Matt:

You did an excellent job in developing and organizing this study. You've obtained some good, new information and have thoughtfully discussed it.

John

Possible Effects of Chloroplast Endosymbionts on the Ecology of the Marine Slug, *Tridachia crispata*

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Abstract

The marine slug, *Tridachia crispata* sequesters endosymbiotic chloroplasts by ingestion from siphonaceous algae. The effect this has on aspects of its ecology was studied. Significant weight change was found between slugs grown under light and dark conditions. Slugs exhibit strong phototaxis when presented with a light gradient. Substrate selection by individuals may involve two factors: 1) ease ~~of~~ mobility on the substrate, 2) food quality of substrate. Day to day position of individuals was remarkably stable, though nighttime observations indicated a decrease in exposed individuals. Diurnal fish did not ~~prey~~ on *Tridachia*, though the slugs were highly palatible to the sea anemone, *Condylactis*. Possible adaptive values for many of the observations were discussed

Introduction

The marine slug, Tridachia crispata (Opisthobranchia, Sacoglossa) is a common inhabitant of the shallow reef waters in Discovery Bay, Jamaica. This organism is of primary interest due to the endosymbiosis which it establishes with ingested chloroplasts from many algae, especially the Siphonales (Trench 1973a). Much has been done with the formation and distribution of photosynthates within Tridachia (Trench, Trench, + Muscatine, 1972, Trench 1973a and 1975). Little, however, is known of its overall ecology - principally photo-related behavioral and physiological adaptations to its endosymbiotic chloroplasts. Trench (1973a and 1975) and others have shown that most photosynthetically fixed carbon compounds are translocated to the pedal gland and are integral in mucus secretion. Trench (1973a) postulates that ~~that~~ this export of photosynthetic products, in the form of a mucopolysaccharide, is an important aspect of organic matter recycling in the coral-reef ecosystem. This is one possible, direct, ecological consequence of the Tridachia - chloroplast symbiosis. It is the purpose of this study to examine some other aspects of Tridachia ecology related to this symbiosis.

Morphologically, T. crispata is distinctive among the marine slugs (Figure 1.). The dorsal surface is covered with numerous, convoluted cerata, green or blue at the base due to chloroplast pigmentation. The cerata are outpocketings of the digestive system and also increase surface area for gas-exchange across the body wall (Barnes, 1968). Colin (1978) states that, in addition to fixed carbon compounds, Tridachia obtains oxygen from its chloroplasts. The mouth parts are adapted to suctorial feeding on marine algae, the head is characterized by a large pair of rhinophores — possibly aiding in chemoreception — and the long, slender foot is closely applied to the head (George, 1979).

The unusual association of an animal with functioning plant organelles undoubtedly had effects on its ecological development. A variety of hypotheses were formulated to investigate possible photo-adaptations to this form of existence:

1) Tridachia will show weight loss when ~~grown~~ starved? in a light-deprived environment,
2.) A change in coloration of Tridachia will occur when individuals are ~~grown~~ kept in the dark,
3.) Individuals will show significant phototaxis when given a range of light conditions,
4.) The amount of time individuals spend on "non-food" substrates will be greater than that spent on substrates considered as food,
5.) Distributional patterns should change both diurnally and with the quality of preferred substrate at depth in the water column,
6.) Fish will not constitute a serious predatory threat to Tridachia

These hypotheses arise from some preliminary observations and the nature of the *Tridachia* – chloroplast relationship. Chloroplasts do not function in total darkness and their production is maximized ~~at~~ over some specific range in light intensity. From information obtained in terrestrial systems, it is evident that chloroplasts become more highly pigmented and numerous at lowering light levels, to a threshold, at which point their function degrades. This may be partially responsible for changes in *Tridachia* coloration when held at low light levels. On the reef, *Tridachia* are observed to spend the most time on filamentous algae-covered rocks near the surface and are rarely seen feeding on their preferred foods, siphonaceous algae (Trench, 1975). Presumably, they derive much nutritional benefit from their endosymbionts and thus need to "feed" only to replenish chloroplasts and essential nutrients. Nudibranch sea slugs are often found to be distasteful to fish. This may be the case here, as *Tridachia* is quite exposed diurnally on the reef and yet remains very abundant. If predation is limiting *Tridachia* at all, then a change in distribution might be expected on a diel cycle. Finally, during the day, *Tridachia* is normally exposed in what may be called its "basking mode". Differences in the available substrate may determine the optimum depth for exposing oneself.

explain

This study was conducted from February 23 – March 3, 1981 in the shallow lagoon off Discovery Bay, Jamaica. Figure 2 contains a map locating the two study sites and approximate reef profiles at those points.

Methods

Laboratory

Light/dark incubation. Twenty Tridachia were collected in 1-3 meters of water on the East Back Reef. A tank was set up beforehand to receive them (Figure 3.). The tank was square in area (62cm x 62cm x 31cm) and sea water was circulated (constant temp. = 27°C) continuously throughout the experiment. Two treatments were designed: 1) Food - contained Caulerpa and algae-covered rocks, 2) No Food. Each treatment was run in the dark and under normal light. The dark side was covered with black plastic. The light treatment was covered with clear plastic — to prevent fresh water influx. Five Tridachia, preweighed, were introduced into each treatment at 11 a.m. on February 24. They were recovered seven days later (11a.m., March 2). Weights, color condition, and condition of food resources was noted.

