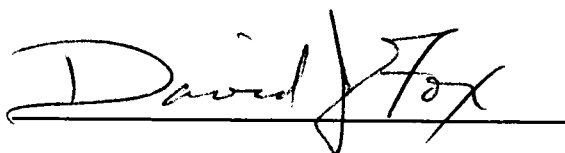


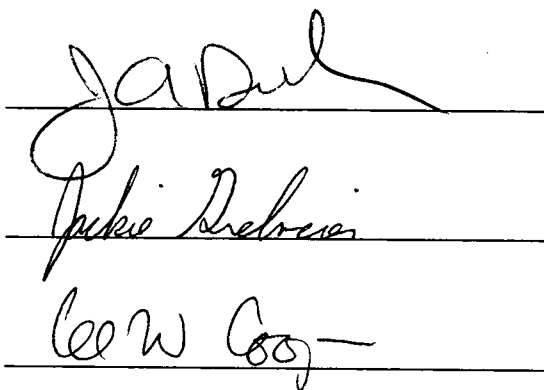
To the Graduate Council:

I am submitting herewith a thesis written by Matteson Todd Bowles entitled: "Behavior and Ecology of *Holothuria floridana* in the Florida Keys: Movement Patterns and Feeding Preferences." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology.

A handwritten signature in cursive script, reading "David J. Fox", written over a horizontal line.

David J. Fox, Major Professor

We have read this thesis and
recommend its acceptance:

Three handwritten signatures in cursive script, each written over a horizontal line. The signatures appear to be "J. A. Dul", "J. K. B. B.", and "C. W. C.", though the last one is partially cut off.

Accepted for the Council:

A handwritten signature in cursive script, reading "C. W. C.", written over a horizontal line.

Associate Vice Chancellor and
Dean of the Graduate School

**BEHAVIOR AND ECOLOGY OF *HOLOTHURIA FLORIDANA* IN THE
FLORIDA KEYS: MOVEMENT PATTERNS AND FEEDING PREFERENCES**

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Matteson Todd Bowles
May 1998

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A special thanks to my parents for their encouragement and support that allowed me to complete this degree.

O Lord, how manifold are Your works!
In wisdom You have made them all.
The earth is full of your possessions-
This great and wide sea,
In which are innumerable teeming things,
Living things both small and great....
Psalm 104:24,25 (NKJV)

Soli Deo Gloria

Abstract

A behavioral study of the sea cucumber, *Holothuria floridana* was conducted in a mangrove channel in the Florida Keys to ascertain its foraging patterns and feeding preferences. The study took place during March, May, July, and September of 1997. Sixty holothurians were tagged and released during each of these months in order to observe foraging behavior and preferences for substrates. Measurements of holothurian movement were made at 3-6 hour intervals after their release. Samples of the sediment were collected for grain-size analysis to determine particle size preferences. Sediment organic carbon, holothurian tissue, particulate organic carbon, and biota from the site were collected for $\delta^{13}\text{C}$ analysis so as to determine the primary carbon source. Current direction and water temperatures were also recorded during each study period. *H. floridana* foraging activity was observed within a 18m^2 area during the study. Little difference was observed between the number of individuals migrating into seagrass beds and those migrating into the open sandy bottom from the seagrass beds. There was no detectable sediment gradient in either seagrass beds or sandy areas, as grain size composition was uniform throughout the entire study site. There was a visible organic flocculant layer trapped within the seagrass beds. A sediment composition comparison grouped fecal pellet samples together, and suggested some particle selectivity by *H. floridana*. *H. floridana* preferred the seagrass beds instead of the open sand (71% in September). Carbon isotope analysis suggested the primary carbon source was the seagrass, *Thalassia testudinum*. Evidence for a preference of the mid-channel areas could

not be clearly shown (seagrass bed locations having sediment organic carbon $\delta^{13}\text{C}$ values from -18‰ to -19‰), but an increasing number each month of the study were found in the mid-channel areas. A light gradient experiment revealed no preference for specific light intensities. At temperatures above 33°C, *H. floridana* leaves seagrass beds for open sandy areas, presumably for cooler temperatures from faster moving water. The foraging behavior of *H. floridana* is directly related to the availability of the primary food source *Thalassia testudinum*, with no significant relationship to sediment grain size or light intensity.

Table of Contents

I. INTRODUCTION	1
HOLOTHURIAN ECOLOGY	1
SEDIMENT GRAIN SIZING	5
STABLE CARBON ISOTOPES	7
LIGHT RESPONSES	10
TEMPERATURE RESPONSES	10
SUMMARY	11
II. MATERIALS AND METHODS	12
FORAGING BEHAVIOR	12
SEDIMENT SAMPLES	16
STABLE CARBON ISOTOPE SAMPLES	18
LIGHT RESPONSE ASSESSMENTS	19
STATISTICAL ANALYSIS	20
III. RESULTS	23
CHARACTERIZATION OF THE STUDY SITE	23
FORAGING BEHAVIOR	24
SEDIMENT GRAIN-SIZE ANALYSIS	24
STABLE ISOTOPE ANALYSIS	30
HOLOTHURIAN MOVEMENT WITH RESPECT TO SUBSTRATE	30
SUBSTRATE PREFERENCE WITH RESPECT TO $\delta^{13}\text{C}$ VALUES	34
LIGHT RESPONSES	40
SUBSTRATE PREFERENCE WITH RESPECT TO TEMPERATURE	40
OTHER VARIABLES AFFECTING SUBSTRATE PREFERENCE	42
IV. DISCUSSION	44
FORAGING BEHAVIOR: PATTERNS OF MOVEMENT	44
RELATIONSHIP BETWEEN SEDIMENT GRAIN-SIZE PREFERENCE AND MOVEMENT	45
RELATIONSHIP BETWEEN FOOD SOURCE AND MOVEMENT:	
STABLE ISOTOPE EVIDENCE	46
RELATIONSHIP BETWEEN FOOD SOURCE AND MOVEMENT: SUBSTRATE PREFERENCE	49
RELATIONSHIP BETWEEN OTHER VARIABLES AND MOVEMENT:	
TEMPERATURE AND LIGHT	51
SUMMARY	52
V. CONCLUSION	53

REFERENCES	56
APPENDICES	63
VITA.....	71

List of Figures

Figure 1. Location of the study site in a mangrove channel of Zane Grey Creek.	3
Figure 2. The 14 areas of the study site.....	12
Figure 3. Holothurian identification tag.....	13
Figure 4. Site map showing the points of holothurian release.	15
Figure 5. Location of sediment samples within study site.	17
Figure 6. Experimental setup for light sensitivity assessment.	21
Figure 7: Locations of three holothurians labeled A, B, and C over 5 time periods.....	25
Figure 8. Locations of random sediment samples collected in March 1997, with the corresponding $\delta^{13}\text{C}$ values.....	32
Figure 9. Locations of transect sediment samples collected in May and July 1997 with the corresponding $\delta^{13}\text{C}$ values of the organic component of the sediments.....	33
Figure 10. Comparison of holothurian distribution between seagrass beds and open sand in 1997, with Chi-square significance values.....	35
Figure 11. Study of areas 6, 7, 8, and 9 showing the zone with sediment organic carbon with $\delta^{13}\text{C}$ values of -20‰ or less (12% of study area).	37
Figure 12. Study of areas 6, 7, 8, and 9 showing the zone with sediment organic carbon with $\delta^{13}\text{C}$ values of -20‰ or less (16% of study area).	38
Figure 13. Percentages of holothurians found in sediments with $\delta^{13}\text{C}$ values either -18‰ to -19‰ or -20‰ or less during the months of March, May, July, and September, 1997. Chi-square chart showing significance of the test.	39
Figure 14. Relationship between holothurian recapture rate and water temperature	41
Figure 15. Percentages of holothurians found in seagrass and open sand with respect to the temperature ranges of 28-32°C and 33-34.5°C.	43

List of Plates

- Plate 1. Photograph of *Holothuria floridana*.....4
- Plate 2. CD-ROM containing Animagic 32-bit GIF Animator and
Holothurian animation files.....In Pocket

I. Introduction

Holothurian Ecology

For more than 1,000 years, members of class Holothuroidea of Phylum Echinodermata have been harvested commercially as a food source by countries of the Far East (Conand & Byrne, 1993). In the last twenty years sea cucumber fisheries have increased rapidly in number. Unfortunately, this increase in fishery numbers has not been paralleled by an increase in research on the ecology of these benthic detritivores.

Holothurians are at great risk of overexploitation, and more information on population density, biology, and ecology of holothurians is needed to improve the efficiency of fishery management (Conand & Byrne, 1993). Earlier publications (e.g., Frizzell & Exline, 1955; Bonham & Held, 1963; Bakus, 1968) characterize holothurians as movers of sediment. By ingesting sediment they disrupt sediment stratification. This process, called bioturbation, exposes previously buried invertebrates and microorganisms to the water column, and mixes surface material to variable depths in the sediment column (Birkeland, 1989).

Holothurians ingest an array of items including sand, shell, coral fragments, marine plant fragments, and detritus in general (Bakus, 1968). More recent studies (Conand, 1991; Rodgers & Bingham, 1996) have focused on understanding movement and aggregative aspects of holothurian behavior. Three species dominate the study region of the Florida Keys: *Holothuria mexicana*, *Holothuria floridana*, and *Isostichopus*

badionotus (Humann, 1992). These species are considered to be deposit feeders, animals that frequently ingest sedimented material of low bulk food value (Jumars *et al.*, 1984; Lopez & Levinton, 1987). In general, detritivores derive the largest portion of their nutritional requirements from the microbial component of detritus within the sediment (Yingst, 1976; Lopez & Levinton, 1987; Cammen, 1980).

Holothuria floridana (Plate 1) is found in large numbers in Zane Grey Creek on Long Key, Florida (Figure 1). Zane Grey Creek is a mangrove channel that is partially bordered by houses. Runoff from these houses and possible leaching from septic systems add to the nutrient load, which promotes and supports bacterial populations that serve as an ideal food source for invertebrate deposit feeders (Hobbie & Cole, 1984). Patches of seagrass are located in the channel, catching and holding decaying material and providing cover for many invertebrates. Numerous *Holothuria floridana* can be observed feeding both within and between the seagrass patches. The abundance of these holothurians may be a response to elevated food levels, as has been shown for copepods and sea urchins (Isaacs & Schwartzlose, 1975; Alldredge *et al.*, 1984; Tegner & Dayton, 1991).

Studies focusing on saponins (common metabolites of holothurians) have been conducted on *Holothuria floridana* (Kerr & Chen, 1995), but few behavioral studies have been conducted. Only one ecological study involving spatial distribution has been conducted (Levin & Gomez, 1976). Questions about *Holothuria floridana*'s pattern of movement and factors that determine foraging behavior have yet to be answered. The present study provides observations on movement patterns of *Holothuria floridana*

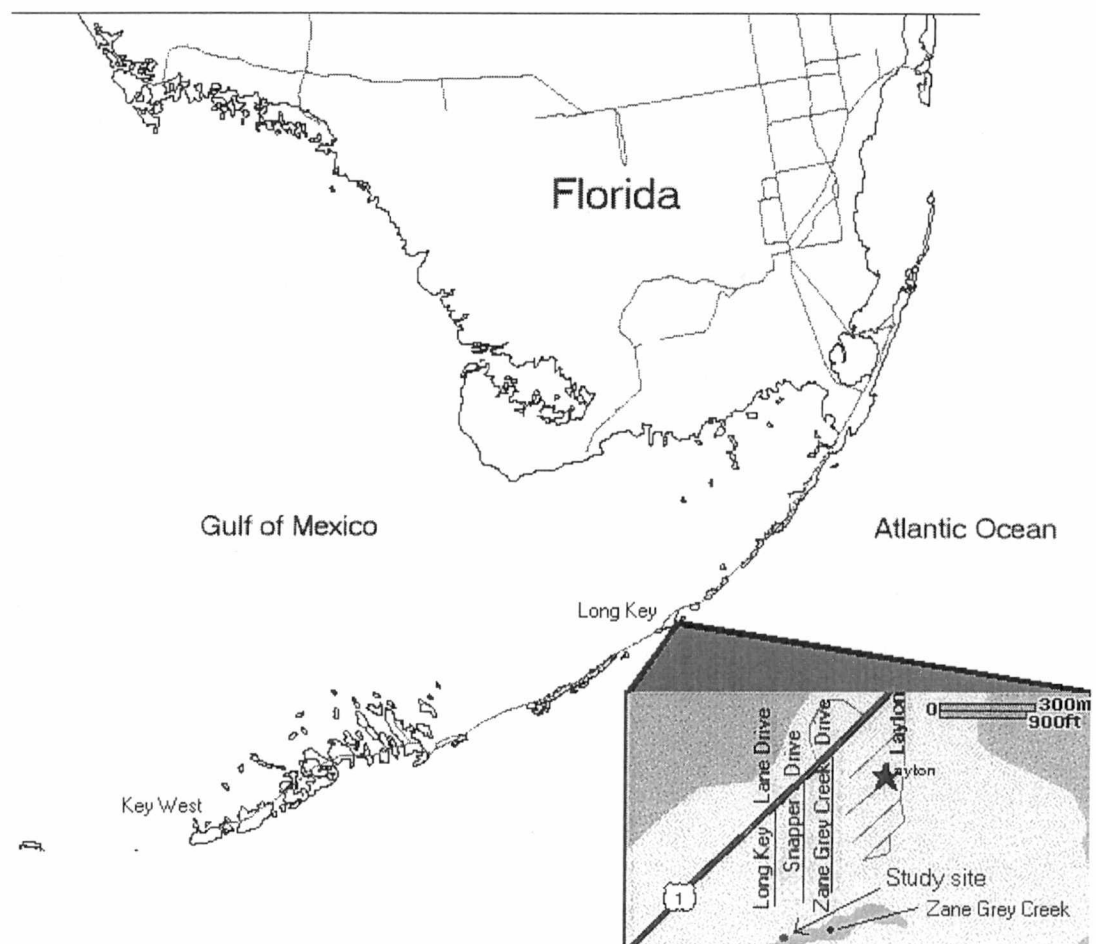


Figure 1. Location of the study site in a mangrove channel of Zane Grey Creek.

Plate 1. Photograph of *Holothuria floridana*

during different seasons of the year in order to determine whether there are any behavioral changes due to reproductive cycles or seasonal environmental variables. Data were also collected on the grain size of the sediment particles being ingested, carbon flow within the ecosystem, and changes in light intensity and seawater temperature during the study. These data were then used in the interpretation of the movement observations.

Sediment Grain Sizing

Foraging behavior, or how an organism acts when searching for food, can affect species movement patterns. How could foraging behavior be the driving force behind observed movement patterns in a species? Foraging Theory attempts to answer questions like this as well as questions on how animals choose their diets, choose foraging techniques, and modify choices according to changing circumstances (Hughes, 1980; Stephens & Krebs, 1986). The question of “where should an animal feed to get the most food?” was asked by the late Robert MacArthur and his colleagues; their studies provided the basic elements of the foraging theory (MacArthur, 1972). A basic premise of the theory, entitled the “Energy Maximization Premise,” is that foraging behaviors that maximize the net rate of intake provide a selective advantage to the organism exhibiting them (Hughes, 1980).

MacArthur (1972) argued that on perceiving a prey item, a predator would either select the prey item or move on in search of prey of greater value. He also proposed that

the predator would select the option that yields the greater resource per unit time. In other words, a predator would only select the prey item, if during the time required to pursue the prey, it would be unlikely to find a better prey item. The time spent actively foraging, or searching the habitat for prey, is called "Search Time." The time spent pursuing and capturing the prey item is called "Pursuit Time" (Hughes, 1980).

