NORTH PACIFIC RESEARCH BOARD PROJECT FINAL REPORT

Assessment of contaminant body burdens and histopathology of fish and shellfish species frequently used for subsistence food by Alaskan Native communities

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NPRB Project 1019 Final Report

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ABSRACT

Subsistence food items can be a health concern in rural Alaska because community members often rely on fish and wildlife resources not routinely monitored for persistent bioaccumulative contaminants and pathogens. Subsistence activities are a large part of the traditional culture, as well as a means of providing protein in the diets for Tribal members. In response to the growing concerns among Native communities, contaminant body burden and histopathological condition of chum and sockeye salmon (*Oncorhynchus keta and Oncorhynchus nerka*) and the shellfish cockles and softshell clams (*Clinocardium nuttallii* and *Mya arenaria*) were assessed. In the Spring of 2010, the fish and shellfish were collected from traditional subsistence harvest areas in the vicinity of Nanwalek, Port Graham, and Seldovia, AK, and were analyzed for trace metals and residues of organic contaminants routinely monitored by the NOAA National Status & Trends Program (NS&T). Additionally, the fish and shellfish were histologically characterized for the presence, prevalence and severity of tissue pathology, disease, and parasite infection. The fish and shellfish sampled showed low tissue contamination, and pathologic effects of the parasites and diseases were absent or minimal. Taken together, the results showed that the fish and shellfish were healthy and pose no safety concern for consumption. This study provides reliable chemistry and histopathology information for local resource managers and Alaska Native people regarding subsistence fish and shellfish use and management needs.

Keywords: Heavy metals, Organic contaminants, Parasites, Disease, Fish, Shellfish, Nanwalek, Port Graham, Seldovia.

Citation

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LIST OF ACRONYMS

As	Arsenic
ASTM	American Society of Testing and Materials
Cd	Cadmium
CIRCAC	Cook Inlet Regional Citizens Advisory Council
Cr	Chromium
CRRC	Chugach Regiona Resource Commission
Cu	Copper
DDT	Dichlorodiphenyltrichloroethane
DEC	Alaska Department of Environmental Conservation
EMAP	Environmental Monitoring and Assessment Program
EPA	Environmental Protection Agency
EVOS	Exxon Valdez Oil Spill
FDA	U.S. Food and Drug Administration
FMP	Fish Monitoring Program
g	Gram
GC/ECD	Gas Chromatography/Electron Capture Detector
GC/MS	Gas Chromatography/Mass Spectroscopy
GOA	Gulf of Alaska
HCH	Hexachord-Cyclohexane
Hg	Mercury
ICP	Inductively Coupled Plasma
KBNERR	Kachemak Bay National Estuarine Research Reserve
MDL	Method Detection Limit
mg	Milligram
Mn	Manganese
MS	Matrix Spike
MSD	Matrix Spike Duplicate
NCCOS	National Centers for Coastal Ocean Science
Ni	Nickel
NIST	National Institute of Standards and Technology
NOAA	National Oceanic and Atmospheric Administration
NS&T	National Status and Trends
PAH	Polycyclic Aromatic Hydrocarbon
Pb	Lead
PCB	Polychlorinated Biphenyl
POP	Persistent Organic Pollutant
PWSRCAC	Prince William Sounds Regional Citizens Advisory Council
SAS	Satistical Analysis System
Se	Selenium

SRM	Standard Reference Material
TBT	Tributyltin
µg	Microgram
Zn	Zinc

STUDY CHRONOLOGY

January 2010: Presentation of the project entitled "Sediment Quality Triad Assessment in Kachemak Bay: Characterization of Soft Bottom Benthic Habitats and Contaminant Bioeffects Assessment" (NPRB Project 726) at the Alaska Marine Science Symposium.

May 2010: NPRB #1019 grant proposal approved.

May 2010: The Chugach Regional Resource Commission (CRRC) coordinated with local leaders in the villages of Port Graham, Nanwalek and Seldovia in organizing community members for field work.

May 2010: Sampling of shellfish was successfully completed at all locations by the community members. Samples shipped to government contracted analytical chemistry and histopathology laboratories for analysis.

July 2010: Sampling of fish was successfully completed at all locations by community members.

July 2010: Fish necropsies were successfully completed by NOAA scientists. Samples for analytical chemistry were shipped to government contracted laboratories. Samples for histopathology were shipped to NOAA's Northwest Fisheries Science Center for analysis.

July 2010: NOAA/NPRB MOA-2010-076/8189 was signed, but first funding installment was late coming from NPRB.

October 2010: Rules on forward funding task orders was changed. Consequently, NOAA could not order chemical analyses.

November 2010: The National Ocean Service (NOS) and the Centers for Coastal Ocean Science (NCCOS) requested a Waiver of Advance Reimbursement Payments for the project.

January 2011: Shellfish histopathology data report was received. Data QA/QC review completed.

January 2011: Progress report was approved.

January 2011: Presentation of the partial results on fish histopathology at the Alaska Marine Science Symposium.

May 2011: Education outreach effort with presentation of preliminary results at Nanwalek, Port Graham, Seldovia, City of Seldovia, and Kachemak Bay NERR. .

May 2011: The Waiver of Advance Reimbursement Payments for the project was approved.

June 2011: Task order for contaminant analysis and shellfish histopathology was issued. Laboratory results were expected in February 2012.

July 2011: No cost extension to February 2013 was granted.

July 2011: Progress report was approved.

December 2011: Fish histopathology data report was received. Data QA/QC review completed.

February 2012: Preliminary analytical data report for organic contaminants (PAHs and chlorinated organic compounds) received.

March 2012: Data QA/QC review completed on organic contaminants.

March 2012: Platform presentation of preliminary results given at the Florida A&M University, NOAA Science Forum.

May 2012: Partial metals analytical report received.

May 2012: Analytical QA/QC review completed on partial metals data set.

February 2013: Submission of NPRB #1019 final report.

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1. INTRODUCTION

The Chugach and Cook Inlet Native communities of Nanwalek, Port Graham and Seldovia are located at the southwestern tip of the Kenai Peninsula near the entrance to Kachemak Bay, an embayment off the lower Cook Inlet. In these villages, subsistence activities are a large part of the traditional culture, as well as a means of providing protein for Tribal members. As defined by the Division of Subsistence, Alaska Department of Fish and Game (ADF&G), subsistence living is the customary and traditional use of wild food gathered through fishing and hunting (http://www.adfg.alaska.gov). In rural Alaska and particularly in Native villages, subsistence activities are a large part of the traditional culture and in many communities hunting and fishing provide the main source of nutritional protein (Wolfe, 1996). Based on the ADF&G most recent comprehensive assessment conducted in the 1990s, the Division of Subsistence estimated an annual per capita average of about 375 pounds of food harvested in rural Alaska statewide. In contrast, for the average American in the contiguous U.S., the estimate is less than 255 pounds of meat, fish and poultry, primarily derived from commercial grocery outlets (http://www.adfg.alaska.gov). Further estimates by Wolfe, (1996) indicated that in some rural Alaskan villages the average per capita harvest reaches well over 600 pounds per person. And drawing from population density, Wolfe estimated that close to two pounds of wild food is consumed per person per day, a figure that highlights the significance of subsistence food consumption in Alaska.

Although wild foods are traditionally considered more nutritious than commercially available food, they may not be any healthier because of potential exposure to environmental stressors. Pollution and other environmental factors such as climate change constitute stressors that are impacting the health of marine and coastal resources in Alaska. Remote Alaskan regions, which were once considered pristine, are now known to be subjected to exposure to contaminants (AMAP, 2005; Wolfe, 1996). Studies have found that arrays of heavy metals and persistent inorganic and organic pollutants including synthetic organic chemicals and, polycyclic aromatic hydrocarbons (PAHs) from natural sources, industrial, and accidental spills are finding their ways into food chains within ecosystems in Alaska (Short *et al.,* 2002; AMAP, 2011). While studying mercury accumulation in fish, MacFarlane, (2004) noted that possible sources of mercury in the south-central Alaska include gold mining activities, and volcanic eruptions. There are five active volcanoes on the western side of Cook Inlet. Intermittent eruptions from these volcanoes periodically contribute volcanic ash to the region. Thus, in addition to the weathering of mineral-rich soil, likely sources of natural inorganic contaminant inputs into area ecosystems could be linked to volcanic eruptions. With better understanding in recent years of global geochemical circulation in the Arctic region, there has been increasing concern about the grasshopper effect by which metals and persistent organic pollutants (POPs) from warmer lower latitudes are being transported and deposited into Alaska's ecosystems (UNEP, 2005). Thus, along with mercury, persistent organic pollutants such as toxic chlorinated pesticides (e.g., DDTs) and industrial contaminants (e.g. polychlorinated biphenyls or PCBs) emitted as results of anthropogenic activities in the Americas, Europe and Asia, could be transported and deposited in the Kachemak Bay ecosystem and stress vital coastal resources.

Most fish and shellfish species harbor a natural array of parasites that can affect their physiological processes , exposure to contaminants is known to impact their immune system and facilitate parasitism and occurrence of diseases (Weis *et al.*, 1995; Johnson *et al.*, 1992; and MacKenzie *et al*., 1995). The Alaska Department of Fish and Game assessed the infection pattern of the unicellular parasite, *Ichthyophonus hoferi*, which was said to be harmless to humans, but was blamed for devastating infections in salmon (Kocan et al., 2004; Dehn, 2008). Recently, a number of biochemical alterations and emergence of disease found in marine and coastal environments have been linked to climate change that is shifting the disease landscape globally (Harvell et al., 2002). Additionally, the presence of biological toxins such as paralytic shellfish poisoning (PSP) in shellfish related to harmful algal bloom events can pose a serious health risk. Recent outbreaks of PSP in Alaska have been linked to the consumption of shellfish (RaLonde, 1996).

Resources used for subsistence foods in Alaska could be potentially exposed to deleterious compounds and biological toxins but there is no systematic wild food testing in Alaska (Wolfe, 1996). Native communities that rely on subsistence foods have minimal information about the safety of their harvest (Wolfe, 1996). In response to the growing concerns within Native communities, this project sampled commonly used subsistence foods -two species of salmon, chum and sockeye (*Oncorhynchus keta and Oncorhynchus nerka*) and cockles and soft-shell clams (*Clinocardium nuttallii* and *Mya arenaria*)- to assess their overall health condition and level of contamination. The fish and shellfish were collected from traditional subsistence harvest areas in the vicinity of Nanwalek, Port Graham and Seldovia, AK, and were analyzed for metals and organic contaminants routinely monitored by the NOAA National Status & Trends Program (NS&T). Additionally, the fish and shellfish were histologically characterized for pathologic parameters such as diseases and parasitic infections.

This report summarizes the findings of the health condition assessment of two species of salmon and two species of shellfish. To put results from this study into perspective, concentration levels in salmon and clams were compared to the Alaska Department of Environment Conservation , Fish Monitoring Program (DEC-FMP) data and, when possible, to the U.S. Food and Drug Administration (FDA) action levels for seafood safety and calculated EPA chronic no effect consumption levels. This study provides useful chemistry and histopathology information on salmon, cockles and clams for concerned native community members and coastal resource managers in Alaska. As the Nation's longest running coastal contaminant monitoring and assessment program, the NS&T program maintains a publically available national database of georeferenced chemical, physical and biological information. The data from this study were incorporated into the NS&T data portal and are available to the public (http://egisws02.nos.noaa.gov/nsandt/index.html#).

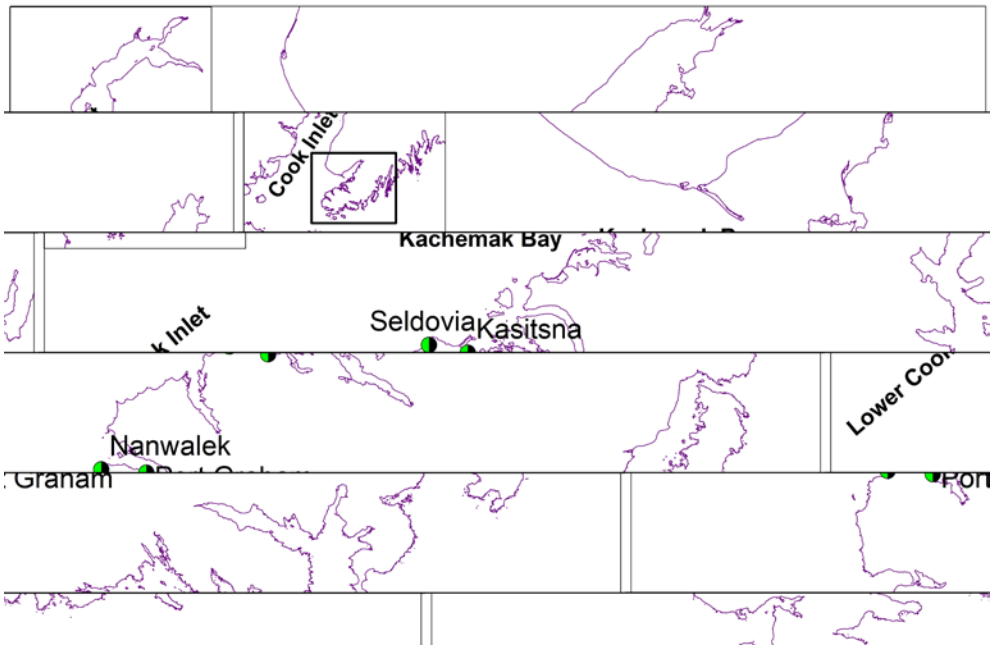


Figure 1. Map showing the geographic location of the villages of Nanwalek, Port Graham and Seldovia in Lower Cook Inlet, and the NOAA Kasitsna Bay lab. The inset depicts the general location of Cook Inlet and Kachemak Bay. Seldovia village is not collocated with the city of Seldovia.

2. OBJECTIVES

The overall goal of the study was to assess the health risks associated with consumption of subsistence food items collected in the traditional harvest ground in Native communities of south-central Alaska. To achieve this goal the following objectives were accomplished:

1. Characterize the potential contamination of sessile (shellfish) vs. mobile (salmon) components of the subsistence fishery, using body burdens and histopathology as metrics.

2. Evaluation of the health condition of the fish and shellfish with analyses of tissue pathogens and occurrences of diseases.

3. Assess the potential consumption risk of these fishery components.

3. METHODS

3.1. **Sampling**

Samples were collected by community members in each village. Sample collectors were trained and assisted by NOAA scientists to conduct quality assured sampling and sample handling. Sampling followed quality controlled and quality assured procedures of the NS&T and national Marine Fisheries Service (NMFS) for sample collection (Apeti et al., 2012; NMFS, 1995). The target shellfish samples were collected in triplicate from each of three harvest areas in May, 2010. Fish were collected in July, 2010, during the salmon run.

3.1.1. Shellfish sampling

Three locations were identified in each of the traditional harvest areas of Nanwalek, Port Graham and Seldovia. At each location, edible sized and co-located cockles and clams were sought for hand collection at low tide. Clams were collected at all locations, while cockles could only be collected at Port Graham and Seldovia. Shellfish were identified in the field based on local traditional knowledge using common (colloquial) names and a scientific name (Foster, 1991). After collection, specimens from each location were kept separate, brushed clean with ambient water and sorted. Organisms of 6 to 7 cm in length were selected into sample composites. For each species, three composite samples were selected for each location, one for each analytical method (trace elements, organics and histopathology). For trace metal and organic contaminant analysis, sample composites consisted of 30 organisms, while only 12 organisms were collected for the histopathology analysis (Apeti et. al, 2012). Due to depleted stock of cockles in Port Graham, samples were only sufficient for organic contaminants and histopathology measurement. The selected samples were put into labeled double Ziploc bags and preserved on ice. The shellfish samples were air-shipped to government contracted analytical laboratories: TDI Brooks in Texas for organic analysis, Texas A&M University for trace element analysis, and Rutgers University, Haskin Shellfish Research Laboratory for histopathology analysis. Histological examination included enumeration of parasites and lesions in the gill, mantle, gonoducts, digestive gland tubules, stomach/digestive gland, and connective tissue.

3.1.2. Fish sampling and necropsy

The two species of salmon were collected from the traditional harvest grounds in each village using gillnets. Sample handling and preparation followed established protocols by Northwest Fisheries Science Center (NWFSC) a division of the NOAA National Marine Fisheries Service (NMFS, 1995; Lauenstein and Cantillo, 1993). For each species of salmon, five males and five females were collected from each village. Immediately after collection the fish samples were delivered to the NOAA/University of Alaska Fairbanks, Kasitsna Bay laboratory for processing and necropsy. Muscle and liver samples were collected for chemical analyses. Liver, kidney, and gill samples were collected for histological assessment.

Fish necropsy occurred as soon after death as possible. The fish were sorted by species and sex, and measured to determine their weight and length. To prevent cross contamination of samples during necropsy, multiple separate sets of dissection tools were used for the removal of fish muscle, liver, kidney and gill tissues for the various analyses, depending on the type of analysis to be performed on the tissue. One set of "external only" dissection tools (that were used only for external procedures) was used to make the initial cuts through the epidermis for access to the fish muscle to be collected for chemical analyses. Using a pair of hemostats, a strip of skin was removed behind the head parallel to and about 5 to 10 mm dorsal to the lateral line, exposing the underlying muscle, and using "external only" dissection tools. Using separate sets of distilled water-rinsed Teflon knife and polyamide forceps (for the metals analyses) and isopropanol-rinsed scalpel and stainless steel scalpel and forceps (for the organic analyses), two separate blocks of muscle tissue were removed from the exposed area, from clearly inside of the margin of the original cut made through the skin, to prevent any contamination of the muscle samples by contact with the external skin or mucus, for the separate analysis of trace metals and organic contaminants. All samples of tissues for organic chemical analysis were collected using dissection tools that had been rinsed with isopropanol between fish; separate samples of tissues for metals analysis were rinsed with distilled water. Samples for chemical analyses were composited by site, species and sex; five fish of separate species and sex at each site consisted of a composite sample, with separate composite for the metals and organics analyses. The "external only" set of dissection scissors, spawning knife and scalpels were then again used to open the abdominal cavity to access the internal organs, without contacting the liver. A separate set of dissection tools were used to collect the histological samples of gill, head and trunk kidney, and liver. When removing liver tissue, care was taken to not puncture the gall bladder, so that bile was not spilled on the liver sample. Liver tissue for the separate chemical samples (organics, metals) was collected using a separate set of dissection tools, consisting of separate distilled water-rinsed Teflon knife and polyamide forceps for the metals sample, and a separate set of stainless steel scalpel, scissors and forceps for the organics sample; these dissection tools were only used to collect samples for chemical analyses from the liver. A total of 90 individual kidney, gill and liver tissues were collected for the histopathology characterization. Liver, kidney and gill tissues were placed into tissue cassettes and immediately preserved in Davidson's fixative (Fournie et al., 2000) until shipped to and analyzed at the Northwest Fisheries Science Center. A total of 24 composite samples of liver and muscle tissues were collected for fish contaminant body burden assessment. The tissue samples destined for contaminants analysis were placed into labeled I-Chem jars and kept frozen until shipped to TDI Brooks and Texas A&M University. The project sought to collect and analyze stomach contents from the salmon to determine fish prey and to quantify the concentration of organic contaminants in prey organisms. However, all attempts to collect stomach contents were unsuccessful as they had stopped feeding in brackish waters during the fish run up the rivers prior to spawning (Pecquerie et. al, 2011). Unused tissues were discarded.

3.2. **Contaminant analysis**

Analyses of organic contaminants and metals in clam and cockles soft tissue and in fish liver and muscle followed the standard NS&T analytical protocols described in Kimbrough and Lauenstein (2006a and 2006b). The shellfish were shucked and the whole soft tissue of the 30 organisms composite were homogenized and freeze-dried. Fish liver and muscle composite samples were also subjected to the same blending and freeze drying processes before digestion for metal analysis and extraction for organics analysis.

During analysis, applicable quality control samples including method blank, duplicates, certified standard reference materials (SRM), and spiked samples were processed with every sample batch. Aliquots of the SRM were digested or extracted and analyzed in a manner identical to actual samples. For quantification of organic contaminants, surrogate standards were also added to samples in order to derive the appropriate surrogate corrected measurements.

