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Determining Heavy Metal Concentrations in the Blueband Hermit Crab (Pagurus samuelis)

Tyler dos Santos

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LOMA LINDA UNIVERSITY
School of Medicine
in conjunction with the
Faculty of Graduate Studies

Determining Heavy Metal Concentrations in the Blueband Hermit Crab (*Pagurus samuelis*)

by

Tyler dos Santos

A Thesis submitted in partial satisfaction of
the requirements for the degree
Master of Science in Biology

June 2019

Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master's.

, Chairperson

Stephen Dunbar, Professor of Biology

David Hessinger

David Hessinger, Professor of Physiology

Danilo Boskovic

Danilo Boskovic, Assistant Professor of Biochemistry

Wolff Kirsch, Professor of Biochemistry

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ABBREVIATIONS

Pb	Lead
Cu	Copper
Cd	Cadmium
Cr	Chromium
Zn	Zinc
MP-AES	Microwave Plasma – Atomic Emission Spectrometer
Ni	Nickel
NS&T	National Status and Trends
Hg	Mercury
Sn	Tin
Ag	Silver
V	Vanadium
Mo	Molybdenum
Fe	Iron
Mn	Manganese
LC_{50}	Lethal concentration for 50% of the population
K_m	Michaelis constant
K_i	Michaelis constant of inhibition
PLA	Port of Los Angeles
Y	Yttrium
MRG	Marine Research Group
LLU	Loma Linda University

SD	Standard Deviation
Ww	Wet weight

ABSTRACT OF THE THESIS

Determining Heavy Metal Concentrations in the Blueband Hermit Crab (*Pagurus samuelis*)

by

Tyler dos Santos

Master of Science, Graduate Program in Biology
Loma Linda University, June 2019
Dr. Stephen G. Dunbar, Chairperson

Although heavy metal concentrations fluctuate naturally in the environment, anthropogenic sources of heavy metal concentrations can mask and override natural fluctuations. Large anthropogenic metal concentrations can lead to deleterious effects in organisms, such as malformation or death. Indicator species can be used to determine environmental concentrations of certain metals to help understand the impacts of anthropogenic sources of heavy metal concentrations. The aim of this study was to determine if *Pagurus samuelis* may play a role as an indicator species by analyzing specimens for Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), and Zinc (Zn) found in tidepool seawater in locations along the Southern California coast. Seawater and *P. samuelis* samples were collected from both Cabrillo Beach and White Point Beach locations and analyzed using Microwave Plasma – Atomic Emission Spectrometer (MP-AES). Pb concentrations were higher at Cabrillo Beach in seawater, while at White Point Beach, Zn concentrations were higher than at Cabrillo. All other metals were not significantly different between locations. In hermit crab samples, Cd was higher in crabs collected from Cabrillo Beach and Cr was higher in crabs collected from White Point Beach. In comparisons of seawater with hermit crab samples, seawater had higher concentrations of Zn and Cd, while hermit crab samples had higher concentrations of Cu for both locations. Hermit crabs had higher concentrations of Cr at White Point Beach. While this study does not provide conclusive evidence of *P. samuelis* as an indicator species, it does demonstrate differences in metal concentrations between locations and populations of *P. samuelis*. Although *P. samuelis* may not be negatively impacted by the heavy metal concentrations found in this study, bioaccumulation is a potential threat to predators higher up the trophic pyramid. Expanded studies are required to confirm or deny if *P. samuelis* is an acceptable, wide-ranging indicator species along the west coast of North America.

CHAPTER 1

INTRODUCTION

Goal, Objectives, and Specific Aims

Goal

The goal of my research was to determine the comparative concentrations of heavy metals in two populations of *Pagurus samuelis* on the coast of Southern California.

Objectives

First Objective

My first objective was to quantify heavy metal concentrations in *P. samuelis* to determine if the Cabrillo Beach and White Point Beach populations had significantly different concentrations of heavy metals.

Specific Aim 1

My first specific aim was to determine internal concentrations of Lead (Pb), Copper (Cu), Cadmium (Cd), Chromium (Cr), and Zinc (Zn) in hermit crab whole homogenate tissue samples using a Microwave Plasma – Atomic Emission Spectrometer (MP-AES). Since Cabrillo Beach is closer to the Los Angeles Harbor than White Point Beach, I hypothesized that:

- H_1, hermit crabs collected from Cabrillo Beach have higher internal concentrations of heavy metals than hermit crabs collected from White Point Beach.

Second Objective

My second objective was to quantify heavy metal concentrations in seawater to determine if concentrations of heavy metals in the two populations of *P. samuelis* were significantly different from concentrations of the same metals in seawater.

Specific Aim 2

My second specific aim was to determine the concentrations of Pb, Cu, Cd, Cr, and Zn in seawater collected from both locations using the MP-AES and compare the concentrations in seawater with the concentrations in hermit crabs from each location. Since the hermit crabs live in tidepools where the water was collected and may accumulate metals, I hypothesized that:

- H_2, *P. samuelis* have higher concentrations of heavy metals than surrounding ambient seawater.

Introduction

Since the Industrial Revolution, the United States has had an increase of anthropogenic heavy metals in the environment (Bruland et al., 1974). During the 1970s, regulations were enacted to begin protecting the environment from pollution, and heavy metal concentrations began dropping. Monitoring is always an important method to ensure anthropogenic concentrations of heavy metals in the environment do not exceed regulated levels.

Natural Metal Fluxes in the Environment

Heavy metal deposits in sediment have natural variations over time based on the location and soil composition of the site and potential natural sources. Heavy metal input sources include erosion and weathering of rocks, movement by water and air, volcanoes, and escaping gases and fluids along major faults in the earth's crust (Garrett, 2000). If there are more natural input sources than anthropogenic metal sources then natural fluxes of heavy metals can be mask input from anthropogenic metal sources (Bruland et al., 1974).

Anthropogenic Metals

Heavy metals from anthropogenic sources have been an environmental problem since the industrial revolution (Finney & Huh, 1989). Because of rain, watersheds, and rivers, the majority of many kinds of pollution eventually ends up in the ocean. While some metals do occur naturally in the ocean, numerous studies have shown metal concentrations in the marine environment above expected levels (Dung et al., 2013; Everaarts & Nieuwenhuize, 1995; Guns et al., 1999; Huh, 1996).

In most cases, natural fluxes of heavy metals are dwarfed by anthropogenic inputs of metals. Culshaw et al. (2002) showed that in the Severn Estuary and Bristol Channel of Great Britain, concentrations of Cd, Zn, and Cu were positively correlated with proximity to metal input sites. The Severn Estuary and Bristol Channel also has a significant pattern of anthropogenic heavy metal concentrations of Zn > Cr > Pb > Cu > Nickel (Ni) > Cd (Duquesne et al., 2006).

Daskalakis and O'Connor (1995) designed a database to find trends in coastal sediments from the United States of nearly 13,500 samples and over 80 analytes. They determined a “high” concentration of an analyte to be the geometric mean plus one standard deviation of the National Status and Trends (NS&T) Mussel Watch site means. The greatest number of sites that were five times higher than “high” (“5× high”) concentrations were near densely populated areas with poor flushed water bodies. The most common chemicals at “5× high” were Mercury (Hg), Cd, Tin (Sn), and Silver (Ag).

Off the coast of Southern California, Bruland (1974) demonstrated that Pb, Cr, Cd, Zn, Cu, Ag, Vanadium (V), and Molybdenum (Mo) all accumulated faster from anthropogenic sources than background natural fluxes in the San Pedro, Santa Monica, and Santa Barbara basins, while in contrast, the natural fluxes of Al, Iron (Fe), Co, Ni, and Manganese (Mn) were larger than any anthropogenic sources, making it difficult to study anthropogenic inputs. This agrees with previous studies that have demonstrated a pattern of increasing anthropogenic heavy metal inputs starting during the industrial revolution and increasing steadily until around the 1970s, when environmental regulations were initially enforced (Bertine & Goldberg, 1977; Bruland et al., 1974; Duquesne et al., 2006; Finney & Huh, 1989; Huh, 1996).

