Interactions Between Endosymbiont-Bearing Infaunal Bivalves and the Biogeochemistry of *Thalassia testudinum* Sediments

Laura Kay Reynolds
Galax, VA

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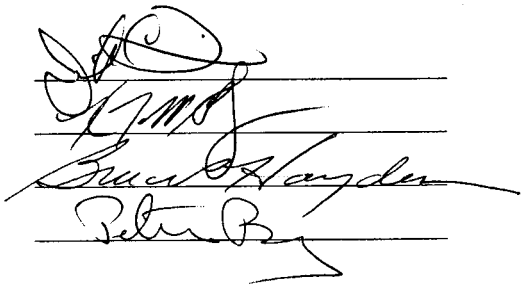

Abstract

Lucinid bivalves dominate the infauna of seagrass sediments. While the effect of seagrass on lucinids has been studied, the reverse effect has been ignored. Lucinids can alter porewater chemistry (i.e. increase porewater nutrients by suspension feeding and decrease porewater sulfides by oxygen introduction and bacterial oxidation), which can potentially change seagrass productivity and growth morphology.

To observe correlations between porewater chemistry and lucinid presence, a survey and a laboratory microcosm experiment were conducted. Survey sampling sites with clams had a significantly lower sulfide and higher ammonium concentrations than sampling sites without clams. There was no difference is phosphate concentration among sampling sites. Both clam species used in the microcosm experiment (*Ctena orbiculata* and *Lucinesca nassula*) significantly lowered sulfide concentrations in the sediment porewater.

Microcosm and field survey results were incorporated into a model sulfide budget. In seagrass sediments, lucinid clams remove 2-10% of the total sulfide lost, and if that sulfide was added back into the sediment porewater, sulfide concentration could increase 0.62 $\mu M\ day^{-1}$. Sulfide is a major stressor to both plants and animals in Florida Bay sediments; therefore, this removal may be important to maintaining seagrass productivity and health.

C. orbiculata sediment oxygen introduction sediments was estimated with a dye experiment. *C. orbiculata* were added to small tubes containing sieved mud and incubated in a bath of seawater with a 3% Rhodamine WT concentration. Rhodamine WT accumulation in the sediment was measured. A model showed that oxygen introduction can only account for 5% of *C. orbiculata* sulfide removal.

Acknowledgements

Graduate degrees are awarded to individuals, but a community shares the labor. I would like to thank some members of my community.

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Chapter 1 Introduction: Endosymbiont bearing infaunal bivalve relationships with *Thalassia testudinum* via sediment biogeochemistry

1.1 Introduction

Clams belonging to the family Lucinidae are the most abundant and diverse of the infaunal mollusks living within the sediments of tropical seagrass meadows. These sediments are also the primary habitat for shallow water lucinid clams (Barnes, 1996, Jackson 1972, 1973, Moore, 1968). The association between lucinid clams and seagrasses is strong enough that the shells of dead lucinid clams are used to age seagrass meadows and to locate relic seagrass meadows, and the association is typically considered a result of positive seagrass influence on lucinid clams. (Barnes, 1996; Bretsky, 1976, 1978; Jackson, 1972, 1973). Seagrasses provide an ideal habitat for lucinid clams. The dense root and rhizome mat, created by the grass, protects the clams from encountering predators (Barnes 1996, Barnes and Hickman, 1999, Jackson, 1972). For most relatively immobile mollusks, seagrass sediments are harsh environments, because they are highly reduced and rich in sulfide, which is toxic to most animals. (Hemminga and Duarte, 2001). Lucinid clams, unlike most animals, thrive in sulfide rich sediments (Barnes, 1996; Barnes and Hickman, 1999, Cavanaugh, 1983). The purpose of this thesis is to demonstrate that not only do seagrasses positively affect lucinid clams, but these clams can potentially positively affect lucinid clams by altering porewater biogeochemistry. Lucinid activity can decrease sulfide and increase nutrient concentrations in the sediment interstitial water. These chemical changes are of potential benefit to the seagrasses:

allowing for increased productivity and even altering above to below ground biomass ratios (Figure 1.1).

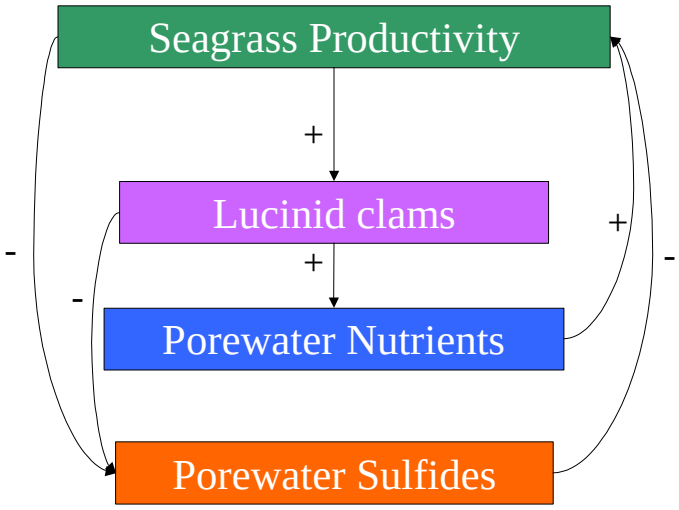

Figure 1.1 **Interactions Occurring in Seagrass Vegetated Sediments.** Rectangular boxes describe standing stocks. Pluses and minuses describe positive and negative interactions among the standing stocks. Positive interactions increase standing stocks, while negative interactions decrease standing stocks. The model describes the following interactions: the presence of seagrass increases lucinid survival, which increases porewater nutrients and decreases porewater sulfides. Both increased nutrients and decreased sulfides increase the standing stock of seagrass

Lucinid clams are unique bivalves. Most bivalves have siphons, which they use to pump water column water into their bodies. They filter the water column water for feeding purposes and use oxygen dissolved in the water column for respiration. Lucinid clams also require water column water for nutrition and for oxygen; however they have no true siphons. They filter the water column instead by building hollow tubes to the sediment water interface and utilizing ciliary currents to bring the water into their bodies (Jackson, 1972). Lucinid clams also have a unique symbiosis. All species of lucinid clams analyzed

to date harbor chemoautotrophic-endosymbiotic bacteria living within their gills. These bacteria enzymatically oxidize sulfides, which are found in abundance in the sediment, to provide energy to run the Calvin cycle and thus create sugars. Lucinid clams have a reduced filtering capacity and a reduced digestive system, so these sugars are an important food source for not only the bacteria but also for the clam (Distel and Feldbeck, 1987).

1.2 Lucinid Sulfide Removal

Seagrass vegetated sediments typically have high sulfide concentrations. Sulfides accumulate as a standing stock in seagrass vegetated sediments as an end product of microbial decomposition (Fenchel and Riedel, 1970, Fenchel, King, and Blackburn 1998) and spontaneously exit the system by one of two methods: (a) chemically reacting with oxygen to form sulfate or (b) chemically reacting with hydrogen to form gaseous hydrogen sulfide, which can escape from the sediment system (Equation 1.1 and Equation 1.2).

$$S^{2-} + 2O_2 \rightarrow SO_4^{2-}$$ **Equation 1.1**

$$2H^+ + S^{2-} \rightarrow H_2S \uparrow$$ **Equation 1.2**

Lucinid clams can further increase the rate of sulfide removal by two methods: bacterial oxidation or oxygen introduction. Since the bacteria living within the gills of the clam enzymatically oxidize sulfide to elemental sulfur or to sulfate in order to create sugars (Distel and Feldbeck, 1987), they remove a finite amount of the available sulfide. While sulfides must be present at some level for these clams to survive, locally, the clams seemingly keep the levels relatively low. Lucinid clams also reduce sulfide levels by

oxygen introduction. Sulfides and oxygen cannot coexist for very long. Oxygen will spontaneously react with sulfide to produce sulfate until one of those reactants is no longer available (Equation 1.1). Paradoxically, these clams require both oxygen and sulfides to survive. Lucinid clams obtain oxygen from the water column using ciliary currents to transport oxygenated water through hollow tubes (Allen, 1958; Jackson, 1972). Not all oxygen brought into the tubes reaches the clam's body. Oxygen can naturally diffuse through the tube walls into the anoxic sediments, where it may encounter and react with sulfide.

The oxidation of sulfides by lucinid bacteria and by oxygen introduction results in elemental sulfur, thiosulfate, or sulfate production and thus the removal of sulfide from the sediment system. While sulfides must be present at some level for these clams to survive, locally the presence and activities of the clams should act to keep the levels relatively low. If sulfide levels get too low to support bacterial demands, the bacteria have the ability to use elemental sulfur stored in the gills of the clam until sulfide levels again increase (Barnes, 1992).

1.3 Lucinid Nutrient Interactions

The process of bringing water column water into the lucinid clam's body is also used as a feeding mechanism. While the clams get sugars from their endosymbiotic bacteria, they still require other nutrition, which they obtain from organic material suspended in the water column (i.e. suspension feeding) (Allen, 1958; Jackson, 1972). Other suspension feeders living in seagrass meadows have been shown to increase nutrients in the interstitial water of the sediments (Peterson and Heck, 1999, 2001, 2001a; Reusch et, al.,

1994). Suspension feeding bivalves transfer planktonic production in the water column to the sediments by means of fecal and pesudofecal production. Thus clam activity can increase the sediment nutrient pools.

1.4 Seagrass Porewater Chemistry Interactions

These chemical changes (i.e. increased nutrients and decreased sulfides) are changes that have the potential to increase seagrass productivity. Seagrasses tend to be nutrient limited. In Florida Bay, phosphorous is typically the limiting nutrient (Fourqurean et. al., 1992). *T. testudinum,* the most abundant seagrass in Florida Bay is more efficient at gaining nutrients from the sediment porewater as opposed to the water column. (McGlathery, 2001; Zieman, 1982). Therefore, an addition of these nutrients or a transfer of these nutrients from the water column to the sediments should increase seagrass productivity. This increase should allow the seagrasses to allocate more resources to above ground as opposed to below ground production, therefore increasing the above to below ground biomass (Hemminga and Duarte, 2000).

Sulfide is toxic to seagrasses and other plants (Carlson et. al., 1994; Koch and Erskine, 2001; Peterson et. al. 2003). Seagrasses, however, have mechanisms for surviving in sulfide rich marine sediments. Oxygen gas is an end product of photosynthesis, which can diffuse from the leaves though lacunae to the roots. Some of that oxygen escapes through the roots into the sediments where it reacts with and reduces the amount of toxic sulfide in close proximity to the seagrass tissue (Equation 1.1) (Pederson et. al., 1998). While seagrasses have this mechanism, sulfide at high but natural levels has been shown to reduce photosynthetic capacity and even induce die-off (Goodman et. al., 1994; Koch

and Eskrine, 2001; Pederson et. al. 2003). Reducing the ambient sulfide levels further by means of clam activity reduces the toxic stress on the seagrasses allowing productivity to increase. Lower sulfide levels also allow the seagrass to allocate more resources to above as opposed to below ground biomass, since they do not need excess below ground surface areas from which to introduce oxygen. (See Figure 1.2 for summary.)