Light intensity gradient. A gradient of light intensity was constructed over a tank (152cm x 45cm x 31cm) using window screening and black plastic (Figure 4.). Fresh sea water was circulated continuously. Five distinct light intensity regimes were established: no screen, 1 screen, 2 screens, 8 screens, no light (black plastic). The tank was aligned north to south in order to minimize shading by tank walls. Five Tridachia were introduced under each light intensity and allowed to establish themselves over a three hour period. The number of individuals in each section was counted after the three hour period. The experiment was replicated twice, from 9-12am and 1-4pm on a clear, windy day (March 1).

Substrate selection. In the laboratory, a tank was filled with various patches of substrates — some food sources, others not: sand, *Penicillus*, *Avrainvillea*, *Caulerpa*, *Dictyota*, *Halimeda*, *Halicystis* and walls of the container (Figure 6.). The tank was used primarily as a holding basin, so the numbers of *Tridachia* in it during each sample interval varied from 8-15 individuals. The number of slugs present on different substrates was counted at varying time intervals over the course of several days.

Field

Movement patterns. In order to quantitatively assess the distributional changes of *Tridachia* in the field under differing light regimes, the depths of individuals were estimated. Day/night changes of individuals were measured by staking out eight slugs in Columbus Park and looking at their movement in 24 and 48 hours (Figure 8.). Depth of individuals was measured using a float attached to a 10 meter, weighted line marked off in .5 meter increments. In a second experiment, the distributional differences between two sites were to be evaluated — Columbus Park and the East Back Reef. The two sites differed in the microhabitat presented to *Tridachia*. Measurements of depth for individual *Tridachia* were made on random transects taken from approximately 8 meters depth to the reef crest. All measurements were made in the course of one morning.

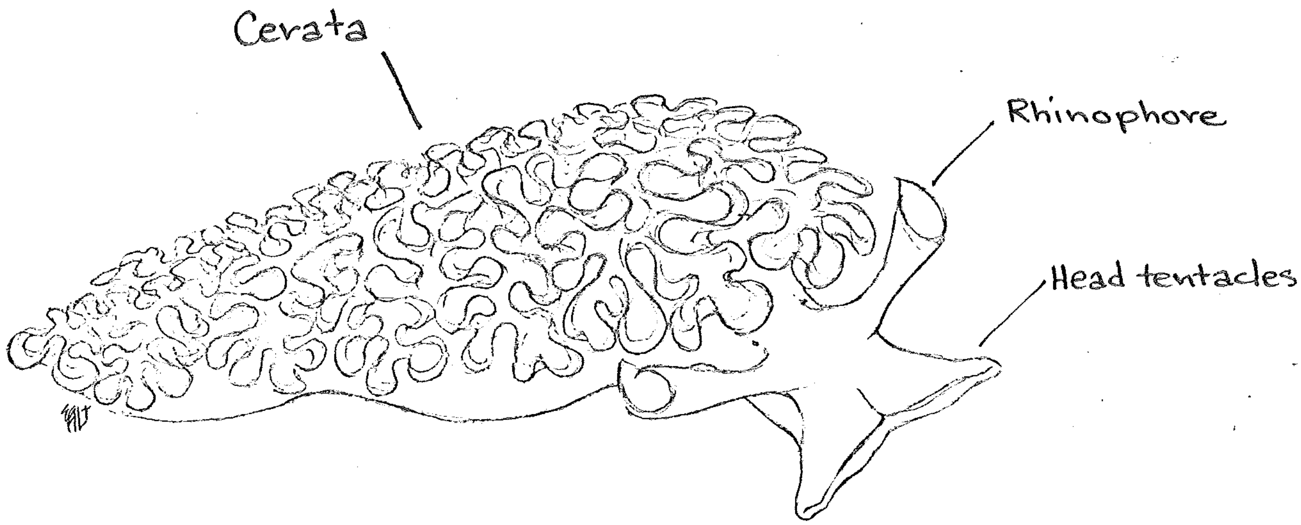


Fig. 1. Tridachia crispata. (Courtesy of Walt Cressler.)

