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Locational differences in heavy metals and metalloids in Pacific Blue Mussels *Mytilus* [*edulis*] *trossulus* from Adak Island in the Aleutian Chain, Alaska

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Abstract

Increasingly there is a need to implement biomonitoring plans that can be sustained cost-effectively, focusing on single widespread (or closely-related species) in different parts of the world to detect exposure, potential damage to the organisms themselves, and risk to their consumers, including humans. Blue Mussels (*Mytilus edulis* and its relatives) have been widely used for environmental monitoring. One successful program that has achieved great coverage in time and space is “Mussel Watch”, and related programs exist in several regions. In this paper we use the Pacific Blue Mussel *Mytilus* [*edulis*] *trossulus* collected from five locations on Adak Island in the Aleutian Chain to examine five heavy metals and two metalloids, to test for locational differences as a function of anthropogenic activities, and to consider potential human health risks. Until the late 1990s Adak hosted a large U.S. military base, with multiple areas of contamination, some of which have been remediated. In June 2004 we identified four presumably human-impacted sites and a presumed unimpacted reference site, the latter on Clam Lagoon Beach, about 3 km from former military activity. No single site had the highest level of more than two metals, and the reference site had the highest levels of chromium and manganese. We subsequently found historic records of a former landfill within 1 km of the reference site. All of the locational differences were less than an order of magnitude, the greatest difference between the highest and lowest values being 4.5 times for lead. The highest correlations were between mercury and arsenic, mercury and lead, arsenic and lead, and chromium and manganese. Shell length was a better indicator of metals' levels than soft body weight, but the relationships were weak. There was no significant correlation between body size or weight with arsenic, lead, or selenium levels. There is substantial comparative data on these metals in mussels. Our results from Adak are generally within the range of mean values reported in the literature, except for the consistently elevated levels of chromium.

Keywords: Metals; Mytilus; Adak; Size relationships; Aleutians; Mercury; Chromium

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1. Introduction

Environmental managers, policy makers and the public are increasingly interested in assessing environmental quality over various geographical and temporal scales. Biomonitoring and surveillance are key methods for assessing the status or well-being of ecological receptors within functioning ecosystems (Cairns, 1990; Burger and Gochfeld, 2001, 2004). Monitoring can provide early warning of any changes that could result in significant risk to individual species (including humans), populations, communities and ecosystems. There are several paradigms to assess human and ecological health (NRC, 1983, 1993; Burger, 1997), including regulatory frameworks for risk management (NRC, 1986; Norton et al., 1992; EPA, 1997; Rand and Zeeman, 1998).

Evaluating environmental change, or performing risk assessments, involves establishing monitoring plans that use indicator species and biomarkers of exposure and effects. Since the hundreds of species in an ecosystem cannot all be monitored, it is essential to develop a suite of bioindicators that can be used to assess status and trends within that ecosystem and across regions (Piotrowski, 1985; O'Connor and Dewling, 1986; NRC, 1991; Peakall, 1992; EPA, 1997; Burger and Gochfeld, 2001, 2004; Carignan and Villard, 2001). While monitoring data for contaminants are useful for risk assessment (Suter, 1990, 1993, 1997), such data are also useful for managers and policy makers to assess status and trends without formal risk assessment.

The Blue Mussel (*M. edulis* and its relatives) have been widely used to monitor metals (O'Connor, 2002) and organics, and more recently radionuclides, endocrine active substances (Ortiz-Zarragoitia and Cajaraville, 2006), and marine toxins after algal blooms (Ciminiello et al., 2006). In the United States, the National Status and Trends Program, run by the National Oceanic and Atmospheric Administrations (NOAA) is an extensive biomonitoring program (O'Connor and Ehler, 1991), a key element of which is "Mussel Watch", initiated in the 1970s (O'Connor, 1996, 2002). It offers the most comprehensive measure of coastal marine metal pollution in the United States (Sarver et al., 2004). Similar programs have been instituted independently in some states (California, Gunther et al., 1999; New Hampshire, Jones et al., 2001), as well as other countries, such as France (Cossa et al., 2002), India (Senthilnathan et al., 1999), Russia (Tkalin et al., 1998), South Africa (Albertus and Kiviets, 2002), and Taiwan (Hung et al., 2001).

In this study, we examine metal levels in Pacific Blue Mussels *Mytilus* [*edulis*] *trossulus* from Adak in the Aleutian Chain to examine levels of seven elements, to test for locational differences as a function of anthropogenic activities, and to examine potential human health risks. In addition to the current local resident population (c100 people), Adak served as a U.S. military base and air station from the early 1940s until 1997, when most military activity ceased, except for site monitoring and remediation. At one time more than 10,000 military personnel lived there. Adak Village (51°52′ × 176°38′) is located about 1900 km southwest of Anchorage. The island is bordered by the Pacific Ocean to the south and the Bering Sea to the north. We test the null hypotheses that there are no differences in metal levels in different sites around northeastern Adak (where the level of military and other human activities varied) and that Adak metal levels do not differ from those reported in mussels elsewhere.

