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Taxonomy, Ecology, and Behavior of the Kleptoplastic Sea Slug Elysia papillosa

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Taxonomy, Ecology, and Behavior of the Kleptoplastic Sea Slug *Elysia papillosa*

by

William Alan Gowacki

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Integrative Biology with a concentration in Ecology & Evolution

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DEDICATION

This thesis is dedicated to my entire family, especially my parents, Alicia M. Gowacki and William C. Gowacki. Without their support, guidance, and love, I would not be where I am today. It is also dedicated to the many doctors and nurses responsible for me surviving two battles with cancer. Finally, I also dedicate this thesis to my bone marrow donor, George, whose selfless donation provided me with a second chance at life.
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ABSTRACT

Sacoglossan sea slugs are one of the best known examples of specialist herbivores in the marine environment and can be found strongly associated with their algal hosts and food sources. Perhaps the most intriguing characteristic of many sacoglossans is their ability to sequester functional chloroplasts from their algal food sources in a process called kleptoplasting. Despite this, there continues to be issues regarding taxonomic identification of species. In turn, the ecological characteristics of many of these slugs, such as algal host and food source preference, as well as their behavioral aspects, have received little attention. A prime example of these issues arises in one such kleptoplastic sacoglossan found at Sunset Beach, Tarpon Springs, USA. The slug had previously been identified as *Elysia patina* based on a recent description, but later evidence suggested this was incorrect. Furthermore, despite the evidence found for the slug’s photosynthetic capabilities, little was known of its ecological relationships and behavior. The purposes of this study were to: 1) correct the uncertain taxonomy of the Tarpon Springs slug previously identified as *Elysia patina*, and 2) explore the ecological and behavioral characteristics of the slug.

First, a comparative study was performed between the Tarpon Springs slug and its original description, as well as descriptions for the superficially-similar congener slug, *Elysia papillosa*. The gross anatomy, dorsal surface vascular morphology, radular morphology, egg mass morphology, and developmental timeline of the Tarpon Springs slug were used as means of comparison with the previous descriptions. The results of the comparison show that the Tarpon Springs slug was in fact *E. papillosa* rather than *E. patina*, and that the most recent description used to identify the slug as *E. patina* was incorrect and should not be used.
Second, a descriptive study of the ecological and behavioral aspects of *E. papillosa* at the Sunset Beach site were performed. From October 2014 to September 2015, bi-weekly algal collections were made to determine the seasonal abundance of the slug and a possible relationship between slug abundance and algal abundance. Next, a second collection study was performed bi-monthly from April to July 2016 to identify the preferred algal host of *E. papillosa* between the three most abundant rhizophytic algae at the site, *Penicillus capitatus*, *Penicillus lamourouxii*, and *Halimeda incrassata*, all of which have been previously reported as being hosts of *E. papillosa*.

The results of these studies showed no relationship between slug abundance and algal abundance, however *E. papillosa* was found to have a seasonal population fluctuation, with the fewest slugs found in winter and summer months and the most slugs found in the spring and fall months, especially in April and May. *Elysia papillosa* was also found in significantly higher numbers on the alga *P. capitatus* compared to the other two algal species, although some slugs were found on *P. lamourouxii*. Only one slug was found on *H. incrassata*, indicating it is not being used as a host despite previous reports. Further exploration into the genetics of sequestered chloroplasts would provide critical details into *E. papillosa*’s feeding behavior *in situ*. Lastly, because of *E. papillosa*’s photosynthetic abilities, an experiment was performed to determine if *E. papillosa* exhibited phototactic behavior. Fully-fed slugs were found to have no preference for either light or dark conditions, indicating their behavior was not being influenced by their photosynthetic abilities. This information provides a means of comparison with future studies of the phototactic behavior of kleptoplastic slugs, and could provide insight into how the longevity of functional chloroplasts in each species of slug could influence their behavior.
CHAPTER ONE:  
CORRECTING TAXONOMIC CONFUSION FOR THE KLEPTOPLASTIC SEA SLUG  
ELYSSIA PAPILLOSA VERRILL (1901)

Introduction

Some of the most intriguing and ubiquitous members of the benthic macroalgal community are the sacoglossans. The molluscan order Sacoglossa Ihering 1876 (Gastropoda: Heterobranchia) is found globally, and is comprised of approximately 300 accepted taxa of mainly herbivorous sea slugs (Jensen 2007). Perhaps the greatest interest in sacoglossans comes from the ability of many sacoglossan species to sequester functioning chloroplasts from their algal food sources into their digestive cells, a process known as kleptoplasty (Pierce and Curtis 2012, Pierce et al. 2015). It has also been shown that kleptoplastic slugs are able to utilize the metabolites created from photosynthesis as a food source (Trench 1970, Pierce and Curtis 2012, Curtis et al. 2015, Pierce et al. 2015). However, not all sacoglossans are equal in this regard, and the longevity of sequestered chloroplasts varies greatly between species (Curtis et al. 2015, Pierce et al. 2015). There is also evidence of the presence of algal genetic code within the genomes of some slugs capable of long-term chloroplast retention and maintenance, hypothesized to be the result of horizontal gene transfer, the first such case among multicellular organisms (Mujer et al. 1997, Pierce et al. 2003, Pierce et al. 2007, Pierce and Curtis 2012). While these findings are somewhat controversial (Wägele et al. 2011, Bhattacharya et al. 2014), other possible examples of horizontal gene transfer among multicellular eukaryotes have been found (Boto 2014).
While there are many interesting avenues of study in regard to sacoglossans, there are still many issues associated with these sea slugs. Perhaps the most critical problem comes with taxonomic classification within the order. The classification of Sacoglossa as an order is still debated, and the taxonomy is frequently being revised as new genetic evidence arises (Krug 2016). This is especially true with the genus *Elysia* Risso 1818, the most speciose genus within Sacoglossa (Jensen 2007, Krug 2016). Many of the members of *Elysia* are relatively small in size, cryptic, and can be similar in appearance and anatomy, making it difficult to distinguish between species (Marcus 1980). In many cases, the original descriptions of these species were performed by a handful of individuals and took place before 1950 (Jensen 2007). Additionally, these descriptions can be cursory, lacking critical details on distinctive characteristics, which leads to confusion of identity.

