Heavy metals in the blueband hermit crab, Pagurus samuelis (Stimpson, 1857) (Decapoda: Anomura: Paguridae), from two Southern California habitats

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ABSTRACT

Although heavy metal concentrations naturally vary in the environment, anthropogenic sources of heavy metals can mask and override such fluctuations. Indicator species can be used to determine environmental concentrations of certain metals and evaluate the impacts of metals from associated anthropogenic sources. We sought to determine if the hermit crab Pagurus samuelis (Stimpson, 1857) 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 rocky intertidal locations and analyzed for concentrations of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) using microwave plasma-atomic emission spectrometry (MP-AES). Lead concentrations were higher in seawater at Cabrillo Beach compared to White Point Beach, whereas Zn concentrations were higher at White Point Beach than at Cabrillo Beach. All other metals were not significantly different between these locations. Cd was higher in hermit crabs collected from Cabrillo Beach than those from White Point Beach, whereas Cr was higher in crabs collected from White Point Beach compared with those from Cabrillo Beach. In comparisons of seawater with samples of hermit crabs, seawater had higher concentrations of Zn and Cd, whereas hermit crabs had higher concentrations of Cu at both locations, and higher levels of Cr at White Point Beach. While this study does not provide conclusive evidence that P. samuelis is an indicator species, it does demonstrate differences in metal concentrations between tested locations and populations of P. samuelis. Although P. samuelis may not be negatively impacted by the heavy metal concentrations we found, the risks of potential bioaccumulation at higher trophic levels is of concern.

Key Words: bioaccumulation, Crustacea, ecology, intertidal zone, metal pollution

INTRODUCTION

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

Natural fluxes of heavy metals are dwarfed by anthropogenic inputs in most cases. Culshaw et al. (2002) demonstrated that in the Severn Estuary and Bristol Channel of Great Britain, concentrations of cadmium (Cd), zinc (Zn), and copper (Cu) were positively correlated with proximity to metal input sites, such as water treatment plants, or densely populated cities. These two locations...
also had significant patterns of anthropogenic heavy metal concentrations of Zn, chromium (Cr), lead (Pb), Cu, nickel (Ni), and Cd (Duquesne et al., 2006).

Daskalakis & O’Connor (1995) created a database to find trends in coastal sediments in the United States, with nearly 13,300 samples and over 80 analytes, including heavy metals. Those authors determined a “high” concentration of an analyte to be the geometric mean plus one standard deviation above the mean in the National Status and Trends (NS&T) Mussel Watch site (Hartwell, 2014). The majority of sites that had concentrations of analytes more than five times higher than concentrations those authors labelled as “high,” were near densely populated areas with poorly flushed water bodies. The most common metals they found to be five times “high” were mercury (Hg), Cd, Tin (Sn), and silver (Ag).

Off Southern California, Bruland et al. (1974) demonstrated that Pb, Cr, Cd, Zn, Cu, Ag, vanadium (V), and molybdenum (Mo) accumulated faster in marine sediments than background natural fluxes in the San Pedro, Santa Monica, and Santa Barbara basins. In contrast, the natural fluctuations of aluminium (Al), iron (Fe), cobalt (Co), Ni, and manganese (Mn) were greater than any anthropogenic sources, making it difficult to study anthropogenic inputs. This agrees with several studies reporting a pattern of increasing anthropogenic heavy metal inputs in Southern California (Bruland et al., 1974; Bertine & Goldberg, 1977; Finney & Huh, 1989; Huh, 1996; Duquesne et al., 2006). These inputs began during the industrial revolution and increased steadily until the 1970s, when the enforcement of environmental regulations began in earnest.

Understanding the sources of anthropogenic metal inputs is critical to the proper analyses of wildlife and their environments. Multiple studies have examined sites at different proximities from point sources for heavy metal pollution (Daskalakis & O’Connor, 1995; Guns et al., 1999; Bay et al., 2003; Cohen et al., 2001), in a study of three different wetlands in Southern California, demonstrated that the least polluted wetland had limited public access, while the most polluted was surrounded by densely populated areas.