Mussels of the genus *Mytilus* and related genera are useful as bioindicators because they have wide distribution on all continents from tropical to high latitudes. They are filter feeders extracting metals from water and particulate matter, and they store some metals at levels high above those in the abiotic environment (high biological concentration factor or BCF) (Perez et al., 2001). They are readily accessible by hand picking in the intertidal or by dredging in the subtidal zones. Although the taxonomy of *Mytilus* is controversial (Seed, 1992), several closely-related species with similar physiologic properties, have received extensive study, and are sometimes treated as a single species, *M. edulis*. These include *M. trossulus* at high latitudes, *M. galloprovincialis* in most of Europe, *M. californianus* on the Pacific Coast of North America, and *M. edulis* (sensu stricto) along Atlantic coasts. Mussels have also been transplanted to various waters for commercial aquaculture. Enclosing *edulis* in square brackets, as in *Mytilus* [*edulis*] *trossulus*, indicates that *M. trossulus* is a member of the *edulis* superspecies, a way of representing both its separate species status as well as its close relationship to Blue Mussels elsewhere.

Metal uptake for mussels from the aqueous and dietary phase is additive (Wang, 2002). Uptake from water may be less efficient than from food; transplant studies have shown that only about 1% of the total mercury content, and 20–50% of the methylmercury content in water is accumulated in the shellfish tissues (Mikac et al., 1996). Further, absorption efficiency is negatively correlated with amount of water that mussels pumped (Wang, 2001). Levels of some metals in mussels correlate with metal levels in sediment, (Puente et al., 1996), however, the relationship is complex (Yap et al., 2002) and non-linear. Mussels are usually attached to rocks, and are not in direct contact with the sediment which is resuspended and wafted by them by wave action.

The Mussel Watch data have been analyzed for spatial and temporal patterns within U.S. waters (O'Connor, 1998), as well as comparing levels in U.S. waters with those from elsewhere (Beliaeff et al., 1998). The U.S. Mussel Watch results from coastal U.S. waters show a decreasing trend in cadmium from 1986 to 1996 (O'Connor, 1998). Cadmium levels are higher around U.S. waters than in France, but mercury levels are higher in France (Beliaeff et al., 1998). However, Blue Mussels are generally limited to temperate waters, limiting their usefulness in tropical environments (Manly et al., 1996), where other species are found (i.e. *Perna* spp. Otchere et al., 2003; Sarver et al., 2004; *Crenomytilus grayanus* Tkalin et al., 1998, *Geukensia demissa*, Park and Presley, 1997).

Mussels have been used to examine a range of contaminant issues, including temporal (Chiu et al., 2000) and seasonal (Otchere et al., 2003) patterns, spatial patterns (Julshamn et al., 2001; Gutierrez Galindo and Muñoz-Barbosa, 2003; Sun et al., 2004; Chou et al., 2004), geographic gradients in contamination (Muñoz-Barbosa et al., 2000), and effects that relate to age (Krolak and Zdanowski, 2001; Otchere et al., 2003), size (Riget et al., 1996; Tewari et al., 2000; Wiesner et al., 2001; Szefer et al., 2002), sex (Sidoumou et al., 1999), and salinity (Struck et al., 1998; Blackmore and Wang, 2003). Green-lipped mussels *Perna viridis* show declines in lead levels in Hong Kong, reflecting the introduction of lead-free gas in 1991 (Chiu et al., 2000). In general, metal levels are higher in winter (Avelar et al., 2000; Unsal, 2001; Odzak, 2002) or at the end of winter (Kaimoussi et al., 2000), and in older animals (Odzak, 2002). The increase in winter or late winter may be due to life cycle differences that influence uptake, storage, and/or excretion or body condition. Metals are higher just before spawning (Nasci et al., 1998). Some studies report metal levels increasing with size of the mussel (Tewari et al., 2000; Wiesner et al., 2001), although copper, lead, and in some cases mercury, decrease with shell size. Wright and Mason (1999) found that mercury levels increased with shell size, but cadmium and zinc did not.

Mussels are also useful because they can be translocated to different habitats with different degrees of contamination, thus examining uptake rates and effects (Romeo et al., 2003; Geffard et al., 2004), as well as assessing contaminants levels at different distances from point sources (Ruus and Klungsoeyr, 2002; St-Jean et al., 2003). While the ability to transplant them has been useful for their domestication and farming (Andersen et al., 1996; Anon., 2003), it is critical to understand uptake rates of pollutants such as heavy metals.

Contaminants in mussels have also been examined from the standpoint of food safety, and in some places levels of contaminants exceeded health standards, including in Chile (cadmium, copper and zinc, de Gregori et al., 1996), China (Sun et al., 2004; Fung et al., 2004), Hong Kong (cadmium and chromium, Fang et al., 2003), Adriatic Sea (arsenic, Jureša and Blanuša, 2003), Italy (arsenic, Giusti and Zhang, 2002), and even in the U.S. (Gulf of Maine, lead, Jones et al., 2001). Usually, however, metal levels are well below safety standards (Brazil, Baraj et al., 2003; Newfoundland, Veinott et al., 2003; Greece, Catsiki et al., 2001; Ireland, Bloxham et al., 1998; Trinidad and Venezuela, de Astudillo et al., 2002). Sometimes, levels of metals can be higher in mussels than in the local edible fish (e.g. lead and cadmium, Adriatic Sea, Jureša and Blanuša, 2003; several elements, Julshamn and Grahl-Nielsen, 1996), bolstering their importance as a bioindicator. In the Adriatic Sea, lead and cadmium levels in mussels were up to 10 times higher than those in fish (Jureša and Blanuša, 2003), and in the Philippines where mercury-contaminated river water was used to irrigate paddy fields, mussels had 5 fold higher mercury levels than fish (Appleton et al., 2006).

