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The Bioaccumulation of Heavy Metals in Trematode *Euhaplorchis californiensis*: Use of Parasites in Environmental Monitoring and Impact Assessments

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**The bioaccumulation of heavy metals in trematode
Euhaplorchis californiensis: use of parasites in
environmental monitoring and impact assessments**



Capstone Project, June 2013
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Abstract

It is informative to understand the effects of pollutants on the host-parasite interactions between parasites and their aquatic host organisms. The effects of pollutants on parasites can alter their role in an ecosystem, affecting host populations, biodiversity, and food webs, with potential cascading effects throughout the ecosystem. Furthermore, parasites are good proxies for monitoring the health of an ecosystem and can be used for environmental health assessments. Here, I selected few heavy metals such as Cd, Ag, Cr and Fe to explain the results for chapter 1 of the study, from the 20 metals tested. Samples of sediments, shell and snails' soft tissues were analyzed using the ICP-OES. Concentrations of heavy metals for sediments were in the same range across the different sites within the marsh. The concentrations were also found to be in the same range for the different sections of the snail shell within the same individuals as well as two different populations. However the concentrations within the tissues varied where foot > digestive tract > mantle. Also, the population closer to the point source had higher level of heavy metals in all tissues compared to the population further away from the polluted site. This information will allow us to use parasites as bioindicators of marine pollution. While this study won't get to the stage of analyzing the implications for the whole ecosystem, it is the first step to an extensive study in which the life cycle of one parasite is being looked at.

1. Introduction

In the study of the health and function of an ecosystem, major biological components are often neglected. One such component is the role of parasites. The understanding of an ecosystem is incomplete if the ecology of parasites is not taken into consideration. Over the past two decades, researchers have found that parasites, once thought trivial, have a significant effect on ecosystem function. Parasites can affect host population dynamics, change interspecific competition, impact the flow of energy and increase biodiversity (Hudson et. al., 2006). Additionally, they can be used as good biomarkers. This is due to the fact that some species of parasites remove toxic pollutants when they are ingested by their host, limiting (Hudson et. al., 2006), and altering the uptake of metals by their hosts (Evans et al., 2001 and Bergey et al., 2002).

Parasite is an organism living in, with, or on another organism, that is dependent on others for existence or support without making a useful or adequate return to an organism living in (Margolis, 1982). However, this relationship could be an uncovered mutualism in certain cases. Many parasites appear to be more sensitive to anthropogenic pollutants than their hosts or other free-living organisms, making them excellent candidate indicators of anthropogenic pollutant levels and effects (Sures, 2004). Pollutants can change the transmission rate of parasites through physiological impacts to the parasite and host. This can change the population dynamics in the ecosystem especially when the parasite has a complex and multi-host life cycles such as trematodes (Koprivnikar, 2001). One of the most common species of trematode present in the estuaries in southern California, *Euhaplorchis californiensis*, has a complex life cycle, which starts in the feces of a bird (Lafferty, 2008), figure 1.

This trophically transmitted trematode lives in three hosts: shorebirds, horn snails, and killifish and modifies the behavior of the host to increase the transmission to the next host in order to complete its life cycle. *E. californiensis* lives in the intestine of several piscivorous bird species as a short-lived adult. The adult trematode lays eggs that are defecated by the bird. Snails are the first intermediate host, usually becoming infected when they ingest the worm's eggs. Alternately, the miracidium, a flat, ciliated, free-swimming larvae stage of the trematode, may infect the snail. After developing in the snail, causing parasitic castration (Baudoin, 1975), the parasite produces and releases the larval stage of the trematode called the cercariae, a free-swimming form which searches for and infects the second intermediate host, the California killifish (*Fundulus parvipinnis*) either by ingestion or invasion through the gills. The cercariae migrate to the brain along blood vessels or nerve tracts. There, the parasite creates cysts on the brain of the fish (McNeff, 1978), altering the behavior and making it more susceptible to predation (Lafferty, 2008). In order for the worm to complete its life cycle, a bird must eat the infected fish (Lafferty, 2008).

Environmental pollution affects the trematodes directly through pollutant loads in the parasites' tissues and indirectly through effects on the health of its hosts. Parasites that are physiologically resistant to the direct effects of pollutants are able to concentrate environmental pollutants in their tissues, making them good bioindicators for heavy metals and some organic pollutants (Sures, 2004). These combined factors make trematodes (Lafferty, 2008), like *E. californiensis* a good indicator of environmental pollution.

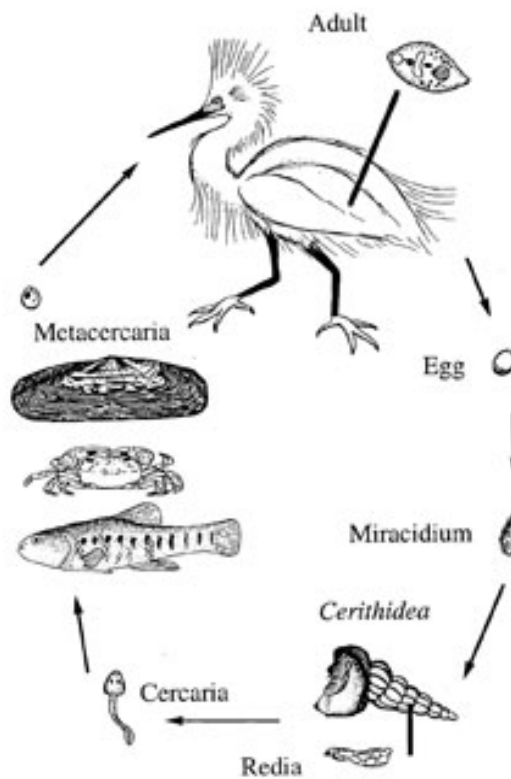


Figure 1. Life cycle of trematodes that develop in snail *Cerithidea* California, including *Euhaplorchis californiensis*.
Credit: T. Huspeni and K. Lafferty, UCSB

This paper will be divided in four complementary chapters looking at the bioaccumulation of heavy metals in the immediate environment, the host's tissue, and the metacercariae and cercariae stages of the trematode, *E. californiensis*. The study seeks to link concentrations of trace metals to the longevity of *E. californiensis*.

- I. Chapter one examines the pollutant levels in:
 - 1) Sediment from several sites
 - 2) The shell rings

- 3) Different tissues (foot, mantle, digestive tract) between two populations of snails.
- II. Chapter two will be studying the native concentration of heavy metals in the cercariae and metacercariae tissue and the immediate surrounding at three life stages: egg, cercariae, and metacercariae (bird feces, snail, killifish), figure 2.

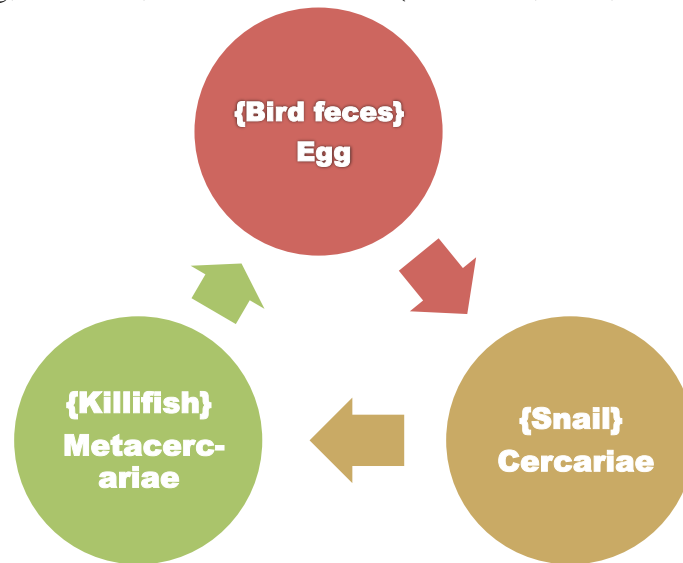


Figure 2. The three life stages of *E. californiensis* and their respective environment in which the parasite is found.

The egg samples to test for trace elements assay could not be collected due to several reasons and complications such as: time restriction of the project, number of feces available in the marsh upon the sample collection, number of eggs per droppings collected, different parasite eggs and identifications within the droppings, enough dry weight egg samples for processing the trace metal assay.

- III. Chapter three will investigate the pollutant load of parasitized versus un-parasitized snail tissues of different individuals within the same population.