1.5 Thesis Goals and Objectives

Previous lucinid research has focused on clam physiology or the influence of seagrass presence and water column conditions (e.g. salinity) on lucinid distributions. At this date, there are no published studies investigating the influence these bivalves have on the sediment biogeochemistry and on the seagrass itself. The main objective of this study was to use Florida Bay or basins within Florida Bay as model systems to demonstrate that the presence and activities of lucinid clams alter the chemistry of the sediments and potentially the seagrass habitat. The goal of chapter 2 is to observe natural populations of lucinid clams and the correlation of their presence with porewater chemistry. Chapter 3 uses a microcosm approach to demonstrate that *Ctena orbiculata* and *Lucinesca nassula* (family: Lucinidae) decrease the sulfide levels in their sediment habitat. Additionally, a model is used to extrapolate the changes observed in the microcosm to natural basins within Florida Bay. Chapter 4 uses a dye experiment and a model to determine the predominant method by which *C. orbiculata* alters porewater sulfide: microbial oxidation or oxygen introduction.

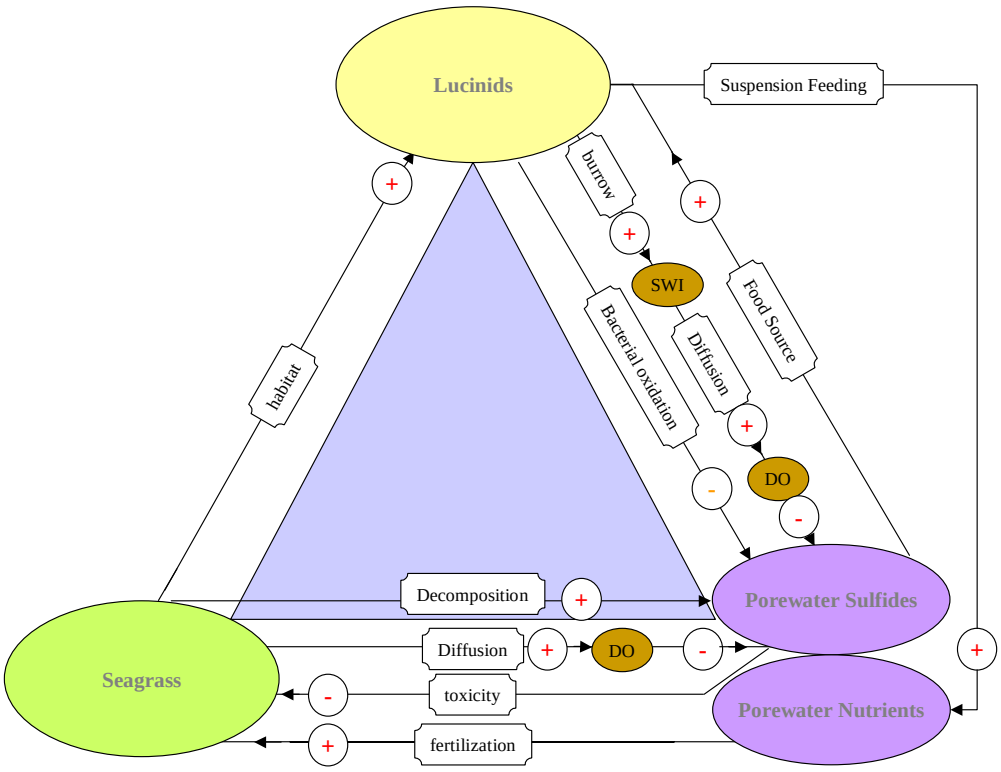

Figure 1.2 Conceptual Model Describing Interactions in Seagrass Vegetated Sediments.

The rectangular boxes describe processes. The ovals describe standing stocks. Pluses and minus describe increases or decreases respectively in the standing stocks symbol following. The model describes the following interactions: the presence of seagrass increases lucinid survival, which increases porewater nutrients and decreases porewater sulfides. Both increased nutrients and decreased sulfides increase the standing stock of seagrass. The following is an example of how to read this diagram using the outside connection between Lucinids and Porewater Sulfides: Lucinid clams create burrows, which increase the sediment water interface. The increase sediment water interface allows for a greater diffusion and thus a larger introduction of dissolved oxygen into the sediments. Dissolved oxygen will react with porewater sulfides and decrease the ambient pool. Abbreviation Definitions: DO = Dissolved Oxygen; SWI = Sediment Water Interface.

Table 1.1 Summary of Thesis Objectives and Hypotheses.

Chapter 2:	Lucinid Distributions and Correlations with Environmental Characteristics within Florida Bay
Objective:	Determine the natural distributions and correlations between seagrass density, lucinid density, and porewater chemistry.
Working Hypotheses:	a) Lucinids will be ubiquitous in Florida Bay but will have higher densities in areas with high sulfide levels. High sulfide levels will occur with large standing stocks of seagrasses. b) Locally (i.e. within basins) patches of high lucinid density will co-occur with patches of lower sulfide standing stock, since clam activity will remove sulfide. c) In all areas, clam-feeding activity will increase porewater nutrients.
Chapter 3:	Lucinid Influence on the Sulfide Concentration of Interstitial Water
Objective:	Quantify the influence of lucinid presence and activity on porewater sulfides.
Working Hypothesis:	The activities of lucinid clams (i.e. bacterial oxidation and oxygen introduction via clam burrows) remove sulfide and decrease the standing stock in the sediment interstitial water.
Chapter 4:	Mechanisms by which *Ctena orbiculata* (family Lucinidae) Alter Porewater Sulfide Levels in Seagrass Vegetated Sediments.
Objective:	Determine if bacterial oxidation or oxygen introduction is more important in decreasing sediment sulfide concentration.
Working Hypothesis:	Both sulfide-reducing activities have an effect on standing stock of sulfide in the interstitial waters. Bacterial oxidation is a larger remover, since clams will use a large portion of the oxygen introduced for respiration.

1.6 Study Site Description

Florida Bay is a small, shallow, triangular estuary. It is bordered by the southern tip of the Florida peninsula on the north, by the Florida Keys on the south and east, and it is open to the Gulf of Mexico on the West. The bay contains numerous mangrove islands and meandering mud banks subdividing the bay into many distinct basins. The sediment typically consists of carbonate muds. The most common benthic cover is seagrass varying in density from very sparse to quite dense. Three species of seagrasses are found in Florida Bay: *Halodule wrightii, Syringodium filiforme, and T. testudinum. T. testudinum* is the most abundant (Zieman, 1982).

1.7 Significance

Previous lucinid research has focused on clam physiology or the influence of seagrass presence and water column conditions (e.g. salinity) on lucinid distributions. The effect of seagrass on lucinid clams has been studied, but the potential for reverse effects has been ignored. In the age of increased human activity altering seagrass and near-shore ecosystems, successful management of these ecosystems will require a detailed knowledge of the flora, fauna, and their interactions.

Chapter 2 Lucinid Distributions and Correlations with Environmental Characteristics within Florida Bay

2.1 Objective

The goal of this study is to observe lucinid clams and the correlation of their presence to the seagrass morphology and the biogeochemistry of their sediment habitat. A bay wide survey and two more intensive surveys were used to make these observations on two different scales.

2.2 Methods

2.2.1 Bay Wide Survey

This study utilized nine specific study sites within Florida Bay: Bob Allen LTER (BA), Barnes Key (BNS), the wind-ward and lee-ward sides of Cross Bank (CBW and CBL), Duck Key (DUK), Peterson Key (PET), Rabbit Key Basin (RKB), Ranking Lake (RAN), and Sprigger Bank LTER (SPG) (Figure 2.1).

At these nine study sites in the bay, seagrass, infauna, and porewater samples were collected. Three cores (diameter = 20 cm, depth = bedrock or 80 cm maximum) were extracted and sieved to 1mm. The samples were frozen until analysis could occur; at which point the grass was divided into the following sub-samples: dead material, live roots, live vertical rhizomes and non-photosynthetic leaves, and photosynthetic leaves. Each sub-sample was dried at a temperature of 60°C and then weighed. All intact clams were removed from the core, identified, and measured. Identification was made using the following references: Abbott, 1974; Abbott, 1996; Anders 1994; Redfern, 2001.

Bay Wide Survey Sampling Sites

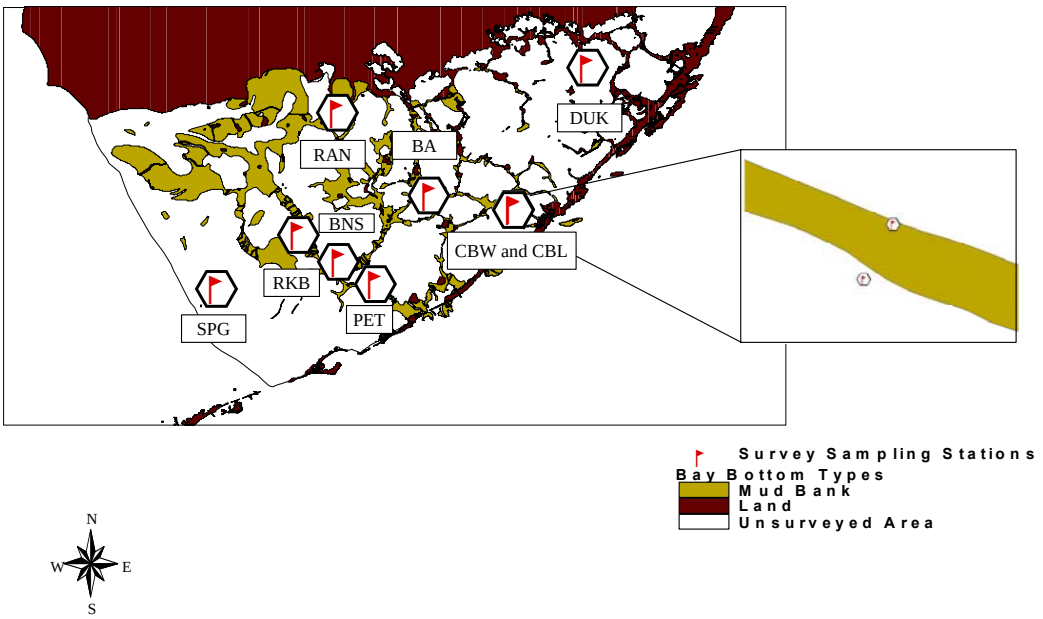

Figure 2.1 Bay-Wide Sampling Site Map.
The above map depicts Florida Bay and the eight sampling sites used in the survey. BA=Bob Allen Key; BNS = Barnes Key; CBL = the leeward side of Cross Bank; CBW = the windward side of Cross Bank; DUK = Duck Key; PET = Peterson Keys; RAN = Rankin Lake; RKB = Rabbit Key Basin; SPG = Sprigger Bank.

In close proximity to the core site, a porewater sample of approximately 30 ml was taken using a sampling probe (Berg and McGlathery, 2000). The sample was filtered through a 0.45 μm Millipore filter and analyzed for sulfide, ammonium, and phosphate concentration. Sulfide concentration was analyzed using the Cline method (1994). Sulfides are converted to zinc sulfide with zinc acetate. The zinc sulfide then reacts with a dimethyl solution producing a blue color, which can be analyzed spectrophotometrically. Ammonium concentration was analyzed spectrophotometrically using a method based on the formation of a blue color (indophonol) by the reaction of hypochlorite and phenol in the presence of ammonium (Strickland and Parsons, 1972). Phosphate was analyzed spectrophotometrically using a composite reagent containing molybdic acid, ascorbic acid, and trivalent antimony that reacts with phosphate and results in a blue color (Stainton et. al., 1974).

