Metal concentrations in native Yupik foodstuffs from St. Lawrence Island, Alaska

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Metal concentrations in native Yupik foodstuffs
from St. Lawrence Island, Alaska

A thesis presented to the Faculty
of the University at Albany, State University of New York
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for the degree of

Master of Science
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Judith Kricheff
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Abstract

The Yupik people of St. Lawrence Island, Alaska have a traditional subsistence lifestyle with the majority of their diet consisting of local birds, fish, seal, walrus, and whale. Diets that are based on fish and marine mammals, such as the Yupik diet, potentially have high levels of mercury and other contaminants. Potential food web contaminant sources include atmospheric deposition; local rocks and soils; two abandoned U.S. military bases and remote foodwebs through seasonal migration of animals to St. Lawrence Island.

The main goals of this research were to report the concentrations of mercury and other heavy metals in foodstuffs of the traditional Yupik diet and to use carbon (C) stable isotope ratios to quantify trophic levels and biomagnification within the ecosystem. Samples were collected by Yupik hunters at the time of kill or shortly thereafter during the years of 2005, 2006, and 2007. For this study, a total of 216 samples were analyzed from 28 different species and 14 different types of tissue. This study focuses on the fat, kidney, liver, and muscle tissues of bearded seal, polar bear, reindeer, and walrus. The metals analyzed are copper (Cu), arsenic (As), selenium (Se), cadmium (Cd), mercury (Hg), and lead (Pb). Results indicate that metals are generally more concentrated in the liver and kidney tissues of organisms and they can become biomagnified at higher levels of the food chain.

The results, together with dietary surveys, can be used to determine how much of the Yupik’s exposure to environmental contaminants is from traditional foods, and to provide a basis for the members of the Yupik community to make informed decisions about dietary choices.
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**Introduction**

St. Lawrence Island is located in the Bering Sea halfway between mainland Alaska and Siberia, Russia (Figure 1). The Yupik people living there have a traditional subsistence hunter/gatherer lifestyle. The majority of their diet, which is collected locally, consists of birds, fish, seal, walrus, and whale. There is evidence that the Yupik have been exposed to persistent organic contaminants, including polychlorinated biphenyl (PCB). PCB blood serum levels of the Yupik are five times higher than unexposed U.S. populations. Diet may be a major exposure pathway. This gives reason to explore why their levels are so high and to what other pollutants they might be exposed.

Figure 1 St Lawrence Island, Alaska. Gambell and Savoonga are permanent settlements. The Northeast Cape is a hunting ground. Abandoned military installations are located at Gambell and the Northeast Cape. (Modified from Fay and Rausch, 1992)
Other pollutants that people of St. Lawrence Island could be exposed to through diet are trace metals such as Cu, As, Se, Cd, Hg, and Pb. There are four potential sources of contamination on St. Lawrence Island: 1) Two formerly used defense sites (FUDS) on the island were active during the cold war and are polluted with PCBs, pesticide, fuel, and heavy metals; 2) atmospheric transport and deposition; 3) Naturally-occurring metals from rocks and soils; 4) Animals that seasonally migrate to St. Lawrence Island might bring pollutants from other areas of the world.

Heavy metals such as As, Cd, Hg, and Pb in fish and seafood are a concern to the general public due to their high toxicity and bioavailability. They can become biomagnified through the food chain. Because of its extreme toxicity and tendency for biomagnification, mercury is of special health concern to humans. The EPA recommendation for unrestricted fish consumption is <50ppb Hg. The FDA limit is 1 ppm. There is some controversy as to what constitutes a dangerous concentration of mercury and whether it is necessary to restrict fish consumption (Alaska, 2001). The state of Alaska does not recommend restricted fish consumption for its residents. There are many communities, especially in the arctic region, with a subsistence lifestyle that rely on fish and marine mammals for food. Mercury biomagnification is especially a concern in these communities. To understand biomagnification of mercury in arctic marine ecosystems, one must understand the sources and biogeochemistry of mercury in the complex food webs of these ecosystems.

In addition, it is important to understand in which animals and animal tissues it becomes most concentrated. This information is necessary to make predictions
about mercury concentration in foods and to give people the ability to make more informed food choices.

The objectives of this study are 1) to determine the concentrations of Hg and other metals in native Yupik foodstuffs of St. Lawrence Island; 2) to determine trophic levels of foodstuff organisms by application of stable C isotope ratios and by comparison to a similar arctic marine ecosystem; and 3) to estimate biomagnification rates of Hg and other metals in the St. Lawrence Island food web.

Sources of mercury

Mercury occurs naturally in the Earth’s crust in coal, soils, sediments, and mineral deposits such as mercury sulfide (HgS) cinnabar ore. The average concentration of mercury in Earth’s crust is 0.08 mg/kg (Krauskopf and Bird, 1995). Mercury is released into the atmosphere by volcanoes, forest fires, oceanic emission, and crustal degassing (Renzoni, 1998). Mercury also enters the atmosphere through human activities such as mining, mineral processing, municipal waste incineration, and fossil fuel combustion (Renzoni, 1998). Some common uses are in products such as batteries, light bulbs, and thermometers. A main source of mercury to the pristine arctic environment is atmospheric transport from industrialized regions and deposition. The arctic acts as a sink for contaminants, which accumulate in this region. Mercury travels quickly through the atmosphere and can reach the arctic from Europe within days. It remains there due to stable atmospheric conditions during the winter season. After atmospheric deposition mercury enters the food chain where it becomes biomagnified.
It can be difficult to identify point sources of contaminants that may also be present in the environment through atmospheric deposition and other natural sources. Two ways to do this are to measure contaminant concentrations in sediment cores from the locality in question and compare the results to a location with similar geologic and climatic characteristics. Using these techniques, Scrudato et al. (submitted) determined that PCB, DDE, Hg, and mirex concentrations are significantly higher at the Northeast Cape than at remote lakes in the Canadian Arctic and Eastern North America. Their findings show that the FUDS at Northeast Cape are significant local sources of these contaminants.

