

# SOLAR POWERED SLUGS: BIOLOGY OF SACOGLOSSANS ON MO'OREA, FRENCH POLYNESIA

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*Abstract.* Sacoglossans are the only metazoans on earth to exhibit kleptoplasty, or the ability to retain functional chloroplasts from the algae they consume. While these unique herbivores have sparked interest in ecological and evolutionary studies, many aspects of their distribution and biology in tropical regions remain poorly understood. Sacoglossan diversity was surveyed on Mo'orea, French Polynesia to understand distribution, host associations, and abundance of sacoglossans in tropical marine environments. In addition, habitat preference experiments were conducted on *Elysia pusilla* and *Elysia stylifera*, two species co-occurring on calcareous algae, *Halimeda*. The role of kleptoplasty in the fitness of three sacoglossans with short-term chloroplast retention was quantified through light and starvation experiments. Diversity survey and habitat preference experiments revealed that ecological factors, rather than presence of sufficient food sources, have greater influence on the distribution and abundance of sacoglossans. Starvation experiments showed that the role of photosynthesis may confer momentary fitness advantages, but does not play a major role in the life-history of sacoglossans studied. Results indicate that the generalities of the biological and ecological aspects of this group remain unstable.

*Key words:* *Sacoglossa; kleptoplasty; biodiversity; habitat preference; competitive interactions; Mo'orea, French polynesia*

## INTRODUCTION

The Sacoglossa (Mollusca: Opisthobranchia) are a unique group of marine gastropods that comprise of at least 300 species (Jensen 2007). Most species are suctorial herbivores that feed on algae, while 2-3 species feed on opisthobranch eggs (Jensen 1993). Many species in this group exhibit kleptoplasty, or the ability to retain chloroplasts from another organism (Rumpho et al. 2006). The chloroplasts may remain functional from a few hours to several months, allowing the organism to use photosynthetic metabolites as an energy resource (Ros and Marin 1992). Due to this trait, they are able to display cryptic coloration when living on their food source (Händeler et al. 2009). Coupled with their apparent low densities in the field and small size (<30mm), species of this group are often overlooked by collectors and researchers (Millen 1980).

While sacoglossans have sparked much research in evolution and ecology, current

faunal lists greatly underestimate the amount of biodiversity in this group. (Carlson and Hoff 2003). Basic information such as distribution and taxonomy are unstable, with many species remaining undescribed and other species becoming synonymized (Millen 1980, Jensen 2007, Wirtz and Anker 2009). Only half of the identified species (n=94) of sacoglossans found in Guam and the Commonwealth of the Northern Marianas have been formally described (Carlson and Hoff 2003). The amount of activity in documenting the biology and diversity of sacoglossans is low, with the 10 most productive authors formally describing nearly half of the 387 named species (Jensen 2007).

Many generalizations about this group are often debated in the literature. For example, Miller (1961) suggests that these herbivores are generally restricted to habitats within the limits of the distribution of their host alga. While sacoglossans are typically referred to as specialist herbivores (Trowbridge 1992, Krug 2001), another study suggests that tropical

sacoglossans may be non-specific feeders, feeding on a variety of species because of the wide availability of different food sources (Clark and Busacca 1978). However, due to the low number of studies that investigate host associations in sacoglossans, assumptions about the group are not resolved. Additionally, the role of photosynthesis in the fitness of many species remains unknown. Typically, studies focus on species with long-term chloroplast retention, which represent only a small proportion of sacoglossan diversity (Hinde and Smith 1975, Casalduero and Muniain 2008). Thus, many areas of sacoglossan biology remain poorly studied, with many scientists calling for greater research in areas such as feeding ecology, biogeography, and taxonomy (Williams and Walker 1999, Jensen 2007).

Much less is known about the biology and distribution of tropical sacoglossans (Jensen 1992). Many of the gaps in sacoglossan biogeography lie within the Indo-West Pacific region (Jensen 2006). Tropical islands like Mo'orea, French Polynesia offer an interesting opportunity to study and describe the biology of undocumented biodiversity. Characterizing the diversity and biology of sacoglossans on Mo'orea would lay a foundation for future investigations in host-herbivore interactions, co-evolutionary relationships, and further ecological ventures.

The first component of this study was to survey the diversity of sacoglossans on Mo'orea and characterize host associations. Green macroalgae from different habitats were sampled to document the distribution and abundance of sacoglossans. Sacoglossans are hypothesized to be limited to their algal food source, and are expected to have varying degrees of host preference depending on the species of algae. The second component of this study investigated the habitat preference of two sister species recovered during sampling of *Halimeda* spp. A recent phylogeny showed that *Elysia pusilla* (Bergh, 1872) and *Elysia stylifera* (Jensen, 1997) are each other's closest living relative (Händeler et al. 2009). Interspecific interactions are hypothesized to be the factor that explains the distributional pattern of these two elysiid species. The final goal of this study determined the influence of

photosynthesis on the fitness of *E. stylifera*, *Ercolania* Species A, and *Ercolania* Species B, species that have short chloroplast retention times. The effects of light and starvation were tested on fitness parameters measured as changes in mass, fecundity, and survival to quantify the benefits of kleptoplasty. It was hypothesized that photosynthesis would confer a benefit in fitness and survival during periods of starvation.

