FORMERLY USED DEFENSE SITES ON ISLANDS IN THE BERING SEA: HOTSPOTS OF CONTAMINATION AND HEALTH RISKS TO LOCAL COMMUNITIES AND WILDLIFE

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ABSTRACT

FORMERLY USED DEFENSE SITES ON ISLANDS IN THE BERING SEA: HOTSPOTS OF CONTAMINATION AND HEALTH RISKS TO LOCAL COMMUNITIES AND WILDLIFE

RENEE R. JORDAN WARD

The Arctic is an important indicator region for assessing properties and effects of persistent organic pollutants (POPs). The Arctic is subject to atmospheric deposition of globally distilled POPs, acting as a hemispheric sink for POPs that are transported from lower latitudes. Additionally, the Arctic contains thousands of contaminated formerly used defense (FUD) sites dating from World War II and the Cold War, many of which are co-located with rural communities and remain significant sources of POPs. The Arctic is therefore a repository of persistent chemicals that are readily transported through the atmosphere or that are released from FUD sites. Once POPs enter the Arctic, low temperatures and low intensity sunlight slow their deterioration, which makes them available for long-term incorporation into biological systems, especially in lipid-rich arctic food webs. As a result, concentrations of some POPs, including polychlorinated biphenyls (PCBs), in the blood of people in certain arctic regions continue to be higher than in general populations of North America and Europe. The Arctic is the home of many Indigenous peoples who rely on a traditional subsistence diet that includes a high proportion of lipid-rich foods such as fish and marine mammals; thus, they may be chronically exposed to dangerous levels of POPs. Because POPs are often endocrine disruptors, carcinogenic, and/or neurotoxic, exposures present important public health concerns for Arctic Indigenous Peoples. My dissertation research focused on health risks posed by FUD sites on Sivuqaq (St. Lawrence Island) and Unalaska Island, Alaska. These islands were used extensively by the U.S. military during WWII and the Cold War, and FUD sites on the islands may contribute to health disparities reported by residents, including high incidence of cancers, thyroid diseases, and reproductive disorders. My dissertation research on Sivuqaq followed a community-based participatory research (CBPR) approach and utilized sentinel fishes living near two FUD sites to examine contaminant concentrations and health effects at multiple levels of biological organization. My results demonstrate that FUD site contamination continues to pose a health risk to local wildlife and Sivuqaq residents despite large-scale remediation efforts. I found that PCB and Hg concentrations in a subsistence fish collected near the Northeast Cape FUD site exceed the Environmental Protection Agency's regulatory guidelines for safe consumption of fish. I found differential expression of genes related to ribosomal and metabolic functions in sentinel fish collected near Sivuqaq FUD sites. At the Gambell FUD site, I demonstrated that ninespine stickleback exposed to FUD site contamination exhibit suppressed gonadal maturation and two distinct liver phenotypes, indicating that some fish may be more resistant to POP toxicity. On Unalaska Island, I modelled distributions of contaminants to identify hotspots of contamination at FUD sites remediated by the Army Corps of Engineers. I found that contaminant concentrations remain above state cleanup thresholds at more than half of Unalaska FUD sites and that the City of Unalaska is a pollution hotspot. Collectively, the results of my dissertation research demonstrate that Alaskan FUD sites continue to serve as point sources of pollution and potentiate the risk of disease for local wildlife and rural communities living near these sites.

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TABLE OF CONTENTS

DEDICATION vii

PREFACE viii

CHAPTERS 1

CHAPTER 1: Introduction 1

REFERENCES 9

CHAPTER 2: Elevated mercury and PCB concentrations in Dolly Varden (*Salvelinus malma*) collected near a formerly used defense site on Sivuqaq, Alaska 15

REFERENCES 43

LIST OF TABLES 49

LIST OF FIGURES 50

LIST OF SUPPLEMENTARY MATERIALS 57

CHAPTER 3: Transcriptomic and developmental effects of persistent organic pollutants in sentinel fishes collected near an arctic formerly used defense site 62

REFERENCES 83

LIST OF FIGURES 88

CHAPTER 4: Differential gene expression and developmental pathologies associated with persistent organic pollutants in sentinel fish in Troutman Lake, Sivuqaq, Alaska 92

REFERENCES 124

LIST OF TABLES 136

LIST OF FIGURES 137

LIST OF SUPPLEMENTARY MATERIALS 145

CHAPTER 5: Formerly used defense sites on Unalaska Island, Alaska: mapping a legacy of environmental pollution 147

REFERENCES 166

LIST OF TABLES 170

LIST OF FIGURES 172

LIST OF SUPPLEMENTARY MATERIALS 176

CHAPTER 6: Conclusions 179

REFERENCES 184

DEDICATION

I dedicate my dissertation research to the people of Sivuqaq, Alaska.

PREFACE

Chapters 2-5 are written in manuscript format for publication in scientific peer-reviewed journals. As such, there may be some redundancy in content and formatting differences across chapters based on journal requirements and to adhere to Northern Arizona University's dissertation guidelines.

CHAPTERS

CHAPTER 1: Introduction

"*It's the great, big, broad land 'way up yonder,*
It's the forests where silence has lease;
It's the beauty that thrills me with wonder,
It's the stillness that fills me with peace."

- Excerpt from "The Spell of the Yukon" by Robert Service, 1907

Alaska is home to incredible landscapes, ecosystems, and biota. The Tongass National Forest in the Alaskan Panhandle is the largest intact rainforest in the world stretching over 68 thousand square kilometers (Orians and Schoen, 2017). The Copper River Delta hosts the highest spring concentration of migratory shorebirds in the Western Hemisphere (Bishop et al., 2000). The vast Bristol Bay supports the largest sockeye salmon fishery in the world (Clark et al., 2006) and is valued at over $1.5 billion dollars per year (Knapp et al., 2013). Central Alaska is home to the highest mountain peak in North America at 6,190 meters above sea level. Above the Arctic Circle, the Gates of the Arctic National Park supports Alaska's largest caribou herd. The expansive environments and biological diversity across Alaska provide both economic and ecosystem services paramount to national and global interests (Vynne et al., 2021).

