Detection of Heavy Metals in Rocky Mountain Tailed Frog (*Ascaphus montanus*) Tadpoles near Abandoned Mines in Northern Idaho

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ABSTRACT

DETECTION OF HEAVY METALS IN ROCKY MOUNTAIN TAILED FROG (*ASCAPHUS MONTANUS*) TADPOLES NEAR ABANDONED MINES IN NORTHERN IDAHO
by

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Amphibians are important bioindicators in environmental assessment. This highly diverse group of vertebrates is experiencing unprecedented declines worldwide due to a complex array of factors, including disease, habitat loss, invasive species, and environmental pollution. Heavy metals are especially problematic due to their persistence and ability to present a localized hazard even at sublethal levels. Northern Idaho has a long history of mining activity, and many watersheds have experienced heavy metal contamination. These streams contain many sensitive species, including the Rocky Mountain Tailed Frog (*Ascaphus montanus*). While their populations are known to be especially vulnerable to logging and road building, the effects of local mining have not been documented. In order to assess the vulnerability of this species to heavy metal pollution, tadpoles were collected from three distinct populations across the Idaho Panhandle National Forest (IPNF). Two sampling sites (Gold Creek and Beauty Creek) were characterized by abandoned mines in the headwaters of the streams, while a stream absent of any local historic mining (Bumblebee Creek) served as a reference site. Whole tadpoles were pooled and treated using EPA Method 3050B and analyzed for total metal concentration (ug/g) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Concentrations of metals in tadpoles (ug/g) at mining sites were generally higher than the reference site. Cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) were significantly higher in tadpoles at Gold Creek when compared with the reference site. These results are consistent with other studies, indicating metal pollution from historic mining are still evident in these stream communities.

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CHAPTER I

INTRODUCTION

Amphibians are one of the fastest declining animal groups worldwide due to complex, nonrandom local causes (Blaustein *et al.* 1994; Alford and Richards 1999; Stuart *et al.* 2004). According to the first Global Amphibian Assessment in 2004, almost half of the world's current amphibian species (2468) are declining or threatened with extinction since the 1980's (Stuart *et al.* 2004). Many scientists will agree that we are in the midst of yet another mass extinction event in Earth's history (Wake and Vredenburg 2008; Blaustein *et al.* 2011). The likely and confirmed threats to amphibians globally include habitat degradation, introduced species, predation, over-exploitation, climate change, ultraviolet radiation, environmental acidity and toxicants, and diseases (Blaustein *et al.* 1994; Alford and Richards 1999; Young *et al.* 2001; Stuart *et al.* 2004), with pollution being the second leading cause (Figure 1). Moreover, population declines are often multifactorial and complex and can vary among species, populations, and life stages (Blaustein 2011).

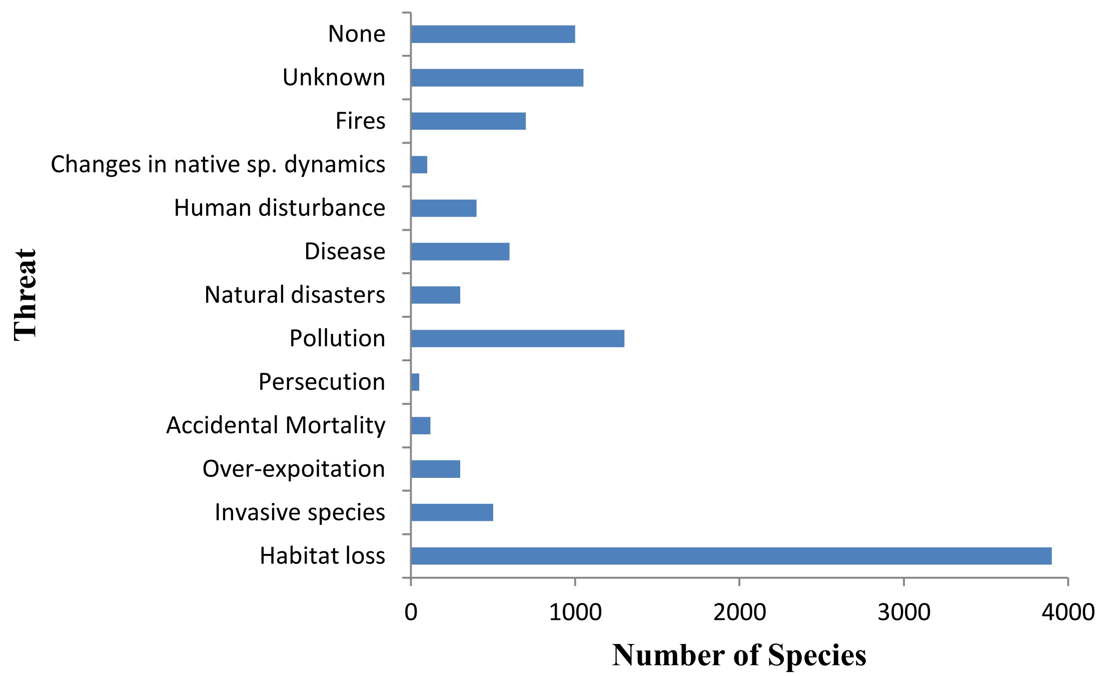

Figure 1. Summary of global threats to amphibians. *Source: IUCN Red List 2008*

Amphibians are often used as bioindicators in environmental assessment. This means they can be used to monitor an ecosystem's health and stability. Furthermore, the integral role of amphibians is of particular ecological importance that may impact other organisms through their natural trophic dynamics. In many systems, adults are vital carnivores and prey species, and in aquatic systems, larvae may also be critical herbivores and prey (Morin *et al.* 1990; Blaustein *et al.* 1994). The risk of direct contact with pollutants is greater in amphibians than many other animals due to their unique life history, which is dependent on both aquatic and terrestrial habitats (Blaustein and Wake 1990; Vitt *et al.* 1990; Alford and Richards 1999; Arrieta *et al.* 2004). Contrary to amniotes, the egg of an amphibian is not covered by a semi-impervious shell, which increases exposure to water-bound pollutants (Read and Tyler 1994). Amphibians may absorb toxicants both through their semipermeable skin and by ingestion making them much more susceptible to environmental toxicants than other animal groups (Burger and Snodgrass 1998). The growing biodiversity loss in amphibians is likely to lead to complex and persistent changes in primary production, energy transfer between terrestrial and aquatic systems, and ecosystem structure (Blaustein *et al.* 2011).

Nearly all ecosystems have been influenced to some degree by human activity frequently resulting in a loss of biodiversity and valuable ecosystem services. Pollution is often so pervasive that it is likely one of the most widespread environmental issues of today. Pollution can alter the chemistry of water or soils, lead to direct mortality of organisms, and affect ecosystem function (Groom *et al.* 2006). Major threatening pollutants include heavy metals which are of particular concern because they do not degrade like organic pollutants and therefore require a much more serious type of remediation (Pepper *et al.* 1996; Arrieta *et al.* 2004). In fact, heavy metals were found to be more prevalent than any other contaminants in remedial action sites documented in a 1984 U.S. EPA survey (Pepper *et al.* 1996).