Since deposit feeders collect and process their food simultaneously, there is no need to distinguish between "Search Time" and "Pursuit Time" as one would when evaluating predators, so the model was modified by Lehman (1976) to account for this difference (Hughes, 1980). Lehman's model for filter feeders emphasizes the rate of filtration and mechanism of particle selection. This model was later modified by Taghon *et al.* (1978) to apply to deposit feeders by simply changing the assumptions.

The assumptions were that the gut was always filled to capacity and that the food was either bacteria or an organic coating of ingested sediment particles. The dietary values of the particles would thus increase as the surface area to volume ratio increases with decreasing particle diameter (Hughes, 1980). So even though smaller particles carry a smaller mass of food than larger particles, more can be packed into the gut, yielding a larger mass of food per unit volume. A deposit feeder could respond to the dietary value of the sediment by selecting for particle size.

Seagrasses have been shown to reduce the rate of bottom sediment transport by acting as a canopy for baffling of currents, and stabilizing sediments by roots and rhizomes (Ginsburg & Lowenstam, 1958; Rhoads & Young, 1970; Scoffin, 1970; Fonseca *et al.*, 1982; Gambi *et al.*, 1990). The range of sediment sizes caught by seagrass

beds has been dominated by grains of 4 phi (very fine sands) and 5 phi (silts and clays) in some cases (Irlandi & Peterson, 1991).

An earlier study has shown that holothurians select for sediments within a certain range of particle sizes (Trefz, 1958), but the question of whether it was by chemosensory or mechanical means is still unanswered. If holothurians were to select for grain size, it might affect their movement. If an area of suitable grain sizes was found, the animal could slow its movement to ingest as much of the sediment as possible. Conversely, if an animal were in an area of unsuitable grain size, it might increase its movement rate, testing each new area until a more suitable patch was found. In the end, the animals should spend more time in suitable areas, and this should be accounted for in the movement observations. Sediment grain size is only one variable that might affect feeding preferences and therefore impact movement patterns.

Stable Carbon Isotopes

Isotopic ratios have been used frequently in the study of marine food webs (e.g., Hobson & Welch, 1992; Rau *et al.*, 1992; Newell *et al.*, 1995). Naturally occurring isotopic ratios of $^{13}\text{C}/^{12}\text{C}$ can be used as a powerful indicator of sources of nutrition for consumers, often revealing the trophic relationships among organisms (e.g., Marguillier *et al.*, 1997). Stable carbon isotopic ratios of animal tissues are largely determined by diet (DeNiro & Epstein, 1978). The animals will incorporate carbon from food sources, and

assume that carbon isotope signature (e.g., DeNiro & Epstein, 1978; Haines & Montague, 1979; Macko *et al.*, 1982; Zieman, 1984; Fry & Sherr, 1984; Loneragan *et al.*, 1997).

Carbon isotope ratios are normally expressed by a δ value. The δ value is the difference from an international standard (PeeDee Belemnite carbonate), and is determined using the equation:

$$\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}) / ({}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}) - 1] \times 10^3$$

The resultant value is indicative of the amount of the heavier ^{13}C isotope in a sample. If more ^{13}C is present, then the value will be less negative, while a sample with little ^{13}C will be more negative.

The source of carbon for plants, atmospheric CO_2 , has a $\delta^{13}\text{C}$ value of approximately -8.0‰ but terrestrial plants that utilize the Calvin cycle to fix carbon have an average $\delta^{13}\text{C}$ value of -27‰ (Peterson & Fry, 1987). Fractionation occurs because of the isotopic selectivity of the enzyme ribulose-1,5-bisphosphate carboxylase, which is responsible for fixing carbon dioxide (Estep *et al.*, 1978). Fractionations associated with diffusion resistance are also a minor factor (Farquhar & Richards, 1984).

Carbon fixation in aquatic plants, such as seagrasses, is a different matter because they are substantially affected by rate-limiting diffusion barriers that decrease isotopic selectivity. Bicarbonate within the seawater incorporates more of the heavier ^{13}C isotope, and can act as a possible carbon source for seagrasses. These factors can account for the

differences between the mean $\delta^{13}\text{C}$ values for mangrove leaves ($-26.7 \pm 0.3\text{‰}$), and seagrass leaves in mangrove areas ($-15.3 \pm 1.2\text{‰}$) from southern Florida (Fleming *et al.*, 1990).

Nonoverlapping signature ranges for $\delta^{13}\text{C}$ have also been reported for mangrove and seagrass leaves in other research areas (Hemminga *et al.*, 1994; Rao *et al.*, 1994). Sampling of the holothurians, as well as their fecal pellets for $\delta^{13}\text{C}$ values should indicate nutritional preferences that will determine the source of the preferred carbon in the area that sea cucumbers are feeding upon as either mangrove or seagrass derived.

Hemminga *et al.* (1994) also reported a carbon isotope ratio gradient for organic carbon in sediment samples beginning with mangroves on the coastline and ending with the deeper water of a coral reef. Sediments near the mangroves showed a $\delta^{13}\text{C}$ mean of -22.9‰ , while sediments collected from the mid-channel were closer to a $\delta^{13}\text{C}$ mean of -18.1‰ .

The research area for the current study is similar in some respects to Hemminga study area (1994). It is a channel bordered by mangrove trees, with patches of seagrass throughout the middle of the channel. With thorough sampling of the area, these $\delta^{13}\text{C}$ sediment values for the organic carbon fraction of the sediment permit the mapping of the carbon isotope ratio gradient throughout the channel. Preference for a particular carbon isotope value should become evident based on the movements of the holothurians corresponding to certain ranges of $\delta^{13}\text{C}$ for organic carbon in the sediments of the research area.

Light Responses

There are many known cases of marine invertebrates responding to light (Miller & Hadfield, 1986; Sulkin, 1990; Bingham & Young, 1993). Light, and the preference for or against varying intensities of light, is yet another variable that can affect the foraging behavior of holothurians. These animals have a broad spectrum of photosensitivity (Willows & Corning, 1975), and in a recent study (Rodgers & Bingham, 1996), light was a factor in the aggregative behavior of holothurians.

Some holothurians have organized photoreceptors as cited by Hyman (1959). Seagrass beds provide protection for organisms from direct sunlight because of the shading effect of the seagrass blades. The result of this canopy effect is that it produces differing light gradients corresponding to the presence or absence of seagrass beds throughout the study area. Holothurian preferences for a particular light intensity range is testable by experimentation, and may help delineate environmental factors influencing foraging behavior.

Temperature Responses

A change in foraging patterns with respect to light might actually be a response to changing seawater temperature. Glynn (1965) showed that an unusual bounding movement (similar to the movement of an inchworm) of the holothurian *Astichopus multifidus*, is likely to be in response to high temperature stress. The baffling effect of the

seagrass blades results in reduced water movement within the beds and greater efficiency of solar heating of the water column. This baffling effect produces areas of varying water temperature throughout the study site. Behavioral reactions to varying temperatures will be evident in the movement data.

Summary

Foraging theory is still in a developmental stage and deposit feeding models based on the theory are still being tested. The testing of foraging models will require the simultaneous measurement of several aspects of food acquisition and metabolism (Lopez & Levinton, 1987) and case studies supporting or rejecting the tenets of the theory are still needed.

The explicit hypotheses tested in this study are:

1. *Holothuria floridana* will have a greater number of migrations into seagrass beds than into open sand.
2. *Holothuria floridana* will spend a significantly greater amount of its foraging time in areas characterized by a greater percentage of smaller grain sizes (in the 4 and 5 phi range).
3. *Holothuria floridana* will have a preference for seagrass beds and will be found more frequently in areas characterized by a sediment organic carbon $\delta^{13}\text{C}$ range influenced by seagrass carbon input.
4. *Holothuria floridana* will have a preference for low-light intensities.
5. *Holothuria floridana* foraging behavior will be interrupted only when temperatures increase, and they will leave the seagrass beds for cooler, faster moving water.

II. Materials and Methods

Foraging Behavior

A section of the study site in Zane Grey Creek was marked off into areas to facilitate mapping of the channel bottom and to aid in tracking holothurian movement (Figure 2). Fourteen areas were mapped, measuring 3 meters on each side, and extending from the shore. Areas 1, 3, 5, 7, and 9 were extended in length to 4.5 meters in order to include part of the shoreline that was submerged during high tide. Area 10 included all the sandy bottom found under overhanging mangrove branches. The study area consists of a total of 155m².

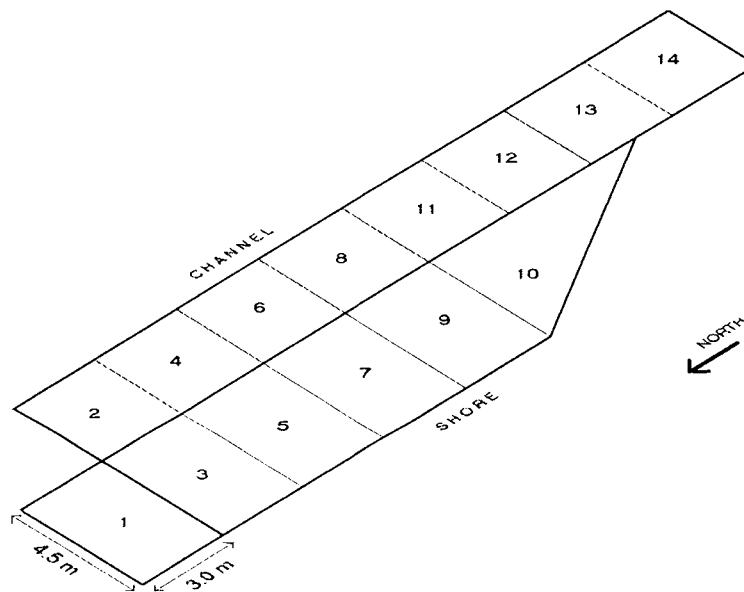


Figure 2. The 14 areas of the study site.

Sixty holothurians were collected from the study area and tagged with an identification string consisting of a color combination of up to six different beads (Figure 3). Each holothurian had a distinct color code (Blue, Blue, Yellow, Red, Yellow, Blue or BBYRYB, for example). The beads were strung onto ultra-thin Stren monofilament, and anchored at both ends with a double knot.

The tag was then attached with a suture by threading it under the body wall of the holothurian and bringing the end out from the other side. Another knot was then tied to anchor the tag to prevent it from slipping back inside. Preliminary tagging trials showed some of the tags were released after 2-3 days, but the majority of the specimens remained

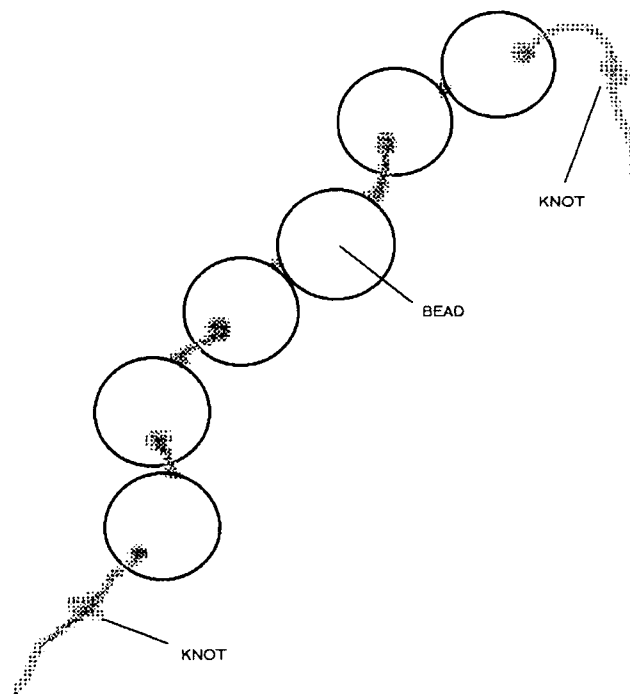


Figure 3. Holothurian identification tag.

tagged for 4-5 days. The area of the body wall that was tagged became brittle, allowing the tag eventually to fall out. The torn area was quickly regenerated, leaving only a small scar.

The tagged holothurians were returned to the site, and released in groups of 15 at 4 specific areas as shown in Figure 4. The holothurians were allowed to adjust to the tag overnight, with their locations recorded the following morning. The tagging process was repeated at the beginning of each study. Four studies were conducted: March 19-22, May 27-30, July 2-4, and September 19-23 of 1997. All four studies were conducted using the facilities of the Keys Marine Lab on Long Key, Layton, Florida. During each study, observations were made at 3-4 hour intervals except during stormy weather and once during the hours of midnight to 0600 EST.

During each observation period the following data were collected: time, water temperature, direction of current flow, and prevailing weather conditions. Temperature and salinity were measured using a Yellow-Springs Instruments Model 33 S-C-T meter. One assistant would stand on the shore to record measurements being made by the search team members in the water. The search team was composed of two people except during September, when only this author conducted the search. Search time was limited to sixty minutes per interval. Each corner of a quadrat was marked by a PVC pole extending above the water, clearly indicating the location of the grid. Tagged holothurians were identified by bead combination and their location was recorded by triangulation

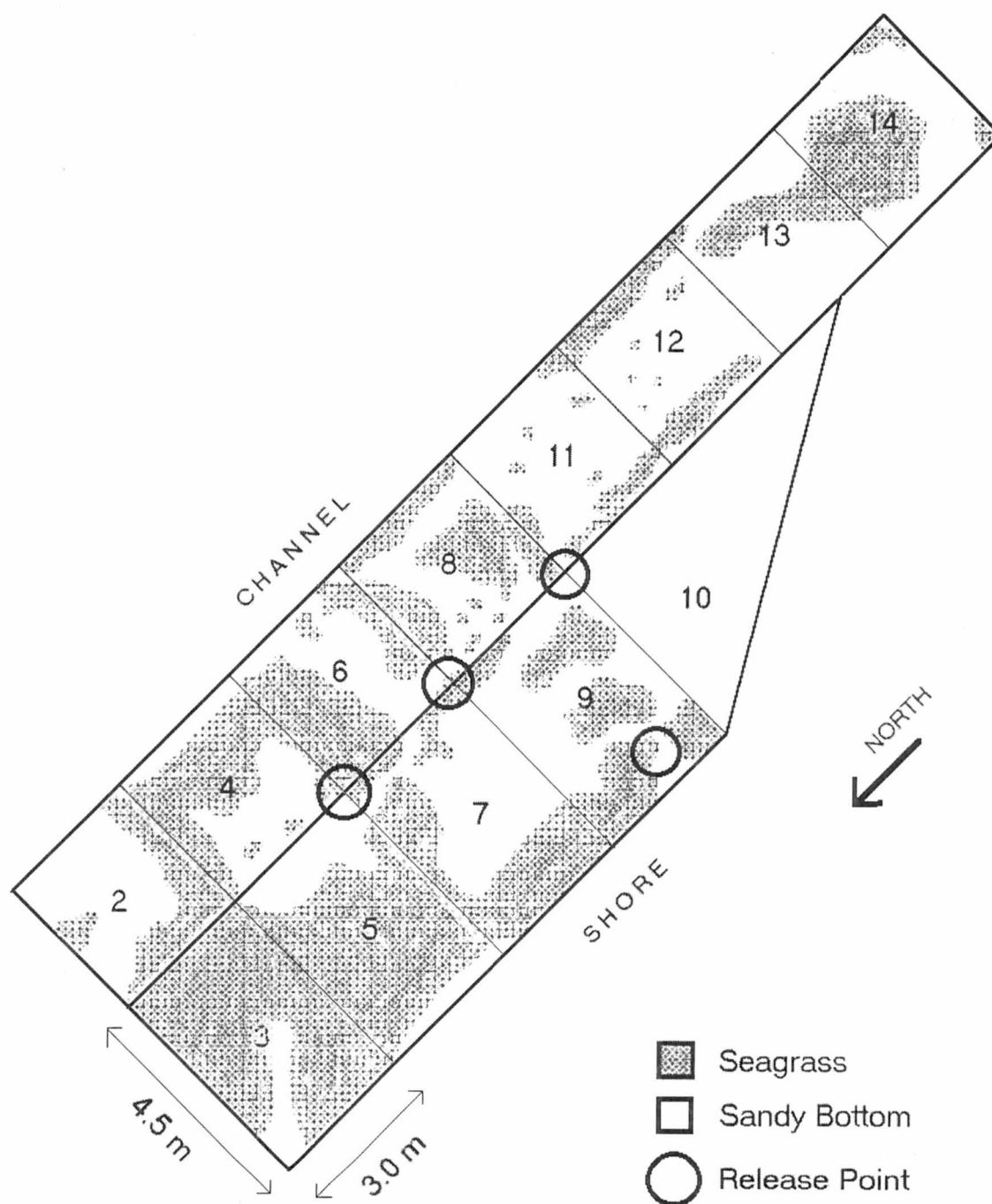


Figure 4. Site map showing the points of holothurian release.

from the three closest poles. The distances from these poles for each tagged individual found were recorded. These measurements were used to accurately identify the location of each animal on a map of the site. The nature of the substrate occupied by each holothurian was recorded as "seagrass", "open-sand", or "algae."