3.2.1. Major and trace metals analysis

Major and trace metals measured in the fish and shellfish tissue are listed in Table 1. All tissue types were subjected to the same digestion and analytical methods (Kimbrough and Lauenstein, 2006). After freeze-drying the samples to constant weight, aliquots of 0.10-0.45 g dried tissue were homogenized, weighed and digested in Teflon bombs. For all metals except Hg, the tissue samples were digested with HNO_3, H_2O_2 and, HCl. After transferring the digestates into polyethylene screw cap bottles for the solution density determination by weight and volume, the digestates were prepared for inductively couple plasma mass spectrometric (ICP-MS) analysis. NS&T routinely measures Hg content in biota as total mercury, which is the aggregate of all forms of mercury present in the biota tissue matrix. For Hg quantification, tissue homogenates were acid digested based on a modified version of the Environmental Protection Agency (EPA) method 245.5. Samples were digested using concentrated H_2SO_4 and HNO_3 and the addition of $KMnO_4$ and $K_2S_2O_8$, followed by a second heated digestion step. Before analysis by cold vapor atomic absorption spectroscopy, 5mL of 10% (w/w) NH_2OH HCL were added to the digestates to reduce excess permanganate and the volumes were brought to 40 mL with distilled water. Metals can exist in the environment in several forms, but the analytical methods used by the NS&T do not distinguish between these various forms. Instead, analytical results are reported as total metal concentration (aggregation of all species of a metal) in microgram per gram ($\mu g.g^{-1}$) for dry tissue weight (dw).

3.2.2. Organic contaminants analysis

Organic contaminants analyzed as part of this study are listed in Table 1. Chlorinated pesticides such as dichlorodiphenyltrichloroethanes (DDTs), dieldrin, hexachlorocyclohexanes (HCHs) and chlordanes as well as polychlorinated biphenyls (PCBs) were analyzed in fish and shellfish tissue. Polycyclic aromatic hydrocarbons (PAHs) were analyzed in shellfish tissue only, because fish effectively metabolize PAHs to

compounds not detectable by routine analytical procedures. Concentrations of all organic contaminant were reported in nanogram per gram ($ng.g^{-1}$) dry tissue weight (dw).

-PAHs, PCBs, chlorinated pesticides

Aliquots of approximately 1 g of sample were weighed and oven-dried at 63 - 56 °C to constant weight in order to determine tissue wet and dry weights. Separate aliquots of about 30 g of tissue samples were homogenized and chemically dried with Hydromatix®. Tissue/Hydromatix mixtures were then extracted with 100% dichloromethane using 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.

Quantification of PAHs and their alkylated homologues were conducted using gas chromatography mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM). Chlorinated hydrocarbons (chlorinated pesticides and PCBs, Table 1) were quantitatively determined by capillary gas chromatography with an electron capture detector (ECD). Calibration solutions analyzed as part of the GC/MS and GC/ECD procedures were run after each six or less samples.

-Organotins (butyltin)

Homogenized sample aliquots were extracted three times by agitation with tropolone in dichloromethane using tissumizer. The sample extracts were then concentrated in a hot water bath and the extracts were centrifuged. The supernate solutions were then further concentrated. The supernate solutions were then back extracted into 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 extracts were dried by addition of anhydrous Na_2SO_4 and then concentrated. The extracts were purified using silica gel/alumina column chromatography. The eluents were collected and concentrated in a water bath. The quantitative method was high resolution, capillary gas chromatography using flame photometric detection (GC/FPD). This method quantitatively determined tetrabutyltin (4BT), tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT).

3.2.3. Method detection limits

For each metals and organic compound measured, an analytical method's limits of detection (MDL) were determined. Determination of MDL followed procedures described by the Environmental Protection Agency in 40 CFR Part 136, (EPA, 2005) and it was defined as the Student's t for 99%

confidence level times the standard deviation of seven or more replicate measurement of the same low level spiked samples.

Table 1. List of organic pollutants and metals analyzed by the NS&T program.

Metals: Silver (Ag), Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Mercury (Hg), Manganese (Mn), Nickel (Ni), Selenium (Se), Tin (Sn), Zinc (Zn)
Butyltins: monobutyltin, dibutyltin, tributyltin, tetrabutyltin
Chlordanes: *alpha*-chlordane, *gamma*-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor, Heptachlor-Epoxide
Chlorpyrifos
DDTs: *ortho* and *para* forms of parent 2,4'DDT and 4,4'DDT and metabolites 2,4'DDE; 4,4'DDE; 2,4'DDD; 4,4'DDD
Dieldrins: aldrin, dieldrin and endrin
Chlorobenzenes: 1,2,3,4-Tetrachlorobenzene, 1,2,4,5-Tetrachlorobenzene, Hexachlorobenzene, Pentachlorobenzene, Pentachloroanisole
Hexachlorocyclohexanes (HCHs): Alpha-Hexachlorocyclohexane, Beta-Hexachlorocyclohexane, Delta-Hexachlorocyclohexane, Gamma-Hexachlorocyclohexane
Endosulfans: Endosulfan I, Endosulfan II, Endosulfan sulfate
PAHs: Naphthalene, C1-Naphthalenes, C2-Naphthalenes, C3-Naphthalenes, C4-Naphthalenes, Benzothiophene, C1-Benzothiophenes, C2-Benzothiophenes, C3-Benzothiophenes, Biphenyl, Acenaphthylene, Acenaphthene, Dibenzofuran, Fluorene, C1-Fluorenes, C2-Fluorenes, C3-Fluorenes, Anthracene, Phenanthrene, C1-Phenanthrenes/Anthracenes, C2-Phenanthrenes/Anthracenes, C3-Phenanthrenes/Anthracenes, C4-Phenanthrenes/Anthracenes, Dibenzothiophene, C1-Dibenzothiophenes, C2-Dibenzothiophenes, C3-Dibenzothiophenes, Fluoranthene, Pyrene, C1-Fluoranthenes/Pyrenes, C2-Fluoranthenes/Pyrenes, C3-Fluoranthenes/Pyrenes, Naphthobenzothiophene, C1-Naphthobenzothiophenes, C2-Naphthobenzothiophenes, C3-Naphthobenzothiophenes, Benz(a)anthracene, Chrysene/Triphenylene, C1-Chrysenes, C2-Chrysenes, C3-Chrysenes, C4-Chrysenes, Benzo(b)fluoranthene, Benzo(k,j)fluoranthene, Benzo(e)pyrene, Benzo(a)pyrene, Perylene, Indeno(1,2,3-c,d)pyrene, Dibenzo(a,h)anthracene, Benzo(g,h,i)perylene Individual Alkyl Isomers, , 2-Methylnaphthalene, 1-Methylnaphthalene, 2,6-Dimethylnaphthalene, 1,6,7-Trimethylnaphthalene, 1-Methylphenanthrene, C29-Hopane, 18a-Oleanane, C30-Hopane
PCBs: PCB8/5, PCB18, PCB28, PCB29, PCB31, PCB44, PCB45, PCB49, PCB52, PCB56/60, PCB66, PCB70, PCB74/61, PCB87/115, PCB95, PCB99, PCB101/90, PCB105, PCB110/77, PCB118, PCB128, PCB138/160, PCB146, PCB149/123, PCB151, PCB153/132, PCB156/171/202, PCB158, PCB170/190, PCB174, PCB180, PCB183, PCB187, PCB194, PCB195/208, PCB199, PCB201/157/173, PCB206, PCB209
Mirex

3.3. **Histopathology analysis**

The histopathology analyses are a set of quantitative and semiquantitative measurements that determine the presence of parasites and degree of infection as well as the occurrence of pathologies in fish and shellfish tissues.

3.3.1. Shellfish histopathology

The histological analyses of shellfish were performed at Rutgers University's Haskin Shellfish Laboratory. A detailed account of the protocol is described in the NOAA's NOS/NCCOS technical memorandum number 27 (Kim *et al*. 2006). From each location subsets of 5 individual organisms of legal harvestable size (38 mm) were randomly selected and prepared for the analysis.

The adductor muscles of organisms were cut with a sharp knife so that the valves remained open. The entire animal was placed in Davidson's fixative for 1 week and then transferred to 70% alcohol for storage. A sharp knife or scalpel was carefully run between the shell and the mantle to separate the meat from the shell. This procedure was repeated for the other shell to completely detach both sides of the mantle from the shell.

For all shellfish, the fixed tissue samples were embedded in paraffin after dehydration and clearing. The tissue-paraffin block was then placed in a freezer overnight before sectioning. The paraffin-embedded tissue blocks were first sliced at 20 µm to expose an entire tissue cross-section, and then sectioned at 5 µm. Tissue sections were deparaffinized and hydrated using a xylene-ethanol series. Following hydration, slides were stained in a pentachrome series, dehydrated in a series of acetic acid dips followed by acetone, cleared in xylene and mounted in Permount®. Each slide was examined microscopically using 10× ocular and a 10× objective to determine gross histopathology parameters (Table 2). Major tissue types examined included gill, mantle, gonoducts, digestive gland tubules, stomach/digestive gland, and connective tissue.

Table 2. List of parameters measured for the histopathological assessment of bivalves. Top: list of parasitic species. Bottom: list of diseases and tissue conditions. Parameters measured semi-quantitatively are in **bold**; all other parameters were measured quantitatively.

Parasite category	Parasites
Cestodes	Body cestode, Gill cestode, Mantle cestode, Cestode metacercariea
Copepods	Body copepod, Gill copepod, Gut copepod
Ciliates	Digestive tract ciliate, Large gill ciliate, Small gill ciliate, Gut ciliate
Protozoan	Digestive tubule protozoan, Gut protozoan
Nematode	Nematodes

Trematodes	*Trematode sporocyst gut, Bucephalid* trematode spore, **Trematode sporocyst** gill, Trematode metacercariea, Protoeces
Gregarines Nematopsis	Nematopsis body, Nematopsis gill, Nematopsis mantle
Rickettsia	Digestive tubule rickettsia, Gut rickettsia, *Chlamydia, Prokaryotic bodies*
Coccidian	*Pseudoklossia*
Hydra	Gill hydra
Nemertines	Gill nemertine
Pea crab	Pinnotherid crab
Unidentified organism	Unidentified gonoduct organism, Unidentified organism

Disease category	Diseases
Tissue Inflammation	Focal inflammation, Diffuse inflammation
Necrosis	Necrosis diffuse, Necrosis focal, Ceroid bodies
Digestive tubule conditions	**Digestive tubule atrophy**, **Unusual digestive tubule**
Edema	Edema
Gonads	**Gonad abnormalities**
Neoplasm	**Neoplasm**
Tumor	Tumor
Xenoma	Xenoma

Quantitative Measures: Conditions scored quantitatively (Table 2) were evaluated by keeping a running count of occurrences of the condition as the slide is scanned to avoid re-examining each incident multiple times. Quantitative scores were used for parasites, pathologies, and selected morphological conditions that could be tallied individually (Kim et al., 2006). Parasites counted quantitatively included prokaryotic inclusion bodies (rickettsia, chlamydia, etc.), various ciliates, gregarines, other protozoans, nematodes, encysted cestodes and metacercariae of trematodes, copepods and other unidentified organisms. Ciliates were quantified by tissue type (gill and digestive tract), as were the gregarines (body, gill, and mantle). Nematodes were also subjected to quantitative count based on their observed cross-sections. A number of tissue pathological conditions were also evaluated quantitatively, including the number of ceroid bodies, cases of hemocytic infiltration that were scored separately as focal and diffuse incidences of tissue inflammation, and tumors.

Semi-quantitative Measures: Some conditions are assigned to a semi-quantitative scale relative to the intensity or the extent of the affected area (Tables 2). Definitions of scale values can be found in Kim *et al.* (2006). A semiquantitative 0-to-4-point scale is used for invasive trematode sporocysts (*Fellodistomidae* and *Bucephalidae*). For each specimen examined, the presence of neoplasia and

unusual digestive tubules is recorded semi-quantitatively using the 0-to- 4-point scale. Abnormal gonadal development characterized by unusual development is given a semiquantitative 0-to-4-point score relative to the spatial coverage of the condition (Kim *et al.,* 2006). For digestive gland atrophy, a condition known to be caused by a variety of stressors, most likely related to poor nutrition (Winstead, 1995), the average degree of thinning of the digestive tubule walls was assigned a numerical rating on a 0-to-4-point scale (Kim *et al.,* 2006). Semi-quantitative procedures for the assessment of the magnitude of parasitic infection and tissue diseases are exemplified in this document using scales for trematode sporocyst infection (Table 3) and histological condition of digestive gland atrophy (Table 4).

Table 3. Semi-quantitative scale for trematode sporocyst infection.

Score	Description
0	Uninfected
1	Present in the gonads only (some gametic tissue still present)
2	Completely filling the gonads (no gametic tissue present); may be present in digestive gland or gills in very limited amount
3	Completely filling the gonads; extensive invasion of the digestive gland and/or the gills
4	Completely filling the gonad; substantially filling the digestive gland or gill; individuals appear to be a sac of sporocyst

Table 4. Semi-quantitative scale for digestive gland atrophy.

Score	Description
0	Normal wall thickness in most tubules (0% atrophy), lumen nearly occluded, few tubules even slightly atrophied
1	Average wall thickness less than normal, but greater than one-half normal thickness, most tubules showing some atrophy, some tubules still normal
2	Wall thickness averaging about one-half as thick as normal
3	Wall thickness less than one-half of normal, most tubules walls significantly atrophied, some walls extremely thin (fully atrophied)
4	Wall extremely thin (100% atrophied), nearly all tubules affected

3.3.2. Fish histopathology

The histopathological analysis of salmon was performed at the NOAA Northwest Fisheries Science Center, in Seattle, WA. Histopathologic diagnosis was performed on fish liver, head and trunk kidney, and gill tissues. Sections of liver, head and trunk kidney (1cm in thickness) and two gill arches collected from individual salmon were preserved in Davidson's fixative (Fournie et al., 2000) at a volume:tissue ratio of at least 10:1 for at least two full days, then transferred to 70% ethanol for storage and transfer to the histopathology laboratory in Seattle. In the laboratory, tissues were processed by an automated tissue processing center, embedded in paraffin, sectioned at a 5*u*m thickness, stained with hematoxylin and eosin and examined by light microscopy with the presence any lesions or detected parasites documented and scored as described for adult salmon in Fairgrieve et al., (2005). Lesions and parasites in tissue sections were identified and classified according to the criteria specified in Meyers and Hendricks, (1985), Cotran et al. (1999) Chitwood and Lichtenfeld (1972) and Bruno et al. (2006).

3.4. **Data analysis**

3.4.1. Contaminant compounds data analysis

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 compound that were smaller than the MDL were qualified as undetected and were assigned a value of zero. For organics, the "totals" were derived as the arithmetic sum of all the individual congeners or homologues of the same group of compounds as listed in Table 1. Contaminant body burdens of toxic metals and organic compounds in salmon and clams were compared to FDA action levels, EPA chronic consumption limits, and to monitoring data from the Alaska DEC, Fish Monitoring Program. Alaska DEC and FDA both report concentration levels on wet weight basis. Assuming average percent moisture of 76% for the salmon and 86% for clam (values were derived from this study), factors of 4 and 7 respectively for salmon and clam were used to convert wet weight concentrations into dry weight concentrations.

3.4.2. Shellfish histopathology data analysis

The severity of parasitic infections and that of pathological conditions were assessed by calculating prevalence and intensity of the condition.

Prevalence describes the proportion of individuals in the population that are infected by a specific parasite or pathology and is calculated as:

$$Prevalence = \frac{number\ of\ hosts\ with\ parasite\ or\ pathology}{number\ of\ hosts\ analyzed}$$

Infection intensity is calculated as the average number of occurrences of the parasite or pathology in infected hosts. This is a measure of the intensity of infection in infected individuals.

$$ntensity = \frac{total\ number\ of\ occurrences\ of\ parasite\ or\ pathology}{number\ of\ hosts\ with\ parasites\ or\ pathology}$$

For conditions measured semi-quantitatively, the scale rating replaced the number of occurrences in this computation. The protocol for the biological component of the NS&T Program stipulates analysis of five individuals per site.

For this study, parasites of the same taxa were pooled by class as indicated in Table 2 and the resulting prevalence and intensity values were determined as the sum of the prevalence and intensity values of the individual parasites. For instance, the class of Cestoda or tapeworms includes body cestodes, gill cestodes, mantle cestodes and cestode metacercariea. The class of Ciliates includes the digestive track ciliates, large gill ciliates, small gill ciliates and gut ciliates. The class of Gregarinia includes the gregarines nematopsis in the body, gill and mantle. The class of Trematodes or flatworms includes *Bucephalid* trematodes spore, trematode sporocyst gill, trematode metacercariea and *Protoeces*.

3.4.3. Fish histopathology data analysis

Conditions 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 in fish gill, liver and kidney for individual. For each site and each salmon species the severity of parasitic infections and that of pathological conditions were assessed by calculating prevalence values separately for the male and female fish. Prevalence describes the proportion of individuals in the population that are infected by a specific parasite or affected by a specific pathological lesion and was calculated as above.

3.4.4. Statistical analysis

SAS and JMP statistical packages were used for data processing and analysis. For the histopathology parameters the severity of parasitic infections and occurrence of disease or histologic conditions were assessed by deriving the prevalence and intensity of each or group of parameters measured. Both prevalence and intensity were derived for parameters measured in clams and cockles. Due to the nature of the parameters measured in the salmon species, only prevalence were calculated for these parameters. For both contaminant concentrations and prevalence/intensity values, Wilcoxon/Kruskal

Wallis and the Fisher's Exact Test was used to assess data comparability and degree of difference between values. Significance of statistical tests were reported at a probability level of 0.05.

4. *RESULTS*

Results are presented for the contaminant analyses and the histological assessments. Contaminant results are presented for shellfish and fish together because all tissues were analyzed for the same constituents. Histological results are presented by taxonomic group because the specific parasites and etiologies are different between organism classes.

The overall analytical results describing levels of the metal and organic contaminants measured in fish and shellfish tissues are presented in Tables 5, 6 (a,b, c). Location-specific assessment and data variation among the fish and shellfish species as well as comparison between different tissue types are discussed below for those chemicals with potential human health significance. Where possible, concentration levels of this study were put into context by comparison to reported safety threshold values from FDA and EPA. An FDA action level represents the limit at or above which FDA may take legal action to remove products from the marketplace. EPA-recommended values typically range from 2 to 120 times lower than the corresponding FDA action levels as the EPA's values are derived from a risk-based approach to initiate local fish consumption advisories and are much more protective (EPA, 2000). For comparative purposes, the values presented here use the EPA chronic reference dose (RfD) and assume an average person's weight of 80 kg (176 lb) and a meal of fish to be 0.227 kg (1/2 lb). The chronic reference dose assumes consumption of a meal of fish every day. For comparative purposes, all values were converted into concentration based on dry weights.

$$\text{No Effect Chronic concentration} = \frac{(\text{RfD X weight})}{\text{meal weight}}$$

Other assumptions for specific groups can be used (e.g. children). The tables also list concentration values derived for different bivalve species from the NOAA NS&T Mussel Watch and the AK DEC Fish Monitoring Programs (FMP) from the region. Bivalves were collected from various locations in and around Kachemak Bay. Salmon filet values from the FMP are from fish captured in the Mantanusk River above the north end of Cook Inlet, the Kenai River in the middle of Cook Inlet, or at Kodiak. Note that metals concentrations are expressed in ppm and organic contaminants are expressed in ppb. All values are presented as dry weight (dw).

4.1 **Metal Contaminant concentrations in fish and shellfish**

The results of contaminant body burdens including concentrations of the major and trace metals measured in in clams and cockles from the three villages are presented in Table 5a. Although results of all metals measured are presented, graphical representations and discussion of the most toxic and/or carcinogenic heavy metals (arsenics, cadmium, chromium, lead, mercury, nickel, and selenium) are examined in detail.

4.1.1. Arsenic

The concentrations of arsenic in the shellfish from the different villages are shown in Figure 2. Concentrations varied from 6.57 to 12.0 $\mu g.g^{-1}$ dw with the minimum and maximum concentration values found in cockles and clams, respectively collected from the Seldovia village harvest grounds (Table 5a). The result showed little variation between arsenic concentration in clams from Nanwalek and Port Graham and cockles from Seldovia. The average arsenic concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 9.22 $\mu g.g^{-1}$. The Alaska DEC-FMP (http://dec.alaska.gov/eh/vet/fish.htm) report tissue concentrations ranging from 3.5 to 22.75 $\mu g.g^{-1}$ in bivalves from the Kachemak Bay area. These results indicate that arsenic concentration in the shellfish used as subsistence food is within the regional concentration range found in shellfish.

Arsenic body burdens in fish showed little variation between the two species or sex of salmon (Tables 6a, 6b and 6c). Body burdens varied from 0.73 to 1.66 $\mu g.g^{-1}$ dw. The Wilcoxon/Kruskal Wallis test showed no differences among the three locations ($p > 0.05$). These values were an order of magnitude lower than concentrations found in the shellfish. Arsenic levels in the salmon were comparable to average value of 1.2 $\mu g.g^{-1}$ dw derived from Alaska DEC-FMP respectively for sockeye from Kenia R. and the Mantanuska R. in 2012. Results of the Wilcoxon/Kruskal Wallis test indicated concentration differences between the two types of fish tissue analyzed ($p < 0.05$) with elevated concentration of arsenic in fish liver relative to fish muscle (figure 3).

