Differences in Morphology and Behavior in Green Frogs (*Lithobates clamitans*) from Urban and Rural Sites in New York and New Jersey

By

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Abstract

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Anthropogenic disturbances to freshwater ecosystems are intensifying with the continued growth and expansion of the human population. Urbanization is associated with increased anthropogenic land use and pollution compared to rural areas. Freshwater ecosystems in particular are altered by the input of nutrients and wastes resulting from run-off in urban areas. Pollutants of particular concern to urban ecosystems are metals, polycyclic aromatic hydrocarbons (PAHs), particulate matter, and radioactive nuclides. Metals from anthropogenic activities constitute a serious threat to the environment due to their toxic effects on plants, animals and humans and their potential to accumulate up food-chains. Animal behavior is a useful individual-level response that acts as critical link between animal physiology and overall population effects. Therefore, differences behavior within a species impacted by varying degrees of urbanization may be useful in predicting overall population effects. Morphology, likewise, may differ between urban and rural environments as animal growth rates are highly dependent upon survival-related behaviors. Efficient acquisition of prey provides energy necessary for individual growth. When prey capture is deficient, growth rates are reduced which in turn impacts survival.

I assess differences in green frog, *Lithobates clamitans* behavior and morphology in frogs from urban and rural sites. I examine the relationship between anthropogenic metals, one measure of urban pollution, and several levels of biological organization within green frogs. I determine which levels of biological organization are influenced by urbanization and if a link exists between lower levels of biological organization by assessing metal accumulation, and upper levels of biological organization, by measuring feeding behavior, advertisement call, morphology, and population composition. With the exception of cobalt (Co), no relationship was observed between metals present in the environment (sediment and water) and *L. clamitans*. Prey capture efficiency and prey capture latency were significantly different between frogs from urban and rural sites. Feeding efficiency was negatively associated with total metal concentration of water. Frogs from urban sites were smaller in size than frogs from rural sites. This may be due in part to lower prey capture efficiencies. I did not observe differences in *L. clamitans* population abundance in urban and rural sites. Therefore, the negative impacts to green frog behavior and morphology may not be severe enough to result in population declines.

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Introduction

Focus of this Study

In this dissertation, I will assess differences in morphology and behavior in green frogs, *Lithobates clamitans* from urban and rural habitats. Urbanization is associated with increased anthropogenic land use and pollution (Grimm et al. 2008). Freshwater ecosystems in particular are altered by the input of nutrients and wastes resulting from run-off in urban areas (Riley et al. 2005). Amphibians rely on these freshwater habitats for critical life stage-specific processes (Herczeg et al. 2012) and therefore may experience negative impacts at multiple biological levels. In chapter one, I will examine the relationship between metals, one kind of pollutant found in urban areas, in sediment and water and those present in the body burdens of frogs from urban and rural sites. Next, in chapter two I will examine differences in survival-related and reproductive-related behaviors, feeding efficiency and call rate, in frogs from these sites. Lastly, in chapter three, given that survival-related behaviors have consequences on the overall size and survival of frogs, I will evaluate differences in green frog morphology and population abundance and make comparisons to behavior.

I address how green frogs might be impacted, at both the individual and population levels, by human-mediated habitat degradation. I used green frogs, *Lithobates clamitans* as an indicator of pollution in urban environments, with metals as one such pollutant. Biological indicators are species whose condition correlates with that of other taxa in the same location (Hilty and Merenlender 2000). This allows for observation of proxy organisms to reflect the condition of others sharing the same habitat. By monitoring several levels of biological organization in a species recognized as a biological indicator, early detection of adverse responses to hazardous conditions may be possible (McCarthy and Shugart 1990). Numerous studies assess species responses to habitat degradation at single biological levels, yet few studies have established the relationships between altered survival and reproductive-related behaviors and the overall fitness of these species (Clements 2000). I asked 1) could I detect differences in behavior and morphology in green frogs from urban and rural sites 2) IF yes, then do these differences influence the overall fitness of these frogs as expressed by population abundance?

Relevance

The rapid growth and expansion of the human population has led to the persistent process of land and resource acquisition. The large scale consumption of resources and subsequent environmental deterioration is of growing concern for scientists, policy makers and the public alike (Mora and Zapata 2013). This concern is twofold, due largely to the dependence of the human population on the renewable and nonrenewable resources provided by the biodiversity of ecosystems (Díaz et al. 2006) and the adverse effects to human health that arise due to habitat degradation (Corvalán et al. 2005). The relationship between the ever-growing human population and resource depletion and habitat degradation is well established; as the human population continues to grow, the negative impacts on resource availability and ecosystem health intensify (Ehrlich and Holdren 1971, Holdren and Ehrlich 1974). Although the growth of the human population is slowing (Lutz et al. 2004), projections of world population growth estimate growth will continue until a peak population of 9.22 billion is reached at 2075 (United Nations. Department of Economic and Social Affairs. Population Division. 2004) making these concerns a global crisis. Assessments of biodiversity and ecosystem health therefore have become essential to identifying areas impacted by anthropogenic disturbances that require immediate remediation (Paoletti 1999).

Habitat destruction and degradation comprise the majority of anthropogenic disturbance, though the methods by which they occur vary. Habitat degradation may occur through industrial accidents some of which, are oil spills (Cohen 1993, Law and Hellou 1999, Colten 2012), mining accidents (Grimalt et al. 1999, Kemper and Sommer 2002) and radiation exposure (Asmolov et al. 1988, Saenko et al. 2011), whose damages are traumatic but acute and isolated, or more commonly as a byproduct of exploitation of resources by the expanding human population (Dobson et al. 1997). The consequences of human exploitation result in a larger scale disruptions of habitats. These perturbations include pollution; caused by industrial production or urban/suburban expansion (Albasel and Cottenie 1985, N. Mollazadeh et al. 2013), deforestation (Brooks et al. 2006, Sauve 2013), and through introduction of monocultures due to development of land for industrial or agricultural purposes (Tilman 1999, Singh 2000).

Urbanization

Urban environments are characterized by an increase in human population density, resource consumption and land use (McDonnel and Pickett 1990). The effects of urbanization are not limited to areas of increased human population but also to locations influenced by the consequences of the needs of the growing human population. Urban environments differ from rural environments in that urban environments have greater concentrations of pollutants such as metals, polycyclic aromatic hydrocarbons (PAHs), particulate matter, and radioactive nuclides (White and McDonnel 1988, Madrid et al. 2008, Pouyat et al. 2010). Hazardous waste sites often exhibit co-contamination with several metal and organic pollutants (Sandrin and Maier 2003). Organic pollutants present include pesticides, herbicides, petroleum, and chlorinated solvents

(Sandrin and Maier 2003). In addition to pollutants, these habitats exhibit greater native plant species extinctions and non-native plant species introductions relative to rural habitats (Pouyat et al. 2010). Urban roadways hinder species migration required for acquisition of more suitable habitats and critical life stage processes (Gibbs 1998, Herczeg et al. 2012).

Soils perform a number of functions in ecosystems from the storage of minerals to the immobilization of pollutants (Pouyat et al. 2010). Soil pH, organic matter, in addition to other soil properties, limit the bioavailability of metals as well as other pollutants to plant and animal inhabitants (Pouyat et al. 2010). In disturbed environments, these properties are altered thus potentially increasing the bioavailability of pollutants. While the type and concentration of pollutants vary in urban environments, urban soils are generally regarded as "drastically disturbed" (Pouyat et al. 2010, Bain et al. 2012).

Urban environments are characterized by greater areas of impervious surface (Riley et al. 2005). Ponds and streams in urban environments are susceptible to the addition of impurities such as nutrients and pollutants from runoff (Sartor et al. 1974, Riley et al. 2005). Urban stormwater acts as a source of metals and organics for receiving water bodies (Field et al. 1995). With the continued expansion of urbanization, the negative influence on freshwater ecosystems will inadvertently grow (Bolund and Hunhammar 1999, Herczeg et al. 2012). Therefore, it is critical to understand species responses to human modified environments, particularly those impacted by urbanization, to determine the full impact of anthropogenic activities.

Metals

Metals are present naturally in the environment in low or “trace” concentrations (Roesijadi and Ronbinson 1994). Many trace metals such as chromium (Cr), cobalt (Co), copper (Cu), iron (Pb), magnesium (Mg), manganese (Mn), selenium (Se), and zinc (Zn) are biologically essential (Iyengar et al. 2002). Non-essential metals such as lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg) also naturally occur in low concentrations (Mejare and Bulow 2001). All metals can be biologically harmful when present in excess quantities (Hanna et al. 1997). Metals are persistent in the environment, meaning they cannot be degraded into less toxic subunits (Athar and Vohra 1995). Therefore, problems arise when industrial processes release metals as waste into the environment in excess of background concentrations (Nriagu 1988).

Metals are transferred from soil and water to food webs when assimilated by plants (Nanda Kumar et al. 1995). Plants bioaccumulate essential and non-essential metals in roots and shoots in concentrations higher than originally present in the environment (Raskin et al. 1994). Bioaccumulation of metals in plants can inhibit plant growth, cause plant death, and result in the trophic transfer of metals to animals and humans (Clijsters and Van Assche 1985, Nanda Kumar et al. 1995). In addition to plants, soil consuming macro invertebrates also make metals from the environment available to higher trophic level consumers (Linder et al. 1998). Animals in polluted environments may also be exposed to metals by dermal contact, inhalation, ingestion of contaminated soil and water, and across gill surfaces in addition to ingestion of contaminated plants and soil consuming invertebrates (James and Kleinow 1994).

Behavior

Animals respond to environments through variations in behavior (activity or inactivity) (Levitis et al. 2009). Urban environments differ significantly in the presence of pollutants, human density, and habitat fragmentation, all of which may act as stressors to native species (Herczeg et al. 2012). The impact of these stressors is determined by the organism's ability to cope (Koolhaas et al. 1999). Individual coping capacity may be expressed as both physiological and behavioral responses to stress (Koolhaas et al. 1999). These physiological and behavioral responses are not mutually exclusive in that physiological responses (uptake of pollutants) may influence behavioral responses (survival-related behaviors). Altered behaviors may further negatively impact organisms at the population and community levels (Weis et al. 2001).

Behaviors that contribute to the overall fitness of organisms can be subdivided into survival-related and reproductive-related. Survival-related behaviors are responses to environmental stimuli that contribute to individual growth and survival (Weis et al. 2001). Feeding efficiency and predator avoidance can be monitored to reflect interactions between species within a community (Reichmuth et al. 2009). Efficient consumption of prey contributes to the energy transfer between trophic levels (Reichmuth et al. 2009). Impairments to feeding behavior may reduce energy available for individual growth (overall size and development of the organism) and survival, therefore negatively impacting population size (Weis et al. 2001).

Animals that live in social groups benefit from the rapid identification of conspecifics (Wenrick Boughman and Wilkinson 1998). Vocalizations convey species identity, aggression, sex and location (Bee and Perrill 1996). These vocalizations come at a great cost to the signaling individual in that they are energetically expensive (Burk 1988) and identify the location of the caller to potential predators (Ryan et al. 1981). Due to the energetic costs to the caller, these vocalizations are often considered “honest” indicators of the callers condition (Bee et al. 2000). Females frogs prefer mates whose calls are lower in frequency (an indicator of mate size), longer in duration, and repeated more frequently (Welch et al. 1998). Since these calls are energy expensive, deficiencies in prey capture efficiency may limit the energy available to produce desired call parameters. In gray tree frogs, *Hyla versicolor*, males frogs with longer calls produced higher quality offspring compared to conspecifics with shorter calls (Welch et al. 1998).

Morphology and Survival

Pollutants common in urban environments negatively affect morphology and survival of native species. Dissolved organic carbons and low pH delay time to metamorphosis in larval wood frogs, *Rana sylvatica* (Horne and Dunson 1995). Wood frogs were smaller at time of metamorphosis and had decreased survival (Horne and Dunson 1995). Exposure to the pesticide Roundup reduced tadpole survival and biomass in Gray tree frogs, *Hyla vericolor*, American toads, *Bufo americanus*, and Northern leopard frogs, *Rana pipiens* (Relyea et al. 2005). Exposure to metal pollutants delay time to metamorphosis in spotted frogs, *Rana luteiventris* (Lefcort et al. 1998). Eastern narrow-mouth toads, *Gastrophryne carolinensis*, collected from industrial areas had offspring with higher occurrences of developmental abnormalities, including craniofacial abnormalities, than offspring of toads collected from a reference site (Hopkins et al. 2006). Carbonyl exposure to Southern leopard frog, *Rana sphenocephala*, tadpoles induces developmental deformities, including visceral and limb malformations (Bridges 2000). Delays in metamorphosis, reduced growth rates, and developmental malformations in amphibian species exposed to pollutants in urban environments can result in the ultimate mortality of the amphibian.

Biological Indicators

Due to the rapid growth of urbanized environments and the adverse effects to human and animal health that arise due to habitat degradation (Corvalán et al. 2005), the application of biological indicators serves as an effective and necessary means of rapid assessment of the vulnerability of multiple species and habitats. Focal species used to assay the environmental health of other sympatric species are termed biological indicators (Hilty and Merenlender 2000). Indicator species are ones that mirror fluctuations in the community as well as reflecting changes in the environment (Simberloff 1998). Desirable properties in biological indicators vary with regards to the objectives of the management plan. Different qualities are needed in indicators tasked at assessing general trends in ecosystems over time versus providing early warning signs of declining ecological integrity versus those used to pinpoint the source of habitat degradation (Cairns Jr. et al. 1993, Dale and Beyeler 2001). Categories of biological indicators include 1) keystone species, whose effect on other species is greater than predicted by abundance (Mills et al. 1993, Power et al. 1996, Simberloff 1998), 2) umbrella species, species requirements umbrella the requirements of numerous less demanding species in the community such that protecting their minimum requirements indirectly protects other species in the community (Roberge and Angelstam 2004) , 3) dispersal limited species, with limited ability to migrate from one population to another which decreases the probability of re-colonization in the event of local extinction, 4) resource limited species, the limiting factor being some nutrient or habitat that is rare, 5) process limited species, sensitive to timing of ecological processes such as floods or fires (Noss 1999), and lastly 6) flagship or charismatic or emblematic species that galvanize public support and funding.

The benefit of indicator species lies not only in decreasing the time required for environmental assessments, allowing for early indication of habitat degradation, but also in using fewer resources (Dale and Beyeler 2001). The disadvantage of biological indicator species is their limited predictive power. While a species may fit one or more criteria listed above, this does not guarantee the entire community will echo this species' response to anthropogenic influences (Landres et al. 2005). The designated species may be more tolerant to some perturbations than others, resulting either in over or under-sensitivity in their predictions. Since no two species occupy exactly the same niche (Carignan and Villard 2002), it becomes increasingly difficult to choose a single species as an indicator. However, if a species both fits one or more of the criteria and its condition correlates with altered environmental states on one or more biological levels, useful predictions can be made from indicator species.

Recently, amphibians, particularly frogs, have been proposed as useful biological indicators (Carignan and Villard 2002). Amphibians have limited dispersal abilities, and are even more limited by human impacts such as fragmentation. Many populations have become isolated and have limited gene flow with other populations (Blaustein et al. 1994). They often qualify as flagship species due to their charismatic appeal and interesting life history. Some frogs also function as keystone species due to their consumption of otherwise problematic insects (Wake 1991).

Amphibians

Frogs are process-limited species that require adequate rainfall. In the event of drought these populations decline significantly, some waiting years to mate (Stuart et al. 2004). Aside from fitting multiple indicator criteria, amphibians have unique life cycle traits that make them desirable as indicators. Amphibians maintain contact with the world through their skin. For amphibians, permeable skin serves a number of functions including protection against pathogens, absorption and secretion of water and nutrients and functioning as a respiratory membrane (Stebbins and Cohen 1995). Additionally, the amphibian life cycle usually consists of an aquatic juvenile stage and a semi-terrestrial adult stage resulting in significant exposure to the environment by both water and land sources. During these distinct stages, amphibians utilize different food sources from their habitats. Tadpoles are non-discriminate continuous feeders and primarily consume vegetation (Jenssen 1967) while adults tend to be sit and wait carnivores (Hamilton 1948). Further, due to the permeable nature of amphibian skin, they not only have the potential to ingest contaminants through diet, but absorb them through their skin (Heyer 1994). Amphibians are also sensitive to changes in UV radiation, predation, disease, and changes in climate and pH (Alford and Richards 1999) all of which support the use of amphibians as indicators.

Worldwide Amphibian Decline

Numerous accounts of local and global extinction of frog species across 6 continents have been reported over the past 40 years (Carey and Bryant 1995, Stuart et al. 2004). The initial lack of concern regarding these declines was likely due to the large natural fluctuations that occur in amphibian populations (Pechmann et al. 1991, Drost and Fellers 1996). Therefore, it is difficult to disentangle normal fluctuations or declines from those caused by anthropogenic factors (Blaustein et al. 1994). Amphibian decline is likely a combination of these natural fluctuations and human-mediated habitat loss and degradation (Blaustein et al. 1994). Previous studies have not been able to identify any one underlying environmental factor as the primary cause of these declines (Corn and Fogleman 1984, Drost and Fellers 1996). Direct factors of decline include UV-B radiation, fluctuations in pH and temperature, pollution and loss of habitat (Donnelly and Crump 1998, Storrs and Kiesecker 2004, Bancroft et al. 2008). Indirect effects resulting from habitat degradation include reduction in ability to capture prey items and avoid predators, disease epidemics, diminished mate recognition and increased road fatalities (Laurance et al. 1996, Bridges 1999, Hels and Buchwald 2001, Weldon et al. 2004, Kaiser and Hammers 2009b). The underlying theme of these studies is that upon introduction of contaminants, amphibian declines are exacerbated.

While contaminants present in water are more readily available for uptake by aquatic organisms due to the solvent properties of water and the dissolved solids present in water, amphibian larvae also have the ability to make less biologically available metals present in substrates trophically available to food webs (James and Kleinow 1994, Unrine et al. 2007). Adult amphibians may also be exposed to dietary sources of pollutants by consumption of contaminated prey items. Linder et al. (1998) demonstrated bioaccumulation of metal in the earthworm, *Eisenia foetida*, with cadmium (Cd), chromium (Cr), zinc (Zn), copper (Cu) and lead (Pb) elevated relative to soil concentrations. Copper (Cu) and cadmium (Cd) were found to increase in concentration in the grasshopper, *Chorthippus brunneus* relative to environmental concentrations (Hunter et al. 1987). In a simulated food chain analysis, crickets, *Acheta domestica*, exposed to dietary selenium (Se) were found to contain trophically available selenium (Se) concentrations (Hopkins et al. 2005). In addition to invertebrates, the adult amphibian diet also often consists of larval amphibians. Tadpoles can accumulate large concentrations of metals through feeding habits as well as dermal and gill exposure (Unrine et al. 2007). Therefore consumption of contaminated larvae may also act as a means of dietary exposure in adult amphibians.

Amphibians are negatively impacted by anthropogenic metals in the environment. Roe et al. (2005) observed life stage specific variations in accumulation of arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), selenium (Se), strontium (Sr) and zinc (Zn) in the southern toad, *Bufo terrestris* and the southern leopard frog *Rana sphenocephala*. Tadpoles of both species had the highest concentrations of arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn), while selenium (Se) and strontium (Sr) remained elevated in adults. Chronic cadmium (Cd) exposure decreases survival in American toads, *Bufo americanus*, and southern leopard frogs, *Rana sphenocephala*, as well as delays metamorphosis in *B. americanus* (James et al. 2005). Exposure of spotted frog, *Rana luteiventris*, tadpoles to solutions containing lead (Pb), zinc (Zn), and cadmium (Cd), individually and combined, demonstrated a higher toxicity at lower metal concentrations with combined metal exposure (Lefcort et al. 1998). Additionally, exposure to superfund soil contaminated by mining activities lead to delayed metamorphosis and decreased fright response when presented with predator chemical cues. Hopkins et al. (1998) measured metal concentrations in adult southern toads, *Bufo terrestris* from a coal ash contaminated site and a reference site. The metals, arsenic (As), selenium (Se), and vanadium (V), all of which are toxic in elevated quantities, were elevated in frogs collected from the contaminated sites. These findings as well as others demonstrate the potential significance of metal pollutants in amphibian decline.

Study Species

I selected frogs of the species *Lithobates clamitans melanota* (Fig. 1) for this study because they inhabit a wide array of habitats in the Northeast United States, from ponds to marshes in rural as well as urban areas. This variation in habitat type allowed for study site selection to be based upon intensity of anthropogenic impacts as measured by urbanization. Due to the permeable nature of amphibian skin, this organism is a model for not only dietary exposure to environmental contaminants, but also to dermal exposure (soil, water) (Heyer 1994). Additionally, since amphibians are more susceptible to fluctuations in UV-B radiation exposure, responses to abiotic environmental factors could be evaluated (Alford and Richards 1999, Bancroft et al. 2008)

Adult green frogs, *Lithobates clamitans*, are approximately 2-4 inches in length from snout to vent, with mature females slightly larger in size than males (Ryan 1953, Martof 1956b). Adult coloration ranges from green to brown/bronze (Conant and Collins 1998). Male green frogs exhibit several morphological differences from females of the species, such as yellow coloration of the vocal sac region, large tympanum diameter with relation to the diameter of the eye and sizeable forelimbs (Harding 1997, Schulte-Hostedde and Schank 2009).

Their habitat spans most of eastern North America, extending from Canada through northern Florida (Conant and Collins 1998). They can typically be found on shorelines of permanent ponds, swamps and marshes (Gibbs 2007). Currently, two subspecies of *Lithobates clamitans* are recognized; *L. c. melanota*, the northern green frog and *L. c. clamitans*, the bronze frog or southern green frog. The boundary between these two subspecies distribution extends through Georgia and South Carolina (Conant and Collins 1998). However, based on mtDNA, Austin and

Zamudio (2008) identified two phylogeographic lineages that split east and west of the Appalachian Mountains.

Green frogs typically begin to surface from hibernation anywhere from late March through April, when the temperature consistently exceeds 60°F for more than 3-4 days in conjunction with adequate precipitation (Martof 1953). Hibernation begins in early November or when temperatures have not exceeded 60°F for several days successively (Martof 1953, 1956a). Adults and juveniles overwinter in aquatic habitats in their home range. Rarely, some adults will hibernate in soil patches beneath leaf litter (Lamoureux and Madison 1999). Green frogs are known to travel approximately 200 m to and from breeding ponds (Martof 1953).

The breeding season of *Lithobates clamitans* extends from June through August depending on weather (Martof 1956a, Wells 1976). Males are territorial, usually occupying and defending territories of 3.4 m during breeding season (Wells 1977, Shepard 2002). During this time, males will vocalize to defend territories against other males (Ramer et al. 1983, Bee and Perrill 1996, Bee et al. 2000), or to advertise to potential mates (Bee et al. 2001). Eggs are deposited in flat, sheet-like masses on the surface of shallow water amongst vegetation. Wells (1976) observed that females that laid eggs prior to July tended to lay a second clutch later in the season. These clutches typically consist of 1,000 to 7,000 eggs and 1,000 to 1,500 eggs that require 3-5 days to hatch (Wells 1976). Tadpoles from early clutches may transform to adults in the same season, though most larval green frogs will over winter before maturation (Lamoureux and Madison 1999).

Green frogs have an opportunistic diet, usually consuming whatever invertebrates are present in their surroundings. Typical contents of the green frog stomach include grasshoppers, crickets and caterpillars (Hamilton 1948). Previous work by Hamilton (1948) found *L. clamitans* to be similarly undiscriminating in the time of day it feeds, feeding as often nightly as daily. Tadpoles survive on a vegetarian diet predominantly consisting of algae (Gibbs 2007).

Green frogs, specifically, have been used as model organisms to study the effects of exposure to metal pollution, one form of pollution present in urban habitats. McDaniel et al. (2004) found *L. clamitans* tadpoles raised from egg masses exposed to study site water containing elevated concentrations of aluminum (Al), cadmium (Cd), chromium (Cr), and copper (Cu) were more likely to be deformed than compared to those raised at a reference site. In a study assessing copper (Cu) toxicity in the presence of hard water and salt (NaCl) on egg mass survivorship, copper (Cu) was lethal to embryos in the presence of hard water (Brown et al. 2012). Bank et al. (2009) found *L. clamitans* and bullfrog, *Lithobates catesbeiana* tadpoles to similarly accumulate mercury (Hg) in tissues. Mercury (Hg) accumulation was found to increase with water concentrations of mercury (Hg). Lead (Pb) concentrations in sediment of highway drainage ditches was also demonstrated to positively correlate with *L. clamitans* tadpole lead (Pb) tissue burdens (Birdsall et al. 1986). Snodgrass et al. (2004b) exposed *L. clamitans* tadpoles to three treatments, control sand, sediment from an abandoned surface mine, or sediment contaminated with coal combustion waste (CCW) to determine effects of trace metal contamination on overwintering tadpoles. Aside from metal accumulation associated with both contaminated sites (abandoned mine sediment: elevated concentrations of lead (Pb) and zinc (Zn); CCW: elevated concentrations of arsenic (As), selenium (Se), strontium (Sr), and vanadium (V)), frogs exposed to mine sediment successfully transformed to adults but at a reduced size compared to control frogs, while CCW exposed frogs were significantly less successful at metamorphosis with mortality occurring close to time of metamorphosis. In a similar study, Snodgrass et al. (2004a) observed that when *L. clamitans* larvae were exposed to CCW sediment they required longer to transform and were smaller at the time of transformation. In adult *L. clamitans*, leg tissue arsenic (As) concentrations were elevated in frogs collected from a contaminated site compared to the reference site. Both water and diet were found to contribute to *L. clamitans* arsenic (As) accumulation (Moriarty et al. 2013).

Site Descriptions

I selected study sites (Fig. 2) in both urban and rural environments. The urban sites, with the exception of one site, are located in New York City, NY. In the urban category, I also included one site in Freehold New Jersey located directly parallel to a landfill previously investigated by the EPA for the presence of hazardous materials by anthropogenic activities. The rural sites are all located in Ulster County, NY. For each site, I measured the sum and individual trace metals in water (Table 1 and 6), sum and individual trace metals in sediment (Table 2 and 5) and other abiotic factors (Table 3).

Urban

1) **Mariner's Marsh Park, Staten Island, NY**

- Mariner's Marsh (Fig. 3a) is a 107 acre park on the North shore of Staten Island. It contains approximately 10 ponds, bogs and marshes. In the 1920's the site was used for steel processing, ship building and housed a rail line. During the 1930's-40's the site was predominantly used to process steel for ship building with slag from these activities disposed of on site. In 1997 the property was acquired by NYC Parks Department. Shortly after acquisition, the park was closed for a health assessment by the U.S. Department of Health and Services. Department of Health discovered heavy metals, polycyclic aromatic hydrocarbons (PAHs) and insecticides present in amounts above EPA/DEC background values. The brownfield site is on location (Eslinger et al. 2006). The park has remained closed since.

2**) Turkey Swamp WMA, Freehold, NJ**

- This pond (Fig. 3b) is approximately 175 meters in circumference and is located adjacent to Lone Pine Landfill, EPA Superfund site #: NJD980505424. The property is owned by the Freehold Fin Fur and Feather Sportsman Club and borders the Turkey Swamp wildlife management area. The Lone Pine landfill was in operation from 1959-1979, at which time it was closed down by NJDEP. During operation, the landfill received over 17,000 drums of chemical waste. EPA investigations discovered severe contamination of surface and ground water with a variety of organic substances, heavy metals, and pesticides (USEPA 2012).

3) **Willowbrook Park, Staten Island, NY**

- Willowbrook park (Fig. 3c) is a 164 acre park within the Greenbelt park system (NYCParks 2013). Willowbrook Park began as 105.41 acres in 1929 with land obtained from Staten Island Water Supply. Additional parcels of land were added in the late 1930’s early 1940’s. The park is most well-known for its lake, the first man made pond on Staten Island, and Carousel. Less known to the public is the runoff collecting pond well hidden by the parks wooded area. This pond acts as a receptacle to the overflow of water from Richmond

Avenue, a busy six lane thoroughfare. NYC Parks has acted to enhance the pond by introducing native plants to the pond.

4) High Rock Park, Staten Island, NY

- High Rock Park (Fig. 3d) is a 90 acre preserve centrally located on Staten Island. The site contains five ponds and various wetlands. High Rock has always been a relatively undisturbed area. Throughout the late 1800's and early 1900's, the property was divided between several landowners. In the 1940's the site became a Boy Scout camp and later sold to the Girl Scouts. In 1965 the property became a NYC park.

Rural

1) Dupre Property, Ulster County, NY

- The Dupre pond (Fig. 3e) is privately owned. The property was developed in 2006. On the property is a house and man-made pond. The pond is located in a relatively open area. The foundation of the pond is naturally occurring stone covered in leaf litter and the pond is surrounded on two sides by stone that extend upward approximately fifty feet. There is no history of contamination, industrial or otherwise at this site.

2) Quarry Property, Ulster County, NY

- The Quarry pond (Fig. 3f) is privately owned. This property was developed in 2006. On the property are a house and a pond. The pond is a man-made extension of a naturally occurring smaller pond.

3) Joy Property, Ulster County, NY

- The Joy pond (Fig. 3g) is located on privately owned property. The land has been the property of a single owner for over 20 years and the house and pond were constructed in 1998. The foundation of the pond is naturally occurring stone covered in a thick layer of leaf litter. High sloping ground makes up two sides of the pond.

Chapter 1: Total Body Metal Concentrations

Bioaccumulation of Metals in Green Frogs, *Lithobates clamitans*, from Urban and Rural Sites

Introduction

The effects of anthropogenic habitat disturbances are complex. Large-scale losses of biodiversity include classes of plants and animals across oceans and continents alike (Blaustein and Belden 2003, Dirzo and Raven 2003). This "biodiversity crisis" has lead to the utilization of biological or ecological indicators to provide an early warning of organisms and ecosystems most at risk to these changes in the environment (Dale and Beyeler 2001, Blaustein and Belden 2003). The challenge arises, when selecting an appropriate biological indicator, of finding one sensitive enough to detect anthropogenic environmental variations (Noss 1999), yet not overtly vulnerable to natural environmental fluctuations (Cairns Jr. et al. 1993). These indicator species should accurately reflect the state of the environment and the organisms within (Neimi and McDonald 2004).

Amphibians, specifically frogs, are commonly used as biological indicators of environmental degradation (Blaustein and Belden 2003, Stoyler et al. 2008). Amphibians are ideal biological indicators due to their wide- ranges, complex lifecycles, and permeable skin and eggs (Blaustein and Belden 2003, Hopkins et al. 2006, Stoyler et al. 2008). Exposure to, and subsequent accumulation of, pollutants, particularly metals, may occur through the consumption of contaminated benthic substrate and algae by tadpoles (Lefcort et al. 1998) and through the ingestion of invertebrates (Linder et al. 1998, Stoyler et al. 2008) as well as cannibalism of juvenile frog species by adult frogs (Crump 1983). In addition to dietary exposure, amphibians may also absorb metals through their skin (Papadimitriou and Loumbourdis 2003), eggs, and in the case of tadpoles, across gill tissue (Mann and Bidwell 2001). This exposure may occur on land as well as in water depending upon the amphibian life stage.

Several anthropogenic factors have been associated with amphibian declines, such as environmental toxicants (Carey and Bryant 1995), habitat destruction (Beebee and Griffiths 2005), UV-B radiation (Blaustein and Belden 2003), introduced species (Garner et al. 2006) and disease (Daszak et al. 1999). It is probable that no one factor nor combination of environmental factors can be directly linked to all declines (Heatwole 2013). Threats to amphibians likely vary from one location to the next with multiple synergisms occurring.

Urban environments pose a unique threat to wildlife. These environments are marked by high human densities and subsequent habitat alterations (Hamer and McDonnel 2008). Habitat alterations include addition of metals (Pouyat et al. 2010), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (Cotham and Bidleman 1995), introduction of invasive plant species (Pouyat et al. 2010), and habitat fragmentation (Dickman 1987). Species richness or biodiversity of both plant and animal species are lower in areas of extreme urbanization (Mckinney 2008).

Trace metals are termed "potentially toxic elements" (PTE) because their toxicity is determined by their cumulative concentration over time (Alloway 1990). Metals, one kind of pollutant present in urbanized habitats, play a key role in industrial processes such as burning of fossil fuels, mining, agriculture and industrial fabrication (Nriagu 1988, Sharma and Agrawal 2005). Wastes from these processes contaminate soil and freshwater ecosystems (Sparling and Lowe 1995, Madrid et al. 2008). Bioaccumulation of some metals are possible due their storage in fatty tissues (Madrid et al. 2008). Trace metals in the environment do not degrade and are not metabolized, rather they cycle from soil, water and air to vegetation, to herbivore and so on up food-chains (Linder et al. 1998, Seebaugh et al. 2005). Each link in these chains represents the transfer of elements from one level to the next with accumulation occurring at each level. Anthropogenic activities have exacerbated this natural cycle by increasing the quantity of trace elements in circulation (Nriagu 1988). At the organismal level, multiple factors affect the intensity of toxicity resulting from metal exposure (Aldridge 1996). Factors such as means of exposure (i.e. source of contamination and state of the contaminant), entry (i.e. through dermal, oral or inhalant) and subsequent delivery of the toxin are contingent on the nature of the contaminant (Aldridge 1996). Consequences to the organism are dependent upon cumulative concentrations of these chemicals that are available to disrupt normal biological processes. Availability is largely determined by metabolism and binding of the potentially toxic element, rendering it inactive, as well as rate of excretion of the element by the organism (Aldridge 1996). Conversely, interactions between multiple trace elements and/or trace elements and varying environmental conditions can result in synergistic consequences to the organism (Howard 1997).