## METHODS

### *Study site*

The present study was conducted in reef flat and bay habitats on Mo'orea, French Polynesia (Fig. 1). Mo'orea is apart of the Society Island Archipelago located in the South Pacific. Three sites were located in reef flat habitats, located in adjacent proximity to the boat channel that connects Cook's Bay and Opunohu Bay (Fig. 1, Table 1: sites 1-3). All habitats consisted of primarily sandy substrate, with large, live and dead coral heads. One site was located on a degraded reef flat in Cook's bay (Fig. 1, Table 1: site 4). The substrate was mostly silt and sand, with very dispersed live and dead coral heads. This site is normally flooded with freshwater input from streams and man-made channels after rainstorms. The water was generally turbid. The last site was located at the edge of a fringing reef at Vaiare Bay (Fig. 1, Table 1: site 5). Substrate was primarily sandy with dispersed live and dead coral heads.

### *Diversity survey*

Survey data was obtained between October 11th, 2010 to November 11th, 2010. At each site, green macro-algae was collected and identified to the lowest taxonomic level possible based on Payri et al. (2000). For each algal sample, a wet weight was obtained. Each algal sample was thoroughly shaken in fresh seawater through a filter, and the species and number of slugs per sample were recorded. Voucher specimens of algae were collected and deposited into the University Herbarium, University of California, Berkeley. Data from all sites were combined, and a relative



Figure 1. Map depicting the location of the 5 field sites sampled for green macro-algae on Mo'orea. Numbers correspond to GPS coordinates and field site names on Table 1.

abundance (A) of each sacoglossan species on each species of algae was calculated with the following formula:

$$A = \text{wet weight of algae (g)} / \# \text{ of individuals}$$

Sacoglossans were identified to the lowest taxonomic level possible by Dr. Patrick Krug from Cal State University, Los Angeles, and Dr. Cynthia Trowbridge from Oregon State University. Voucher specimens were collected for future genetic analyses.

#### Habitat Preference

To assess habitat, or substrate, preferences of *E. pusilla* and *E. stylifera* in the field, a chi-square test was used to analyze the relative abundance of both species on the 5 species of *Halimeda*.

To assess the substrate preference of each species excluding environmental limitations, individuals (n = 14) of each species were placed into a container with 5 species of *Halimeda*. *Halimeda* spp. used were: *H. incrassata*, *H. taenicola*, *H. distorta*, *H. discoidea*, and *H. opuntia*. After two days, the number of individuals on each algal species was scored. The experiment was replicated three times with the same 14 individuals, but individuals were starved for 24 hr before presented newly collected algae. If individuals looked

Table 1. List of GPS coordinates for each study site.

Site #	Longitude	Latitude	Site Name
1	149° 50' 48" S	17° 29' 07" W	Opunohu Fringing Reef
2	149° 50' 51" S	17° 28' 54" W	Opunohu Barrier Reef
3	149° 50' 34" S	17° 28' 47" W	Opunohu Barrier Reef
4	149° 49' 24" S	17° 30' 12" W	Cook's Bay
5	149° 46' 34" S	17° 31' 29" W	Vaiare Bay

unhealthy or died during the experiment, they were not included in the total for the replicate, and were replaced following replication.

To assess the effects of competitive or inhibitory interactions between species on substrate preference, individuals (n = 7) of each species were placed together into a container with the same 5 species of *Halimeda* and scored after 2 days. Individuals were starved for 24 hr before replication and the experiment was replicated 4 times. The percentage of substrate preference for both treatments was calculated, arcsin square-root-transformed, and analyzed using a 2-way ANOVA.

#### Quantifying short-term kleptoplasty

Short-term kleptoplasty was defined as chloroplast retention time of 1-2 wks. *E. stylifera* was observed to retain chloroplasts for that amount of time. Specimens were collected from various reef flat habitats around the island. To quantify the benefit of photosynthesis, weight changes were tracked over an 18 d period. Individuals (n = 9) were placed into one of two treatments: A) starved under a regular photoperiod (12 hr light, 12 hr dark) and B) starved under complete darkness. For each experimental treatment, slugs were in individual replicate dishes. The light treatment was obtained by suspending a 75W fluorescent light bulb ~1 m above the dishes. Slugs were blotted dry and weighed to the nearest mg. Individual weight gain/loss in both treatments were traced over time. Slugs

were weighed at the beginning of the experiment to obtain an original mass and weighed every 3 d after to minimize the frequency of stressful drying conditions. A percentage weight change for each weighing event was calculated, and subsequent data was log-transformed and analyzed with a repeated measures ANOVA.

#### Quantifying no appreciable kleptoplasty

No appreciable kleptoplasty was defined as chloroplast retention that lasted 1-3 days. Individuals of *E. Species A* and *E. Species B* were observed to exhibit this trait. Specimens were collected from various reef flat habitats around the island. Individuals (n = 7 for *E. Species A*, n = 10 for *E. Species B*) were placed into replicate dishes for each treatment. The four treatments were: A) starved under a regular photoperiod (12 hr light, 12 hr dark), B) starved under complete darkness, C) fed under a regular photoperiod, and D) fed under complete darkness. The light treatment was obtained by suspending a 75W fluorescent light bulb ~1 m above the dishes. Fecundity and survival rate were scored every day for ~3 wks. Experiments were terminated when a majority (>90%) of individuals in all starved treatments died. Total number of eggs laid by each individual were summed for the first 10 days and analyzed using a 2-way ANOVA. Survival rate was analyzed for starved treatments using a LogRank test.