Sediment samples were also collected from each study site. Three small sediment cores were taken with a 60 cc syringe to determine sediment organic content. The sediment was dried at 60°C and weighed. It was then ashed for 5 hours at 600°C, and again weighed. Sediment organic content was determined using Equation 2.1. All sites were sampled within a two-week period during August of 2002.

$$\text{Sediment organic content (\%)} = \frac{\text{Dry weight - Ash free dry weight}}{\text{Dry weight}} \qquad \textbf{Equation 2.1}$$

2.2.2 Within Site Survey

This study utilized two specific sites: Rabbit Key Basin and Sunset Cove (Figure 2.2).

At these sites, a more intense survey was conducted. Approximately biweekly during the spring and summer of 2003 (March until August), twelve 20 ml porewater samples were collected using a sampling probe (Berg and McGlathery, 2001). Each porewater sampling site was marked with a numbered flag. The sample was filtered through a 0.45 μm Millipore filter, and one 5 ml sub-sample was fixed with 5 ml zinc acetate for sulfide analysis. Samples were transported on ice to laboratory facilities where they were analyzed for sulfide, ammonium, and phosphate concentration using the methods described previously (Section 2.2.1). Directly around each porewater sampling site (marked with a flag), a core (diameter = 7.6 cm, depth = bedrock or 50 cm maximum) was taken. The twelve cores were sieved in 5 cm segments though a 1 mm mesh. All seagrass tissue was discarded, but all live clams from the family Lucinidae were kept, identified, and measured.

Since Sunset Cove was not studied in the bay wide survey, and since the within site surveys occurred a year following the bay wide survey, the bay wide survey methods were repeated at both sites during the summer of 2003.

2.3 Results

2.3.1 Bay Wide Survey

Eight out of the nine studied sites had lucinid clams. The one site that lacked these clams was the leeward side of Cross Bank. This site interestingly showed evidence of significant sedimentation (i.e. the vertical short shoots were very long and had long vertical short shoot internodes) and had recently experienced some die-off episodes (pers. obs.).

Within Site Survey Sampling Sites

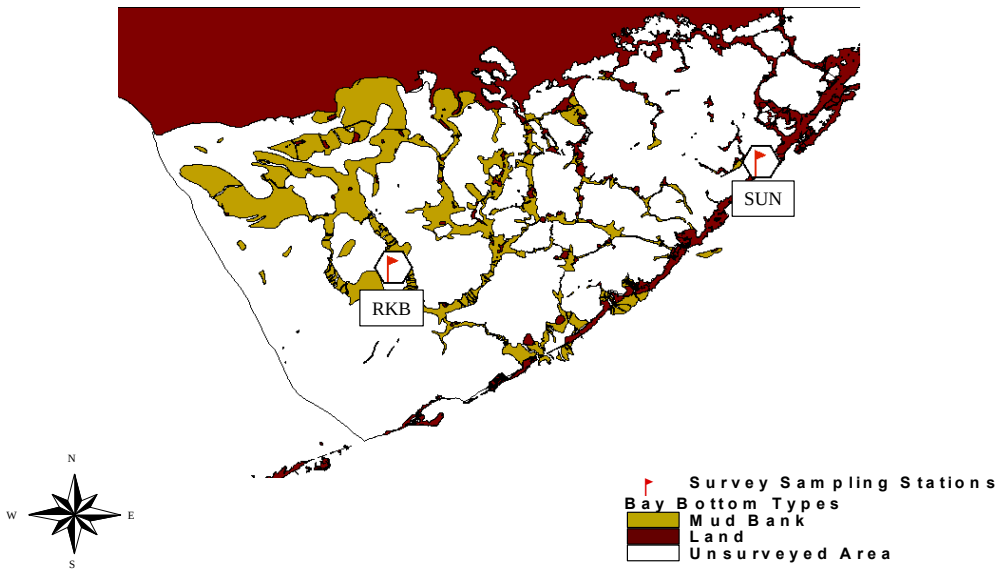

Figure 2.2 Within Sampling Site Map

The above map depicts Florida Bay and the sites surveyed for within site analysis. RKB = Rabbit Key Basin, and SUN = Sunset Cove.

Seagrass biomass, above to below ground biomass ratio, seagrass and lucinid density, and porewater sulfide, ammonium, and phosphate concentration measurements are displayed in Table 2.1. Ammonium concentrations are omitted from this table, because the measured concentrations were very low and sporadic. It appears that there was a problem with the method; therefore the values are not reliable. The sites showed considerable variability in each of the measurements, but there did seem to be some appreciable variation between sites. The presence of lucinid clams and sulfide concentrations were positively correlated, just as hypothesized, but there was no observable bay-wide trend linking lucinid presence to nutrient concentrations (Figure 2.3). Due to the limited size of the survey and high variability in site location and in physical structure, the statistical results of variable correlations between sites were not powerful.

2.3.2 Within Site Survey

2.3.2.1 Sunset Cove

Sunset Cove is located in the northeastern part of Florida Bay very close to the main line of the keys, adjacent to Key Largo. Maximum water depth is no more than 2 m. Sunset Cove is characterized by patches of hard bottom interspersed with patches of relatively dense *T. testudinum* (742 shoots m^{-2}) with low densities of *Halodule wrightii* (95 shoots m^{-2}) in a very thin sediment layer (10 to 50 cm). The sediment wet density is 1.08 g ml^{-1}, and the percentage of water is 74%. The average above to below ground biomass ratio of *T. testudinum* within Sunset Cove is 0.22. The average porewater sulfide concentration is 103.8 μM. Average phosphate porewater concentration is 2.94 μM, and average

	Species	total biomass (g/m^2)	above: below ground biomass ratio	Shoots / m^2	# of Lucinids/ m^2	sulfide concentration (µM)	Ammonium concentration (µM)	Phosphate concentration (µM)
BA	Tt	343 ± 187	0.05 ± .04	297 ± 204	10 ± 18	148 ± 139	1.33 ± .2	0.04 ± .01
BNS	Tt	1652 ± 250	0.17 ± .02	1379 ± 405	63 ± 63	1642 ± 607	.59 ± .3	0.02 ± .00
CBL	Tt	2163 ± 691	0.27 ± .24	1495 ± 135	0 ± 0	1069 ± 1123	2.43 ± .5	0.02 ± .01
CBW	Tt	1233 ± 296	0.08 ± .04	2079 ± 361	106 ± 80	384 ± 281	2.21 ±.2	0.02 ± .01
DUK	Tt	640 ± 235	0.04 ± .00	689 ± 207	21 ± 18	559 ± 437	.76 ± 1	0.01 ± .01
PET	Tt	983 ± 300	0.1 ± .00	1220 ± 265	21 ± 36	356 ± 209	15.31 ± 6	0.03 ± .01
RAN	Tt	1850 ± 318	0.2 ± .02	933 ± 255	10 ± 18	1323 ± 832	1.35 ± .07	0.05 ± .01
RKB	Tt	1273 ± 183	0.12 ± .03	1145 ± 198	222 ± 63	2333 ± 1700	2.89 ± 1	0.03 ± .01
SPG	Tt	642 ± 165	0.27 ± .08	286 ± 48	21 ± 18	151 ± 27	.67 ± .2	0.03 ± .01
SPG	Sf	102 ± 42	0.22 ± .08	625 ± 186				

Table 2.1 Bay wide survey Data Summary

A summer 2002 survey of 9 sites in Florida Bay resulted in the following data. Data is displayed as an average ± standard deviation. Site abbreviations are as follows: BA=Bob Allen Key; BNS = Barnes Key; CBL = the leeward side of Cross Bank; CBW = the windward side of Cross Bank; DUK = Duck Key; PET = Peterson Keys; RAN = Rankin Lake; RKB = Rabbit Key Basin; SPG = Sprigger Bank.

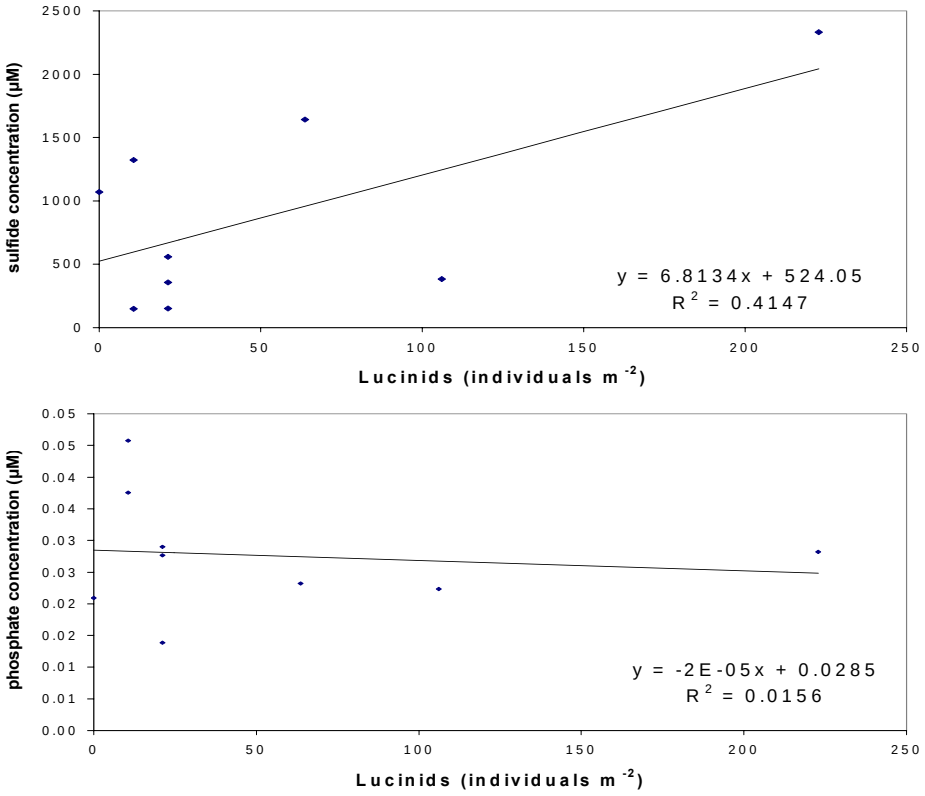

Figure 2.3 Bay Wide Survey Trends

A survey of 9 sites in Florida Bay during the summer of 2002 showed a positive correlation between porewater sulfide concentration and lucinid density, and no correlation between porewater phosphate concentration and lucinid density.

ammonium concentration is 6.39 μM. The lucinid population is dominated by *Ctena orbiculata* (80 m^{-2}). All *C. orbiculata* were found from 0 to 25 cm sediment depth, and most *C. orbiculata* were found at a depth of 5 to 10 cm.

Cores in which clams were found had a mean sulfide concentration of 69 μM, a mean ammonium concentration of 8.8 μM, and a mean phosphate concentration of 2.5 μM. Cores where clams were absent had a mean sulfide concentration of 119 μM, a mean ammonium concentration of 8.4 μM, and an average phosphate concentration of 2.4 μM. The difference in concentrations was analyzed using a t test. Sulfide concentration was lower in cores with clams as opposed to cores without clams using a 95% confidence interval. Ammonium concentration was higher in cores with clams as opposed to cores without clams using a 90% confidence interval, and there was no correlation between clam presence and phosphate concentration (Figure 2.4).