One example of these taxonomic difficulties is apparent in a slug species found in Tarpon Springs, Florida, USA (Figure 1.1), that has been the subject of multiple recent studies (Curtis *et al.* 2010, Pierce and Curtis 2012, Curtis *et al.* 2015, Pierce *et al.* 2015). These studies identified the slug as *Elysia patina* based on the most recent detailed redescription of the species by Ortea *et al.* (2005). Ortea *et al.* (2005) sought to correct the taxonomic confusion between *E. patina* and its somewhat superficially-similar relative, *Elysia papillosa*. They provided very detailed anatomical descriptions of both species, and further expanded the taxonomy to include two new species. However, recent genetic evidence suggested these classifications were incorrect (Krug *et al.* 2016). The first chapter of this study is devoted to the correction of this mistake and the proper identification and description of the Tarpon Springs sea slug previously identified as *E. patina*. To do so, comparisons of various anatomical and developmental aspects of the Tarpon Springs slug were made alongside both original descriptions and Ortea *et al.*’s (2005) descriptions of *E. patina* and *E. papillosa*.
Materials and Methods

Study Site

The primary collection site was a large, dense bed of mixed rhizophytic algae located on the north side of Sunset Beach, Tarpon Springs, Florida, USA (28.1445 N, -82.7903 W). The bed consisted mainly of algae *Penicillus capitatus*, *Penicillus lamourouxii*, and *Halimeda incrassatta*, all growing in close proximity to one another. Because of a seasonal die-off of standing algal stock, a secondary location with similar community structure was used for collections of food algae, *P. capitatus* and *P. lamourouxii*, in order to maintain slugs in the lab. This site was located on the ocean-facing side of Anne’s Beach, Islamorada, Florida, USA (24.8486 N, -80.7409 W). Some slugs were also found in the Keys collections, but these had identical features to those found in Tarpon Springs. Therefore, this study focuses on the slugs found in Tarpon Springs.

Sea Slug Collection and Maintenance

The Tarpon Springs slugs were obtained by carefully collecting stalks of *P. capitatus*, which the slugs have been shown to use as a food and symbiotic chloroplast source (Curtis et al. 2010), as well as the congener alga *Penicillus lamourouxii*, which grows alongside *P. capitatus*. During this study the Tarpon Springs slugs have been observed readily feeding on *P. lamourouxii* in the lab, but it had not been shown before that they use it as either a host or symbiotic chloroplast source. The algae were returned to the lab and placed in aquaria filled with artificial seawater [ASW, ~35 ppt (Instant Ocean, Blacksburg, VA)] treated with 0.1 g/L each of penicillin-G (Fisher Scientific, Pittsburgh, PA) and streptomycin sulfate (Sigma-Aldrich, St. Louis, MO). Paratype specimens of these slugs were deposited in the Marine Invertebrate Museum at the Rosenstiel School of Marine and Atmospheric Science at the University of Miami at Key Biscayne, Florida (UMML 30.16085).
Due to their small size and cryptic nature the slugs are very difficult to see on their host algae, so they were allowed to crawl out of the algae and onto the walls of the aquaria before being collected and placed into a large beaker filled with the same antibiotic-treated ASW and a few thalli of *P. capitatus* or *P. lamourouxii* to feed on. The slugs were kept at 23°C under fluorescent tubes at a 14/10 h light/dark cycle. The slugs’ water and food algae were replaced weekly.

**Means of Comparison with Previous Descriptions**

Evaluation of the Tarpon Spring slug’s identity was done by comparing various aspects of their anatomy and development with previous descriptions of *E. patina* (Marcus 1980) and *E. papillosa* (Verrill 1901, Marcus and Marcus 1967, 1970, Marcus 1980, Clark 1984) and with Ortea *et al.*’s (2005) descriptions. The general external morphology, dorsal surface vessel morphology (DSVM), radular tooth morphology, egg mass morphology, and development timeline of the Tarpon Springs slug were observed. Although various other anatomical features can be used to distinguish species of *Elysia*, the original descriptions commonly use DSVM and radular tooth morphology as the means of differentiation (Marcus and Marcus 1970, Marcus 1980). Therefore, these were the primary characteristics used in this study for comparison.

**Dorsal Surface Vessel Morphology**

The DSVM of the Tarpon Springs slug was visualized by partially anesthetizing individual slugs in ASW containing 0.35 mM MgCl$_2$ and then pinning their parapodia apart to expose the dorsal surface. Images were taken using a dissecting microscope (Leica M80) equipped with a digital camera (Leica DFC 295). The DSVM of the Tarpon Springs slug was then compared to the previous descriptions and drawings of *E. patina* (Marcus 1980, Ortea *et al.* 2005) and *E. papillosa* (Marcus 1980, Clark 1984, Ortea *et al.* 2005).
**Radular Tooth Morphology**

Radulae were dissected from two Tarpon Springs slugs approximately 8 mm in length. After being euthanized, the buccal mass of each slug was dissected under a dissecting microscope using fine insect pins. The tissue surrounding the radula was removed as much as possible without damaging the radula. Each radula was then left overnight in 5M NaOH to dissolve any remaining tissue. The next day, the radula was rinsed in deionized water to remove the NaOH before being placed on aluminum stubs covered with double-faced conductive carbon adhesive pads. The sample was then dried in a vacuum oven, coated with 20 nm Au-Pd alloy, and imaged using a scanning electron microscope (Topcon Aquila Compact SEM). The morphology of the radular teeth was compared with the previous descriptions and drawings of the radular teeth of *E. patina* (Marcus 1980, Ortea *et al.* 2005) and *E. papillosa* (Marcus and Marcus 1967, 1970, Marcus 1980, Ortea *et al.* 2005).

The mean number of teeth in the radulae of adult Tarpon Springs slugs was determined from five adult slugs (8-11 mm in length). The slugs were flattened, but not killed, between a glass microscope slide and a glass cover slip. This made the radula clearly visible under a compound microscope at 100x total magnification without further dissection or harm to the slugs. The number of teeth in the radula of each slug was counted and the average and standard deviation were calculated.

**Egg Mass Morphology**

Larger captive slugs would occasionally lay spiral egg masses on the walls of the culture beaker or the surface tension of the water. The egg masses were carefully scraped from the wall of the culture beaker with a fine blade or sucked from the surface of the water using the wide end of a glass Pasteur pipette and then transferred into a sterilized 50 mm diameter glass culture dish filled with sterile 0.1 μm filtered ASW treated with 5 mg/L of rifampicin (Sigma-
Aldrich). The egg masses were monitored daily and rinsed every two days with rifampicin-treated ASW on a 125 μm Nitex filter before being transferred into a new dish of rifampicin-treated ASW.

The average diameter of individual eggs was determined by measuring 10 eggs in each of 5 egg masses using an ocular micrometer on a compound microscope (40x total magnification) and calculating the average egg diameter of all eggs ($n = 50$). The average development times of embryos to reach trochophore and veliger stages were determined for 8 egg masses. The time to hatching was determined for 6 of these egg masses, as the veligers in 2 of the egg masses did not survive to hatching. Upon hatching, filaments of the caps of $P$. capitatus were added to some of the dishes, as the presence of chemical cues from algal hosts has been shown to initiate settling and metamorphic behavior in some slugs (Krug 2001, 2009). Veligers were monitored for metamorphosis, but were not fed.