Amphibians can experience delayed metamorphosis, growth, altered behavior, such as predator avoidance, and prevalence of disease associated with metals (Lefcort et al. 1998, Parris and Baud 2004). Bull frog, *Lithobates catesbeiana,* tadpoles accumulate higher concentrations of trace elements (vanadium (V), manganese (Mn), iron (Fe), zinc (Zn), arsenic (As), selenium (Se) and lead (Pb)) than do other aquatic organisms (Unrine et al. 2007). Mercury (Hg) also bioaccumulates in Bull frog and Green frog, *Lithobates clamitans,* tadpoles with accumulation increasing with concentration in the water (Bank et al. 2009). Barium (Ba), beryllium (Be), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb), and strontium (Sr) concentrations in Northern cricket frogs, *Acris crepitans,* Gray tree frogs, *Hyla versicolor*, and Green frogs, *Lithobates clamitans,* tadpoles increase with increasing soil concentrations (Sparling and Lowe 1995). Exposure to high concentrations of cadmium (Cd) through diet in

Adult African clawed frogs, *Xenopus laevis*, lead to elevated levels of cadmium (Cd) in frog liver and egg samples (Linder et al. 1998). Adult Southern toads, *Bufo terrestris*, exposed to coal combustion waste also had elevated concentrations of aluminum (Al), cadmium (Cd), strontium (Sr), thallium (Tl), arsenic (As), selenium (Se) and vanadium (V) when compared to unexposed toads (Hopkins et al. 1998). Previous studies with regards to trace metals also found metal contaminants assimilated in liver, kidney, gut and brain tissue (Papadimitriou and Loumbourdis 2003, Loumbourdis et al. 2007). Transmission electron microscopy (TEM) has successfully been used to identify areas of sequestration of metal in tissue (Morgan and Turner 2005) and demonstrate morphological changes associated with metal toxicity (Pawert et al. 1996).

Limited studies investigate impacts on freshwater ecosystems in urban environments with low-level concentrations of metal (Stoyler et al. 2008). Studies thus far primarily focus on single metal exposures or specific combinations of metals in controlled laboratory environments (Vogiatzis and Loumbourdis 1997, Lefcort et al. 1998, Kostaropoulos et al. 2005, Loumbourdis et al. 2007) or examine the consequences of severe pollution by specific anthropogenic sources (Hopkins et al. 1998, Loumbourdis 1998, Barra et al. 2001, de Mahiques et al. 2013). Few, if any, studies to date have assessed the use of adult Green frogs, *Lithobates clamitans*, as a biological indicator of environmental health. The Green frog's expansive range (Conant and Collins 1998), complex lifecycle (Hammond et al. 2013), and demonstrated ability to take up trace elements in high concentrations (Sparling and Lowe 1995, Bank et al. 2009), make it an ideal candidate as a biological indicator of urban metal exposure.

I selected study sites in both urban and rural environments. The urban sites, with the exception of one site, are located in New York City, NY. In the urban category, I also included one site in

Freehold New Jersey located directly parallel to a landfill previously investigated by the EPA for the presence of hazardous materials by anthropogenic activities. The rural sites are all located in Ulster County, NY. To my knowledge, the ponds themselves have not previously been assessed for contaminants.

I use metal concentrations in sediment and water as one measure of pollutants present in these urban and rural habitats. Additionally, I measure abiotic factors, such as UV-B readings, distance to nearest road, water pH, dissolved oxygen, salinity, conductivity and nitrate concentration as additional measures of urbanization. Lastly, I compare individual metal concentrations in sediment and water with metal concentrations in frog total body burdens to determine if *Lithobates clamitans* accumulates heavy metals in proportion with water or sediment metal concentrations. I will also evaluate the use of transmission electron microscopy (TEM) with energy dispersive spectroscopy (EDS) and scanning electron microscopy (SEM) with X-Ray microanalysis to isolate specific locations of trace metal in frog tissue. Based upon these results, I will determine if *Lithobates clamitans* is an appropriate biological indicator of trace metals in urban and rural ponds.

Methods

1) Study Site Description

I selected study sites in both urban and rural environments. The urban sites, with the exception of one site, are located in New York City, NY. The sites sampled were Mariner's Marsh Park, Staten Island, NY, Turkey Swamp Wildlife Management Area, Freehold, NJ, Willowbrook Park, Staten Island, NY, High Rock Park, Staten Island, NY, Dupre Property, Ulster County, NY, Quarry Property, Ulster County, NY, Joy Property, Ulster County, NY. Table 4 lists the sites, site history, and designation as urban or rural.

2) *Sediment and Water Analysis*Sediment processing

I obtained sediment samples from two locations at each site and kept at -20°C pending further processing. Sediment samples were thawed and filtered through 74µm mesh screen (Mc Master-CARR Supply Co.). I took three replicates from each sample to account for variability within the sample. Coarse and fine sediment samples were dried for 48-72 hrs at 65°C then weighed. I reserved approximately 1g of ground fine sediment for acid digestion. All collection and processing tools were acid washed prior to use.

Acid digestion

I placed approximately 1g of sediment in pre-weighed 20ml vials. Trace metal grade Nitric Acid (5ml) was added to each sample. Vials were topped with glass watch glasses and placed under the hood for 24 hrs. I allowed samples to reflux on a hotplate approximately 3-4 days, until clear. Watch glasses were removed and fluid was allowed to evaporate until samples were dry. I reconstituted the samples in 2% Nitric Acid for 24 hrs. Each sample was then filtered through a 45µm filter (Millipore Corp.). Filtrate was collected and analyzed with a Perkin Elmer Elan DRC-e ICP-MS, with HPLC and GC (Brown and Luoma 1995) in the Brooklyn College Environmental Sciences Analytical Center. Metals for which concentrations were obtained were chromium (Cr), cobalt (Co), nickel (Ni), copper (Co), zinc (Zn), arsenic (As), cadmium (Cd), mercury (Hg), Titanium (Ti), lead (Pb) and uranium (U).

Water Processing

I collected water samples from each location. All collection and processing tools were acid washed prior to use. Samples were refrigerated until processing. Samples were processed by the associate laboratory director of the Interstate Environmental Commission located within the College of Staten Island. Samples were analyzed for metals by ICP. The procedure followed for metal analysis was as follows: First, 100 mL well-mixed, acid preserved sample was transferred to a 250 mL beaker. Under a fume hood, 2 mL 1:1 HNO_3 and 1 mL 1:1 HCl were added to the sample. The beaker was placed on a hot plate and cautiously evaporated at 90°C to a volume of 25 mL. The lip of the beaker was covered completely with the watch glass and refluxed for an additional 30 minutes. The samples were cooled and walls of the beaker and watch glass rinsed down with reagent grade water. The acid extracts were transferred to a 100 mL volumetric flask. The volume was adjusted back to 100 mL and mixed. Metals were analyzed using EPA 200.7 Rev. 4.4 and Perkin Elmer ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrophotometer Model 3300XL). Metals for which concentrations were obtained were aluminum (Al), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), titanium (Ti), lead (Pb) and iron.

On Site Water Analysis

On site water sampling was conducted using both a YSI Professional Plus (ProPlus) multimeter calibrated to standards and an IQ160 pH meter calibrated to standards for collection of temperature, pH, dissolved oxygen, salinity, conductivity and nitrate concentration data.

3) *Other site properties*

I collected UV-B readings with a Solarmeter model 6.2 UV meter. I collected measurements from four approximately equidistant locations at each site between twelve and one pm.

4) Total Body Burden/Cellular Analysis

Capture

I captured approximately ten adult green frogs, *Lithobates clamitans* from each study site during breeding seasons, June-August. I obtained both male and female frogs from ponds in both New Jersey and New York. Permits to collect frogs were obtained from NYC Parks department, New York State Department of Environmental Conservation, Division of Fish, Wildlife and Marine Resources, and New Jersey Department of Environmental Protection, Division of Fish and Wildlife. I collected frogs at night either manually or using dip nets. Once frogs were captured they were placed in a ventilated plastic aquarium for transport to the laboratory.

Euthanasia

I euthanized frogs by first cooling them to 4°C, followed by decapitation by guillotine and double pithing, following AVMA guidelines for euthanasia (AVMA 1993) and guillotine maintenance (AVMA 2007). Frogs were to be euthanized when they were no longer required for experiments, if open wounds appear or if they were unable to feed.

ICP

I sacrificed five frogs from each site for total body concentrations of metals. Frogs were depurated prior to euthanasia. Euthanized frogs were homogenized in Tris buffer solution and dried in an oven at 60 ° C for approximately 3-4 days. All processing tools were acid washed prior to use. Samples were ground by mortar and pestle and three subsamples (~.25g) were taken from each. Metal grade Nitric acid (5ml) was added to each sub sample and samples were digested by microwave acid digestion (Milestone EHTOS EZ Microwave Assisted Digested System). Once digested, a 1:10 dilution of sample to ultrapure water was analyzed by a Perkin Elmer Elan DRC-e ICP-MS, with HPLC and GC (Brown and Luoma 1995) in the Brooklyn College Environmental Sciences Analytical Center. Metals for which concentrations were obtained were aluminum (Al), valium (V), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb).

TEM

I sacrificed five individuals from each study site for transmission electron microscopy (TEM) and Energy-dispersive X-ray spectroscopy (EDS). Tissue samples from the brain and liver were processed following a modified electron microscopy procedure (Hayat 1972). Images were obtained with a FEI Tecnai GZ Twin (2KX2K digital camera, AMT) analyzed using an EDAX Si(Li) detector with Genesis X-ray microanalysis software.

SEM

I harvested tissue samples from frogs euthanized for TEM analysis. Liver samples were stored in 3.7% paraformaldehyde, 2.5% glutaraldehyde in Millonig phosphate buffer at 4° C. I dehydrated samples in an ascending ethanol series and dried at the critical point using liquid CO_2 transition fluid (Autosamdri-815A, Tousimis Research Corp.). The membranes were coated with approximately 10 nm of Carbon with a MED-020 coating system (Bal-Tec)(Hayat 1972). SEM images were obtained with an AMRAY 1910 FE-SEM.

5) Statistical Analysis

I used Statistica 8.0 (StatSoft Inc, 1984-2007). I used a Mann Whitney-U test to compare total metal concentrations in the water by site and chose a significance level of 0.05. I used principal components analysis (PCA) to organize urban and rural sites by abiotic variables. PCA is a visual demonstration of the pattern of the data. This test does not yield a p value. Principal components analysis summarizes the variation within the data to several factors that function as axes. This analysis included individual sediment and water metal concentrations as well as additional site variables such as distance to nearest road, UV-B (high, low and average), water properties (pH, salinity, dissolved oxygen (DO) and nitrogen. I then used a discriminant function analysis using the PC scores provided by PCA to determine which factors contain variables that significantly group urban and rural sites. I chose a significance level of 0.05.

I calculated mean metal concentration of the five frogs sampled at each site, and compared those means to water and sediment concentrations for the corresponding site. I used multiple regressions to determine if total body metal concentrations of individual metals correlated more closely with water or soil concentrations. Using scatterplots, I visually assessed if any groupings between urban and rural sites could be statistically supported. I used whisker plots surrounded by variance to demonstrate the variance within populations for each metal.

Results

The urban and rural sites and their relative total water (ppm) and sediment (ppm) concentrations of metal (Table 1 and 2) and individual sediment (ppm) and water (ppm) concentrations of metal (Table 5 and 6) are located in the "tables" section of this document. Urban sites have significantly greater total water metal concentrations than do rural sites (Figure 4; $U = 0.00$, $p =$

0.03). The rural sites are, Dupre Property, Ulster County, NY, Quarry Property, Ulster County, NY, Joy Property, Ulster County, NY. The urban sites are, Mariner's Marsh Park, Staten Island, NY, Turkey Swamp Wildlife Management Area, Freehold, NJ, Willowbrook Park, Staten Island, NY, High Rock Park, Staten Island, NY.

Discriminant function analysis of PC scores from PCA revealed factor 1 ($p = 0.04$) and factor 3 ($p = 0.04$) can be used to significantly distinguish between urban and rural sites. The variables with the highest loading (above 0.75 correlation) for factor 1 are, aluminum (Al) in water (ppm), zinc (Zn) in soil (ppm), arsenic (As) in soil (ppm), distance to main road (m), and pH. The variables with the highest loading (above 0.75 correlation) for factor 3 are, iron (Fe) in water (ppm), lead (Pb) in soil (ppm), and dissolved oxygen (DO mg/l). Approximately 54% of the variation (Figure 5) in the variables can be grouped into two factors (factor 1 = 34.87; factor 3 = 19.75) by principal components analysis. Projection of cases on a factor plane organized the sites by the site variable data into separate groupings (Figure 6). Projection of the variables on the factor plane can be seen in figure 7**.** Dupre Property, Ulster County, NY, Quarry Property, Ulster County, NY, and Joy Property, Ulster County, NY were grouped closely together (listed as sites 1, 2 and 3) in quadrant one with these sites organized by farther distance to roads, high cobalt (Co), thallium (Tl), and arsenic (As) in sediment, as well as low levels of metals in water (aluminum (Al), manganese (Mn), and iron (Fe)) and the remaining sediment metals (chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg), thallium (Tl), and lead (pb)) relative to the other sites. One urban site, High Rock Park, Staten Island, NY (designated as 7) was separated from all other urban sites in quadrant II. This site is distinct from others, as it is defined by high water concentrations of manganese (Mn), high water concentrations of arsenic (As), variable UVB (high and low), high water nitrogen, salinity, and dissolved oxygen relative to the other sites. The urban sites, Turkey Swamp WMA, Freehold, NJ (5) and Mariners Marsh Park, Staten Island, NY (4) both fall into quadrant III. These sites are defined by high water concentrations of, iron (Fe), higher pH and high sediment concentrations of nickel (Ni), copper (Cu), lead (Pb) and zinc (Zn) relative to other sites. Lastly, The Willowbrook Park, Staten Island, NY (6) site (quadrant IV) is distinguished by high sediment levels of cadmium (Cd) and chromium (Cr) relative to the other sites.

There does not appear to be any clear relationship between any of the metals in the water and metals in the frog or metals in the sediment and metals in the frog (Figures 8;10-18) with the exception of cobalt in sediment and cobalt in frogs (Figure 9; $p = 0.005$). Additionally, no clear patterns or groupings appeared between urban and rural sites.

Both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) methods were not sensitive enough to detect the low-level concentrations of metal present in *Lithobates clamitans* tissue. Likewise, cellular anomalies were not observed.

Discussion

I selected study sites in both urban and rural locations. Proximity to sources of contamination greatly increase the likelihood of exposure by the environment and its inhabitants to pollutants (Wong et al. 2006). Urban soil and freshwater habitats are more likely to be exposed to pollutants from anthropogenic activities than more rural habitats (Madrid et al. 2008). I found this was true of total metal concentrations present in water samples. Total water metal concentrations were greater in urban sites than rural sites. I used principal components analysis (PCA) to further visually organize the sites by variable composition. Within urban sites, I found elevations of different environmental variables that contributed to separate groupings of these sites by PCA. This coincides with the Pouyat et al. (2010) assessment of urban habitats, particularly of urban soils, to be heterogeneous with little in common with regards to the type and concentration of pollutants. However, these habitats are consistently viewed as highly disturbed (Pouyat et al. 2010). The rural sites used for reference were grouped closely by principal components analysis.

I found cobalt in sediment to significantly correspond to cobalt concentrations in frogs. However, contrary to expectations, cobalt concentrations in sediment were greater for rural sites than for urban sites. Cobalt has many industrial uses and may be introduced to the environment through anthropogenic processes (Gal et al. 2008). Cobalt may also be introduced to the environment through a number of natural processes such as erosion of sediment and rocks (Gal et al. 2008). Cobalt in low concentrations is an essential element for many animals. However in excessive quantities is toxic (Bulog et al. 2001). Oral cobalt exposure is associated with polycythemia, inhibited growth and greater food consumption in rats (Orten and Bucciero 1948). In vertebrate studies on fish species LC50 values for cobalt in water ranged from 470-225,000 μg/L (Nagpal 2004). The effects of cobalt in sediment on the uptake and subsequent toxicity in amphibians are unavailable. There are no known sources of anthropogenic cobalt in the rural sites, therefore these sites may be naturally cobalt enriched.

The results of this study show no clear relationship between the remaining metals in the sediment and water for each site with total metal accumulation in adult frogs from the corresponding sites. Host factors such as age, sex and size contribute to the likelihood of metal assimilation (Peakall and Burger 2003). Juvenile *Lithobates clamitans* demonstrate this species has the ability to accumulate metals from substrate and water (Sparling and Lowe 1995, Bank et al. 2009). However, the concentrations of metals were higher than the metals occurring at the sites currently being sampled. In addition, since tadpoles are continuous feeders, feeding on aquatic vegetation, they are limited in sources of exposure outside their immediate aquatic environment (Lefcort et al. 1998). Therefore, water and sediment samples alone may accurately reflect the metal available for uptake. Juvenile *L. clamitans* are exclusively aquatic compared to semi-aquatic adults. Total mercury concentrations (THg) in American toad, *Bufo americanus*, tadpoles are greater than adult *B. americanus* THg concentrations (Bergeron et al. 2010b). Adult *L. clamitans* are not limited only to absorption of metals through water and sediment from the immediate pond, but also consume invertebrates and larval vertebrates of other amphibian species (Crump 1983, Linder et al. 1998). Adults are less dependent upon constant water contact and are therefore less exposed to dissolved aquatic metals than juveniles. As a result adult *L. clamitans* may have lower metal concentrations than juveniles. Green frog tadpoles may more accurately reflect the low-level metal concentrations in sediment and water at the current study sites.

Metal concentrations in adult green frogs may more closely reflect environmental metal concentrations of areas other than their current breeding pond. Adult *L. clamitans* migrate from breeding ponds to explore their home range (as far as 110 meters (Martof 1953)), to increase foraging opportunities, and to overwintering sites (Martof 1953, 1956b, Lamoureux et al. 2002). Overwintering sites may be as far as 560 meters from breeding ponds (Lamoureux and Madison 1999). Newly transformed frogs disperse from larval ponds in search of suitable breeding territories with dispersal anywhere from approximately 180 meters to 4.8 kilometers (Schroeder 1976). While habitat fragmentation in urban environments may inhibit long distance migration, some migration may still occur. Water and sediment samples for trace metal analysis were collected from ponds where the frogs were captured. As is the case with Mariner's Marsh Park and Turkey Swamp WMA, sources of contamination are well within the foray range of *L. clamitans*. Likewise, trace metals present at the sampled ponds may be higher than the ponds from which the frogs analyzed in this study originated. Future studies should include mark and recapture of individuals from study ponds and surrounding ponds to determine the extent of migration in these populations.

The lack of relationship between metals in the sediment and water with total metal accumulation in adult *L. clamitans* may also be due to the low number of amphibians sampled from each site. Wide ranges in metal concentration were observed in individuals within each site. A larger sample size may more accurately reflect the sediment and water metal concentrations. This is somewhat difficult to ethically obtain with whole body samples. Therefore nondestructive methods such as toe clippings and blood samples should be developed for *L. clamitans.* Blood concentrations of selenium (Se) in green sea turtles, *Chelonia mydas*, are positively correlated with liver, muscle, kidney and blood concentrations of selenium (Se) (van de Merwe et al. 2010). Blood concentrations of cobalt (Co), arsenic (As), cadmium (Cd), and mercury (Hg) are correlated with liver and kidney cobalt (Co), arsenic (As), cadmium (Cd), and mercury (Hg) concentrations in *C. mydas* (van de Merwe et al. 2010). Strong positive correlations exist between blood and whole body concentrations of total mercury (THg) in American toads, *B. americanus* (Bergeron et al. 2010b). Total mercury (THg) concentrations of toe clippings are also positively correlated to blood total mercury (THg) levels in *B. americanus* (Todd et al. 2012) and should therefore reflect whole body concentrations. Additionally, toe clippings are predictive of maternal mercury (Hg) transfer (Todd et al. 2012). Unlike whole body sampling, large numbers of frogs could be marked, sampled and released by nondestructive methods for yearly or seasonal sampling.

My results suggest that adult *L. clamitans* metal accumulation does not reflect the concentrations of the majority of metals present in water and sediment of their breeding ponds. This again may be due to small sample size, decreased contact with water and sediment of the immediate pond with age of the frog, and lifetime contact with metals present at other breeding ponds, foraging sites, and overwintering sites not sampled in the present study. Future areas of study should include non-destructive methods of assessing a larger sample of frogs to determine if a relationship exists and if it is being masked by the variation in accumulation within populations, mark and recapture studies to determine the extent of migration to surrounding ponds, and sampling of *L. clamitans* tadpoles to determine if they more closely reflect environmental metal concentrations.

Chapter 2: Behavior

Differences in Green Frog Behavior in Urban Environments: Evaluating a Non-Destructive Method to Quantify Effects of Urbanization

Introduction

In this chapter, I evaluate behavior in Green frogs, *Lithobates clamitans,* from both urban and rural sites. I measured feeding efficiency, a survival-related behavior, and male advertisement call, a reproductive-related behavior, for their responses to urbanization. Behavior is an organism's response to the surrounding environment (Tinbergen 1963, Koolhaas et al. 1999). These responses evolve within species and each have particular survival values (Tinbergen 1963). Behavioral responses may modify through interactions with severely anthropogenically altered environments. Therefore the study of behavior in natural settings, through comparisons of behavior in urban and rural habitats, is necessary to determine if these altered behaviors hold survival value or function as vulnerability (Koolhaas et al. 1999).

Urban environments are characterized by an increase in human population density, resource consumption and land use (McDonnel and Pickett 1990) which contribute to the increased prevalence of pollutants such as metals, polycyclic aromatic hydrocarbons (PAHs), particulate matter, and radioactive nuclides (White and McDonnel 1988, Madrid et al. 2008, Pouyat et al. 2010). These pollutants permeate soil and water in urban environments (Sartor et al. 1974, Pouyat et al. 2010). While these pollutants may also be present in rural environments, there number and concentrations are less than those of urban areas.

Amphibians, particularly frogs, have been used extensively as biological indicators of environmental pollutants due to their aquatic habitats and permeable skins. Animals differ in the extent to which they experience the negative impacts of pollutants due to their differential exposure as a result of their varying life history strategies (Peakall and Burger 2003). Amphibian life history strategies consist of complex life cycles, in which aquatic juveniles continuously filter feed and obtain oxygen through gills and permeable membrane, while semi-aquatic adults are predatory feeders and acquire oxygen through lungs and permeable skin (Lefcort et al. 1998, Stoyler et al. 2008). At any point in their life cycles, amphibians may be exposed to numerous pollutants present in water, sediment and diet.

Dramatic declines in biodiversity affect ecosystems worldwide (McNeely 1992). To assess ecological integrity, the complexity of whole ecosystems should be represented (Dale and Beyeler 2001). Whole system ecological integrity consists of abiotic factors, species richness, population stability, and interactions between organisms and the environment (Dale and Beyeler 2001, Chapin et al. 2002). Yet, monitoring all aspects of an ecosystem is costly and unrealistic (Simberloff 1998, Neimi and McDonald 2004) and likewise, concentrating time and resources on the preservation of threatened and endangered species alone limits our knowledge of species yet to be disturbed (Lindenmayer et al. 2002). Rather, a simple, inexpensive method is needed to quantify the relative stress experienced by ecosystems. Implementation of biological indicator species from naturally occurring populations allows scientists to use surrogate species to quantify exposure to environmental contaminants and monitor overall ecosystem health (Fossi 1994, Dale and Beyeler 2001).

Negative impacts to species resulting from anthropogenic activities are not limited to species declines by direct lethal effects of contaminants (Fleeger et al. 2003). Sensitive species may experience sub-lethal effects that also ultimately lead to population declines (Fleeger et al. 2003). Sub-lethal effects may be observed in different levels of biological organization nested within a species acting as a biological indicator. These levels of biological organization include cellular, individual, population and community (Weis et al. 2001). Since these levels of organization are hierarchical, it is expected that adverse effects of toxicity would be observed at lower levels prior to observations at higher levels (Weis et al. 2001). Therefore, sub-lethal effects at each of these levels may be used as early warning signals, or biomarkers, prior to population declines (Forbes et al. 2006).

Trace metals from anthropogenic activities constitute a serious threat to the environment due to their toxic effects on plants, animals and humans (Sharma and Agrawal 2005). Metals are more prevalent in soil and water of urban environments due to the reliance on transportation and industry (Field et al. 1995, Pouyat et al. 2010). Metals are persistent in nature and therefore have the ability to bioaccumulate in organisms, potentially resulting in the build up of toxic elements in food chains (Nriagu 1988). While the effect of high concentrations of metal are frequently fatal, sub-lethal effects are often less obvious. Traditional sampling techniques often require the sacrifice of large numbers of individuals to attain analytical accuracy (Ohba et al. 2012). These methods may not be feasible or desirable when the organisms under evaluation are in decline, threatened or endangered. For this reason, researchers are exploring non-destructive methods, such as behavior, to assess the effects of pollutants on organisms (Fossi 1994).

While biological indicator species on the whole are not limited to those categorized as threatened or endangered, indicators have often been sensitive species whose populations are experiencing declines (Carignan and Villard 2002). With traditional destructive/invasive methods, the researcher may impair the population under observation as much or greater than the effects of the contaminants under evaluation (Fossi 1994). For this reason, non-destructive techniques are being developed to replace traditional destructive methods of assessing impacts of contaminants on indicator species (Fossi 1994, Sanchez and Porcher 2009). The benefits of non-destructive biomarker methods include reducing/eliminating researcher mediated population reductions, the ability to increase sample size and therefore increase statistical strength, and the option of repeated sampling in the same individual over a period of time (Fossi 1994, Joshi et al. 2013). Alternatives to conventional cellular and physiological-level analyses are conducted using blood, tissue biopsy, feathers, fur, feces, and tail, scale and toe clippings (Fossi et al. 1999, Dauwe et al. 2005, Simon et al. 2010, Aguilera et al. 2012). Individual-level biomarkers such as behavior may also be used as a non-destructive indicator of toxicity (Depledge and Fossi 1994, Depledge et al. 1995).

Behavior is a useful biomarker of toxicological effects not only because of its close ties to survival, but also due to its connection to the biochemical level (Weis et al. 2001). Behavioral biomarkers are also valuable tools in quantifying low-level exposures to environmental pollutants such as trace metals, with behaviors of multiple organisms demonstrated to be highly responsive to slight environmental modifications (Lefcort et al. 2000). In addition, behavior is reasonably straightforward to evaluate and is therefore a useful tool for ecological management (Wallace and Estephan 2004). The successful use of behavior as a biomarker of pollution include observations of altered competitive interactions between Columbia spotted frog tadpoles (*Rana luteiventris*) and snails (*Lymnaea pulustris*), and decreased predator avoidance response to chemical cues in snails exposed to lead (Pb), zinc (Zn) and cadmium (Cd) (Lefcort et al. 1999); impaired swimming performance in Bull frog tadpoles (*Lithobates catesbeiana*) when exposed to coal ash (Raimondo et al. 1998); and hampered vertical swimming in the amphipod, *Gammarus lawrencianus*, when exposed to cadmium (Cd) (Wallace and Estephan 2004). However, some behaviors may be more sensitive to effects of pollutants, while others exhibit only minor divergences between pollutant exposed and non-exposed conspecifics (Wallace and Estephan

2004). Therefore, it is necessary to not only determine *if* behavior serves as an effective non-destructive biomarker, but *which* behaviors are most sensitive to environmental contamination.

The ability of an organism to acquire and consume food items is closely tied to that organism's survival (Weis et al. 2001). Surplus energy expended during this process reduces energy stored for metabolic processes (Anderson and Karasov 1981). Bridges (1999) found red spotted newts to consume half as many prey items when exposed to insecticide than groups that were not. Weis et al. (2001) examined the effect of contaminants on killifish, finding intoxicated killifish slower to capture prey items and avoid predators. Khoury et al. (2009) found fiddler crabs (*Una pugnax*) from impacted sites containing higher levels of cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), silver (Ag), zinc (Zn), and mercury (Hg), made fewer foraging attempts than crabs from the non-impacted reference site. Perez and Wallace (2004) observed lower feeding efficiency in Grass shrimp (*Palaemonetes pugio*) exposed to water and sediment collected from an impacted site (a creek that flows through a large municipal landfill) compared to shrimp exposed to water and sediment from a relatively unimpacted site. These results were further reinforced by a laboratory study in which shrimp from a pristine site were then exposed to sediment and water from the impacted site and exhibited similar declines in feeding efficiency (Perez and Wallace 2004). These studies demonstrate the potential of feeding efficiency as an index of behavioral toxicity across multiple species.

The diet of *L. clamitans* and amphibian natural feeding behavior has been well documented. In an extensive study by Hamilton (1948), *L. clamitans* was observed to be a sit and wait predator actively feeding day and night. The majority of their diets, established by stomach contents, consist of Coleoptera, caterpillars (Lepidoptera) and Orthoptera. Sight and detection of prey movement plays a large role in this species prey selection rather than any major prey preference (Hamilton 1948). Amphibian vision adds a layer of complexity to prey acquisition due to the absence of eye movement and fovea (area of sharp central vision) (Lettvin et al. 1959). In amphibians, visual acuity is dependent upon the field-of-view (Fite 1973). Field-of-view is determined by eye position and orientation of visual axes (Fite 1973). Visual acuity in fish increases with size (Breck and Gitter 1983). With the increase in fish size there is a corresponding increase in visual volume and decrease in reactive distance for prey acquisition (Breck and Gitter 1983). In avian species, relative eye size and brain size are believed to have coevolved for the capture of moving prey items (Garamszegi et al. 2002). Amphibian feeding in response to prey movement in its field of view begins with orientation of the amphibian in the direction of the prey, followed by stalking towards the prey, binocular fixation on the prey item and subsequent snapping and swallowing of the prey item (Zupanc 2010). Similar feeding strategies have been observed in the *L. clamitans* by Hamilton (1948). Since this feeding behavior appears to be universal among *L. clamitans*, feeding efficiency should serve as a valid measure of behavior.

Energy conservation and budgeting are important in dictating the development and overall size of the organism (Sih 1980, Morin et al. 1990). These differences are expressed in different components of the advertisement call. Body size, temperature and phylogeny have all been found to have an effect on advertisement call structure (Zweifel 1959, 1968, Ramer et al. 1983, Gayou 1984, Moriarty-Lemon 2009). Acoustic signals serve many communication functions ranging from territory defense, health quality, and mate acquisition (Ramer et al. 1983, Bee and Perrill 1996, Welch et al. 1998, Bee et al. 2000, Tomaszyski and Adkins-Regan 2005). Studies pertaining to variations in male mating call due to anthropogenic factors focus primarily on the impacts of anthropogenic noise (Sun and Narins 2005, Kaiser and Hammers 2009a). However, few studies assess alterations in mating behavior in response to physical pollutants such as endocrine disruptors and trace metals. One of the few studies available documented an increase in sexual arousal in the frog *Xenopus laevis* quantified by call rate when exposed to endocrine disrupting androgens (Hoffmann 2012). Based upon the endocrine disrupting abilities of some metals (Davey et al. 2007), similar results may have yet to be observed for trace metal exposure. Since these as well as other pollutants are present in higher concentrations in urban environments (White and McDonnel 1988), we may observe differences in advertisement call between frogs from urban and rural sites.

Phenotypic variation in behavioral traits between individuals is important in species recognition as well as sexual selection (Ryan and Rand 1993, Howard and Young 1998). The call of *L. clamitans* has been likened to the plucking of a loose banjo string (Bee et al. 2008). Vocal communications in *L. clamitans* may serve as male-male communication of aggression or in advertisement calls to females for the purpose of attracting a mate (Bee and Perrill 1996, Shepard 2002). Both purposes are somewhat intertwined in that males will aggressively defend territories against conspecific males and females select mates based on territory quality (Wells 1977). Male advertisement call is of particular interest due to the variation of components in call within individuals used for the acquisition of mates. Advertisement calls will often convey information on the callers size, fighting ability and relative health (Bee et al. 2000). While there are many components within male call that can be measured, for the purpose of this study, call rate will be analyzed in relation to urban and rural environments.

Methods

6) Study Site Description

I selected study sites (Fig. 2) in both urban and rural environments. The urban sites, with the exception of one site, are located in New York City, NY. In the urban category, I also included one site in Freehold New Jersey located directly parallel to a landfill previously investigated by the EPA for the presence of hazardous materials by anthropogenic activities. The rural sites are all located in Ulster County, NY. For each site, I measured the sum and individual trace metals in water (Table 1 and 6), sum and(Morin et al. 1990) individual trace metals in sediment (Table 2 and 5) and other abiotic factors (Table 3).

7) Frog Care

Capture

I collected Approximately 40 adult green frogs, *Lithobates clamitans*, from each study site during breeding seasons, June-August over two consecutive years. Permits to collect frogs were obtained from NYC Parks department, New York State Department of Environmental Conservation, Division of Fish, Wildlife and Marine Resources, and New Jersey Department of Environmental Protection, Division of Fish and Wildlife. Collection was done at night either manually or using dip nets. I placed frogs in a ventilated plastic aquarium for transport to the laboratory.

Maintenance

I followed the protocol for housing, care and use of animals approved by the College of Staten Island Institutional Animal Care and Use Committee (IACUC # CSI-09-007). I held all captured frogs in an amphibian room and exposed to a constant temperature range of 20-25°C, as selected by temperature ranges recorded in the field (Pough 2007). I simulated daily light cycles by natural light. I housed frogs in pairs in glass aquariums and frogs from each site were not in contact with subjects from other locations. Housing chambers were filled with enough water to adequately cover each frog (approximately five centimeters). I used a plastic storage container as a retreat from water as well as for concealment. I selected live crickets as prey items due to their availability in *L. clamitans* natural habitat in addition to being a recognized component of their diet (Hamilton 1948). I disinfected enclosures between study groups with a 70% ethanol solution (Browne et al. 2007).

Release

I released the majority of the frogs at the site of capture once the trial period was complete. The remaining frogs (approximately five per site) were humanely sacrificed by first chilling to 4°C, as an anesthetic, then decapitation followed by double pithing (AVMA 1993), for later analysis of the brain and liver by transmission electron microscopy and scanning electron microscopy. I sacrificed an additional five frogs per site for total metal analysis by inductively coupled plasma mass spectrometry (*ICP-MS*).

