was measured in the light and dark. Oxygen consumption was again measured after the isopod was euthanized and their gut removed.

Dissection

To assess the proportion of total photosynthesis was due to external diatoms growing on the cuticle of the isopod as compared to possible photosynthesis within the gut, the isopods were anesthetized following live respirometry and their gut removed. Isopods were anesthetized by placing them in a solution of carbonated seawater which was made by adding the appropriate amount of aquarium salt to carbonated water to obtain a salinity of 29.5 0/00. Once individuals were unresponsive, incisions were made dorsally across the width of the pleotelson and anteriorly up the sides of the dorsal surface through to the first pereon, lateral to the gut. The dorsal cuticle was then peeled forward and the gut carefully removed from the body. At this point the isopods were considered to be dead.

Exposure to CO_2 was not considered enough to alter the photosynthetic rate of the diatoms remaining on the cuticle surface. While there is variation among species of diatoms, in general prolonged exposure of several days to CO_2 causes an increase in their photosynthetic efficiency (Gao & Campbell, 2014). If this were true of this species of diatom and if it was also of sufficient magnitude, it may cause my results to appear less significant. However, in this case the isopods, and subsequently the diatoms growing on their cuticle, were exposed to elevated levels of CO_2 for approximately an hour and were removed from the elevated CO_2 and placed back into normal filtered seawater for respirometry immediately following dissection.

ANALYSIS

Isopod activity in the light and the dark was quantified by the number of times the cephalon (head) crossed a blue line on the plastic grid. The total number of lines crossed during each 10 min interval by an individual isopod was considered representative of its activity level. Results were analyzed using a paired randomization t-test with 10,000 randomizations.

For the respirometry portion of the study the micromoles of dissolved oxygen per liter were calculated based on formulas from Colt (1984). All data were tested for homoscedasticity with a Bartlett's test. In the case of parametric data, one and two way ANOVAs were used with a Tukey HSD post -test. Nonparametric data were analyzed using a paired randomization t test with 10,000 randomizations or a Kruskal-Wallis test. In a few cases data were transformed using the square root or log in order to make it parametric. A two one-sided test was used to compare the mean difference in light and dark respirometry between living and dead isopods. Statistical analysis was done using R software. Alpha for all tests was 0.05.

RESULTS

Length and Mass

All individuals collected for the purpose of this study ranged in length from 45 to 54 mm and ranged in mass from 0.72 g to 1.45 g (Figure 6). The largest individual collected, weighing 1.45 g, was quite a bit heavier than the majority of the specimens; however its mass-specific metabolism was within the range seen in the smaller individuals. Length of individuals did not differ among groups at the time of capture and no significant difference was observed in length among the weeks (ANOVA, P=0.141). Additionally, no significant difference was found in isopod mass between weeks in live individuals (Kruskal-Wallis, P=0.08, Figure 7).

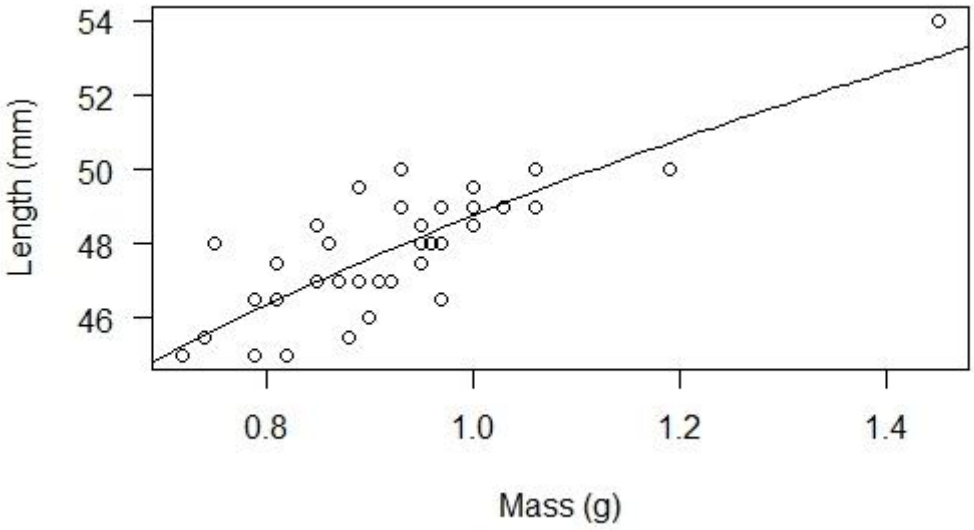

Figure 6. Total length and wet mass of isopods at the time of capture. Mass varied from 0.72 g to 1.45 g with only one individual above 1.19 g. Length varied from 45 to 54 mm with only one individual above 50 mm. The line was fit using the nl2sol algorithm. Line equation: Mass = $49(\text{Length})^{0.23}$. Residual standard error: 1.06 mm on 34 degrees of freedom, $P<0.001$.