2. Methods

2.1. Study areas

Adak is a treeless island in the Andreanof Island group of the Aleutian Chain, approximately 1900 km southwest of Anchorage. The Aleutians separate the Bering Sea from the North Pacific. Most of its population resides in the town of Adak (Fig. 1), which has an airport and seaport (51°51′ N; 176° 37′ W). The island is partly owned by the U.S. Fish and Wildlife Service, and partly by the Aleut Corporation. The Aleutian Islands were established as a National Wildlife Refuge in 1913 by the executive order of President Taft (ATSDR, 2002). Formerly a Naval Air Facility occupied the northeastern portion of the island from 1942, but the base operations closed in 1997 (ATSDR, 2002), and military activities have been phased out. Cleanup of hazardous waste has occurred in some areas, and removal of unexploded ordnance was ongoing during our sojourn in 2004.

Early in World War II, over 100,000 military personnel staged at Adak prior to an assault on Japanese-held Kiska Island. Subsequently about 10,000 troops and

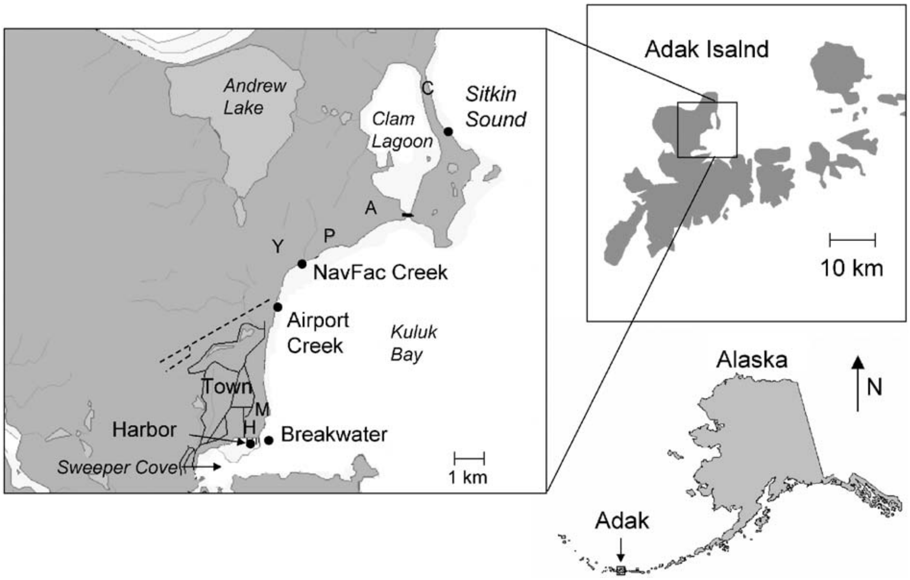

Fig. 1. Map of northeastern Adak Island. Heavy dots indicate the five sampling areas: Harbor, Breakwater, Airport Creek, NavFac Creek, and Clam Lagoon Beach. Letters indicate possibly relevant contaminated sites: H = hazardous waste, M = metal dump, Y = maintenance yard along NavFac Creek, P = Palisades dump, A = Antenna Field, C = Causeway Landfill. Dashed line indicates airport runway.

their dependents were stationed on the island; thus the potential exists for historic contamination of the marine environment, particularly near the seaport and airport areas of Akak. In 1994 the Naval Air Station at Adak was placed on the National Priorities List based on an Environmental Protection Agency (EPA) Resource Conservation and Recovery Act (RCRA) inspection (ATSDR, 2002).

Under appropriate permits from the State of Alaska Department of Fish and Game (CF-04-043), we collected Blue Mussels in June 2004 from five locations in northeastern Adak (see Fig. 1): 1) the Adak harbor in Sweeper Cove, 2) the breakwater (just outside the harbor), 3) Airport Creek mouth at east end of airport runway, 4) NavFac creek mouth near a maintenance area, and 5) at a relatively remote site on the Sitkin Bay/Bering Sea coast at Clam Lagoon Beach. Sites 2, 3, and 4 are on Kuluk Bay. We predicted that metals' levels would be highest in mussels from sites 1 and 2, intermediate at sites 3 and 4, and lowest at site 5, based on the current relative levels of human activity and our observations of military use and debris.

2.2. Mussel collection and preparation

Our overall protocol was to collect mussels representing the range of sizes available at a site, by hand-picking from the intertidal rocks. We collected at randomly selected points along a transect parallel to the shore. Since most published studies of metals in mussels do not mention depuration, and since we were interested in the exposure of ecologic as well as human consumers, we did not depurate the mussels. Samples were stored in plastic bags, labelled, and frozen for transportation to the Environmental and Occupational Health Sciences Institute for dissection and elemental analysis. We recorded the length and width of the shell, weight of the whole body, and weight of the soft tissue (Tables 1 and 2), and then removed the soft tissue for analysis. We measured the moisture content of a subset of mussels by desiccation in an oven at 57 °C for 24 h. The dry weight ranged from 13.7% to 21.3% (mean 18.5%) of the wet weight, yielding an average conversion factor of 5.4 (dry:wet). Thus, for Table 4, comparing the Adak results to other studies, we multiplied all our values by 5.4, since most studies report results on a dry weight basis.

All mussels were analyzed individually rather than as composites. Approximately 2 g (wet weight) of mussel tissue was weighed to 0.1 mg and digested in ultrex ultrapure nitric acid in a microwave (MD 2000 CEM), using a digestion protocol of three stages of 10 min each under 50, 100 and 150 lbs per square inch (3.5, 7, and 10.6 kg/cm^2) at 80X power. Digested samples were subsequently diluted in 100 ml deionized water. All laboratory equipment and containers were washed in 10% HNO_3 solution and deionized water (18 MΩ) rinse, prior to each use (Burger et al., 2002).