Understanding the source of anthropogenic metal input is critical to studying different locations. Multiple studies have examined sites at different proximities from point sources for heavy metal pollution (Bay et al., 2003; Daskalakis & O'Connor, 1995; Guns et al., 1999). Cohen et al. (2001) studied three Southern California wetlands and suggested the least polluted wetland was because it had limited human proximity since it was near a military base, while the most polluted wetland had heavily populated areas nearby. Duquesne et al. (2006) found sediment metal concentrations were highest at sites close to industrial centers in the Severn Estuary and Bristol Channel. Some studies may demonstrate all of a particular metal is coming from a single point source (Culshaw et al., 2002; Fink & Manley, 2011), while other studies suggest multiple point sources that add up to the heavy metal concentrations found at the testing site (Bruland et al., 1974; Zhang et al., 2009). Point sources can be water treatment plants, watersheds, dust, as well as high motor traffic areas. It is important to understand potential point sources when researching pollution. Knowing anthropogenic sources can determine which heavy metals to study to help us understand their environmental effects.

Effects of Heavy Metals

Heavy metals can have varying effects on organisms depending on the metal and the animal species. Tests can be used to determine a mortality rate, such as an acute or prolonged Lethal Concentration for 50% of the sample (LC_{50}), of heavy metal concentrations for a population. Bay et al. (2003) collected storm water runoff from the mouths of rivers after storms, as an anthropogenic heavy metal source, and grew sea urchin embryos and amphipods in the storm water to determine survival rates. Other studies have examined how metal concentrations effect organisms within a polluted habitat. Warwick (2001) noted that in areas of high metal contamination there was a loss of two crustacean species and a high abundance of small opportunistic annelid species in the Fal estuarine system in England. Heavy metals can also create multiple nonlethal problems in organisms. When the winter flounder, *Pseudopleuronectes americanus,* was chronically exposed to Cu at 1 ppm, they suffered a change of gill appearance, fatty metamorphosis of the liver, and necrosis of the kidney, while at only 0.18 ppm mucus cells of the gill epithelium were replaced with chloride cells that may help excrete Cu (Bryan, 1971). Cu, at concentrations as low as 0.03 ppm prevent spawning, and concentrations of 0.10 ppm can be lethal to the fathead minnow, *Pimephales promelas*, (Bryan, 1971). When the mussel *Mytilus galloprovincialis*, is exposed to Hg, Cu, and Cd at different concentrations over 5 days in controlled laboratory conditions, these metals were shown to cause DNA damage (Bolognesi et al., 1999). Cd and Pb can also inhibit renal Ca uptake in freshwater pearl mussels (*Margaritifera margaritifera*) leading to weaker shells (Hartmut & Gerstmann, 2007). In *M. margaritifera* Cu is known to increase locomotion, raise respiratory currents, increase oxygen consumption, reduce feeding rates, and reduce growth rates (Hartmut & Gerstmann, 2007). Calcium uptake by brush border membrane vesicles from pyloric caeca in the starfish, *Pycnopodium helianthoides*, (Zhuang et al., 1995), and hepatopancreas in the American lobster, *Homarus americanus*, (G. A. Ahearn et al., 1994) were significantly reduced by the addition of Zn or Cd to the external medium. Both metals acted as competitive inhibitors of calcium uptake by the respective membrane preparations and the authors suggested that both calcium and the metal cations shared at least one transport protein carrier for ion uptake at the cell border. Based on the Michaelis constant (K_m) of uptake of Ca in lobster and the Cd inhibition of Ca influx (K_i), it was determined by Zhuang et al. (1995) that the transport systems in the cell borders had a higher binding affinity for the metals over Ca. Cooper et al. (2009) determined that in the water flea, *Ceriodaphnia dubia*, even when concentrations of Cu and Zn were lower than US and Australia guidelines, these metals nevertheless inhibited reproduction. In the hermit crab, *Clibanarius longitarsus*, in labartory controlled conditons, the 96 h LC_{50} for 100 larvae was determined for both Cu (50 ppb) and Zn (90 ppb). To test effects of non-lethal levels, concentrations were chosen at 50%, 25%, and 10% of the LC_{50}. As concentrations of Cu and Zn increased, survival rate decreased and the time at each zoeal stage increased. At similar concentrations, Cu was more toxic than Zn, and when combined together, Cu and Zn were more deleterious to larval stage development than individual concentrations (Lyla & Ajmal Khan, 2010).

Uptake and Removal

Uptake of heavy metals can take place across multiple pathways and at different rates depending on the organism (Bryan, 1971; Nott & Nicolaidou, 1994; Rainbow, 2007). Heavy metals can enter an organism from food ingested, across the gills, or through skin membranes. After uptake, the majority of heavy metals are detoxified and converted into insoluble granules. Detrimental effects occur when uptake occurs more rapidly than detoxification (Rainbow, 2007). Some metals are also used naturally. For example, Cu is a functional part of the respiratory protein haemocyanin found in certain molluscs and arthropods, and Zn is a key component of many enzymes including carbonic anhydrase (Rainbow, 2002).

After uptake, the organism requires mechanisms for preventing damage from the toxins. Organisms possess multiple cellular detoxification pathways. Each of these pathways may help reduce the active concentrations of potentially toxic metals circulating in the blood. One pathway is the physiological regulatory mechanisms balancing metal uptake rates from the environment with excretion rates (G. A. Ahearn et al., 2004). Toxic heavy metals may potentially enter animals across the integument (H. R. H. Ahearn et al., 2000; Peppler & Ahearn, 2003) by way of the gills (Bury et al., 1999; Bury et al., 2003; Grosell & Wood, 2002; Verbost et al., 1989), or through the gut after food consumption. There are three ways an organism can excrete metals: back across the gills, excreted into the gut, or excreted through the kidneys in the urine. There has been evidence of the shore crab, *Carcinus maenas* (Bryan, 1967), and the rainbow trout, *Salmo gairdnerii* (Nakatani, 1966) excreting metals back across the gills. However, not all species are able to secrete metals back into the gut. For instance, in the lobster genus *Homarus*, gut excretion has not been demonstrated to be an important route for removing metals. In contrast, the freshwater crayfish, *Austropotamobius pallipes pallipes* is able to use this route for metal elimination (Bryan, 1967). There are multiple ways for excretion to occur into the gut. The cyprid larva of the barnacle *Balanus amphitrite nivevus* excretes excess Cu into the lumen of the gut (Bernard & Lane, 1961), while *Octopus dofleini* excretes both Cu and Zn into the rectal fluid (Potts & Todd, 1965). *Octopus dofleini* is also able to excrete Cu and Zn through the urine (Potts & Todd, 1965). Crustaceans have been shown to excrete Zn, Cu, Co, Mn, and Hg in the urine. Few studies have determined if fish are able to excrete metals through urine, although one study suggests that rainbow trout do not appear to be able to do so (Nakatani, 1966). Excretion by intracellular sequestration of metals can be accomplished by two main mechanisms. Excretion can involve high-affinity binding sites on low molecular weight proteins, known as metallothioneins, followed by their elimination through the lysosomal endomembrane system (G. A. Ahearn et al., 2004). Excretion can also involve vacuoles producing solid metallic phosphorus or sulfur granules which subsequently undergo exocytosis for elimination (G. A. Ahearn et al., 2004).

Another method of dealing with metals is storage. In lobsters, it has been demonstrated that excess Zn found in the blood can be partially removed by being absorbed into the hepatopancreas and then lost gradually through urine or across the body surface (Bryan, 1971). To store the metals without them being active in the body means they are usually stored in granules to keep them from interfering with natural processes. In the brush border or luminal membrane of lobster hepatopancreatic epithelium, metallic ions may associate with metallothionien or glutathione (Viarengo, 1989), be transported into mitochondria (Chavez-Crooker et al., 2002; Klein & Ahearn, 1999), accumulated by lysosomes (Chavez-Crooker et al., 2003), transferred into the endoplasmic reticulum, or undergo efflux across the basolateral cell membrane to the blood. It has also been shown that the shrimp, *Chlamya opercularis,* stores excess Cu as granules in the cells of the hepatopancreas. The mollusk, *Scrobicularia plana* stores excess Zn, Pb, and Cd in the hepatopancreas, while the scallop, *Chlamys opercularis* stores metals in the renal glands (Bryan, 1971). In the scallop, the renal glands are about 0.5% of the body mass without the shell, yet these glands contain more than 50% of the body burden of the metals, especially Zn and Mn. It is possible that the scallop can later excrete the metals as granules. Oysters will store excess Cu in granules in wandering leucocytes that are gradually eliminated (Galtsoff, 1964). It is likely that different species employ metal detoxification strategies to differing degrees, and there may be changes in their relative importance within a single organism throughout its life cycle.