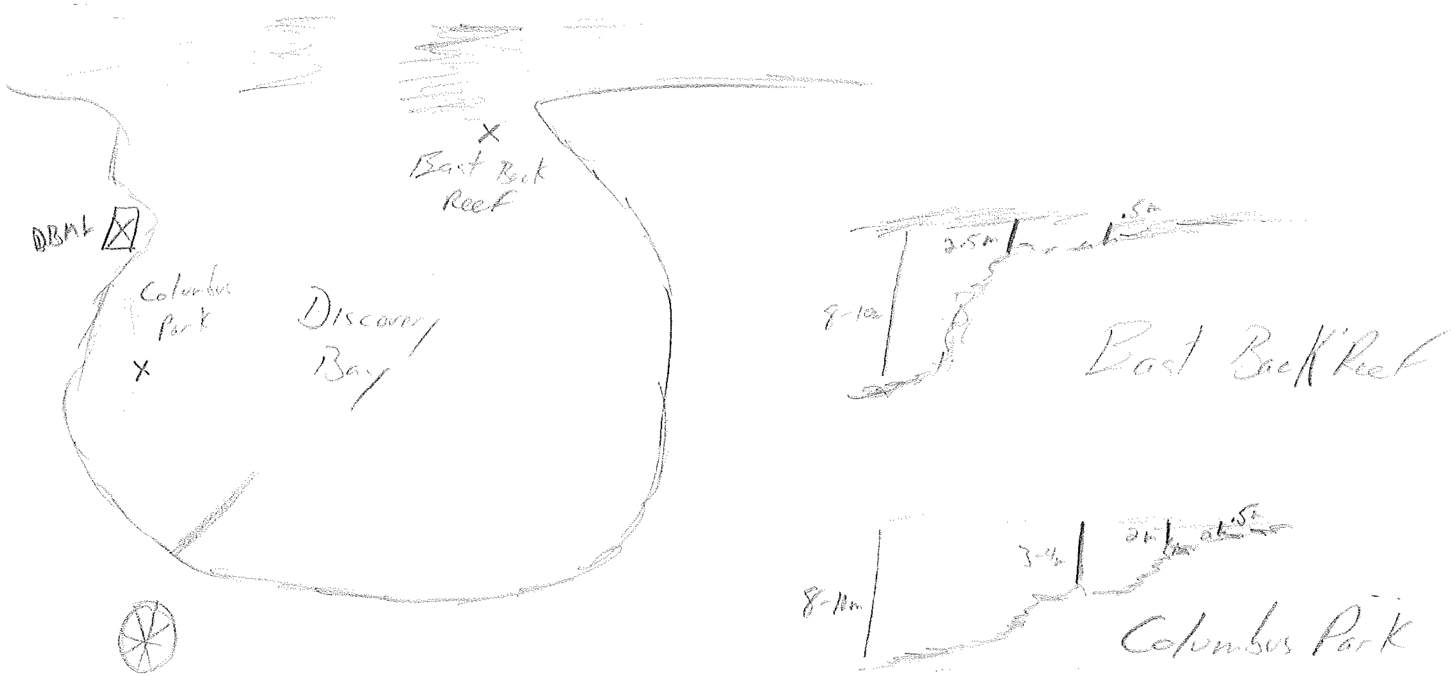


Figure 2. Study sites and approximate reef profiles

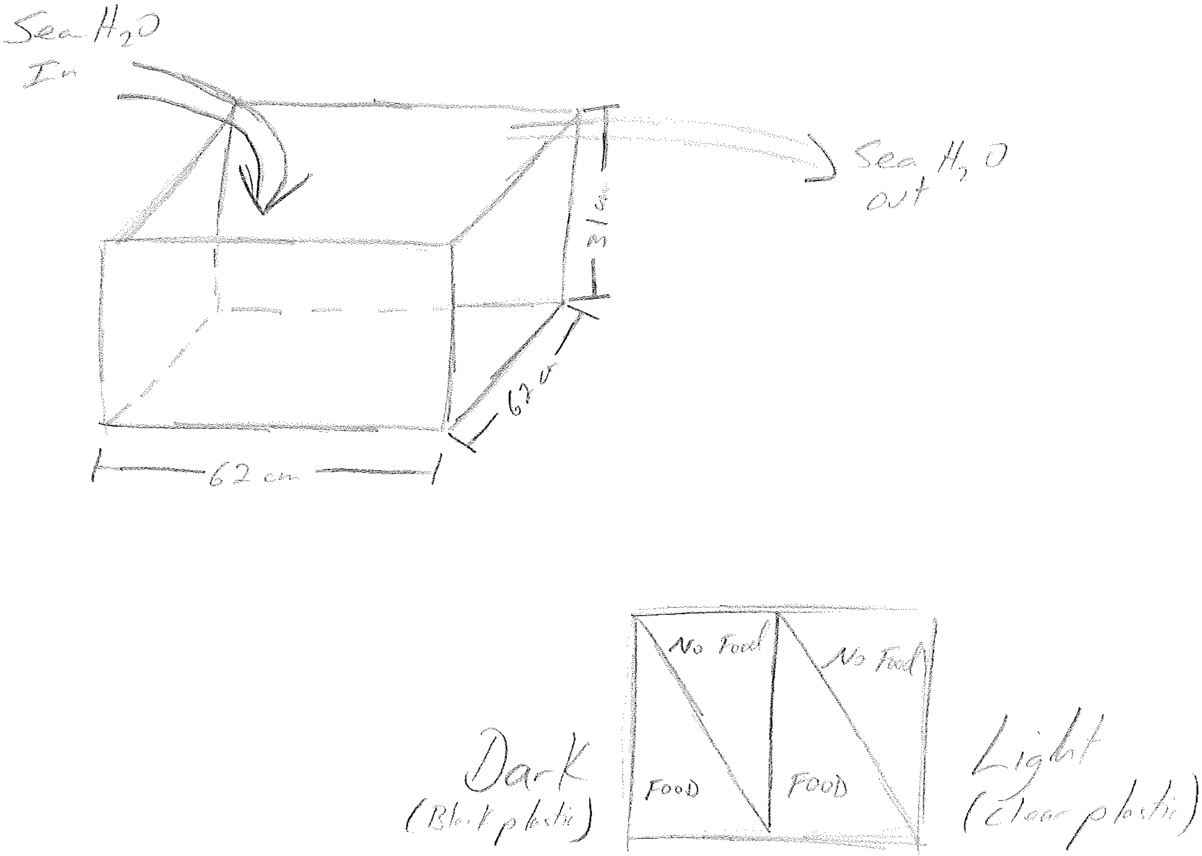
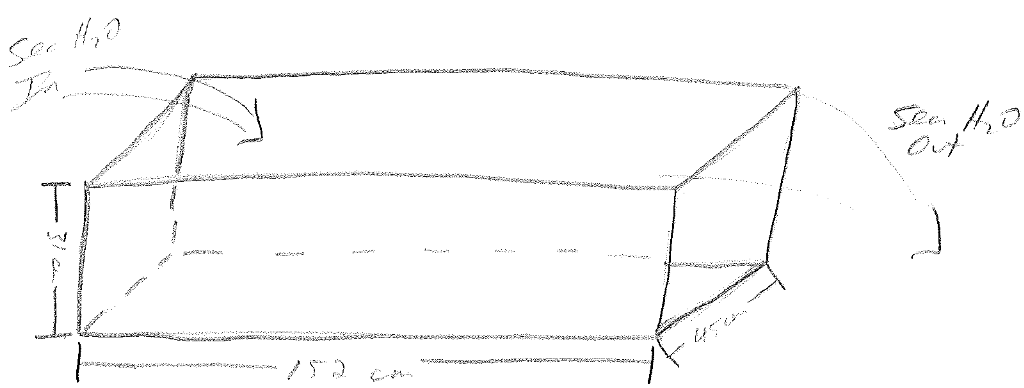


Figure 3. Experimental set-up for light/dark incubation.