The FDA has set the maximum permissible action level of 76 and 86 $\mu g.g^{-1}$ arsenic wet weight (ww) in crustaceans and molluscan shellfish respectively. Using the measured 86 % moisture content in shellfish we derived an equivalence value of 602 $\mu g.g^{-1}$ arsenic dw in shellfish. The highest arsenic concentrations found in the clams, cockles and salmon from the villages were very low relative to the FDA criterion. EPA (2000) has calculated a reference dose for inorganic arsenic, whereas the data presented here are for total arsenic.

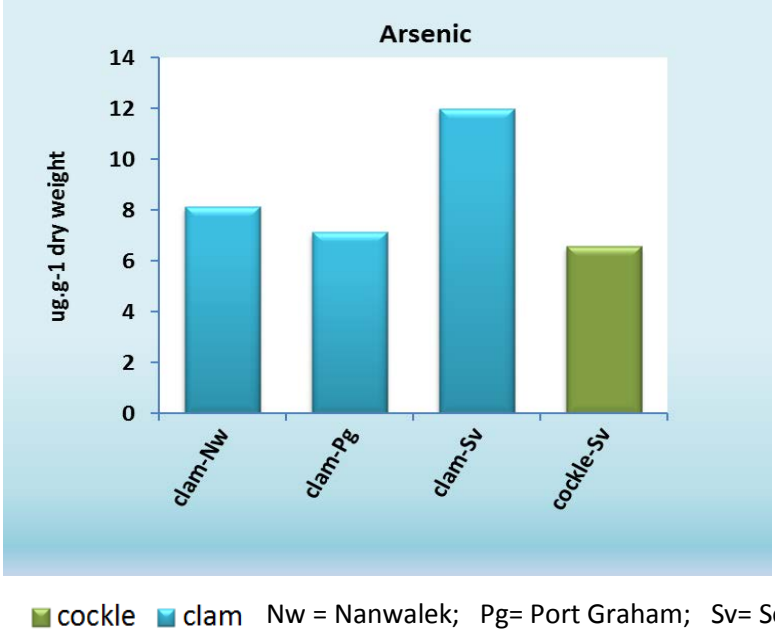


Figure 2. Concentration of arsenic in clam and cockle collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

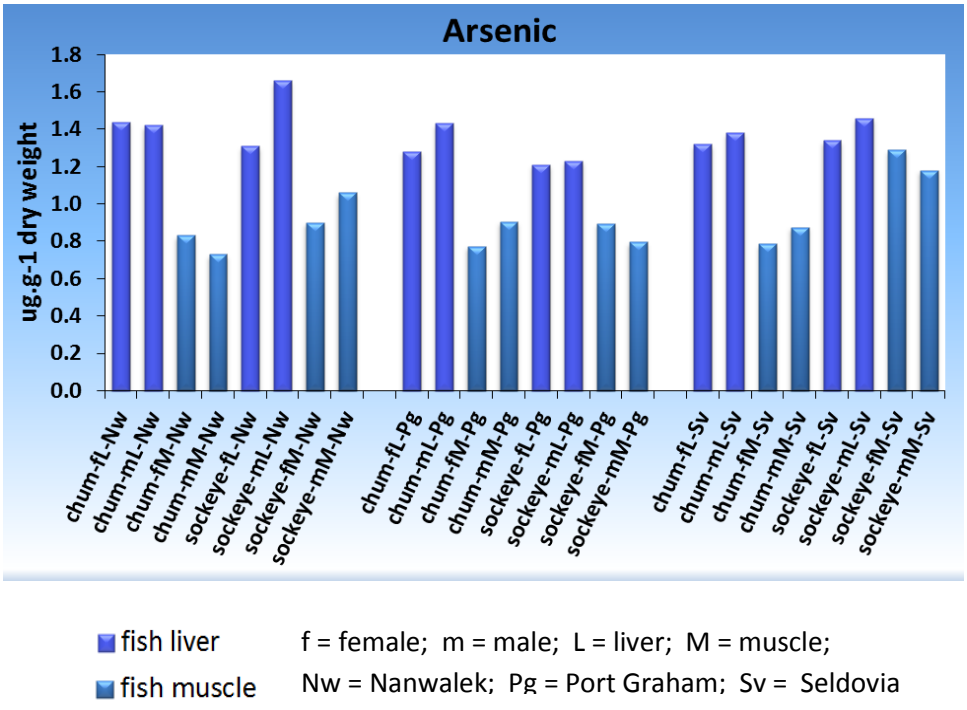


Figure 3. Concentration of arsenic in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

4.1.2. Cadmium

The concentrations of cadmium in the shellfish from the different villages are shown in Figure 4. The highest cadmium concentration (1.27 $\mu g.g^{-1}$ dw) was found in clams from Port Graham while the lowest concentration was measured in cockles from the Seldovia harvest grounds. In the 2011 survey, the Alaska DEC-FMP reported tissue values of 0.0 to 2.18 $\mu g.g^{-1}$ dw cadmium in bivalves from Kachemak Bay. The average cadmium concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 2.62 $\mu g.g^{-1}$ dw. As in the case of arsenic, cadmium concentrations in littleneck clams and cockles used for subsistence food were within the regional concentration range found in shellfish.

As illustrated in Tables 6 (a, b, and c), cadmium concentration varied from 0.010 to 5.45 $\mu g.g^{-1}$ with no obvious concentration differences among the two species of salmon. Wilcoxon/Kruskal Wallis test applied to the combined data indicated significant differences for cadmium concentration between fish gender and tissue types ($p < 0.05$). For both salmon species, cadmium concentrations in liver were more than 500 times higher than concentrations found in muscle (Figure 5). Also, at all locations, liver tissue of male fish had higher cadmium content compared to liver tissue from female fish ($p < 0.05$). The low cadmium values found in fish muscle were consistent with those of the Alaska DEC-FMP, which reported cadmium concentrations that were at or below reporting limits.

The FDA action level for cadmium in shellfish is 4 $\mu g.g^{-1}$ wet weight. Using the measured 86 % moisture content in shellfish we derived an equivalence value of 28 $\mu g.g^{-1}$ cadmium dw in shellfish. There is no FDA action level for cadmium in fish tissue. Using the measured 76% moisture content of the fish muscle, and the EPA reference dose value for cadmium in fish filets, the concentration which would be expected to cause no adverse effects for an average person consuming fish daily, is 1.41 $\mu g.g^{-1}$ dry weight. The average measured concentration in fish muscle was 0.012 $\mu g.g^{-1}$. There is no comparable reference for fish liver from any source. Thus, concentrations of cadmium in clams and fish tissue are one to two orders of magnitude below applicable safety thresholds.

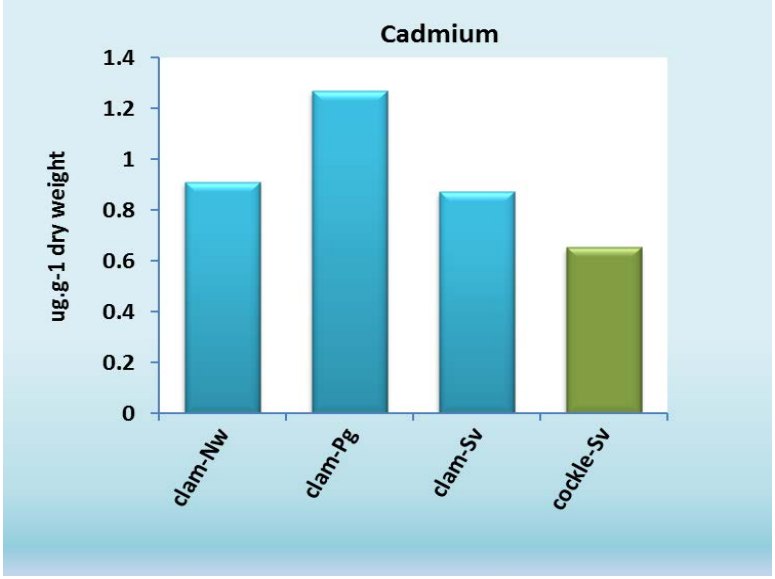


Figure 4. Concentration of cadmium in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

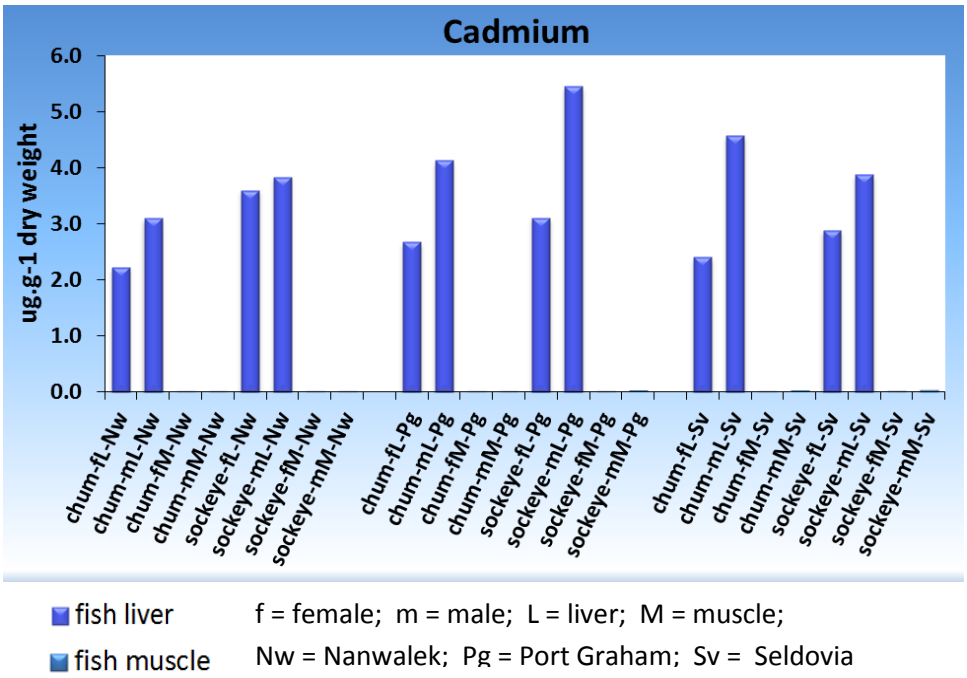


Figure 5. Concentration of cadmium in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

4.1.3. Chromium

The concentrations of chromium in the shellfish from the different villages are shown in Figure 6. The highest chromium concentration (4.65 µg.g^{-1} dw) was found in clams from Seldovia while the lowest concentration (2.02 µg.g -1 dw) was measured in cockles from the Seldovia harvest grounds. In the 2012 survey, the Alaska DEC-FMP reported tissue values from 1.23 to 4.24 µg.g^{-1} dw chromium in bivalves from Kachemak Bay. The average chromium concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 1.26 µg.g^{-1} dw. Chromium concentrations in littleneck clams and cockles used for subsistence food were within the regional concentration range found in shellfish.

Chromium was below detection limits in all fish samples. The low chromium values are consistent with those of the Alaska DEC-FMP, which reported chromium concentrations that were below reporting limits at all locations.

The FDA action level for chromium in shellfish is 13 µg.g^{-1}. Using the measured 86 % moisture content in shellfish we derived an equivalence value of 91 µg.g^{-1} chromium dw in shellfish. There is no FDA action level or EPA reference dose value for chromium in fish tissue.

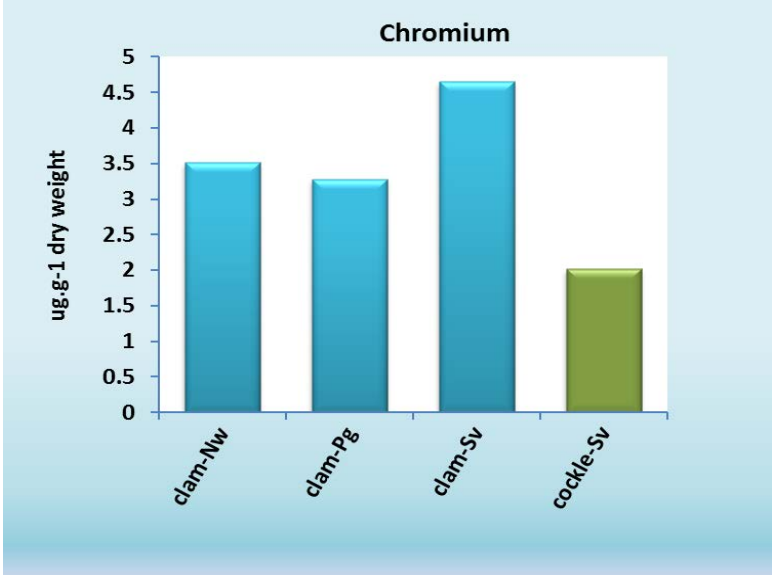


Figure 6. Concentration of chromium in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

4.1.4. Lead

The concentrations of lead in the shellfish from the different villages are shown in Figure 7. The highest lead concentration (1.49 µg.g^{-1} dw) was found in cockles from Seldovia while the lowest concentration (

0.27 µg.g^{-1} dw) was measured in clams from the Nanwalek harvest grounds. Both species had higher concentrations in the Seldovia samples. In the 2012 survey, the Alaska DEC-FMP reported tissue values from 0.31 to 0.70 µg.g^{-1} dw lead in bivalves from Kachemak Bay. The average lead concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 0.59 µg.g^{-1} dw. Concentrations in littleneck clams and cockles used for subsistence food were slightly above the regional concentration range found in shellfish.

As illustrated in Tables 6 (a, b and c), lead concentrations in fish tissue were either very low or not detectable. Lead was found at detectable concentrations only in chum salmon (Figure 8). The low lead values found in fish muscle were consistent with those of the Alaska DEC-FMP, which reported lead concentrations below detection limits in 2012.

The FDA action level for lead in shellfish is 1.7 µg.g^{-1}. Using the measured 86 % moisture content in shellfish we derived an equivalence value of 11.9 µg.g^{-1} lead dw in shellfish. There is no FDA action level or EPA reference dose value for lead in fish tissue.

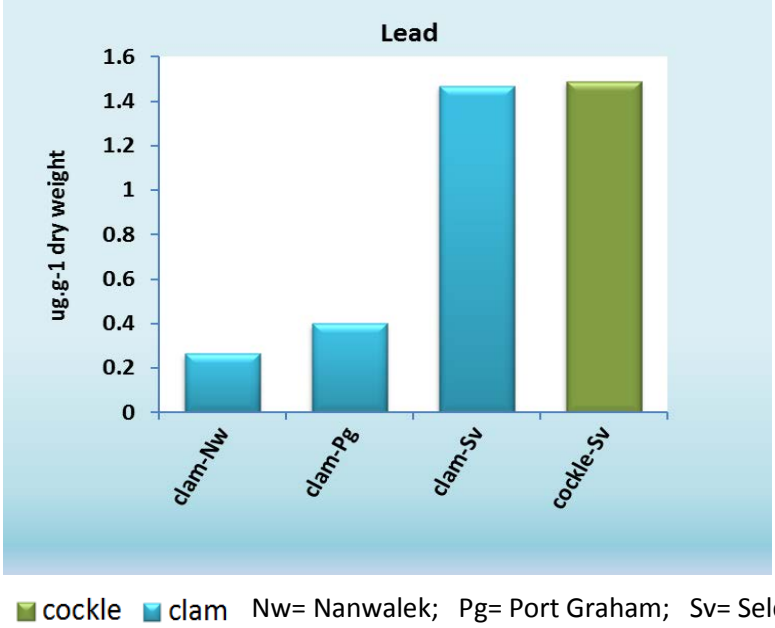


Figure 7. Concentration of lead in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

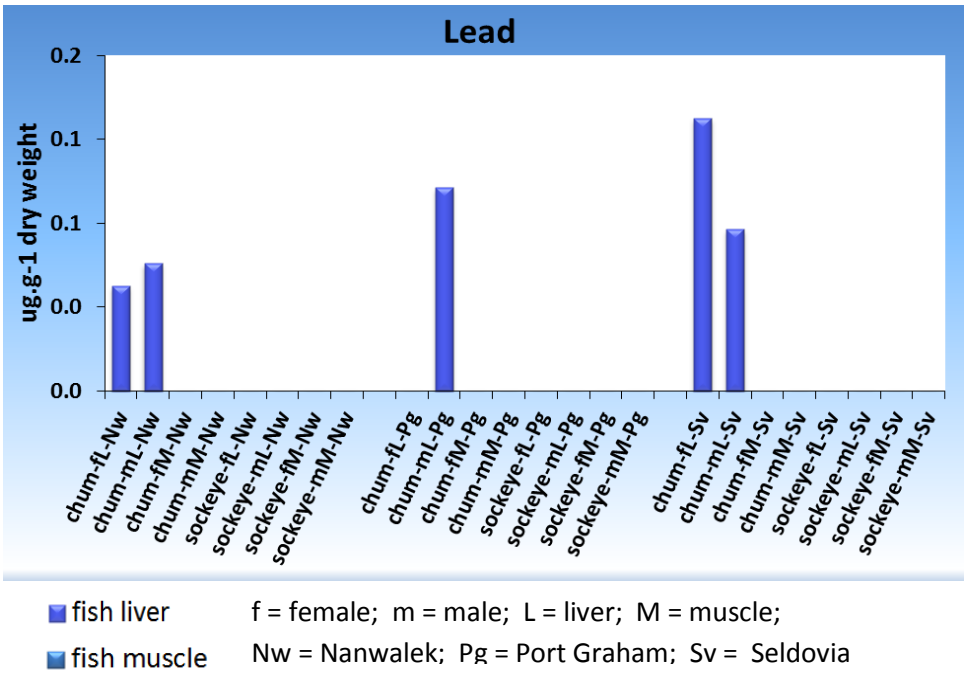


Figure 8. Concentration of lead in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

4.1.5. Mercury

The concentrations of mercury in the shellfish from the different villages are shown in Figure 9. The highest mercury concentration (0.21 $\mu g.g^{-1}$ dw) was found in clams from Seldovia while the lowest concentration (0.03 $\mu g.g^{-1}$ dw) was measured in clams from the Nanwalek harvest grounds. In a 2011 survey, the Alaska DEC-FMP reported an average value of 0.1 µg.g-1 dw total mercury in razor clams from the Lower Cook Inlet . In the 2012 survey, mercury was below detection limits in Razor clams from Redoubt Creek. The average mercury concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 0.082 $\mu g.g^{-1}$ dw.

As illustrated in Tables 6a, 6b and 6c, mercury concentrations in fish tissue were similar to the shellfish levels. Mercury concentrations were significantly higher in liver than in muscle ($p < 0.01$), but only in the sockeye salmon (Figure 10). The mean mercury concentration in muscle was 0.11 $\mu g.g^{-1}$ dw. The mercury values found in fish muscle were consistent with those of the Alaska DEC-FMP, which reported mercury concentrations of 0.100 to 1.120 $\mu g.g^{-1}$ dw.

There is no FDA action level for mercury in shellfish tissue. There is an FDA action level of 1 $\mu g.g^{-1}$ for methylmercury in fish tissue. Using the measured 76% moisture content of the fish tissue, the FDA action level is 4 $\mu g.g^{-1}$ dw. Using the measured 76% moisture content of the fish muscle, and the EPA reference dose value for mercury in fish filets, the concentration which would be expected to cause no

adverse effects for an average person consuming fish daily, is 0.14 µg.g dry weight. It should also be noted that the EPA reference dose is for methylmercury, whereas the data presented here are for total mercury. In fish tissue, the majority of mercury is methyl mercury (EPA, 2000). There is no comparable reference for fish liver from any source.