Alaska's natural resources have been key to human migration, survival, and success over thousands of years. A growing body of genomic research shows that the Americas were populated by early Eurasian nomadic tribes that crossed and settled in Beringia during the Last Glacial Maximum (LGM) as early as 25,000 years ago (Hoffecker et al., 2016; Moreno-Mayar et al., 2018; Vachula et al., 2019). These early settlers relied on the milder temperatures, higher net primary production, and shrub-tundra vegetation in central Beringia (Brubaker et al., 2005; Hoffecker et al., 2014; Westbrook, 2014). At the end of the LGM, deglaciation led to rapid sea level rise which caused the Ancient Beringians to seek higher ground (Yokoyama et al., 2000). Parts of Beringia remained above sea level, including Sivuqaq (St. Lawrence Island) and Unalaska Island, which are the focus of this dissertation. The Alaska Natives inhabiting these islands are the descendants of the Ancient Beringians who colonized the Americas (Mason, 2016; Tackney et al., 2019). The Indigenous people of Sivuqaq are Siberian Yupik, and the Indigenous people of Unalaska are Qawalangin, part of the Unangan people.

In modern times, Alaska's natural resources supported population growth following the U.S. purchase of Alaska from Russia in 1867. The treaty between Alaska and Russia added about 1.5 M km^2 to the United States (U.S.) and was nicknamed "Seward's folly" by critics who believed the land was useless. These attitudes changed in 1896 with the discovery of gold in the Klondike region of the Yukon and in the early 1900s with the discovery of vast petroleum reserves. Alaska's natural resources support several billion-dollar industries that are the economic basis for the state. For example, Alaska's petroleum industry has generated more than $180 billion dollars in revenue since statehood in 1959 (Simonelli, 2018) and supports nearly 85% of the state's budget (alaska.gov). Alaska's fishing industry employs over 100,000 workers each summer and generates about $5 billion dollars in annual economic output (Welch, 2020).

Strategically positioned at the nexus of east and west, Alaska has been and continues to be of great geopolitical importance (Roucek, 1983). Alaska was strategically important to the U.S. military during World War II (WWII) and was the only American territory invaded and occupied by an enemy army since the War of 1812 (Polhamus, 2015). The militarization of Alaska during WWII facilitated further military expansion during the Cold War. Alaska's proximity to the former Union of Soviet Socialist Republics (U.S.S.R.) was fundamental to the U.S. defense plan (Denfeld, 1994). At the start of the Cold War, the U.S. feared a U.S.S.R. attack from the Arctic where bombers would go undetected until they reached the contiguous U.S., allowing little time to mount a counterattack. As tensions escalated between the U.S. and the U.S.S.R. after World War II, the newly formed Department of Defense (DoD) directed major military developments be built across Alaska to provide an early warning system for U.S.S.R. bombers and intercontinental ballistic missiles.

Between 1950 and 1970, the U.S. rapidly expanded its military presence in Alaska and installed numerous defense sites and surveillance systems across the state, including the Distant Early Warning Line, Ballistic Missile Early Warning System, and White Alice Communications System sites (Winkler, 1997). These defense sites included radar towers and supporting facilities for housing and operations, communications, and other functions. Because many locations of strategic importance were in remote regions of Alaska, the military often constructed defense sites at or near rural communities with existing infrastructure. In some cases, villages were relocated for the installation of a defense site (USATSDR, 2017). Collectively, these sites became the U.S.'s first line of defense against attack from the U.S.S.R. and afforded the U.S. the ability to detect and characterize an attack with enough time (estimated to be about 4 hours) to mount an effective counterattack (Hummel, 2005).

By the late 1970s, advancements in satellite technologies rendered radar surveillance stations obsolete and the defense sites were abandoned (Sepez et al., 2007). Many of the buildings and much of the materials used during military operations were left on site, including lumber, paints, electrical wiring, insulation, transformers, and tanks and drums containing hazardous substances (e.g., brake fluid, fuel, anti-freeze, and polychlorinated biphenyls; PCBs; Lubbert and Chu, 2007). Over time, containers deteriorated in the harsh arctic climate and leaked hazardous substances into local environments.

The publication of Rachel Carson's *Silent Spring* in 1962 galvanized the environmental movement in the U.S. (von Hippel, 2020). In 1970, President Richard Nixon established the U.S. Environmental Protection Agency (EPA). The EPA had little authority to enforce cleanup of sites until Congress passed the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA; hereafter referred to as Superfund) of 1980 as a response to the Love Canal disaster. CERCLA was enhanced by the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Lubbert and Chu, 2007). SARA created the Defense Environmental Restoration Program for formerly used defense (FUD) sites and granted the DoD the authority to oversee cleanup activities at former military sites used prior to 1986 (Lubbert and Chu, 2007; USACE, 2021a). This included FUD sites in Alaska constructed during WWII and the Cold War.

Military activity in Alaska during WWII and the Cold War resulted in over 600 FUD sites (USDOI, 2016; USEPA, 2004). Of the FUD sites examined for contamination, the Army Corps of Engineers has identified over 250 sites containing hazardous, toxic, and/or radioactive waste (USACE, 2015). However, many of these are not prioritized for remediation because of the difficulty and cost associated with remediating remote FUD sites and the relatively small populations that are impacted by contamination. As of 2021, 80% of the FUD sites in Alaska listed in the Army Corps of Engineers Defense Environmental Restoration Program portal still do not have cleanup projects (USACE, 2021b), including sites identified as containing hazardous waste (USACE, 2015). As of 2021, Alaska has six sites listed on the Superfund National Priorities List – a list of sites of national priority for cleanup of known or potential releases of hazardous substances (USEPA, 2021). Five of these sites are associated with the military, of which four remain active military bases. Furthermore, sites that do undergo environmental investigation and remediation are often deemed safe for human use based on insufficient sample sizes and budget limitations that prevent comprehensive risk analysis and cleanup measures (Jordan-Ward et al., 2022; von Hippel et al., 2018).