Some metals are essential to life, while others are extremely toxic. Even essential metals can become toxic in high concentrations. While metals cannot be degraded, they can be transformed, much of which is mediated by microorganisms. Toxicity of a metal is largely determined by the chemical form of a metal or elemental speciation rather than total concentration alone (Pepper *et al.* 1996; Lage *et al.* 2006). Moreover, speciation determines water solubility of a metal thus altering its bioavailability to an organism (John and Leventhal 1995). The complex combination of environmental factors may influence uptake by an individual organism (Sparling and Lowe 1996). Exposure to some metals can result in acute and chronic adverse health effects in animals and humans.

Cadmium (Cd) is a metal that is widely released as a byproduct of zinc mining and smelting, although it also has number of industrial and agricultural applications (Coles *et al.* 1995; Martins *et al.* 2004; Herbette *et al.* 2006). Cadmium is not known to have any metabolic role in plants or animals, with the exception of some marine diatoms where natural zinc concentrations are unavailable (Lane and Morel 2000; Lane *et al.* 2005; Herbette *et al.* 2006). Cadmium in soils and water pose major environmental risks. The metal can enter food webs via uptake by plants where it is stored in their tissues (Herbette *et al.* 2006). Cadmium is known to target the kidneys, as well as cardiovascular, renal, gastrointestinal, neurological, reproductive, and respiratory systems once in the body (Kido and Nordberg 1998; Schnellmann 2001).

Lead (Pb) is one of the easiest metals to mine (Pattee and Pain 2003). Its use increased during the Industrial Revolution and again during the 1900's as a gasoline additive. It has no known functional or metabolic role in biological systems (Arrieta *et al.* 2004). The neurological, behavioral, and developmental effects in children are well documented (Goyer 1996). Lead is a carcinogen in study animals, and possibly humans (Arrieta *et al.* 2004; Goyer 1996). Immunosuppression is a result of lead exposure, increasing susceptibility to diseases and altered host resistance (Luster and Rosenthal 1993). The metal tends to enter the body through dietary intake of organisms that have greater contact with soils (Driscoll *et al.* 1988). This is particularly important for lower trophic animals that directly ingest soils or macroinvertebrates.

Zinc (Zn) and copper (Cu) are essential trace metals for biological systems (Irwin 1997; Chen *et al.* 2007). Zinc and copper are released into local environments as a result of both natural processes and human activities. However, excessive amounts of zinc can suppress the body's ability to absorb copper and metabolize iron (Irwin 1997). Likewise, elevated levels of copper can target organs like the liver and kidney and induce neurodegenerative disorders becoming highly toxic to humans and animals (Chen *et al.* 2007; Pal *et al.* 2013). Copper readily binds to dissolved organic matter in the environment at a low pH, thus increasing mobilization from one trophic level to another (Nierop *et al.* 2002).

These metals have radical effects on a variety of biota. Body concentrations of some metals are positively correlated with environmental concentrations (Sparling and Lowe 1996). Since many metals are leachable, the effects can be widespread. When metals enter the body, they do not always accumulate in tissues homogeneously. Some organs are targeted differentially, affecting system function (Arrietta *et al.* 2004). For example, lead has been noted to compete with and replace calcium, making bone a storage site for this metal (Arrieta 2004, Tripp 2012). Studies (Dilling and Healey 1926; Khangarot *et al.* 1985; Perez-Coll *et al.*; Stansley *et al.* 1997; Lefcort 1998; Chen *et al.* 2007) show that tadpoles exposed to metal-contaminated surface water exhibit increased physical abnormalities, decreased/delayed hatching, stunted growth, and decreased survival rates. For example, Pickerel Frogs (*Rana palustris*) which metamorphosed in lead-contaminated water exhibited hind limb deformities that severely hindered movement (Stansley *et al.* 1997).

Among anurans, there is one basal clade, Family Leiopelmatidae, which diverged from all other frogs over 200 million years ago. This family includes four extant species of New Zealand Frogs (*Leiopelma* spp.) all of which are endemic to New Zealand and categorized as endangered, critically endangered, or vulnerable by the International Union for Conservation of Nature (IUCN) Red List. Conversely, there are only two extant species of Tailed Frogs (*Ascaphus* spp.), both of which are endemic to North America. Nielson *et al.* (2001) recommended the genus *Ascaphus* be split into two species because of mitochondrial DNA differences.

Rocky Mountain Tailed Frogs (*Ascaphus montanus)* are endemic to the Pacific Northwest and their ecology is poorly understood, although there are many shared characteristics with the Coastal Tailed Frog (*Ascaphus truei*). This ancient group of frogs requires cold swift-moving mountain streams where they exhibit extreme philopatry (Daugherty and Sheldon 1982; Welsh and Ollivier 1998; Bury and Adams 1999; Ritland *et al.* 2000). Larvae may take up to four years to metamorphose, and still do not reach reproductive maturity for another four years (Daugherty and Sheldon 1982; Bury and Adams 1999). Many females do not lay their first clutch of eggs until their 9th year, and thereafter in alternating years (Daugherty and Sheldon 1982). While there is no known mating call for this species, mating takes place in the summer to fall where females will retain the sperm and oviposit the following summer (Metter 1964; Adams and Frissell 2001). Eggs will hatch in late summer and tadpoles may remain near the nest site for approximately another year (Brown 1975). It is speculated *Ascaphus montanus* can live a remarkable 15 to 20 years. This primitive yet highly specialized frog is extremely vulnerable to local extirpation and population declines following habitat disturbances

(Dupuis and Steventon 1999). Populations that have experienced a catastrophic decline may have limited recovery because of their unique life history which includes long lifespans, low reproductive output, and low dispersal rates (Hammerson and Adams 2004). *Ascaphus montanus* larvae have a large and distinctive suctorial mouth that allows for attachment on rocks in fast-flowing streams (Altig and Brodie 1972). *Ascaphus montanus* tadpoles are herbivores mostly consuming nonfilamentous algae such as diatoms or periphyton by scraping rocks (Metter 1967; Nussbaum *et al.* 1983; Welch and Ollivier 1998). Welsh (1993) reported that the amount of nonfilamentous algae is a positive predictor of the larvae's presence and abundance.

Multiple studies have found *Ascaphus* populations to be especially vulnerable to logging (Corn and Bury 1989; Leonard *et al.* 1993; Bull and Carter 1996; Dupuis and Steventon 1999; Wahbe and Bunnell 2001; Spear and Storfer 2010) and road building (Welsh and Ollivier 1998). The resulting increased sediment load is particularly deleterious to philopatric stream biota including *Ascaphus* species (Wahbe and Ollivier 1998). Corn and Bury (1989) documented a decline in species richness and abundance in Oregon populations of southern torrent salamanders, tailed frog larvae, and pacific giant salamanders in logged streams when compared to unlogged streams. They suggested loss of critical microhabitat was likely the reason for population declines. Infusion of fine sediment in the coarse substrate reduces the amount of surface area available. As a result, an organism's ability to feed and seek refuge is reduced as it is unable to frequent these interstitial spaces due to siltation. Furthermore, the increased sediment load can alter the water turbidity; the high amount of suspended particulate matter can prevent photosynthesis, inhibiting algal growth (Newcombe and MacDonald 1991), the main food source for larvae.