2.3.2.2 Rabbit Key Basin

Rabbit Key Basin is located within the western central portion of Florida Bay. It is further from inhabited land influence than Sunset Cove. Maximum water depth is again no more than 2m. The sediment wet density is 1.2 g ml^{-1}, and the percentage of water is 67%. The bottom is carpeted by dense *T. testudinum* (1146 shoots m^{-2}). The average *T. testudinum* above to below ground biomass ratio is 0.12. The average porewater sulfide concentration is 101.9 μM. The average phosphate porewater concentration is 2.76 μM, and the average ammonium porewater concentration is 10.78 μM. Two species of lucinid clams dominate the population: *C. orbiculata* (25 m^{-2}) and *Lucinesca nassula* (30 m^{-2}). A

third species, *Andontia alba*, is found rarely. All lucinid clams were found between 0 and 25 cm sediment depth, with most individuals burrowing to a depth of 5 to 10 cm.

Cores in which clams were found had a mean sulfide concentration of 72 μM, a mean ammonium concentration of 21 μM, and a mean phosphate concentration of 2.6 μM. Cores where clams were absent had a mean sulfide concentration of 108 μM, a mean ammonium concentration of 8.4 μM, and a mean phosphate concentration of 2.8 μM. The difference in concentrations was analyzed using a t test. Sulfide concentration was lower in cores with clams as opposed to cores without clams, and ammonium concentration was higher in cores with clams as opposed to cores without clams using a 90% confidence interval, and there was no correlation between clam presence and phosphate concentration (Figure 2.4).

RKB p = 0.09
SUN p = 0.01

RKB p = 0.1
SUN p = 0.1

RKB p = 0.3
SUN p = 0.4

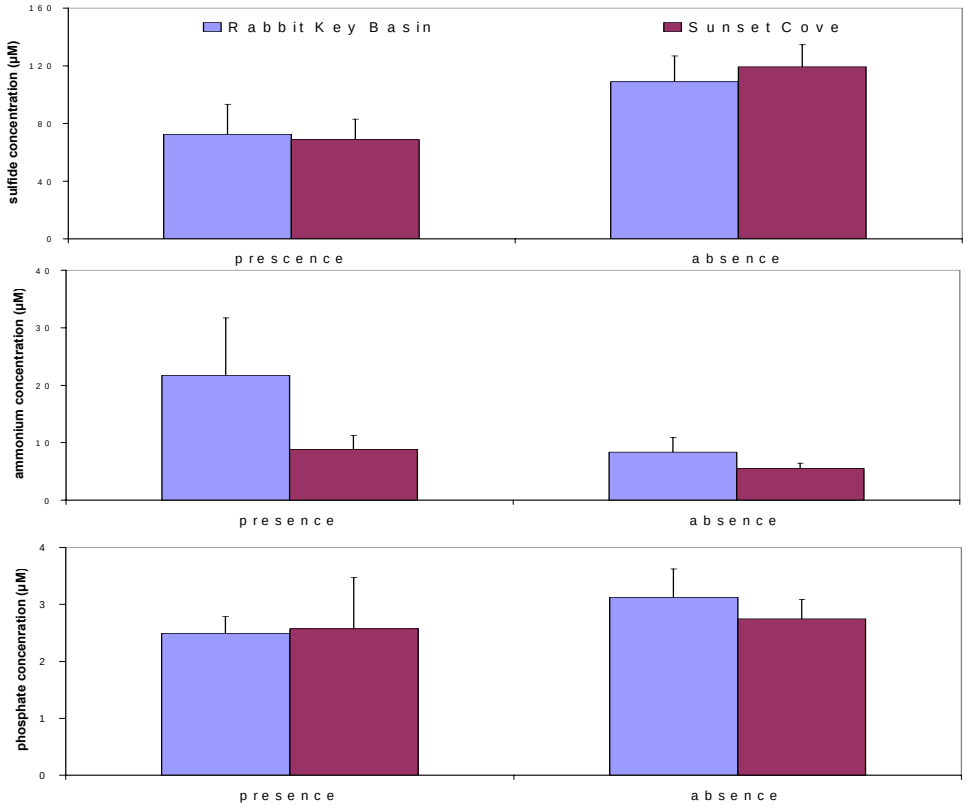

Figure 2.4 Within Site Survey Results

The above bar graphs display the results from a 2003 survey of two Florida Bay basins: Rabbit Key Basin and Sunset Cove. The bars marked presence reflect porewater concentrations in cores which contained lucinid clams, and the bars marked absence reflect porewater concentrations in cores without clams. The displayed p values are the result of a t test.

Chapter 3 Lucinid Influence on the Sulfide Concentration of Interstitial Water

3.1 Objective

The goal of this study is to quantitatively describe the influence of the lucinid clams *Ctena orbiculata* and *Lucinesca nassula* on porewater sulfide concentrations. It was hypothesized that clam activities (i.e. the bacterial sulfide oxidation and the introduction of oxygen due to burrows) would decrease the ambient sulfide levels (Figure 3.1). This study uses a microcosm approach to demonstrate that these clams are affecting sulfide concentration. A model is used to project the results to a 1 m x 1 m x 0.25 m block of sediment within two Florida Bay basins: Sunset Cove and Rabbit Key Basin. These basins are described in section 2.3.2.1.

3.2 Methods

3.2.1 Experimental Design

Fifteen tubes (diameter = 3.8 cm, depth = 10 cm, capped at one end) were filled with coarsely sieved (1 cm) sediment from Rabbit Key Basin. These tubes were placed into a tank of seawater continuously bubbled with air and maintained at a temperature of approximately 28°C using aquarium heaters. After a settling period of at least 24 hours to allow the sediments to become anoxic, the tubes were carefully removed from the water bath, and a 2 ml sample of the porewater was taken using a sampling probe (Berg and McGlathery, 2000). Removing the tubes assured that water column water was not introduced into the sample. Samples were filtered through a 0.45 μm Millipore filter, fixed with 2 ml of zinc acetate, and kept cold (in the refrigerator) until analysis. In the lab, samples were analyzed for sulfides using the Cline method (1994). Sulfides were

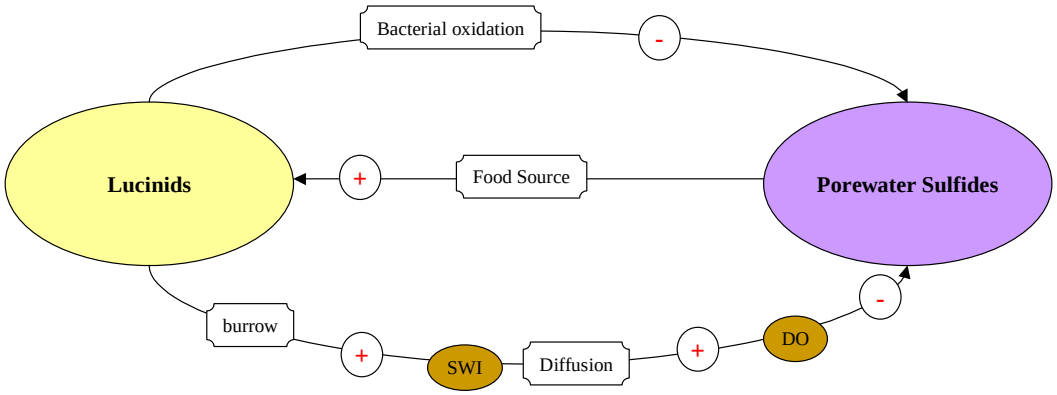

Figure 3.1 Conceptual Model Describing Lucinid Effect on Porewater Sulfides
The rectangular boxes describe processes. The ovals describe standing stocks. Pluses and minus describe increases or decreases respectively in the standing stocks symbol following. The following is an example of how to read this diagram using the bottom connection between Lucinids and Porewater Sulfides: Lucinid clams create burrows, which increase the sediment water interface. The increase sediment water interface allows for a greater diffusion and thus a larger introduction of dissolved oxygen into the sediments. Dissolved oxygen will react with porewater sulfides and decrease the ambient pool. Abbreviation Definitions: DO = Dissolved Oxygen; SWI = Sediment Water Interface.

converted to zinc sulfide with the zinc acetate. The zinc sulfide then reacted with a dimethyl solution producing a blue color, which was analyzed spectrophotometrically. Following initial sampling, the tubes were then carefully returned to the seawater bath, so as to create as small of a disturbance as possible. The tubes were randomly assigned to one of three treatment groups: control, *C. orbiculata*, or *L. nassula*. Control tubes were not manipulated. One live *C. orbiculata* or one live *L. nassula* was added to their respective tubes and allowed to burrow. After an incubation period of 1 to 3 days, porewater was sampled and analyzed using the method described previously.

The sediment used in the experiment was analyzed for wet density and water content. Three 5 ml sub-samples of the sediment used in the experiment were collected, weighed, dried to a constant weight at 60°C, and re-weighed. Wet density was calculated as the wet sample mass divided by the wet sample volume (Equation 3.1). The water content of the sediment was determined as the mass of water lost in drying divided by the mass of the total wet sample (Equation 3.2). Finally, these measurements were used to determine the sediment porosity, which is the volume of porewater divided by the total volume of the wet sample (Equation 3.3).

$$\text{wet density} = \frac{\text{wet mass}}{\text{wet volume}}$$ **Equation 3.1**

$$\text{water content} = \frac{\text{wet mass - dry mass}}{\text{wet mass}}$$ **Equation 3.2**

$$\text{porosity}\,(\varphi) = \frac{\text{wet mass} * \text{water content}}{\text{wet volume} * \text{porewater density}}$$ **Equation 3.3**

3.2.2 Model Projections

The change in sulfide concentration is calculated as the initial sulfide concentration of each tube subtracted from the final concentration. The mean rate of concentration change is simply the quotient of the mean change and the incubation period, and that can be converted to actual (molar) change by multiplying concentration change by the volume of porewater in each tube. The actual consumption rate of each clam was determined by subtracting the mean sulfide change in the control tubes from the mean change in the clam experiment tubes.

The effect of the clams on a 1 m x 1 m x 0.25 m block of natural sediment was analyzed by applying stoichiometry to two model systems: Rabbit Key Basin and Sunset Cove (2.3.2.1 and 2.3.2.2). This analysis made several assumptions. Sediments and sulfide production were assumed to be homogeneous. Clam consumption rates were assumed to be constant with time and with various conditions (i.e. various water column nutrient levels, various porewater sulfide levels) and similar to those consumption rates measured in the microcosm experiment. Sulfide standing stock was assumed to be relatively constant.

To determine the effect of lucinids on sulfide removal, sulfide production was estimated. Sulfide is generated from decomposition using sulfate as a terminal electron acceptor (i.e. sulfate reduction). Sulfate reduction was estimated using literature references (Holmer and Nielson, 1997; Pollard and Moritary, 1991). Assuming that sulfate reduction results in a 1:1 production of sulfide, sulfide production was estimated on a daily basis and

multiplied out to a study period of 180 days. The study period was chosen since chapter 2 monitored these basins for that length of time. Using both model systems, the amount of standing sulfide in the system was subtracted from the amount of sulfide produced in the study period resulting in an amount of sulfide removed from the system. Lucinid consumption in each system was calculated by multiplying the experimental consumption by the natural density. The proportion of the total sulfide removed from the system due to lucinid activity was calculated as a percentage.