Cold War defense sites were installed in strategic locations that granted the U.S. the best ability to detect a USSR attack. These sites tended to be in remote areas near rural communities that traditionally depend on subsistence foods. The delay in removing military buildings, debris and hazardous wastes at remote FUD sites has prolonged environmental contamination and incorporation of toxic chemicals into local food webs (Carpenter et al., 2005; Scrudato et al., 2012; von Hippel et al., 2018). Chemicals of concern at FUD sites include persistent organic pollutants (POPs; e.g., PCBs used as dielectrics in transformers and capacitors and dichlorodiphenyltrichloroethane; DDT; used in insecticides), toxic metals (e.g., mercury used in electronics and lead used in paint), and volatile and semi-volatile organic compounds (VOCs; e.g., petroleum products, benzene, and polycyclic aromatic hydrocarbons used in a variety of products; Scrudato et al., 2012; von Hippel et al., 2018).

Disproportionate exposure to toxic chemicals remains an environmental justice issue for Indigenous communities across the U.S. as these chemicals may contribute to health disparities (Hoover et al., 2012; Welfinger-Smith et al., 2011). Because many POPs, toxic metals, and VOCs are endocrine disruptors, carcinogens, and neurotoxicants, chronic exposure to these compounds presents an important public health concern for the predominantly Indigenous communities living near FUD sites (Faass et al., 2013; Linares et al., 2015; Sonne et al., 2017). Indigenous communities living near FUD sites did not have agency in the selection of these sites by the military, nor did they benefit from the use of chemicals at these sites, but they inherited the burden of environmental pollution in their communities.

Our ability to accurately assess risks posed by FUD sites depends on our understanding of pollution sources, concentrations, and health effects in the local environment. Relatively little is known about the environmental impacts and potential health risks of exposure to FUD site contamination, especially in Alaska. To my knowledge, only 14 Alaska FUD sites have been extensively researched (Appendix A). My doctoral dissertation research explores the impacts of FUD sites on contaminant concentrations and health endpoints in native fishes, which serve as a model for human exposure and disease.

My first three chapters expand on previous studies conducted at two FUD sites on Sivuqaq. Sivuqaq is located off the west coast of Alaska and is only 75 km southeast of the Chukotka Peninsula of Russia. During the Cold War, the U.S. military constructed and operated two defense sites on the island, one near the village of Gambell and one at the former village of Northeast Cape (the village at Northeast Cape was relocated for the construction of the defense site). Previous research shows that the Gambell and Northeast Cape FUD sites are point sources of POP pollution and are associated with adverse health effects in humans and fish (Carpenter et al., 2005; Scrudato et al., 2012; von Hippel et al., 2018). These projects were initiated by the Yupik people of Sivuqaq and we followed a community-based participatory research (CBPR) approach from study design through project completion and publication. The fourth chapter of my dissertation identifies contamination hotspots on Unalaska Island in the Aleutian Archipelago. About 4,500 residents live on Unalaska year-round and rely on local subsistence foods, including sockeye salmon (*Oncorhynchus nerka*), caribou, birds, and marine invertebrates (Scarbrough and Fall, 1997). The island was used extensively by the U.S. military during WWII in the Aleutian Islands Campaign, which resulted in a legacy of pollution that poses a health risk to the local community and to wildlife (Adams et al., 2019; RIDOLFI Inc., 2020).

The first data chapter (Ch. 2) of my dissertation extends work conducted by Scrudato et al. (2012) and von Hippel et al. (2018). Scrudato et al. (2012) showed that mercury concentrations in soils are higher at the Northeast Cape FUD site than at other locations on the Northeast Cape, indicating that the FUD site is a point source of mercury. von Hippel et al. (2018) demonstrated that PCB levels in fish are higher at sites receiving inflow from the Northeast Cape FUD site, and that high molecular weight congeners predominate, which indicates a local source. These studies led to two questions: 1) Do fish living near the FUD site have elevated mercury concentrations? and 2) Do mercury and PCB concentrations in Dolly Varden (*Salvelinus malma*), a subsistence food source, pose a health risk to residents? I utilized contaminant chemistry techniques and stable isotope analyses to test the following hypotheses: 1) Dolly Varden living near the FUD site have higher mercury and PCB concentrations because mercury and PCBs bioaccumulate, and 2) Remediation efforts at the Northeast Cape FUD site were insufficient in that contamination continues to threaten the health of local people and wildlife.

The results from Chapter 2 and from previous research at Northeast Cape show that fish collected near the FUD site have elevated levels of POPs and mercury, and that high concentrations of POPs are associated with altered gene expression and endocrine disruption in ninespine stickleback (*Pungitius pungitius*; hereafter referred to as stickleback) (von Hippel et al., 2018). These results raised two questions: 1) Do we see similar transcriptomic effects in other native fish exposed to Northeast Cape FUD site contamination? and 2) Does FUD site contamination induce developmental pathologies or abnormal endocrine function? My third chapter seeks to answer these questions by assessing differential gene expression in Alaska blackfish (*Dallia pectoralis*), and by examining tissue morphologies and thyroid hormone concentrations in stickleback collected from Northeast Cape.

Byrne et al. (2015) and Zheng et al. (2020) showed that stickleback collected from Troutman Lake near the Gambell FUD site had elevated concentrations of multiple POPs, indicating strong local sources of these contaminants. But are elevated POP concentrations associated with transcriptomic, anatomical, and physiological changes in these fish? To this end, my fourth chapter assesses gene expression differences, tissue morphologies, and hormone level variation, in stickleback collected from Troutman Lake.