Metallothioneins are proteins that are found in nearly all invertebrate phyla, as well as in all vertebrates. They are water soluble and heat stable proteins, that generally possess approximately 60 amino acids rich in cysteine, which creates a high affinity for metal ions and can selectively bind them at very low intracellular concentrations. An increase in environmental metal concentrations will lead to an increased production of metallothionein to keep pace with an accelerated uptake of heavy metals (Roesijadi, 1992). In this role of detoxification, metallothioneins are strongly involved with the lysosomal system of cells. Yet the mechanism by which metals bound to metallothioneins are able to cross lysosomal membranes and become sequestered there from the rest of the cytoplasm is not currently understood.

Heavy metal-containing granules are found in animal cell vacuoles of nearly every invertebrate phylum (Al-Mohanna & Nott, 1985; Brown, 1982; Mason & Simkiss, 1982; Viarengo, 1989). Granules are generally found in epithelial cells of crustacean and mollusk hepatopancreas or kidney where they occur in membrane-bound vesicles or vacuoles. X-ray microprobe analysis of the metal composition of the granules suggests that they may contain either calcium or heavy metal cations, such as Zn, Cu, and Fe, complexed with sulfur or phosphorus (Al-Mohanna & Nott, 1985; Coombs & George, 1978; Mason et al., 1984; Mauri & Orlando, 1982). The result of complexing these heavy metal cations with anions is the removal of the potentially toxic heavy metal from the cytoplasm and the sequestration of them within the vacuolar membrane in an insoluble, detoxified form. It is also possible that some granules may be removed from the organism. Hopkin (1989) describes three different kinds of intracellular vacuoles, that may or may not include metallothionein, for detoxifying heavy metals. Type A vacuoles appear to accumulate Zn and phosphorus. Type B vacuoles appear to contain Cd, Cu, Hg, and Ag along with sulfur. Type C vacuoles appeared to be restricted to Fe although the respective anion in the structures is undescribed. These apparent distinguishing characteristics suggest that specific transport proteins may occur on the membrane of these different vacuoles for both metals and anions.

External heavy metals transported across the cellular plasma membrane of gut, renal, or gill epithelial cells enter the cytoplasm and are either detoxified in the cytoplasrm or are transported across the basolateral membrane to the blood. The mitochondrion is able to detoxify heavy metals by removing them from the epithelial cytoplasm and sequestering them as insoluble precipitates. Hepatopancreatic mitochondria of blue crabs are able to store large amounts of calcium phosphate in the organellar matrix without apparent impairment of mitochondrial function (Becker et al., 1974; Becker et al., 1976). Cu ion can use calcium transport proteins of mitochondria to enter the organelle and presumably interact with calcium regulatory processes. It is possible that processes responsible for forming calcium-phosphate granules in mitochondria would also incorporate Cu or Zn if they were available in the matrix, as well. Under these circumstances, metals can be removed from the soluble pool within the mitochondrion and sequestered in granules. These processes may reduce potential interfering interactions of soluble metals with calcium-regulating mechanisms. If these processes are overwhelmed by very high cytoplasmic metal concentrations, clear toxic effects on oxidative phosphorylation or other mitochondrial processes may occur.

Combining these methods is what allows organisms to regulate the heavy metal concentrations they uptake. For example, in the shore crab, *Carcinus maenas,* after Zn concentrations in the surrounding water were increased 500 fold, the concentrations in the organism as a whole increased by a factor of four (Bryan, 1971).

Pagurus samuelis and the Southern California Coast

The blueband hermit crab, *Pagurus samuelis* (Stimpson, 1857), is a metropolitan species found along most of the Pacific coast of North America. It ranges from Alaska to Baja California and is the most common hermit crab in California (Ricketts et al., 1992). It lives in the upper intertidal zone on rocky coasts. *P. samuelis* prefers to use the discarded shell of the black turban snail, *Tegula funebralis* (Jensen, 1995). *P. samuelis* has the potential to be an indicator species because of its range and abundance. A related hermit crab, *P. bernhardus*, has been shown to be an appropriate indicator species for multiple metals in Belgium (Guns et al., 1999). The cosmopolitan nature of *P. samuelis* makes it likely to be found in polluted areas in Southern California.

Based on the number of shipping containers, the Port of Los Angeles (PLA) is the busiest port in the United States (Anonymous, 2017). The goal of our research was to determine the comparative concentrations of heavy metals in two populations of *P. samuelis* on the coast of Southern California. Both *P. samuelis* and seawater were collected from two locations in Southern California. Our locations of choice are both within 10 miles of the PLA (Figure 1). Cabrillo Beach, which is closer to the PLA, is expected to be more polluted than the more distant location, White Point Beach.

Figure 1. A map of locations where *P. samuelis* and seawater samples were collected for comparisons of Pb, Cu, Cd, Cr, and Zn concentrations.

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CHAPTER TWO

DETERMINING HEAVY METAL CONCENTRATIONS IN THE BLUEBAND HERMIT CRAB (*PAGURUS SAMUELIS*) AND ITS SURROUNDING ENVIRONMENT

dos Santos, T. S.[1], Boskovic, D. S.[2], Shih, W.[3], Dunbar, S. G.[1]

[1]Marine Research Group, Department of Earth and Biological Sciences, Loma Linda University, Loma Linda, CA 92350
[2]Division of Biochemistry, Department of Basic Sciences, School of Medicine, Loma Linda University, Loma Linda, CA 92350, USA
[3]Research Consulting Group, Loma Linda University, Loma Linda, CA 92350, USA

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Abstract

Although heavy metal concentrations fluctuate naturally in the environment, anthropogenic sources of heavy metal concentrations can mask and override natural fluctuations. Indicator species can be used to determine environmental concentrations of certain metals to help us understand impacts of anthropogenic sources of heavy metals by analyzing specimens for concentrations of Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), and Zinc (Zn) found in tidepool seawater. We analyzed specimens to determine if *Pagurus samuelis* may play a role as an indicator species along the Southern California coast. Seawater and *P. samuelis* samples were collected from both Cabrillo Beach and White Point Beach locations and analyzed using Microwave Plasma – Atomic Emission Spectrometery (MP-AES). Pb concentrations were higher at Cabrillo Beach in seawater, while at White Point Beach, Zn concentrations were higher. All other metals were not significantly different between locations. In hermit crab samples, Cd was higher in crabs collected from Cabrillo Beach and Cr was higher in crabs collected from White Point Beach. In comparisons of seawater with hermit crab samples, seawater had higher concentrations of Zn and Cd, while hermit crab samples had higher concentrations of Cu at both locations. Hermit crabs had higher concentrations of Cr at White Point Beach. While this study does not provide conclusive evidence of an indicator species, it does demonstrate differences in metal concentrations between locations and populations of *P. samuelis*. Although *P. samuelis* may not be negatively impacted by the heavy metal concentrations found in this study, the potential for bioaccumulation at higher trophic levels may be of concern. More work is required to confirm or deny if *P. samuelis* is an acceptable indicator species.

Introduction

Heavy metal concentrations have a natural rise and fall over time based on site location and potential natural sources. Sources include erosion and weathering of rocks, movement by water or air, volcanoes, and escaping gases and fluids along major faults in the earth's crust (Garrett, 2000). In areas where there are multiple natural input sources for a location, natural fluxes of heavy metals may exceed anthropogenic sources (Bruland et al., 1974). However, heavy metals from anthropogenic sources have been of environmental concern since the industrial revolution (Finney & Huh, 1989). As a result of rain, watersheds, and rivers, a majority of pollutants eventually wash into marine ecosystems. While some metals do occur naturally in oceans, numerous studies have shown metal concentrations in marine environments surpass expected natural levels (Dung et al., 2013; Everaarts & Nieuwenhuize, 1995; Guns et al., 1999; Huh, 1996).

In most cases, natural fluxes of heavy metals are dwarfed by anthropogenic inputs. Culshaw et al. (2002) demonstrated that in the Severn Estuary and Bristol Channel of Great Britain, concentrations of Cd, Zn, and Cu were positively correlated with proximity to metal input sites. The Severn Estuary and Bristol Channel also had significant patterns of anthropogenic heavy metal concentrations of Zinc (Zn), Chromium (Cr), Lead (Pb), Copper (Cu), Nickel (Ni), and Cadmium (Cd) (Duquesne et al., 2006).

Daskalakis and O'Connor (1995) created a database to find trends in coastal sediments in the United States with nearly 13,500 samples and over 80 analytes, including heavy metals. They determined a "high" concentration of the analyte to be the geometric mean plus one standard deviation of the NS&T Mussel Watch site means (Hartwell & Assessment, 2014). The greatest number of sites that were five times higher than “high” concentrations were near densely populated areas with poorly flushed water bodies. The most common chemicals at “5× high” were Mercury (Hg), Cd, Tin (Sn), and Silver (Ag).