2. Materials and methods

2.1. Description of study site

Carpinteria Salt Marsh Reserve, established in 1977, is a 93-hactare estuarine wetland located 12 km east of Santa Barbara, along the South Coast of Santa Barbara County (Cao et al., 2006). The reserve provides habitat for at least 190 bird's species, 37 fish species, 11 mammal species, 5 herpetofauna species, and over 100 invertebrate species. The estuary is important for resident species including (1) birds such as many shorebirds, wading birds, gulls and terns, and passerines; (2) fish such as Arrow Gobies, and California Killifish; and estuarine-restricted crustaceans and molluscs. The estuary also provides important habitat for migratory birds and habitat for seasonal use by species of

special interest such as regionally declining and threatened or endangered bird species including Long-billed Curlews, Least Terns, and Snowy Plovers. Carpinteria Salt Marsh also provides important nursery functions for various marine fish including Diamond Turbot, Stary Flounder, and the economically important California Halibut (Carpinteria Salt Marsh Reserve, 2013).

The reserve is surrounded by developed land, fed by channelized urban-creeks and receives large amount of agricultural runoff as well as road run off from the major highway and rail road track directly next to the marsh. Other potential inputs of contaminants are from atmospheric inputs, urban development and industries in the surrounding area (figure 3).

Carpinteria Salt Marsh is a geomorphologically mature system that consists of creek channels and tidal flats (8–13% of area). The regularly flooded tidal marsh is vegetated mainly by *Salicornia virginica* (50–60%) and by mixed species associations (Page et al., 1995). The climate is semiarid Mediterranean, which is typical of southern California with annual average temperatures ranging from 10.2 to 21.7 °C and an annual average rainfall of 44.7 cm (Cao et al., 2006).

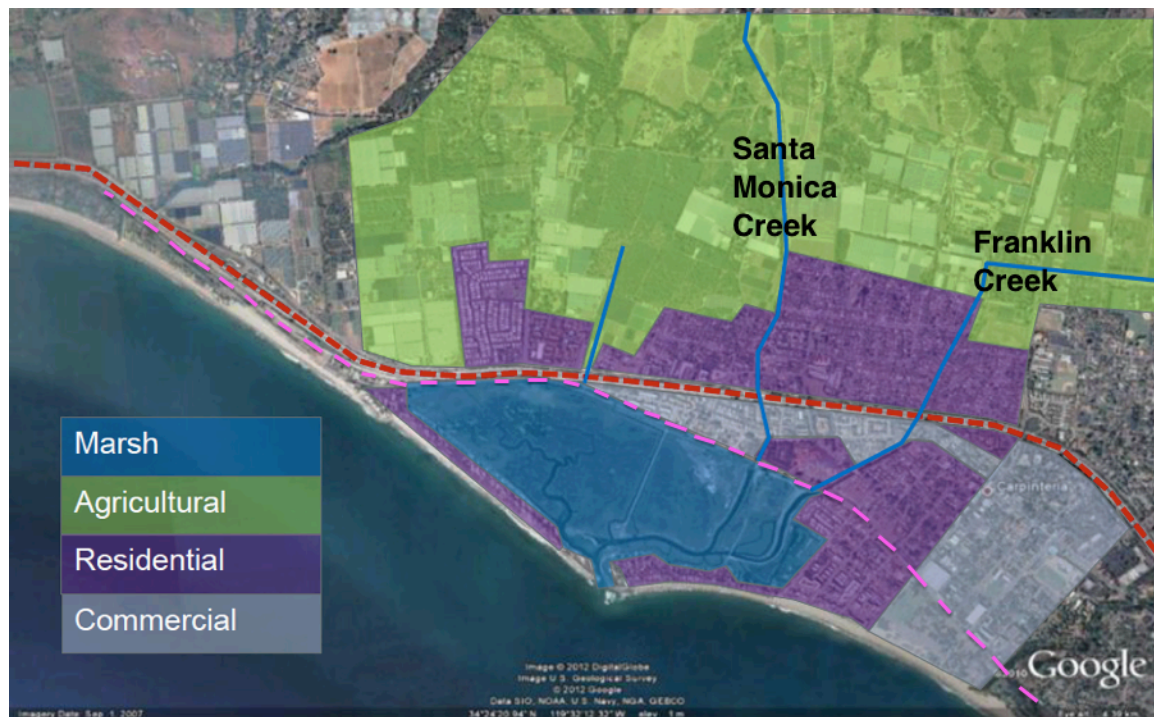


Figure 3. Carpinteria land use and pollutant sources.

Dashed red line and pink line represent major highway and railroad track respectively.

Credit: ERM foundation

2.2. Collection of biological material

Samples were collected from the Carpinteria Salt Marsh Reserve (CSMR) in Santa Barbara, CA. The snails from sites C3 and C7 (figure 4) were collected in February 2013 and mailed to San Diego overnight. All other samples of killifish, snails, bird feces and sediments were collected on April 3 and 4th, 2013.

The specimens collected in April were harvested during a single week to avoid variability of ambient contaminant concentration from uncontrolled sources such as rain and temperature.

The concentration of pollutants in different areas of the marsh is suspected to vary according to the distance from the pollutant source. Samples collected closer to the mouth of the estuary are expected to have lower pollutant levels attributable due to regular flushing. Areas closer to the creek inflow such as Santa Monica Creek and Franklin Creek (figure 4) should have higher levels due to contaminants entering via runoff.



Figure 4. Aerial image of Carpinteria Salt Marsh Reserve, Santa Barbara, CA. Yellow marks represent the sites where samples were collected.

Chapter 1: Heavy metal concentration in Sediments and snails

The snails were received on Feb 21, 2013 and were kept alive in a moist paper towel in a Zip-lock bag during the transportation and for the first week in San Diego. The number of snails collected from C3 and C7 was 11 and 10, respectively. The length and width of individuals were measured and recorded using two methods: a caliper and a hyperspectral imaging camera and Q-Capture software (figure 5). After measurements, they were labeled and were kept in 6 well plastic culture plates, wrapped in a paper towel moistened with Artificial Sea Water (ASW) until dissected.

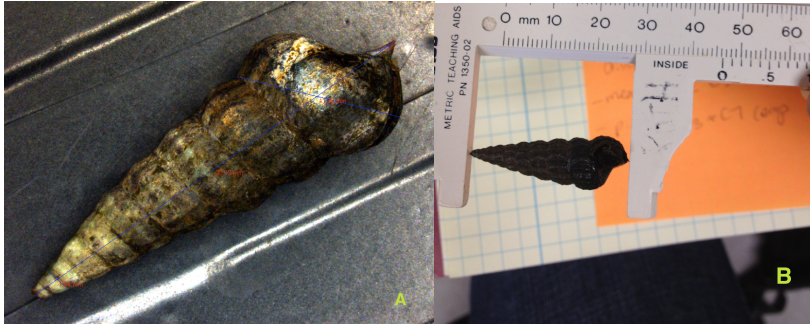


Figure 5. Snail measurement methods, A) Measurements of snail length and width using Q-Capture; B) using caliper.

Chapter 2: Heavy metal concentration of *E. californiensis* at three life stages and their respective environment

Six randomized sediment samples were collected from site X as well as bird feces, snails, and killifish. The fish were collected using a minnow seine technique (figure 6), and were frozen within 2 hours. The snails were kept cool and transported to San Diego where they were housed in the aquarium with access to Fresh Sea Water (FSW) at 21 °C .



Figure 6. Minnow seine for killifish from channel, site X.

Chapter 3: Heavy metal concentration of Parasitized versus un-parasitized snails of same population

The same snails collected from chapter 2 were used. The parasitized snails were detected (refer to sample preparation, chapter 2) and separated from parasitized snails for comparison.



