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Effect of Chromium VI on the Production and Behavior of *Lytechinus variegatus*

(Echinodermata: Echiniodea)

by

Jennifer Rhora

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science
Department of Biology
College of Arts and Sciences
University of South Florida

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Keywords: Sea grass, Algae, Pollution, Heavy Metals, Toxicology

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Dedication

I would like to dedicate this thesis in honor of Lotte Geller who initially inspired me to pursue a career in the sciences. Without her encouragement and example I would not have chosen this path. Thank you Lotte. I would also like to honor my parents Carol and Gerald Rhora for their steadfast belief in me and their encouragement in pursuing my dreams. Lastly I would like to honor my professor Dr. John Lawrence and my committee Dr. James Garey and Dr. Gordon Fox for being so understanding and helpful throughout this entire endeavor.

Table of Contents

List of Tables

List of Figures

Effect of Chromium VI on the Production and Behavior of *Lytechinus variegatus* (Echinodermata: Echinoidea)

Jennifer Rhora

ABSTRACT

Small amounts of chromium (VI) are carcinogenic in mammals. Concentrations of Cr in marine algae and seagrasses range from 0.06-7.17 µg/g DW and 0.1-30.6 µg/g DW respectively. To test for an effect of these concentrations, production (change in organic material), righting response, feeding rates, absorption efficiency and fecal production were measured in *Lytechinus variegatus* from Sarasota fed prepared diets containing 0, 4.1, and 32µg Cr/ g DW and individuals from Ft. DeSoto fed diets containing 0, 41 and 82µg Cr/ g DW. The urchins were fed for 4-5 weeks, with weekly measurements of their feeding rates, absorption efficiency and fecal production. At the end of the experiment the urchins were righted to note any changes in behavior. Their gonads, gut, lantern and test with spines were weighed and ashed to calculate gonadal and gut indices and inorganic and organic percentage and content. After five weeks individuals in all treatments from experiment one showed no significant results. Urchins in all treatments from experiment two showed a significant decrease Individuals in all treatments had a significant increase in wet ($P<0.001$) and dry ($P=0.005$) weights as well as total organic material ($P<0.001$) in the gut of the urchins recieveing 82µg Cr/ g DW. There was significant decrease in the feeding rate ($P<0.001$) and absorption efficiency ($P<0.001$), countered by a significant increase in fecal production. The righting times were significantly different between the 0µg Cr/ g dry weight, 82µg Cr/ g DW and initial ($P=0.031$), but not the 41µg Cr/ g DW. Chromium in the feed at the concentrations used in this experiment does not affect the production or absorption efficiency of *Lytechinus variegatus*, but it does affect feeding rates, fecal production and righting response.

INTRODUCTION

Pollution in the ocean is contamination with man-made waste at levels that cause measurable and deleterious effects on the marine biota (Kennish, 1998). Pollutants include heavy metals, such as cadmium, chromium, lead, and mercury. These heavy metals are in the water column and sediment (Temara *et al.* 1996; Warnau *et al.,* 1999; Gounin *et al.,* 1995). Most information about the effect of heavy metals on aquatic organisms concerns metals in solution. For example, exposure of the sea urchin *Anthocidaris crassispina* to cadmium results in reduction of gamete quality (Au et al. 2001). Skeletogenesis is reduced in the starfish *Asterias rubens* exposed to lead (Temara et al. 1997). Heavy metals also accumulate in organisms (Table 1) where they enter the trophic chain and affect consumers (Temara *et al.,* 1996; Pelletier and Larocque, 1987; Sadiq *et al.,* 1996). *Asterias rubens* takes up cadmium (Temara et al. 1996) and lead (Boisson et al. 2002) and the starfish *Leptasterias polaris* (Békri and Pelletier, 2004) takes up tributyltin from contaminated mussels. Tributyltin is transferred from macroalgae to the sea urchin *Strongylocentrotus droebachiensis* (Mamelona and Pelletier 2003). None of these studies have assessed the effect on the consumer.

Grabe (1997) measured the concentrations of eight heavy metals in the sediment in Tampa Bay because "… they have been associated with reductions in the numbers of species as well as numbers of animals, or, alternatively, with the proliferation of 'pollution tolerant' animals". He found most of the metals were only of marginal concentration (Grabe, 1997), meaning that there is a low probability these metals are toxic to aquatic life. These are arsenic, cadmium, copper, lead, silver and zinc. Chromium and nickel are at levels that have a higher probability than the other heavy metals of being toxic to aquatic life (Grabe, 1997). Toxic effects of the metals at the concentrations observed have not been demonstrated.

Table 1:

A comparison of the heavy metal concentrations found in the sea grass, algae and echinoids.

Organism	Section	Cr concentrations	Source
Algae			
Cystseria barbata		<0.06-7.76 + 0.55 ug/g dry wt.	Topcuoglu *et al.* 2002
Ulva lactuca		<0.06 ug/g dry wt.	
Ulva lactuca		0.33 + 0.02- 1.56 + 0.08 ug/g dry wt.	Muse *et al.* 1999
Enteromorpha prolifera		3.05 + 0.08- 4.60 + 0.08 ug/g dry wt.	
Porphyra columbia		0.30 + 0.18- 0.49 + 0.12 ug/g dry wt.	
Padina pavonica		2.20 + 0.40 - 3.55 + 0.05 ug/g dry wt.	Campanella *et al.* 2001
Padina durvillaei		2.55-4.63 ug/g dry wt.	Sanchez-Rodriguez *et al.* 2001
Codium cuneatum		0.99- 2.44 ug/g dry wt.	
Sargassum sinicola		2.63- 36.2 ug/g dry wt.	
Gracilaria pachidermatica		7.17 ug/g dry wt.	
Hypnea pannosa		5.25 ug/g dry wt.	
Laurencia johnstonii		2.19 ug/g dry wt.	
Laurencia papillosa		3.02 ug/g dry wt.	
Fucus vesiculosus		0.8 + 0.1- 5.0 + 0.6 ug/g dry wt.	Giusti, 2001
Fucus vesiculosus		0.17- 123 ug/g dry wt.	Rigit *et al.* 1997.
Ascophyllum nodosum		0.6 ug/g dry wt.	
Enteromorpha spp.		1.45 + 0.3999- 3.00 + 2.40 ug/g dry wt.	Villares *et al.* 2002
Enteromorpha linza		3.73 + 0.641 ug/g dry wt.	Haritonidis and Malea 1995
Ulva rigida		2.60 + 0.536 ug/g dry wt.	

Cystoseira barbata		0.60 + 0.02- 0.95 + 0.05	Topcuoglu *et al.* 2001
Pterocladia capillacea		1.05 + 0.08- 1.15 + 0.01	
Phyllophora nervosa		0.90 + 0.07- 1.20 + 0.03	
Corallina granifera		1.05 + 0.08- 5.50 + 0.02	
Ceramium rubrum		1.45 + 0.08	
Ulva lactuca		0.50 + 0.03	
Seagrass			
Zostera capricorni	Leaf	5.0- 30.6 ug/g dry wt.	Prange and Dennison 2000
	Root-Rhizome	4.7- 29.7 ug/g dry wt.	
Posidonia oceanica	Rhizome	0.91 + 0.03- 1.38 + 0.02 ug/g dry wt.	Campanella *et al.* 2001
	Leaf tip	0.61 + 0.02- 1.51 + 0.18 ug/g dry wt.	
	Leaf	0.31 + 0.11- 0.94 + 0.08 ug/g dry wt.	
	Leaf basal	0.10 + 0.01- 0.36 + 0.05 ug/g dry wt.	
	Leaf- epiphyte complexes	0.96 + 0.64- 1.67+ 1.68 ug/g dry wt.	Warnau *et al.* 1995
	Rhizomes	1.96 + 1.24- 3.27+ 2.48 ug/g dry wt.	
	Roots	1.52 + 0.89- 1.97+ 1.25 ug/g dry wt.	
Echinoid			
Paracentrotus lividus	Digestive wall	0.86 + 0.17- 1.23 + 0.75 ug/g dry wt.	Warnau et al. 1995
	Gonads	0.88 + 0.34- 1.59 + 0.92 ug/g dry wt.	
	Body wall	0.73 + 0.64- 0.89 + 0.77 ug/g dry wt.	
	Digestive wall	0.78 + 0.12- 1.74 + 1.54	Warnau *et al.* 1998
	Gonads	0.67 + 0.20- 2.16 + 1.13	
	Body wall	0.24 + 0.05- 2.05 + 0.15	
	Skeleton	0.03 + 0.04- 1.35 + 0.19	
	Aristotle's lantern	0.09 + 0.05- 1.33 + 0.09	
Echinometra mathaei	Gonad	nd-4.73 mg/kg wet wt.	Sadiq *et al*., 1996
	Intestine	.26-2.36 mg/kg wet wt.	
	Aristotles' lantern	nd-.19 mg/kg wet wt.	
	Spine	nd-.94 mg/kg wet wt.	
	Test	nd-.44 mg/kg wet wt.	