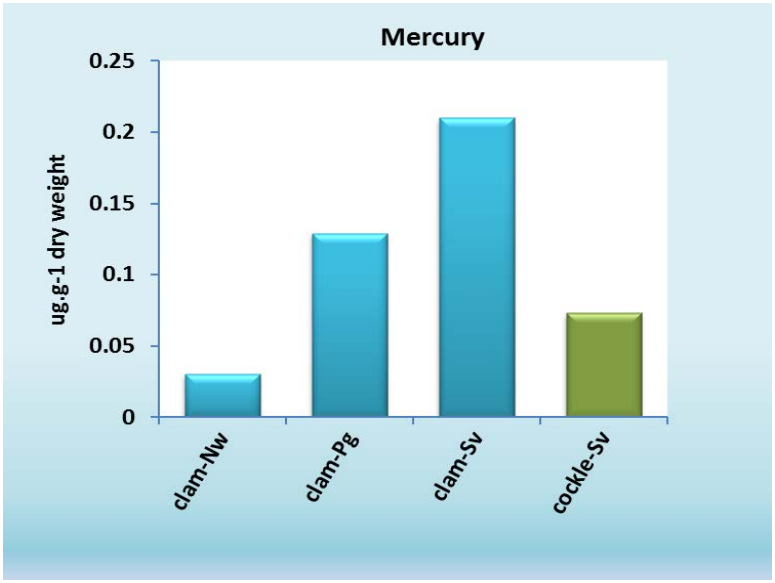


Figure 9. Concentration of mercury in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

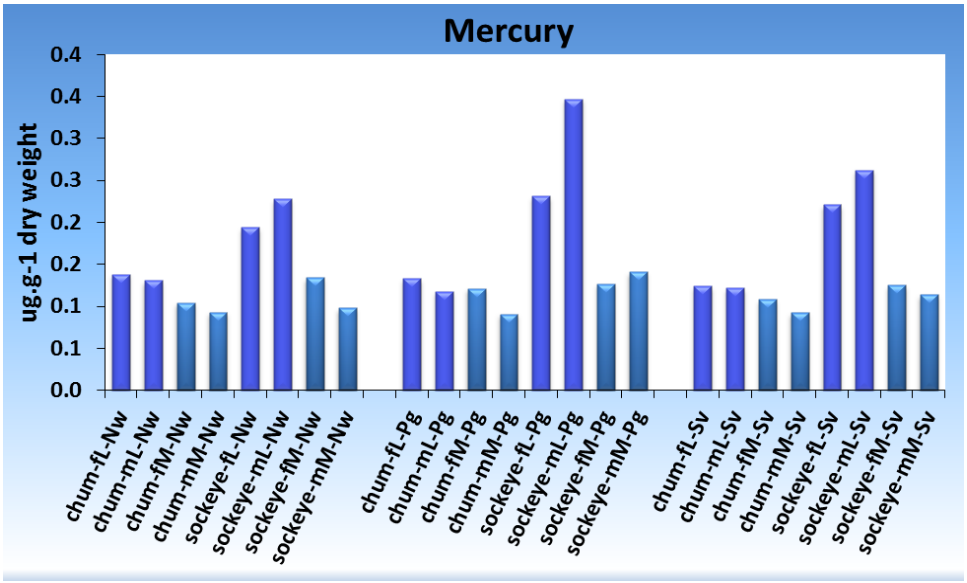


Figure 10. Concentration of mercury in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of the Alaskan Native village of Nanwalek, Port Graham and Seldovia.

4.1.6. Nickel

The concentrations of nickel in the shellfish from the different villages are shown in Figure 11. The highest nickel concentration (12.1 µg.g^{-1} dw) was found in cockles from Seldovia while the lowest concentration (3.27 µg.g^{-1} dw) was measured in clams from the Port Graham harvest grounds. Both species had higher concentrations in the Seldovia samples. In the 2012 survey, the Alaska DEC-FMP reported tissue values of 0.31 to 0.70 µg.g-1 dw nickel in bivalves from Kachemak Bay . The average nickel concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 1.83 µg.g^{-1} dw. Concentrations in littleneck clams and cockles used for subsistence food were slightly above the regional concentration range found in shellfish.

As illustrated in Tables 6 (a, b and c), nickel concentrations in salmon vary by tissue, again, primarily in the sockeye salmon where liver concentrations were significantly higher than muscle ($p < 0.01$). Concentrations were variable with some values at or below the detection limit, but with no obvious pattern (Figure 12). The low nickel values found in fish muscle were consistent with those of the Alaska DEC-FMP, which reported nickel concentrations below detection limits in sockeye salmon.

The FDA action level for nickel in shellfish is 80 µg.g^{-1}. Using the measured 86 % moisture content in shellfish we derived an equivalence value of 560 µg.g^{-1} nickel dw in shellfish. There is no FDA action level or EPA reference dose value for nickel in fish tissue.

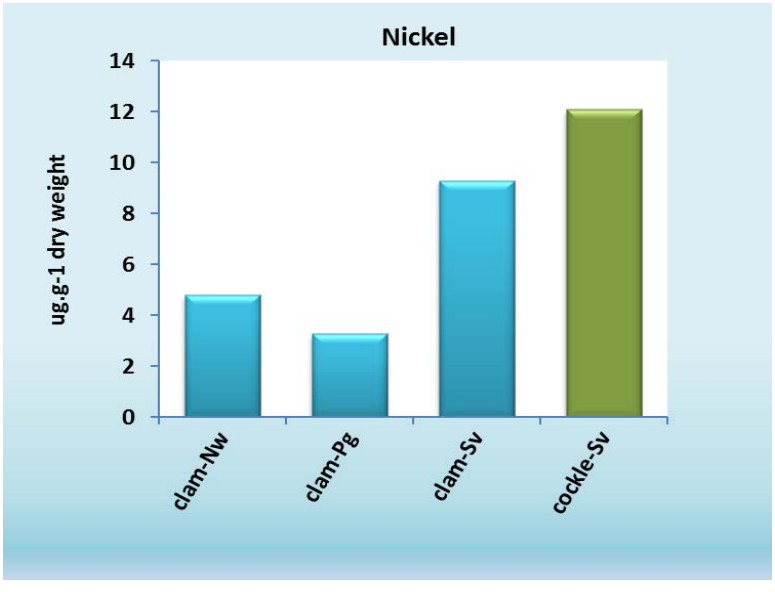


Figure 11. Concentration of nickel in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

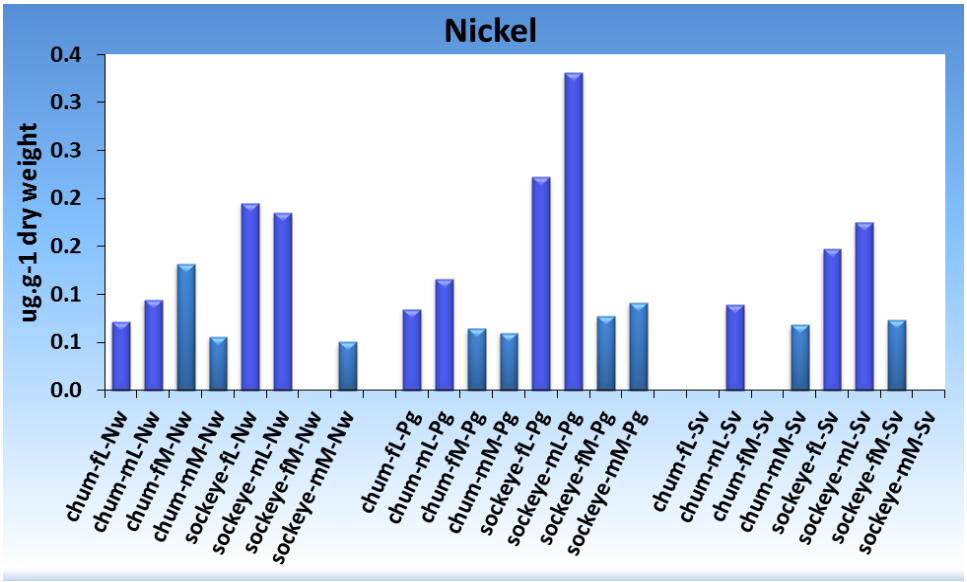


Figure 12. Concentration of nickel in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

4.1.7. Selenium

The concentrations of selenium in the shellfish from the different villages are shown in Figure 13. The highest selenium concentration (3.28 µg.g^{-1} dw) was found in clams from Seldovia while the lowest concentration (1.89 µg.g^{-1} dw) was measured in clams from the Nanwalek harvest grounds. Both species had higher concentrations in the Seldovia samples. In the 2011 survey, the Alaska DEC-FMP reported an average value of 3.8 µg.g^{-1} dw selenium in razor clams from the Lower Cook Inlet. In the 2012 survey, razor clams in Redoubt Cr. Had a mean concentration of 0.53 µg.g^{-1}. The average selenium concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 2.89 µg.g^{-1} dw. Concentrations in littleneck clams and cockles used for subsistence food were within the regional concentration range found in shellfish.

As illustrated in Tables 6 (a, b and c), selenium concentrations vary by tissue, in both species (Figure 14). Liver concentrations were significantly higher than muscle ($p < 0.01$). The low selenium values found in fish muscle were consistent with those of the Alaska DEC-FMP, which reported selenium concentrations of 0.88 and 0.84 µg.g^{-1} dw in sockeye salmon.

There is no FDA action level for selenium in shellfish tissue. Using the measured 76% moisture content of the fish muscle, and the EPA reference dose value for selenium in fish filets, the concentration which would be expected to cause no adverse effects for an average person consuming fish daily, is 7.1 $\mu g.g^{-1}$ dry weight. The average measured concentration in fish muscle was 1.06 $\mu g.g^{-1}$. There is no comparable reference for fish liver from any source.

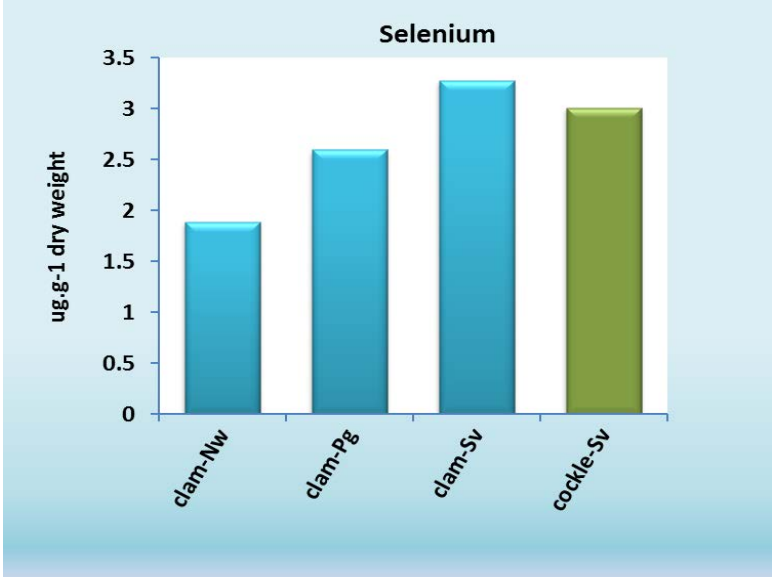


Figure 13. Figure 13. Concentration of selenium in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

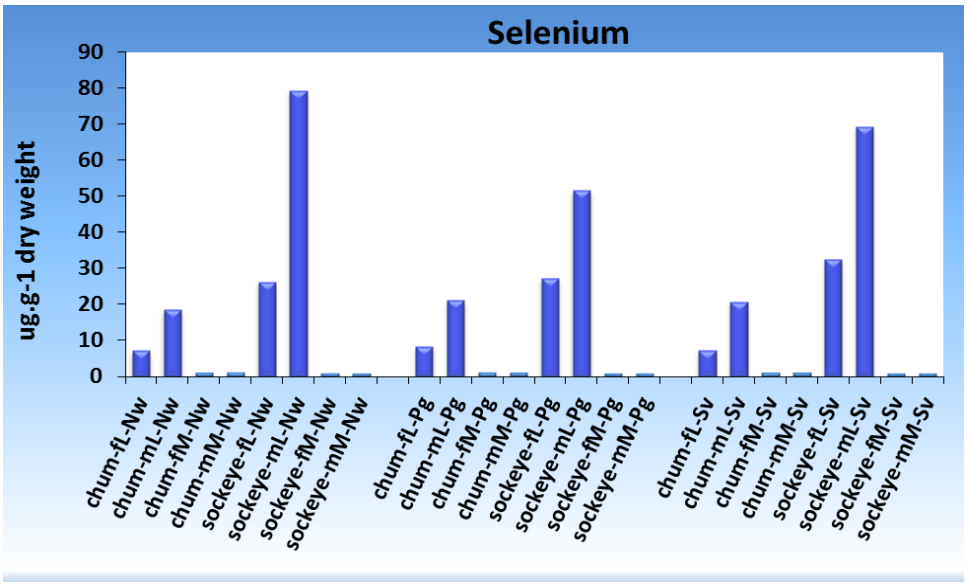


Figure 14. Concentration of selenium in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of the Alaskan Native village of Nanwalek, Port Graham and Seldovia.

4.2. **Organic contaminant concentrations in fish and shellfish**

Detailed descriptions of contaminant concentrations found in molluscs and salmon in this study are limited to well-known and well-studied compounds. Calculated EPA chronic threshold tissue concentrations and FDA action levels for all contaminants are shown in Tables 5 and 6a-c. All of the pesticide thresholds were one to two orders of magnitude greater than any tissue concentration seen in this study.

4.2.1. Chlordanes

The concentrations of chlordane in the shellfish from the different villages are shown in Figure 15. The highest chlordane concentration (1.48 $ng.g^{-1}$ dw) was found in clams from Port Graham while the lowest concentration (0.27 $ng.g^{-1}$ dw) was measured in clams from the Nanwalek harvest grounds. Both species had higher concentrations in the Port Graham samples. The average chlordane concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 1.46 $ng.g^{-1}$ dw. Concentrations in littleneck clams and cockles used for subsistence food were within the regional concentration range found in shellfish.

As illustrated in Tables 6a, 6b and 6c, chlordane concentrations in salmon vary by tissue, in both species (Figure 16). Sockeye salmon tended to have higher levels of chlordane, but this was not true in all cases.

The low chlordane values found in fish muscle were not consistent with those of the Alaska DEC-FMP, which reported higher chlordane concentrations of 11.3 and 6.88 $ng.g^{-1}$ dw in sockeye salmon from Kodiak and Matanuska R. respectively. According to earlier reports, muscle tissue concentrations in Chinook, pink and Coho salmon were 21.52, 1.62, and 3.22 $ng.g^{-1}$ dw, respectively.

There is no FDA action level for chlordane in shellfish tissue. The FDA action level for chlordane in fish tissue is 1200 $ng.g^{-1}$ dw. Using the measured 76% moisture content of the fish muscle, and the EPA reference dose value for chlordane in fish filets, the concentration which would be expected to cause no adverse effects for an average person consuming fish daily, is 704.8 $ng.g^{-1}$ dw. The average measured concentration in fish muscle was 1.90 $ng.g^{-1}$. There is no comparable reference for fish liver from any source.

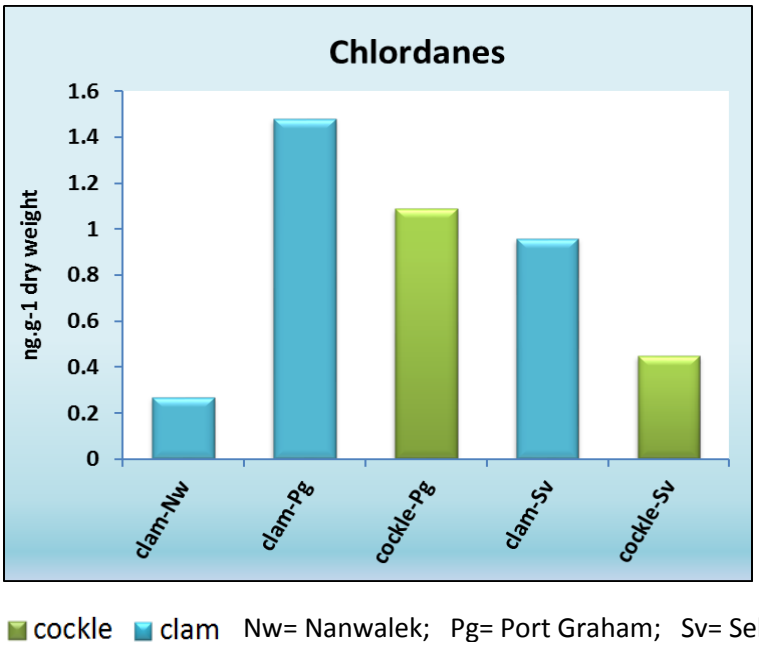


Figure 15. Concentration of total chlordanes in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

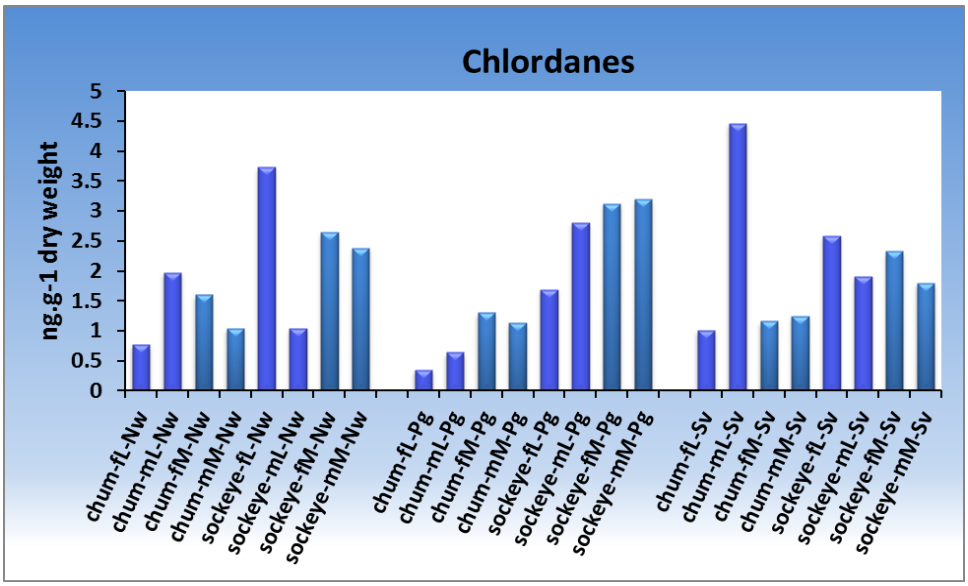


Figure 16. Concentration of total chlordane in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

4.2.2. DDTs

The concentrations of DDT in the shellfish from the different villages are shown in Figure 17. The highest DDT concentration (0.73 ng.g^{-1} dw) was found in clams from Seldovia while the lowest concentration (0.17 ng.g^{-1} dw) was measured in cockles from the Port Graham harvest grounds. Both species had higher concentrations in the Seldovia samples. The average DDT concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 0.47 ng.g^{-1} dw. Concentrations in littleneck clams and cockles used for subsistence food were within the regional concentration range found in shellfish.

As illustrated in Tables 6a, 6b and 6c, DDT concentrations in salmon vary by tissue, in both species (Figure 18). Sockeye salmon tended to have higher levels of DDT, but this was not true in all cases. In muscle tissue however, sockeye had much higher average concentrations (8.46 ng.g $^{-1}$) than the chum salmon (2.44 ng.g $^{-1}$). The differential DDT values found in the two species were consistent with those of the Alaska DEC-FMP, although they report higher values for both locations (19.04 and 38.12 ng.g $^{-1}$) as was seen with chlordane.

There is no FDA action level for DDT in shellfish tissue. Using the measured 76% moisture content of the fish muscle, the FDA action level for DDT in fish tissue is 20,000 ng.g^{-1} dw. Using the measured 76%

moisture content of the fish muscle, and the EPA reference dose value for DDT in fish filets, the concentration which would be expected to cause no adverse effects for an average person consuming fish daily, is 704.8 $ng.g^{-1}$ dw dry weight. There is no comparable reference for fish liver from any source.