Table 1
Overall descriptive statistics for size variables and metal levels in Blue Mussels (*Mytilus* [*edulis*] *trossulus*) from northeastern Adak Island, Aleutians, Alaska ($n=125$)

	Arith. mean (ppm)	Standard deviation	CV (%)	Maximum value (ppm)	Ratio of highest site average to lowest
Shell length (mm)	47.1	7.32	15	67.0	1.16
Shell width (mm)	21.6	3.09	14	27.8	1.15
Shell weight (g)	12.9	5.41	42	27.0	1.54
Soft part weight (g)	4.50	1.99	44	9.0	1.62
Arsenic (ppm)	1.98	2.78	140	32.2	2.98
Cadmium (ppm)	0.99	0.53	45	2.98	1.98
Chromium (ppm)	20.7	23.0	111	148	2.76
Lead (ppm)	0.49	0.98	199	8.29	4.47
Manganese (ppm)	4.59	5.35	117	47.6	1.62
Mercury (ppm)	0.016	0.007	47	0.04	3.01
Selenium (ppm)	0.90	1.50	167	11.1	3.99

All metal levels are in μg/g (ppm wet weight). Multiply by 5.4 to estimate comparable dry-weight concentrations. CV = coefficient of variation (100*standard deviation/mean).

2.3. Analysis and quality control

Mercury was analyzed by the cold vapor technique using the Portable Zeeman Lumex (RA-915) mercury analyzer, with an instrument detection level of 0.2 ng/g (0.2 ppb), and a matrix level of quantification of 2 ppb. All other metals were analyzed with graphite furnace atomic absorption (GFAA). Instrument detection limits on the GFAA were 0.2 ppb for arsenic, 0.1 ppb for cadmium, 1.0 ppb for chromium, 2.0 ppb for lead, 1.0 ppb for manganese, and 0.5 ppb for selenium. Matrix detection levels were about an order of magnitude higher. All concentrations are expressed in parts per million (ppm = μg/g), of total metal on a wet weight basis. All batches included blanks, a standard calibration curve, a spiked sample and a DORM-2 Certified dogfish tissue sample. The correlation for the calibration curves exceeded 0.995 each day. Spike recoveries between 85–115% were accepted and DORM results within 10% were accepted to validate a batch. 10% of samples were digested twice and analyzed as blind replicates (with agreement within 15%). We used Kruskal Wallis non-parametric one way analysis of variance (generating a χ^2 statistic) to examine locational differences. We also used ANOVA with Duncan Multiple Range test on log-transformed data to identify the significant differences (SAS, 1999). Kendall correlations were used to examine relationships among metals and with length and weight. The level of significance was alpha = 0.05.

3. Results

3.1. Locational differences

The overall descriptive statistics are given in Table 1. There were significant inter-location differences for all metals except chromium (which neared significance, Table 2). The highest levels of arsenic and lead occurred along the outer edge of the breakwater just outside the harbor. The harbor had the highest mercury level. The highest level of cadmium was at Airport Creek, and the highest level of selenium was at the NavFac creek. The Bering Sea site at Clam Lagoon Beach had the highest levels of manganese and chromium. Thus, no one site had the highest levels of more than two metals (Table 2). Further, all of the locational differences were within one order of magnitude. Thus the putative reference site had high levels of two metals, although the levels of arsenic, selenium, and mercury were lower there than elsewhere.

3.2. Relationships among metals

We examined inter-metal correlations for all 125 specimens using the non-parametric Kendall tau. Of the 21 possible pairwise comparisons among seven metals, eight were positive ($p<0.02$), two were negative (mercury and manganese, chromium and selenium, Table 3). The highest correlations were between mercury and arsenic, mercury and lead, arsenic and lead, and chromium and manganese. Mercury and selenium were positively correlated (tau = .37, $p<0.0001$).

3.3. Size and metals

There was a significant positive relationship between all size measurements (all Kendall tau correlations > 0.52, $p<0.0001$) and chromium and manganese (Table 3). Shell length was also positively correlated with cadmium and with mercury. Surprisingly, the correlation coefficient between shell length and width was 0.67, which is lower

Table 2
Contaminant levels in Blue Mussels (*Mytilus* [*edulis*] *trossulus* collected from five locations on Adak Island (Aleutians, Alaska; summer 2004)

	Adak Harbor geometric mean± SE n=25	Breakwater geometric mean± SE n=25	Airport Creek geometric mean± SE n=25	NavFac Creek geometric mean± SE n=25	Clam Lagoon geometric mean± SE n=25	Kruskal-Wallis One Way ANOVA among sites χ^2 (p)
Arsenic	2.19±0.06 2.17(A,B)	3.63±1.19 2.66 (A)	1.37±0.05 1.35 (B)	1.52±0.07 1.48(B)	1.22±0.11 1.13 (B)	χ^2=80 p<0.0001
Cadmium	0.995±0.88 0.790(B)	0.807±0.06 0.720(B)	1.51±0.15 0.830(A)	0.76±0.46 0.730(B)	0.875±0.08 0.810(B)	χ^2=28 p<0.0001
Chromium	20.7±3.28 15.2 (B)	19.0±2.17 16.8 (B)	12.5±1.62 10.1 (B)	17.0±3.63 12.4 (B)	34.5±8.20 14.5 (A)	χ^2=8.0 p<0.08
Lead	0.228±0.021 0.157 (B)	1.02±0.141 0.814 (A)	0.289±0.112 0.131 (B)	0.597±0.329 0.248 (A,B)	0.330±0.208 0.129 (B)	χ^2=53 p<0.0001
Manganese	4.47±0.930 3.52 (B)	2.96±0.19 2.82 (B)	3.24±0.26 3.05 (B)	4.71±0.65 4.11 (B)	7.26±1.99 4.66 (A)	χ^2=14 p<0.006
Mercury	0.024±0.001 0.023 (A)	0.020±0.001 0.019 (B)	0.017±0.001 0.016 (C)	0.011±0.001 0.011 (D)	0.008±0.001 0.008 (E)	χ^2=83 p<0.0001
Selenium	0.690±0.019 0.683 (B)	0.702±0.033 0.684 (B)	0.898±0.120 0.790 (B)	1.76±0.635 0.832 (A)	0.441±0.056 0.382 (B)	χ^2=36 p<0.0001

