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Bioeffects Assessment in Bristol Bay, Alaska: Characterization of Soft Bottom Benthic Habitats, Fish Body Burdens and Sediment Contaminant Baseline Assessment in Kvichak and Nushagak Bays

NOAA National Centers for Coastal Ocean Science
Center for Coastal Monitoring and Assessment

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January 2017

N O A A T E C H N I C A L M E M O R A N D U M N O S N C C O S 2 2 7

NOAA National Centers for Coastal Ocean Science

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Bioeffects Assessment in Kvichak and Nushagak Bays, Alaska:

Characterization of Soft Bottom Benthic Habitats, Fish Body Burdens and Sediment Contaminant Baseline Assessment

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Executive Summary

A baseline environmental characterization of the northern reaches of Bristol Bay, Alaska was conducted using the sediment quality triad approach (sediment chemistry, sediment toxicity, and benthic invertebrate community structure), along with measures of contaminant body burdens and the characterization of parasites and disease in starry flounder (*Platichthys stellatus*, Pallas, 1788) and rainbow smelt (*Osmerus mordax*, Mitchill, 1814). The study area was subdivided into 6 strata based on geophysical and hydrodynamic patterns (the upper and lower reaches of Nushagak and Kvichak Bays, Dillingham Harbor and the mouth of the Naknek River). Within each stratum, a stratified random sampling approach was used to select sampling sites for surficial sediment.

Concentrations of over 150 organic contaminants and metals were analyzed. Ambient toxicity was assessed using two bioassays (Microtox® and sea urchin fertilization and development). Habitat conditions (depth, salinity, temperature, dissolved oxygen, sediment grain size, and organic carbon content) were also measured.

The study results indicated that organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), were low relative to NOAA's sediment quality guidelines (SQG). Tributyltin was detected at trace levels only in Dillingham Harbor. Polychlorinated biphenyls (PCBs) and other chlorinated organic contaminants were detected only in trace amounts in the sediment. Sediment metal concentrations were very low; all values were below NOAA sediment quality guidelines (SQGs), except for arsenic. Benthic communities were relatively sparse at most locations due to harsh physical conditions. Species richness and diversity had no correlation to grain size, TOC, depth, or location when outliers were removed. Significant chemical toxicity was virtually absent except for high porewater ammonia levels at selected locations, associated with fish processing waste streams. Contaminant body burdens and histopathological lesions were very low in the fish tested. Except for an occurrence of an external papilloma condition and the observation of mild to moderate accumulation of macrophage aggregates in the spleen and/or kidney in some flounders, the fish were generally healthy and non-contaminated. In general, the bay appeared to be a robust environment with a biologically diverse benthic assemblage.

A follow up study may be needed to assess if the ammonia issue has a significant impact on the overall biological system. We also need to investigate the fate of the fish waste to see whether it is taken up by the benthic or the pelagic food web.

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List of Acronyms

AAS	Atomic Absorption Spectroscopy
ADEC	Alaska Department of Environmental Conservation
ADF&G	Alaska Department of Fish & Game
Ag	silver
Al	aluminum
AMAP	Arctic Monitoring & Assessment Program
AOOS	Alaska Ocean Observation System
APHA	American Public Health Association
As	arsenic
ASTM	American Society of Testing and Materials
Cd	cadmium
CFR	Code of Federal Regulations
Cr	chromium
Cu	copper
DDT	dichlorodiphenyltrichloroethane
DO	dissolved oxygen
EMAP	Environmental Monitoring and Assessment Program
EPA	Environmental Protection Agency
ERL	Effects Range - Low
ERM	Effects Range - Median
EVOS	Exxon Valdez Oil Spill
Fe	iron
GC/ECD	gas chromatography/electron capture detector
GC/MS	gas chromatography/mass spectroscopy
gm	gram
GOA	Gulf of Alaska
H'	diversity (Shannon-Weiner)
HCH	hexachlorocyclohexane
Hg	mercury
HRGS	Human Reporter Gene
ICP	inductively coupled plasma
km	kilometer
L	liter
m	meter
MDL	method detection limit
mg	milligram
Mn	manganese
MS	matrix spike

List of Acronyms (Continued)

MSD	matrix spike duplicate
ng	nanogram
Ni	nickel
NIST	National Institute of Standards and Technology
NPDES	National Pollution Discharge Elimination System
NPRB	North Pacific Research Board
NOAA	National Oceanic and Atmospheric Administration
NS&T	National Status and Trends Program
P	probability
PAH	polycyclic aromatic hydrocarbon
Pb	lead
PCB	polychlorinated biphenyl
POP	persistent organic pollutant
POTW	Publically Owned Treatment Works
ppt	parts per thousand
QA/QC	quality assurance/quality control
Sb	antimony
Se	selenium
Si	silicon
Sn	tin
SQG	Sediment Quality Guidelines
SQT	Sediment Quality Triad
SRM	Standard Reference Material
TBT	tributyltin
TIC	total inorganic carbon
TOC	total organic carbon
ug	microgram
Zn	zinc

INTRODUCTION

1.1 National Status and Trends Bioeffects Program Studies

This report summarizes the results of the National Oceanic and Atmospheric Administration's (NOAA) sediment toxicity, chemistry, benthic community and fish contaminant body burden studies in Kvichak and Nushagak Bays in northern Bristol Bay, Alaska. As part of the National Status and Trends (NS&T) Program, NOAA conducts studies to determine the spatial extent and severity of chemical contamination and associated adverse biological effects in coastal bays and estuaries of the United States. This program encompasses a broad spectrum of research and monitoring studies to evaluate sediment contamination and toxicity in U.S. coastal waters, including the long-term, nationwide monitoring of contaminant concentrations in sediments and bivalves; sediment toxicity assessments in specific coastal areas; the evaluation and application of biomarkers; and the development of ecological indices (Turgeon et al. 1998, Hartwell and Claflin 2005). The NS&T Bioeffects Program has conducted sediment toxicity assessment studies in coastal water bodies since 1991. Results from previous studies in over 20 coastal water bodies and estuaries have been published (Long et al. 1996, Turgeon et al. 1998, Long 2000, Hartwell and Hameedi 2006, Hartwell and Hameedi 2007, Pait et al. 2006, Hartwell et al. 2009). The NS&T Bioeffects Program began doing assessments in Alaska in 2007. This report presents the results of the study which was funded in part by the North Pacific Research Board (NPRB) and NOAA.

In Bristol bay the tide rules: View of low tide in Dillingham Harbor.

Sediment contamination in U.S. coastal areas is a major environmental issue because of potential toxic effects on biological resources and often, indirectly, on human health. A large variety of contaminants from industrial, agricultural, urban, and maritime activities are associated with bottom sediments, including synthetic organic chemicals, polycyclic aromatic hydrocarbons (PAHs), and trace elements. In many instances, fish consumption advisories are coincident with severely degraded sediments in coastal water bodies. Contaminants, particularly those that are lipophilic, can biomagnify in the coastal food chain with increasing concentrations up through the food chain in predatory wildlife and in humans. Thus, characterizing and delineating areas of sediment contamination and toxicity are viewed as important goals of coastal resource management. This is particularly important in Alaska where subsistence food contamination is an emerging health concern, especially in rural areas where large amounts of these foods are consumed as a primary source of protein (Wolfe 1996). Additionally, natural sources of pollution, particularly trace elements, may be associated with river runoff. Excessive levels of contaminants in the sediments, whether of natural or anthropogenic origin, can pose ecological and human-health risks. The presence of contaminants in coastal ecosystems can cause habitat degradation and loss of biodiversity through degraded habitats, loss of fauna, biomagnification of contaminants in the coastal ecosystem, and effects on humans from consumption of contaminated fish and wildlife.

In addition to chemical analyses, NS&T Bioeffects Program studies utilize measures of benthic community condition as indicators. Macrobenthic organisms play an important role in the estuarine environment. Critical habitats and food chains supporting many fish and wildlife species involve the benthic environment. Benthic organisms are secondary consumers in the ecosystem, and represent an important link between primary producers and higher trophic levels for both planktonic and detritus-based food webs. They are composed of diverse taxa with a variety of reproductive modes and life history characteristics. They are a particularly important food source for juvenile fish and crustaceans. Furthermore, most benthic species have limited mobility and cannot physically avoid stressful environmental conditions. Benthic assemblages thus cannot

evade, and must respond to, a variety of stressors, such as toxic contamination, eutrophication, sediment quality, habitat modification, and seasonal weather changes. Biological systems are able to integrate the complexity of natural habitat stressors and ambient pollutant mixtures, through physical contact with sediments, ingesting sediment, bioaccumulating contaminants in food webs, and expressing the synergetic effects of exposure to toxic chemicals.

history of use in regional estuarine monitoring programs and have been proven to serve as an effective indicator for describing the extent and magnitude of pollution impacts and habitat modification in estuarine ecosystems, as well as for assessing the effectiveness of management actions (Llanso et al. 2004, Long et al. 1995).

Several examples exist in which marine benthic communities' response to contaminant and physical stressors have

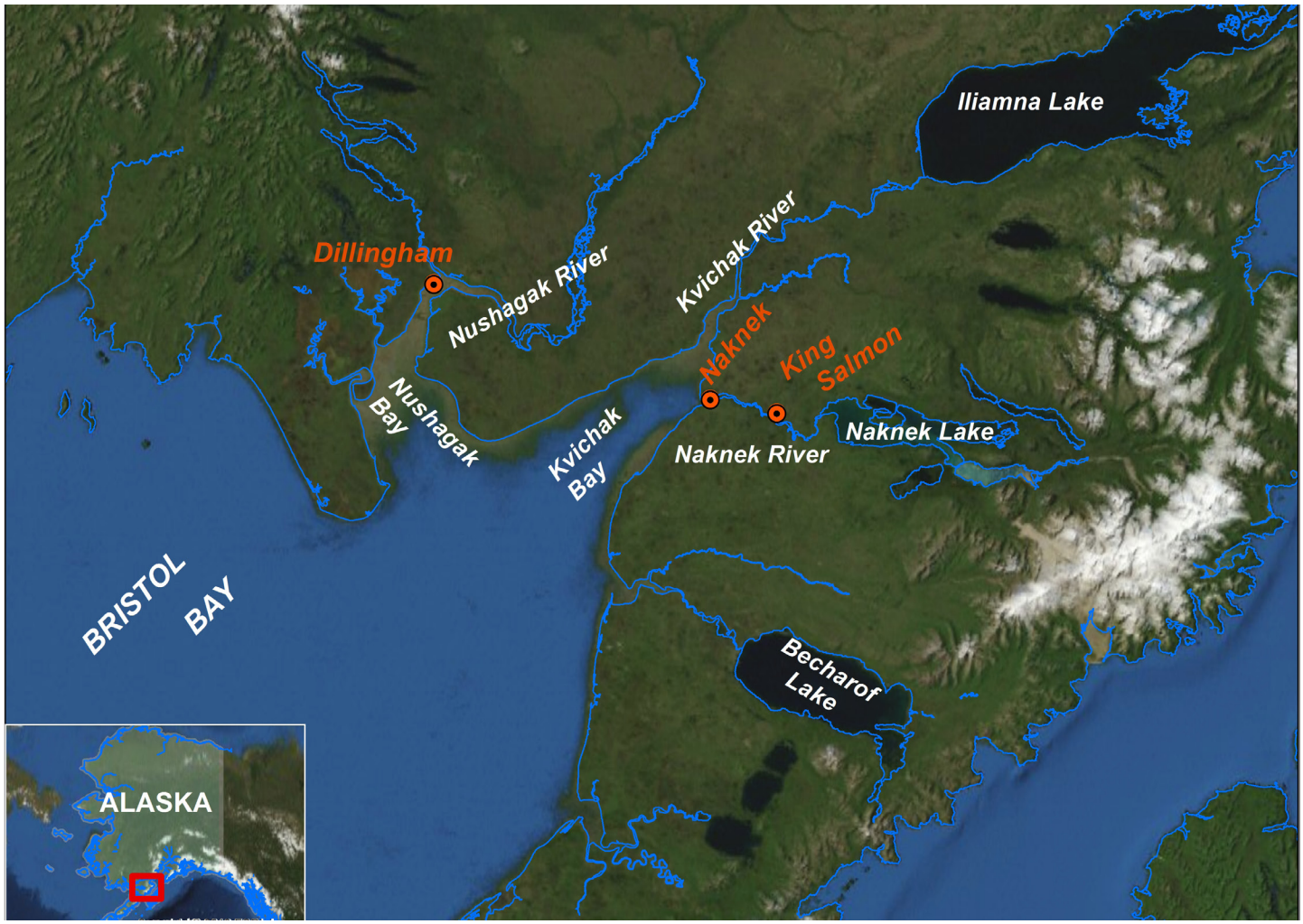

Figure 1. Map of the Bristol Bay area showing the towns of Dillingham, King Salmon, and Naknek, in Alaska (inset).

Distributions of benthic organisms are predictable along estuarine gradients and are characterized by similar groups of species over broad latitudinal ranges. Benthic species composition, abundance, and biomass are influenced by habitat conditions, including salinity, sediment type, and environmental stressors, both natural and anthropogenic (Slim et al. 1997, Nanami et al. 2005). Information on changes in benthic population and community parameters due to habitat change can be useful for separating natural variation from changes associated with human activities. For that purpose, benthic community studies have a long

been documented. Impacts of organic enrichment on marine benthos have shown that total biomass, relative proportion of deposit feeders, and abundance of species with 'opportunistic' life histories (e.g. high fecundity, short generation time, and rapid dispersal) increase. Some opportunistic taxonomic groups are known to be tolerant of chemical toxicants. Others are capable of thriving in physically disturbed habitats (e.g. high sedimentation, dredging operations, etc.), but not necessarily in contaminated areas. In areas impacted by excessive sedimentation from terrestrial runoff, dominant organisms tend toward surface suspension

feeding modes and high reproductive potential regardless of taxonomic relationship, whereas away from the sedimentation stress, feeding modes shift to species that are deep deposit feeders, along with the emergence of filter feeders (Wlodarska-Kowalczuk et al. 2005, Pearson and Rosenberg 1978). Experimental manipulation of habitats has shown that specific taxonomic lines, with opportunistic life history strategies respond positively to organic enrichment (Lenihan et al., 2003). Other taxa respond negatively to both toxicants and excessive organic enrichment. The response of specific species to organic and toxic contamination is mediated by life history and feeding mode characteristics.

National Status and Trends Bioeffects Program studies also utilize measures of toxicity using bioassays that may evaluate different modes of contaminant exposure (bulk sediment, sediment pore water, and chemical extracts of contaminants from sediment) to a variety of species and different assessment end-points (i.e., mortality, impaired reproduction, physiological stress, and biomarker response). Since the test results are usually not necessarily axiomatic and biological effects of contaminants occur at different levels of biological organization, i.e., from cells to ecosystems, results from a suite of toxicity tests are used in the "weight of evidence" context to infer the incidence and severity of environmental toxicity (Chapman 1996). Typically, the amphipod mortality bioassay, the sea urchin fertilization and development impairment bioassay, the Microtox test, and, in recent years, a Human Reporter Gene System (HRGS) P450 tests, are used in each study area. Other tests, based on promising new techniques, e.g. full life-cycle tests, and genotoxicity, have also been used in some areas for test evaluation or to meet a specific information need.

Taken together, all three assessments, sediment chemistry, sediment benthic assemblage, and sediment toxicity constitute what is referred to as the Sediment Quality Triad (SQT). The SQT is an important ecosystem based management tool widely used by coastal managers for coastal resource management.

Despite its extensive coastline of 33,000 miles, greater than the contiguous US (EPA, 2005), and vast natural marine and coastal resources, Alaska lacks adequate spatial data coverage to provide baseline information necessary to assess future trends. More environmental monitoring and research is needed to assess not only areas of known pollution impacts, but also the whole coastal Alaska region. Historically, assessments in the region have been either limited or focused on areas of known impairment. The NS&T Bioeffects Program conducted a sediment quality assessment in Kachemak Bay (Hartwell et al. 2009). Other previous NS&T Mussel Watch Program monitoring efforts involved measurements of contaminants in sediment and mussels collected from a few selected sites in the Gulf of Alaska (O'Connor 2002). The Prince William Sound Regional Citizens Advisory Council (PWSRCAC) has been assessing PAHs and other petroleum-related compounds in Prince William Sound since the Exxon Valdez Oil Spill in 1989 (EVOS) (Page et al. 2001). In collaboration with the U.S. EPA Environmental Monitoring and Assessment Program (EMAP), the Alaska Department of Environmental Conservation has undertaken coastal ecological condition studies of southcentral Alaska that encompasses assessment of contaminants and benthic assemblage in sediment along the Gulf of Alaska and the Aleutian Islands (Saupe et al. 2005), and currently with NOAA on the Kenai peninsula, and in the Chukchi and Beaufort Seas. The Cook Inlet Regional Citizens Advisory Council (CIRCAC) assesses the impacts of oil and gas operations in Cook Inlet, including chemical and benthic community assessments.

The goals of this project included: 1) the assessment of habitat conditions that influence the biodiversity and distribution of benthic infaunal community using the SQT approach, 2) determine the magnitude and spatial patterns or gradients in chemical contamination; 3) characterization of the benthic macroinvertebrate community, 4) quantification of the magnitude of contaminant body burdens and histopathology in fish in the study area, and 5) measurement of sediment toxicity in Nushagak and Kvichak Bays. The present study provides sediment quality baseline information on contaminant concentrations and sediment benthic community condition for future development that may occur in the area, to help evaluate unforeseen spill events or other disasters (e.g. earthquakes), and to supplement ecosystem-based management assessments in the region.

1.2 Site Background

Bristol Bay is a large region in the southeast corner of the Bering Sea, extending from the Alaska Peninsula to Cape Newenham and east into several bays and estuaries. Bristol Bay is one of the most productive fishery bays in the U.S. Its biologically diverse marine wildlife supports important subsistence and commercial fisheries. The sockeye salmon fishery in the bay is the largest in the world, as well as strong runs of chum, coho, pink and king salmon. Two of the most productive rivers flow into Nushagak and Kvichak Bays. The fishery resources and the commercial fishing and seafood processing industries are the backbone of the regional economy and integral to many residents' livelihoods and way of life in outlying villages. Annual subsistence harvest in the Bristol Bay region averages over 1,400 lb per household, up to over 2,200 lb in smaller more remote

communities (ADFG 2009). Winter conditions last about seven to nine months out of the year. The population in the three boroughs surrounding the bay is approximately 7,000 people. Half live in the vicinity of Dillingham, Naknek and King Salmon (Figure 1). The rest are spread out over 40,000 square miles in small villages. During the summer fishing season however, the human population in the region swells by thousands for recreational and commercial fishing. Access to the region is only by air or water. There are no roads that lead to the outside world from either Dillingham or Naknek.

Significant features of the Nushagak and Kvichak Bays that impact habitat patterns include multiple deep channels, multiple tributaries, an extreme tidal range of 20+ feet, freshwater inflow from rivers, and salt water inflow from the Bering Sea from the south. Large fresh water flows from the tributaries render the estuaries to be low salinity systems. Counterclockwise circulation within Bristol Bay also delivers sea water diluted by the Egegik and Ugashik rivers further south (Straty 1977). The upper portions of Nushagak and Kvichak Bays are highly turbid from eroding cliffs of loess soil deposits in the tidal areas, which suppresses benthic diversity. The deposits are derived from late-Wisconsin age glacial outwash deposits subjected to eolian transport 13-23 thousand years ago (Buppert 1994).

The land area draining to Nushagak and Kvichak Bays consists of four major watersheds, the Wood, Nushagak, Kvichak, and Naknek, plus the Egegik, and Ugashik Rivers further south on the Aleutian Peninsula. The Nushagak and Kvichak watersheds encompass approximately 50% of the total watersheds. Kvichak Bay receives fresh water input from the Kvichak and Naknek Rivers. The Kvichak River flows into the northern end of Kvichak Bay with headwaters in Lake Iliamna, Alaska's largest freshwater lake and an important salmon rearing habitat. The State of Alaska recently improved the Williamsport-Pile Bay road access between Lake Iliamna and Cook Inlet. It is used as a short cut for boats, fuel and freight moving between Cook Inlet and Bristol Bay. The Alagnak River merges with the Kvichak at its mouth and drains alpine lakes in the Aleutain Range. The Naknek River drains Naknek Lake and its associated watersheds on the Alaska peninsula.

Nushagak Bay receives the flow of the Nushagak, Wood, Snake, and Igushik Rivers. To the north and west the watersheds lie in the protected areas of the 4.7 million acre Togiak National Wildlife Refuge and the Wood-Tikchik State Park, the largest state park in the nation, at 1.6 million acres. The park encompasses two pristine chains of lakes. All five species of Pacific salmon, along with rainbow trout, arctic char, dolly varden and northern pike, are prolific in the Wood River and Tikchik systems. The park was created in 1978 for the purpose of protecting the area's fish and wildlife breeding and support systems and preserving continued subsistence and recreational activities. The Togiak Refuge is roadless, with primary access only via air or water.

The watersheds are bordered to the west by the Ahklun Mountains and to the east and south by the Alaska and Aleutian Ranges. The Ahklun Mountains are old and eroded by glaciers forming the deep lakes in the Wood-Tikchik Park. East of the mountains the land is mantled with colluvium, alluvium, glacial drift, and moraines. The southern Alaska Range and the Aleutian Range are comprised of a series of high, steep, glaciated mountains covered by rocky slopes, glacial moraines, and glaciers. Soils are shallow or absent and permafrost occurs as isolated masses. Soils have formed in volcanic ash over glacial deposits at lower elevations. Alpine tundra is the predominant vegetation. In the large expanses between the mountains, the land has glacial moraines and ponds, eroded ridges with broad, gentle slopes and broad, flat or gently sloping valleys with low local relief and deep glacial moraine, drift, and outwash deposits. The vegetation is primarily spruce-hardwood forest, scrub, or open tundra. There are active volcanoes on the Alaska Peninsula on the southern boundary of Bristol Bay and the Aleutian archipelago to the southwest. These periodically contribute volcanic ash to the region, and have produced tsunamis that impact the region.

Conditions in the Bristol Bay watershed are highly favorable for Pacific salmon. The Nushagak and Kvichak River watersheds encompass an abundant and diverse array of aquatic habitats and support a diverse salmonid assemblage. Freshwater habitats range from headwater streams to braided rivers, small ponds to large lakes, and side channel alcoves. These watersheds contain over 54,000 km of streams (Johnson and Blanche 2012). Lakes and associated tributary and outlet streams are key spawning and rearing areas for sockeye salmon. Lakes cover a relatively high proportion of the watershed area: 7.9% for the entire Bristol Bay watershed area and 13.7% for the Kvichak River watershed (RAP 2011). This value tends to be much lower in other North Pacific river systems, from northern Russia to western North America, (e.g., 0.2 to 2.9%) (RAP 2011). Relatively low elevations in the watershed, and the absence of dams and roads, mean that not only are streams, lakes, and other aquatic habitats abundant in the Bristol Bay region, but they also tend to be accessible to anadromous fish. With very few exceptions, all major lakes in the watershed are accessible to anadromous salmon (EPA, 2014).

There are no known industrial point source discharges in Nushagak or Kvichak Bays. However, there are a variety of permitted waste discharges from fish processing plants at Dillingham, Ekuk, and Naknek, plus Egegik just south of the study area. In addition, floating fish processing plants anchor outside of Naknek and in the Nushagak at Clarks Point during the salmon run. These plants operate seasonally, producing large volume effluents containing fish waste and process water. Other releases occur from the airports at Dillingham and King Salmon, and Kanakanak Hospital. Previously, small mine sites in the watershed were active but have been closed and remediated. Other sources of pollution to Nushagak and Kvichak Bay may include leaking septic tanks, marine activities associated with commercial and recreational fishing, commercial shipping, stormwater runoff, and long-range atmospheric transport. There are no sewage pumpout facilities for vessels anywhere in the region. The boat harbor at Dillingham is dredged annually, with the dredge spoil being disposed of by simply releasing it into ebb tidal currents to be distributed down the bay. Any contaminants accumulated in the harbor during the year are thus released into the system at large.

Despite its fishery and ecological importance, the bay and its watersheds are threatened by increasing anthropogenic activities. The projected Pebble Mine Project and other proposed mining operations in the headwaters of tributaries of the Kvichak and Nushagak Rivers, which drain into the bay, constitute the most significant pollution threats to water and habitat quality in the bay. Proposed mining development in the watershed poses a risk to the fishery, and an environmental quality database is critically needed, but baseline data is lacking. Baseline data will be essential for monitoring pollution control effectiveness in the watershed.

This study produced a georeferenced chemistry and infaunal dataset that characterizes sediment quality in northern Bristol Bay. Completing a comprehensive taxonomic assessment of infauna and sediment chemistry in Kvichak and Nushagak Bays will assist in achieving the long-term goal of conducting research designed to address pressing fishery management or marine ecosystem information needs. Results contribute to a broader understanding of the marine ecosystems off Alaska that will enable effective management and sustainable use of marine resources. This project also provides important benthic community and sediment toxicity data that can be integrated into the AOOS data base, as well as baseline information for unforeseen events. In addition, climate change and fishing pressure influence the populations of benthic species. Indices of the distribution and abundance of benthic species that are an integral part of the benthic food web, are needed to evaluate these impacts. The research provides the basis for the development of index surveys designed to assess interannual to decadal trends in the distribution and abundance of benthic species, and examines the community structure of the benthic ecosystem. The NS&T Program developed a relational web-portal database on contaminants, toxicity, and benthic infaunal species distribution in coastal United States. The data portal is an “Internet doorway” to data and information products of NS&T. Data from the study in Bristol Bay are incorporated into this database and are available to local managers as well to concerned citizens nationally.

2. MATERIALS AND METHODS

2.1. Study area and sampling locations

The National Status and Trend Program uses a stratified-random design approach for selection of sampling sites to determine the spatial extent of sediment toxicity in U.S. coastal waters (Apeti et al. 2012). One of the design principles of the NS&T Program is to apply the same suite of tests synoptically to all areas so that comparisons can be made without the confounding interference of using different methods in different areas. Thus, comparison of the spatial extent of impact between areas is possible even if the areas are not contiguous.