**Biogeochemistry of Mercury**

Mercury (Hg) is a highly volatile metal that is liquid at room temperature. In the surface environment, it can occur in elemental, inorganic, and organic forms. In natural waters, mercury occurs as elemental Hg (Hg\(^0\)), mercurous ion (Hg\(^+\)), mercuric ion (Hg\(_2^+\)), methyl mercury (CH\(_3\)Hg\(^+\) or MeHg\(^+\)), and ethyl mercury (C\(_2\)H\(_5\)Hg\(^+\)), and it may be complexed to hydroxide, chloride, and sulfide. The pH, chloride, and sulfide concentration in water are important factors that affect the chemical speciation of mercury (Morel, 1998). Atmospheric deposition is the main source of elemental mercury in natural waters. It is deposited in surface water in dissolved inorganic forms where it undergoes chemical changes.

One important change that happens to mercury is methylation, the bonding between Hg and an alkyl anion group, CH\(_3\)\(^-\). Microbial processes in anoxic waters and pH influence the binding of Hg and the formation of MeHg\(^+\) (WHO, 2003). Methylation can also be a result of photochemical processes; however acetate or
humic acids must be present for the reaction to occur (Morel, 1998). When inorganic mercury is consumed by an organism, it can be converted to MeHg in the organism (WHO, 2003). It is known that methylation reactions in the water are caused by sulfate-reducing bacteria, such as Desulfovibrio desulfuricans (Morel, 1998). Mercury in the water is available to these bacteria for methylation unless it has been complexed as the insoluble form of HgS. HgS becomes deposited in sediments and can remain there for long periods of time (D. Caussy et al., 2003). After HgS is deposited, methylation reactions do not occur.

Just as available mercury in water can undergo methylation reactions, it can also undergo demethylation. After methylation occurs, most of the resulting MeHg+ is absorbed by microorganisms and primary producers. Some MeHg however, goes through a demethylation process and returns back to Hg(II). At this point, the mercury can either be complexed, or reverted to MeHg where it is ultimately assimilated into the food chain. Demethylation reactions are more likely to occur near the surface than at lower levels in the water column because they are initiated by photochemical reactions. The demethylation process occurs when sunlight decomposes MeHg in the presence of oxygen (Morel, 1998; King et al., 2002).

Almost all of the mercury that is bioaccumulated is in the organic form, MeHg. In this form it can be taken up by microorganisms and biomagnified in arctic marine food chains. Although mercury is toxic in all of its forms, MeHg is the form most threatening to humans because it more bioavailable than inorganic and elemental mercury and it becomes bioconcentrated.
Bioaccumulation of Mercury

The various types of mercury behave differently in the way that they are bioaccumulated in organisms. Because mercury is methylated in water and biomagnified by aquatic and marine organisms, the main source of MeHg to humans is through consumption of freshwater fish, seafood and picivorous mammals. Methyl mercury is easily absorbed in the body and can be passed to the fetus in pregnant women (Renzoni, 1998). The different species of mercury have different reactions in organisms. MeHg is bioaccumulated while Hg^0, (CH\_3)\_2Hg, and Hg(II) are not. Hg^0 and (CH\_3)\_2Hg are not retained in plankton because they are not reactive. They can easily diffuse in and out of the organism and therefore do not become bioaccumulated. Similarly, Hg(II) does not bioaccumulate because it binds to the cell membrane instead of the soluble portion of the cell. When tissue containing Hg(II) is consumed, Hg(II) is excreted with the cell membrane metabolites and is not absorbed. MeHg, on the other hand, becomes a part of the cell by attaching onto the soluble portion, which is then incorporated into the organism (Morel, 1998). In humans and marine mammals liquid Hg^0 is not absorbed in the GI tract, but almost all of the MeHg that enters the gastrointestinal tract does become absorbed (D. Caussy et al., 2003). In order for mercury to reach higher levels of the food chain and become biomagnified, it must first be converted to MeHg and then become absorbed by organisms at the bottom of the food chain.

Biomagnification of Mercury

Biomagnification occurs as mercury is accumulated in organisms and becomes more concentrated at higher trophic levels. The organic form MeHg is reactive in
water and therefore bioavailable. The concentration in fresh and seawater is on the order of ppt (pg/g) (Morel, 1998). In arctic marine ecosystems, MeHg is taken up from seawater and sediment by primary producers such as particulate organic matter (POM), ice algae, and kelp. These organisms are eaten by invertebrate primary consumers including copepods, mysids, anenomes, and amphipods. Sculpin and other small fish, as well as walrus are secondary consumers that mainly eat invertebrates. These secondary consumers will accumulate 1000 times more mercury than the water, on the order of ppb (ng/g). Secondary consumers are in turn eaten by larger fish, which are eaten by humans and other fish-eating animals. These higher level consumers can accumulate mercury concentrations on the order of ppm (mg/g), which is 1,000,000 times more than in the water. This increase of concentration in animals that are higher on the food chain is called biomagnification.