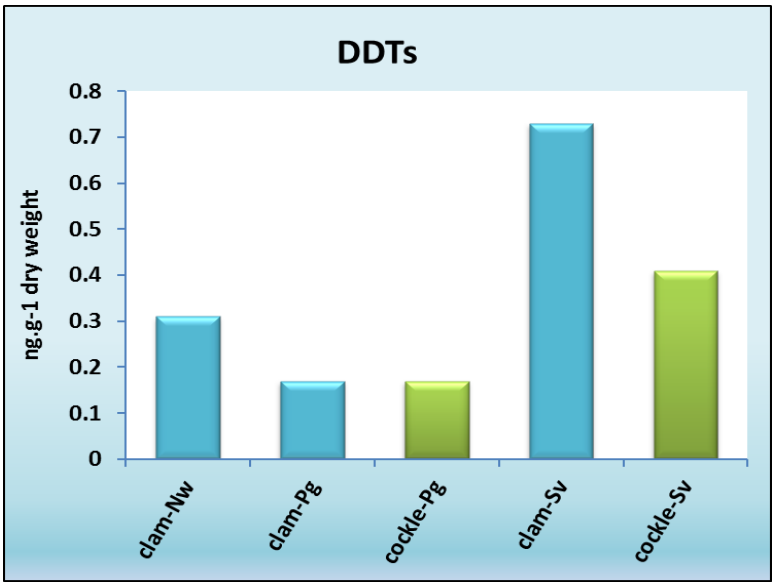


Figure 17. Concentration of total DDTs in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

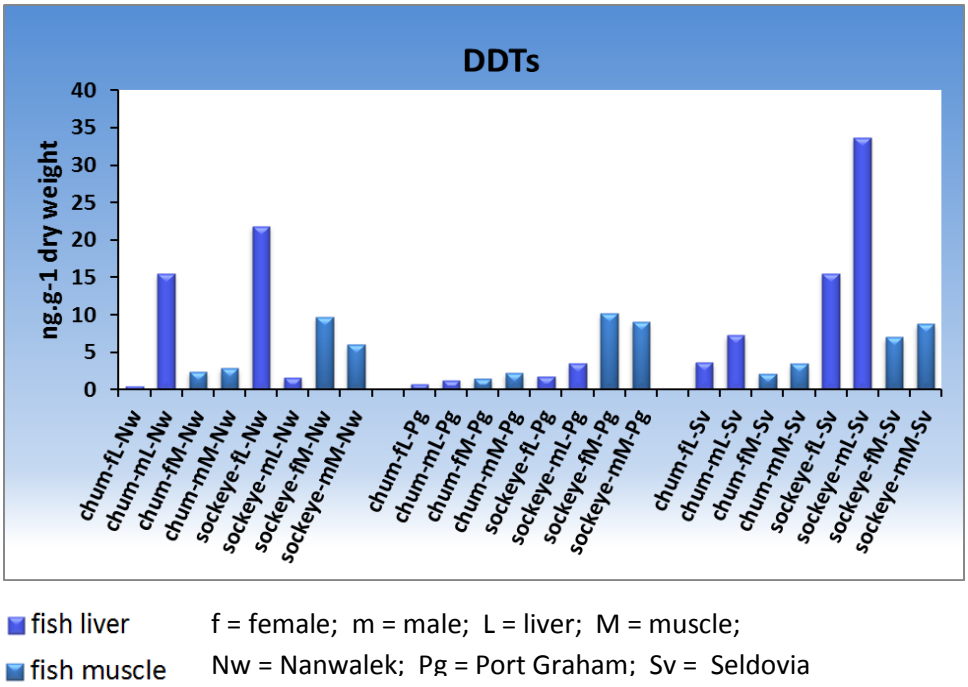


Figure 18. Concentration of total DDTs in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

4.2.3. PAHs

The concentrations of PAHs in the shellfish from the different villages are shown in Figure 19. The highest PAH concentration (302 $ng.g^{-1}$ dw) was found in clams from Port Graham while the lowest concentration (143.8 $ng.g^{-1}$ dw) was measured in clams from Nanwalek harvest grounds. The average PAH concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 167.8 $ng.g^{-1}$ dw, but varied between 72.1 and 263.7 $ng.g^{-1}$ dw. Concentrations in littleneck clams and cockles used for subsistence food overlap the regional concentration range found in shellfish. There are no applicable FDA action level or EPA reference dose for PAHs in shellfish. The average PAH concentration in blue mussels from the entire data set from southcentral and southeast Alaska (15 stations) in the NOAA NS&T data base is 304.84 $ng.g^{-1}$ dw, and varies between 28.13 and 1,026.23 $ng.g^{-1}$ dw. PAHs were not measured in fish tissue because vertebrates are able to metabolize PAHs to a much greater extent than mollusks.

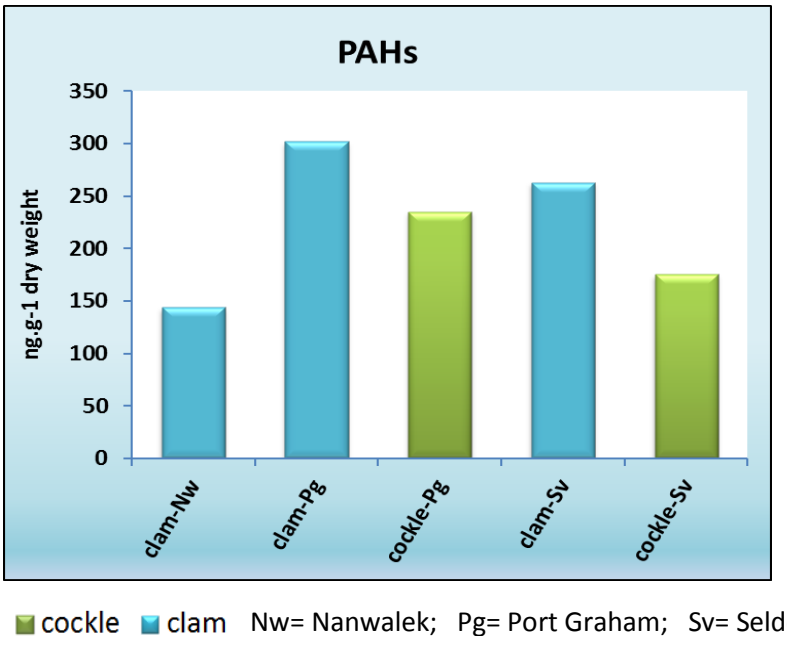


Figure 19. Concentration of total total PAHs in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

4.2.4. PCBs

The concentrations of PCBs in the shellfish from the different villages are shown in Figure 20. The highest PCB concentration (10.1 $ng.g^{-1}$ dw) was found in clams from Seldovia while the lowest concentration (0.92 $ng.g^{-1}$ dw) was measured in cockles from the Port Graham harvest grounds. The average PCB concentration in blue mussels from Kachemak Bay in the NOAA NS&T data base is 2.47 $ng.g^{-1}$ dw. Concentrations in littleneck clams and cockles used for subsistence food overlap the regional concentration range found in shellfish.

As illustrated in Tables 6a, 6b and 6c, PCBS concentrations in salmon vary by tissue, in both species, and by location (Figure 21). Sockeye salmon tended to have higher levels of PCBs , but this was not true in all cases. Port Graham sockeye muscle tissue had much higher average concentrations (13.15 $ng.g^{-1}$) than the other values, but the same was not seen at Nanwalek or Seldovia. The Alaska DEC-FMP reports values of 23.60 and 11.85 $ng.g^{-1}$ in sockeye salmon from Kodiak and the Matanusk R., respectively.

There is no FDA action level for PCBs in shellfish tissue. Using the measured 76% moisture content of the fish muscle, the FDA action level for PCBs in fish tissue is 8,000 $ng.g^{-1}$ dw. Using the measured 76% moisture content of the fish muscle, and the EPA reference dose value for total PCBS in fish filets, the concentration which would be expected to cause no adverse effects for an average person consuming fish daily, is 28 $ng.g^{-1}$ dw dry weight. The EPA reference dose is based on analyses of Aroclors. These are industrial mixtures of PCBs with specific chlorination levels. Once PCBs are released into the environment, they fractionate into different media over space and time and are slowly degraded. Thus, they no longer represent the congener mixtures they originally comprised. The data presented here are the sum of the PCB congeners actually measured. There is no clear relationship at this time between total Aroclors and total PCBs that have been weathered. There is no comparable reference for fish liver from any source.

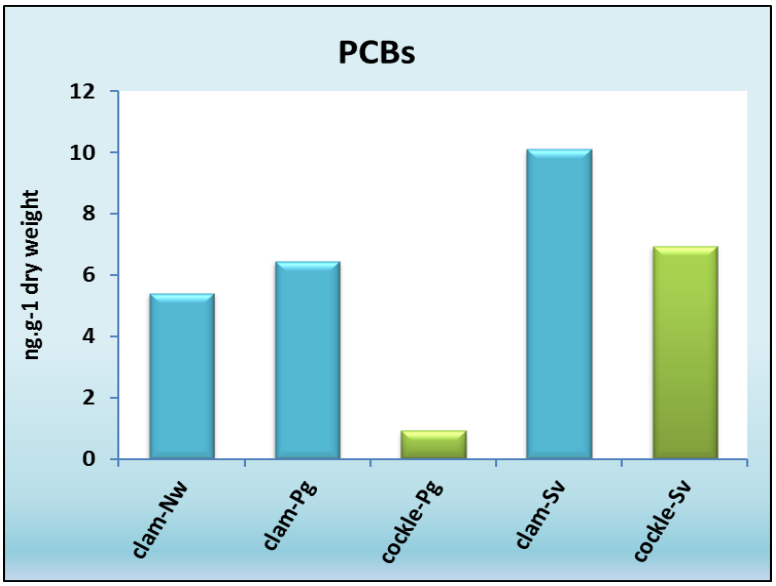


Figure 20. Concentration of total total PCBs in clams and cockles collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

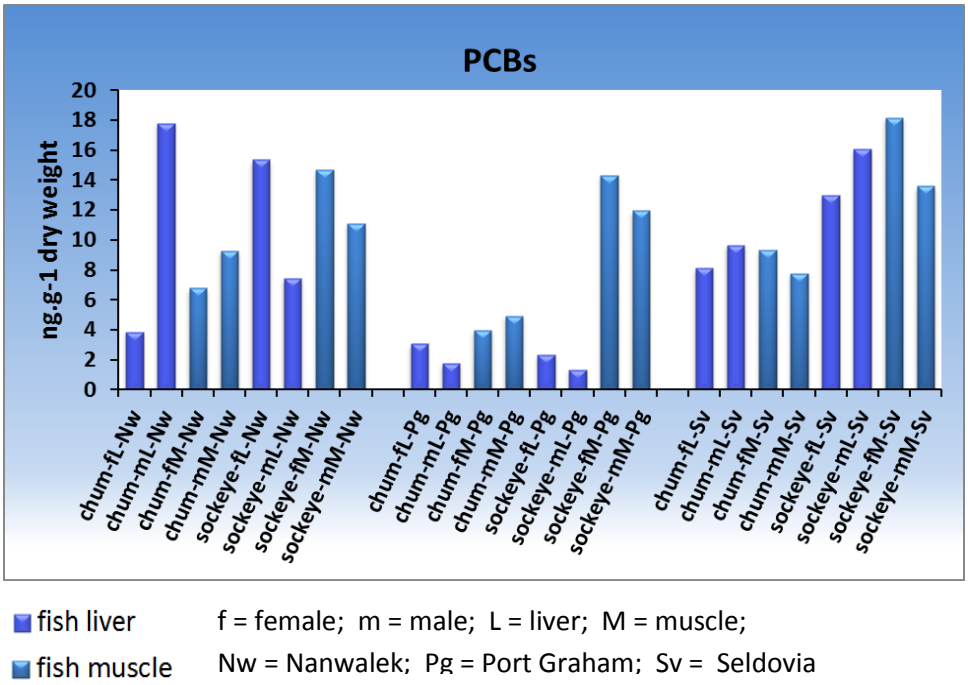


Figure 21. Concentration of total PCBs in liver and muscle tissues of chum and sockeye salmon collected from subsistence harvest grounds of the Alaskan Native villages of Nanwalek, Port Graham and Seldovia.

Table 5a. Contaminant concentration in shellfish. Where available, results from this study were weighed against mean concentration values reported by the NOAA Mussel Watch for blue mussels and the Alaska DEC Fish Monitoring Program for razor clams. Results were also compared to the FDA action levels for shellfish consumption. (Metals $\mu g.g^{-1}$, Organics $ng.g^{-1}$ dry weight).

	Nanwalek	Port Graham		Seldovia		NOAA (2007)	FDA action level
Compound	**Clam**	**Clam**	**Cockle**	**Clam**	**Cockle**	**Blue Mussel**	**shellfish**
Butyl Tin	0	0		0	0	1.18	
Chlordanes	0.27	1.48	1.09	0.96	0.45	1.46	
Chlorobenzenes	1.82	1.5	1.5	1.2	0.94	0.66	
Chlorpyrifos	0.44	0	0.41	0.23	0.37	0	
DDTs	0.31	0.17	0.17	0.73	0.41	0.47	
Dieldrins	0.13	0	0	0.38	0	0.44	
Endosulfans	0	0.26	0.22	0.04	0	0	
HCHs	3.85	5.7	4.32	2.93	3.53	0.71	
Mirex	0.22	0	0	0.5	0	0	
PAHs	143.8	302.0	235.1	262.9	175.4	167.9	
PCBs	5.41	6.45	0.92	10.1	6.94	2.47	
Compound	**Clam**	**Clam**		**Clam**	**Cockle**	**Blue Mussel**	**shellfish**
Arsenic	8.14	7.14		12	6.57	9.22	602
Cadmium	0.912	1.27		0.87	0.65	2.62	28
Chromium	3.51	3.28		4.65	2.02	1.26	91
Copper	18.6	23.1		33.9	38.4	7.96	
Iron	316	1170		2330	1100	670	
Lead	0.27	0.4		1.47	1.49	0.59	11.9
Manganese	12.3	26.7		40.4	18.4	15.37	
Mercury	0.031	0.129		0.210	0.073	0.082	
Nickel	4.79	3.27		9.3	12.1	1.83	560
Selenium	1.89	2.6		3.28	3.01	2.89	
Silver	0.31	0.27		0.50	0.03	0.06	
Tin	0.437	0.13		0.12	0.18	0.0	
Zinc	81.8	140.0		210.0	131.0	100.4	

Table 5b. Contaminant concentrations in shellfish reported by the Alaska DEC Fish Monitoring Program. Results were also compared to the FDA action levels for shellfish consumption. (Metals $\mu g.g^{-1}$ dry weight).

	Butter clam	Littleneck clam	Maya sp.	Razor clam	Redneck clam	Cockle	FDA action level
Arsenic	21.18	22.75	5.60	5.18	3.50	4.90	602
Cadmium	0.74	2.18		1.67	1.09	0.00	32
Chromium	3.64	1.20	3.22	3.71	4.24	1.23	104
Lead	0.35	0.31	0.63	0.70	0.49	0.39	14
Nickel	0.35	0.31	0.63	0.70	0.49	0.39	640

Table 6a: Contaminant concentrations in liver and muscle tissue of salmon collected from Nanwalek. To put concentration values from this study into context, concentration levels reported by the Alaska DEC Fish Monitoring program are presented. Results were also compared to the FDA action levels and calculated EPA chronic consumption thresholds for fish consumption. (Metals $\mu g.g^{-1}$, Organics $ng.g^{-1}$ dry weight). m = male, f=female.

	Chum f		Chum m		Sockeye f		Sockeye m		AK FMP Sockeye		FDA	EPA
	liver	muscle	liver	muscle	liver	muscle	liver	muscle	Kodiak	Matanuska	fish	fish
Chlordanes	0.77	1.6	1.97	1.04	3.73	2.64	1.04	2.38	11.32	6.88	1,200	704.8
Chlorobenzene	2.27	2.78	2.72	2.05	2.99	2.84	3.55	2.22	5.00	4.00		1127.8*
Chlorpyrifos	0	0	0	0	2.59	0	0	0				422.9
DDT	0.46	2.43	15.45	2.88	21.84	9.74	1.58	5.99	38.12	19.04	20,000	704.8
Dieldrins	0.4	0.62	0.56	0.24	0.42	0.45	2.15	0.61	1.76	0.88	1,200	70.5
Endosulfan	0.19	0.12	0.8	0.23	2.05	1.32	0.3	0.17				8458
HCHs	0.63	5.52	3.85	10.08	5.29	12.56	3.32	0.31	6.72	0.52		422.9#
Mirex	0	0	0	0	0	0.6	0	0			400	281.9
PCBs	3.86	6.79	17.78	9.29	15.35	14.7	7.45	11.11	23.60	11.85	8,000	28
									Kenai R.			
Arsenic	1.44	0.83	1.42	0.73	1.31	0.90	1.66	1.06	1.20	1.20		
Cadmium	2.21	0.01	3.09	0.01	3.59	0.01	3.83	0.02	0.00	0.00		1.41
Chromium	0	0	0	0	0	0	0	0	0.00	0.00		
Copper	73.5	2.01	329	2.11	365	1.81	1500	1.83		0.82		
Iron	449	17.6	563	15.4	558	13.9	452	12.4				
Lead	0.05	0	0.06	0	0	0	0	0	0.00	0.00		
Manganese	7.2	0.321	5.19	0.37	9.03	0.374	5.61	0.301				
Mercury	0.138	0.104	0.131	0.093	0.194	0.134	0.228	0.098	0.120	0.100	4000	0.14
Nickel	0.07	0.13	0.09	0.06	0.20	0.00	0.19	0.05	0.00	0.00		
Selenium	7.21	1.14	18.6	1.3	26.1	0.99	79.4	0.89	0.88	0.84		7.05
Silver	2.63	0	4.95	0	6.19	0	7.4	0				
Tin	0	0	0	0	0	0	0	0				
Zinc	98.9	13.6	108.0	13.9	128	12.2	173	14.3				

*Hexachlorobenzene

#gamma HCH

Table 6b: Contaminant concentrations in liver and muscle tissue of salmon collected from Port Graham. To put concentration values from this study into context, concentration levels reported by the Alaska DEC Fish Monitoring program are presented. Results were also compared to the FDA action levels and calculated EPA chronic consumption thresholds for fish consumption. (Metals $\mu g.g^{-1}$, Organics $ng.g^{-1}$ dry weight). m = male, f=female.

	Chum f		Chum m		Sockeye f		Sockeye m		AK FMP Sockeye		FDA	EPA
	liver	muscle	liver	muscle	liver	muscle	liver	muscle	Kodiak	Matanuska	fish	fish
Chlordanes	0.35	1.3	0.64	1.13	1.69	3.12	2.81	3.2	11.32	6.88	1,200	704.8
Chlorobenzene	1.78	1.47	2.01	1.6	1.71	1.83	2.98	2.18	5.00	4.00		1127.8*
Chlorpyrifos	0	0	0	0	0	0	0	0				422.9
DDT	0.8	1.46	1.3	2.22	1.79	10.19	3.49	9.02	38.12	19.04	20,000	704.8
Dieldrins	0.42	0.31	1.82	0.33	0.89	0.6	0.47	0.49	1.76	0.88	1,200	70.5
Endosulfan	0	0	0.44	0	0.66	0.38	0	0.26				8458
HCHs	0.38	0.38	1.17	0.24	0.79	0.35	0.74	2.92	6.72	0.52		422.9#
Mirex	0	0	0	0	0	0	0	0			400	281.9
PCBs	3.07	3.98	1.76	4.92	2.35	14.33	1.35	11.97	23.6	11.85	8,000	28
									Kenai R.			
Arsenic	1.28	0.77	1.43	0.91	1.21	0.90	1.23	0.80	1.20	1.20		
Cadmium	2.67	0.02	4.13	0.02	3.10	0.02	5.45	0.02	0.00	0.00		1.41
Chromium	0	0	0	0	0	0	0	0	0.00	0.00		
Copper	151	2.1	423	1.97	415	1.94	1440	1.68		0.82		
Iron	627	16	1130	14.9	649	13.2	518	12.9				
Lead	0	0	0.10	0	0	0	0	0	0.00	0.00		
Manganese	6.93	0.35	5.21	0.39	7.57	0.39	4.28	0.37				
Mercury	0.133	0.121	0.118	0.091	0.231	0.127	0.347	0.141	0.120	0.100	4000	0.14
Nickel	0.08	0.06	0.12	0.06	0.22	0.08	0.33	0.09	0.00	0.00		
Selenium	8.29	1.23	21.10	1.14	27.30	0.92	51.60	0.95	0.88	0.84		7.05
Silver	3.84	0	6.46	0	5.56	0	7.84	0				
Tin	0	0	0	0	0	0	0	0				
Zinc	101.0	12.6	106.0	15.1	125.0	13.2	152.0	18.6				

*Hexachlorobenzene
#gamma HCH

Table 6c: Contaminant concentrations in liver and muscle tissue of salmon collected from Seldovia. To put concentration values from this study into context, concentration levels reported by the Alaska DEC Fish Monitoring program are presented. Results were also compared to the FDA action levels and calculated EPA chronic consumption thresholds for fish consumption. (Metals $\mu g.g^{-1}$, Organics $ng.g^{-1}$ dry weight). m = male, f=female.