8) Behavior

Feeding Efficiency

I captured twenty frogs per site for behavioral analysis per year over two consecutive years. Frogs were observed and recorded for differences in prey capture efficiency. I fasted each individual for 24 hours prior to feeding trials to ensure individuals were motivated to participate. I conducted trials on three consecutive days with an average efficiency taken across all days. I introduced each subject at the same position in the chamber (figure 19a & 19b) during the trials. Upon introduction, a recorded 10-minute habituation phase began. I recorded trials using a Sony Handycam Vision DCR-SX44. I introduced five crickets through tubing entering the chamber's side once the habituation phase was complete. Again a 10-minute recording period began. The recording chamber was cleaned and a new disposable liner used between sites. I determined efficiency by number of attempts to capture prey items versus successful attempts as well as the duration of time required to capture prey items including latency between captures.

Recovery

I also recorded recovery efficiency from exposure to contaminants over a five week period in addition to recording feeding efficiency and latency between captures. I retained five frogs from the urban site, Mariner's Marsh Park, Staten Island, NY in the lab over five weeks. Each week feeding trials were repeated as previously described and I recorded feeding efficiency one day each week. I compared feeding efficiency to frogs from the rural site, Dupre property, Ulster County, NY, also over a five week period.

Advertisement Call Survey

I recorded frog calls in the field using a SONY ICD-PX720 digital recording device. I also recorded both water and air temperatures at the time of recording using a MinnKota MKA-38 Fisherman's Air & Water Temperature Sensor. I transferred recordings to a computer by a SONY Digital Voice Editor (Version 3.2.00) and analyzed them using Raven Pro 64 1.4 console (Cornell Lab of Ornithology). I tested the call variables, call rate and latency between calls (Howard and Young 1998, Welch et al. 1998, Moriarty-Lemon 2009). Since results from previous studies suggest that all populations within the range of this study belong to a single monophyletic lineage of *Lithobates clamitans* (Austin and Zamudio 2008), phylogeny should not influence these call variables.

Statistical Analysis

Feeding Efficiency

I compared feeding efficiency using a Mann-Whitney U-test by site groupings of urban and rural. I chose a threshold of $p = 0.05$ for statistical significance. I used a scatterplot and linear regression with associated p values to determine if feeding efficiency was associated with the size of the frog (snout to vent lengths (SVL) and body mass (BM)) to determine if larger frogs were more efficient at capturing prey items. I used Scatterplots and linear regressions with associated p values to determine if feeding efficiency was related to total metal in the frog, sediment and water. I used a Mann-Whitney U-test to compare latency between cricket captures among urban and rural sites with a threshold of $p = 0.05$ for statistical significance. I used scatterplots and linear regressions with associated p values to determine if latency between cricket captures was related to total metal in the frog, sediment and water. I used a repeated measures analysis of variance to determine if feeding efficiency improved in the absence of environmental contaminants, with a threshold of $p = 0.05$ for statistical significance.

Call Rate

I compared mean call rate using an ANCOVA by site groupings of urban and rural with a threshold of $p = 0.05$ for statistical significance. I corrected for temperature using air temperature as a covariate. I used scatterplots and linear regressions with associated p values to determine if average call rate was related to total metal in the frog, soil and water. I used a multiple regression to correct for temperature. I compared average call latency using an ANCOVA by site groupings of urban and rural with a threshold of $p = 0.05$ for statistical significance. I corrected for temperature using air temperature as a covariate. I used scatter plots and linear regressions with associated p values to determine if average call latency was related to total metal in the frog, sediment and water. I used a multiple regression to correct for temperature. I used linear regressions and scatterplots as described above to determine if frog size affected call rate or call latency.

Results

Feeding Efficiency

Frogs from rural and urban sites demonstrated significant differences in prey capturing abilities. Frogs from the rural sites were significantly better at capturing prey items compared to frogs from urban sites (Figure 20; $U = 5425.00$, $p = 0.000006$). Prey capture efficiency was not found to improve with the size of the frog (Figures 21 & 22; BM, $p = 0.317$; SVL, $p = 0.625$). When feeding efficiency was compared to site sediment total metal concentrations, no relationship was observed (Figure 23; $p = 0.51$). I found a negative relationship between water metal concentration and feeding efficiency (Figure 24; $p = 0.007$). As total metal in the water increases, feeding efficiency decreases. I found no significant relationship between average feeding efficiency compared to average total metal concentrations in frogs from each site (Figure 25; $p = 0.11$). I found a significant difference in feeding efficiency in frogs from Mariner's Marsh park compared to frogs from the Dupre property in the recovery trial, with frogs from Dupre significantly more efficient at capturing prey items (Figure 26; $p = 0.047$). There was no significant difference in efficiency by week ($p = 0.88$) for either site.

I found a significant difference in latency between prey captures between rural sites and urban sites (Figure 27; $U = 37388.00$, $p < 0.0001$). Frogs from urban sites required more time between prey captures. I did not find any relationships when I compared average latency to total concentration of metal in water, sediment, or frog (Figures 28-30; $p = 0.06$; $p = 0.21$; $p = 0.12$).

Frog Call

I found a non significant difference in call rate in frogs from urban and rural sites (Figure 31; $p = 0.53$). I did not find any relationship between average call rate and water, sediment, and frog metal concentrations (Figures 32-34; $p = 0.32$, $p = 0.92$; $p = 0.51$). When corrected for temperature, I did not find any relationship between average call rate and water, sediment, and frog metal concentrations ($p = 0.37$; $p = 0.97$; $p = 0.18$). Frogs from urban sites also have less lag time between calls than frogs from rural sites, though results are not significant (Figure 35; $p = 0.07$). I did not find any relationship between average call latency and water, sediment, and frog metal concentrations (Figures 36-38; $p = 0.31$; $p = 0.83$; $p = 0.37$). When corrected for temperature, I did not find any relationship between average call latency and water, soil, and frog metal concentrations ($p = 0.42$; $p = 0.83$; $p = 0.28$). Lastly, I did not observe any relationship between average call rate or average latency between calls and body mass (BM) or snout to vent length (SVL), therefore body size did not affect the number of calls per minute (Figures 39-42; call rate: BM, $p = 0.11$; SVL, $p = 0.13$; call latency: BM, $p = 0.34$; SVL, $p = 0.30$). When corrected for temperature, I did not find any relationship between average call rate or average latency between calls and body mass (BM) or snout to vent length (SVL) (call rate: BM, $p = 0.75$; SVL, $p = 0.68$; call latency: BM, $p = 0.66$; SVL, $p = 0.59$).

Discussion

The purpose of this study was to determine if behavior could be used to measure effects of urbanization in the Green frog by comparing urban to rural sites. Additionally, I wanted to determine which behaviors could serve to appropriately reflect effects of urbanization. Frogs from rural sites (Dupre Property, Ulster County, NY, Quarry Property, Ulster County, NY, and Joy Property, Ulster County, NY) were compared to urban sites (Mariner’s Marsh Park, Staten Island, NY, Turkey Swamp Wildlife Management Area, Willowbrook Park, Staten Island, NY, and High Rock Park, Staten Island, NY) in measures of prey capture (feeding efficiency and latency between prey captures) and male advertisement call (call rate/min and latency between calls). I also compared the previously mentioned behaviors to total concentrations of metals, one type of pollutant typically present in greater concentrations in urbanized environments, present in the water, soil, and frogs sampled from each study site.

Green frogs from urban sites (Mariner's Marsh Park, Turkey swamp WMA, Willowbrook Park, and High Rock Park) significantly differed in feeding efficiency and latency between prey captures from frogs from rural sites (Dupre property, Quarry Property and Joy Property), with frogs from urban sites feeding less efficiently relative to frogs from rural sites. Frogs from urban sites strike at and miss prey items more frequently than do frogs from rural sites. Prey capture latency likewise is often longer in frogs from urban sites. This is most likely due to the energy exertion during the capture of prey in frogs from these sites. Prey capture in amphibians is visually motivated. Amphibian feeding behavior may be viewed as a fixed-action pattern in that the response to prey in the visual field is highly stereotyped (Weerasuriya 1989). The release of this fixed-action pattern is the presence of moving prey in the field of view. The stereotyped behavior that follows consists of orienting the body towards the prey item to compensate for lack of eye movement, approaching the prey item, binocular fixation to better gauge prey distance, and finally snapping at the prey item (Weerasuriya 1989, Zupanc 2004). Exposure to environmental pollutants has been demonstrated to negatively impact prey capture in a number of species (Bridges 1999, Weis et al. 2001, Perez and Wallace 2004, Khoury et al. 2009). The mechanisms behind these deficiencies may vary by pollutant and species. In green frogs, since feeding is visually driven, some aspect of the visual system may alter the frog's perception of prey location thus accounting for the increased observance of missed strikes. Further analysis of green frog morphology may provide insight into the observed variations in prey capture behavior.

I found deficiencies in prey capture efficiency to be proportionately associated with elevated total metal concentrations in the water. Metals dissolved in water are more likely to be absorbed by organisms through dermal exposure and ingestion due to the formation of ions in aquatic solutions, which make metals more bioavailable (James and Kleinow 1994, Roesijadi and Ronbinson 1994). Skin on the ventral surface, in particular, acts as a site of water absorption (Stoyler et al. 2008). Lead (Pb) accumulation in ventral skin as well as other organs was found to positively correlate with lead (Pb) exposure by water in *Rana ridibunda* (Vogiatzis and Loumbourdis 1999). Juvenile *L. clamitans* also have the ability to assimilate metals from the environment to tissue (Sparling and Lowe 1995, Bank et al. 2009). In similar studies using the Columbia spotted frog, *Rana luteiventris*, these assimilations were associated with adverse behavioral responses (Lefcort et al. 1998).

Prey capture was not associated with total metal concentrations in the sediment collected from either site category. Bioavailability of metal in soil is dependent on chemical factors such as redox potential, pH, speciation and concentration (Ernst 1996). Therefore total metal concentration in soil may not reflect the true bioavailability of metal (Roesijadi and Ronbinson 1994).

Additionally, prey capture was not associated with total metal concentrations in frogs. These results are surprising due to contrary evidence in the literature in other species, such as decreased prey capture efficiency in fiddler crabs (*Uca pugnax*) associated with higher total body burdens of silver (Ag), cadmium (Cd), copper (Cu), nickel (Ni), and selenium (Se) (Khoury et al. 2009) and decreased rate of prey capture associated with higher brain concentrations of mercury (Hg) in mummuchogs (*Fundulus heteroclitus*) (Smith and Weis 1997). However, the lack of association between metal in the frog and the behaviors observed in the current study could be the result of the small sample size used in the total body metal analysis for each site. The results within site demonstrated wide variance, which may account for the lack of association. Future studies should include a larger sample size for body burden analysis. Toe clippings and blood samples may serve as a non-destructive method to obtain larger sample sizes (Bergeron et al. 2010b, Todd et al. 2012).

Lastly, though prey capture efficiency was significantly different between frogs from the urban site, Mariner's Marsh park and frogs from the rural site, Dupre property, with frogs from Dupre significantly more efficient at capturing prey items, there was no significant improvement over the five week recovery period. Mummichogs from sites contaminated with mercury demonstrate a similar lack of improvement over extended laboratory prey capture studies (Smith and Weis 1997). Green frogs may require longer recovery periods or lack the ability to recover once trace metals are accumulated. Metals are accumulated in higher concentrations during early development than in later life stages (Roe et al. 2005) and these developmental effects may be irreversible.

Feeding behavior in *L. clamitans* does appear differ between urban and rural environments and does correspond to metal concentrations in water. Feeding efficiency was negatively associated with total water metal concentrations. Although the results suggest a relationship between total metal concentrations in water and prey capture efficiency, urban environments also contain numerous other anthropogenic pollutants (White and McDonnel 1988). Therefore, the observed deficient prey capture in urbanized sites may be the result of some other pollutant, not measured in this study.

Regardless of the cause, deficient prey capture has negative consequences to the individual growth and survival of the organism. Weis et al. (1998) found mummichogs, *Fundulus heteroclitus*, from a contaminated estuary were less efficient at capturing prey items and had reduced growth rates and life spans compared fish from unimpacted sites. Therefore we may detect differences between green frog size and survival in populations from rural and urban sites.

In amphibians, acoustic pollution negatively impacts vocal communications (Sun and Narins 2005). I wanted to determine if other aspects of urbanization, such as anthropogenic metal pollution, could also negatively impact male vocal communications. Male advertisement call was found to not differ significantly between rural and urban sites. No relationships were observed when male advertisement call was compared to total metal concentrations in water, sediment or frogs collected from the sites. Previous studies in fishes have found deviations from normal courtship behavior, such as increases or decreases in frequency of courtship displays and increased courtship behavior associated with polluted environments (Jones and Reynolds 1997). Metal exposure in wood ants (*Formica aquilonia*) was found to reduce aggressive (territorial) behavior in males (Sorvari and Eeva 2010). However, in the current study, I did not find any such deviations from normal call behavior in frogs from urbanized sites.

These results support the use of behavior as a valuable, non-destructive, individual-level response that can be quantified to determine the intensity of the effects of urbanization. Behavioral impairments can reflect significant physiological changes resulting from chronic or acute pollutant exposure (Raimondo et al. 1998). These modifications in behavior can affect conspecific interactions (competition) as well as interactions with individuals of other species (predator-prey interactions) (Reichmuth et al. 2009). Therefore, assessment of behavior can be used to reflect overall changes in communities resulting from environmental impacts. However, as observed in the current study, some behaviors are more sensitive to environmental contaminants in urbanized environments than others. Feeding efficiency and latency between prey captures were both sensitive to urbanization. A relationship was observed between elevated concentrations of metal present in water and deficient feeding behavior. Male advertisement call did not vary significantly between urban and rural sites. These results suggest that male advertisement call in *L. clamitans* is not a sensitive to urbanization.

Chapter 3: Population

Differences in Green Frog (*Lithobates clamitans*) Population Structure, Morphology and Subsequent Impacts to Feeding Efficiency in Frogs from Urban and Rural Sites

Introduction

The quantification of amphibian morphology and population abundance, combined with observations of these animals behavior, is potentially a useful means of gauging the impact of environmental pollution present in urban sites. Towards this end I studied the effects urbanization on frogs, both at the population and individual level. I studied effects on morphology of individuals, as well as structure of populations.

Urban areas pose a threat to both terrestrial and aquatic ecosystems. These areas are characterized by greater human population density, land development, and pollution (Hamer and McDonnel 2008). Some pollutants more prevalent in urban environments are, metals, polycyclic aromatic hydrocarbons (PAHs), particulate matter, and radioactive nuclides (White and McDonnel 1988, Madrid et al. 2008, Pouyat et al. 2010). Freshwater ecosystems are especially vulnerable to the input of nutrients and wastes to water and sediment from run-off due to the increased area of impervious surfaces in urban areas (Riley et al. 2005, Bain et al. 2012). In addition to pollution, urban environments have higher incidences of habitat fragmentation resulting from increased land modifications (Sarre 1996). Habitat fragmentation inhibits migration necessary for life-stage-specific processes, recolonization in events of local extinction, and acquisition of more suitable habitat. Urban habitats also exhibit greater native plant species extinctions and non-native plant species introductions relative to rural habitats (Pouyat et al. 2010). Some introduced plants, may produce secondary compounds that are toxic to developing amphibian larvae. American toad, *Bufo americanus,* tadpoles raised with Purple Loosestrife, *Lythrum salicaria,* extract are less developed than tadpoles raised with extracts from native plants (Maerz et al. 2005). Likewise, exposure to Amur honeysuckle, *Lonicera Maackii*, extract in Eastern American toads, *Anaxyrus americanus*, results in decreased respiratory ability and higher mortality (Watling et al. 2011). The combined ecotoxicological effects of pollutants and invasive plant species as well as the inhibition of migration by habitat fragmentation in urban environments may result in negative impacts to all levels of biological organization.

Metals were used as one measure of pollutant present in urban environments. Metals tend to persist in the environment (Nriagu 1988) and some can biomagnify (due to their storage in fatty tissues) in food-chains (Seebaugh et al. 2005). As a result, even low-level environmental concentrations of metals may adversely affect frogs (Linder et al. 1998). Effects may include mortality as well as sub-lethal effects (Wallace and Estephan 2004) that occur at cellular, individual and population levels (Weis et al. 2001). Behaviors, such as feeding efficiency and predator avoidance, have been shown to be affected by toxins such as metals. Survival-related behaviors contribute to the growth and survival of individuals (Weis et al. 2001). Impairments in behaviors such as feeding efficiency and predator avoidance can negatively impact population size, population size structure, and number of individuals present in each age class (Raimondo et al. 1998, Weis et al. 2001, Preston 2002, Khoury et al. 2009). If, for example, feeding efficiency is impaired because of high levels of cadmium within an organism, then clearly such impairment can have impacts at the individual, population and community levels. For example, mummichogs, *Fundulus heteroclitus*, experience significant life stage related sensitivities to contaminants including mercury (Hg) (Weis et al. 2001). While gametes and embryos are tolerant to mercury (Hg) contamination, adults demonstrate reduced growth rates and life spans associated with impaired prey capture rates compared to their non-contaminated counterparts (Weis et al. 2001). The degree to which frogs are influenced by metals depends on the species-specific activity and life history (Peakall and Burger 2003).

Pollutants common in urban environments negatively affect normal morphology of native species. Exposure to pollutants during early development retards growth rates (Horne and Dunson 1995, Relyea et al. 2005), increases occurrences of developmental malformations (Bridges 2000, Hopkins et al. 2006), and delays time to metamorphosis in amphibians (Lefcort et al. 1998). The result of these delays is smaller, sometimes developmentally deformed transformed frogs, more vulnerable to predation. Retarded growth rates may also impact feeding efficiency. In amphibians, feeding behavior is highly stereotyped and visually motivated. Visual acuity governed by field-of-view is critical in the acquisition of prey (Fite 1973). As frogs lack fovea and eye movement, the process of prey capture consists of orientation of the amphibian toward the direction of the prey item, followed by stalking towards the prey, binocular fixation and finally the snapping and swallowing of the prey item (Zupanc 2010). Successful prey capture is dependent on the frog’s ability to gauge prey distance (Gerhardt 1994). In fish, reactive distance, the distance in which they engage prey items, is significantly less in larger fish due in part to the larger visual volume (Breck and Gitter 1983). In birds, eye diameter was found to evolve to effectively capture moving prey items (Ryan et al. 1981). To accommodate for increased sensory input, increase brain size was believed to co-evolve. Garamszegi et al. (1981) found a positive correlation between eye size, brain size and subsequent prey capture. As frogs exposed to pollutants have slower growth rates, visual volume may also be inhibited. Deficient prey capture may therefore reduce energy required for continued growth (Sih 1980, Morin et al. 1990) and survival over winter (Fitzpatrick 1976).

Amphibians have complex life cycles which require close contact with both aquatic and terrestrial habitats (Fontenot et al. 2000). Reports of worldwide amphibian decline have placed amphibian species in the highest proportion of vertebrates at risk of extinction (Cushman 2006).

Anthropogenic activities are, at least in part, responsible for these rapid declines (Carey and Bryant 1995). Amphibian declines may forecast negative community-level impacts. To determine if declines are occurring locally we must 1) identify species appropriately sensitive to changes in environmental conditions and 2) understand normal behavior and life history strategies in this species, and 3) compare deviations from these baselines to predict impacts to these ecosystems.

Green Frog Life History Strategies

Green frogs, *Lithobates clamitans*, have a broad range and often occur in high abundance (Bergeron et al. 2010b) and therefore play an integral role in energy transfer between aquatic and terrestrial communities. Green Frogs inhabit riparian zones and margins of ponds. They have home ranges averaging 60 square meters (Martof 1953). Newly transformed frogs disperse from breeding ponds in search of suitable territories (Schroeder 1976). Acquisition of suitable territory reduces competition and increases the likelihood of obtaining shelter from predators in dense vegetation (Martof 1953, Lamoureux et al. 2002). Adult Green Frogs, *L. clamitans*, display intense male-male competition for breeding territories (Wells 1977). Females select mates based on territory quality, and prefer sites with adequate vegetative cover for eggs (Wells 1977). Defense of territory often occurs stepwise with a sequence of escalating deterring events from vocalization of the defending male to a physical defense of the territory (Ramer et al. 1983, Bee and Perrill 1996). Once a mate is selected, females deposit eggs in a single sheet, consisting of approximately 1,000 to 4,000 eggs, on the surface of the pond, often attached to vegetation (Wells 1977, Wright and Wright 1995). If a clutch is deposited early in the breeding season (May-August), a second clutch may be laid during the same season (Martof 1956b, Wells 1976).

Larvae often require one full year as tadpoles prior to transformation (Martof 1956a). However, some transform in their first year. Because of this prolonged aquatic stage, during drought periods *L. clamitans* populations may experience great losses of tadpoles (Martof 1956b).

Mortality by predation is common, with eggs and tadpoles consumed by birds, fish, turtles, snakes, insects, and other frogs (Martof 1956a). Larval frogs are especially vulnerable to predation since they tend to inhabit shallow areas of ponds. Adult *L. clamitans* are also at risk of predation from a host of predators shared by larval frogs such as birds, turtles, snakes, and frogs, as well as consumption by mammals (Martof 1956a). Predator mediated limb deformities observed in adult *L. clamitans*, result from partial predation during metamorphosis from tadpole to adult when hind limbs are exposed (Martof 1956a).

Amphibian population structure most closely resembles that of a metapopulation (Marsh and Trenham 2001). Amphibians have limited dispersal abilities dictated by reliance on water and inability to travel long distances over dry land (Cushman 2006). Frogs migrate short distances from wintering sites to breeding ponds and from breeding sites to surrounding areas for foraging and dispersal (Schroeder 1976, Lamoureux et al. 2002). Longer migrations are possible during heavy rain (Lamoureux et al. 2002). Dispersal can enable recolonization following local extirpation, though recovery may take many years (Alford and Richards 1999).

Amphibian Sensitivity to Metals

Exposure to pollutants during early developmental stages and adulthood lead to the differential health and survival of each life stage. Pollutants occur in greater number and concentrations in urbanized environments than rural (White and McDonnel 1988). Exposure to metals during particularly sensitive life stages result in stage specific adverse effects (Horne and Dunson 1995, Boone et al. 2001, Hopkins et al. 2006, Bergeron et al. 2010a). Maternal transfer of mercury (Hg) and selenium (Se) has been observed between female American toads (*Bufo americanus*) and eggs (Bergeron et al. 2010a). Maternal transfer of strontium (Sr) and selenium (Se) has also been observed in eastern narrow mouthed toads (*Gastrophryne carolinensis*), with females transferring significant metal concentrations to eggs (Hopkins et al. 2006). Maternal transfer of metal as well as environmental metal exposure to eggs is associated with reduced egg size and hatching success compared to non-exposed eggs (Hopkins et al. 2006, Clark and Lazerte 2011). Phenotypic variation in hatching time has been observed in *L. clamitans* eggs in response to chemical cues from stage specific predators. Egg predator chemical cues trigger rapid transition from egg to larvae, while eggs exposed to larval predator cues prolong incubation, and transition at larger body sizes (Ireland et al. 2007). The phenotypic plasticity involved in anti-predator response is crucial to the survival of larval amphibians; therefore, metal induced size effects may reduce egg and tadpole survival.

Amphibian larvae exposed to metal also experience negative effects that impact survival and reproduction. Tadpole exposure to metal is associated with decreased size (mass and length) and survival, and increased incidence of developmental abnormalities (Lefcort et al. 1998, Hopkins et al. 2006, Weir et al. 2010). Experimental exposure of larvae to metals as well as low pH and dissolved organic carbon result in prolonged time to metamorphosis (Horne and Dunson 1995, Lefcort et al. 1998, Davey et al. 2008) with exposure to multiple metals at low concentrations more disruptive to tadpoles than high concentrations of single metals (Lefcort et al. 1998). Prolonged transition time increases the vulnerable period for which hind limbs are exposed to predators potentially intensifying predator mediated limb deformities. Additionally, delayed time to metamorphosis reduces recruitment rate of tadpoles to the adult stage, therefore decreasing the number of adults present in the population (Horne and Dunson 1995, Bridges 2000).

Metals may also induce limb deformities. Limb malformations drastically increase the vulnerability of transformed frogs to predators (Cohen Jr. 2001). Missing/additional limbs and digits, craniofacial abnormalities, tail malformations and skin lesions may also result from metal contamination (Bridges 2000, Blaustein and Johnson 2003, Hopkins et al. 2006). Limb malformations have been induced in laboratory environments primarily through exposure to UV-B radiation, chemical contaminants and pesticides (Rosenshield et al. 1999, Bridges 2000, Blaustein and Johnson 2003). The endocrine disruptive capacity of metals such as arsenic (As) (Davey et al. 2008) may also contribute to malformations in natural populations.

The impacts of urbanization on adult frog morphology, survivorship of each life stage and its demographic impact are still uncertain. I analyzed morphology (Appendix I) of individual frogs in these populations to determine if frogs from urban sites differ in size or occurrence of malformations from those in rural sites. There is evidence that amphibians exposed to toxic levels of metals and other pollutants experience decreased fright response, loss of balance, slowness in motion, and reduced swimming speed (Lefcort et al. 1998, Savage et al. 2001, Selvi et al. 2003, Chen et al. 2009). In addition, tadpoles may develop axial malformations that impact swimming efficiency (hopkins et al. 2000). Accurate and lightning-fast responses to moving prey are likely to be critically important to frog survival. Therefore, I set out to test whether frogs from urban sites suffered impairment in their prey capture ability resulting from retarded growth or malformations. To assess the overall population impact of urbanization, I assess population abundance in Green Frog populations from urban and rural sites.

Methods

Study Site Description

I selected study sites (Fig. 2) in both urban and rural environments. The urban sites, with the exception of one site, are located in New York City, NY. In the urban category, I also included one site in Freehold New Jersey located directly parallel to a landfill previously investigated by the EPA for the presence of hazardous materials by anthropogenic activities. The rural sites are all located in Ulster County, NY. For each site, I measured the sum and individual trace metals in water (Table 1 and 6), sum and individual trace metals in sediment (Table 2 and 5) and other abiotic factors (Table 3).

Population structure

I collected census data by walking the perimeter of the ponds at each selected site (Heyer 1994). Adult frogs were located visually and left undisturbed. The census ended when the point of count initiation was reached. I recorded the time to complete the circuit. I used frog per unit time search to assess adult numbers. I collected tadpoles by minnow trap sampling (Heyer 1994). Two traps were placed approximately 15 meters apart. I baited traps with rabbit food pellets in mesh and left out for approximately 24-hours. Tadpoles were removed by hand and counted, but not marked. Egg masses were located visually, counted and left undisturbed.

Morphometric Analysis

Capture

I collected approximately 40 adult green frogs from each study site during breeding seasons, June-August, over two consecutive years. Permits to collect frogs were obtained from NYC

Parks department, New York State Department of Environmental Conservation, Division of Fish, Wildlife and Marine Resources, and New Jersey Department of Environmental Protection, Division of Fish and Wildlife. I collected frogs at night either by hand or with dip nets. I placed frogs in a ventilated plastic aquarium for transport to the laboratory.

Morphology

I sexed frogs by measuring the tympanum diameter with relation to the diameter of the eye (males tending to have a larger tympanum) and the presence of yellow coloration of the vocal sac in adult males (Harding 1997). If one tympana was misshapen the abnormality was noted and the measurement of the other tympanum was used (Martof 1956b). I took all measurements from the right side unless otherwise noted. Body mass was taken by placing each frog individually in a pre-weighed cup on a top loading balance. Frogs were depurated prior to taking of body mass. I recorded morphological measurements using vernier calipers to establish differences in individuals between sites. Frogs were manually restrained during measuring for as little time as necessary in order to minimize stress. Measurements included (Appendix I) snout to vent length (SVL), head width (HW), head length (HL), tympanum diameter (TD), eye width (EW), snout length (Snout), femur length (FeL), tibia length (TL), foot length (FoL) and snout angle (SA) (Moriarty-Lemon 2009). Significant differences in forelimb size resulting from sexual dimorphism were taken into consideration (Schulte-Hostedde and Schank 2009).

Statistical Analysis

I conducted three analyses. First, to determine if there was a difference in frog population structure between urban and rural sites, I compared sites using Mann-Whitney U-tests with $\alpha = 0.05$. To assess if urban and rural sites could accurately be grouped by any morphological characteristics, I used a discriminant function analysis (DFA). Lastly, to determine if a relationship existed between behavior and morphology, I compared morphological characters to feeding efficiency by multiple regression.

Results

I did not observe any egg masses during 2012. Because of this I could not calculate a ratio of egg mass to tadpoles to adults. Instead, I used a Mann-Whitney U-test to compare adult count per unit time (seconds) and average tadpole count per minnow trap independently of one another. No significant differences were observed in adult count per unit time between urban and rural sites ($U = 3.00$, $p = 0.28$). Non-significant results were also obtained for average tadpole count ($U = 4.00$, $p = 0.46$).

I measured morphological characters (Appendix I) for each frog used in feeding trials. The discriminant function analysis (DFA) grouped urban and rural sites by several of these variables. Body mass ($p = 0.03$), snout to vent length ($p = 0.046$), eye width ($p = 0.002$), snout length ($p = 0.005$), femur length ($p = 0.03$), tibia length ($p = 0.002$), and foot length ($p = 0.001$) were significantly different between urban and rural sites.

I found that eye width ($p = 0.027$) predicted feeding efficiency. Frogs from rural sites had greater eye widths (Figure 43; $U = 4589.00$, $p < 0.0001$).

Discussion

Morphology

Malformations during amphibian development negatively impact amphibian survival by increasing vulnerability to predators as well as affecting their ability to capture prey (Cohen Jr. 2001, Levitis et al. 2009). Whole-body deformities such as missing/additional limbs and digits, craniofacial abnormalities, tail malformations and skin lesions have been observed in association with anthropogenic factors including UV-B radiation and chemical contaminants (Bridges 2000, Blaustein and Johnson 2003, Hopkins et al. 2006). I did not observe any obvious developmental anomalies in frogs from urban or rural sites.

I measured snout to vent length (SVL), head width (HW), head length (HL), tympanum diameter (TD), eye width (EW), snout length (Snout), femur length (FeL), tibia length (TL), foot length (FoL) and snout angle (SA) for each frog used in feeding trials to determine if any size differences existed between urban and rural sites. Of these morphological characters, urban and rural sites were found to significantly differ in body mass (BM), snout to vent length (SVL), eye width (EW), snout length (Snout), femur length (FL), tibia length (TL) and foot length (FoL). In general frogs from rural sites were larger than frogs from urban sites. The size of the frogs from this study (snout to vent lengths), were generally with within the normal size range for green frog adults (2-4 inches) (Martof 1956b). However, urban sites had a greater number of frogs below the normal adult size range. This difference in size composition in frogs from urban and rural sites may be due to the significant disparity in frog prey capture ability as discussed in chapter 2. Energy derived from the consumption of prey is required for individual growth, production of gametes and survival over winter inactivity periods (Fitzpatrick 1976). Therefore, smaller frogs from urban sites may have a lower fitness than larger frogs from rural sites.

The variation in size of individuals from urban and rural sites may also be due to the age class composition of frogs from each site. I did not classify transformed frogs as juveniles or breeding adults. It is possible that the population structure of transformed frogs at urban sites consisted primarily of newly recruited juveniles or of first year breeding adults rather than mature breeding frogs. The absence of larger more competitive males may free up territories to smaller males therefore attributing to the observed size difference in frogs from urban sites. Subsets of the transformed population would need to be measured to determine if juvenile frogs are in fact supplementing the breeding adult population.

Comparisons between Morphology and Behavior

I compared morphological character measurements to feeding efficiency to determine the potential influence frog size in frogs from urban and rural sites on behavior. I found that of all the morphological characters measured, only eye width was significantly related to feeding efficiency. Frogs with greater eye widths were more successful at capturing prey items. Greater eye widths were observed more often in frogs from rural sites than urban sites. Due to the green frogs' reliance on visual cues for capturing prey items, large eye diameter may contribute to the success of capturing prey. In studies on both fish and birds, eye width has been found to increase visual acuity necessary for successful capture of moving prey (Breck and Gitter 1983, Garamszegi et al. 2002). The presence of pollutants in the environment has been shown to retard growth rate and development, thus reducing overall amphibian size (Pérez-Coll et al. 1985, Carey and Bryant 1995). However, eye width was not found to be significantly related to total metal concentrations present in water in the current study. These results may reflect that as a byproduct of delayed development, pollutants other than or in addition to metals present environment may negatively impact prey capture efficiency by reducing sensory input required to gauge prey distance. In salamanders, *Ambystoma talpoideum*, female size at metamorphosis was related to age of reproduction, with larger females reproducing at an earlier age (Semlitsch 1988). Therefore, reduced frog size in urban environments may have direct fitness consequences.

Population Structure

I found no significant differences in the abundance of *L. clamitants* from urban and rural sites assessed in this study. Adult *L. clamitans* as measured by count per unit time and average tadpole count per trap did not vary significantly in urban and rural sites. While these results may indicate that *L. clamitans* populations in urban environments are not impacted at the population level by the observed differences in morphology and feeding behavior, they may not accurately capture the population make-up that could be better represented by further age class sub sectioning.

Male green frogs are territorial; competition over prime breeding territories and refuge from predators influence the number of transformed males that remain in a territory versus disperse after metamorphosis (Shepard 2002). Delayed growth and reduced life span may reduce the number of breeding individuals. It is possible that in the event of decline of individuals in the breeding adult age class, fewer newly transformed frogs will migrate from the breeding pond in which they originated. These immature frogs may then supplement the missing individuals. In the population census, I considered all transformed frogs as adults and did not distinguish between juveniles and adults. To determine if juvenile frogs are supplementing a loss of breeding adults, additional subsets of the transformed population would need to be measured.

Overall I found that both frog size and feeding efficiency are reduced in green frog populations in urban habitats compared to frogs in rural habitats. These deficiencies may be the result of increased pollution associated with urbanized environments. Deficiencies in both size and feeding efficiency have negative consequences to frog fitness. Smaller amphibians may be subject to predation, delayed time to reproductive age, as well having shorter life spans (Semlitsch 1988, Weis et al. 2001). While overall population size differences were not observed, the negative effects of these deficiencies may not discernible by the population census methods used in the current study.