Sediment Samples

Thirty-three sediment samples from the study area were collected (Figure 5) with a 6.5cm diameter hand core. Surface sediment samples (0-2cm) were collected from the top of each core for grain size analysis. Samples of holothurian fecal pellets were also collected for grain size analysis. The samples were placed in Whirl-pak bags, frozen, and returned to Oak Ridge National Laboratory for sediment grain size analysis. The complete sediment grain sizing procedure is outlined in Appendix A.

The method of analysis was adapted from Jackson (1969), Ingram (1971), Gee and Bauder (1986), and Kunze and Dixon (1986). The samples were dried, then weighed out in 20g sub-samples and placed in 250mL beakers. Organic matter was removed by the addition of approximately 40mL of 30% hydrogen peroxide. The samples were placed in a warm water bath between 75-80°C and stirred until the reaction had subsided. The samples were then removed and centrifuged at 1600 rpm for 10 minutes.

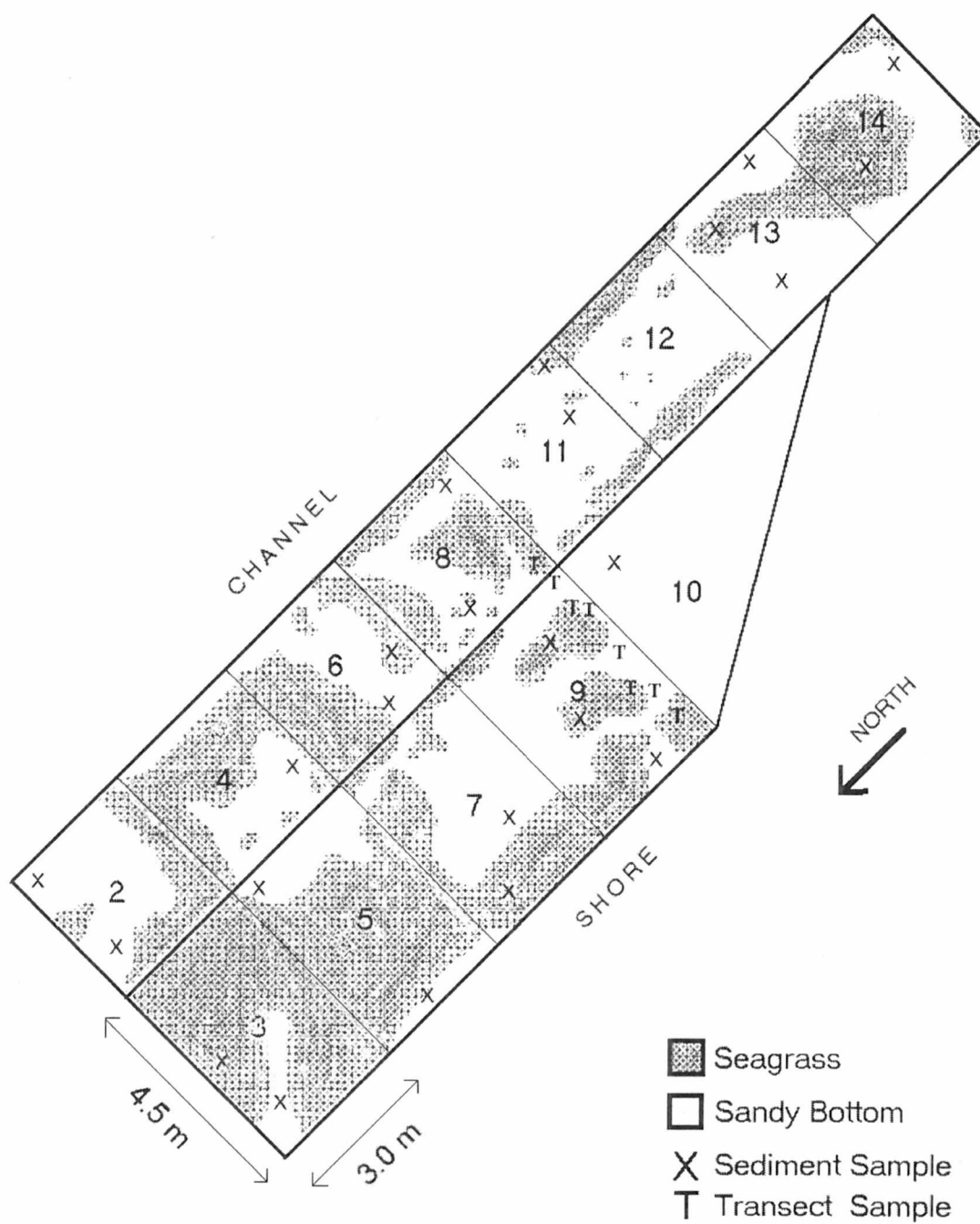


Figure 5. Location of sediment samples within study site.

Iron oxides were removed by adding a sodium citrate buffer solution, and again heating the samples in a water bath of 75-80°C. The samples were again centrifuged at 1600 rpm for 10 minutes. Sediments of gravel size were removed from the sands and smaller clays by wet-sieving using geological sieves. The sand sizes (<5 phi) were further separated from smaller sizes (≥5 phi) by wet- sieving.

The sand sediments were then sorted into standard sizes of 0,1, 2, 3, and 4 phi using dry sieving on a sieve shaker. Grains of 5 phi and larger were separated into clay and silt fractions by the addition of magnesium chloride. The clay and silt particles flocculated out of solution and were captured by centrifugation. Next, samples were dried and weights for individual phi sizes and individual weight percents for each sample were recorded.

Stable Carbon Isotope Samples

A total of 95 samples were collected for isotopic analysis, including 60 sediment samples collected in November 1996 (n = 18), March 1997 (n = 10), May 1997 (n = 18), and July 1997 (n = 14). The remaining 35 samples for isotopic analysis included holothurian body wall, seagrass leaves, mangrove leaves, filtered particulate organic carbon, and algae. These samples were placed in Whirl-pak bags, frozen, and returned to Oak Ridge National Laboratory for analysis. Epiphytes were removed from seagrass leaves and 10 mL of 1N HCl was added to each of the 10 g subsamples in order to obtain

carbonate-free sediments. Particulate organic carbon (POC) samples were collected in 250 mL bottles from the surface of the water. The samples were brought back to Keys Marine Laboratory where they were filtered using Gelman™ glass fiber microfilters. The filters were then frozen for the return trip to Oak Ridge.

The carbonate-free sediments were subsequently analyzed for stable carbon isotope composition adapting methods from Handley *et al.* (1991) and Lajtha and Michener (1994). A 0.005 g aliquot of the sediment sample was added to a 9 mm O.D. Vycor® glass tube. One g of cupric oxide, 1 g of copper, and 1 mm² silver foil were also added, and the sealed tube was combusted in a vacuum at 875 °C for two hours. The samples were combusted in a programmable furnace with a slow cool-down function used to prevent the production of oxides of nitrogen (Lajtha & Michener, 1994).

The CO₂ was isolated using the methods of Handley *et al.* (1991) and was then analyzed using a VG SIRA Series II dual inlet stable isotope mass spectrometer at the Environmental Sciences Division, Oak Ridge National Laboratory. The precision of the analysis was determined using an internal replicate which yielded a standard deviation of +/- 0.20 ‰. Calibration to international standards was assured by analysis of NBS-22 oil (National Institute of Standards and Technology).

Light Response Assessments

Determination of the holothurians' response to light was made in the wet lab of Keys Marine Laboratory using a tank with dimensions of 2.44 m by 1.22 m, and a depth

of 15 cm with a thin layer of sand covering the bottom of the tank. Three 60 watt incandescent light bulbs were placed at one end of the tank and fine window screening was spread over the sink tank into layers in order to produce a four-part step-wise light gradient. Light intensity was measured in EV factors with a Minolta Auto Meter IVF series light meter. EV factors were then converted into joules/sec. Twenty holothurians were placed into a 1 L bowl, and the bowl was submerged so that two different light intensities bisected the bowl (See Figure 6). The holothurians were allowed to roam freely throughout the sink tank. The number of holothurians observed in each part of the gradient was recorded every 30 minutes. Five different thirty-minute observations were made in succession (2.5 hours total).

Statistical Analysis

All data were analyzed using a statistical software package (SPSS 7.5., 1996). Observations of the number of holothurians found in seagrass versus other substrates were analyzed using contingency tables. Counts of holothurians were categorized as to whether or not they were found in seagrass or open sand. The numbers in one category were then tested to see if they were significantly different from the numbers in another category. Factors including time of day, temperature and current were also considered in the contingency tables to determine if substrate preference was affected. Sediment samples were analyzed with multivariate techniques of cluster analysis to investigate: 1) whether grouping between fecal pellets and sediment samples occurred in the study site,

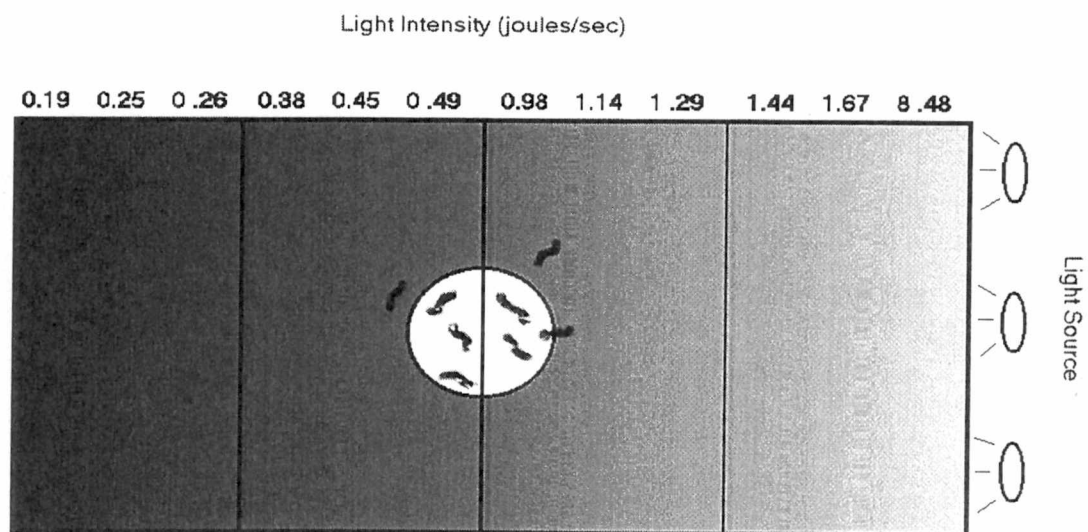


Figure 6. Experimental setup for light sensitivity assessment. Twenty holothurians were placed in a bowl bisected by different light gradients and allowed to freely roam the tank. Observations of the number found in each gradient were recorded every 30 minutes for a total 2.5 hour observational period.

and 2) whether groupings of sediment samples closely resembled each other with respect to the percentages of phi sizes within samples.

Holothurian movement within the study site was recorded on maps of the site, and then converted to a digital file. A Graphic Interchange Format™ (GIF) file was produced for every time interval of every study month. These images were then animated with Animagic, 1.03a, 32-bit GIF animator (Right to Left Software Inc., 1997), so that the movement of all the tagged holothurians could be reviewed in order to record the number of migrations into and out of the seagrass beds. A migration into a seagrass bed was recorded if a holothurian in the open sand had a movement point that ended within a seagrass bed. If a holothurian was in a seagrass bed, and the next movement point ended in the open sand, a migration out of the seagrass was recorded.

III. Results

Characterization of the Study Site

Zane Grey Creek is dominated on both sides by the mangrove tree *Rhizophora mangle*. Two other mangrove trees, *Avicennia germinans* and *Laguncularia racemosa*, are also significant components of the community, but are further inland than *R. mangle*. This tidal creek reaches a maximum depth of 1 m, and experiences tidal flushing, which contributes to fluctuating water temperatures. Current flow is diminished by seagrass patches and mangrove branches that descend into the water near the shoreline. As a result, areas of slow moving water warm up quickly and can vary in temperature 2-3°C from temperatures in the middle of the channel. The shallow nature of the study site allows for excellent light penetration, allowing rapid heating of the water column; temperatures ranged from 29-34 °C during the study period.

The seagrasses that dominate the area are *Thalassia testudinum* and *Halodule wrightii*. These occur in patches throughout the study area with *Halodule wrightii* growing inshore and under the shade of the mangrove trees. *Thalassia testudinum* is found in abundance away from the shoreline and in areas of stronger current flow. There is a thick organic mat trapped within the seagrass beds that floats within the blades, and on top of the bottom sediment. Holothurians can go undetected by moving within this large flocculant mass of organic matter. Algae that are common to the study area include species of the genera *Batophora*, *Lawrencia*, *Cladophora*, and *Acetabularia*.

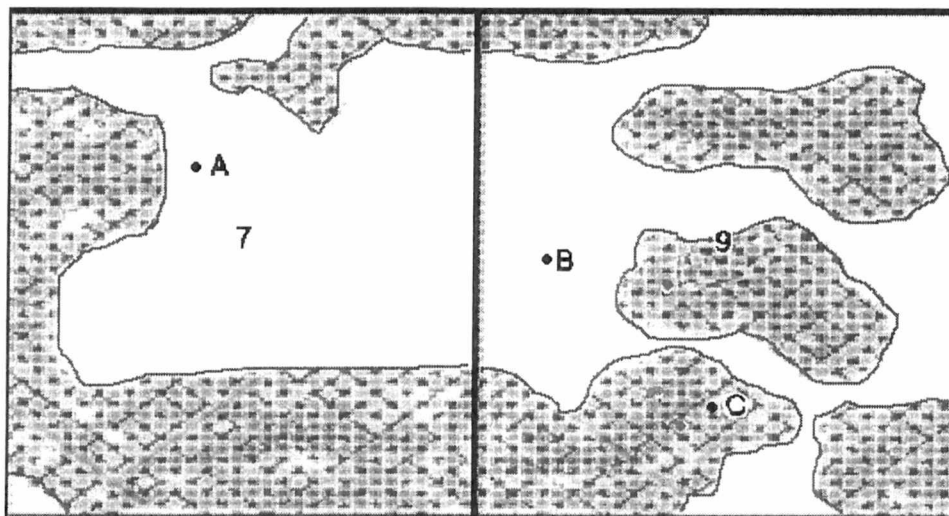
Foraging Behavior

After plotting each tagged individual on a site map for each observation period and day of the month, the maps were viewed as a GIF slide animation to see if any pattern of movement was apparent. Figure 7 illustrates the typical holothurian movement patterns observed over a 2-3 day period. Many of the individuals changed direction, sometimes crossing back and forth within a small area. Few tagged individuals moved out of the study area. Holothurian "C" in Figure 7 gives an example of the distance that can be traveled if there are few changes of direction. The majority of animals remained within a quadrat during the entire study. Holothurians were observed moving in and out of seagrass patches and some remained in one location for hours.