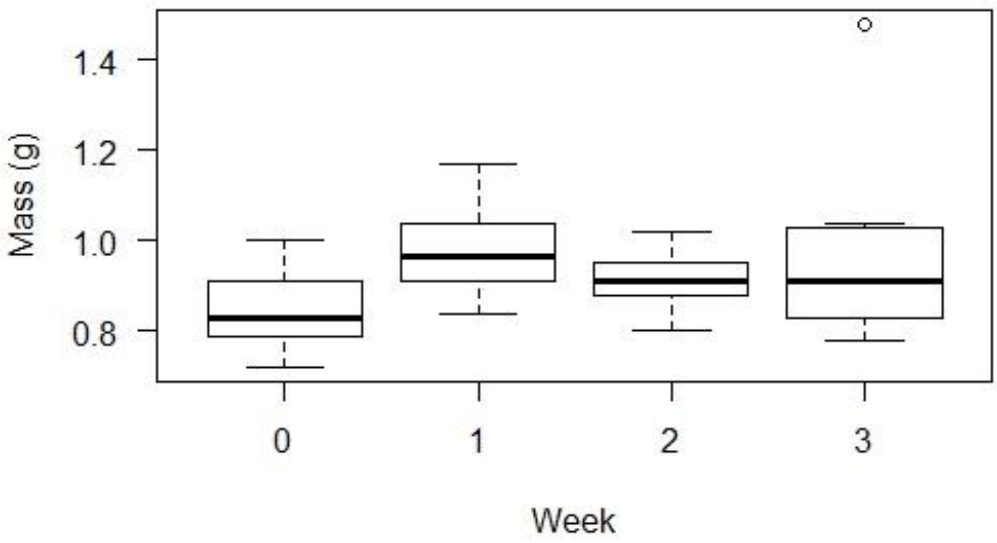

Figure 7. The mass of live isopod by week as measured at the end of their captive period, just prior to respirometry. No significant difference was found among the weeks (Kruskal - Wallis, P>0.08, Df=3, n=8, 10, 14, 10 for weeks 0, 1, 2, and 3 respectively.) In this boxplot the dark central bar is the median. The upper and lower bounds of the box represent the upper and lower quartile, and box height represents the interquartile range. Error bars indicate the most extreme value which is less than or equal to the distance to the upper or lower quartile plus 1.5 times the interquartile range. Unfilled circles indicate outliers and are defined as numerical values which are more than 1.5 times the interquartile distance above or below the upper or lower quartile range.

Although “starved” individuals were not provided with eelgrass, diatoms grew very quickly in the outdoor seawater tank and could have been utilized as a food source by the eelgrass-deprived isopods. During isopod dissection it was noted that the majority of isopods in the “starved” subgroups still had a full gut. Diatoms have been observed within the gut of *P. resecata*, and at least one of the examined specimens which had been eelgrass deprived contained diatoms within the gut (McLarty, 2015). No significant difference was found in mass between fed and starved groups in any week (Two way ANOVA, $P>0.96$). Additionally, no significant difference was found in oxygen consumption between starved and fed live isopods in the dark in any week (Two way ANOVA, $P>0.2$). The change in mass of individuals between their collection date and when they were used for respirometry was calculated for both fed and starved individuals and the before-and-after differences were minimal. The mean mass at capture and at the time of respirometry as well as the change in mass between those two dates was calculated for each week (Table 1A). Data were also normalized to the mean mass at capture for each week (Table 1B.)

Table 1. A.) The change in mean mass of fed and starved isopods by week. B.) The proportional change in mean mass with data normalized to isopod mass at capture. For both, the capture mass indicates mass at the time of capture, the experimental mass indicates mass at the time of respirometry, and the change indicates the percent difference between the two. The standard deviation of experimental mass is given in parentheses.

A)

Fed					
		Average mass (g)			
Week	Date captured	At capture	Experimental	Change	% change
0	10-Aug-14	0.86 (0.11)	0.86 (0.11)	0.00	0.00%
1	10-Aug-14	0.96 (0.17)	0.99 (0.14)	0.03	3.56%
2	7-Aug-14	0.89 (0.04)	0.93 (0.04)	0.04	4.72%
3	14-Jul-14	1.03 (0.26)	1.04 (0.27)	0.01	0.97%

Starved					
		Average mass (g)			
Week	Date captured	At capture	Experimental	Change	% change
0	10-Aug-14	-	-	-	-
1	10-Aug-14	0.97 (0.05)	0.96 (0.06)	-0.01	-1.23%
2	7-Aug-14	0.88 (0.08)	0.91 (0.09)	0.02	2.49%
3	14-Jul-14	0.89 (0.07)	0.87 (0.07)	-0.02	-1.80%

B)

Fed					
		Average mass (g)			
	Date captured	At capture	Experimental	Change	% change
Week 0	10-Aug-14	1.00	1 (0.00)	0.00	0%
Week 1	10-Aug-14	1.00	1.04 (0.10)	0.04	4%
Week 2	7-Aug-14	1.00	1.05 (0.04)	0.05	5%
Week 3	14-Jul-14	1.00	1.01 (0.04)	-0.02	1%

Starved					
		Average mass (g)			
	Date captured	At capture	Experimental	Change	% change
Week 0	10-Aug-14	-	-	-	-
Week 1	10-Aug-14	1.00	0.99 (0.03)	-0.01	-1%
Week 2	7-Aug-14	1.00	1.02 (0.02)	0.02	2%
Week 3	14-Jul-14	1.00	0.98 (0.04)	-0.02	-2%

Activity

P. resecata crossed more lines in the light than in the dark; however, many of the isopods did not move at all, particularly while in the dark. The mean activity level of the isopods during the light portion of each trial was 7.30 lines crossed per individual during the 10 minute trial period with a median of 1.0 lines crossed. For the dark portion of each trial, the mean activity level was 4.09 lines crossed with a median of 0 lines crossed. Although the activity level varied among individual isopods, nonparametric statistical analysis found that isopods were significantly more active in the light than in the dark (Paired randomization t-test, P=0.0.04, Figure 8). While some of the isopods were fairly active, 11 of the 23 isopods did not cross any lines on the grid in either light condition. Based on these results, activity will be discounted as a potential variable in further analysis of respirometry results.