Results

Gross Morphology

$Elysia papillosa$ was originally described as a “… distinctly papilllose species. Body rather elongated in extension ; head large ; neck long ; rhinophores large… Whole surface of body, head, and outside of flaps thickly covered with small conical papillae… Rhinophores… with two indistinct transverse bands of orange-brown,” and accompanied by an artistic rendering (Verrill 1901) (Figure 1.2). Subsequent accounts of $E. papillosa$ confirmed these features (Marcus and Marcus 1967, 1970, Clark 1984). They are also present in the Tarpon Springs slug (Figure 1.1). However, Ortea et al. (2005) described these features in $Elysia patina$ (Figure 1.3).
Dorsal Surface Vessel Morphology

The pericardial complex in *E. papillosa* was described as “… globular,” with a “straight renal sac [extending] backwards twice as long as this [pericardial] swelling,” with simple branching in the lateral vessels (Marcus and Marcus 1967, Marcus 1980). Marcus (1980) subsequently provided a simple line drawing of *E. papillosa*’s DSVM that curiously does not match her earlier descriptions (Marcus and Marcus 1967) (Figure 1.4a). Furthermore, the DSVM of *E. patina* was described as “… far different from all the other known Atlantic [elysiid] species,” so that it was “easily recognized as a new species” (Marcus 1980). In her description, the main renal vessel of *E. patina* measured approximately half the length of the parapodia with seven vessels entering from the right and six from the left (Figure 1.4b) (Marcus 1980).

In comparison, the DSVM of the Tarpon Springs slug has a globular pericardial complex with the main renal vessel extending straight back half the length of the parapodia (Figure 1.5). Four to five lateral vessels branch out from both sides of the main renal vessel towards the edge of the parapodia. The posterior-most end of the main renal vessel splits into two longer vessels that extend towards the posterior end of the slug (Figure 1.5). The branching of the vessels is simple, matching Marcus and Marcus’ (1967) description of *E. papillosa*’s DSVM, but is quite unlike the DSVM of Ortea et al.’s (2005) *E. papillosa*, which features a much more complex branching (Figure 1.6a). Likewise, this complex branching distinguishes it from the more simply-branched DSVM of Marcus’ (1980) *E. patina*. The Tarpon Springs slug had been previously identified as *E. patina* because its morphology most closely resembled that of *E. patina* as described by Ortea et al. (2005). However, Ortea et al.’s (2005) description of *E. patina* does not match earlier description (Marcus 1980), but rather those of *E. papillosa* (Marcus and Marcus 1967, 1970, Clark 1984).
Gametolytic Vesicles

Some of the Tarpon Springs slugs had two large, round, white structures on their dorsal surface located on either side of the main renal vessel (Figure 1.5). These structures appear to be the gametolytic vesicles described on *E. patina* by Marcus (1980) (Figure 1.4b) and Ortea et al. (2005) (Figure 1.6b). However, these vessels had also been described on *E. papillosa* (Clark 1984). The presence of the vesicles on both species, as well as their temporary nature, indicate that they cannot be used as a character to distinguish the two species.

Radular Teeth

The Tarpon Springs slug's teeth are blade-shaped with a deep dorsal groove, a bent shaft, pointed cusp, and coarse serrulation on the midline ridge of the ventral surface, with each denticle measuring 2-3 μm wide (Figure 1.7). These features match Ortea et al.'s (2005) description of *E. patina*'s teeth, but are drastically different from Marcus’ (1980) description. According to Marcus (1980), *E. patina*'s teeth have a dorsal groove, a thin and slightly curved shaft, a sharp pointed cusp, and no serrulation (Figure 1.8c). In contrast, the teeth of the Tarpon Springs slug exactly match Marcus and Marcus’ (1967) description of *E. papillosa*'s teeth (Figure 1.8a). Strangely, Ortea et al.’s (2005) description of *E. papillosa*'s teeth match neither those of the Tarpon Springs slug, Marcus and Marcus’ (1967) *E. papillosa*, or Marcus’ (1980) *E. patina* (Figure 1.8d). The average number of teeth in the radula of the Tarpon Springs slug was 12 (± 1.5 SD). Marcus and Marcus (1967) described the radula of *E. papillosa* as consisting of 13 teeth. In Ortea et al.’s (2005) account, *E. papillosa*'s radula had 22 teeth while *E. patina* had 12 teeth.
**Egg Mass Morphology and Development**

The egg mass of *E. patina* described by Ortea *et al.* (2005) had two turns in its spiral, one or two eggs adjacent to one another, and a band of yellow extracapsular yolk (ECY). An image was not provided. Similarly, Ortea *et al.*’s (2005) *E. papillosa* egg mass was described as having three turns in the spiral and a yellow band of ECY. A drawing of this egg mass portrayed many small eggs within the egg mass arranged in no order. The diameters of individual eggs was not reported for either species. No descriptions of the egg masses of *E. patina* or *E. papillosa* were included in their previous descriptions (Verrill 1901, Marcus and Marcus 1967, 1970, Marcus 1980).

The Tarpon Springs slug egg mass was most similar to Ortea *et al.*’s (2005) description of the egg mass of *E. patina*. The individual eggs were lined up one-by-one through the spiral egg mass. However, unlike Ortea *et al.*’s (2005) *E. patina*, the Tarpon Springs slug’s egg mass had a thin white band of ECY that came in contact with each egg (Figure 1.9). The first Tarpon Springs egg mass had been obtained in April 2015, but did not develop properly. From late August to September 2015, the Tarpon Springs slugs began laying eggs much more rapidly. A total of 8 egg masses were collected. These egg masses had the same overall morphology as the first. The mean diameter (± SD) of the eggs was 109 μm (±8.3; n = 50 eggs from 5 egg masses). For the 8 egg masses, the mean time to trochophore development was 2 days and the mean time to veliger stage was 5 days. From the 8 egg masses, only 6 managed to hatch a mean of 15 days after deposition. From 4 of the 6 hatched egg masses, some veligers metamorphosed into juvenile slugs an average of 5 days after hatching, but the majority died before metamorphosis. The presence of *P. capitatus* filaments in the culture dishes had no effect on metamorphosis.
Swimming Behavior

On several occasions, handling the Tarpon Springs slugs would induce them to swim by peristaltically flapping their parapodia. This behavior was noted in the original description of *E. papillosa* (Verrill 1901). While Marcus (1980) reiterated this claim, no other papers describing *E. patina* or *E. papillosa* mention observing swimming behavior in either species.