## RESULTS

### Diversity Survey

12 species of green macro-algae were sampled and 7 species of sacoglossans were encountered (Appendix A). 6 species of algae did not yield any slugs. *Elysia obtusa* (Baba 1938) was found on *H. opuntia*, but was starved when recovered. Both *Ercolania* spp. were found in high abundance (Species A: A=2.2, Species B: A=1.1) on *Boodlea kaeneana* (Appendix A). *Oxynoe viridis* (Pease 1861) and *Volvatella ventricosa* (Jensen & Wells 1990) were found on *Caulerpa serrulata*.

### Habitat Preference

In field surveys, *E. pusilla* preferred *H. incrassata* and *E. stylifera* preferred *H. opuntia* to all other *Halimeda* spp. (Table 2, Chi-square test: DF = 4,  $\chi^2 = 402.65$ ,  $p < 0.0001$ ). *E. pusilla* was found on all 5 species and *E. stylifera* was never found on *H. discoidea*, *H. incrassata*, and *H. taenicola*. *E. pusilla* and *E. stylifera* were rarely found to co-occur on the same clump of *Halimeda*.

Under laboratory conditions, *E. pusilla* preferred *H. taenicola* and *H. discoidea* to all other species (Fig. 2, 2-way ANOVA: F = 3.54,  $p < 0.05$ ). *E. stylifera* did not have an inhibitory

Table 2. Relative abundances (A) of *Elysia* sp. on *Halimeda* spp. from field surveys.

Algal Species	<i>E. pusilla</i> algae (g) to slug ratio	<i>E. stylifera</i> algae (g) to slug ratio
<i>H. distorta</i>	151	377.8
<i>H. opuntia</i>	200.2	120.1
<i>H. discoidea</i>	157.1	0
<i>H. incrassata</i>	8.8	0
<i>H. taenicola</i>	82.3	0

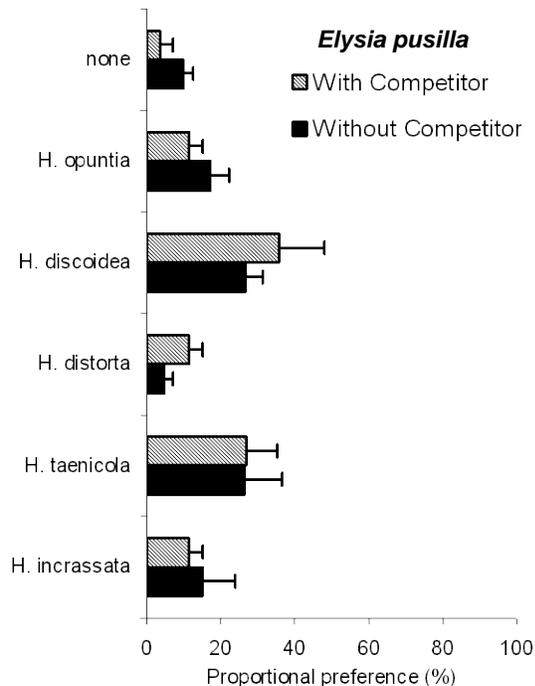


Figure 2. Habitat preference of *Elysia pusilla* on *Halimeda* spp. Data are mean preference percentages (+Standard Error, n=4 with competitor, n=3 without competitor).

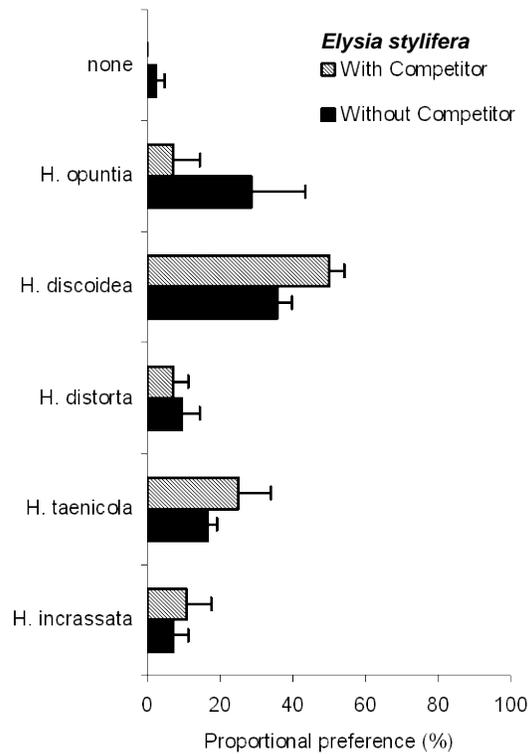


Figure 3. Habitat preference of *Elysia stylifera* on *Halimeda* spp. Data are mean preference percentages (+Standard Error, n=4 with competitor, n=3 without competitor).

effect on the preferences of *E. pusilla* (Fig. 2, 2-way ANOVA:  $F = 0.27$ ,  $p = 0.61$ ). *E. stylifera* was able to live and feed on all 5 spp. of *Halimeda*, but preferred *H. discoidea* over all other species (Fig. 3, 2-way ANOVA:  $F = 7.61$ ,  $p < 0.0001$ ). The presence of *E. pusilla* did not have inhibitory effects on the preferences of *E. stylifera* (Fig. 3, 2-way ANOVA:  $F = 0.45$ ,  $p = 0.51$ ).