Analyses were performed on individual (non-composited) specimens (n=25 per site) (All results are in µg/g = parts per million on wet weight basis, rounded to three significant figures). Each cell includes the arithmetic mean+standard error, over the geometric mean. (Multiply values by 5.4 to estimate equivalent concentrations on dry weight basis). Different letters in parentheses indicate significant differences among means within the row, using Duncan post-hoc test.

than expected for such linear measurements (typically closer to 0.9), indicating wide shape variation in the growth form of the mussels. Shape may be constrained by the densely-packed growth form. There were slight size differences among the sites.

There was no significant correlation between body size or weight with arsenic, lead, or selenium levels (Table 3). The strongest correlations among metals' levels in soft tissue and size or weight measurements was between: 1) soft tissue mass and chromium (negative), and 2) shell length and cadmium (negative), manganese (negative), and mercury (positive). It is surprising that shell length was a better indicator of metals' levels than soft body weight, but perhaps shell measurements more accurately reflect both size and quantities of water that pass over the body, while body weight may reflect variable conditions.

4. Discussion

4.1. Methodological issues

There is a large and variable literature on metals in mussels, dating back to the 1970s. Several long-term studies show the utility (and also complexity) of using mussels to assess long-term trends (O'Connor, 1996). Many studies have used mussels for comparing contaminated with uncontaminated sites or for examining spatial patterns of contamination and the contribution of point source pollution. Care must be taken, however, because of the relationships among metals. For example, in green mussels (*P. viridis*) the assimilation efficiency of methylmercury from their foods is independent of the inorganic selenium loadings, while selenomethionine inhibits the uptake of methylmercury

Table 3
Intermetal correlations in Blue Mussels (*Mytilus* [*edulis*] *trossulus*) from Adak (Aleutians, Alaska) using non-parametric Kendall tau (p values)

	Arsenic	Cadmium	Chromium	Lead	Manganese	Mercury	Selenium	Shell length	Soft weight
Arsenic	–	NS	NS	0.40 (0.0001)	NS	0.5 (0.0001)	0.3 (0.0001)	NS	NS
Cadmium		–	NS	NS	−0.01 (0.05)	0.2 (0.0003)	0.2 (0.004)	0.2 (0.0002)	NS
Chromium			–	NS	0.4 (0.0001)	NS	−0.1 (0.05)	−0.2 (0.0004)	−0.3 (0.0001)
Lead				–	NS	0.3 (0.0001)	0.2 (0.002)	NS	NS
Manganese					–	NS	−0.4 (0.0001)	−0.3 (0.0001)	−0.3 (0.0001)
Mercury						–	0.2 (0.0009)	0.2 (0.0009)	NS
Selenium							–	NS	NS
Shell length								–	0.6 (0.0001)
Soft tissue weight									–

N=125 individual samples.

(Wang et al., 2004). The effect of selenium on metal uptake and effects, however, is complicated, and in some cases the presence of selenium may decrease the effects of cadmium also (Ferrarello et al., 2002). Further, care must be taken in interpreting levels of some metals, such as zinc and copper, which are internally regulated (Shulkin et al., 2002, 2003). Mussels and bivalves in general are known to be efficient accumulators of certain metals, particularly zinc, which can be stored against a concentration gradient of orders of magnitude. Other metals, particularly lead and mercury, are not bioconcentrated efficiently by bivalves (Zachariadis et al., 2001).

In this study we used the soft tissue, mainly because that is the tissue consumed by people and other predators. While some authors have reported that the byssus organ (the hairs formed at the base of attachment) and the shell are better accumulators than the soft tissue for some metals (Szefer et al., 1999; Yap et al., 2003a,b), other studies have found the shell to be less useful than the soft tissues (Szefer et al., 2002). Moreover, there is a high positive correlation between the metal concentrations of the byssal and soft tissue (Szefer et al., 2002), making the soft tissue useful as a general bioindicator of environmental quality.

4.2. Locational differences in metal levels

Mussels take up metals through the gills from the water column and through ingestion of food and particulates. Concentrations in water are seldom reported, and concentrations in sediments have variable relationships to mussel levels, probably because the mussels are growing mainly on rocks, and are only exposed to fine sediments resuspended by wave action. Ultimately, the tissue levels can be attributed to natural metal levels in seawater, local point sources, tide and current transport, and atmospheric deposition. For example, in the Arctic mercury is removed from the atmosphere and deposited in snow, which can enter the marine environment as snowmelt, thereby transferring atmospheric mercury to Arctic and sub-arctic food webs (AMAP, 1998). Volcanic activity and erosion of mercury ores (cinnabar) also contribute to environmental transport of mercury. Metals associated with organic-coated particles are generally absorbed to a greater extent than those associated with uncoated particles (Gagnon and Fisher, 1997).