There are two main species of chromium, chromium III (Cr(III)) and chromium VI (Cr(VI)). Cr(III) is the more stable of the two species and as a micronutrient in mammals aids in the metabolism of glucose, cholesterol, lipids, and insulin (Burrows, 1983; Barceloux, 1999; Kotaś and Stasicka, 2000). It occurs naturally from the weathering of chromite as well as run-off from tanneries and is found in pigments such as chrome yellow (Cohen *et al.,* 1993; Kotaś and Stasicka, 2000; Barceloux, 1999). Cr(VI) is a strong oxidizer, which has been shown to damage DNA and to be carcinogenic in mammals. (Bagchi *et al.*, 2001) It passes readily through cell membranes whereas Cr(III) does not. Cr(VI) is reduced to Cr(III), which generates free radicals, theorized to be the cause of the toxicity of Cr(VI). This process can be reversed by manganese oxide causing Cr(III) to oxidize into Cr(VI) (Kotas and Stasicka, 2000). These two species occur in equilibrium in the water column (Schroeder and Lee, 1975). Cr(VI) is introduced to the environment via oxidation of Cr(III) and anthropogenically discharged as liquid, solid and gaseous waste from a variety of industries. Refractory industries use it as a catalyst to form bricks; metallurgical industries use it to harden steel, manufacture stainless steel and other alloys; textile industries use it in mordants and pigments; aircraft industries use it to anodize aluminum and chemical laboratories use it as a catalyst for quantitative analyses. It is also used to create green glass (Barceloux, 1999, Bagchi *et al.,* 2001; Cohen *et al.*, 1993).

Heavy metals have deleterious effects on aquatic life. Some of these occur at the molecular level, which can have physiological and behavioral consequences. Cadmium and PCBs accumulate in the gonads and cause embryological abnormalities in the sea star *Asterias rubens* (den Besten *et al.*, 1989). Sublethal levels of nickel in the lake whitefish *Coregonus clupeaformis* and lake trout *Salvelinus namaycush* affect the blood glucose and electrolyte concentrations. Lake trout fed high dose diets of nickel in the laboratory lost a considerable amount of weight (Ptashynski *et al.*, 2001).

Dallinger and Rainbow (1992) stated, "…trace metal uptake via food has been largely ignored". Research has focused on whether metals in the water column accumulate in organisms or what effect these metals have on the embryonic stages, not on what effect the metals accumulated in food have on the organisms which ingest them. Sea urchins accumulate the majority of ingested heavy metals in their gut and gonads (Sadiq *et al.,* 1996).

Lead accumulation in the skeleton of the sea star *Asterias rubens* disrupts the growth and regeneration of the skeleton (Temara *et al.,* 1997). Mercury accumulation in the sea urchin *Strongylocentrotus intermedius* causes embryonic and gametogenic abnormalities that result in a marked decrease in viable embryos (Vashchenko *et al.*, 1995). Accumulation of cadmium in the blue mussel, *Mytilis edulis,* and the soft-shelled clam, *Mya arenaria,* causes a slower filtration rate and slower, more erratic movement of the gills (Capuzzo *et al.,* 1977). Heavy metals accumulated by the limpet *Crepidula fornicata* are trophically transferred to their predator, *Asterias rubens* (Temara *et al.,* 1997). Duquesne and Riddle (2001) showed that lead is trophically transferred from the bivalve, *Laternula elliptica* to its predator, the sea star *Notasterias armata*. No studies have investigated the sublethal effects of heavy metals in food on the behavior or production of the sea urchin *Lytechinus variegatus.*

Sea urchins are important in maintaining system integrity (Vadas *et al.,* 1992; Vadas and Steneck, 1995; Edmunds and Carpenter, 2001). If, for example, the algae on coral reefs are allowed to grow unchecked the corals die and an algal reef is formed. This is the case in the Florida Keys where a massive die off of the sea urchin *Diadema antillarum* has resulted in an overgrowth of algae (Williams *et al.,* 2001; Lessios *et al.,* 2001).

Lytechinus variegatus is a major herbivore, detritivore and food source where it occurs (Valentine *et al.,* 2000*;* Vadas and Elner, 2000; Ruitton *et al.,* 2000). *Lytechinus variegatus* is common in the Gulf of Mexico and from South Carolina to Brazil and Bermuda on rocky outcroppings, sandy bottom and seagrass beds (Serafy, 1979) It ranges in depth from 0-250m, but is mostly found

at depths of 50m and less (Hendler *et al.,* 1995) *Lytechinus variegatus* feeds mostly on drift algae and seagrasses, but also encrusting algae on rocks (Maciá, 2000; Rose *et al.,* 1999; Valentine *et al.,* 2000). Many types of fish, shore birds, helmet shells and crabs eat *L. variegatus* (Hendler, *et al.*, 1995), which in turn are themselves predated upon by other predators, including sharks, whales and humans. Humans also eat sea urchin gonads, including those of *L. variegatus* (Lawrence, 2001). In some cultures they are a common food source, whereas in others they are considered a delicacy (Lawrence, 2001). The gonads are a long-term storage organ for sea urchins (Lares and Pomory, 1998) and accumulate metals and other toxins (Sadiq *et al.,* 1996; Warnau *et al.,* 1995, 1998). By feeding on sea urchin gonads the chromium and other bioaccumulating pollutants are transferred up the food chain.

I hypothesize that chromium at concentrations reported to occur in the natural food will have deleterious effects on the behavior and production of *Lytechinus variegatus*. Behavior and production have been used to indicate whether a sea urchin is under stress (Lawrence, 1990; Böttger *et al.,* 2001).

MATERIALS AND METHODS

Experiment 1

Eighty-three sea urchins were collected at Lido Key, FL on April 26, 2003. The urchins were kept without feeding in 8 aquaria with re-circulating filters at a salinity of 35 ± 2 ppt and temperature of 22°C for a week before beginning the experiment. Salinity and temperature were kept constant throughout the experiment.

A total of forty-eight urchins were used with sixteen individuals per treatment. Six urchins were placed into each of eight aquaria, each in a 1028 mL plastic container suspended on a plastic grating in the tank with water flow provided by a tube extending from the filter at a flow rate of 102 ± 2 mL/min. Air was bubbled into the aquaria through a suspended air stone. These containers were cleaned and the water changed at each feeding, resulting in one-quarter water change three times a week. The concentration of ammonia and nitrate was measured by the methods given in Strickland and Parsons (1968) for the first three weeks. The concentrations were consistently less than 0.3μmol for ammonia and less than 0.5μmol for nitrate during this period.

Two urchins in each aquarium were fed feed with a chromium concentration of 0, 4.1 or 32 ug Cr/g feed (4.1 ug Cr/ g dry weight being approximately the maximum observed concentration of chromium in both algae and seagrass). The feed was prepared from 5% formulated meal and 4% agar in seawater. The feeds containing 4.1 and 32 ug Cr/g dry weight were made using 1% and 8% respectively of a stock solution of potassium dichromate (0.05 mg Cr/mL). 0.05 g potassium dichromate was weighed on an OHAUS balance with readability of 0.01 g and an accuracy of ± 0.01 g. This was then added to 1 L of DI water. This solution was serial diluted from 0.05 g Cr/mL to 0.05 mg Cr/ mL before being added to the feed. A coin was tossed to randomly select which level of chromium each urchin was fed. The urchins were fed 6-7 g feed every other

day. Uneaten food and feces were removed before the urchins were fed and collected and measured once a week.

At the beginning of the experiment and after five weeks, the righting time of the urchins was measured to ascertain the well-being of the sea urchins (Bőttger *et al.,* 2001) The urchins were placed on their aboral surface in a clean glass aquarium and the time it took them to half right themselves was measured. The times were converted into seconds and the righting coefficient was calculated by dividing 1000 by righting time (Percy, 1973).

At the end of the experiment each sea urchin was weighed, its diameter measured and dissected into the gut, gonads, Aristotle's lantern, and test and spines. These were weighed, dried at 60°C for 48 hours and then reweighed. Approximately 50 mg of each dried component was combusted at 400°C for 5 hours. The amount of organic matter was calculated by subtracting the weight of the ash from the weight of the dry component (Paine, 1971). The percent organic matter was calculated as (mg organic matter/mg dry component)(100). The total amount of organic matter in each body component was calculated by multiplying the percent organic matter in the body component by the dry weight of the body component: (percent organic matter)(dry weight of body component. The gonadal, gut and lantern indices were calculated as [(dry weight of body component/ diameter of urchin) $*$ 100]. The initial values for the wet and dry weights, and total organic content were subtracted from the end results to calculate the net change per treatment for the experiment.