Tables

Table 1. Sum of trace metals in water for each site and distinction as urban or rural

Site	Sum of Trace Metals (PPM)	Urban or Rural
Dupre Porperty	0.26	Rural
Quarry Property	0.49	Rural
Joy Property	0.11	Rural
Mariner’s Marsh Park	1.59	Urban
Turkey Swamp WMA	2.05	Urban
Willowbrook Park	2.21	Urban
High Rock Park	0.98	Urban

Samples are corrected to laboratory blanks

Table 2. Sum of trace metals in sediment for each site and distinction as urban or rural

Site	Sum of Trace Metals (PPM)	Urban or Rural
Dupre Porperty	904.2	Rural
Quarry Property	604.2	Rural
Joy Property	818.9	Rural
Mariner’s Marsh Park	705.15	Urban
Turkey Swamp WMA	610.45	Urban
Willowbrook Park	271.5	Urban
High Rock Park	312.96	Urban

Samples are corrected to laboratory blanks

Table 3. Environmental variables by site

Category	Site	Mean UVB	High UVB	Low UVB	Distance to Road (m)	Distance to Main Road (m)	DO% Saturation	DO Mg/L	Salinity (PPT)	pH	Nitrogen Mg/L
Rural	Dupre Porperty	226.25	318	44	124.24	832.51	59.9	5.85	0.04	6.64	0.24
Rural	Quarry Property	280.75	356	77	410.96	1659.39	91.3	8.92	0.06	6.44	0.34
Rural	Joy Property	215	293	72	572.54	1989.77	86.5	9.08	0.02	6.19	0.13
Urban	Mariner's Marsh Park	262.5	322	121	27.28	348.16	105.7	9.77	0.1	8.49	0.4
Urban	Turkey Swamp WMA	207.25	317	102	20.98	1595.26	35	3.41	0.03	6.93	0.22
Urban	Willowbrook Park	255.5	344	129	97.48	97.48	126.9	10.56	0.23	7.59	1.7
Urban	High Rock Park	241.375	201	37	483.52	497.71	49.1	4.73	0.06	6.85	0.33

Table 4. Site history and designation as urban or rural

Site	Potential Contamination Source	Urban or Rural
Mariner's Marsh Park, Staten Island, NY	• 1930's-40's site was used in shipbuilding • Slag from activities dumped on site • Park is considered a Brownfield site.	Urban
Turkey Swamp WMA, Freehold, NJ	• Adjacent to Lone Pine Landfill • Received over 17,000 drums of chemical waste • Landfill listed as an EPA Superfund site	Urban
Willowbrook Park, Staten Island, NY	• Collecting reservoir for excess roadway water from busy six lane road	Urban
High Rock Park, Staten Island, NY	• Located in an urban city • Relatively undisturbed • Girl/boy scout camp prior to becoming a NYC park	Urban
Dupre Property, Ulster County, NY	• Privately owned • In a relatively rural area • Developed in 2006 • Has no history of pollution	Rural
Quarry Property, Ulster County, NY	• Privately owned • In a relatively rural area • Developed in 2006 • No history of pollution	Rural
Joy Property, Ulster County, NY	• Privately owned • In a relatively rural area • Developed in 1998 • No history of pollution	Rural

Table 5. Individual metal concentrations (PPM) in sediment samples

Category	Site	Cr	Co	Ni	Cu	Zn	As	Cd	Hg	Ti	Pb
Rural	Dupre Property	21	703	15	26	17	82	6.9	0.4	0.2	32
Rural	Quarry Property	27	447	12	22	12	62	7.7	0.3	0.2	13
Rural	Joy Property	40	632	9	26	12	79	6.6	0.5	0.4	12
Urban	Mariner’s Marsh Park	39.5	96.3	25.95	68.42	327.98	52.48	3.1	0.37	0.15	89.48
Urban	Turkey Swamp WMA	325	74.5	2.5	9	20.5	77.5	43.45	0.65	0.25	54.5
Urban	Willowbrook Park	29	109	5	22	19	68	1.8	0.7	0.2	14
Urban	High Rock Park	25.63	87.9	30.97	61.63	77.33	49.53	1.93	0.33	0.27	99.87

Table 6. Individual metal concentrations (PPM) in water samples

Site	Al	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Tl	Pb	Fe
Dupre Porperty	0.0081	<DL	0.0644	<DL	<DL	<DL	<DL	<DL	0.0003	<DL	<DL	0.1889
Quarry Property	0.0904	<DL	0.2596	<DL	<DL	0.002	<DL	<DL	0.0003	<DL	<DL	0.1417
Joy Property	0.012	<DL	0.0387	0.0007	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.0554
*Mariner's Marsh Park	0.4381	<DL	0.2174	0.0021	0.0087	0.0026	0.0502	<DL	0.0005	<DL	<DL	1.3946
*Turkey Swamp WMA	0.0373	<DL	0.0041	<DL	<DL	0.0009	<DL	0.0142	0.0003	<DL	<DL	2.1672
*Willowbrook Park	0.3571	<DL	0.0377	<DL	<DL	0.011	<DL	<DL	<DL	0.0087	0.0155	0.5876
*High Rock Park	0.0973	<DL	0.0739	<DL	<DL	0.0035	<DL	<DL	<DL	<DL	<DL	1.4223

Sites with (*) are categorized as urban

Figures

Fig 1 Adult male *Lithobates clamitans*

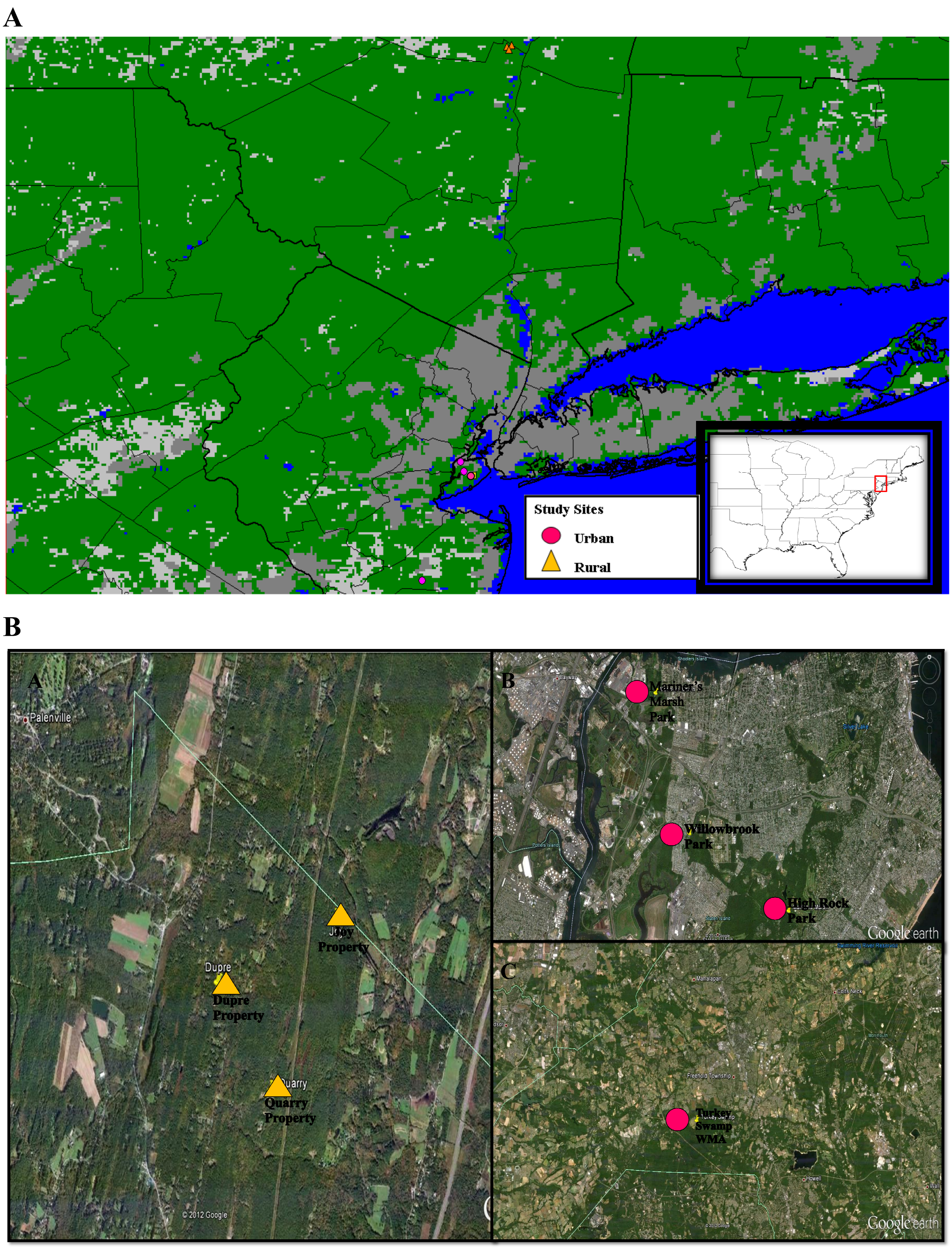
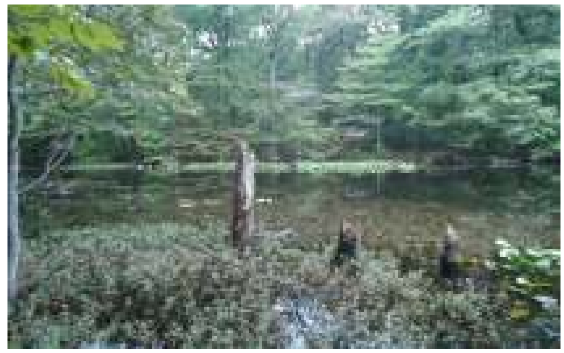


Fig 2 a) Map of Eastern U.S. with study sites b) An aerial view of study sites

A. Mariner's Marsh Park, Staten Island, NY

B. Turkey Swamp WMA, Freehold, NJ

C. Willowbrook Park, Staten Island, NY



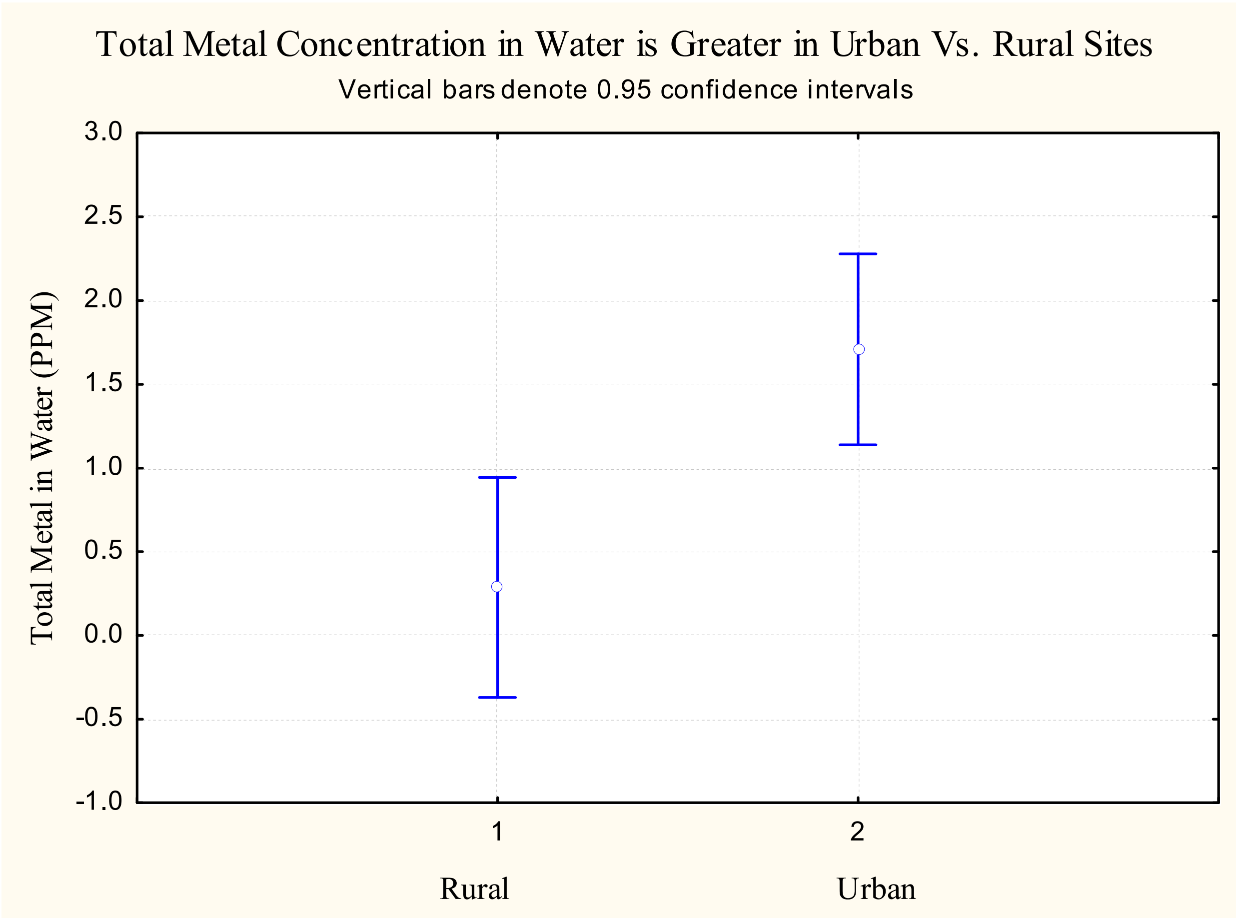
D. High Rock Park, Staten Island, NY

E. Dupre Property, Ulster County, NY

F. Quarry Property, Ulster County, NY

G. Joy Property, Ulster County, NY

Fig 3



Total Metal Concentration in Water is Greater in Urban Vs. Rural Sites
Vertical bars denote 0.95 confidence intervals
Total Metal in Water (PPM)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
1
2
Rural
Urban

Fig 4

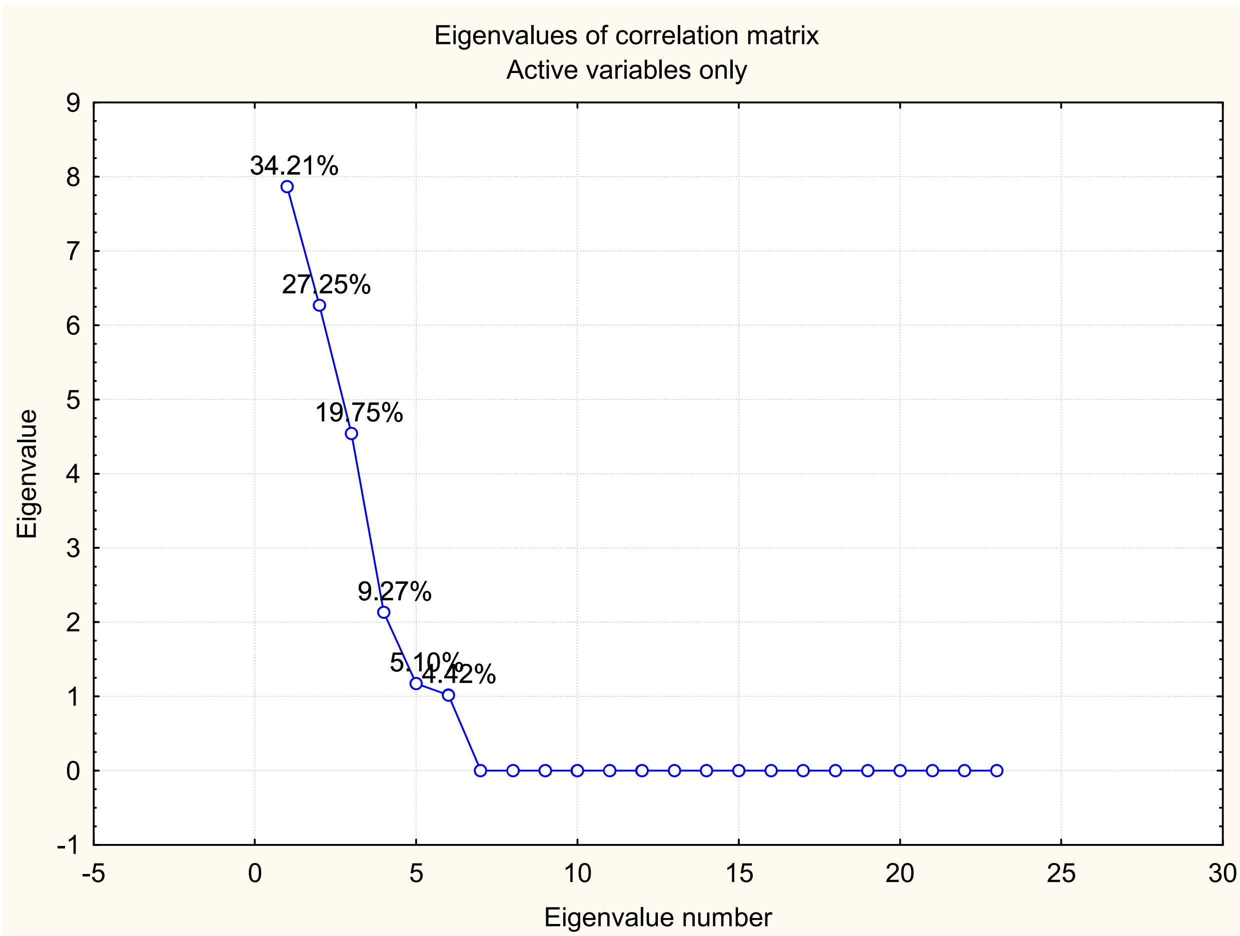

Eigenvalues of correlation matrix
Active variables only
Eigenvalue
Eigenvalue number
34.21%
27.25%
19.75%
9.27%
5.10%
4.42%
9
8
7
6
5
4
3
2
1
0
-1
-5
0
5
10
15
20
25
30

Fig 5

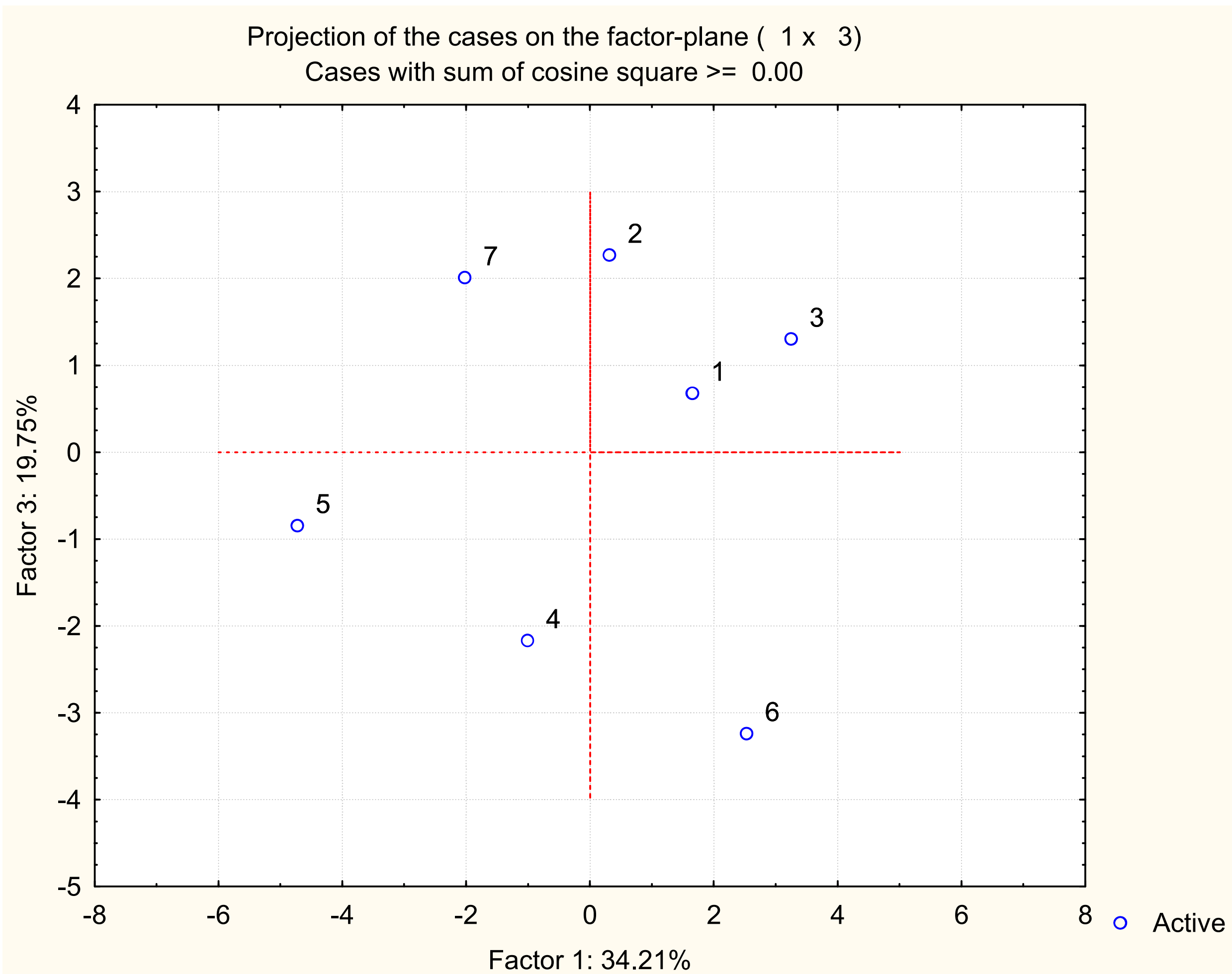

Projection of the cases on the factor-plane (1 x 3)
Cases with sum of cosine square >= 0.00
Factor 3: 19.75%
Factor 1: 34.21%
4
3
2
1
0
-1
-2
-3
-4
-5
-8
-6
-4
-2
0
2
4
6
8
1
2
3
4
5
6
7
Active

Fig 6

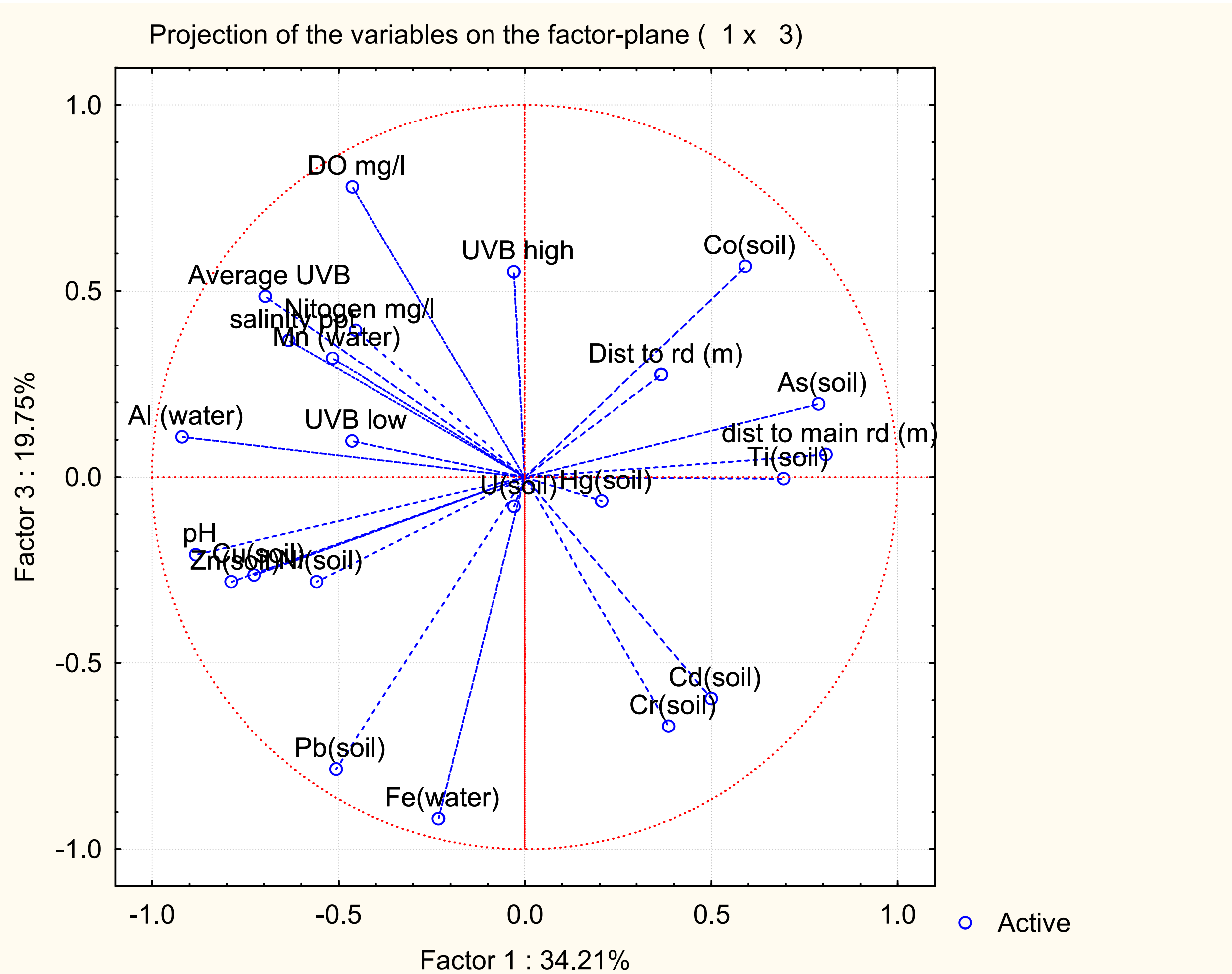
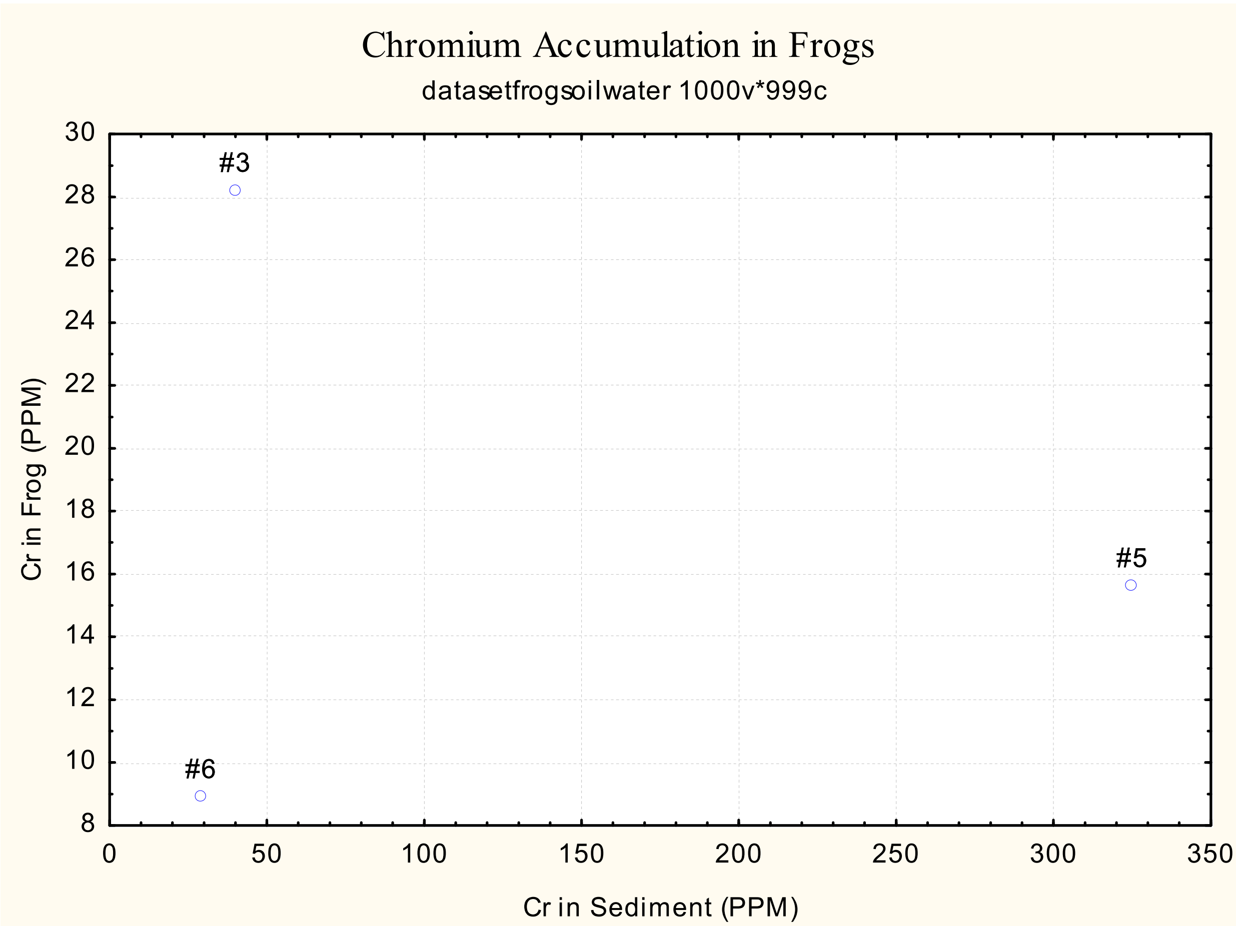

Projection of the variables on the factor-plane (1 x 3)
Factor 1 : 34.21%
Factor 3 : 19.75%
DO mg/l
UVB high
Co(soil)
Average UVB
Nitogen mg/l
salinity ppt
Mn (water)
Dist to rd (m)
As(soil)
Al (water)
UVB low
dist to main rd (m)
Ti(soil)
U(soil)
Hg(soil)
pH
Cu(soil)
Zn(soil)
Ni(soil)
Cd(soil)
Cr(soil)
Pb(soil)
Fe(water)
Active

Fig 7



Chromium Accumulation in Frogs
datasetfrogsoilwater 1000v*999c
Cr in Frog (PPM)
Cr in Sediment (PPM)
#3
#5
#6
30
28
26
24
22
20
18
16
14
12
10
8
0
50
100
150
200
250
300
350

Fig 8

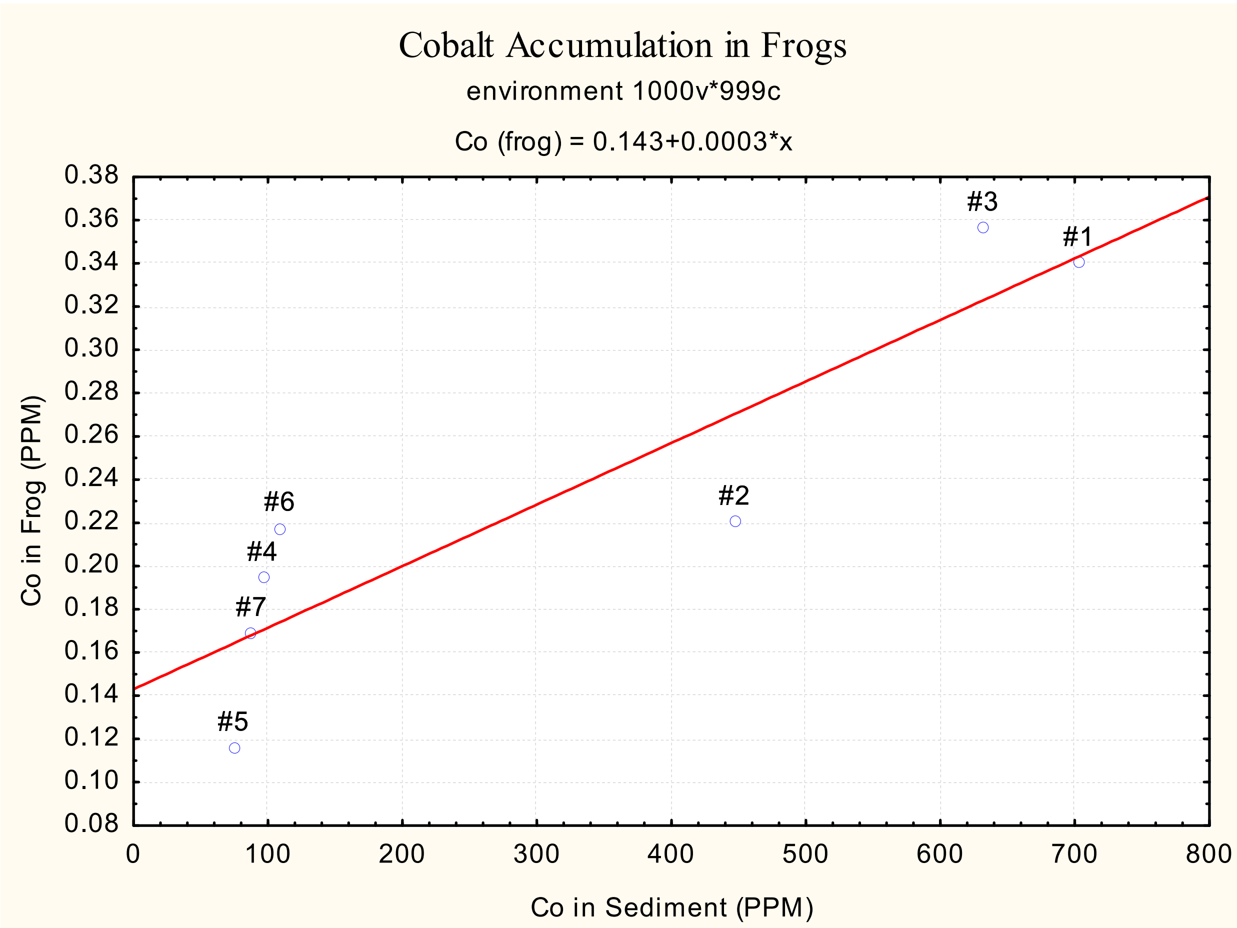

Cobalt Accumulation in Frogs
environment 1000v*999c
Co (frog) = 0.143+0.0003*x
Co in Frog (PPM)
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
#3
#1
#6
#2
#4
#7
#5
0
100
200
300
400
500
600
700
800
Co in Sediment (PPM)

