SUURJ: Seattle University Undergraduate Research Journal

Volume 2 Article 13

2018

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Sub-lethal Effects of Heavy Metal Pollution on Intertidal Crustaceans in the Duwamish Waterway

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Introduction

The Green River starts in the Wenatchee National Forest on the eastern slopes of the Cascade Mountain Range. It twists and turns through central Washington and as it approaches the city of Seattle, its name changes to the Duwamish Waterway. Indigenous groups have inhabited areas along the Duwamish Waterway for as many as 12,000 years, making it a cultural, spiritual, and economically significant area (Burke Museum, 2009).

Shortly after white settler colonizers arrived in the West in 1851, the Klondike gold boom in Canada caused Seattle to become a center of industrialization and development (Burke Museum, 2009). By 1920, 4.5 miles of the mouth of the river had been dredged 50 feet deep to straighten its mouth in order to allow for large ship navigation and industrial development (Wilma 2001). Dredging and development of the waterway led to extreme industrial pollution. Industrial toxins include, but are not limited to, elemental toxins (cadmium, zinc, copper, and lead) and toxic organic compounds like polychlorinated biphenyl (PCB), polycyclic hydrocarbons (PAH), and phthalates (Lower Duwamish Waterway Site History, 2017). In 2001 the Environmental Protection Agency (EPA) declared the Duwamish Waterway a Superfund site, an area that has been declared a risk to human and/or environmental health due to contamination, and that receives federal funds for cleanup (Lower Duwamish Waterway Site History, 2017). As Seattle continues to be one of the fastest growing cities in the United States, the resulting pollution from this growth will impact the surrounding ecosystems and the organisms that inhabit them.

Throughout its history there has been specific recording of the heavy industrial pollution along the Duwamish Waterway. According to a 1945 state report there was a recorded 500 pounds per day of acetylene generator waste from Todd Dry Docks and other sources, 200–250 pounds per day of "highly toxic chromic acid solution" from Boeing Plants 1 and 2, and 1,200–1,500 gallons of acid dip dumped monthly from National Steel Construction (Lacitis, 2014). The Washington Department of Ecology lists the currently operating industry along the Duwamish Waterway as cargo handling and storage, marine construction, boat manufacturing, marina operations, concrete manufacturing, paper and metals fabrication, food processing, and airplane parts manufacturing (Lower Duwamish Waterway Site History, 2017).

Industrial and urban activity like dredging, toxic runoff, and dumping of wastewater can have a damaging effect on marine organisms when the deposited contaminants accumulate in sediment. While some metals, like zinc, copper, and manganese, which are naturally present in aquatic environments, are required for the physiological processes of organisms, the majority of heavy metals can cause extreme toxicity and harm to the biological processes of organisms. Heavy metals accumulate in sediments, algae, and the tissues of organisms and are not flushed out of systems efficiently (Frisch, 2011). Crabs are in direct contact with pollutants since they live in, and feed on prey living in, contaminated sediment

(Zhao et al., 2012). It has been found that decapods could not regulate non-essential heavy metals on any bodily level (Rainbow, 1985). Lead was found to be associated with toxicity and retention in benthic organisms over time (Ringenary et al., 2006), and such contamination has lethal or sub-lethal effects on crustaceans. Lethal effects result in the quick mortality of an organism before it is able to reproduce, while sub-lethal effects change the performance of an organism but do not completely hinder its ability to reproduce (Ross, 2002). Examples of sub-lethal effects are reduced fecundity, reduced ability to find food, or reduced biological functions. Heavy metal contamination of estuaries could cause additional stresses on aquatic organisms, which may exhibit changes in respiratory processes, and thus the ability to carry out physiological processes.

Like lead, other toxins can have long residence times and cause sub-lethal effects on organisms. For instance, increased naphthalene, a polycyclic aromatic hydrocarbon and a byproduct of coal refinement, when introduced to crabs caused an increase in oxygen consumption, an indicator of increased stress (Vijayavel et al., 2006). Crabs were observed to have the highest concentration of heavy metals in the lungs (Zhao, 2012). Marine species subjected to heavy metals and toxins similar to those of the Duwamish Waterway are likely affected by such contaminants.