Off the coast of Southern California, Bruland (1974) demonstrated that Pb, Cr, Cd, Zn, Cu, Ag, Vanadium (V), and Molybdenum (Mo) all accumulated faster than background natural fluxes in the San Pedro, Santa Monica, and Santa Barbara basins, while in contrast, the natural fluxes of Al, Iron (Fe), Co, Ni, and Manganese (Mn) were larger than any anthropogenic sources, making it difficult to study anthropogenic inputs. This agrees with several studies that have demonstrated a pattern of increasing anthropogenic heavy metal inputs beginning during the industrial revolution and increasing steadily until the 1970s, when environmental regulations were initially enforced (Bertine & Goldberg, 1977; Bruland et al., 1974; Duquesne et al., 2006; Finney & Huh, 1989; Huh, 1996).

Understanding the sources of anthropogenic metal inputs is critical to properly analyze organisms and the environments they inhabit. Multiple studies have examined sites at different proximities from point sources for heavy metal pollution (Bay et al., 2003; Daskalakis & O'Connor, 1995; Guns et al., 1999). Cohen et al. (2001) studied three Southern California wetlands and suggested the least polluted wetland was because it had limited public access since it was near a military base, while the most polluted wetland were in close proximity to heavily populated areas.

Heavy metals may have varying effects on organisms depending on the metal and the animal species. Tests may be used to determine a mortality rate from heavy metal concentrations for a population, such as an acute or prolonged Lethal Concentration for

50% of the sample (LC_{50}). When the mussel *Mytilus galloprovincialis*, was exposed to Hg, Cu, and Cd at different concentrations over 5 days in controlled laboratory conditions, these metals were shown to cause DNA damage (Bolognesi et al., 1999). Both Cd and Pb can also inhibit renal Ca uptake in freshwater pearl mussels (*Margaritifera margaritifera*) leading to weaker shells (Hartmut & Gerstmann, 2007). In *M. margaritifera* Cu is known to increase locomotion, raise respiratory currents, increase oxygen consumption, reduce feeding rates, and reduce growth rates (Hartmut & Gerstmann, 2007). In the hermit crab, *Clibanarius longitarsus,* in labartory controlled conditons, Lyla and Ajmal Khan (2010) determined the 96 h LC50 of Cu (50 ppb) and Zn (90 ppb) in 100 larvae. To test effects of non-lethal levels, concentrations were chosen at 50%, 25%, and 10% of the LC50. As concentrations of Cu and Zn increased, survival rate decreased and the time at each zoeal stage increased. At similar concentrations Cu was more toxic than Zn, and when combined together, Cu and Zn were more deleterious to larval stage development than individual concentrations.

Uptake of heavy metals can take place at different rates and across multiple pathways depending on the organism (Bryan, 1971; Nott & Nicolaidou, 1994; Rainbow, 2007). Heavy metals can enter an organism from food ingested, across the gills, or through skin membranes. After uptake the majority of heavy metals are detoxified and converted into insoluble granules. Some metals are used naturally, such as Cu for oxygen transport in crustaceans. However, detrimental effects occur when uptake occurs more rapidly than detoxification (Rainbow, 2007).

After uptake, mechanisms are required for preventing tissue damage from toxins. Organisms possess multiple cellular detoxification pathways. Each of these may help reduce the active concentrations of potentially toxic metals circulating in the blood. It is likely that different species employ metal detoxification strategies to differing degrees, and there may be changes in their relative importance within a single organism throughout its life cycle.

The blueband hermit crab, *Pagurus samuelis* (Stimpson, 1857), is a metropolitan species found along most of the Pacific coast of North America. It ranges from Alaska to Baja California and is the most common hermit crab in California (Ricketts et al., 1992). It lives in the upper intertidal zone on rocky coasts. *Pagurus samuelis* preferentially uses the discarded shell of the black turban snail, *Chlorostoma funebralis* (Jensen, 1995). This hermit crab species has the potential to act as an indicator species for heavy metals because of its range and abundance. A related hermit crab, *P. bernhardus*, has been shown to be an appropriate indicator species for multiple metals in Belgium (Guns et al., 1999). As a result of the cosmopolitan nature of *P. samuelis*, it is likely to be found in polluted areas in Southern California. One such potential site is the Port of Los Angeles (PLA).

Based on the number of shipping containers moved in a year, the PLA is the busiest port in the United States (Anonymous, 2017). As such, the PLA area is likely to demonstrate anomalous levels of metal toxicity, and is likely to impact marine invertebrates in that area. The goal of our research was to determine the comparative concentrations of heavy metals in two populations of *P. samuelis* on the coast of Southern California, as well as in ambient tide pool water. This was done to determine if there were relationships between concentrations of heavy metals in ambient tide pool water and *P. samuelis*. If a pattern or association could be found, *P. samuelis* may have the potential to be an appropriate indicator species.

Methods & Materials

Collection

Samples of the blueband hermit crab (*Pagurus samuelis*) and seawater were collected from tide pools in two locations; Cabrillo Beach (33°42'25.18"N 118°17'9.09"W) approximately 4 km from the opening of the PLA, and White Point Beach (33°42'56.86"N 118°19'10.55"W) approximately 8 km from the opening of the PLA (Figure 1). These locations were chosen to investigate differences in heavy metal concentrations at different distances from the potential point source of the PLA. We considered Cabrillo a possible heavily polluted site because of its close proximity to the PLA, while we considered White Point Beach a potentially less affected area, due to its more distal location from the PLA. At both sites, *P. samuelis* and seawater samples were collected between August 2016 and February 2018. All samples were collected in Environmental Express metal digestion test tubes (UC474-WH, Charleston, SC) and frozen at -20 C until analysis.

Figure 1. Collection locations of *P. samuelis* and seawater samples for analysis of heavy metal concentrations. Samples were analyzed for Pb, Zn, Cu, Cd, and Cr using MP-AES.

Seawater

Seawater was collected by dipping 50 ml Environmental Express test tubes (UC474-WH, Environmental Express, Charleston, SC) into tide pools, then adding ~5 ml of HNO_3 to acidify the samples. Samples were prepared through standard addition using MilliQ water as a background. Each sample was split into 5 ml aliquots in separate test tubes. A 2.5 ml aliquot of the stock multielement 1000 ppm of Cd, Cr, Cu, Pb, and Zn standard (Environmental Express, Charleston, SC) was added to one tube of 5 ml sample to make a 50 ppm standard, and 0.5 ml was added from the same 1000 ppm multielement stock to another 5 ml of sample to make a 10 ppm standard. Each sample was then filled to 50 ml with MilliQ water to create a series of concentrations from which a standard curve was derived from the samples. Three subsamples were also made to verify consistent instrument measurements for each sample to insure accuracy.

Hermit Crabs

After collection, hermit crabs were gently removed from their shells with a bench vice and frozen. After freezing they were thawed and rinsed with MilliQ water then refrozen to insure they were solid before using the desiccator. Samples were then dried using a desiccator overnight, then weighed on a balance to accurately record dry weight (± 0.01 g). We followed methods of Huang et al. (1985) to prepare whole animal homogenate hermit crab samples. Samples were then separated into test tubes. Each sample had 1 ml of 50 ppm Yttrium (Y) (Environmental Express, Charleston, SC) added as an internal standard. Five ml of concentrated HNO_3 was added to each sample for digestion and samples were then placed in a hot block (12-well HotBlock, Environmental Express, Charleston, SC) with a watch glass on each tube and gradually raised to 60 ºC over a 30 min period. Three ml of 30 % H_2O_2 were added to each warm sample, and samples were then re-heated to 120 ºC until approximately 3 ml of sample was left in each test tube. This process took approximately 3.5 h. Each sample was then diluted to 50 ml with MilliQ water and vortexed to mix.

Analysis

After preparation, hermit crab samples were analyzed by Microwave Plasma-Atomic Emission Spectrometer (MP-AES) (4200 MP-AES, Agilent, Santa Clara, CA) using a standard curve made before sample analysis. Seawater samples were analyzed with standard addition curves. The standard used was a multielement standard consisting of Pb, Zn, Cd, Cr, and Cu. All metals in the standard were at 1000 ppm. Standards were acid matched with the samples by adding HNO_3 until the pH was within 1 pH unit of the samples. Each sample type was optimized by the MP-AES before use. Quality control for the MP-AES was established by analyzing a preparation blank, initial calibration blank and verification, and continuing calibration blank and verification throughout analysis. In hermit crab samples, an internal standard of Y was also used for calibrating results.