Figure 7. A) Un-parasitized snail digestive tract
Dissecting microscope magnification: 30X

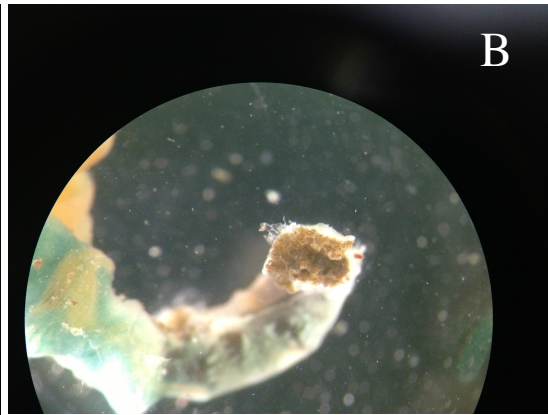


Figure 7. B) Un-parasitized digestive tract
Dissecting microscope magnification: 60X



Figure 8. A) Parasitized snail digestive tract
Dissecting microscope magnification: 30X

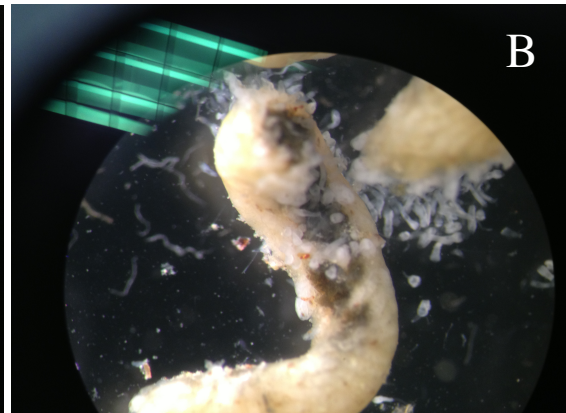


Figure 8. B) Parasitized snail digestive tract
Dissecting microscope magnification: 60X

2.3. Sample preparation

Chapter 1: Heavy metal concentration in Sediments and snails

The snails were anesthetized in Magnesium Chloride (MgCl_2), 100% for 30-60 minutes prior to dissection (figure 9). The shell was cracked using hammer and the last ring (largest ring) and first ring (smallest ring) were collected. The snail's body was then frozen in a -20°C freezer for dissection later. The metal forceps and scissors were acid washed using 1%, diluted Nitric Acid, followed by Methanol wipe for each individual to prevent contamination from the dissecting tools and cross contamination between samples. A section of foot, mantle, and digestive tract, was dissected from each individual and was placed in a labeled container. The samples were then put in the dryer oven at 46°C for two nights to dry.

One dried, the samples were transferred to labeled copolymer plastic vial and weighed using win-wedge software (reference the make and model of the scale?). Shell samples mass ranged from 0.01000-0.02626 g and tissues from 0.00001-0.00292g. Next, 250 μ l of 70% concentrated Nitric acid was added to each sample and the weight of the acid was recorded. This process is called acid digestion and the snail tissue and shells were dissolved over the two days at room temperature, 23 °C . A 200 μ l aliquot of digested acid solution was transferred to labeled polystyrene tubes. The full 250 μ l was not used to avoid inclusion of solid and undigested residues left at the bottom of the digestion tube. The mass of the aliquot was recorded and then mixed with 2.2 mL of MilliQ-water (filtered and deionized water), and weighed again. The samples were diluted to 7% concentration, and analyzed with an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Three blanks with the same concentration of acid and MilliQ-water were used and the instrument was calibrated before running the samples.



Figure 9. Anesthetizing snails in $MgCl_2$

Chapter 2: Heavy metal concentration of *E. californiensis* at three life stages and their respective environment

A. Sediments

The sediments were dried for 12 days at 10 °C and later weighed. Sample masses ranged from 0.010000 g- 0.03460 g. Subsequently, 3.5mL of 1M (3%) Hydrochloric acid (HCl) was added and the samples were placed on the shaker at room temperature, 23 °C , for 24 hours. After five days and when the sediment particles settled, 3 mL of supernatant was transferred into labeled ICP tubes. Three blanks with the same concentration of acid and MilliQ-water were used and the machine was calibrated before running the samples.

HCl is the preferred acid used for the sediments instead of Nitric Acid since the goal is to measure the bioavailable amount of heavy metals, leachable concentration that can enter an organism. HCl will mimic the conditions, enzymes and organic acids in the stomach and small intestine fluids of the snail after ingesting the sediments. The acid concentration used also prevents the digestion of sediment to the point of extracting minerals from the inorganic components of the silt.

B. Bird feces

The bird feces were dried for 15 days and average of 0.03g dry weights for each sample was recorded. 280 μ L of 70% nitric acid was used, and weighed for acid digestion and the samples sat under the hood for 8 days followed by microwave digestion (80 °C for 2 hours). Microwave digestion was necessary to dissolve bird feces material that remained undigested after the preliminary 8 days. A diluted nitric acid (250 μ l HNO_3 and 250 μ l MilliQ-water) was then added to the bird feces samples, weighed, and left over

night. 3.0 mL of MilliQ-water was then added and the samples incubated for and additional 6 days (final volume, 3.78 mL). 3.0 mL of supernatant was then extracted and transferred to the ICP tubes.

*Data are currently processed for analysis.



Figure 10. The first transmission, snail contaminated with bird feces most likely infected with *E. californiensis*.



Figure11. Channel near F2 site with large snail.

*Snails are currently in the process of being dissected.

C. Keeping and condition for the snails

The snails collected from SB were kept on a plastic grid with flowing water underneath it, in the aquarium at 69-70 °C and 70-90% humidity.

They were dipped in the cool fresh seawater for 20 minutes every 2-3 days to mimic the high tide exposure.

The snails have not been dissected yet since more cercariae samples are needed and preferred to be collected. The snail dissection and sample preparation will be similar to chapter 1 (refer to chapter 1 for more information).

D. Cercariae shedding

The snails were dried for 2 days prior to shedding. They were then placed in a labeled small beaker with a small amount of ASW, exposed to 60 W light for minimum 2 hours. The light was turned off for 15-30 minutes every hour to keep the water temperature under 35°C to prevent snails from overheating and dying (Ecological parasitology lab UCSB, personal communication). This light and heat stress method releases the cercariae in the snails so they could get identified using (Martin, 1972 and Hechinger, 2013) key.

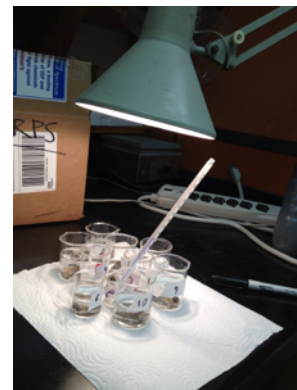


Figure12. Shedding snails for cercariae using heat and light as stressor.

Snails with the cercariae, *E. californiensis* were separated and kept in a labeled mesh bag. The cercariae released from the individual snail were pipet out in labeled tube and put in the drier so the ASW could evaporate, leaving the *E. californiensis* (figure 13).

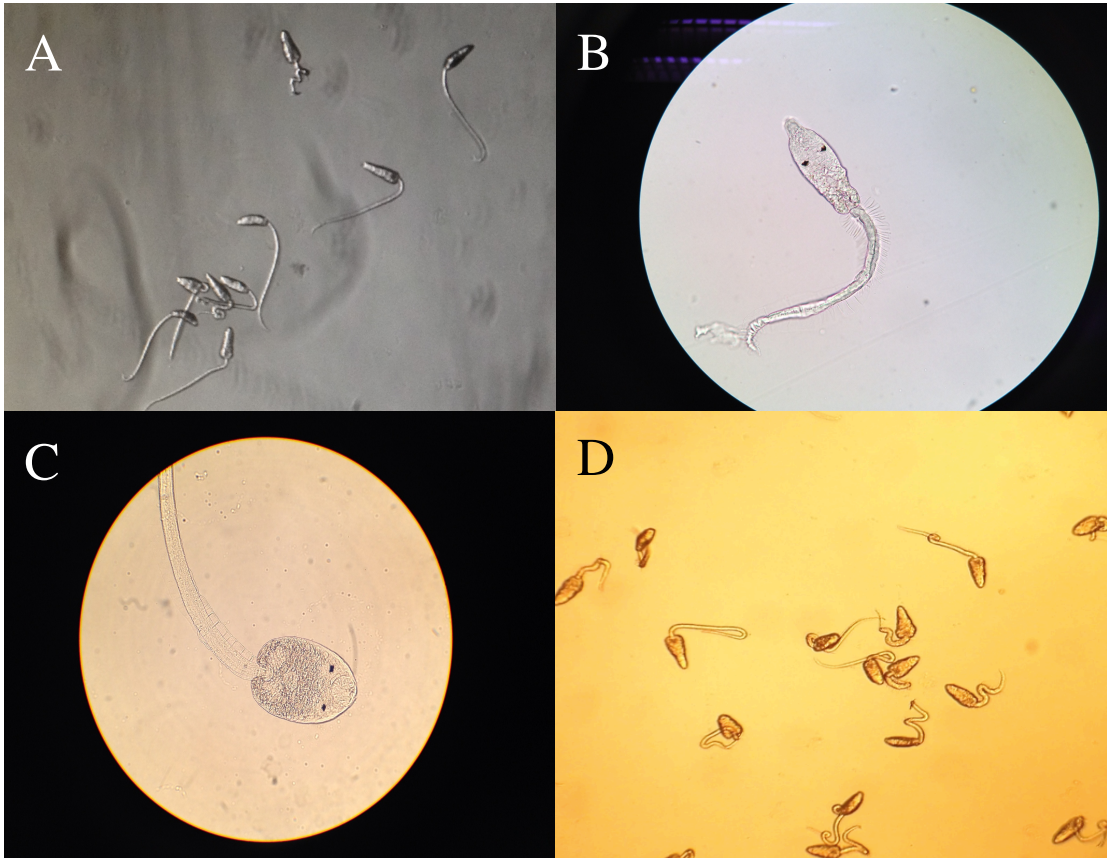


Figure 13. *E. californiensis* magnified under compound microscope. A and D) Picture taken under 200X magnification. B and C) General physiological characteristics of *E. californiensis* entails of finned tail, eye spots, oral sucker, with pen gland distributed from anterior of genital anlagen laterally around excretory bladder to posterior of the body, size (200 μ m), (Martin, 1950).