We had predicted that, in general, metal levels in mussels should be highest in the harbor, where both natural currents and direct deposition from ship and land-based runoff occurs, and lowest at Clam Lagoon Beach. The harbor experienced contamination, not only during the years of naval occupation, but in the post-occupation cleanup. Metal debris was evident along the shoreline and in the intertidal at many points along the harbor and Sweeper Cove. Ship and vehicle maintenance sheds, military warehouses and derelict equipment occupied much of the shoreline. The two creeks would have had intermediate impacts, while the Clam Lagoon Beach site had no visible habitation, industry, or other activities nearby that might result in direct anthropogenic sources.

Although there were significant locational differences in levels for most metals, the two harbor sites (inner harbor, and breakwater) had the highest levels for only three analytes (arsenic, lead, mercury). Cadmium was highest at Airport Creek; selenium was highest at Navfac Creek, and chromium and manganese were highest at Clam Lagoon Beach (refer back to Table 1). A similar anomaly has been reported for mussels analyzed from St.Paul Island in the Bering Sea; samples from the seemingly least-impacted area had the highest metal levels (Lauenstein et al., 2001). However, for all metals the differences were less than an order of magnitude. The greatest comparative difference was in lead, which was highest at the breakwater, which might reflect past use of leaded fuel on the dock, disposal of batteries, or lead shot, accumulating in organisms living on the rocky surfaces near the jetty. While there was a locational difference for mercury, the overall levels in mussels were very low.

Cadmium was highest at the Airport Creek, and cadmium levels were generally high (see below), reflecting a trend noted by other authors of high cadmium levels at high latitudes. Cadmium levels in mussels (*M. trossulus*) from near the Kurile Islands in the North-west Pacific Ocean were also unusually elevated, but were attributed to regional upwelling (Kavun et al., 2002). Cadmium levels in some invertebrates from the Barents Sea were also high, but cadmium levels for *M. edulis* were within the worldwide range of 1–2 μg/g (Zauke et al., 2003). The U.S. Mussel Watch results from coastal U.S. waters show a decreasing trend in cadmium from 1986 to 1996 (O'Connor, 1998).

Metallothionein levels are usually correlated with cadmium levels (Geffard et al., 2002), and in some cases exposure to cadmium and subsequent induction of metallothionein-like proteins affected cadmium uptake (Blackmore and Wang, 2002). Other factors, such as particle size affect assimilation efficiency of metals, such as selenium and zinc (Ke and Wang, 2002). Cadmium and zinc inhibit each other's uptake rates, but the mechanism is unclear (Vercauteren and Blust, 1999).

4.3. Current evidence of contamination

Even today the Adak Harbor is a functional seaport with a small resident fishing fleet, as well as visiting ships. Some of the large warehouses are still in use. Abundant naval debris as well as heavy equipment was sunk in parts of the harbor, and scrap metal and cable can be seen on both the Kuluk Bay and Sweeper Cove side of the breakwater. Debris, scrap metal, and rusting machinery and vehicles are stored at the maintenance yard close to NavFac Creek. The main residential community, occupying much of the former naval housing, is about 1–2 km from the harbor itself. On the other hand, the Clam Lagoon site appears relatively undisturbed, and is about 3 km from abandoned military facilities; we did not detect surface evidence of contamination there.

4.4. Historic evidence of contamination

Faced by a perplexing pattern of metal levels, we sought historical documentation of contamination. In 1986 the U.S. Navy identified 32 areas on Adak contaminated with hazardous wastes including storage areas, drum disposal sites, oil depots, petrochemical spills, and landfills including metals waste pile. After the base was placed on the NPL, investigations expanded until 128 contaminated sites were identified involving soil, sediment, and ground and surface water, as well as unexploded ordnance. Partial remediation activities were completed as part of base closure. However, search for unexploded ordnance was ongoing during our visit in 2004, and areas of residual contamination remain in several areas. Sweeper Cove and Kuluk Bay are considered contaminated and are under fish advisories, mainly related to polychlorinated biphenyls (ATSDR, 2002). Other institutional controls are in effect for many other sites in northeastern Adak (U.S. Navy, 2005). The distribution of waste sites readily accounts for contamination at the harbor, airstrip and NavFAC creek, and we were surprised to learn that there was a waste dump site on the causeway at Clam Lagoon, about km from our sampling site (see letter C on Fig. 1).

Several former waste sites are of particular interest. The former Pesticide Disposal Area in Adak town (1950–1987) is a likely source of lead, arsenic, and manganese. Today the site is covered with gravel and sparse vegetation. A hazardous waste storage facility was located on the edge of a town close to the breakwater, and had received chemical and petrochemical waste, mostly uncharacterized (ATSDR, 2002). Runoff and leachate from this site would have impacted mainly the breakwater site. The Metals Landfill, a CERCLA site, is located on the edge of Adak Town and drains into Kuluk Bay just north of the Breakwater. Estimates of the former dump size range from about 8 to 20 ha (ATSDR, 2002; U.S. Navy, 2005). The records indicate that a variety of scrap metal, metal waste, cutting oils and petrochemical products (lubricating oil, grease) were disposed of.