An increase due to lucinid removal was calculated, assuming that if the lucinids were removed, the sulfide that they currently remove would stay in the system. Their consumption rate was converted to a concentration change using the sediment characteristics (i.e. the volume of porewater) of each system. This concentration change was added to the normal sulfide concentration in each system, and a percent increase was calculated.

3.3 Results

3.3.1 Experimental Results

The sediment used in the experiment had a density of 1.09 g ml^{-1} and a water content of 70.6%. The sulfide concentration in the control tubes increased an average of 69.4 μM per day. The sulfide concentration in the *C. orbiculata* and *L. nassula* tubes decreased an average of 99.8 μM day^{-1} and 58.3 μM day^{-1} respectively (Figure 3.2).

The volume of the experimental tubes was 113.95 ml. Using the density of the experimental sediment, the weight of the sediment was calculated to be 126.45 g, and

using the water content of the sediment, the weight of the porewater was 89.2 g. Using a seawater density of 1.02 g ml^{-1}, the volume of the porewater in each tube was 80.4 ml. Molar amount of change was determined by multiplying the concentration change by the volume of porewater. Consumption rate was calculated as the increase in control tubes subtracted from the decrease in the *C. orbiculata* and the *L. nassula* tubes. Mean daily consumption for one *C. orbiculata* was 13.58 μmol. Mean daily consumption for one *L. nassula* was 10.27 μmol.

3.3.2 Model Projections

The range of sulfate production determined from a literature review was 12 – 60 mmol m^{-2} day^{-1} (Holmer and Nielson, 1997; Pollard and Moritary, 1991). This was assumed to be the sulfide production rate. Over a 180 day study period, these rates would result in a production of 2160 – 10800 mmol m^{-2} of sulfide. In both Sunset Cove and in Rabbit Key Basin (ambient sulfide concentration 102 μM and 104 μM respectively), that results in a loss of 1980 – 10620 mmol m^{-2} study $period^{-1}$. In Sunset Cove (*C. orbiculata* density of 80 m^{-2}) total lucinid consumption is 1.02 mmol m^{-2} day^{-1} or 184 mmols m^{-2} study $period^{-1}$. In Rabbit Key Basin (*C. orbiculata* density 26 m^{-2} and *L. nassula* density 28 m^{-2}) total lucinid consumption is 0.65mmol m^{-2} day^{-1} or 117 mmol m^{-2} study $period^{-1}$. That means that sulfide removal due to lucinid activity is between 2% and 10% of the total sulfide removal in both systems.

Assuming that all sulfide removal systems are removing sulfide at their highest capacity, if lucinids were removed from the system, more sulfide would accumulate. In Sunset

Cove, 1.02 mmol of sulfide would accumulate. Using the sediment characteristics to convert an amount to a concentration, that results in a 0.62 μM m^{-2} day^{-1} increase. At this rate, sulfide concentration would double in 160 days. In Rabbit Key Basin 0.65 mmol m^{-2} day^{-1} would be added to the system. Using the field sediment characteristics, that results in a 0.39 μM m^{-2} day^{-1} increase. At this rate, sulfide concentration would double in 265 days (Summarized in Figure 3.3).

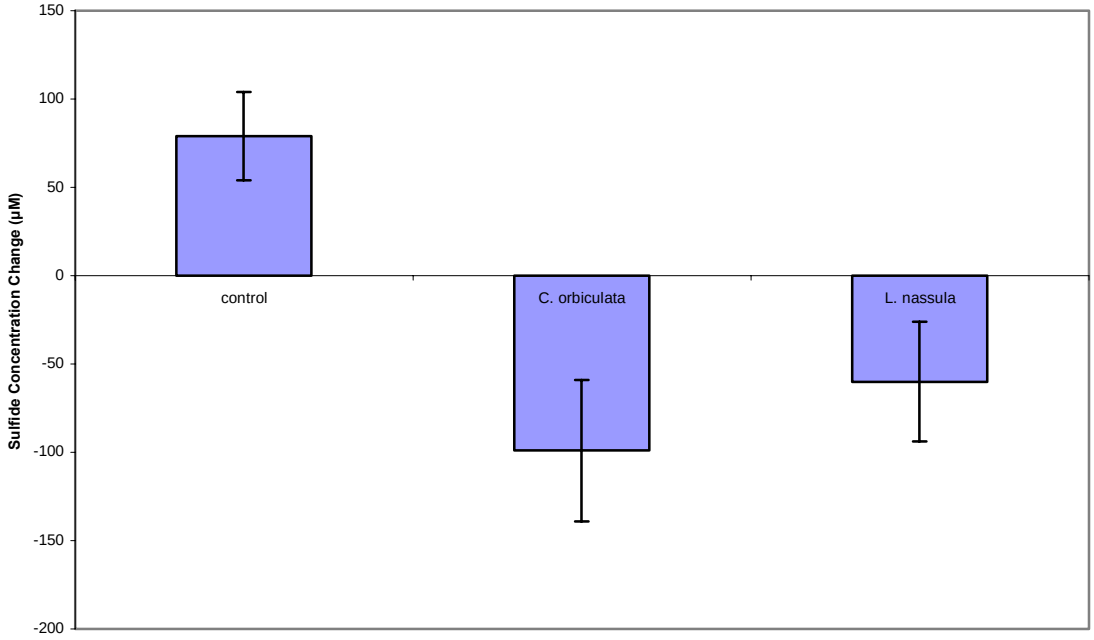

Figure 3.2 Lucinid Influence on Porewater Sulfide Concentration: Microcosm Experiment Results

The above chart describes the sulfide concentration change is small tubes incubated under one of the following treatments: control, +1 *Ctena orbiculata*, +1 *Lucinesca nassula*. Decreases in both clam tubes are significantly different from the control increases, but the changes in the clam tubes were not different from each other. The error bars represent standard error.

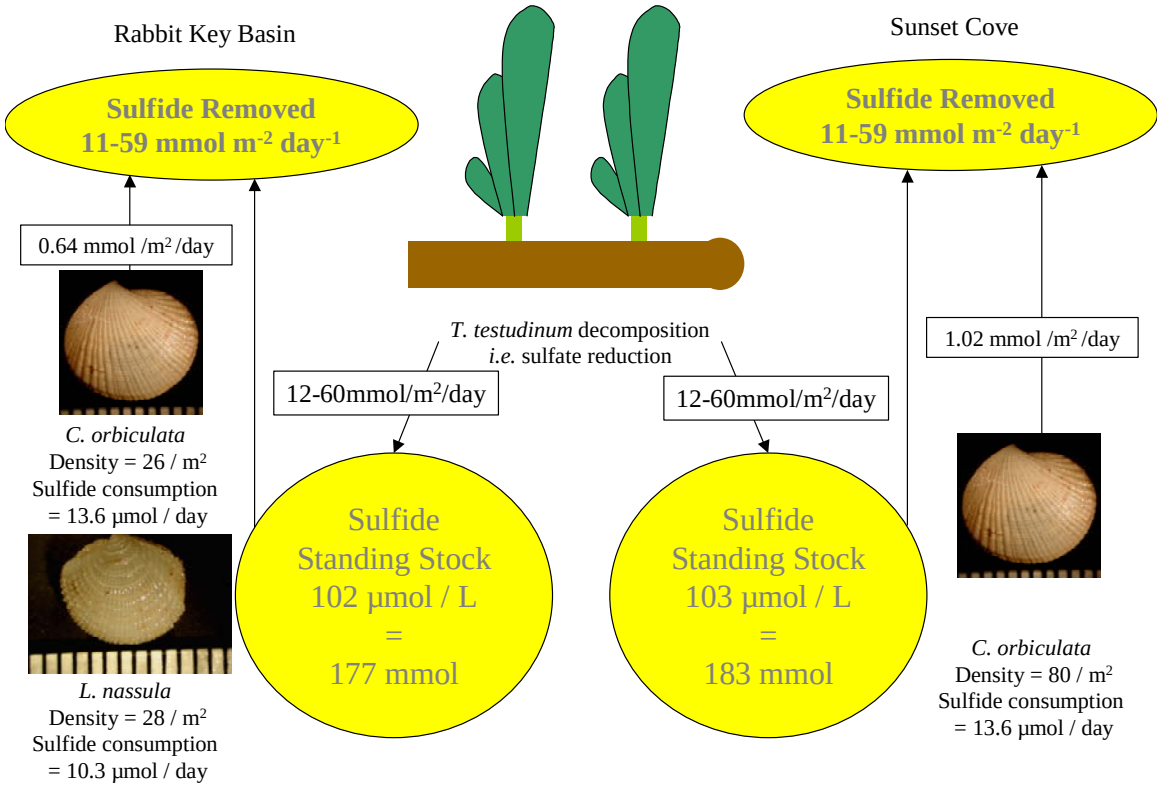

Figure 3.3 **Model Sulfide Budgets for Rabbit Key Basin and Sunset Cove**

Sulfide production in both systems was estimated using published sulfate reduction rates. Sulfide standing stocks were assumed to be relatively constant at a measured concentration. And sulfide removal rate was determined with stoichiometry. Lucinid removal rate was determined using measured lucinid density and species composition for each basin as well as measured individual sulfide oxidation rates for each species.

Chapter 4 Mechanisms by which *Ctena orbiculata* (family Lucinidae) Alter Porewater Sulfide Levels in Seagrass Vegetated Sediments.

4.1 Objective

In previous studies, clams in the family Lucinidae have been shown to decrease ambient sulfide standing stocks (Chapter 2 and Chapter 3). This reduction was assumed to be the result of bacterial oxidation as well as oxygen introduction via burrowing tubes. The goal of this study was to estimate dissolved oxygen introduction in an effort to determine which of the two lucinid sulfide removal mechanisms, bacterial oxidation or oxygen introduction, is more influential.

4.2 Methods

4.2.1 Experimental Design

Eight tubes (diameter = 2.5 cm, depth = 15 cm, capped at one end) were filled with coarsely sieved (1 cm) sediment. These tubes were placed in a tub containing 7 L of seawater continuously bubbled with air and maintained at a temperature of approximately 28°C using aquarium heaters. After a settling period of approximately 24 hours, the tubes were randomly assigned to one of two treatment groups: control or *C. orbiculata*. Controls were not manipulated. To the *C. orbiculata* tubes, one live *C. orbiculata* was placed on the surface and allowed to burrow. Once all clams were covered with sediment, 200 ml of concentrated Rhodamine WT dye was added to the water column, resulting in a water column with a concentration of 3% Rhodamine WT. The experiment was allowed to incubate for 5 days.

After 5 days, the tubes were removed, covered with parafilm, and frozen. Once completely frozen, the tubes were segmented into 2 cm sections. The sediment from each section was placed into a centrifuge tube and centrifuged on the highest setting for ten minutes. The resulting supernatant was pulled off with a syringe, filtered though a 0.45 μm Millipore filter, and analyzed spectrophotometrically for Rhodamine WT concentration.