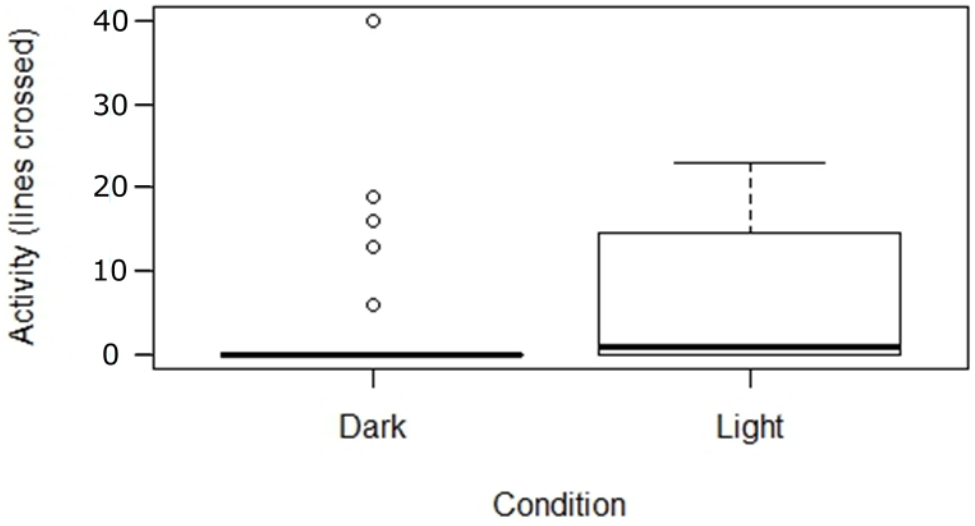

Figure 8. The activity level of 23 isopods measured by the number of lines crossed during the light and dark experimental conditions. Of the isopods in the dark only five showed any movement at all, resulting in the five apparent outliers in that category. Statistical analysis showed that isopods were significantly more active in the light than they were in the dark (Paired randomization t test with 10,000 randomizations, P=0.04).

Oxyconformity

No oxyconformity was observed within the narrow range of partial pressures used in this study. When comparing the trend in individual live animals in the dark, 36% showed a downward trend in metabolism with decreasing oxygen pressure and 60% had a metabolic rate which stayed essentially the same throughout their time in the chamber, while 5% of the individuals had a net increase in metabolism over their period of time in the chamber. A change was defined as an increase or decrease in metabolic rate which was greater than one standard deviation. The partial pressure of oxygen at the beginning of the run ranged from 152.7 mm Hg to 131.7 mm Hg among all the runs. The lowest partial pressure of oxygen reached by any individual at the end of a run was 99.6 mm Hg. A representative example of an individual with a metabolic rate which stayed within one standard deviation of the mean is shown (Figure 9).

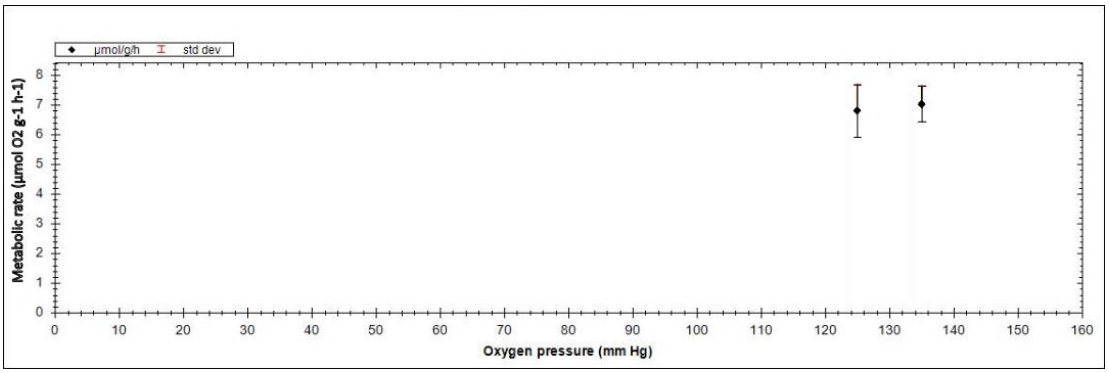

Figure 9. Representative change in metabolic rate. Note that metabolic rate for all animals was measured over a narrow range of oxygen pressure which was close to air saturation. For 60% of the individual isopods, metabolic rate stayed within one standard deviation of the mean when measured alive and in the dark. This graph was taken from an individual in the week 3 group.

Weekly Trends in Respirometry

The rate of oxygen consumption between fed and starved individuals was not significantly different in any week, therefore fed and starved individuals were analyzed together (Two way ANOVA, $P>0.3$).

A comparison of oxygen consumption in live animals in the dark between the weeks of captivity showed no significant difference among any of the weeks (Two way ANOVA, $P>0.06$, Figure 10 and Appendix I). This was done to determine whether there was any change in oxygen consumption over time. When this analysis was repeated for live animals in the light (which therefore included both photosynthesis and respiration, and which I refer to as the 'apparent consumption), individuals in week 0 (measured immediately after capture) had significantly higher rates of oxygen consumption than those in any other week (Two way ANOVA, $P<0.03$, Figure 11 and Appendix I).

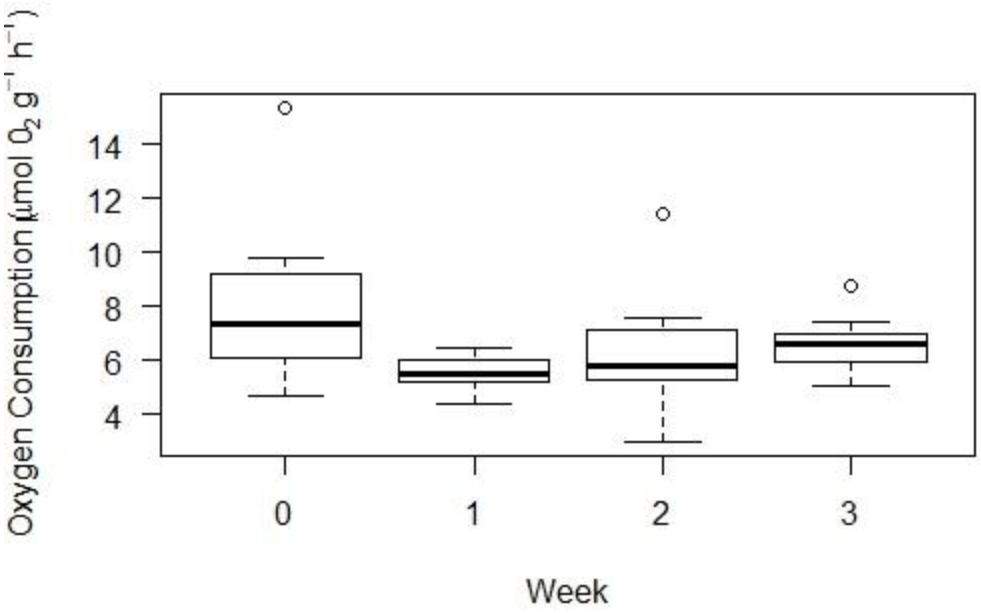

Figure 10. The oxygen consumption of live animals in the dark over the experimental period. There was no significant difference in oxygen consumption between any of the weeks (Two way ANOVA, P<0.06, Df=3, F value=2.76, n=8, 10, 14, 10 for weeks 0, 1, 2, and 3 respectively).