	Chum f		Chum m		Sockeye f		Sockeye m		AK FMP Sockeye		FDA	EPA
	liver	muscle	liver	muscle	liver	muscle	liver	muscle	Kodiak	Matanuska	fish	fish
Chlordanes	1.01	1.16	4.46	1.25	2.59	2.33	1.91	1.8	11.32	6.88	1,200	704.8
Chlorobenzene	3.01	1.72	1.94	3.65	1.49	1.96	2.1	1.56	5.00	4.00		1127.8*
Chlorpyrifos	0.79	0	0	0	0	0	3.48	0				422.9
DDT	3.67	2.14	7.24	3.47	15.54	6.99	33.67	8.79	38.12	19.04	20,000	704.8
Dieldrins	0.47	0.26	1.72	0.31	0.45	0.4	0.63	0.4	1.76	0.88	1,200	70.5
Endosulfan	0.61	0.57	0	0.42	2.16	1.1	1.67	0.98				8458
HCHs	5.39	10.58	3.68	15.45	5.84	16.72	6.17	14.35	6.72	0.52		422.9#
Mirex	0	0.18	0	0.24	0	0.52	0	0.46			400	281.9
PCBs	8.15	9.33	9.65	7.76	12.99	18.16	16.05	13.61	23.6	11.85	8,000	28
									Kenai R.			
Arsenic	1.32	0.79	1.38	0.88	1.34	1.29	1.46	1.18	1.20	1.20		
Cadmium	2.40	0.01	4.57	0.03	2.88	0.02	3.87	0.04	0.00	0.00		1.41
Chromium	0	0	0	0	0	0	0	0	0.00	0.00		
Copper	86.3	2.23	404	2	593	2.19	1570	1.86		0.82		
Iron	685	17.7	1060	15	249	12.1	427	13.9				
Lead	0.13	0	0.08	0	0	0	0	0	0.00	0.00		
Manganese	7.21	0.52	6.38	0.67	7.62	0.36	4.33	0.304				
Mercury	0.124	0.108	0.122	0.092	0.221	0.125	0.262	0.114	0.120	0.100	4000	0.14
Nickel	0	0	0.09	0.07	0.15	0.07	0.18	0	0.00	0.00		
Selenium	7.26	1.18	20.70	1.13	32.50	0.93	69.30	0.93	0.88	0.84		7.05
Silver	3.03	0	6.33	0	5.56	0	8.13	0				
Tin	0	0.04	0	0	0	0	0	0				
Zinc	98.3	13.8	119.0	16.2	134.0	12.8	135.0	12.7				

*Hexachlorobenzene

#gamma HCH

4.3 Histopathology characterization in shellfish

Results of prevalences (% affected) and intensity of parasitic infections and pathological/disease in cockles and softshell clams sampled from subsistence harvest grounds in Nanwalek, Port Graham, and Seldovia, Alaska are summarized in Table 7 and presented graphically in Figures 22 and 23.

4.3.1. Parasitic infection

Parasitic copepods infection: Copepod infections were observed in clams and cockles sampled from the harvest grounds of Seldovia (Figure 22). Copepods are aquatic microcrustaceans related to crabs and shrimp. There are two groups of parasitic copepods within the order Cyclopoida which infect bivalves. These are obligate endoparasites which affect the digestive tract of bivalves and ectoparasites, which affect the mantle and gills (Heegaard, 1962; Darwin and Stefanich, 1966). In bivalves such as clams and cockles, parasitic copepods are typically found in the digestive tract but, rarely in the gills (Johnson et al., 2004; Kim et al., 2006). In this study the microcrustaceans were found at about a 40% prevalence (Figure 22) with intensity values of 1 to 2.5 (Figure 23) in the softshell clams and cockles from the Seldovia harvest grounds.

Nematode (roundworms) infection: Nematodes, or roundworms, were detected in clams from the harvest grounds of Seldovia (Figure 22). With over a million species, roundworms are very diverse, with many thousands of species described as pathogenic (Hugot et al., 2001). Roundworms have multiple development stages. According to Cheng (1978), roundworms that infect molluscan shellfish such clams and cockles are mainly in larval stages, while adults can be found in the predators of the mollusks. As illustrated in Figure 22, prevalence values for the occurrence of the roundworms in the softshell clams from this study were low (only 20% prevalence).

Gregarine infection: Also found in the softshell clams from the harvest grounds of Nanwalek and Seldovia were gregarines, which are sporozoan microbes (parasitic protozoans) (Figure 22). The gregarines are generally parasites of arthropods and mollusks. They have several life stages with larval stages frequently found in bivalves while mud and stone crabs were found to be the most common final hosts (Kim et al., 2006). Gregarines are often found in the digestive region of their hosts but may invade the other tissues (Kim et al., 2006). The results indicated that gregarine infections were relatively more intense in clams from Seldovia than those from Nanwalek (Figure 23). However, with prevalence of occurrence of only 20% at both locations, gregarine infections appeared to be relatively low in the clams.

Ciliates, cestodes, trematodes, Rickettsia, xenoma and MSX infections were not observed in any specimen.

4.3.2. Disease conditions

Pathological and disease conditions were detected at various degrees in the softshell clams and cockles, depending on species and location (Figures 22 and 23, and Table 7). Xenoma, which usually results from

enlargement of tissue infected by parasites were not observed, but ceroid deposition, digestive tubule atrophy, inflammation and neoplasms were detected in the shellfish.

Ceroid bodies: Ceroid bodies, a manifestation of cellular disease, are primarily an accumulation of fats (lipofuscinosis) resulting from cellular damage and/ or metabolic unbalance, were detected at about a 40 % prevalence in clams from Port Graham (Figure 22 and Table 7).

Digestive tubule atrophy: The condition of digestive tubule atrophy was the most common condition in the shellfish, occurring at a 100% prevalence with intensity of occurrence reaching 4, particularly in cockles from the Seldovia harvest grounds (Figures 22 and 23, and Table 7).

Tissue inflammation: Cases of tissue inflammation were also observed in the softshell clams from the Seldovia harvest grounds. Tissue inflammation conditions were not detected shellfish from Nanwalek or Port Graham, however, the condition was frequent in softshell clams from Seldovia with a prevalence value of 80% (Figure 22 and Table 7).

Tissue necrosis: Conditions of cell death in living tissue, or tissue necrosis were detected at various degrees in shellfish from virtually all of the three harvest grounds (Figure 23 and Table 7). While tissue necrosis was measured at 20% prevalence in clams from Seldovia and at 60% in clams from Nanwalek and Port Graham, the condition reached 100% prevalence in cockles from Seldovia (Figures 22 and 23, and Table 7).

Tissue neoplasia: Cases of tissue neoplasia were observed in both clam and cockle species collected from the Seldovia harvest grounds (Figures 22 and 23, and Table 7). A neoplasm is an mass of tissue resulting from an abnormal cell proliferation characterized by high nucleus-to-cytoplasm ratios in the cells (Ford et al., 1997). Tissue neoplasms were relatively low from the Seldovia harvest grounds, at about 33% and 20% prevalence in cockles and softshell clams respectively (Figure 22).

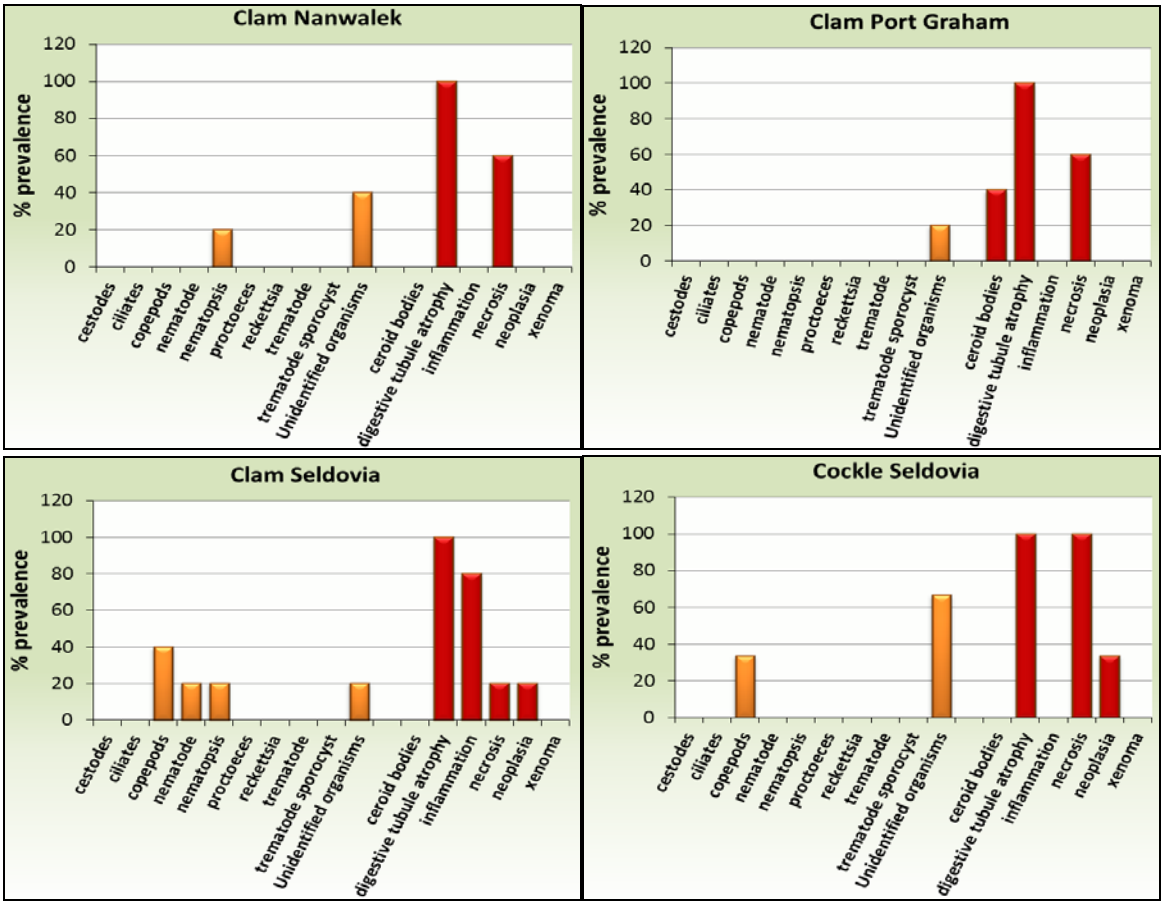


Figure 22. Prevalence (%) of parasite infections and histological lesions in cockles and softshell clams collected from subsistence harvest grounds of the Native villages of Nanwalek, Port Graham and Seldovia.

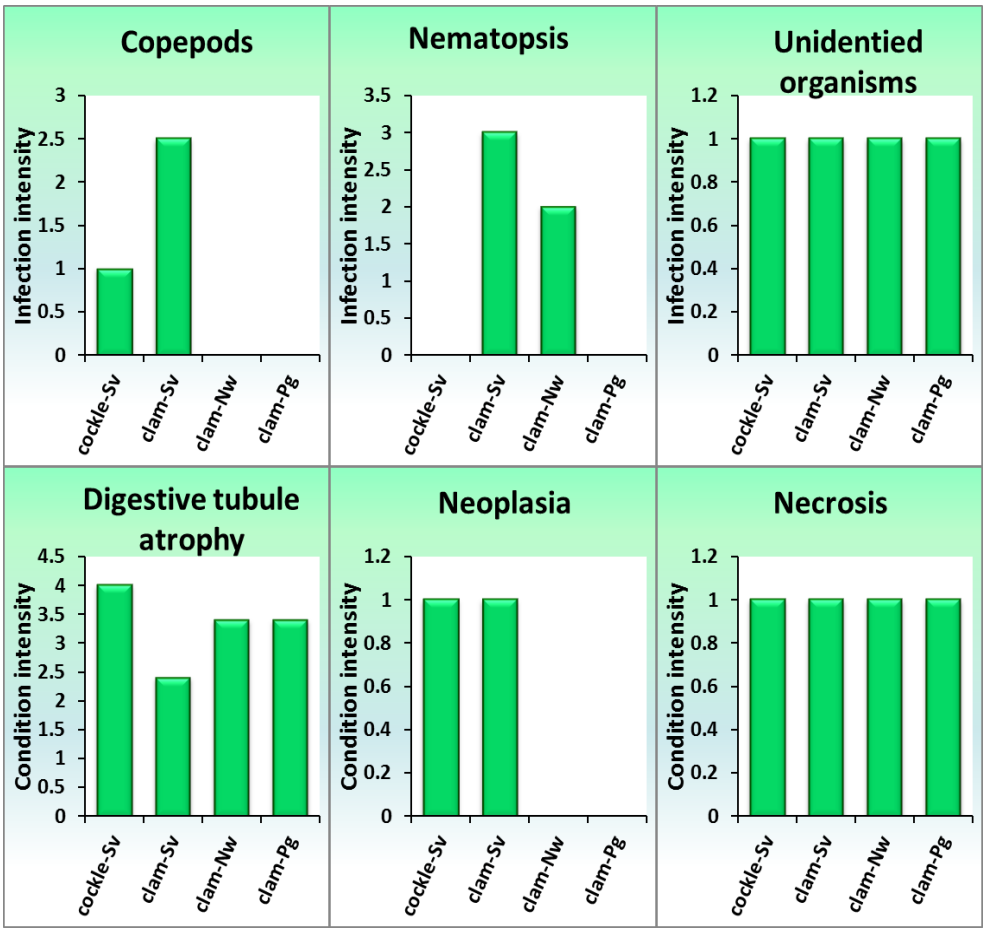


Figure 23. Intensity of parasite infections and histological lesions in cockles and softshell clams collected from subsistence harvest grounds of the Native villages of Nanwalek, Port Graham and Seldovia.

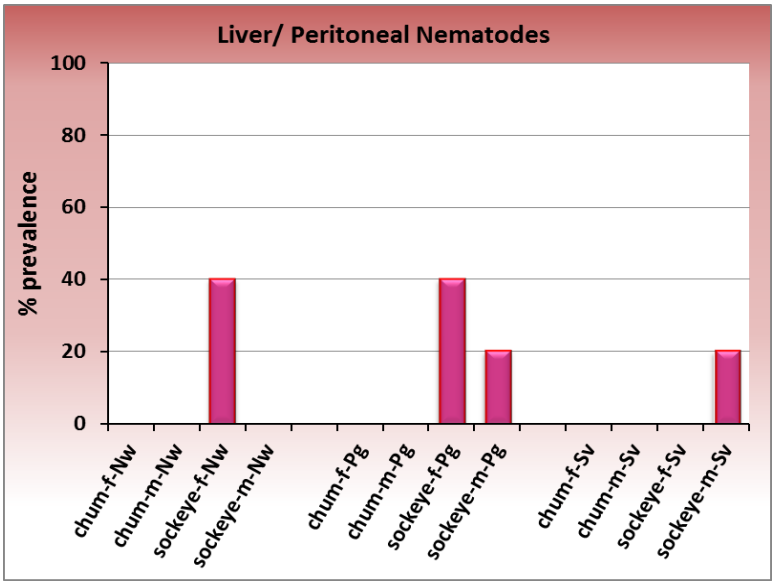
Table 7. Prevalences (% affected) and intensity of histological conditions and parasitic infections in cockles and softshell clams sampled in May 2010 from subsistence harvest grounds in Nanwalek, Port Graham, and Seldovia in Alaska.

	Nanwalek clams		Port Graham clams		Seldovia clams		Seldovia cockles	
Histopathology parameter	**Prevalence (%)**	**Intensity**	**Prevalence (%)**	**Intensity**	**Prevalence (%)**	**Intensity**	**Prevalence (%)**	**Intensity**
cestodes	0	0	0	0	0	0	0	0
ciliates	0	0	0	0	0	0	0	0
copepods	0	0	0	0	40	2.5	33.3	1
nematode	0	0	0	0	20	2	0	0
reckettsia	0	0	0	0	0	0	0	0
trematode	0	0	0	0	0	0	0	0
trematode sporocyst	0	0	0	0	0	0	0	0
proctoeces	0	0	0	0	0	0	0	0
nematopsis	20	2	0	0	20	3	0	0
xenoma	0	0	0	0	0	0	0	0
Unidentified organisms	40	1	20	1	20	1	66.7	1
unidentified foll org	0	0	0	0	0	0	0	0
MSX	0	0	0	0	0	0	0	0
ceroid bodies	0	0	40	20	0	0	0	0
digestive tubule atrophy	100	3.4	100	3.4	100	2.4	100	4
inflammation	0	0	0	0	80	1	0	0
necrosis	60	1	60	1	20	1	100	1
neoplasia	0	0	0	0	20	1	33.3	1
unusual digestive tract	0	0	0	0	0	0	0	0
Replicates (N)	5		5		5		3	
wet weight (g) whole	10.5		17.66		17.02		13.43	
shell length (cm)	5.44		7.32		7.28		5.03	

4.4 Histopathology characterization in fish

4.4.1 Parasitic infection

Nematodes (roundworms) infection: The histopathological examination of adult chum and sockeye salmon captured from traditional harvest grounds of Nanwalek, Port Graham and Seldovia were limited to parasitic infections/infestations of the gills, kidney and liver tissues, and a single noninfectious condition observed in the gills (Figure 24 and Table 8). Tissue inflammations were observed where roundworms infections occurred in the fish liver. These inflammations are a typical chronic host response to nematode infections. In female sockeye salmon, the prevalence of nematode infections ranged from 0% at Seldovia to 40% at Port Graham and Nanwalek. In male sockeye, prevalences ranged from 0% at Nanwalek to 20% at Seldovia and Port Graham. Overall prevalences of the nematode infections in sockeye were low with 10% at Seldovia , 30% at Port Graham, and 20% at Nanwalek. These prevalences were not significantly different by the Fisher's Exact Test ($p < 0.05$). The vast majority of livers in both species were normal; in fact in chum salmon, all livers were histologically normal.

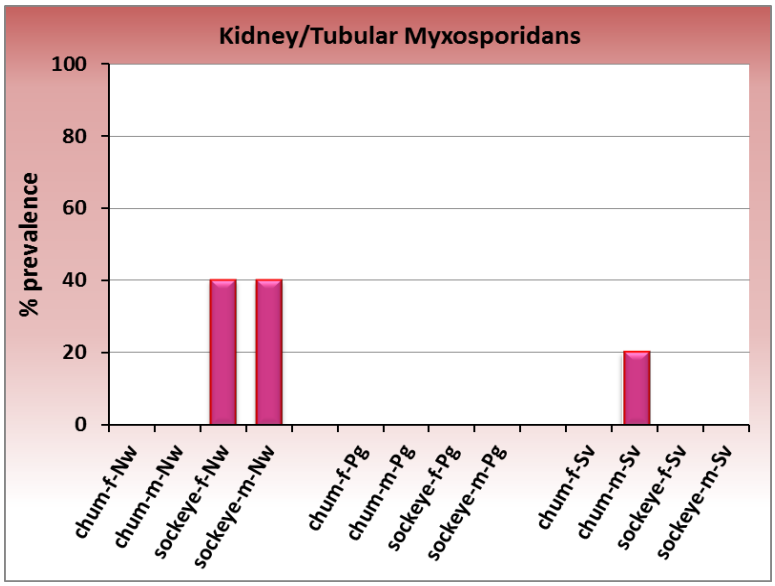


f = female; m = male
Nw = Nanwalek; Pg = Port Graham; Sv = Seldovia

Figure 24. Prevalence of nematodes in liver peritoneal cavities of chum and sockeye salmon collected from subsistence harvest grounds of the Alaska Native villages of Nanwalek, Port Graham and Seldovia.

Myxosporidans (*Myxidium sp*.) infection: In this study, myxosporidan parasites were detected in the chum and sockeye salmon (Figure 25) at various levels in the kidney tissue of the fish. They were present in the tubular epithelium and lumen of the kidney. Prevalence values were 20% in the kidney of male chum salmon from Seldovia and 40% in both female and male sockeye from Nanwalek. The results

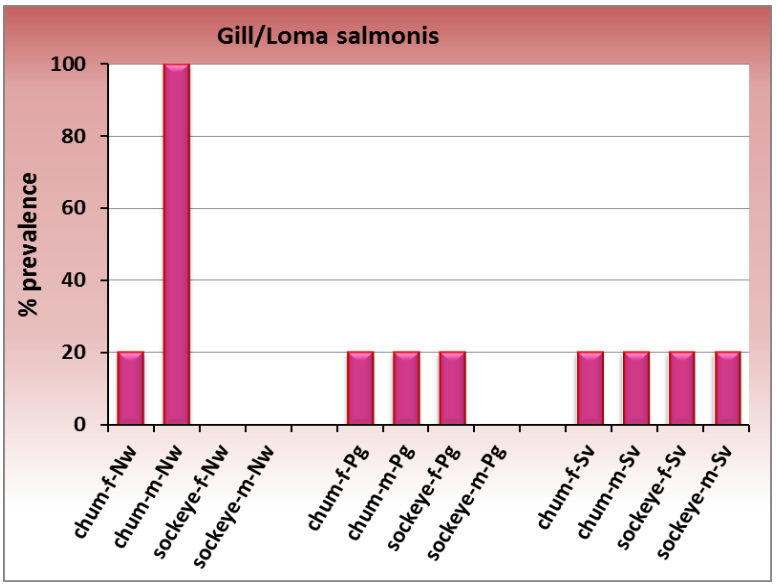
indicated that the myxosporidan infections were minor in severity, and were not associated with any tissue pathology.



f = female; m = male
Nw = Nanwalek; Pg = Port Graham; Sv = Seldovia

Figure 25. Prevalence of myxosporidan parasites in kidney of chum and sockeye salmon collected from subsistence harvest grounds of the Alaska Native villages of Nanwalek, Port Graham and Seldovia.