About 80 snails were induced to shed over two weeks time period to collect 9 highly parasitized snails with a significant burden of *E. californiensis*. The infected snails were shed twice a week to collect enough cercariae for dry weight of 0.01 g, or close to that weight if possible, in order to run for ICP. Six snails will be chosen based on the highest rate of infection for the further analysis and assay.

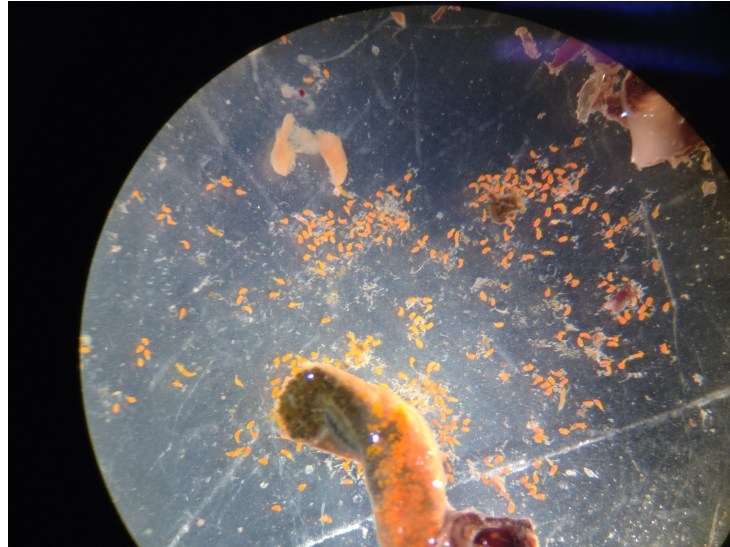


Figure14 .*E. californiensis* rediae, sac shaped structures devoid of ambulatory process (Martin 1950) with orange membrane
Magnification under dissection microscope: 30X

E. Killifish

After the frozen fish samples were room temperature, they were dissected. The forceps and scissors were acid washed, using a dilute nitric acid. The surface of Styrofoam used for dissection was wiped with methanol. Samples from liver, gills, mussel, brain, and blood brain barrier (BBB) was taken and placed on microscope slides to dry. The BBB samples were checked under the compound microscope for presence of metacercariae cysts. Figure16. The samples were dried for 10 days and weighed.



Figure15. Brain of the killifish
Magnification under dissection microscope: 250X

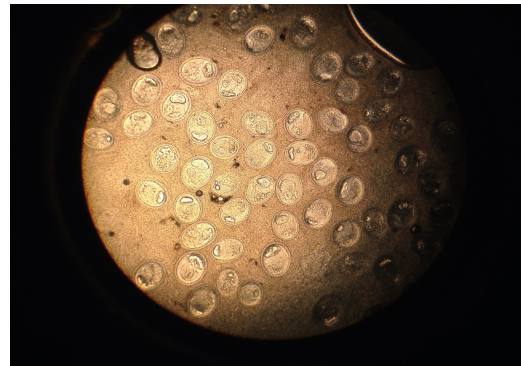


Figure 16. Metacercariae cysts collected from the blood brain barrier. Size 100 μ m

*The samples are in progress and will be analyzed in July, 2013.

The cyst sacs that cover the metacercariae are beneficial in protecting the parasite while it completes its life cycle. The sacs allow the metacercariae to survive the acid wash of the bird stomach in order to settle in the digestive tract and metamorphose to adult, releasing the eggs that fall with the feces (Hechinger, personal communication).

Chapter 3: Heavy metal concentration of Parasitized versus un-parasitized snails of same population

The un-parasitized snails will be dissected and prepared similar to the snails from chapter 1. The data and body load from different tissues will be compared and analyzed with the parasitized snails data from chapter 2. This chapter will be completed in July 2013.

Areas with large bird populations are likely to have correspondingly high prevalence of parasitized gastropods (James, 1968; Irwin, 1983).

2.4. Statistical analysis

Chapter 1: Heavy metal concentration in Sediments and snails

Descriptive statistics such as boxplot, regression, and histogram will be used first to analyze the data for the 20 heavy metals and to compare distributions between several groups or sets of data. This will allow us to show the distribution of the data set at a glance and find patterns before further analysis.

Chapter 2: Heavy metal concentration of *E. californiensis* at three life stages and their respective environment

The effect of environmental pollutants can vary at different developmental stages of the parasite, because the characteristics of larvae and adult are significantly different; they have unique hosts, different physiology, different physical forms, and varied longevity (Sures, 2004). For example, the toxicity level of Zinc and Cadmium can effect survival (Morley et. al., 2001) and tail loss in the free-swimming cercariae of specific trematodes (Morley et. al., 2002). These changes in the swimming behavior of cercariae are important in the transmission to the next host, fish, and reduce their susceptibility to parasitism (Cross et al., 2001). Therefore, the effects of toxins on parasites can alter their role in an ecosystem, affecting host populations, biodiversity, and food webs, with potential cascading effects throughout the ecosystem (Hudson et. al., 2006).

Parasites can be used as sentinel organisms since some species have the ability to accumulate heavy metals in their tissue (Sures & Siddall (2003), Sures et. al., (2003)). They are valuable environmental pollution effect indicators if the changes of populations or communities are monitored. Additionally, they can be used as long-term sustainable monitoring of ecosystems (Sures, 2004).

This will allow me to assess whether the pre-exposure of the parasite to contaminants from the tissue of the host, sediments, water, and bird feces, give the organism a resistance or less susceptibility to exposure of the toxin. This is done by running an assay for each sample using ICP-OES.

*This section is in progress and will be analyzed in July 2013.

Chapter 3: Heavy metal concentration of Parasitized versus un-parasitized snails of same population

This study will examine the inter-relationship between mollusk, heavy metal concentration and trematode parasite. Based on a study done on *Littorina littorea*, it was shown that the infected periwinkles had significantly lower levels of iron, copper and nickel than uninfected periwinkles. The underlying reason for the difference is thought to

be pathological effects of digenean infection on the hepatopancrease of the organism, storing the HMs in membrane-bound insoluble granules occupying up to 20% of the volume of hepatopancrease cells (Bebianno and Langston, 1995).

*This chapter is under process and will be completed in July 2013.

3. Results

Chapter 1: Heavy metal concentration in sediments and snails

The sediment, snail shell and tissue samples were measured for 20 heavy metal concentrations: Ag, Al, As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Pb, Se, Sr, Ti, V, and Zn. Few elements that show the trends more clearly for the questions asked are discussed below and shown in graphs for better understanding.

A. Sediments concentration

Hypothesis:

The bioavailability of heavy metal concentrations in the sediments changes within different sites in the marsh.

Results:

The results show that there is no significant variability in heavy metal concentrations in the sediments within the different sites in the marsh (figure 17).

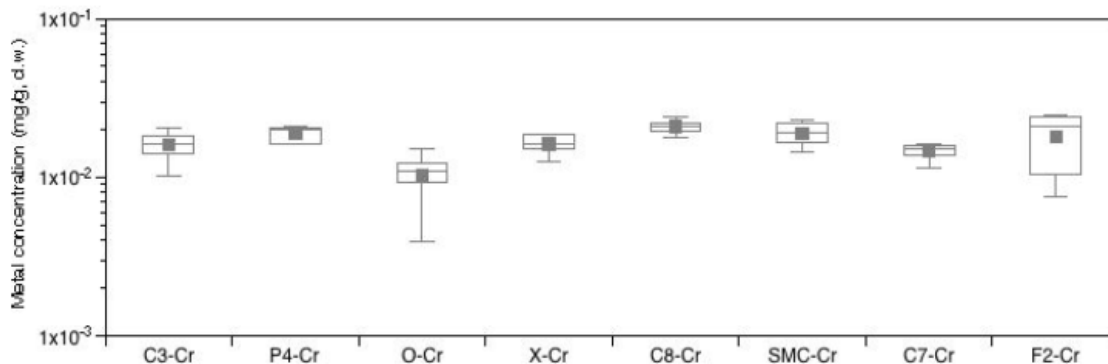


Figure 17. Concentration of Chromium over eight sites in the marsh shows similar range.