Despite the well-documented history of lead contamination in the Duwamish Waterway, the effect of such contamination on the aquatic organisms has scarcely been studied. Investigation of the aquatic system's ability to recover naturally from a stressor has been neglected considerably in environmental assessments. This dearth of research is primarily due to lack of attention to the recovery from a stressor of an aquatic system and lack of pre-disturbance data to properly assess if a system has recovered (Neimi et al., 1990). Using organisms as monitors would allow for examination of recovery from a stressor, such as sediment contamination.

Studying organisms as pollutant monitors has many advantages over the chemical analysis of abiotic comportments (Karadede-Akin et al., 2007). It has been seen in mussels, oysters, and clams that sediment contamination has effects ranging from changes in the community structure to life history alterations, such as impairment of reproduction and age selective toxicity. In the Puget Sound, sediment was assayed with regards to the life cycle of a marine polychaete worm. Exposure to contaminated sediment resulted in slower growth rates, non-egg bearing females (indicating impaired reproduction), and higher initial mortality rates. Similarly, the impairment of reproduction and development was seen in oyster larvae with sediment contamination (Karadede-Akin et al., 2007).

Examining sediment contamination in the benthos can be separated into two parts: the impacts of sediment contaminants on the benthos and the process by which benthic invertebrates can affect contaminant movement. Organisms can ingest copious amounts of lead and survive, yet the physiological toll and cycling of these contaminants to the next generation in the ecosystem is largely unknown. The most obvious effect of contaminated sediment of marine invertebrates is direct acute toxicity. More subtle chronic impacts may be observed, such as changes to physiological processes, brooding, and feeding. (Reynoldson, 1987).

The aim of this study was to investigate the impacts of sediment contaminants on commonly found benthic organisms along the Seattle, Washington coastline. We examined lethal and sub-lethal effects of lead contamination on three benthic crustaceans (*Idotea wosnesenskii, Gnorimosphaeroma oregonensis, and Hemigrapsus oregonensis*) along two sites in the Duwamish Waterway in comparison to two other less contaminated sites. We also examined brooding proportions of *H. oregonensis* during the regular brooding season at contaminated and non-contaminated sites. There is significant research on decapods, but we added isopods because they were another species found consistently throughout our contaminated and non-contaminated sites. We also examined *Fucus*, an intertidal algae, because, like our crabs and isopods, it was consistently found and serves as a food source for both species. Examining the effect of sediment contamination on *Fucus* would serve to investigate food source preferences among isopods.

First, we used field surveys to investigate preliminary heavy metal levels in sediment and *Fucus* at the four sites, and to examine the proportion of brooding female crabs, *H. oregonensis*, at the sites. Next, we examined survival of the isopod *G. oregonensis* in contaminated vs. uncontaminated sediments. Finally, we tested whether the herbivorous isopod *I. wosnesenskii* preferred contaminated vs. uncontaminated algae (*Fucus* sp.) for a food source. The purpose of these examinations were to see to what effect, if any, heavy metal contamination in the sediment of the Duwamish Waterway had on the most common benthic-dwelling organisms as an indicator of how the ecosystem has been changed due to repeated industrial pollution.

Methods

<u>Study Area and Focal Species</u>

Contaminated sites included two locations along the Duwamish Waterway, close to the entrance to the Puget Sound. Herring's House is a 15.5-acre area currently under restoration efforts to remediate the Duwamish Waterway as part of the Superfund cleanup effort (Herring's House Park, 2017). Diagonal Marsh is a 1.2-acre park located across the river from Herring's House that has experienced dredging and runoff pollution (Terminal 108 Park, 2017). Both sites consist of a mixture of silty and muddy substrate with small rocks and boulders littered with large amounts of organic debris and human trash. These sites have exposure to freshwater from the Duwamish River, making their water brackish.

The two uncontaminated sites were Golden Gardens in North Seattle and Alki Beach in West Seattle, two beaches associated with public parks. Golden Gardens and Alki Beach differ mostly in substrate, with Golden Gardens providing a fine grain sand beach and Alki Beach providing a rocky pebble beach. Both beaches have constant human disturbance but no industrial activity or presence. Neither beach has any significant exposure to freshwater.

We examined three benthic crustacean species. *H. oregonensis,* known as the shore crab, is a common crab that feeds mostly on diatoms and algae. *Idotea wosnesenskii* is an isopod species that resides on the underside of rocks and eats the intertidal algae *Fucus* sp. (*Pentidotea wosnesenskii*, 2017). *Gnorimosphaeroma oregonensis*, commonly known as the Oregon pill bug, is an intertidal isopod found under rocks that feeds on detritus (Light and Carlton 2007).