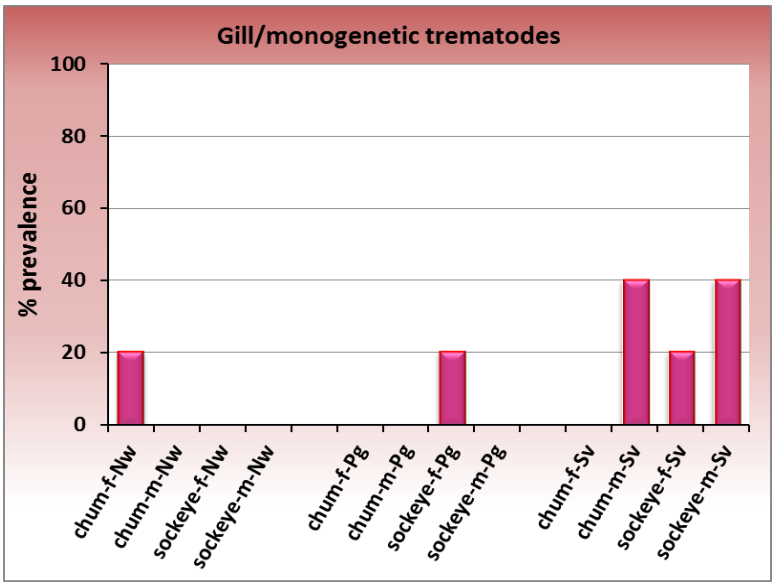
Microsporidan (*Loma salmonis*) infection: Infection by this microsporidan (spore-forming eukaryotic, intracellular parasites), were detected in the gill tissue of fish of both species and sexes from virtually all of the areas sampled, with the exception of Nanwalek sockeye (Figure 26 and Table 8). In male chum salmon, prevalences of the microsporidan infection ranged from 20% at Seldovia and Port Graham to 100% at Nanwalek (N=2 male chum at Nanwalek). In female chum, prevalences at all three sites were 20%. The prevalences of the microsporidan infection in chum were 20% at Seldovia and Port Graham, and 43% at Nanwalek. In male sockeye, prevalences of the infection ranged from 0% at Port Graham and Nanwalek to 20% at Seldovia In female sockeye, prevalences ranged from 0% at Nanwalek to 20% at both Seldovia and Port Graham. Overall prevalences were 0% at Nanwalek, 10% at Port Graham, and 20% at Seldovia Bay. Overall the differences among these prevalences were not statistically significant with Fisher's Exact test ($p < 0.05$).



f = female; m = male
Nw = Nanwalek; Pg = Port Graham; Sv = Seldovia

Figure 26. Prevalence of *Loma salmonis* in gill of chum and sockeye salmon collected from subsistence harvest grounds of the Alaska Native villages of Nanwalek, Port Graham and Seldovia.

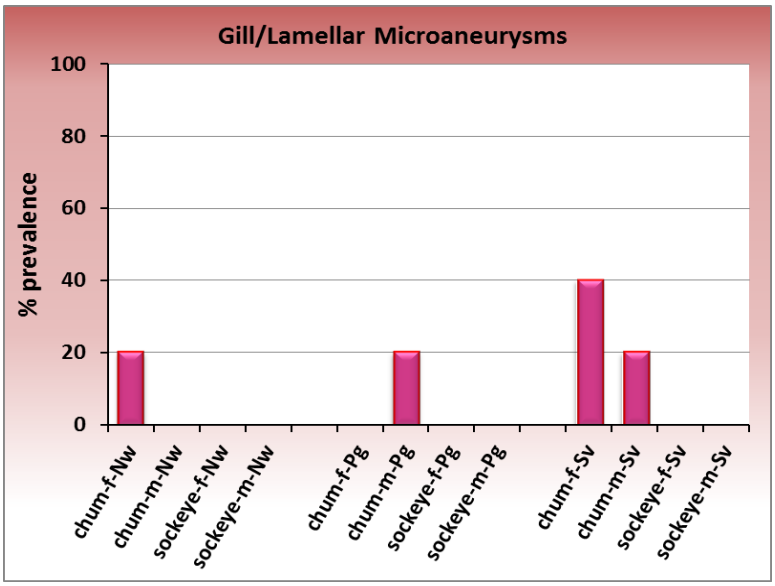
Trematodes (flukes) infection: External infestations of the gill by monogenetic trematode parasites, probably of the family Gyrodactylidae, were observed in both species of salmon (Figure 27 and Table 8). In male chum, prevalences ranged from 0% at Port Graham and Nanwalek to 40% at Seldovia Bay. In female chum, prevalences ranged from 0% at Seldovia and Port Graham to 20% at Nanwalek. Overall prevalences of gill monogenetic trematodes in chum were 20% at Seldovia, 0% at Port Graham, and 14% at Nanwalek. In male sockeye, prevalences ranged from 0% at Port Graham and Nanwalek to 40% at Seldovia. In female sockeye, prevalences ranged from 0% at Port Graham and Nanwalek to 20% at Seldovia. Overall prevalences of this parasitic infestation in sockeye were 30% at Seldovia, 10% at Port Graham, and 0% at Nanwalek. These prevalences were not significantly different from one another with Fisher's Exact test ($p > 0.05$).



f = female; m = male
Nw = Nanwalek; Pg = Port Graham; Sv = Seldovia

Figure 27. Prevalence of trematodes in gill of chum and sockeye salmon collected from subsistence harvest grounds of the Alaska Native villages of Nanwalek, Port Graham and Seldovia.

Gill microaneurym lesions: Microaneurysms were only observed in the lamellae of gills of chum salmon (Figure 28 and Table 8). Microaneurysms are localized distensions or outpocketings of the capillaries in the lamellae of the gills. Microaneuryms in the gill lamellae were detected at low overall prevalences among the sites, but only at a minor degree of severity. Overall prevalences ranged from 10% at Port Graham, 14% at Nanwalek, to 30% at Seldovia. These prevalences were not significantly different from one another with Fisher's Exact test ($p < 0.05$).



f = female; m = male
Nw = Nanwalek; Pg = Port Graham; Sv = Seldovia

Figure 28. Prevalence of microaneurysms in gill of chum and sockeye salmon collected from subsistence harvest grounds of the Alaska Native villages of Nanwalek, Port Graham and Seldovia.

Musculature lesions

The photographic image in Figure 29 illustrates a gross lesion in the musculature of a single male sockeye from Nanwalek. The lesion, which exhibited significant hemorrhage in the musculature of both posterior lateral flanks, was detected at gross necropsy. The histological appearance of this lesion did not show any evidence of bacterial or other infections or any systemic disease, and there was no significant inflammation, and it was concluded that this lesion was the result of capture-related trauma.

Figure 29. An unusual muscular lesion in a sockeye salmon.

Table 8. Prevalences (% affected) of histological conditions and parasitic infections in adult chum and sockeye salmon (female, f and male m) sampled in July 2010 from subsistence fisheries in Nanwalek, Port Graham, and Seldovia in Alaska.

	Nanwalek				Port Graham				Seldovia			
organ lesion/parasites	**Chum**		**sockeye**		**Chum**		**sockeye**		**Chum**		**sockeye**	
Sex	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**
Liver, Normal	100	100	60	100	100	100	60	80	100	100	100	80
Liver, Peritoneal Nematodes	0	0	40	0	0	0	40	20	0	0	0	20
Kidney, Normal	100	100	40	60	100	100	100	100	100	80	100	100
Kidney, Tubular Myxosporidan	0	0	40	40	0	0	0	0	0	20	0	0
Gill, Normal	60	0	100	100	80	60	60	100	60	20	60	40
Gill, Loma salmonis (microsporidan)	20	100	0	0	20	20	20	0	20	20	20	20
Gill, monogenetic trematodes (external)	20	0	0	0	0	0	20	0	0	40	20	40
Gill, Lamellar Microaneurysms	20	0	0	0	0	20	0	0	40	20	0	0
Replicates (N)	5	2	5	5	5	5	5	5	5	5	5	5
Average fish length (cm)	60.7	64	56.8	55.3	61.5	62.5	55.3	59.7	60.7	60.8	51.9	54.3
Weight (g) whole fish	2946	3420	2132	2212	3140	3202	2165	2632	2950	2650	1700	1924

5. DISCUSSION

5.1. *Contaminant body burden*

Trace and major elements are present everywhere because they are naturally occurring elements derived from surface soil and rock. Elevated concentrations may be the result of natural weathering of mineral-rich source rock, volcanic eruptions, or anthropogenic sources such as industrial activity or mining. Many metals are essential micronutrients at low levels, even some metals that are considered to be toxic at higher exposures. Organisms will absorb metallic elements and attain equilibrium concentrations in their tissues in proportion to their exposure and their depuration processes. Exposure may be by direct contact with environmental media such as sediment or water, ingestion of sediment, or via the food chain. The metals concentrations on the Kenai peninsula are reflective of metals eroded from rocks that have been subjected to tectonic uplift and folding of crustal rock, volcanic intrusions, and deposition of atmospheric fallout both from volcanic eruptions and long range atmospheric transport. It is a highly variable environment in spatial terms, and local conditions may vary even between adjacent embayment. Thus the local geology influences the metals exposure of resident organisms in different locations, and therefore their species-specific equilibrium tissue body burdens. Variation in local sediment contaminant concentrations have been documented in Port Graham for example (Hartwell et al., 2009). In a cove near the village, sediment chromium and mercury concentrations were 50.9 and 0.353 $ug.g^{-1}$, respectively. In the adjacent cove at the head of the bay only 3 km away, the concentrations were 334 and 0.143 $ug.g^{-1}$ respectively.

The iron and zinc concentrations in clams from Seldovia are twice as high as in Port Graham, which in turn are two to three times as high as neighboring Nanwalek (Table 5). Iron and zinc are essentially non-toxic to humans so there is no issue with consumption, but it illustrates the importance of local geology. The pattern of elevated metals at Seldovia relative to the other locations is seen with virtually all of the trace metals. Port Graham concentrations exceed those of Nanwalek in half of the elements. Given the range of tissue concentrations in studies at wider spatial scales (NOAA NS&T and AK DEC FMP), the values on the Kenai are not greatly different from regional observations. The important point is that for those elements warranting FDA action levels (arsenic, cadmium, chromium, lead, nickel), the observed tissue concentrations are far below FDA thresholds (Table 5), some by more than an order of magnitude.

Chromium and coal mining activity has occurred historically on the Kenai Peninsula and the Kachemak Bay region. Chromite mines were located at Red Mountain in the interior of the peninsula, and at Chrome Bay at the lower tip of the Kenai. Ore from Red Mountain was transported to Kasitsna Bay where it was loaded onto transport vessels for shipping. Coal mining has been carried out at various times throughout Kachemak Bay. Coal mining typically exposes sulfide-bearing rock and ground water. Upon exposure to the atmosphere, the sulfides are oxidized into sulfuric acid, releasing bound metals, and the acid leaches more metals from the rock and soil through which it flows. Upon entry into marine water, the acid is buffered, many of the metals are chelated by salts or adsorbed by particulates in the water, and free sulfates bind many of the others, all of which will accumulate in local sediment deposits. These sediments may accumulate and become a source of heavy metal contamination in resident

organisms, may be dispersed by tidal currents to settle elsewhere, or be diluted to insignificant concentrations. The data presented in this report does not indicate any accumulation of chromium, or other metal-laden sediments in any of the three study areas, based on the observed tissue concentrations in the mollusks. Concentrations are within the ranges seen in other bivalves in Kachemak Bay, and the lower Cook Inlet in other monitoring data sets.

Mobile animals (such as salmon) which spend significant periods of their lives in other habitats will reflect the chemical makeup of those other habitats, influenced by their exposure to local conditions only as they move through them. It is well documented that Pacific salmon stop feeding on their spawning migration from the open ocean into coastal estuaries and rivers. None of the fish that were examined in this study had anything in their stomachs. Thus, their body burdens are primarily a result of their exposure in the open ocean from their diet, injestion of sea water, and ion exchange across their gills. Within the range of overall variation in muscle tissue, there is very little difference in individual metal concentrations between locations or species. Muscle tissue levels are below calculated chronic no adverse effects levels for cadmium and selenium. Mercury levels are on the same order of magnitude, but still below the EPA chronic level. The reference dose for mercury is an order of magnitude below the other metals. Again, the standard is for methylmercury, and the measured value is total mercury, potentially providing an additional degree of safety relative to the standard. There is a large difference between muscle and liver tissue. Liver concentrations are considerably higher than muscle in all cases except arsenic and mercury. Unlike mammals, fish do not have the metabolic enzymes to regulate many metals levels. Consequently they accumulate in the liver and cannot be expelled. There are no consumption standards for liver tissue, and the liver constitutes a much smaller proportion of the fishs' mass compared to the muscle. Mercury does not tend to accumulate in the liver. Most mercury in tissues is converted into methylmercury and remains in the tissues.

All of the organic contaminants assessed in this study are synthetic chlorinated compounds, except the PAHs. They are of interest due to their persistence, toxicity and tendency to bioaccumulate. PAHs may originate from natural seeps or human activities, while most of the chlorinated compounds are synthetic chemicals banned or severely restricted in the U.S. With a few exceptions, the pesticides have likely never been used in the vicinity of the study area. Their presence indicates contamination from outside the region. The PCBs were used in a variety of industrial applications and may have local sources from previous uses and old machinery. The original mixtures, called Aroclors, contained specific mixtures of PCB congeners. Some Aroclors contained congener mixes that were more heavily chlorinated than others. Each Aroclor mixture had different uses. The Aroclors were identified by measuring a subset of specific peaks in their chromatogram. Mixtures of PCBs released to the environment will proceed through several transformations. The individual congeners will fractionate into different media. Each has a different vapor pressure, solubility coefficient, octanol/water partition coefficient, etc. Thus, they will accumulate more or less strongly at different sediment depths, in sediments with differing organic content, in different grain size environments, and so on. In addition, they are slowly degraded into compounds with lower chlorination levels. Thus, measuring the specific peaks for a given Aroclor in an

environmental sample that has undergone transformation and fractionation does not reflect the true content of the PCB mixture. Only by measuring individual congeners can an analyst determine what is actually in the sample. This is why fish consumption reference doses based on analysis of Aroclors are not entirely reliable for environmental samples.

Some compounds degrade into distinct by-products that can aid in assessing the relative proximity to sources. For example DDT breaks down into DDE and DDD. The higher the relative proportion of DDT in the mixture the fresher the source. DDT is still used in Asia for example, which is up wind from Alaska. DDT was frequently below detection limits in our samples, and DDE and DDD were found at higher concentrations than DDT, indicating old sources. Tri-butyl tin, formerly used in anti-fouling paint, also goes through a degradation sequence of decreasing butyl content, which can be used to infer age of release. None of the concentrations were above detection limits in the mollusks.

PAHs are derived from natural and man-made sources. Natural sources include coal, decaying vegetation, and natural oil seeps. Anthropogenic sources are spilled fuel and oil, and burning organic material including fuel, wood or plastics. All of these substances are transported long distances by the atmosphere and on ocean currents. Different classes of organisms have differing ability to metabolize PAHs. Vertebrates can metabolize or conjugate them and excrete them. It is the metabolism of certain compounds (e.g. benzo[a]pyrene) that generates carcinogenic by-products which renders them dangerous to health. Bivalve mollusks cannot effectively metabolize PAHs, which makes them good indicators of local PAH contamination.

Usually, high proportions of low molecular weight PAHs are associated with oil and petroleum releases (petrogenic source). A high proportion of high weight PAHs is often linked to combustion by-products and/or long-term weathering. With the exception of naphthalene, the low weight PAHs were found at concentrations comparable to the higher weight fractions (Figure 30). The patterns of higher concentrations of parent compound PAHs (Dibenzofuran, Phenanthrene, Pyrene/Fluoranthene, Chrysene) with lower concentrations of alkyl-substituted analogs is typical of burned fuel residue. The fact that the substituted naphthalenes are found at comparable concentrations to the higher weight PAHs indicates a mixture of sources from spilled fuel and atmospheric drift of exhaust fumes from diffuse sources. Naphthalene itself is derived from a variety of sources, chiefly combustion and off-gassing from natural hydrocarbon sources and fuel, but is a commonly used chemical and is also emitted from a variety of substances from building materials, to tobacco smoke. It is the primary ingredient in moth balls. One of the largest components of the suite of PAHs was perylene. This is a harmless natural by-product of the breakdown of terrestrial plant material (NRC 1985). This indicates that naturally occurring PAHs are as large or larger component to the body burden of the clams as anthropogenic sources. Considering that, and the overall very low concentrations, the contribution of anthropogenic PAHs in the harvest areas appears to be extremely limited. In other shellfish, such as blue mussels from remote locations in the Gulf of Alaska and the Shelikof Strait, similar patterns of elevated concentrations of parent PAHs relative to the concentrations of their alkyl-substituted analogs were observed (Figures 31 and 32). These samples were collected by the National Park Service (NPS) as part of their coastal water monitoring program (unpublished NPS data). The concentrations of the higher molecular weight

PAHs were lower in the NPS data relative to the Kachemak Bay data because Shelikof Strait and the eastern shore of the Kenai Peninsula are subjected to strong currents and are flushed with open water from the Gulf of Alaska Current.

The levels of organic contaminants in the fish tissues were far below FDA action levels and EPA chronic no-effect concentrations. There are only a few FDA action levels for organic compounds in clam tissue. Concentrations in the clams were less than concentrations in the fish muscle and liver. The fish had much higher concentrations of DDT and PCBs, most likely as a consequence of their much higher trophic position in the food chain. The chemistry data suggests there are interspecies and intersite differences. The levels of DDT and PCBs in liver are higher in the Nanwalek and Seldovia fish, relative to fish of the same species from Port Graham. This is not seen in the muscle tissue. There is no obvious reason for this and may simply be a consequence of small sample sizes and low absolute concentrations. Also, muscle tissue levels in the sockeye salmon were consistently higher than in the chum salmon. This is likely due to different feeding habits of the two species (Davis et al., 2005).

Figure 30. Individual PAH concentrations in clams collected from subsistence harvest grounds of the Alaska Native villages of Nanwalek, Port Graham and Seldovia. The PAHs are arranged from low molecular weight compounds (left) to high molecular compounds (right).