The study area was originally divided into eight strata; upper, middle, and lower reaches of Nushagak and Kvichak Bays, plus Dillingham Harbor and the mouth of the Naknek River. in the vicinity of the town of Naknek and the fish packing industries. Four sampling sites were located on a random basis within each Bay stratum and three in the much smaller strata at Dillingham and Naknek. This approach combines the strengths of a stratified design with the random-probabilistic selection of sampling locations, allowing the data generated within each stratum to be attributed to the dimensions of that stratum with a quantifiable degree of confidence (Heimbuch et al. 1995). Strata boundaries were established in consultation with regional UAF and NGO scientists and AK DF&G resource managers, and were based on bathymetric, hydrographic, and regional environmental considerations, and limited previous studies on benthic invertebrate communities. Within each stratum, four randomly selected alternate sites were also selected for each primary sampling site. In instances where the primary site could not be sampled due to non-accessibility or an unsuitable substrate, the next sequential alternate site was sampled.

Field work was initiated in September 2013 and samples were collected in Dillingham Harbor and a subset of stations in upper Nushagak Bay. However, sampling was suspended due to poor weather conditions. Subsequently, the sampling design was altered to reduce the strata number to two (upper and lower) in the main Bays with four samples each. Samples in the harbor strata at Dillingham and Naknek were unchanged. In July 2014, sampling resumed, starting in the Kvichak estuary and finishing in the Nushagak estuary. A total of 23 sites were sampled for sediments and benthic infauna (Figure 2, Table 1) during 2013 and 2014. An extra sample was opportunistically collected at the mouth of the Kvichak River because the sediment

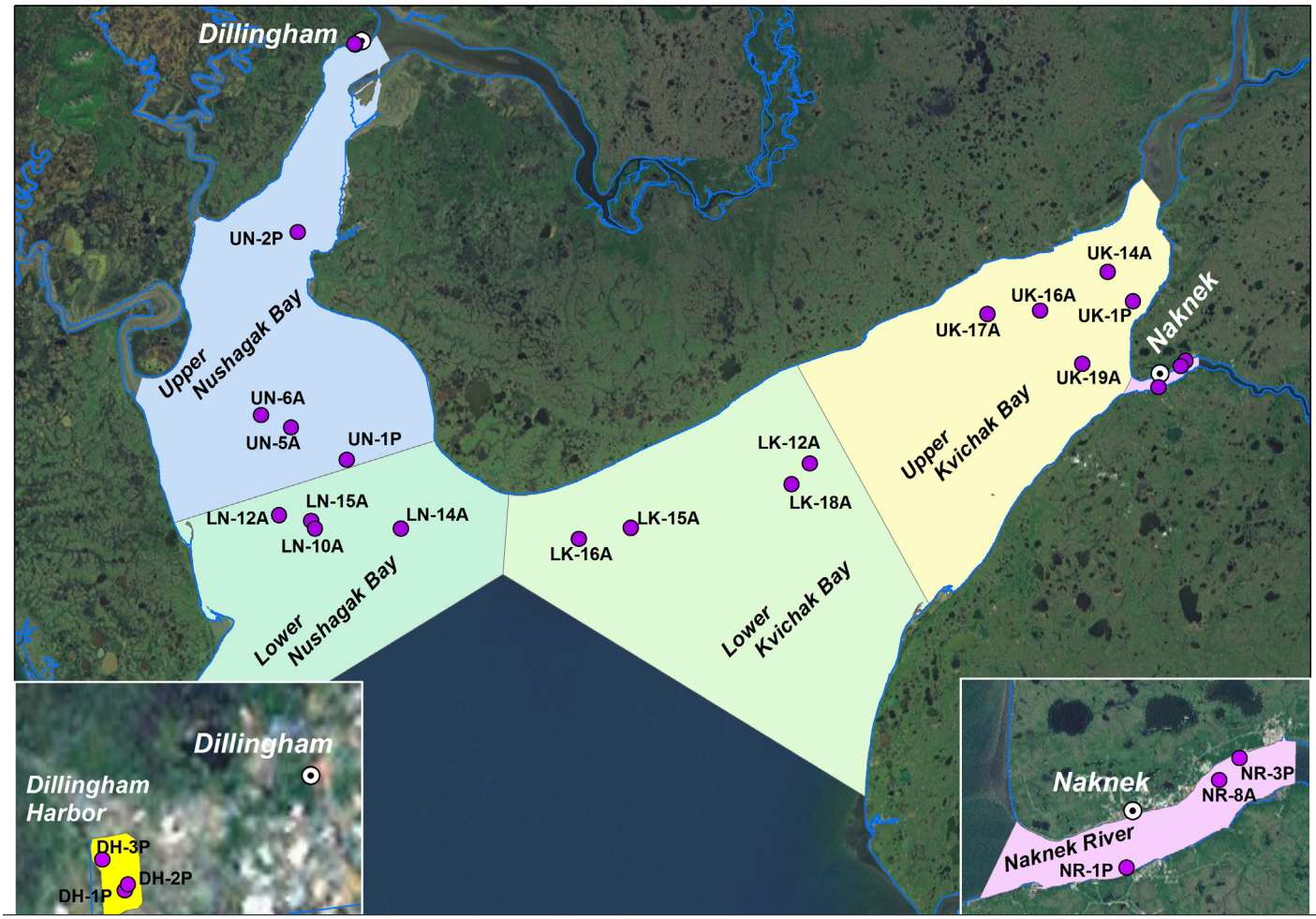

Table 1. Location of sediment sampling stations in Nushagak and Kvichak Bays in 2013 and 2014

Location/year	Site	Longitude (DD)	Latitude (DD)	MLW Depth (m)
Dillingham Harbor (2013)	DH-1P	-158.4770	59.0383	4.3
Dillingham Harbor (2013)	DH-2P	-158.4768	59.0385	4.0
Dillingham Harbor (2013)	DH-3P	-158.4784	59.0393	3.4
Upper Nushagak Bay (2014)	UN-1P	-158.4923	58.6463	16.2
Upper Nushagak Bay (2013)	UN-2P	-158.5814	58.8619	6.1
Upper Nushagak Bay (2014)	UN-5A	-158.5935	58.6770	14.3
Upper Nushagak Bay (2013)	UN-6A	-158.6423	58.6853	9.1
Upper Nushagak Bay (2014)	UN-6A	-158.6483	58.6887	7.6
Lower Nushagak Bay (2014)	LN-10A	-158.5570	58.5881	16.8
Lower Nushagak Bay (2014)	LN-12A	-158.6154	58.5938	12.8
Lower Nushagak Bay (2014)	LN-14A	-158.3938	58.5809	11.6
Lower Nushagak Bay (2014)	LN-15A	-158.5501	58.5809	14.6
Naknek River (2014)	NR-1P	-157.0131	58.7152	11.0
Naknek River (2014)	NR-3P	-156.9637	58.7402	5.5
Naknek River (2014)	NR-8A	-156.9726	58.7351	3.0
Upper Kvichak Bay (2014)	UK-14A	-157.1060	58.8245	7.0
Upper Kvichak Bay (2014)	UK-1P	-157.0594	58.7965	7.6
Upper Kvichak Bay (2014)	UK-16A	-157.2291	58.7876	2.6
Upper Kvichak Bay (2014)	UK-17A	-157.3249	58.7847	9.1
Upper Kvichak Bay (2014)	UK-19A	-157.1520	58.7376	7.0
Lower Kvichak Bay (2014)	LK-12A	-157.6485	58.6428	9.1
Lower Kvichak Bay (2014)	LK-15A	-157.9745	58.5816	4.9
Lower Kvichak Bay (2014)	LK-16A	-158.0695	58.5711	4.9
Lower Kvichak Bay (2014)	LK-18A	-157.6816	58.6231	3.3

texture was so different (muddy) than the rest of the study area. Station 6A in the upper Nushagak was sampled in 2013 and 2014 as a qualitative inter-annual comparison. Fish were collected by trawl at Dillingham and Naknek and at 1-3 locations in two of the open-bay strata.

2.2. Sampling procedures

2.2.1 Sediment and water sampling

Samples were collected on locally chartered fishing boats. A standard 32' drift gillnetter (Kasota) was used in 2013. The sampler was a stainless steel, 0.1 m-2 Smith-McIntyre grab sampler. It was deployed off the starboard side from a block mounted on the main boom swung over the side and retrieved using the net wheel. In 2014, a 47' fish tender/ seiner (Namorada) was employed which had a larger deck and no net wheel or roller. The sampler was deployed off the boom using the power block. Trawling was conducted off the stern, again using the power block to retrieve the net.

Two sediment samples were taken at each site in addition to water quality measurements for salinity, temperature and dissolved oxygen with a YSI meter at the surface and bottom of the water column. The sampler was initially washed, rinsed with acetone and deionized water, followed by an acid wash with 10% HCl and again rinsed with deionized water. At each site, the sampler was rinsed with acetone and deionized water immediately prior to sampling. Only the upper 2-3 cm of the sediment was retained in order to assure collection of recently deposited materials. A sediment sample was discarded if the jaws of the grab were open, the sample was partly washed out, or if the sediment sample in the grab was less than 5 cm deep. Sediments were removed with a Teflon coated stainless steel scoop. Sediment was composited from multiple grabs in a bucket with an acetone rinsed, high-density polyethylene (HDPE) liner. Between each deployment of the sampler, the bucket was covered with an HDPE lid to minimize sample oxidation and exposure to atmospheric contamination. Additional grab samples were taken and the top layer of sediment was collected and composited until sufficient volume (3-4 L) of sediment for all the toxicity bioassays, microbial assays, and chemical analyses was collected.

The sediment samples were thoroughly homogenized in the field with an acetone-rinsed, stainless steel mixer attachment on an electric drill. This composite sample was

subdivided for distribution to various testing laboratories. Separate subsamples were collected for grain size characterization and fecal coliform measurements. Samples for chemical analyses and *Clostridium perfringens* assays were frozen in pre-cleaned glass jars with Teflon® liners. Samples for pore water toxicity testing were stored in 1 L polyethylene jars with Teflon® coated lids. Samples for Microtox® testing were stored in pre-cleaned glass jars

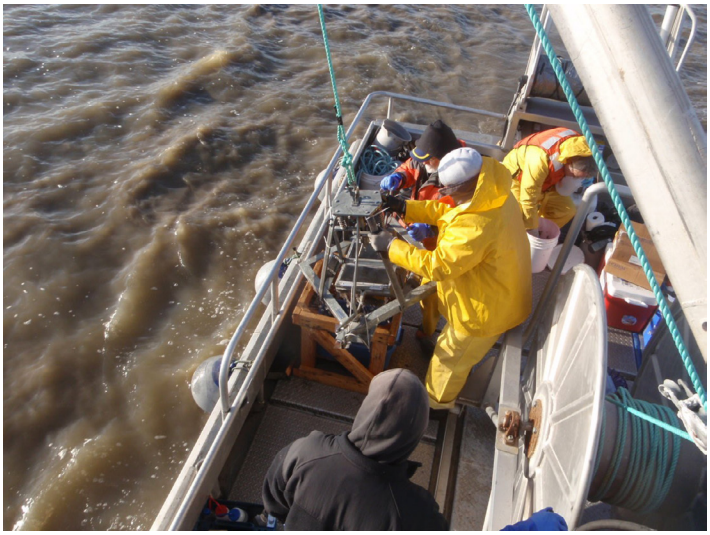

Figure 3. Illustration of sediment collection using stainless steel, 0.1 m^2 Smith-Macintyre sediment grab sampler (above) and fish sampling using otter trawl (right). (Note water turbidity).

with Teflon® liners. Bioassay samples were collected in Dillingham Harbor, at Naknek, and at a subset of randomly chosen sites in the open portions of Nushagak and Kvichak Bays. All subsamples were either refrigerated or frozen, as appropriate, prior to shipment to analytical laboratories. The bucket liners were not reused between sampling sites. A second sample was taken for benthic community analysis. The entire contents of an acceptable sample (at least 5 cm deep) were sieved on site through 0.5 mm mesh. All organisms were retained in Nalgene bottles and preserved in buffered formalin containing Rose Bengal stain.

2.2.2 Fish sampling

Trawling for fish and invertebrates was done at Dillingham Harbor, Naknek R. and in the open bays using a 3 m otter trawl. One trawl was taken each at Dillingham and Naknek. Four trawls were pulled in the Kvichak and five in the Nushagak to obtain smelt and two size classes of starry flounders. A variety of fish species were caught and released (yellowfin sole, sculpin spp., Pacific cod, pipefish sp, blackline prickleback, and stickleback, in addition to large numbers of shrimp, amphipods and isopods) but rainbow smelt (*Osmerus mordax*) and starry flounder (*Platichthys stellatus*) (Fig 4) were captured at all locations and kept for contamination and histopathology analyses. We were unable to find substantial population of bivalve species, thus bivalves were not included in this study as originally planned. Fish used for chemical contaminant analyses were composited and frozen in zip-lock bags. Fish for histological examination were injected with 10% buffered formalin and also stored in plastic bags but were not frozen. They were sent to the NOAA Oxford lab (Oxford, MD) for assessment of pathological conditions and parasite burden.

2.3. Sediment analyses

Chemical analyses followed procedures routinely used in the NOAA NS&T Program (Kimbrough and Lauenstein 2007 and American Society for Testing and Materials (ASTM 2003)). A broad suite of sediment contaminants were analyzed from each station, including 59 PAHs, 15 chlorinated pesticides including DDT and its metabolites, 25 polychlorinated biphenyls (PCBs), 16 major and trace elements, and three butyltins (Tables 2 – 6). Other parameters included grain size analysis, total organic/inorganic carbon (TOC/TIC), and percent solids.

2.3.1 Metals

The list of major and trace metals analyzed is illustrated in Table 2. Samples were shipped frozen to the laboratory and stored at -20 °C until analysis. Samples were prepared for inductively coupled plasma/mass spectrometry analysis (ICP-MS) for major metals while atomic fluorescence spectrometry was utilized to measure arsenic and selenium and atomic absorption spectrometry was used for mercury analysis. In general, samples were homogenized, freeze dried, weighed and digested in a sequence of heating steps with metal grade HNO_3, HF and, boric acid. For analysis of Hg, sediment samples were digested based on a modified

Figure 4. Starry flounder (*Platichthys stellatus*; left), and rainbow smelt (*Osmerus mordax*; right).

version of EPA method 245.5, using a concentrated H2SO4 and HNO_3 digestion, followed by addition of $KMnO_4$, and $K_2S_2O_8$, and the samples were again digested. Before analysis, 5 mL of 10% (w/w) NH2OH . HCl were added to reduce excess permanganate and the volume brought to 40 mL with distilled water.

Quality control samples were processed in a manner identical to actual samples. A method blank was run with every 20 samples, or with every sample set, whichever was more frequent. If corrected blank concentrations for any component were above three times the method detection limit (MDL), the whole sample set was re-extracted and reanalyzed. If insufficient sample was available for re-extraction, the data was reported and appropriately qualified. Matrix spike/matrix spike duplicate (MS/MSD) samples were run with every 20 samples, or with every sample set, whichever was more frequent. Recalibration standards were also run every 12 samples, and matrix modifiers were used as necessary. The appropriate spiking level was ten times the MDL. Reference materials were extracted with each set of samples and analyzed. The MDLs were determined following the procedures outlined in CFR 40, part 136 (1999).

2.3.2 Organics (PAHs, PCBs, chlorinated pesticides)
Samples were shipped frozen to the laboratory and stored at -20 °C until analysis. An aliquot of approximately 1 gm of sample was weighed and oven dried at 63 °C to a constant weight to determine wet/dry weight. Homogenized sample aliquots were chemically dried with Hydromatix®. Sample/Hydromatix® mixtures were spiked with surrogates then extracted with 100% dichloromethane using the accelerated solvent extraction (ASE) method. The extracts were then concentrated to 3 ml by evaporative solvent reduction. Silica gel/alumina column chromatography was utilized to concentrate and purify the samples before analysis. If sediment or other particulates were present in the sample extract, the extracts were filtered through a funnel containing glass wool and sodium sulfate. Quality control samples were processed with each batch of samples in a manner identical to the samples, including matrix spikes. Extracts were stored in the dark at or below 4°C. A method blank was run with every 20 samples, or with every sample set, whichever was more frequent. If blank levels for any component were above three times the MDL, samples analyzed in that sample set were re-extracted and reanalyzed. If insufficient sample was available for extraction, the data were reported and appropriately qualified. Matrix spike/matrix spike duplicate samples were run with every 20 samples, or with every sample set, whichever was more frequent. Surrogate standards were spiked into every sample and quality control sample.

Quantitation of PAHs (Table 3) and their alkylated homologues was performed by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring (SIM) mode. The compounds in the surrogate solution were deuterated naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}. The internal standards were: fluorene-d_{10}, and benzo[a]pyrene-d_{12} at 4 µg mL-1 and were prepared with a certified standard (NIST or equivalent). The GC conditions were set so that the internal standards were resolved, but would elute in close proximity to the analytes of interest.

A solution containing 2- to 5-ring PAH compounds was used to fortify matrix spike samples. A certified solution (NIST SRM 2260) was diluted to the appropriate working concentration. Dibenzothiophene was not present in the SRM and was added to the solution by weighing pure mate-

Table 2. Major and trace elements (metals) measured in the Bristol Bay sediments and tissues. For simplicity the term metal is used without distinction between true metals and metalloids.

Symbol	Element	Symbol	Element	Symbol	Element
Ag	Silver	Fe	Iron	Sb	Antimony
Al	Aluminum	Hg	Mercury	Se	Selenium
As	Arsenic	Mn	Manganese	Sn	Tin
Cd	Cadmium	Ni	Nickel	Si	Silicon
Cr	Chromium	Pb	Lead	Zn	Zinc
Cu	Copper				

rial to make a concentration of 1.00 µg L-1. The spiking solution was used to fortify samples to a final concentration of approximately ten times the MDL. A laboratory reference oil solution was analyzed as an instrument reference solution with each analytical batch. After every 8 - 10 samples, the mass spectrometer response for each PAH relative to the internal standard was determined using check standards. Daily response factors for each compound were compared to the initial calibration curve and recalibration was repeated when necessary. The standard reference oil was analyzed with all analytical batches.

A standard reference material was extracted and analyzed with each batch of samples. Target concentrations were defined as the range of the certified value plus or minus the 95% confidence intervals found in the SRM certification. The measured concentration was within ±30% of the target concentration on average for all analytes either certified or non-certified with concentrations greater than 10 times the MDL. The actual analytical method detection limit (MDL) was determined following procedures outlined in CFR 40, part 136 (1999).

Chlorinated hydrocarbons (chlorinated pesticides (Table 4) and PCBs (Table 5) were quantitatively determined by capillary gas chromatography with an electron capture detector (ECD). If the response for any peak exceeded the highest calibration solution, the extract was diluted, a known amount of surrogate and tetrachloro-m-xylene (TCMX) solution added, and the sample reanalyzed for those analytes that exceeded the calibration range. Analyte concentrations in the samples were based on calculations using the PCB 103 surrogate. The internal standard (TCMX) was used to calculate surrogate recoveries. 4,4'-dibromooctafluorobiphenyl (DBOFB) or PCB 198 was used to calculate selected analyte concentrations, if it was demonstrated that they produced more reliable data (i.e., if matrix interference occurs with PCB 103) based on percent recoveries in spiked blanks, matrix spikes, or reference materials. The calibration solutions that were analyzed as part of the analytical GC/ECD run were preceded by no more than six samples and no more than six samples were run between calibration mixtures.

An acceptable method blank contained no more than two target compounds at concentrations three times greater than the MDL. All samples and quality control samples were spiked with DBOFB, PCB 103 and PCB 198. The surrogate standard solution was spiked into the samples prior to extraction in an attempt to minimize individual sample matrix effects associated with sample preparation and analysis. A ma trix spike and a duplicate were analyzed with each sample set or every 20 field samples, whichever was more frequent. The acceptable matrix spike recovery criteria were 50 - 125% recovery for at least 80% of the analytes. Criterion for duplicates was ≤30% relative percent difference (RPD). The method detection limit was determined following the procedures outlined in CFR 40, part 136 (1999). Most target compounds, surrogates and internal standard were resolved from one another and from interfering compounds. When they were not, coelutions were documented. A standard reference material sample was analyzed per batch of samples or every 20 samples whichever was more frequent.

2.3.3 Butyltins

For the analysis of butyltins (Table 6), an aliquot of freeze dried sediment was weighed and appropriate amounts of surrogate standards (approximately 10 times the method detection limit, MDL) were added to all samples, matrix spikes, and blanks. Samples were extracted three times by agitation with tropolone in dichloromethane. The sample extract was concentrated in a hot water bath, and the extract was centrifuged and further concentrated. The solvent was exchanged to hexane and concentrated to a final volume of about 10 – 20 mL at which point only hexane remained. Hexylmagnesium bromide (2 M; Grignard reagent) was added to the sample extract under nitrogen and heated to hexylate the sample. After separation from the organic phase, pentane:CH_2Cl_2 (3/1, v/v) was added to the aqueous phase and the sample shaken vigorously. The pentane:CH_2Cl_2 extraction was done twice. The hexylated extract was dried by addition of anhydrous Na_2SO_4 and then concentrated. The extract was purified using silica gel/alumina column chromatography. The eluent was collected and concentrated on a water bath.

Table 3. Low and high molecular weight polycyclic aromatic hydrocarbons (PAHs) measured in Bristol Bay sediments and tissues.

Low weight		High weight
Naphthalene		Fluoranthene
C1-Naphthalenes		Pyrene
C2-Naphthalenes		C1-Fluoranthenes/Pyrenes
C3-Naphthalenes		C2-Fluoranthenes/Pyrenes
C4-Naphthalenes		C3-Fluoranthenes/Pyrenes
Benzothiophene		C4-Fluoranthenes/Pyrenes
C1-Benzothiophenes		Naphthobenzothiophene
C2-Benzothiophenes		C1-Naphthobenzothiophenes
C3-Benzothiophenes		C2-Naphthobenzothiophenes
C4-Benzothiophenes		C3-Naphthobenzothiophenes
Biphenyl		C4-Naphthobenzothiophenes
Acenaphthylene		Benz(a)anthracene
Acenaphthene		Chrysene/Triphenylene
Dibenzofuran		C1-Chrysenes
Fluorene		C2-Chrysenes
C1-Fluorenes		C3-Chrysenes
C2-Fluorenes		C4-Chrysenes
C3-Fluorenes		Benzo(b)fluoranthene
Carbazole		Benzo(k,j)fluoranthene
Anthracene		Benzo(a)fluoranthene
Phenanthrene		Benzo(e)pyrene
C1-Phenanthrenes/Anthracenes		Benzo(a)pyrene
C2-Phenanthrenes/Anthracenes		Perylene
C3-Phenanthrenes/Anthracenes		Indeno(1,2,3-c,d)pyrene
C4-Phenanthrenes/Anthracenes		Dibenzo(a,h)anthracene
Dibenzothiophene		C1-Dibenzo(a,h)anthracenes
C1-Dibenzothiophenes		C2-Dibenzo(a,h)anthracenes
C2-Dibenzothiophenes		C3-Dibenzo(a,h)anthracenes
C3-Dibenzothiophenes		Benzo(g,h,i)perylene
C4-Dibenzothiophene		

The quantitative method was based on high resolution, capillary gas chromatography using flame photometric detection (GC/FPD). This method quantitatively determined tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT).

Quality control samples were processed in a manner identical to actual samples. A method blank was run with every 20 samples, or with every sample set, whichever was more frequent. If corrected blank concentrations for any component were above three times MDL, the whole sample set was re-extracted and reanalyzed. If insufficient sample was available for re-extraction, the data was reported and appropriately qualified. Matrix spike/matrix spike duplicate (MS/MSD) samples were run with every 20 samples, or with every sample set, whichever was more frequent. The appropriate spiking level was ten times the MDL. Reference materials were extracted with each set of sample and were analyzed when available. The method detection limit

Table 4. Chlorinated pesticides measured in Bristol Bay sediments and tissues.

Compound class	Compound
Cyclodienes	Aldrin
	Dieldrin
	Endrin
	Heptachlor
	Heptachlor-Epoxide
	Oxychlordane
	Alpha-Chlordane
	Gamma-Chlordane
	Trans-Nonachlor
	Cis-Nonachlor
Hexachlorocyclohexanes	Alpha-HCH
	Beta-HCH
	Delta-HCH
	Gamma-HCH
DDT and breakdown products	2,4'-DDD
	4,4'-DDD
	2,4'-DDE
	4,4'-DDE
	2,4'-DDT
	4,4'-DDT
Chlorinated Benzenes	1,2,3,4-Tetrachlorobenzene
	1,2,4,5-Tetrachlorobenzene
	Hexachlorobenzene
	Pentachloroanisole
	Pentachlorobenzene
Others	Endosulfan II
	Endosulfan I
	Endosulfan Sulfate
	Mirex
	Chlorpyrifos

was determined following the procedures outlined in CFR 40, part 136 (1999).

2.3.4 Sediment particle size and total organic carbon measurement

Sediment physical parameters such as grain size characterization and total organic carbon content (TOC) were measured. Sediment grain size was measured using a series of wet sieving and pipetting techniques. Sediment aliquots were first treated with 30% hydrogen peroxide (H_2O_2) then with deflocculent solution to remove organic matter prior to particle size determination. The treated samples were then poured onto a sieve stack arranged in descending order (i.e., -1 phi and +4 phi), which removed the coarser and sand particles respectively. The remaining solutions with sediment grain <63µm (silt and clay) were allowed to sit undisturbed for 20 seconds, after which the clay fraction is separated from the silt fraction by pipetting. For the final classification of sediment grain size NS&T program uses

Table 5. Polychlorinated biphenyls (PCBs) measured in Bristol Bay sediments and tissues.

Congeners		Congeners
PCB8/5		PCB128
PCB18		PCB138/160
PCB28		PCB146
PCB29		PCB149/123
PCB31		PCB151
PCB44		PCB153/132
PCB45		PCB156/171/202
PCB49		PCB158
PCB52		PCB170/190
PCB56/60		PCB174
PCB66		PCB180
PCB70		PCB183
PCB74/61		PCB187
PCB87/115		PCB194
PCB95		PCB195/208
PCB99		PCB199
PCB101/90		PCB201/157/173
PCB105		PCB206
PCB110/77		PCB209
PCB118		

Table 6. Butyltins measured in Bristol Bay sediments and tissues.