Airport Creek and NavFac Creek empty into Kuluk Bay, which had extensive contamination. The paved airstrip itself would have been a source of stormwater runoff, particularly of lead from certain aviation fuels. Improper disposal of batteries operating landing field lights was also a potential source of local contamination. Nearby Palisades Creek also empties into the Bay, and drains the former Palisades Landfill (1940s to 1970), a multipurpose disposal site. This landfill was a known source of chromium which impacted Blue Mussels at the Creek mouth (U.S. Navy, 2005). NavFac Creek drains an area impacted by maintenance yards and currently by scrap metal, derelict vehicles, construction debris, and abandoned buildings. It may also be impacted by leachate from the “Antenna Field” waste site, which was believed to contain oil and gasoline from leaking underground storage sites. This field was not excavated and is considered a “Monitored Natural Attenuation Site”.

The surprise was identification of the Clam Lagoon “Causeway Landfill”, about 1 km from our sampling site, which operated from mid-1950s to 1960s, receiving sanitary waste, construction debris, scrap equipment and “other refuse” (U.S. Navy, 2005). The landfill was not excavated, but was covered with dirt, and eventually with tundra vegetation. This 3 ha site was not apparent to us in June 2004. Thus the historic record provides ample evidence of contamination sources for metals (and organics) for all five of the sites were surveyed on Adak in 2004.

4.5. Geographical differences in metal levels

There are few comparative data for mussels in the Aleutian Islands. Although the U.S. Fish and Wildlife Service examined contaminants, including heavy metals, in some wildlife at Amchitka Island (Crayton, 2000), they did not examine contaminant levels in any invertebrates. Thus our data contribute to the overall data base on metals in mussels.

Table 4 summarizes published data on the levels of the metals we studied in mussels from various parts of the world. The literature review covered several named “species” of *Mytilus* including *M. californianus* and *M. edulis* in North America, *M. galloprovincialis* in

Table 4
Ranges of mean values of metal concentrations in Blue Mussels by geographic region

Geographic region	# of studies	Arsenic	Cadmium	Chromium	Lead	Manganese	Mercury	Selenium
Adak, Alaska[a]	This paper	6.0–19.0 $n=5$	3.7–7.5 $n=5$	62–170 $n=5$	1.2–5.1 $n=5$	15–36 $n=5$	0.4–.12 $n=5$	2.2–8.8 $n=5$
Northeast Asia and Northwest Pacific[b]	5[c]	0.01–0.14 $n=2$	0.48–34(66) $n=7$	0.04–15.7 $n=5$	0.73–5.6 (283) $n=9$	2.8–6.7 $n=3$	0.2–2.6 $n=6$	3–6.9 $n=2$
Greenland and northern Europe to White Sea[d]	8[e]	1.0–13 $n=2$	0.4–5.0(100) $n=9$	1.3 $n=1$	0.35–15(800) $n=9$	5–98 $n=2$	0.08–5.0 $n=1$	4.1 $n=1$
Britain, Southern and Eastern Europe[f]	13[g]	nd–18	0.2–6.4(134) $n=4$	nd–7.6 (42) $n=11$	nd–11.6(510) $n=4$	4.3–41 (170) $n=13$	0.03–0.6 $n=5$	no data $n=7$
North America[h]	7[i]	3.7–10.5 $n=3$	0.6–10 (67) $n=11$	0.4–19(310)[j] $n=7$	0.04–4.8(270) $n=9$	4–22 $n=3$	.07–.43 $n=7$	.23–3. $n=42$

All values are in μg/g (parts per million, dry weight basis). The number of data points contributing to each cell is indicated by n (not all studies covered all metals). Outlier maxima are given in parentheses. Based on moisture content from this study, Adak results in Table 1 have been multiplied by 5.4 to achieve a dry weight estimate. nd = non-detectable.

[a] *Mytilus trossulus*.

[b] *Mytilus edulis, M. trossulus, Crenomytilus grayanus*.

[c] Szefer et al. 1999; Tkalin et al. 1998; Shulkin et al. 2003; Kavun et al. 2002; Fung et al. 2004.

[d] *Mytilus edulis* and *M. trossulus*.

[e] Riget et al. 1996; Rainbow et al. 2004; Green and Knutzen 2003; Andersen et al. 1996; Julshamn and Grahl-Nielsen 1996; Zauke et al. 2003; Herut et al. 1999; Millward et al. 1999.

[f] mainly *M. galloprovincialis*.

[g] Puente et al. 1996; Beiras et al. 2003; Beliaeff et al. 1998; Giustia and Zhang 2003; Licata et al. 2004; Jureša and Blanuša 2003; Zachariadis et al. 2001; Rainbow et al. 2000; Odzak 2002; Topcuoglu et al. 2002; Wright and Mason 1999; Zauke et al. 1995a; Szefer et al. 2002.

[h] *Mytilus edulis*, *M. californicanus, Modiolus demissus*.

[i] O'Connor 1998, 2002; Muñoz-Barbosa et al. 2000; Park and Presley 1997; Paulson et al. 2003; Galloway et al. 2002.

[j] New Bedford Harbor, MA, USA.

southern Europe, and *M. trossulus* at high latitudes. Mussels from east and southeast Asia were identified as *P. viridis*, with *Perna perna* from Ghana and *Perumytilus purpuratus* from South America. Some studies included *Modiolus demissus*=*G. demissa*, the Ribbed Mussel.