Discussion

These comparisons indicate that all of the anatomical characteristics of the Tarpon Springs slug matches those previously used to describe *E. papillosa* (Verrill 1901, Marcus and Marcus 1967, 1970, Marcus 1980). Confusingly, the anatomy and drawings used by Ortea et al. (2005) to re-describe *E. patina* also match previous descriptions of *E. papillosa*. Therefore, the re-description of *E. patina* by Ortea et al. (2005) is incorrect and their taxonomy should not be used. The reasons for Ortea et al.’s (2005) errors are not clear. However, it is now evident that the Tarpon Springs slug are *E. papillosa*, not *E. patina*. In turn, previous papers which reported having used *E. patina* based on Ortea et al.’s (2005) descriptions (Curtis et al. 2010, Pierce and Curtis 2012, Curtis et al. 2015, Pierce et al. 2015) were actually done using *E. papillosa*. It is not possible to comment on the species Ortea et al. (2005) labeled *E. papillosa*, but it is clear that it was also incorrectly labeled. Furthermore, while the species has been reported as occurring across the Caribbean, the Florida Keys, Bahamas, and Bermuda (Verrill 1901, Marcus and Marcus 1967, 1970, Marcus 1980), the presence of *E. papillosa* in Tarpon Springs indicates a range extension northwards into the Gulf of Mexico. Also, the previously unreported ability of *E. papillosa* to consume the alga *P. lamourouxii* is of special interest in regards to *E. papillosa*’s algal host range, which is somewhat ambiguous.

Generally, sacoglossan species with large egg diameters (200 μm) hatch as fully-formed juvenile slugs while those with smaller egg diameters (≤ 70 μm) tend to hatch as long-lasting
plankotrophic veligers (Clark and Jensen 1981). The Tarpon Springs slug egg diameters fall within the range of lecithotrophic species (65-120 μm), which have a relatively short planktonic veliger stage and do not need to feed before settling out as juveniles (Clark and Jensen 1981). It is not clear at this point if the low level of metamorphosis by the Tarpon Springs slug veligers is actually an example of lecithotrophy, but their overall development timeline matches previous reports of *E. papillosa*’s development (Clark and Goetzfried 1978, Clark and Jensen 1981). It is possible the few metamorphoses witnessed were caused by stress from a lack of food or other culture-related issues (Krug 2009). Likewise, what role the ECY plays in the development of the Tarpon Springs slug is uncertain.

Finally, it should be noted that there is no doubt that genetic analysis is the most reliable means of species identification. However, it is useful to have non-destructive, anatomical markers for rapid species identification, especially in field situations. Likely the most obvious trait for *E. papillosa* is the brown transverse band located midway up each of the long rhinophores. This appears to be a unique feature of the species that is easily identified by the naked eye, even on small specimens.

References


Figure 1.1. Tarpon Springs slug on the alga *Penicillus lamourouxii*. Scale bar = 0.5 cm.
Figure 1.2. Original drawing of *Elysia papillosa* accompanying the species description (Verrill 1901). No scale bar was included.
Figure 1.3. Ortea et al.’s (2005) drawing of the head region of “Elysia patina”. No scale bar was provided. Image used with permission from Vieraea (Appendix C).
**Figure 1.4.** Dorsal view drawings of (A) *Elysia papillosa* (Marcus 1980), and (B) *Elysia patina* (Marcus 1980). No scale bars were provided. Images used with permission from University of Miami (Appendix D).
Figure 1.5. DSVM of Tarpon Springs slug. Arrow A indicates the left gametolytic vesicle. Arrow B indicates location of the pericardial prominence. Scale bar = 1 mm.
Figure 1.6. Dorsal view drawings of (A) "Elysia papillosa" and (B) "Elysia patina", from Ortea et al. (2005). Arrow on (B) indicates left gametolytic vesicle. Scale bars = 2 mm. Images used with permission from Vieraea (Appendix C)
Figure 1.7. SEM images of (A) the entire radula of the Tarpon Springs slug (scale bar = 50 μm), and (B) a close up of a single radular tooth of the Tarpon Springs slug (scale bar = 15 μm).
Figure 1.8. Side-by-side comparison of drawings of the radular teeth for (A) *E. papillosa* (Marcus and Marcus 1967), (B) “*E. patina*” (Ortea et al. 2005), (C) *E. patina* (Marcus 1980), and (D) “*E. papillosa*” (Ortea et al. 2005). All scale bars = 50 μm. No scale bar was provided for (C). (A) and (C) used with permission from University of Miami (Appendix C). (B) and (D) used with permission from Vieraea (Appendix D).
Figure 1.9. Newly deposited egg mass of a Tarpon Springs slug. Thin white string of ECY is visible. Scale bar = 0.25 mm.
CHAPTER 2:
ECOLOGY AND BEHAVIOR OF THE KLEPTOPLASTIC SEA SLUG, ELYSIA PAPILLOSA

Introduction

In chapter 1, the issue of proper taxonomic classification of the kleptoplastic sea slug *Elysia papillosa* was rectified. However, previous literature still reports inconsistent facts on *E. papillosa*, particularly in its interactions and associations with the algae it consumes. *Elysia papillosa* can be found in the rhizophytic algae beds located throughout the Caribbean, the Bahamas, Bermuda, and as far north into the Gulf of Mexico as Tarpon Springs, FL, U.S.A. This small-sized, cryptic slug can be found, on and consumes, at least one species of rhizophytic algae, *Penicillus capitatus* (Curtis *et al.* 2010, Pierce and Curtis 2012), although other reports suggest that *E. papillosa* can be associated with rhizophytic algae from the genera *Caulerpa*, *Halimeda*, and *Udotea* (Marcus and Marcus 1967, Jensen 1983, Clark 1984). Curiously, the original description of *E. papillosa* by Verrill (1901) reports *E. papillosa* as having been found under a rock.

This wide range of algal substrata from which *E. papillosa* has been reported to be collected may indicate that it is not a truly specialist herbivore, as is often the case with sacoglossan sea slugs. Sacoglossans are often stenophagous, specializing in consuming one or two species of green algae, typically from the same genus (Jensen 1983, 1997, Trowbridge and Todd 2001, Poore *et al.* 2007). However, there are some examples of euryphagy, where the slug can consume multiple algal species (Jensen 1994, Curtis *et al.* 2006, Curtis *et al.* 2010, Handeler *et al.* 2010, Middlebrooks *et al.* 2014). In fact, the sacoglossans represent the majority
of well-known marine specialist herbivores (Trowbridge and Todd 2001, Poore et al. 2007, Baumgartner and Toth 2014). Herbivore specialization has been extensively studied in terrestrial environments, particularly in insects, and some similarities and contrasts can be made between them and their marine counterparts (Hay et al. 1989, Poore et al. 2007, Trowbridge et al. 2009). However, little knowledge of the detailed interactions of marine specialist herbivores with their algal hosts and food sources currently exists (Poore et al. 2007).