All results were analyzed with a Wilcoxon rank sum test to calculate differences among groups. Wilcoxon rank sum test was used because of many strong outliers that would affect means and standard deviations. We set the significance value at 0.05.

Results

Seawater

In seawater, Pb and Zn concentrations were significantly different between Cabrillo Beach and White Point Beach (Pb $p < 0.001$, Zn < 0.001). Pb was higher in Cabrillo tide pools while Zn was higher in White Point Tide Pools. Neither Cd, Cr, or Cu were significantly different between sites. Concentrations of Cr in 52 of 55 samples from Cabrillo beach and 52 of 53 samples from White Point beach were below detection limit. Because of this we do not here report results of Cr for seawater samples. Results for seawater samples can be found in Table 1, and Figure 2.

Table 1. Data collected from both Cabrillo and White Point Beaches. All values are in ppm. P-values were calculated using a Wilcoxon rank sum test for comparing samples of *Pagurus samuelis* and seawater collected from tidepools in the same location.

		Crab					Water					
Site	**Metal Type**	**N**	**Median**	**Q1**	**Q3**	**Mean (SD)**	**N**	**Median**	**Q1**	**Q3**	**Mean (SD)**	**p-value**
Cabrillo Beach												
	Cd	53	2.9	1.43	3.95	3.05 (2.48)	55	15.21	14.33	15.96	15.31 (1.61)	< 0.001
	Cr	53	0.75	0.11	1.63	1.1 (1.09)	55	0.53	0.44	0.61	0.56 (0.18)	0.092
	Cu	53	141.25	102.41	160.9	135.85 (46.71)	55	1.8	1.67	1.98	1.8 (0.28)	< 0.001
	Pb	53	8.61	5.19	12.54	21.9 (51.75)	55	7.18	6.72	7.7	7.3 (0.89)	0.553
	Zn	53	128.1	115.89	133.68	129.91 (41.86)	55	130.53	124.14	136.95	128.75 (13.1)	0.091
White Point Beach												
	Cd	55	0.31	0.1	3.01	1.57 (2.02)	53	15.42	14.82	16.06	15.28 (1.07)	< 0.001
	Cr	55	1.41	0.87	2.09	2.94 (7.28)	53	0.46	0.37	0.63	0.51 (0.14)	< 0.001
	Cu	55	150.19	135.06	171.14	153.48 (31.42)	53	1.76	1.66	1.86	1.78 (0.16)	< 0.001
	Pb	55	7.85	4.71	13.53	10.68 (9.25)	53	6.78	6.46	6.94	6.77 (0.36)	0.191
	Zn	55	130.47	117.06	148.65	130.89 (37.18)	53	140.92	133.03	151.02	142.31 (10.29)	0.002

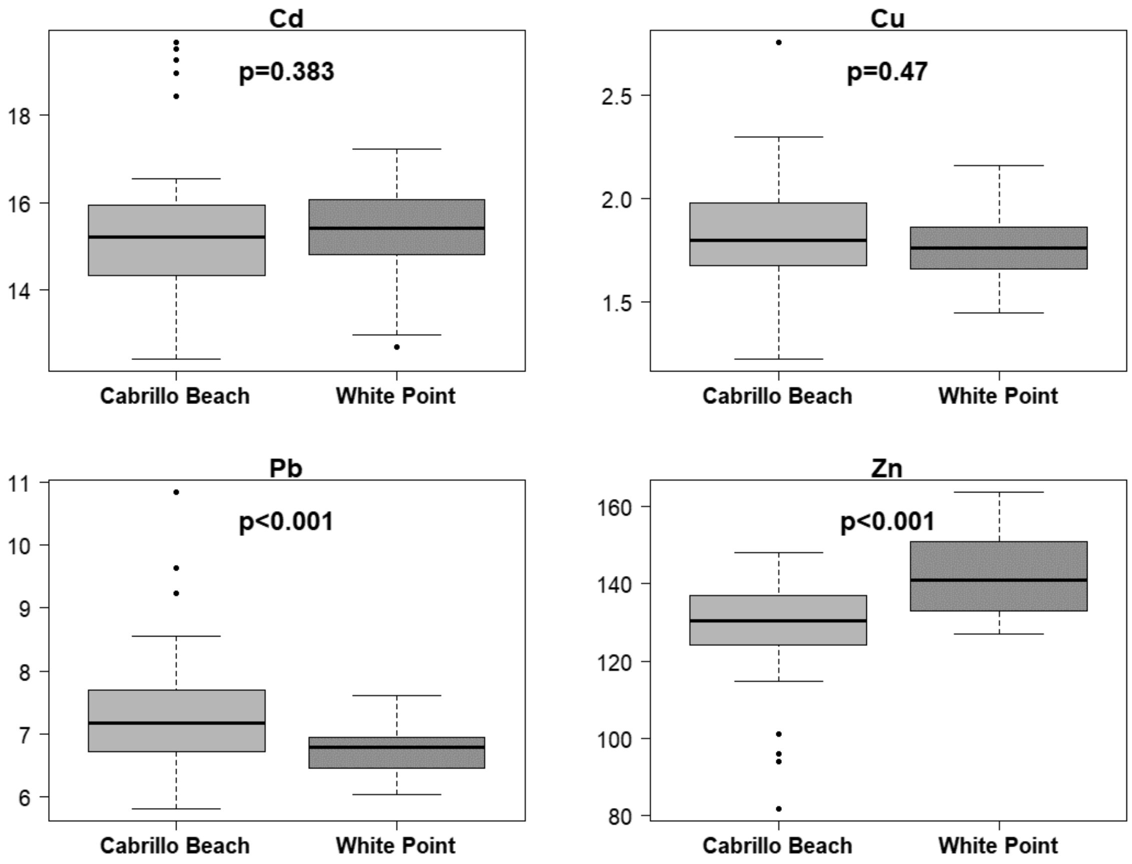

Figure 2. Concentrations of metals found in seawater samples from Cabrillo and White Point beaches. Data were analyzed using a Wilcoxon rank sum test to determine significance. Results are in ppm.

Hermit Crabs

In hermit crab samples, concentrations of Cd ($p < 0.001$) and Cr ($p = 0.048$) were significantly different between hermit crab populations. Concentrations of Cd were higher in hermit crabs found in Cabrillo tidepools while concentrations of Cr were higher in crabs found in White Point tide pools. For the 53 crabs collected from Cabrillo Beach, 9 crabs had Cd concentrations below detection limit, and 17 crabs had Cr concentrations below detection limit. For the 55 crabs collected from White Point beach, 28 crabs had Cd concentrations below detection limit, and 10 crabs had Cr concentrations below detection limit. While not significant, Cu approached significance ($p = 0.0793$) with White Point Beach trending toward higher concentrations. For the 53 crabs collected at Cabrillo Beach, 1 crab had Cu concentrations below detection limit, and 3 crabs had Pb concentrations below detection limit. For the 55 crabs collected from White Point Beach, 1 crab had Pb concentrations below detection limit. Results for hermit crab samples are presented in Table 1 and Figure 3. Comparisons of hermit crab samples to other decapod species can be seen in Table 2.

Hermit Crabs vs Water

Hermit crab concentrations were significantly lower than seawater for Cd ($p < 0.001$ for both locations) and Zn (Cabrillo $p = 0.045$, White Point $p < 0.001$), and higher for Cu ($p < 0.001$ for both locations) and Cr (White Point $p < 0.001$). Table 1 and Figure 4 present results of comparisons between hermit crabs and seawater for concentrations of detected metals.

Table 2. A comparison of heavy metal concentrations found in *Pagurus samuelis* in this study and other decapod species found in other studies. All results are in ppm. Results from this study are the median while other studies are mean and standard deviation.