Heavy metals may have varying effects on organisms, depending on the metal and the animal species. A variety of tests may be used to determine mortality rates of animal populations due to high concentrations of heavy metals. For example, a test designated as the LC50 can be performed to determine an acute or prolonged lethal concentration impacting 50% of the sample population. Impairment effects of metals on organisms may be seen at sub-lethal concentrations, as well. Bolognesi et al. (1999) demonstrated that the intertidal mussel, Mytilus galloprovincialis Lamarck, 1819, exposed to Hg, Cu, and Cd at different concentrations over 5 d in controlled laboratory conditions, caused DNA damage. Hartmut & Gerstmann (2007) demonstrated that both Cd and Pb inhibited renal Ca uptake in freshwater pearl mussels (Margaritifera margaritifera (Linnaeus, 1758)) leading to weaker shells, and Cu increased locomotion, respiratory currents, and oxygen consumption, and reduced feeding and growth rates. Lyla & Ajmal Khan (2010) determined the 96 h LC50 of Cu (50 ppb) and Zn (90 ppb) in 100 zoae larvae of the hermit crab Clibanarius longitarsus (De Haan, 1849) in De Haan, 1833–1850. Testing the effects of non-lethal levels, concentrations were chosen at 50%, 25%, and 10% of the LC50. As concentrations of Cu and Zn increased, survival rates decreased, while duration of larval stage increased. At similar treatment concentrations, Cu was more toxic to C. longitarsus than Zn. Moreover, when combined, Cu and Zn together were more deleterious to the larval stage development than if tested individually.

Uptake of heavy metals may occur via multiple pathways and at different rates, depending on the organism (Bryan, 1971; Nott & Nicolaidou, 1994; Rainbow, 2007). Heavy metals may enter an animal through ingested food, across the gills, or through membranes of the skin. The majority of heavy metals are detoxified and converted into insoluble granules after uptake. Some metals are essential with specific biological functions, such as Cu, which is used for oxygen transport in crustaceans. Such essential metals often employ specific transport and carrier systems. Even for these essential metals, however, there may be detrimental effects when the uptake rate exceeds the handling capacity or rate of detoxification (Rainbow, 2007).

After uptake, mechanisms are required to prevent tissue damage from toxins. Animals possess multiple cellular detoxification pathways, each reducing the concentrations of potentially toxic metals circulating in the blood. While it is likely that different species employ particular metal detoxification strategies to varying degrees, it is also likely there may be changes in the relative importance of these strategies throughout the life cycle of a particular organism.

The blueband hermit crab, Pagurus samuelis (Stimpson, 1857), is 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), living in the upper intertidal zone on rocky coasts. Pagurus samuelis preferentially uses discarded shells of the black turban snail, Chlorostoma funebralis (Adams, 1885) (Jensen, 1995). Like many anomurans, this hermit crab is a generalist omnivore (Britton & Morton, 1994; Greggor & Lairde, 2016), which although with a wide range and abundance, enables it to potentially serve as an indicator species for heavy metals. A related hermit crab, P. bernhardus (Linnaeus, 1758), was shown to be an appropriate indicator species for multiple metals in Belgium (Guns et al., 1999). As a result of the wide distribution of P. samuelis, it is likely that it is also 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 per year, PLA is the busiest port in the United States (Anonymous, 2017), and as such, is likely a substantial anthropogenic point source for a variety of metals that are introduced into the environment (Anonymous, 2009; Fink & Manley, 2011), impacting marine invertebrates in neighboring intertidal zones. The goal of our study was to determine the comparative concentrations of heavy metals in two populations of P. samuelis in the rocky intertidal in Southern California, as well as in the water found in their respective ambient tidepools. This was done to determine if relationships existed between concentrations of heavy metals in ambient tidepool water and in P. samuelis. If patterns or associations could be found, P. samuelis may have the potential to serve as an appropriate indicator species for heavy metal contamination in intertidal ecosystems where it is distributed.