Heavy Metal Assay

Heavy metal levels were measured in dried sediment (n = 4 per site) and *Fucus* samples (n = 3 per site) from each of the four sites. Sediment and *Fucus* samples were dried at 120 °C for 24 hours then ground into a fine powder in either a mortar and pestle or an electric coffee grinder. A trace metal analysis using an ICP-MS trace metal analyzer was conducted to test for differences in concentration (parts per billion, ppb) of copper and lead. We conducted a one-way analysis of variance (ANOVA) to test whether heavy metal accumulation in sediments or algae differed among sites.

Seasonal Brooding Proportions

To examine whether heavy metal contamination affected the reproductive potential of crabs (*H. oregonensis),* we surveyed the proportion of brooding females from our four locations over late spring to midsummer (May-August). At each site we collected and sexed the crab from under randomly chosen rocks and noted the number of ovigerous (brooding eggs) and non-ovigerous females from a sample of 60 total crabs at each site. We used Chi-Square tests to measure any significant variation of proportions within a given month. If the Chi-Square test came up significant ($P \geq 0.05$), all sites were compared individually using 2x2 Fisher's exact tests to determine which sites were significantly different from each other. We then corrected for repeating measures using a Bonferroni corrected P-value of 0.0083.

28-day Mortality Bioassay

We used a 28-day mortality bioassay to assess the lethal effects of lead levels in sediment from contaminated sites on the mortality of the isopod *G. oregonensis.* A 28-day bioassay is the standard measurement to assess chronic effects of a contaminant in a bioassay (Ringenary et al., 2007). In two-inch glass petri dishes, 5 mL of sediment was added to the bottom with salt water (35 ppt Instant Ocean) filled 1 cm from the top. We obtained lead-contaminated sediment from the Herring's House site (mean lead concentration = 21.62 ppb) and uncontaminated sediment from Padilla Bay (lead = 1.18 ppb), an additional site that lacked isopods and crabs but did have sediment of a similar size to that of Herring's House. We used two isopod populations, Golden Gardens (uncontaminated) and Herring's House (contaminated), resulting in a 2x2 experiment (isopod population x sediment type). We filled 20 dishes with contaminated sediment, 10 received an isopod from a contaminated site and 10 received an isopod from an uncontaminated site. We prepared 20 more dishes the same way, but filled them with uncontaminated rather than contaminated sediment. One isopod was added to each dish. We used a 2x2 contingency test to examine whether there were any effects on isopod population or lead contamination level on isopod survival.

Fucus Preference

We tested the food preference of two populations of *I. wosnesenskii* for contaminated vs. uncontaminated *Fucus* using a 4-hour habitat choice assay. Isopods were collected from Whidbey Island (uncontaminated site) and Diagonal Marsh (contaminated site). An isopod was placed into a container with contaminated *Fucus* at one end and uncontaminated *Fucus* at the other, separated by 10 cm. At 30-min intervals, we scored if the isopods were on the contaminated algae (score = -1) or uncontaminated algae (score = 1) for a total of 4 hours (n = 8 observations). We then averaged together the score of each individual isopod and conducted a non-parametric Wilcoxon test to see whether there were differences between populations in their preference. Since there were no differences between isopod populations in their mean preference (Wilcoxon test, $P = 0.4359$), we pooled together populations. We then tested whether mean preference values differed from 0 (the null expectation if preference for uncontaminated and contaminated sediment was equal) using a one-sample t-test.

Results

Heavy Metal Assay

There were significantly higher levels of lead (Figure 1a) and copper (Figure 1b) in the Herring's House sediment compared to all other sites ($P < 0.05$, Tukey post-hoc tests). Diagonal Marsh had intermediate lead levels, but was not significantly different from Alki or Golden Gardens ($P > 0.05$). There were significantly higher concentrations of lead in *Fucus* from Herring's House than from Alki and Golden Gardens ($P < 0.0053$). Diagonal Marsh had intermediate lead levels (Figure 1c), but not significantly different from Herring's House, Alki, or Golden Gardens ($P > 0.05$).