Species	Location	Cd	Cr	Cu	Pb	Zn	Source
Pagurus samuelis	White Point Beach	0.31	1.41	150.19	7.85	130.47	This Study
	Cabrillo Beach	2.9	0.75	141.25	8.61	128.1	This Study
Pagurus bernhardus (ww)*	Belgian Coast	0.072 ± 0.027	0.28 ± 0.14	29 ± 6.6	0.28 ± 0.16	31 ± 5.9	(Guns et al., 1999)
Liocarcinus holsatus (ww)*	Belgian Coast	0.055 ± 0.021	0.21 ± 0.14	9.9 ± 5	0.28 ± 0.2	25 ± 8.5	(Guns et al., 1999)
Crangon crangon (ww)*	Belgian Coast	0.034 ± 0.019	0.17 ± 0.12	8.7 ± 2.9	0.19 ± 0.15	29 ± 4.6	(Guns et al., 1999)
Gennadas valens	N.E. Atlantic Coast	1.9	40.2	-	-	47.2	(Ridout et al., 1989)
Sergia robustus	N.E. Atlantic Coast	2.3	25.2	-	-	53.7	(Ridout et al., 1989)
Systellaspis debilis	N.E. Atlantic Coast	8.7	49	-	-	46.9	(Ridout et al., 1989)
Acanthephyra purpurea	N.E. Atlantic Coast	2.2	29.3	-	-	43.6	(Ridout et al., 1989)

*ww is wet weight measurements.

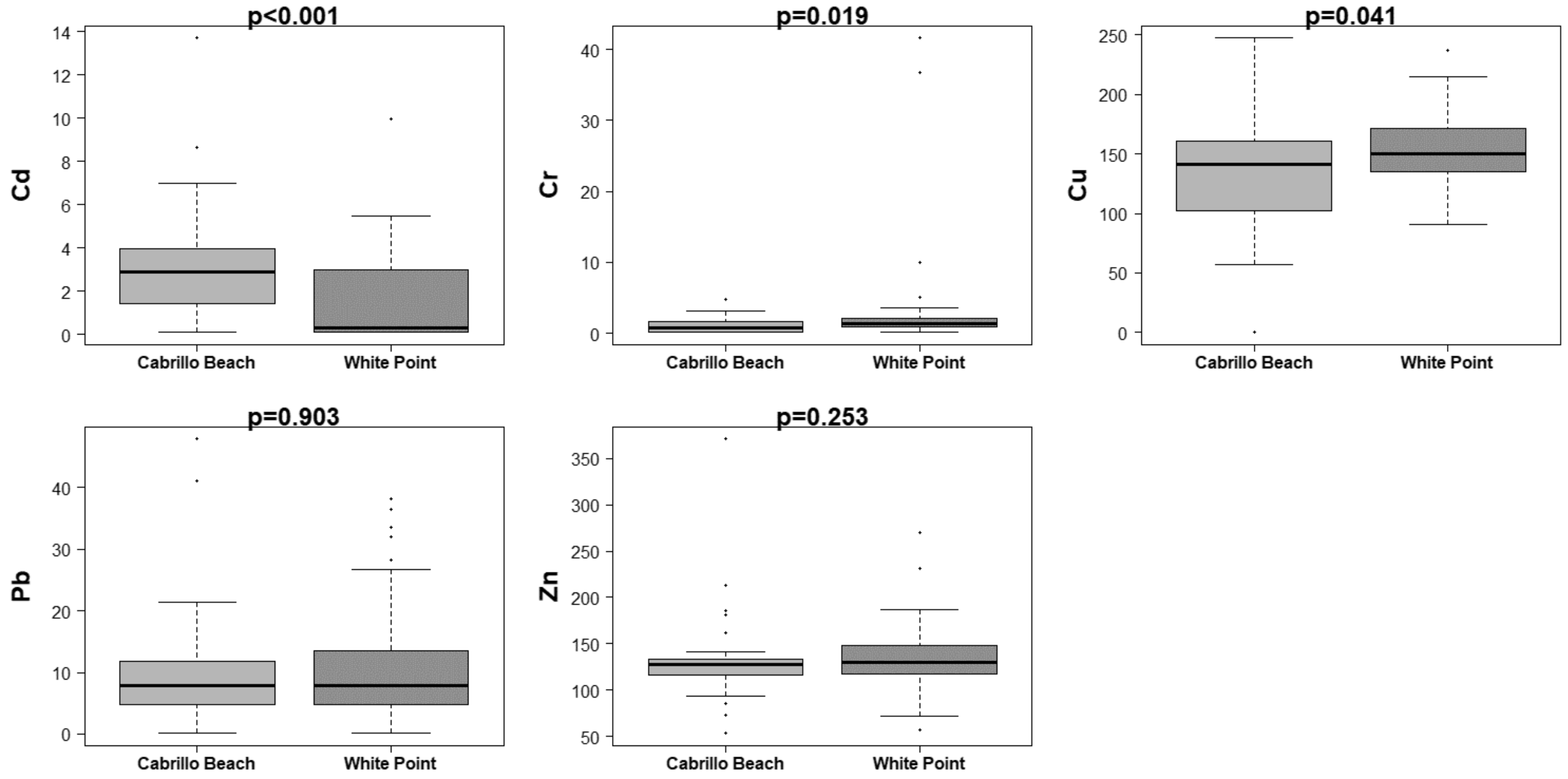

Figure 3. Concentrations of metals found in *P. samuelis* samples collected from Cabrillo and White Point beaches. Data were analyzed using a Wilcoxon rank sum test to determine significance. Results are in ppm.

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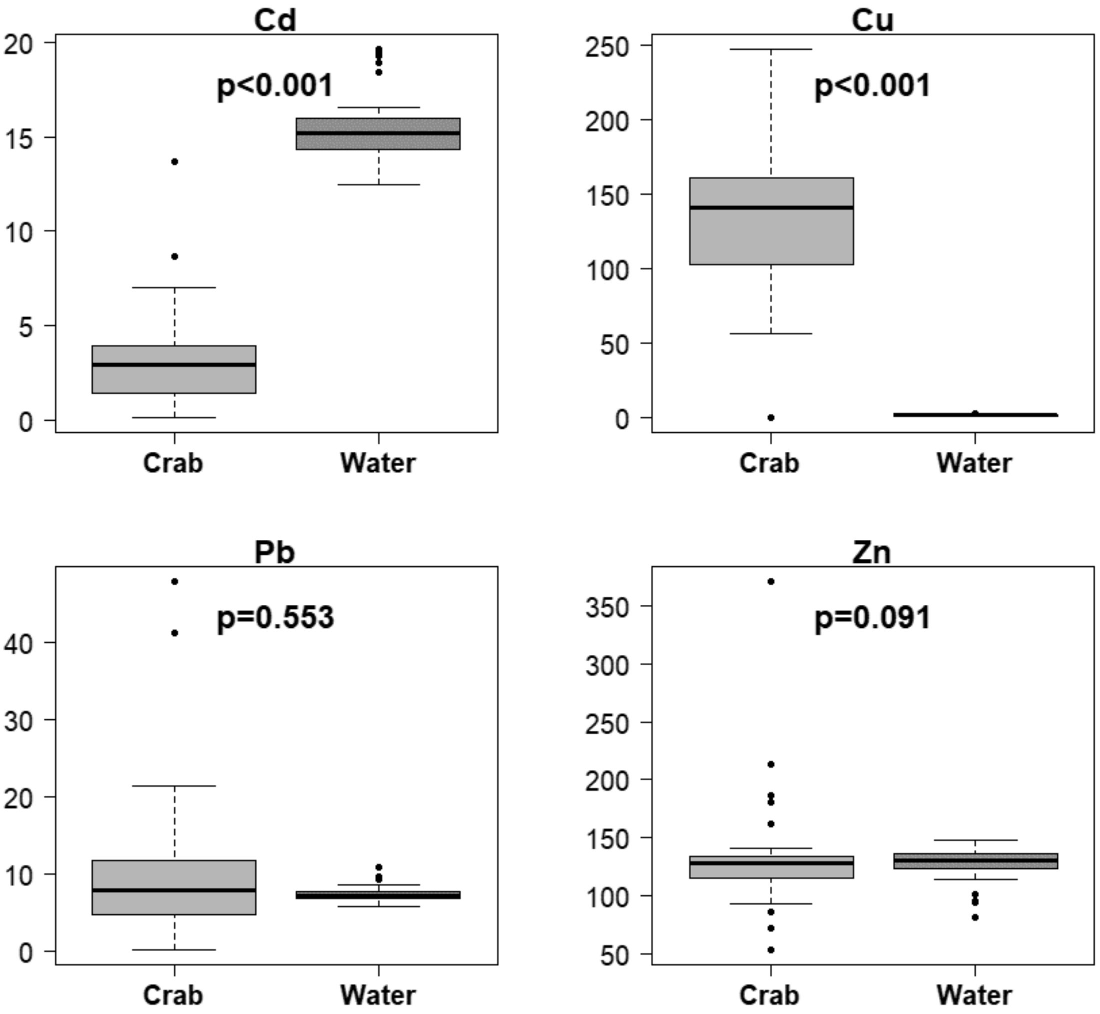

Figure 4a. Comparison of heavy metal concentrations found in *P. samuelis* and seawater samples found at Cabrillo Beach. Results are in ppm.