Collectively, Chapters 2-4 of my dissertation provide a comprehensive understanding of the adverse health effects in model fish exposed to contaminants on Sivuqaq. These contaminants originate from FUD sites, atmospheric deposition, and potentially other sources (e.g., the Gambell landfill). As I worked through these projects, I became interested in modelling contamination across multiple FUD sites to identify areas that may pose a health risk to communities. For my fifth chapter, I mined data from investigation and remediation reports of the Army Corps of Engineers and mapped contamination across 18 FUD sites on Unalaska Island. In this investigation, I identify priority areas for further remediation, and highlight environmental justice issues for remote communities exposed to FUD site pollution.

Overall, my doctoral research employed a CBPR approach and a One Health approach employing fish models to examine health risks associated with exposure to FUD site contamination. The following chapters explore a legacy of contamination left behind after World War II and the Cold War in the Bering Sea region, and the risks posed to rural communities and local wildlife. My hope is that this body of work inspires further action – community, governmental and academic – to assess risk and remediate these sites for future generations.

REFERENCES

Adams, E.M., von Hippel, F.A., Hungate, B.A., Buck, C.L., 2019. Polychlorinated biphenyl (PCB) contamination of subsistence species on Unalaska Island in the Aleutian Archipelago. Heliyon 5, e02989.

alaska.gov, Alaska Kid's Corner. State of Alaska. Online. https://alaska.gov/Kids/learn/economy.htm. Accessed November 16, 2021.

Anthony, R.G., Miles, A.K., Estes, J.A., Isaacs, F.B., 1999. Productivity, diets, and environmental contaminants in nesting bald eagles from the Aleutian Archipelago. Environ Toxicol Chem 18, 2054-2062.

Anthony, R.G., Miles, A.K., Ricca, M.A., Estes, J.A., 2007. Environmental contaminants in bald eagle eggs from the Aleutian Archipelago. Environ Toxicol Chem 26, 1843-1855.

Bishop, M.A., Meyers, P.M., McNeley, P.F., 2000. A method to estimate migrant shorebird numbers on the Copper River Delta, Alaska. J Field Ornithol 71, 627-637.

Brubaker, L.B., Anderson, P.M., Edwards, M.E., Lozhkin, A.V., 2005. Beringia as a glacial refugium for boreal trees and shrubs: new perspectives from mapped pollen data. J Biogeogr 32, 833-848.

Burger, J., Gochfeld, M., 2006. Locational differences in heavy metals and metalloids in Pacific blue mussels *Mytilus* [*edulis*] *trossulus* from Adak Island in the Aleutian Chain, Alaska. Sci Total Environ 368, 937-950.

Burger, J., Gochfeld, M., Shukla, T., Jeitner, C., Burke, S., Donio, M., Shukla, S., Snigaroff, R., Snigaroff, D., Stamm, T., Volz, C., 2007. Heavy metals in Pacific cod (*Gadus macrocephalus*) from the Aleutians: location, age, size, and risk. J Toxicol Environ Health Part A 70, 1897-1911.

Busby, R.R., Douglas, T.A., LeMonte, J.J., Ringelberg, D.B., Indest, K.J., 2020. Metal accumulation capacity in indigenous Alaska vegetation growing on military training lands. Int J Phytoremediation 22, 259-266.

Byrne, S., Miller, P.K., Seguinot-Medina, S., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2018a. Associations between serum polybrominated diphenyl ethers and thyroid hormones in a cross sectional study of a remote Alaska Native population. Sci Rep 8, 2198.

Byrne, S., Miller, P.K., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2015. Persistent organochlorine pesticide exposure related to a formerly used defense site on St. Lawrence Island, Alaska: data from sentinel fish and human sera. J Toxicol Environ Health Part A 78, 976-992.

Byrne, S., Seguinot-Medina, S., Miller, P.K., Waghiyi, V., von Hippel, F.A., Buck, C.L., Carpenter, D.O., 2017. Exposure to polybrominated diphenyl ethers and perfluoroalkyl substances in a remote population of Alaska Natives. Environ Pollut 231, 387-395.

Byrne, S.C., Miller, P.K., Seguinot-Medina, S., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2018b. Exposure to perfluoroalkyl substances and associations with serum thyroid hormones in a remote population of Alaska Natives. Environ Res 166, 537-543.

Carpenter, D.O., DeCaprio, A.P., O'Hehir, D., Akhtar, F., Johnson, G., Scrudato, R.J., Apatiki, L., Kava, J., Gologergen, J., Miller, P.K., Eckstein, L., 2005. Polychlorinated biphenyls in serum of the Siberian Yupik people from St. Lawrence Island, Alaska. Int J Circumpolar Health 64, 322-335.

Clark, J.H., McGregor, A., Mecum, R.D., Krasnowski, P., Carroll, A.M., 2006. The commercial salmon fishery in Alaska. Alsk Fish Res Bull 12, 1-146.

Denfeld, D.C., 1994. The Cold War in Alaska: a management plan for cultural resources, 1994-1999. US Army Corps of Engineers, Alaska District.

Faass, O., Ceccatelli, R., Schlumpf, M., Lichtensteiger, W., 2013. Developmental effects of perinatal exposure to PBDE and PCB on gene expression in sexually dimorphic rat brain regions and female sexual behavior. Gen Comp Endocrinol 188, 232-241.

Hardell, S., Tilander, H., Welfinger-Smith, G., Burger, J., Carpenter, D.O., 2010. Levels of polychlorinated biphenyls (PCBs) and three organochlorine pesticides in fish from the Aleutian Islands of Alaska. PLoS One 5, e12396.

Hoffecker, J.F., Elias, S.A., O'Rourke, D.H., 2014. Out of Beringia? Science 343, 979-980.

Hoffecker, J.F., Elias, S.A., O'Rourke, D.H., Scott, G.R., Bigelow, N.H., 2016. Beringia and the global dispersal of modern humans. Evol Anthropol 25, 64-78.