B. The C3 and C7 ring shells heavy metal concentration

Hypothesis:

The heavy metal concentrations in different parts of the shell vary.

Results:

The concentration of heavy metals in different parts of the shell, oldest ring and youngest ring, within the same population and different populations do not vary and have a similar range (Figure 18). However the Iron concentration in the shells from C3 population show a significant concentration of ten times higher than the C7 population (Figure 19).

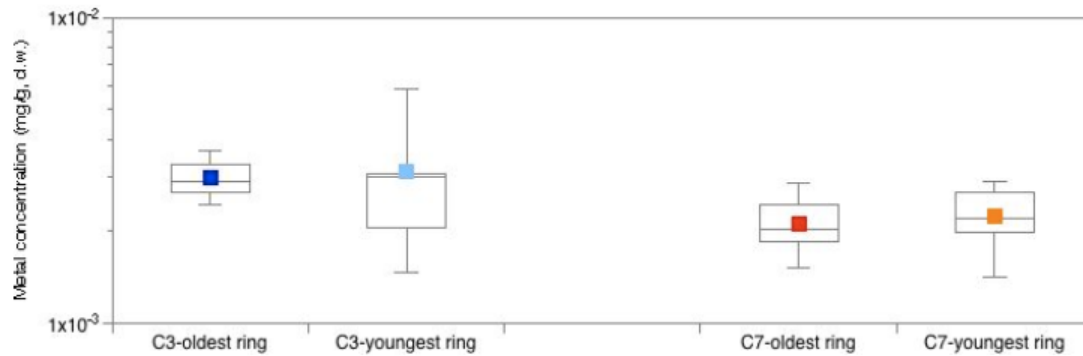


Figure 18. Concentration of Cadmium from the oldest and youngest ring, between the C3 and C7 populations shows a relatively similar range.

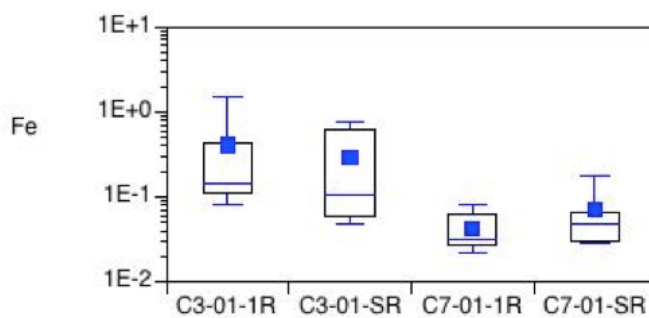


Figure 19. The concentration of Iron in shell from the C3 population is greater than the C7 population.

C. The C3 and C7 snail body tissues concentration

Hypothesis:

The snail tissues from population C3 and C7 have different heavy metal concentrations.

Results:

The snail tissues from the two populations show a significant difference in heavy metal concentrations. The concentrations in the C7 population are ten times higher than the C3 population. Additionally, the concentrations in the different tissues of the snail decrease from foot > digestive tract > mantle. The elements Aluminum and Arsenic are greatly enriched in snail tissues.

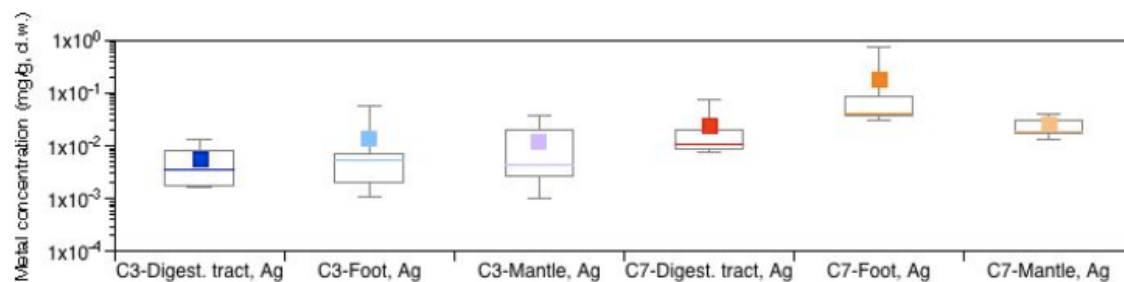


Figure 20. Silver concentrations in snail body tissues from C3 and C7 population, where C7 concentrations are higher than C3. The tissues accumulate at different rates, foot > digestive tract > mantle.

The concentration of heavy metals from the digestive tract provides information about the heavy metal levels from the snails diet and ingested sediment. The levels from

the foot will give a better understanding about the heavy metal concentration in the sediment and water. Since the snails' skin has pores that can uptake chemicals from its surrounding environment, direct contact with sediment and water, allows for higher bioaccumulation of trace metals (figure 20). Furthermore, the mantle is the organ responsible for producing the shell and depositing heavy metals in it. It should be noted that the levels in the shells are clearly lower than the mantle, supporting that the shell has a saturation point. This along with muscle levels may represent a potential biomarker for environmental metal pollution.

Moreover the concentrations for the tissues from the C3 site were below detection point for the following elements: Cd, Cr, Pb, Ti, and V in addition to the digestive tract and mantle for element Se.

Also, the pattern of valley shaped hyperbola for the C3 population tissue concentration and mountain shaped hyperbola for the C7 samples is seen for Al, Cu, Fe, Se, and Zn, as well as, below detection pattern for the C3 population and mountain hyperbola for the C7 population that repeats for the elements: Cd, Cr, Pb, Ti, and V.

4. Discussion

The distribution of heavy metals in different sites within the marsh were relatively similar and in the same range and did not show significant variation. The expected locations with higher concentration of metals (C7, F2, SMC) due to geographical location and distance from the point source had relatively similar levels as the predicted lower concentrated sites (X, O, P4, C3, C8), located in the middle of the marsh and away from a point source, as well as the mouth of the marsh (figure 3). This could be due to several factors such as similar grain size, regular tidal flushing, and saturated sediments where the trace metals have precipitated on the grains of sand (Deheyn, personal communication).

The heavy metal concentration in the oldest ring of the snail shell (smallest ring), compare to the youngest ring of the shell (widest ring), within the same individual, did not exhibit a difference. This could be explained by the fact that the shells are saturated, possibly from heavy metals excreted from the mantle and after being stored in the shell, it cannot accumulate more.

Additionally the similar concentrations between the shells was observed within the two populations from site C3 and C7, where the shell rings concentration between the populations were at the same level for all metals. The example of element, Cadmium is used in figure 18 to demonstrate this.

However, the Iron concentration between the populations varied. The concentrations for all trace metals were in the similar and very close range except Iron. The load in the first and last ring from population in the C3 site was higher than the level in population from the C7 site (figure 19). Higher abundance of algae in the C3 site and presence of it on the shells could explain this difference. Since Iron, is one of the component elements making the Chlorophyll (Boekema et al., 2001).

Even though the distribution of metal around the marsh sediment did not show the trend expected, the hypothesis that the C7 site is more polluted than the C3 site due to its location next to a point source, Franklin Creek, is supported by the snail tissue samples. The three body tissues levels, digestive tract (D), foot (F), and mantle (M) from the C7 site were all higher than the levels in the C3 site (figure 20). Since the sediment

concentration for both sites is lower than the tissue load, it is likely that the snail uptake the heavy metal through the water and ingestion of algae, since algae in marine and estuaries accumulate significant concentrations of heavy metals in their tissues (Evans et al., 2001) versus the sediment. This is likely due to presence of Franklin Creek as a source of heavy metals and could also be confirmed after the gill analysis from the killifish since the gills are in direct contact with the water and accumulate heavy metals. This bio-accumulative property renders mollusks useful monitors of metal pollution (Evans et al., 2001).

It is important to note that the heavy metal accumulation in snail tissue is influenced by the metabolism of the snail as well as the availability of the different metals and therefore slightly selective (Huanxin et al., 1999).

4. 1. Heavy metals in nature

Even though marine organisms do need very small quantities of heavy metals, for instance copper forms part of haemocyanin, the respiratory pigment of crustaceans (Crabs, prawns etc), large quantities of copper as well as other heavy metals, will inhibit their vital functions and will severely impair or kill them (Clarks, 2001).