Compound
Monobutyltin
Dibutyltin
Tributyltin

the Wentworth scale method with the following major particle size classes: gravel (-2 phi to -5 phi), sand (+4 phi to -1 phi), silt (+5 phi to +7 phi), and clay (+8 phi and smaller). Total organic carbon was determined using a carbon analyzer. Dried sediment aliquot were treated with phosphoric acid (1:1) to remove inorganic carbon, then combusted at 1,350°C in an oxygen atmosphere. The gaseous phase was then let to flow through a non-dispersive infrared (NDIR) detection cell to measure CO_2 which is converted to %carbon. Grain size and TOC measurements are reported as percentages of the total sample weight. Detailed descriptions of the NS&T methods for grain size and TOC measurements in marine sediment are described in McDaonald et al. (2006).

2.3.5 Fecal coliform and Clostridium perfringens measurements

The bacterium *Clostridium perfringens* has been used as an indicator of fecal pollution and was analyzed in sediment samples from Bristol Bay using ASTM method D5916-96 (ASTM, 2003). This bacterium occurs in the intestines of humans and other animals, and is a common cause of food poisoning. Samples were segregated based on grain size

characteristics so as to determine proper dilutions. The initial sample analysis aids in the determination of subsequent dilutions necessary for similar samples. When weighing samples the consistency and particle size were recorded and taken into consideration for determining how much sample was used. Approximately 1.0 gram of sample was used for loose, sandy samples and 0.5 gram was typically used for thick, muddy samples. Laboratory procedure specifies doing at least two and sometimes three and four dilutions on the first samples depending on grain size. These were always done in duplicate. The highest dilution was 10 ml and in some cases, if the sediment was heavy, less than 10 ml was used. To assess the presence of viable *C. perfringens*, sediment extracts were plated on growth medium and the number of colonies that develop counted.

The analysis of sediment samples for fecal coliform bacteria (FCB) was carried out using Standard Method 9221E (APHA, 1998), which requires the addition of a dilution series of sample to tubes of Lauryl tryptose broth. After incubation, production of gas is a positive presumptive reaction. Positive samples were then incubated for 24-48 in Brilliant Green Bile (BGB) and E. coli (EC) growth media. The production of gas in the BGB and EC tubes confirms the presence of total and fecal coliforms, respectfully, in the sample. The number of tubes that confirm positive and using the Most Probable Number (MPN) chart in Standard Methods, Table 9221.IV was used to calculate the MPN Index. Positive and negative control tubes with *E. coli* and Pseudomonas aeruginosa, respectively, were run daily.

2.4. Benthic community characterization

In the laboratory, samples were inventoried, rinsed gently through a 0.5 mm mesh sieve to remove preservatives and residual sediment, stained with Rose Bengal, and stored in 70% isopropanol solution until processing. Sample material (sediment, detritus, and organisms) were placed in white enamel trays for sorting under Wild M 5A dissecting microscopes. All macroinvertebrates were carefully segregated into major taxonomic groups (e.g. Polychaeta, Mollusca, and Arthropoda). The macroinvertebrates were then identified to the lowest practical identification level, which in most cases is to species level unless the specimen is a juvenile, damaged, or otherwise unidentifiable. The number of individuals of each taxon, excluding fragments was recorded. Data were synthesized into a data summary report for each site, which includes a taxonomic species list and benthic community parameters list. At a minimum, 10 percent of all samples were resorted and recounted on a regular basis. Also 10% of samples were randomly selected and re-identified. The minimum acceptable sorting and taxonomic efficiency was 95%. A voucher collection composed of representative individuals of each species encountered in the project was accumulated and retained.

Taxa are distributed along environmental gradients, so there are generally no distinct boundaries between communities. However, the relationships between habitats and species assemblages reflect the interactions of physical and biological factors and can indicate major ecological trends. Quantitatively, the benthic communities were characterized as enumeration by abundance, species richness, evenness, and diversity, followed by pattern and classification analysis for delineation of taxa assemblages. Abundance was calculated as the total number of individuals per square meter; taxa richness as the total number of taxa represented at a given site; and taxa diversity was calculated with the Shannon-Weiner Index (Shannon and Weaver 1949), using the following formula:

Eqn1

$$H' = -\sum_{i=1}^{S} p_i (\ln p_i)$$

where, S = is the number of taxa in the sample, $_i$ is the ith taxa in the sample, and pi is the number of individuals of the ith taxa divided by the total number of individuals in the sample.

2.5. Sediment toxicity bioassays

Sediment quality was assessed using two bioassays: sea urchin fertilization test and the Microtox® response test. All methods are based on standard methods promulgated by the EPA and/or APHA. The proposed bioassays measure sensitive sublethal endpoints, and assess both water soluble and organic phases of the sediment. Microtox® tests were run on sediment pore water in 2013 and 2014. Sea urchin fertilization bioassays were run using *Strongylocentrotus purpuratus* in 2013. Sea urchin fertilization and development bioassays were run using *Arbacia punctulata* in 2014.

2.5.1. Sea urchin toxicity bioassay

The sea urchin fertilization toxicity bioassay involves exposing sea urchin sperm to pore water followed by the addition of eggs. This test is used extensively in assessments of ambient water quality, toxicity of industrial and municipal effluents, and sediment toxicity in coastal waters. It combines the features of testing sediment pore waters (the phase of sediments in which dissolved toxicants may be bioavailable) and exposure of gametes which often are more sensitive than adults. A reference sample is included with each test as a negative control, and a reference toxicant test as a positive control. Adult urchins are stimulated to spawn and the gametes are collected. After an additional 30 minutes of incubation, the test is terminated. An aliquot of the suspension is examined to determine the presence or absence of a fertilization membrane surrounding the eggs, and percent fertilization is recorded for each replicate. Each pore water sample is tested with five replicates. Reduction in mean fertilization success after exposure to pore water,

in comparison with the negative control, is the experimental end-point. Statistical treatments of data include analysis of variance and Dunnett's one-tailed t-test on arcsine square root transformed data. In addition to statistically significant differences from control sediment, a detectable significance criterion is used to determine the 95% confidence value based on power analysis of data from similar tests (n=3,110). This value is the percent minimum difference from the reference that is necessary to detect a significant response: at ($\alpha = 0.05$, it is 15.5%, and at $\alpha = 0.01$, it is 19% (Carr and Biedenbach 1999).

In 2014, an additional bioassay was run to measure embryological development of the sea urchins. For this test, 150 µl of a 1:1250 sperm dilution in filtered artificial seawater was used to fertilize 30 ml of the egg solution. After 15 minutes the fertilization rate was checked and determined to be approximately 91%. Each pore water replicate promptly received 100 µl of the fertilized egg suspension (~200 eggs per replicate). The test was terminated after incubating 48 hours at 20 ± 0.5 °C by adding 2 ml of 2X Z-fix (1X final in 30 ppt FASW), a proprietary zinc-buffered formaldehyde fixative. All but the last 1 ml of fixed sample was removed, the remaining 1 ml was transferred to a Sedgewick-Rafter counting slide, and ~100 embryos scored according to normal and degrees of abnormal development under 100X magnification. Scoring included normal fully developed 4-arm plutei (N), retarded plutei <1/2 the normal size (R), pathologic malformed plutei (P1), and pathologic embryos (P2) unable to differentiate to the pluteus larval stages. Percent normal development (normal plutei) in each treatment was compared to the reference treatment.

2.5.2. Microtox® toxicity bioassay

This test uses a strain of the bacterium *Vibrio fischeri* (B-11177), in which bioluminescence is closely tied to cellular respiration. Inhibition of cellular activity results in a decreased rate of respiration and a corresponding decrease in luminescence. The test is simple, rapid, reproducible and inexpensive; there are published data on the Microtox® response (EC^{50} values) upon exposure to over 1,000 chemicals. For these reasons, this test is used worldwide, mostly as a screening test but in some instances as a government-approved regulatory test as well. NOAA uses Microtox® response to an organic extract of sediment. Sediment is extracted and processed in accordance with EPA Method 3550. The extraction procedure is well suited for extraction of neutral, non-ionic organic compounds, such as aromatic and chlorinated hydrocarbons. Light emission is measured following incubation periods of 5 and 15 minutes. Percent decrease in luminescence relative to the reagent blank is calculated. A standard dose-response curve method is used to determine EC50 values: EC50 denotes the concentration that is effective in causing a 50% reduction in light production and expressed as mg equivalent sediment wet weight mL-1. All EC50 values are based on average readings with 95% confidence intervals for the replicates. Each sample is tested in triplicate. Tests of sediment extract from clean sediment are used as a reference standard. A phenol spiked sediment is used as the negative control standard. Sample EC50 s are normalized to the reference extract EC50. Any sample with an EC50 significantly ($P< 0.05$) lower than the controls indicates marginal toxicity. Samples with an EC50 significantly below the phenol-spiked standard are considered highly toxic.

2.6 Histopathology characterization

Fish were weighed, measured, examined grossly, and necropsied with sections of liver, skin/muscle (if lesion present), kidney, gill, and gonad preserved in 10% neutral buffered formalin for routine histological processing (Ribelin and Migaki 1975). Conditions (Table 7) were scored quantitatively by keeping a running count of occurrences of the condition as the slide is visually scanned to avoid re-examining each incident multiple times. Quantitative scores were used for parasites based on their observed cross-sections. Tissue pathological conditions (lesions) were also evaluated quantitatively. Parasite and pathologic conditions were tallied and scored for gill, liver and kidney for individual fish.

2.7 Data analysis

2.7.1 Chemical contaminants

Laboratory concentration results were subject to regular NS&T performance-based quality assessment and quality control for data accuracy and precision. Concentration values for individual compounds that were smaller than the MDL were qualified as undetected and were assigned a value of zero. For organic contaminants, "totals" were derived as the arithmetic sum of all the individual congeners or homologues of the same group of compounds as listed in Tables 2 - 6.

Contaminant concentrations in sediment were evaluated against NOAA's numerical Sediment Quality Guidelines (SQG) developed by Long and Morgan (1990) and Long et al. (1995) known as ERM and ERL (effects range-median, effects range-low) which express statistically derived levels of contamination, above which toxic effects would be expected to be observed with at least a 50% frequency (ERM), and below which effects were rarely (<10 %) expected (ERL). The mean ERM quotient (Long et al. 1998) is the average of the ratio of ERM value to sediment

concentration for each chemical. The mean quotient of the ERMs and observed contaminant concentrations were calculated on a site by site basis. The calculation included all the individual metals for which SQG exist, as well as total PAHs, total PCBs, and total DDT.

Body burdens of toxic metals and organic compounds in fish were compared to monitoring data from the Alaska DEC, Fish Monitoring Program. Alaska DEC report concentration levels on a wet weight basis. Assuming an average percent moisture content of 76% for fish tissue (values were derived from this study), a factor of 4 was used to convert wet weight concentrations into dry weight concentrations (100% tot.-76% wet ~25% dry, 25 x 4 =100). Primary statistical analyses were conducted using SAS and the JMP-11TM system statistical package. The data were tested for normality using Shapiro Wilks "goodness of fit" test. Because the data were not normally distributed, robust nonparametric statistical approaches were utilized for data comparison and spatial distribution. Wilcoxon and Kruskal-Wallis rank sum were applied to assess data comparability and degree of difference between values. Spearman rank correlation was used to test relationships between diverse parameters including physical and chemical characteristics of all sediment, water column, and biological parameters. The approach uses the range of concentration distributions in each stratum based on quartiles and Chi-square approximation for inter-stratum comparisons. The plots show the median, the 25th and 75th percentile (bottom and top of the box) and the whiskers above and below the box represent the 10th and 90th percentiles. Significance of statistical tests was reported at a probability level of 0.05. Box-plot statistics were used to assess concentration variations among strata.

2.7.2 Benthic community analysis

Multivariate cluster analysis was employed to group site and species data. The objective was to produce a coherent pattern of association between sites and species. Cluster analysis is a two-step process including; 1) creation of a resemblance data matrix from the raw data, and 2) clustering the resemblance coefficients in the matrix. The input resemblance (similarity or dissimilarity) matrix can be created by a number of methods. Input data may or may not be standardized or transformed depending on the requirements of the method (e.g. Bray Curtis). Based on previous research (Hartwell and Claflin 2005) the Jaccard method (Goodall 1973) was used to generate the similarity matrix.

The Jaccard method is a binary method based only on presence/absence data, and thus ignores abundance values. Cluster analyses were calculated from the matrices using the Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) procedure which clusters coefficients based on arithmetic mean distance calculations (Sneath and Sokal 1973). To optimize the cluster analysis results, several manipulations of the input data were performed to remove confounding effects and bias.

1- Epiphytic species such as sea anemones and tunicates were eliminated from the data set as they are not truly infauna.

2- Artificial species (resulting from failure to identify some specimens all the way down to species) were identified as a data bias. For example, if specimens of 2-3 species were identified in genus A, and other specimens were identified only to genus A, this tends to artificially increase species richness and diversity of the sample when in fact that diversity is an artifact of imperfect taxonomic identification. In some instances, specimens were only identifiable to family, order or class. To address this problem, specimens not identified to species level were eliminated, unless they were identified to a taxonomic level below which no other specimens in the collection belonged. That is, even though they were not identified to species, they were the only representative of that taxonomic line and did represent a non-redundant taxon. In other cases where a specimen was identified to genus and there was only one species identified in that genus, they were combined at the genus level.

Table 7. List of parasites and pathologies that were measured as a part of the histopathology assessment of fish.

Parasite	Nematode	**Pathology**
Body cestode	Nematopsis body	Ceroid
Body copepod	Nematopsis gill	Inflammation
Bucephalus	Protozoan Gut	Edema
Chlamydia	Rickettsia gut	Necrosis diffuse
Ciliate gut	Trematode metacercariae	Necrosis focal
Gill cestode	Unidentified organism	Neoplasm
Gill copepod		Tumor
Gill nemertine		Xenoma
Large gill ciliate		
Metacercaria		

3- Rare and unique taxa were defined as those species that were found at no more than two stations. Although they do contribute to the overall assessment of biodiversity, they were eliminated from the cluster analysis data set. Because of their limited distribution, by definition, they do not provide information on the impact of contaminant or other stressors gradients in the environment because they do not occur across the entire gradient.

After the data set had been finalized, a nodal analysis routine was applied to the data (Lambert and Williams 1962). This consisted of combining independent cluster analyses in a graphical array. The first analysis clustered sites using species occurrence data. The second calculation clustered species together into groups. The intersection of site clusters on the abscissa and species clusters on the ordinate axis yields a pattern of species associations with site clusters, termed nodes. In practice, this is done on large 3'x4' plots of the cluster analysis output. Reduction to normal text page size sacrifices a significant amount of detail. The site and species clusters were also characterized by physicochemical habitat parameters, contaminant concentrations, and other site-specific data. For each species, the parameters were normalized to their abundance at each site. For example, if 100 specimens of species A were found at a site with a TOC value of 1.5% and 10 were found at a site where TOC was 2%, the abundance normalized TOC preference for species A would be [(100*1.5)+(10*2)]/110=1.55.

2.7.3 Spatial distribution of habitat condition and benthic community parameters.

To evaluate the spatial distribution of benthic community (e.g. taxa, species abundance), and habitat parameters that influence them (e.g. grain size, salinity and toxicity), a three groups classification scheme was applied using ArcGIS 9.2. In ArcGIS, data classification is based on the Jenks' grouping method, which uses natural break points inherent in the data. ArcGIS identifies break points that best divide the data into the specified number of classes. The resulting classes are made of relatively similar values, while the differences between them are maximized.

3. Results and Discussion

3.1 Habitat Conditions

3.1.1 Water quality

Water clarity was uniformly low in the study area. The highest clarity was observed at locations in the middle of the lower Nushagak Bay (Figure 5). The Bristol Bay watershed is mainly comprised of unconsolidated soils (colluvium, alluvium, glacial deposits, moraines and volcanic ash deposits). The tidal areas are bordered by eroding cliffs of loess soil deposits. The constant input of eroded materials into the system and the extreme tidal range that continuously scours the bottom and mixes the water column keeps the turbidity high. Water clarity is a critical water quality parameter. The combination of the highly murky water and the constant erosion and deposition of silt material on the bottom of the bay system may suppress sensitive benthic species to where only tolerant communities can survive.

NOAA nautical charts indicate bathymetry with variable depth (up to 31m) in the open reaches of Nushagak and Kvichak Bays. Sampled locations were relatively flat with depth varying from 2.6 – 16.8 m (Table 1). The water column was very well mixed in the study area with measurements at the bottom and surface showing no significant differences in salinity, temperature or dissolved oxygen (DO) ($p > 0.05$) based on the Wilcoxon non-parametric test.

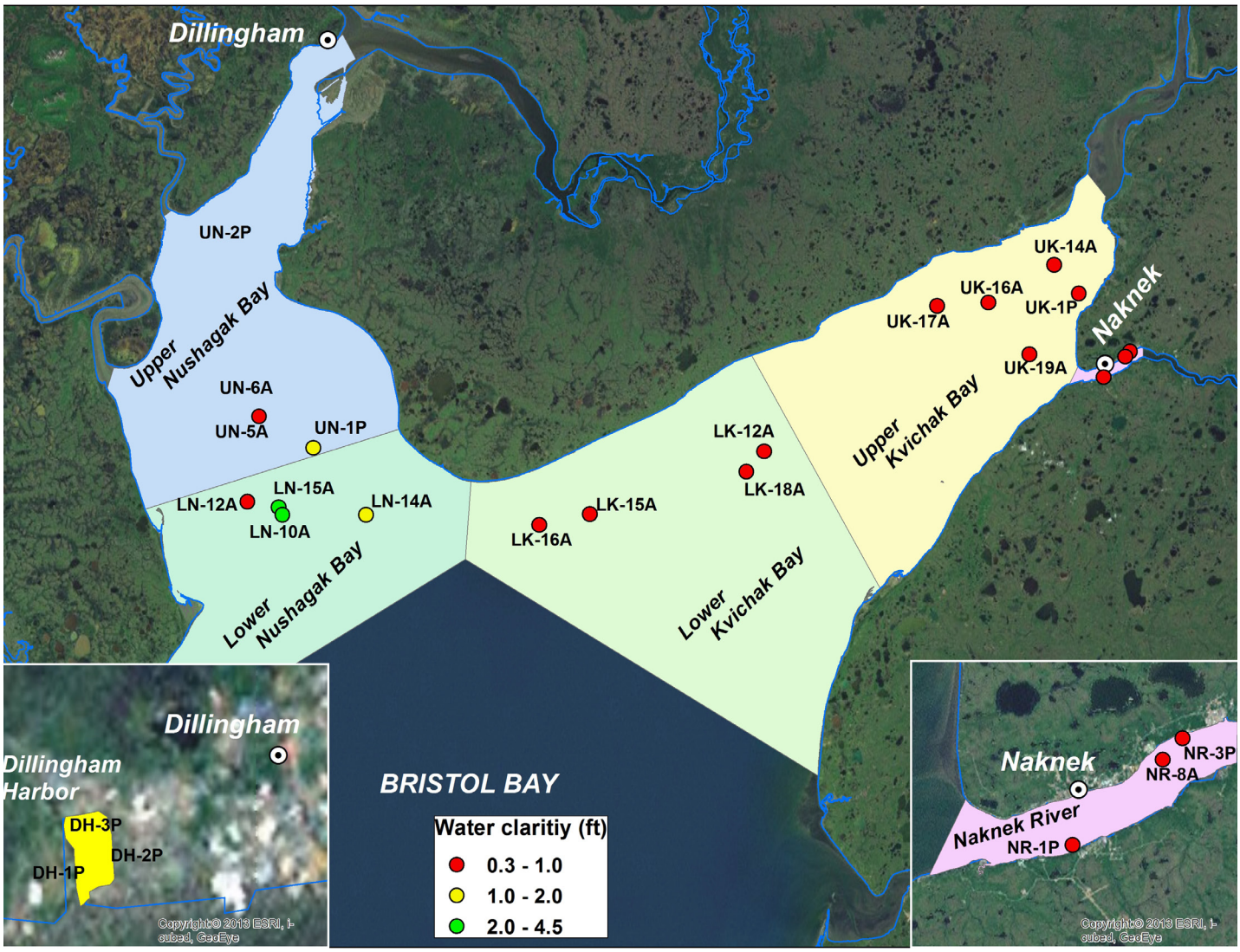

Figure 5. Water clarity measured by Secchi disc showed very turbid conditions.

Water temperature varied from 10.1 to 16.4 ºC (Figure 6a), which is within the seasonal average range of water temperature in the upper reaches of Bristol Bay (NOAA, National Oceanographic Data Center). Water temperatures at the four stations sampled in September 2013 were cooler than July 2014. Spatial variation across the entire study area showed uniformly distributed water column temperature (Chi-square = 5.78, $p = 0.016$) (Figure 6b). The median bottom temperature was 15.4 o^C.

Dissolved oxygen, did not vary by more than a few percent between the surface and bottom (Figure 7a,). Bottom DO varied from 7.7 – 8.4 mg L-1 in most parts of the study area with higher values of 9.5 – 11.4 mg L-1 in the shallowest areas, particularly in Dillingham Harbor (at cooler temperatures) (Figures 7b). The median value was 8.9 mg L-1.

Salinity measurements indicated brackish conditions throughout the study area (2.6‰ – 21.1‰) except in Dillingham Harbor and the upper Naknek at low tide where the water was essentially fresh at the time of collection. Salinity values showed no vertical variation (Chi-Square=0.85; p=0.36) (Figure 8a). The lack of vertical layering of the water mass in the bay is another indication of a well-mixed system, as a consequence of the strong tidal currents. With the tidal flows, fresh water input from the Kvichak and Nushagak tributaries are mixed with saline water inflow from Bristol Bay to produce a well-mixed brackish water column in the bay with a median salinity of 14 ppt. In addition to spatial variations (Figure 8b), tidal stage resulted in large temporal variations in the upper portions of the tributaries where salinity increased, for example, from zero to 8-10 ppt at Naknek at high tide. Salinity was more variable than other water column measures depending on tidal stage at the time of sampling.

3.1.2 Sediment physical characteristics

In marine coastal environments, sediment physical characteristics define not only the bottom habitats, but also strongly influences the types and distribution of the benthic community. Contaminant levels, sediment grain size and organic matter content are among the most important sediment physical parameters that help define sediment quality. Within the study area, sediment texture was mainly gravel and sand, with pockets of mud in Dillingham Harbor and in the upper stratum at the head of the Kvichak Bay (Figure 9, Table 8). The organic carbon content in the sediment was very low. TOC values exceeded 1% at only 4 stations with fine grained sediment in Dillingham Harbor and at the mouth of the Kvichak River (Table 8). One location in the Naknek River (NR-3P) had slightly higher TOC than the rest of that stratum and was in the vicinity of a fish processing plant (Figure 10).

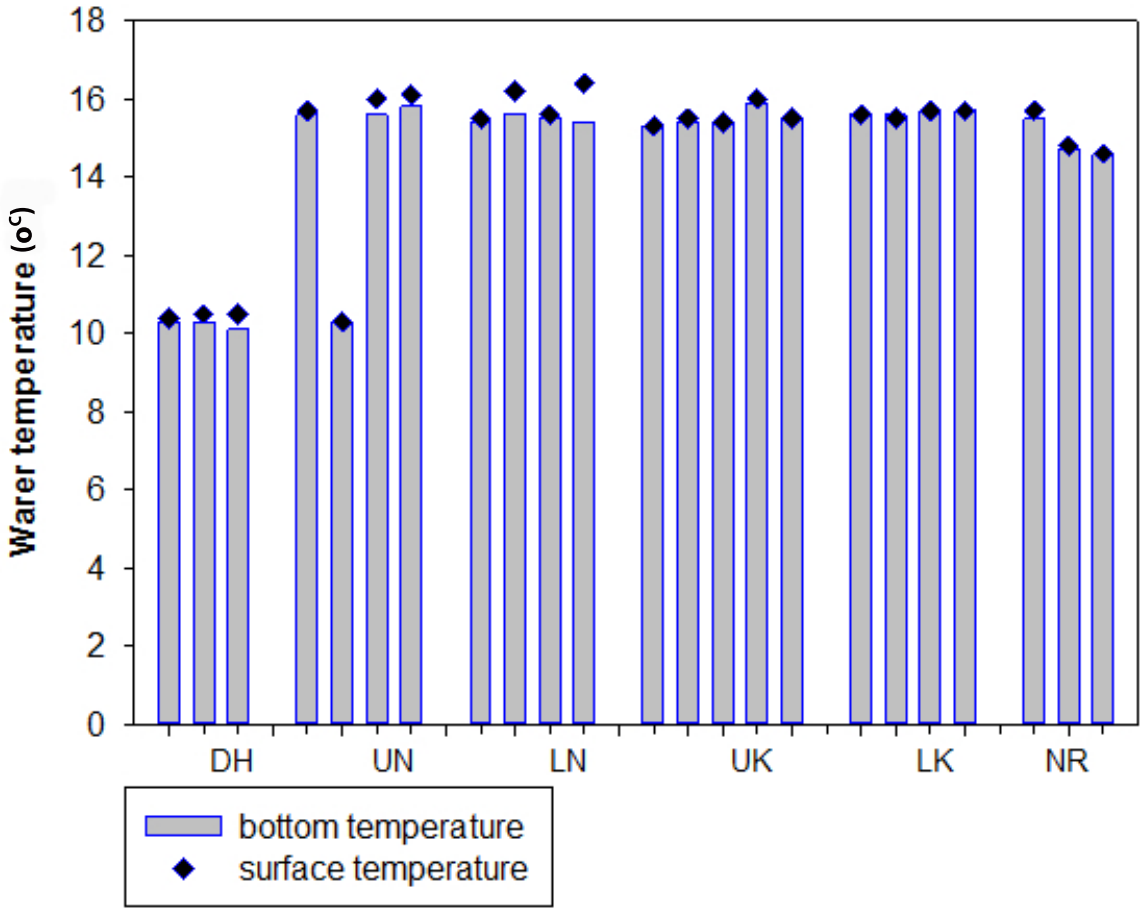

Figure 6a. Contrast between surface and bottom temperature in Nushagak and Kvichak Bays. (DH- Dillingham Harbor; UN and LN- Upper and Lower Nushagak; UK and LK- Upper and Lower Kvichak; NR- Naknek River).