Given the fluid and controversial status of mussel taxonomy, and the fact that several species are recognized as part of the *M. edulis* complex or superspecies, or are even treated as conspecific (Seed, 1992) no distinction among species has been made. It is likely that the ecotypic variation within species, and the selection for resistant phenotypes might have a greater influence on the toxicokinetics of metals in mussels, than any over-riding "species" differences. Indeed, one study, found no "important concentration differences" between two species (*M. edulis* and *M. californianus*) living in close proximity off the Columbia River (Washington State) (O'Connor, 2002).

It is apparent that the mean levels found in mussels from most parts of Adak are within the ranges of means commonly reported in the literature, including both contaminated and non-contaminated sites. However, chromium levels from all five sites were among the highest reported in the literature, exceeded only by New Bedford Harbor, Massachusetts. Since both the urban and remote sites on Adak showed high chromium levels, it is difficult to identify a point source responsible for this elevation. The selenium level of 8.8 ppm at NavFac creek is the highest mean value reported.

4.6. Data limitations

In reviewing the literature on metals in mussels, it became apparent that there is no standard protocol. Most, but not all, studies are reported on dry weight basis. Most give some indication of sample size, but it is not always clear how many individuals went into how many pools. Some, but not most, specify the size of mussels collected. Some papers indicate whether collections were intertidal or subtidal. Some studies allowed depuration for one or more days. And descriptive statistics vary from arithmetic or geometric means to ranges. Maximum values are not always apparent.

Most frustratingly, while the data in the NOAA Mussel Watch program are extremely important for comparing contaminants on a national level among sites, they are less useful for comparisons in individual studies because the mean levels themselves for individual sites are not published (O'Connor 1996, 2002;

Sarver et al., 2004). While this method of presentation makes it immediately clear which harbors, bay, estuaries or other coastal areas have the highest levels comparatively, it does not allow direct comparison with the actual contaminant levels. This suggests that such summary data should be readily and easily available to the World scientific community.

4.7. Metal levels and risk

One important use of data on contaminants in mussels, in addition to serving a useful biomonitoring role because of their extensive use worldwide, is the determination of whether the levels pose a risk to the mussels themselves, or to organisms that consume them (including humans. The *Codex Alimentarius*, a joint FAO/WHO agency, proposes (CODEX, 2005) a cadmium standard of 1.0 ppm and lead standard of 0.5 ppm for molluscs (wet weight). Shellfish are known to be effective at bioconcentrating metals, and the role of metalloproteins is a fertile area of investigation (Leignel and Laulier, 2006).

For human consumption, WHO (1976) sets a limit of 0.5 μg/g (wet) for mercury in shellfish, although the U.S. level is 1 ppm. EPA has set arsenic residues of 1.3 ppm in freshwater fish for human protection (Eisler, 1994). There is no standard for cadmium in fish. The Codex Alimentarius (2005) is standards range from 0.3 to 2.0 ppm. We did not find standards for manganese in seafood. Chromium has virtually no standards, with Hong Kong having the lowest standard at 1 ppm.

Metallothioniens probably play important roles in transporting both essential and toxic cations in mussels as in vertebrates. Preliminary spectropolarimetric study demonstrated unique secondary conformation of cadmium-metallothionein from mussels (Vergani et al., 2005). In vitro studies showed that c-AMP content of mussel gills was stimulated by micromolar concentrations of hexavalent Cr, but inhibited in the millimolar range (Fabbri and Capuzzo, 2006). Risk to organisms has not been well characterized. Mercury levels of 5 ug/g cause disease in fish (Eisler, 1987). Study of these effects must take into account complex interactions with environmental variables and other metals. Thus, although lead affects metabolism in *P. perna* (Pessatti et al., 2002), interactions among lead, cadmium, and copper appear to influence stress responses in *M. edulis* (Radlowska and Pempkowiak, 2002). Likewise, several metals affect growth in this species in the Irish Sea (Widdows et al., 1999). Metals such as cadmium, zinc, and copper altered the response of cell signaling pathways in *Mytilus* mantle tissue to thermal stress (Kefaloyianni et al., 2005). In addition to direct measurement of residues in tissues, it is possible to use response biomarkers such as induction of heat shock protein (HSP) by arsenic (La Porte, 2005).

4.8. In conclusion

The Blue Mussel complex is already in widespread use for biomonitoring, although there seems to be no standardized approach for sampling, analysis, and reporting of results. There is sparse information on moisture content of *Mytilus*, and this is not constant within a population. Studies that analyze on a dry weight basis are well-positioned to provide moisture content data. Studies reporting on a wet weight basis need to provide moisture information so a comparison can be made. Studies using depuration should analyze some samples without depuration so the magnitude of change is documented.

It took extensive historical research to determine that our chosen reference site was not at all pristine, regardless of its current appearance. There are many more papers reporting on copper and zinc levels, despite the fact that these are under some physiologic control. More attention needs to be paid to the toxic metals mercury, lead and cadmium, particularly since the latter is higher in high latitude sea life. A minority of papers report on the size of mussels analyzed and since size reflects both age and growth rate, this information would be useful. The format used for reporting the U.S. Mussel Watch data is not conducive to comparison with other waters. Increased standardization will enhance the usefulness of Mussel watches as a means of biomonitoring the marine environment on all coasts. Experimental studies on growth rate, uptake, and assimilation will make it easier to interpret comparative data among mussel genera: *Mytilus, Crenomytilus, Modiolus, Geukensia*, and *Perna*. Most reports cover a single time frame. Developing sustainable monitoring programs is essential for providing temporal trends which can illuminate both the worsening and improvement of estuarine contamination by metals, organics, and radionuclides.

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