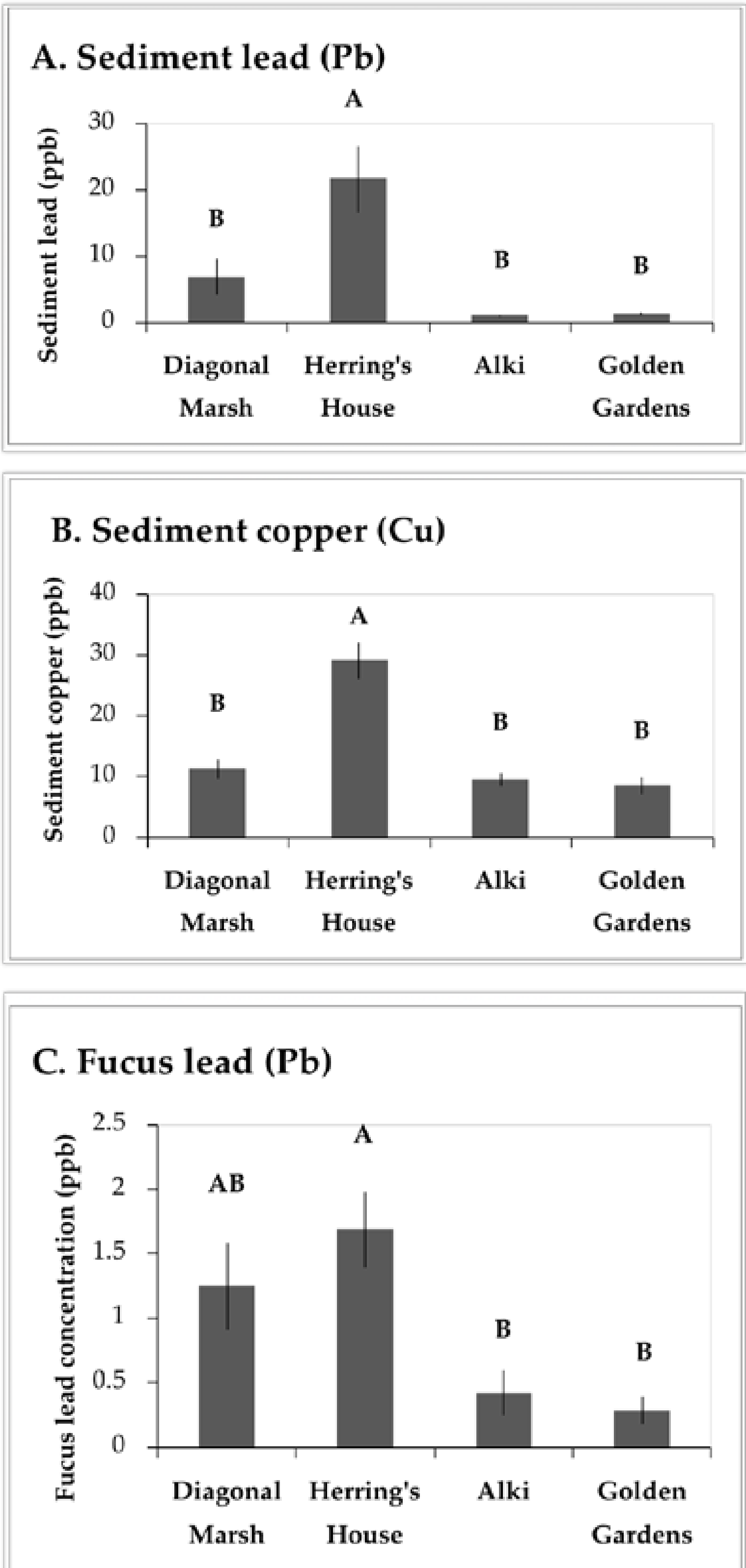

Figure 1 *Average concentrations (ppb) of (a) lead sediment, (b) copper sediment, and (c) lead in Fucus at each of the four study sites: Diagonal Marsh, Herring's House, Alki Beach and Golden Gardens. Different letters over each mean indicate statistically significant differences (Tukey Test). Each sample was collected between May and June of 2017.*

Brooding Proportions

The significant variation in brooding proportions was seen in the months of May and June, with June having the largest proportion of brooding females (Figure 2). In May, Alki Beach was measured having the largest proportion of brooding females and was significantly different from all other sites ($P < 0.0083$).