*Stable isotope analysis: $\delta^{13}C$ and $\delta^{15}N$*

To determine the pattern and rate of biomagnification in a food web, one must determine trophic levels and biomagnification factors of the ecosystem in question. Methods of determining trophic levels are predator/prey relations, stomach content, stable nitrogen isotopes ($\delta^{15}N$) and stable carbon isotopes ($\delta^{13}C$). Stable isotope analysis methods are preferred over stomach content analysis because they show a long term record of accumulation rather than the small period of time that is represented by stomach content. Predator-prey relations are a useful tool in determining trophic levels, but this method can only provide a qualitative assessment of trophic level. To determine trophic levels quantitatively, stable isotopes are the most reliable method. $\delta^{15}N$ and $\delta^{13}C$ can both be used for determining trophic levels.
in an arctic marine ecosystem (Hobson and Welch 1992, Dehn et al. 2006). $\delta^{15}N$ is better at higher levels of the food chain because $\delta^{13}C$ enrichment becomes less pronounced, and it is more difficult to differentiate between trophic levels. $\delta^{13}C$ is useful for studying primary producers and examining spatial distribution and carbon sources. Together, $\delta^{13}C$ and $\delta^{15}N$ are complementary tools for determining trophic levels for a complete food chain (Hobson and Welch 1992, Dehn et al. 2006).

Although $\delta^{13}C$ and $\delta^{15}N$ are the leading methods for trophic level quantification, there are some drawbacks to the each method. Consideration must be taken for the fact that there are several factors that can affect $\delta^{15}N$ such as age, body condition, water stress, and factors associated with starvation and hibernation. In addition, enrichment factors can vary from 2.4‰ to 3.0‰ per trophic level. Variations due to these factors must be considered when assigning trophic levels based on averaged enrichment factors (Dehn et al. 2006). Despite its drawbacks, stable isotope ratio analysis is a widely used and trusted method for determining trophic levels in arctic marine ecosystems.

Stable carbon isotope analysis involves measuring the difference of $^{13}C:^{12}C$ of the sample compared to the $^{13}C:^{12}C$ of a working reference carbon dioxide gas. $\delta^{13}C$ is defined as the following: $[(^{13}C/^{12}C_{sample}-^{13}C/^{12}C_{ref})/(^{13}C/^{12}C_{ref})] \times 1000 = \delta^{13}C_{sample}$, expressed in per mil. A higher $\delta^{13}C$ indicates a higher trophic level as animals become enriched with the $^{13}C$ isotope. This is because as an animal consumes its prey it is also consuming the level of $^{13}C$ that is in its prey. The $^{13}C$ that an organism consumes becomes more concentrated because $^{13}C$ is preferentially retained in the organism while $^{12}C$ is preferentially excreted. When $^{13}C$ becomes more concentrated
in relation to $^{12}\text{C}$, the value of $^{13}\text{C}:{^{12}\text{C}}$ increases. This increasing concentration of $^{13}\text{C}$ is an example of biomagnification. In the arctic marine ecosystem of the Northwater Polynya, Baffin Bay, $\delta^{13}\text{C}$ has an enrichment factor of 0.8‰ to 1‰ per trophic level (Campbell et al., 2005). When the enrichment factor is low, there is less difference between trophic levels and therefore it is more difficult to distinguish between them.

Stable nitrogen isotope analysis involves measuring the difference of $^{15}\text{N}:{^{14}\text{N}}$ of the sample compared to the $^{15}\text{N}:{^{14}\text{N}}$ of a working reference gas. $\delta^{15}\text{N}$ is defined as the following: 

$$
\delta^{15}\text{N}_{\text{sample}} = \left( \frac{^{15}\text{N}/^{14}\text{N}_{\text{sample}} - ^{15}\text{N}/^{14}\text{N}_{\text{ref}}}{^{15}\text{N}/^{14}\text{N}_{\text{ref}}} \right) \times 1000
$$

$\delta^{15}\text{N}_{\text{sample}}$, expressed in per mil. Higher $^{15}\text{N}:{^{14}\text{N}}$ ratio indicates a higher trophic level as animals become enriched with the $^{15}\text{N}$ isotope. The ratio of $^{15}\text{N}:{^{14}\text{N}}$ increases because $^{15}\text{N}$ is preferentially taken up during protein synthesis and $^{14}\text{N}$ is preferentially excreted. The bonds formed by the heavier $^{15}\text{N}$ isotope are higher energy bonds than the bonds formed by $^{14}\text{N}$ (Adams and Sterner, 1987). The result is increasing values of $^{15}\text{N}:{^{14}\text{N}}$ with increasing levels of the food chain.

At higher trophic levels, $\delta^{15}\text{N}$ is used to discriminate trophic levels more than $\delta^{13}\text{C}$ because $^{15}\text{N}$ has a higher enrichment factor than $^{13}\text{C}$ (Fry, 1988; Hobson and Welch, 1992). For example, in the arctic marine ecosystem of the Northwater Polynya, Baffin Bay, $\delta^{15}\text{N}$ has an enrichment factor of 2‰ to 4‰ per trophic level as opposed to the enrichment factor of 0.8‰ to 1‰ for $\delta^{13}\text{C}$. The C and N isotopes were measured with errors of ± 0.1 ‰ and ± 0.3 ‰, respectively (Campbell et al., 2005).

When examining trophic levels ranging from primary producers to carnivorous predators a combination of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ methods can be used.
Primary producers can be identified with $\delta^{13}$C analysis while higher trophic levels are quantified by $\delta^{15}$N analysis. The relationship of $\delta^{13}$C and $\delta^{15}$N in the Northwater Polynya is shown in figure 2. There is larger range for $\delta^{15}$N (~4.5‰ to ~18‰) and greater variation in high trophic level organisms, while the range for $\delta^{13}$C is smaller (~22‰ to ~17.5‰). The low trophic level organisms are easily identified by $\delta^{13}$C, but there is less variation of $\delta^{13}$C in the higher trophic organisms.