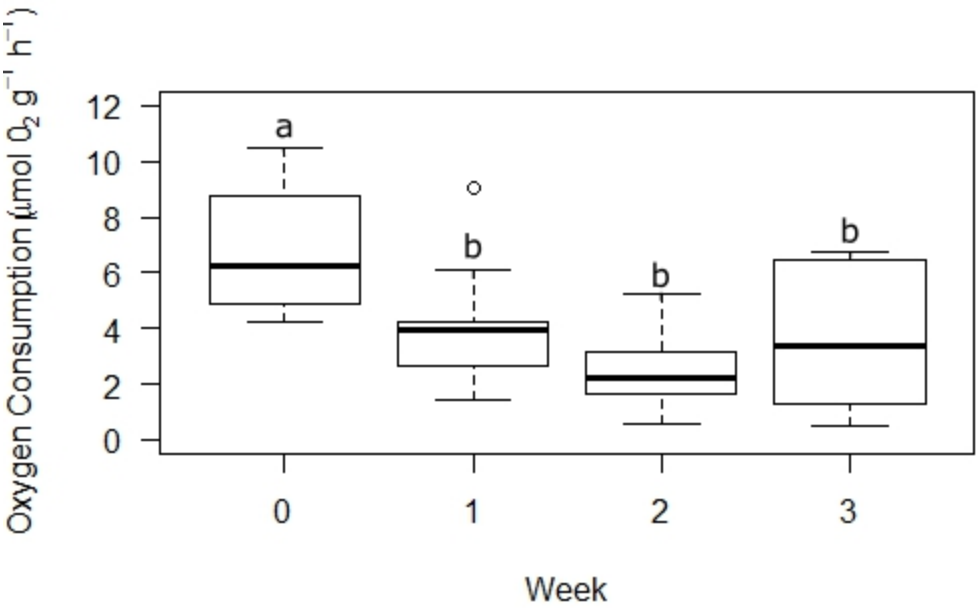

Figure 11. The apparent oxygen consumption of live individuals in the light over the experimental period. Since light was present, these rates include both respiration and any photosynthesis that was taking place from within the gut or from epiphytes growing on the cuticle. The apparent oxygen consumption was significantly higher in individuals from week 0 than in any other week (Two way ANOVA, P<0.03, Df=3, F value=8.21, n=8, 10, 14, 10 for weeks 0, 1, 2, and 3 respectively).

Dead isopods (with gut removed) from week 3 had a higher metabolic rate in the dark than those in any other week (Two way ANOVA, Tukey post-test $P<0.04$). In addition, week 2 was significantly lower than week 1 (Two way ANOVA, Tukey post-test $P=0.02$, Figure 12). Results were similar for dead isopods in the light, with isopods in week 3 having a significantly higher apparent metabolic rate than that of isopods in any other week (ANOVA, $P<0.03$, Figure 13). Actual values for dead animals in both the light and the dark are provided in table form (Table 2 in Appendix I).

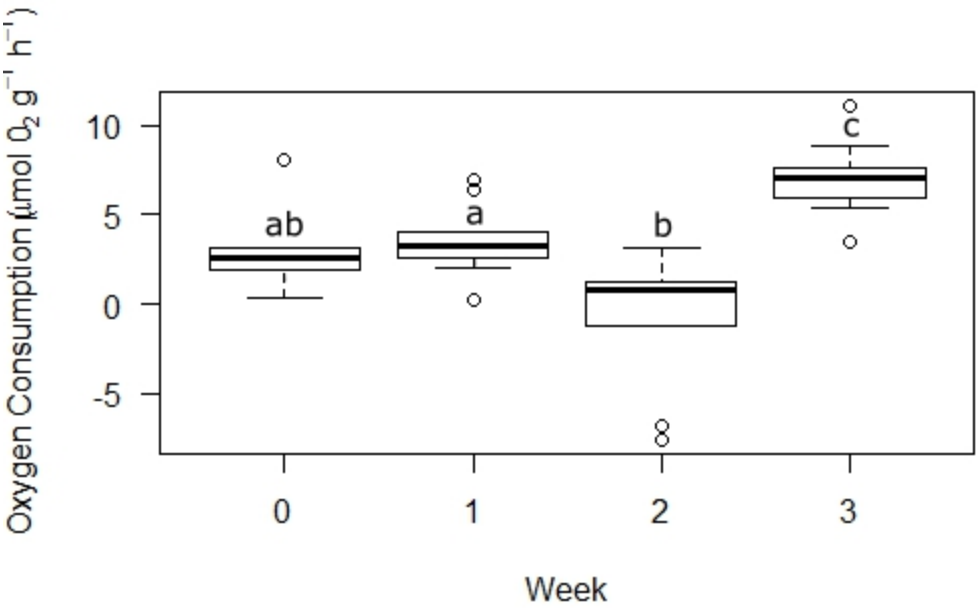

Figure 12. The rate of oxygen consumption for dead animals in the dark over the experimental period. Oxygen consumption was significantly higher in individuals from week 3 than any other week. In addition, week 2 was significantly lower than week 1 (Two way ANOVA P<0.04, Df=3, F value=12.343, n=6, 10, 10, 10 for weeks 0, 1, 2, and 3 respectively). Letters indicate significance.