Hoover, E., Cook, K., Plain, R., Sanchez, K., Waghiyi, V., Miller, P., Dufault, R., Sislin, C., Carpenter, D.O., 2012. Indigenous peoples of North America: environmental exposures and reproductive justice. Environ Health Perspect 120, 1645.

Hummel, L.J., 2005. The U.S. military as geographical agent: the case of Cold War Alaska. Geogr Rev 95, 47-72.

Jessup, D.A., Johnson, C.K., Estes, J., Carlson-Bremer, D., Jarman, W.M., Reese, S., Dodd, E., Tinker, M.T., Ziccardi, M.H., 2010. Persistent organic pollutants in the blood of free-ranging sea otters (*Enhydra lutris ssp*.) in Alaska and California. J Wildl Dis 46, 1214-1233.

Jordan-Ward, R., von Hippel, F.A., Zheng, G., Salamova, A., Dillon, D., Gologergen, J., Immingan, T., Dominguez, E., Miller, P., Carpenter, D.O., Postlethwait, J.H., Byrne, S., Buck, C.L., 2022. Elevated mercury and PCB concentrations in Dolly Varden (*Salvelinus malma*) collected near a formerly used defense site on Sivuqaq, Alaska. Sci Total Environ.

Kenney, L.A., Eagles-Smith, C.A., Ackerman, J.T., von Hippel, F.A., 2014. Temporal variation in fish mercury concentrations within lakes from the western Aleutian Archipelago, Alaska. PLoS One 9, e102244.

Kenney, L.A., von Hippel, F.A., 2014. Morphological asymmetry of insular freshwater populations of threespine stickleback. Environ Biol Fishes 97, 225-232.

Kenney, L.A., von Hippel, F.A., Willacker, J.J., O'Hara, T.M., 2012. Mercury concentrations of a resident freshwater forage fish at Adak Island, Aleutian Archipelago, Alaska. Environ Toxicol Chem 31, 2647-2652.

Knapp, G., Guettabi, M., Goldsmith, O.S., 2013. The economic importance of the Bristol Bay salmon industry. Institute of Social and Economic Research, University of Alaska Anchorage.

Linares, V., Belles, M., Domingo, J.L., 2015. Human exposure to PBDE and critical evaluation of health hazards. Arch Toxicol 89, 335-356.

Lubbert, R., Chu, T., 2007. Challenges to cleaning up formerly used defense sites in the twenty-first century. Fed Facil Environ J 11, 5-18.

Mason, O.K., 2016. The old Bering Sea florescence about Bering Strait. The Oxford Handbook of the Prehistoric Arctic, Chapter 17, 417-442.

Miles, A.K., Ricca, M.A., Anthony, R.G., Estes, J.A., 2009. Organochlorine contaminants in fishes from coastal waters west of Amukta Pass, Aleutian Islands, Alaska, USA. Environ Toxicol Chem 28, 1643-1654.

Moreno-Mayar, J.V., Potter, B.A., Vinner, L., Steinrücken, M., Rasmussen, S., Terhorst, J., Kamm, J.A., Albrechtsen, A., Malaspinas, A.-S., Sikora, M., Reuther, J.D., Irish, J.D., Malhi, R.S., Orlando, L., Song, Y.S., Nielsen, R., Meltzer, D.J., Willerslev, E., 2018. Terminal Pleistocene Alaskan genome reveals first founding population of Native Americans. Nature 553, 203-207.

Orians, G., Schoen, J., 2017. North Pacific temperate rainforests: ecology and conservation. University of Washington Press.

Polhamus, J.A., 2015. Aleutian Campaign In World War II: A Strategic Perspective. CreateSpace Independent Publishing Platform.

Ricca, M.A., Keith Miles, A., Anthony, R.G., 2008. Sources of organochlorine contaminants and mercury in seabirds from the Aleutian Archipelago of Alaska: inferences from spatial and trophic variation. Sci Total Environ 406, 308-323.

RIDOLFI Inc., 2020. Strategic project implementation plan, Unalaska, Alaska Native American Lands Environmental Mitigation Program. Prepared by RIDOLFI Inc. for the Qawalangin Tribe of Unalaska.

Roucek, J.S., 1983. The geopolitics of the Arctic. Am J Econ Sociol 42, 463-471.

Scarbrough, L., Fall, J., 1997. Unalaska: subsistence harvest and use information. Alaska Department of Fish and Game Subsistence Division

Scrudato, R., Chiarenzelli, J., Miller, P.K., Alexander, J.C., Arnason, J., Zamzow, K., Zweifel, K., Gologergen, J., Kava, J., Waghiyi, V., Carpenter, D., 2012. Contaminants at arctic formerly used defense sites. J Local Glob Health Sci 2, 1-12.

Sepez, J., Package, C., Malcolm, P.E., Poole, A., 2007. Unalaska, Alaska: memory and denial in the globalization of the Aleutian landscape. Polar Geography 30, 193-209.

Simonelli, I.S., 2018. Where does all that oil go?, Alaska Business, Online.

Sonne, C., Torjesen, P.A., Fuglei, E., Muir, D.C.G., Jenssen, B.M., Jørgensen, E.H., Dietz, R., Ahlstrøm, Ø., 2017. Exposure to persistent organic pollutants reduces testosterone concentrations and affects sperm viability and morphology during the mating peak period in a controlled experiment on farmed arctic foxes (*Vulpes lagopus*). Environ Sci Technol 51, 4673-4680.

Steele, M., Griffith, C., Duran, C., 2018. Monthly variations in perfluorinated compound concentrations in groundwater. Toxics 6.

Stout, J.H., Trust, K.A., 2002. Elemental and organochlorine residues in bald eagles from Adak Island, Alaska. J Wildl Dis 38, 511-517.

Tackney, J., Jensen, A.M., Kisielinski, C., O'Rourke, D.H., 2019. Molecular analysis of an ancient Thule population at Nuvuk, Point Barrow, Alaska. American Journal of Physical Anthropology 168, 303-317.