A peculiar spine behavior was first observed after two weeks. This behavior consisted of the urchins spreading their spines away from the ambulacral grooves and towards each other, creating the appearance of spikes around the test. The occurrence of this behavior, which urchin was performing it and the treatment were recorded by presence/absence.

The urchins were fed every two days. Uneaten feed and feces were collected and dried weekly. The feed and feces were dried and ashed by the same methods used for the body components. The amount eaten was calculated

by subtracting the uneaten food, which was removed and patted dry with a paper towel and then weighed, from the amount originally fed to the urchin. To calculate the organic absorption efficiency the food and feces were dried and ashed at 400°C for 5 hours (Lowe and Lawrence, 1976). The organic material and remaining ash were entered into the following equation:

$$U'_N = 100 \cdot ((N'_f / A'_f) - (N'_e / A'_e) \ / (N'_f / A'_f))$$

Where U'_N is the absorption efficiency for the nutrient, N'_f and N'_e are the nutrient levels in the food and feces, respectively and A'_f and A'_e are the levels of ash in the food and feces.

Experiment 2

Eighty-three sea urchins were collected by SCUBA from Ft. DeSoto state park in St. Petersburg, FL on July 19, 2003. The experimental design was the same as that of experiment 1, except that each aquarium contained a different treatment and the experiment continued for four weeks. There were still 16 urchins per treatment. This design contrasts with experiment 1 in which all treatments were present in each aquarium. This change was made to eliminate the possibility that leaching of chromium from the feeds affected exposure. The treatments contained the following concentrations of chromium in the feed: 0, 41 or 82 ug Cr/g DW. These higher concentrations were chosen because no effects were found at the lower concentrations in experiment 1. The urchins were fed three times a week. The same observations as made in experiment 1 were made at the beginning and end of experiment 2.

Statistics

A one-way ANOVA was used to test for significant differences in wet and dry weight, body indices, and total organic content between chromium concentrations (Zar, 1999). The ANOVA tests were conducted after testing for normality and homogeneity of variance. 2-way repeated ANOVAS were used for feces production, feeding activity, and absorption efficiency. Unusual spine

behavior was analyzed using linear regression. Righting behavior was tested using K-M probability, Cox and parametric models.

RESULTS

Experiment 1

The dry and wet weights, respectively, of the gut, gonads, lantern and the test and spines did not change significantly (Table 3). The total organic content of the lantern (P=0.612), test and spines (P=0.458), gonads (P=0.593) and gut (P=0.360) of the sea urchins did not change significantly in any treatment (Table 5).

The concentration of organic (P=0.275, P=0.356, P=0.623 and P=0.113) and inorganic (P=0.275, P=0.356, P=0.623 and P=0.113) material did not differ significantly for the gonad, gut, lantern or test and spines, respectively (Table 4). Although the wet weight of the gut increased from 2.5 to 3.5 g the percent water in the gut did not change. These non-significant results are listed in Table 2 and the ANOVAs are listed in Table 5.

The mean feeding rates of urchins in the 0 and 4.1μg/g treatments decreased as the experiment progressed, but the variance increased (Figure 1). The feeding rates of the urchins in the 32μg/g treatment remained constant throughout the experiment. There was a significant difference in feeding rate over time (P=0.009) but not between treatments (P= 0.661) or treatments over time (P=0.845) (Table 6). There was no significant difference in the righting times of the urchins between treatments (P=0.901).

The dry weight of the feces differed significantly between treatments (P=0.011), over time (P<0.001) but not between treatments over time (P=0.283) (Table 7). The dry weight of the feces in the 0 and 32 μgCr/g dry weight treatments was not significantly different until week five. The dry weight of feces in the 4.1 μgCr/g dry weight treatment was significantly lower than the other treatments until the fourth week when it was not significantly different from the other treatments. In the fifth week the dry weight of the feces in the 32 μgCr/g dry

Table 2: Non-significant results from experiment one after exposure to 0, 4.1 and 32 μg chromium/g dry weight for five weeks. (mean ± standard error 0μg/g n=15, 4.1μg/g n=13, 32μg/g n=16)

	Gut			Gonad		
	0	4.1	32	0	4.1	32
Wet Weight	3.56 ± 0.10	3.48 ± 0.20	3.70 ± 0.13	10.60 ± 1.02	10.32 ± 1.07	10.31 ± 1.02
Dry Weight	0.65 ± 003	0.58 ± 0.04	0.63 ± 0.03	2.79 ± 0.362	2.87 ± 0.36	2.42 ± 0.26
Percent Inorganic material	11.45 ± 0.41	12.97 ± 1.78	11.35 ± 0.50	10.40 ± 0.94	12.92 ± 1.08	11.81 ± 0.91
Percent Organic Material	88.55 ± 0.41	87.03 ± 1.78	88.65 ± 0.50	89.61 ± 0.94	87.08 ± 1.08	88.19 ± 0.91
Total Organic Material	34.96 ± 1.77	31.13 ± 2.34	32.71 ± 1.49	145.89 ± 21.97	155.24 ± 20.87	128.12 ± 15.14

	Lantern			Test and Spines		
	0	4.1	32	0	4.1	32
Wet Weight	N/A	N/A	N/A	N/A	N/A	N/A
Dry Weight	3.07 ± 0.13	2.95 ± 0.10	2.98 ± 0.08	N/A	N/A	N/A
Percent Inorganic material	76.02 ± 1.86	77.83 ± 1.65	75.27 ± 1.22	82.12 ± 1.18	81.14 ± 1.19	78.93 ± 1.32
Percent Organic Material	23.98 ± 1.86	22.17 ± 1.65	24.73 ± 1.22	17.86 ± 1.18	18.86 ± 1.19	21.07 ± 1.32
Total Organic Material	43.97 ± 3.57	39.87 ± 3.19	43.39 ± 2.33	352.71 ± 26.61	379.91 ± 24.13	395.48 ± 23.44

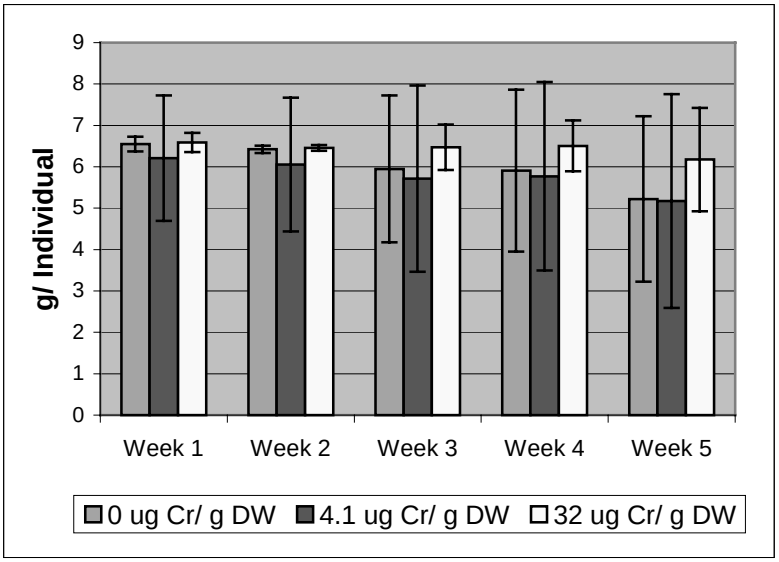


Figure 1: Amount of food eaten per urchin per treatment during exposure to 0, 4.1 and 32μg Cr/g DW for five weeks. (means ± standard error 0μg/g n=15, 4.1μg/g n=14, 32μg/g n=16).

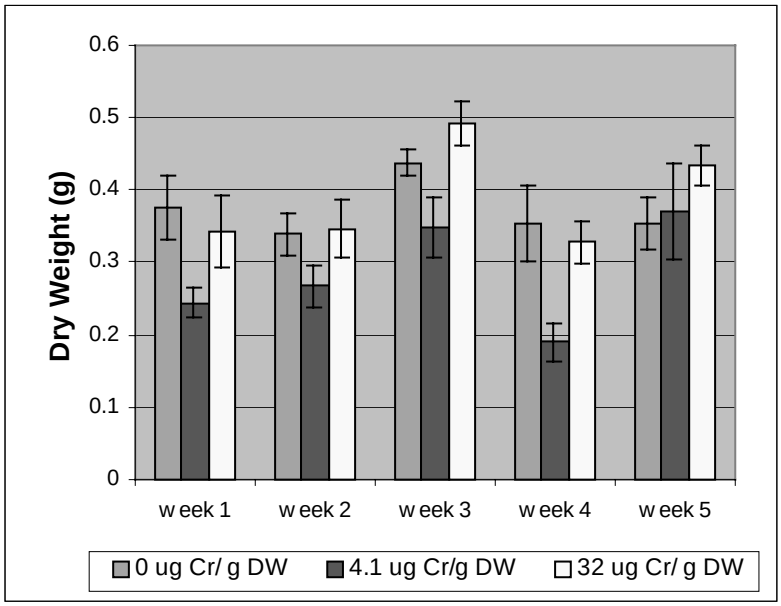


Figure 2: Dry weight of the feces during exposure to 0, 4.1 and 32 μg Cr/g DW for five weeks. (mean ± standard error 0μg/g n=15, 4.1μg/g n=13, 32μg/g n=16)

weight treatment was significantly higher than those of the 0 and 4.1 μgCr/g dry weight (Figure 2). The total organic content of the feces differed significantly between treatments (P=0.018) and over time (P<0.001) but not between treatments over time (P=0.409) (Table 7). The total organic content of the feces of urchins fed 0 and 32μgCr/g dry weight did not significantly differ from each other at any time during the experiment. The total organic content of the feces of urchins in the 4.1μgCr/g dry weight treatment was significantly lower than those of the other treatments until week five, when it increased. The treatments were not significantly different from each other at the end of the experiment (Figure 3).