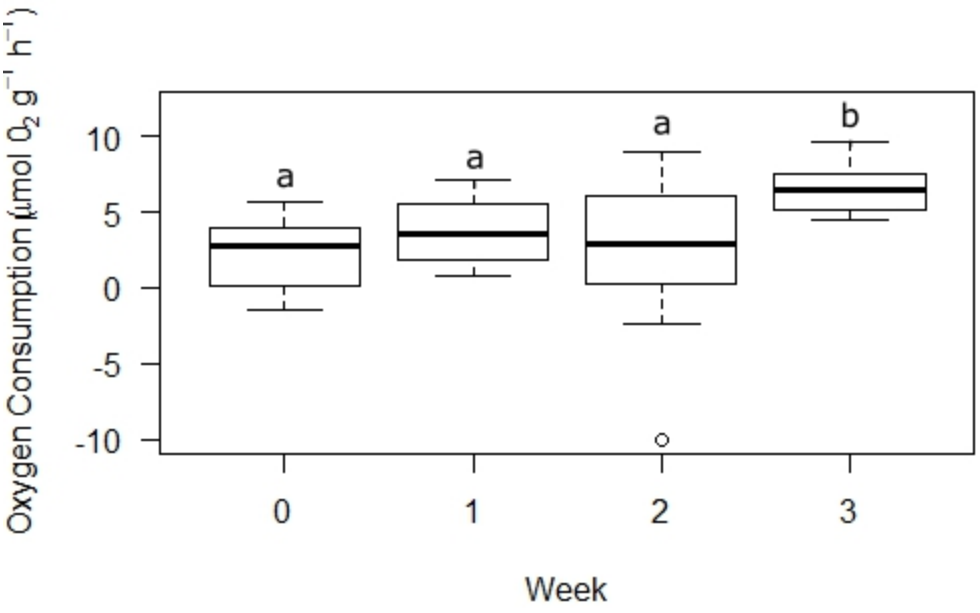

Figure 13. The apparent oxygen consumption of dead animals in the light over the experimental period. Since light was present, these rates include both respiration and any photosynthesis that was taking place. The apparent oxygen consumption was significantly higher in individuals from week 3 than in any other week (Two way ANOVA, P<0.03, Df=3, F value=5.633, n=6, 10, 10, 10 for weeks 0, 1, 2, and 3 respectively). No other significance was observed between weeks. Letters indicate significance.

Analysis of Light vs. Dark Oxygen Consumption

Since no consistent changes were seen in the rate of oxygen consumption over the time course of the experiments when measured in either the light or the dark, nor between fed and starved individuals, analyses were made by combining data for the different weeks and for fed and starved individuals. This left two sets of independent 'treatment' variables: "Light" and "Dark", and "Dead" and "Alive". Metabolic rates of live individuals in the dark were significantly higher than those of dead individuals in the dark, as would be expected (paired randomization t test, P=0.0001, Figure 14). Unpaired data was excluded from this analysis.

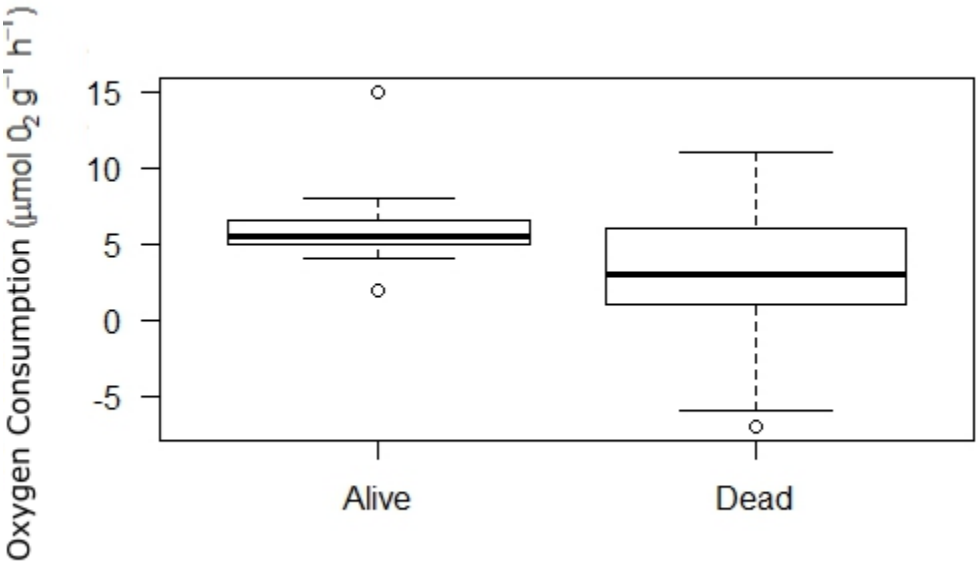

Figure 14. A comparison between the oxygen consumption of living individuals in the dark and dead individuals in the dark. As expected, living individuals had a significantly higher consumption of oxygen than those that were dead (Paired randomization t test, P=0.0001). Unpaired data was excluded from this analysis.

The rates of oxygen consumption for live isopods in the dark were significantly higher than that of the same live isopods in the light (ANOVA, $P< 0.0001$, Figure 15). No significance was found in a comparison between light and dark environments in dead isopods (ANOVA, $P=0.6$, Figure 16). Additionally, the mean difference between light and dark respirometry in dead individuals (with their gut removed) was less than the mean difference between light and dark respirometry in live individuals (Two one-sided test, $P=0.009$).