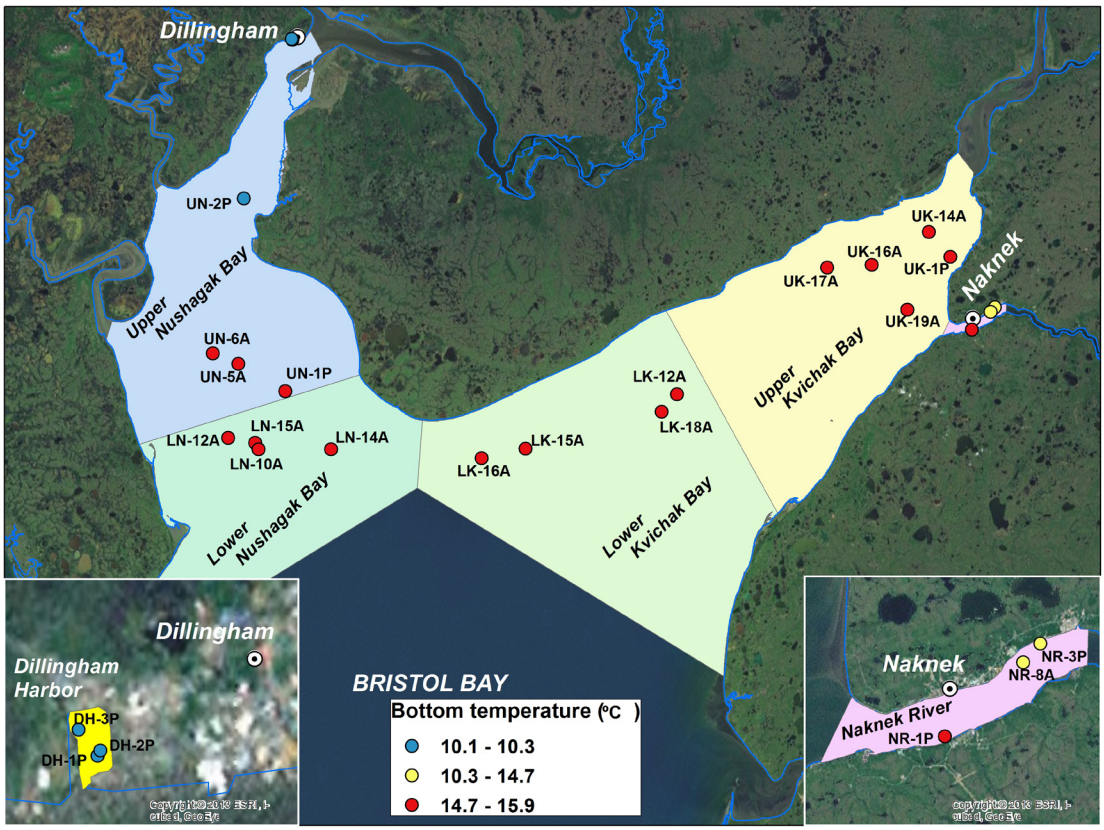

Figure 6b: Spatial distribution of bottom water temperature in Nushagak and Kvichak Bays.

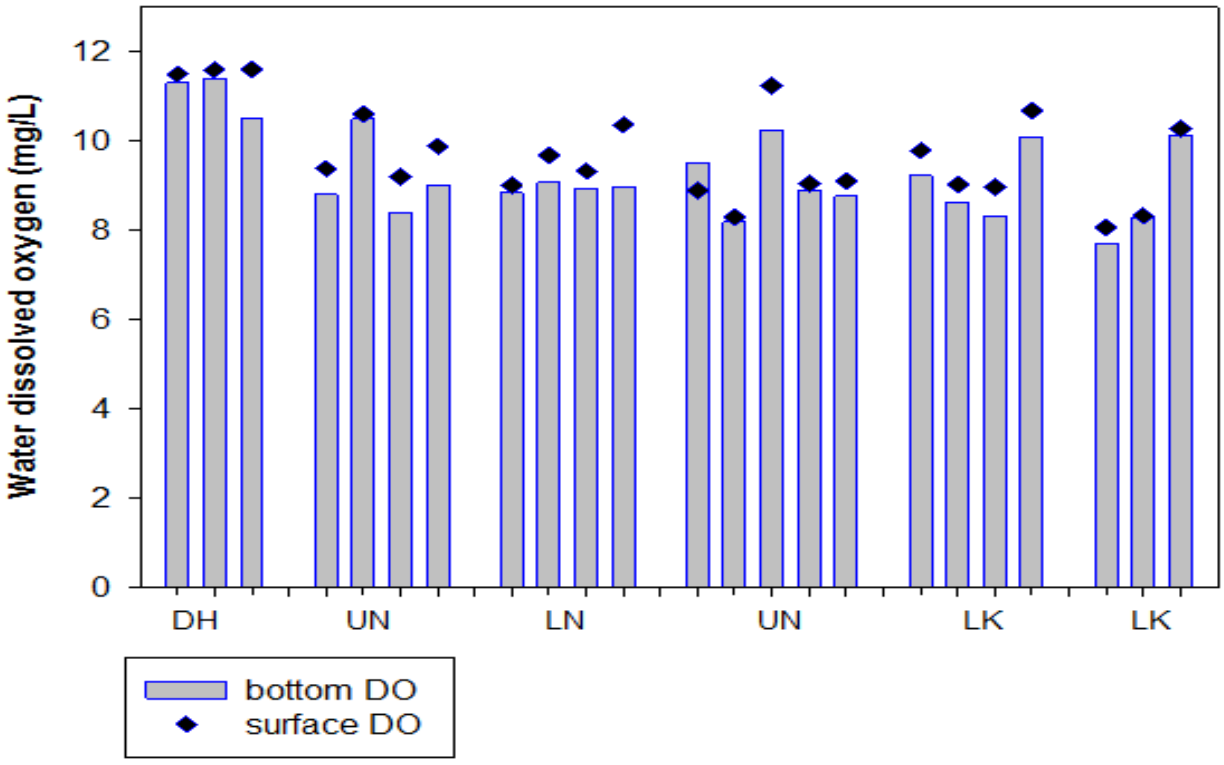

Figure 7a. Contrast between surface and bottom water column dissolved oxygen in Nushagak and Kvichak Bays. No significant difference was found (Chi-Square=2.86; p=0.09). (DH- Dillingham Harbor; UN and LN- Upper and Lower Nushagak; UK and LK- Upper and Lower Kvichak; NR- Naknek River).

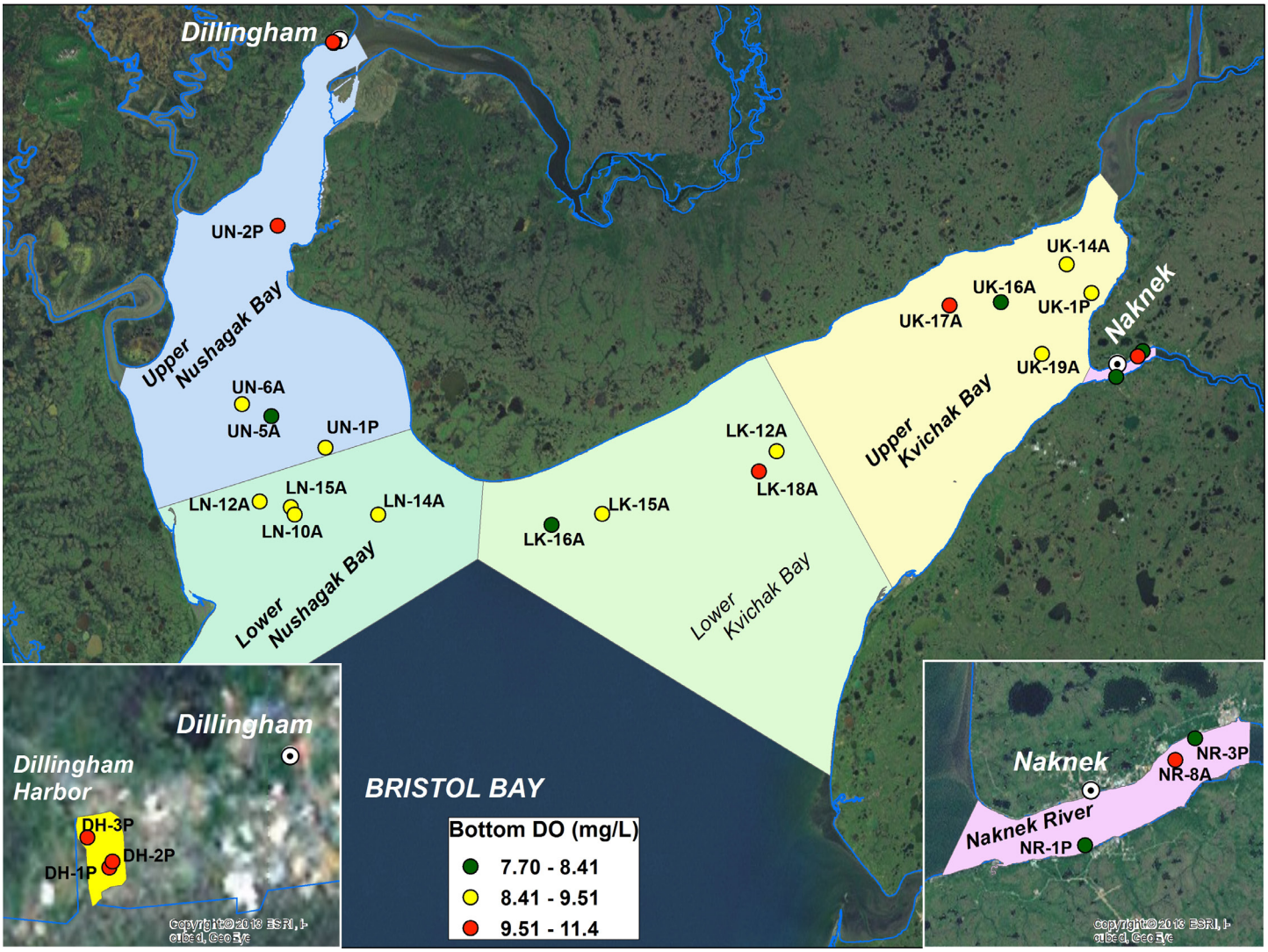

Figure 7b: Spatial distribution of bottom dissolved oxygen in Nushagak and Kvichak Bays.

TOC content correlated strongly with fine fraction of sediment (Figure 11) indicating that although TOC is low in the bottom sediment, the distribution of the organic matter was mainly determined by the finer sediment content. The distribution of TOC and the fine fraction of sediment affects chemical and biological community distributions as contaminants are chemically more likely to adsorb onto the finer sediment particles and organic matter, and benthos tend to forage and cluster in organically rich sediments.

3.2 Concentration of fecal coliform and *Clostridium perfringens* bacteria in sediment

The results of the analysis of sediments for *C. perfringens* and fecal coliform are shown in Table 9. Spearman non-parametric tests indicated that both *C. perfringens* and fecal coliform bacteria were positively correlated ($p<0.05$) with the % fines fraction of the sediment, and negatively correlated with the coarse fractions. The highest levels of *C. perfringens* were found in the sediments in Upper Kvichak Bay, at Site UK-14A and at Site UK-1P (Figure 12) with colony forming units of 1,450 CFU g-1 and 1,095 CFU g-1 respectively. As noted, *C. perfringens* occurs in the intestines of animals, including birds (FDA, 2012). Kvichak Bay is an important stopover area for migrating waterfowl such as king eiders and black scoters. In addition, Kvichak Bay is used by seasonally resident plovers, yellowlegs and dunlin. Kvichak Bay also receives terrestrial runoff. *C. perfringens* in this part of the Bay would not appear to be derived from human sources, however, as the nearest town (Levelock) is nearly 500 km upstream. An elevated concentration of *C. perfringens* was also found in the Naknek river.

3.2 1. River stratum at site NR-3P (952 CFU g-1), and may be related to the presence of wildlife or humans, as it is just upstream of the town of Naknek, and adjacent to salmon processing facilities. NOAA's NS&T Program has monitored levels in *C. perfringens* as part of its regular monitoring of contaminants throughout the coastal US. Values

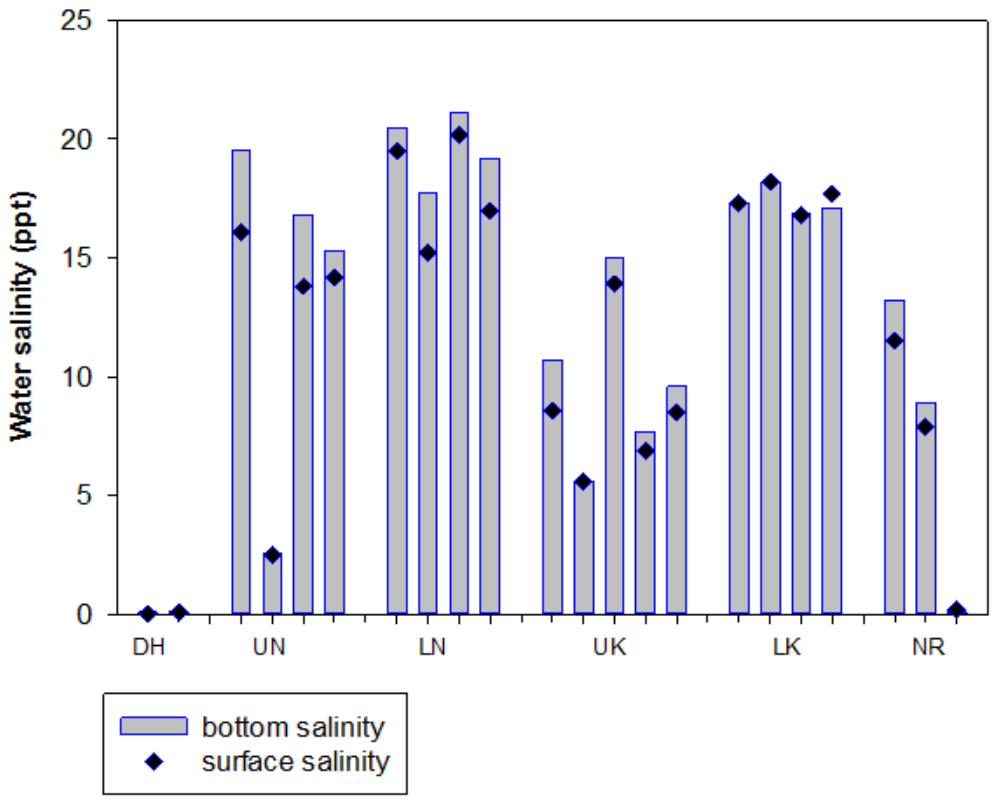

Figure 8a. Contrast between surface and bottom water salinity in Nushagak and Kvichak Bays. No significant difference was found (Chi-Square=0.85; p=0.36). (DH- Dillingham Harbor; UN and LN- Upper and Lower Nushagak; UK and LK- Upper and Lower Kvichak; NR- Naknek River).

range as high as 41,000 CFU g-1 in polluted urban harbors. The 90th percentile is 588 CFU g-1 and the median value is 11. The median from the sediment samples taken in Bristol Bay was 18 CFU g-1, comparable to the NS&T median value, although four sites from Bristol Bay were above the NS&T 90th percentile. No NOAA or health guidelines exist for *C. perfringens* in sediments. *C. perfringens* is a common cause of foodborne illnesses (FDA, 2012). A rare but severe form of enteritis necroticans can be fatal and results from ingesting large numbers of the active bacteria, typically from uncooked food. *C. perfringens* also has the capability of forming spores which can persist in soils and sediments.

The concentrations of fecal coliform bacteria found in the sediments from Bristol Bay are also included in Table 9. As indicated in the spatial distribution assessment (Figure 13), the highest levels (230 MPN g-1) were found in Dillingham Harbor (Site DH-3P), and downstream from Dillingham Harbor (UN-2P) in Upper Nushagak Bay, which may indicate human influences. The third highest level (170 MPN g-1) was also found in Dillingham Harbor (DH-2P), with the fourth highest level being found at Site NR-3P (130 MPN g-1), near the town of Naknek (Figure 13). Like *C. perfringens*, sources of fecal coliform bacteria can be humans as well as wildlife.

There was a significant ($p<0.05$) positive correlation between concentrations of fecal coliform bacteria and *C. perfringens*. However, the highest levels of fecal coliform bacteria were not found in the Upper Kvichak Bay. Unlike *C. perfringens*, fecal coliform bacteria do not have the ability to form cysts, which can persist in the environment. The measurement of fecal coliform bacteria has not been a common parameter in NOAA's NS&T Program, and so comparisons with national medians are not possible. There are numerical guidelines for fecal coliform bacteria in water, but not sediments.

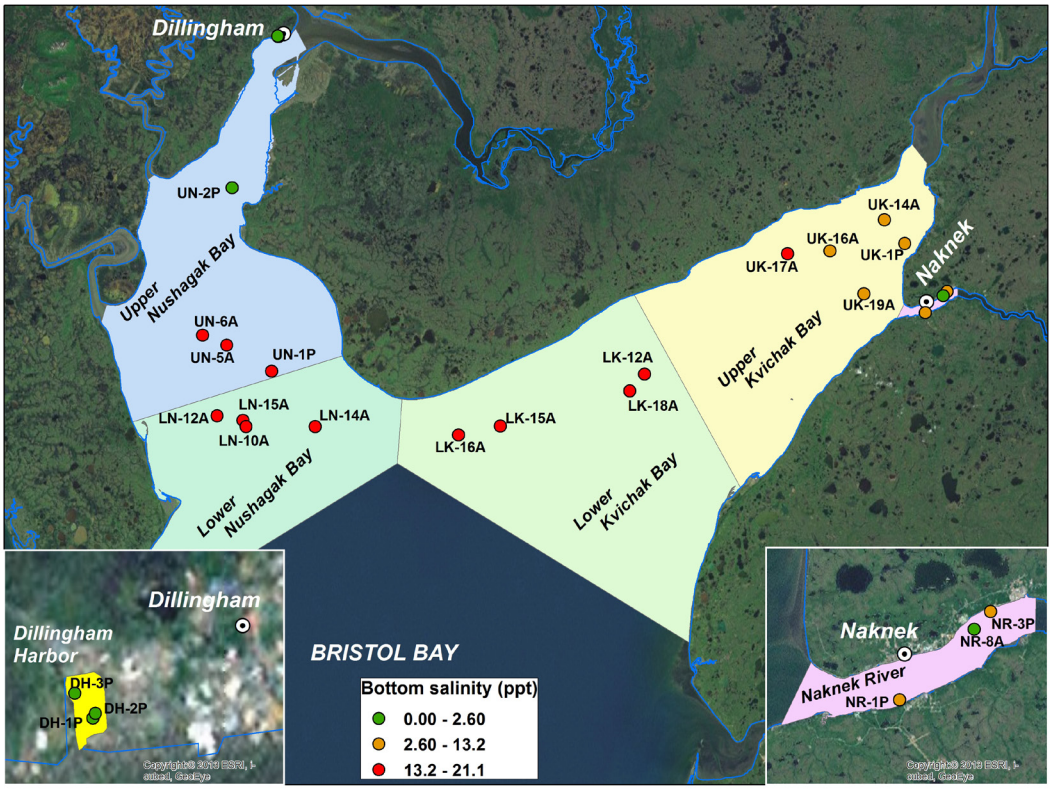

Figure 8b. Spatial distribution of bottom salinity in Nushagak and Kvichak Bays.

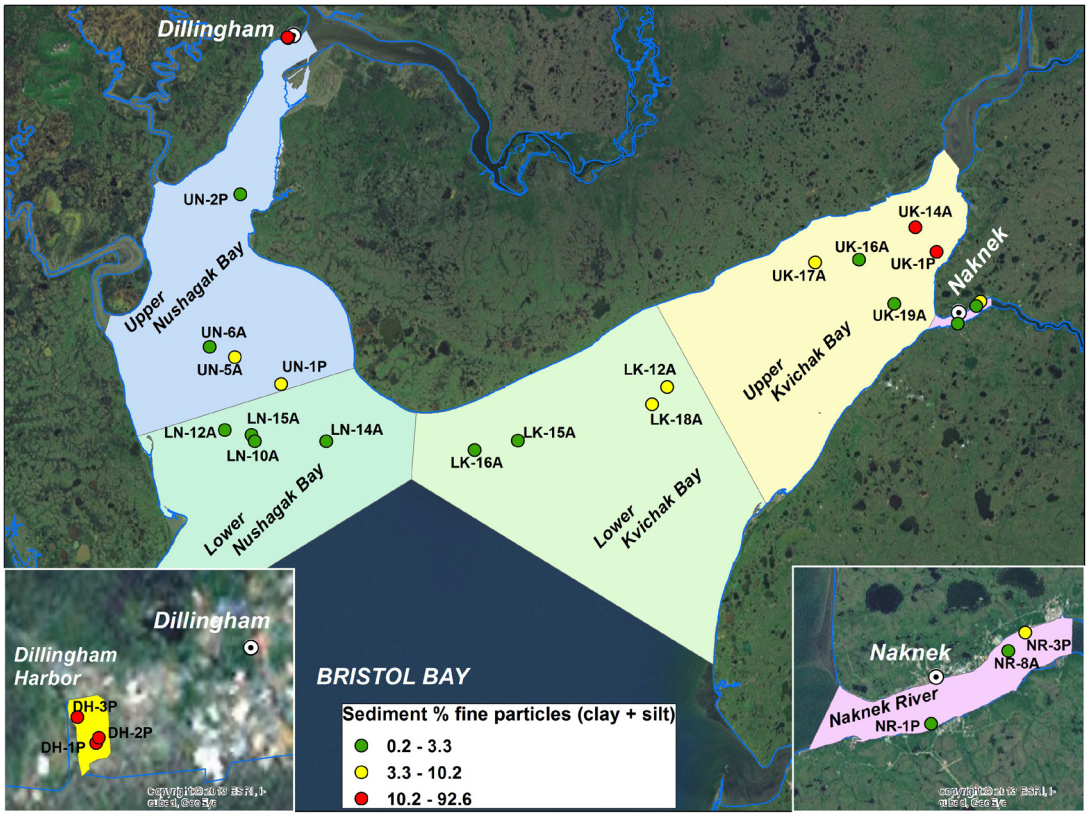

Figure 9. Spatial distribution of fine grained sediment (percent silt + clay) in Nushagak and Kvichak Bays.

Table 8. Grain size characteristics and TOC in sediment from Nushagak and Kvichak Bays.

Station_ID	Location	% Gravel	% Sand	Tot % Coarse	% Silt	% Clay	Tot % Fine	% TOC
DH-1P	Dillingham Harbor	0.0	8.8	8.8	67.8	23.4	91.2	2.47
DH-2P	Dillingham Harbor	0.0	7.4	7.4	66.5	26.1	92.6	2.25
DH-3P	Dillingham Harbor	0.0	22.3	22.3	56.0	21.7	77.7	
UN-2P	Upper Nushagak Bay	3.8	94.6	98.4	0.5	1.1	1.6	0.05
UN-1P	Upper Nushagak Bay	65.2	27.5	92.7	3.0	4.3	7.3	0.17
UN-5A	Upper Nushagak Bay	30.1	65.8	95.8	1.7	2.5	4.2	0.22
UN-6A	Upper Nushagak Bay	2.2	95.9	98.1	0.2	1.7	1.9	0.05
UN-6A	Upper Nushagak Bay	6.2	92.5	98.7	0.4	0.9	1.3	0.07
LN-10A	Lower Nushagak Bay	0.0	99.5	99.5	0.2	0.3	0.5	0.09
LN-12A	Lower Nushagak Bay	8.0	90.3	98.3	0.2	1.5	1.7	0.11
LN-14A	Lower Nushagak Bay	10.8	88.3	99.2	0.2	0.6	0.8	0.08
LN-15A	Lower Nushagak Bay	8.4	89.8	98.2	0.1	1.7	1.8	0.08
NR-1P	Naknek R.	41.9	57.1	99.0	1.0	0.0	1.0	0.11
NR-3P	Naknek R.	2.3	87.5	89.8	4.9	5.4	10.2	0.52
NR-8A	Naknek R.	0.0	99.5	99.5	0.4	0.1	0.5	0.09
UK-14A	Upper Kvichak Bay	0.0	23.8	23.8	54.4	21.8	76.2	1.64
UK-1P	Upper Kvichak Bay	0.0	35.6	35.6	43.3	21.1	64.4	1.37
UK-16A	Upper Kvichak Bay	27.4	72.2	99.6	0.3	0.1	0.4	0.10
UK-17A	Upper Kvichak Bay	0.0	91.2	91.2	4.5	4.3	8.8	0.18
UK-19A	Upper Kvichak Bay	60.1	39.7	99.8	0.1	0.1	0.2	0.11
LK-12A	Lower Kvichak Bay	47.3	48.2	95.5	1.5	3.0	4.5	0.16
LK-15A	Lower Kvichak Bay	0.0	96.7	96.7	0.7	2.7	3.3	0.11
LK-16A	Lower Kvichak Bay	12.5	87.1	99.7	0.2	0.1	0.3	0.12
LK-18A	Lower Kvichak Bay	35.3	60.2	95.5	1.4	3.2	4.5	0.22

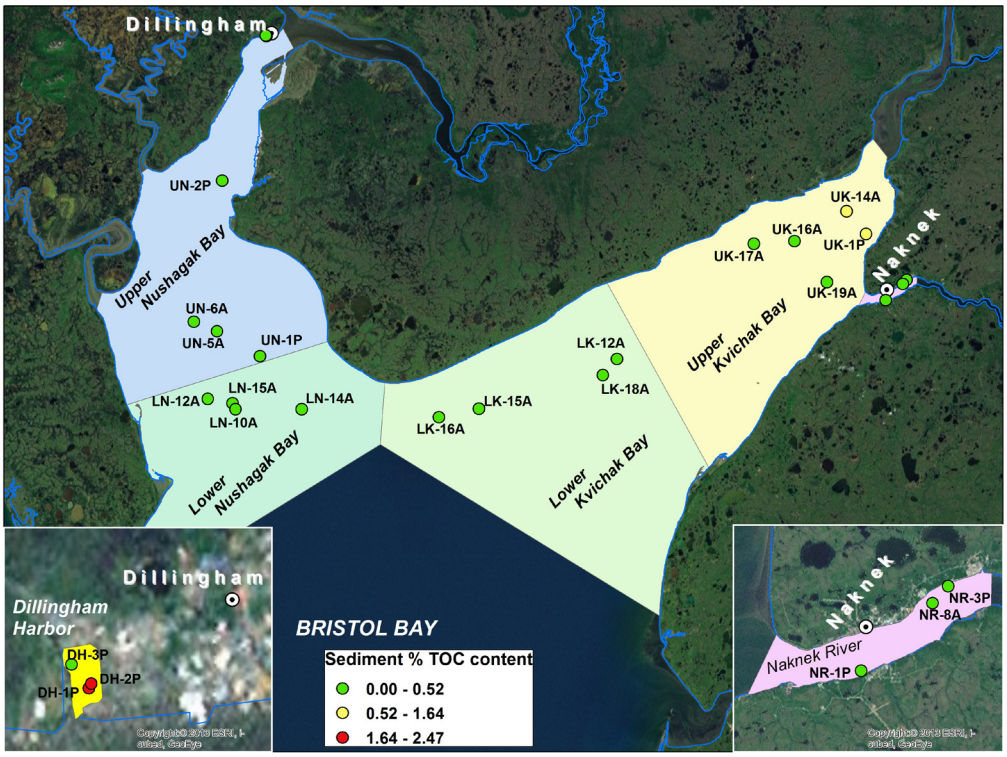

Figure 10. Spatial distribution of TOC content in sediment in Nushagak and Kvichak Bays.