The total percentage of migrations into the seagrass over the entire study period was only slightly larger than the percentage moving into open sand (Table 1). March had a greater percentage of holothurians moving into the seagrass beds, while the results in May show a larger percentage moving into the open sand. The months of July and September favor migrations of animals into the seagrass, but not with any margin of significance.

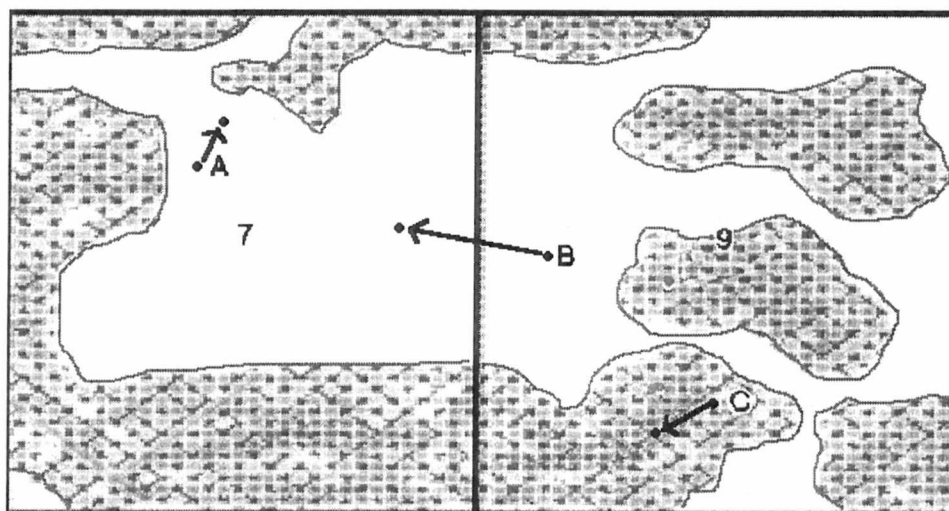
Sediment Grain-Size Analysis

The various sediment sample types collected over the study period were separated by sample site. A modal phi size was determined for each sample site. With a few



 Seagrass
 Sandy Bottom

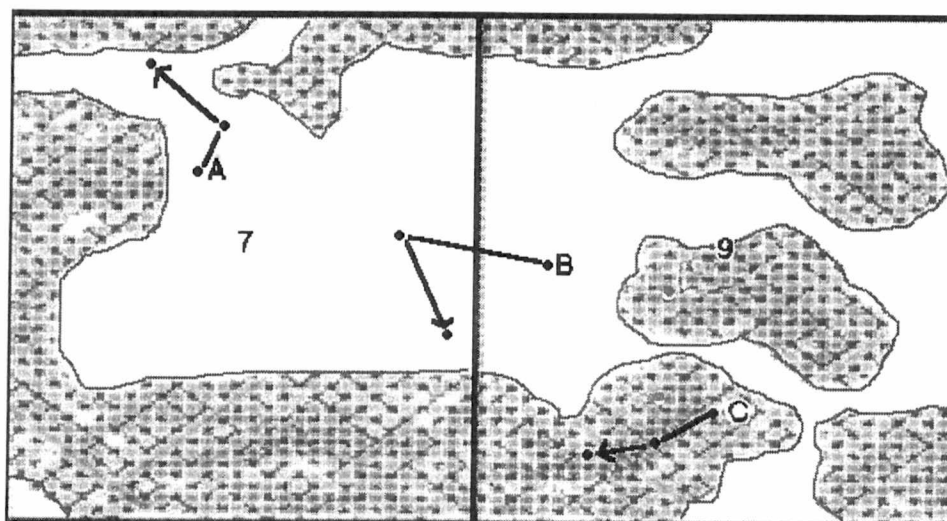
March 19, 1997- 2050 EST



 Seagrass
 Sandy Bottom

March 20, 1997- 0850 EST

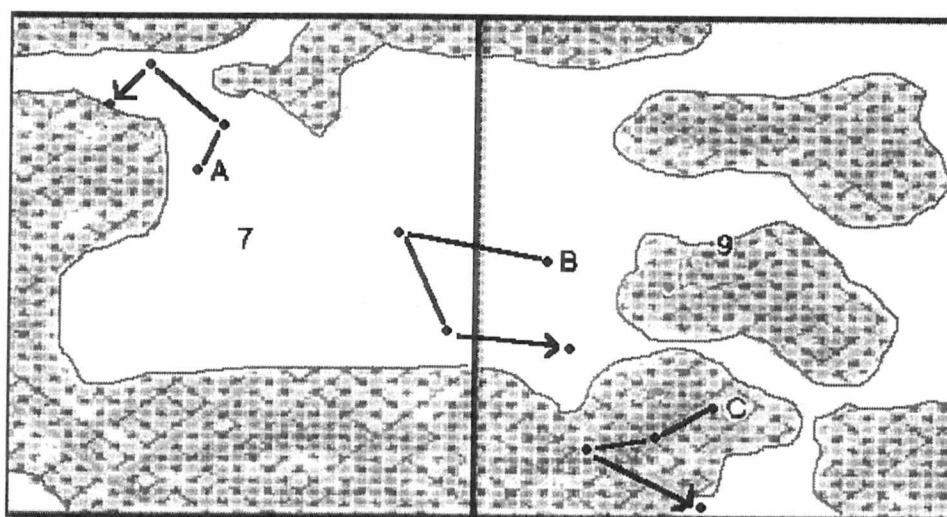
Figure 7: Locations of three holothurians labeled A, B, and C over 5 time periods; March 19, 1997- 2050 EST, and March 20, 1997- 0850 EST (continued on next page).



Seagrass

Sandy Bottom

March 20, 1997- 1140 EST

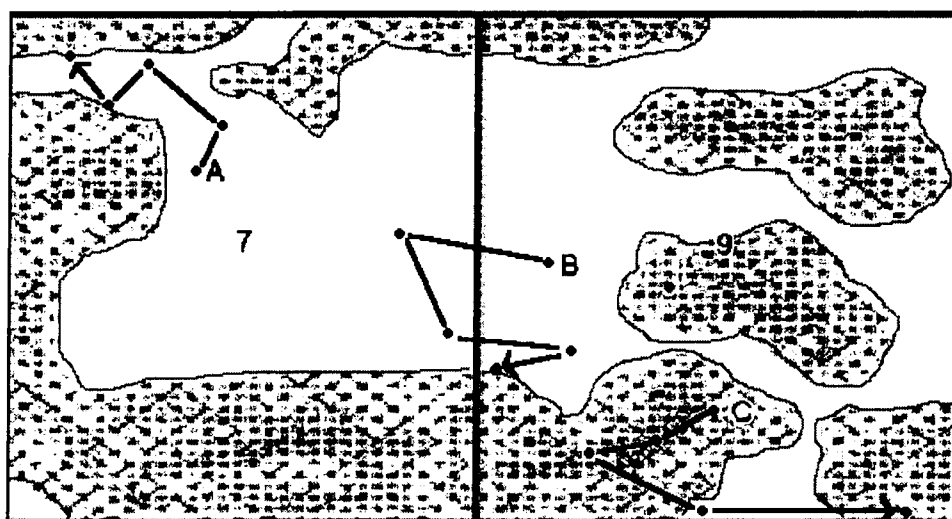


Seagrass

Sandy Bottom

March 20, 1997- 1440 EST

Figure 7: (continued) Locations of three holothurians labeled A, B, and C over 5 time periods; March 20, 1997- 1140 EST, and March 20, 1997- 1440 EST (continued on next page).



■ Seagrass

□ Sandy Bottom

March 20, 1997- 2150 EST

Figure 7: (continued) Locations of three holothurians labeled A, B, and C over 5 time periods; March 20, 1997- 2150 EST.

Table 1. Percentages and actual counts (in parentheses) of holothurians observed moving from one substrate and ending their movement in either seagrass or open sand.

	<i>March</i>	<i>May</i>	<i>July</i>	<i>September</i>	<i>Year</i>
<i>Seagrass</i>	61% (25)	39% (12)	52% (16)	55% (21)	52% (74)
<i>Open sand</i>	39% (16)	61% (19)	48% (15)	45% (17)	48% (67)

exceptions, most sediment samples had a modal phi size of 1.0 (Table 2). This indicates that there is a uniform sediment size composition dominated by coarse sand across the study area. Organic particles were observed in higher concentrations within the seagrass patches, but underneath this organic layer was a sediment size composition indistinguishable from that of open sand areas.

A hierarchical cluster analysis examined the percentage of differing phi sizes within each sample, and ranked each sample in order of its similarity in composition to each other sample. Seagrass sediment samples showed a particular percentage of phi size that was also mirrored in samples taken from the open sand. The same is true of samples taken from the interface of seagrass and sand. Holothurian fecal pellets could not be clustered with any specific grouping of sediment from seagrass, sand, shore or edge. The percentage of phi sizes in the fecal samples was similar enough that the best match for fecal pellets was other fecal pellet samples.

Table 2. Summary of sediment sample measurements. SG: seagrass; OS: open sand; EDGE: edge of seagrass and open sand; SHORE: area exposed at low tide; PT: Holothurian fecal pellets (CGP: collection of 20 individual fecal pellets). A7 designates a sample from area 7; T1 designates the first of a transect sample.

<i>Sample</i>	<i>Silt and clay ($\geq 5 \phi$)</i>	<i>Very fine sand (4ϕ)</i>	<i>Fine sand (3ϕ)</i>	<i>Medium Sand (2ϕ)</i>	<i>Coarse sand (1ϕ)</i>	<i>Gravel ($\leq 0 \phi$)</i>	<i>Modal size class (ϕ)</i>
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	
T1 SG	13.9	1.4	4.6	6.5	45.8	27.8	1.0
T3 SG	0.0	0.9	7.1	11.3	53.9	26.8	1.0
T5 SG	5.7	0.5	2.6	11.2	53.9	26.0	1.0
T8 SG	7.3	1.7	8.3	10.9	44.3	27.5	1.0
A10 SG	58.2	4.8	13.2	8.2	13.0	2.6	≥ 5.0
A14 SG	28.7	3.9	13.2	13.8	34.3	6.1	1.0
A8 SG	4.8	6.1	19.5	14.3	36.4	19.1	1.0
A8 SG	13.8	2.1	10.0	12.1	39.0	23.1	1.0
A1 SG	10.2	3.2	9.0	2.4	33.8	41.4	≤ 0.0
A13 SG	20.7	2.8	8.8	11.8	44.0	11.8	1.0
A9 SG	6.5	0.7	2.7	6.6	53.1	30.4	1.0
T4 OS	10.0	8.9	1.5	3.8	42.2	33.6	1.0
A5 OS	7.5	0.8	4.0	6.1	44.1	37.7	1.0
A8 OS	8.9	1.4	4.4	6.9	44.3	34.0	1.0
A8 OS	6.4	2.1	5.1	11.6	48.1	26.8	1.0
A2 OS	11.4	1.5	5.2	6.4	36.7	38.8	≤ 0.0
A14 OS	6.0	1.0	6.5	3.3	60.0	23.3	1.0
A7 OS	12.3	2.0	8.4	10.7	42.3	24.3	1.0
A3 OS	17.4	2.3	7.1	9.8	38.8	24.5	1.0
A8 OS	6.4	2.1	5.1	11.6	48.1	26.8	1.0
A9 OS	6.7	13.6	30.5	17.3	23.0	8.9	3.0
A9 OS	2.9	3.1	4.8	9.0	50.0	30.6	1.0
A13 OS	7.2	0.9	2.8	5.0	48.1	36.1	1.0
A4 OS	25.5	6.0	17.5	12.8	23.3	15.0	≥ 5.0
T2 EDGE	7.9	0.5	2.8	7.7	44.9	36.2	1.0
T6 EDGE	5.3	0.75	2.8	6.7	53.7	30.8	1.0
T7 EDGE	6.7	0.3	1.9	5.5	51.1	34.6	1.0
A2 EDGE	4.9	0.6	3.9	7.4	45.1	38.1	1.0
A1 EDGE	6.5	26.0	36.2	21.6	10.0	0.1	3.0
A1 SHORE	63.3	3.3	5.0	5.0	13.3	10.0	≥ 5.0
A2 SHORE	19.7	2.9	10.8	12.1	33.3	21.3	1.0
CGP PT	33.3	0.2	3.7	7.3	36.3	19.3	1.0
A13 PT	25.3	1.4	3.7	5.8	42.2	21.6	1.0

Stable Isotope Analysis

The $\delta^{13}\text{C}$ values for organic carbon in sediment collected during March, May, and July of 1997 ranged from -17.4‰ to -22.5‰ . The March samples were collected during a survey of the site and results are presented in Figure 8. Values for sediment samples collected during May and July are shown in Figure 9 and depict two transects across the areas of the study site most frequented by large numbers of holothurians. More negative sediment $\delta^{13}\text{C}$ values tend to occur closer to mangrove trees, and in regions of mangrove detrital deposition. Sediment areas closer to the middle of the channel were characterized by $\delta^{13}\text{C}$ values ranging from -17.4‰ to -19.0‰ .

Samples of holothurian body wall and leaves of the seagrass *Thalassia testudinum* had $\delta^{13}\text{C}$ values averaging -15.7‰ , and -15.3‰ , respectively. The remaining $\delta^{13}\text{C}$ values for algae, fecal pellets, *Halodule wrightii*, and mangrove leaves were more negative ($\leq -18\text{‰}$) (Tables 3 and 4). Particulate organic carbon samples from seawater collected near shore had a mean of -19.0‰ ; $\text{sd} = \pm 0.8$; $n = 2$. POC samples collected in water from mid-channel areas had a mean of -18.6‰ ; $\text{sd} = \pm 0.1$; $n = 3$.

Holothurian Movement with Respect to Substrate

The recapture frequency of tagged holothurians is shown in Appendix B, and is divided into the study months of March, May, July and September. The recapture rate for holothurians ranged from no recaptures to a maximum of 11 recaptures of an individual

Table 3. Values of $\delta^{13}\text{C}$ for seagrass, algae and mangrove samples. NA denotes that a sample was not taken in that area.

<i>Sample</i>	<i>Area 6</i>	<i>Area 7</i>	<i>Area 8</i>	<i>Area 9</i>
<i>T. testudinum</i>	-14.4	-15.8	-15.4	-15.7
<i>H. wrightii</i>	-18.0	-18.4	-20.0	-19.7
<i>Cladophora sp.</i>	-24.3	-27.6	NA	-21.7
<i>Lawrencina sp.</i>	-21.0	-23.5	-23.2	-22.5
<i>Batophora sp.</i>	-23.1	-21.4	NA	-23.1
<i>Acetabularia sp.</i>	-20.3	NA	-19.3	-15.8
<i>Mangrove</i>	NA	NA	-25.6	-27.3

Table 4. Values of $\delta^{13}\text{C}$ for samples of holothurian body wall, and corresponding fecal pellets.