MATERIALS AND METHODS

Study area

Our investigation was conducted at two locations on the Southern California coast near PLA, Cabrillo Beach (33°42′25.18″N, 118°17′7.09″W), approximately 4 km from the opening of PLA, and White Point Beach (33°42′56.86″N, 118°19′10.55″W) approximately 8 km from the opening of PLA (Fig. 1). These locations were chosen because they represent typical rocky intertidal zones along the Southern California coast, and provide opportunities to investigate differences in heavy metal concentrations at specific distances from PLA as the principal potential point source. Both sites are characterized by underlying bedrock beds, overlaid with fine sandy sediments. We considered Cabrillo Beach to possibly be a heavily polluted site because of its close proximity to PLA, and White Point Beach a potentially less affected area, due to its more distant location from PLA. We did not account for the sex of hermit crabs, since previous studies demonstrated no effects.
Samples of P. samuelis were collected from rocky tidepools at both locations between August 2016 and February 2018. All samples were collected in Environmental Express metal tidepools, collecting 45 ml seawater, then adding ~5 ml of HNO3 (UC474-WH, Environmental Express, Charleston, SC, USA) with a watch glass on each tube, and gradually heated to 60 °C for at least 30 min. We added 3 ml of 30% H2O2 to each warm sample, followed by further heating to 120 °C until approximately 3 ml of sample were 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.

Hermit crabs. All hermit crabs were gently removed from their shells with a bench vice and frozen. After freezing, hermit crabs were thawed, rinsed with MilliQ water, then refrozen to ensure they were solid. This was followed by drying with a desiccator overnight, prior to weighing to accurately record dry weight (± 0.01 g). We followed Huang & Schulte (1985) to prepare whole-animal homogenate samples. Samples were then separated into test tubes. Each sample had 1 ml of 50 ml of 30% H2O2 to each sample for digestion. Samples were then placed in a hot block (12-well HotBlock; Environmental Express, Charleston, SC, USA) with a watch glass on each tube, and gradually heated to 60 °C for at least 30 min. We added 3 ml of 30% H2O2, to each warm sample, followed by further heating to 120 °C until approximately 3 ml of sample were 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.

RESULTS

Seawater
We collected 55 water samples from Cabrillo Beach, 53 from White Point Beach. Pb concentrations were significantly higher at Cabrillo Beach compared to White Point Beach (P < 0.001).
Conversely, Zn was higher in White Point tidepools compared to those at Cabrillo Beach \( (P < 0.001) \). Neither Cd nor Cu were significantly different between these sites \( (P < 0.001) \). Concentrations of Cr in 52 of 55 samples from Cabrillo Beach, and 52 of 55 samples from White Point Beach were below detection limit. Because of this, Cr data for seawater samples are not reported. Results for seawater samples can be found in Table 1 and Figure 2.

**Hermit crabs**

We collected a total of 108 individuals of *P. samuelis*, 53 from Cabrillo Beach and 55 from White Point Beach. Concentrations of Cd were significantly higher in hermit crabs found in Cabrillo compared to those from White Point \( (P = 0.019) \), whereas Cr levels were higher in crabs from White Point compared to those from Cabrillo Beach \( (P = 0.019) \) \( (\text{Fig. 3}) \). Of the 53 crabs collected from Cabrillo Beach, 9 had Cd concentrations below detection limit, and 17 had Cr concentrations below detection limit. Of the 55 crabs from White Point Beach, 28 had Cd concentrations below detection limit, and 10 had Cr concentrations below detection limit. Concentrations of Cu were significantly higher in hermit crabs from White Point Beach compared to those from Cabrillo Beach \( (P = 0.041) \). For the 53 crabs from Cabrillo Beach, 1 had Cu concentrations below detection limit, and 3 had Pb concentrations below detection limit. Results for hermit crab samples are summarized in Table 1 and Figure 3.

**Hermit crabs versus water**

Hermit crabs from Cabrillo Beach had a significantly lower concentration of Cd than seawater \( (P < 0.001) \), but a significantly higher concentration of Cu than seawater \( (P < 0.001) \) \( (\text{Fig. 4}) \). There were no significant differences between crabs and seawater for concentrations of Pb \( (p = 0.553) \) or Zn \( (p = 0.091) \). These results are summarized in Table 1 and Figure 4.