This dearth of information on marine specialist herbivores may in part be the result of the relative scarcity and rarity of such species (Hay et al. 1989, 1990, Trowbridge and Todd 2001), but the difficulties associated with collection and proper identification of sacoglossans also present a significant obstacle. Despite this, studies on sacoglossans present a unique opportunity to examine the tightly-associated relationships between herbivore and food source, if both can be readily collected. Patterns in abundance of the possible algal hosts of *E. papillosa* have been documented at Sunset Beach, Tarpon Springs, FL, USA, (Demès et al. 2009, 2010, Bedinger 2012, Bedinger et al. 2013). However, information regarding the distribution and abundance of *E. papillosa* is limited, and to date no studies have focused on the interactions between *E. papillosa* and their potential algal hosts.

Behavioral aspects of sacoglossans, such as response to physical and chemical stimuli or predation, are also poorly studied. The kleptoplastic and photosynthetic abilities of the sacoglossans, particular those of the genus *Elysia*, are well documented (Trench 1970, Ireland and Scheuer 1979, Clark et al. 1990, Evertsen et al. 2007, Pierce and Curtis 2012). However, the effects of this photosynthetic capability on the slugs’ behavior have only just recently begun to be explored (Giménez and Muniaín 2006, Jesus et al. 2010, Schmitt & Wägele 2011, Yamamoto et al. 2013, Miyamoto et al. 2015).

Recent studies have expanded on the phototactic behavior of kleptoplastic slug, and have shown that multiple kleptoplastic sacoglossans exhibit positive phototactic behavior that
varies depending on whether the slug possesses functional sequestered chloroplasts and the intensity of the light source (Schmitt & Wägele 2011, Miyamoto et al. 2015). Conversely, some non-kleptoplastic sacoglossans have been found to have neutral or negative phototactic behavior (Miyamoto et al. 2010). It has also been shown that photosynthetic production can have an effect on the foraging behavior of kleptoplastic slug *Elysia clarki*, which has long-lived sequestered chloroplasts (Middlebrooks et al. 2011). However, behavioral data for the majority of kleptoplastic slugs remains lacking. *Elysia papillosa* has been shown to sequester and retain functional chloroplasts for about one week before they degrade, and the slug eventually dies within two weeks of the onset of starvation (Curtis et al. 2010, Pierce and Curtis 2012, Curtis et al. 2015). In contrast, other species of *Elysia* have been shown to maintain sequestered chloroplasts and survive exclusively on photosynthesis for up to 9 months (West et al. 1984, Middlebrooks et al. 2011, 2012, Curtis et al. 2015).

Positive phototaxy may imply preferential behavior aimed at maximizing photosynthetic productivity while negative phototaxy may indicate behavior aimed at increasing the longevity of sequestered chloroplasts by avoiding degradation associated with photosynthesis (Schmitt & Wägele 2011, Middlebrooks et al. 2014, Miyamoto et al. 2015). However, a lack of preference for either light or dark conditions may indicate that photosynthetic production does not influence a slug’s behavior. Considering *E. papillosa*’s relatively short window of chloroplast functionality, it could be predicted that they would show no preference for either the light or the dark. Determining the extent of *E. papillosa*’s phototactic behavior can provide insight into the nature of the slug’s interaction with its food sources, and how the different capacities for chloroplast retention and maintenance of kleptoplastic slugs may affect behavior.

Host preference, seasonality, abundance, and behavior of specialist herbivores like sacoglossans can be used to explore the broader ecological dynamics of the systems they inhabit (Trowbridge 1992). Therefore, the objectives of this chapter are to: (1) determine the
patterns of seasonality and abundance of *E. papillosa*, (2) explore relationships between the abundance of *E. papillosa* and the biomass of rhizophytic algae at the Sunset Beach site, (3) identify the preferred algal host of *E. papillosa*, and (4) experimentally investigate the phototactic behavior of *E. papillosa*.

**Materials and Methods**

**Seasonality and Abundance Collections**

From October 2014 to October 2015, bi-weekly collections of algae were made in a large, dense, contiguous mixed rhizophytic algae bed on the north side of Sunset Beach (28.1445 N, -82.7903 W) in Tarpon Springs, FL, U.S.A. to estimate the density of *E. papillosa*. A 0.25 m² PVC quadrat was haphazardly thrown into a section of an algal bed approximately 400 m² in size and allowed to settle to the substrate. All above-ground algal biomass within the quadrat was carefully harvested. Eight quadrats were taken for a total sample area of 4 m² per sample date. Additional samples of *P. capitatus* and *P. lamourouxii* were collected for feeding slugs in the lab. Each quadrat sample was placed in a Ziploc bag and transported to the laboratory where all algae were placed by quadrat into separate aquaria filled with filtered artificial seawater [ASW, ~35 ppt (Instant Ocean, Blacksburg, VA)]. The ASW was treated with 0.1 g/L each of penicillin-G (Fisher Scientific, Pittsburgh, PA) and streptomycin sulfate (Sigma-Aldrich, St. Louis, MO) in order to prevent bacterial growth. The aquaria were kept at 23°C under fluorescent tubes on a 14/10 h light/dark cycle.

Some sacoglossans are rather conspicuous and can be collected individually in the field (West *et al.* 1984, Trowbridge 1992, Middlebrooks *et al.* 2014). However, many species, including *E. papillosa*, are very small and cryptic, requiring indirect collection methods (Baumgartner *et al.* 2009, Rasher *et al.* 2015). Therefore, slugs were collected from each algal sample after they crawled out of the algae and onto the walls of the aquaria. All *E. papillosa*
found within two weeks of collection were removed from the aquaria and placed into a large beaker filled with the same ASW and a few thalli of *Penicillus* species on which to feed, and retained for later experiments. The number of slugs found in each sample was recorded. After 14 days, the algae from each quadrat sample were removed from the aquaria, separated by sample and species, and left to air dry at 23°C for 5 days. The biomass (g dry weight m⁻²) of each algal species was recorded. The density of *E. papillosa* (# slugs m⁻²) was calculated for each sample date. Correlation analyses were performed comparing the number of *E. papillosa* with grams dry weight⁻¹ of the three most dominant algae, *P. capitatus, P. lamourouxii, H. incrassata*, as well as total biomass of algal species (Baumgartner et al. 2009, Baumgartner and Toth 2014, Rusher et al. 2015).

**Host Preference Collections**

*Elysia papillosa* has been described as feeding on the rhizophytic alga *P. capitatus* (Curtis et al. 2010). However, they have also been seen readily consuming *P. lamourouxii* in the lab. *Penicillus lamourouxii* can be found in high densities adjacent to *P. capitatus* at the Sunset Beach site, sometimes even growing from within the same rhizoidal "clump". *Elysia papillosa* has also previously been reported to be found on other rhizophytic algae, such as *Udotea* and *Halimeda* (Marcus and Marcus 1967, Clark 1984), but there are no reports of the slug actually eating either species. Although no *Udotea* species are present at the Sunset Beach site, *H. incrassata* is abundant in close proximity to the *Penicillus* species. Therefore, a potential diversity of hosts and food sources are available to *E. papillosa* at the study site.