In June, Alki Beach and Golden Gardens had the highest proportions (40%-60%) of brooding females, though after statistical analysis, only Alki Beach had brooding proportions significantly different from a contaminated site. The brooding proportions at Alki Beach were significantly higher than Diagonal Marsh ($P = 0.0023$). Golden Gardens did not significantly differ from any of the other sites in terms of number of brooding females, possibly because the sample size was low (n=7 female crabs examined). There were no significant differences among sites in August ($P > 0.0083$). In May, only Alki differed significantly from other sites ($P < 0.0083$).

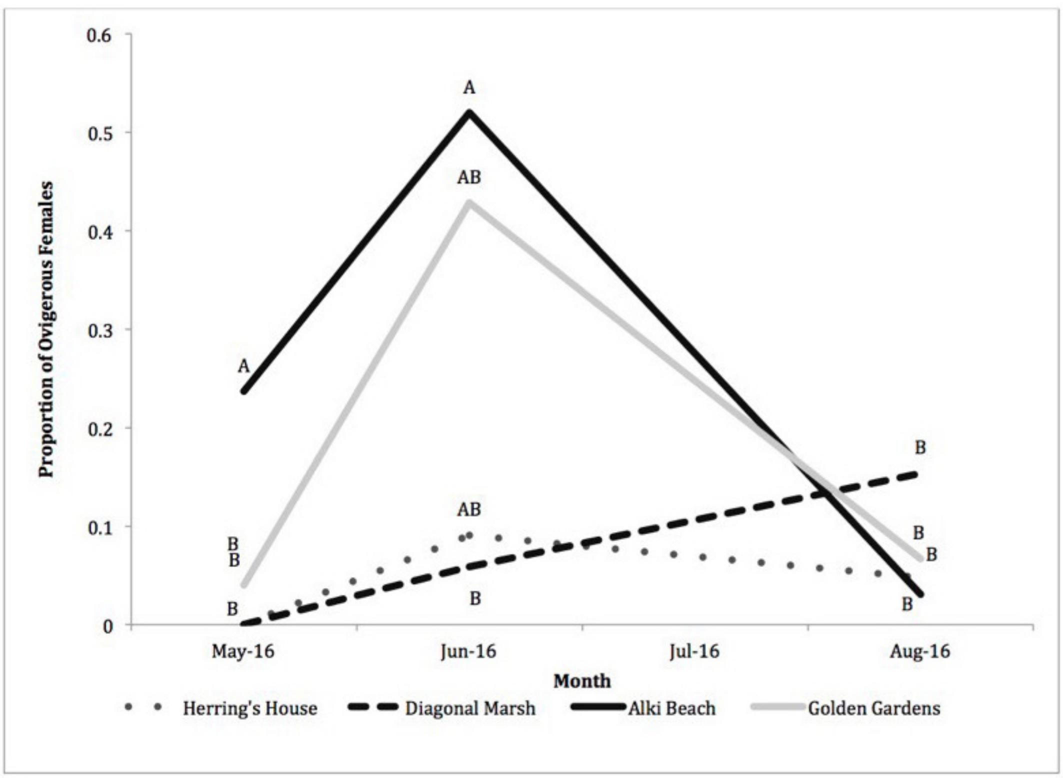

Figure 2 *The proportion of ovigerous females crabs (H. Oregonensis) during the seasonal brooding months (May to August) at each of the four study sites. Different letters by each site indicate significant differences among sites for a sampling date.*

28-day Bioassay

There were no significant differences among treatments in days of survival of *Gnorimosphaeroma* (ANOVA, P = 0.2085, Figure 3). There did appear to be high rates of fatality in juvenile isopods in contaminated sediment in our experiments, but officially recorded bioassays were never conducted, only informal tests. Juveniles were so sensitive to isolated conditions that they rarely made it past 24 hours in test conditions.