USACE, 2015. Formerly Used Defense Sites per State - Alaska. United States Army Corps of Engineers.

USACE, 2021a. Formerly Used Defense Site Projects. United States Army Corps of Engineers https://www.hnc.usace.army.mil/Media/Fact-Sheets/Fact-Sheet-Article-View/Article/482110/formerly-used-defense-sites/

USACE, 2021b. FUDS Portal. United States Army Corps of Engineers. Accessed Nov 15, 2021. https://www.usace.army.mil/Missions/Environmental/Formerly-Used-Defense-Sites/FUDS-Inventory/.

USATSDR, 2017. Health Consultation: Northeast Cape Formerly Used Defense Site. United States Agency for Toxic Substances and Disease Registry.

USDOI, 2016. Report to Congress: Hazardous Substance Contamination of Alaska Native Claim Settlement Act Lands in Alaska. United States Department of the Interior Bureau of Land Management.

USEPA, 2004. Making Environmental Progress, Improving Local Communities: Accomplishments of the EPA Region 10 Superfund Program. United States Environmental Protection Agency Superfund Program.

USEPA, 2021. National Priorities List (NPL) sites by state. United States Environmental Protection Agency, Superfund Division.

Vachula, R.S., Huang, Y., Longo, W.M., Dee, S.G., Daniels, W.C., Russell, J.M., 2019. Evidence of Ice Age humans in eastern Beringia suggests early migration to North America. Quat Sci Rev 205, 35-44.

von Hippel, F.A., 2020. The Chemical Age: How Chemists Fought Famine and Disease, Killed Millions, and Changed Our Relationship with the Earth. University of Chicago Press.

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: health implications for indigenous residents. Environ Pollut 234, 279-287.

Vynne, C., Dovichin, E., Fresco, N., Dawson, N., Joshi, A., Law, B.E., Lertzman, K., Rupp, S., Schmiegelow, F., Trammell, E.J., 2021. The importance of Alaska for climate stabilization, resilience, and biodiversity conservation. Front For Glob Change 4, 701277.

Wagner, A.M., Barker, A.J., 2019. Distribution of polycyclic aromatic hydrocarbons (PAHs) from legacy spills at an Alaskan Arctic site underlain by permafrost. Cold Reg Sci Technol 158, 154-165.

Walsh, M., Walsh, M., Voie, Ø., 2014. Presence and persistence of white phosphorus on military Ttaining ranges. Propellants Explos Pyrotech 39, 922-931.

Walsh, M.E., Collins, C.M., Jenkins, T.F., Hewitt, A.D., Stark, J., Myers, K., 2003. Sampling for explosives -residues at Fort Greely, Alaska. Soil Sediment Contam 12, 631-645.

Welch, L., 2020. Breaking down Alaska seafood’s economic value, Anchorage Daily News.

Welfinger-Smith, G., Minholz, J.L., Byrne, S., Waghiyi, V., Gologergen, J., Kava, J., Apatiki, M., Ungott, E., Miller, P.K., Arnason, J.G., Carpenter, D.O., 2011. Organochlorine and metal contaminants in traditional foods from St. Lawrence Island, Alaska. J Toxicol Environ Health Part A 74, 1195-1214.

Westbrook, R.E., 2014. Evidence for a glacial refugium in south-central Beringia using modern analogs: a 152.2 kyr palynological record from IODP Expedition 323 sediment.

Winkler, D.F., 1997. Searching the skies: the legacy of the United States Cold War defense radar program. Library of Congress.

Yokoyama, Y., Lambeck, K., De Deckker, P., Johnston, P., Fifield, L.K., 2000. Timing of the Last Glacial Maximum from observed sea-level minima. Nature 406, 713-716.

Zheng, G., Miller, P., von Hippel, F.A., Buck, C.L., Carpenter, D.O., Salamova, A., 2020. Legacy and emerging semi-volatile organic compounds in sentinel fish from an arctic formerly used defense site in Alaska. Environ Pollut 259, 113872.

Appendix A

Appendix A. Studies conducted at formerly used defense (FUD) sites in Alaska. Studies were compiled using Web of Science advanced search with any field containing the key words "Alaska", "Military", and "Concentration". DDE = dichlorodiphenyldichloroethylene, DDT = dichlorodiphenyltrichloroethane, HCB = Hexachlorobenzene, HCH = hexachlorocyclohexane, OCs = organochlorines, OPEs = organophosphate esters, PAH = polycyclic aromatic hydrocarbons, PBDEs = polybrominated diphenyl ethers, PCBs = polychlorinated biphenyls, PFAS = perfluoroalkyl and polyfluoroalkyl substances.

Location	FUD site(s)	Contaminant(s) of interest	Source
Aleutian Archipelago	Adak, Amchitka, Kiska, Tanaga	DDT, HCB, mirex, PCBs, toxic metals	Anthony et al. (1999)
Aleutian Archipelago	Adak	OCs, toxic metals	Stout and Trust (2002)
Interior Alaska	Fort Greely	Explosives	Walsh et al. (2003)
Sivuqaq	Gambell, Northeast Cape	PCBs	Carpenter et al. (2005)
Aleutian Archipelago	Adak	Toxic metals and metalloids	Burger and Gochfeld (2006)
Aleutian Archipelago	Adak, Amchitka, Buldir, Amaknak	Chlordane, DDE, HCB, HCH, mirex, mercury, PCBs	Anthony et al. (2007)
Aleutian Archipelago	Adak, Amchitka Kiska	Toxic metals	Burger et al. (2007b)
Aleutian Archipelago	Adak, Amchitka Kiska	Mercury, OCs	Ricca et al. (2008)
Aleutian Archipelago	Adak, Attu, Kiska, Amchitka	OCs, PCBs	Miles et al. (2009)
Aleutian Archipelago	Adak, Amchitka, Kiska	Various	Jessup et al. (2010)
Aleutian Archipelago	Adak, Amchitka, Atka, Kiska	PCBs, DDE, HCB, mirex	Hardell et al. (2010)
Sivuqaq	Gambell, Northeast Cape	OCs, PCBs, toxic metals	Welfinger-Smith et al. (2011)
Sivuqaq	Northeast Cape	Cesium-137, DDE, HCB, mercury, mirex, PCBs	Scrudato et al. (2012)
Aleutian Archipelago	Adak	Mercury	Kenney et al. (2012)
Aleutian Archipelago	Agattu	Mercury	Kenney et al. (2014)