To determine if *E. papillosa* displayed a preference for *P. capitatus, P. lamourouxii, or H. incrassata* as a host, additional bi-monthly collections were made at the Sunset Beach site in 2016. Given that the greatest number of slugs were collected at the site in 2015 between April and May during seasonality and abundance collections (see Results), host preference
collections were conducted from April to July 2016 over a total of 9 collection dates. On each collection date, 10 individual thalli each of *P. capitatus*, *P. lamourouxii*, and *H. incrassata*, were carefully collected at 10 haphazardly chosen sample locations. Each sample of 10 thalli was placed into a separate press-seal bags with seawater and transported back to the laboratory, where the algal samples were placed in 1 L clear plastic containers filled with filtered artificial seawater (ASW) at 23°C under fluorescent lights set to a 14/10 h light/dark cycle. The samples were monitored daily for emerging slugs. When a slug was found, it was removed from the sample container, measured for length (mm) at full extension from the end of the head to the tip of the “tail”, and placed into an 80 mm specimen dish filled with filtered ASW along with other slugs from the corresponding sample date. The mean length (mm, ±SD) of all slugs was determined for each sample date. The slugs were maintained in the lab for the phototaxis experiment by being given 1-2 thalli of *P. capitatus* or *P. lamourouxii* as sources of food. After one week, the algae from which slugs were collected were separated by species and sample (*n* = 10 per algal species) and air dried at 23°C for 5 days. The dry weight of each algal sample was recorded. The percent composition of algae was calculated for each sample date. Densities of *E. papillosa* were determined as the number of slugs x gram algal dry weight\(^{-1}\) for each of *P. capitatus*, *P. lamourouxii*, and *H. incrassata*.

In order to determine whether *E. papillosa* displayed any preference for a particular algal host, the proportion of total number of slugs found on *P. capitatus*, *P. lamourouxii*, and *H. incrassata* was calculated for each sample date. Collections made on and after July 3 produced no slugs, so those dates were not used for analyses. A two-sample t-test for unequal variances was performed to determine if a significant difference in the number of slugs between the two *Penicillus* species could be found. Correlation analyses were performed on the number of *E. papillosa* recovered per gram dry weight of algae for each of *P. capitatus* and *P. lamourouxii*. *Halimeda incrassata* was excluded from these analyses because only one slug was found on
the alga over the entire sampling period. Finally, these data were used to examine *E. papillosa*'s host preference by comparing the total algal biomass (g dry weight) for each species of algae with the total number of slugs found on each species during collections from April to July 2016 using the Pearre’s $C$ selectivity index (Pearre 1982):

$$C = \pm \left[ \frac{(a_d b_e - b_d a_e)^2 - \frac{n}{2}}{(a_d + a_e)(b_d + b_e)(a_d + b_d)(a_e + b_e)} \right]^{\frac{1}{2}}$$

where $a_d$ is the total number of slugs found on a given algal species (*P. capitatus*, *P. lamourouxii*, or *H. incrassata*), $b_d$ is the number of slugs on the two other algal species combined, $a_e$ is the total number of samples of a given algal species (40 for each species), $b_e$ is the sum of all other algal biomass in grams, and $n$ is the sum of $a_d$, $b_d$, $a_e$, and $b_e$.

**Phototactic Behavior**

A series of laboratory experiments was performed in order to elucidate whether *E. papillosa* showed preferential phototactic behavior for light or dark conditions. All experiments were performed using adult ($\geq 8$ mm length) slugs. Slugs were given access to *Penicillus* species to feed *ad libidum* for 7 days before the experiments were run to ensure sequestration of functional chloroplasts. A deep green coloration was observed in each slug, indicating successful chloroplast sequestration (Curtis et al. 2015). All trials were performed in a large, dark incubator at 23 °C in order to prevent other light sources from influencing the experiment. Half of the exterior base and sides of a sterilized 50 mm culture dish was covered in a double layer of duct tape to prevent light from penetrating one side of the dish. The dishes were filled with 100 mL of filtered ASW and an individual slug was added to each dish. A total of 21 slugs were tested individually in separate dishes. A hole approximately 50 mm in diameter was cut into the top of a 30 x 30 cm box to hold the experimental dish while an LED aquarium lamp
(5616 µmol s\(^{-1}\) m\(^{-2}\)) was placed inside the box facing upward to ensure only half of the dish was illuminated (Appendix A). This created an environment with both light and dark conditions through which the slugs were able to move freely. The orientation of each dish with a slug was determined by a coin flip in order to prevent any possible directional bias in the slugs' behavior.

In order to record the slugs’ behavior, a GoPro\textsuperscript{TM} camera (GoPro Hero 3) was suspended above each dish so that the entire dish was visible within the frame. The camera was set to take images every 30 sec over a 3 h period for a total of 360 images per trial. Each trial was performed at the same time each day at 12:00 to prevent possible diurnal behavior changes in the slugs from influencing the results. All images were reviewed, and the percentage of time spent by the slugs in the light vs. the dark side of the dish, as well as the approximate time (mins) spent on either condition, were calculated for each slug. A two-sample t-test for equal variances was performed to compare the mean time spent by the slugs in light vs. dark conditions.

**Results**

**Seasonal Abundance**

*Elysia papillosa* was present in all 20 collections of rhizophytic algae from the Sunset Beach site between October 2014 and September 2015 (Figure 2.1). Slug density (±SE) was lowest in September 2015 with only one slug found (0.50 slugs m\(^{-2}\) ±0.50), while the highest density of slugs occurred in April 2015 (27.43 slugs m\(^{-2}\) ±8.96). The mean (±SD) length of all *E. papillosa* found during host preference collections was 2.72 mm (±0.15). Overall abundance peaked sharply in April and dropped quickly to low levels through May. Biomass (g dry weight m\(^{-2}\)) of the algae *P. capitatus* and *P. lamourouxii* fluctuated seasonally and peaked during different seasons, but the density of *H. incrassata* increased from January to June (Figure 2.2). *Halimeda incrassata* had the highest overall mean biomass (g dry weight m\(^{-2}\) ±SE) (15.24
when compared to *P. capitatus* (11.86 ±1.22) and *P. lamourouxii* (14.18 ±1.07). However, a single-factor ANOVA found no statistically significant difference between the mean biomass of all three algae throughout the year (all dates combined) \[F(2,57) = 1.48, p = 0.24\]. No correlation between slug abundance (slugs m⁻²) and algal biomass (g dry weight m⁻²) for any algal species was detected (Figure 2.3). Sea temperature at the Sunset Beach site ranged from 15 °C in January 2015 to 35.5 °C in August 2015, however no correlation between slug abundance and sea temperature was detected (Appendix B).