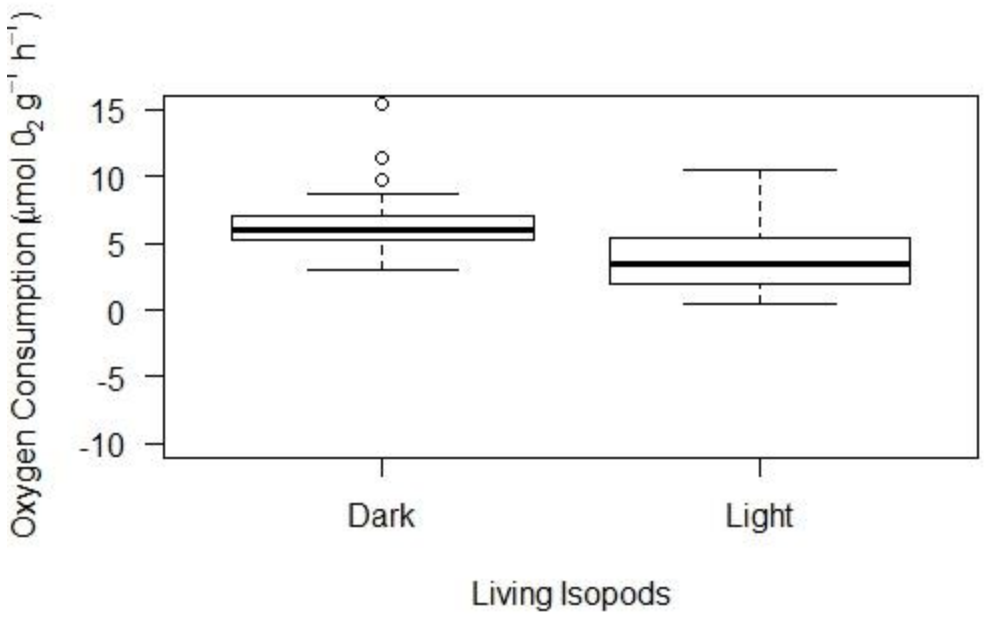

Figure 15. A comparison of oxygen consumption in live isopods between light and dark environments. Oxygen consumption of isopods in the dark was significantly higher than it was in the light (ANOVA, P<0.0001, Df=1, F value=26.72, n=40).

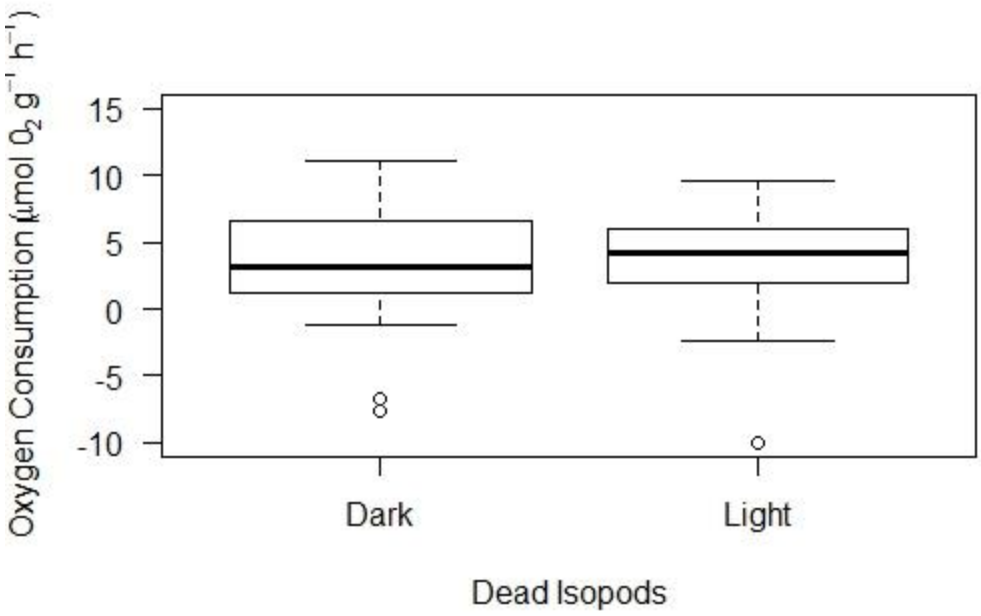

Figure 16. A comparison of oxygen consumption in dead isopods between light and dark environments. There was no significant difference found in metabolic rate between the two light conditions (ANOVA, P=0.6, Df=1, F value=0.277, n=36). A Two one-sided test indicated that the mean difference between light and dark respirometry in dead individuals (with their gut removed) was less than the mean difference between light and dark respirometry in live individuals. Epsilon was 2.6, the mean difference between light and dark respirometry in live isopods (Two one-sided test, P=0.009, Df=69.84, n=36)

Additional Observations

Over the course of my research I made several additional observations regarding the behavior and development of *P. resecata* which were not quantified. This information, although not directly related to this project, may be useful in further understanding the natural history of *P. resecata* and can be read in Appendix II.

DISCUSSION

The results obtained in this study suggest that photosynthesis is associated with *Pentidotea resecata* and a substantial proportion of this photosynthesis may be taking place within the gut. The primary support for this hypothesis comes from the comparison of metabolic rate between light and dark environments for both living (intact) isopods and dead isopods (with their gut removed.) The apparent oxygen consumption of individuals while they were alive and in the dark was significantly higher than while they were alive and in the light (Figure 15). Since individuals in the dark were actually less active than those in the light (Figure 8), this difference in oxygen consumption can be attributed to photosynthesis that may have been taking place on or in individuals in the light, counteracting and reducing the measured oxygen consumption rate. It should be noted that the oxygen consumption observed in the dark was due to animal metabolism as well as any metabolism occurring within algal or plant cells. No significant difference was found between light and dark groups of dead isopods (ANOVA, P=0.06). The mean difference between light and dark respirometry in dead isopods with their gut removed was significantly lower than the mean difference in living isopods. Since dead individuals had their gut removed but their cuticle and epiphytes remained, the primary contribution of photosynthesis may be coming from the gut rather than from the external epiphytes which were present on the cuticle. These data also suggest that viable plant or algal cells are either passing through the gut

(Epp & Lewis, 1981, Jansen & Bathmann, 2007) or being sequestered by the animal (Chang & Jenkins, 2000) as seen in other crustaceans.