Holothurian	Fecal Pellets
-16.1	-19.0
-17.1	-19.0
-15.7	-19.6
-15.1	-18.8
-15.6	-19.6
-15.0	-19.7
-16.2	-20.3
-15.0	-19.8
Mean = -15.7, n= 8, SD = +/- 0.7	Mean = -19.5, n=8, SD = +/- 0.5

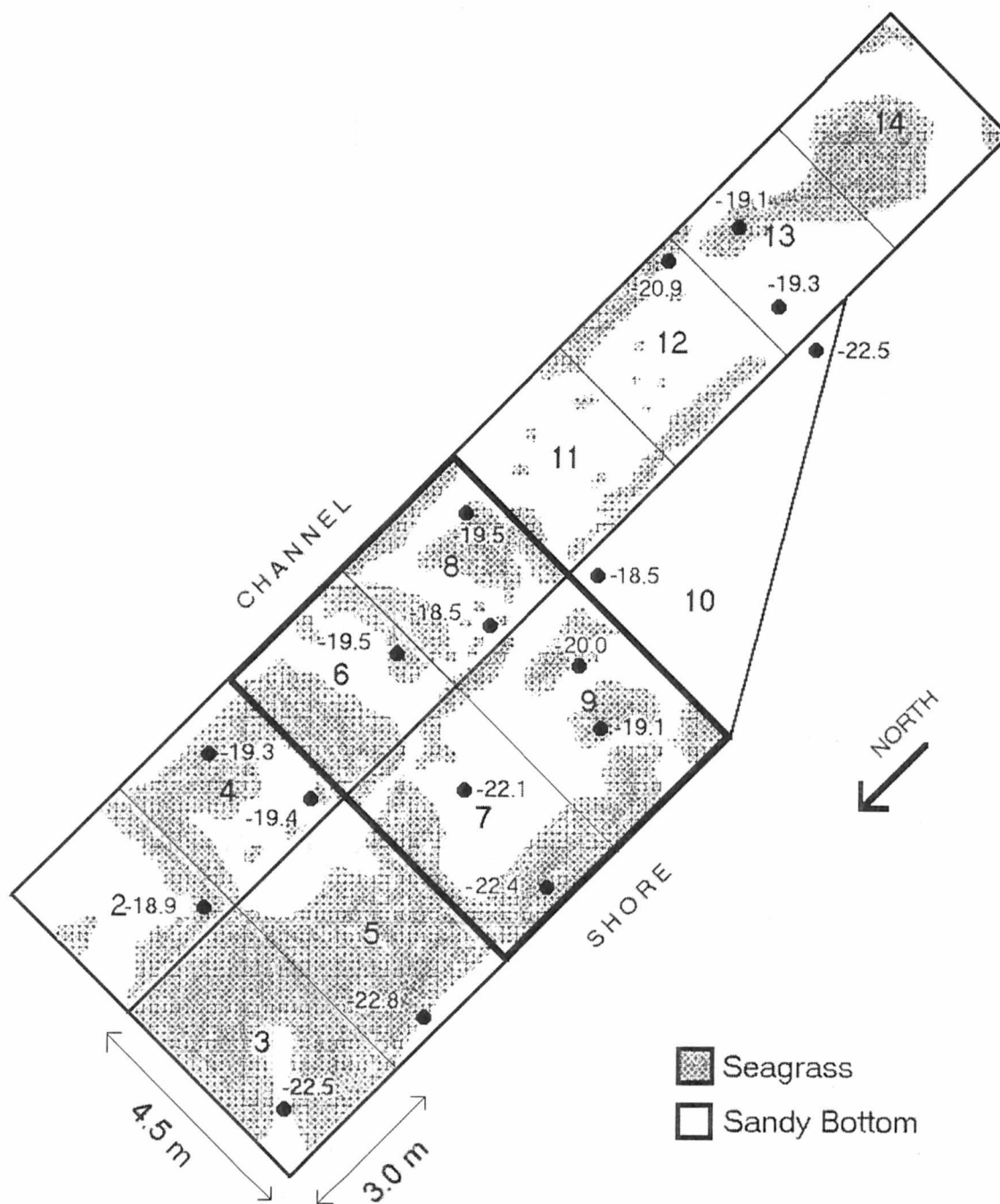


Figure 8. Locations of random sediment samples collected in March 1997, with the corresponding $\delta^{13}\text{C}$ values.

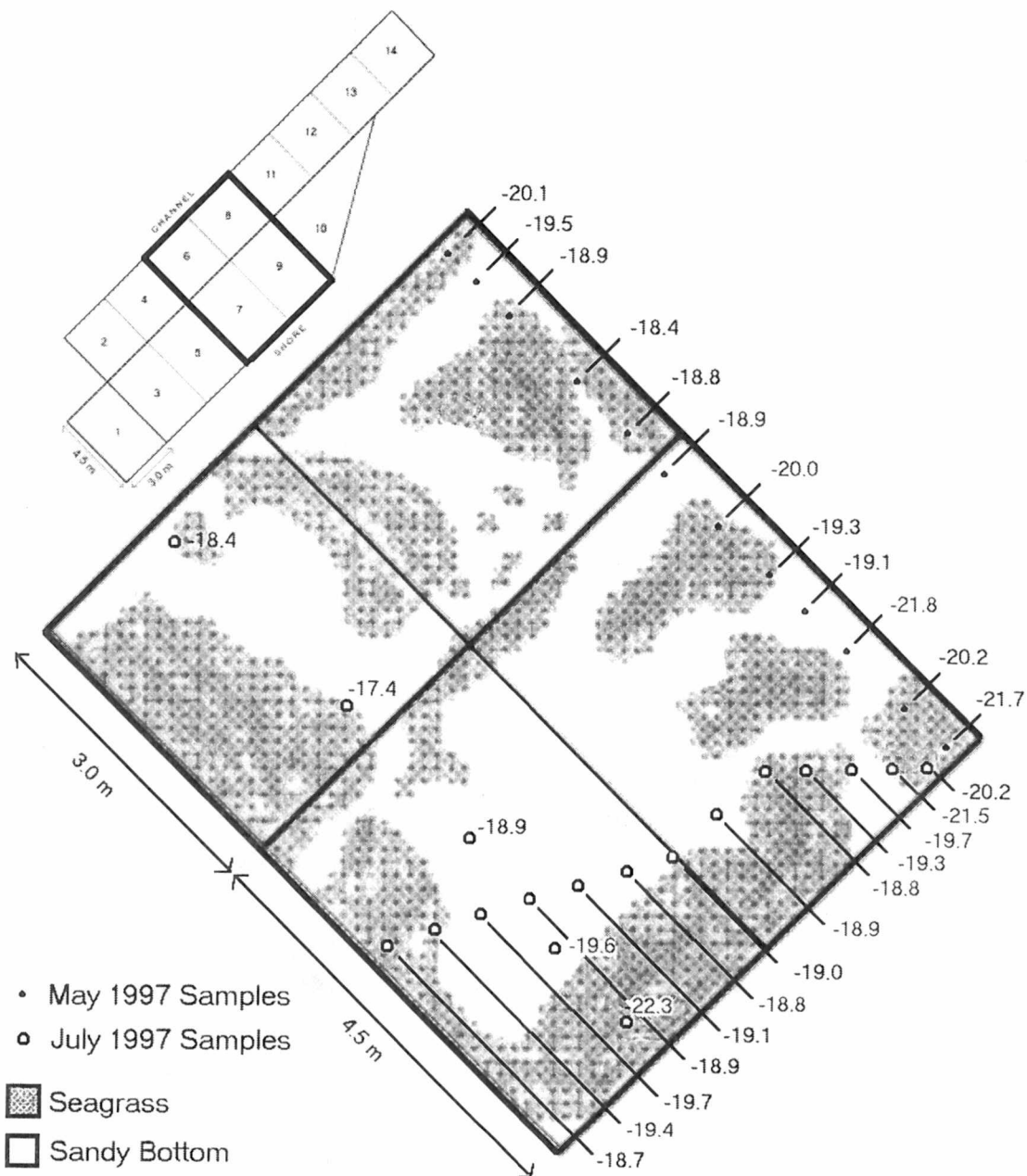


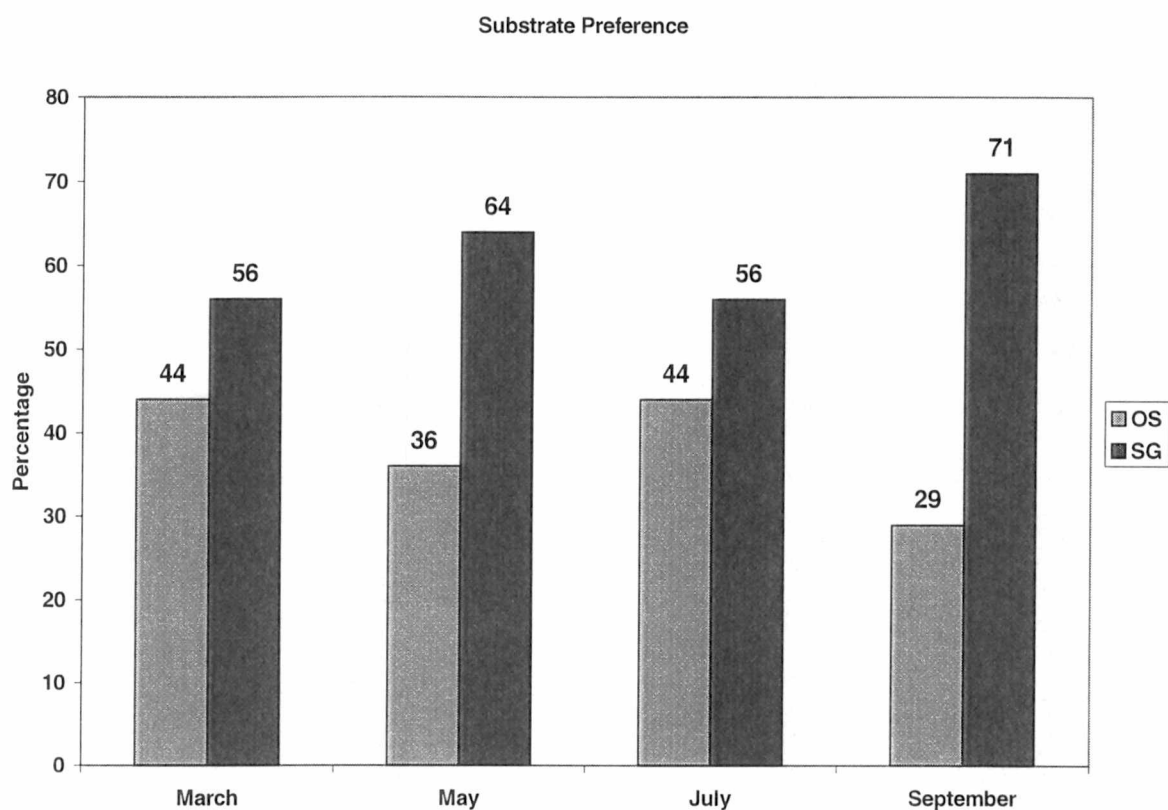
Figure 9. Locations of transect sediment samples collected in May and July 1997 with the corresponding $\delta^{13}\text{C}$ values of the organic component of the sediments.

holothurian in September. Some holothurians were not as active as others and remained in place for numerous observation periods and were recovered more often. Tagged individuals that moved readily in and out of the seagrass patches were not recaptured with consistency.

The percentage of times a tagged individual was found in either seagrass or open sand was then entered into a contingency table. Analysis included a Pearson Chi-square test which determined independence of the row (months) and column (substrate) variables. The study site was divided evenly between seagrass and open sand. The percentage counts and their corresponding Chi-square test is presented in Figure 10. This Chi-square test indicated that holothurians were found in the seagrass areas more often than they were found in the open sand areas ($\chi^2 = 9.045$, $p = 0.029$, $n = 491$). The greatest difference in holothurian location occurred in the month of September, with 70.8% of the holothurians recaptured in seagrass beds compared with 29.2% observed in open sand regions.

Substrate Preference with Respect to $\delta^{13}\text{C}$ Values

Sediment samples collected from the months of March, May, and July, 1997 indicated a pattern of more negative $\delta^{13}\text{C}$ values occurring close to mangrove trees (Figure 11). This gradient incorporates sediment samples collected directly adjacent to mangrove trees on the shore, or in the case of a sample in Area 8, collected next to a mid-



	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	9.045^a	3	0.029
N of Valid Cases	491		

a. 0 cells have expected count less than 5. The minimum expected count is 31.10.

Figure 10. Comparison of holothurian distribution between seagrass beds and open sand in 1997, with Chi-square significance values. OS: open sand areas, SG: seagrass areas.

channel mangrove tree. Two zones of $\delta^{13}\text{C}$ values for sediment organic carbon were arbitrarily separated along scattered data points: -18‰ to -19‰ and -20‰ or less (Figure 11). The -18‰ to -19‰ zone was determined to be 88% of the available study area, while the -20‰ or less zone was the remaining 12%.

Frequencies of occurrence of holothurians in each zone were analyzed by Pearson Chi-square analysis using expected values corresponding to the percentage of each zone available in the study area (88% and 12%). More holothurians than expected were found in areas closer to the mangrove trees where sediment organic carbon $\delta^{13}\text{C}$ values were less than -20‰ ($\chi^2 = 8.50$, $p = < 0.04$, $n = 491$). This indicates a significant relationship between holothurian location and preference for locations closer to mangrove areas.

The two zones were then redefined by extending beyond the -20‰ or less data points so that the -18‰ to -19‰ zone decreased to 84% of the study area, and the -20‰ or less zone increased to 16% (Figure 12). Frequencies of occurrence of holothurians in each zone were analyzed; this time with no significance ($\chi^2 = 0.06$, $p = < 0.80$, $n = 491$).

Analyzing the data on a monthly scale using a contingency table reveals a significant relationship between the month, and the number of holothurians recaptured in a given zone ($\chi^2 = 20.281$, $p = < 0.05$, $n = 491$). From March to September of 1997 there is an increase in the numbers of holothurians found in the -18‰ to -19‰ zone, and a consequent decrease of holothurians in the -20‰ or less zone (Figure 13).