Hermit crabs from White Point Beach had levels of Cd \( (P < 0.001) \) and Zn \( (P = 0.002) \) significantly lower than seawater \( (\text{Fig. 5}) \). Conversely, levels of Cu in hermit crabs were significantly higher \( (P < 0.001) \) than in seawater. Table 1 and Figure 5 present comparisons of metal levels between hermit crabs and seawater.

**DISCUSSION**

Although the analyzed sites where water and hermit crab samples were taken are characterized by underlying bedrock substrate, it is clear from our results that the tidepools have distinct environmental conditions with respect to metals, and that *P. samuelis* populations differ in their internal concentrations of the metals measured in this study.

Differences in tidepool concentrations of metals are unlikely to be influenced by the natural release of metals from rock substrates into pools, since both sites are located along the same bedrock feature of the Palos Verdes Peninsula. Bedrock of the peninsula is dominated by sedimentary units of Middle and Upper Miocene age. Below the Middle Miocene Monterey Formation are Miocene submarine basalts that are part of the Conejo Volcanics. These basalts have relatively low Mg, Cr, and Ni values \( \text{Weigand \\ & \text{Savage, 1993}} \). Because of these low values and because they crop out at a distance from the coast, the Conejo volcanics are unlikely to be a local source of metals in the intertidal area of the current study. Natural substrate release of metals is thus less likely to impact rocky intertidal habitats than it is in sand or mud bottom.

**Figure 2.** Concentrations of metals found in seawater samples from Cabrillo and White Point beaches, Southern California. Data were analyzed using a Wilcoxon rank sum test to determine significance. Data is shown in a box and whisker plot in ppm values. Note differences in y-axis scales.
habitats. While currents and tides may also influence the distribution of suspended metals in aquatic contexts, data on local water movements in the areas of the two sampled beaches suggest these locations are influenced more by on-shore/off-shore tidally dominated flows than by long-shore current movements (Anonymous, 2006). Still, currents may carry suspended metals out from land-based sources, to eventually be brought back towards shore by small-scale on-shore water movements (Anonymous, 2006).

Cu was significantly higher in hermit crabs than in seawater in both locations, suggesting that, if there is accumulation of Cu above essential concentrations, this accumulation may be from either the intake of ambient water across the gills, or from sediment or food intake over time. Measuring metal concentrations in both sediment and food samples was nevertheless beyond the scope of our study. In Pagurus samuelis, as in most crustaceans, Cu is used as an essential mineral in several ways, including for O₂ transport by hemocyanin in the blood. Rainbow (1993) studied necessary Cu in the caridean decapod, Pandalus montagui Leach, 1814, and estimated that total requirement for Cu was 38.1 ppm. His collected samples of P. montagui from the Firth of Clyde, Scotland measured 57.4 ± 18.9 ppm, showing the field concentration of Cu fell within the calculated concentration range. Pandalus montagui, however, is not an intertidal species, and P. samuelis would be expected to have a higher total necessary concentration of Cu for survival in periodically hypoxic tidepool environments. It is possible that P. samuelis may be absorbing Cu from food rather than from solution in water. Weeks & Rainbow (1993) investigated two talitrid amphipods, Orchestia gammarellus (Pallas, 1766) and Orchestia mediterranea Costa, 1853, and concluded that food was a more important source for Cu accumulation in O. gammarellus.

Table 1. Results from seawater and Pagurus samuelis samples collected from Cabrillo and White Point beaches, Southern California. All values are in ppm. Probability values (P) were calculated using a Wilcoxon rank sum test for comparing seawater and crab samples collected from tidepools in the same location. Q1 and Q3 are for the first and third quartiles of the data. *, 96 h LC₅₀ concentrations for dissolved Cu and Zn (ppm) in Clibanarius longitarsus from Lyla & Ajmal Khan (2011); CTR, California Toxics Rule (CTR) Water Quality Criteria for Dissolved Metals, chronic concentrations (Anonymous, 2000).