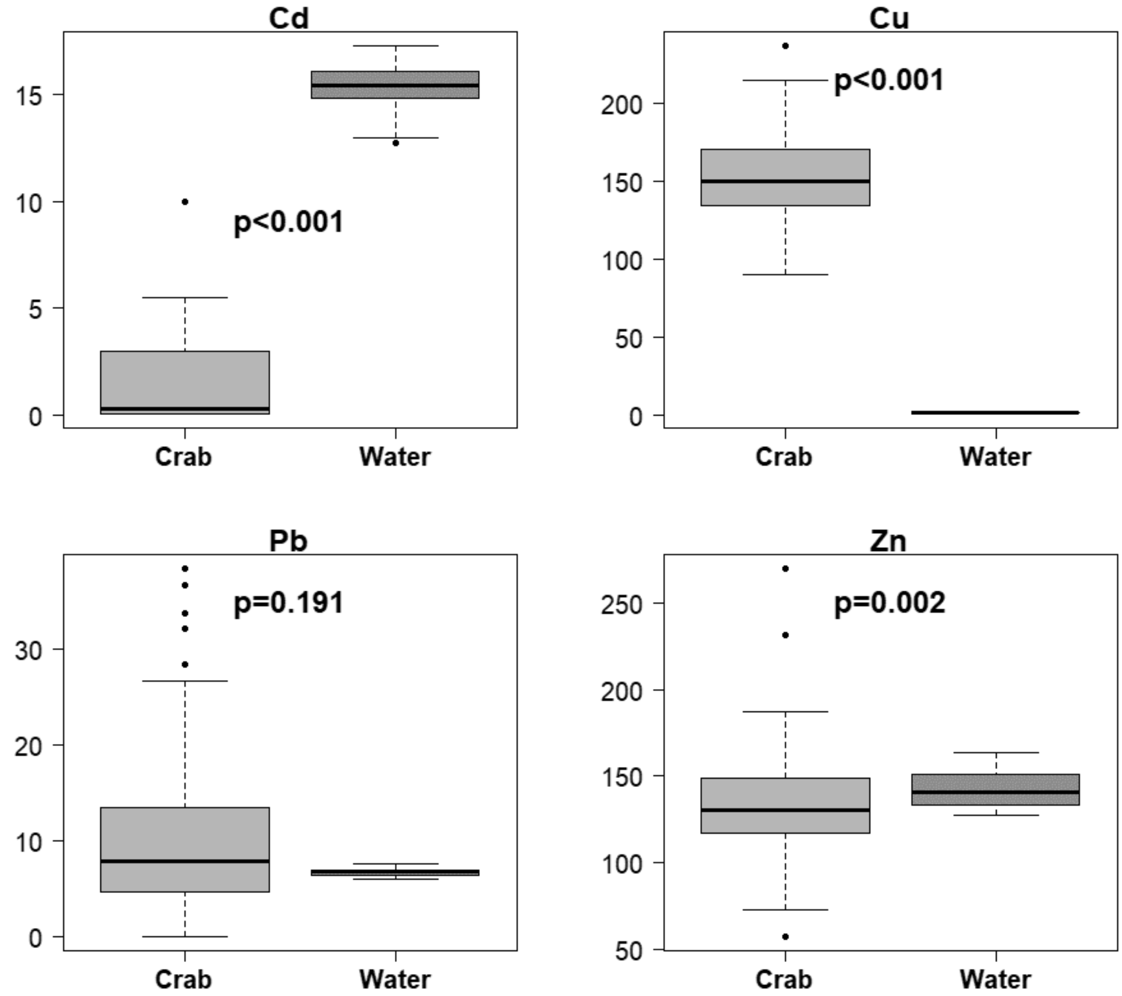

Figure 4b. Comparison of heavy metal concentrations found in *P. samuelis* and seawater samples found at White Point Beach. Results are in ppm.

Discussion

Cabrillo Beach and White Point Beach have distinct environmental conditions and *P. samuelis* populations. In both locations, Cu was significantly higher in hermit crabs than seawater, suggesting if there is any accumulation of Cu, the source is not likely from seawater transported across the gills. *Pagurus samuelis*, as in most crustaceans, uses hemocyanin in the blood, which uses Cu to bind O_2 for transport. Rainbow (1993) studied necessary Cu in the caridean decapod, *Pandalus montagui*, and estimated that total requirement for Cu was 38.1 ppm. His collected samples of *P. montagui* from the Firth of Clyde, Scotland measured at 57.4 ± 18.9 ppm, showing the field concentration was within range of the theoretical concentration. However, *P. montagui* is not an intertidal species, and thus, we expect that *P. samuelis* may have a higher total necessary concentration of Cu for survival in hypoxic tide pool environments. It is possible that *P. samuelis* is absorbing Cu from food rather than solution. Weeks and Rainbow (1993) investigated two species of Talitrid amphipods, *Orchestia gammarellus* (Pallas) and *Orchestia mediterranea* (Costa), and concluded that for *O. gammarellus*, food was a more important source for Cu accumulation than solution, while in contrast *O. mediterranea* was unable to satisfy its Cu needs from food sources, yet was able to achieve all its Cu needs from solution. Since food sources were not tested for heavy metals in the current study, we are unable to conclude if accumulation through diet has taken place in these populations. However, in studies on the decapod *Palaemon elegans*, White and Rainbow (1982) found they were able to internally regulate concentrations from the surrounding water of essential metals Cu and Zn up to 100 ppb without storage. When higher than 100 ppb, *P. elegans* stored excess Cu and Zn. They also found that for Cu, *P. elegans* could tolerate internal concentrations up to 5× base level and up to 2× base level for Zn.

In Cabrillo Beach tide pools, we found a significantly higher concentration of Pb in the water when compared to water in White Point tide pools. This may be because of the potential source of Pb from the PLA which is in close proximity to the Cabrillo tide pools.

Sabin et al. (2006) studied airborne heavy metals in the coastal Los Angeles area and found ranging levels of Pb. Heavy metals in air particles can enter water sources through deposition either directly through the water surface (Golomb et al., 1997; Lawlor & Tipping, 2003), or indirectly through the watershed as runoff during rainfall (Bay et al., 2003; Fink & Manley, 2011; Göbel et al., 2007).

In the White Point Beach tide pools, there was a significantly higher concentration of Zn in the water when compared with water in Cabrillo Beach tide pools. There may be a specific source of Zn near White Point Beach of which we are unaware. The most likely source appears to be roadside runoff. Sabin et al. (2006) found that mean concentrations and fluxes of Cr, Cu, Pb, and Zn were significantly higher at urban sites compared with a nonurban site in Los Angeles, CA area. These urban areas can act as point sources of heavy metals that may be deposited directly from the air to the water, or through runoff from streets and walkways. White Point Beach has a road and parking lot next to the tide pools, potentially increasing the potential for roadside runoff and anthropogenic pollution to enter the water.

While concentrations of both Pb and Zn were significantly different (Pb $p < 0.001$, Zn < 0.001) between the two tide pool locations, Pb and Zn were not significantly different between the *P. samuelis* populations collected. This suggests that hermit crabs may be regulating their internal Pb and Zn concentrations. Hermit crabs may be able to filter out metals that come across the gills from the environment (Gonçalves et al., 2006), or there may be a currently unaccounted source for these two metals, such as food (Fink & Manley, 2011) or sediment (Huh, 1996). Rainbow (2002) discussed different mechanisms for removal of heavy metals by invertebrates from their tissues, such as pumping metals back across the gills, or shedding stored metals into the gut. It is possible that *P. samuelis* uses methods similar to these to regulate heavy metal tissue loads. Further study is needed to determine if they are capable of shedding certain metals, since even closely related species within the same genus can have different internal concentrations of metals when studied under the same heavy metal conditions (Moore & Rainbow, 1987; Rainbow, 1993, 1998).

At Cabrillo Beach, hermit crabs had significantly higher concentrations of Cd when compared with hermit crabs from White Point Beach. While Cd concentrations were different in the two hermit crab populations, they were both significantly lower than the inshore seawater in which they live. This suggests that hermit crabs can regulate excess Cd from their system by removal. Bjerregaard et al. (2005) investigated seasonal variation of Cd in the shore crab *Carcinus maenas* and discovered they were able to eliminate some of their internal Cd concentration across the gut membrane, thus allowing the crab to have a lower internal Cd concentration than if they only stored Cd in tissues.