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- May 1997 Samples
- July 1997 Samples

■ Seagrass

□ Sandy Bottom

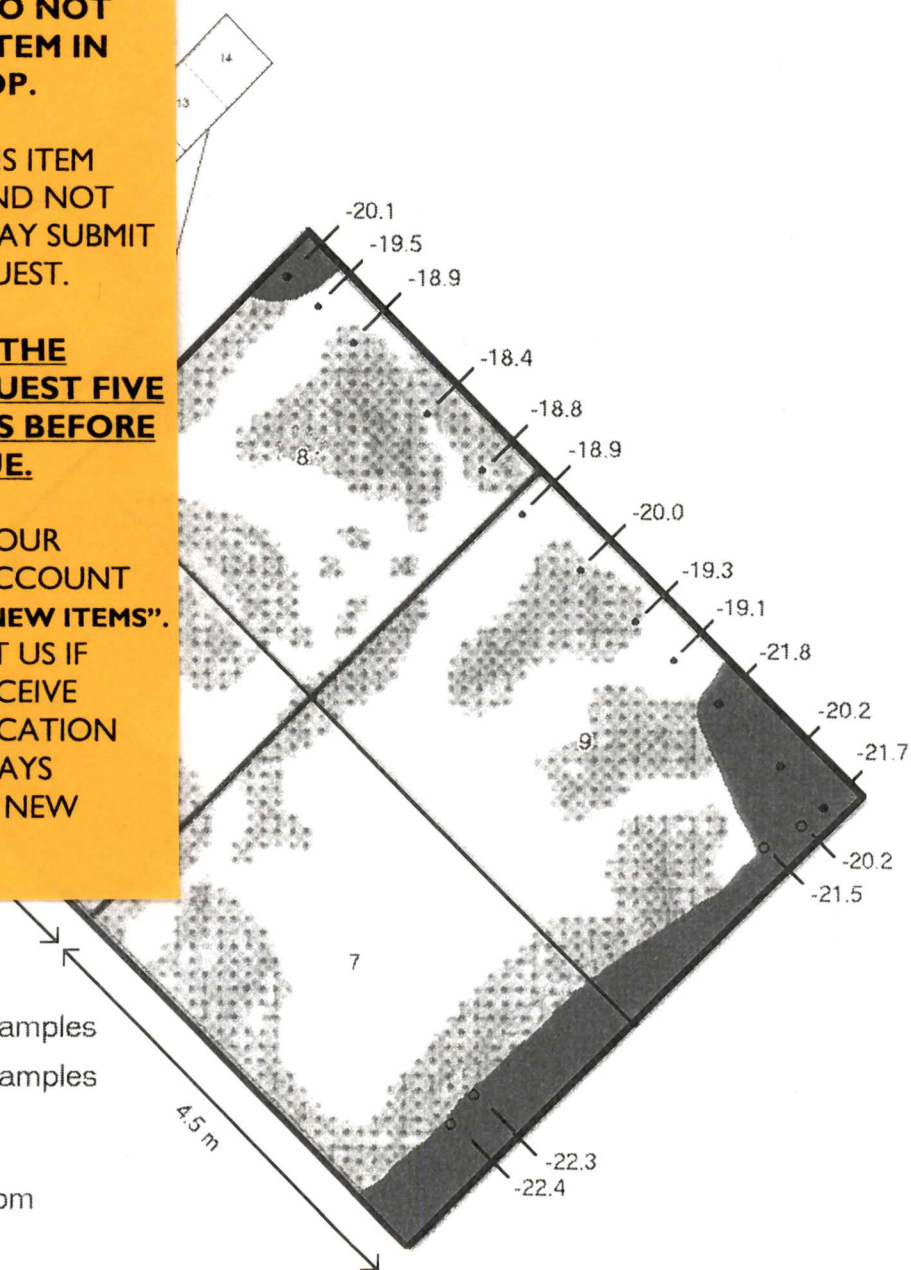


Figure 11. Study of areas 6, 7, 8, and 9 showing the zone with sediment organic carbon with $\delta^{13}\text{C}$ values of -20‰ or less (12% of study area).

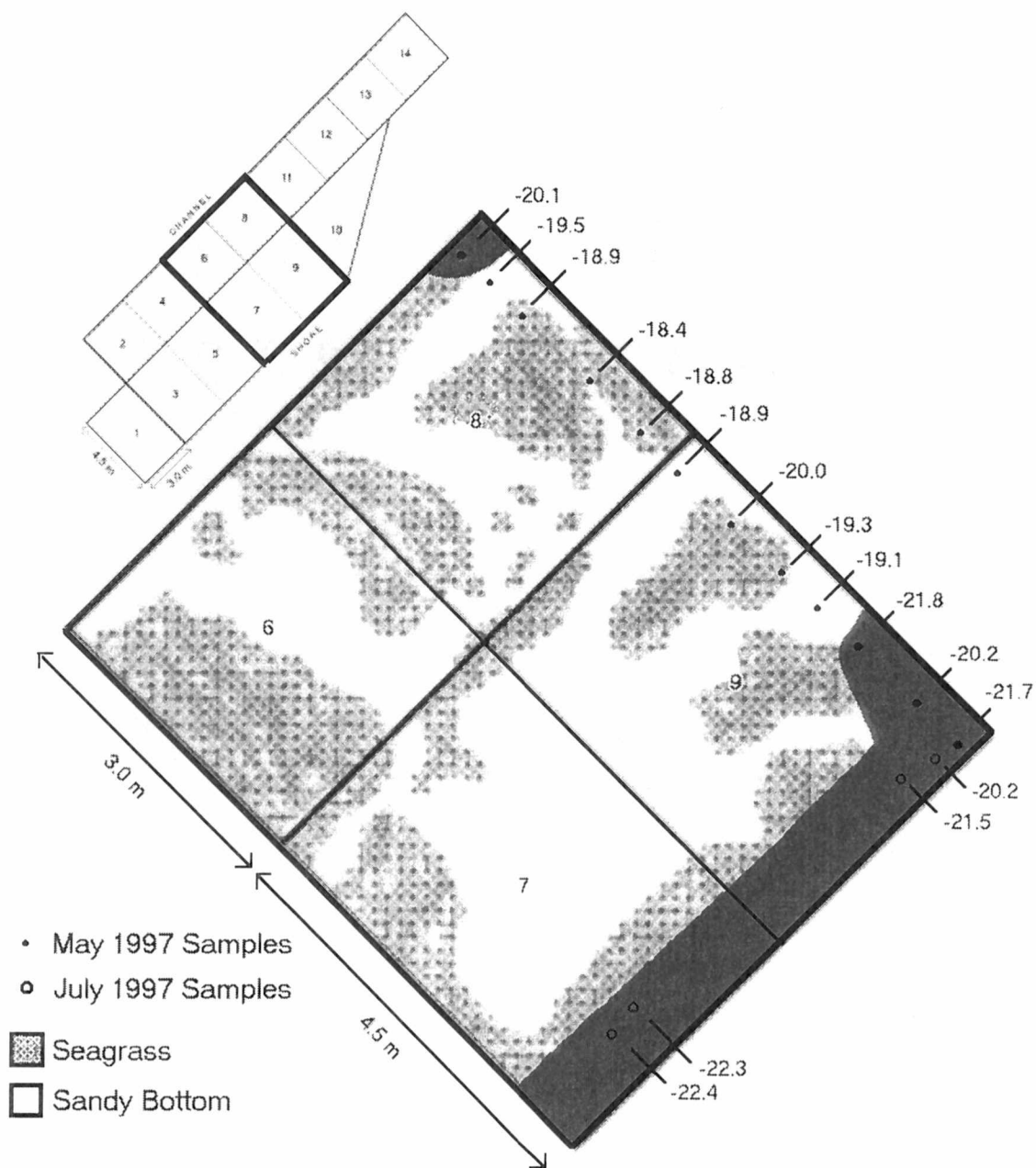
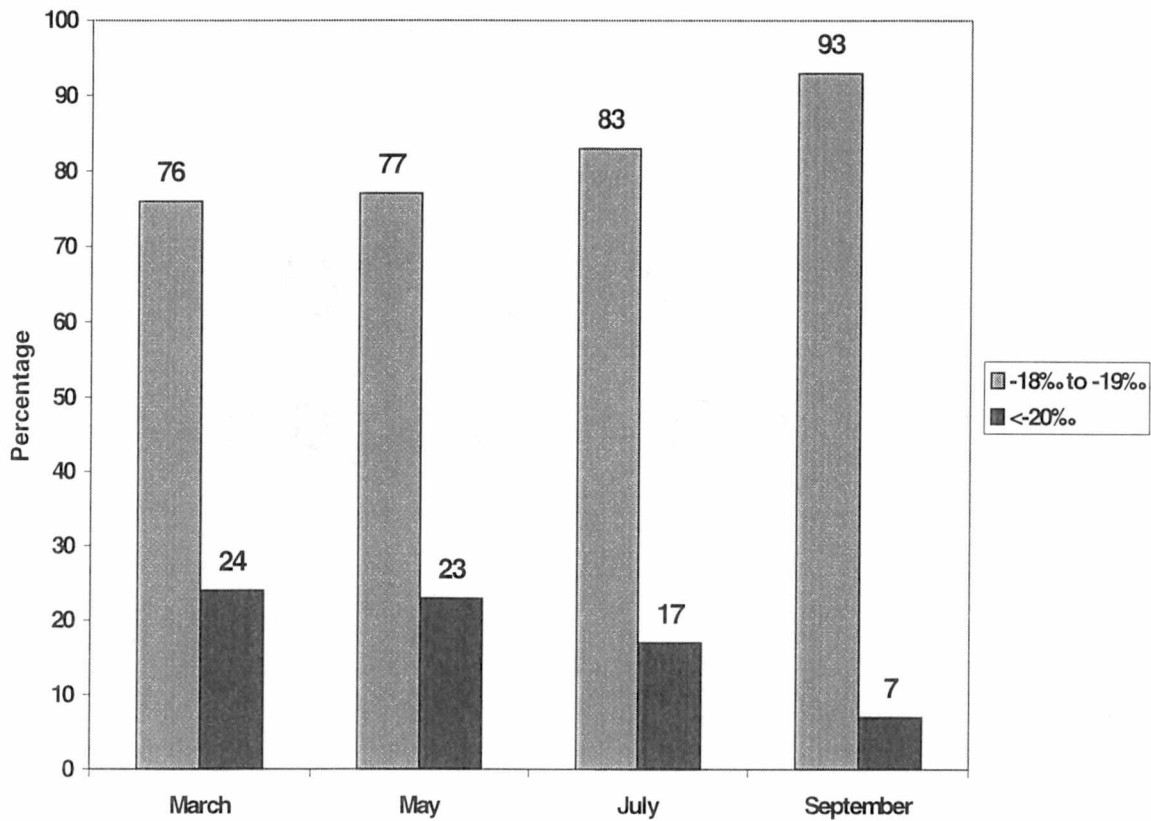


Figure 12. Study of areas 6, 7, 8, and 9 showing the zone with sediment organic carbon with $\delta^{13}\text{C}$ values of -20‰ or less (16% of study area).

Delta Carbon Isotope Preferences



	Value	df	Asymptotic Significance (2- sided)
Pearson Chi-Square	20.281 ^a	3	0.001
N of Valid Cases	491		

a. 0 cells have expected count less than 5. The minimum expected count is 13.52.

Figure 13. Percentages of holothurians found in sediments with $\delta^{13}\text{C}$ values either -18‰ to -19‰ or -20‰ or less during the months of March, May, July, and September, 1997. Chi-square chart showing significance of the test.

Light Responses

The number of holothurians observed within each of the four light gradients resulted in sample sizes too small for a strong test of significance. Therefore, light gradients of 0.19-0.26 joules/sec and 0.38-0.49 joules/sec, and their accompanying holothurian counts were clustered as one group. Gradients of 0.98-1.29 joules/sec and 1.44-8.48 joules/sec and their counts were clustered as a second group. The twenty holothurians showed no preference for either light gradient, as numbers observed on either side of the sampling platform during the 5 sequential observation periods showed no observable bias ($\chi^2 = 2.400$, $p = 0.663$, $n = 92$). Movement patterns do not seem to be correlated with preferences for light intensities.

Substrate Preference with Respect to Temperature

Water temperatures measured during the study period ranged from 28.89°C to 34.44°C. Water temperature readings were taken during the months of May, July, and September 1997. Observations of holothurians during March were excluded from this comparison due to incomplete water temperature data. Figure 14 shows the frequency of holothurians found with respect to the water temperature at the time of recapture. The difference in the number of holothurians in seagrass and open sand for specific water temperatures at the time of collection were only marginally significant ($\chi^2 = 15.151$,

Percentage Recapture versus Temperature

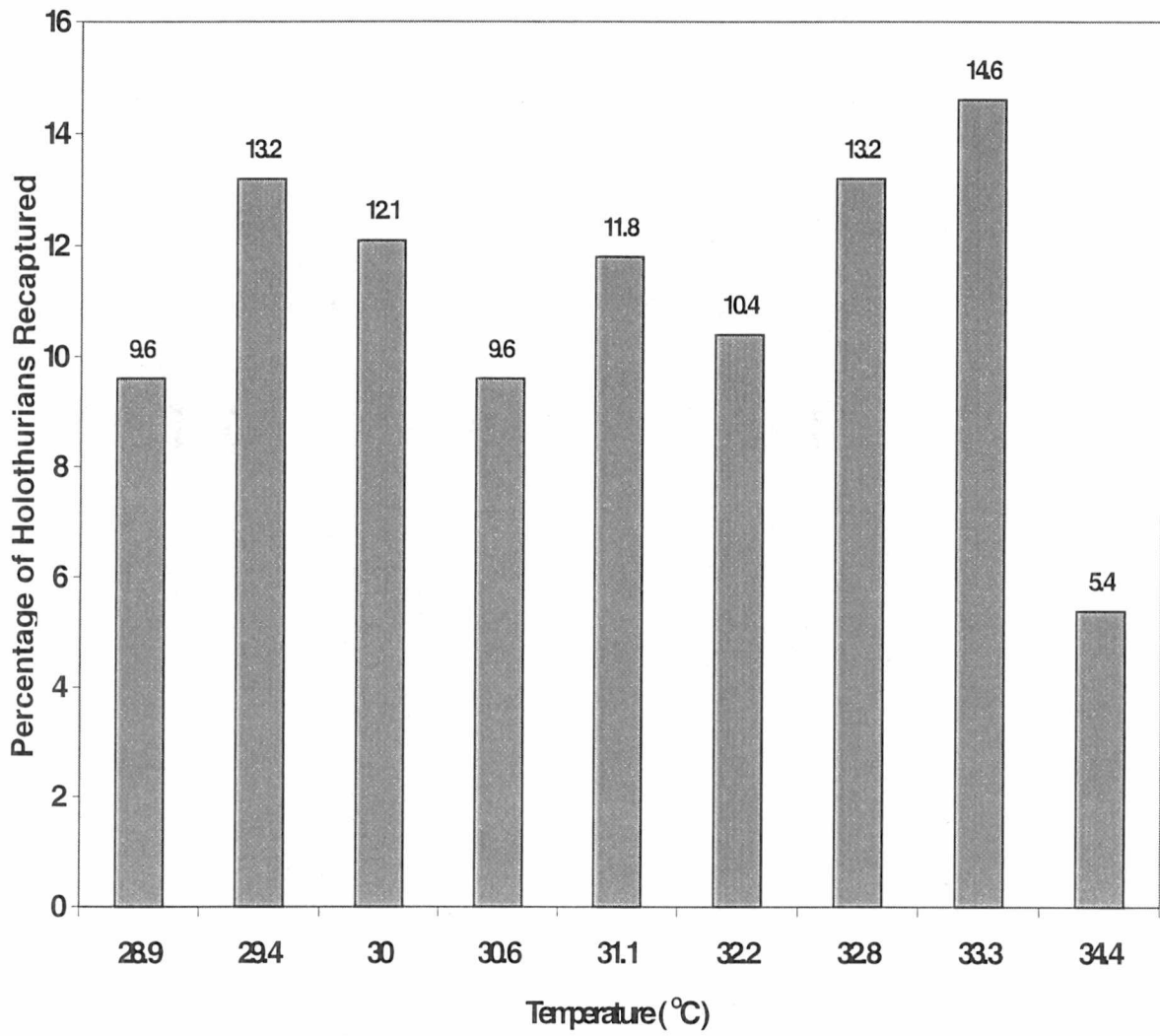


Figure 14. Relationship between holothurian recapture rate and water temperature (n = 280).