Figure 2. The relationship of $\delta^{13}$C and $\delta^{15}$N for species of the Northwater Polynya, Baffin Bay. Species codes are listed below (Campell et al, 2005). Particulate organic matter (POM) were from Hobson et al. (2002).
Determining trophic levels and biomagnification rates from $\delta^{15}\text{N}$

Once the $\delta^{15}\text{N}$ ratio is determined, it can be used in an equation to determine the trophic level of that animal. With a set of $\delta^{15}\text{N}$ data from an arctic marine ecosystem, the food web can be illustrated based on each organism that has been analyzed. If every trophic level is represented, a complete food web from primary producers to carnivorous predators can be displayed. In some instances it is not possible to obtain samples representative of every trophic level. Even when data is not available, trophic level assessment through $\delta^{15}\text{N}$ makes it possible to make predictions about the trophic level of an organism and expected levels of mercury (or other contaminants) in that organism based on identified predator/prey relationships and the concentration of organisms above and below it in the food web reconstruction.

In order to create a model of trophic levels in the Barrow Strait, Lancaster Sound arctic marine food web, Hobson and Welch used the equation:

$$TL = 1 + (D_m - 5.4)/3.8,$$
Where the value of 5.4 is the $\delta^{15}\text{N}$ value of particulate organic matter (POM) in that ecosystem, $\text{TL} = \text{trophic level of the consumer}$, $\text{D}_m = \delta^{15}\text{N}$ of the consumers muscle tissue, and $3.8\text{‰}$ is the $\delta^{15}\text{N}$ enrichment factor for all animals of this food web except birds. The enrichment factor $3.8\text{‰}$ is used throughout the food web because this relationship was found between Polar bears and their primary prey, ringed seals, as well as between copepods and their main food source POM (particulate organic matter). Because it was found at these different levels of the food chain, it is suitable for use throughout. For birds, a $\delta^{15}\text{N}$ enrichment factor of $2.4\text{‰}$ was used because it has been shown that there is less enrichment between birds and their food. This enrichment factor of $2.4\text{‰}$ was determined by Mizutani et al. (1991) from a study based on the tissues of a captive adult cormorant with a known diet for 23yr (Hobson and Welch, 1992). The trophic levels derived from $\delta^{15}\text{N}$ as determined by Hobson and Welch is displayed in figure 3. As figure 3 illustrates, the quantification of trophic levels can be complicated. It is often difficult to assign one species to one specific trophic level. Instead, there is often a wide variation in $\delta^{15}\text{N}$, and therefore, of trophic levels for individual species and a significant amount of overlap among different species.
The trend of trophic level increase with $\delta^{15}N$ increase applies to other arctic marine ecosystems as well (Campbell et al., 2005; Fry, 1988). Determining trophic levels provides a detailed look at the relationships within a food web and an understanding of the complexities of an ecosystem. There are many variables such as migrations, region, and human impact that can influence the dynamics of a food web making each one unique. For example, human development may drive a particular type of species, or several different species, away from an area. If they migrate to a different location, this could change the general structure of these food webs. Some animals, such as birds and some seals undergo extreme seasonal migrations. In their
travels, they could consume chemicals and pollutants and bring them back to a pristine arctic environment in their bodies, thus altering the natural state of that food web.

Application of $\delta^{15}N$ and $\delta^{13}C$ to biomagnification studies

Trophic level constructions from $\delta^{15}N$ are useful in assessing the transfer of metals and organic pollutants in a food web. A comparison of trophic levels or $\delta^{15}N$ of animals to measured mercury concentrations reveals biomagnification relationships. Atwell et al. (1998) and Campbell et al. (2005) both showed that mercury concentration increases with trophic level. In figure 4, Hg concentration as a function of trophic level is shown graphically. The rate of Hg transfer is small at the lowest trophic levels and increases at higher levels in the Northwater Polynya, Baffin Bay and the Lancaster Sound, Northwest Territories ecosystems. Chemicals such as mercury that are taken up by primary producers, and absorbed by the bodies of predators, become biomagnified. In contrast, if a chemical is not taken up by microorganisms, or not absorbed efficiently by predators, it will undergo biodilution. For example, Fe has been shown to become diluted at increasing levels of an aquatic food chain in Montana (Quinn et al., 2002). Biomagnification of mercury in aquatic ecosystems is an important concern for humans because of its high toxicity and the potential of mercury poisoning from seafood.

Mercury biomagnification rates vary among different species, trophic levels, and location. Variation in isotope enrichment factors and mercury contamination among ecosystems can be caused by different animal species, migration patterns, or concentration in the water due to a local source of mercury contamination. The
marine food web of Lancaster Sound, Northwest Territories has a $\delta^{15}$N enrichment factor of 3‰ to 5‰, while the food web of Georges Bank, an Atlantic fishing ground, had $\delta^{15}$N enrichment as high as 10.1‰ in some fish at higher trophic levels (Atwell et al., 1998; Fry, 1988). Trophic enrichment factors this high are not ordinary though, and the most common range is 3‰ to 5‰; the trophic enrichment constant used in most equations fall within this range. As a result of this tendency toward relatively high and predictable enrichment factors, many studies use the method of $\delta^{15}$N in determining trophic levels in arctic marine food webs. Quantifying trophic levels with stable nitrogen isotopes provides a basis for predicting biomagnification patterns and animal relationships in a food web and determining biomagnification rates.

Figure 4 (Campbell et al, 2005)
Total mercury concentration (circles) compared to $\delta^{15}$N (triangles) in the Northwater Polynya and Lancaster Sound ecosystems
Human health impacts from consuming mercury-contaminated fish

Mercury is known to be toxic to humans. The FDA recommendation for unlimited fish consumption is a concentration at or below 1ppm Hg. The EPA recommendation for unlimited fish consumption is 50 ppb Hg (or .05ppm), but it is the responsibility of each state to post advisories and recommendations. In Alaska, the department of public health does not recommend restricted fish consumption for any of its residents, including pregnant women and children. This is because fish and marine mammals are a main staple of many native diets, especially those with subsistence lifestyles. The Alaska Division of Public Health states that the known benefits of eating fish outweigh the controversial dangers (Alaska Public Health, 2001).