#### Short-term kleptoplasty

There were no significant differences between light and dark treatments (Fig. 4, Repeated measures ANOVA:  $F = 0.74$ ,  $p = 0.19$ ). Individuals in both treatments gained mass proportionally in the first 6 days, and then slowly decreased for the rest of the experimental period due.

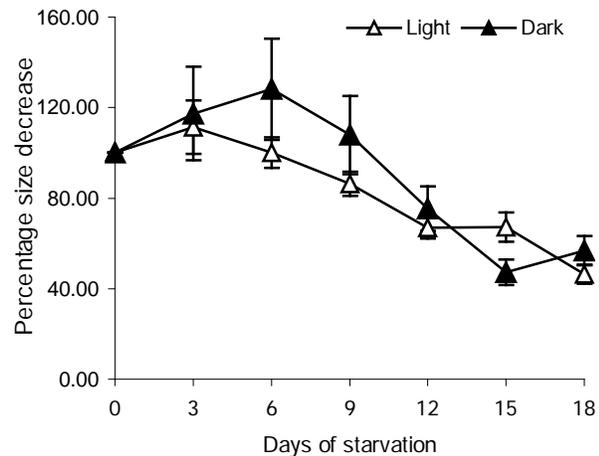


Figure 4. Effect of starvation under light (12 hr light; 12 hr dark) and dark treatments on *Elysia stylifera*. Mean percentage rate of size decrease ( $\pm$  Standard Error,  $n = 9$ ).

#### No appreciable kleptoplasty

During the experiment, 1 replicate from the light, fed treatment and 2 replicates from the light, starved treatment for *E. Species B* were contaminated and not used in data analysis.

There was no significant difference in fecundity between light and dark treatments

#### Ercolania Species A

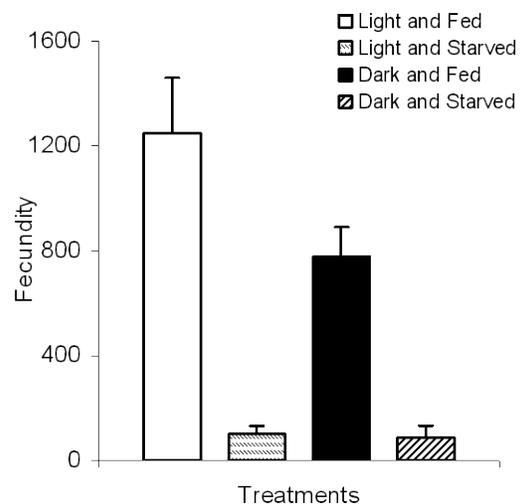


Figure 5. Mean number of eggs laid within a 10 d period (+ Standard Error,  $n = 7$ ) for *Ercolania Species A* under fed/starved, light/dark treatments.

### *Ercolania* Species B

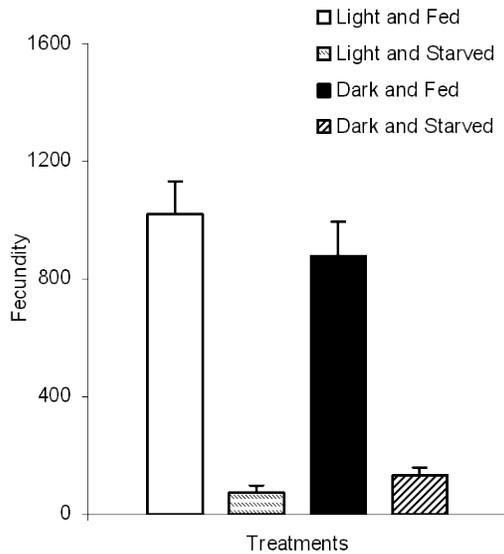


Figure 6. Mean number of eggs laid within a 10 d period (+ Standard Error, n = 9 for light, fed; n = 8 for light, starved; n = 10 for other treatments) for *Ercolania* Species B under fed/starved, light/dark treatments.

for both species (*E. Species A*, Fig. 5, 2-way ANOVA:  $F = 3.57$ ,  $p = 0.07$ ; *E. Species B*, Fig. 6, 2-way ANOVA:  $F = 0.22$ ,  $p = 0.64$ ). For both species, fecundity was lower for all starved treatments (*E. Species A*, 2-way ANOVA:  $F = 51.34$ ,  $p < 0.0001$ ; *Species B*, 2-way ANOVA:  $F = 104.46$ ,  $p < 0.0001$ ). Most slugs stopped laying egg masses after 10 d. Both species in light treatments were able to lay an average >1000 eggs after isolation from mates over a 10 d period.

### Survival rate

No individuals in fed treatments for both species died throughout the duration of the experiment. For *E. Species A*, individuals kept in a light treatment lived an average of ~3 d longer than those kept in complete darkness (Fig. 7, LogRank test:  $\chi^2 = 5.63$ ,  $p < 0.05$ ). For *Species A*, individuals in the dark and starved treatment started dying off on day 15 while those in the light treatment started dying off on day 18 (Fig. 7). For *E. Species B*, there was no difference between light conditions (Fig. 8, LogRank test:  $\chi^2 = 2.83$ ,  $p = 0.09$ ). For *Species B*, individuals in the dark and starved