Light meter conversion table.

Shutter	# Screens	F	F^2 [1]	% trans. [illegible]
1/250	0	16	256	100.0
	1	13	169	66.0
	2	9.5	90.2	35.0
	8	2.6	6.8	2.6
	∞	—	—	0.0

[1] (F^2 = exposure)

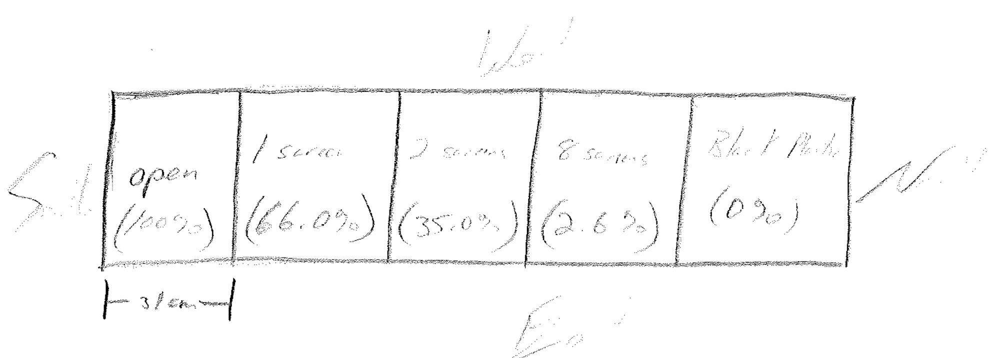


Figure 4. Design for light intensity tank.

Table 1: *Tridachia* Weight Change Data[1]

	Weight Before Incubation (2/24)		Weight After Incubation[2]	Weight Change (After − Before)
Dark	6.88 g		6.24 g	−.64 g
	6.79		5.42	−1.37
	4.05	(Food)	4.15	+.10
	3.70	(Food)	3.48	−.22
	3.28	(Food)	3.20	−.08
	2.60	(Food)	2.81	+.21
	2.53		2.41	−.12
	2.21		1.91	−.30
	1.79	(Food)	1.02	−.77
	1.22		×	—
$\bar{X}$ =	3.50 (±1.55)		3.40 (±1.66)	
Light	3.62	(Food)	4.03	+.41
	2.94		3.50	+.56
	2.48		3.30	+.82
	2.41	(Food)	2.75	+.34
	2.40	(Food)	2.55	+.15
	2.31	(Food)	2.48	+.17
	2.30		2.35	+.05
	2.25	(Food)	2.12	−.13
	2.10		×	—
	1.52		1.87	+.35
$\bar{X}$ =	2.43 (±.54)		2.77 (±.70)	

[1] The pairing of these weights may be somewhat artificial. The five individuals in each section could not be distinguished. The match-up of before/after pairs may not be exact and the scatter in weight changes would be increased to some degree. This may weaken the results of the Mann-Whitney U-test performed on the data.

[2] Two individuals ~~[illegible]~~ inexplicably disappeared at some point in the experiment.