The characteristics of the experimental sediment were analyzed. Three 5 ml sub-samples of the sediment used in the experiment were taken, weighed, dried to a constant weight at 60°C, and re-weighed. Wet density was calculated as the wet sample mass divided by the wet sample volume. The water content of the sediment was determined as the mass of water lost in drying divided by the mass of the total wet sample. Finally, these measurements were used to determine the sediment porosity, which is the volume of porewater divided by the total volume of the wet sample (Equation 3.1,Equation 3.2, and Equation 3.3).

4.2.2 Calculations

4.2.2.1 Bulk Calculation

The amount of Rhodamine WT added to the sediment by one *C. orbiculata* was determined by subtracting the bulk amount of Rhodamine WT found in the control tubes from the bulk amount of Rhodamine WT found in the clam tubes. The amount of water column intrusion due to the clam activity was determined by dividing the amount of Rhodamine WT added to the sediments by the experimental water column concentration.

Results were depth integrated to estimate a total amount of introduced water due to one clam.

Using the assumption that clams react similarly in the natural environment, the results were scaled up to a 1 m^2 area within a natural basin (Sunset Cove) in Florida Bay. Depth of influence was estimated to 12 cm, since that was the depth of influence in the experimental tubes. The total water column intrusion was determined by multiplying the water column intrusion of one experimental *C. orbiculata* by the density of *C. orbiculata* in Sunset Cove. The amount of oxygen introduction was determined by multiplying the average water column dissolved oxygen concentration by the volume of water column water introduced. Finally, it was assumed that all of this oxygen chemically reacted with the sulfide in the sediment, and the amount of sulfide used in this reaction was determined stoichiometrically. Since the sediment characteristics of Sunset Cove are known (sect. 2.3.2.1), a concentration change was also determined. This assumption is not valid. Oxygen introduced into anoxic sediments can have different fates (i.e. consumption by bacteria, reaction with reduced nitrogen species (NH_4^+)). Therefore, the sulfide removal rates will be overestimated.

4.2.2.2 Irrigation Calculation

The process of introducing oxygen into the sediments is more complicated than the bulk calculation suggests. Instead introduction is a 3D diffusive processes complicated by changing dissolved oxygen concentrations. Water from the water column is transported into the clam burrow by ciliated currents. Those ciliated currents are probably not steady, so the dissolved oxygen concentration in the tube is not constant with time. Dissolved

oxygen is continually diffusing from the water filled tube into and through the sediments and being consumed in the sediments by both chemical reactions and by microbes. The rate of diffusion depends on the concentration in the water filling the clam burrow. This process results in a profile where oxygen concentration is greatest close to the tube and exponentially decreases with distance from the tube. This decrease is due to diffusion and to consumption. This process is described by Boudreau (1984) and by Emmerson et. al (1984) by the following equation (Equation 4.3):

$$\varphi D' \frac{\partial^2 \overline{C}}{\partial x^2} + \varphi \alpha(x)\left(C_0 - \overline{C}\right) + \overline{R} = \varphi \frac{\partial C}{\partial t}$$

Equation 4.1

The above equation describes the concentration change of a species (C) around an irrigated tube. x is sediment depth. D' is the diffusivity of that species, and R is the strength of species reaction. t is time, and φ is sediment porosity. α(x) is a depth dependent constant describing the irrigation process.

The first term in this equation describes vertical molecular diffusion. Since only the top few millimeters of seagrass system contain significant amounts of oxygen (Hemminga and Duarte, 2000) and since the vertical molecular diffusion of Rhodamine was found to be small, diffusion from the sediment water interface is considered negligible, and that term is disregarded. The equation can therefore be simplified as:

$$\overline{R} + \varphi \alpha(x)\left(C_0 - \overline{C}\right) = \varphi \frac{dC}{dt}$$

Equation 4.2

The above equation is a modification of a 1D irrigation model by Emmerson et. al (1984) and by Bourdreau (1984). This modification is an assumption of no vertical molecular diffusion and assuming sediments with a species concentration of 0.

The alpha value was calculated by applying the experimental data to Equation 4.2. Since Rhodamine WT was not produced or consumed in the experimental tubes, $\overline{R}$ becomes zero. C_0 is the concentration of Rhodamine WT in the water column and was considered

constant throughout the experiment at 2.8%. The change in sediment Rhodamine concentration over the course of the experiment $\left(\frac{dC}{dt}\right)$ is calculated as an absolute change. $\overline{C}$ is the average Rhodamine concentration in the sediment over the experiment. This term was estimated as half of the difference in concentration at the beginning and end of the experiment.

Since an alpha value incorporates a density (i.e. one individual per tube (4.9 cm^2) or 2000 individuals per m^2), the value must be corrected for natural densities in Sunset Cove, Florida Bay (80 individuals per m^2 or 1 individual per 125 cm^2). In the experimental tubes, concentration change was measured in 2 cm deep segments with an area of 4.9 cm^2. The same absolute addition of Rhodamine WT would occur by *C. orbiculata* in Sunset Cove, but the concentration change would be spread over 2 cm deep segments with an area of 125 cm^2. To make this correction, the concentration change in the tube segment was multiplied by the volume of porewater in the tube segment (7.5 ml) to get an absolute molar change, which can be divided by the volume of porewater in a Sunset Cove segment (190 ml) to get a corrected concentration change (Equation 4.3).

$$\alpha(x) = \frac{\frac{C_{final} - C_{intial}}{t} * \frac{7.5ml}{190ml}}{C_0 - \left[\frac{C_{final} + C_{initial}}{2} * \frac{7.5ml}{109ml}\right]}$$

Equation 4.3

The above equation is a modification of a 1D irrigation model by Emmerson et. al (1984) and by Bourdreau (1984) rearranged to solve for α(x), the irrigation constant. The alpha value is corrected from an experimental density of 1 individual per 4.9cm^2 (7.5 ml porewater) to a natural density of 1 individual per 125 cm^2 (109 ml porewater).

To estimate the amount of oxygen introduced into the sediments by clam irrigation, Equation 4.2 was solved using the corrected alpha estimates and oxygen conditions in Sunset Cove. Since sediments are nearly always anoxic $\left(\overline{C} = 0\right)$, steady state was assumed. The concentration of oxygen does not change with time $\left(\frac{dC}{dt} = 0\right)$. C_0 was assumed to be constant at 6 mg L^{-1} in both the water column and in the clam tubes. $\overline{R}$ is calculated as the accumulation of oxygen at depth. Since it was assumed that the average concentration of oxygen at depth is negligible, $\overline{R}$ will be negative, describing a consumption of oxygen. For simplicity's sake, it was assumed that all oxygen was consumed by reaction with sulfide (Equation 1.1), so the calculated $\overline{R}$ value is half of the sulfide concentration decrease at depth (when expressed as mol per volume as opposed to mass per volume). Consumption can be expressed as sulfide concentration decrease using the characteristics of the sediment used in the experiment. Again the resulting oxygen

consumption was depth integrated to estimate the total oxygen introduction due to one clam.

4.3 Results

4.3.1 Experimental Results

The sediment used in the experiment had a density of 1.09 g ml^{-1} and a water content of 70.6%. The sediment in the tubes incubated without a clam contained little Rhodamine WT below 2 cm depth. The sediment in the tubes incubated with a clam contained Rhodamine WT to a depth of at least 8 cm. The concentration of Rhodamine in the sediment was statistically significant (t test at $p \leq 0.05$) in each segment above 10 cm depth (See Figure 4.1). Assuming that all Rhodamine WT entering the sediments without clams was due to molecular diffusion, and assuming that Rhodamine entering sediments with clams resulted from both molecular diffusion and clam introduction, molecular diffusion was never more than 16% of total Rhodamine introduction. Below the first measured segment, clam introduction of Rhodamine consisted of at least 95% of the total Rhodamine introduction.

4.3.2 Calculation

4.3.2.1 Bulk Calculation

In the experiment, one *C. orbiculata* introduced 0.7 μl of Rhodamine WT per day. Since the Rhodamine WT concentration in the water column was 2.8%, water column water introduction is 2.3 ml day^{-1}. Assuming that the average daily dissolved oxygen concentration of the water column is 6 mg L^{-1}, each clam introduces 0.37 μl of dissolved oxygen day^{-1}.

In a 1 m x 1 m x 0.12 m block of Sunset Cove sediment (*C. orbiculata* density = 80 individuals m^{-2}), clams cause an increase in dissolved oxygen of 35 μmol day^{-1}. Using natural Sunset Cove sediment characteristics, sediment DO increase was calculated 0.37 μM day^{-1} or 67 μM 180 day study period. A 180 day study period was used because Sunset Cove was monitored for 180 days in Chapter 2. Assuming that all of this oxygen introduced reacts with sulfide and converts it to sulfate, sulfide concentration will decrease 34 μM per 180 day study period.

4.3.2.2 Irrigation Calculation

In the experimental tubes the maximum corrected irrigation constant (α) of Rhodamine was 2 $year^{-1}$, and alpha reached approximately zero by a depth of approximately nine centimeters. Oxygen consumption ranged from 16 to 0 μmol day^{-1} in a 1 m x 1 m x 2 cm sediment segment depending upon depth (Figure 4.2). In a 1 m x 1 m x 0.12 m block of Sunset Cove sediment, consumption of dissolved oxygen is 42 μmol day or an introduction of approximately 0.44 μmol dissolved oxygen per individual. If all of the introduced oxygen reacts with sulfide, a sulfide concentration decrease of 78 μM would occur every 180 day study period.

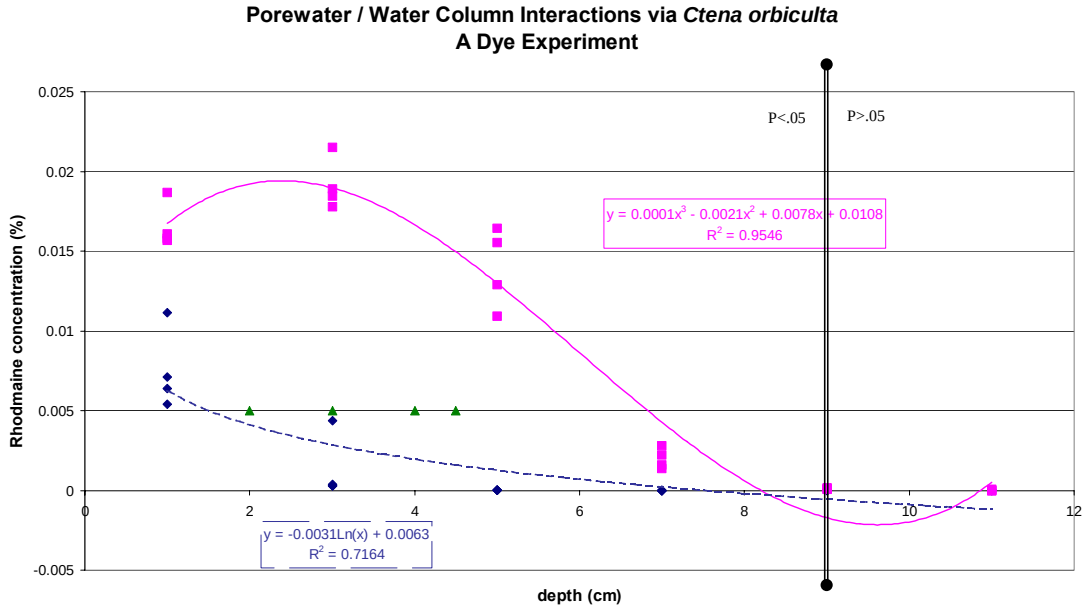

Figure 4.1 **Porewater / Water Column Interactions via *C. orbiculata*: Dye Experiment Results**

The above chart describes the Rhodamine WT concentration change of tubes containing either 0 or 1 *Ctena orbiculata.* All tubes were incubated for 5 days in a bath of seawater with a 3% Rhodamine WT concentration.