Ascaphus species are extremely temperature sensitive (de Vlaming and Bury 1970; Claussen 1973; Hawkins *et al.* 1998). Reduction in the forest canopy can increase sunlight penetration and temperature of the stream. Laboratory experiments show that their eggs have the narrowest temperature tolerance range of all North American anurans, requiring 5° to 18.5ºC for successful hatching (Brown 1975), although nests sites can be found in slightly warmer water in the wild (Adams and Frissell 2001). Tadpoles behaviorally avoid high temperatures and are seldom found in streams above 16°C. The tendency to congregate to specific temperature ranges varies with tadpole age (de Vlaming and Bury 1970). Larvae have been observed to migrate with the stream current, although clear cutting, log jams, and high gradient streams can reduce these movements and therefore impede dispersal and population recovery (Wahbe and Bunnell 2001). Metter (1964) suggested adults migrate to smaller and shadier streams to avoid warm temperatures in the summer months. Adams and Frissell (2001) also found evidence that adults migrate seasonally to avoid high temperatures in the summer while moving downstream in the fall to overwinter. Although some studies (Daugherty and Sheldon 1982) found no evidence of seasonal movements, frogs congregating near cool groundwater seepage may likely be utilizing thermal microhabitats and exhibiting behavioral thermoregulation (Adams and Frissell 2001). It has also been documented that gravid females may travel to smaller tributaries together to oviposit their eggs (Brown 1975; McEwan 2013). Little is known about the movement patterns of juveniles, but Bury and Corn (1987) captured recent metamorphs in pitfall traps in forested stands suggesting fall migrations of juveniles. Nonetheless, it is clear the movements of *Ascaphus* are in response to local conditions and can be diverse among populations and locations.

Other threats to *Ascaphus montanus* are not well studied, but likely concerns are any activities across their range that might alter their habitat, microhabitat, or microclimate conditions. Anthropogenic additives such as toxic pesticides, herbicides, fertilizers, and fire retardants may adversely affect these critical conditions. In addition, cattle grazing, climate change, fire, flood, disease, invasive species, and habitat fragmentation may pose an increased risk to this sensitive species (Olson 2011). Welsh and Ollivier (1998) argued that long-lived stream amphibians that exhibit philopatry are more reliable indicators of ecosystem stress than anadromous fish and macroinvertebrates. Utilization of streambed interstices is a shared characteristic among amphibians, fish, and macroinvertebrates; however, other factors such as longevity, movement patterns, and breeding habits in other taxa may complicate their reliable use as bioindicators.

The Idaho Panhandle National Forest (IPNF) of northern Idaho is considered one of the world's richest heavy metal mining locations. Mining largely galena, a natural mineral form of lead sulfide with significant amounts of silver sulfide, the Coeur d'Alene Basin has produced approximately 1.2 billion ounces of silver, 8 million tons of lead, and 3.2 million tons of zinc, beginning over 100 years ago (Adams 2004; Lefcort *et al.* 2010). An estimated 62 million tons of mine tailings, containing 880,000 tons of lead and more than 720,000 tons of zinc were directly discharged into the Coeur d'Alene River and its tributaries prior to the 1970's (Adams 2004). However, practices post-

1970's contribute relatively little to the contamination. Most of these ore tailings have been widely distributed over thousands of hectares by water particularly during high-flow events. When soils are disturbed, especially during flood years, heavy metals from tailing and contaminated sediments continue to enter ecosystems and adversely affect biota (Lefcort *et al.* 1998). Additional distribution of ore and concentrates were a result of construction and railroad cars spills during transport (Adams 2004). Despite cleanup efforts, the impacts of the mine waste containing arsenic, cadmium, lead, zinc, and other metals that were deposited still dramatically impact terrestrial and aquatic life of the northern Rockies roughly a century later (Lefcort *et al.* 1998, Adams 2004; Lefcort *et al.* 2010).

In order to investigate mining related activities on *Ascaphus montanus,* tadpoles were sampled from three different streams in Idaho Panhandle National Forest (IPNF) in May of 2013. To associate heavy metal concentrations in tadpoles from historic mining sites, a reference site with no known mining history was used for comparison. The aim of this research was to contribute to the ecological understanding and conservation of a sensitive species belonging to a globally declining vertebrate group, while the goal was to determine if abandoned mines still impact stream biota, specifically *Ascaphus montanus*. The objective of this study was to determine if heavy metal concentrations in tadpoles at chosen mining sites were greater than those of the reference site with no mining.

CHAPTER II

MATERIALS AND METHODS

Study Location

An Institutional Animal Care and Use Protocol (IACUC) and United States Forest Service (USFS) collection permits were obtained to collect and research *Ascaphus montanus*.

Northern Idaho (Figure 2) was chosen due to species occurrence and rich local mining history. Sampling sites in this study were characterized by established populations and by historic mines located in the headwaters of the streams. Samples were collected from Bonner, Kootenai, and Shoshone counties (Figure 3). There are known historic mining sites situated in the Idaho Panhandle National Forest both in the Lake Coeur d'Alene watershed and Lake Pend Oreille watershed. Documented and non-documented sites of *A. montanus* populations were investigated by visual sightings and hand-searching of tadpoles on rocks in streams.