Higher activity in the dark could also potentially explain these data; however my results showed that isopods were significantly more active in the light than in the dark. If the isopods were more active within the respirometry chamber during the light, the apparent effect of photosynthesis would be reduced in analysis of oxygen consumption, making the potential amount of photosynthesis appear less significant. However, due to the relatively small size of the chamber, very little movement was observed when the isopods were in the respirometry chamber under either light or dark conditions.

Other species of isopod such as *Idotea baltica* and *Tylos europaeus* have been shown to maintain greater activity levels during the night rather than during the day (Jormalainen & Tuomi, 1989; Bohli-Abderrazak et al., 2012). Several species within the genus *Tylos* are active foragers in the upper intertidal of sandy beaches and are most active at night when the tide is low (Bohli-Abderrazak et al., 2012). Why *P. resecata* is more active during the light is unknown, however as an herbivore which feeds on eelgrass and associated epiphytes (Lee & Gilchrist, 1972), foraging is likely not limited to a certain time of day.

The higher rate of oxygen consumption of live individuals in the light from week 0 compared with other weeks (Figure 11) can potentially be explained by two possible factors. The freshly caught individuals from week 0 had fewer diatoms growing on their

outer cuticle and inside their valve chamber. Fewer diatoms would lead to lower levels of photosynthesis contributing oxygen into the system. In individuals with abundant diatoms this photosynthesis would lead to an apparent lower oxygen consumption rate, while individuals with few or no diatoms would appear to have a higher oxygen consumption rate, as was observed. In addition, while not quantified, individuals from week 0 appeared to be slightly more active than those from subsequent weeks. This may also have contributed to their increased metabolic rate.

Several anomalous respirometry values were present in dead isopods in the dark. Results from two individuals indicated a net production of oxygen, which does not seem biologically possible under those conditions. It is likely that these anomalous values were a result of the residual hydrogen peroxide from rinsing the chamber breaking down and increasing the partial pressure of oxygen within the chamber. While the chambers were rinsed multiple times with fresh and filtered sea water following the hydrogen peroxide, it is possible that in some cases it was not all successfully removed. Other possible explanations for the anomalous values which were observed could be drift in the actual oxygen optodes or a leak in the chamber. Having been used constantly over the course of several weeks, water may have been absorbed into the optode. The two anomalous values showing net oxygen production by dead isopods in the dark were some of the last data points to be collected. However, the two individuals were tested at different times in different chambers using different oxygen optodes. This suggests that while drift in the equipment or a chamber leak could have occurred, the effect of

hydrogen peroxide is a more likely explanation since it could easily affect both chambers simultaneously. While these two data points may represent anomalous data, they were retained rather than removed as outliers since it was unknown to what extent the hydrogen peroxide may have affected the results of the rest of the data.

CONCLUSION

While these results do not conclusively show that photosynthesis is mainly occurring within the gut of *P. resecata*, they provide further evidence that photosynthesis is taking place and suggest the possibility that much of it may be occurring within the gut. External diatoms proved to be more difficult to remove than expected; however, the fact that no significant difference was found between light and dark metabolism in dead isopods with their gut removed and the mean difference between light and dark respirometry in living isopods was significantly greater than the mean difference in dead isopods with their gut removed, suggests that photosynthesis within the gut may be the most important contributing factor to photosynthesis. Photosynthesizing plant or algal material may be within the lumen of the gut as was observed in copepods (Epp & Lewis, 1981) or within gut-associated tissue, as has been observed in *Daphnia* and sacoglossans (Chang & Jenkins, 2000, Clark, 1992). While it would be particularly interesting if chloroplasts were being sequestered within isopod cells, microscopy of the gut and associated tissues has shown no evidence of algae or

chloroplasts outside of the lumen itself (McLarty, 2015). Due to the buildup of diatoms within the holding tank and their probable subsequent consumption by eelgrass-deprived isopods, this study was not able to examine the effects of starvation on photosynthetic rates. However, evidence of photosynthesis occurring for at least a period of 24 hours in the gut of copepods (Epp & Lewis, 1981) and the fact that food material remains in the gut of other herbivorous isopods for extended periods of time during starvation (Holdrich & Ratcliffe, 1970) would suggest the possibility that photosynthesis occurring within the gut of *P. resecata* may persist for some period of time as well. Further research needs to be done in this area with *P. resecata* under a more effective experimental setup to further establish that photosynthesis is occurring within the gut, as well as to determine how long material within the gut is functionally capable of photosynthesis.

ACKNOWLEDGEMENTS

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To my committee members, Dr. Nester and Dr. Onthank, I would like to express my gratitude for all of your support, comments and constructive criticism throughout the process of my research and the writing of my thesis. Dr. Onthank in particular was very helpful in assisting me with the analysis portion of my research. I want to extend a special thank you to my fellow graduate student, Shelley McLarty, who gave a lot of advice and feedback throughout my research and shared much of the responsibility of capturing and caring for the isopods. I couldn't have asked for anyone better to share the joys of dragging a net through the cold water and frantically scooping up isopods whenever they popped to the surface.

My sincere gratitude goes to my major professor, Dr. Cowles, for all of his support and expert advice throughout the process of my research. As my mentor he has provided me with the tools, knowledge, and guidance I needed; and as my dad he has

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APPENDIX I

Weekly Metabolic Rates

Table 1. A). Oxygen consumption of live animals in the dark over the experimental period. Contribution of photosynthesis was eliminated by analyzing only the oxygen consumption during dark respirometry. There was no significant difference in oxygen consumption between any of the weeks (ANOVA, P<0.2). B) Apparent oxygen consumption of live individuals in the light over the experimental period. Since these individuals were in the light, the apparent rates include both respiration and any photosynthesis that was taking place. Apparent oxygen consumption was significantly higher in individuals from week 0 than it was in any other week (ANOVA, P<0.03).