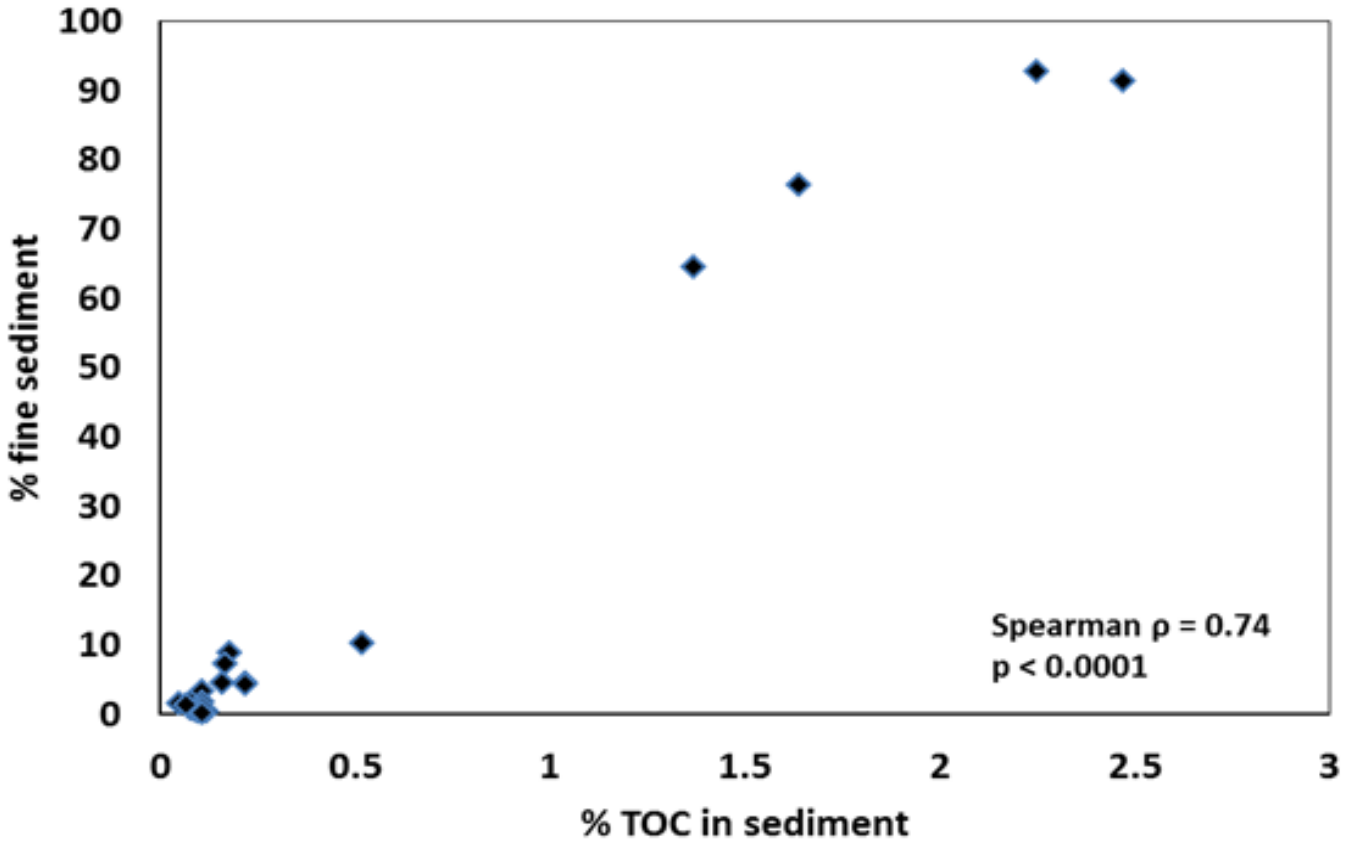

Figure 11. Plot showing positive correlation between % fine grain sediment and %TOC content in sediment from Nushagak and Kvichak Bays, and Spearman rank correlation.

3.3 Contaminant concentrations in sediment

3.3.1 Organic contaminants

PAHs were at higher concentrations in Dillingham Harbor than the open bays, but at very low concentrations overall, and were dominated in all samples by perylene, a natural degradation by-product from decaying vegetation (Figure 14). The same was true for the mouth of the Kvichak and Naknek Rivers (Figure 15, Figure 16). Differences in PAH levels between locations are likely a by-product of site specific watershed characteristics. No sample site had indications of spilled fuel, combustion by-products, or oil. For those chemicals with ERLs and ERMs, the concentrations of organic contaminants were at least an order of magnitude below the ERL levels (Table 10). Overall, the concentrations of organic contaminants in Bristol Bay were well below the NOAA's ERL values suggesting that when considered individually or together, organic contaminants are unlikely to cause any toxic effects in the sediment

Chlorinated pesticides, PCBs, and butyltins (an organometallic compound formerly used in boat antifoulant paints) were either below detection limits or were found only at trace levels, and in widely separated locations (Table 10). Except for Dillingham Harbor, there was only one instance where a single PCB congener was above the method detection limit (0.04 ppb).

In Dillingham Harbor, the sediment contained higher levels of PCBs than other places, but still were only at trace concentrations. All concentrations, even summed concentrations, were below the one part per billion range. Only two congeners were above the method detection limits (PCB 28 and 101/90) at Dillingham. The sediments there were very muddy, and with relatively high TOC levels. In the fall of 2011, an 11,000 lb sack of PCB contaminated soil from a clean-up site at Port Heiden sank off the city dock while in temporary storage there (ADN, 2012). The soil PCB concentration was less than 50 parts per million. The contents were never recovered and presumably were dispersed by the extreme tidal currents. Some PCB residue may have been trapped within the harbor and is slowly being degraded and/or lost by currents and dredging. Total PCB concentrations were more than 2 orders of magnitude below the ERL. A comparable sampling effort in Kachemak Bay (Hartwell et al. 2009) found higher concentrations of all the organic contaminants than those seen in Nushagak

Table 9. Results of fecal coliform and Clostridium perfringens analysis in Bristol Bay sediments. (MPN – most probable number; CFU – colony forming units)

Station ID	Location	%Fine	Fecal coliforms MPN g-1	*C. perfringens* (CFU g-1)
DH-1P	Dillingham Harbor	91.2	70	417
DH-2P	Dillingham Harbor	92.6	170	310
DH-3P	Dillingham Harbor	77.7	230	764
UN-2P	Upper Nushagak Bay	1.60	230	7
UN-1P	Upper Nushagak Bay	7.31	2	70
UN-5A	Upper Nushagak Bay	4.15	2	146
UN-6A 2013	Upper Nushagak Bay	1.92	2	0
UN-6A 2014	Upper Nushagak Bay	1.30	130	0
LN-10A	Lower Nushagak Bay	0.46	2	0
LN-12A	Lower Nushagak Bay	1.70	2	0
LN-14A	Lower Nushagak Bay	0.83	2	0
LN-15A	Lower Nushagak Bay	1.81	2	0
NR-1P	Naknek River	0.98	2	18
NR-3P	Naknek River	10.2	130	952
NR-8A	Naknek River	0.52	2	0
UK-14A	Upper Kvichak Bay	76.2	23	1450
UK-1P	Upper Kvichak Bay	64.4	30	1095
UK-16A	Upper Kvichak Bay	0.40	2	0
UK-17A	Upper Kvichak Bay	8.80	2	54
UK-19A	Upper Kvichak Bay	0.19	2	18
LK-12A	Lower Kvichak Bay	4.52	2	51
LK-15A	Lower Kvichak Bay	3.32	2	0
LK-16A	Lower Kvichak Bay	0.31	2	0
LK-18A	Lower Kvichak Bay	4.54	2	84

and Kvichak Bays. Even the PAH concentrations in Dillingham Harbor were comparable to all but the most remote locations in Kachemak Bay.

3.3.2 Metals

Concentrations of major and trace elements in sediment from the study area were generally low (Tables 11 and 12 respectively) except for arsenic. Summary statistics of concentration ranges and median values for each trace and major element are shown in Figures 17a and 17b. Box-plot statistics and Wilcoxon non-parametric tests indicated significant differences ($P < 0.05$) between strata for half of the elements (Figures 17a and b). Relative to the other strata, most of the metals in Dillingham Harbor were elevated although some spikes were recorded at isolated sites in the Upper Kvichak and Nushagak strata (Figures 17a and b). Fine-grained sediment has a high surface to volume ratio. In addition, metals tend to bind to clays as a result of the charge characteristics of the clay particles. Thus, fine grained sediments tend to sequester higher concentrations of particle reactive elements through adsorption. Dillingham Harbor is a depositional environment with a high percentage of fine grain sediments. It is also a center of vessel activity and maintenance which undoubtedly is a source of metals. Sediments at the UK-1P, UK-14A in and UK-17A sites in Upper Kvichak stratum had relatively higher content of fine sediment (Table 8), which may be linked to the spikes in the concentration of cadmium, copper, iron and zinc.

To test whether metal concentrations may be elevated at the head of the Kvichak and Nushagak Bays because of river transport which brings eroded materials to the head of the bays, sediment metal concentrations in the pool of upper strata (excluding Dillingham Harbor) were compared to those of the lower strata. The results revealed no significant

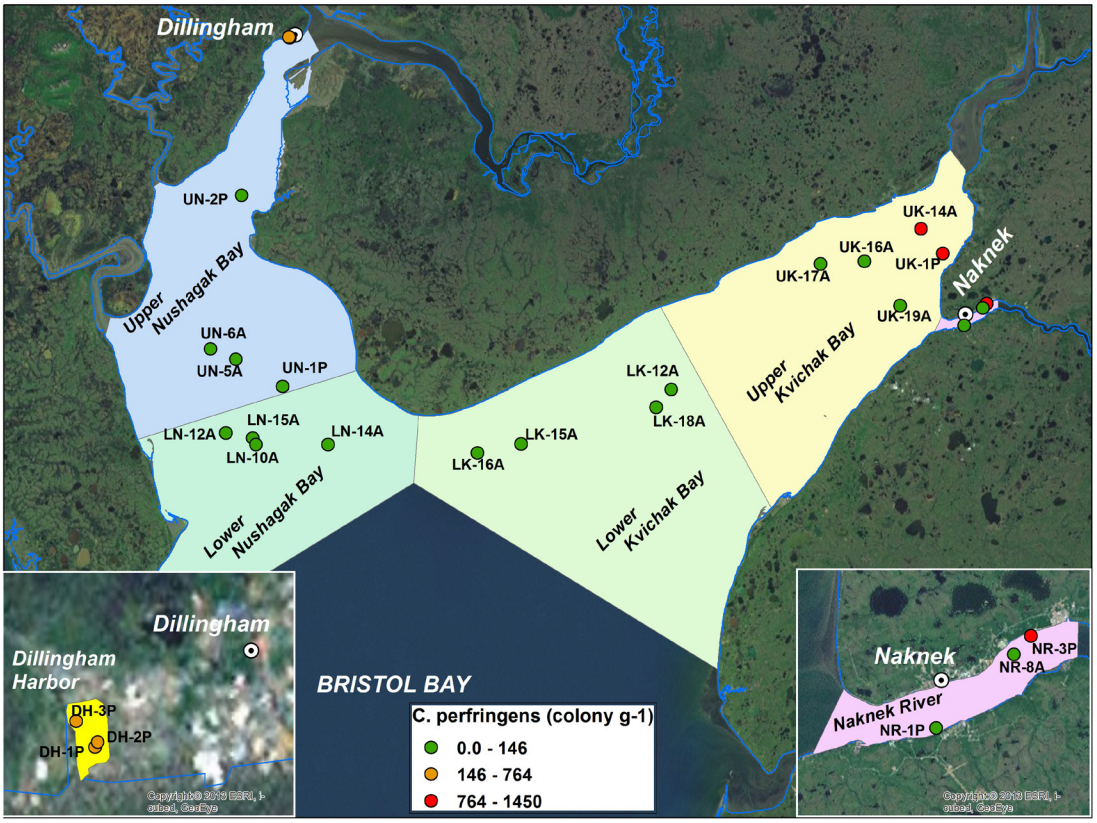

Figure 12. Spatial distribution of colony forming Costridium perfringens in sediment from Nushagak and Kvichak Bays.

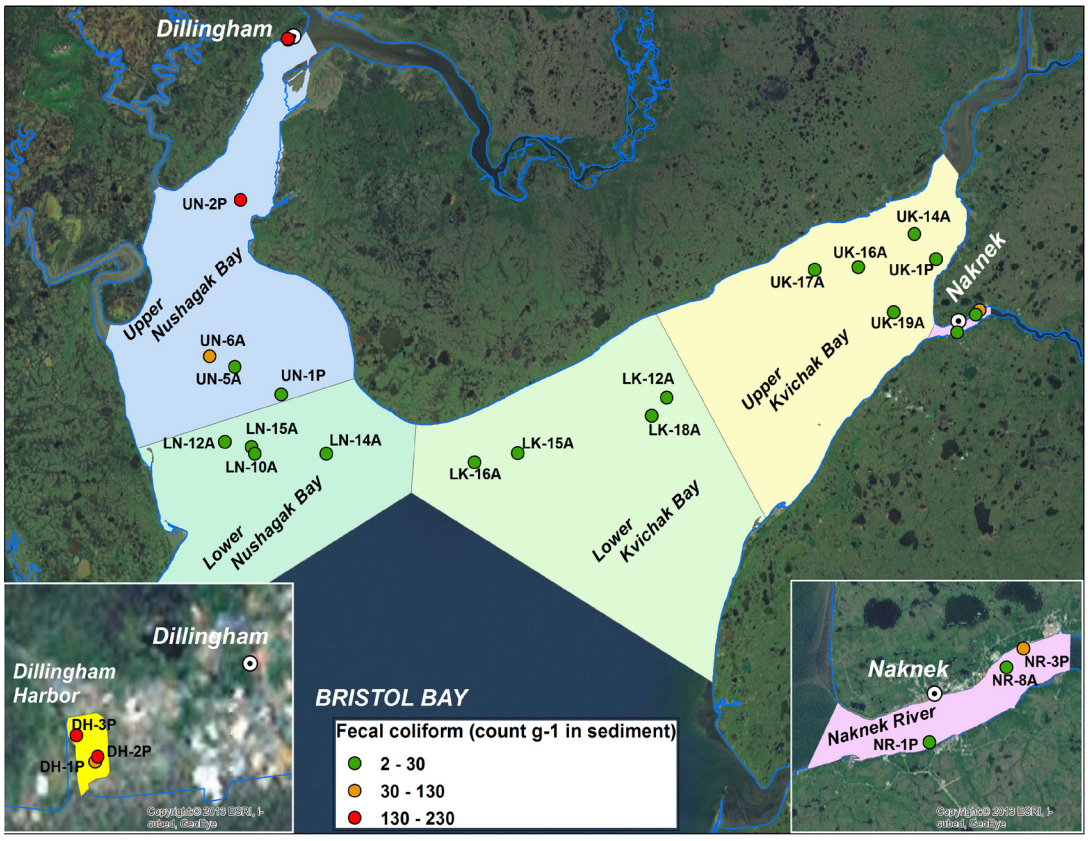

Figure 13. Spatial distribution of Fecal coliform content in sediment from Nushagak and Kvichak Bays.

differences, however, between the upper bay and lower bay strata (Chi-square $p > 0.05$). Metal concentrations were evenly distributed in sediment throughout the study area due to the strong influence of tidal mixing.

In general, trace element concentrations in the study area were lower than regional concentrations reported by other studies in the region. The NS&T Bioeffects Program conducted a sediment quality assessment in Kachemak Bay, Alaska in 2007 (Hartwell et al. 2009). In collaboration with the U.S. EPA Environmental Monitoring and Assessment Program (EMAP), the Alaska Department of Environmental Conservation undertook a coastal ecological condition study in Southcentral Alaska, which encompassed an assessment of contaminants and benthic assemblages in sediments along the Gulf of Alaska and the Aleutian Islands (Saupe et al. 2005). Relative to data reported by the aforementioned studies, metal concentrations in Bristol Bay were very low compared to bays and coastal regions of central and southcentral Alaska. To put this comparison in perspective, the median metal concentration values reported by Hartwell et al. (2009) in Kachemak Bay were plotted against median values in Bristol Bay and are illustrated in Figures 17a and b. For virtually all metals, median metal concentration values were lower in Bristol Bay except for aluminum, which showed similar concentrations in both regions (Figure 17a).

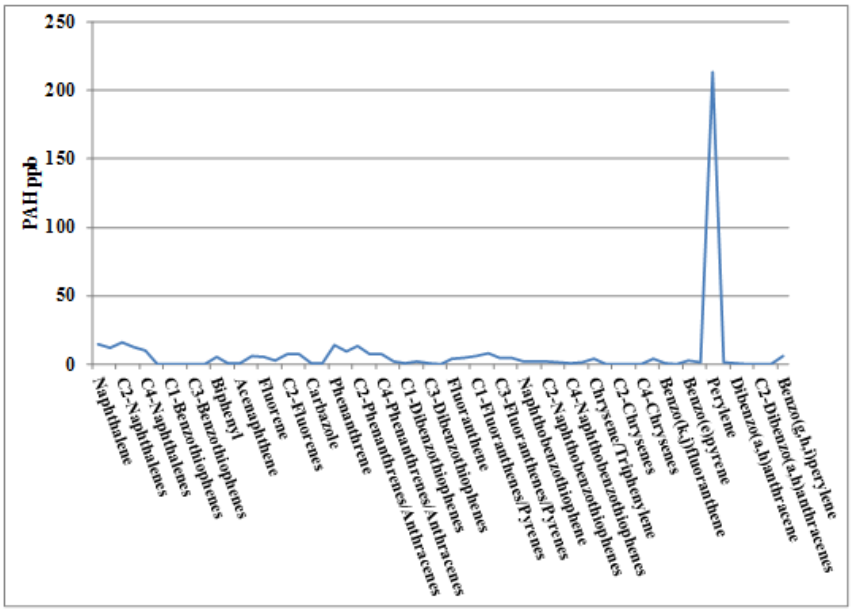

Figure 14. Concentrations of individual PAHs in sediment from Dillingham Harbor.

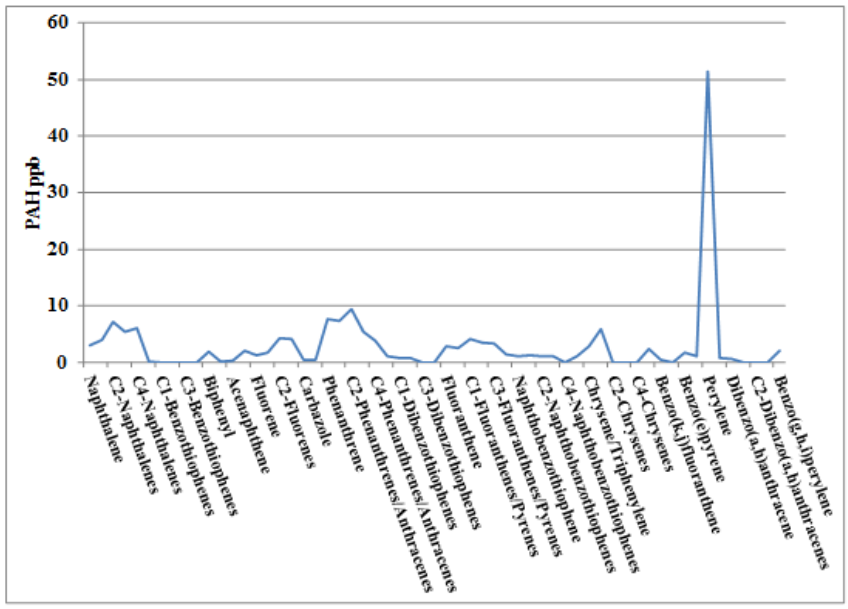

Figure 15. Concentrations of individual PAHs in sediment from the mouth of the Kvichak River.

There are no Alaska State criteria for sediment quality. The quality of the sediment pertaining to chemical contamination was assessed based on the NOAA sediment quality guidelines ERL and ERM (Long et al. 1995). None of the toxic trace elements exceeded any ERL or ERM, except arsenic (Table 12). Arsenic was present at virtually all stations at or above the ERL level, and appears to be related to naturally occurring background concentrations in the watersheds. Elevated arsenic concentrations were observed in bioeffects data sets in other regions, particularly Kachemak Bay, Alaska (Hartwell et al. 2009) and Southcentral, Alaska (Saupe et al. 2005).

There was not an inverse relationship between aluminum and silica as is normally seen in other places (Figure 18). In fact the relationship between aluminum and the other elements was relatively poor, including iron (Figure 19a) which is normally strongly positively correlated with aluminum. This phenomenon has not been seen in other areas (Figure 19b), but the observed range of Al con-

Table 10. Organic contaminant concentration in sediments from Bristol Bay. Where available ERM and ERL values are included.

Station_ID	Location	Tot PAHs	Perylene	% Perelyene	Tot PCBs	Tot HCH	Tot Cyclodienes	Tot DDTs	tot BTs
DH-1P	Dillingham Harbor	489	213.6	43.7	0.42	0.05	0.21	0.15	0.00
DH-2P	Dillingham Harbor	476	210.7	44.3	0.29	0.25	0.18	0.68	0.00
DH-3P	Dillingham Harbor	587	198.6	33.8	0.19	0.00	0.00	0.32	0.65
UN-2P	Nushagak Bay	16.9	8.4	49.6	0.00	0.00	0.00	0.00	0.00
UN-1P	Nushagak Bay	26.8	7.4	27.7	0.05	0.09	0.00	0.00	0.00
UN-5A	Nushagak Bay	21.1	8.3	39.5	0.07	0.09	0.00	0.00	0.00
UN-6A	Nushagak Bay	10.2	3.0	29.5	0.05	0.01	0.00	0.00	0.00
UN-6A	Nushagak Bay	13.5	5.6	41.1	0.03	0.10	0.00	0.00	0.00
LN-10A	Nushagak Bay	9.87	1.7	17.7	0.03	0.08	0.00	0.00	0.00
LN-12A	Nushagak Bay	11.5	2.3	20.1	0.04	0.08	0.00	0.02	0.14
LN-14A	Nushagak Bay	6.83	1.4	20.8	0.03	0.07	0.00	0.07	0.00
LN-15A	Nushagak Bay	7.18	1.4	19.0	0.02	0.09	0.00	0.05	0.00
NR-1P	Naknek R.	15.4	2.5	16.6	0.04	0.08	0.00	0.00	0.00
NR-3P	Naknek R.	56.4	12.5	22.2	0.08	0.09	0.00	0.00	0.00
NR-8A	Naknek R.	14.3	2.6	18.1	0.02	0.08	0.00	0.08	0.00
UK-14A	Kvichak Bay	201	69.3	34.4	0.00	0.00	0.00	0.00	0.00
UK-1P	Kvichak Bay	183	51.3	28.0	0.00	0.04	0.00	0.10	0.00
UK-16A	Kvichak Bay	8.15	2.3	28.4	0.03	0.08	0.00	0.08	0.00
UK-17A	Kvichak Bay	25.5	9.8	38.6	0.03	0.00	0.00	0.00	0.00
UK-19A	Kvichak Bay	11.5	2.0	17.7	0.04	0.08	0.00	0.08	0.00
LK-12A	Kvichak Bay	15.8	2.7	16.9	0.02	0.00	0.00	0.00	0.00
LK-15A	Kvichak Bay	14.4	2.1	14.4	0.01	0.00	0.00	0.22	0.00
LK-16A	Kvichak Bay	7.49	1.3	17.5	0.02	0.09	0.00	0.02	0.00
LK-18A	Kvichak Bay	15.1	2.3	15.3	0.02	0.00	0.00	0.00	0.00
ERL		4022			22.7			1.58	
ERM		44792			180			46.1	

centrations in the Nushagak and Kvichak is relatively small ranging only between 6 and 8% (60,000 – 80,000 ppm) of sediment.

Copper was elevated in Dillingham Harbor relative to other areas. Few boats in the harbor use copper-based bottom paint, but fishing boats from other regions that migrate there for salmon season may. The harbor is dredged annually and dredge spoil was reportedly dumped on land adjacent to the harbor in the past, and may serve as a reservoir. On the other hand, the sediments in the harbor are ~90% fine-grained material which tends to accumulate higher metals levels (Figure 20).

Benthic Community Characterization

A total of 1,887 organisms representing 79 taxa were collected (excluding 8 epizoic taxa, comprising only 39 individuals). Total number of taxa, abundance per m-2 and diversity at each station and stratum are listed in Table 13. A complete species list is presented in Appendix 1. Benthic community condition was highly variable, with pockets of diverse communities throughout the study area, but with no obvious gradients or spatial patterns (Figures 21 and 22). Diversity and species richness were correlated with higher salinity and Secchi depth and negatively correlated with latitude. This would indicate that there is increasing diversity away from the river mouths, and all three parameters are confounded. However, this is driven by the extremely low diversity at Dillingham and Naknek (all less than 1). Station NR-3P near the fish packing plant within Naknek River stratum had only 1 taxon (Oligochaete sp.). Diversity and species richness did not show consistently increasing gradients going down the estuaries (Figure 23). Previous reports state that the upper Nushagak Bay has a benthic community Shannon Diversity (H') value of 1.54, ranking it below similar subarctic estuaries (Radenbaugh and Pederson 2011) which is consistent with the current data. Sampling data from the lower bays is virtually non-existent.