In general, the effects of heavy metals toxicity can be classified into three main categories (Ochiai, 1977):

- the blocking of essential function groups of the biomolecules (e.g. proteins, enzymes)
- the displacement of a metal ion from a biomolecule
- the modification of the structure of the biomolecules in space which is very important for their function

There are only a few existing studies concerning the effect of heavy metals at the level of the ..ecosystem... According to Connell & Miller (1984) their impact can be said to show the following general characteristics.

Mollusks, crustacean, and oligochaetes are most vulnerable to Zinc. Despite the fact that algae show a certain degree of inconsistency toward the effect of heavy metals, it is important to note that in general marine plants and invertebrates are more resistance than fish and less influenced by heavy metals. The adaptations caused by their presence mostly entail a decrease in the number of species, the complete elimination of sensitive species, and a corresponding reduction in the number of individuals in the species that survive. Some species are even able to develop resistance mechanisms and therefore accumulate higher metal concentrations. This resistance of invertebrates toward heavy metals makes them less adequate organisms for environmental assessments especially in dose response tests. Therefore, the health assessment routines need to be modified accordingly and more sensitive organisms such as parasites or parasites within the invertebrates could be a valuable resource in monitoring and providing environmental assessments in the near future.

The aquatic environment determines fate of heavy metals in natural water, the molecular weight of organic compound in the water column, bed sediment movement, suspended solids, and sedimentation (Clarks, 2001).

4. 2. Input routes

Besides the natural inputs from erosion of ore-bearing rocks, wind-blown dust, volcanic activity, and forest fires (Clark, 2001), additional quantities of metals are being added to estuaries and coastal regions by human activities from industrial effluents, sewage, ship traffic, and from atmospheric pollution. For example high concentrations of zinc come from the wastes from synthetic fiber production, mining, electroplating or from sewage (Bryan, 1971).

- Atmosphere input

An important way that some metals enter the sea is through the atmosphere, where there are large natural imputes of metals like aluminum in wind-blown dust derived from rocks and shale's, and mercury from volcanic activity and degassing of the earths crust. Metal discharged to the atmosphere may exist as a gas (Hg, Se, or B) or Aerosols (most other metals) and are deposited by gas exchange at the sea surface, by the fall out of particles, dry deposition, or are scavenged from air column by precipitation, wet deposition (Clark, 2001).

- River input

Most rivers and creeks (figure 3) are the major contribution of metals to the sea and in this case the marsh, especially if agricultural lands are the upper basin. The amount of metal discharged is higher when the river passes through urban areas by human waste and discharges. Additionally sedimentation in estuaries traps large quantities of metals, which gets adsorbed on to the sediment particles and gets carried to the bottom. The sediments in industrialized areas with major ports contain larger amounts and more waste discharge due to dredging of shipping channels (Clark, 2001).

- Road runoff contributing to heavy metal pollution

Today, roadways and automobiles are considered to be one the largest sources of heavy metals. Zinc, copper, and lead are the three most common heavy metals released from road travel, accounting for at least 90 percent of the total metals in road runoff. Other metals such as nickel and cadmium are also found in road runoff and exhaust at smaller amount (Moore, 1947). However, the lead concentrations have been decreasing (Zevenhoven and Kilpinen, 2001) since leaded gasoline was discontinued in 1995 (EPA, 2013).

Around half of the zinc and copper source to the environment from urbanization is from automobiles. Brakes release copper, while tire wear releases zinc. Motor oil also tends to accumulate metals when it comes into contact with surrounding parts as engine runs; therefore oil leaks become another source by which metals enter the environment (Clarks, 2001).

Most heavy metals bound to the surfaces of road dust or other particulates and during precipitation they will either become soluble or swept off the roadway with the dust, entering the soil or are channeled into a storm drain.

Since most metals are cations (ie. Zn, Co have 2+ charges), the negative charges of the soil particles tend to attract and bind to the metal positive charges and prevent them from becoming soluble. The soluble form of metals is thought to be more dangerous since its easily transported and more and more readily available to plants and animals.

Common metals in road runoff from highway next to the marsh

1. Lead: leaded gasoline, tire wear, lubricating oil and grease, bearing wear
2. Zinc: tire wear, motor oil, grease, brake emissions, corrosion of galvanized parts
3. Iron: auto body rust, engine parts
4. Copper: bearing wear, engine parts, brake emissions
5. Cadmium: tire wear, fuel burning, batteries
6. Chromium: air conditioning coolants, engine parts, brake emissions
7. Nickel: diesel fuel and gasoline, lubricating oil, brake emissions
8. Aluminum: auto body corrosion

(Heavy metal pollution is more common than you think, 2013)

4.3. Parasites as biomarkers for the assessment of marine pollution

One way to assess the health of an ecosystem is the use of effect indicators or biomarkers to assess marine pollution (Everts, 2013). Parasites could be used as good proxies for monitoring the health of environment (Sures, 2004), as well as improving the ecological health assessments. The use of biomarkers lies as a foundation for evaluation of ecosystem health and allows for early detection of biological changes due to chemical pollutants exposure. These introductions could have toxic or chronic effects and in long term may result in long-term physiological disturbances.

Previous research has shown the risk of results falsified and being lower when the organism used has been parasitized (Moller, 1987). This especially brings attention to the bio-monitoring programs and that they should take into account the influence of trematode infections on the heavy metal concentrations of their gastropod hosts (Evans et al., 2001).

- **Chemical monitoring**

The chemical monitoring of organisms mainly evaluates the presence and concentrations of pollutants in tissues by chemical analysis. Mussels such as *Mytilus edulis* and other marine bivalves, as well as invertebrates are widely used in monitoring programs as indicator species, or sentinel organisms due to their sensitivities to exposure and biological effects of metals and organic pollutants (Everts, 2013). The reason why mollusks are mostly used in monitoring programs is due to their wide geographic distribution, abundance and accessibility in the field as well as in aquaculture.

Even though some fish such as *Mullus sp.*, *Platichthys flesus* L., *Zoarcetes viviparus*, *Perca sp.* are widely used in monitoring programs, they are not used as often due to the fact that sampling is expensive. The importance of their use however is linked to their position in the trophic chain and high commercial value. It is important to note that parasites as well have a relatively high trophic level in the food web, supporting why they could be valuable source in environmental monitoring (Thompson et al., 2005).

- Dose response

In contrary to chemical monitoring of organisms, biomonitoring methods assess not only the presence of pollutants but what is more important, the response of animals to these pollutants by evaluating the biomarkers. For example the parameters that show their effects at the molecular, cellular, organ, and organism level. However, the use of these biomarkers in monitoring should not replace chemical monitoring or population studies, instead, they should be integrated in order to determine the toxic effects of pollutants (Everts, 2013). This will display the presence of low and sub-lethal concentrations. This could be assessed in the following section, where the survival rate and longevity of cercariae is determined by dose response to heavy metal.

5. Future Direction

We could evaluate how varying the concentration of a chosen heavy metal in a controlled lab experiment affects the longevity of cercariae.

This will be a concentration-dependent effect over time response, or so called dose response experiment. This could be determined by the survival rate at acute vs. chronic toxicity of the cercariae. This data from the dose response as well as the body load levels will provide information in order to determine if *E. californiensis* gains resistance to the pollutant caused by previous exposure.

Additionally it will help make predictions of how the free-swimming stage of the trematode larvae, cercariae, could be affected by the environmental contamination (Hudson et. al., 2006). These will impact the *E. californiensis* transmission to fish, and fish parasites could potentially be used as indicators of biological integrity or anthropogenic impairment of waters (Pietro, 2001).

For example the toxicity level of Zinc and Cadmium can affect survival (Morley et. al., 2001) and tail loss in the free-swimming cercariae of specific trematodes (Morley et. al., 2002). These changes in the swimming behavior of cercariae are important in the transmission to the next host, fish, and reduce their susceptibility to parasitism (Cross et al., 2001).

The heavy metal(s) (for example, cadmium and zinc) will be chosen based on the preliminary analysis. Mixture of cercariae will be placed in three different concentrations of pollutant(s). One concentration will be the same level as the snails' tissue load for a control sample, one concentration will be higher, and one lower than the level the cercariae naturally experience. Additionally a solution of seawater will be used as a control experiment. The concentrations will be determined after the first section of the project is analyzed. According to a previous experiment, a 0.1ug/L, 10ug/L, 100ug/L of zinc concentrations is appropriate (Morley et. al., 2003). The temperature and pH of each concentration will be measured and controlled since temperature and pH affect the cercariae (Morley et al., 2001). Three pipet samples of known volume (for example 500 μ l) from the mixture of cercariae and solution will be placed in plastic containers for behavioral observations.