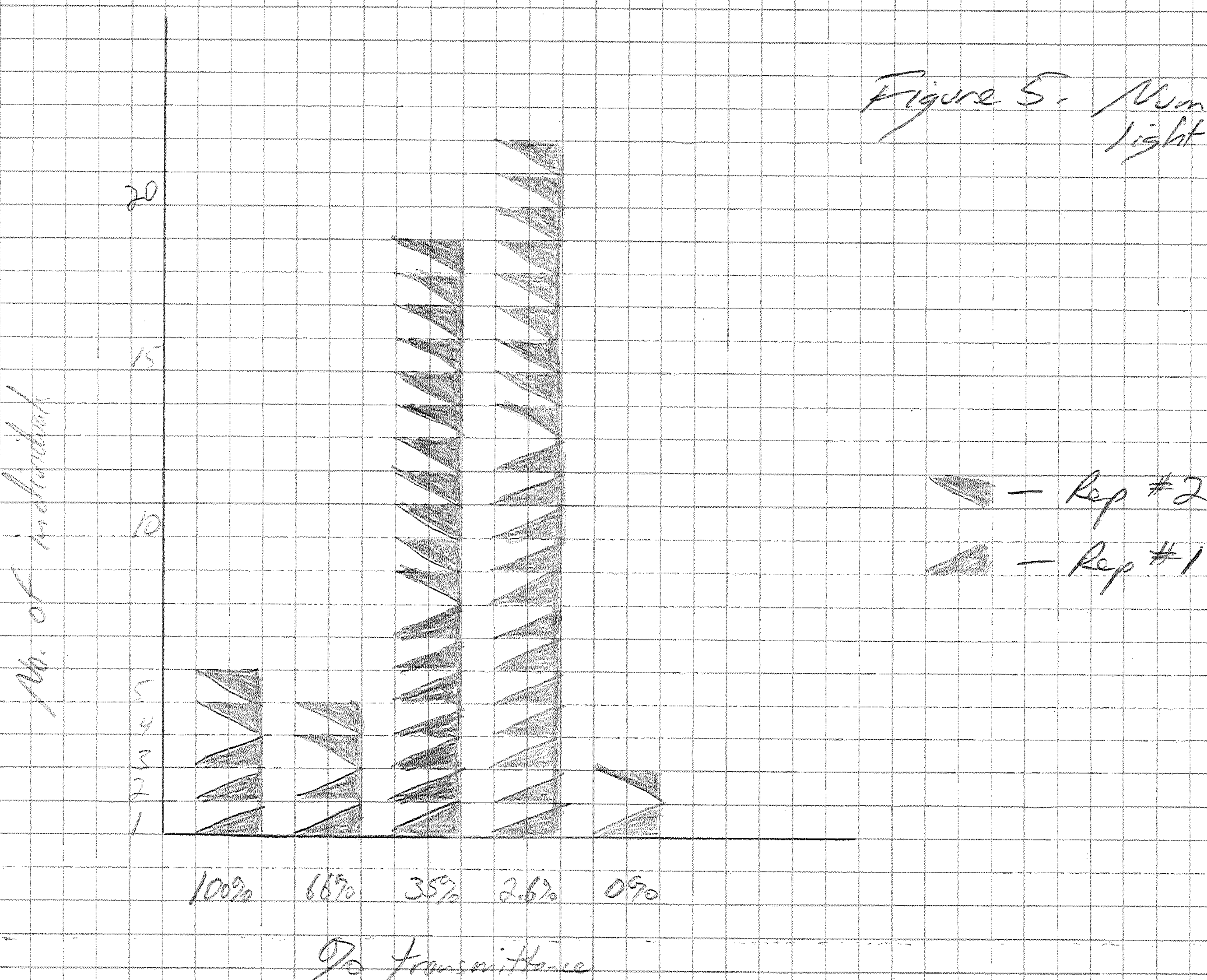


Figure 5. Number of individuals vs. light intensity (2 reps pooled)

Table 2. Data for light intensity step-gradient.

	Light intensity: Number of individuals in each section				
	Open	1 screen	2 screens	8 screens	Opaque
Rep. 1 :	3 (1-shade)	2	7	12	1
Rep. 2 :	2 (2-shade)	2 (2-shade)	11	9	1

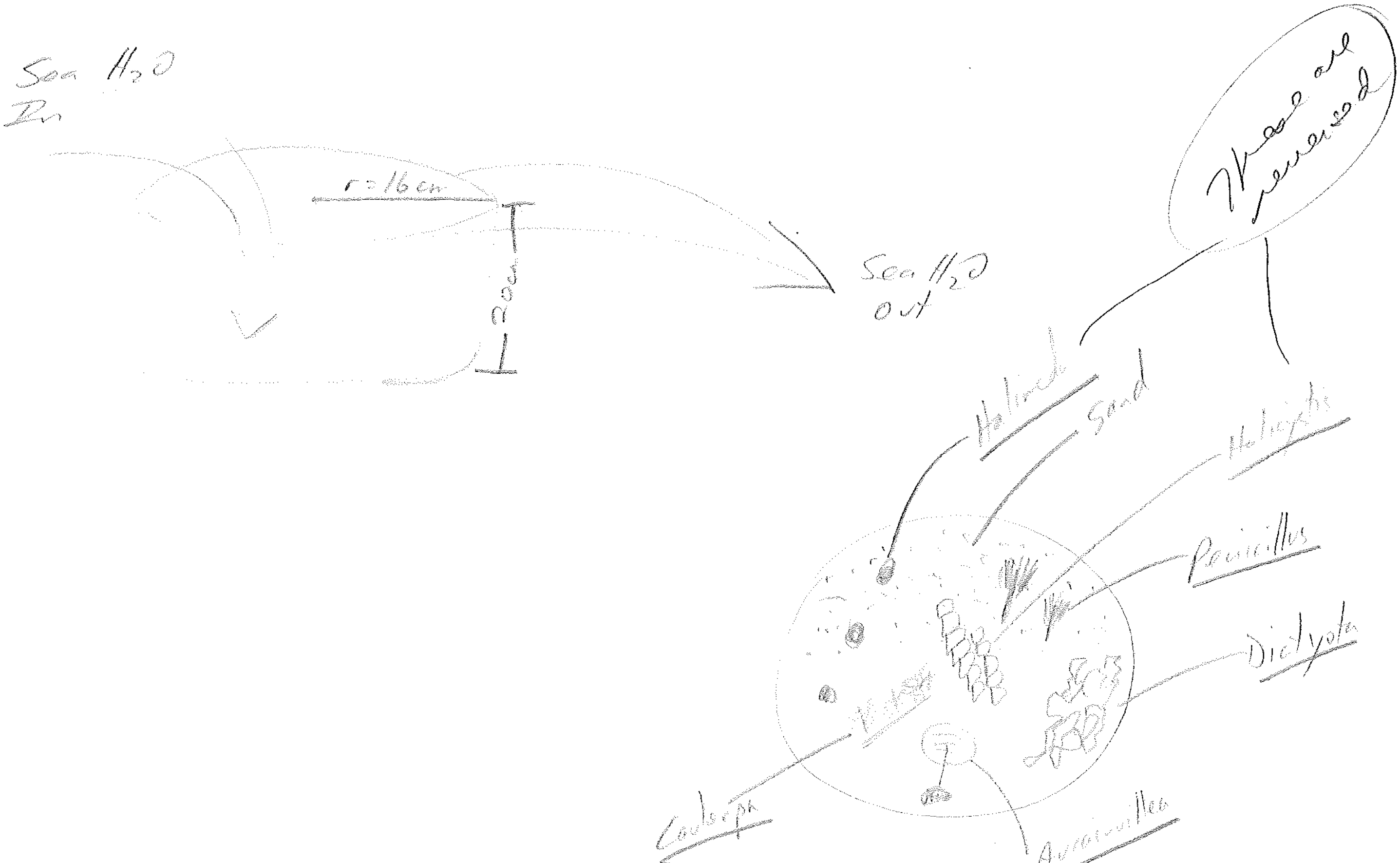


Figure 6. Tridachia holding basin with different substrates.