Abundance did not correlate to any habitat parameter, except % silt (ρ =0.42854, p= 0.0367 (but not % clay or % fines) and was negatively correlated to % sand (ρ = -0.65695, p =0.0005 (but not % gravel or % coarse). Again, these statistical results are attributed to very low values at Dillingham which was anomalously muddy, along with the outlier value at Naknek station 8_1P.

The nodal analysis did not identify any discernable pattern or gradient of locations beyond grouping those stations with relatively high numbers of taxa vs low numbers. Similar results were obtained in 2010 by a USGS survey of the western side of Kvichak Bay to assess benthic food

Table 11. Concentration (ppm) of major metals in sediment from Bristol Bay. Where available ERM and ERL values are included.

Station ID	Location	Aluminum	Chromium	Copper	Iron	Manganese	Silicon	Zinc
DH-1P	Dillingham Harbor	74900	41.8	23.8	44600	1140	272000	82.7
DH-2P	Dillingham Harbor	77200	44.3	25.2	45600	1220	286000	84
DH-3P	Dillingham Harbor	77400	35.6	23.5	41200	971	288000	76.1
UN-1P	Upper Nushagak	65500	32.2	9.23	40100	719	301000	56.5
UN-2P	Upper Nushagak	65100	21.3	8.79	25100	412	321000	42.5
UN-5A	Upper Nushagak	67500	19.1	16.9	31700	557	299000	50.7
UN-6A 2013	Upper Nushagak	67200	15.5	8.44	26400	477	328000	45.3
UN-6A 2014	Upper Nushagak	70600	21.4	6.98	26900	562	314000	45
LN-10A	Lower Nushagak	65300	17.7	7.59	26800	630	313000	40.1
LN-12A	Lower Nushagak	65500	29.3	7.69	35600	696	310000	51
LN-14A	Lower Nushagak	68600	21.4	9.3	27900	501	320000	43.7
LN-15A	Lower Nushagak	68600	16.6	10.9	31000	900	314000	49
NR-1P	Upper Kvichak	66200	29	19.7	35100	817	247000	62.2
NR-3P	Upper Kvichak	73500	19.8	7.56	26400	466	312000	42
NR-8A	Upper Kvichak	70900	32	10.8	44000	717	298000	58.7
UK-14A	Upper Kvichak	62000	35.1	9.59	54900	1130	293000	74.3
UK-1P	Upper Kvichak	71500	30.1	17.4	37900	857	277000	64.7
UK-16A	Lower Kvichak	64400	29.2	8.77	42900	804	298000	59.7
UK-17A	Lower Kvichak	69400	26	7.31	31300	680	316000	45.7
UK-19A	Lower Kvichak	73300	18.3	6.96	27400	498	324000	42.2
LK-12A	Lower Kvichak	68300	24.8	9.09	33500	615	310000	50.9
LK-15A	Naknek River	68300	32	10.5	40500	797	287000	59.6
LK-16A	Naknek River	74600	23	14.9	31200	751	296000	55.2
LK-18A	Naknek River	69500	25.5	9.15	31800	624	305000	47.2
ERL			**81**	**34**				**150**
ERM			**370**	**270**				**410**

resources for migratory birds (unpublished data). They identified a total of 98 taxa. The number of taxa per station ranged from 0 to 26, with a median of 6.

3.5 Sediment toxicity assessment

The toxicity bioassays did not indicate acute toxicity due to anthropogenic contamination. The Microtox® bioassays did not result in negative responses at any location (Table 14). In fact, only one station in the Lower Nushagak stratum exceeded the control sample response. The sea urchin fertilization results indicated toxicity at two adjacent stations (LK-12A and LK18A) in the Lower Kvichak stratum (Table 15). The development endpoint is generally a more sensitive indicator than the fertilization endpoint (Carr, 1997). That test indicated significant impacts at those same two sites in the Lower Kvichak stratum, and also in the Naknek River and Lower Nushagak strata (Table 15).

The impacts appear to be related, in part, to unionized ammonia concentrations in the pore water (Figures 24 and 25). Sulfide was below detection limits in all cases. There is a gradient of impact in the Lower Kvichak stratum from station LK-12A and LK-18A toward the downstream direction (downstream being a relative term, depending on whether the tide was rising or falling). These were located below where the fleet of factory ships are required to anchor. Station NR-3P in the Naknek River stratum was directly in front of a fish packing plant. All three locations had very high unionized ammonia concentrations. The development endpoint is more sensitive than the fertilization endpoint, and thus the possibility for false positive results is greater. Also, unionized ammonia is not a measured value. It is calculated based on the pH, salinity and total ammonia measures. It cannot be known what chemical equilibria are changed, nor how fast they occur in the samples, when the salinity is adjusted from ambient to the standard 30 ppt test level in the testing laboratory. Furthermore, pore water samples from stations LK-12A, LK-18A in the Lower Kvichak and NR-3P in Naknek River were too muddy to extract through air stones with a syringe, but were extracted by centrifugation (as were the 2013 samples). Thus, some uncertainty exists as to the cause of developmental effects,

Table 12. Concentration (ppm) of trace metals in sediment from Bristol Bay. Where available ERM and ERL values are included

Station ID	Location	Antimony	Arsenic	Cadmium	Lead	Mercury	Nickel	Selenium	Silver	Tin
DH-1P	Dillingham Harbor	0.54	10.8	0.174	9.13	0.0306	9.76	0.447	0.0785	2.81
DH-2P	Dillingham Harbor	0.537	10.8	0.18	9.03	0.0321	9.78	0.657	0.133	1.17
DH-3P	Dillingham Harbor	0.488	9.94	0.165	7.6	0.0309	7.9	0.448	0	1.24
UN-2P	Upper Nushagak	0.527	7.99	0	5.81	0.007	7.83	0	0	0.792
UN-1P	Upper Nushagak	0.648	13.9	0	6.64	0.007	5.14	0	0	0.796
UN-5A	Upper Nushagak	0.578	15.3	0.086	5.93	0.01	10	0.462	0	0.765
UN-6A 2013	Upper Nushagak	0.68	18.6	0	6.25	0.0054	6.0	0.248	0	0.701
UN-6A 2014	Upper Nushagak	0.61	11.6	0	6.28	0.0079	4.44	0	0	0.67
LN-10A	Lower Nushagak	0.54	14.1	0	6.03	0.006	5.63	0	0	0.667
LN-12A	Lower Nushagak	0.643	8.26	0	5.77	0.005	6.94	0	0	0.82
LN-14A	Lower Nushagak	0.587	14.4	0	5.66	0.005	6.84	0.374	0	0.583
LN-15A	Lower Nushagak	0.714	20.5	0	6.19	0.006	6.16	0.263	0	0.851
NR-1P	Upper Kvichak	0.41	9.81	0.153	6.89	0.025	7.88	0.413	0.132	0.887
NR-3P	Upper Kvichak	0.525	10.7	0	6.31	0.006	6.38	0.232	0	1.3
NR-8A	Upper Kvichak	0.526	8.37	0.087	6.44	0.005	6.91	0.12	0	0.848
UK-14A	Upper Kvichak	0.538	10.4	0	5.28	0.003	11	0.099	0.085	0.845
UK-1P	Upper Kvichak	0.516	11.1	0.133	7.2	0.02	8.32	0.437	0.097	0.87
UK-16A	Lower Kvichak	0.503	10.5	0	5.33	0.003	7.12	0	0	1.03
UK-17A	Lower Kvichak	0.531	12	0	5.6	0.004	6.58	0.294	0	0.84
UK-19A	Lower Kvichak	0.56	16.7	0	5.82	0.004	6.39	0.341	0	0.675
LK-12A	Lower Kvichak	0.46	12.9	0.083	5.43	0.004	6.89	0.537	0	0.636
LK-15A	Naknek River	0.456	6.54	0	5.36	0.004	7.56	0	0	0.688
LK-16A	Naknek River	0.923	8.56	0.095	5.81	0.009	8.8	0.382	0.115	0.696
LK-18A	Nanek River	0.484	6.29	0	5.15	0.003	7.39	0	0	0.585
ERL			**8.2**	**1.2**	**46.7**		**20.9**		**1**	
ERM			**70**	**9.6**	**218**		**51.6**		**3.7**	

but it is clear that some sublethal impacts are occurring in specific locations due to either natural or anthropogenic causes.

NPDES permit data from 2014 from the shore-based processing plants and the floating facilities are available from Alaska DEC (ADEC) (Figure 26). Over forty three million pounds of shredded fish waste were dumped into Nushagak and Kvichak Bays in 2014, mostly in the Kvichak. The land-based plants discharged over 28.4 million pounds and the floating processing plants dumped 14.7 million pounds of waste. Additional waste may be dumped outside the mouth of both Kvichak and Nushagak Bays that is barged up from the processing plants located at Egegik. This huge influx of organic matter introduced over a relatively short period must affect the habitat. Whether the net effect is harmful (e.g. toxicity, local hypoxia) or beneficial (e.g. food resource) needs investigation.

3.6 Contaminant body burdens in fish

Contaminant body burdens in fish were relatively low. Only strongly lipophilic contaminants (organic compounds and methyl-mercury) were consistently detected at or above instrument detection limits (Table 16). Rainbow smelt accumulated relatively higher levels of mercury, PCBs and chlorinated pesticides than young starry flounder in Dillingham Harbor, due in part to higher lipid contents (Figures 27 and 28). The fish from Dillingham and Naknek had higher lipid levels than fish from the open bays. Whether or not the fish waste dumped at those areas is a contributing factor is unclear.

Larger (older) flounder had accumulated higher levels of mercury than younger fish. Given the relatively uniform spatial distributions of mercury, location is unlikely to have affected this. For inter-stratum comparison, contaminant concentrations in fish were lipid-normalized. The number of PCB congeners in fish tissues was much larger than that seen in the sediments and is very similar in fish from separate locations, indicating that the source(s) are probably not local but due to long-range water and/or atmospheric dispersal. However, body burdens were higher in fish from Dillingham and Naknek than in the open bays. Overall, all tissue concentrations are in the same range as those report-

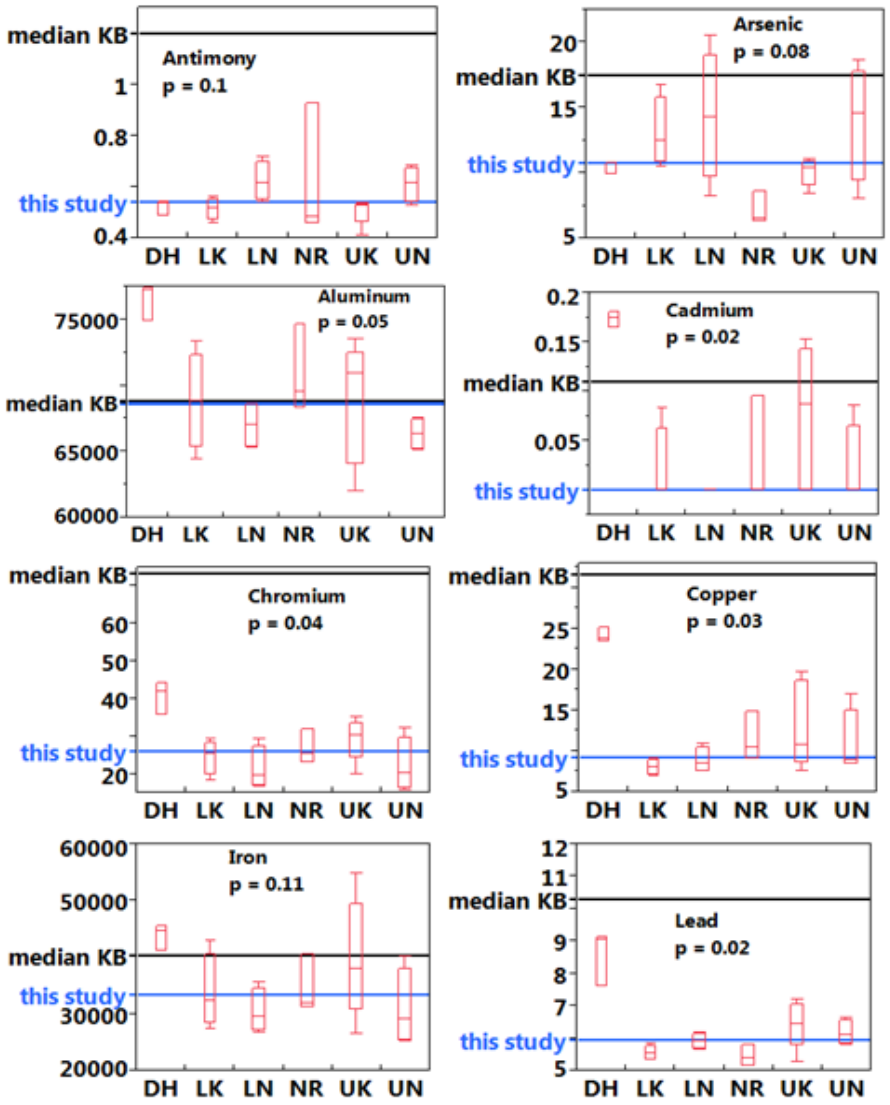

Figure 17a. Box-plot statistics illustrating elemental (mg g-1 in sediment) distribution in each stratum (DH = Dillingham Harbor, LK = Lower Kvichak, LN = Lower Nushagak, UK = Upper Kvichak, and UN = Upper Nushagak). The box shows the data range in each stratum as the 25th, median and 75th percentiles, while the top and bottom whiskers of the box represent the 10th and 90th percentiles. The p-value indicates the significance of nonparametric inter-stratum comparison based on the Wilcoxon test. The horizontal lines contrast the overall median between metal concentration in sediment from the Bristol Bay study area (blue) and Kachemak Bay (KB, black).

ed by the Alaska Fish Monitoring Program (AFMP, 2011) in a variety of species from around the state. It should also be noted that the AFMP reports concentrations on skinless fish fillets. The values reported in this report are from whole body homogenates which would be expected to yield higher values than fillets due to fatty tissue in the viscera.

3.7. Fish Histopathology

Starry flounder and rainbow smelt were collected by trawl in Dillingham Harbor in 2013. Fish were also collected in 2014 for body burden and histology, but the histology specimens were not preserved with enough formalin to use for histological examination.

3.7.1 Starry flounder

The Dillingham flounder specimens were small and ranged in size from 11.0-12.5 cm and 13-25g. Externally, all fish appeared normal with the exception of a single flounder with a papilloma extending from the dorsal side, maxillary region of the mouth (Figure 29). The papilloma was off white in color and cauliflower in texture measuring ~20 X 20 mm.

Tumors in Pacific flatfish are fairly common, and well documented. McArn et al. (1968) reported an incidence rate of 5.4% in collections from Port Susan, Port Gardner, and Bellingham Bay during the period of 1965-1966 (N

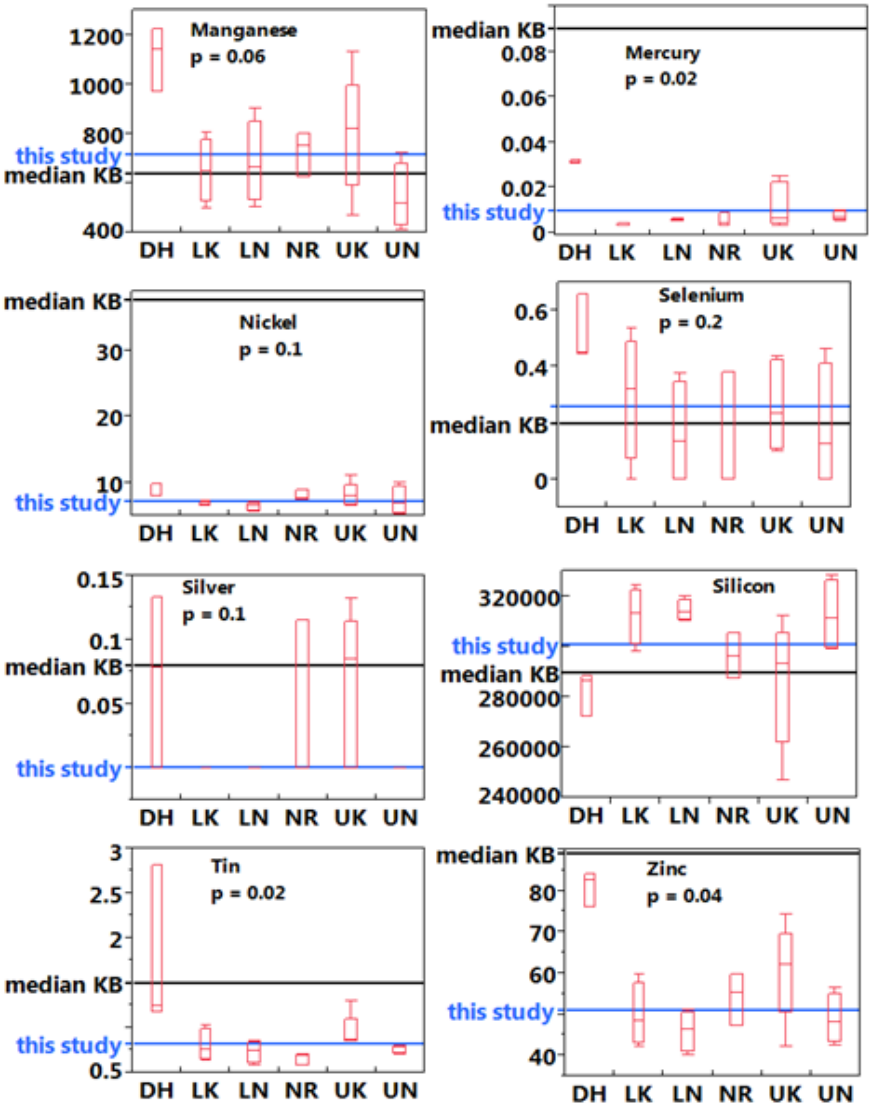

Figure 17b. Box-plot statistics illustrating elemental (mg g-1 in sediment) distribution in each stratum (DH = Dillingham Harbor, LK = Lower Kvichak, LN = Lower Nushagak, UK = Upper Kvichak, and UN = Upper Nushagak). The box shows the data range in each stratum as the 25th, median and 75th percentiles, while the top and bottom whiskers of the box represent the 10th and 90th percentiles. The p-value indicates the significance of nonparametric inter-stratum comparison based on the Wilcoxon test. The horizontal lines contrast the overall median between metal concentration in sediment from the Bristol Bay study area (blue) and Kachemak Bay (KB, black).

= 1063). Similarly, a survey by Campana (1983) found a high incidence in age 1 flounder, which declined with age. The author concluded that the most likely reason was mortality associated with the pathology and stress. Many papillomas from flatfish and cod have been associated with the finding of protozoan parasites know as X-cells (Freeman et al. 2011). No X-cells were observed in this particular case. Internal gross observation revealed the presence of helminth nematodes (roundworms) in 3 of the 8 flounder (38%). In all cases, they were free in the visceral cavity and only a single nematode was found per fish.

Tissue sections of skin, muscle, spleen, kidney, heart, liver, and intestine were assessed for pathological conditions. Four of the eight fish had mild to moderate accumulation of macrophage aggregates in the spleen and/or kidney. This is generally considered a non-specific stress response and not unusual. Without comparative fish from other locations of similar age, the significance of the finding is unknown.

Necrosis was noted in several organs, however, this could be the result of insufficient preservation. Thrombosis, or cellular accumulation and swelling, was noted in the gill lamellae of a single fish, which most likely reflects post-mortem change. The remainder of the fish gills were unremarkable with the exception of the flounder with the papilloma.

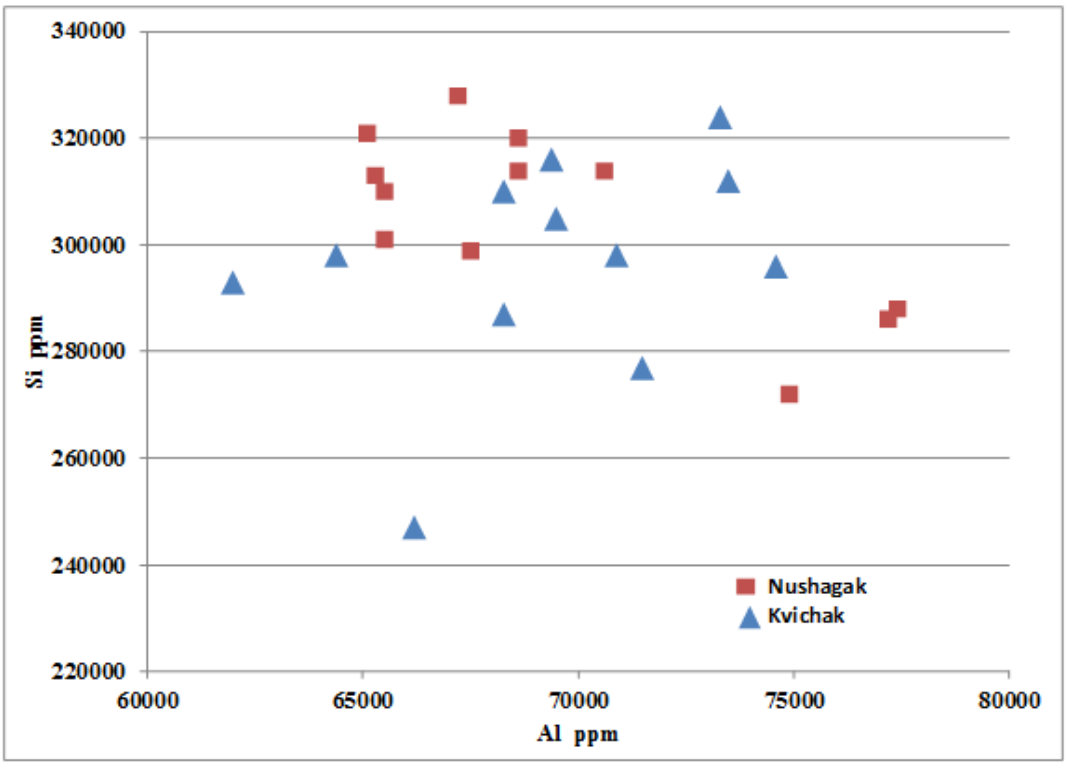

Figure 18. Plots of silicon (mg kg-1) as a function of aluminum concentration in sediments from Nushagak and Kvichak Bays.

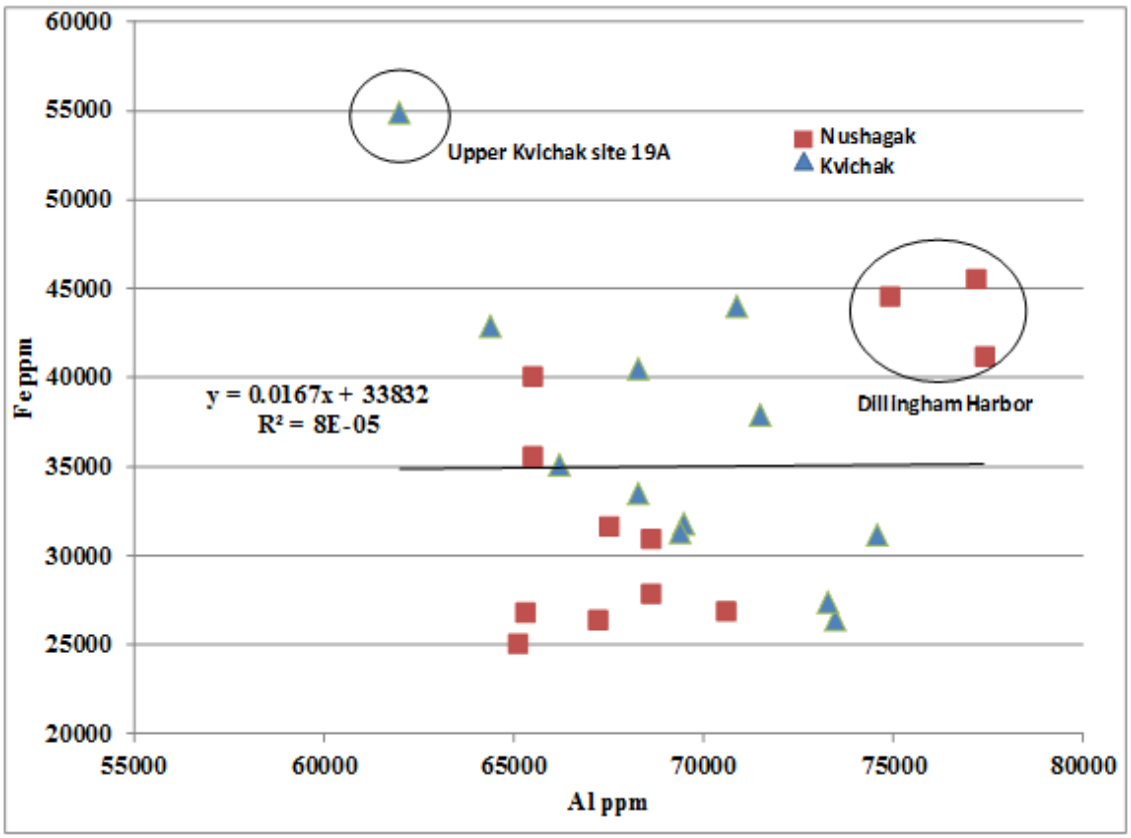

Figure 19a. Plots of iron (mg kg-1) as a function of aluminum concentration in sediments from Nushagak and Kvichak Bay.