The kidneys are most affected by mercury because they are a filter for the human body. Mercury accumulation can decrease kidney function, a serious and potentially life threatening condition (D. Caussy et al., 2003). Another effect of mercury poisoning is neurological disorder. One extreme example of this occurred in Minimata, Japan when Minimata Bay, a major fishing source, was heavily polluted with methyl-mercury (Renzoni et al., 1998). The largest exposure pathway of mercury in humans is through fish consumption. This is especially of concern to pregnant or breastfeeding women because mercury is passed along to the fetus or baby (Burger et al., 2005). Fish is a major nutritional staple and culturally important to many societies. It is a low fat, high quality protein that contains omega 3 fatty acids and B vitamins that promote good health. Fisheries are also vital to the
economy as many people depend on them for their livelihood. For all of these reasons, mercury contamination in fish and seafood is an important topic to address.

_Selenium and Mercury Interactions_

There may be biochemical interactions between selenium and mercury that can mitigate the negative neurological effects of mercury. These two elements form complexes resulting in insoluble mercury selenides. The result is that both mercury and selenium become less bioavailable in an organism. Se is essential for many functions in the body including growth, thyroid hormone regulation, immunity, fertility and amino acid protein synthesis. Se can also lessen the negative effects of Hg in organisms (Jin, 1997). Because Hg and Se become complexed, there is also the potential for Hg to interfere with the critical role that Se has in the developing fetal brain. Se and Hg bind together so the presence of Hg can also make Se less bioavailable to perform its biochemical function. One of the functions of Se is that it regulates thyroid hormone. A disturbance to the thyroid and thyroid hormone can cause neurological damage because it is essential in the process of neurological development (Raymond, 2004). It is the consequences of Se and Hg interaction that make Se:Hg ratios significant.

The interaction of Se and Hg causes each to become less bioavailable therefore it is possible for the presence of Se to slow the rate of bioaccumulation of Hg in fish (Raymond, 2004). Studies have shown that there can be a negative correlation between Se and Hg in fish muscle (Chen, 2001). In contrast, there have been studies that revealed no significant relationship between the two (Barghigiani, 1991; Chen, 2001). The topic is still one of debate as these systems are very complex and can be
altered by a number of external factors such as outside sources of contaminants, metabolic processes in the organisms, disease, diet and availability of food. Chen et al. (2001) have shown that in fish populations in lakes near the Sudbury smelters in Canada, there is an exponential decline in Hg concentration with increasing Se concentration in the fish. They found that the concentration of Se in the fish increased as the concentration of Se in the water increased. The lakes closest to the smelters had the highest Se concentrations. Higher Se concentration resulted in lower Hg concentrations in fish tissue and, therefore, the lakes closer to the smelters had consistently higher Se:Hg ratios. The results of this study suggest that Se can inhibit Hg bioaccumulation, because if Hg retention is reduced at lower levels, this will affect higher levels in the food chain. Trends in the Se:Hg ratio in an ecosystem may be useful in determining which foods are likely to contain more Hg, when trying to make educated food choices.

**Materials and Methods**

*Sampling methods*

Samples were collected by Yupik hunters at the time of kill or shortly thereafter during the years of 2005, 2006, and 2007. The animals were hunted at or near the villages of Gambell and Savoonga. Savoonga hunters also hunted at the NE Cape. For this study, there were a total of 216 samples analyzed, 122 from Savoonga and the NE Cape, 84 from Gambell, and 10 of unknown origin. It was not possible to distinguish samples from Savoonga versus NE Cape, but these were both collected by Savoonga hunters. Because the traditional St. Lawrence Island diet is inconsistent throughout the year and from year to year, this sample set represents the diet as
completely as was possible during the period of study. The samples that were provided for this study are what were available to these families at that time. Appendix 1 lists the number of each species and types of tissue in the entire sample set. For this study a small piece of the animal was cut or dissected from the carcass at either the time of kill or the time of consumption. Samples were stored in zip top plastic bags and kept frozen at approximately -15°C. The samples were shipped frozen to Anchorage, AK and then to Albany, NY.

Analytical methods

Samples were thawed and homogenized with a stainless steel Waring commercial Quik Stik® immersion blender at the University at Albany Department of Earth and Atmospheric Science. The Quik Stik® was cleaned between each sample by removing all solid material with a paper towel then submersion in soapy de-ionized water, followed by submersion in deionized water, followed by a rinse with 18.3Ω ultrapure de-ionized water. After homogenization, each sample was transferred to a polyethylene specimen storage container with lid. For each sample, 0.25±0.01g (wet weight) was digested in a CEM MARS-5 microwave following EPA method 3052. For this method, weighed sample is placed in Teflon liners and 9ml of high purity nitric acid is added. The solution is subjected to a temperature of 180°C and pressure of up to 207,000 Pascals in a microwave. After digestion, the solution was quantitatively transferred to a 50 mL Digitube®, 0.1ml of ICP-MS internal standard containing 50 ppm of Rh, In, Re, and Bi in 5% nitric acid was added, and the solution was diluted to 50ml with 18.3Ω ultrapure de-ionized water.
Analysis for mercury was done by cold vapor atomic absorption spectrometry (CV-AAS) with a Leeman Labs Hydra AA automated mercury analysis system. 2% HCl rinse solutions, SnCl₂, and calibration standards were prepared from high purity reagents at the time of analysis. Standard Reference Materials (NIST 1566b, DOLT 3, and DORM 3) were used for each run to evaluate the method accuracy. Method blanks, duplicates, and matrix spikes were also run for quality control.

Trace metal analysis (other than Hg) was done by inductively coupled plasma mass spectrometry (ICP-MS) with a PerkinElmer/Sciex Elan 6100 DRC. Atomic masses analyzed are 63.546, 65.38, 74.921, 78.96, 112.41, 200.59, and 207.2. Rinse and standard solutions were prepared at the time of analysis. Method blanks, duplicates, matrix spikes, and certified standards were used for quality control.