The diamonds and dotted line represent control tube results. The squares and solid line represent results of tubes incubated with *Ctena orbiculata.* Triangles represent the depths where *Ctena orbiculata* were found at the end of the experiment. The vertical double line displays the depth at which the differences in mean Rhodamine WT concentration between treatments becomes statically insignificant.

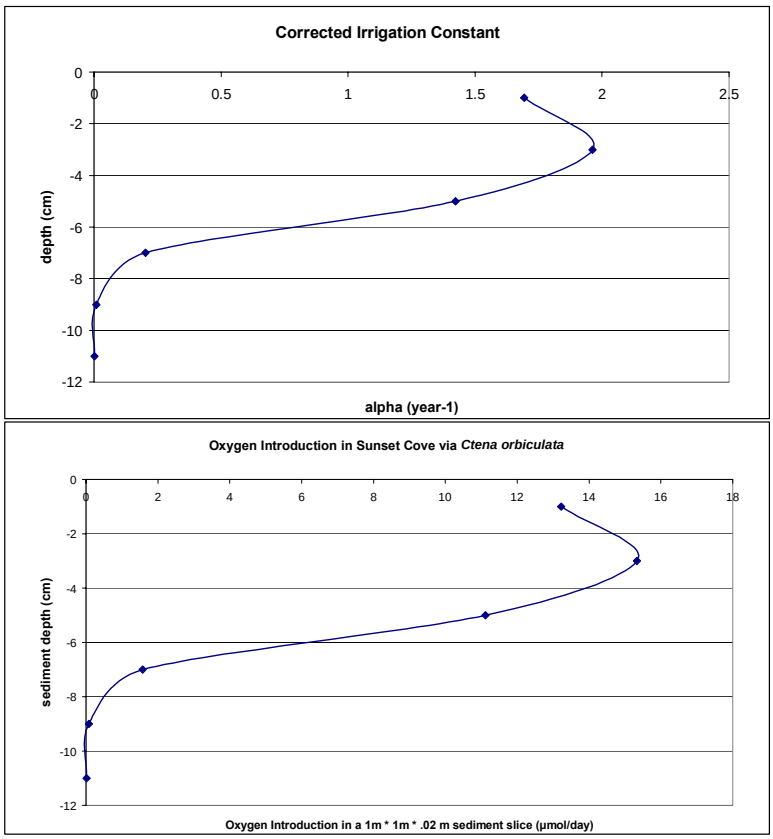

Figure 4.2 Alpha and oxygen introduction sediment distribution
The top chart shows the modeled Rhodamine WT irrigation constant for *C. orbiculata* corrected for a density of 80 individuals m^{-2}. The lower chart shows modeled oxygen additions via *Ctena orbiculata* at a density of 80 individuals m^{-2}.

Chapter 5 Discussion: The effect of Endosymbiont Bearing Infaunal Bivalves on the Biogeochemistry of *Thalassia testudinum* Sediments

5.1 Bay Wide Survey

A literature review determined that fifteen species of Lucinids have a high probability of being found in the seagrass sediment of Florida Bay (Tabb and Manning, 1961; Turney and Perkins 1972; Mikkelson et. al. 1995; Mikkelson and Bieler 2000). This bay-wide survey of nine Florida Bay sites found 8 species. None of the published surveys studied exactly the same area, and some studies were conducted many years ago. Therefore, differences in those studies and this one are to be expected.

Table 5.1 compares the results of previously published mollusk surveys and the results of this bay-wide survey. Since this survey found lucinid populations similar to those found in past studies of different estuaries, experiments performed in Florida Bay have a potential bearing on other systems.

Table 5.1 Species of Lucinid clams found in the literature and in this bay-wide survey

BA=Bob Allen Key; BNS = Barnes Key; CBL = the leeward side of Cross Bank; CBW = the windward side of Cross Bank; DUK = Duck Key; PET = Peterson Keys; RAN = Rankin Lake; RKB = Rabbit Key Basin; SPG = Sprigger Bank. Checks mark sites where species were found.

Species	Reference	BA	BNS	CBL	CBW	DUK	PET	RAN	RKB	SPG
Andontia alba	2,3,4								√	
Codakia orbicularis	2,3,4				√					√
Ctena orbiculata	1,2,3,4		√		√		√		√	√
Divalina quadrisulcata	2,3,4									
Divaricella denta	4									
Lucina pectinata	1,3,4									
Lucina pensylvancia	2,3,4		√							
Lucina radians	4									
Lucina sombrerensis	4									
Lucina trisulcata	4									
Lucinesca nassula	1,3,4		√						√	
Parvalucina multilineata	2,3,4	√			√					
Lucina amiantus	1,3						√			
Pseudomiltha floridana					√	√		√		
Parvalucina costa			√							

1- Tabb and Manning, 1961
2- Turney and Perkins 1972
3- Mikkelson et. al. 1995
4- Mikkelson and Bieler 2000

5.2 Within Site Survey

The survey of Sunset Cove showed that the presence of clams in the family Lucinidae is correlated with lower porewater sulfide concentration in adjacent sediments (95% confidence). A similar relationship was present but not as evident in the survey of Rabbit Key Basin (90% confidence). While both surveys show that there is a correlation, the Sunset Cove survey describes a stronger relationship. This difference may simply be a sampling anomaly, but there are several hypotheses supporting a real difference. There were more observations in Sunset Cove than there were in Rabbit Key Basin. In the extremely variable natural environment, it may take more observations to see an accurate correlation.

The lucinid population differs in the two basins. Sunset Cove (80 lucinid individuals m^{-2}) has a slightly larger density of lucinid clams than Rabbit Key Basin (60 lucinid individuals m^{-2}). Furthermore, the lucinid population in Sunset Cove is dominated by one single species (*C. orbiculata*) as opposed to the two species (*C. orbiculata* and *L. nassula*) that dominate the lucinid population of Rabbit Key Basin. The two species may act differently and therefore effect different results. One species could build larger tubes and increase more oxygen to the sediments, or one species might house more bacteria, which can oxidize more sulfides.

The two basins physically differ. There is more water in the sediments of Sunset Cove (74%) than in the sediments of Rabbit Key Basin (67%). More porous sediment systems allow for more diffusion. Therefore, the effect of clam activity can be seen over a larger

area. If this is the case, using smaller cores in Rabbit Key Basin might reveal a more significant correlation. Additionally, there is a larger density and diversity of infauna in the sediments of Rabbit Key Basin (i.e. medusa worms) bioturbating the sediments, introducing oxygen, and potentially masking the effects of Lucinid clams.

Although the larger standing crop of seagrass in Rabbit Key Basin might otherwise induce more decomposition and thus more sulfide production, Rabbit Key Basin is better flushed than Sunset Cove. Therefore, there may be more mechanical removal of litter, less decomposition, and less sulfide production. Additionally, the sampling sites in Sunset Cove are closer to mangrove trees than the sampling sites in Rabbit Key Basin. Introduction of additional litter may increase decomposition and sulfide production. Lucinid clams do not produce sulfides; they only alter what is already in the system. Therefore, differences in sulfide production might alter the degree to which the clams can take advantage of and manipulate sulfide levels.

There are problems affecting the observation of sulfide removal at both sites. Sulfide is a very transient molecule. Measuring the ambient pool might not accurately depict alterations made by lucinid clams. Accurately measuring sulfide oxidation would reveal a better understanding of lucinid effect on porewater sulfide. However, measuring sulfide pools is much easier and less time consuming that measuring sulfide oxidation. Despite the complications with these measurements and the problems associated with surveying a complex system, it is apparent that the presence and activity of lucinid clams is correlated

to some extent with decreased sulfide concentrations in the sediment. Altering the sulfide levels even to a small degree in the systems has many implications for the seagrasses in these sediments.

In both systems, the presence of lucinid clams was weakly associated with increased ammonium concentrations in the sediment. The correlation is expected, since other suspension feeders have been shown to increase porewater nutrients (Peterson and Heck, 1999, 2001, 2001a; Reusch et, al., 1994). The alterations in porewater ammonium concentration appear somewhat lower than the increases found with mussels in seagrass beds, but that is again expected. Lucinids rely to some degree on the bacteria living in their gills for food and therefore have a reduced filtering capacity. The small changes however can be detected since ammonium is relatively labile in the sediments of Florida Bay. Florida Bay is typically considered phosphorous limited; therefore, nitrogen additions are not taken up quickly. That implies that those additions are not of as much importance. However, lucinids and seagrass exist in systems that are not necessarily phosphorous limited, and in other areas of the world, this interaction between lucinids and sediment ammonium levels might be more significant. Furthermore, this nitrogen addition might be underestimated. This nitrogen addition is occurring in close proximity to oxygen introduction (via diffusion through hollow tubes). Therefore, some of the ammonium might be nitrified to nitrate. Nitrate was not measured in this survey, since nitrate is typically considered below the detection level in Florida Bay sediments.

In phosphorous limited systems, an addition of phosphate will be immediately taken up by plants and bacteria in the sediments. Also, the carbonate muds of these systems are able to adsorb phosphate and remove it from the porewater pool. Therefore, it is not unexpected that slight phosphate additions due to suspension feeding to the sediment are difficult to detect, especially since lucinid suspension feeders have a reduced filtering capacity and presumably introduce only a small amount of phosphorous to the sediments.

5.3 Microcosm Experiment

Chapter 3 data show that the clams *C. orbiculata* and *L. nassula* have the potential to considerably alter the sediment chemistry of their environment. While their activity is certainly not the only method for removing toxic sulfide from the environment, it does appear to be important. These clams may be more important to total sulfide removal than the 2 – 10% calculated, since sulfide production was most likely overestimated. While most sulfate reduction yields sulfide, some reactions might not run to completion and form only elemental sulfur or thiosulfate. If total sulfide production and thus total sulfide removal is less than estimated and if removal by the clams is not, total clam influence would be underestimated.

The model sulfide budgets created to project these results to a 1 m x 1 m area suggest that the removal of lucinid clams and the subsequent addition of the sulfide they currently remove back into the sediment standing stock would increase porewater sulfide concentrations 0.62 μM day^{-1}. That increase, summed daily would double the sulfide concentration in only 160 days. Additional sulfide removal mechanisms might

compensate for the loss of lucinids and decrease the sulfide accumulation rate if these clams were removed. Seagrass above to below ground biomass ratio could decrease, allowing for a larger below ground surface area from which oxygen can diffuse and react with sulfide. In areas with higher sulfide concentrations, the reaction of sulfide with hydrogen ions might increase resulting in the formation of more hydrogen sulfide gas, which can escape from the sediments (Equation 1.2). The latter appears less likely since in the laboratory microcosm experiment, sulfide accumulated in the sediments. Regardless, lucinid clams are removing a lot of sulfide from these sediments, and since sulfide has been linked to seagrass decline (Carlson et. al., 1994; Koch and Erskine, 2001; Peterson et. al. 2003), this removal potentially has a large impact on seagrass productivity and health.

