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A Comparison of Bioaccumulation and Digestive Enzyme Solubilization of Copper in Two Species of Sea Cucumbers with Different Feeding Habits.

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A Comparison of Bioaccumulation and Digestive Enzyme Solubilization of Copper in Two Species of Sea Cucumbers with Different Feeding Habits.

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ABSTRACT

A Comparison of Bioaccumulation and Digestive Enzyme Solubilization of Copper in Two Species of Sea Cucumbers With Different Feeding Habits.

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The mode of feeding exhibited by different organisms may influence the form or quantity of copper that is bioaccumulated. This hypothesis has been tested by exposing 2 species of sea cucumbers, *Pentacta anceps* and *Stichopus chloronotus*, which possess different feeding modes, to varying concentrations of copper.

The digestive tract and body wall were dissected and analyzed for copper concentration using atomic absorption spectroscopy. A trend was present, exhibiting a small dose dependent curve. The results did not show a significant difference between species or treatments. This study indicates that feeding mode may influence the amount of copper accumulated but it could not be concluded because of a small sample size. The model used in this study demonstrated that copper was being actively precipitated out of the water and deposited into sediment. Future studies focusing on pollutant uptake may find this useful when evaluating the role of feeding mode or habitat.

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CHAPTER 1

INTRODUCTION

Pollution in aquatic environments has received considerable attention. The contamination of ecosystems from anthropogenic pollutants such as polycyclic aromatic hydrocarbons, organic nutrients, and heavy metals has led researchers to examine how these pollutants enter an organism, interact with other molecules and tissues, and advance up food chains (Fowler et al.1978; VanDover et al.1992; Hope et al.1997). The majority of the organisms taken from aquatic environments for human consumption come from marine ecosystems. For this reason pollution impact studies that focus on the marine environment are receiving more attention.

Heavy metal pollutants in the marine environment have been shown to bioaccumulate in marine organisms and many marine animals can accumulate heavy metals such as cadmium, copper, and zinc (Greig et al.1976; Martin 1979; Davies 1992). These accumulated metals can originate from food, water, or sediment, and their relative importance varies with the metal and the nature of the organism (Week and Rainbow 1993). The ultimate fate for most metal contaminants that enter an aquatic ecosystem is to be chemically or organically bound in the sediment, which reduces its dispersion and bioavailability. The toxicants in solution are typically regarded as the most bioavailable form in aquatic environments, but they seldom stay in the water column for long periods of time since most contaminants are strongly adsorbed onto sediments, which often causes a reduction in concentrations of the toxicants in the water (Landrum and Robbins 1989).

Heavy Metals - Copper

Heavy metals such as Pb, Cd, Zn, and Cu pollutants in an aquatic habitat can enter

as a form of run-off or discharge from domestic, industrial, agricultural, and mining activities. Many of these metals are found in marine waters at levels that are toxic to aquatic organisms. Of these, copper is the most common metal found at toxic concentrations in marine waters (NCDEM 1991).

Copper refining is the largest source of environmental contamination, primarily as airborne particulates and aqueous copper solutions released into sewage waters (Young et al. 1979). Copper has been used extensively in the manufacture of electrical equipment, a large number of alloys, coinage, and chemical apparatus. Different forms of copper are also widely used for industrial purposes. Copper salts are used in antifouling paints, insecticides, fungicides, and algaecides. All of these uses produce copper as a waste product at some point during their manufacturing, usage, or disposal.

An increasing problem in the marine environment is the copper contamination connected with antifouling paints. The input of copper via bottom paints and scrapings has been shown to be quite significant (Young et al. 1979). On ocean-going vessels a copper-containing paint is applied to the hull in order to prevent organisms from attaching and building up in sheets. These organisms are usually sessile suspension feeding or filter feeding invertebrates like mussels, oysters, sponges, and barnacles. Although these paints do keep organisms from attaching, they also release copper salts and free copper ions into the water. Copper ions are then able to bind to proteins or chelators, which allows copper to precipitate out of the water column and settle to the sediments.

Marine Vertebrates as Bioindicators

The effect of copper and its accumulation has been very well documented in

many marine vertebrates (Hardisty et al. 1974; Greig et al. 1976; Baker et al. 1998). Greig showed that muscle tissue samples taken from deep-water fish had substantially lower levels of copper when compared to coastal fish. These results were also verified by other researchers (Hardisty et al. 1974; Hellou et al. 1992). Another form of exposure comes from the digestion of food items that contain high concentrations of copper. Baker et al. (1998) demonstrated that food intake and growth of the juvenile grey mullet was significantly reduced when copper dosed food was given. Liver tissue concentrations for exposed fish were also significant exhibiting 4 times the amount copper found in the controls. Copper accumulation in vertebrates resulting from deposit feeding modes has not yet been evaluated, but those few that are active bottom feeders may also show a direct correlation to increased copper levels.

Marine Invertebrates as Bioindicators

Marine invertebrates such as mussels, polycheates, and crustaceans have been used as assay organisms for heavy metals and polychlorinated biphenyl pollution. These invertebrates have been found to be reliable bioindicator organisms for some pollutants found in the water column, but not for sediment quality assays (Fowler et al. 1978; Landrum and Robbins 1989; Week and Rainbow 1993; Warnau et al. 1995; Nicholas et al. 1997). Invertebrate organisms dominate the marine benthos and it is these organisms that are continually in contact with and sometimes ingesting sediment along with any bound forms of pollution. The substrate is an important sink for suspended matter and associated land-derived contaminants with a large amount of the input ultimately accumulating in the sediment (Morrisey et al. 1995). These elevated concentrations of heavy metals, such as copper, in the sediments have been found to be toxic to benthic

organisms such as the marine amphipod *Hyella azteca* by inhibiting enzyme systems or growth (Kubitz et al. 1995).

Deposit feeding organisms ingest large quantities of sediments to supply their metabolic requirements. The digestive enzymatic action on the sediments release ionic forms of copper directly into the gut, which would then be available for absorption and bioaccumulation (Mayer et al. 1996). Copper that was ingested with sediments was also found to inhibit the digestive proteases of the lugworm *Arenicola marina* (Chen and Mayer 1998). For some invertebrate species, it is clear that digestive solubilization is the principal route of exposure for some sedimentary contaminants (Fowler et al. 1978; Landrum et al. 1989; Mayer et al. 1996; Nicholas et al. 1997).

Establishing sediment contamination as an uptake route for pollutants leads to the question that sediment-feeding organisms may accumulate contaminants from a combination of routes such as absorption from the water column and digestion from the sediment. This may result in a greater bioaccumulation than that of absorption alone. This was demonstrated by Nicholas et al. (1997) when the sediment feeding lugworm *Arenicola mariana* was found to contain a greater concentration of PCBs when compared to 2 filter-feeding mollusks.