Fig 9

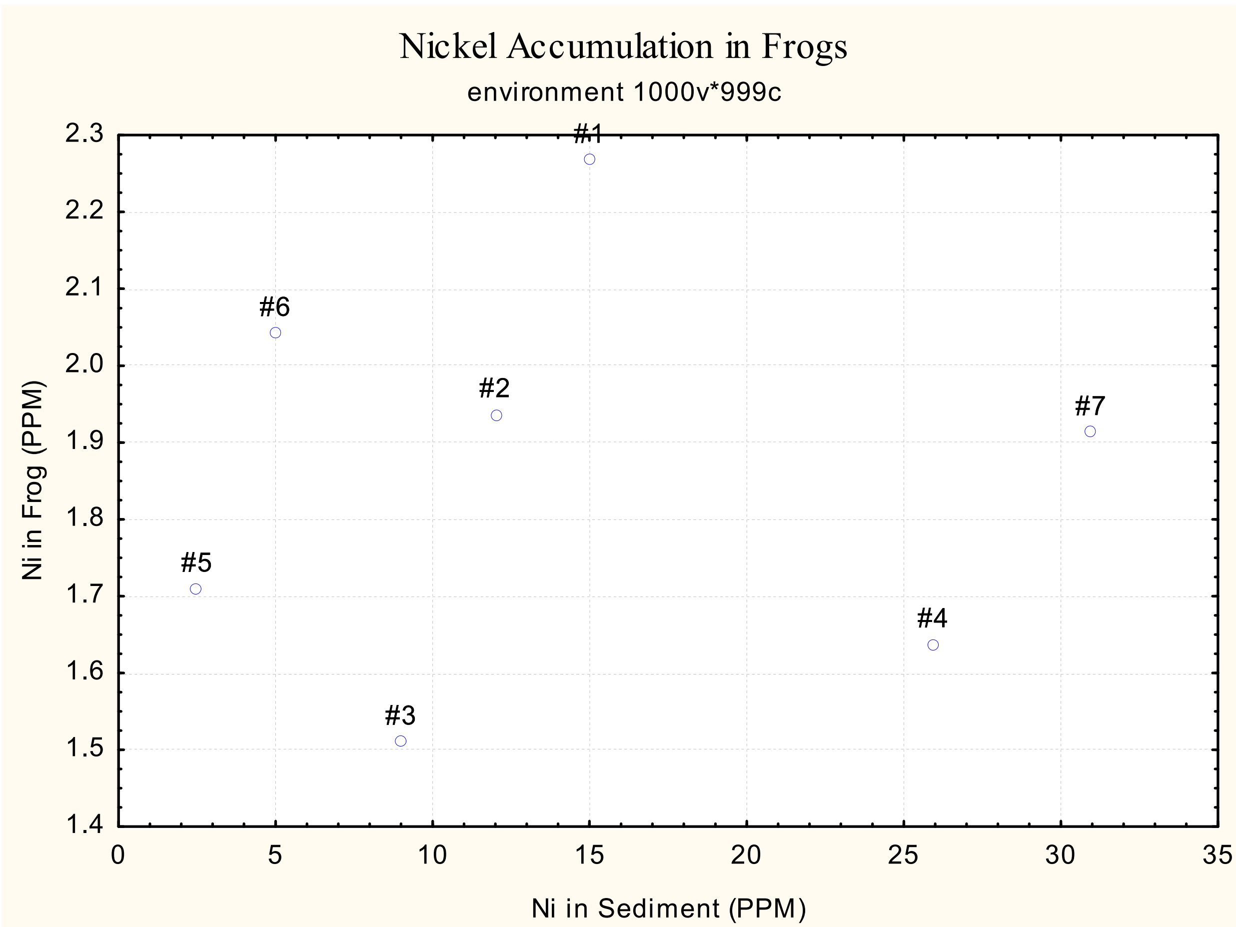

Nickel Accumulation in Frogs
environment 1000v*999c
#1
#2
#3
#4
#5
#6
#7
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
Ni in Frog (PPM)
0
5
10
15
20
25
30
35
Ni in Sediment (PPM)

Fig 10

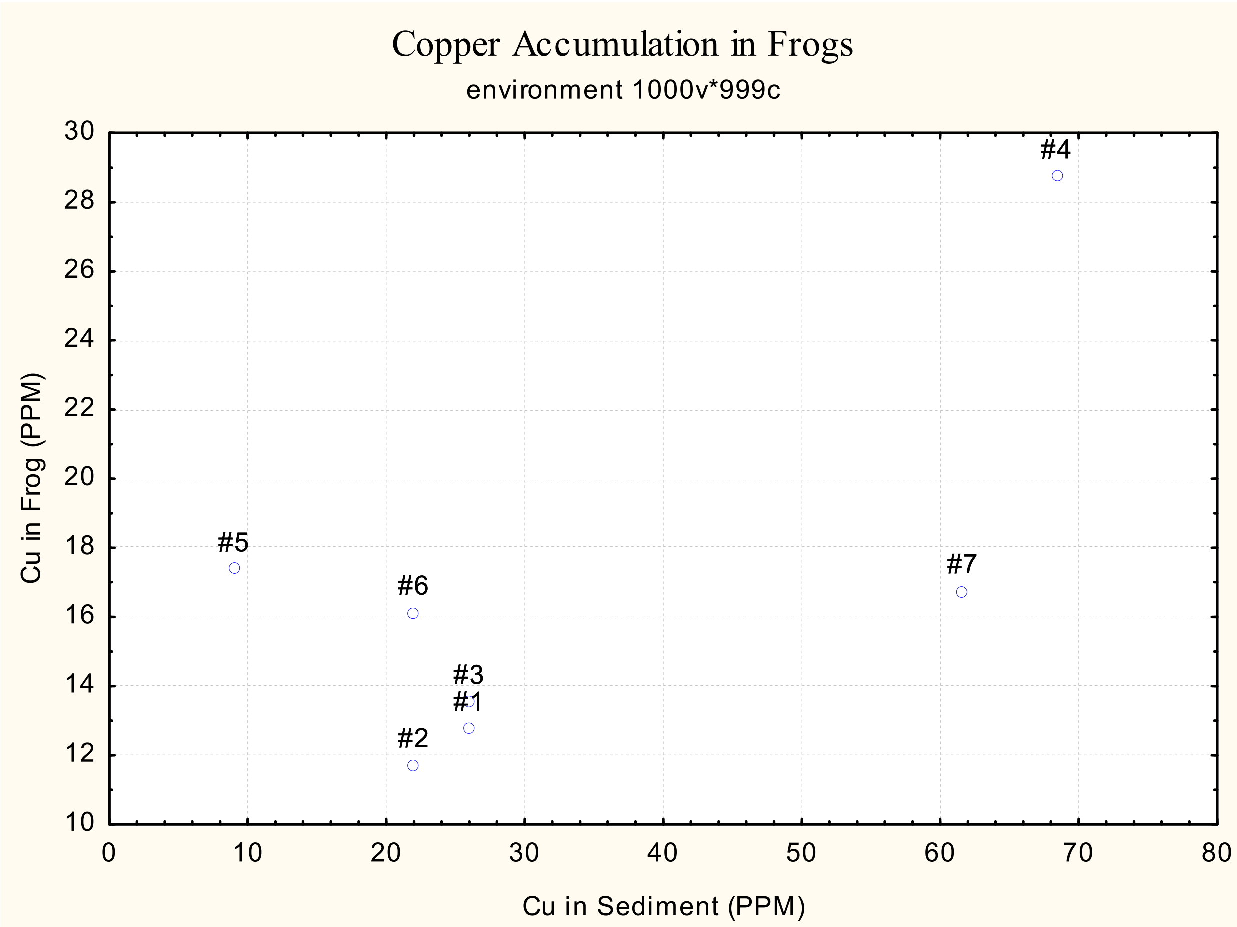

Copper Accumulation in Frogs
environment 1000v*999c
Cu in Frog (PPM)
Cu in Sediment (PPM)
#1
#2
#3
#4
#5
#6
#7
0
10
20
30
40
50
60
70
80
10
12
14
16
18
20
22
24
26
28
30

Fig 11

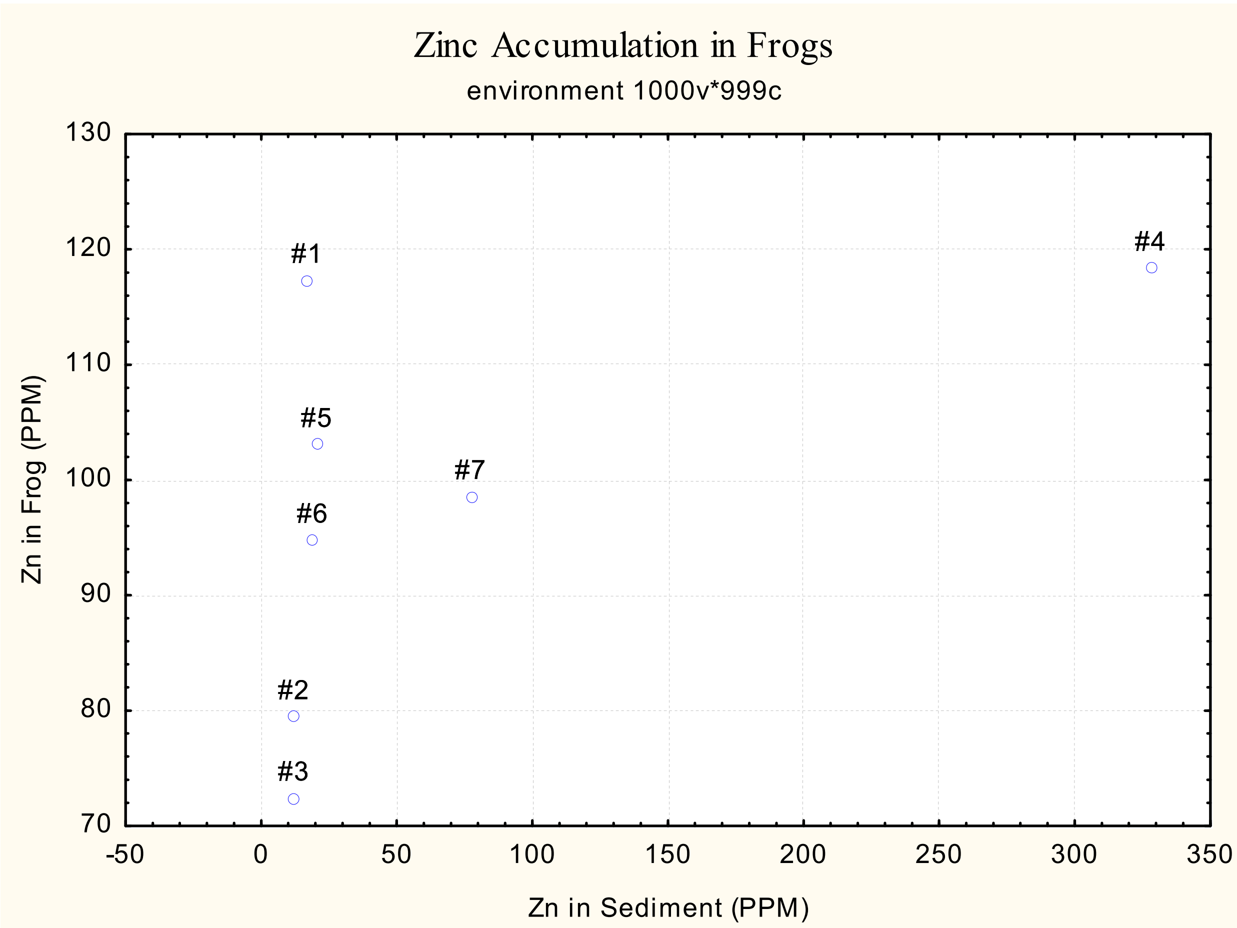
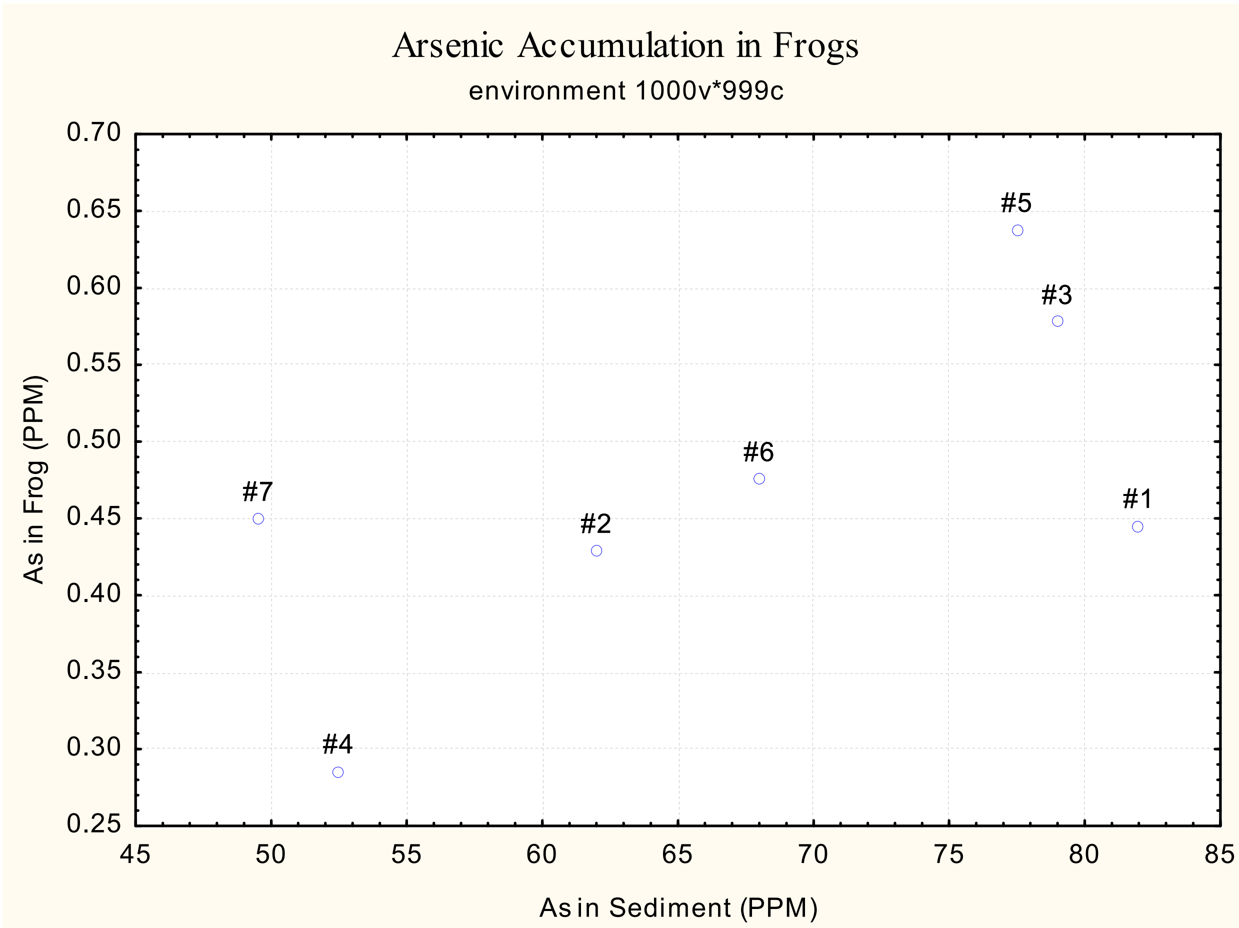
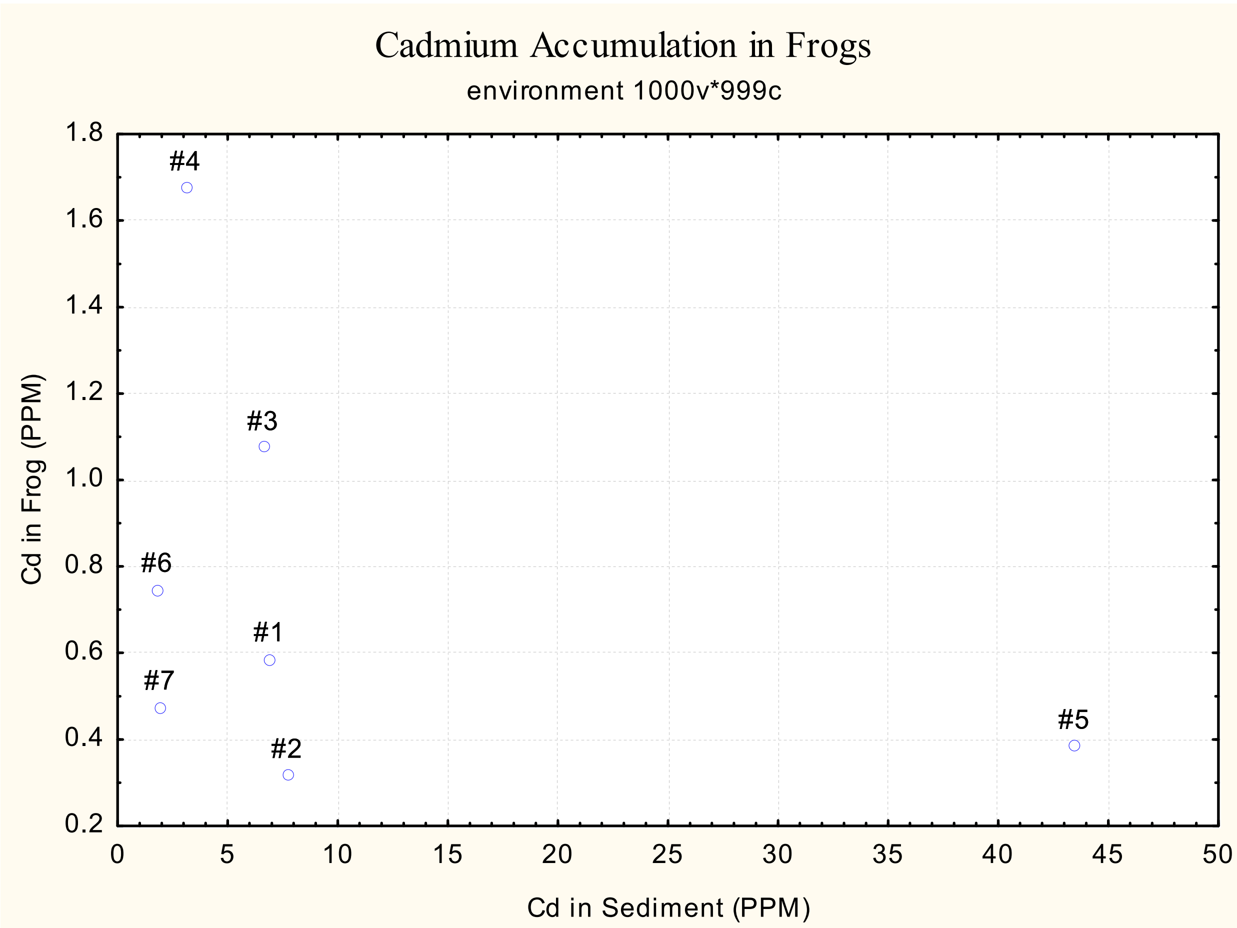

Zinc Accumulation in Frogs
environment 1000v*999c
130
120
110
100
90
80
70
Zn in Frog (PPM)
-50
0
50
100
150
200
250
300
350
Zn in Sediment (PPM)
#1
#2
#3
#4
#5
#6
#7

Fig 12



Arsenic Accumulation in Frogs
environment 1000v*999c
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
As in Frog (PPM)
45
50
55
60
65
70
75
80
85
As in Sediment (PPM)
#5
#3
#6
#7
#2
#1
#4

Fig 13



Cadmium Accumulation in Frogs
environment 1000v*999c
Cd in Frog (PPM)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
40
45
50
Cd in Sediment (PPM)
#4
#3
#6
#1
#7
#2
#5

Fig 14

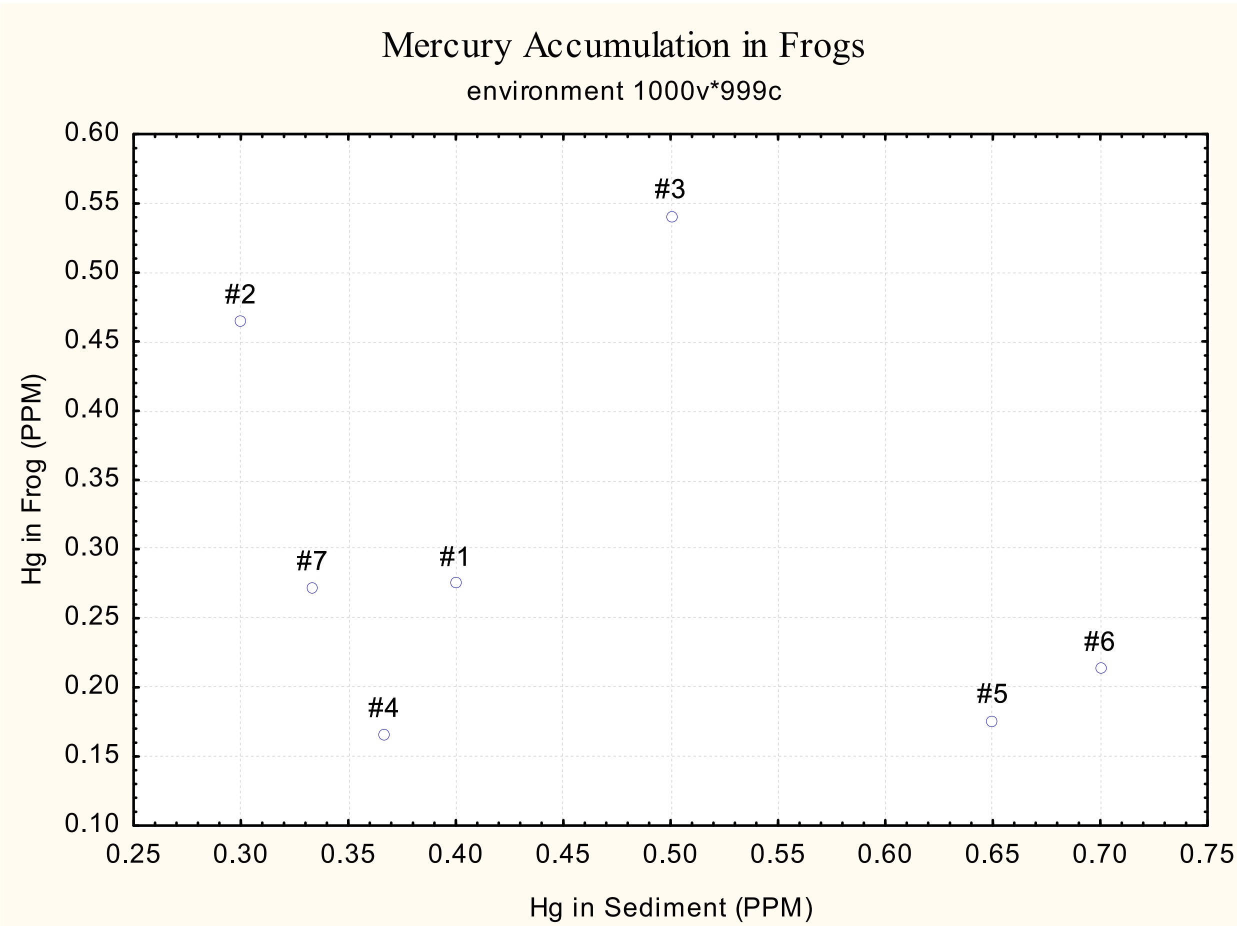

Mercury Accumulation in Frogs
environment 1000v*999c
Hg in Frog (PPM)
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
#3
#2
#7
#1
#6
#5
#4
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
Hg in Sediment (PPM)

Fig 15

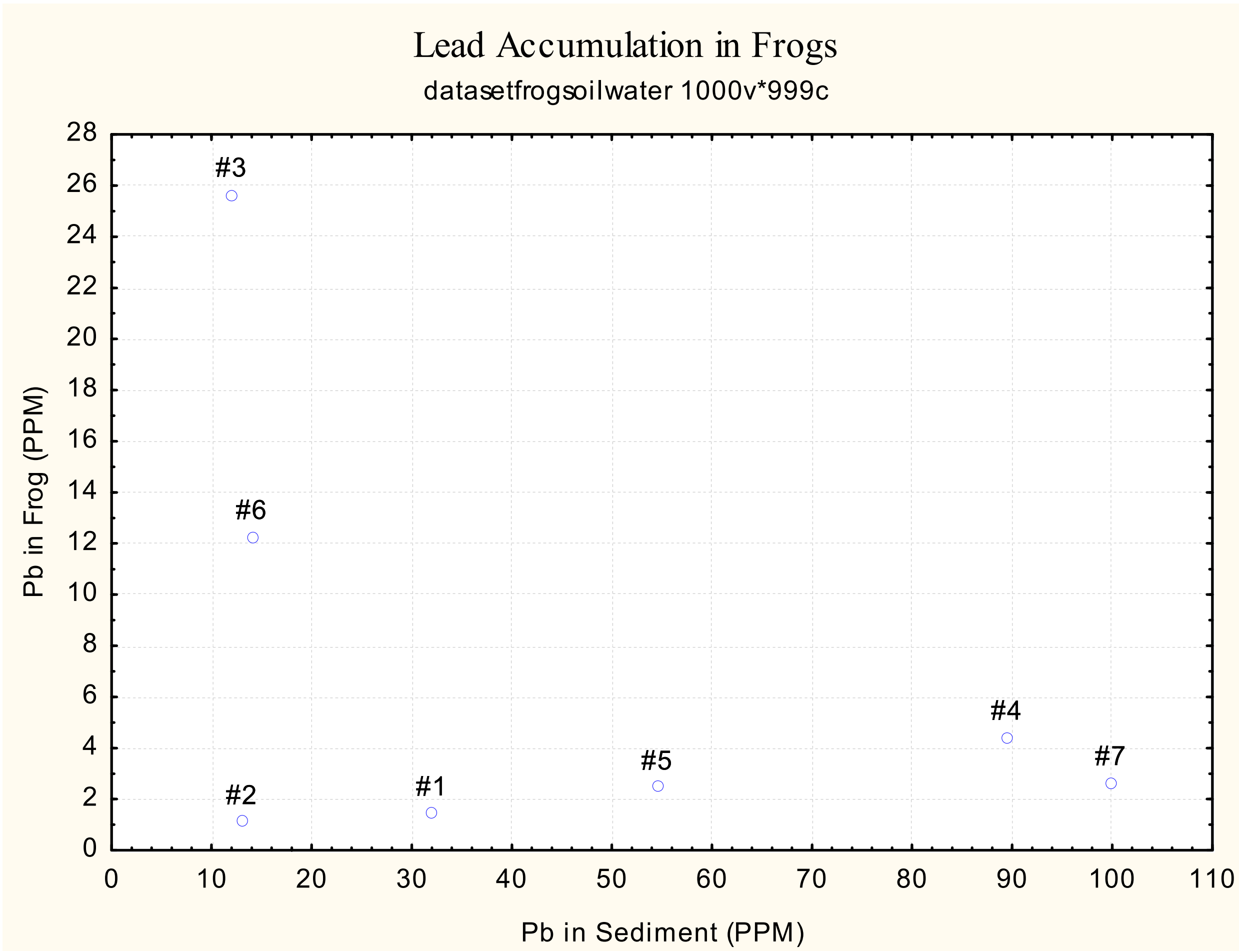
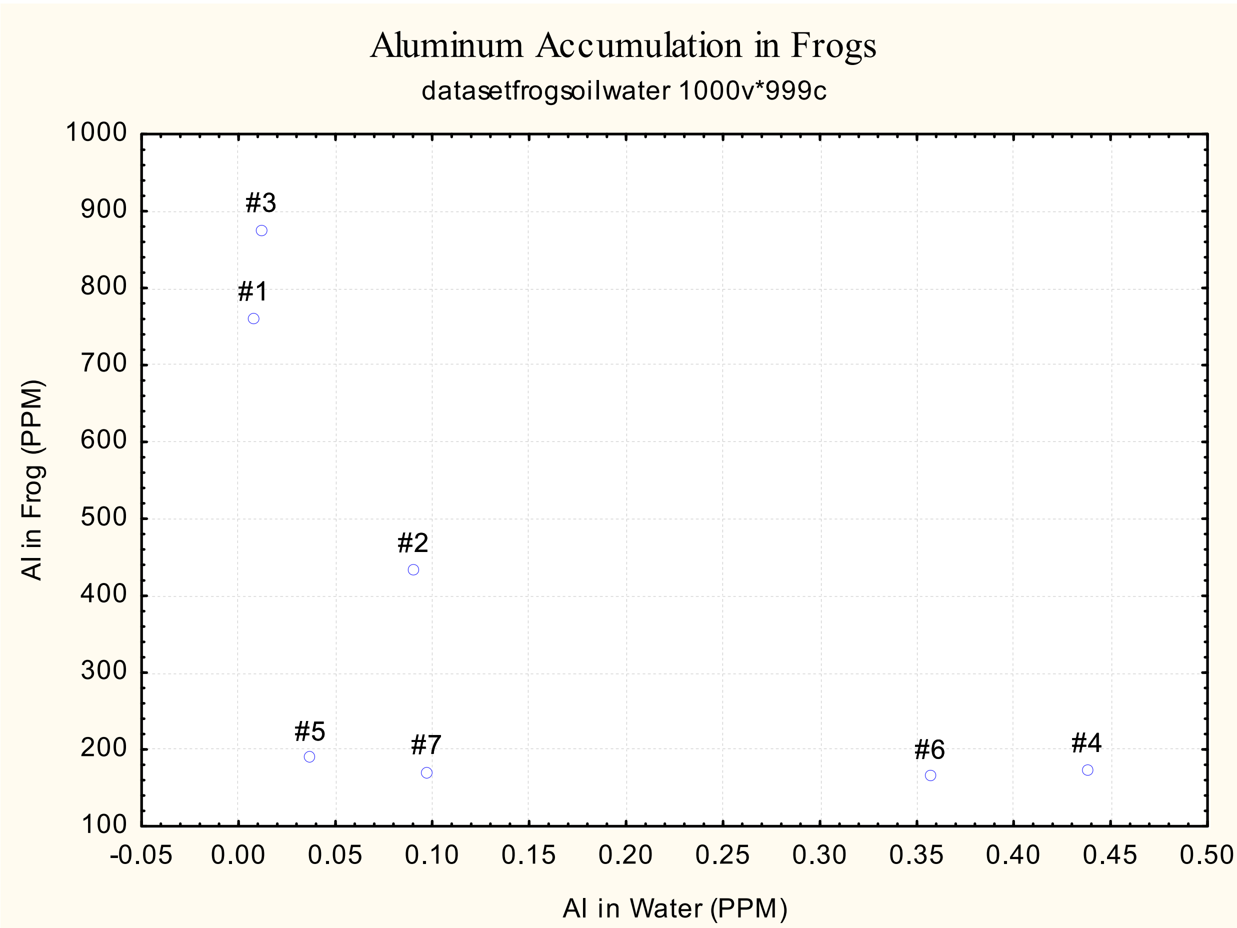

Lead Accumulation in Frogs
datasetfrogsoilwater 1000v*999c
Pb in Frog (PPM)
Pb in Sediment (PPM)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
0
10
20
30
40
50
60
70
80
90
100
110
#1
#2
#3
#4
#5
#6
#7

Fig 16



Aluminum Accumulation in Frogs
datasetfrogsoilwater 1000v*999c
Al in Frog (PPM)
Al in Water (PPM)
1000
900
800
700
600
500
400
300
200
100
-0.05
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
#3
#1
#2
#5
#7
#6
#4