To prepare samples for isotope analysis, an aliquot of homogenized sample (around 1 tsp size) was freeze dried in a VirTis benchtop freeze drier at Union College. The freeze dried portion was ground up with a mortar and pestle and stored in a glass vial. Lipids were extracted from the freeze dried samples by adding 10mL hexane. The samples were then shaken for five minutes with a wrist action shaker and then centrifuged for 5 minutes at 2500 rpm. This lipid extraction process was performed three times for each sample with hexane decanted each time. Samples were analyzed in the Stable Isotope Ratio Mass Spectrometer (SIRMS) Laboratory in the University at Albany Dept. of Earth and Atmospheric Science with an Optima gas-source triple-collector mass spectrometer equipped with a dual inlet hooked up with a MultiPrep automated sample preparation device and a Carlo Erba NA 1500 Series II NC elemental analyzer.
Quality control for metals analysis was maintained with duplicates, method blanks, matrix spikes, and standard reference materials (SRM). NIST SRM’s 1566b oyster tissue, DOLT 3 dogfish liver, and DORM 3 fish protein were measured in 8, 4, and 4 replicates, respectively to determine the relative precision of the CV-AAS method (appendix 2). The coefficient of variation, expressed as relative standard deviation (%RSD) for ng/g (ppb) analysis of NIST1566b, DOLT 3 and DORM 3 were 8, 3, and 13% respectively. The average measured concentration of the NIST 1566b, DOLT 3, and DORM 3 standards was not significantly different from the certified value within a 95% confidence interval. NIST 1566b was used for quality control with ICP-MS. The mean, standard deviation, %RSD, confidence intervals, and bias are shown in appendix 3 for Cu, As, Se, Cd, and Pb.

For δ13C analysis, the NIST 1547 peach leaves standard was used for quality control. Seven replicates of the standard were run during analysis with an average δ13C of -25.81 ± 0.15 per mil (appendix 3). Although this standard has not yet been certified as an isotopic standard, its carbon and nitrogen isotopic compositions have been fairly well established through multiple analyses by several different laboratories. For example, the University of Arkansas obtained δ13C = -25.89 ± 0.13 per mil for over 150 analyses of NIST 1547. The unofficial accepted value obtained by other labs is -25.88 to -25.99 per mil.

Method blanks were analyzed for both the CV-AAS Hg analysis and the ICP-MS heavy metal analysis. Method blanks were used to determine the minimum detection limit (MDL) and minimum reporting limit (MRL) in the sample for each protocol (appendix 4).
The formulas used to calculate these are:

\[ \text{MDL} = 3\sigma, \quad \text{and} \]
\[ \text{MRL} = 10\sigma, \]

where \( \sigma \) is the standard deviation of the blank replicates.

For the ng/g protocol of Hg analysis, the MDL was .14 ng/g Hg in sample and the MRL was .46ng/g Hg in sample.

**Data analysis**

Statistical analyses were handled with Microsoft Excel and S-Plus software. Quality control formulas were calculated with Microsoft Excel and metals concentrations were plotted with S-Plus. Data were organized according to species and tissue. Stable isotope calculations and plots were created with Microsoft Excel.

**Selenium:Mercury**

The molar concentration of Hg and Se were calculated from the wet weight \( \mu g/g \) concentration as determined for this study. Molar concentration was determined with the following equation:

\[
\text{Molar concentration (nmol/g)} = \frac{(\text{concentration (\( \mu g/g \)) x 1000})}{\text{atomic weight}}
\]

Atomic weight Hg = 200.59

Atomic weight Se = 78.96

Scatter plots were made on a log scale with Microsoft Excel for each of the following components: liver/kidney, muscle/skin, heart/intestine, and blubber and/or fat/oil.
Results and Discussion

Mercury

A comparison of mercury concentration in muscle tissue of different species is shown in Figure 5. Animals of higher trophic levels are expected to have higher mercury concentration in muscle/meat tissue. There is an apparent range of mercury concentrations within each species, and in some cases, an individual of a lower trophic level species might have more mercury than an individual of a higher trophic level species even if the average concentration is similar. For example, this occurs between polar bears (TL=5) and bearded seal (TL=4). These trophic levels are based on Hobson and Welch, 1992. There is variation of mercury concentration within these individual species. Bearded seal is from a lower trophic level than polar bear but individual bearded seal might have more mercury than an individual polar bear. This variation occurs because natural ecosystems are complex and often unpredictable. Bearded seal feed mostly on crabs, shrimp, clams, and snails. Polar bear mainly eat ringed seal, which are the smallest of the arctic seals. When looking at a complicated ecosystem such as St. Lawrence Island it is useful to compare the sample averages rather than individuals. Factors that contribute to the natural variation in mercury bioaccumulation of individuals within a species are age and migration patterns.
**FDA recommendation for unlimited mercury consumption = 1ppm (mg/g)**

**EPA recommendation for unlimited mercury consumption < 50 ppb (ng/g)**

*The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.*

Figure 6 is a comparison of different types of tissue from walruses. The concentration of Hg in walrus tissues ranged from <14 ng/g to 1350 ng/g. The range for walrus muscle was n/d to 179 ng/g. Blubber, heart, intestine, oil, and skin concentrations were in the range of n/d to 200 ng/g. Walrus liver contains a higher concentration of mercury than the other tissues that were tested. It was also more variable, ranging from 79 ng/g to 1350 ng/g.
Figure 6
Mercury in Walrus Tissue

FDA recommendation for unlimited mercury consumption = 1 ppm (mg/g)
EPA recommendation for unlimited mercury consumption < 50 ppb (ng/g)

The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.