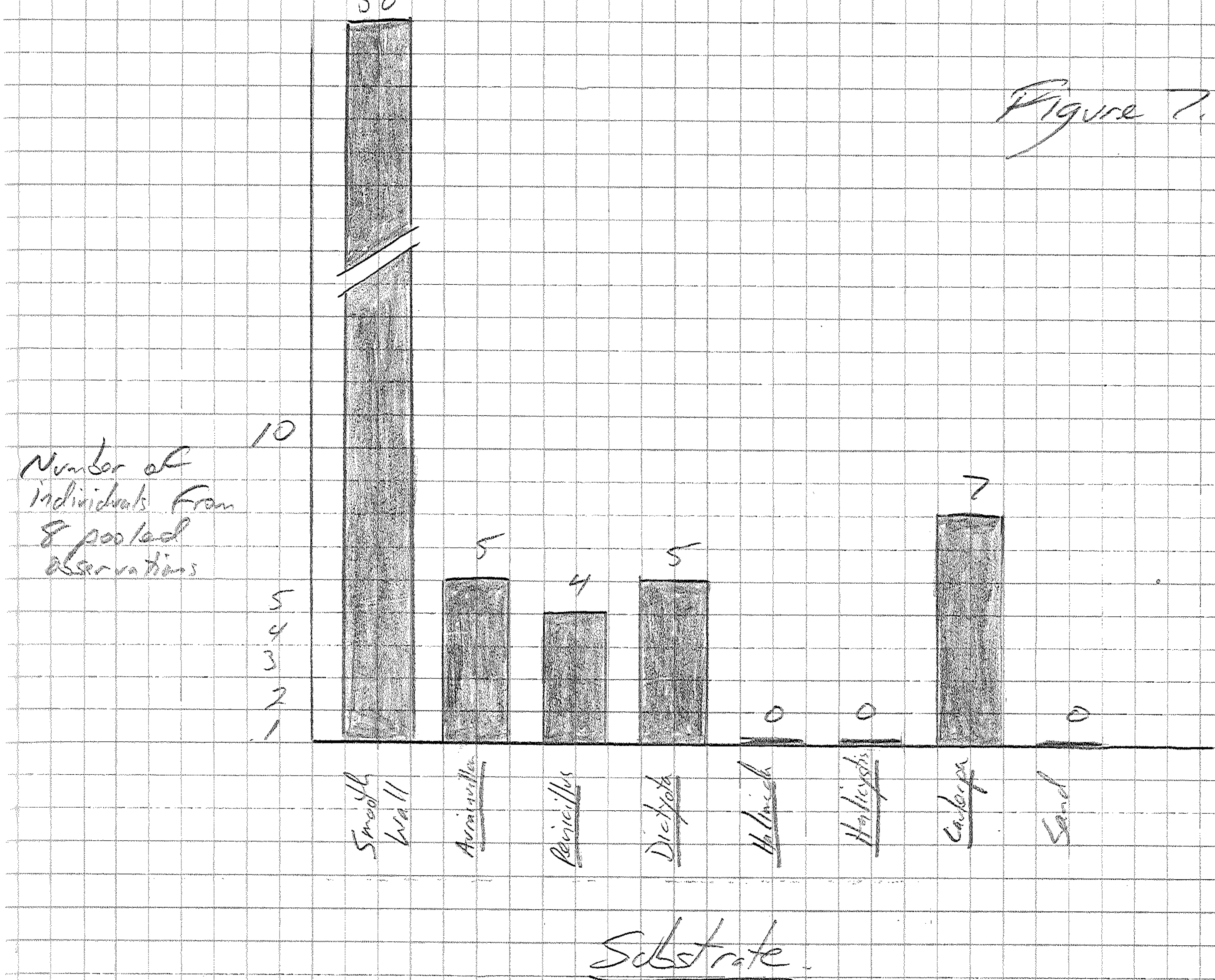
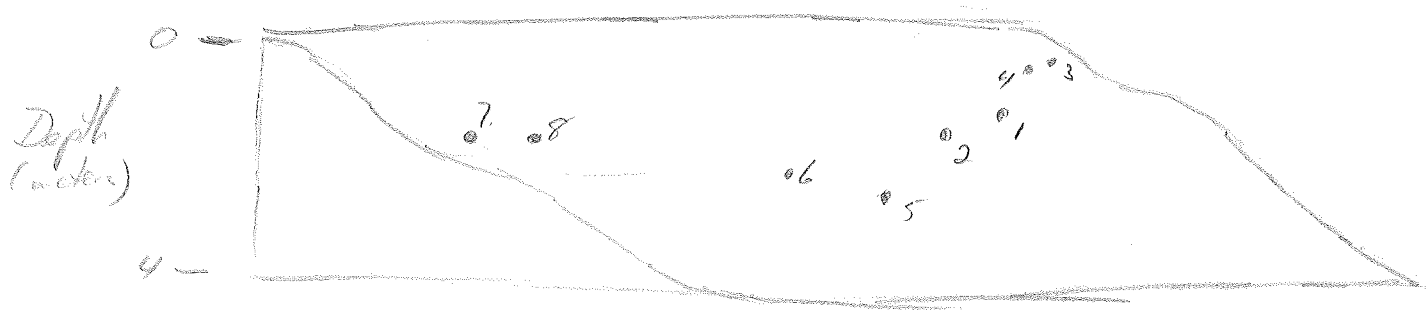


Figure 7. Number of individuals found on a variety of substrates.