The absorption efficiency (AE) of the urchins differed significantly over time (P=0.025), but not between treatments (P=0.415) or between treatments over time (P=0.900) (Figure 4) (Table 7).

During week 2 some urchins began exhibiting an unusual spine behavior. The spines were moved away from the ambulacra to touch each other over the interambulacra (Figure 5). This behavior did not differ significantly between the treatments (P=0.884) (Figure 6).

Experiment 2

The wet weight (P=0.039), dry weight (P=0.025) and total organic content (P=0.037) of the gut significantly increased in the urchins receiving 82μgCr/g. (Figures 7, 8 and 9 respectively). Neither the wet and dry weights nor the total organic content of the gonad, lantern, and test and spines significantly differ between treatments. These data are shown in Table 8. The ANOVAs for the wet and dry weights are shown in Table 10. The ANOVAs for the total organic content are in Table 11.

There was a significant decrease (P=0.050) in the concentration of inorganic material, but not in the organic material (P=0.403) of the gonad between the urchins that received 0 and 82μgCr/g dry weight and those who received 41μgCr/g dry weight (Table 9). The concentrations of inorganic

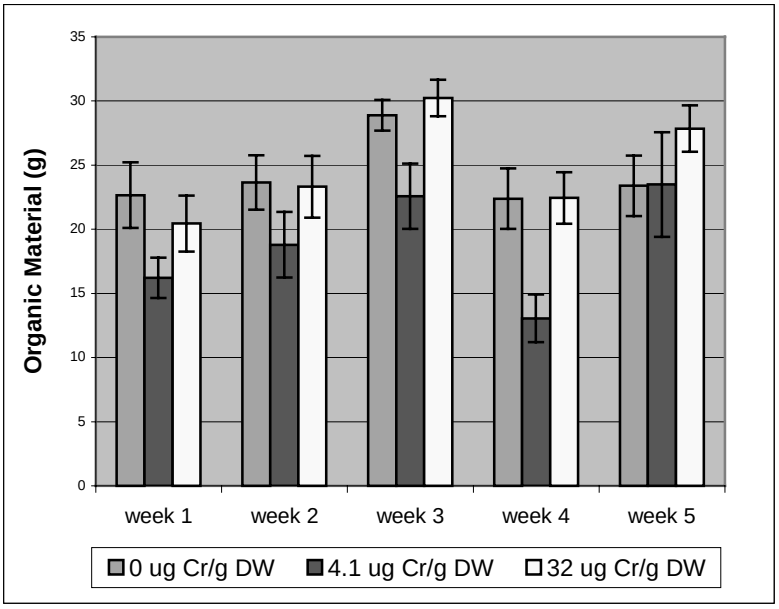


Figure 3: Total organic content of feces during exposure to 0, 4.1 and 32μg Cr/g feed for five weeks. (mean ± standard error, 0μg/g n=15, 4.1μg/g n=13, 32μg/g n=16)

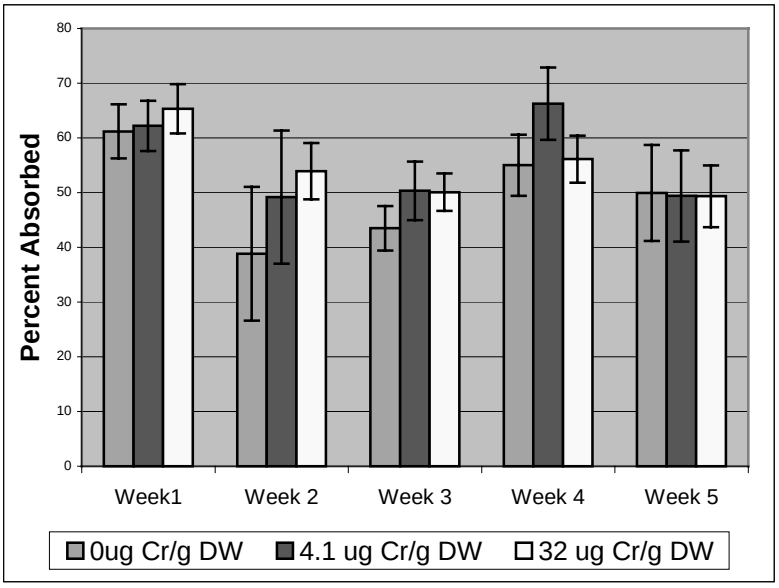
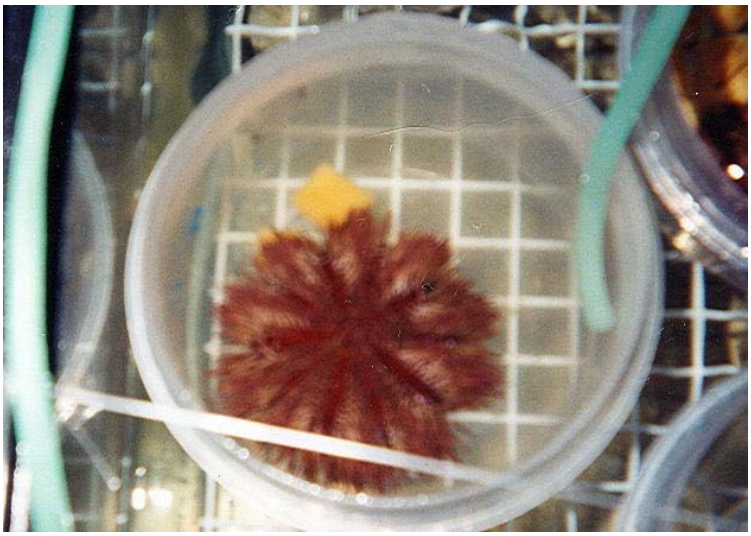


Figure 4: Absorption efficiency of organic material by the urchins during exposure to 0, 4.1 and 32μg /g feed for five weeks. (mean ± standard error, 0μg/g n=15, 4.1μg/g n=13, 32μg/g n=16)

Figure 5: A sea urchin exhibiting unusual spine formation.



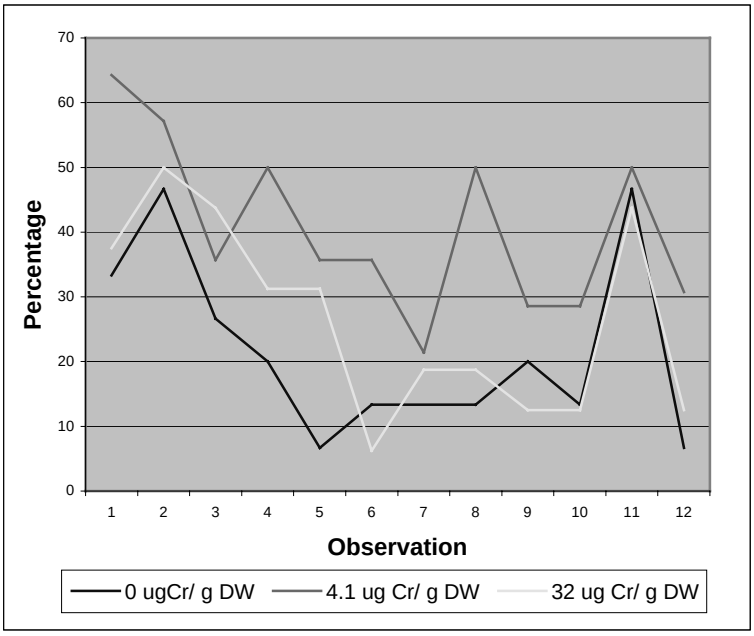


Figure 6: The occurrence of the unusual spine behavior within treatments during exposure to 0,4.1 and 32 μg Cr/g feed for five weeks measured as presence/absence. (means ± standard error 0μg/g n=15, 4.1μg/g n=13, 32μg/g n=16).