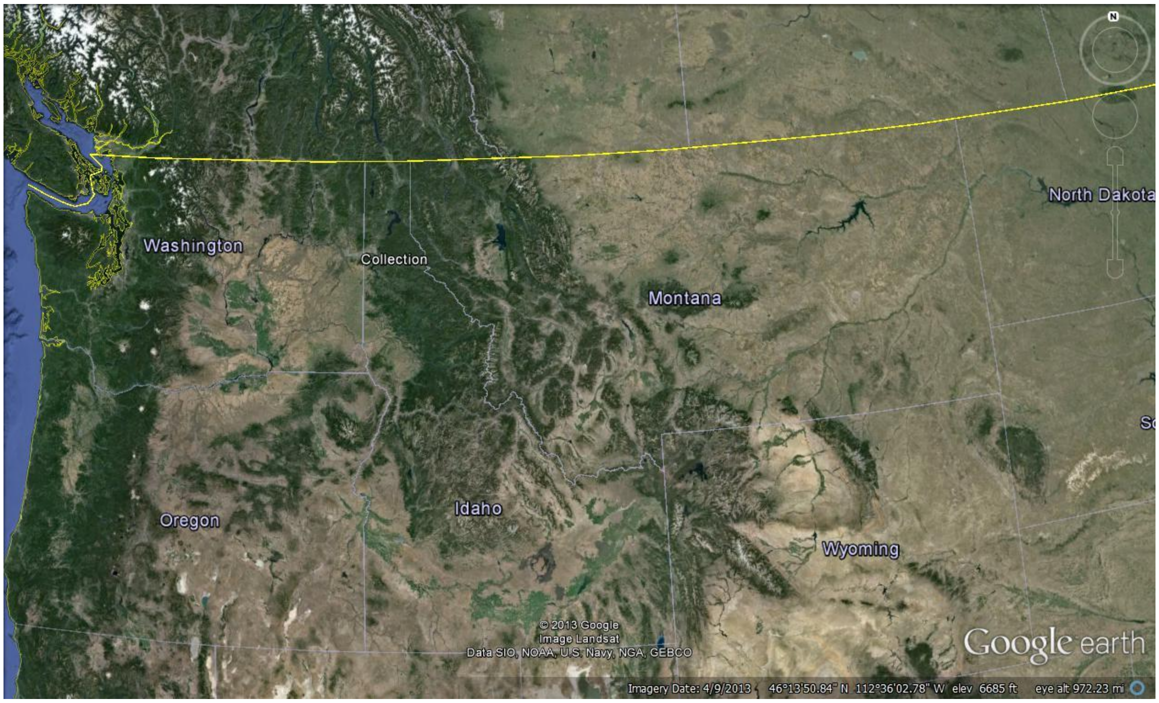

Figure 2. Regional map of study area in northern Idaho. Rocky Mountain Tailed Frogs (*Ascaphus montanus*) are endemic to the Pacific Northwest.

Figure 3. County map of study area. Coeur d'Alene Lake watershed and Lake Pend Oreille watershed are situated in the Idaho Panhandle National Forest. Specimens were collected from Bonner, Kootenai, and Shoshone counties.

A large population of adults and tadpoles exists at Gold Creek (lat. 47.90846667, long. -116.4317833) which flows north into Lake Pend Oreille (Figure 4). This stream is considered of ecological importance because it is the second most productive spawning and rearing site within Lake Pend Oreille Basin for the threatened Bull Trout (*Salvelinus confluentus;* Kiser *et al.* 2010). However, in the headwaters of this drainage are several abandoned mine sites, mining mostly lead and silver that produced wastes increasing the risk to aquatic biota (Kiser *et al.* 2010).

Figure 4. Map of Gold Creek. Mining sites are situated along Gold Creek in Bonner County flowing north into Lake Pend Oreille.

Beauty Creek (lat. 47.60245, long. -116.6624806) is a third order stream that also flows north into Beauty Bay on the northeast end of Coeur d'Alene Lake. The lower part of the stream is limited to sub-surface flows during the summer months due to channelization to protect a USFS campground (Corsi 2007; Keith and Steed 2010). The stream is utilized by Westslope Cutthroat Trout (*Oncorhynchus clarkii*) for spawning and rearing, although habitat degradation has reduced these populations (Wasden 2007). Inactive mines are located upstream of this drainage (Figure 5).

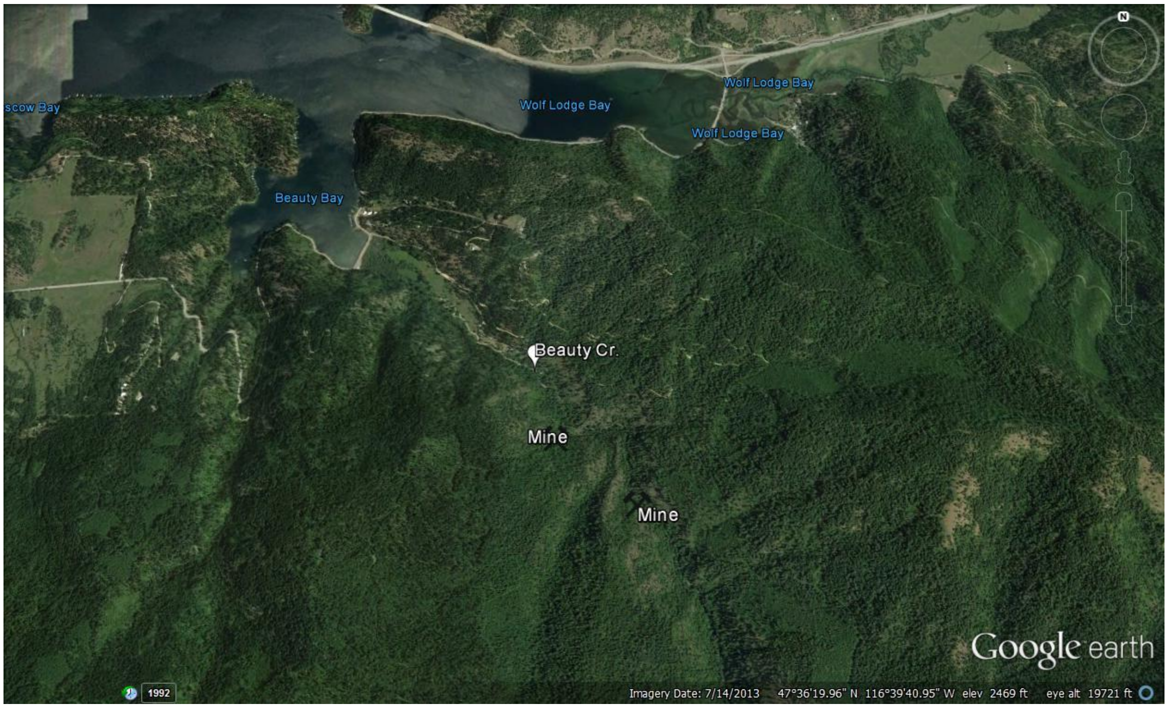

Figure 5. Map of Beauty Creek. Mining sites are situated along Beauty Creek in Kootenai County flowing north into Beauty Bay of Coeur d'Alene Lake.

Bumblebee Creek (lat. 47.64508333, long. -116.2699) drains south into Little North Fork Coeur d'Alene River. A USFS campground exists in the lower end of the drainage. This site served as a reference site due to the lack of apparent mining activity in the area of the headwaters.

Collection

Once presence was established, individuals were collected by hand. One-year old tadpoles were targeted for the study (Figure 6) because of their relative abundance. The age of the tadpole was determined by the Gosner stage (Gosner 1960); young tadpoles (~GS25) were characterized by free-swimming and feeding behavior, often smaller in size, and usually lacking limb buds. Temperature and elevation of sampling locations were documented (Table 1). All tadpoles were then placed in separate Whirl-Pak® bags filled with local stream water and were allowed to depurate (intestinal clearing) for 48 hours (Burger an Snodgrass 1998). Individuals were then euthanized by overdose of tricaine mesylate (MS-222) and placed in sterile scintillation vials and frozen until metal analysis.