Stake #	Depth (2/27)	status (2/28)	status (3/1)
1	1.50 m	Present	Present
2	1.75 m	Present +2	"
3	1.25	Present	"
4	1.25	"	"
5	2.50	"	"
6	2.00	"	"
7	1.75	"	Absent
8	1.75	"	"

Figure 8. Stake positions marking locations of individuals on portion of reef face at Columbus Park.

Table 3. *Tridachia* depth at two sites on same day.

Tridachia #	EBR	Col. Park
1	.50 m	1.00 m
2	1.00	1.00
3	1.25	1.50
4	.75	1.75
5	.50	1.00
6	1.50	.75
7	2.25	1.50
8	3.00	1.00
9	2.50	1.25
10	2.50	
11	.50	
12	.50	
13	.50	
14	.75	
15	.25	
16	1.00	
17	1.25	
18	1.50	
19	1.50	
20	2.00	
21	1.75	
22	1.00	
$\bar{X}$	1.28 m	1.19
S.D.	.78	.33

Predation: The palatibility of Tridachia to species of fish common in its diurnal habitat was estimated in this experiment. Before going into the field, several individuals were cut in 1-2 cm longitudinal strips and placed in a specimen bottle. Another bottle contained live individuals. A sea urchin (Tripneustes) was hemisected and its vital entrails removed. In the field a live Tridachia was dropped through the water column, followed by a few pieces. Observations on the number and kind of fish attacks was made. Next the urchin shell was placed on the reef in 1-2 meters of water in order to precipitate a "feeding frenzy." ~~First~~ A live Tridachia, and then several pieces were added to the half-urchin shell. Finally, several pieces were added to the water above the feeding fish. Again, observations as to the numbers and kinds of attacking fishes were made.

Results

The Mann-Whitney U-test was used to compare the weight change for the 10 normal-light individuals to the 10 light-deprived individuals. Results were very highly significant ($p < .001$). The test, however, may have been biased by artificial pairing of data points (Table 1). This will be further explained in the Discussion. No significant difference was found between individuals in the food/no-food regimes. There appeared to be a deepening of color in all experimental animals in comparison with open-water controls.

Phototactic response of Tridachia was first noticed when individuals succeeded in crawling under partitions from the dark to light side in the light/dark incubation experiments. Figure 5 shows dramatic peaks in individuals preferring intensities of 35% and 26% transmitted light. Pooling of the data in Table 2 and comparison of the two mid-range intensities with the remaining intensities proved very highly significant ($p < .001$, Mann-Whitney U-statistic).

The informal testing of substrate preferences yielded some interesting results. The histogram in Figure 7 shows a 7-10 fold preference by Tridachia for the smooth walls of their tank over any other substrate. Tridachia showed nearly violent intolerance to sand. Forced introduction to sand initiates thrashing behavior in Tridachia and difficulty in uprighting themselves when turned over. Individuals were never found in the sand, on Halimeda, nor on Halicystis of their own accord. In comparison, they were often found on the other algal substrates (Avrainvillea, Penicillus, Dictyota, Caulerpa) to approximately the same degree. One case of feeding on Caulerpa sertularioides was actually noted. When examined under the dissection microscope an individual was attached to the algae by means of its radular mouthparts. Upon removal of the algal substrate from the light/dark incubation, signs of deterioration in both C. sertularioides and C. racemosa were noticed. This was best described as "drainage" of cellular matrix; plant parts were blanched of chloroplasts, though the epidermis remained basically intact.

Marked individuals in Columbus Park showed remarkable positional stability over a 48 hour period (Figure 8.). 8 of 8 individuals were in a 30cm radius of their initial position after 24 hours and 7 of 8 were similarly present after 48 hours. The #7 individual was missing after 48 hours, while the #2 individual had gained a partner after 24 hours. The positions of these individuals were not assessed at night. Night observations, however, done at the East Back Reef indicate a decrease in the number of individuals readily visible in the same locations during the day. One area of rock (1 meter water depth) harbored a large number of Tridachia during two day observations that were done on either side of the night observations. Comparison of individuals at two different sites — Columbus Park and East Back Reef — showed no significant variation in depth of individuals between populations (Table 3.).

Attempts to entice feeding on Tridachia by various reef fishes were unsuccessful. One squirrelfish darted from a hole ~~a~~ and took a nip at the first Tridachia dropped intact through the water column. No feeding response was elicited, by pieces of Tridachia, in the yellowhead wrasse, bluehead, beaugregory, or three-spot damselfish; though many fish swam to the proximity of the pieces and then turned away. Placement of a fresh urchin shell started a feeding frenzy among the wrasses which disturbed the damselfish nearby. No pieces or whole Tridachia, placed in the urchin shell and dropped from above, was ever touched by any fish. The observation was made (Charles Leistan, personal communication) that the level of frenzy may even have decreased after the addition of _Tridachia_ to the urchin shell.

Several specimens of the sea anemone, _Condylactis_, were available in the laboratory. _Tridachia_ dropped into their tentacles were quickly and voraciously consumed. Two other anemones, _Heteractis_ and _Stoichactis_ rejected individuals within a minute or two. On March 2, an unusual event occurred. A _Tridachia_ that had escaped from its holding bin was crawling in the vicinity of a _Condylactis_. The tentacles of the anemone were obviously orienting towards the slug. When the slug had passed beyond 1-2 cm of the outermost tentacles, the anemone suddenly enveloped it and drew it onto the oral disc. The individual did not reappear within the next hour.