Table 3: Analysis of variance for the net difference of the wet and dry weights of the gut, gonads, lantern and test and spines after exposure to 0, 4.1 and 32μg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*
		Wet Weight		
Gut				
Treatment	2	0.378	0.611	0.548
Error	41	12.676		
Gonad				
Treatment	2	0.806	0.026	0.975
Error	41	646.288		
		Dry Weight		
Gut				
Treatment	2	0.033	1.195	0.313
Error	41	0.571		
Gonad				
Treatment	2	1.679	0.543	0.585
Error	41	63.36		
Lantern				
Treatment	2	0.113	0.361	0.699
Error	41	6.444		

Table 4: Analysis of variance for the net difference of the percent inorganic and organic material in the gut, gonads, lantern and test and spines after exposure to 0, 4.1 and 32μg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*
		Percent Inorganic Material		
Gut				
Treatment	2	30.049	1.059	0.356
Error	41	581.503		
Gonad				
Treatment	2	34.122	1.338	0.275
Error	37	471.683		
Lantern				
Treatment	2	35.492	0.478	0.623
Error	41	1522.228		
Test and Spines				
Treatment	2	102.468	2.304	0.113
Error	41	911.704		
		Percent Organic Material		
Gut				
Treatment	2	30.049	1.059	0.356
Error	41	581.503		
Gonad				
Treatment	2	34.122	1.338	0.275
Error	37	471.683		
Lantern				
Treatment	2	35.492	0.478	0.623
Error	41	1522.228		
Test and Spines				
Treatment	2	102.468	2.304	0.113
Error	41	911.704		

Table 5: Analysis of variance for the net difference of the total organic content of the gut, gonads, lantern and test and spines after exposure to 0, 4.1 and 32μg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*
		Total Organic Content		
Gut				
Treatment	2	104.292	1.047	0.36
Error	41	2042.912		
Gonad				
Treatment	2	5191.18	0.531	0.593
Error	37	180933.2		
Lantern				
Treatment	2	134.869		
Error	41	5564.336	0.497	0.612
Test and Spines				
Treatment	2	14400.67	0.795	0.458
Error	41	371400.7		

Table 6: Repeated measures analysis of variance for the feeding rate of the urchins after exposure to 0, 4.1 and 32μg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*	*G-G*	*H-F*
		Between Subjects				
Treatment	2	0.688	0.419	0.661	N/A	N/A
Error	42	34.527				
		Within Subjects				
Time	4	9.619	5.292	0.000	0.011	0.009
Between subjects over time	8	1.151	0.317	0.959	0.830	0.845
Error	168	76.34				

Table 7: Repeated measures analysis of variance for the dry weight of feces produced, total organic content of feces produced by urchins and absorption efficiency of urchins exposed to 0, 4.1 and 32µg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*	*G-G*	*H-F*
Dry Weight of Feces Between Subjects						
Treatment	2	0.257	5.581	0.011	N/A	N/A
Error	21	0.484				
Dry Weight of Feces Within Subjects						
Time	4	0.299	8.391	0.000	0.000	0.000
Between subjects over time	8	0.089	1.245	0.283	0.293	0.283
Error	84	0.749				
Total Organic Content of Feces Between Subjects						
Treatment	2	876.029	4.872	0.018	N/A	N/A
Error	21	1887.962				
Total Organic Content of Feces Within Subjects						
Time	4	1108.915	9.172	0.000	0.000	0.000
Between subjects over time	8	252.897	1.046	0.409	0.405	0.409
Error	84	2538.980				
Absorption Efficiency Between Subjects						
Treatment	2	820.045	0.917	0.415	N/A	N/A
Error	21	9392.537				
Absorption Efficiency Within Subjects						
Time	4	4909.446	3.346	0.014	0.035	0.025
Between subjects over time	8	1064.431	0.363	0.937	0.866	0.900
Error	84	30809.276				

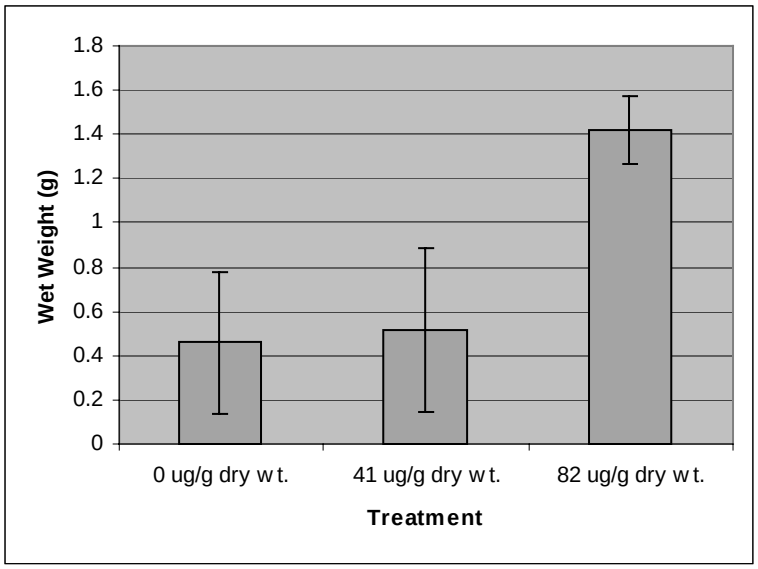


Figure 7: Net change in the wet weight of the gut after exposure to 0, 41 and 82 μg Cr/ g DW for four weeks (means ± standard error n=15 except for 41μg/g where n=13).

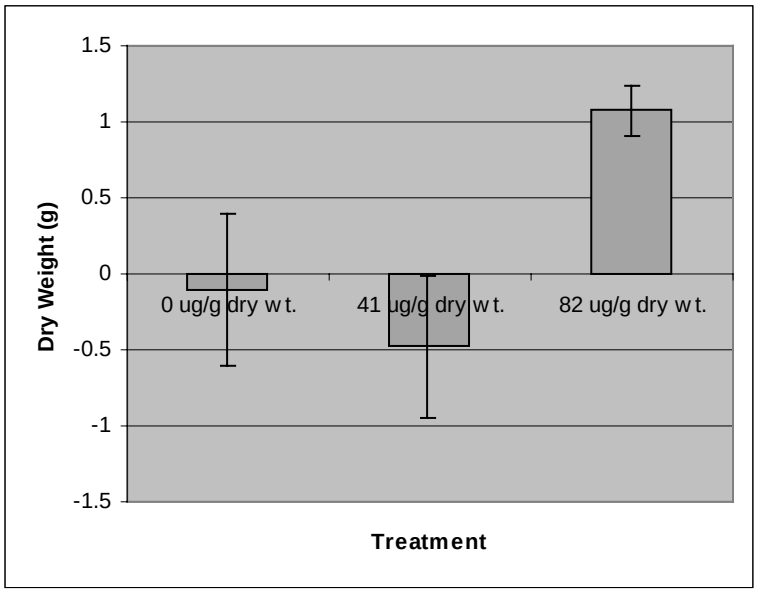


Figure 8: Net change in dry weight of the gut after exposure to 0, 41 and 82μg Cr/g dry weight for four weeks. (means ± standard error n=15 except for 41μg/g where n=13).

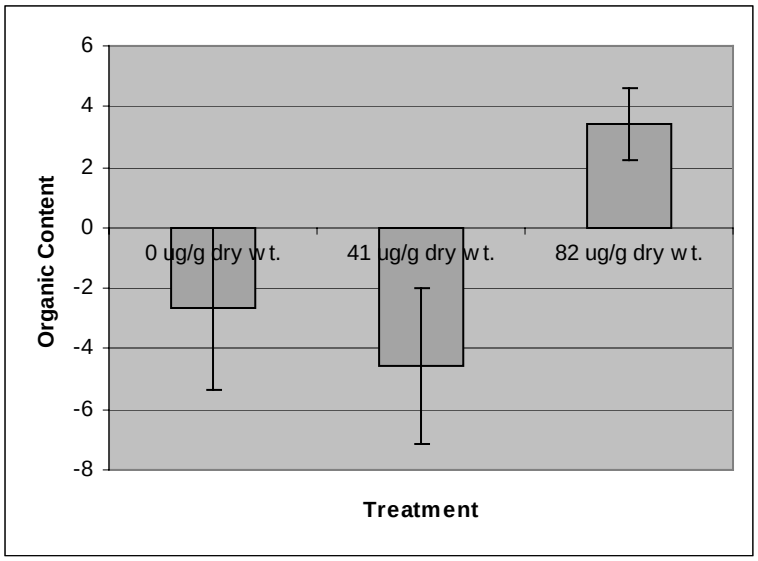


Figure 9: Net change in total organic content of the gut after exposure to 0, 41 and 82μg Cr/g dry weight for four weeks. (means ± standard error n=15, except for 41μg/g where n=13).