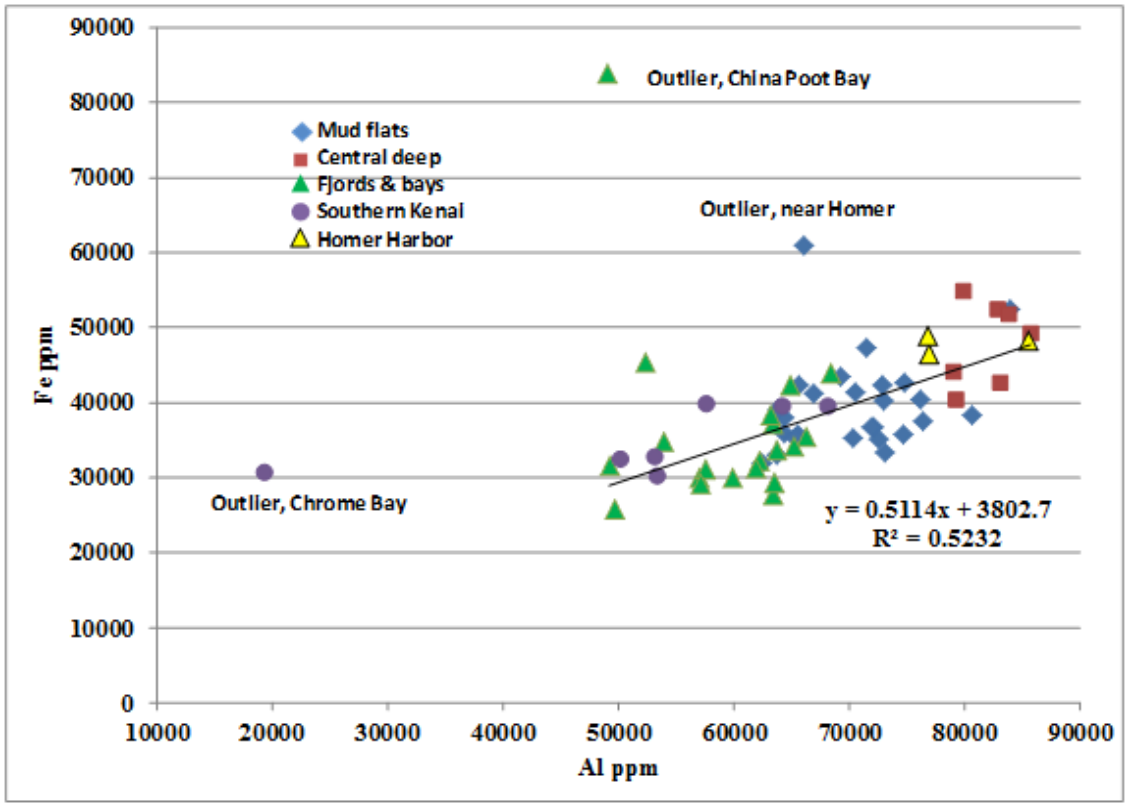

Figure 19b. Plots of iron (mg kg-1) as a function of aluminum concentration in sediments from Kachemak Bay (Hartwell et al., 2009).

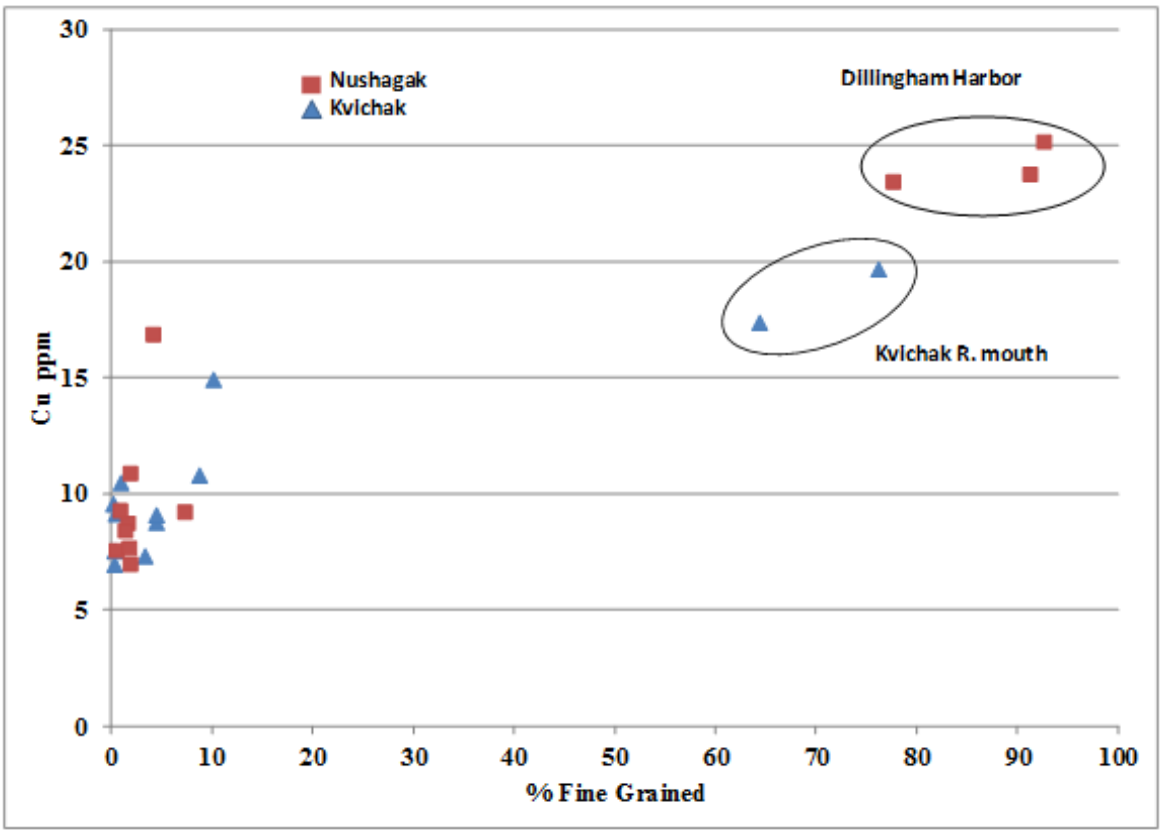

Figure 20. Plots of copper (mg kg-1) vs. % fine grained sediment in Nushagak and Kvichak Bays.

Table 13. Total number of taxa and abundance (# m^2) for stations in the Bristol Bay study area.

Station	Location	# Taxa	Abundance (# m^2-1)	Ln Diversity
DH-1P	Dillingham Harbor	3	3,475	0.26
DH-2P	Dillingham Harbor	4	2,725	0.47
DH-3P	Dillingham Harbor	4	1,225	0.76
UN-2P	Upper Nushagak Bay	0		
UN-1P	Upper Nushagak Bay	32	2,200	2.94
UN-5A	Upper Nushagak Bay	31	1,580	2.59
UN-6A 2013	Upper Nushagak Bay	2	80	0.56
UN-6A 2014	Upper Nushagak Bay	8	400	1.88
LN-10A	Lower Nushagak Bay	7	140	1.73
LN-12A	Lower Nushagak Bay	5	80	1.49
LN-14A	Lower Nushagak Bay	9	410	1.71
LN-15A	Lower Nushagak Bay	6	180	1.45
NR-1P	Naknek River	5	14,280	0.5
NR-3P	Naknek River	1	30	0
NR-8A	Naknek River	3	140	1.08
UK-14A	Upper Kvichak Bay	7	440	1.04
UK-1P	Upper Kvichak Bay	4	460	0.75
UK-16A	Upper Kvichak Bay	5	430	0.91
UK-17A	Upper Kvichak Bay	16	1,170	2.07
UK-19A	Upper Kvichak Bay	15	890	1.57
LK-12A	Lower Kvichak Bay	36	1,800	3.2
LK-15A	Lower Kvichak Bay	9	410	1.05
LK-16A	Lower Kvichak Bay	9	1,280	0.74
LK-18A	Lower Kvichak Bay	32	1,100	2.89

A marked accumulation of eosinophilic granular cells was noted. This is considered an innate immune response and may reflect antimicrobial activity in the gill lamellae.

3.7.2 Rainbow smelt

Six specimens ranged in size from 5.7-12.4 cm and 0.8 – 12.8 g. Externally, the fish appeared normal with the exception of a single smelt with an unusual growth extending from the opercular cavity on the right side of the fish (Figure 30). The operculum was moderately deformed to accommodate this growth, which appeared grayish white, with liver-like consistency.

Histological examination revealed the presence of hepatocytes but with structural alteration and fibrous tissue. The attachment site is the muscle with no epithelial cells in between. Degradation of the tissue may preclude a more detailed designation of the neoplasia, however, no mitotic figures were noted.

Internal gross observation revealed nothing remarkable in any of the rainbow smelts. Histologically, necrosis was more pronounced in the rainbow smelts than in starry flounders, particularly in gill sections. No pathologies that could be separated from preservation artifacts were noted. Liver sections generally contained high concentrations of vacuoles suggestive of high glycogen storage in the organ.

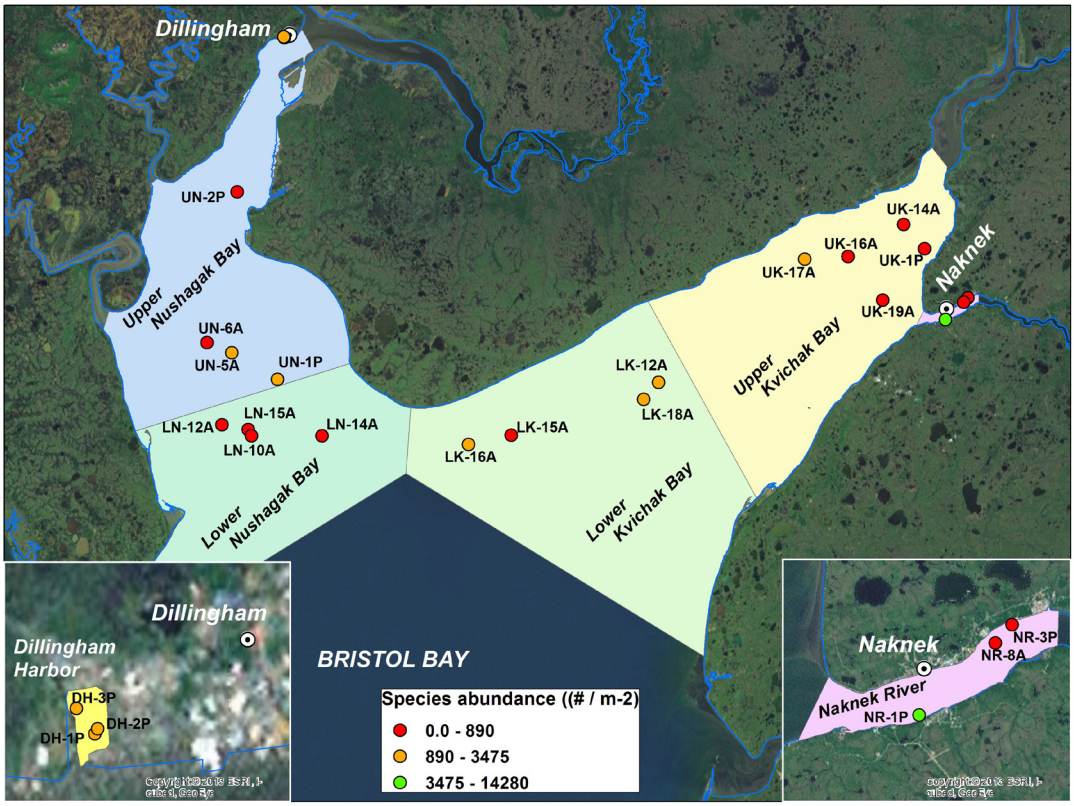

Figure 21. Benthic species abundance distribution in Nushagak and Kvichak Bays

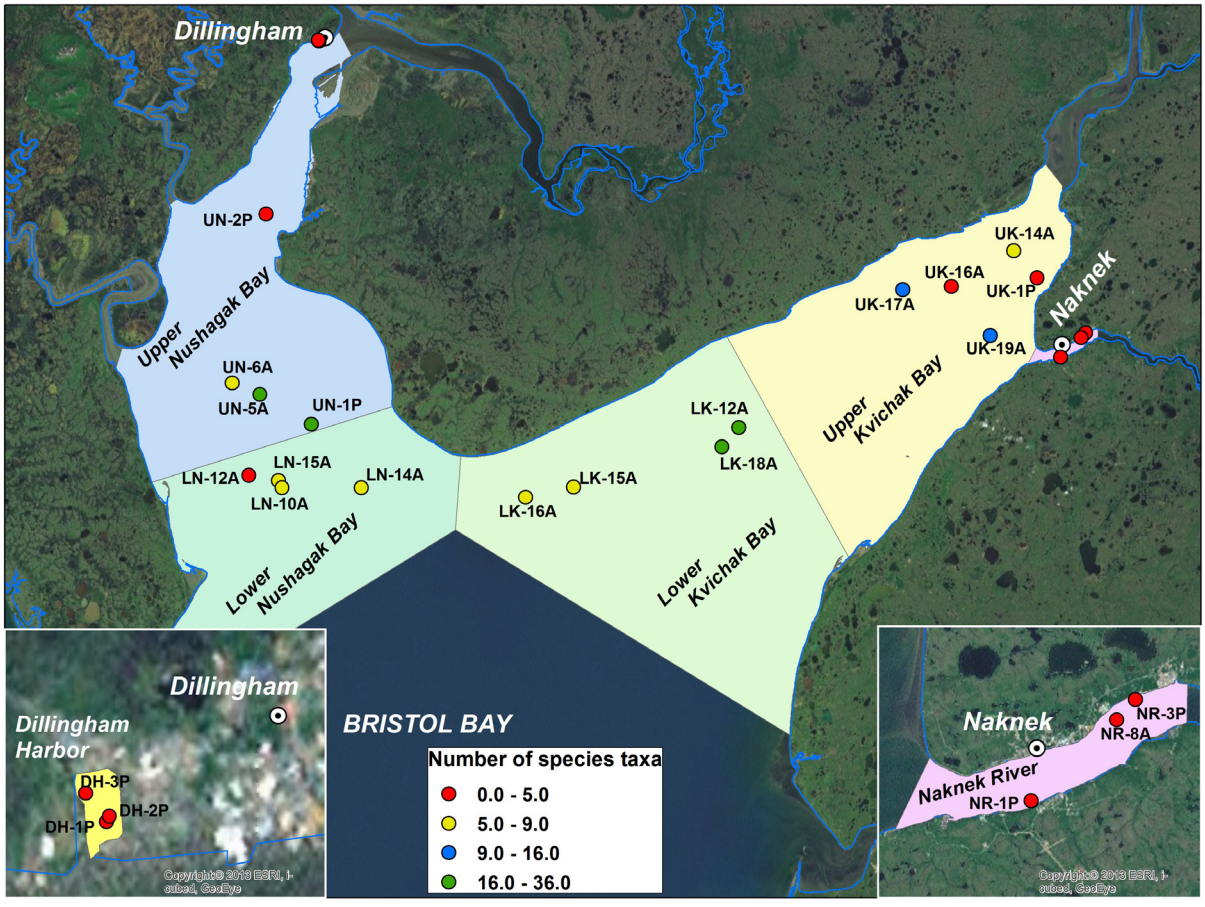

Figure 22. Benthic species number of taxa distribution in Nushagak and Kvichak Bays.

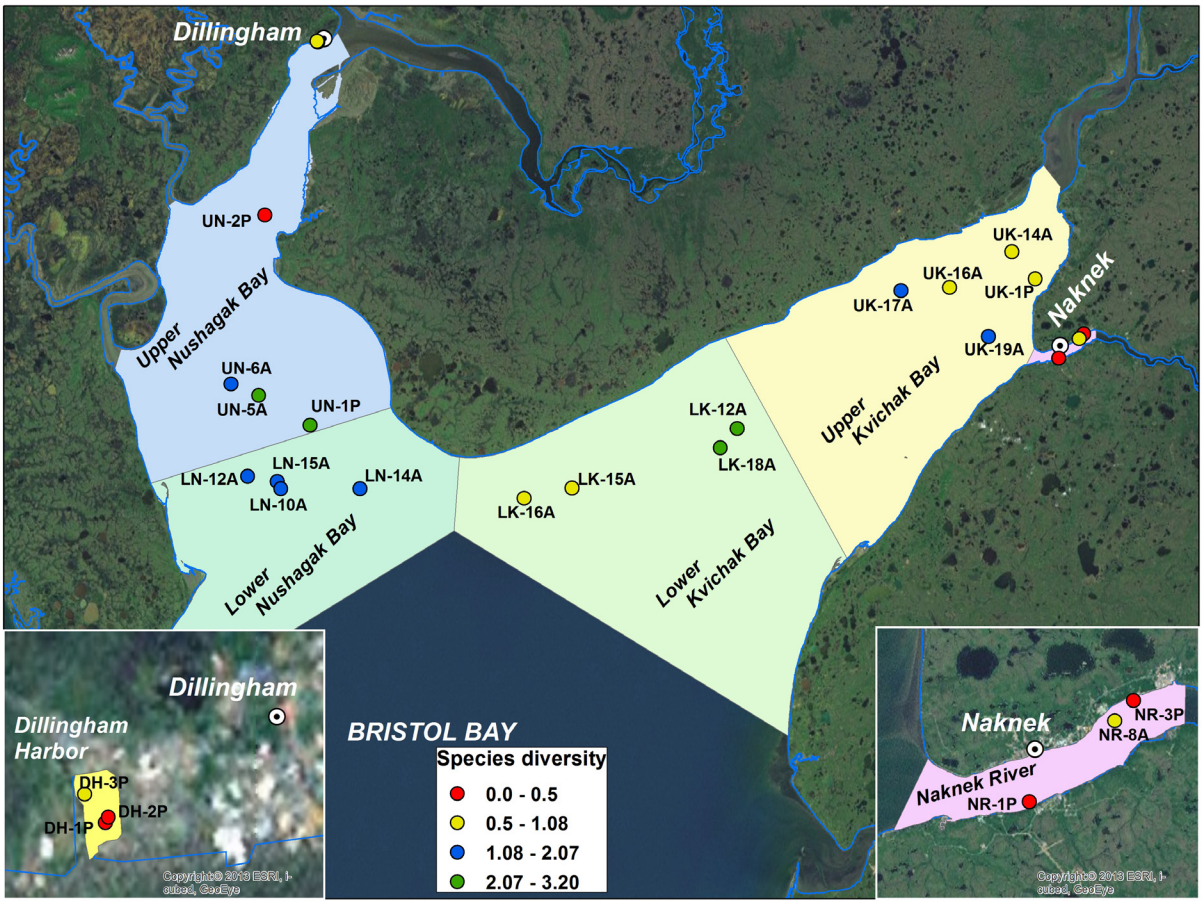

Figure 23. Benthic species diversity distribution in Nushagak and Kvichak Bays.

Table 14. Bristol Bay sediment toxicity results from the Microtox bioassay. One station in the Lower Nushagak stratum exceeded the control sample.

	5 min incubation				15 min incubation		
	Station	Mean EC50 mg ml-1	%Control		Station ID	Mean EC50 mg ml-1	% Control
Dillingham Harbor	DH-1P	4.058	50.4		DH-1P	4.678	77.7
Dillingham Harbor	DH-2P	2.318	28.8		DH-2P	2.631	43.7
Dillingham Harbor	DH-3P	4.869	60.5		DH-3P	5.516	91.6
Upper Nushagak Bay	UN-2P	3.310	41.2		UN-2P	4.584	76.1
Upper Nushagak Bay	UN-6A	3.899	48.5		UN-6A	5.370	89.1
Upper Nushagak Bay	UN-6A	3.582	44.5		UN-6A	4.375	72.6
Lower Nushagak Bay	LN-10A	1.089	13.5		LN-10A	1.404	23.3
Lower Nushagak Bay	LN-12A	1.734	21.6		LN-12A	2.233	37.1
Lower Nushagak Bay	LN-14A	4.851	60.3		LN-14A	6.188	102.7
Naknek R.	NR-1P	0.884	11.0		NR-1P	0.945	15.7
Naknek R.	NR-3P	0.529	6.6		NR-3P	0.548	9.1
Naknek R.	NR-8A	1.072	13.3		NR-8A	1.210	20.1
Lower Kvichak Bay	LK-12A	0.353	4.4		LK-12A	0.360	6.0
Lower Kvichak Bay	LK-15A	0.250	3.1		LK-15A	0.234	3.9
Lower Kvichak Bay	LK-16A	0.394	4.9		LK-16A	0.427	7.1
Lower Kvichak Bay	LK-18A	0.304	3.8		LK-18A	0.314	5.2
	Control	8.044				6.025	

Table 15. Bristol Bay sediment toxicity results from the sea urchin fertilization and development bioassay. Development measurements were not conducted in 2013. Toxicity at two adjacent stations in the Lower Kvichak stratum exhibited significant toxicity. Fertilization and development are expressed as raw numbers and as a percentage of the control (% Cont). (TAN - Total ammonia as nitrogen; UAN - Un-ionized ammonia; LaP - Control pore water (La Perouse Bay); SDS - Positive control (3 mg L-1 sodium dodecyl sulfate).

Sample	Location	Initial Salinity (ppt)	Test Salinity (ppt)	TAN (µg/L)	UAN (µg/L)	% Fert	Fert % Cont	Normal Devel	Devel % Cont	Retarded	Malformed	A
SDS			30.0	26	1	87		36.4	51.7	50.4	1.5	
LaP		36	30.0	78	3	92		70.4		0.3	15.6	
DH-1P	Dillingham	0.5	30.0	1,100	16	98	101.9					
DH-2P	Dillingham	<0.5	30.0	1,500	9	98	101.7					
DH-3P	Dillingham	0.5	30.0	1,800	11	99	102.1					
UN-2P	Nushagak	2	30.0	400	45	99	102.3					
UN-6A	Nushagak	10	30.0	300	40	98	101.7					
LN-10A	Nushagak	20.5	30.0	0	0	93	101.1	81.3	88.4	0.5	6.6	
LN-12A	Nushagak	16.5	30.2	194	8	94	102.2	22.8	24.8	3.8	43.6	
LN-14A	Nushagak	20.5	31.0	8	0	94	102.2	72.5	78.8	0.8	12.2	
LN-15A	Nushagak	18	30.5	34	1	89	96.7	29.0	31.5	7.8	39.8	
LK-12A	Kvichak	19	30.0	**>8000**	**393**	60	**65**	**0**	**0**	0.0	0.0	
LK-15A	Kvichak	18	30.5	134	9	95	103.3	**3.0**	**3.3**	3.3	38.5	
LK-16A	Kvichak	17.5	30.5	14	0	94	102.2	45.3	49.2	1.8	34.7	
LK-18A	Kvichak	19	30.0	**4,157**	**308**	57	**62**	**0**	**0**	0.0	0.0	
NR-1P	Naknek	11	30.5	948	39	96	104.3	25.8	28.0	15.1	38.8	
NR-3P	Naknek	2	30.2	**3,544**	**298**	98	106.5	**0**	**0**	0.0	0.0	
NR-8A	Naknek	0	30.2	0	0	89	96.7	76.7	83.4	0.5	14.5	

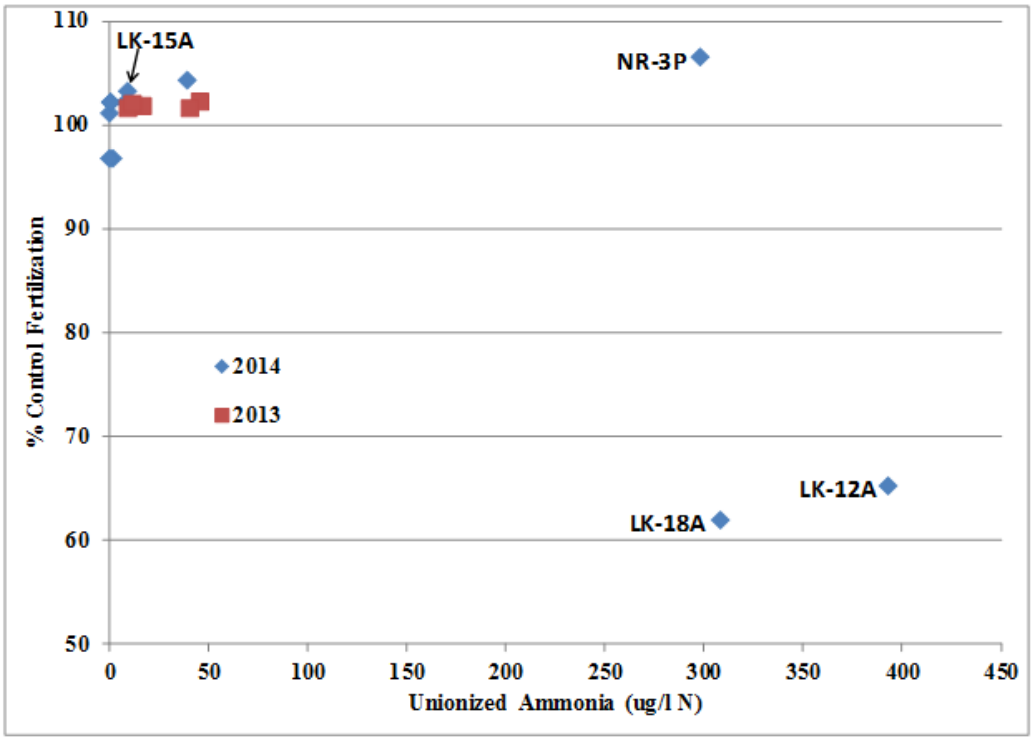

Figure 24. Bristol Bay sediment toxicity assessment with the sea urchin fertilization.

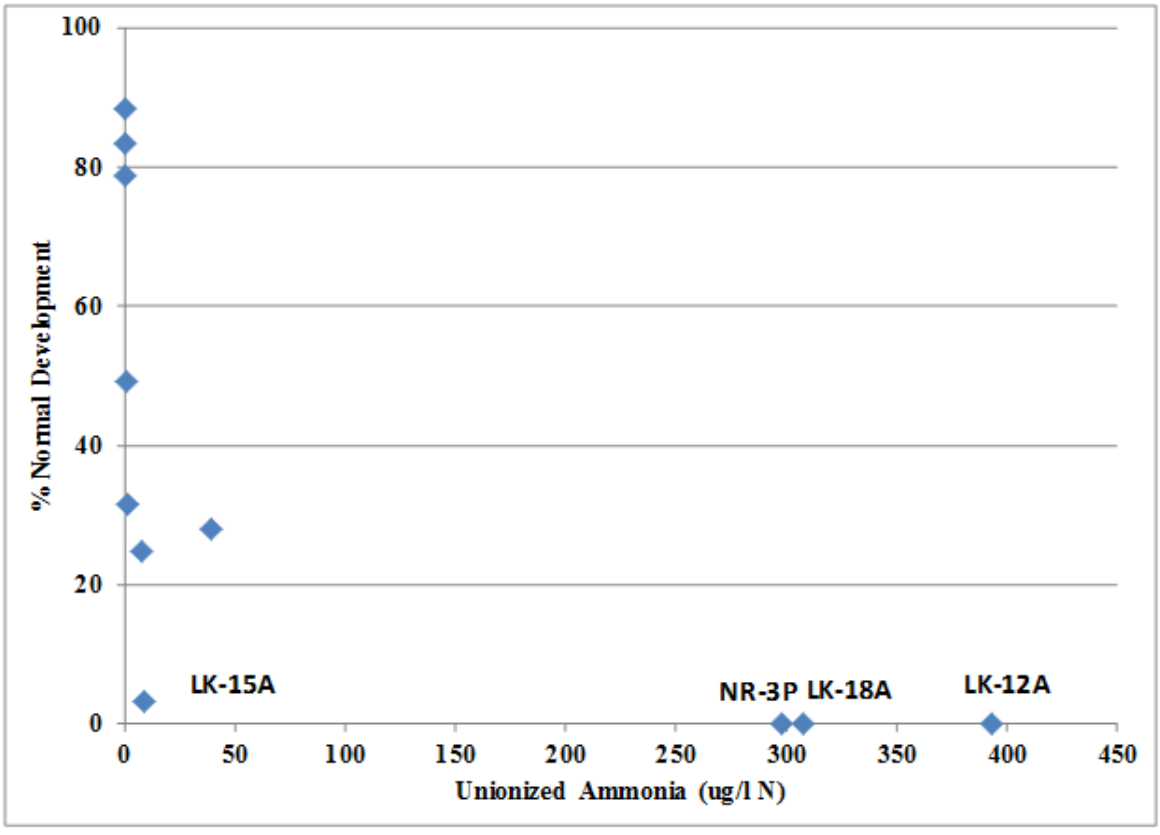

Figure 25. Bristol Bay sediment toxicity assessment with the sea urchin development.