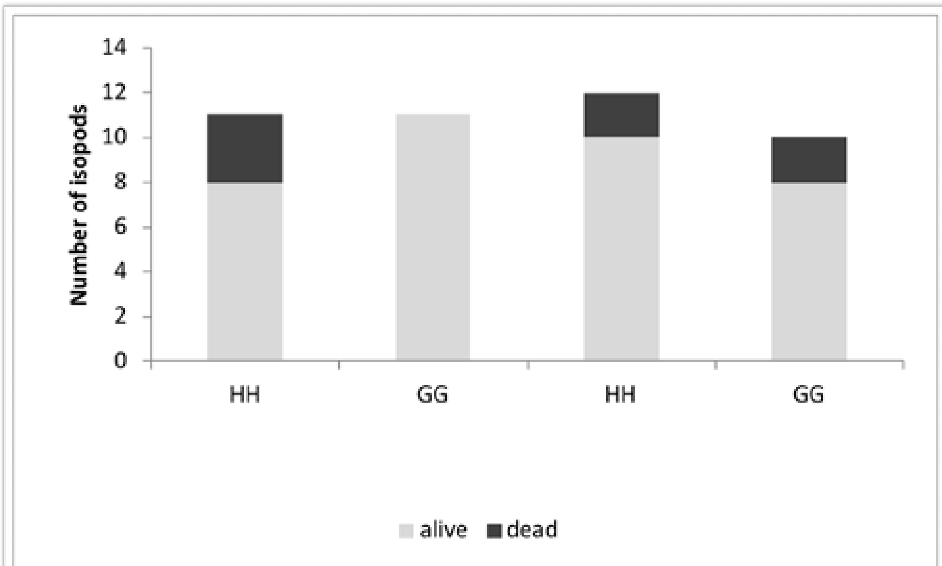

Figure 3 *Mean survival in Gnorimosphaeroma in contaminated and uncontaminated sediments, and from different isopod populations (GG = Golden Gardens, uncontaminated; HH = Herring's House, contaminated).*

Fucus Preference

I. wonsnesenskii isopod populations did not differ in their preference for contaminated or uncontaminated *Fucus* (T-Test, P = 0.3696, Figure 4). Isopods from contaminated sites appeared to have a stronger preference for contaminated *Fucus*, but after statistical analysis they did not differ significantly from the preferences of the isopods collected at the uncontaminated site.

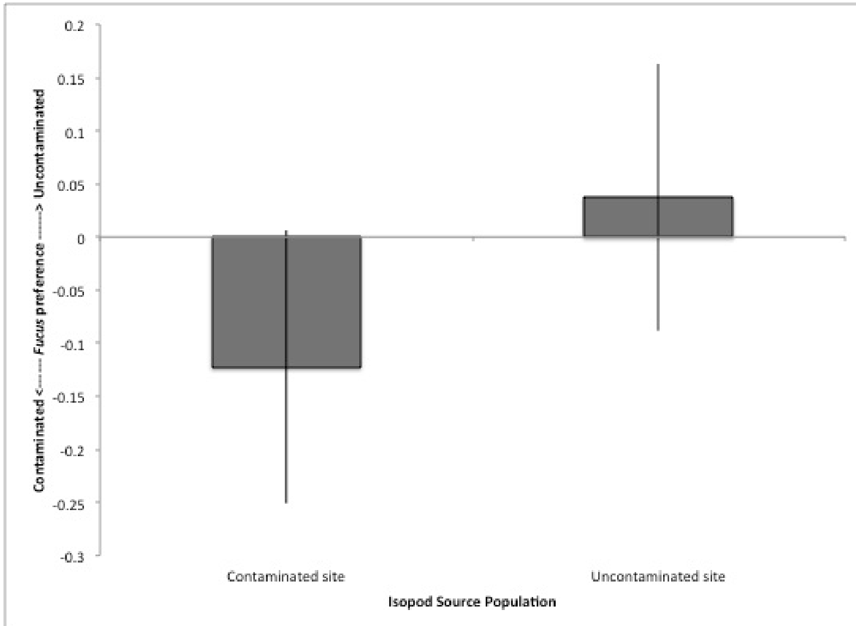

Figure 4 *I. wosnesenskii did not exhibit any significant preferences for either uncontaminated or contaminated Fucus.*

Discussion

The metal assay detected significant lead concentrations among sites in the Seattle area with significantly higher concentrations in both sediment and *Fucus* at sites in the Duwamish Waterway, especially Herring's House. At these same sites, we measured differences in brooding proportions, a sub-lethal effect that has the potential to significantly alter the health of an ecosystem.

The data analysis from our studies points to few measurable effects of heavy metal contamination among populations of *I. wosnesenskii* and *G. oregonensis.* We measured no significant lethal effects among mature populations, and sub-lethal effects remained hard to measure.