<table>
<thead>
<tr>
<th>Site</th>
<th>Metal</th>
<th>N</th>
<th>Median</th>
<th>Q1</th>
<th>Q3</th>
<th>Mean (SD)</th>
<th>N</th>
<th>Median</th>
<th>Q1</th>
<th>Q3</th>
<th>Mean (SD)</th>
<th>P</th>
<th>96 h LC₅₀ for C. longitarsus*</th>
<th>CTR</th>
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<tr>
<td>Cabrillo Beach</td>
<td>Cd</td>
<td>53</td>
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<td>1.43</td>
<td>3.95</td>
<td>3.05 (2.48)</td>
<td>55</td>
<td>15.21</td>
<td>14.33</td>
<td>15.96</td>
<td>15.31 (1.61)</td>
<td>&lt; 0.001</td>
<td>8.8</td>
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<tr>
<td></td>
<td>Cr</td>
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<td>0.11</td>
<td>1.63</td>
<td>1.1 (1.09)</td>
<td>50</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>53</td>
<td>141.25</td>
<td>102.41</td>
<td>160.9</td>
<td>135.85 (46.71)</td>
<td>55</td>
<td>1.8</td>
<td>1.67</td>
<td>1.98</td>
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<td>8.61</td>
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<td>55</td>
<td>7.18</td>
<td>6.72</td>
<td>7.7</td>
<td>7.3 (0.89)</td>
<td>0.553</td>
<td>81</td>
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<tr>
<td></td>
<td>Zn</td>
<td>53</td>
<td>128.1</td>
<td>115.89</td>
<td>133.68</td>
<td>129.91 (41.86)</td>
<td>55</td>
<td>130.53</td>
<td>124.14</td>
<td>136.95</td>
<td>128.75 (13.1)</td>
<td>0.091</td>
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<tr>
<td>White Point Beach</td>
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<td>0.1</td>
<td>3.01</td>
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<td>14.82</td>
<td>16.06</td>
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<td></td>
<td>Cr</td>
<td>55</td>
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<td>0.87</td>
<td>2.09</td>
<td>2.94 (7.28)</td>
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<td>140.92</td>
<td>133.03</td>
<td>151.02</td>
<td>142.31 (10.29)</td>
<td>0.002</td>
<td>81</td>
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</table>

Figure 3. Heavy-metal concentrations in samples of Pagurus samuelis collected from Cabrillo and White Point beaches. Data were analyzed using a Wilcoxon rank sum test to determine significance. Data is shown in a box and whisker plot in ppm values. Note differences in y-axis scales.
compared to the surrounding water. By contrast, *O. mediterranea* was unable to satisfy its Cu needs from food sources, yet was able to meet all its Cu requirements from the ambient water. Since we did not test food sources for heavy metals, we are unable to verify if accumulation through diet was a factor in our populations. White & Rainbow (1982) nevertheless reported that the caridean shrimp *Palaemon elegans* Rathke, 1836 was able to regulate concentrations of the essential metals Cu and Zn up to 100 ppb from the surrounding water without storage. By contrast, when concentrations were higher than 100 ppb, *P. elegans* was able to store excess Cu and Zn. Those authors also found that *P. elegans* could tolerate internal concentrations up to five times the base level of Cu, but up to two times the base level in the case of Zn.

We found a significantly higher concentration of Pb in the water of Cabrillo Beach tidepools compared to White Point tidepools. This may be because of the potential source of Pb from PLA, which is in close proximity to Cabrillo Beach. 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; Gobel et al., 2007; Fink & Manley, 2011).

Water in White Point Beach tidepools had significantly higher concentrations of Zn compared with Cabrillo Beach tidepools. Although there may be a specific source of Zn near White Point Beach of which we are unaware, it is unlikely Zn is being carried up-shore to be deposited in rocky intertidal pools, since prevailing currents along this coast are southward (Hickey, 2003), and because Zn in solution is quickly oxidized or adsorbs to other molecules and settles out of the water column. The most likely source, however, 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 the Los Angeles area. These urban areas can act as point sources of heavy metals which are deposited directly from the air into water, or through runoff from streets and walkways. White Point Beach has a road and a parking lot next to the tidepool beach area, increasing the potential for roadside runoff and other anthropogenic pollution to enter the intertidal ecosystem.