Effects of Copper on Holothurians

Eisler (1981) stated that echinoderms are the "primary movers of sediments and detritus in the sea and are probably very important in the cycling of trace metals" and "show promise of becoming suitable indicators". Xing and Chia (1997) showed that copper was being accumulated in the tissues of the sea cucumber *Holothuria leucospilota* and the exposure was originating from contaminated sediments that the organism was

feeding on. The sea cucumber *Stichopus japonicus* and its embryos were found to be highly sensitive to increased levels of copper when exposed to small concentrations of copper added to their water (Shcheglov et al. 1990). The 96-hour LC_{50} value was 70 ppm for adults and 40 ppm for embryos. Embryos exhibited mortality at a fraction of the dosage needed to kill an adult. Although no tissue samples were taken to confirm metal uptake, it can be concluded that the copper concentrations were significant enough to cause death. Sea cucumbers posses 2 different modes of feeding, deposit feeding and suspension feeding. These species may be present in the same habitat because their feeding preferences and food items are different from each other, which means that competition between the 2 species is not a factor. For a study comparing the uptake of metals between organisms this would make them suitable candidates. A demonstration of substrate contamination verses waterborne contamination would be a practical step in determining if some organisms are at a higher risk for toxicity in a polluted environment.

Objectives

Evidence has shown that digestion of contaminated sediment by marine organisms is an important pathway for contaminant bioaccumulation (Mayer et al. 1996). Also, that the mode of feeding exhibited by different organisms may influence the form or quantity of contaminant that is present in the ecosystem (Nicholas et al. 1997). These 2 reasons have lead to the following hypothesis. Will an organism's mode of feeding affect the quantity of contaminant bioaccumulation in tissues?

To test this, 2 species of sea cucumbers possessing different modes of feeding were exposed to a high and a low dose of copper in replicate microcosms. The objectives of this study were as follows: (1) to determine whether the mode of feeding in the 2 species

of sea cucumbers influences the amount of bioaccumulation that occurs in their tissues and organs, and (2) to compare the amount of copper that is solubilized by stomach enzymes from contaminated sediment between the 2 species of sea cucumbers.

CHAPTER 2

MATERIALS AND METHODS

Aquaria Setup

9 38-liter all-glass aquaria containing 0.5 cm of substrate (consisting of a granular mix of calcareous Bermuda sand purchased from CaribSea Inc.) were filled with synthetic seawater (Instant Ocean). Microcosms were cycled for 3 months with a bacterial culture (Cycle vital, Two Little Fishes, Pensacola, FL) to establish a nitrifying and denitrifying bed for nitrogenous waste removal. Each tank was equipped with a power head (Hagen model #125) to oxygenate and circulate the water at approximately 125 gal/hr, which keeps food particles suspended for the suspension feeders.

Water Quality

The temperature in each of the tanks was controlled by 9 100-watt Acura digital aquarium heaters (Tetra, Oakland, CA). Salinity for each of the 9 tanks was measured with a SeaTest hydrometer (Aquarium Systems, Mentor, OH). The salinity for each tank was kept constant and water was added to adjust for evaporation. The pH was measured by an Orion pH probe and any variance from the desired value of 8.2-8.4 was corrected by the addition of a small amount of Marine and dKH buffer (Kent marine, Acworth, GA). Organic waste including ammonia, nitrite, and nitrate was measured by a SeaTest saltwater test kit (Aquarium systems, Mentor, OH).

Experimental Procedures

Sea cucumbers were added after the nitrogen cycle had been established and ammonia, nitrite, and nitrate were below harmful levels. The sea cucumbers in this study

consisted of 2 species, *Pentacta anceps* and *Stichopus chloronotus*. *Pentacta anceps* is a non-selective suspension-feeding sea cucumber that adheres to substrate above the sediment and extends its buccal tentacles for feeding. This mode of feeding captures only particles that are suspended in the water column above the sediment. *Stichopus chloronotus* is a non-selective sediment-feeding sea cucumber that crawls along the substrate and ingests whole particles of detritus and sand, eliminating the substrate. These species were collected from the reefs surrounding the Pacific Island of Bali and obtained commercially from Pet Gallery, a retail aquarium supply shop located in Gray, TN. These 2 species occur together in the same habitat and were exposed to the same water quality parameters while in captivity.

The addition of sea cucumbers was hindered because the supplier could not deliver the requested number of individuals at one time. The study called for 27 of each species be placed in the aquaria at the same time. The first shipment contained only 19 suspension feeders and 22 sediment feeders. The remaining individuals were added in a separate shipment a month later. During this period, spawnings and deaths of some individuals occurred, as noted in the results section. All individuals were allowed to freely move and feed. Sizes of individuals varied but length and weight were not measured due to the contractile body of the sea cucumbers and potential damage due to excess handling of individuals. Once a day, specimens were fed a mixture of ground fish food and brine shrimp.

The initial undosed copper levels in all tanks plus a dosed liter of aquarium water calculated to be at 1.0 ppm copper were measured using atomic absorption spectroscopy (Appendix C). The dosed liter of saltwater served as an experimental control to confirm

both the calculation that yielded the dosage amount, and the integrity of the A.A. unit.

Stichopus japonicus has an LC_{50} for copper of 70 ppm (Shcheglov et al. 1990). A lethality test was performed to confirm the effects of copper on these species. Three individuals of each species were given an acute exposure of 100.0 ppm copper for 48 hours. This dosage was made by adding 39.3 mg of $CuSO_4$ to 1 liter of deionized water and thoroughly mixing on a stir plate until the $CuSO_4$ was dissolved. This solution was then added to the aquaria slowly over the next 30 minutes.

A lethal concentration of copper was not desired. Therefore, the established LC50 value of 70 ppm was adjusted to several lesser values. There were 3 replicate tanks for each treatment (control, low dose, and high dose). The aquaria were dosed with copper sulfate at the following copper concentrations: 0.0 ppm, control; 10.0 ppm, low dosage tank; and 40.0 ppm, high dosage tank (Appendix A). The purpose of the low copper dosage was not to kill the sea cucumbers but to allow copper to accumulate in the sediments and tissues over a 2-month exposure time.

$CuSO_4$ ($5H_2O$) was mixed with deionized water until completely dissolved and then slowly added to the test aquaria, this slow addition of copper was easier on the organisms and allowed for proper mixing of the 2 liquids. The addition of $CuSO_4$ to water releases Cu^{+2} ions and SO_4^{-2},, which is oxidized and released as gas. The quantity of $CuSO_4$ needed to reach the desired copper level was determined by the following equation.

$$\frac{[\text{Cu+2 desired in ppm}]}{106\ \text{ppm}} \times \frac{*249.62\ (\text{g/mol})}{**63.55\ (\text{g/mol})} \times \frac{1\ \text{gram}}{\text{ml}} \times \frac{3897\ \text{ml}}{1\ \text{gallon}} \times 10\ \text{gallons}$$

*Molecular weight of $CuSO_4$ = 249.61 **Molecular weight of Cu^{+2} = 63.55

The amount of $CuSO_4$ used for each treatment was the same at the start of the study. The copper that was available in the water column decreased over time, accumulating in the sediments. This process required additional amounts of $CuSO_4$ based on the amount of copper present in the water column (Appendix A).