Aleutian Archipelago	Attu, Kiska, Adak	Various	Kenney and von Hippel (2014)
Near Anchorage	Eagle River Flats	White phosphorous	Walsh et al. (2014)
Sivuqaq	Northeast Cape	Chlordane, DDT, HCB, mirex	Byrne et al. (2015)
Sivuqaq	Gambell, Northeast Cape	PBDEs, PFAS	Byrne et al. (2017)
Sivuqaq	Gambell, Northeast Cape	PBDEs	Byrne et al. (2018a)
Sivuqaq	Gambell, Northeast Cape	PFAS	Byrne et al. (2018b)
Sivuqaq	Northeast Cape	PCBs	von Hippel et al. (2018)
Not specified	Not specified	PFAS	Steele et al. (2018)
Unalaska	Amaknak, Captain’s Bay	PCBs	Adams et al. (2019)
Point Barrow	Naval Arctic Research Laboratory	PAHs	Wagner and Barker (2019)
Sivuqaq	Gambell	OPEs, PBDEs, PCBs, PFAS,	Zheng et al. (2020)
Interior Alaska	Fort Greely	Toxic metals	Busby et al. (2020)

CHAPTER 2: Elevated mercury and PCB concentrations in Dolly Varden (*Salvelinus malma*) collected near a formerly used defense site on Sivuqaq, Alaska

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KEYWORDS

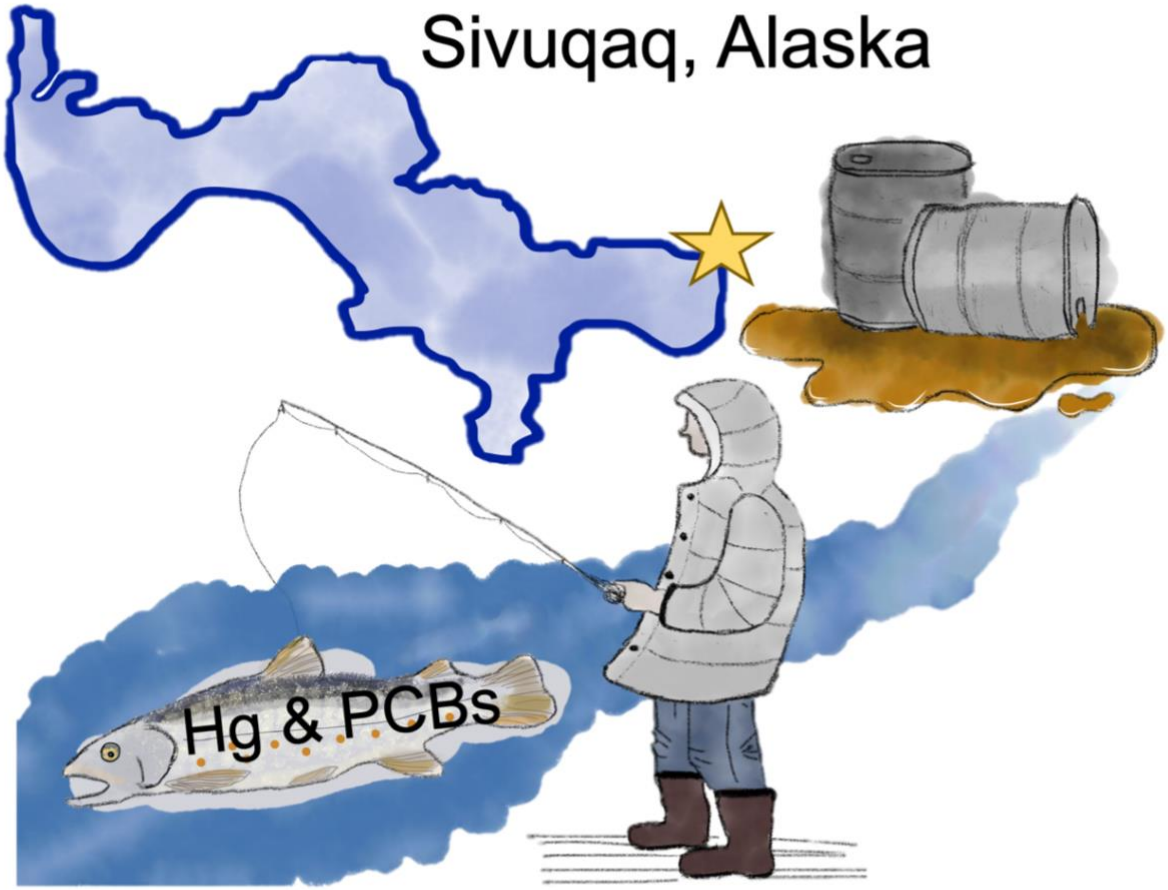
Agency for Toxic Substances and Disease Registry, Arctic Indigenous People, Military contamination, St. Lawrence Island, Subsistence foods, Yupik

HIGHLIGHTS

- Sivuqaq has 2 formerly used defense (FUD) sites, including at Northeast Cape (NEC)
- 89% of fish near the NEC FUD site exceeded the EPA's 0.049 µg/g Hg screening level

- All fish near the NEC FUD site exceeded the EPA's 0.0015 µg/g PCB screening level
- Relationships between δ13C, δ15N, [Hg], and [PCBs] were driven by the NEC FUD site
- The NEC FUD site is a point source for Hg and PCB pollution of the local food web