**Algal Host Preference**

The alga *H. incrassata* accounted for the largest proportion of total algal biomass (dry weight, g) making up a mean (±SE) of 45% (±3.6%) of all algal biomass, while the algae *P. capitatus* and *P. lamourouxii* each accounted for a mean of 27% (±1.5 and 2.5% respectively) of total algal biomass (Figure 2.4a). A single-factor ANOVA found these differences in algal biomass among the three algal species to be highly significant \[F(2,145) = 99.96, p < 0.001\]. Despite the comparatively high biomass of *H. incrassata*, only one *E. papillosa* was found on any samples of this alga. In total, 305 *E. papillosa* were found, with 257 emerging from *P. capitatus* and 48 from *P. lamourouxii*. A mean (±SE) of 93% (±3.5%) of all *E. papillosa* were found on *P. capitatus*, with 7% (±3.5%) found on *P. lamourouxii* (Figure 2.4b). A two-sample t-test for unequal variances detected significantly higher number of slugs g dry weight⁻¹ (±SE, \(n = 40, p < 0.001\)) for *P. capitatus* (1.39 ± 0.17) than *P. lamourouxii* (0.31 ±0.09). However, no significant correlations between the number of slugs found and algal biomass (g dry weight m⁻²) of either *Penicillus* species were found (Figure 2.5). As seen in Fig. 2.5, comparatively high numbers of *E. papillosa* were sometimes recorded from low algal biomass, with the converse also being true. *Elysia papillosa* showed strong positive selectivity for *P. capitatus* (Pearre’s \(C = \))
+0.77), but weak positive selectivity for *P. lamourouxii* (Pearre’s $C = +0.13$) and weak negative selectivity for *H. incassata* (Pearre’s $C = -0.20$).

**Phototaxis**

*Elysia papillosa* showed no evidence of preference for either light or dark condition in the phototaxis experiments (Figure 2.6). The slugs spent on average 50.6% of the experimental time in the lighted side of the dish ($n = 21$). A two-sample paired t-test for equal variance showed no significant difference in the average time (mins) spent by slugs in the light or dark sides of the dishes ($t_{40} = 0.09$, $p = 0.93$).

**Discussion**

The abundance of *E. papillosa* appears to be seasonally driven, with lower abundance in the winter and summer and higher abundance in the fall and spring (30.5% increase), with particularly high numbers of slugs in April and May. Although water temperature was not found to be a significant factor with respect to slug abundance, the seasonality of *E. papillosa* at Sunset Beach may be tied to seasonal minimum and maximum thermal conditions, at least in shallower areas. Algal biomass did not show any relationship with the abundance of *E. papillosa* at the Sunset Beach site. The lack of association between abundance of food source and abundance of herbivore is not unexpected (Middlebrooks *et al.* 2014), and points towards the abundance of algal food sources as being a poor predictor of slug abundance.

Algal abundance also fluctuated seasonally and clear degradation of algae was visible in the field, especially during winter months. These patterns are similar to previous reports of the condition of *Penicillus* and *Halimeda* species in the Caribbean, with algal turnover rates of 4-6 weeks, with seasonal growth peaking in late summer and being reduced in midwinter (Biber and Irlandi 2006). Despite *H. incrassata* being the most abundant rhizophytic algae at Sunset
Beach, *E. papillosa* displayed no association with this algal species. The one slug found on a single *H. incrassata* sample was likely due to the close proximity in which *H. incrassata*, *P. capitatus*, *P. lamourouxii* grow at Sunset Beach. The close spatial association of the two *Penicillus* algae should conceivably allow for a slug to move from one algal thallus to another. However, the degree to which this may occur is unknown. Furthermore, the possibility that *E. papillosa* has the capacity to switch hosts, which has been seen in other slug species (Trowbridge and Todd 2001, Trowbridge *et al.* 2008), has not yet been explored. Regardless, *E. papillosa*’s significantly higher abundance on *P. capitatus* and the strong positive Pearre’s *C* selectivity index supports this alga as its primary host. Accordingly, *P. lamourouxii* may also be considered a secondary host.

The host preference of *P. capitatus* by *E. papillosa* does not necessarily reflect the dietary preference of *E. papillosa* in situ. Previous studies have shown *E. papillosa* will consume and sequester the chloroplasts of *P. capitatus* (Curtis *et al.* 2010, Curtis *et al.* 2015, Krug *et al.* 2016), but currently no evidence has been shown that *P. lamourouxii* was consumed by the slug. Sacoglossans exhibit specific morphological characteristics, particularly in the shape of the radular teeth, that allow them to specialize in certain food sources (Jensen 1993, 1994). The denticulated blade shape of *E. papillosa*’s radular teeth would indicate a specialization on filamentous algae like *P. capitatus* (Jensen 1993), so consumption of congener algae could be expected. However, in order to obtain a truly accurate account of the *in situ* diet of *E. papillosa*, genetic analysis of sequestered chloroplast DNA is crucial. The diets of slugs have been extensively explored using analysis of the chloroplast-encoded *rbcL* gene, as well as the chemical identities of secondary algal metabolites (Gavignon *et al.* 2000, Curtis *et al.* 2006, Baumgartner *et al.* 2009, Curtis *et al.* 2010, Händeler *et al.* 2010, Middlebrooks *et al.* 2014). Future investigations using such techniques would provide invaluable insight into *E. papillosa*’s natural feeding preferences outside of their host associations.
Two other sacoglossans associated with rhizophytic algae, *Elysia velutinus* and *Cyerce antillensis*, were also found during collections at Sunset Beach. *Elysia velutinus* is also kleptoplastic, and is reported to be associated with *Halimeda* species (Gavignon *et al.* 2000, Krug *et al.* 2016). *Cyerce antillensis*, which is not kleptoplastic, can be found on both *Penicillus* species in tandem with *E. papillosa* (Jensen 1993, Krug *et al.* 2016). Since *E. papillosa* and *E. velutinus* do not share hosts or food sources, it is unlikely that they experience interspecific competition. However, the possible interactions and competition between *E. papillosa* and *C. antillensis* are unknown. It has been shown that there can be indirect effects between herbivores utilizing the same host (Long *et al.* 2007, Ali and Agrawal 2014). Trowbridge *et al.* (2009), however, found no evidence of interspecific interactions between multiple sacoglossans that utilized the same algal species, and even the same individual algal thalli, as food sources, but these interactions have not been widely explored. The difference in kleptoplastic ability between *E. papillosa* and *C. antillensis* may also play a role in the extent of their interactions and may merit further investigation.