Figure 6. *Ascaphus montanus* larva. Notice the large number of denticles and sucker-like mouth used to scrape and adhere to rocks in swift streams.

Table 1. Collection site data. Temperature and elevation for the three sample locations in the USFS Idaho Panhandle National Forest of northern Idaho.

Creek Name	No. of Pooled Samples	Elevation (M)	Water Temp (°C)
Gold Cr.	2	1023.5	5.1
Beauty Cr.	3	704.4	6.8
Bumblebee Cr.	4	710.5	7.1

Metal Analysis

Whole tadpoles were kept in scintillation vials and dried in a Fisher Scientific Isotemp drying oven at 45°C for 72 hours. Tadpoles were pooled within collecting sites to increase sample dry weight (Sparling and Lowe 1996). Dry weights of pooled samples were weighed using an analytical balance to the nearest microgram. Dried samples were transferred to digestion vials and acid digested following a modified EPA method 3050B (Shrestha *et al.* 2006) using a Hot Block (Environmental Express, Charleston, SC). The final volume of each digested sample was 15 ml. The digested samples were then filtered using 0.7 um filters and were further diluted using double deionized water in order to maintain approximately 2% nitric and hydrochloric acids. Each diluted sample was then analyzed using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Agilent 7500ce). Standard Reference Material (SRM) 2782 (industrial sludge) was included for quality control.

Statistical Analysis

Weight differences in the samples for metal concentrations were corrected by converting to ppm (ug/g). Data was then adjusted by using the SRM recovery rate for the specific metal. A student's t-test was performed on the data (Lefcort *et al.* 1998; Gupta and Sinha 2006; Ernst *et al.* 2008). Pooled values from each mining site were compared to the reference site using t-statistics (Baker *et al.* 1997). The null (H_0) is $\mu 1 = \mu 2$, and the alternative hypothesis is (H_1) $\mu 1 > \mu 2$ where the null hypothesis is rejected if the probability of this occurring strictly due to chance (*P*) is <0.05 (Wehr *et al.* 1983).

CHAPTER III

RESULTS OF THE STUDY

Metal concentrations in tadpoles (ug/g) at mining sites were generally higher than the reference site (Figure 7). All analyzed metal concentrations including cadmium (p=0.025), copper (p=0.008), lead (p=0.026), and zinc (p<0.001) in Gold Creek tadpoles were significantly higher compared with the reference site (Table 2). The average lead concentration in tadpoles at Gold Creek was more than ten times greater than the reference site (Figure 8), and the average zinc concentration in tadpoles was more than three times greater at Gold Creek when compared to the reference site (Figure 9). The average cadmium concentration was also twice as high in Gold Creek samples when compared to the reference site (Figure 10).

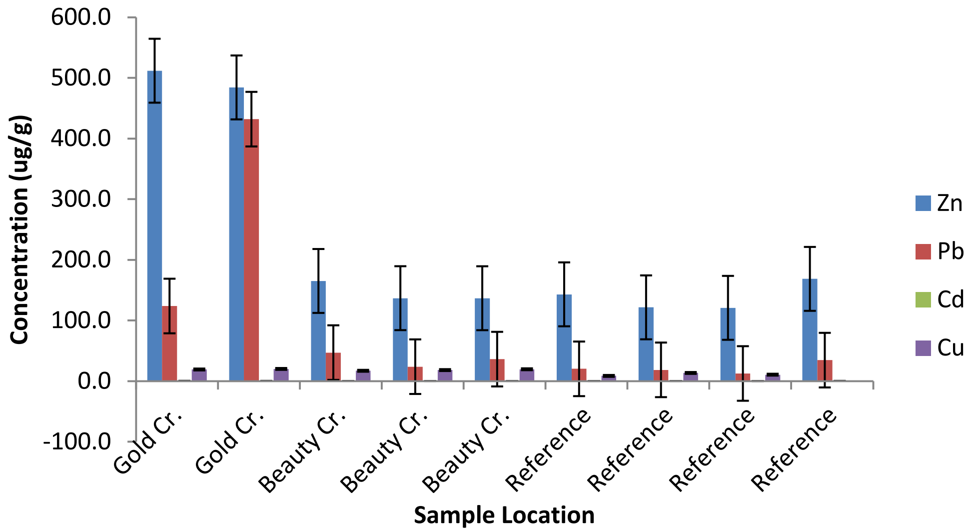

Figure 7. Metal concentrations in pooled samples. Metal concentrations in ug/g and +/- standard error measured in pooled *Ascaphus montanus* tadpoles sampled from three streams in Idaho Panhandle National Forest.

Table 2. Mean tadpole metal concentration. The mean tadpole metal concentrations (ug/g dry wt.) collected from northern Idaho (mean with standard deviation in parentheses).

Metal	Gold Creek	Beauty Creek	Reference
Cd	0.29 (0.02)*	0.15 (0.04)	0.14 (0.07)
Cu	19.62 (0.64)*	18.23 (1.25)*	10.87 (2.44)
Pb	277.85 (217.84)*	35.67 (11.63)	21.48 (9.32)
Zn	497.98 (19.44)*	146.16 (16.4)	138.49 (22.52)

*Significant difference compared with reference site. All tests considered statically significant at $\alpha \leq 0.05$.

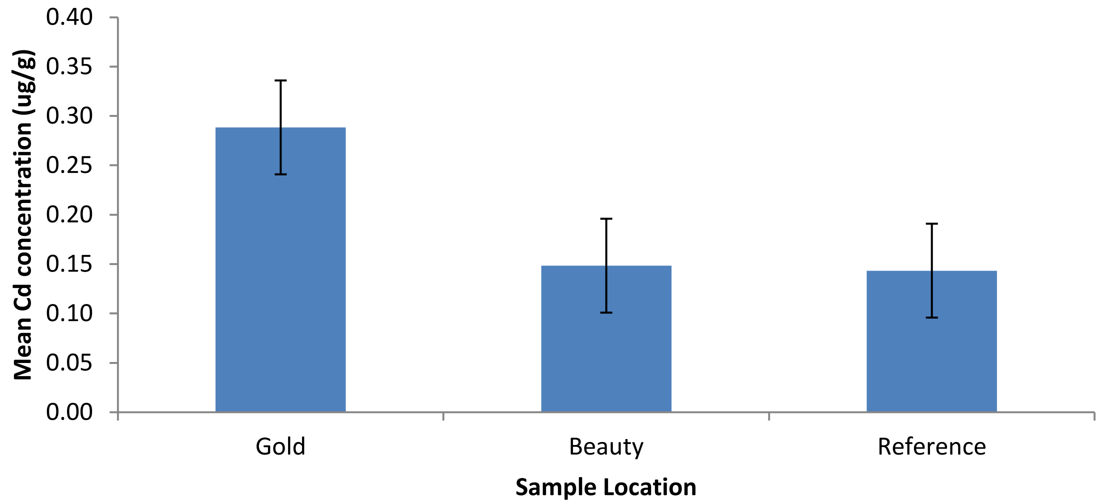

Figure 8. Average cadmium concentrations. Mean tadpole cadmium concentrations (ug/g) and +/- standard error sampled from three streams in Idaho Panhandle National Forest.