Sediment Sampling and Analysis

Sediment samples were taken at the beginning and the end of the study for copper content analysis. These samples were dried at 110 ^{o}C until a constant dry weight was obtained (40-48 hours). All samples were digested with 5 ml 65% heavy metal grade nitric acid in a 30 ml beaker under a laminar flow hood. All glassware used was stored after washing and rinsing with deionized water in a 5% solution of nitric acid made with high purity H_20. The beakers were heated gradually to 125 ^{o}C for 30-60 minutes in the fume hood. . After the solution was cooled to room temperature, 1 ml of 30% hydrogen peroxide was added to each beaker and the solutions were further digested at 135-140^{o}C until the color of the sample had stabilized. The samples were then transferred to holding vials and refrigerated until all samples were digested.

Tissue Sampling and Analysis

Specimens were removed from the aquarium and relaxed in a 6.7% MgCl solution for 30 minutes (Xing and Chia 1997) and dissected by a longitudinal incision from the cloaca to the tentacles. Major organs and tissues in the body of each specimen were removed for analysis: digestive tract, gonads, and longitudinal muscles. All dissected structures were washed thoroughly with filtered seawater and placed in a labeled glass petri dish. The rinsing removed any of the residual copper that was left on

the surface of the tissue from the dosed aquarium water. After rinsing, the samples were dried in an oven until a constant weight was reached. The tissues of each species were pooled to make one sample for each tank. This method yielded 3 samples from each species per tank for a total of 54 tissue samples.

A modified procedure of Agemian et al. (1980) was followed for tissue and organ digestion. A sample weighing between .05 - 0.5 g dry weight was placed in a 30 ml beaker containing 5 ml of 65% nitric acid and was heated at 60 ^{o}C on a hot plate in the hood for 30 minutes, followed by an additional 5 ml of 65% nitric acid. The temperature was gradually increased to 135-140^{o}C, while the solution was steadily refluxing, and the temperature maintained there for 4-6 hours (depending on the tissue weight) or until the disappearance of brown fume. The brown fume that rose from the sample served as an indicator for completion of the tissue digestion. After the solution was cooled to room temperature, 1 ml of 30% hydrogen peroxide was added to each beaker and the solutions were further digested at 135-140^{o}C until the color of the sample had stabilized. The process of adding hydrogen peroxide and heating the solution was repeated 2-4 times until the solution in the beaker became clear.

Each of the digested animal tissue/organs and sediment samples was then diluted to 25 ml in a volumetric flask. The solution was then filtered through a #25 Wattman filter paper and transferred into holding bottles (Xing and Chia 1997). These solutions were stored at 5^{O}C until all samples were digested and analyzed by standard atomic absorption methods to determine the concentration of copper that had accumulated in the various tissues.

Analysis for Enzyme Solubilization of Copper

Enzyme solubilization analysis was done in collaboration with Zen Chen at the Darling Marine Institute in Walpole, Maine. At the end of the study 3 individuals of each species and samples of wet sediments from each microcosm were sent to Zen for solubilization analysis. The organisms sent were not treated specimens and were shipped out days after receiving them from the supplier. Each of the sediment treatments was pooled to make one representative sample for each treatment. These were packed in plastic bags for shipment and were sent by overnight airmail in styrofoam-insulated boxes containing heat packs to keep the temperature in a tolerable range.

Incubation of gut fluids with polluted sediments and analysis of metals in gut fluids were done by Zen according to Mayer et al. (1996). Briefly, 3 replicates of wet sediments were incubated with gut fluids at a ratio of about 1g wet weight to 2 ml fluid in plastic centrifuge tubes for 240 min at room temperature. Centrifuging the mixture at 8,000g for 30 min stopped the incubation period, and the supernatant fraction was used for measurements of metals. Experiments included gut fluids without sediments, seawater with sediments, and gut fluids with sediments from the cucumber's respective microcosms.

CHAPTER 3

RESULTS

Water Quality of Aquaria

Water quality parameters were measured before and during the experiment (Appendix B). The water quality parameters that were monitored consisted of temperature, salinity, pH, ammonia, nitrite, and nitrate. The average and range of the results are given in Table 1. All of the environmental conditions measured fall within the typical range for sea cucumbers in their normal reef habitat (Table 1). These critical factors showed little variation between aquaria or testing periods.

Table 1. Water Quality Measurements of Test Aquaria

Parameters	Mean ψ	Range ψ	Typical Range*
Salinity (ppt)	33.9	32.0 - 35.0	30 - 36
Temperature (C°)	25.6	24.5 - 26.3	21 - 27
pH	8.28	8.12 - 8.42	8.0 - 8.4
Ammonia (ppm)	0.03	0.0-0.1	0.0 - 0.3
Nitrite (ppm)	0.05	0.0-0.2	0.0 - 0.5
Nitrate (ppm)	7.2	0.0-15.0	0.0 – 20.0

Table 1. ppt = parts per thousand, ppm = parts per million. ψ Values expressed here are derived from water chemistry taken throughout the study, all values may be found in Appendix B.
* Typical range of environmental conditions was taken from Delbeek and Sprung (1995).

Addition to Aquaria, Spawnings, and Mortalities of Sea Cucumbers

Spawnings occurred before and during the study. *Pentacta anceps* was the only species to exhibit spawning behavior, which when observed was a fine spray being released from the oral opening. This event coincided with the addition of freshwater while adjusting the salinity because of evaporation. The first spawning resulted in an

accumulation of organics in the aquaria and the subsequent death of 4 suspension feeders and 5 sediment feeders. The following spawnings resulted in no deaths because the gametes were removed from the aquaria by a micron filter and were not allowed to foul the tank.

Lethality Testing

Copper exposure at 100.0 ppm resulted in the death of all 3 sediment feeding and 3 filter feeding individuals in less than 36 hours. Both species were covered in a thick mucus coat.

Water Concentrations of Copper

All of the treatments were monitored for the water concentration of copper. Samples were analyzed at the beginning on undosed water, during and at the end of the study on dosed water (Table 2). There was not a significant difference found between the initial undosed copper concentrations obtained from 1-way ANOVA, $p = 0.4043$ (Appendix F). After dosing, a significant difference occurred between treatments, obtained by a 1-way ANOVA, $p = 0.0001$), (Appendix G), (Figure 1). The Fisher and Scheffe multiple comparison tests indicated all treatments significantly differed from each other (Appendix F).

Table 2. H_20 Copper Concentrations of Treatment Aquaria

Treatment	C #1	C #2	C #3	L #1	L #2	L #3	H #1	H #2	H #3
10/20/98	4.9	4.5	4.6	4.3	6.2	8.9	7.4	1.7	2.6
11/24/98	4.8	7.6	5.5	12.3	7.3	8.5	33.4	14.0	9.2
12/14/98	7.9	8.2	9.3	13.3	22.5	12.4	30.2	37.1	38.4
1/10/99	6.1	5.2	4.7	12.8	10.8	19.1	29.2	24.4	22.7

Table 2. All values are expressed in ppm; Key: C = Control, L = Low dose and H = High dose test aquaria. Copper concentrations of each aquaria over the study period. Copper dosages for aquaria to keep them at desired levels were calculated from these values. Exact amounts are found in Appendix A.