Swimming behavior of cercariae will be monitored over a 5 hours time frame as the first recording in the 0 minutes, 20minutes, one hours, then every hour time frame. The species of the trematode determines the life expectancy of the cercariae since some

species live up to 23 hours (Pietroock et. al., 2001). The contaminant uptake of the cercariae in these samples will then be measured and calculated from each trial.

6. Supplementary data

This section provides additional graphs and findings generated, which could provide more information about the system but is in the process of further analysis. This will be completed and added to the appropriate chapters once finalized.

6.1. The C3 versus C7 population for Silver concentrations in snail tissues

In the study of size versus snail tissue concentration, a trend could be seen for most metals tested. Silver is used as an example to show this pattern. The concentration of heavy metals in digestive tract decrease with an increase in length (figure 21a), for mantle it remain almost the same with a small gradual decrease (figure 21b), and for the foot tissue there is a slight decrease in the C3 population with an increase in length versus the C7 population which the concentration remain the same (figure 21c).

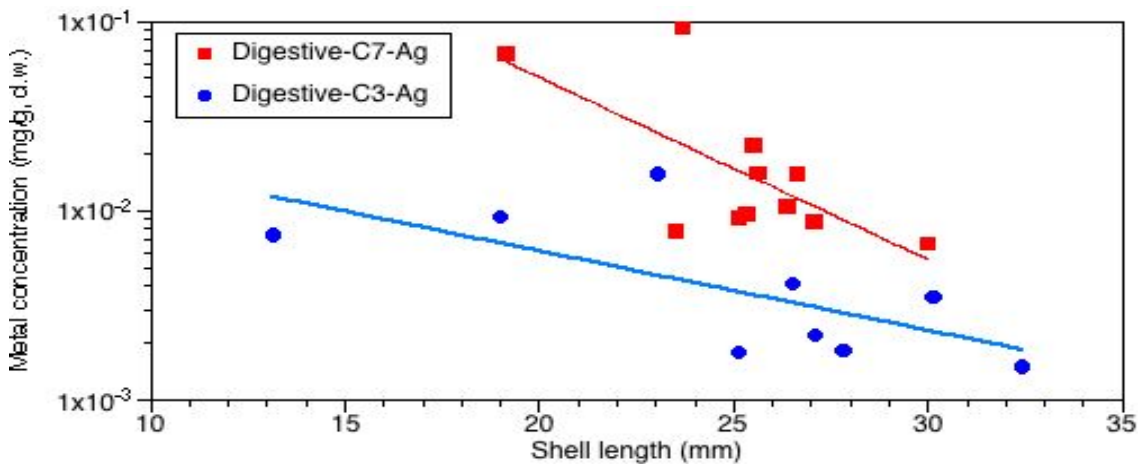


Figure 21a. Snail length versus digestive tract's Silver concentration.

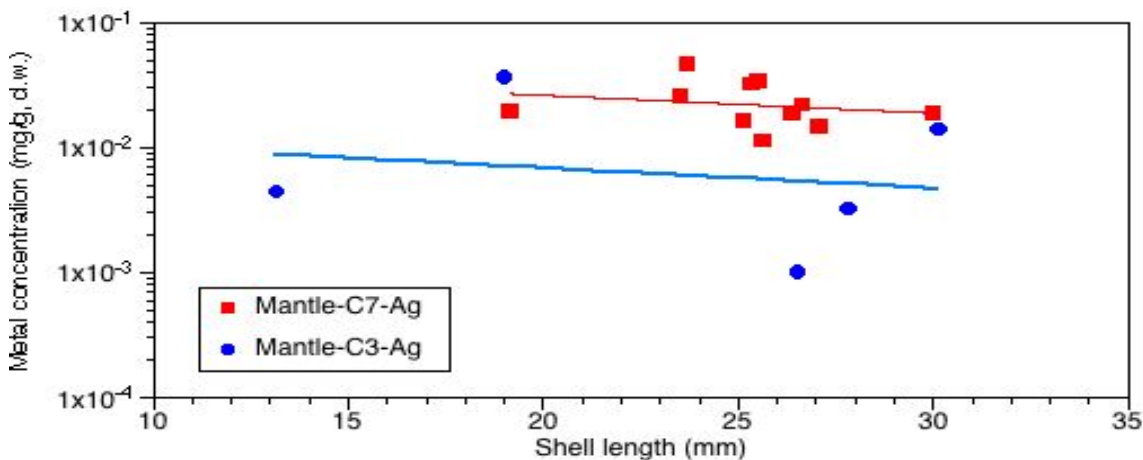


Figure 21b. Snail length versus mantle's Silver concentration.

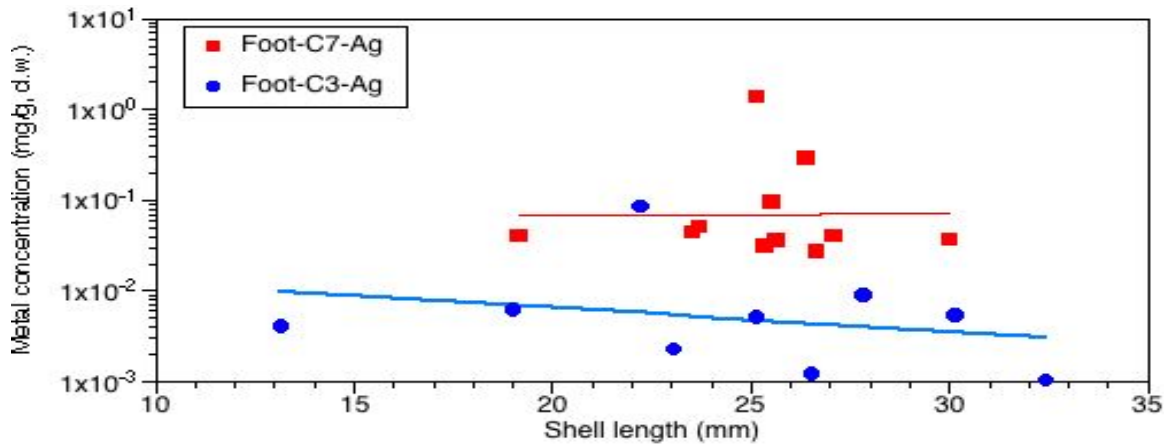


Figure 21c. Snail length versus foot's Silver concentration.

6.2. The C3 versus C7 population for Ag concentrations in snail shells

A trend could be seen as the length of the snail increases, the concentration of heavy metals in the shell for population C3 remains the same. For population C7 there is a slow decrease in heavy metal concentration for the youngest ring, largest ring, with an increase in the size (figure 22a). Additionally, this relationship is reversed for the heavy metal concentration in the oldest ring for population C7 in which it increases with the snail size (figure 22b).

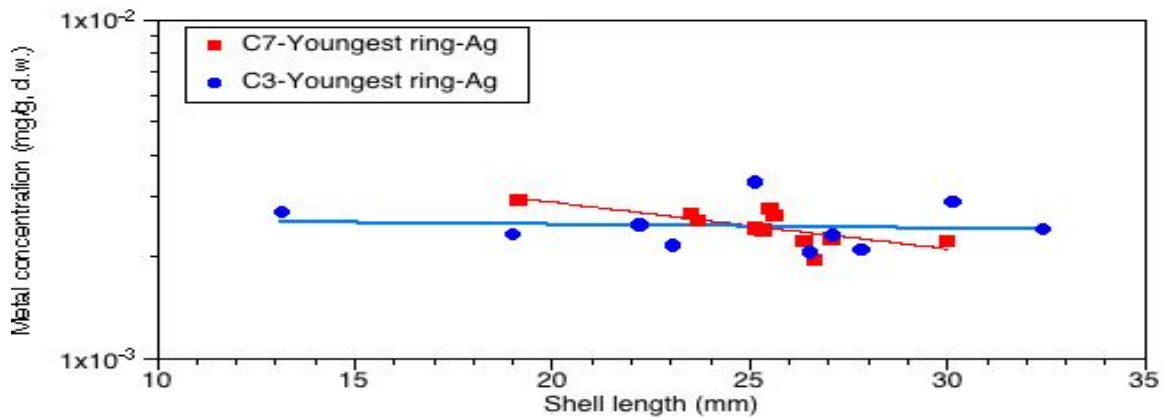


Figure 22a. Snail length versus shell's youngest ring Silver concentration.