Reindeer tissue are shown in figure 7. This graph is a representation of the St. Lawrence Island terrestrial ecosystem. The range for reindeer liver and kidney are n/d to 615 ng/g and 673-2200 ng/g, respectively. Heart and muscle concentrations were < 8.35ng/g. This shows that there is more mercury in the liver and kidney tissues, most likely due to their physiological roles in sequestering and eliminating toxic metals.
FDA recommendation for unlimited mercury consumption = 1ppm (mg/g)
EPA recommendation for unlimited mercury consumption < 50 ppb (ng/g)

The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.

Mercury concentration in the different tissue of bearded seal is shown in figure 8. These results show a similar pattern to those of walrus and reindeer tissues (Figures 6 and 7). The liver tissue of bearded seal has much higher concentrations of mercury than the blubber, muscle, prepared meat, and rendered oil. The concentration of liver ranged from 667 ng/g to 5860 ng/g (5.86ppm), blubber from n/d to 190 ng/g, and muscle from n/d to 237 ng/g. There was only one sample of
prepared meat (not shown), which had a concentration of 100 ng/g, and the two oil samples were 127 ng/g and 134 ng/g.

![Figure 8: Mercury in Bearded Seal Tissue](image)

**FDA recommendation for unlimited mercury consumption = 1ppm (mg/g)**  
**EPA recommendation for unlimited mercury consumption < 50 ppb (ng/g)**

The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.

A comparison of blubber from six different species is shown in figure 9. Most of the samples that were available for this type of tissue were from bearded seal and walrus. Bearded Seal and walrus blubber are similar in Hg concentration, and have a variation of n/d to 190 ng/g and n/d to 151 ng/g, respectively. The greater variability found in bearded seal and walrus blubber than in spotted seal and walrus calf blubber.
is likely the result of sample size differences. It is interesting to note that while there is little difference between the average muscle Hg concentrations in walrus and bearded seal, the average blubber Hg concentration in bearded seal appears to be significantly higher than that of walrus.

Figure 9
Mercury in Blubber Tissue

*FDA recommendation for unlimited mercury consumption = 1ppm (mg/g)*
*EPA recommendation for unlimited mercury consumption < 50 ppb (ng/g)*

*The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.*
Cadmium

Another heavy metal of interest is cadmium (Cd). Figure 10 shows a comparison of Cd in the muscle tissue of different species. This graph shows that in all of the species represented, mean muscle Cd concentration is less than 45 ng/g. Figure 11 shows Cd concentration in different tissues of walrus and Figure 12 is Cd in tissues of reindeer and plants. Both of these graphs indicate that Cd is concentrated in the liver and kidneys of walrus and reindeer. Like Hg, Cd is a non-essential element. The major objects of Cd toxicity are the kidney, lung, and cancers (Caussey et al., 2003).

The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.
Figure 11
Cadmium in Walrus Tissue

The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.
The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.
Copper, Arsenic, Selenium, and Lead

Comparisons of Cu, As, Se, and Pb in the different tissues of walrus are shown in figures 13, 14, 15, and 16. Walrus is the most abundant species in the sample set for this study and provides good representation of metals in food from St. Lawrence Island. The average concentration of As (figure 13) tends to be higher in the blubber and oil tissue than other tissues, while Cu (figure 14) is more concentrated in the liver tissue, with an average concentration of 15 ng/g. Pb and Se (figures 15 and 16, respectively) both have average concentrations at or near the detection limits, but the average Se concentration in heart and muscle are slightly higher, reaching 2-2.5 ng/g.

Figure 13
As in Walrus Tissues

The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQR) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.
Figure 14
Cu in Walrus Tissues

The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.
The horizontal line represents the median of the data. The height of the box is the interquartile distance (IQD) or the difference between the third and first quartiles. Whiskers at the top and bottom of the box go to the nearest value within the standard span of the quartiles. Any points outside the whiskers and outliers.
Selenium: Mercury

Selenium and mercury form complexes resulting in the formation of insoluble mercury selenides. The result is that both mercury and selenium become less bioavailable in an organism. This is important because Se is essential for many functions in the body including growth, thyroid hormone regulation, immunity, fertility and amino acid protein synthesis. Figure 17 shows Se:Hg ratios in liver of sea birds, seals, polar bear, walrus, and reindeer. Reindeer kidney is also included in this graph because it is comparable to the liver tissue. All of these Se:Hg ratios were greater than 1:1. One polar bear liver sample was close to 1, with a Se:Hg value of 1.2. The molar concentration of Hg in this sample was 31.66 and the molar concentration of Se was 37.74. This single sample of polar bear liver has both the highest Hg concentration and the lowest Se:Hg ratio as compared to the seal and walrus livers. Two of three seal livers have higher total Hg and lower Se:Hg than all of the walrus livers. These data suggest that both total Hg and total Se increase with trophic level, but that Hg increases faster, and consequently, Se:Hg ratios decrease. More data are needed to evaluate this hypothesis.
Figure 18 shows Se:Hg results from sculpin and walrus intestine and the heart tissue from polar bear, reindeer and walrus. These samples all had an excess of Se over Hg. Values were all higher than 100:1 with the exception of one walrus heart sample, which was lower than the others, but still greater than 10:1.
Figure 19 shows the Se:Hg results from muscle and skin tissue from seals, sea birds, polar bear, reindeer, walrus, fish, and whale. Muscle and skin were all above 1:1, they ranged from just above 1:1 up to over 100:1. All species had some variation showing no particular patterns. Despite the high variation in Se:Hg ratios in muscle/skin tissue, within each species, muscle and skin tissues have higher average Se:Hg ratios than liver tissues.
Figure 20 is the Se:Hg results for fat/blubber/oil from seals, walrus, whale, seabirds, polar bear, and seabirds. Most of the samples had Se values at or below the detection limit. Of the samples that are above the detection limits, Se:Hg was close to 10:1.
Although each group of tissue had Se:Hg greater than one, there were some differences between the groups. The muscle/skin tissue and the heart/intestine tissue both had generally higher ratios than the other groups. These two groups had ratios falling between 100:1 and 1000:1. The blubber/fat/oil group was generally lower and had some points approaching 1:1. Similarly, the liver tissue was low, with samples near 10:1 and approaching 1:1 as well, with one polar bear sample having ratio of 1.2:1. Heavy metals are generally more concentrated in the liver and kidney tissues of animals than in other organs and tissue, and fat/blubber/oil tissue concentrations are generally lower.