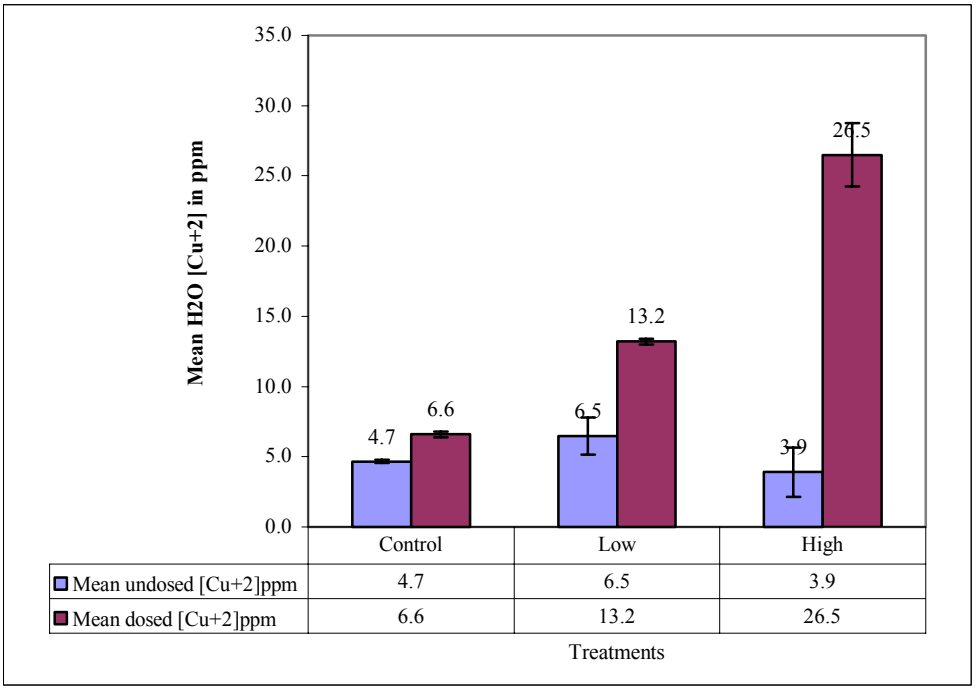

FIGURE 1. H_20 COPPER CONCENTRATIONS OF TREATMENT AQUARIA. Comparison of the mean copper concentrations of water samples. Samples were taken at the beginning on dosed and undosed aquaria and throughout the study on dosed aquaria. Error bars present showing that there is no significant difference present between undosed aquaria, but a significant difference is present between the dosed aquaria.

Sediment Concentrations of Copper

The concentrations of Cu present at the start of the study were not significantly different from each other obtained from 1-way ANOVA, $p = 0.5845$ (Appendix H). The control tanks showed little if no increase in sediment Cu concentrations, while all of the

dosed tanks accumulated some Cu in concentrations that were greater than the starting concentration (Table 3). There was a significant difference found between the treatments that were analyzed at the end of the study, obtained from a one-way ANOVA, $p = 0.0149$ (Appendix I) (Figure 2). The Fisher and Scheffe multiple comparison tests indicated that their was a significantly difference between the control vs. high treatments and the low vs. high treatments (Appendix I).

Table 3. Sediment Copper Concentrations.

Treatment #	Starting [Cu^{+2}]	Ending [Cu^{+2}]
Control #1	4.02	3.57
Control #2	3.37	3.61
Control #3	5.30	3.42
Low #1	5.08	6.81
Low #2	5.34	6.10
Low #3	2.82	4.96
High #1	5.44	16.30
High #2	4.18	9.30
High #3	5.83	9.38

Table 3. Copper concentrations of treatment tanks taken at the beginning of the study and at the end of the study, all values expressed in ppm. Note the increase in copper present at the end of the study in the dosed aquaria.

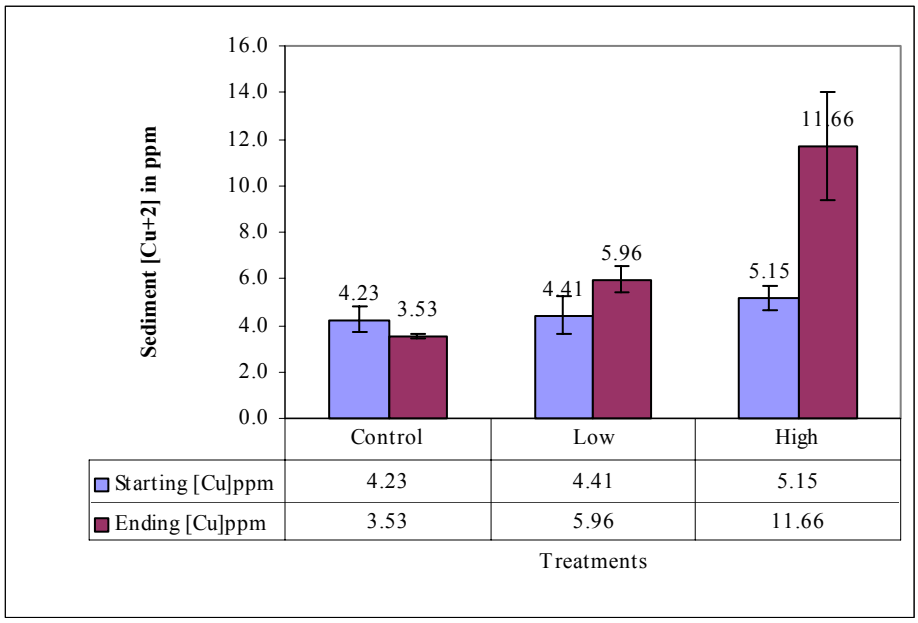

FIGURE 2. MEAN SEDIMENT COPPER CONCENTRATIONS
Copper concentrations of the 3 treatment tanks. Samples taken at the beginning and end of the experiment. All values expressed in ppm. Error bars present showing that there is no significant difference present between undosed aquaria, but a significant difference is present between the dosed aquaria.

Tissue Analysis for Copper

Minitab statistical software package version 13.32 was the software used for all data analysis. The amount of copper detected varied between the species, tissue type, and dosage (Table 4, Table 5). All treatments exhibited a high standard deviation (Table 6). The wide ranges of values are apparent when individual data points were plotted and the medians of each treatment were denoted (Figure 4, Figure 5).

Table 4. Copper Concentration of Body Wall Tissues

Pentacta anceps	[Cu] µg/g	Stichopus chloronotus	[Cu] µg/g
Control tank #1	13.78	Control tank #1	57.51
Control tank #2	12.47	Control tank #2	98.65
Control tank #3	18.95	Control tank #3	26.44
Low tank #1	8.57	Low tank #1	25.19
Low tank #2	6.70	Low tank #2	10.06
Low tank #3	11.67	Low tank #3	10.56
High tank #1	13.56	High tank #1	29.42
High tank #2	8.34	High tank #2	18.97
High tank #3	21.58	High tank #3	52.61

Table 4. Copper concentrations found in the body wall tissues of *Pentacta anceps* and *Stichopus chloronotus.* Expressed in µg/g.