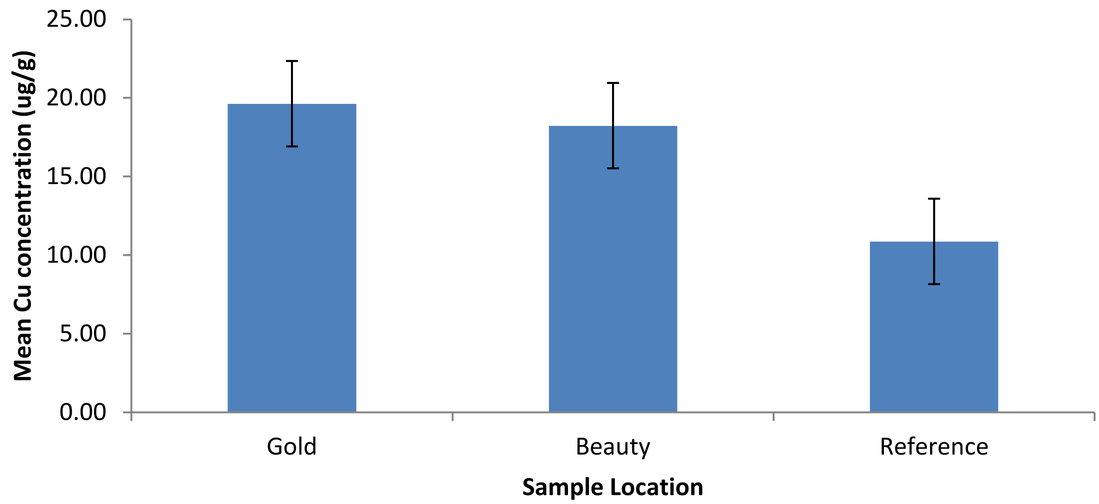

Figure 9. Average copper concentrations. Mean tadpole copper concentrations (ug/g) and +/- standard error sampled from three streams in Idaho Panhandle National Forest.

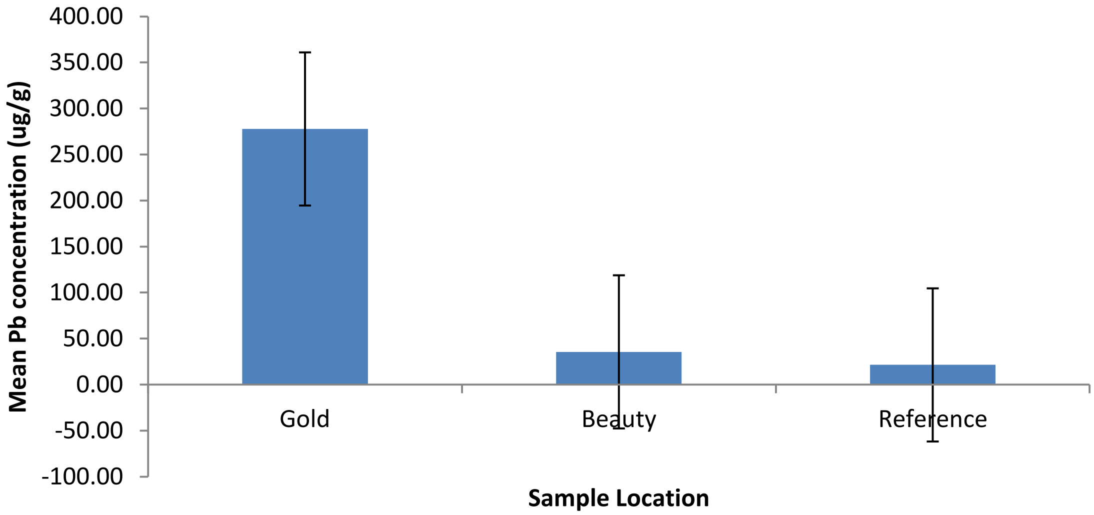

Figure 10. Average lead concentrations. Mean tadpole lead concentrations (ug/g) and +/- standard error sampled from three streams in Idaho Panhandle National Forest.

Metal concentrations of tadpoles at Beauty Creek were generally not as high as Gold Creek. The tadpole metal concentration for copper (p=0.004) at Beauty Creek was significantly higher than the reference site (Figure 11). Although the other metals were not significantly different, the mean levels of cadmium, lead, and zinc concentrations still exceeded the reference site.

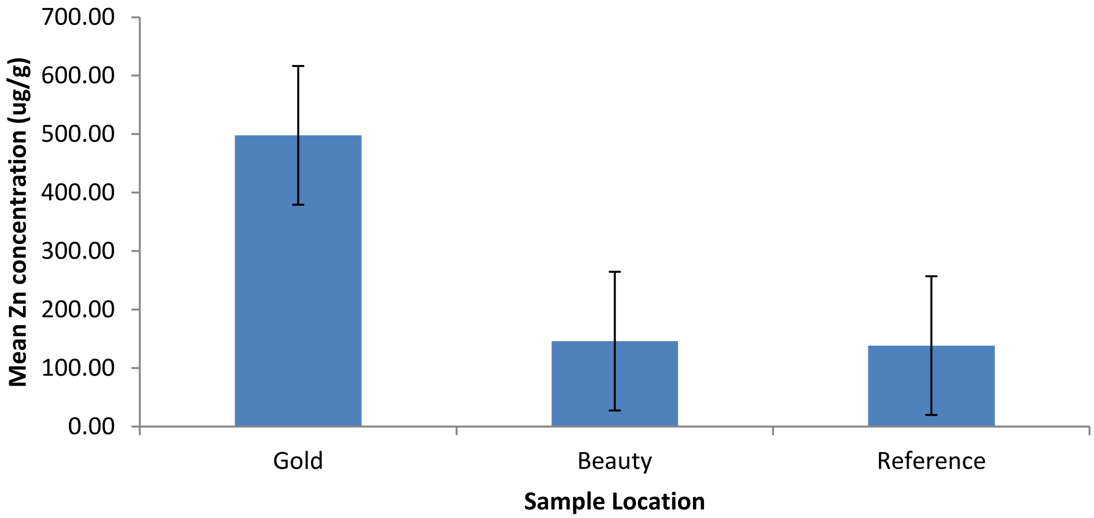

Figure 11. Average zinc concentrations. Mean tadpole zinc concentrations (ug/g) and +/- standard error sampled from three streams in Idaho Panhandle National Forest.

CHAPTER IV

DISCUSSION

My observations suggest that *Ascaphus montanus* populations are still exposed to metals from abandoned mines. This is consistent with other studies (Chupp and Dalke 1964; Blus *et al.* 1987; Lefcort *et al.* 2000; Maret and MacCoy 2002) that found elevated metals downstream of mining sites in northern Idaho. These heavy metals can widely impact terrestrial and aquatic life of the local area.