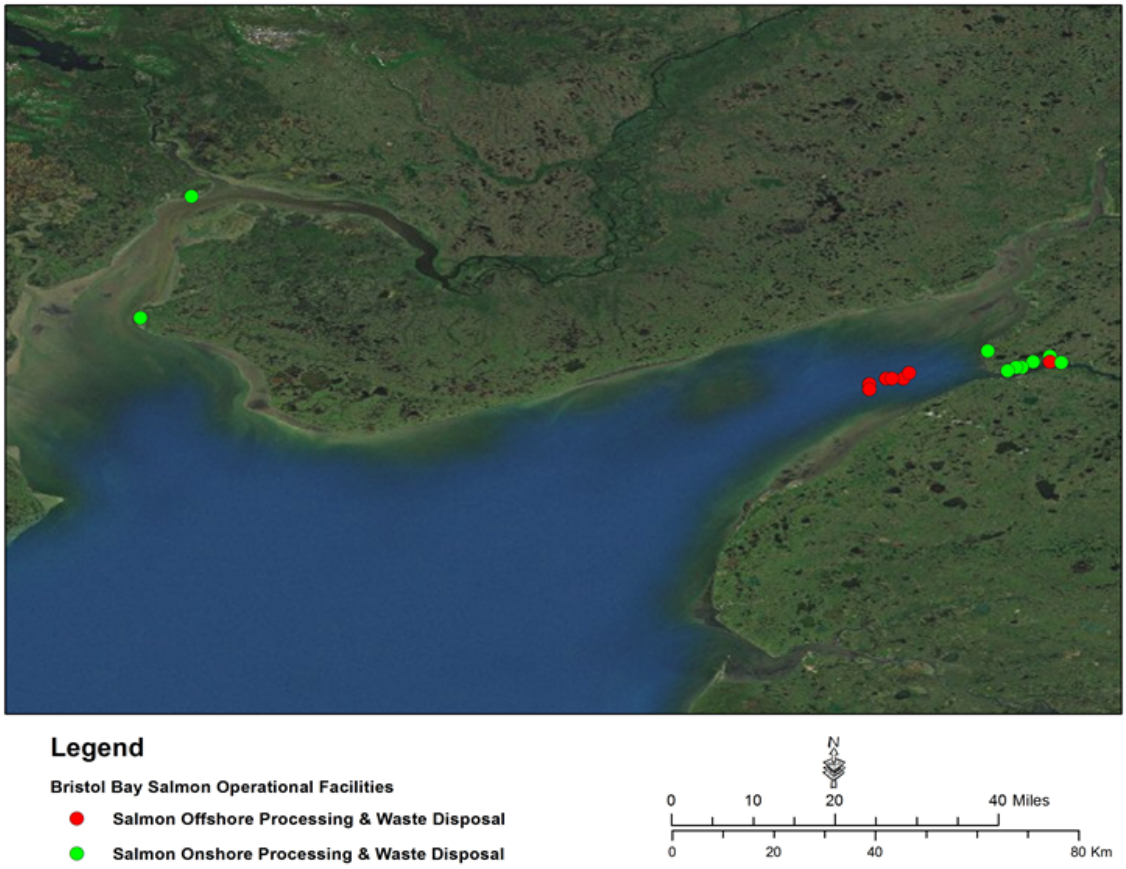

Figure 26. Chart showing permited discharge locations for shore-based (green circles), and floating fish processing factories (red circles) in 2014

Table 16. Concentration of contaminants in fish starry flounder (*Platichthys stellatus*), rainbow smelt (*Osmerus mordax*)

Location	Fish	Ave Length cm	% Lipid (dry)	Total PCB ppb	Total HCH ppb	Total Cyclodienes ppb	Total DDT ppb	Hg
Naknek River	large flounder	23.0	22.25	32.99	0.00	8.50	29.81	0.157
Lower Kvichak	large flounder	28.4	10.46	4.28	0.00	1.00	1.36	0.127
Upper Nushagak	large flounder	28.4	10.40	5.19	0.00	1.13	1.41	0.126
Naknek River	small flounder	7.8	16.60	21.47	0.00	4.13	18.92	0.088
Dillingham Harbor	small flounder	10.5	12.91	13.68	5.33	0.93	4.50	0.079
Naknek River	Smelt	9.2	11.73	11.60	0.00	2.07	9.69	0.046
Lower Kvichak	Smelt	10.6	7.05	1.47	0.00	0.00	0.52	0.040
Dillingham Harbor	Smelt	9.4	32.44	47.50	11.54	4.33	33.62	0.146
Upper Nushagak	Smelt	8.6	9.35	2.57	0.00	0.00	1.33	0.029

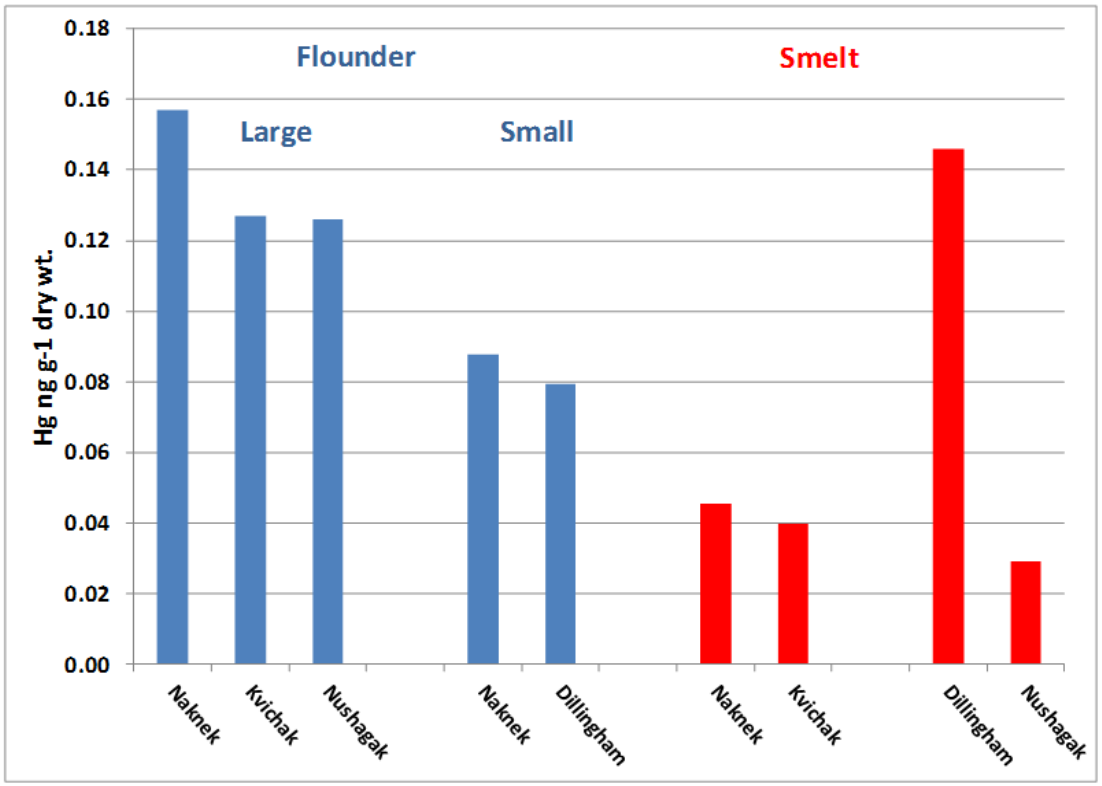

Figure 27. Mercury body burdens in starry flounder (*Platichthys stellatus*) and rainbow smelt (*Osmerus mordax*) from Nushagak and Kvichak Bays.

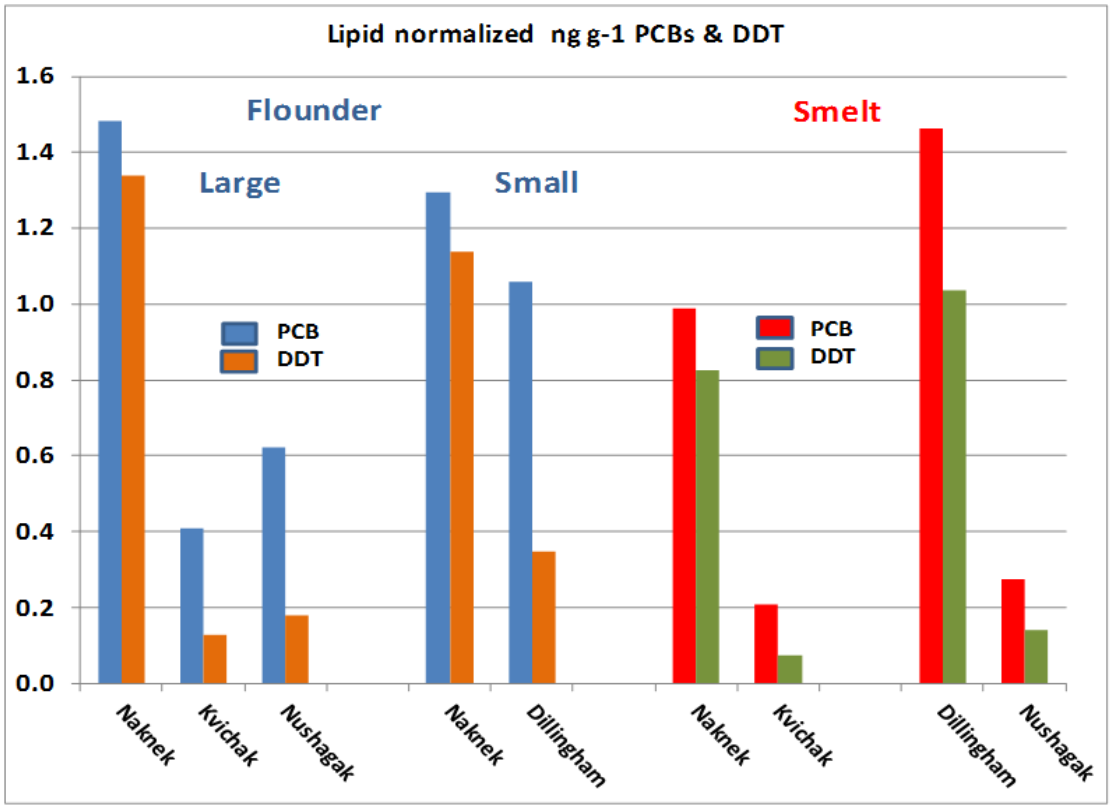

Figure 28. Lipid normalized PCB and DDT body burdens in starry flounder (*Platichthys stellatus*) and rainbow smelt (*Osmerus mordax*) from Nushagak and Kvichak Bays.

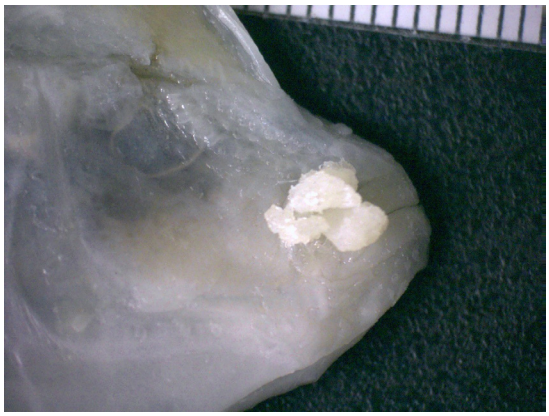

Figure 29. Image of papilloma observed on a flounder from Dillingham Harbor. The papillomatous epithelial lesions on the fish extended from the dorsal side, maxillary region of the mouth.

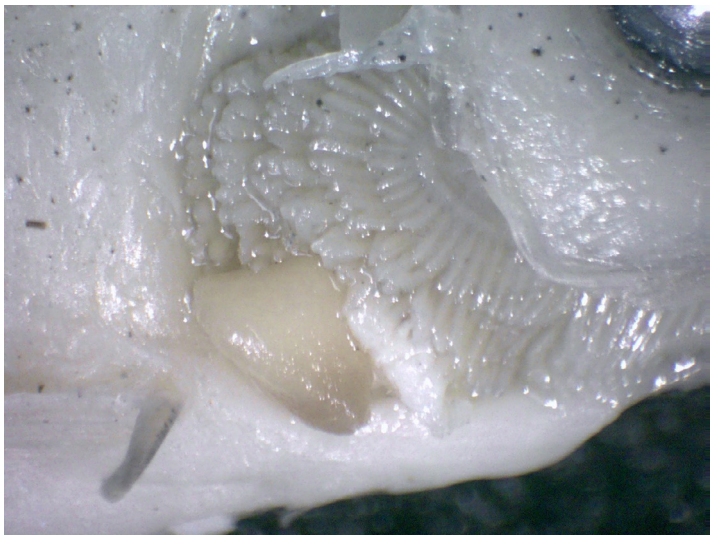

Figure 30. Image of an unusual growth (tumor-like) extending from the opercular cavity on the right side of a rainbow smelt from Dillingham Harbor.

4. CONCLUSIONS

The goals of this project included: 1) the assessment of habitat conditions that influence biodiversity and distribution of benthic infaunal community using the SQT approach, 2) determine the magnitude and spatial patterns or gradients in chemical contamination; 3) characterization of the benthic macroinvertebrate community, 4) characterization of the magnitude of contaminant body burdens and histopathology in fish in the study area, and 5) measures of sediment toxicity in Nushagak and Kvichak Bays.

Organic contaminants were detectable throughout the study area but at very low concentrations. Dillingham Harbor sediments did contain elevated levels of organic contaminants relative to the other locations in the study area but, the levels were well below acutely toxic levels based on NOAA SQGs. Sediments in the harbor were very fine grained with higher organic carbon content, relative to areas outside the harbor. The physical characteristics of the harbor sediment probably account for the chemical results. Tributyltin was only found in the Harbor, but like the other organic contaminants including PAHs, concentrations were very low. Metal concentrations were also very low in the sediment and all concentrations were well below SQG except for arsenic. Arsenic levels were above the ERL throughout the system, which is probably a results of diffuse, natural sources in the watersheds.

Contaminant body burdens and histopathological lesions were very low in the flounder and smelt tested. Older fish had accumulated higher levels as expected. Fish from Naknek and Dillingham tended to have higher lipid levels, possibly related to food availability in the vicinity of fish processing plants. Except for one occurrence of an external papilloma, and the observation of mild to moderate accumulation of macrophage aggregates in the spleen and/or kidney in some flounders, the fish were generally healthy and non-contaminated.

While trace metals and, to some extent, PAHs are naturally occurring chemicals in sediment, the presence of organic compounds of anthropogenic origin (e.g. synthetic pesticides) also indicate a low degree of pollution of the bay. With no significant known point sources of contaminant discharge, the presence of manmade organic chemicals in the region may result from non-point sources and/or long-range oceanic or atmospheric transport.

The benthic habitat appeared to be a robust environment with a biologically diverse benthic assemblage, but is spotty in nature. The habitat is a physically harsh environment. Very strong tidal currents result in highly turbid water, physical scouring and large salinity changes, particularly toward the heads of the bays and the tributaries. Abundance and species richness varied by one or more orders of magnitude among sample locations, but with no apparent spatial pattern. Abundance was lower in locations at Dillingham and the head of Kvichak Bay probably as a result of the high turbidity and daily salinity stress.

Significant anthropogenic chemical toxicity was virtually absent. Compared to data from other studies in the region, Bristol Bay, including Dillingham Harbor, could be characterized as being pristine. Only the most sensitive bioassay endpoints revealed impacts. These impacts appear to be related to ammonia concentrations in selected locations. The source of the ammonia is likely the discharge of fish processing waste from shore-based and floating factories. The discharge of millions of pounds of viscera, and unused salmon body parts which are shredded to 1/2” sized pieces introduces a large flux of organic matter including protein, in a relatively short time span. This material sinks to the bottom and decomposes resulting in toxic levels of ammonia in the sediment. One of the shore-based facilities is under a consent order to construct a conversion plant to generate a fish meal product rather than discharging wastes to the water. Not all plants currently feed into the facility, and the floating factories currently still discharge to open water.

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APPENDIX 1

List of benthic species collected in sediment grabs, excluding epizoic species. Table lists taxonomic classification and frequency of occurrence (number of stations), plus abundances.

Phylum	Class	Order	Family	Genus	Species	Freq	MIN	MAX	MEAN
Annelida	Oligochaeta				sp.	19	1	307	36.4
Annelida	Polychaeta	Archiannelida	Polygordiidae	Polygordius	sp.	1	1	1	1.0
Annelida	Polychaeta	Canalipalpata	Ampharetidae		sp.	3	5	15	9.7
Annelida	Polychaeta	Canalipalpata	Ampharetidae	Ampharete	sp.	2	1	4	2.5
Annelida	Polychaeta	Canalipalpata	Ampharetidae	Amphicteis	sp.	1	1	1	1.0
Annelida	Polychaeta	Canalipalpata	Cirratulidae	Aphelochaeta	sp.	3	1	13	6.7
Annelida	Polychaeta	Canalipalpata	Oweniidae	Owenia	fusiformis	1	43	43	43.0
Annelida	Polychaeta	Canalipalpata	Spionidae		sp.	3	1	4	2.3
Annelida	Polychaeta	Canalipalpata	Spionidae	Dipolydora	quadrilobata	2	1	1	1.0
Annelida	Polychaeta	Canalipalpata	Spionidae	Marenzelleria	wireni	11	1	38	9.9
Annelida	Polychaeta	Canalipalpata	Spionidae	Pygospio	elegans	4	1	4	2.5
Annelida	Polychaeta	Canalipalpata	Spionidae	Rhynchospio	glutaea	3	1	11	5.3
Annelida	Polychaeta	Canalipalpata	Terebellidae	Polycirrus	sp.	3	1	3	2.3
Annelida	Polychaeta	Harmothoinae			sp.	4	1	6	3.5
Annelida	Polychaeta	Phyllodocida	Goniadidae	Glycinde	picta	1	3	3	3.0
Annelida	Polychaeta	Phyllodocida	Goniadidae	Glycinde	sp.	5	1	7	3.0
Annelida	Polychaeta	Phyllodocida	Nephtyidae	Nephtys	caeca	5	1	2	1.4
Annelida	Polychaeta	Phyllodocida	Nephtyidae	Nephtys	ciliata	4	1	12	4.3
Annelida	Polychaeta	Phyllodocida	Nephtyidae	Nephtys	sp.	6	1	4	2.0
Annelida	Polychaeta	Phyllodocida	Nereididae	Nereis	vexillosa	1	1	1	1.0
Annelida	Polychaeta	Phyllodocida	Pholoidae	Pholoe	assimilis	4	2	6	4.5
Annelida	Polychaeta	Phyllodocida	Pholoidae	Pholoe	minuta	3	1	3	2.0
Annelida	Polychaeta	Phyllodocida	Phyllodocidae	Eteone	sp.	3	1	1	1.0
Annelida	Polychaeta	Phyllodocida	Polynoidae	Bylgides	macrolepidus	1	1	1	1.0
Annelida	Polychaeta	Phyllodocida	Polynoidae	Harmothoe	imbricata	4	1	32	12.5
Annelida	Polychaeta	Phyllodocida	Sphaerodoridae	Sphaerodoropsis	minuta	1	3	3	3.0
Annelida	Polychaeta	Phyllodocida	Syllidae	Sphaerosyllis	californiensis	1	2	2	2.0
Annelida	Polychaeta	Phyllodocida	Syllidae	Syllides	sp.	4	1	3	1.5
Annelida	Polychaeta	Scolecida	Capitellidae	Heteromastus	filiformis	1	1	1	1.0
Annelida	Polychaeta	Scolecida	Opheliidae		sp.	1	4	4	4.0
Annelida	Polychaeta	Scolecida	Opheliidae	Ophelia	limacina	9	2	19	7.6
Annelida	Polychaeta	Scolecida	Opheliidae	Thoracophelia	sp.	3	1	2	1.3
Annelida	Polychaeta	Scolecida	Opheliidae	Travisia	sp.	1	2	2	2.0
Annelida	Polychaeta	Scolecida	Orbiniidae	Leitoscoloplos	pugettensis	1	3	3	3.0
Annelida	Polychaeta	Scolecida	Orbiniidae	Scoloplos	sp.	3	1	2	1.3
Arthropoda	Collembola				sp.	1	2	2	2.0
Arthropoda	Insecta	Diptera	Chironomidae		sp.	1	1	1	1.0
Arthropoda	Malacostraca	Amphipoda	Caprellidae	Caprella	sp.	5	1	16	8.0
Arthropoda	Malacostraca	Amphipoda	Corophiidae	Crassicorophium	crassicorne	3	1	5	2.3
Arthropoda	Malacostraca	Amphipoda	Corophiidae	Monocorophium	sp.	2	1	1	1.0
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Lagunogammarus	setosus	4	1	6	2.3
Arthropoda	Malacostraca	Amphipoda	Haustoriidae	Eohaustorius	eous	2	1	31	16.0
Arthropoda	Malacostraca	Amphipoda	Isaeidae	Cheirimedeia	sp.	2	1	2	1.5
Arthropoda	Malacostraca	Amphipoda	Isaeidae	Photis	sp.	1	1	1	1.0
Arthropoda	Malacostraca	Amphipoda	Ischyroceridae	Ischyrocerus	sp.	1	2	2	2.0
Arthropoda	Malacostraca	Amphipoda	Phoxocephalidae	Foxiphalus	sp.	1	1	1	1.0
Arthropoda	Malacostraca	Amphipoda	Podoceridae	Dyopedos	monacanthus	2	5	14	9.5
Arthropoda	Malacostraca	Amphipoda	Pontoporeiidae	Monoporeia	affinis	2	8	11	9.5
Arthropoda	Malacostraca	Amphipoda	Pontoporeiidae	Pontoporeia	affinis	3	1	18	7.0
Arthropoda	Malacostraca	Cumacea	Lampropidae		sp.	2	1	1	1.0

APPENDIX 1 (Continued)

Phylum	Class	Order	Family	Genus	Species	Freq	MIN	MAX	MEAN
Arthropoda	Malacostraca	Decapoda	Crangonidae		sp.	2	2	2	2.0
Arthropoda	Malacostraca	Decapoda	Crangonidae	Crangon	septemspinosa	1	1	1	1.0
Arthropoda	Malacostraca	Isopoda	Chaetiliidae	Saduria	entomon	3	1	3	1.7
Arthropoda	Malacostraca	Isopoda	Munnidae	Munna	sp.	1	1	1	1.0
Arthropoda	Malacostraca	Mysida	Mysidae	Neomysis	awatschensis	3	2	5	3.3
Arthropoda	Malacostraca	Mysida	Mysidae	Neomysis	czerniavskii	8	1	4	1.6
Arthropoda	Malacostraca	Tanaidacea			sp.	1	3	3	3.0
Arthropoda	Pycnogonida	Pantopoda	Ammotheidae	Achelia	alaskensis	4	1	22	9.3
Arthropoda	Pycnogonida	Pantopoda	Ammotheidae	Achelia	gracilipes	2	13	17	15.0
Arthropoda	Pycnogonida	Pantopoda	Ammotheidae	Nymphon	sp.	1	2	2	2.0
Cnidaria	Anthozoa	Actiniaria	Halcampidae	Halcampa	crypta	2	3	3	3.0
Echinodermat	Echinoidea	Clypeasteroida	Dendrasteridae	Dendraster	excentricus	2	1	15	8.0
Echinodermat	Holothuroidea	Dendrochirotida	Phyllophoridae	Pentamera	lissoplaca	1	1	1	1.0
Echinodermat	Holothuroidea	Dendrochirotida	Phyllophoridae	Pentamera	sp.	1	1	1	1.0
Mollusca	Bivalvia	Mytiloida	Mytilidae		sp.	1	1	1	1.0
Mollusca	Bivalvia	Mytiloida	Mytilidae	Mytilus	trossulus	6	1	38	9.2
Mollusca	Bivalvia	Veneroida	Cardiidae	Clinocardium	sp.	2	12	105	58.5
Mollusca	Bivalvia	Veneroida	Tellinidae	Macoma	balthica	3	1	3	2.0
Mollusca	Bivalvia	Veneroida	Tellinidae	Macoma	sp.	3	1	4	3.0
Mollusca	Gastropoda	Heterostropha	Pyramidellidae	Odostomia	sp.	5	1	8	4.4
Mollusca	Gastropoda	Neotaenioglossa	Littorinidae	Lacuna	sp.	6	1	22	10.0
Mollusca	Gastropoda	Nudibranchia			sp.	1	1	1	1.0
Nematoda					sp.	15	1	55	6.8
Nemertea	Anopla	Heteronemertea	Lineidae		sp.	2	1	2	1.5
Nemertea	Anopla	Heteronemertea	Lineidae	Micrura	sp.	2	1	5	3.0
Nemertea	Anopla	Paleonemertea	Tubulanidae	Tubulanus	sp.	4	1	16	5.5
Nemertea	Enopla	Hoplonemertea	Tetrastemmatida	Tetrastemma	sp.	1	4	4	4.0
Platyhelminthe	Turbellaria	Polycladida	Leptoplanidae		sp.	1	2	2	2.0
Platyhelminthe	Turbellaria	Proseriata			sp.	1	1	1	1.0

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