Table 5. Copper Concentration of Digestive Tract Tissues

Pentacta pygmaia	[Cu] µg/g	Stichopus chloronotus	[Cu] µg/g
Control tank #1	22.87	Control tank #1	9.56
Control tank #2	44.07	Control tank #2	27.67
Control tank #3	36.02	Control tank #3	42.29
Low tank #1	21.99	Low tank #1	75.18
Low tank #2	12.67	Low tank #2	10.16
Low tank #3	32.57	Low tank #3	11.67
High tank #1	35.96	High tank #1	90.92
High tank #2	16.82	High tank #2	58.08
High tank #3	42.57	High tank #3	111.34

Table 5. Copper concentrations found in the body wall tissues of *Pentacta anceps* and *Stichopus chloronotus.* Expressed in µg/g.

Table 6. Mean & SD of Tissue Copper Concentrations

	Control means	Control SD	Low dosed means	Low dosed SD	High dosed means	High dosed SD
Stichopus – DT	26.51	13.39	32.34	30.30	86.78	21.94
Pentacta – DT	34.51	8.74	22.41	8.13	31.78	10.92
Stichopus – BW	60.87	29.58	15.27	7.02	33.67	14.06
Pentacta - BW	15.07	2.80	11.67	2.05	21.58	5.45

Table 6. Values expressed in µg/g, DT = Digestive tract. BW = Body Wall. The mean copper concentrations found for each species by treatment are given above. 3 Tissue samples were pooled to make one sample per aquaria. These results were then averaged to yield the above means.

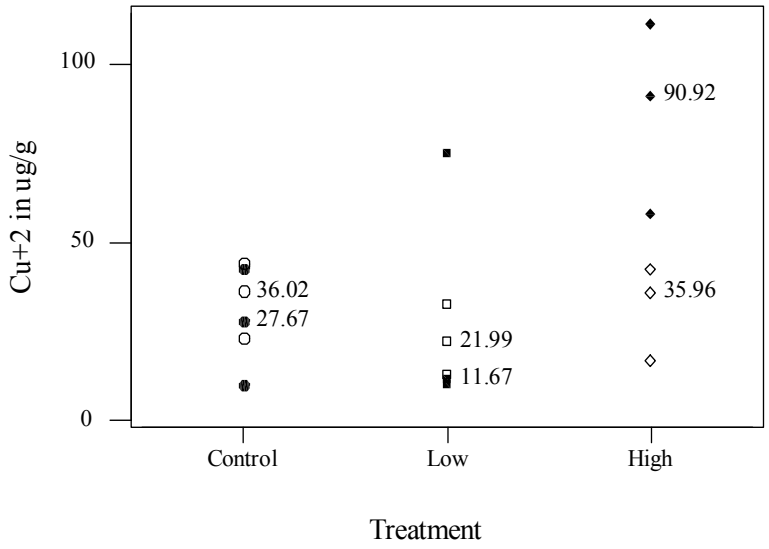

FIGURE 3. DIGESTIVE TRACT TISSUE COPPER CONCENTRATIONS

Values expressed in µg/g. Solid symbols = Stichopus, Open symbols = Pentacta

Medians listed for each species and treatment. Note the wide spread of data pointsand also the skewed data for Stichopus that is present on the high treatment indicating an increased amount of copper accumulated when visually compared to Pentacta.

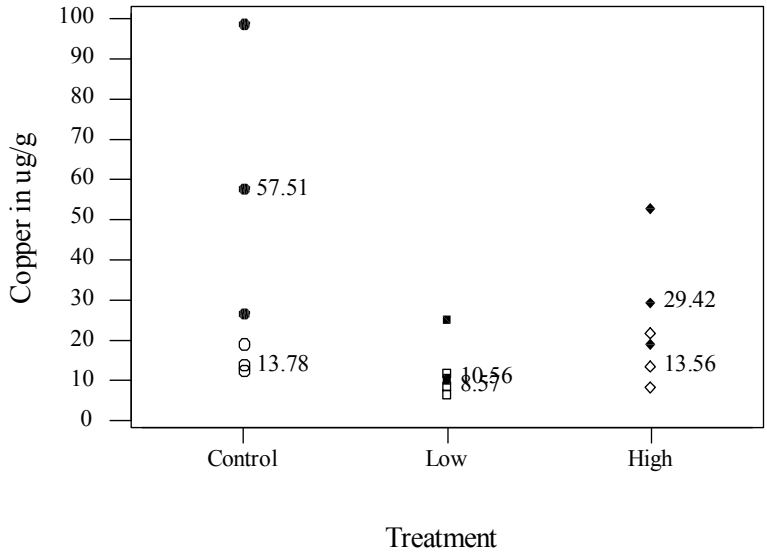

FIGURE 4. BODY WALL TISSUE COPPER CONCENTRATIONS

Values expressed in µg/g. Solid symbols = Stichopus, Open symbols = Pentacta Medians listed for each species and treatment. Note the smaller spread of data points than that of the digestive tract tissue. The same skewed data for Stichopus is present on the high treatment indicating an increased amount of copper accumulated when visually compared to Pentacta.

Tissue concentrations were compared between species and also within species to determine if a statistical difference was present in either. Data obtained from tissue samples were analyzed using the Mann-Whitney nonparametric analysis comparing the median values of each treatment and species (Appendix G). The concentrations of copper present in the body wall tissue samples were not significantly different from each other when compared between species (Table 7). The concentration of copper for body wall tissue within a species was also found to be not significant (Table 8, Table 9).

Table 7. Comparison of Body Wall Tissues Between Species Using The Mann-Whitney Nonparametric Analysis

SPECIES	Pentacta - C	Pentacta – L	Pentacta – H
Stichopus – C	0.08	0.08	0.08
Stichopus - L	0.66	0.38	1.00
Stichopus - H	0.08	0.08	0.19

Table 7. N = 3. C = Control Treatments, L = Low Treatments, H = High Treatments.

Table 8. Comparison of Body Wall Tissues Within Species Using The Mann-Whitney Nonparametric Analysis

SPECIES	Pentacta - C	Pentacta – L
Pentacta – L	0.08	-
Pentacta – H	0.38	0.38

Table 8. N = 3. C = Control Treatments, L = Low Treatments, H = High Treatments.

Table 9. Comparison of Body Wall Tissues Within Species Using The Mann-Whitney Nonparametric Analysis

SPECIES	Stichopus - C	Stichopus – L
Stichopus - L	0.08	-
Stichopus - H	0.38	0.19

Table 9. N = 3. C = Control Treatments, L = Low Treatments, H = High Treatments

Concentrations of copper present in the digestive tract tissue samples were not significantly different from each other when compared between species (Table 10). The concentration of copper for digestive tract tissue within a species was also found to be not significant (Table 11, Table 12).