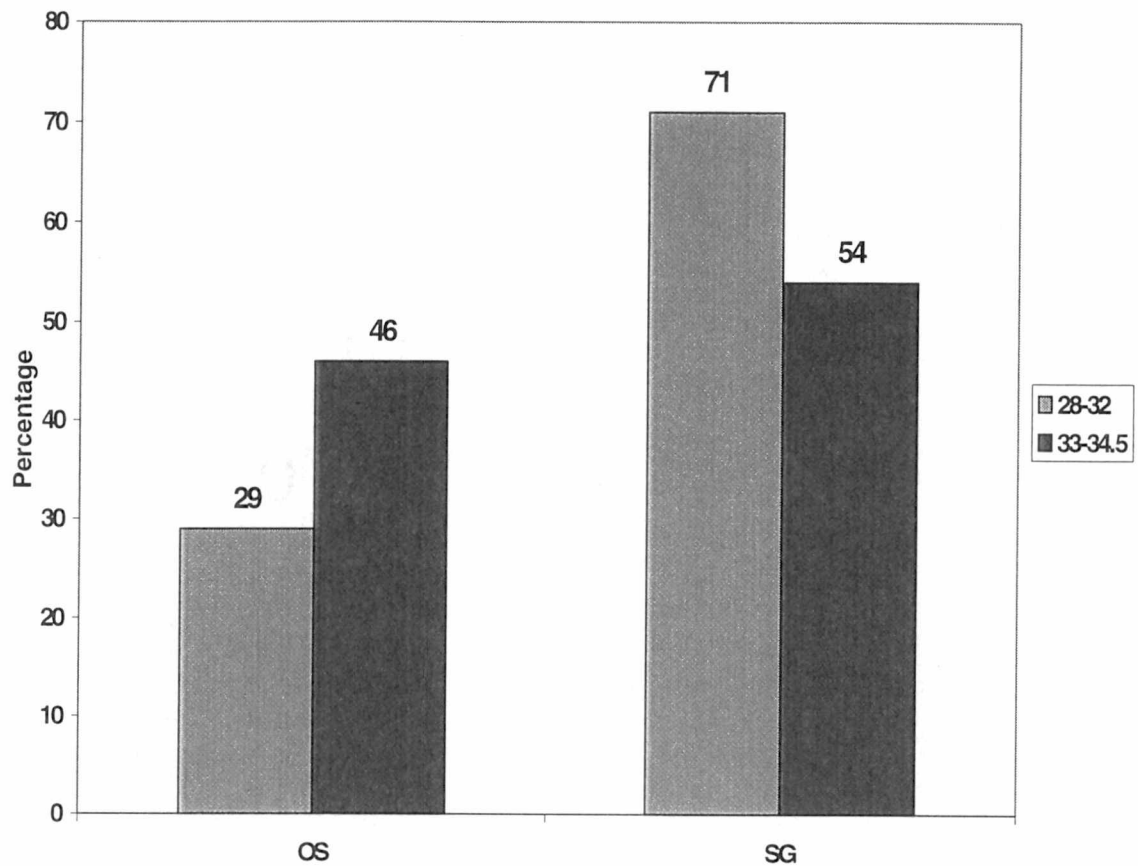
$p = 0.056$, $n = 280$). Clustering the number of counts for temperatures into two temperature groups does produce a Chi-square statistic that is stronger ($\chi^2 = 7.728$, $p = 0.05$, $n = 280$). Temperatures ranging from 29°C to 32°C were classified as group one, while temperatures ranging from 33°C to 34.5°C were classified as group two.

The number of times a holothurian was found in seagrass and open sand was then compared between the two temperature groups (Figure 15). Holothurians move out of the seagrass and into the open sand as temperatures begin to rise above 33°C. The number found in open sand never exceeds the number found in seagrass, but the drop from 71% to 54% found in the seagrass is significant ($\chi^2 = 7.728$, $p = 0.05$, $n = 280$).

Other Variables Affecting Substrate Preference

Current direction and time of day were two other variables analyzed in order to determine their corresponding effect on holothurian substrate preference. Current direction records were only completed for July and September 1997, and there was no significant differences in substrate preferences between the two months ($\chi^2 = 5.174$, $p = 0.639$, $n = 472$). Time of day observations were recorded for all four months of the study, but again the results between months, and the results within months (specific time of day) did not show a trend in substrate preferences that was significant ($\chi^2 = 1.319$, $p = 0.725$, $n = 472$).

Substrate Preference According to Temperature



	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	7.728 ^a	1	0.005
N of Valid Cases	280		

a. 0 cells have expected count less than 5. The minimum expected count is 32.55.

Figure 15. Percentages of holothurians found in seagrass and open sand with respect to the temperature ranges of 28-32°C and 33-34.5°C.

IV. Discussion

Foraging Behavior: Patterns of Movement

The greatest difficulty in accurately observing movement patterns is the need for continuous observation of the individuals. This study observed movements at 3 hour intervals, so movements between these intervals are not resolved. Plots of the holothurian movement are not complete, as only a straight line could be drawn from the initial resting place of a holothurian, to sequential intermediate sites, to its final location at the end of the observation period. However, adult individuals of *Holothuria floridana* appear to be localized, as no individuals were observed moving through more than two areas (18m²) during the study.

Many of the tagged individuals showed movement behavior within a small area of the site, and a frequent change of direction that would prevent the individual from ultimately leaving a given area. The metabolic costs of covering greater distance must be weighed against the available resources to be collected in the immediate area. If greater gains are returned from traveling longer distances in chance of finding optimal food patches, adaptations in behavior for covering greater distances should have been seen.

There was not a significantly greater percentage of recorded migrations into the seagrass areas than into the sandy areas over the study period (Table 1). This observation would seem to contradict the finding of larger numbers of *Holothuria floridana* within the seagrass beds. The lack of migrations into the seagrass beds points out, however, that

the majority of the holothurians were already in the seagrass beds, and probably remained in the seagrass beds throughout the duration of the study.

Relationship Between Sediment Grain-Size Preference and Movement

Regardless of sediment category (SG, OS, SHORE, EDGE) there was no grain size gradient in the study site. This result is surprising since there is presumably a reduction of water flow in the seagrasses that might lead to smaller sediment size fractions where seagrasses were growing (Irlandi & Peterson, 1991).

However, in this study, these patterns were not observed. The field observation of a higher percentage of smaller grain-sized particles trapped within seagrass patches included organic materials that would have been removed during the grain-size procedure with the addition of 30% hydrogen peroxide. Beneath this deep organic monolayer was the sediment layer that had a uniform sediment size composition throughout the study area (Table 2). This may explain why percentages of fines, and smaller phi sizes are not greater within seagrass than within open sandy bottom.

Completely non-selective deposit feeding organisms probably do not occur in nature because of the great metabolic advantages offered by selection. Selection allows the sorting of food-rich particles from those particles that are undesirable, which produces a lower feeding rate based on the fact that the enriched ingested food has a longer residence time in the gut (Lopez and Levinton, 1987). *Holothuria floridana* feeds by means of tentacles, allowing for considerable selective activity (Lopez and Levinton,

1987). Many particles can be handled simultaneously by the holothurian (one particle per feeding tentacle), while also being handled individually by a complex system of muscles, cilia, and mucus. This heterogenous selectivity allows various sized particles to be collected by the tentacles, with some particles rejected before ingestion; thus selectivity does occur at some level in a "non-selective deposit feeder" (Jumars, *et al.*, 1982).

The phi size composition of the fecal pellet samples were similar enough that they were always grouped together in cluster analysis. They shared a modal phi size of 1.0 (coarse sand), and the similarity in percentages of other phi sizes is also evident (Table 2). The similarity of fecal pellet composition provides evidence for some size selection by *Holothuria floridana*. Since the particle size composition of the fecal samples cannot be correlated with any one particular substrate (seagrass, open sand), some feeding selection is occurring regardless of location. If feeding preference is not based entirely on particle size, and the desired particle sizes can be found throughout the area, then greater selection will be made for areas of food-rich particles over areas of food-depleted particles. The carbon source that the holothurians feed upon now becomes the focus because food-rich particles will be defined as those particles found in areas containing the preferred carbon source.

Relationship Between Food Source and Movement: Stable Isotope Evidence

The $\delta^{13}\text{C}$ values of seagrass in this study are more negative than typical $\delta^{13}\text{C}$ seagrass values due to the influence of mangrove carbon, as has been concluded from

previous studies (e.g., Zieman *et al.*, 1984; Fleming *et al.*, 1990; Lin *et al.*, 1991). Values of $\delta^{13}\text{C}$ change little during decomposition of mangrove leaves and seagrass, so isotopic identity is preserved (Zieman *et al.*, 1984). Holothurians feeding on sediment in the study site had an average $\delta^{13}\text{C}$ value of -15.7‰ . The nearest isotopic value is found in the leaves of *T. testudinum* (mean $\delta^{13}\text{C} = 15.3\text{‰}$), and this observation suggests that seagrass leaves were the digested carbon source. From previous studies, *T. testudinum* also shows no significant isotopic variability between young and mature leaves (Lin *et al.*, 1991).

The digestion of algae was avoided by the holothurian *Parasticopus parvimensis* in a study by Yingst (1976), which further showed that material that the animal digested was decaying plant material and the associated microbes. This result may also hold true for *Holothuria floridana*. A review of benthic deposit feeders indicate that they digest different percentages of detritus and microbes, thus acquiring necessary resources from a combination of resources available (Lopez and Levinton, 1987). Even though the current study did not differentiate between microbes and plant material, it does indicate that *T. testudinum* is probably more important as a primary carbon source for *Holothuria floridana* in the study area. Based upon the significant difference in $\delta^{13}\text{C}$ values between individual *Holothuria floridana* and fecal pellets the mangrove carbon is not digested, and passes through the organisms digestive system resulting in fecal pellets with organic carbon $\delta^{13}\text{C}$ values typical of sediments from the study area (Table 4).

The study site in Zane Grey Creek is characterized by organic carbon sediment fractions with $\delta^{13}\text{C}$ values of -18.0‰ , and less, due to the influence of terrestrial carbon, and mangrove detritus. There is a localized area with $\delta^{13}\text{C}$ values $< -21.0\text{‰}$ found near

the roots of the mangrove trees that line the channel (Figure 11), but the $\delta^{13}\text{C}$ values increase toward the center of the channel. This change in sediment $\delta^{13}\text{C}$ values along a gradient was also observed on a much larger scale by Hemminga et al. (1994), who described a carbon $\delta^{13}\text{C}$ gradient that depicted the loss of mangrove carbon influence over 3 km.

In the Hemminga et al. (1994) study, as water flowed over seagrass beds that had trapped mangrove carbon, the trapped material was resuspended in the water column resulting in $\delta^{13}\text{C}$ values for seston (phytoplankton and zooplankton) decreasing from -18.10‰ to -22.88‰ . Even though this occurred on a much larger scale in the Hemminga et al (1994) study, the reverse may be true of the present study site as current reversals may carry excess mangrove carbon away from the bottom sediment. This would increase $\delta^{13}\text{C}$ values for sediment organic carbon from -21‰ to -18‰ at the location of strongest current flow.

There is little water flow near the roots of the mangroves, causing mangrove carbon to be retained or deposited in large quantities in this area. One would think that the holothurians would prefer locations closer to the channel where there is less influence of mangrove carbon. Mid-channel also has stronger current flow which is likely to be responsible for sediment $\delta^{13}\text{C}$ values ranging from -17.4‰ to -19.0‰ . This area is also the location of *T. testudinum* patches, and as mentioned earlier, is the more important source for the carbon isotope ratios observed in the holothurian body.

Contrary to expectations, the holothurians showed a preference for areas closer to the mangroves in the first test with 88% of the study area consisting of the first zone

(-18‰ to -19‰), and 12% of the second zone (-20‰ and less)(Figure 11). When these zones were redrawn (84%, and 16%, respectively, Figure 12), the significance was lost. More samples are likely needed to accurately pinpoint where the gradient shifts between these two zones.

When one looks at the data on a monthly time scale, however, the preference for areas closer to the channel (characterized by *T. testudinum*) increases from March to September (Figure 13), with the maximum of a 71% preference for sediment areas with -18‰ to -19‰ $\delta^{13}\text{C}$ values in September. This is true of both of the two different zone divisions. The 71% preference in September coincides with the spawning season of *Holothuria floridana* which occurs in late summer (Engstrom, 1979). It is possible that there is a selective advantage for sea cucumbers to move from the mangroves to the open channel so that gametes being dispersed would be easily carried in the water column and transported over a greater distance for colonization.

Relationship Between Food Source and Movement: Substrate Preference

Holothuria floridana were found more often in seagrass areas than in open sand. This result was especially true during the month of September when 71% of holothurians recovered were found in the seagrass beds. These locational findings are correlated strongly with the results of the isotope aspects of this study indicating *T. testudinum* is the probable carbon source ingested by these organisms. *T. testudinum* patches were observed away from the mangroves, and were observed in greater densities toward the center of the

channel. *Halodule wrightii* is found in small patches next to shore, and near mangrove trees. As the results indicate, however, the holothurians spent most of their time in areas of sediment with organic carbon $\delta^{13}\text{C}$ values of -17.4‰ to -19.0‰ which does not include patches of *Halodule wrightii* (Table 3).

It is possible that *Holothuria floridana* is slowing its movement rate once it is in an area of a preferred food source like *T. testudinum*. Studies on *Uca pugnax* revealed that the crab spent more time foraging in patches manipulated to have higher concentrations of benthic diatoms (Robertson et al., 1980). Forbes and Lopez (1986) discovered that the snail *Hydrobia truncata* spent more time foraging for food in patches rich in chlorophyll. These snails slowed their rates of crawling, but did not change their egestion rates.

Foraging theory predicts that individuals will forage in a patch only until the mean rate of gain falls to the mean level that can be expected from the environment as a whole (Jumars, 1993). This behavior would explain why *Holothuria floridana* was found with greater frequency in seagrass patches than in open sandy bottom. Patches of *Thalassia* not only provide the carbon source, but the baffling effect of the leaves trap greater concentrations of the food, and prevent large amounts of organic carbon from being carried to other areas.

Relationship Between Other Variables and Movement: Temperature and Light

It is hard to differentiate between the effects of light and temperature on holothurian movement because temperature can be the result of enhanced thermal heating from light and its penetration into the water column. Even though it was hypothesized that the holothurians would prefer low-light gradients, the light gradient experiment showed that the holothurians have no significant preference for the observed light intensities, and this is consistent with the results of Rodgers and Bingham (1996) who studied *Cucumaria lubrica*. *Holothuria floridana* did show a response to increasing water temperature as it was found with greater frequency in the open sandy areas when the water temperature was between 33-34.5 °C. The open sandy areas are subjected to stronger current flow from tidal flushing. Tidal flushing keeps the water in motion and prevents it from being easily heated. The baffling effect of the seagrass blades slows water movement and temperatures reach higher levels within the seagrass beds.

Glynn (1969) examined the temperature tolerances of *Asticopus* and *Leptosynapta* (Holothuroidea). When subjected to high water temperatures (29-32.4°C) the holothurians reacted with quick, bounding movements that allowed them to cover larger distances to move out of the high temperature zone, and presumably into areas of cooler water. Even though *Holothuria floridana* was not observed to react in this fashion, the escape behavior observed by Glynn (1969) may be similar to observations in this study of holothurian movement out of the seagrass beds and into the sandy bottom where current velocity is greater, and water temperatures are cooler.

Summary

It is possible to construct a simple model to account for the $\delta^{13}\text{C}$ values that were found for *Holothuria floridana* and sediments in the study site. Mangrove detritus is deposited throughout the study site, in greater concentrations closer to the shore and near the mangrove branches. This results in relatively negative $\delta^{13}\text{C}$ values for the sediment organic carbon. *T. testudinum* detritus, with more positive $\delta^{13}\text{C}$ values, is also deposited and spread throughout the site by currents, but is in greatest concentration in the seagrass patches due to the baffling of the current by the seagrass leaves.

During the time between change in current direction, water movement over the study area is stifled, allowing the settling of mangrove carbon throughout the site, including open sandy bottom and seagrass areas. Seagrass patches also trap greater amounts of settling mangrove carbon than the open sandy areas, so that the average $\delta^{13}\text{C}$ values of organic sediment fractions resemble those of open sandy areas.