Fig 17

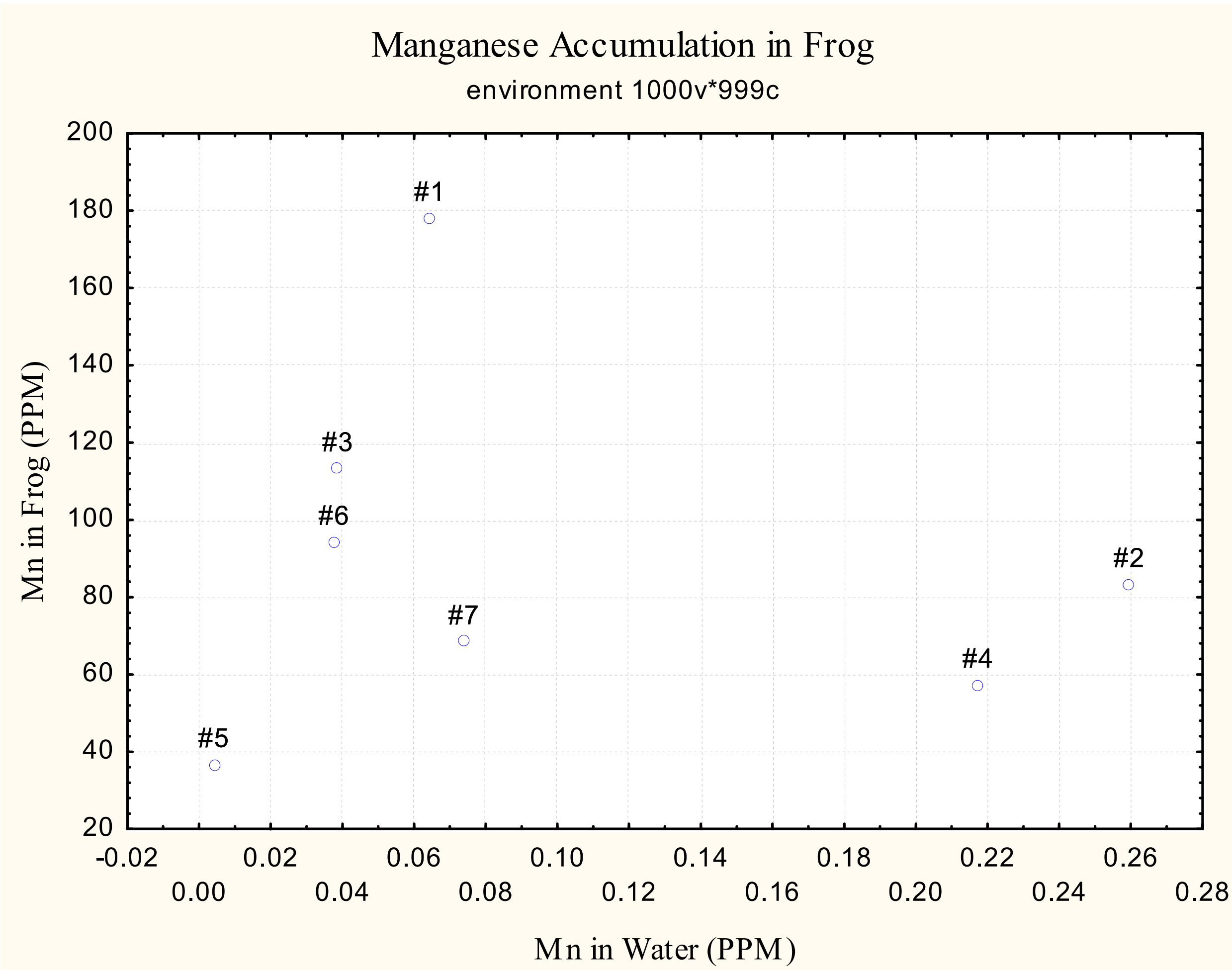
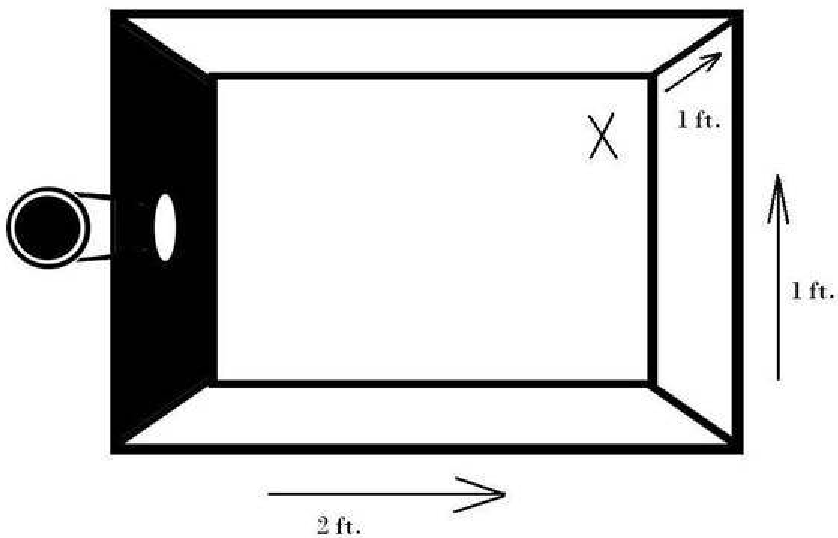

Manganese Accumulation in Frog
environment 1000v*999c
Mn in Frog (PPM)
Mn in Water (PPM)
200
180
160
140
120
100
80
60
40
20
-0.02
0.02
0.06
0.10
0.14
0.18
0.22
0.26
0.00
0.04
0.08
0.12
0.16
0.20
0.24
0.28
#1
#3
#6
#7
#2
#4
#5

Fig 18

A



B

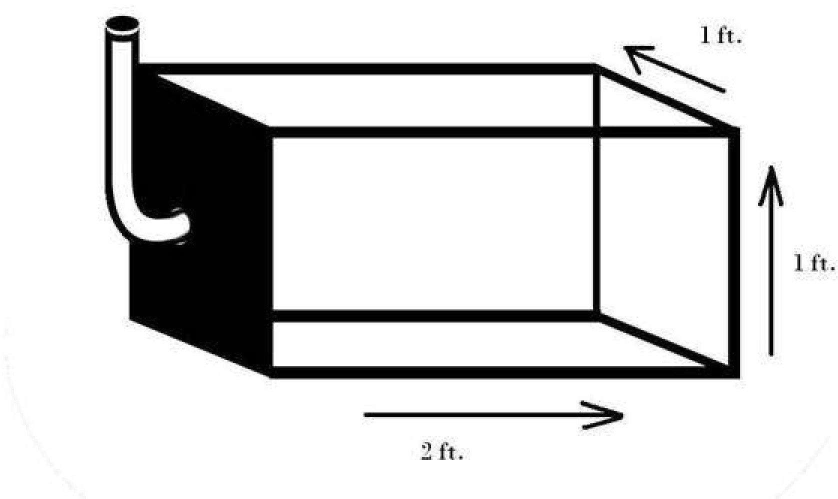
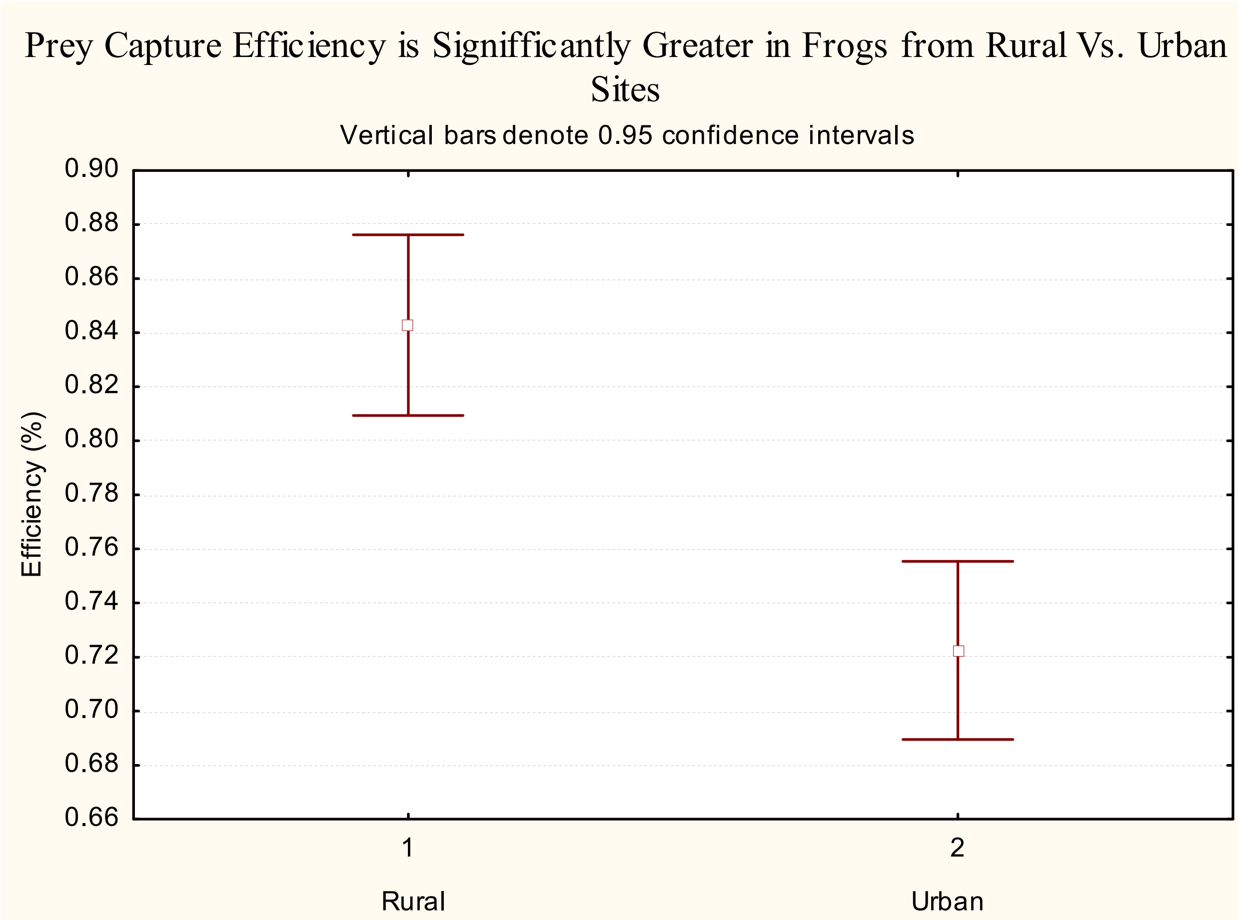


Fig 19. (A) Top view of the amphibian recording chamber. (B) Side view of the amphibian recording chamber.



Prey Capture Efficiency is Signifficantly Greater in Frogs from Rural Vs. Urban Sites
Vertical bars denote 0.95 confidence intervals
Efficiency (%)
0.90
0.88
0.86
0.84
0.82
0.80
0.78
0.76
0.74
0.72
0.70
0.68
0.66
1
2
Rural
Urban

Fig 20

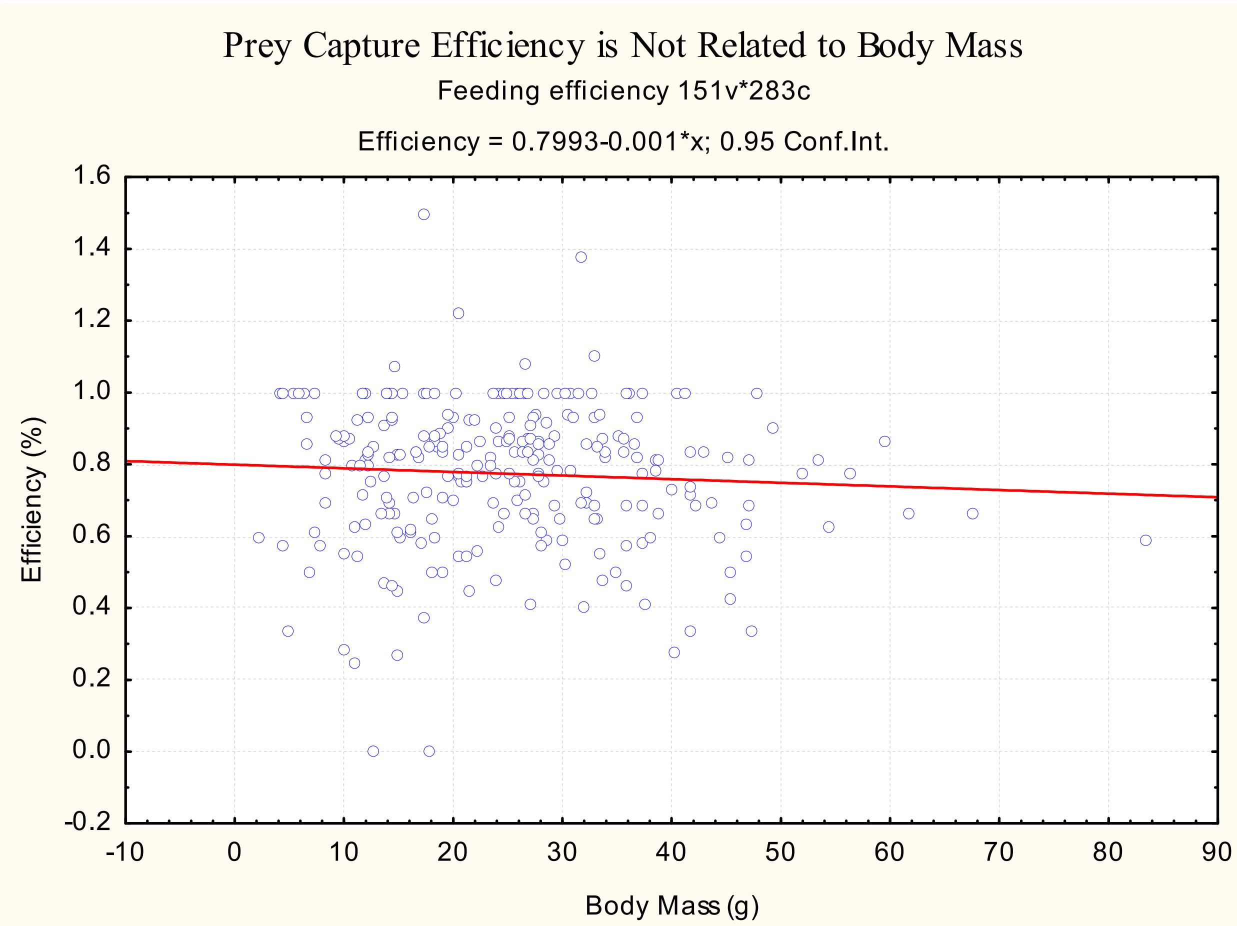
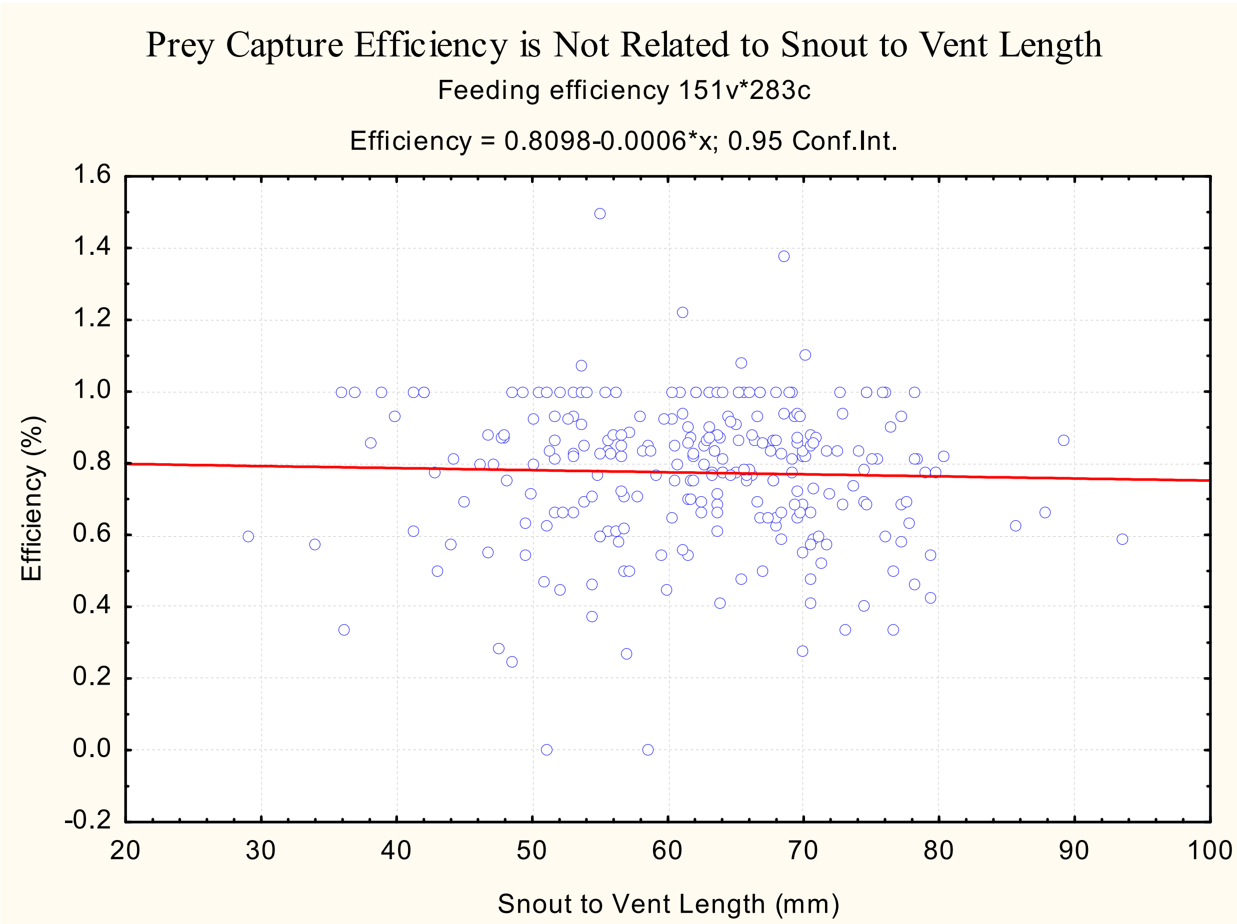

Prey Capture Efficiency is Not Related to Body Mass
Feeding efficiency 151v*283c
Efficiency = 0.7993-0.001*x; 0.95 Conf.Int.
Efficiency (%)
Body Mass (g)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-10
0
10
20
30
40
50
60
70
80
90

Fig 21



Prey Capture Efficiency is Not Related to Snout to Vent Length
Feeding efficiency 151v*283c
Efficiency = 0.8098-0.0006*x; 0.95 Conf.Int.
Efficiency (%)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
20
30
40
50
60
70
80
90
100
Snout to Vent Length (mm)

Fig 22

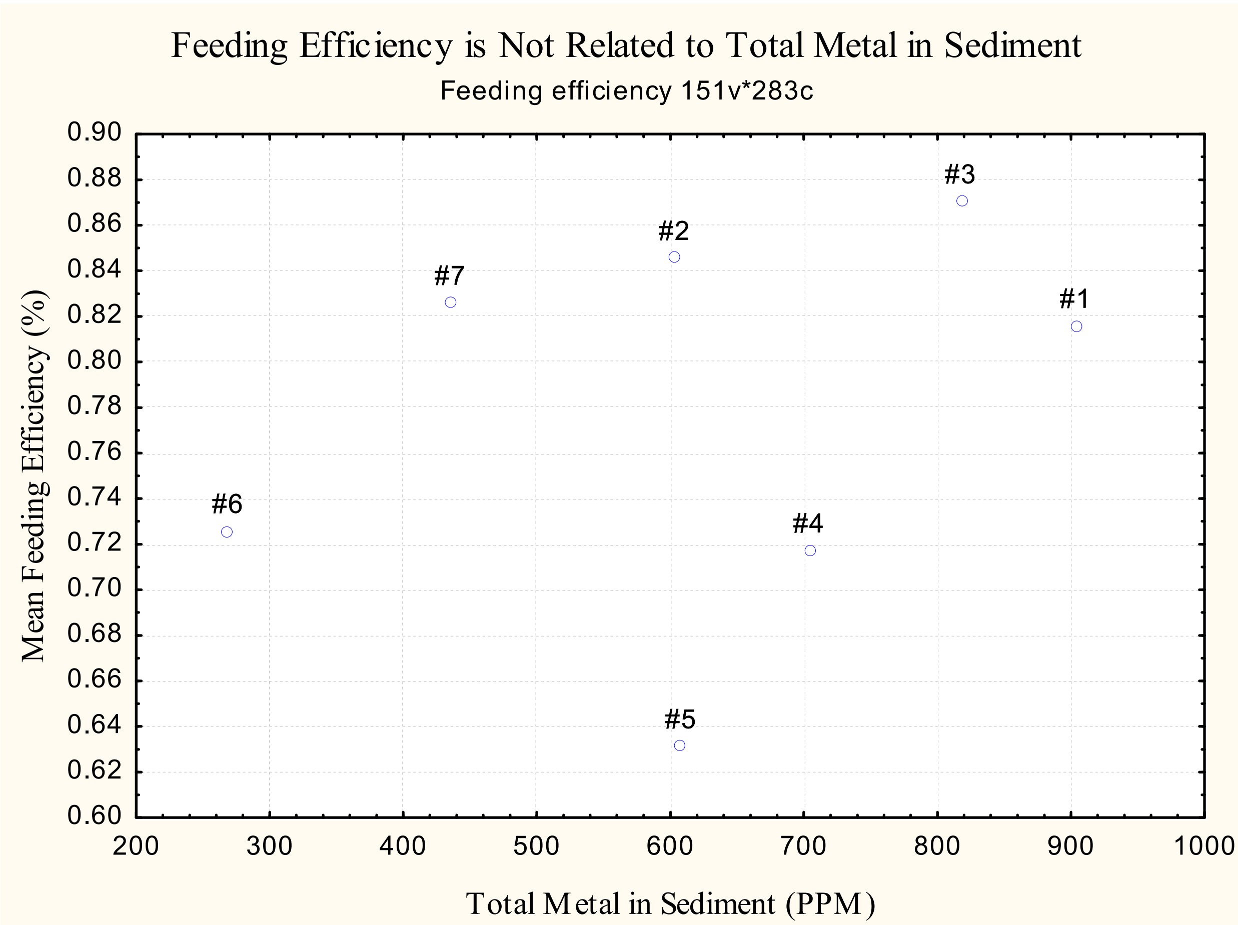

Feeding Efficiency is Not Related to Total Metal in Sediment
Feeding efficiency 151v*283c
Mean Feeding Efficiency (%)
0.90
0.88
0.86
0.84
0.82
0.80
0.78
0.76
0.74
0.72
0.70
0.68
0.66
0.64
0.62
0.60
200
300
400
500
600
700
800
900
1000
Total Metal in Sediment (PPM)
#1
#2
#3
#4
#5
#6
#7

Fig 23

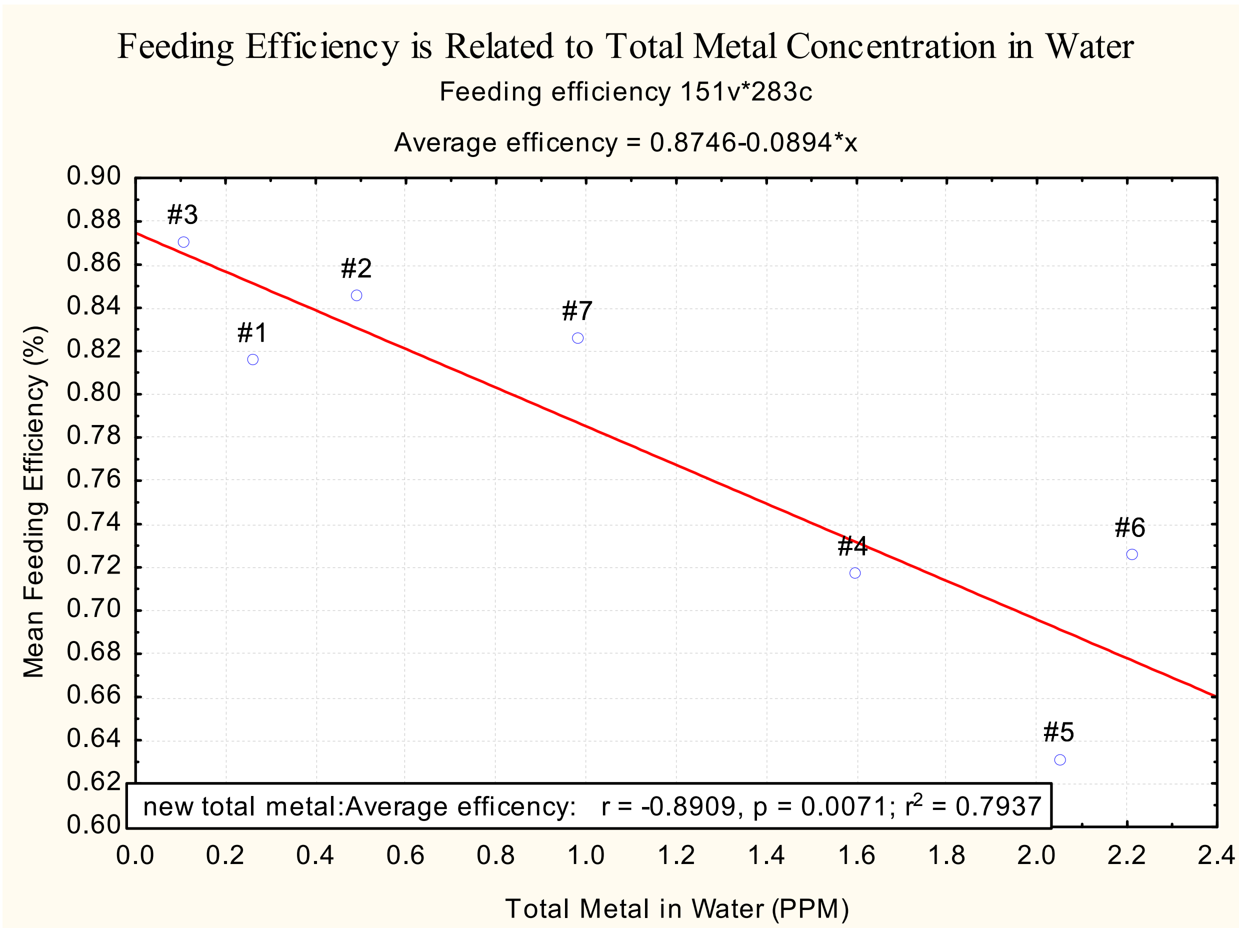

Feeding Efficiency is Related to Total Metal Concentration in Water
Feeding efficiency 151v*283c
Average efficency = 0.8746-0.0894*x
Mean Feeding Efficiency (%)
Total Metal in Water (PPM)
#1
#2
#3
#4
#5
#6
#7
new total metal:Average efficency: r = -0.8909, p = 0.0071; r2 = 0.7937
0.90
0.88
0.86
0.84
0.82
0.80
0.78
0.76
0.74
0.72
0.70
0.68
0.66
0.64
0.62
0.60
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4

Fig 24

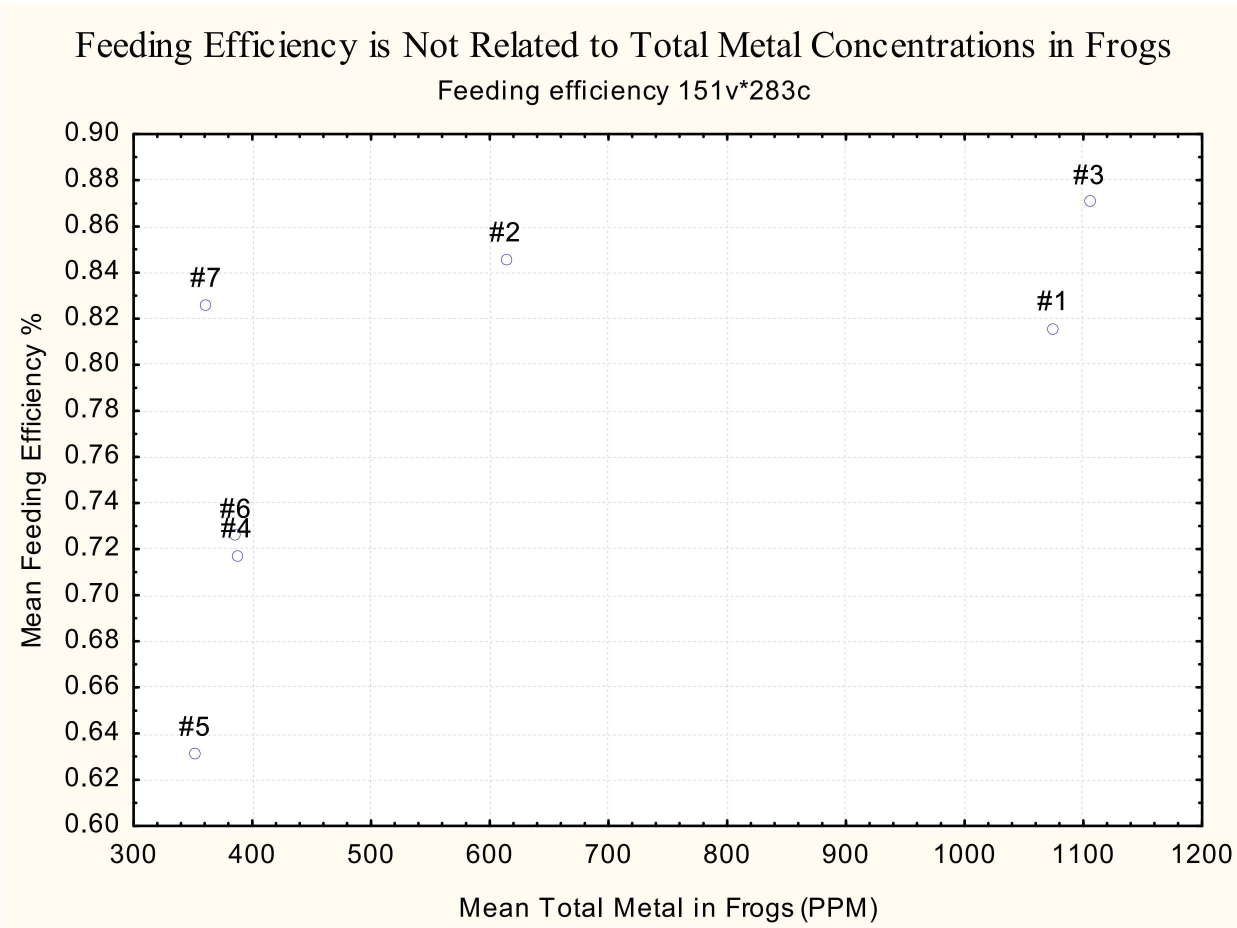

Feeding Efficiency is Not Related to Total Metal Concentrations in Frogs
Feeding efficiency 151v*283c
Mean Feeding Efficiency %
Mean Total Metal in Frogs (PPM)
#1
#2
#3
#4
#5
#6
#7
0.60
0.62
0.64
0.66
0.68
0.70
0.72
0.74
0.76
0.78
0.80
0.82
0.84
0.86
0.88
0.90
300
400
500
600
700
800
900
1000
1100
1200

Fig 25

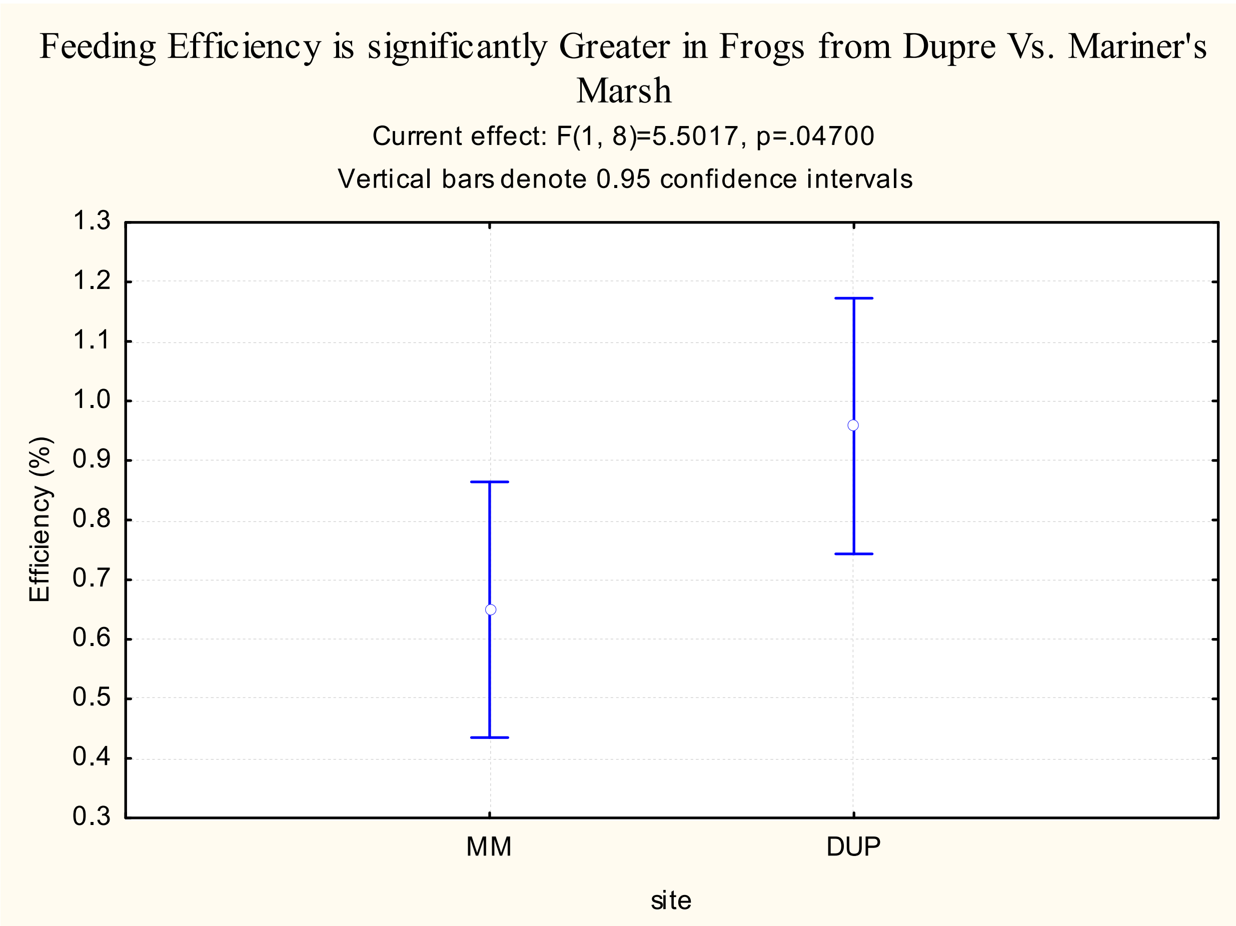

Feeding Efficiency is significantly Greater in Frogs from Dupre Vs. Mariner's Marsh
Current effect: F(1, 8)=5.5017, p=.04700
Vertical bars denote 0.95 confidence intervals
Efficiency (%)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
MM
DUP
site