The specific location of these two species makes them especially important. The average depth of these clams is 7-9 cm (Chapter 2). This is above the bulk of the rhizosphere, closer to the basal meristem area. Pederson et al. demonstrated by means of microelectrode sensors that sustained sulfide intrusion into these specific tissues is most detrimental and most likely to initially induce die off.

5.4 Dye Experiment

Estimating oxygen introduction due to *C. orbiculata* using a depth integrated bulk calculation approach and an irrigation model approach yields similar results (approximately .4 μmol day^{-1} $individual^{-1}$). The bulk calculation was slightly lower. (Figure 5.1). The small difference is most likely due to vertical molecular diffusion. The bulk calculation approach recognizes and compensates for vertical molecular diffusion

from the surface, while the irrigation model disregards this introduction. Vertical molecular diffusion can be omitted from the bulk calculation approach by considering clam Rhodamine WT introduction as the amount of Rhodamine WT found in the clam tubes as opposed to the amount of Rhodamine WT found in the control tubes subtracted from the amount of Rhodamine WT found in the clam tubes. (Refer to section 4.2.2.1 for review.) This results in a value closer to the value estimated by the irrigation calculation (0.44 μmol dissolved oxygen introduced per day).

The microcosm experiment in chapter 3 showed that one *C. orbiculata* can remove 14 μmol of sulfide per day. If all of that sulfide was removed by oxygen, one *C. orbiculata* individual would need to add 28 μmol of oxygen per day in addition to the oxygen that it introduces for its own respiration. Each model of *C. orbiculata* oxygen introduction projects a much smaller oxygen introduction. These estimates of oxygen introduction suggest that oxygen introduction can only remove about 1.5% of the sulfide removed by lucinid activity. Therefore, one must assume that most of the sulfide removal is achieved by means of bacterial oxidation or by another method (Figure 5.1).

Both calculations are subject to some measurement errors. Both approaches use Rhodamine WT diffusion to approximate dissolved oxygen diffusion. However, the diffusivities of the two compounds are not equal. Rhodamine WT diffusivity is $2.3 * 10^{-6}$ cm^2 s^{-1} (Glud and Fenchel, 1999), and oxygen diffusivity is approximately $1.9 * 10^{-5}$ cm^2 s^{-1}.

The sulfide removal achieved by clam burrows might be underestimated in this study. This study only acknowledges sulfide removal by oxygen introduced into the sediments. Sulfide could be diffusing out of the sediments as well as being removed by introduced oxygen. The clam tubes are assumed to be filled with water that is very similar to the overlying water column: water with some dissolved oxygen and a zero sulfide concentration. Therefore, while oxygen is diffusing out of the tube into the sediment, sulfide can be diffusing into the tubes from the sediment. The sulfide in the sediments near the clam burrows will react with oxygen introduced, but since sulfide is much more abundant than oxygen and since spontaneous rates of the reaction are slow, there is likely to be excess sulfide. Excess sulfide can enter the tube and diffuse down a concentration gradient into the water column, where abundant oxygen will react with it to form sulfate, thus removing it from the sediment porewater system. This is less likely to occur at the sediment water interface since a relatively large area is covered by oxygenated water making oxygen introduction greater and less patchy.

While this oxygen introduction via lucinid burrows is small and may not be the predominant method for sulfide removal, it may still be important. Oxygen typically enters the sediment by means of diffusion from the overlying water column. That oxygen is depleted in the top few millimeters of sediment by bacterial respiration (Hemminga and Duarte, 2000). *C. orbiculata* burrows to an average depth of 7.5 cm (Chapter 2).

Therefore, these clams are introducing oxygen in small amounts to a deeper depth. This creates or enhances a sediment complex that while mostly anoxic has small transient patches of oxygenated sediment.

Small oxygenated zones have a large impact on the sediment environment. Microbes are much more efficient decomposers when they use oxygen as opposed to sulfate as a terminal electron acceptor. More energy is produced, and more of the available carbon is assimilated (Alongi, 1998). While seagrass meadows are some of the most productive systems in the world, most of the production is passed to higher trophic levels only via the detrital food web (Zieman, 1982). More efficient assimilation by bacteria will result in more efficient transfer of energy up the food chain.

5.5 Overall Conclusions and Significance

Sulfide dynamics in seagrass systems are highly studied; however, most studies of sulfide dynamics do not incorporate lucinid sulfide removal. This study demonstrates that these clams remove a significant amount of sulfide from these sediments, and in order to get a full and accurate understanding of sulfide dynamics, the activities of these clams must be considered.

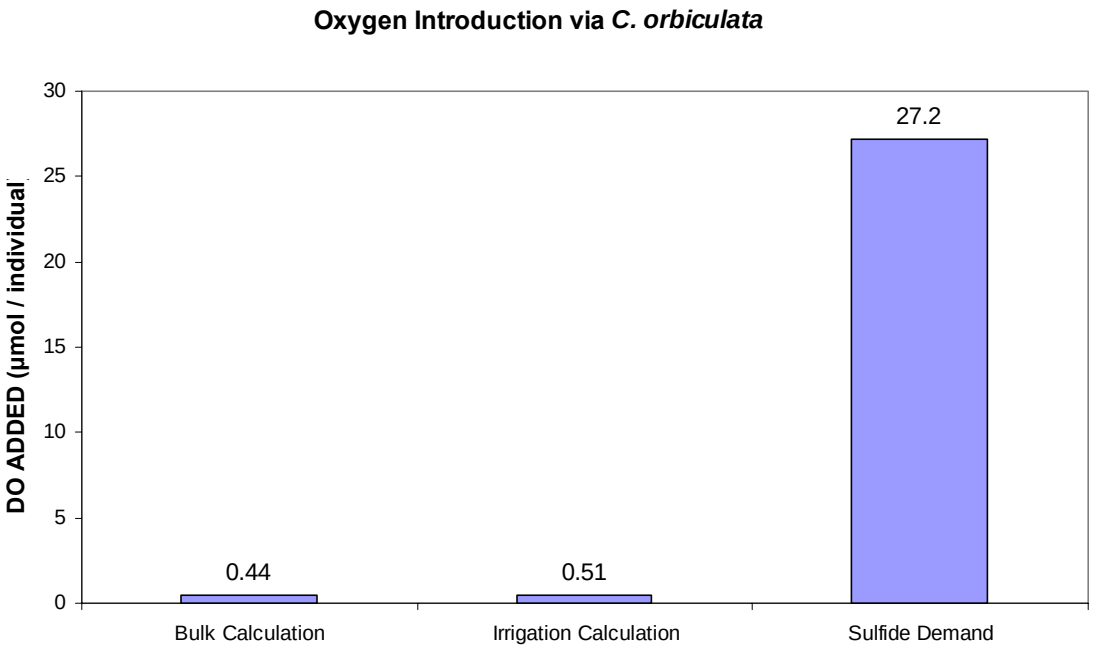

Figure 5.1 Comparison of Oxygen Introduction Estimates

The above figure shows the estimates of oxygen introduction via *Ctena orbiculata* burrows using a bulk calculation approach and an irrigation approach. The final bar shows the amount of introduced oxygen needed to remove 13.6µmol of sulfide. This value was estimated as potential sulfide removal using a microcosm experiment.

Seagrass sediments are very reduced and high in sulfide. Seagrasses form large meadows and create a sizeable biomass which senesces and must be decomposed on or in the sediments. These sediments, like many marine sediments, are mostly anoxic; therefore, most decomposition occurs using sulfate, an ion abundant in seawater, as a terminal electron acceptor, resulting in sulfide production (Fenchel et. al., 1998; Hemminga and Duarte, 2000). Seagrasses have evolved in this environment and are efficient at dealing with toxic sulfide. Oxygen produced photosynthetically in the leaves can diffuse throughout the plant to the below-ground tissues. Diffusion of oxygen into the sediments from the below-ground tissues protects the seagrass from toxic intrusion by spontaneously reacting with sulfide to form sulfate, thus removing the toxin from the environment (Pederson et. al., 1998). However, when seagrass biomass becomes greater and the subsequent decomposition produces larger amounts of sulfide, this oxygen transport cannot compensate for sulfide intrusion. When sulfide intrudes into plants, photosynthetic capacity decreases and seagrass die-off is potentially induced (Goodman et. al., 1994; Koch and Eskrine, 2001; Pederson et. al. 2003) (Figure 5.2).

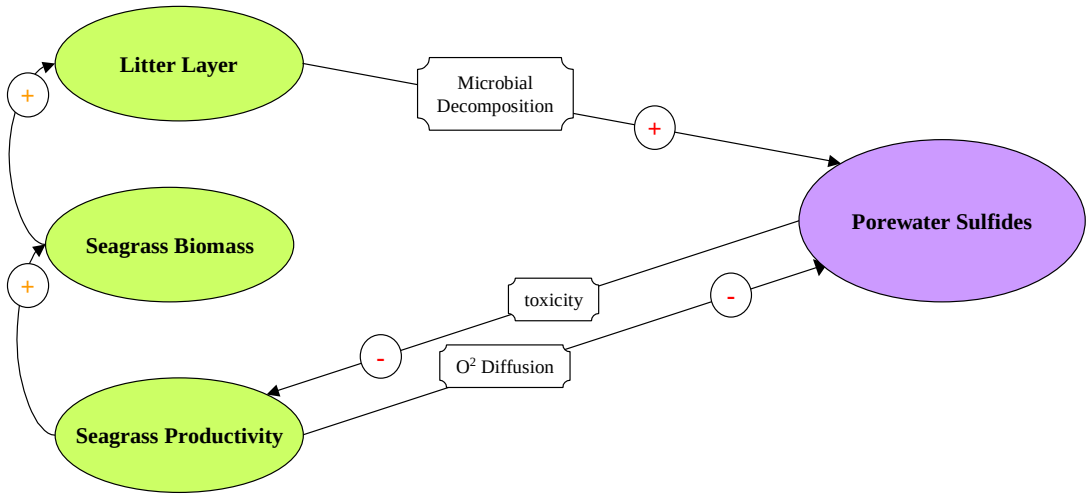

Figure 5.2 Conceptual model describing the Porewater Sulfide Concentration and Seagrass Productivity and Biomass Interactions.

The rectangular boxes describe processes. The ovals describe standing stocks. Pluses and minus describe increases or decreases respectively in the standing stocks symbol following. The following is an explanation of how to read this diagram using the outside connection between seagrass productivity and porewater sulfides as an example: Seagrass productivity decreases porewater sulfides by producing oxygen and facilitating its diffusion into the sediments where it will react with sulfides.

Lucinid clams have also evolved in seagrass beds (Bretsky, 1976; Jackson, 1972,1973), and these clams are acting to reduce porewater sulfides. This removal allows seagrasses to create a large biomass without being impacted by intruding sulfide. However, the sulfide removal capacity is limited. And while these clams might buffer some of the harmful results of anthropogenic impacts such as nutrient additions and decreased salinity (i.e. wetland draining) which increase seagrass productivity and biomass to an unnatural and unsustainable size, these clams, in their current densities, cannot deter these effects completely.

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