Although *E. papillosa*’s photosynthetic ability and longevity have been previously measured (Curtis *et al.* 2010, 2015), their phototactic behavior has not. In this study the slugs showed an overall lack of preference for either light or dark conditions. This lack of behavioral response suggests no influence on *E. papillosa*’s behavior from photosynthetic activity. However, this conclusion is based on experiments run using fully-fed *E. papillosa*. All slugs that were tested had been allowed to eat algae for seven days before the phototaxis experiments took place. This, presumptively, allowed the slugs to refresh their sequestered chloroplast supply with fully-functional chloroplasts. If the photosynthetic abilities of *E. papillosa* had a positive phototactic effect on its behavior, the slugs could have been expected to maximize their photosynthetic productivity by spending more time in the light. Alternatively, since *E. papillosa* has such a relatively short time frame for chloroplast functionality, the slug may have displayed
the opposite behavior and spent more time in the dark in order to maximize the longevity of the chloroplasts. There is evidence of slugs with longer-lived chloroplasts displaying shifts in foraging behavior over time due to chloroplast degradation and reduced photosynthetic productivity (Middlebrooks et al. 2011). Investigating whether starvation can affect the foraging behavior of *E. papillosa* would aid in predicting such behavior in other kleptoplastic slugs in the field, a subject that has been poorly studied (Marín and Ros 1992).

As one of the best-known examples of specialist marine herbivores (Poore et al. 2007), sacoglossans present a unique opportunity to study the ecological and behavioral characteristics that accompany such strong relationships between food source and herbivore. A greater understanding of these characteristics will, in turn, lead to a greater understanding of the evolutionary relationship between herbivore and food source. This study provides descriptive ecological information for one such herbivore that has up to now been mostly ignored despite it being one of the most numerically abundant elysiid in the Caribbean (Krug et al. 2016). As a result, this study serves as a basis for future research on behavior, host range, phylogeny, and evolutionary history of not only *E. papillosa*, but other sacoglossans as well.

**References**


Figure 2.1. Abundance of *Elysia papillosa* from October 2014 to September 2015. Standard error bars provided. $N = 8$ for each sample except April 2015 ($n = 7$).
Figure 2.2. Mean algal biomass (g dry weight m$^{-2}$) for the three algal species, *Penicillus capitatus* (solid line), *Penicillus lamourouxii* (dashed line), and *Halimeda incrassata* (dotted line) from October 2014 to September 2015.
Figure 2.3. Mean number of *Elysia papillosa* m$^{-2}$ as a function of algal biomass (g dry weight m$^{-2}$) for (A) total combined algae, (B) *Penicillus capitatus*, (C) *Penicillus lamourouxii*, and (D) *Halimeda incrassata*. Data from algal collections made from October 2014 to September 2015. X-axes vary between algal species. $N = 20$ for each algal species.
Figure 2.4. (A) Percent composition of algal biomass (g dry weight) from April to June 2016. N = 100 stalks of each species of algae. Number above bar represents total algal biomass (g dry weight) collected on each date. (B) Proportion of Elysia papillosa found on each algal species from April to June 2016. Number above bar represents number of slugs collected on corresponding date. Black = Halimeda incrassata, white = Penicillus lamourouxi, grey = Penicillus capitatus.
Figure 2.5. Number of *E. papillosa* as a function of algal biomass (g dry weight) for collections made from April to July 2016. Scale of x-axes adjusted for both species of algae. Regression lines and $R^2$ values provided.
Figure 2.6. Range of time (mins) spent by *E. papillosa* (*n* = 21) in light and dark conditions. The minimum (0 mins) and maximum (180 mins) time spent by the slugs in each condition were identical. Median time spent in the light was 64.44 mins (IQR = 20.99 – 176.51), while the median time spent in the dark was 115.56 mins (IQR = 3.49 – 159.01).
APPENDIX A:

DIAGRAM OF PHOTOTAXIS CHAMBER

Figure A1. Diagram of phototaxis chamber used during phototaxis experiment.
APPENDIX B:

ABUNDANCE OF *ELYSSIA PAPILLOSA* AS A FUNCTION OF SEA TEMPERATURE

**Figure A2.** Total number of *Elysia papillosa* per sample date as a function of sea temperature (°C). Data from algal collections made from October 2014 to September 2015.
APPENDIX C:

PERMISSION TO REPRODUCE FIGURES FROM ORTEA ET AL. (2005)

Alejandro de Vera Hernández

De: William Gowacki [gowacki@mail.usf.edu]
Enviar el: miércoles, 10 de mayo de 2017 10:39
Para: Alejandro de Vera Hernández
Asunto: SPAM Solicitud de permiso de imagen

Buenos días,

Mi nombre es Bill Gowacki, un estudiante graduado en el Universidad del Sur de Florida. Te estoy contactando hoy porque necesito tu permiso para usar imágenes de varias figuras de una de sus publicaciones de Vieraea. Recientemente recibí tu permiso para usar estas imágenes de Ortea et al. (2005) en mi propia publicación (Figuras 3, 6, 8b, y 8d), que se adjunta. Sin embargo, mi universidad requiere que yo reciba su permiso para utilizar también estas figuras (2a, 2d, 4b, y 4g) de Ortea et al. (2005), que también he adjuntando, en mi tesis de posgrado. Muchas gracias por tu pronta asistencia en este asunto.

--

Bill Gowacki
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"I accept chaos, I'm not sure whether it accepts me."
- Bob Dylan
Figura 2.- *Elysia papillosa* Verrill, 1901: Detalle de los vasos del manto en un ejemplar fijado (A); dientes en el asca (B); pene (C); dientes radulares, último ascendente y primero descendente (D).
Figura 4.- *Elysia patina* Marcus, 1980: Vista dorsal de un animal vivo de 12 mm con los parapodios abiertos (A); forma de los rinóforos cuando se desplaza (B) y en reposo (C); suela del pie y cabeza (D); detalle de las papilas del cuerpo y borde de los parapodios (E); detalle de los vasos del manto en el animal fijado (F); dientes radulares último ascendente y primero descendente (G); esitete peneal (H).
APPENDIX D:

PERMISSION TO REPRODUCE FIGURES FROM MARCUS AND MARCUS (1967)
AND MARCUS (1980)

William Gowacki <gowacki@mail.usf.edu>
to Geoffrey

Good afternoon,

My name is Bill Gowacki. I am a graduate student at the University of South Florida. A few years ago I requested and received permission to use figures found in publications from the UM Institute of Marine Science for use in my own publication (attached). I have since also included these findings in my graduate thesis. However, the University now requires me to receive additional permission to use these figures in my thesis. The figures in question are:

Figure 23 (pg 26) from "American Opisthobranch Mollusks" in Studies in Tropical Oceanography No. 6 by Eveline and Ernst Marcus.

And Figures 9, and 41-42 (pgs. 57 and 71 respectively) from:


Thank you very much for your assistance in this matter.

Shideler, Geoffrey Scott
to me

Bill,

No problem to use them in your thesis. Please simply make full reference and citation to the original Bulletin of Marine Science article, as before.

Good luck.

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Geoffrey & Shideler
Assistant Editor
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University of Miami