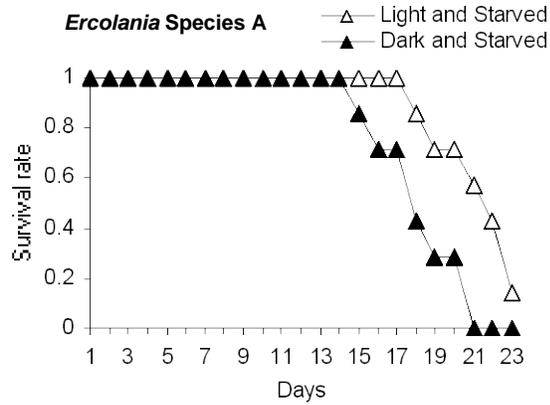


Figure 7. Effect of light (12 hr light; 12 hr dark) and dark treatments on the survival rate of *Ercolania* Species A. Data are percentages of survival (n = 7).

treatment started dying off on day 5 and individuals in the light treatment started dying off on day 8 (Fig. 8). However, all individuals did not survive past day 14.

## DISCUSSION

### Diversity Survey

Many aspects of sacoglossan biology remain debated in the literature including host specificity (Miller 1961, Clark and Busacca 1978, Jensen 2003, Krug 2001), and habitat requirements (Trowbridge 1992, Jensen 2003).

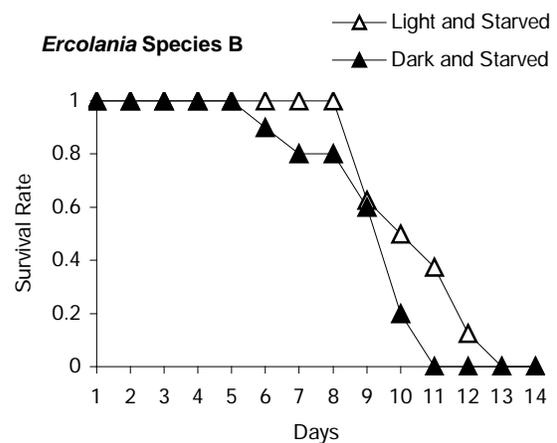


Figure 8. Effect of light (12 hr light; 12 hr dark) and dark treatments on the survival rate of *Ercolania* Species B. Data are percentages of survival (light, n = 8; dark n = 10)

While the present study attempted to address some of these issues, diversity surveys of sacoglossans on Mo'orea were not exhaustive, but general assumptions about the group as a whole can be addressed. Sacoglossans are known to be host specific or highly stenophagus, but host associations are usually not as highly specific as in insect-plant interactions (Jaenike 1990, Jensen 2003). *E. pusilla* and *E. stylifera* were found to feed on a wide variety of *Halimeda* spp., suggesting that interactions between sacoglossans and their food source may not be specific as species to species interactions. This compares with studies done by Clark and Busacca (1978), in which sacoglossan species studied were able to feed on at least one genus or more.

Sacoglossans were considered to be rare animals, but populations can be large in the presence of suitable habitat or abundant populations can form during certain times of the year (Trowbridge 1992, Jensen 2003). *Ercolania* spp. were found in very high densities during collections, suggesting that optimal habitat may lead to eruptive populations. Additionally, *Ercolania* spp. were observed to have lecithotrophic, or non-feeding larvae, potentially allowing rapid colonization of optimal habitats due to shortened planktonic larval phases. This is congruent with surveys of sacoglossans in Hong Kong by Jensen (2003), indicating the influence of other habitat requirements than the presence of abundant food sources.

#### *Habitat preference*

Closely related species, like *E. pusilla* and *E. stylifera*, are predicted to share similar ecological requirements, but may avoid each other along fine gradients (Pfenninger and Nowak 2008, Händeler et al. 2009). While it was proven that both species are capable of feeding on the same 5 species of *Halimeda*, they were rarely found co-occurring on the same clump of algae. Indicated by the disparity between host preferences for both species between lab experiments and field surveys, other environmental or biological factors may be shaping the distribution and abundance of slug species. Lab experiment preferences showed that both species of *Elysia*

prefer the least calcareous algae, potentially due to the increased accessibility to food from the algae due to the typically highly calcified nature of species within the *Halimeda* genus.

Since sister-species presence did not inhibit preferences, other biological factors, such as competition from other organisms living in *Halimeda* may prevent the two species from co-occurring more often. The two slug species may also have different ecological requirements that allow them to avoid competition as well. *E. pusilla* may prefer more sandy substrate habitats due to the high preference of *H. incrassata*, an algal species that prefers to grow in sandy sediment (Payri et al. 2000). Through field observations, *E. stylifera* may prefer more marginal habitats, typically found most abundant in degraded reefs. Additionally, the distribution and abundance of the different species of *Halimeda* may play a role in the relative abundances on each species of algae. Finch (2007) found that generally, only one or two species of *Halimeda* dominate reef ecosystems, which may affect the abundance of these two slug species.

#### *Benefits of short-term/no appreciable kleptoplasty*

Due to the lack of difference between light and dark treatments for *E. stylifera*, photosynthesis did not play a large role in its energetic needs. Individual weight changes generally increased during the first 2 weighing events, suggesting a potential source of error in weight measurement throughout the experiment. While *E. stylifera* has evolved structures (e.g., parapodial flaps) that allow it to photosynthesize effectively, short-term kleptoplasty may not be sufficient amount of time to confer a large benefit in fitness. However, it may confer a fitness benefit in survival calcification of algae impedes feeding (Händeler et al. 2009). Since survival was not measured, kleptoplasty could have potentially played a role in the fitness of this species.