A)

Dark				
	Week 0	**Week 1**	**Week 2**	**Week 3**
n	8	10	14	10
Oxygen consumption (µmol O_2/g/h)	8.16	5.50	6.14	6.60
standard deviation	3.35	0.69	1.97	1.07

B)

Light				
	Week 0	**Week 1**	**Week 2**	**Week 3**
n	8	10	14	10
Oxygen consumption (µmol O_2/g/h)	6.80	3.99	2.39	3.52
standard deviation	2.28	2.26	1.39	2.38

Table 2. A) Oxygen consumption of dead individuals in the dark over the experimental period. Oxygen consumption was significantly higher in individuals from week 3 than it was in any other week and week 2 was significantly lower than week 1 (ANOVA, P<0.04). B) Apparent oxygen consumption of dead individuals in the light over the experimental period. Oxygen consumption from week 3 were significantly higher than in any other week (ANOVA, P<0.03).

A)

Dark				
	Week 0	**Week 1**	**Week 2**	**Week 3**
n	6	10	10	10
Oxygen consumption (µmol O_2/g/h)	3.10	3.52	-0.53	7.06
standard deviation	2.62	1.97	3.72	2.01

B)

Light				
	Week 0	**Week 1**	**Week 2**	**Week 3**
n	6	10	10	10
Oxygen consumption (µmol O_2/g/h)	2.30	3.65	2.05	6.52
standard deviation	2.58	2.15	5.31	1.67

APPENDIX II

General Observations

Relatively little information is known about *Pentidotea resecata,* with the majority of the information available in the literature being several decades old. Over the course of my research I made several interesting observations which were not directly related to my research. While these observations were not quantified, they may help to increase the small pool of knowledge that is available on this species. My fellow graduate student, Shelley McLarty, also made observations which can be found in her thesis (McLarty, 2015)

One of the major observations that were made during the course of this study was that the size range of *P. resecata* is much greater than that reported in the literature. While looking for background information on the species, no reference could be found in either handbooks or primary literature which gave a size range which exceeded 4 cm (Kozloff, 1983, Lamb & Hanby, 2005). However, over the course of this research project many individuals were found which exceeded 4 cm in length when measured from the tip of the telson to the base of the antenna. In fact, when collecting individuals for research all specimens collected which were less than 4 cm in length were released where they were captured. The majority of the larger individuals were male, however some large females were found as well. The largest individual collected was a male measuring 64.5 mm from the tip of the telson to the base of the antenna.

In addition to their greater size several other observations were made. Males, particularly the largest ones, will clasp the females by the edges of the first few pereons and hold the female underneath themselves. In some cases it was very difficult to disengage the females without causing injury. When placed in a container where males outnumbered females the females were often mobbed with several males attempting to grasp and hold them. No attempted mating was ever observed. When capturing isopods it was not uncommon to find a large male swimming through the water with a female clasped underneath him. In at least one case the female was in the process of molting and was quite soft. However this was not always the case and it is unknown whether or not the females were nearing a molting phase.

Cannibalism was clearly observed in this species. Individuals will consume other individuals within a container, particularly if the other individual are weak or sickly. When placed into a smooth sided container without eelgrass for transport they will grab onto anything available, whether that be a thermometer, a bubbler hose, or each other. This can sometimes be quite aggressive and in some cases one individual will be grabbed by several others. It was not initially known whether they were directly trying to kill each other or if it happened as a consequence of their tendency to grab onto anything they can. However, when examining gut contents under a compound microscope, body parts of other isopods were clearly observed. They are either directly attacking and killing each other or simply consuming the bodies of dead isopods. In one case observed by a fellow researcher two healthy juveniles were left alone in a finger

bowl together. After less than half an hour of the researcher being away one isopod had killed the other (McLarty, 2015).

During dissection when the gut was removed for dead respirometry it was also observed that the hemolymph has a very bright green coloration. It was not uncommon for small amounts of hemolymph to leak from the dissected isopod and stain the paper towel underneath them a bright green color.

Toward the end of the summer the size of the isopods in the field seemed to decrease. Although there were still some isopods available during our last collection date on August 10 which within my needed size range, many of the isopods we found were much smaller and had to be released. Although I did use one larger individual in my research, those that I collected were typically between 45 mm and 50 mm. The very large individuals were much less common on that date and proved difficult to find. I was not able to quantify this since I only recorded the measurements of the isopods I collected, which were all within a set size range.

P. resecata reportedly disappears in the fall and is not seen again until later the next spring (Lee & Gilchrist, 1972). During the spring before my research began we made several attempts to find them; however we not able to collect or even locate any isopods in Padilla Bay until May 29. It is possible that we observed nearly the full duration of time during which this *P. resecata* can be found and their population size was beginning to decrease, with the larger individuals disappearing first in the beginning

of August. It was also noted during our last collection that the eelgrass appeared less healthy, and had copious amounts of diatoms growing along the blades.

Observation of juvenile *P. resecata* also showed the variability in their development as previously reported in the literature (Menzies & Waidzunas, 1948). The most notable of these occur within the palp of the maxilliped and the shape of the telson. A palp of five segments on the adult is a major characteristic in identification of this species, while the concave telson is often regarded as one of the most diagnostic features. However, juveniles possess only four segments in the palp of the maxilliped and the telson appears to be convex early on in juvenile development and only change slowly to the concave telson which is so unique to this species. This may lead to misidentification in very small individuals. More information is available in McLarty, 2015.

A few isopods which were collected during the summer were transported to the main campus in College Place, WA and maintained in seawater tanks for several weeks. Several of the collected isopods were ovigerous females. One of these females released 153 juveniles of approximately 3 mm in length from her marsupium on September 12, 2014. Maintaining the juveniles proved somewhat difficult due to their small size and tendency toward cannibalism when housed together. One juvenile molted 31 days after leaving the marsupium. It is unknown if this was the first molt. The last adult, the

mother who had released the juveniles, died on October 10, 2014. The last juvenile died on October 20, 2014.