Fig 26

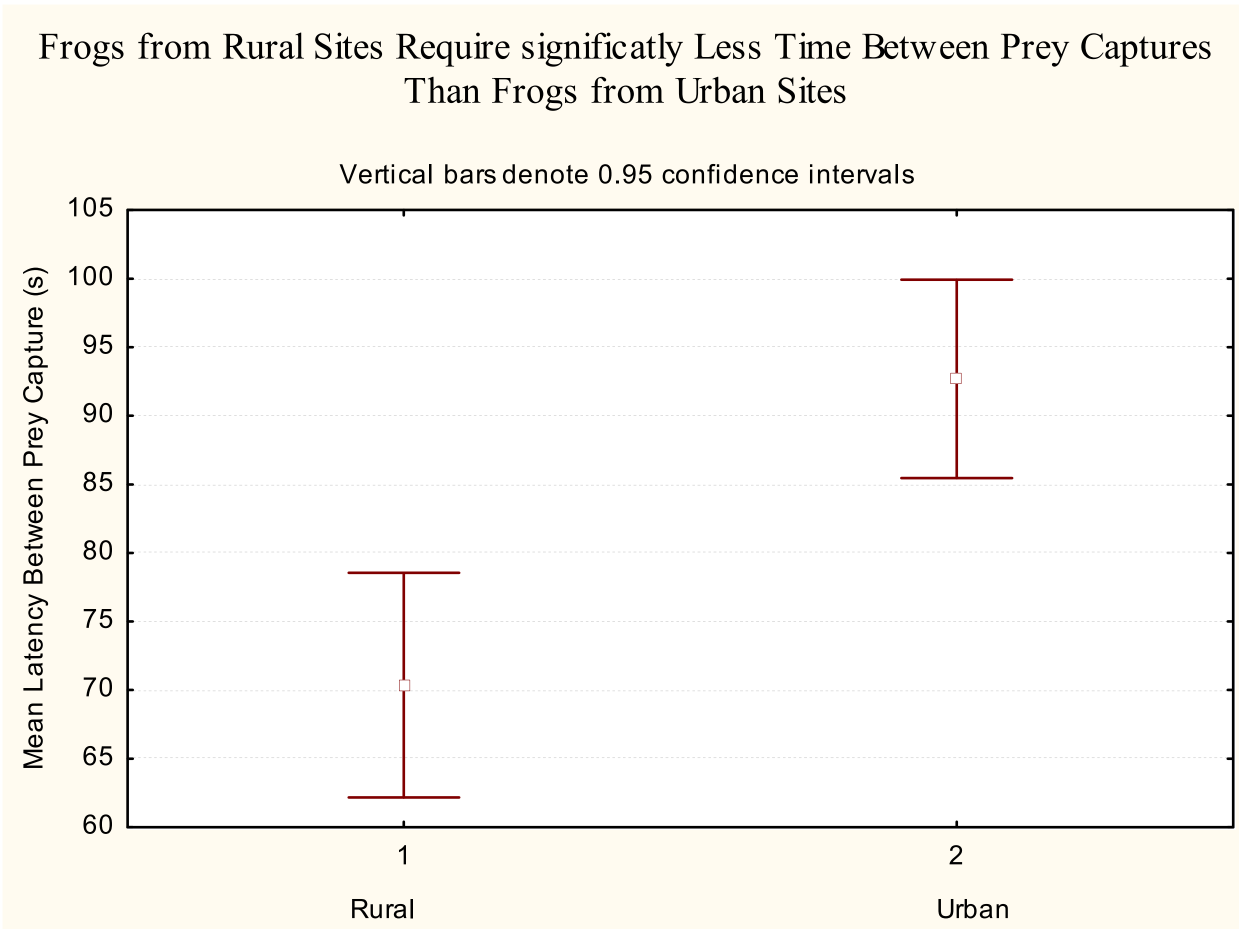

Frogs from Rural Sites Require significatly Less Time Between Prey Captures Than Frogs from Urban Sites
Vertical bars denote 0.95 confidence intervals
Mean Latency Between Prey Capture (s)
105
100
95
90
85
80
75
70
65
60
1
2
Rural
Urban

Fig 27

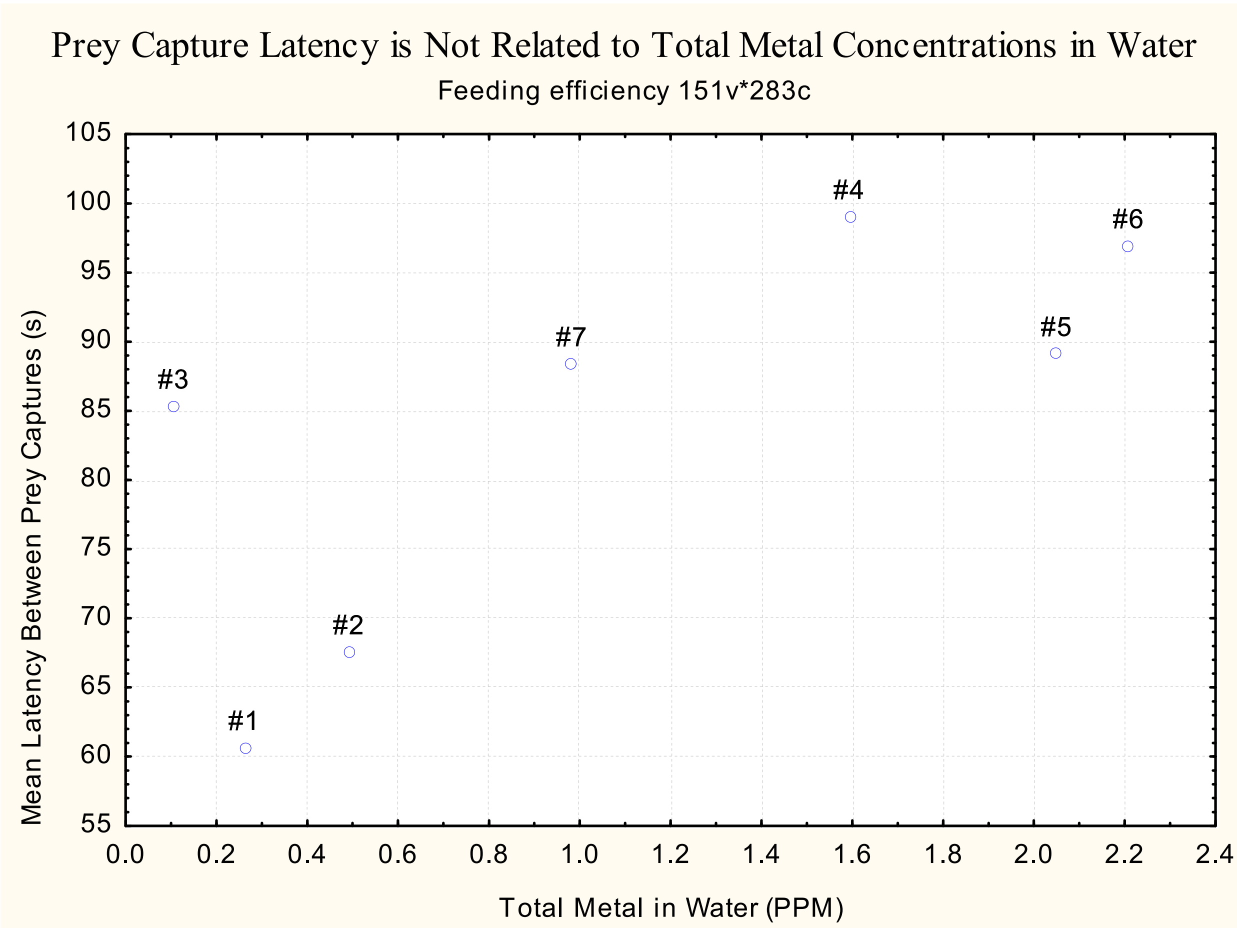

Prey Capture Latency is Not Related to Total Metal Concentrations in Water
Feeding efficiency 151v*283c
Mean Latency Between Prey Captures (s)
Total Metal in Water (PPM)
105
100
95
90
85
80
75
70
65
60
55
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
#1
#2
#3
#4
#5
#6
#7

Fig 28

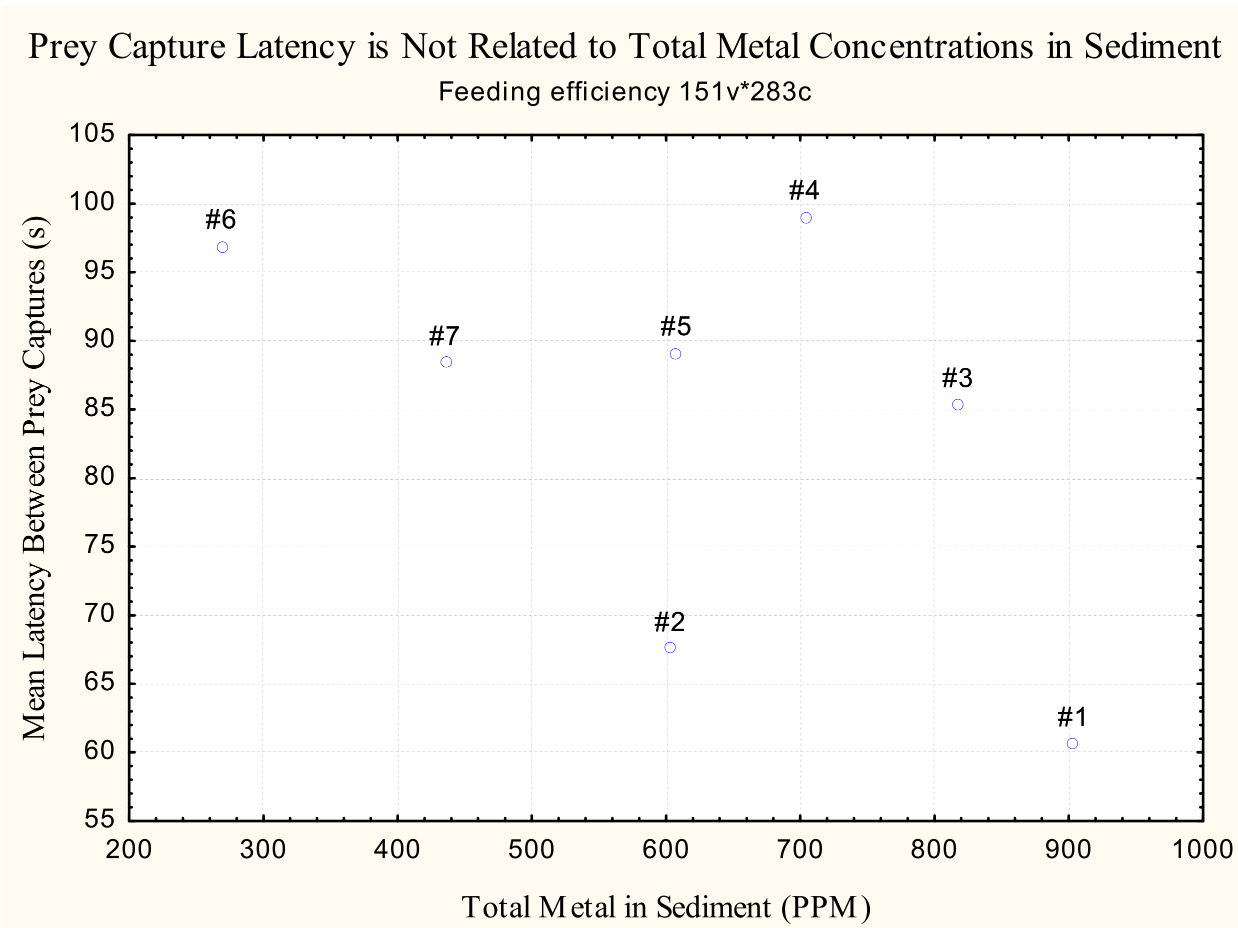

Prey Capture Latency is Not Related to Total Metal Concentrations in Sediment
Feeding efficiency 151v*283c
Mean Latency Between Prey Captures (s)
Total Metal in Sediment (PPM)
105
100
95
90
85
80
75
70
65
60
55
200
300
400
500
600
700
800
900
1000
#1
#2
#3
#4
#5
#6
#7

Fig 29

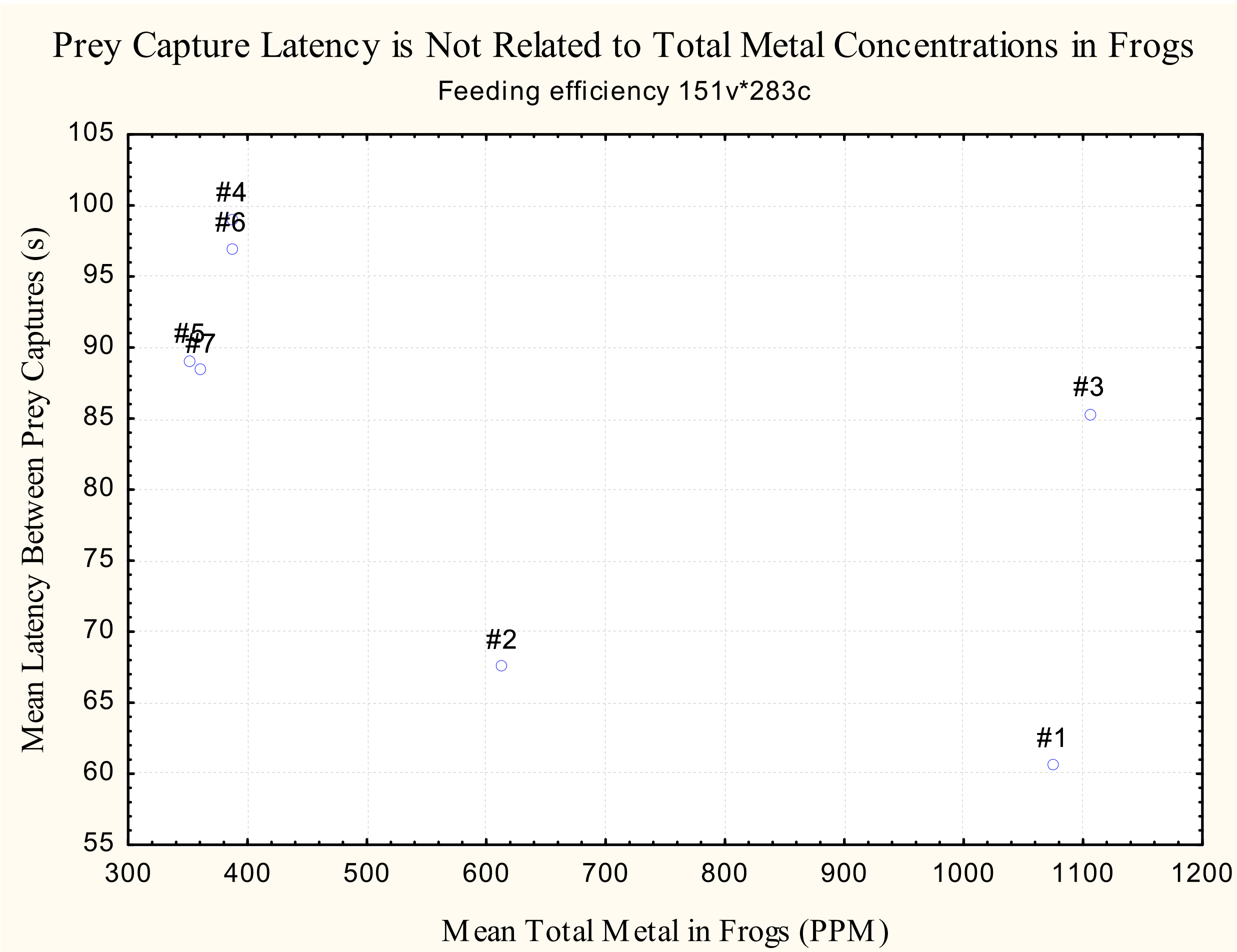

Prey Capture Latency is Not Related to Total Metal Concentrations in Frogs
Feeding efficiency 151v*283c
Mean Latency Between Prey Captures (s)
Mean Total Metal in Frogs (PPM)
105
100
95
90
85
80
75
70
65
60
55
300
400
500
600
700
800
900
1000
1100
1200
#1
#2
#3
#4
#5
#6
#7

Fig 30

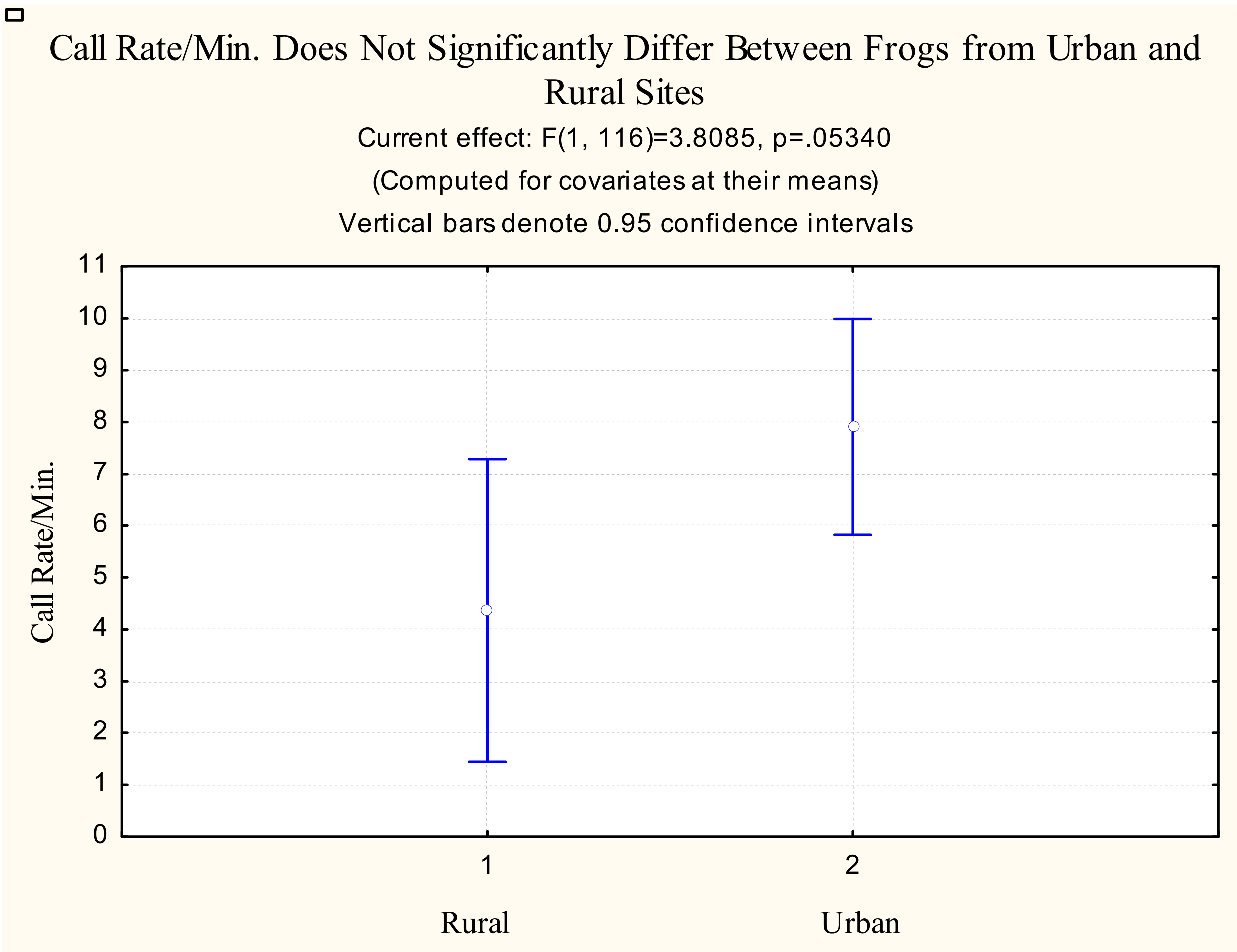

Call Rate/Min. Does Not Significantly Differ Between Frogs from Urban and Rural Sites
Current effect: F(1, 116)=3.8085, p=.05340
(Computed for covariates at their means)
Vertical bars denote 0.95 confidence intervals
Call Rate/Min.
0
1
2
3
4
5
6
7
8
9
10
11
1
2
Rural
Urban

Fig 31

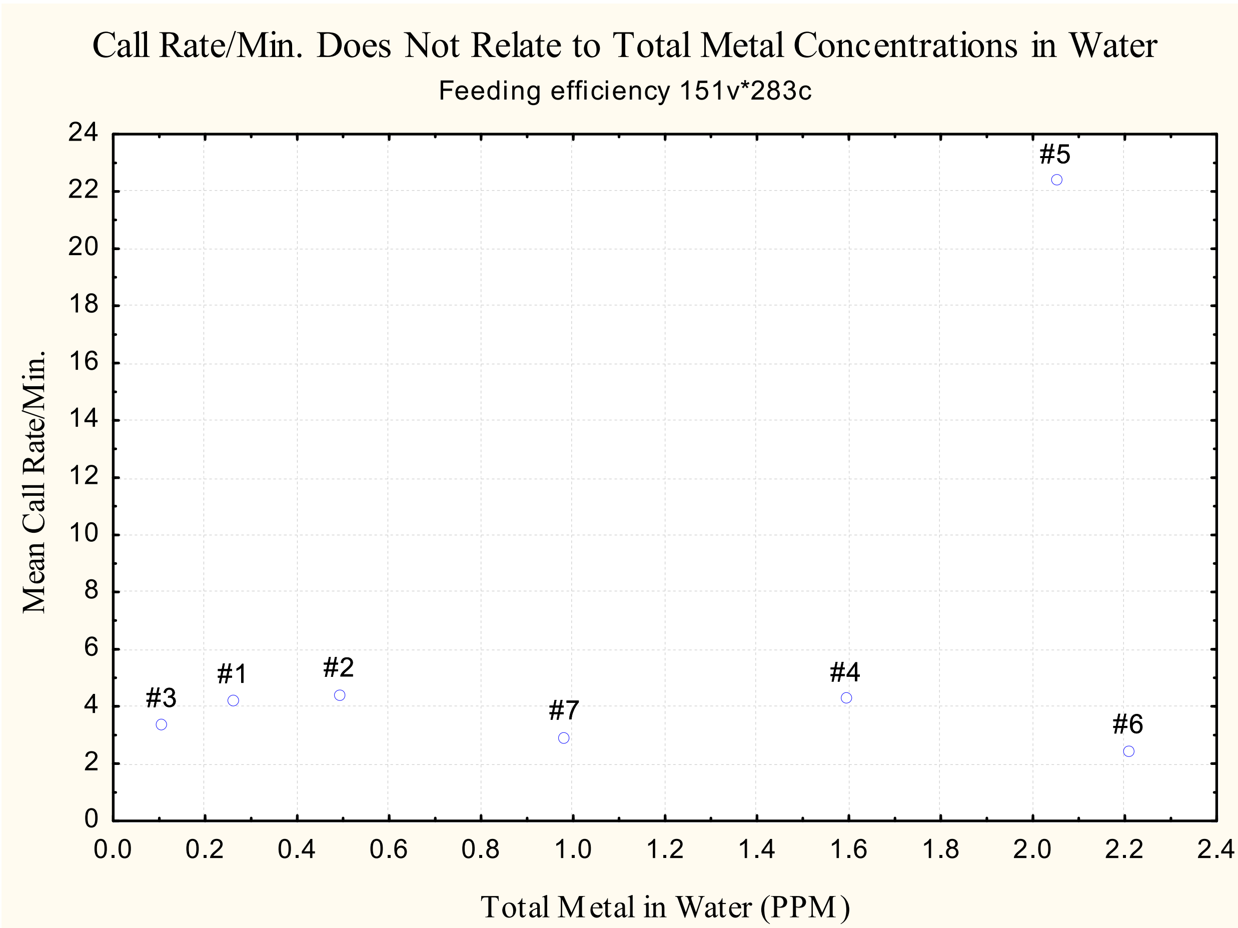

Call Rate/Min. Does Not Relate to Total Metal Concentrations in Water
Feeding efficiency 151v*283c
Mean Call Rate/Min.
Total Metal in Water (PPM)
#1
#2
#3
#4
#5
#6
#7
0
2
4
6
8
10
12
14
16
18
20
22
24
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4

Fig 32

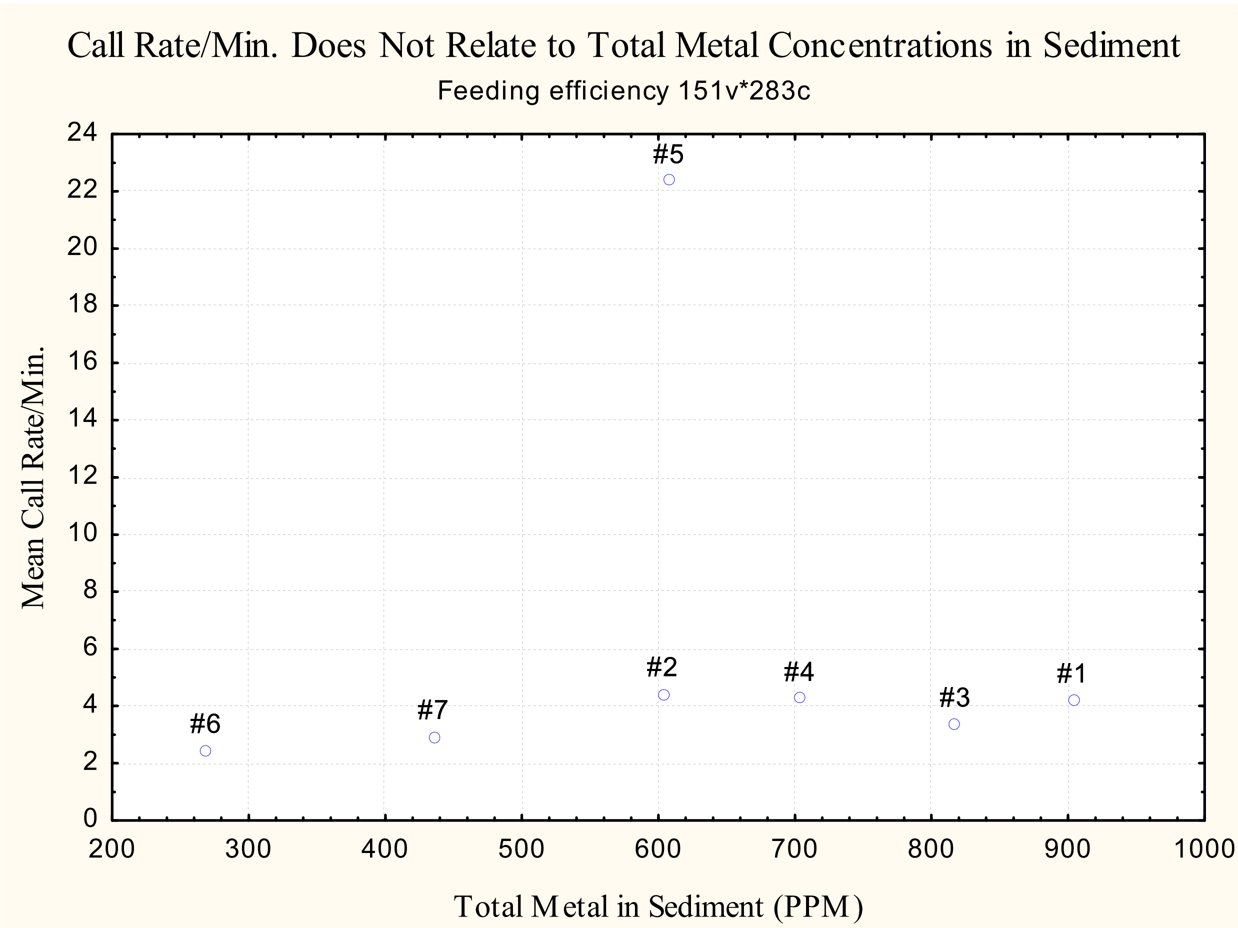

Call Rate/Min. Does Not Relate to Total Metal Concentrations in Sediment
Feeding efficiency 151v*283c
Mean Call Rate/Min.
Total Metal in Sediment (PPM)
#1
#2
#3
#4
#5
#6
#7
0
2
4
6
8
10
12
14
16
18
20
22
24
200
300
400
500
600
700
800
900
1000

Fig 33

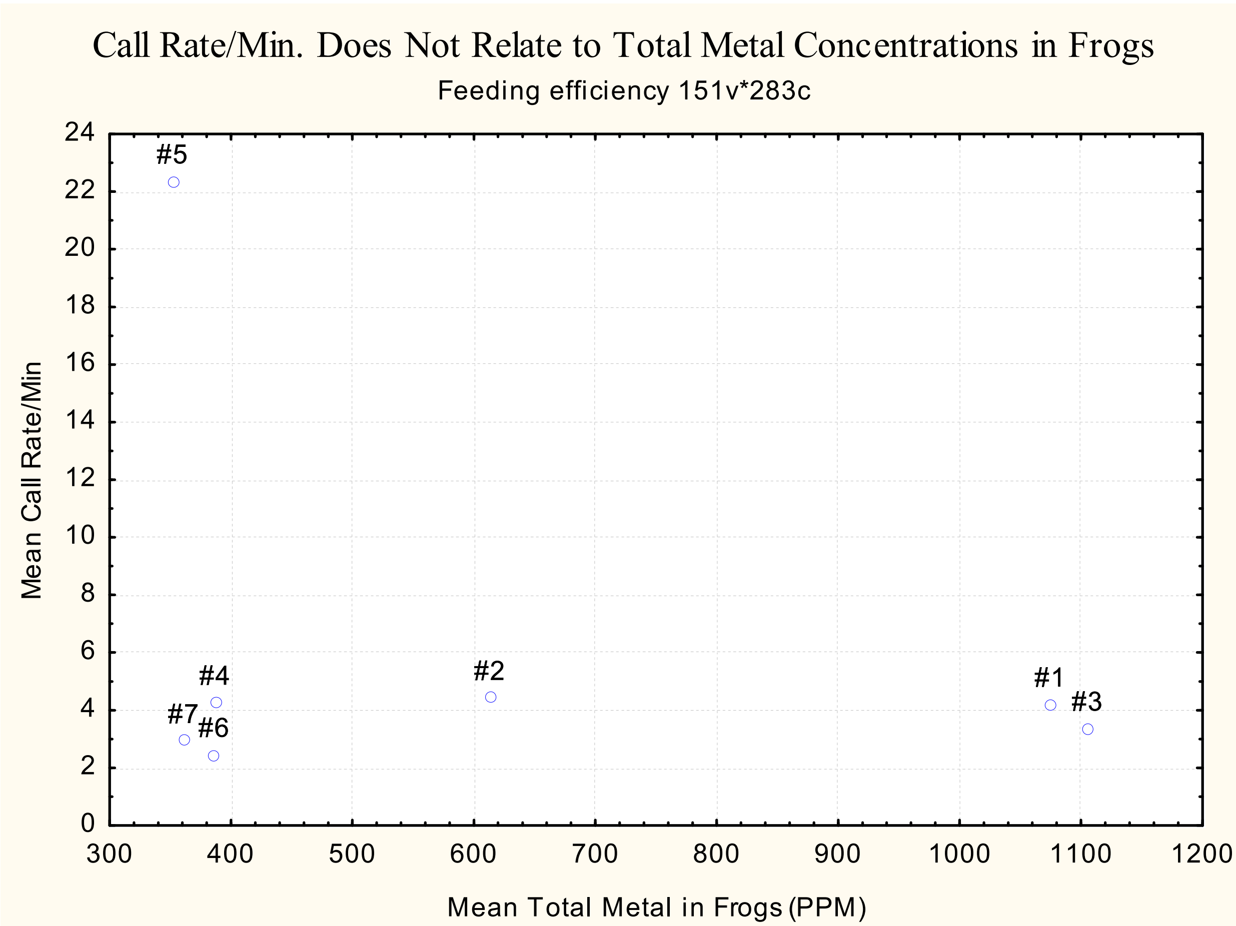

Call Rate/Min. Does Not Relate to Total Metal Concentrations in Frogs
Feeding efficiency 151v*283c
Mean Call Rate/Min
Mean Total Metal in Frogs (PPM)
0
2
4
6
8
10
12
14
16
18
20
22
24
300
400
500
600
700
800
900
1000
1100
1200
#1
#2
#3
#4
#5
#6
#7

Fig 34

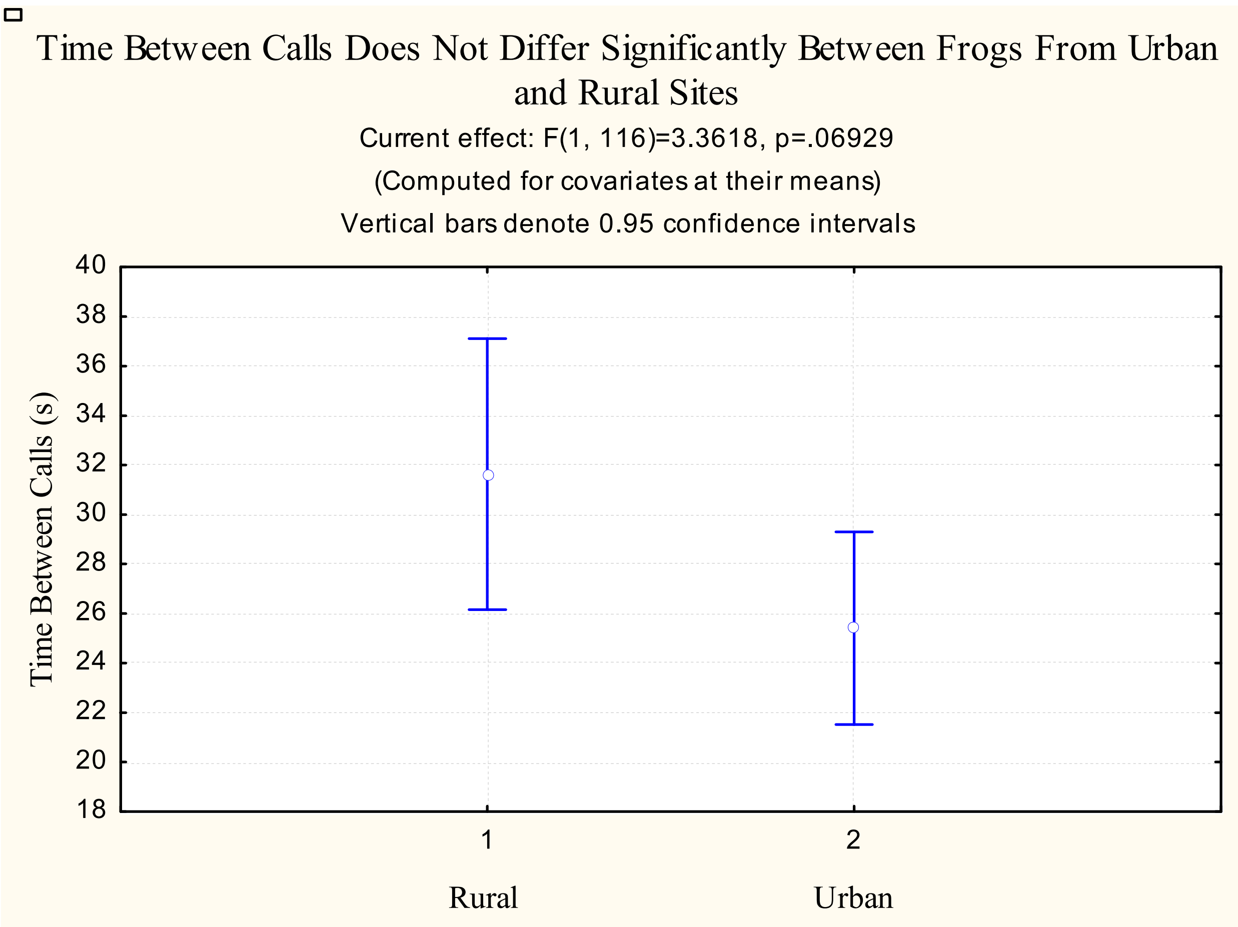

Time Between Calls Does Not Differ Significantly Between Frogs From Urban and Rural Sites
Current effect: F(1, 116)=3.3618, p=.06929
(Computed for covariates at their means)
Vertical bars denote 0.95 confidence intervals
Time Between Calls (s)
40
38
36
34
32
30
28
26
24
22
20
18
1
2
Rural
Urban