With *Ercolania* spp., species with no appreciable kleptoplasty, both species did not show significant differences between light and dark treatments relating to fecundity, indicating that metabolites gained from photosynthesis may not be used for maternal investment, but potentially as a supplement to

their diet. Since both species showed a decrease in fecundity in starved treatments compared to fed treatments, fecundity may depend more on energy from food sources rather than photosynthetic energy.

*E. Species A* showed a slight survival advantage when under the light treatment than in the dark treatment. While the advantage is short (~3 d), and chloroplast retention is limited, kleptoplasty may confer a momentary benefit when needed by the individual. *E. Species B* did not show a difference between light and dark treatments, suggesting the lack of influence of photosynthesis in the fitness of this species. While the influence of kleptoplasty remains small in these three slug species, they may confer slight advantages in routine activities that were not measured in this study.

While many studies focus on chloroplast retention times (Evertsen 2007), few studies have looked at the role of kleptoplasty in the fitness of sacoglossans. In comparison with Casalduero and Muniain (2008), slugs in this study did not show strong signatures of advantages conferred by photosynthesis. This is due to the differences in chloroplast retention times, with *E. timida* being about to retain functional chloroplasts for 45 d (Casalduero and Muniain 2008). Although the role of kleptoplasty in the energy budgets of sacoglossans are poorly understood, this study suggests that photosynthesis may play minor roles in many sacoglossan species because there are few species that retain chloroplasts for long periods of time (Händeler et al. 2009).

### Conclusions

The patterns exhibited by the diversity survey and habitat preference experiments underscore the importance of understanding ecological and habitat factors that influence the distribution of sacoglossans. Though collectors have often sampled algae in a variety of habitats, quantifying the ecological requirements of species has received limited attention (Jensen 1997). The presence of a particular host species may have minimal effects on predicting the presence of the slug species, evidenced by the high abundance of

*Ercolania* spp. and *E. pusilla* in certain habitats in Mo'orea.

Although evolution of structures that increase the efficiency suggests that selective pressures favored benefits from kleptoplasty, individuals with short-term kleptoplasty may not be completely dependent upon photosynthetic energy (Händeler et al. 2009). However, although the benefits are variable between species, momentary advantages may be important to the ecology of sacoglossans in general. Further research in sacoglossan biology and ecology are necessary to understand the general ecological and evolutionary aspects of these unique herbivores.

### Future research

While this study attempted to understand sacoglossan biology, many aspects still remain unanswered. Habitat requirements seemed to be an important factor in driving the distribution and abundance of sacoglossan species. Investigating the habitat differences and ecological requirements between *E. pusilla* and *E. stylifera* may possibly explain the niche partitioning between these two species. In addition, *Ercolania* spp. was found in high abundance, which suggests that there may be intraspecific and interspecific interactions between individuals of both species. Sexual selection theory in opisthobranchs suggests that intraspecific mating through hypodermic insemination lowers fecundity due to the cost of mating (Smolensky et al. 2009). This could be investigated in these two species to understand how high densities play a role in shaping maternal investment.

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## APPENDIX A

Field Survey Summary. Data from all sites were combined. Algae specimens were identified using Payri et al. (2000). Slugs were identified by Patrick Krug from Cal State University, Los Angeles, and Cynthia Trowbridge from Oregon State University.

Slug Species	Algal Species	Total # Found	Total wet weight of algae	Algae (g) to slug ratio (A)
<i>Elysia pusilla</i> (Bergh, 1872)	<i>H. distorta</i>	5	755.5	151
	<i>H. opuntia</i>	3	600.5	200.2
	<i>H. discoidea</i>	2	314.2	157.1
	<i>H. incrassata</i>	35	306.29	8.8
	<i>H. taenicola</i>	2	164.53	82.3
<i>Elysia stylifera</i> (Jensen, 1997)	<i>H. distorta</i>	2	755.5	377.8
	<i>H. opuntia</i>	5	600.5	120.1
<i>Elysia obtusa</i> (Baba, 1938)	<i>H. opuntia</i> *	1	600.5	600.5
<i>Ercolania</i> Species A (undescribed)	<i>B. kaeneana</i>	43	94.98	2.2
<i>Ercolania</i> Species B (undescribed)	<i>B. kaeneana</i>	78	94.98	1.1
<i>Oxynoe viridis</i> (Pease 1861)	<i>C. serrulata</i>	5	117.74	23.5
<i>Volvatella ventricosa</i> (Jensen & Wells 1990)	<i>C. serrulata</i>	1	117.74	117.7
None	Species B <sup>1</sup>	0	22.8	N/A
	<i>H. macroloba</i>	0	124.4	N/A
	<i>Avrainvillea</i> sp.	0	8.2	N/A
	<i>Cladophora</i> sp.	0	26.1	N/A
	<i>N. umbosaeae</i>	0	0.9	N/A

\**Elysia obtusa* does not feed on *Halimeda* spp.

<sup>1</sup>Photos of unidentified algae are found in Appendix C

## APPENDIX B

Descriptions and pictures of sacoglossans. All individuals pictured are at least 4 mm in length, but no greater than 30 mm.