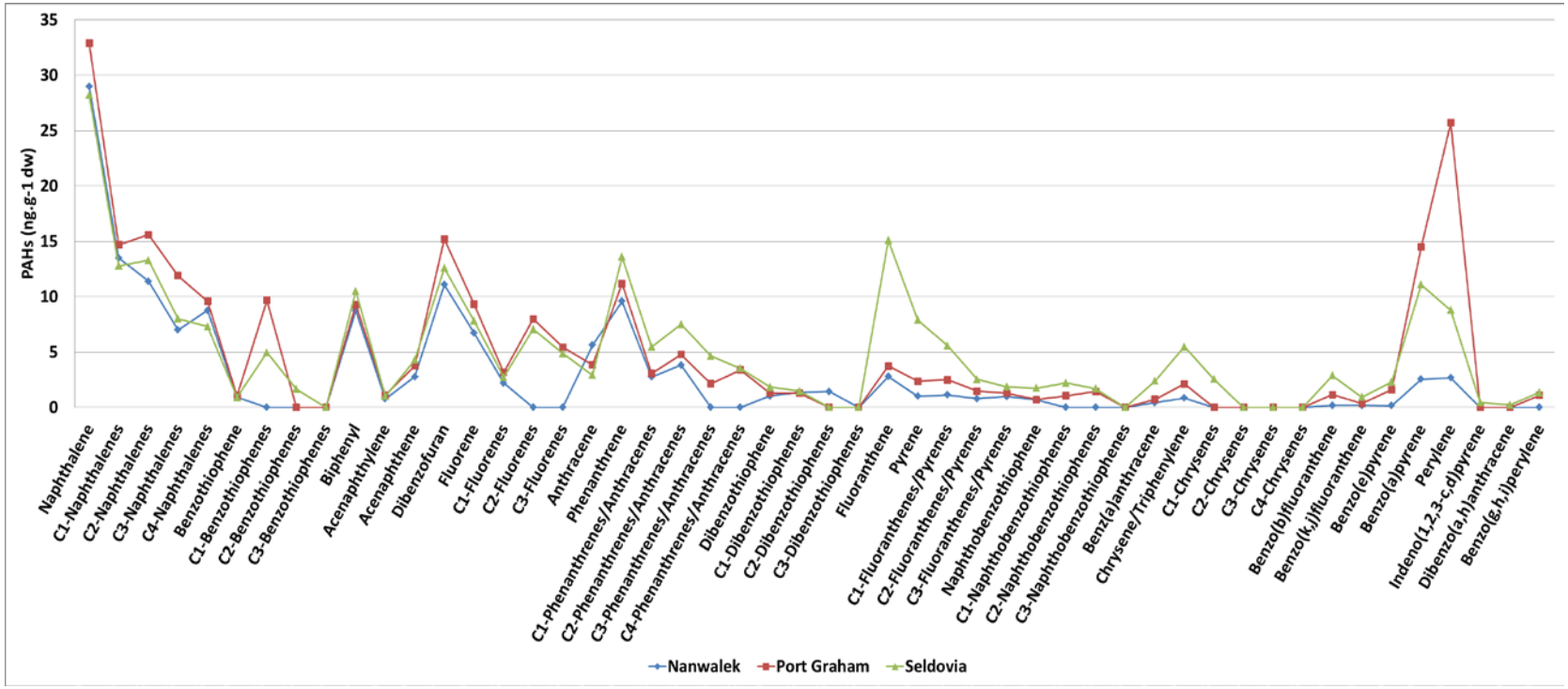


Figure 31. Individual PAH concentrations in blue mussels collected in 2007 from NPS, Southeast Network monitoring locations along the eastern Kenai Peninsula fjords. The PAHs are arranged from low molecular weight compounds (left) to high molecular compounds (right)

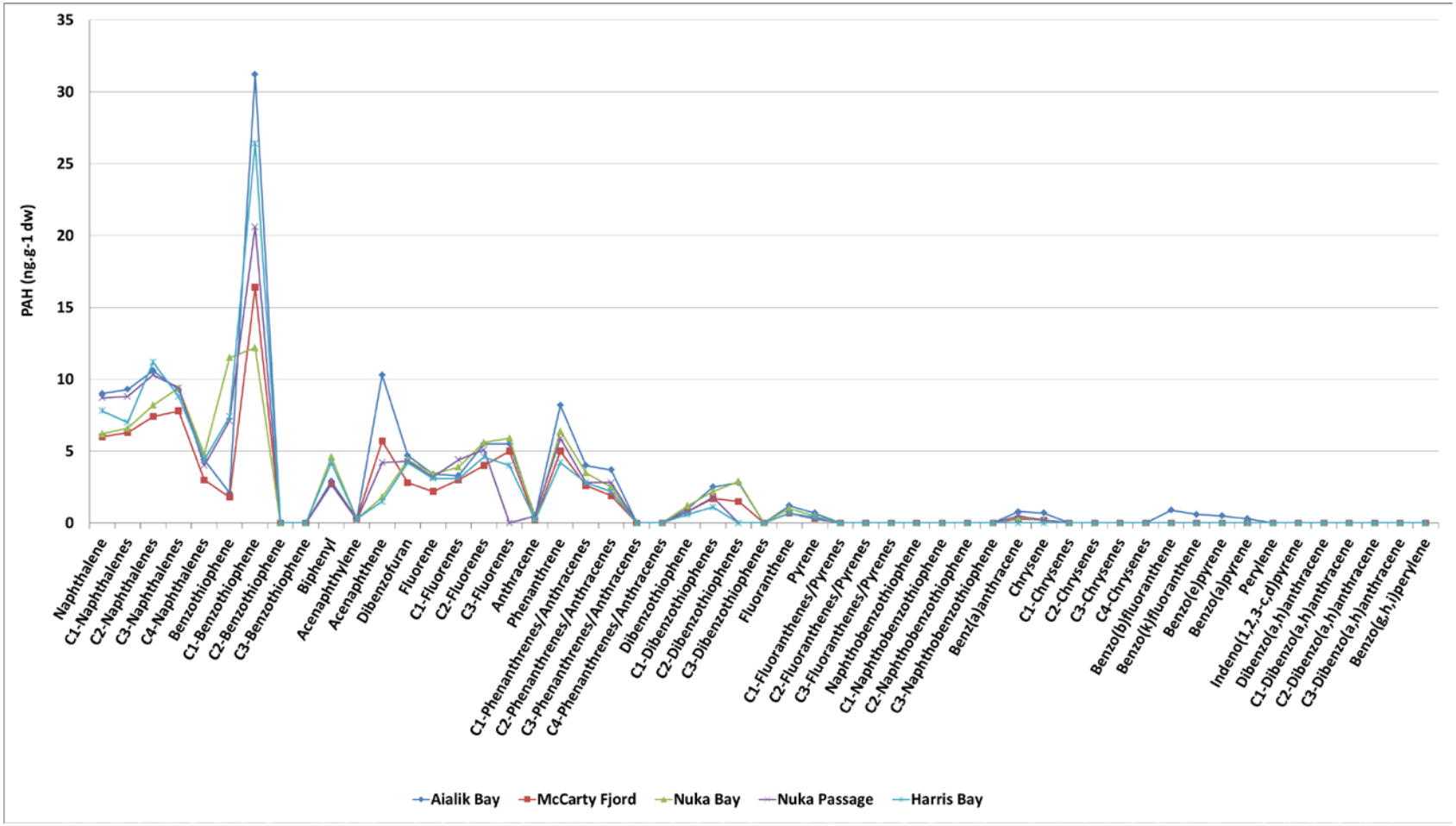
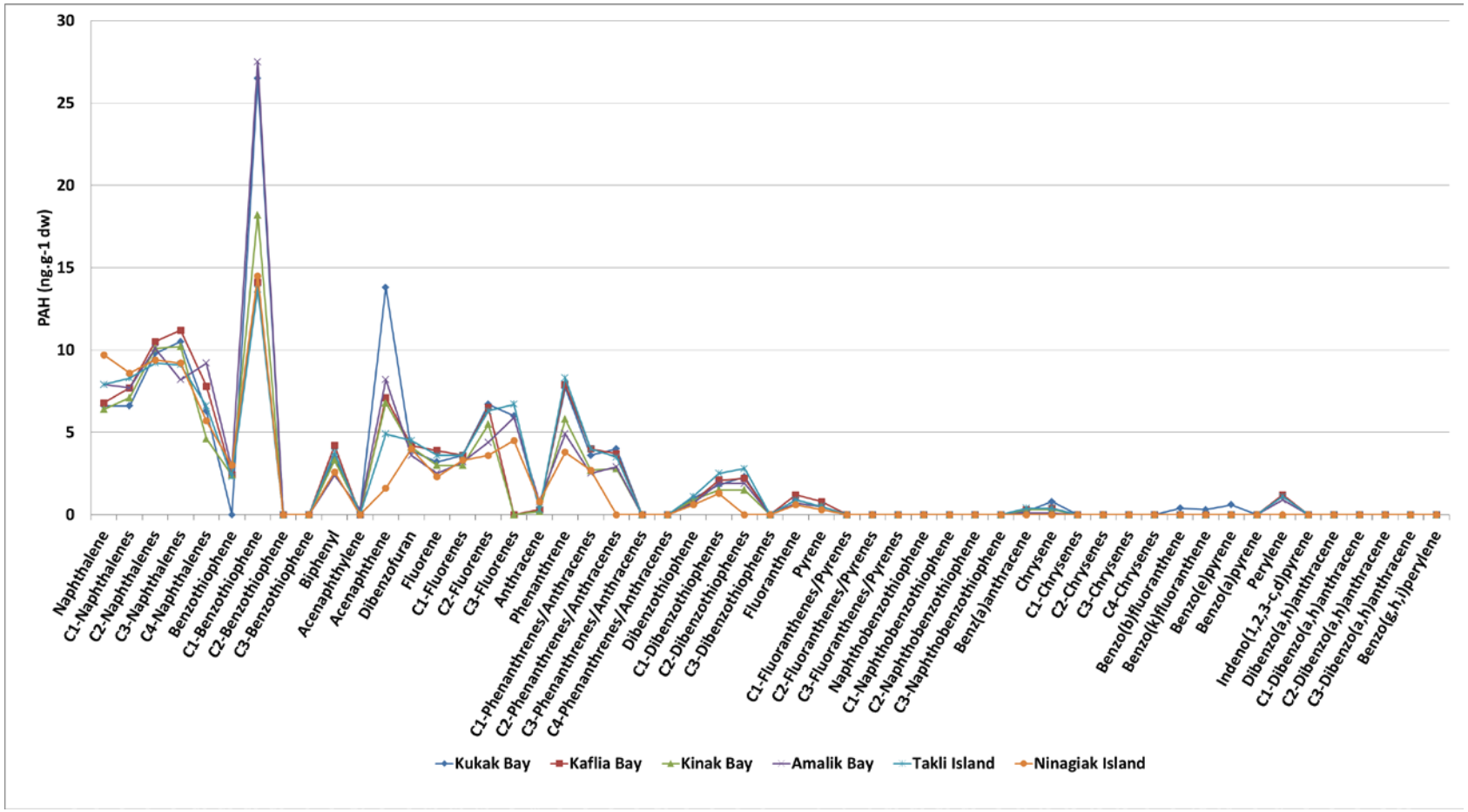


Figure 32. Individual PAH concentrations in blue mussels collected in 2007 from NPS, Southeast Network monitoring locations along the western shoreline of the Shelikof Strait. The PAHs are arranged from low molecular weight compounds (left) to high molecular compounds (right).



5.2. **Histopathology**

Every organism living today has some level of parasitic infection. In normal healthy organisms, this is of little consequence unless the parasites grow or proliferate to levels capable of producing significant pathology or disease (pathogenic) in the host. Among the parasites analyzed in the shellfish, only microcrustacean copepods, gregarines (nematopsis) and roundworms (nematodes) were detected. Although there are no human health concerns with the occurrence of parasitic copepods in shellfish, many of these parasitic microcrustaceans can have potential for ecological effect such as the growth, fecundity, and survival of their hosts (Johnson et al., 2004). Roundworm infections in mollusks can cause destruction of adjacent tissues, and in some cases, cellular responses in the host shellfish include infiltration of hemocytes around the area where the worm is located (Kim et al. 2006). Additionally, many roundworms infect humans with the most common ones include hookworms (ascaris) and trichina worm, which can cause trichinosis if raw or undercooked meat is ingested. Thus, roundworms infections of the shellfish could be harmful to the shellfish, but also to human consuming these resources. Gregarines were the other parasites detected in the shellfish. Heavy infections of gregarines has been suggested to have some harmful effects on the physiology of infested shellfish (Sindermann, 1990) however, Cheng (1967) concluded that, in general gregarine infections have low pathogenicity in bivalve mollusks. Although some of the parasites detected in the shellfish can cause ecological health and human health effects, the measurements indicated that the infections of virtually all parasites were relatively minor and may not have any significant health impacts on the shellfish or people.

The presence of diseases and other histopathologic conditions in the shellfish analyzed were limited to cellular metabolic disease (ceroid), abnormal tissue inflammations, tissue necrosis, digestive tubule atrophy and tissue neoplasms (Table 8). The conditions of digestive tubule atrophy detected in the shellfish are generally characterized by the thinning of the digestive tubule walls and the conditions have been linked to a variety of stressors including exposure to contaminants and poor nutrition (Kim et al., 2006). Although digestive tubule atrophy is not necessarily a pathologic condition, it can impact food uptake and potentially growth (Kim et al., 2006). Tissue inflammations, which were observed in some of the shellfish, are usually the result of intense infiltration of hemocytes (phagocytic cells in hemolymph of the shellfish). Tissue inflammation in shellfish can occur as diffuse or focal inflammation. Diffuse inflammation is differentiated from focal inflammation when the affected area does not appear to have a clear center or focal point of highest hemolytic concentration and hemocytes are abundant and distributed broadly over a large section of tissue (Kim et al., 2006). Although tissue inflammation was not detected in shellfish from Nanwalek and Port Graham, the condition was very prominent in softshell clams from Seldovia (Figure 22). Although necrosis was seen at all locations, measurement of intensity indicated that occurrence of the condition was not pronounced (figure 23). In bivalve mollusks, most cases of tissue necrosis were observed in the visceral connective tissue and is sometimes associated with the presence of parasites (Kim et al., 2006). Cases of tissue neoplasm conditions were observed in both clams and cockles collected from the Seldovia harvest ground (Figures 22 and 23). In bivalve mollusks like clams and cockles, neoplastic sarcomas usually occur in vesicular connective tissues and could be harmful to the overall health of the bivalves.

The gender difference of the roundworm infections in fish did not appear to be universal since, in Seldovia, the parasites were detected in liver tissue of male sockeye, but not in liver tissue of female sockeye (Figure 24). Additionally, the Fisher's Exact Test results indicated that prevalence values were not significantly different between the genders of the fish ($p < 0.05$). Roundworm infections at high intensity in fish can impact fish reproduction, although in the majority of cases only larval stages are present in marine fish (Cheng, 1978). The final hosts for the parasitic roundworms are fish-eating birds or mammals including humans. Certain roundworm taxa found in fish can infect humans and sometimes cause damage to stomach and intestinal tissue in humans (Darwin and Stefanich, 1966). In this study, the vast majority of liver tissues in both species were normal; in fact in chum salmon, all livers were histologically normal. Thus the presence of the parasitic roundworms in the fish was not likely to be pathogenic.

Myxosporea, a class of microscopic, protozoan parasites were also detected in the fish tissue (Figure 25). Myxosporidans are characterized by the presence of complex spores and having infective amoeboid sporoplasm (Noble, 1944). Although they are primarily parasites of fish, some species of myxosporidians also infect amphibians and reptiles (Noble, 1944). According to Jirk et al., (2006), most Myxidium species are coelozoic parasites, which usually infect the gallbladder, urinary bladder, or urinary tubules in the kidneys of fish hosts. The myxosporidans, detected in the kidney tubules of the adult chum and sockeye salmon were likely to be of the Myxidium genus. Overall prevalences of myxosporidan infection in the kidney tubules of salmon showed variable infection frequencies (Figure 25), with sockeye salmon from Nanwalek being significantly more infested than either the Port Graham or Seldovia harvest grounds (Fisher's Exact Test, $p < 0.05$). Myxidium infection can be debilitating or even deadly to the fish (Alvarez-Pellitero and Sitja-Bobadilla, 1993). However, in this study all infections were minor in severity and did not involve significant pathological change to the affected tubules or nephrons. Consequently, the higher prevalence of this condition at Nanwalek is unlikely to have significant physiological impacts on the affected fish.

The spore-forming microsporidan parasitic infections (*Loma salmonis)* can be pathogenic. They commonly infect fish gills and can cause serious xenomas (massively hypertrophic lesions in infected cells) in salmon species because they undergo intracellular division (sporogony) in host cells (Higgins et al., 1998). In this study, the *Loma salmonis* infections were found at relatively low prevalences in salmon of both species and sexes collected from Port Graham and Nanwalek's traditional harvest grounds (Figure 26). No infected sockeye were found at Nanwalek. Prevalence of the *Loma salmonis* infection was higher in male than female chum from Nanwalek, but this 100% prevalence was in a sample size of only two male fish. However, the statistical assessment indicated no significance difference in prevalences of infection among fish from different harvest grounds (Fisher's Exact Test, $p < 0.05$). Although infection in fish by *Loma salmonis* can be severely pathogenic, (Higgins et al., 1998), in

the present study, infections were generally low (Figure 26), and the infections represented by the typically small xenomas were all minor in severity and nonpathogenic.

The monogenetic trematodes found in salmon from this study are likely of the family of Gyrodactylidae. Trematodes mainly infect mollusks like clams and cockles as a first host, but intermediate and final hosts may include animal ranging from invertebrates to mammal and humans (Kumar, 1999). There are two types of parasitic trematodes; digenetic trematodes are endoparasites of mammals and humans, while monogenetic trematodes are ectoparasites in fish, mollusks and reptiles (Kumar, 1999; Darwin and Stefanich, 1966). Trematodes detected in the chum and sockeye salmon analyzed in this study were low in prevalence at nearly all three traditional harvest grounds (Figure 27). The infections that were present did not appear to induce any significant host response or result in any significant lesions (nonpathogenic).

Microaneurysms are small saccular distentions or swellings usually found in vascular tubules, such as the capillaries of the gill lamellae as reported in this study. These lesions are quite commonly observed in histological preparations of fish gills sampled from the wild (Landolt and Busch, 1991). In fish, microaneurysm lesions are frequently associated with a variety of infectious diseases (Landolt and Busch, 1991). In this study, the microaneurym lesions in the gill lamellae were observed only in chum salmon (Figure 28). The lesions were minor in severity and were probably an artefactual result of sampling trauma such as capture by gillnet.

A gross lesion in the muscular tissue, characterized by bilateral hemorrhage in left and right lateral posterior flanks of the musculature near the posterior extent of the peritoneum (Figure 29) was observed in a single male sockeye salmon from Nanwalek. However, careful histopathologic assessment indicated that there was no significant inflammation associated with this lesion ,and there was no evidence of bacterial or other infections or any systemic disease. It was concluded that the lesion was most probably caused by gill net capture and possibly trauma resulting from extraction from the gill net.

Histopathological conditions were characterized in the softshell clams and cockles, as well as in chum and sockeye salmon used for subsistence food in the Chugach communities of Nanwalek, Port Graham and Seldovia. Among the histologic parameters measured, only parasitic infections/infestations were detected with consistency in both the fish and shellfish specimens. Occurrences of noninfectious histologic conditions or diseases were limited and found mainly in the two shellfish species. In general, parasitic infections in the fish and shellfish were relatively few in type and minor in severity, resulting in a very low parasitic impact. Many parasitic infections are often associated with actual disease (pathogenic); however, in this study parasitic taxon richness and intensity/severity of infection were low and not adequate for assessing the parasite-disease linkages. The results indicated that parasitic infections and the rare noninfectious histologic conditions in the subsistence salmon and shellfish species were nonpathogenic, and no toxicopathic lesions (those likely to possess an etiology related to

toxic chemical exposure) were detected in salmon. We conclude that none of the infections or noninfectious histologic conditions constitute a health hazard for the fish or shellfish analyzed, or to humans.

6. CONCLUSION AND RECOMMENDATIONS

Across the three sampling sites, the fish and shellfish sampled showed low tissue contamination. Pathological effects in shellfish and fish tissues for the parasites and diseases measured were absent or minimal. Taken together, our results showed that they were healthy and non-contaminated. These findings do not preclude the possibility of other factors synergistically impacting these coastal resources in the region. The mere presence of the synthetic contaminants at detectable levels in the tissues suggested some minimal exposure from remote sources.

Contaminant body data and information about histopathology characterization in coastal and marine biota are important for resource managers. Chemistry and histopathology data from this study represent useful information for concerned native community members and coastal resource managers in Alaska. The data from this study were georeferenced and incorporated into the NS&T data portal and are available to the public.

Fish and shellfish have high nutritional value as they are excellent sources of essential protein, antioxidants, fatty acids (lipid), and vitamins. Of a particular importance for human health are omega-3 fatty acids, which provide many health benefits including protection from diabetes and cardiovascular disease. Omega-3 lipids also help improve maternal nutrition and neonatal/infant brain development. With low contamination and presence of few to no toxicopathic lesions (especially in salmon), this assessment indicated that the clams, cockle and salmon from the traditional harvest grounds in Nanwalek, Port Graham and Seldovia are safe for consumption by Native communities. However, we recommend the following:

- *Because fish and shellfish harbor potentially harmful pathogenic parasites, it is a good practice to always freeze the harvest unless it is to be preccessed or cooked promptly.*
- *To avoid the possibility of migration of intestinal worms into the edible parts, thoroughly clean the fish and shellfish as soon after catching as possible.*
- *During cleaning and processing of fish in particular, if lesions or ectoparasites are observed, it is recommended to always remove the entire organ where the parasites were found (Darwing and Stefanich, 1966). Most parasitic worms die when heated. It is recommended to refrain from consuming raw seafood of any kind.*

7. OUTREACH

Tribal community involvement

The Chugach Regional Resource Commission (CRRC) coordinated with local leaders in the villages of Port Graham, Nanwalek and Seldovia in organizing community members for field work. The project provided community-based organizations such as CRRC and individual tribe members with the chance to gain experience in conducting research projects and to address their research needs. Village 'Elders" and students were involved and learn how to conduct sampling for environmental studies.

Presentations

-2011 Alaska marine science symposium

-2011 Town hall meetings with village council in Port Graham, Nanwalek and Seldovia.

-2011 Presentation at K-12 school in Port Graham and Nanwalek

-2011 Kachemak Bay National Estuarine Research Reserve, City of Seldovia

-2012 Alaska marine science symposium

-2012 Florida A&M University, NOAA Science Forum

The town hall meetings provided the opportunity to village Elders to offer inputs and comments about the outcome of the project. In addition to presenting the results of the study in K-12 schools, a simplified version of the report is being developed as pamphlets handouts that will be distributed in school within the villages.

Web Pages

Internet publication project page: http://coastalscience.noaa.gov/projects/detail?key=138

Internet publication data portal: http://egisws02.nos.noaa.gov/nsandt/#

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