Fig 35

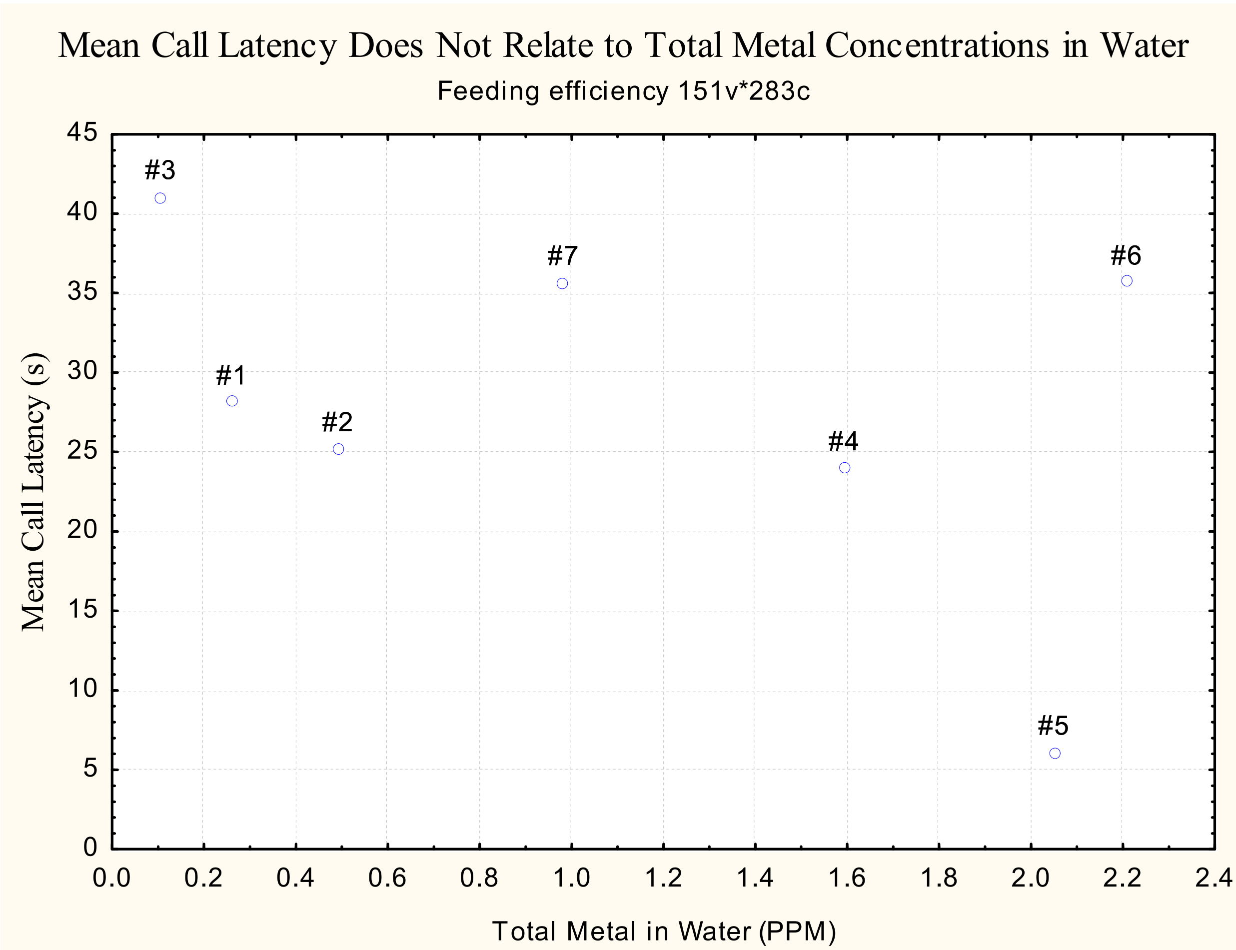
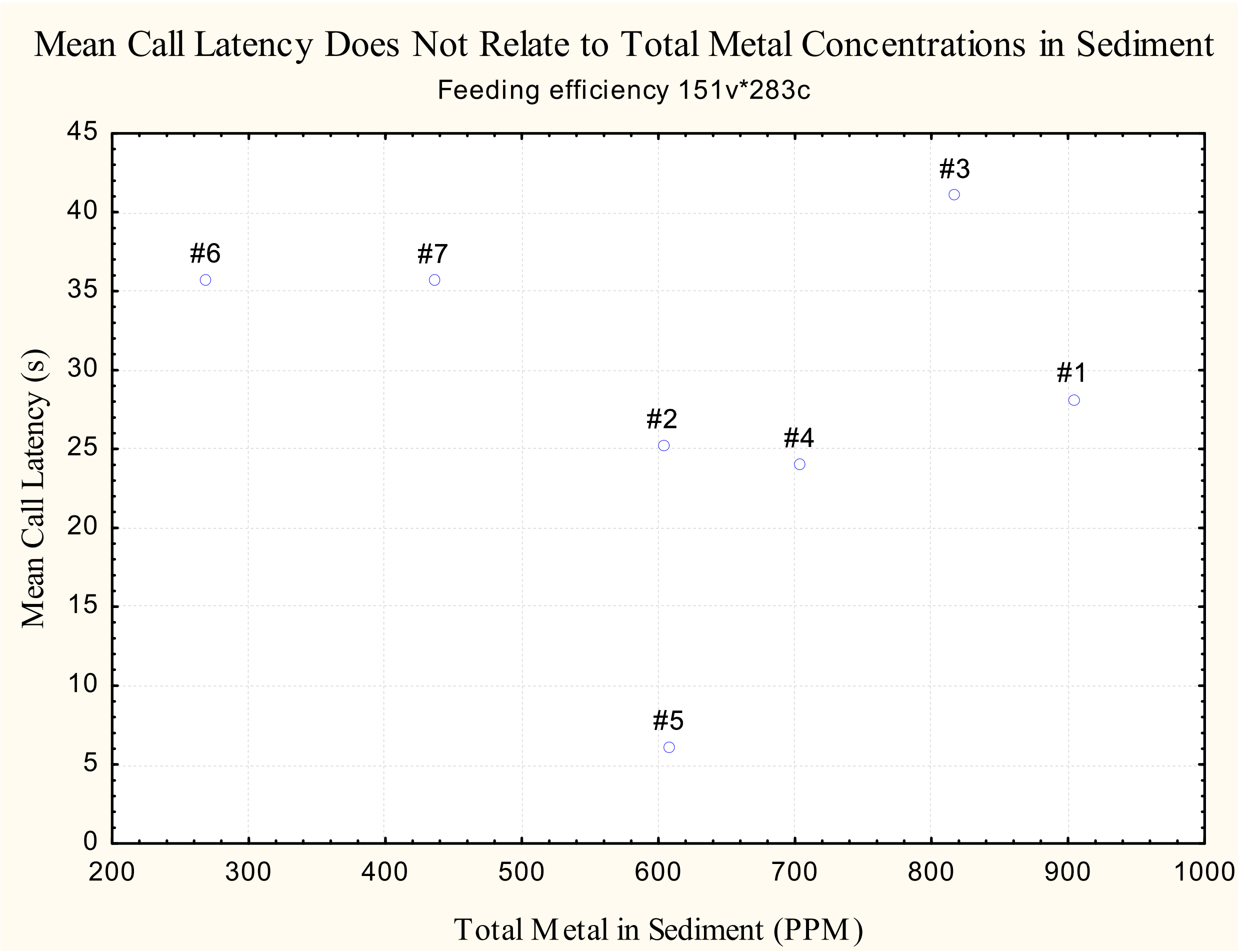

Mean Call Latency Does Not Relate to Total Metal Concentrations in Water
Feeding efficiency 151v*283c
Mean Call Latency (s)
Total Metal in Water (PPM)
#1
#2
#3
#4
#5
#6
#7
0
5
10
15
20
25
30
35
40
45
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4

Fig 36



Mean Call Latency Does Not Relate to Total Metal Concentrations in Sediment
Feeding efficiency 151v*283c
Mean Call Latency (s)
Total Metal in Sediment (PPM)
0
5
10
15
20
25
30
35
40
45
200
300
400
500
600
700
800
900
1000
#1
#2
#3
#4
#5
#6
#7

Fig 37

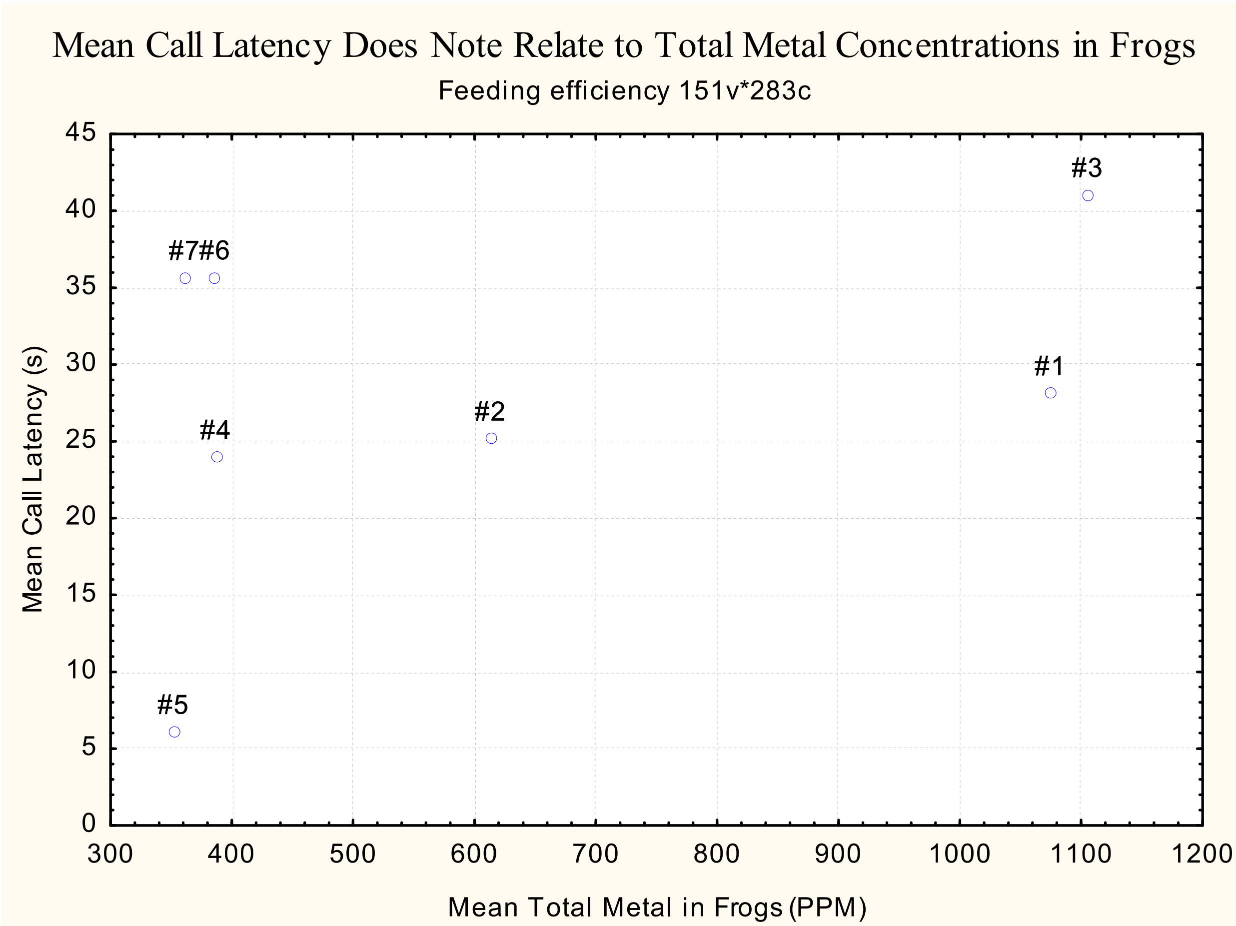

Mean Call Latency Does Note Relate to Total Metal Concentrations in Frogs
Feeding efficiency 151v*283c
Mean Call Latency (s)
Mean Total Metal in Frogs (PPM)
#1
#2
#3
#4
#5
#7#6
0
5
10
15
20
25
30
35
40
45
300
400
500
600
700
800
900
1000
1100
1200

Fig 38

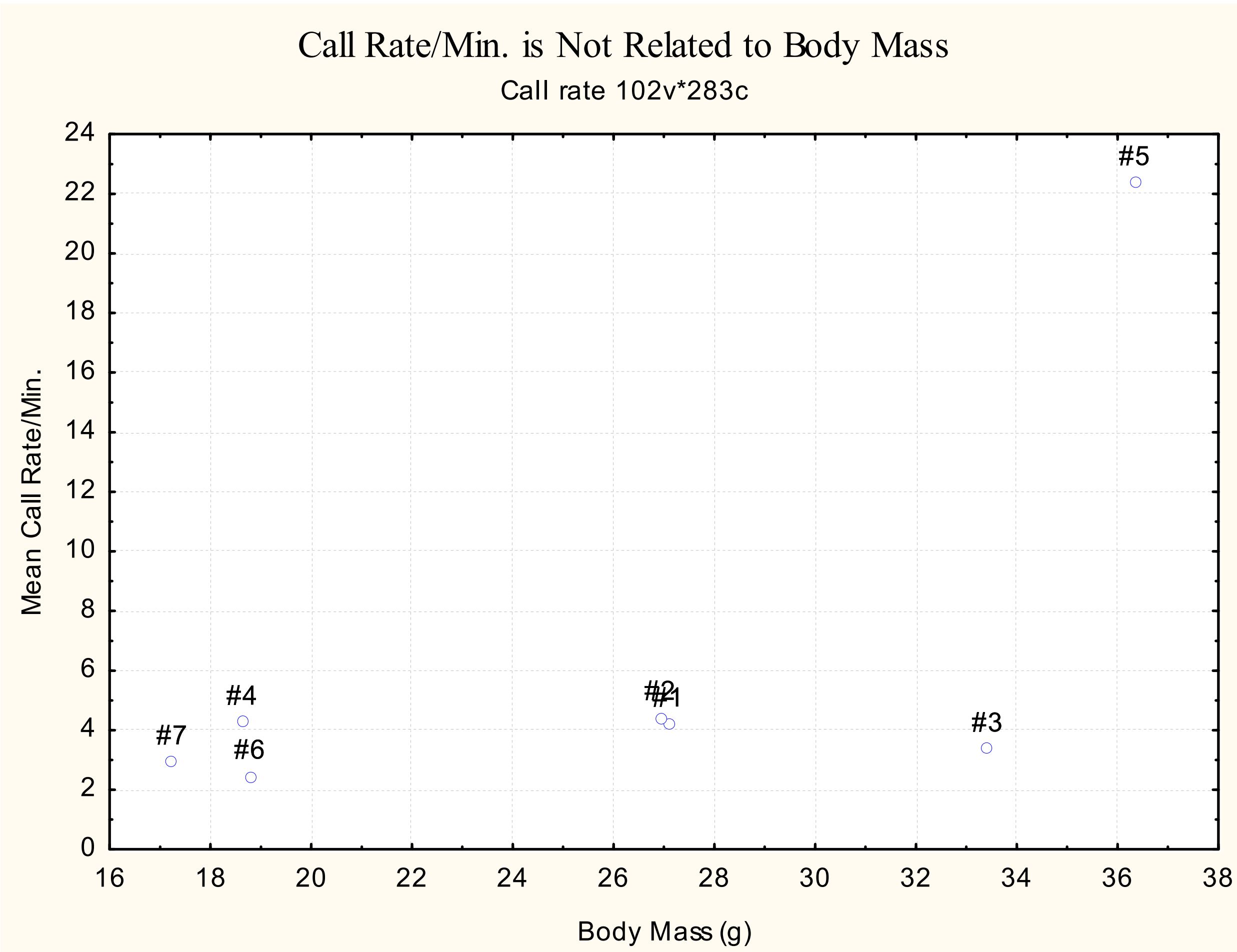

Call Rate/Min. is Not Related to Body Mass
Call rate 102v*283c
Mean Call Rate/Min.
Body Mass (g)
#1
#2
#3
#4
#5
#6
#7
0
2
4
6
8
10
12
14
16
18
20
22
24
16
18
20
22
24
26
28
30
32
34
36
38

Fig 39

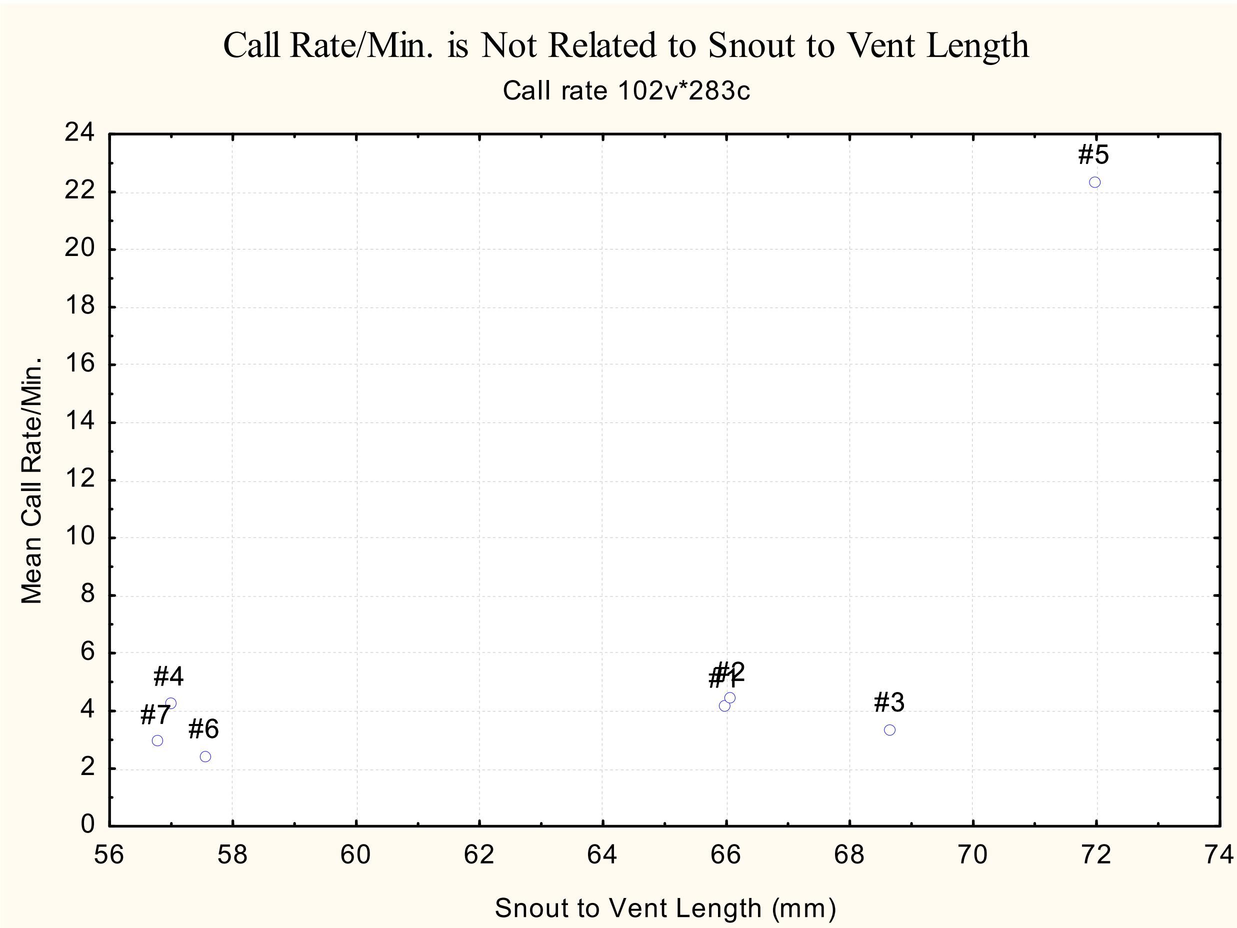

Call Rate/Min. is Not Related to Snout to Vent Length
Call rate 102v*283c
Mean Call Rate/Min.
Snout to Vent Length (mm)
0
2
4
6
8
10
12
14
16
18
20
22
24
56
58
60
62
64
66
68
70
72
74
#1
#2
#3
#4
#5
#6
#7

Fig 40

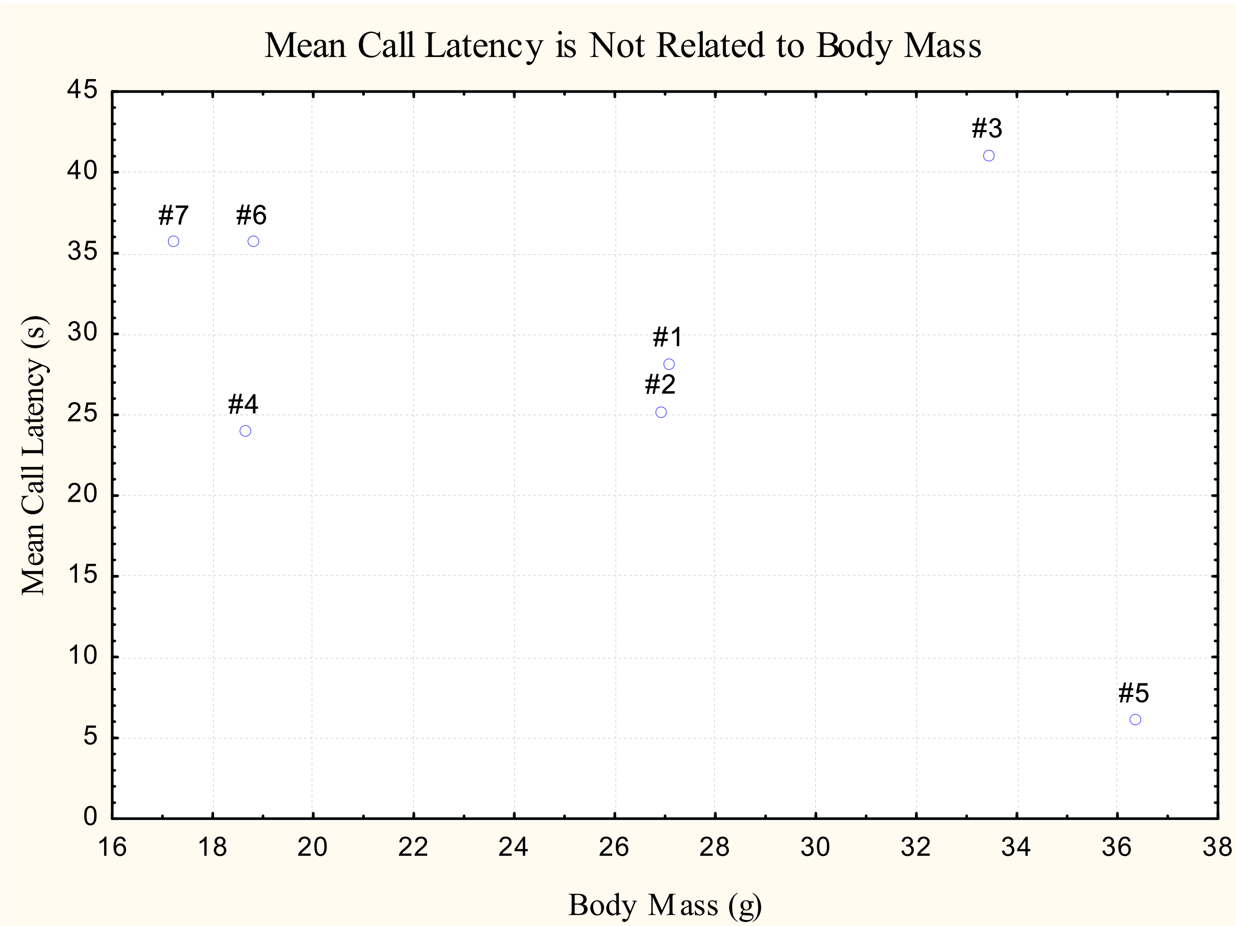

Mean Call Latency is Not Related to Body Mass
Mean Call Latency (s)
Body Mass (g)
45
40
35
30
25
20
15
10
5
0
16
18
20
22
24
26
28
30
32
34
36
38
#1
#2
#3
#4
#5
#6
#7

Fig 41

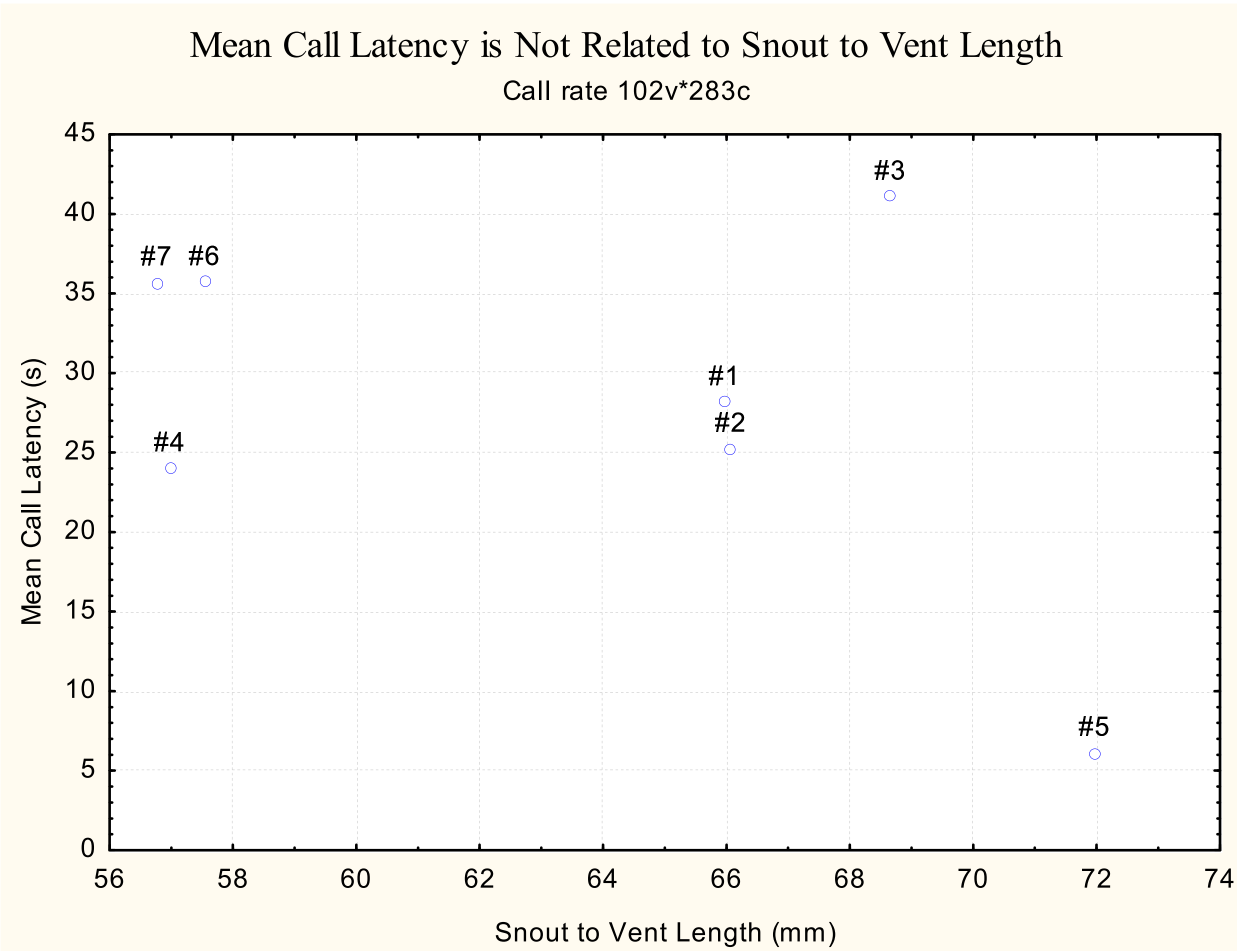

Mean Call Latency is Not Related to Snout to Vent Length
Call rate 102v*283c
Mean Call Latency (s)
Snout to Vent Length (mm)
0
5
10
15
20
25
30
35
40
45
56
58
60
62
64
66
68
70
72
74
#1
#2
#3
#4
#5
#6
#7

Fig 42

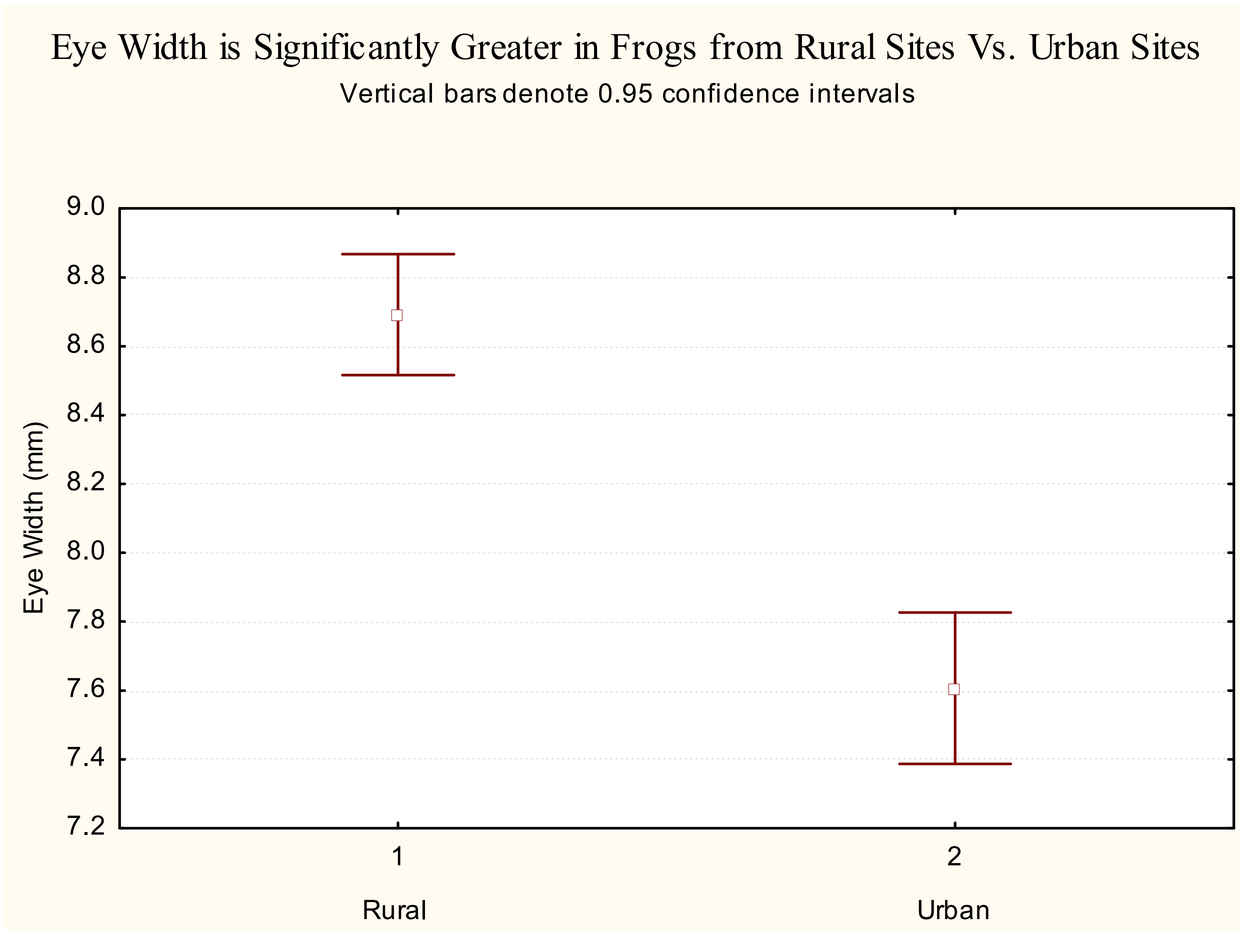

Eye Width is Significantly Greater in Frogs from Rural Sites Vs. Urban Sites
Vertical bars denote 0.95 confidence intervals
Eye Width (mm)
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
1
2
Rural
Urban

Fig 43

Appendix I

List of morphological characters used in this study

1. Snout-Vent Length (SVL). Measured from the tip of snout to the posterior end of the urostyle.

2. Head Width (HW). Measured as the width at the posterior end of the jaw.

3. Head Length (HL). Measured from the tip of the snout to the posterior end of the jaw.

4. Tympanum Diameter (TD). Measured as the diameter of the tympanum, horizontally, at the widest point.

5. Eye Width (EW). Measured as the diameter of the eye, horizontally, at the widest point.

6. Snout Length (Snout). Measured from the anterior end of the eye to the naris.

7. Femor Length (FeL). Measured from the urostyle to the knee.

8. Tibia Length (TL). Measured as the straight distance from the knee to the foot.

9. Foot Length (FoL). Measured from the proximal edge of the inner metatarsal tubercle to the tip of the longest toe.

10. Snout Angle (SA). Calculated using the formula: $\left[\text{arcsine}\left(\frac{\frac{\text{HW}}{2}}{\text{HL}}\right)\right] * 2$

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