Sediment influenced by mangrove detritus is ingested by the holothurians. Selection may be occurring at this point as the holothurian selects specific particle sizes and composition or they may be ingesting everything and are just unable to digest the mangrove carbon. Holothurians are spending more time in seagrass patches because of the greater concentration of higher quality food. Sediment and associated organic carbon is then ingested and $\delta^{13}\text{C}$ values that are less negative are incorporated into the body (digested), while particles with $\delta^{13}\text{C}$ values closer to mangrove carbon values are rejected and passed from the system. The typical sediment values of these rejected lower quality

particles are found in the fecal pellets reflecting the influence of mangrove carbon.

During periods of high temperature stress, the holothurians apparently move out of the areas of warm water within the seagrass patches and into the flowing water of the open sandy bottom with lower water temperatures.

V. Conclusion

This study evaluated foraging behavior patterns of *Holothuria floridana*. These animals were found to cover small distances and forage in very local areas. Few tagged specimens were observed leaving the study area. Migrations from open sand into the seagrass beds were only slightly higher than migrations from seagrass into open sand. *Holothuria floridana* fecal pellets were of uniform grain size composition providing evidence of particle selection taking place by this deposit feeder. Different portions of the study site were not characterized by specific sediment size compositions because the sedimentary layer is almost uniform in composition throughout the site, although visual observation indicated more flocculate particulate carbon within seagrass beds. Foraging preferences for some areas appears not be a function of available sediment sizes, but rather the food quality of the sediment particles.

The ultimate carbon source for *Holothuria floridana* appears to be predominantly the seagrass *T. testudinum*, based upon carbon isotope analyses. This conclusion is supported by a greater amount of foraging time spent by the animals in the seagrass beds. The evidence is inconclusive with respect to holothurian preference for sediments influenced by seagrass detritus over those heavily influenced by mangrove detritus. Light appears to have little effect on the foraging patterns of *Holothuria floridana*. At water temperatures above 33°C, holothurians were stimulated to move out of the seagrass beds and into the open sand, where a greater current flow and cooler water temperatures prevail.

There are very few studies of deposit feeding species that detail a complete foraging strategy. *Holothuria floridana* is no exception, and this study has presented only fragmentary aspects of its overall behavior and foraging strategy. Measurements of total organic carbon in and outside of the seagrass beds would be helpful in quantifying the organic flocculate that was visually observed in the study area. More sediment samples need to be collected for $\delta^{13}\text{C}$ analysis of the organic carbon content so that the exact border between changes in carbon gradients throughout the study site can be isolated.

There is need for more information on possible chemical cues that initiate ingestion, processing rate, and patch selection. For example, understanding of insect behavior was greatly enhanced by improvement of understanding of chemical cues (Bernays, 1985). Similar approaches have been suggested for deposit feeders (Jumars, 1993). Future studies could also include effects of current flow on patch selection. Studies on the seastar, *Oreaster reticulatus* (Scheibling, 1981), have shown that current flow helped determine which areas were preferred for foraging activity, and prevented the seastar from searching areas it had already foraged. This finding raises an interesting possibility that the same may be true for other deposit feeding echinoderms.

Finally, studies on gut selection, ingestion rate, and detailed particle selection of *Holothuria floridana* will be necessary to complete the picture of how physiology may be driving behavior. The results of this study contribute toward an understanding not only of *Holothuria floridana*, but lead to inferences about the foraging behavior of other species of holothurians, and perhaps deposit feeders in general.

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Appendices

Appendix A

Sediment Grain Size Procedure

According to Jackson (1969), Ingram (1971), Gee and Bauder (1986), Kunze and Dixon (1986).

Organic Removal via H_2O_2 (rough procedure)

Procedure

1. Initial sample dry wt. at room temperature from 40°C drying.
2. Put sample into 250 mL LDPE bottle.
3. Place bottle into 500 mL beaker, place beaker into an ice bath.
4. Stir in 10 mL aliquots of 30% H_2O_2 allowing sample to efferves.
5. Continue stirring in aliquots of H_2O_2 at 10 mL/20 g until effervescence is completed.
6. Watch closely, as not to allow to overflow.
7. Remove from ice bath allowing to equilibrate to room temperature. Watch for overflowing.
8. Add 100 mL of water to 500 mL beaker and warm to 80 °C on hot plate. Place 250 mL bottle of sample into water bath and warm for one hour stirring occasionally; watch for overflowing.
9. Remove and allow to cool to room temperature.
10. Add final aliquot of H_2O_2 and allow to stand over night (at least 12 hours).
11. Test with aliquot H_2O_2 , if efferevesence occurs (+ test), bring to volume with distilled water and centrifuge at 1600 rpm for 10 mins, decanting off excess.
12. If tested "+," then repeat steps 5 through 11; if test results in negative then continue.
13. Repeat centrifuge at 1600 rpm for 10 mins with distilled water as rinse.
14. Ready for iron treatment.

Equipment

500 mL beakers
250 mL LDPE centrifuge bottles
30% H_2O_2
glass stir rods
hot plate

Fe₂O₃ Removal for Grain Size Separation

Procedure

1. Pre-heat water bath to 75-80 °C, not more than 80 °C.
2. Place sample in 250 mL LDPE wide mouth bottle.
3. If sample dry, moist with minimal amount of distilled water to make as sludge.
4. Warm samples in water bath for 10 mins.
5. Add pre-mixed citrate buffer solution to samples at prescribed amounts. For every 10 g of sample add 45 mL of solution.
6. Heat for 5 mins.
7. Add sodium-dithionite at prescribed amounts. For every 45 mL of solution add 1 g of sodium-dithionite.
8. Heat for a total of six minutes, stirring for 1 min, then occasionally for 5.
9. Repeat steps 7 and 8.
10. For large samples, ≥50 g, add a third sodium-dithionite aliquot before removing from bath.
11. Bring to volume.
12. Centrifuge for 15 mins. At 1600 rpm.

Reagents

Sodium-citrate/Sodium-bicarbonate solution mixed at 8:1

1. 40 mL of citrate and 5 mL of bicarbonate or 3 L of 0.3 M citrate solution with 375 mL of 1 M bicarbonate solution buffered to pH 7.3 with ~9 g citric acid.
2. 0.3 M sodium-citrate solution
88 g sodium-citrate/ L of distilled water or
264 g of sodium-citrate/ 3 L of distilled water
3. 1M sodium-bicarbonate solution
1 L of distilled water
84 g sodium-bicarbonate

Weight Percent Distributions via Wet Sieve Analysis

Gravel (>2mm), Sand (2mm<63 μ) and Fines (<63 μ)

Procedure

1. Prior to initiating any treatment or sieving, first dry the sample via a drying oven at 40 °C.
2. Cool to room temperature in a dessicator and determine an initial dry weight.
3. Soak the sample (completely covered) in distilled water and 10 mL of sodium-metaphosphate for 24 hours.
4. Place the nested sieves into a large plastic funnel and have the funnel resting in a large 4 L beaker or catch basin of this size (e.g. Gallon milk jug)
5. Pre-wet the sieve screen and then place the soaked sample onto the top sieve.
6. Using the distilled water, spray the sample until the fines have completely passed through. Use care not to overflow the catch basin.
7. Place the sand fraction sieve into the oven drying the sample.
8. Place 10 g of MgCl in the fines fraction. This encourages flocculation of the sample so excess water can be readily removed.
9. After setting 24 hours, use the vacuum pump to remove excess water from the fines fractions, being careful not to disturb the sample.
10. Using a minimal amount of distilled water, transfer the fines fraction to a clean pre-tared plastic 500 mL container for drying in the oven.
11. After the fines fractions have re-settled in the drying oven, excess water may again be removed to expedite drying and to decrease the salt content.
12. Using a large sheet of white paper dump the sand fraction out of the sieve onto it with a tapping motion and a clean brush.
13. Obtain dry weights for all fractions, gravel (if present), sand, and fines.

Calculations

Fraction weight/total sample weight (sum of fractions) x 100 = Size Fraction Weight Percent

Equipment

Drying oven	Large Funnel
Dessicator	Sieves
MgCl	500 mL plastic dishes
4 L beaker	Vacuum pump system

Appendix B

Frequency of Holothurian Recapture

March 1997

<i>Specimens ID</i>	<i>Frequency</i>	<i>Percent</i>
BBBBB	3	2.2
BBBBBB	1	0.7
BBRBB	2	1.5
BBRYB	4	3.0
BBYRB	2	1.5
BBYYB	3	2.2
BBYYBY	4	3.0
BRBRB	4	3.0
BYBBB	5	3.7
BYRRB	2	1.5
RBRBR	8	5.9
RBRRR	4	3.0
RBRYR	5	3.7
RBYBR	2	1.5
RBYYR	3	2.2
RRBRR	2	1.5
RRRR	1	0.7
RRRRR	2	1.5
RRRRRR	3	2.2
RRYRR	4	3.0
RYBBR	1	0.7
RYBRR	10	7.4
RYYR	1	0.7
RYYYR	2	1.5
YBBBY	3	2.2
YBBRRY	2	1.5
YBBY	8	5.9
YBRBBY	1	0.7
YBRRRY	3	2.2
YBRRY	3	2.2
YBRY	2	1.5
YBYBY	4	3.0
YBYYY	1	0.7
YELLOW	5	3.7
YRBBBY	1	0.7
YRBY	4	3.0
YRRY	3	2.2
YRYBYY	2	1.5
YRYRY	6	4.4
YRYRYR	1	0.7
YYBY	1	0.7
YYRBY	1	0.7
YYRY	1	0.7
YYYRBY	1	0.7
YYYYY	2	1.5
YYYYYY	2	1.5
Total	135	100.0

May 1997

<i>Specimen ID</i>	<i>Frequency</i>	<i>Percent</i>
B-BBBYY	1	1.2
B-RBBYY	2	2.4
B-RYYBB	3	3.6
B-YYYRR	2	2.4
B-YYYYY	3	3.6
B-YYYYYY	1	1.2
BBBYB-B	1	1.2
BBRBY-R	2	2.4
BBRRB-R	3	3.6
BBYRR-R	1	1.2
BBYYR-Y	1	1.2
BBYYY-R	2	2.4
BRBRB-Y	1	1.2
BRRBB-R	1	1.2
BYRRY-B	1	1.2
BYRYB-Y	1	1.2
R-BBBRR	5	6.0
R-BBRBY	2	2.4
R-BBRRB	2	2.4
R-BBYYY	1	1.2
R-BYBBR	4	4.8
R-RBYYY	6	7.2
R-YBYBR	1	1.2
R-YYRYY	1	1.2
R-YYYYR	3	3.6
RBBBR-B	1	1.2
RBRBR-B	1	1.2
RBYYY-R	1	1.2
RRRRR-B	1	1.2
RRYBB-Y	1	1.2
RYBBR-Y	1	1.2
RYYBB	1	1.2
Y-BBBBB	2	2.4
Y-BBYRR	1	1.2
Y-BBYRR	1	1.2
Y-BRBRB	3	3.6
Y-BRYBR	2	2.4
Y-RBBYR	1	1.2
Y-RBRBR	2	2.4
Y-RRRBY	2	2.4
YBRBB-R	1	1.2
YBRRRY	1	1.2
YBYBR-R	1	1.2
YYRYY-R	1	1.2
YYYBB-B	2	2.4
YYYBR-R	1	1.2
YYYYR-R	2	2.4
YYYYY-B	2	2.4
Total	83	100.0

July 1997

<i>Specimen ID</i>	<i>Frequency</i>	<i>Percent</i>
BBBBB	2	1.8
BBBBBB	2	1.8
BBBBBBB	3	2.7
BBBBRR	1	0.9
BBRYB	3	2.7
BBRYR	3	2.7
BBY	1	0.9
BBYBBY	1	0.9
BBYYBBR	3	2.7
BRRYBY	2	1.8
BRYBY	4	3.6
BYBRR	1	0.9
BYRYBY	1	0.9
RBBBY	1	0.9
RBBRY	2	1.8
RBBYR	2	1.8
RBRBRBY	2	1.8
RBRYB	6	5.4
RYBR	2	1.8
RYYY	3	2.7
RYYYR	1	0.9
RRBBRYR	4	3.6
RRBYYY	1	0.9
RRRRB	3	2.7
RYBBR	2	1.8
RYBRYBB	1	0.9
RYBRYBBB	1	0.9
RYRRY	5	4.5
RYRYB	3	2.7
YBB	1	0.9
YBBBBBB	6	5.4
YBBBBBYR	2	1.8
YBBBY	3	2.7
YBBR	1	0.9
YBBYBBYB	2	1.8
YBBYBBYBB	1	0.9
YBRBYR	3	2.7
YBYRB	2	1.8
YRBBRY	1	0.9
YRBBRYR	2	1.8
YRBRRYR	1	0.9
YRRBB	5	4.5
YRRBR	2	1.8
YRRRB	2	1.8
YRRRR	1	0.9
YRYRY	1	0.9
YYBBR	3	2.7
YYRRB	2	1.8
YYYBBR	1	0.9
YYYRR	4	3.6
Total	112	100.0

September 1997

<i>Specimen ID</i>	<i>Frequency</i>	<i>Percent</i>
B-BBNNPP	3	1.6
B-BNBPPP	1	0.5
B-NBBPPP	12	6.3
B-PBBPPP	1	0.5
B-PNNPPP	2	1.0
B-PPBBBB	1	0.5
B-PPBPPP	2	1.0
B-PPPNB	2	1.0
B-PPPBNN	5	2.6
B-PPPNBN	5	2.6
B-PPPNNB	2	1.0
B-PPPNNP	6	3.1
B-PPPNPP	1	0.5
B-PPPPNBN	1	0.5
BBBBPP	1	0.5
BBNBBB	1	0.5
N-BBNNNN	2	1.0
N-BNBNNN	4	2.1
N-BNNNNN	2	1.0
N-NBBNNN	4	2.1
N-NBNBBB	1	0.5
N-NBNNNN	6	3.1
N-NNBBPP	5	2.6
N-NNNBBN	1	0.5
N-NNNBNN	2	1.0
N-NNNBPP	1	0.5
N-NNNN	1	0.5
N-NNNNBB	3	1.6
N-NNNNBN	1	0.5
N-NNNNN	1	0.5
N-NNNNNB	5	2.6
N-NNNNNN	6	3.1
N-NNNNPP	1	0.5
N-PPBNNN	4	2.1
N-PPNNNNN	1	0.5
N-PPPPPP	5	2.6
P-BBBBBN	1	0.5
P-BBBBBB	4	2.1
P-BBBBPP	7	3.6
P-BBBNBB	1	0.5
P-BBBNBN	4	2.1
P-BBBNNB	9	4.7
P-BBBNNN	4	2.1
P-BBBNPP	5	2.6
P-BBNBBB	11	5.7
P-BBNNBB	6	3.1
P-BBNNPP	1	0.5
P-BNB BBB	4	2.1
P-BNNBBB	2	1.0
P-NBBBBB	7	3.6
P-NBNBBB	2	1.0
P-NNNBBB	8	4.2
P-PPBBBB	6	3.1
P-PPBPPP	1	0.5
P-PPNBBB	5	2.6
P-PPPPPB	1	0.5
PPPNB	1	0.5
Total	192	100.0

Vita

Matt Bowles was born in Knoxville, Tennessee on June 7, 1972. He attended schools in the public system of Knoxville, Tennessee, the Knox County Public School System, where he graduated from Farragut High School in May, 1990. He entered the University of Tennessee, Knoxville during August of 1990 where in December, 1993 he received the Bachelor of Arts in Anthropology. He then entered the Graduate Certificate program in January, 1994 at Covenant Theological Seminary in St. Louis, Missouri. After completing the one year certificate in St. Louis, he returned to Knoxville in January, 1995 to begin coursework toward a Master's program in Ecology at the University of Tennessee, Knoxville. He officially entered the Master's program in August, 1995, and received the Master's degree in May, 1998.