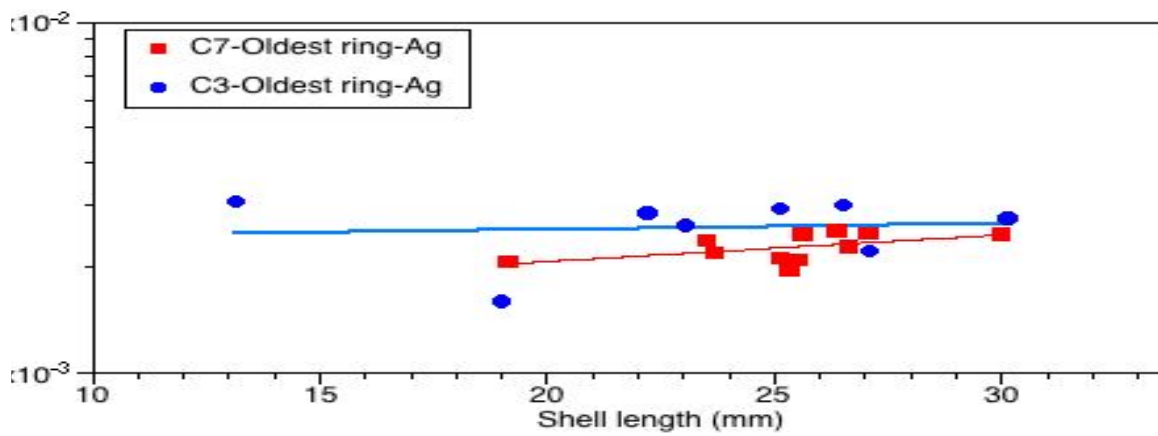
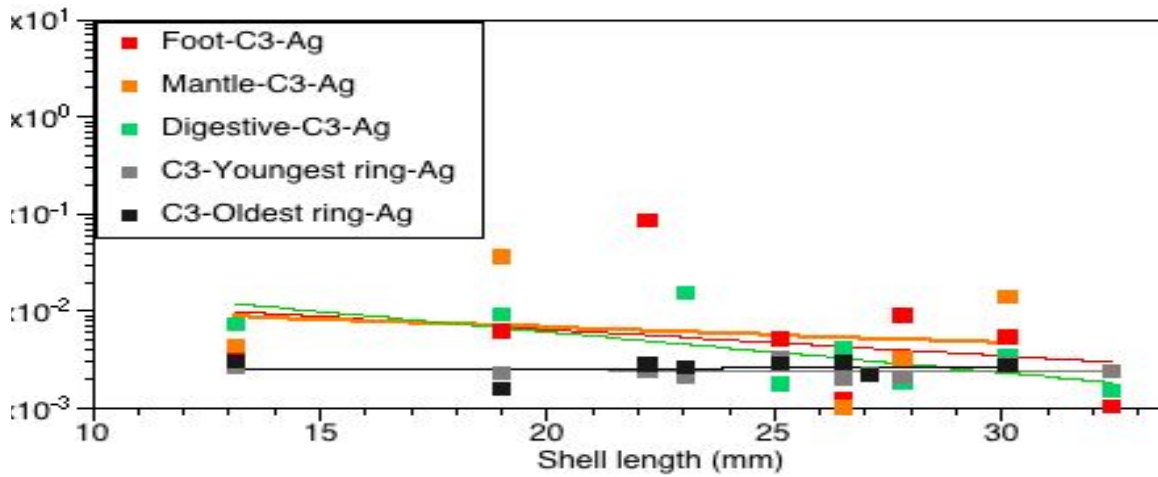
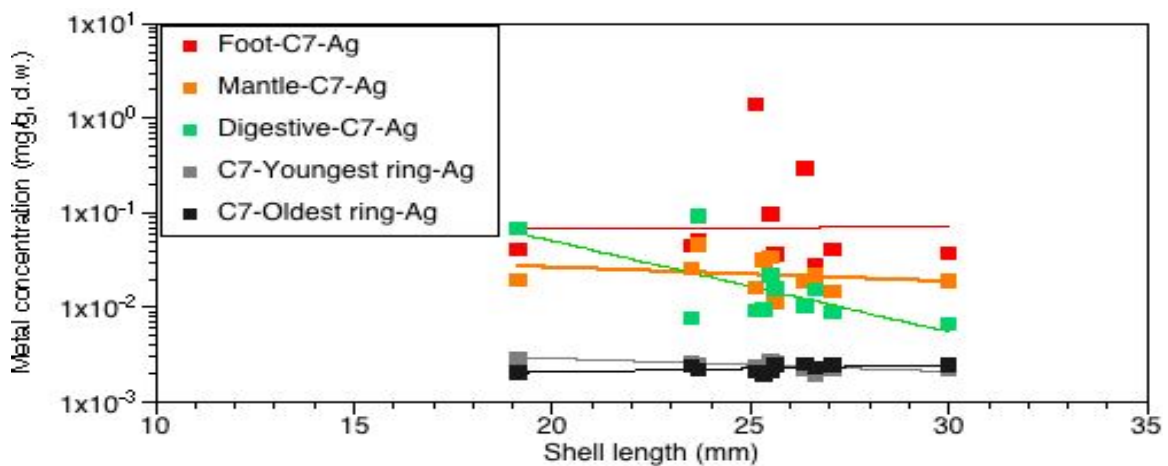


Figure 22b. Snail length versus shell's oldest ring Silver concentration.

The combination graph for the C3 tissues as well as shell concentrations below:



The combination graph for the C7 tissues as well as shell concentrations below:



6.3. Heavy metal concentration in cercariae versus digestive tract

From the experimental samples ran, it could be observed that the heavy metal concentration for most metals in cercariae tissue is relatively higher or at the same level as the average of snail digestive tract levels (figure 23). Note that the data for cercariae is from one replicate, from an individual snail in the C7 population and is compared to the average of digestive tract levels from 11 replicates in the same population. However, it is worth mentioning that cercariae tissue can accumulate a significant amount of heavy metals compare to tissues. This information will be very beneficial once the data from chapter 2 is generated and finalized. The data in progress will be finished by the end of summer.

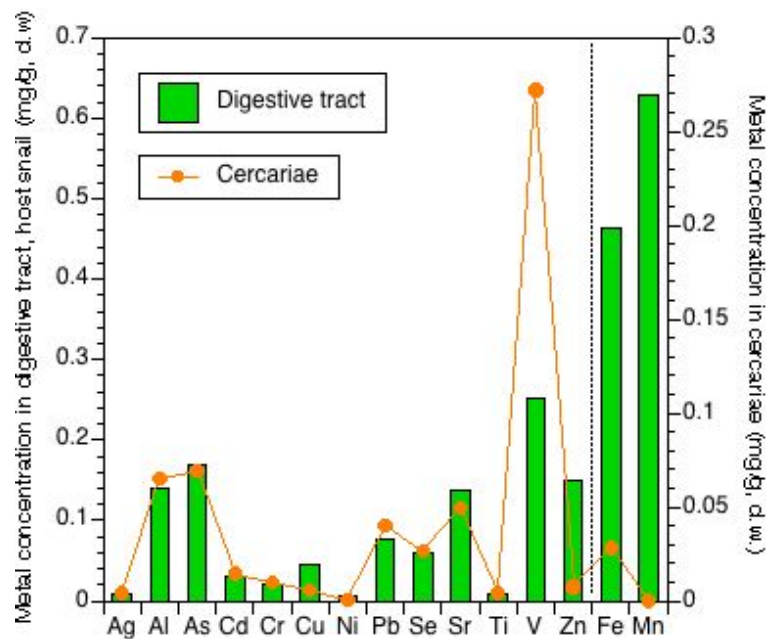


Figure 22. Metal concentration in cercariae correlated with metal concentration in digestive tract

7. Conclusion

Salt marshes are one of the most productive ecosystems and are usually the last barriers between the coastal oceans and developments (Cao et al., 2006). Because they function as buffer zones by blocking, stabilizing, and removing pollutants (Cao et al., 2006) and excessive nutrients they are critical to maintaining healthy coastal ecosystems that in turn are critical to human health (Niemi et al., 2004). Heavy metals can effect and change the density, diversity, community structure, and species composition of the populations (Moore and Ramamoorthy, 1984). Parasites could be used to monitor the health of marine ecosystems and identify the “hot spots” of chemical contamination along the nations coastline (Huanxin et al., 1999).

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