Table 10. Comparison of Digestive Tract Tissues Between Species Using The Mann-Whitney Nonparametric Analysis

SPECIES	Pentacta - C	Pentacta – L	Pentacta – H
Stichopus – C	0.66	1.00	0.66
Stichopus - L	0.66	0.08	0.66
Stichopus - H	0.08	0.08	0.66

Table 10. N = 3. C = Control Treatments, L = Low Treatments, H = High Treatments.

Table 11. Comparison of Digestive Tract Tissues Within Species Using The Mann-Whitney Nonparametric Analysis

SPECIES	Pentacta - C	Pentacta – L
Pentacta - L	0.19	-
Pentacta - H	0.66	0.38

Table 11. N = 3. C = Control Treatments, L = Low Treatments, H = High Treatments.

Table 12. Comparison of Digestive Tract Tissues Within Species Using The Mann-Whitney Nonparametric Analysis

SPECIES	Stichopus - C	Stichopus – L
Stichopus - L	1.00	-
Stichopus - H	0.08	0.19

Table 12. N = 3. C = Control Treatments, L = Low Treatments, H = High Treatments

There was no apparent dose relationship present in either species (Table 4). Dissection of the test subjects revealed that the gonads of *Stichopus chloronotus* were immature and not fully developed. At least .05 grams of the pooled gonads were needed for analysis. The amount of tissue recovered after drying was less than .01 grams and, therefore, the analysis could not be performed appropriate for that tissue type.

Enzyme Solubilization of Copper

The organisms sent to the Darling Marine Center were found to be placid with no apparent reactions upon arrival. The temperature of the shipping water was 42^{O}F, which is far below the optimum temperature of 70-80^{O}F. The animals could not be revived but an effort was made to retrieve gut fluids.

The amount of gut fluid available was not sufficient to perform the analysis on any of the animals. The sediments were unaffected by the cold temperatures and retained for a later attempt.

CHAPTER 4

CONCLUSIONS

Lethality testing

The sea cucumbers expelled a thick mucus covering and tightly contracted their body walls. Davies (1992) described the production of a thick protective mucus coat by the limpet *Patella vulgata* when exposed to water copper concentrations of 20 ppm. Although the concentration of copper was not lethal to the limpets the results obtained in this study also indicate that the mucus coat expelled by both species of sea cucumber was a protective barrier against the excess copper. The 96-hour LC_{50} value of 70 ppm for adults of *Stichopus japonicus* (Shcheglov et al. 1990) was found to be comparable with the results of this study in which a 36-hour LC_{100} value was found for both species.

Water and Sediment Concentrations of Copper

The results obtained from the analysis of water copper concentrations were as expected. The initial undosed water treatments did not have a significantly different water copper concentration (Appendix F), but a significant difference was detected between the dosed treatments assayed at the end of the study (Appendix G). The concentration of copper in the water continued to fall during the study and had to be replenished accordingly. The copper is presumed to have precipitated out of the water column and settled into the sediment. The substrate for the precipitation of copper is unknown; likely it could have been any number of the organics present in the systems or one of the salts in the water. This could be a possible area for further research. The presence or absence of certain organics in the field could affect the rate at which the free

copper becomes bound and less toxic.

The analysis of the sediment showed that it was the place in which the copper was accumulating. There was significant increase in copper concentrations in the high treatments from that of the control and the low dosed treatments (Appendix I). This suggests that the system used for this study adequately demonstrated the precipitation of copper into the sediment and, therefore, was an appropriate model for the uptake of copper by sediment feeding organisms.

Enzyme Solubilization of Copper

No data were gathered due to the death of the test subjects. The animals were no longer available at the time of this study and a replacement set could not be sent. The study was designed to be correlated to the solubilization results. Hopes were that the results showed that Stichopus because of its sediment feeding nature had a more robust stomach enzyme mechanism than that of Pentacta. This digestive action would be used to strip sediment of organic nutrients while at the same time releasing bound copper to a free state. It would be assumed that Pentacta also possesses similar digestive enzymes but because it is only needed to digest food and not sediment, then it would not release as much free copper as Stichopus.

Tissues Bioaccumulation of Copper

Copper analysis was expected to show an increase in concentrations present in individuals as dosed levels increased from 0.00 ppm to 40.0 ppm. The wide range of values found in each data set did not permit for a comparison of means. The Man-Whitney nonparametric test of medians did not find any statistical differences present

between treatments or species. Visually there was a trend that was apparent. The high treatments of that of Stichopus chloronotus were noticeably higher that that of Pentacta anceps in both digestive tract and body wall tissues. Statistically there was no difference.

The body wall tissue copper concentration found in the control treatment of Stichopus was considerably higher than all the other groups (Figure 4). This was not the result that was anticipated and did not correlate with the results obtained from the digestive tissue. The cause of such an increase was not found and could not be explained. A likely cause may have been analyst error or preparation error. If this data set was excluded, the remaining data do visually posses a slight dose dependent curve with the highest concentrations found in the high treatments (Figure 4). This curve is also visually apparent in the digestive tract tissue data (Figure 3).

Although the differences between the species are visually apparent the data sets had high standard deviations present (Table 4). This may be due to the way in which the samples were pooled. Each treatment contained 9 individuals per species. These 9 were pooled into 3 samples, 1 sample for each tank. This was done because of the high cost of tissue prepping and analysis. This high deviation is the probable cause for the lack of statistical evidence. Individual assayed data may have statistically shown a more consistent data set and, therefore, yielded better results.

Further studies could use the model developed in this study to deliver a bound form of copper to sediment feeding organisms. The form of copper present in the sediment would be an appropriate extension of this work focusing on how the copper was absorbed onto the sediment itself or precipitated out of the water column into a detritus type material.

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APPENDICES

APPENDIX A

$CuSO_4$ USED TO REACH THE DESIRED CU CONCENTRATIONS

Treatment	Cu desired (ppm)	$CuSO_4$ added Time 1	Cu, 35 days after addition	$CuSO_4$ added Time 2	Cu, 20 days after 2nd addition	$CuSO_4$ added Time 3	Cu 27 days after 3rd addition
Control #1	0.0	0.0	4.8	0.0	7.9	0.0	6.1
Control #2	0.0	0.0	7.6	0.0	8.2	0.0	5.2
Control #3	0.0	0.0	5.5	0.0	9.3	0.0	4.7
Low #1	10.0	1.53 g	12.3	0.0	13.3	0.0	12.8
Low #2	10.0	1.53 g	7.3	0.41 g	22.5	0.0	10.8
Low #3	10.0	1.53 g	8.5	0.23 g	12.4	0.0	19.1
High #1	40.0	6.12 g	33.4	1.01 g	30.2	1.49 g	29.2
High #2	40.0	6.12 g	14.0	3.98 g	37.1	0.44 g	24.4
High #3	40.0	6.12 g	9.2	4.7 g	38.4	0.24 g	22.7

APPENDIX B

WATER QUALITY DATA OF STUDY AQUARIA

Water quality data for 10/29/98

Treatment	C #1	C #2	C #3	L #1	L #2	L #3	H #1	H #2	H #3
Salinity (ppt)	32	34	35	34	33	34	35	35	34
Temp. (^{0}C)	26.3	25.9	25.6	26.0	26.0	26.2	25.7	24.5	25.7
pH	8.2	8.3	8.3	8.3	8.2	8.2	8.3	8.12	8.3

Water quality of all treatments before and during the study. C = Control, L = Low dose and H = High dose.