Both populations of hermit crabs live near dense urban populations. As an intertidal species, *P. samuelis* is able to survive many short term acute environment changes, such as hypoxia (Dunbar et al., 2017; Valère-Rivet et al., 2017), burial (Shives & Dunbar, 2010), and rapid fluctuations of temperature (Jokumsen & Weber, 1982) and salinity (Davenport et al., 1980). While both populations of *P. samuelis* have concentrations of nonessential metals found in their tissues, these populations are thriving.

Although results from the current study do not directly suggest that *P. samuelis* is an adequate indicator species for heavy metal concentrations, expanded investigations may demonstrate this species is, indeed, an appropriate indicator species. Guns et al.

(1999) found that the closely related, *Pagurus bernhardus* was an adequate indicator species for heavy metals in their study. Rainbow (2002) demonstrated that internal metal concentrations above baseline in organisms does not inherently negatively impact the quality of life for them. For example, Lyla and Ajmal Khan (2011) found the pattern of accumulation in tissue for both Cu and Zn in the hermit crab *Clibanarius longitarsus* to be hepatopancreas, ovary, and then muscle, suggesting that the preferable storage of metals in the ovaries over muscle could allow for removal of unwanted metal concentrations by shedding of metal-laden eggs. Bjerregaard et al. (2005) found seasonal differences in Cd concentrations in *Carcinus maenas*, suggesting there is some regulation involved. Organisms are only negatively affected by internal concentrations of heavy metals when they exceed the ability to regulate them by storage or excretion. At lower toxic concentrations, effects can be seen in slower development of the hermit crab, *Clibanarius longitarsus*, (Lyla & Ajmal Khan, 2010) and crayfish, *Orconectes rusticus* (Hubschman, 1967). At higher toxic concentrations, heavy metal concentrations can lead to death (Cooper et al., 2009; Hartmut & Gerstmann, 2007; Lyla & Ajmal Khan, 2010). Biomagnification may also be of concern, as seen in the edible crab species, *Carcinus maenas*, with Hg in Portugal (Cardoso et al., 2014).

We found differences in heavy metal concentrations between the two populations of hermit crabs, suggesting the populations are different based on environmental pressures. While *P. samuelis* may not be negatively affected by these heavy metal concentrations, animals that prey on *P. samuelis* may bioaccumulate heavy metals to sublethal or lethal concentrations. Further studies are needed to investigate internal regulation of heavy metal concentrations, lethal dosage and nonlethal effects on growth or development of *P. samuelis*, and the potential of biomagnification on organisms that prey on this species.

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CHAPTER THREE

DISCUSSION

The goal of my research was to determine the comparative concentrations of heavy metals in two populations of *Pagurus samuelis* on the coast of Southern California. I collected 108 samples of both *P. samuelis* and seawater, and analyzed them for heavy metal concentrations with the Microwave Plasma – Atomic Emission Spectrometer (MP-AES). I have accomplished my goals by fulfilling my objectives and specific aims. My two hypotheses were only partially correct.

I first hypothesized that hermit crabs collected from Cabrillo Beach would have higher concentrations of heavy metals than hermit crabs collected from White Point Beach. Although crabs from Cabrillo beach did have higher concentrations of Cd, they also had lower concentrations of Cr and Cu. This suggests that there are two populations of *P. samuelis* and they are facing different stressors from their surrounding environments. There is potential that there is more than one point source for heavy metals near the sampling locations, which may explain the lower levels of Cr and Cu at Cabrillo.

My second hypothesis was that hermit crab samples would have higher concentrations of heavy metals than the surrounding ambient seawater. *P. samuelis* only had a higher concentration of Cu when compared to seawater. This suggests that *P. samuelis* is able to regulate heavy metals internally to keep them lower than the surrounding environment, as suggested by multiple studies (Bryan, 1971; Rainbow, 1985, 1998; White & Rainbow, 1982). The information garnered from the current study is important for understanding interactions between intertidal species and metals within their environments.

I also discovered that seawater in tide pools at Cabrillo Beach had higher concentrations of Pb than seawater in White Point Beach tide pools. These data support our suggestion that the Port of Los Angeles (PLA) could be a point source for heavy metals, since Cabrillo Beach is closer to the PLA than White Point Beach. However, White Point Beach seawater had higher levels of Zn than seawater from Cabrillo Beach. This may be from a point source I was unable to identify prior to, or during the study, or may potentially be caused by roadside runoff from the parking lot in close proximity to the tide pools.

While I did find evidence of nonessential heavy metal concentrations in *P. samuelis*, it is important to note that high concentrations do not necessarily indicate that organisms are suffering any deleterious effects. Storage of unnecessary metals is a common method to avoid toxic effects in crustaceans (Rainbow, 2002). Heavy metal concentrations only become deleterious when uptake rates becomes faster than detoxification rates (Rainbow, 2007). The populations of *P. samuelis* in Cabrillo Beach and White Point Beach tide pools were both thriving, thus, I do not suspect that deleterious effects are being caused in *P. samuelis* by the levels of heavy metals found in this study. However, animals that prey on *P. samuelis* may be affected through biomagnification from consuming large numbers of organisms containing the levels of metals found in this study.

Limitations

There were some limitations to this work. I did not standardize for sex of crab samples or time of year which may have indicated patterns of storage or differences in concentrations that were unseen here. It would also have been beneficial to observe different types of tissues. Previous research done by Lyla and Ajmal Khan (2011) determined the storage site preference of Cu and Zn was in the hepatopancreas, muscle, and ovary tissues in the hermit crab *Clibanarius longitarsus*. Being able to test *P. samuelis* specimens from a wide variety of areas including less populated areas to compare with high population density areas such as the Cabrillo Beach tide pools to contrast a site very close to a point source and one that is expected to have much lower concentrations. Fink and Manley (2011) found that in giant kelp (*Macrocystis pyrifera*) sieve tube sap, lower concentrations of heavy metals were found closest to less urbanized areas, such as Santa Catalina Island and Malibu.

Future Works

1. *Heavy Metal Concentrations Found in Pagurus samuelis Along the West Coast of the Continental United States*

It would be advantageous to measure similar data from other locations along the Pacific Coast of the United States. As a cosmopolitan species on the west coast of North America, *Pagurus samuelis* has great potential to be an indicator species for most of the Pacific coast. Samples of *Pagurus samuelis* could be collected in Northern California, Oregon, and Washington. Hermit crab samples would then be compared with habitat samples to assess the possibility of *P. samuelis* being an indicator species over a large latitudinal gradient.

2. *Heavy Metal Concentrations Found in Pagurus samuelis and Their Surrounding Environment*

It would be beneficial to collect other environmental samples to be compared with *P. samuelis* samples to determine its potential as an indicator species. Samples of food and sediment could be collected to understand the pathways of uptake for heavy metals in *P. samuelis*. Certain heavy metals may be absorbed through the gut from the diet or through the sediment during burial. Testing seawater samples is only one potential input source of heavy metals for these organisms. To understand the ecology of these organisms, more factors should be studied.

3. *Differences in Storage of Heavy Metal Concentrations in Males and Females of Pagurus samuelis*

It is likely that *P. samuelis* females may store heavy metals in the ovaries. Lyla and Ajmal Khan (2011) found that in the hermit crab, *Clibanarius longitarsus*, females stored Zn and Cu in the ovaries. However, Rainbow (1998) demonstrated that even in similar taxa organisms can have different methods of storage and detoxification of heavy metals. Studies should be done to compare males and females to determine if there is a difference between heavy metal concentrations. If females are able to store heavy metals in the ovaries it is likely that heavy metals could be shunted into eggs allowing the females to lower their internal concentrations in ways males are unable to do. It is expected that this may also have an impact on how well offspring are able to develop, if heavy metal concentrations in the egg are above certain thresholds.

4. ***Variation of Heavy Metal Concentrations over time found in Pagurus samuelis***

Long term studies should be conducted to determine if *P. samuelis* has fluxes in heavy metal concentrations during the year. It is possible that *P. samuelis* is able to remove certain heavy metals from their tissues. Previous work has shown that the shore crab *Carcinus maenas* has had seasonal changes of Cd concentrations (Bjerregaard et al., 2005). One possible way would be to shunt heavy metals into the old carapace before molting. A study could compare one month before and one month after the time of molting in *P. samuelis*.

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