Table 8: Non-significant results after exposure to 0, 41 and 82μg Cr/g DW for four weeks. (means ± standard error n=15 except for 41μg Cr/g DW where n=13).

	Gut			Gonad		
	0	41	82	0	41	82
Wet Weight	*	*	*	7.24 ± 0.8	7.52 ± 0.92	8.03 ± 0.52
Dry Weight	*	*	*	11.86 ± 1.23	12.41 ± 1.43	14.56 ± 1.02
Percent Inorganic material	12.93 ± 0.90	11.30 ± 0.55	10.27 ± 0.30	*	*	*
Percent Organic Material	87.07 ± 0.90	88.71 ± 0.55	89.74 ± 0.30	91.91 ± 0.32	90.97 ± 0.79	92.55 ± 0.45
Total Organic Material	*	*	*	55.64 ± 4.24	60.18 ± 6.80	62.99 ± 4.49

	Lantern			Test and Spines		
	0	41	82	0	41	82
Wet Weight	N/A	N/A	N/A	N/A	N/A	N/A
Dry Weight	26.41 ±2.25	24.94 ± 1.27	26.40 ± 1.90	N/A	N/A	N/A
Percent Inorganic material	82.98 ± 1.08	80.98 ± 1.76	81.07 ± 1.34	86.75 ± 0.62	86.05 ± 0.64	85.96 ± 0.61
Percent Organic Material	17.03 ± 1.09	19.02 ± 1.76	18.93 ± 1.34	13.26 ± 0.62	13.95 ± 0.64	14.04 ± 0.61
Total Organic Material	23.83 ± 4.37	24.59 ± 2.16	23.80 ± 3.27	157.45 ± 19.48	150.38 ± 13.43	168.38 ± 14.41

* = significant values

Table 9: Net difference of the percent inorganic material in the gonads after exposure to 0, 41 and 82 μg Cr/ g DW. (means ± standard error n=14, except for 41μg Cr/g DW where n=13).

Treatment	Percent Inorganic Material ± Standard Error
0μg Cr/ g dry weight	-2.851 ± 0.618
41μg Cr/ g dry weight	-0.976 ± 0.859
82μg Cr/ g dry weight	-3.093 ± 0.402

Table 10: Analysis of variance for the net difference of the wet and dry weights of the gut, gonads, lantern and test and spines after exposure to 0, 41 and 82μg Cr/ g DW for four weeks.

Source	*df*	SS	*F*	*P*
		Wet Weight		
Gut				
Treatment	2	8.547	3.523	0.039
Error	40	48.517		
Gonad				
Treatment	2	4.74	0.294	0.747
Error	40	322.785		
		Dry Weight		
Gut				
Treatment	2	18.823	4.048	0.025
Error	40	93.008		
Gonad				
Treatment	2	60.2	1.409	0.256
Error	40	854.4		
Lantern				
Treatment	2	30.769	0.298	0.744
Error	40	2064.054		

(P= 0.797, P=0.533 and P=0.518) and organic (P=0.235, P=0.533 and P=0.518) materials were not significantly different for the gut, lantern and test and spines, respectively. These data are also shown in Table 8. The ANOVAs are shown in Table 11.

The amount of feed eaten significantly differed over time (P<0.001), between treatments (P<0.001) and between treatments over time (P<0.001) (Table 13). There was no significant difference in the amount of food eaten between treatments for the first three weeks. The amount of food eaten by urchins fed 0 and 82μgCr/g dry weight were not significantly different from each other. The amount of food eaten by those fed 41μgCr/g dry weight treatment was significantly lower than those fed 0 and 82μgCr/g dry weight, decreasing significantly from week three to week four (Figure 10).

The dry weight of the feces significantly differed between treatments (P=0.003) over time (P<0.001) and between treatments over time (P=0.045) (Table 14). For the first three weeks the three treatments did not significantly differ. In the fourth week dry weight of the feces of urchins fed 0 and 82μgCr/g dry weight significantly increased, though they were not significantly different from each other (Figure 11).

The total organic content of the feces significantly increased over time (P<0.001) and between treatments over time (P=0.041), but not between treatments (P=0.209) (Table 14). The treatments did not significantly differ for the first three weeks, but in the fourth week the organic content of the feces in the 0 and 82μgCr/g dry weight treatments were significantly higher than the 41μgCr/g dry weight treatment (Figure 12).

There was a significant decrease (P<0.001) in absorption efficiency (AE) of the urchins in all treatments over time. No difference was found between treatments (P=0.895) or between treatments over time (P=0.104) (Table 11). The AE of urchins in the 82 Cr/ g dry weight treatment decreased until week 2, whereas the AE of the urchins in the other two treatments slowly decreased

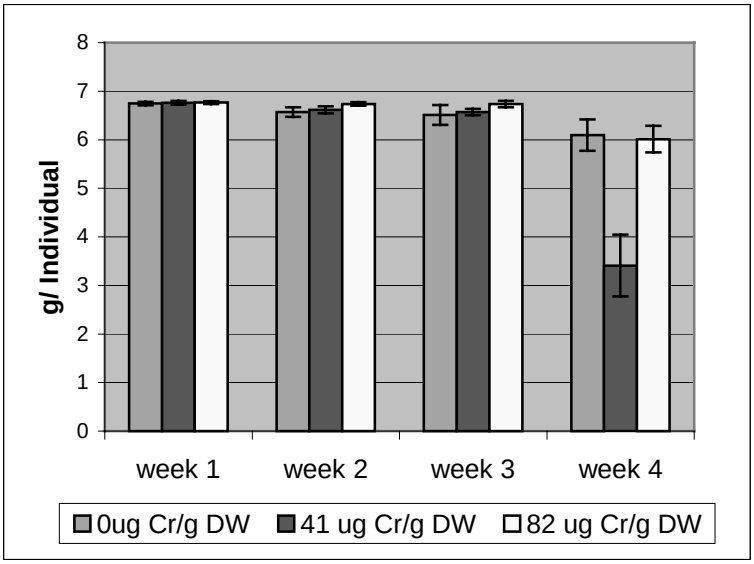


Figure 10: Amount of food eaten per urchin per treatment during exposure to 0, 41 and 82μg Cr/g DW for four weeks. (means ± standard error n=15 except for 41μg/g where n=13).

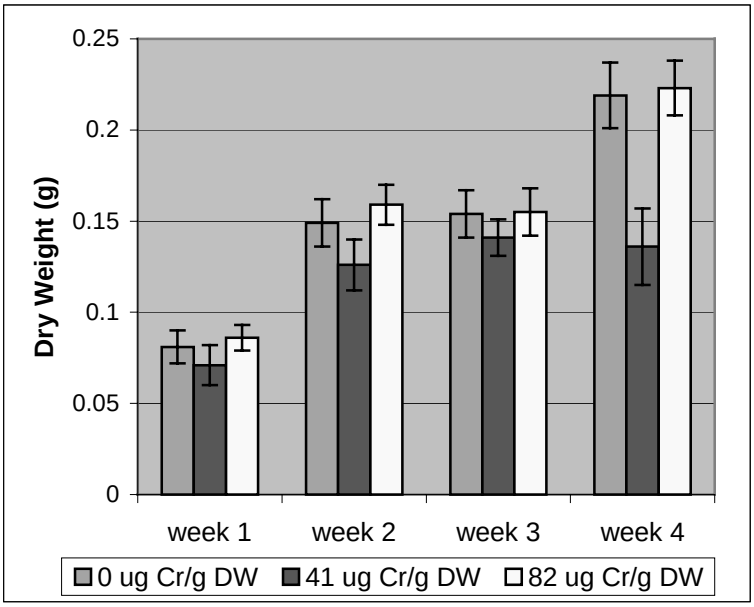


Figure 11: Dry weight of the feces during exposure to 0, 41 and 82μg chromium/g feed for four weeks. (means ± standard error n=15 except for 41μg/g where n=13).

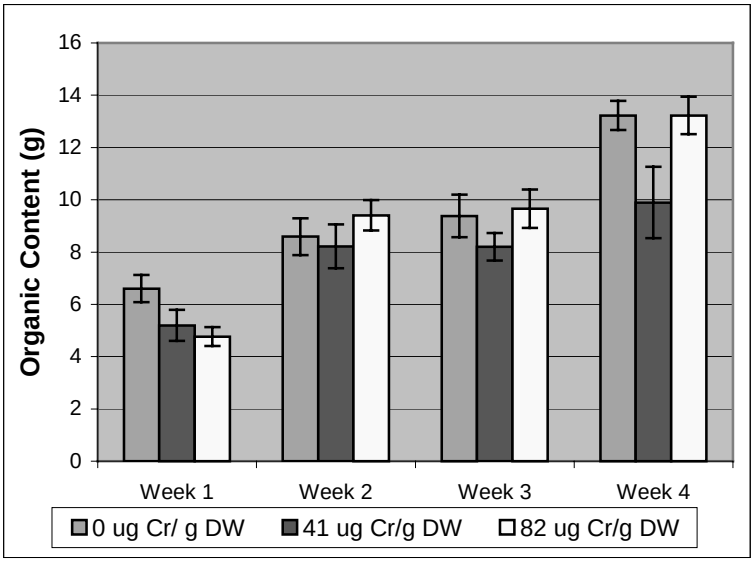


Figure 12: Total organic content of the feces during exposure to 0, 41 and 82μg chromium/g feed for four weeks. (means ± standard error n=15 except for 41μg/g where n=13).