Water quality data for 11/13/98

Treatment	C #1	C #2	C #3	L #1	L #2	L #3	H #1	H #2	H #3
Salinity (ppt)	34	34	34	34	34	34	34	34	34
Temp. (^{0}C)	25.7	25.0	25.6	25.6	25.6	25.6	25.7	25.4	25.6
pH	8.18	8.25	8.25	8.31	8.23	8.34	8.4	8.21	8.34

Water quality of all treatments before and during the study. C = Control, L = Low dose and H = High dose.

Water quality data for 12/13/98

Treatment	C #1	C #2	C #3	L #1	L #2	L #3	H #1	H #2	H #3
Salinity (ppt)	34	34	34	34	34	34	33	33	34
Temp. (^{0}C)	26.3	25.9	26.1	26.1	25.6	25.9	26.0	25.4	25.0
pH	8.36	8.31	8.38	8.27	8.38	8.29	8.42	8.40	8.36

Water quality of all treatments before and during the study. C = Control, L = Low dose and H = High dose.

Water quality data for 1/05/99

Treatment	C #1	C #2	C #3	L #1	L #2	L #3	H #1	H #2	H #3
Salinity (ppt)	34	34	34	34	34	34	34	34	33
Temp. (^{0}C)	25.7	25.0	25.6	25.6	25.6	25.6	25.7	25.4	25.6
pH	8.25	8.19	8.31	8.20	8.32	8.20	8.23	8.32	8.17

Water quality of all treatments before and during the study. C = Control, L = Low dose and H = High dose.

Water quality data for 11/13/98

Treatment	C #1	C #2	C #3	L #1	L #2	L #3	H #1	H #2	H #3
Ammonia (ppm)	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0
Nitrite (ppm)	0.2	0.0	0.2	0.2	0.0	0.0	0.2	0.0	0.0
Nitrate (ppm)	<5.0	<5.0	10.0	<5.0	<5.0	<5.0	10.0	<5.0	10.0

Water quality of all treatments before and during the study. C = Control, L = Low dose and H = High dose.

Water quality data for 12/20/99

Treatment	C #1	C #2	C #3	L #1	L #2	L #3	H #1	H #2	H #3
Ammonia (ppm)	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0
Nitrite (ppm)	0.2	0.0	0.2	0.2	0.0	0.0	0.2	0.0	0.0
Nitrate (ppm)	<5.0	<5.0	10.0	<5.0	<5.0	<5.0	10.0	<5.0	10.0

Water quality of all treatments before and during the study. C = Control, L = Low dose and H = High dose.

Water quality data for 1/05/99

Treatment	C #1	C #2	C #3	L #1	L #2	L #3	H #1	H #2	H #3
Ammonia (ppm)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrite (ppm)	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Nitrate (ppm)	15.0	5.0	15.0	10.0	10.0	10.0	15.0	10.0	10.0

Water quality of all treatments before and during the study. C = Control, L = Low dose and H = High dose.

APPENDIX C

ONE WAY ANOVA FOR WATER COPPER CONCENTRATIONS

Source	DF	Sum of Squares	Mean Square	F-Test
Between Groups	2	10.416	5.208	1.057
Within Groups	6	29.553	4.926	p=.4043
Total	8	39.969		

Group	Count	Mean	Std. Deviation	Std. Error
Control	3	4.667	.208	.12
Low	3	6.467	2.312	1.335
High	3	3.9	3.064	1.769

COMPARISON	DIFF. OF MEANS	FISHER PLSD	SCHEFFE F-TESTS:	DUNNETT T:
Control vs. Low	-1.8	4.435	.493	.993
Control vs. High	.767	4.435	.089	.423
Low vs. High	2.567	4.435	1.003	1.416

APPENDIX D

ONE WAY ANOVA FOR DOSED WATER COPPER CONCENTRATIONS

Source	DF	Sum of Squares	Mean Square	F-Test
Between Groups	2	616.46	308.23	59.313
Within Groups	6	31.18	5.197	p=.0001
Total	8	647.64		

Group	Count	Mean	Std. Deviation	Std. Error
Control	3	6.6	.361	.208
Low	3	13.2	.361	.208
High	3	26.5	3.915	2.261

COMPARISON	DIFF. OF MEANS	FISHER PLSD	SCHEFFE F-TESTS:	DUNNETT T:
Control vs. Low	-6.6	4.555*	6.287*	3.546
Control vs. High	-19.9	4.555*	57.153*	10.691
Low vs. High	-13.3	4.555*	25.529*	7.146

APPENDIX E

ONE WAY ANOVA FOR STARTING SEDIMENT COPPER CONCENTRATIONS

Source	DF	Sum of Squares	Mean Square	F-Test
Between Groups	2	1.423	.711	.588
Within Groups	6	7.258	1.21	p=.5845
Total	8	8.681		

Group	Count	Mean	Std. Deviation	Std. Error
Control	3	.423	.982	.567
Low	3	4.413	1.386	.8
High	3	5.15	.862	.498

COMPARISON	DIFF. OF MEANS	FISHER PLSD	SCHEFFE F-TESTS:	DUNNETT T:
Control vs. Low	-.183	2.198	.021	.204
Control vs. High	-.92	2.198	.525	1.024
Low vs. High	-.737	2.198	.336	.82

APPENDIX F

ONE WAY ANOVA FOR ENDING SEDIMENT COPPER CONCENTRATIONS

Source	DF	Sum of Squares	Mean Square	F-Test
Between Groups	2	104.443	52.222	9.199
Within Groups	6	34.06	5.677	p=.0149
Total	8	138.503		

Group	Count	Mean	Std. Deviation	Std. Error
Control	3	3.533	.1	.058
Low	3	5.957	.933	.539
High	3	1.66	4.019	2.32

COMPARISON	DIFFERENCE OF MEANS	FISHER PLSD	SCHEFFE F-TESTS:	DUNNETT T:
Control vs. Low	-2.423	4.761	.776	1.246
Control vs. High	-8.127	4.761*	8.726*	4.177
Low vs. High	-5.703	4.761*	4.298	2.932

APPENDIX G

MEDIAN COMPARISON USING THE
MANN-WHITNEY NONPARAMETRIC TEST

Mann-Whitney Test and CI:
Body Wall - Control Treatment
Pentacta anceps vs. Stichopus chloronotus

Pentacta N = 3 Median = 13.78
Stichopus N = 3 Median = 57.51
Point estimate for ETA1-ETA2 is -43.73
91.9 Percent CI for ETA1-ETA2 is (-86.16,-7.51)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI:
Body Wall - Low Treatment
Pentacta anceps vs. Stichopus chloronotus