While concentrations of both Pb and Zn were significantly different (Pb $P < 0.001$, Zn $P < 0.001$) between the two tidepool locations, they were not significantly different in the respective *P. samuelis* populations. 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 the uptake of these two metals, such as food (Fink & Manley, 2011) or sediment (Huh, 1996). Rainbow (2002) discussed various mechanisms for removal of heavy metals from tissues by invertebrates. These strategies may include 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 studies are needed to determine if these hermit crabs are capable of shedding particular metals, since even closely related species within the same genus may have different internal levels of metals when studied under the same heavy metal conditions (Moore & Rainbow, 1987; Rainbow, 1993; 1998). Cabrillo Beach hermit crabs had significantly higher concentrations of Cd compared with those from White Point Beach. While Cd levels were different in the two hermit crab populations, they were both significantly lower than the inshore seawater in which they live. This

![Comparison of heavy-metal concentrations in samples of Pagurus samuelis and seawater from Cabrillo Beach. Data is shown in a box and whisker plot in ppm values. Note differences in y-axis scales.](https://static.oup.com/upc/8100/tifs/1810039159d08635937f7bb8b.png)
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* (Linnaeus, 1758) and discovered they were able to excrete some of their internal Cd concentration across the gut membrane, thus allowing individuals to have a lower internal Cd concentration than if they only stored Cd in tissues.

Seawater concentrations of Cd and Zn in both studies locations were higher than the Environmental Protection Agency’s (EPA) California toxins rule for chronic concentrations (Anonymous, 2000). Concentrations of metals in solution may increase in shallow pools at low tide due to rapid evaporation. EPA’s California toxins rule for acute concentrations has a maximum concentration of 40 ppm for Cd and 90 ppm for Zn (Anonymous, 2000). While, this does appear to suggest that *P. samuelis* may be facing toxic concentrations of Zn, these hermit crab populations, nonetheless, appear to be thriving in spite of the circumstances.

Both populations of hermit crabs live near dense urban populations. As an intertidal species, *P. samuelis* is able to survive many short-term acute environmental changes such as hypoxia (Dunbar et al., 2017; Valère-Rivet et al., 2017), burial (Shives & Dunbar, 2010), and we studied rapid fluctuations in temperature (Jokumsen & Weber, 1982) and salinity (Davenport et al., 1980). The *P. samuelis* populations appear to be thriving despite having accumulated nonessential metals in their tissues.

Although our results do not directly demonstrate that *P. samuelis* is an adequate indicator species for marine heavy-metal pollution, expanded investigations may do so. Guns et al. (1999) found that congeneric *P. herbsti* was an adequate indicator species for heavy metals in Belgium. Still, Rainbow (2002) demonstrated that internal metal concentrations in some invertebrates above baseline do not inherently negatively impact their quality of life. For example, Lyla & Ajmal Khan (2011) found the pattern of preferential accumulation in tissue for both Cu and Zn to be hepatopancreas, ovary, and muscle in that order in the hermit crab *Clibanarius longitarsus*. Those authors suggested that the preferred storage of metals in the ovaries over muscle could allow for removal of unwanted metal concentrations. Bjerregaard et al. (2005) found seasonal differences in Cd concentrations in *Carcinus maenas*, suggesting there is some regulation involved. Invertebrates may well be negatively affected by internal buildup of heavy metals only when these exceed the regulatory capacities for storage or excretion. At less toxic concentrations, effects may be seen in the slowed development of the hermit crab *Clibanarius longitarsus* (Lyla & Ajmal Khan, 2010) and in the crayfish *Faxonius rusticus* (Girard, 1852) (Hubschman, 1967). At highly toxic concentrations, heavy metals can lead to death (Hartmut & Gerstmann, 2007; Cooper et al., 2009; Lyla & Ajmal Khan, 2010).

We found differences in heavy metal concentrations between two populations of hermit crabs, suggesting that these populations are experiencing different environmental pressures. While *P. samuelis* may not be negatively affected by these levels, animals that prey on *P. samuelis* may bio-accumulate metals to sub-lethal or lethal levels, as seen with respect to mercury in the edible crab, *Carcinus maenas*, in Portugal (Cardoso et al., 2014). Further studies are needed to investigate internal regulation of heavy metals, lethal dosages, and nonlethal effects on the development and growth of *P. samuelis*, and the potential for biomagnification in animals that prey on this common intertidal species.

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