Lefcort *et al.* (2010) found mine wastes in stream communities where no active mining had occurred for at least 70 years. In their study, decreased insect community diversity (total number of organisms and families) was associated with increased levels of cadmium and zinc. Contrary to their hypothesis and previous reports (Malmqvist and Hoffsten 1999), they did not find a reduction in Ephemeroptera, a sensitive family, suggesting that most of the streams had lower levels of metals than earlier reports or perhaps metal tolerance had evolved in this species. However, using taxonomic genera assemblages to indicate metal pollution may be difficult as biotic and abiotic factors may affect metal-tolerant genera (Pollard and Yuan 2006). Northern Idaho has high patches of background levels of heavy metals, making strong associations difficult. Regardless if measured metals are occurring naturally or occurring from anthropogenic causes, small abandoned mines seem to be acting as a point-source. The hypothesis that organisms have evolved tolerance to metals in these local isolated populations can provide a significant challenge.

Collection of *Ascaphus* should be conservative, as development is slow. This caveat in my study restricted the sample size making it difficult to identify strong associations. Larger tadpoles were collected when smaller ones were unavailable. This size difference could account for misrepresented metal accumulation. For instance, larger tadpoles tend to be older thus increasing exposure duration. However, I did find that increased mass per organism may be helpful for future studies. For example, I found that the mass (dry weight) of a single tadpole can vary from 0.003 to 0.398 g. The greater the mass of the samples, the less need there is to pool samples to maintain detectable limits of concentrated metals. On the contrary, it is uncertain if older tadpoles would respond similarly. Behavioral avoidance, enhanced metal excretion, and biological resistance may preferentially alter survival rates potentially reducing the concentration per unit of mass. Alternatively, mortality may increase in sensitive individuals, in which the most fit and resistant individuals of the population remain. Adult frogs are likely accumulating metals by ingesting small amounts of soils directly from their invertebrate prey; they also spend much time in the water and near the streambeds where many pollutants are often sequestered. However, larvae and juveniles tend to be more susceptible to metal exposure than adults. This is likely due to the difference in habitat use, feeding habits, and physiology (Buhl and Hamilton 1990; Roe *et al.* 2005). Furthermore, Sparling and Lowe (1996) found individual organisms can selectively uptake metals, where concentrations of specific metals may be above or below ambient levels, which may change through the animal's lifespan.

Farag *et al.* (1998) defined biofilm as attached algae, bacteria, and associated fine detrital material to substrates in water bodies, although pollen and minute insect larvae can likely extend this definition. In their study, metal concentrations were equal in biofilm as they were in the sediment collected in the Coeur d'Alene River Basin. They suggested scrapers-shredders accumulate higher concentrated metals than other functional feeding groups. In general, concentrations of metals increase from biofilm and sediment to macroinvertebrates to fish. Kiffney and Clements (1993) found that invertebrates feeding on metal-rich biofilm had greater concentrations of metals relative to other invertebrates. In fact, approximately 30 to 40% of all gut contents of *Ascaphus* tadpoles are fine sand grains (Metter 1964). This pathway is likely an important movement of metals from one trophic level to another, although concentrations directly ingested as food in combination with environmental levels that are absorbed by the gills and skin may difficult to differentiate. The tadpoles collected at Gold Creek in this study suggest metals are still bioavailable.

Kiser *et al.* (2010) found sediment metal concentrations (Table 3) at Gold Creek to be higher than the local background levels (Maxim Technologies Inc.® 2002), yet dissolved metals within water (ug/l) were below US Environmental Protection Agency (EPA) chronic criteria for the protection of aquatic life except cadmium and zinc. Their results indicated strong correlations with metal concentrations in sediment and benthic macroinvertebrates tissues, and again with macroinverebrates and fish tissues in Gold Creek, indicating a biotransfer of metals through a dietary pathway. Although, metal concentrations were greatest below mine sites, they collected bull trout exhibiting symptoms consistent with contamination exposure as far as 8 km downstream of the mines. Much variability in my samples is likely from this concentration gradient of metals from the point source downstream. Metals do not tend to biomagnify between trophic levels, but they may bioaccumulate (Farag *et al.* 1998) to concentrations that can cause deleterious effects to biota. Bioaccumulation is a process by which a pollutant is accumulated in the tissues of an organism at a rate quicker than it can be eliminated resulting in higher concentrations than ambient levels. For instance, significant amounts of methylmercury (MeHg) can bioaccumulate in fish (Hall *et al.* 1997) and amphibians (Bank *et al.* 2007) even when water samples are quite low, thus water samples alone are not sufficient in environmental sampling. Contrarily, biomagnification is a process by which a pollutant's concentration is increased as a result of a larger organism consuming a smaller one (Pepper *et al.* 1996). Metals accumulated in *Ascaphus* tadpoles may also adversely affect several potential predators including garter snakes, giant salamanders, American Dippers, shrews, raccoons, sculpins, and variety of trout species (Feminella and Hawkins 1994). Animals utilizing heavy metal contaminated waters may have an ecologic impact on other wildlife eating the animal whole, while having a minimal impact on those that discard internal organs (Tripp 2012). Metal concentrations in whole fish collected from the Coeur d'Alene River notably exceed the average metal concentrations throughout the entire United States (Farag *et al.* 1998).

Table 3. Sediment metal concentration range. Concentrations measured in mg/kg (dry wt.) at sampled mining sites.

Mining Site	Cd	Cu	Pb	Zn
Gold Creek	0.06-3.56	9.6-51.3	5.1-304.0	7.5-836.0
Beauty Creek	<0.001-2.1	-------	16.3-250.0	92.0-262.0

Source: Kiser et al. 2010; Lefcort et al. 2010; no data found for Cu at Beauty Cr.

Conjecture Mine, one of the mines along Gold Creek, operated periodically in the first half of the twentieth century which resulted in its classification as a CERCLA or superfund site, but has since been remediated. In 2007, the US Environmental Protection Agency (EPA) excavated approximately 31,000 cubic yards of contaminated soils from the rock dumps and another 2,000 cubic yards of metal rich sediment from the bed and banks of Gold Creek (EPA Environmental Bulletin 2007). Kiser *et al.* (2010) collected biological samples from Gold Creek that exhibited elevated levels of heavy metals in 2006 (Table 4). Indeed more than a half decade later, there is evidence of elevated metals in biological samples in this study. However, it is difficult to comment on the success of the cleanup without knowing the population dynamics and physical parameters from both before and after remediation. Further studies are needed at Gold Creek to determine survival and recruitment of *Ascaphus montanus* and other local fauna. It should be noted however, when collecting tadpoles at this location, an adult female was found with an abnormal lump on the side of its abdomen. Contrarily, little information exists about the intensity of mining operations at Beauty Creek, which may in part explain why most of the metals found in tadpoles at Beauty Creek were not significantly higher than the reference site, suggesting metals are below ecologically relevant levels. Spears *et al.* (2006) analyzed several of Lake Coeur d'Alene's bays, including Beauty Bay, that showed elevated sediment levels of lead (Pb); waterfowl utilizing some of these wetlands had high blood lead levels that were similar those with severe clinical lead poisoning. Regardless of metal concentrations detected at study locations, I do not know what levels will start to cause physiological implications without further studies and therefore cannot currently make a determination on the metal burdens for *Ascaphus montanus*.