Pentacta N = 3 Median = 8.57
Stichopus N = 3 Median = 10.56
Point estimate for ETA1-ETA2 is -3.36
91.9 Percent CI for ETA1-ETA2 is (-18.49,1.62)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.3827
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI:
Body Wall - High Treatment
Pentacta anceps vs. Stichopus chloronotus

Pentacta N = 3 Median = 13.56
Stichopus N = 3 Median = 29.42
Point estimate for ETA1-ETA2 is -15.86
91.9 Percent CI for ETA1-ETA2 is (-44.26,2.61)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.1904
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps control vs. Pentacta anceps low

Pentacta control N = 3 Median = 13.780
Pentacta low N = 3 Median = 8.570
Point estimate for ETA1-ETA2 is 5.770
91.9 Percent CI for ETA1-ETA2 is (0.800,12.248)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps control vs. Pentacta anceps high

Pentacta control N = 3 Median = 13.78
Pentacta high N = 3 Median = 13.56
Point estimate for ETA1-ETA2 is 0.22
91.9 Percent CI for ETA1-ETA2 is (-9.11,10.61)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 1.0000
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps low vs. Pentacta anceps high

Pentacta low N = 3 Median = 8.57
Pentacta high N = 3 Median = 13.56
Point estimate for ETA1-ETA2 is -4.99
91.9 Percent CI for ETA1-ETA2 is (-14.88,3.33)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.3827
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Stichopus chloronotos control vs. Stichopus chloronotus low

Stichopus control N = 3 Median = 57.51
Stichopus low N = 3 Median = 10.56
Point estimate for ETA1-ETA2 is 46.95
91.9 Percent CI for ETA1-ETA2 is (1.26,88.57)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Stichopus chloronotos control vs. Stichopus chloronotus high

Stichopus control N = 3 Median = 57.51
Stichopus high N = 3 Median = 29.42
Point estimate for ETA1-ETA2 is 28.09
91.9 Percent CI for ETA1-ETA2 is (-26.18,79.68)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.3827
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Stichopus chloronotus low vs. Stichopus chloronotus high

Stichopus low N = 3 Median = 10.56
Stichopus high N = 3 Median = 29.42
Point estimate for ETA1-ETA2 is -18.86
91.9 Percent CI for ETA1-ETA2 is (-42.53,6.22)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.1904
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI:
Digestive Tract Tissue - Control Treatments
Pentacta anceps vs. Stichopus chloronotus

Pentacta control N = 3 Median = 36.02
Stichopus control N = 3 Median = 27.67
Point estimate for ETA1-ETA2 is 8.35
91.9 Percent CI for ETA1-ETA2 is (-19.43,34.51)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.6625
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI:
Digestive Tract Tissue - Low Treatments
Pentacta anceps vs. Stichopus chloronotus

Pentacta low N = 3 Median = 35.96
Stichopus low N = 3 Median = 90.92
Point estimate for ETA1-ETA2 is -54.96
91.9 Percent CI for ETA1-ETA2 is (-94.51,-15.52)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI:
Digestive Tract Tissue - High Treatments
Pentacta anceps vs. Stichopus chloronotus

Pentacta high N = 3 Median = 21.99
Stichopus high N = 3 Median = 11.67
Point estimate for ETA1-ETA2 is 2.51
91.9 Percent CI for ETA1-ETA2 is (-62.52,22.42)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.6625
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Digestive Tract Tissue
Pentacta anceps control treatment vs. Pentacta anceps low treatment

Pentacta control N = 3 Median = 36.02
Pentacta low N = 3 Median = 21.99
Point estimate for ETA1-ETA2 is 11.50
91.9 Percent CI for ETA1-ETA2 is (-9.70,31.40)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.1904
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Digestive Tract Tissue
Pentacta anceps control treatment vs. Pentacta anceps high treatment

Pentacta control N = 3 Median = 36.02
Pentacta high N = 3 Median = 35.96
Point estimate for ETA1-ETA2 is 1.50
91.9 Percent CI for ETA1-ETA2 is (-19.70,27.25)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.6625
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Digestive Tract Tissue
Pentacta anceps low treatment vs. Pentacta anceps high treatment

Pentacta low N = 3 Median = 21.99
Pentacta high N = 3 Median = 35.96
Point estimate for ETA1-ETA2 is -10.00
91.9 Percent CI for ETA1-ETA2 is (-29.90,15.74)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.3827
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Digestive Tract Tissue
Stichopus chloronotus control treatment vs. Stichopus chloronotus low treatment

Stichopus control N = 3 Median = 27.67
Stichopus low N = 3 Median = 11.67
Point estimate for ETA1-ETA2 is -0.60
91.9 Percent CI for ETA1-ETA2 is (-65.64,32.14)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 1.0000
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Digestive Tract Tissue
Stichopus chloronotus control treatment vs. Stichopus chloronotus high treatment

Stichopus control N = 3 Median = 27.67
Stichopus high N = 3 Median = 90.92
Point estimate for ETA1-ETA2 is -63.25
91.9 Percent CI for ETA1-ETA2 is (-101.78,-15.77)
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Digestive Tract Tissue
Stichopus chloronotus low treatment vs. Stichopus chloronotus high treatment

Stichopus low N = 3 Median = 11.67
Stichopus high N = 3 Median = 90.92
Point estimate for ETA1-ETA2 is -47.92
91.9 Percent CI for ETA1-ETA2 is (-101.20,17.09)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.1904
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps Low vs. Stichopus chloronotus Control

Pentacta N = 3 Median = 8.57
Stichopus N = 3 Median = 57.51
Point estimate for ETA1-ETA2 is -48.94
91.9 Percent CI for ETA1-ETA2 is (-91.94,-14.79)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps Low vs. Stichopus chloronotus Control

Pentacta N = 3 Median = 13.56
Stichopus N = 3 Median = 57.51
Point estimate for ETA1-ETA2 is -43.95
91.9 Percent CI for ETA1-ETA2 is (-90.32,-4.86)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps Control vs. Stichopus chloronotus Low

Copper i N = 3 Median = 13.78
Stichopu N = 3 Median = 10.56
Point estimate for ETA1-ETA2 is 2.41
91.9 Percent CI for ETA1-ETA2 is (-12.72,8.88)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.6625
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps Control vs. Stichopus chloronotus High

Copper i N = 3 Median = 13.78
Stichopu N = 3 Median = 29.42
Point estimate for ETA1-ETA2 is -15.64
91.9 Percent CI for ETA1-ETA2 is (-40.14,-0.02)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps High vs. Stichopus chloronotus Low

Pentacta N = 3 Median = 13.56
Stichopu N = 3 Median = 10.56
Point estimate for ETA1-ETA2 is -1.72
91.9 Percent CI for ETA1-ETA2 is (-16.85,11.52)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 1.0000
Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: Body Wall
Pentacta anceps High vs. Stichopus chloronotus Low

Pentacta N = 3 Median = 8.57
Stichopu N = 3 Median = 29.42
Point estimate for ETA1-ETA2 is -20.85
91.9 Percent CI for ETA1-ETA2 is (-45.92,-7.30)
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is not significant at 0.0809
Cannot reject at alpha = 0.05

VITA

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