### Glossary:

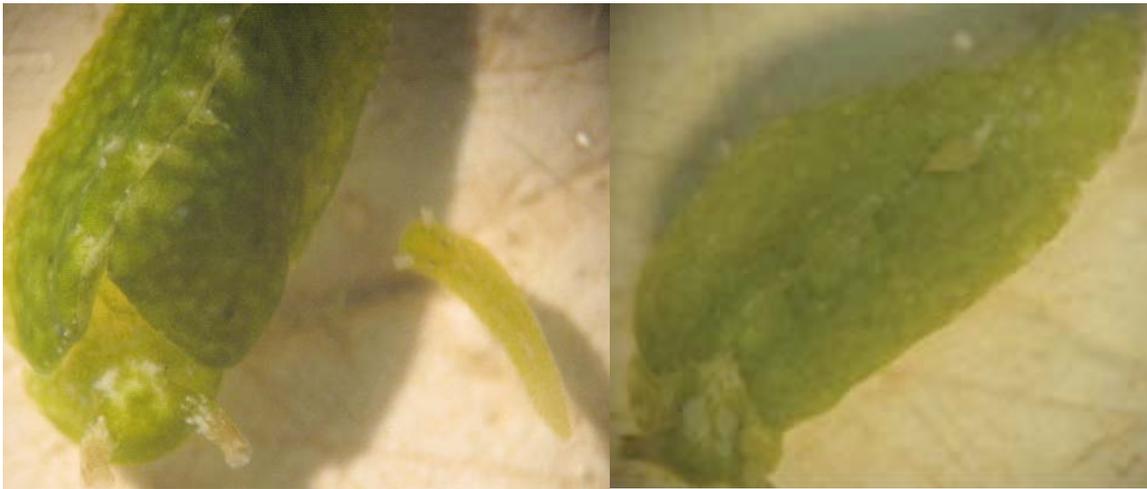
*Rhinophore*: Club-shaped structures located on the head of most sea slugs. Used for sensory (e.g., scent, taste).

*Parapodial flap*: Wing like flaps that are common among elysiid sacoglossans.

*Diverticula*: Outpouching of a fluid-filled structures. Typically, sacoglossans have extensive digestive diverticula to store chloroplasts.

*Lecithotrophy*: Non-feeding larvae, nutrients supplied through the yolk before hatching.

*Planktotrophy*: Feeding larvae, larvae, once hatched, must feed on plankton before metamorphosing.



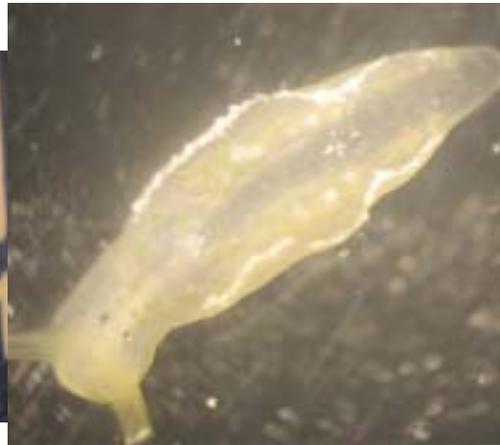
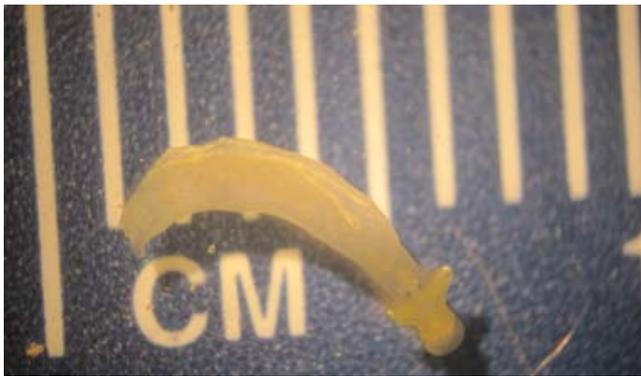
### *Elysia pusilla* (Bergh, 1872)

Description: Individuals are generally dark green, sometimes with white speckles. Parapodial flaps are partially fused. Rhinophores are rolled, and generally white in coloration. This species may potentially be poecilogonous.



*Elysia stylifera* (Jensen 1997)

Description: Individuals are generally dark green with red speckles. Color morphs range from dark green to rosy red. Rhinophores are rolled. This species lays planktotrophic egg masses with extra-cellular yolk.



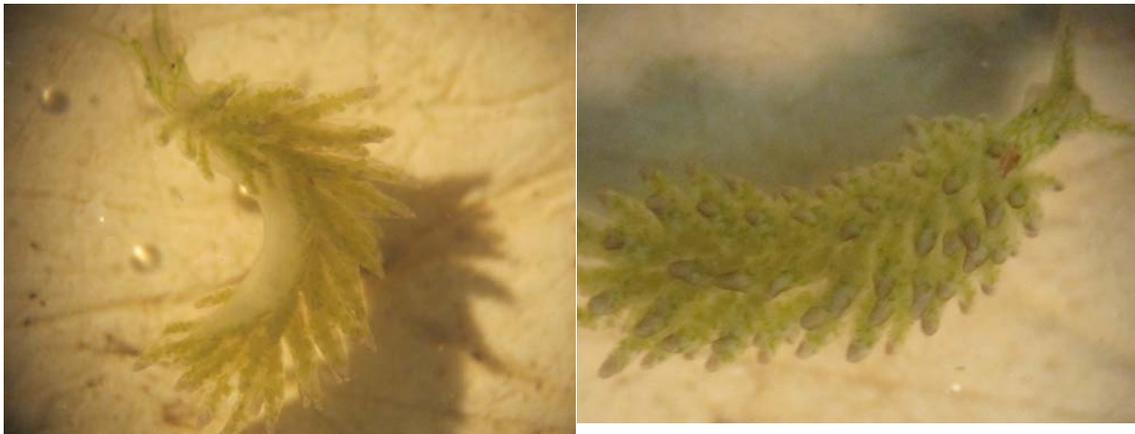
*Elysia obtusa* (Baba, 1938)

Description: Presence of parapodial flaps and rolled rhinophores. Individual was starved during collection. Adult food source is unknown. When fed, generally a pale-yellow to yellow green color.