Table 4. Gold Creek comparative biological samples. Metal concentrations (ug/g) in biological tissues collected from Gold Creek, Idaho sampled in 2006 (*Kiser et al. 2010*) and 2013 (mean with standard deviation in parentheses).

Sample	Cd	Cu	Pb	Zn
Tadpole tissues	0.29(0.02)	19.62(0.64)	277.85(217.84)	497.98(19.44)
Macroinvertebrates	3.23(0.35)	43.9(2.55)	68.5(13.2)	990(157)
Fish tissues	0.899(0.236)	6.3(0.873)	3.10(2.51)	312(98.1)

Bioavailability of a metal is a complex function of several environmental factors. In terrestrial and aquatic systems, these factors include total concentration of the metal, speciation of the metal, mineralogy including physical and chemical structure of the soil, pH, redox potential, temperature, salinity, organic matter, other suspended particulate matter, removal and transport by wind or rain, water velocity and volume, and duration of available water (John and Leventhal 1995; Qiu *et al.* 2007). Metal solubility increases with decreasing pH and increasing water velocity thus enhancing metal mobilization (Stansley *et al.* 1992). This may be especially important in these mountain streams where the pH can range from slightly acidic to near-neutral and water velocity is especially high. As solubility of the metal increases, its bioavailability thus increases to plants and animals. Some metals such as lead largely tend to settle at the bottom of water bodies, but suspended particles or dissolved metals may remain in the water column as acidity increases (Irwin 1997). Cadmium and zinc tend to be more widely dispersed and can readily be mobilized through different trophic levels (John and Leventhal 1995; Lefcort *et al.* 2010). Even when metals are bioavailable, it may have differing toxicological results on organisms (Lage *et al.* 2006) and may be compounded by environmental factors. Biotic factors include tolerance of the organism (or organ), size, life stage, species, and nutrition (Wang 1987). Abiotic factors influencing toxicity include pH, temperature, alkalinity and hardness, inorganic ligands, interactions, and chemistry balance of soil or water (Wang 1987; Irwin 1997).

Laboratory studies extend to acute metal toxicities which may underestimate the effects of chronic heavy metals in the field. Chen *et al.* (2007) found that chronic levels of copper had differing effects on tadpoles depending on the Gosner stage (GS). Although the survival rate was high in younger tadpoles (<GS25), it dropped to less than 10% by the end of the experiment. Tadpoles that did survive were found to exhibit decreased growth rates. In the field, this can increase the individual's vulnerability to gape-limited predators while decreasing their competitive advantage. Moreover, the study found a significant reduction in the number of tadpoles that successfully

metamorphosed and a delayed time to reach metamorphosis. Successful metamorphosis directly implies the number of individuals recruited from the larval aquatic stage to the adult terrestrial stage and therefore influences the number who can reproduce (Snodgrass *et al.* 2004). Although it has been argued there is a tradeoff between size and age at the time of metamorphosis, they did not observe any increase size advantage with the delayed metamorphs. This may lead to serious fitness consequences that may also delay or reduce the size of the breeding population. Recent metamorphs with increased concentration metal burdens from the larvae stage may also increase trace metal mobilization therefore affecting possible uncontaminated food webs by their dispersal (Roe *et al.* 2005).

Successful recruitment is especially crucial for *Ascaphus montanus* populations. *Ascaphus* exhibit extreme delayed sexual maturity relative to other anurans. This long-lived species is restricted to low order streams, many of them presumably near small overlooked mining operations that may not always bear fish and therefore lack standard federal or state protection. Any minute change in microhabitat conditions places them at risk for extirpation. It is hypothesized that *Ascaphus* populations have a long evolutionary relationship with aquatic predators forcing them upstream and restricting biogeographic ranges (Metter 1964; Feminella and Hawkins 1994), therefore limiting available habitat. Because this cold-adapted species has reduced lungs, the skin likely aids in respiration relying on the cold turbulent oxygen-rich water (Olson 2011). This extreme narrow temperature range even further restricts their habitat and development. Any disturbances may result in this highly specialized species inability to recolonize their historical distribution. It is unclear how metal pollution from the release of anthropogenic activities may impact populations, without an understanding of their natural biological tolerance. A long-term monitoring program of *Ascaphus montanus* populations may determine their natural tolerance of heavy metals and the level of ecological relevance across the landscape.

Sublethal levels of metals presented together can also have an additive effect. For example, low levels of heavy metals reduced predator avoidance behavior in Bull Frog (*Rana catesbeiana*) tadpoles (Raimondo *et al.* 1998). Likewise, Colombian Spotted Frog (*Rana luteiventris*) tadpoles failed to detect and seek refuge in the presence of predacious Rainbow Trout when exposed to a combination of non-lethal levels of heavy metals (Lefcort *et al.* 1998). In addition, tadpoles living in metal-rich habitats have reduced number of teeth and increased oral deformities, limiting their ability to graze which may have ecological ramifications (Rowe *et al.* 1996), particularly with *Ascaphus montanus* tadpoles. Metal exposure may also have a negative effect on tadpole locomotion. For instance, Northern Leopard Frog (*Rana pipiens*) tadpoles experienced spinal deformities associated with abnormal swimming behavior when exposed to lead (Chen *et al.* 2006), yet without any visible morphological abnormalities they still showed reduced swimming speed when exposed to copper (Chen *et al.* 2007). Susceptibility to pathogens and diseases may increase in the presence of heavy metal pollution. *Batrachochytrium dendrobatidis* (Chytrid) is an emerging infectious disease attributable to the worldwide amphibian declines. Indeed, in both the presence of copper and *B. dendrobatidis,* Gray Treefrog (*Hyla chrysoscelis*) tadpoles exhibited lengthened larval periods when compared to the control group (Parris and Baud 2004). A study on a harbor porpoises (*Phocoena phocoena*) showed that individuals that died from infectious diseases had higher levels of mercury, selenium, and zinc than did healthy individuals (Bennett *et al.* 2001). The synergistic and interactive effects of metals have differing impacts on biota and may present a steep conservation challenge.

In summary, I found that metals at abandoned mining sites, particularly at Gold Creek, are still persistent even after remediation efforts. In addition, my evidence suggests *Ascaphus montanus* populations are exposed to metals via anthropogenic activities. Further studies should investigate tadpole survival, fitness, and recruitment and possible metal tolerance. These studies will yield a greater understanding of conservation measures for this sensitive species, especially those inhabiting streams that do not bear fish. Moreover, future studies should focus on all possible exposure pathways for this species in order to estimate metal transfer to higher trophic levels.

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