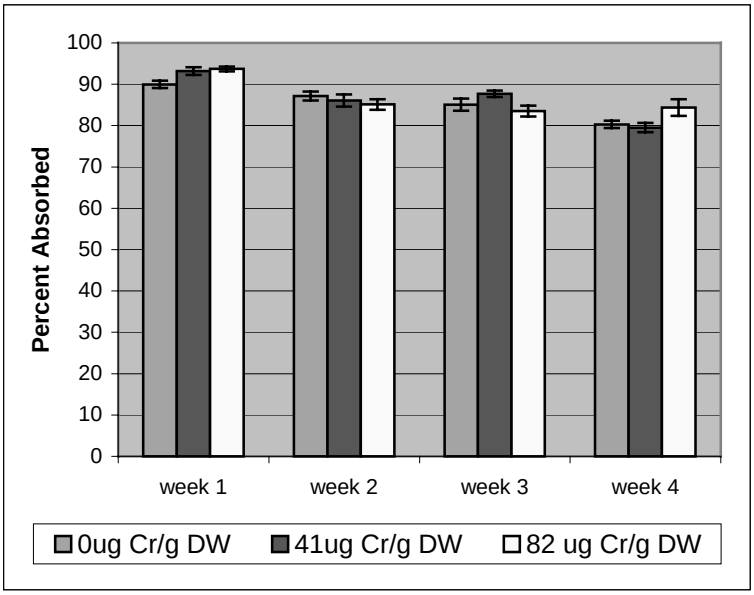


Figure 13: Absorption efficiency of organic material by the urchins during exposure to 0, 41 and 82μg chromium/g feed for four weeks. (means ± standard error n=15 except for 41μg/g where n=13).

Table 11: Analysis of variance for the net difference of the percent inorganic and organic material in the gut, gonads, lantern and test and spines after exposure to 0, 41 and 82μg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*
		Percent Organic Material		
Gut				
Treatment	2	2003.51	1.501	0.235
Error	40	26692.54		
Gonad				
Treatment	2	374.266	0.93	0.403
Error	40	8050.665	12.748	
Lantern				
Treatment	2	37.673	0.639	0.533
Error	40	1178.924		
Test and Spines				
Treatment	2	7.431	0.669	0.518
Error	40	222.094		
		Percent Inorganic Material		
Gut				
Treatment	2	7.062	0.229	0.797
Error	40	617.362		
Gonad				
Treatment	2	36.57	3.191	0.052
Error	40	229.198		
Lantern				
Treatment	2	37.673	0.639	0.533
Error	40	1178.924		
Test and Spines				
Treatment	2	7.431	0.669	0.518
Error	40	222.094		

Table 12: Analysis of variance for the net difference of the total organic content in the gut, gonads, lantern and test and spines after exposure to 0, 41 and 82μg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*
		Total Organic Content		
Gut				
Treatment	2	500.773	3.57	0.037
Error	40	2805.727		
Gonad				
Treatment	2	1893.543	2.096	0.136
Error	40	18070.53		
Lantern				
Treatment	2	1.731	0.005	0.995
Error	40	6670.676		
Test and Spines				
Treatment	2	2580.761	0.345	0.71
Error	40	149711.1		

Table 13: Repeated measures analysis of variance for the amount eaten by the urchins exposed to 0, 41 and 82μg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*	*G-G*	*H-F*
		Between Subjects				
Treatment	2	18.189	10.221	0.000	N/A	N/A
Error	42	37.372				
		Within Subjects				
Time	3	75.354	33.652	0.000	0.000	0.000
Between subjects over time	6	50.028	11.171	0.000	0.000	0.000
Error	126	94.048				

Table 14: Repeated measures analysis of variance for the dry weight of feces produced, total organic content of feces produced by urchins and absorption efficiency of urchins exposed to 0, 41 and 82μg Cr/ g DW for five weeks.

Source	*df*	SS	*F*	*P*	*G-G*	*H-F*
Dry Weight of Feces Between Subjects						
Treatment	2	0.042	6.593	0.003	N/A	N/A
Error	43	0.138				
Dry Weight of Feces Within Subjects						
Time	3	0.311	38.939	0.000	0.000	0.000
Between subjects over time	6	0.038	2.379	0.033	0.051	0.045
Error	129	0.343				
Total Organic Content of Feces Between Subjects						
Treatment	2	23.226	1.689	0.209	N/A	N/A
Error	21	144.388				
Total Organic Content of Feces Within Subjects						
Time	3	538.846	38.585	0.000	0.000	0.000
Between subjects over time	6	68.082	2.438	0.035	0.055	0.041
Error	63	293.270				
Absorption Efficiency Between Subjects						
Treatment	2	5.048	0.011	0.895	N/A	N/A
Error	19	431.404				
Absorption Efficiency Within Subjects						
Time	3	1780.983	33.295	0.000	0.000	0.000
Between subjects over time	6	211.849	1.980	0.084	0.121	0.104
Error	57	1016.316				

throughout the experiment (Figure 13). The righting time showed no significant difference between treatments.

The unusual spine formation observed in experiment one began on the second week of experiment two and did not continue past the third week. 18% of the urchins in the 0μgCr/g dry weight, 37.5% of those in the 41μgCr/g dry weight and 25% of those in the 82μgCr/g dry weight were performing this behavior for one week. None if the urchins in any of the treatments spread their spines again for the duration of the experiment.

Discussion

Experiment 1

The average concentration of chromium found in seagrasses and algae is 4 μg/g dry wt (Topcuoglu *et al.* 2002; Muse *et al.* 1999; Campanella *et al.* 2001; Sanchez-Rodriguez *et al.* 2001; Giusti, 2001; Rigit *et al.*1997; Villares *et al.* 2002; Haritonidis and Malea 1995; Topcuoglu *et al.* 2001; Prange and Dennison 2000; Campanella *et al.* 2001; and Warnau *et al.* 1995). Sanchez-Rodriguez *et al.* reported the highest level of 30.6μg C/g dry weight for *Sargassum sinicola*. This was the basis for the decision to test the effects of 4.1 and 32 μg Cr/ g dry weight. Sea urchins have a difficult time breaking down and digesting plant and algal cell walls (Lawrence, 1982). Consequently, chromium in artificial feeds should be more biologically available to the urchins. Even though the concentrations fed the urchins were 4.1 and 32 μg Cr/ g dry wt the amount they were actually able to access should be higher.

There was no significant change in the total organic content of any of the components for the urchins. This indicates production was similar for individuals in all treatments. The gut however had an increase in the wet weight, which suggests the urchins in the 32μg Cr/g dry weight treatments were retaining water in the gut.

Lawrence *et al.* (2003) found that sea urchins in good health maintain a consistent feeding rate, depending on the frequency with which they are fed. The feeding rates for the urchins fed 0 and 4.1μgCr/g dry weight in this experiment decreased by 20 and 17%, respectively, suggesting that these urchins were not in good health. The urchins receiving the 32μgCr/g dry weight treatment had a 6% decrease in feeding rate. Sea urchins fed 0 and 4.1μgCr/g dry weight had lower feeding rates from week 3 until the end of the experiment, though the variation increased with the duration of the experiment.

This decrease in feeding rates does not correlate with a decrease in fecal production. Fecal production for sea urchins fed 0 and 32μgCr/g dry weight treatments were the same, whereas that of sea urchins fed 4.1μgCr/g dry weight was significantly lower throughout the experiment, until week 5 when fecal production was not significantly different from those fed 0 and 32μgCr/g dry weight. The urchins fed 4.1μgCr/g dry weight did not absorb more than the urchins in the other treatments during the experiment, except for week 4, yet they had consistently lower feces production and total organic content. Sea urchins in all treatments showed a large drop in both feces production and organic content of the feces on week 4, but there was no correlating decrease or increase in feeding at that time. There was, however, a significant increase in the absorption efficiency (A.E.) for the urchins fed 4.1μgCr/g dry weight. This was followed by a return to previous weeks' feces production, total organic content and AE in week 5. The urchins fed 4.1 and 32μgCr/g dry weight were able to digest 5% more of their feed, on average, than those fed 0μgCr/g dry weight.