Results for St. Lawrence Island were similar to those found by Dietz et al. (2000) in Greenland marine animals. In both locations, seal ratios were generally closer to 1:1 than polar bear ratios. Birds displayed a wider range of Se:Hg ratio than the other species, which is to be expected given the span of trophic levels that birds occupy. Fish ratios tend to be higher with Hg and Se concentrations near the limit of detection (LOD) in both studies. One difference is that the data from Dietz et al. had some Se:Hg ratios at or below 1:1, although the greater majority were higher, and comparable to St. Lawrence Island. Dietz et al. were working with a larger data set of 2510 samples, collected over two decades, compared to our data set of 233 samples, collected over 3 years. The large data set and greater time span from Greenland may contribute to the greater variation of observed Se:Hg ratios.

δ^{13}C and Trophic level analysis

δ^{13}C analysis is not the ideal method for trophic level identification at higher levels of the food chain. However, paired with known eating habits it can be
sufficient for comparative purposes. $\delta^{13}$C from muscle tissue of animals from St. Lawrence Island are plotted in figure 21 with the $\delta^{13}$C of the same species from the Hobson and Welch (1992) study of Lancaster Sound. Although there is a linear relationship and these data suggest that the St Lawrence Island ecosystem has a similar trophic level distribution as Lancaster Sound, there is not enough evidence here to determine trophic level quantitatively. However, one can compare the $\delta^{13}$C and metal concentration distribution of these two ecosystems to derive qualitative trophic relationships among the organisms represented. The value of this is the ability to recognize organisms that are likely to have higher concentrations of metals in them. Figure 22 shows the average Hg concentration of arctic marine organisms plotted with the trophic level assignments that Hobson and Welch (1992) have given to the same species from Lancaster Sound. Calculations for average Hg concentration and standard deviations were calculated with Microsoft Excel. Arctic birds are not shown here because birds have a different trophic enrichment factor and wide range of trophic levels ranging from ~3-5 (Hobson and Welch, 1992). The x-axis error bars represent the range of trophic level that each species occupies as shown in figure 3 (Hobson and Welch, 1992). The y-axis error bars show the standard deviation of the Hg concentration data. Based on average Hg concentration, the data in figure 22 suggest that bearded seal should occupy a higher trophic level than polar bear. However, based on known diets of these animals, this is not the case. Bearded seal diets consist mainly of crabs, shrimp, clams, and snails while polar bears eat mostly ringed seals and sometimes walrus and beluga whale. Hg concentration in an organism can be affected by many factors including age and migration patterns of
the animal (note the variation of the bearded seal Hg data). More information, specifically $\delta^{15}N$ data, is needed to make any determinative trophic level assignments for the St. Lawrence Island ecosystem.

Figure 21
Comparison of d13C from two different ecosystems

Hobson and Welch, 1992

Arctic birds

Walrus

Polar bear

St. Lawrence Island

Bearded seal
Summary and Implications

The information provided from this analysis may be useful to the Yupik people when making choices about their diet. They are concerned about their exposure to mercury and heavy metal contaminants and can use the data for reference purposes. Based on the findings of this study residents can know which of the foods in their diet are likely to be safer for eating, in terms of heavy metal exposure. For example, if a pregnant or breastfeeding mother is looking to restrict her exposure to heavy metal contaminants, she could use this information to make educated food decisions, and know which types of foods to eat and to avoid. The data from this study show that muscle and fat tissue are generally safer to eat than the liver and kidney if one is concerned about heavy metals and the negative effects of Hg. Also, there is generally less potential for heavy metal exposure from consuming the tissue of animals that are lower on the food chain such as plants, crabs, fish, and walrus.
In the future this data could be compared to results from other studies that have been done on arctic marine food webs to see variation among these different ecosystems. This could help us get a better understanding of the sources of contamination. If the mercury concentration in foodstuffs is similar from one location to the next, we can assume that the mercury at these locations comes mostly from atmospheric deposition and not from a local source of pollution. Higher mercury levels in one location would indicate that there is a source of local contamination in that area. Statistical tests such as ANOVA (analysis of variance) could be used to determine if one location is significantly higher than another. Once identified, actions can be taken to remediate the source of pollution.

Conclusion

The overall conclusions of this study are: 1) Copper (Cu), Cadmium (Cd), and Mercury (Hg) are generally more concentrated in the liver and kidney tissues of St. Lawrence Island animals than in the fat and muscle tissues. 2) Arsenic (As) becomes concentrated in the fat and blubber tissues and rendered oil. 3) There is a general surplus of Selenium (Se) relative to Mercury (Hg) in all of the tissues analyzed; however, in fat, blubber, oil, liver, and kidney ratios may approach 1:1. 4) Based on the available data, metals appear to become biomagnified in the St. Lawrence arctic marine ecosystem. Further investigation into this topic is required to obtain a normally distributed data set for determination of statistically significant trends in metals bioaccumulation and biomagnification, and to use stable isotopes for quantitative assessment of trophic level transfer in this ecosystem.


Hobson KA and Welch HE. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}$C and $\delta^{15}$N analysis. Mar. Ecol.Prog. Ser 84 (1992) 9-18.