*Ercolania* Species A (undescribed)

Description: Cerata-bearing sea slug. Rhinophores are simple in form. Diverticula does not extend into the rhinophores. Most of body is baige-clear color, while diverticula is generally dark green. This species deposits lecithotrophic egg masses.



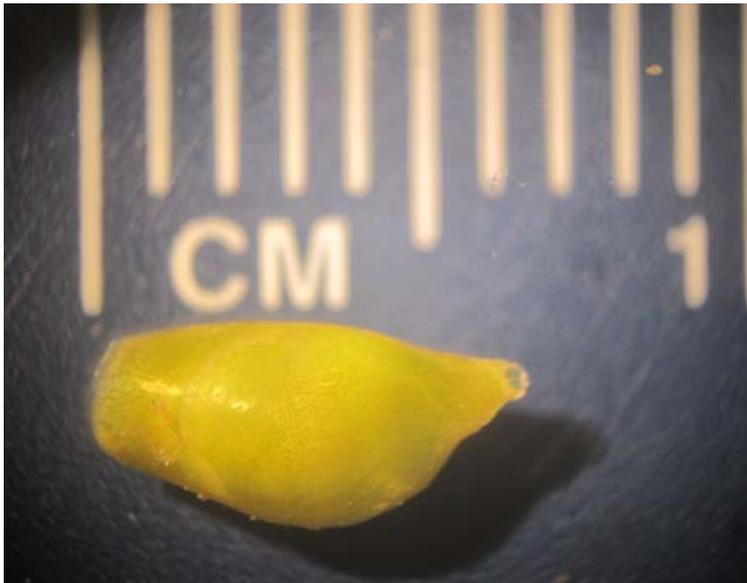
*Ercolania* Species B (undescribed)

Description: Cerata-bearing sea slug. Rhinophores are simple in form. Diverticula does extend into the rhinophores. Most of body is baige-clear color, while diverticula is generally light green. This species deposits lecithotrophic egg masses.



*Oxynoe viridis* (Pease 1861)

Description: Shelled sacoglossan species. Rhinophores are rolled, with alternating blue and yellow color pattering. Individuals are generally dark yellowish-green. This species deposits planktotrophic egg masses. Found on the leafy parts of *Caulerpa*

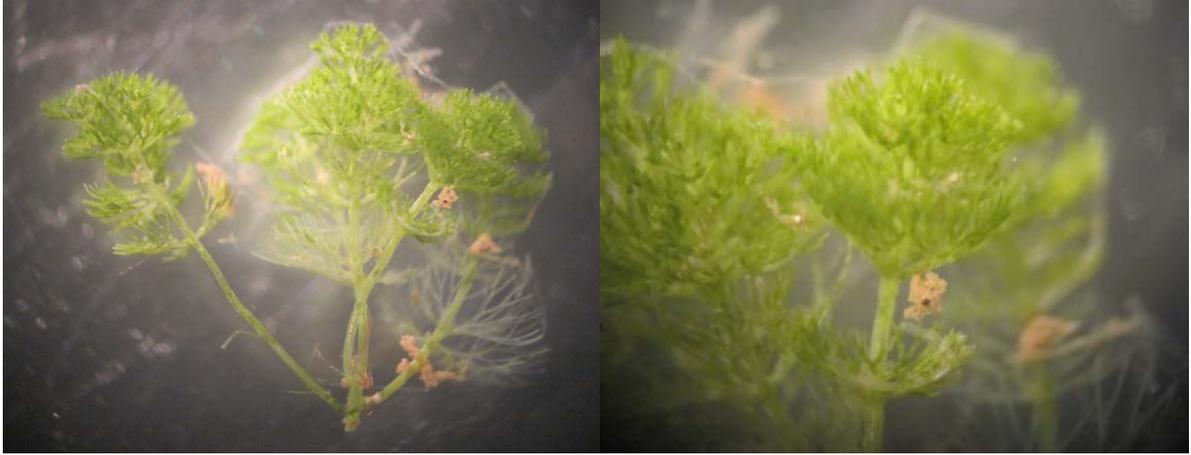


*Volvatella ventricosa* (Jensen & Wells 1990)

Description: Shelled sacoglossan species. Shell is generally yellow green, with soft tissue being white to clear coloration. Generally found on the thalli of *Caulerpa* spp.

## APPENDIX C

### Unidentified algal species



Species B: Green algae growing in whorls. Generally found in silty habitats attached to hard substrates (e.g., rocks, clam shells, etc.).

#### *Oxynoe viridis* (undescribed)

Description: Shelled sacoglossan species. Rhinophores are rolled, with alternating blue and yellow color patteration. Individuals are generally dark yellowish-green. This species deposits planktotrophic egg masses. Found on the leafy parts of *Caulerpa* spp.