A gut content analysis was to be run on several _Tridachia_. This proved difficult, as little detail could be discerned. The body was entirely green throughout. Tubes and filaments transected this viscera, but no digestive tract was evident. An interesting observation was made after the conclusion of the light/dark incubation. The ~~[illegible]~~ ten _Tridachia_ grown in darkness all secreted copious quantities of mucus upon removal, while the light-side individuals exhibited normal secretions. Finally, a "frilling out" behavior was often noticed in laboratory animals, but was strictly absent from animals in nature. This consisted of the spreading of the dorsal cerata, such that the highly pigmented bases of the digestive diverticulae were exposed.

Discussion

The incubation of Tridachia under light versus dark conditions may point towards some interesting conclusions. Results suggest that animals in dark conditions lose weight, while those in light gain weight. As mentioned previously, the pairing of before and after weights could only be followed over each group of five individuals and not individually. For each group of five, before and after weights were ranked and opposite pairs assumed to denote the same individual. This may not be the case - Each before weight could actually correspond to any one of five after weights. It seems unlikely, however, that in seven days large weight changes could occur. Thus, a 2-gram slug does not grow to 6 grams nor a 6 gram individual shrink to 2 grams. The smallest weight changes, over this time period, are in fact likely to represent the same individual. With this in mind, the results of the Mann-Whitney U-statistic are weakened, but not likely beyond significance. Larger sample size would be useful in further clarifying this issue. The result, however, is logical if animals depend to a large degree on their symbiotic chloroplasts for nutrition. Chloroplasts in the dark would require maintenance energy while showing an enormous drop over their previous production. More surprising here is the active growth of light-side individuals in an experimental situation that is assumed to be less than optimum. The color change of all incubated individuals is rather inexplicable. As mentioned

I wonder chloroplast activity is reduced in dark.

in the introduction, it is possibly related to chloroplast increase at low light levels akin to the increase noted in terrestrial systems under ~~[illegible]~~ low light conditions. Another anomalous response noted in the lab, but absent in nature is the "frilling out" of cerata in many individuals at reduced light intensity. Could this possibly help to maximize the photosynthetic area available?

Phototaxis in Tridachia is clearly demonstrated by the movement of individuals in a light intensity gradient. Individuals would not tolerate complete darkness, nor full sunlight under a 30 cm water column. In the 100% and 66% illumination areas, slugs were found in the shadow created by the walls – effectively reducing the light intensity they experienced. This result points to an optimal range of light intensity for the photopigments. Tridachia are possibly selected for their ability to maximize their optimum exposure. Tridachia, which are frilled out in the laboratory, show an extremely fast response to an increase in light intensity from a flash camera. They respond by rapidly contracting their exposed cerata so only the dense white surface remains exposed. Is this a photoadaptation for rapid changes in solar flux? This experiment also demonstrates the ability of Tridachia to change its position fairly rapidly in response to light gradients. If displaced on the reef, it should quickly be able to ~~[illegible]~~ return to optimal exposure range.

The testing of substrate preference was rather informal and certainly ~~a more~~ rigorous methodology can be devised. Several results, however, are salient. *Tridachia* noticeably prefers smooth substrates over fine-grained, irregular surfaces (sand). Possibly this relates to mucus secretion and ease of movement. Filamentous green algae is also prevalent on smooth substrates. This may be an unrecognized food resource of *Tridachia*. Filamentous greens cultured on a glass plate and then exposed to *Tridachia* would be a simple way to observe ~~this~~ possible feeding. Of the other algae on which *Tridachia* was found, only *Dictyota* is not in the order Siphonales. Trench (1973a) feels that absorbance of chloroplast pigments of the siphonaceous algae matches most closely those found in *Tridachia*. Signs of deterioration in the two *Caulerpa* species used in the light/dark incubation appear to be due to suctorial grazing by *Tridachia*. This might corroborate the observation of an individual attached to *C. sertularioides* via its mouth — an observation that has, heretofore, not been made, to my knowledge. As previously thought (Trench, 1975), it may not be the case that *Tridachia* spends little time on its "food" substrates. Perhaps its diet is actually more expansive than realized.

The positional stability noted for the eight staked individuals in Columbus Park is probably indicative of little diel movement by *Tridachia*. This stands in contrast to the lower abundance of individuals found at night in the East Back Reef. Where do the individuals why hide at night?

go at night? A likely explanation is that the absence of animals at night is due to local hiding and not any form of extreme diel migration. *Tridachia* might be moving to the nearby crevices or the undersides of rocks. A large scale, diel migration to depth seems out of the question in light of the stability of individuals on the reef. It is logical that *Tridachia* would stay in proximity to preferred basking spots and move only short distances to hiding places if night predators are a problem.

The lack of predation by diurnal fishes is not surprising. Nudibranch sea slugs, in general, are often distasteful or toxic to fish. Often nematocysts or defensive glands are present in other sea slugs. Possibly *Tridachia* sequesters some secondary algal toxin from its chloroplasts, that makes it unattractive to fish. The extreme palatability of *Tridachia* to the anemone *Condylactis* is interesting. As seen in the lab, *Tridachia* is capable of unsuspectingly wandering into a *Condylactis*. Perhaps this is not so in nature; ~~—~~ possibly moving sea anemones and gastropods act as nighttime predators on inactive *Tridachia*.

The ecology of many marine animals seems to be less understood than in most terrestrial systems. The marine slug, *Tridachia* provides a salient case in point. Certainly this study succeeds in barely elucidating a few of the areas of ecological interest in this symbiotic relationship and raises many more questions than it answers. Ecologically, aspects of the Tridachia-chloroplast symbiosis may be important in man's future. An animal that is at least partially autotrophic merits some scientific interest.

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