Studies involving the juvenile *I. wosnesenskii* were not officially recorded, but they showed high levels of sensitivity in the lab to isolation, regardless of exposure to contaminated sediment. When juveniles were isolated on both sediments, all died within 24 hours. A further study into relative sensitivities and responses to contaminated sediments could improve the resolution of sub-lethal effects on the populations.

Our experiments indicated that contaminated sediment and algae had few measurable, detrimental effects on isopods. Together with the heavy metal assay data and the recorded industrial chemical dumping, our study shows that there is indeed industrial disturbance and pollution. The organisms we studied may be the most resistant within their ecosystems, while other species may have died out long ago. A species presence and absence assay may reveal species that were once present in these spaces and therefore were more sensitive to local pollutants.

In order to continue this project, a more established understanding of the variation in contamination among sites is key. While these sites have a well-documented history of contamination and disturbance, more heavy metal concentration assays of lead need to be established in order to reinforce the data previously gathered. The levels of the heavy metals in this region may be less than those that significantly affect isopod populations. Since contaminants have been around for 100 years already, future studies might consider incorporating more sensitive species that used to occupy Duwamish Waterway habitats but no longer exist in large numbers there. Incorporating sensitive fish groups such as salmon (*Oncorhynchus sp.*), perch (*Perca*), bass (*Micropterus sp.*), and sole (*Solea*) could benefit future studies.

The differences between brooding females at non-contaminated sites compared to contaminated sites was particularly interesting. Higher proportions of brooding females at non-contaminated sites (significant in the month of June) could suggest disturbance of heavy metal resistance. These crabs may have developed a tolerance to such stressors over years of continuous contaminant exposure, expressing different responses to the disturbances. More often than not, contaminated sites did not significantly differ compared to non-contaminated sites. Disturbances on species in aquatic systems can be classified as pulse and press disturbances, as was done in the study by Neimi et al. (1990). Chemical stressors are examples of pulse disturbances while press disturbances are longer in duration and usually involve changes in a stream channel, where residual stresses may remain for long periods of time (Neimi et al., 1990). It is possible that benthic organisms in the Duwamish Waterway are experiencing pulse and press disturbances, and thus these brooding differences for each month are occurring. Pulse disturbances are considered relatively short-term, discrete events in time; common examples of pulse disturbance are drought and fire. Press disturbances are more gradual and long term, with a cumulative pressure on a system. Scientists often determine classification of a disturbance by considering whether it is short or long term. Runoff from the Duwamish Waterway could be considered a pulse disturbance if it clears from a given parameter in less than a year. However, if runoff remains sedentary within the given parameter, then prolonged contamination could occur and thus be described as a pulse disturbance. Both disturbances occur in the Duwamish Waterway, with locality and severity varying throughout the Waterway, and thus making such disturbances difficult to classify.

In a study published by Neimi et al. (1990), the authors give the example of two aquatic systems, A and B, which experience the same stressor. System A may experience substantial effects from the stressor, while system B experiences minor effects. In contrast, system A recovers relatively fast, while recovery of system B is slow or non-existent. Examining such systems in a short-term study limits our understanding because by short-term observation the stressor on system A is determined more detrimental. However, in long-term observation we may see that the overall effects are more detrimental to system B because of the slow or nonexistent recovery relative to system A. Continuing a long-term investigation of sediment contamination effect on benthic species in the Duwamish Waterway will be the only vehicle by which the recovery phase of the system can be understood.

It is possible that the benthic organisms studied in this paper were beginning to present an initial example as in system B. Given that these species were still present in highly contaminated sediment, it appears at first glance that crabs, isopods, and *Fucus* are functioning like system B as in the described example. However, only with continuous long-term observation will we be able to clearly see the effects of pollution on such benthic organisms. With the inception of the Superfund project on the Duwamish Waterway, now would be an ideal time to implement continuous observation. Such long-term studies would allow us to see how these benthic organisms change as the Duwamish Waterway is slowly restored. Though we lack knowledge of how these benthic organisms existed prior to heavy sediment contamination, we expect to see changes in benthic species over time through what we hope will be a successful Superfund project. With this in mind, we urge a long-term environmental assessment examining sediment contamination in the Duwamish Waterway to learn how anthropogenic stressors in aquatic environments alter ecosystems for benthic organisms.

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