These results suggest that the urchins are better able to digest food when it contains at least 4.1μgCr/g dry weight. Chromium (III) is a micronutrient necessary for the metabolism of lipids and carbohydrates in mammals (Vincent 2001, Kotaś and Stasicka 2000, and Barceloux 1999). The fact that urchins fed 4.1μgCr/g dry weight ate less than the others may also contribute to the lower production of feces (Lowe and Lawrence, 1976; Bőttger *et al.,* 2001). I do not know why the total organic content and dry weight of the feces would be lower for the urchins fed food with 4.1μgCr/g dry weight. The difference in the urchins AE's possibly explains the results for week 4, but not the other weeks. The urchins were not eating less or absorbing more than the urchins in the other treatments. It follows that somehow they were retaining the undigested feed. There was evidence of this. Upon dissection most of the urchins had undigested food still in their guts, but how much each urchin had and which treatment it was in was not measured. The effects may exhibit an inverted u relationship, where the lower concentration has more of an effect than the higher concentration because the

organism is better able to eliminate or sequester the higher doses. Chromium had no effect on the urchins' ability to right themselves. This indicates that the levels of chromium ingested did not inhibit the neuromuscular system of the tube feet.

The urchins in all treatments, including the 0:g/g dry weight, began spreading their spines away from the ambulacrum during the second week. Chromium may have been affecting the musculature associated with their spines causing this abnormal posture. The individuals in the 0:g/g dry weight treatment may have been exposed to chromium leached from the other feeds. Fernandes *et al.*, (2002) found that chromium (VI) interferes with mitochondrial respiration, which decreases the amount of ATP formation and inhibits muscle contraction.

Experiment 2

The gonads of urchins in experiment one had 86% more wet weight and 83% more dry weight than the gonads in experiment two. The guts of urchins in experiment two were more comparable to those in experiment one, having only 32% more wet weight and 38% more dry weight. This could be due to the lack of food, the lateness of the season or the urchins having recently spawned. In addition, the urchins from Lido Beach in the first experiment were in a lush seagrass bed and thus had more available food than those in the second experiment, which were from a sand flat at Fort DeSoto Park with little available food.

Sea urchins in all treatments had significantly larger gonads at the end of the experiment than at the beginning. The increase in gonad size did not differ significantly between treatments indicating chromium in the feed had no effect on production. The dry and wet weights as well as the total organic content of the guts significantly increased in the urchins fed 82μg Cr/ g DW. Lares and Pomory (1998) found that upon starvation the gut is the first body component of *Lytechinus variegatus* to decrease in weight and total organic content. The gut also is the first body component to grow after starvation (Bishop and Watts,

1992). This suggests that the gut is utilized for short-term energy storage and would be of a smaller size when the urchin is under nutritive stress. Lawrence *et al.* (2003) found significant increases in the gut and gonad dry weight and indices of *L. variegatus* fed everyday but not if fed every 2 or 4 days. The urchins in this experiment were fed every 3 days, including weekends. The weight of the lantern decreased significantly in all treatments. No other studies have reported changes in the lantern with nutritional condition of sea urchins and this difference is probably an artifact.

The sea urchins fed less in all treatments as the experiment continued. The urchins fed 0 and 82μgCr/g dry weight showed a 10 and 11 % decrease in feeding rates, respectively. Up until week 4 the urchins in the 41μgCr/g dry weight treatment showed only a 3% decrease in feeding rate. However, in week 4 they had a 50% decrease in feeding rate. This did not correlate with a decrease in feces production, total organic content, or an increase in AE. This suggests that these urchins were under stress and were not in good health at the end of the experiment (Lawrence, 1990). *Lytechinus variegatus* starved for nine days and then provided with constant food show a marked increase in feeding followed by a plateau. Urchins fed intermittently have a consistently high feeding rate (Lawrence *et al.,* 2003). This occurred in this experiment suggesting that the urchins, up until week 4, were better able to digest and store the feed given to them than those in the previous experiment.

Urchins fed 0, 41 and 82μgCr/g dry weight showed an 11, 15 and 10% decrease, respectively, in AE over the length of the experiment. The AE's were not different from each other throughout the experiment. Fecal production by sea urchins fed 41μgCr/g dry weight did not increase after week 2, but continued to increase in sea urchins fed 0 and 82μgCr/g dry weight. There was a decrease in feeding in week 4 in all treatments, most notably for urchins fed 41μgCr/g dry weight, yet the feces production by urchins fed 0 and 82μgCr/g dry weight showed the highest overall increase in this week. Urchins fed 41μgCr/g dry weight maintained the same output as in weeks 2 and 3. This suggests that they

were digesting less of the feed and this undigested feed was being passed into the feces, which would account for both the increase in feces production as well as the increase in total organic content.

There was no significant difference in righting time, again suggesting that the chromium did not affect the neuromusculature associated with the tube feet. The urchins at the end of the experiment seemed to be in much better condition than they were at the beginning of the experiment. The gut and gonads were larger and contained more organic material than they did in the beginning, though there was a significant decrease in the AE from the beginning to the end.

The urchins in this experiment exhibited the abnormal spine behavior only in week 2. It is possible chromium was being sequestered or excreted and therefore was no longer affecting the neuromusculature of the spines of the urchins after week 3 but this would not explain the decrease in urchins fed 0:gCr/g dry weight. The amount of chromium in the body components or feces was not measured.

Chromium may be an enhancer. At low concentrations chromium III is a micronutrient for mammals. It is utilized in carbohydrate and lipid metabolism (Vincent, 2001; Bagchi, *et al.,* 2001; Barceloux, 1999). The sea urchins diet consists mostly of carbohydrates (Lowe and Lawrence, 1976) and it is therefore likely that they also require chromium. It is possible that the urchins were converting chromium VI from the food into chromium III and then utilizing this micronutrient (Kotaś and Stasicka, 2000).

Summary

The urchins in the two experiments showed dramatically different results. The urchins of each experiment can be compared, but their differences in food availability in the field and in season must be taken into account. As the urchins are from two separate populations, collected at two different seasons of the year, physiological state and environmental factors and would have different responses to the chromium based on these differences. These differences make using the responses of individuals in the 0μg/g treatments the best way to compare the state of the urchins in the two treatments.

The urchins fed 0μg Cr/g dry weight in experiment one had higher wet and dry weights as well as total organic content of all components and overall weight at the end of the experiment than did those in experiment two. The urchins in experiment two, however, ate 8% more over the course of the experiment than did those in experiment one with an AE of 89%. The urchins in experiment one had an AE of only 50%, yet they produced more feces with a higher total organic content than did the urchins in experiment two.

The urchins used in experiment one had larger gonads and guts when collected than those in experiment two. The gut and gonads of the urchins in experiment two were 86% and 38% smaller than those in experiment one. The smaller size of the gut indicates they had less nutrient reserves that would be expected with a lower availability of food. The smaller size of the gonad could mean they had yet to begin gonadal production associated with the annual reproductive cycle or inadequate food for gonadal production. This suggests these urchins were starved. This would explain the higher feeding rates and AEs in experiment two. An AE of only 50% indicates the urchins in experiment one were not digesting all the food they ingested. Their guts and gonads did not increase throughout the experiment suggesting that they were receiving

adequate amounts of food and the food to maintain themselves but not for production (Lawrence, *et al.,* 2003).

Chromium (VI) is readily converted to chromium (III), which is a micronutrient (Kotaś and Stasicka, 2000; Barceloux, 1999). It is very possible that either the chemical reactions upon making the food, putting it in the water or the urchins made this conversion and so the urchins were not receiving chromium (VI) at all, but chromium (III) which they were able to use to aid in digesting the carbohydrates in their feed.

The presence of heavy metals in the environment does not necessarily mean they are pollutants. Under the conditions of this experiment and at the concentrations tested, chromium in food is not a pollutant for adult *Lytechinus variegatus*. This is not to say that it would not affect juveniles or larvae. The embryological stages are the most susceptible to damage by pollutants (Greco *et al.,* 2001; Vashchenko *et al.,* 1995). This is also not to say that water borne or sediment bound chromium would not negatively affect these urchins.

Though the trend was not significant, the urchins in experiment two who were fed the highest levels of chromium had a greater increase in the size of the gonads than those fed 0μgCr/g dry weight. Those fed 82μg Cr/ g DW did experience a significant increase in the dry and wet weights and total organic content of their guts. Chromium at the levels and conditions of this experiment has no measurable difference on the behavior or production of the adult sea urchin *Lytechinus variegates.*

The increase in the wet and dry weights and total organic content of the guts of the urchins receiving 82μg Cr/ g DW suggests that chromium is an enhancer for these urchins. Even though it is unknown what effects added chromium in a system would have on the embryological or larval stages of *Lytechinus variegatus* it may help the adults. It is also unknown how chromium and other heavy metals and pollutants interact with each other and what effects this would have on organisms.

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