GRAPHICAL ABSTRACT



ABSTRACT

Environmental pollution causes adverse health effects in many organisms and contributes to health disparities for Arctic communities that depend on subsistence foods, including the Yupik residents of Sivuqaq (St. Lawrence Island), Alaska. Sivuqaq's proximity to Russia made it a strategic location for U.S. military defense sites during the Cold War. Two radar surveillance stations were installed on Sivuqaq, including at the Northeast Cape. High levels of persistent organic pollutants and toxic metals continue to leach from the Northeast Cape formerly used defense (FUD) site despite remediation efforts. We quantified total mercury (Hg) and polychlorinated biphenyl (PCB) concentrations, and carbon and nitrogen stable isotope signatures, in skin and muscle samples from Dolly Varden (*Salvelinus malma*), an important subsistence species. We found that Hg and PCB concentrations significantly differed across locations, with the highest concentrations found in fish collected near the FUD site. We found that 89% of fish collected from near the FUD site had Hg concentrations that exceeded the U.S. Environmental Protection Agency‘s (EPA) unlimited Hg-contaminated fish consumption screening level for subsistence fishers (0.049 μg/g). All fish sampled near the FUD site exceeded the EPA's PCB guidelines for cancer risk for unrestricted human consumption (0.0015 μg/g ww). Both Hg and PCB concentrations had a significant negative correlation with $\delta^{13}C$ when sites receiving input from the FUD site were included in the analysis, but these relationships were insignificant when input sites were excluded. $\delta^{15}N$ had a significant negative correlation with Hg concentration, but not with PCB concentration. These results suggest that the Northeast Cape FUD site remains a point source of Hg and PCB pollution and contributes to higher concentrations in resident fish, including subsistence species. Moreover, elevated Hg and PCB levels in fish near the FUD site may pose a health risk for Sivuqaq residents.

INTRODUCTION

Environmental pollution poses adverse health risks to people and may contribute to health disparities for communities that depend on subsistence foods, such as fish and marine mammals (Burger et al., 2007a; Scrudato et al., 2012). Many environmental contaminants, including mercury (Hg) and polychlorinated biphenyls (PCBs), accumulate in arctic environments from both local point sources of pollution and long-range atmospheric transport from lower latitudes (AMAP, 2011; Scheringer et al., 2004; Wania and Mackay, 1993). Alaska contains over 600 formerly used defense (FUD) sites dating from World War II and the Cold War, with over 500 sites identified for environmental cleanup by the U.S. Army Corps of Engineers and the Alaska Department of Environmental Conservation (USDOI, 2016). Many of these contaminated sites are located near rural communities that rely on subsistence foods and these communities may be directly affected by environmental contamination through their diet (Welfinger-Smith et al., 2011; Williams and Cravez, 2018). As a result, communities located near FUD sites may experience elevated risk of adverse health effects (Carpenter et al., 2005; Miller et al., 2013; von Hippel et al., 2018).

Mercury is a toxic metal that enters the environment from natural (e.g., volcanic eruptions and weathering of Hg-containing deposits) and anthropogenic sources (e.g., coal burning, mining, and electronics, including those used by the military at FUD sites) (Wang et al., 2004). Once in the environment, inorganic Hg can be transformed by microbes into the organic form methylmercury (MeHg). MeHg is a potent toxicant that bioaccumulates in animal tissues and biomagnifies within food webs (Peng et al., 2016). Point sources of Hg pollution may have a greater effect on MeHg bioaccumulation patterns at higher latitudes due to increased trophic magnification slopes and slower excretion rates, leading to increased accumulation in biota (Lavoie et al., 2013; Trudel and Rasmussen, 1997). MeHg exposure disrupts a variety of health endpoints in vertebrates, including cognitive, thyroidal, adrenal, and gonadal functions (Wada et al., 2009). High levels of Hg in humans have been linked to low birth weights, cognitive deficiencies, and reproductive impairment (Clarkson et al., 2003; Guallar et al., 2002; Harada, 1995; Oken et al., 2005).

PCBs are highly stable synthetic chemicals that were used in plasticizers, capacitors, sealants, flame retardants, hydraulic lubricants, electrical transformers, and pesticides until bans on their production and use came into force beginning in the 1970s (Safe, 1994). Despite the inclusion of PCBs under provisions of the Stockholm Convention for global elimination, PCBs remain ubiquitous in the environment and are frequently detected in biotic and abiotic samples, including near Cold War era FUD sites (von Hippel et al., 2018). PCBs are lipophilic compounds that readily bioaccumulate and biomagnify within the food web (Aronson et al., 2000; Burreau et al., 2004; Dewailly et al., 1989). PCBs are known carcinogenic, immunotoxicant, and endocrine-disrupting compounds that negatively affect a wide range of organisms, including humans (Schell et al., 2014; Wassermann et al., 1979; Wurgler and Kramers, 1992).

Dietary exposure is the primary route of MeHg bioaccumulation in animals (Bloom, 1992; Hall et al., 1997; Klaverkamp et al., 1983), and fish tend to accumulate MeHg in muscle tissue (Bloom, 1992; Harley et al., 2015). Similarly, dietary exposure is the primary route of PCB accumulation in animals (Ampleman et al., 2015; Crinnion, 2011), but PCBs preferentially accumulate in lipids (Müllerová and Kopecký, 2007). Thus, exposure to MeHg and PCBs via consumption of fish poses a direct risk for higher trophic level, piscivorous organisms (Peng et al., 2016; Van Oostdam et al., 2005). People who consume large quantities of fish are at higher risk of toxicity due to chronic dietary exposure. Many Alaska Native communities rely on high trophic level, lipid-rich subsistence foods, including fish and marine mammals. For instance, rural communities in Western Alaska consume an average of 183 g fish per adult per day while the general U.S. population consumes only 22-24 g fish/adult/day (Polissar and Neradilek, 2019;

USEPA, 2014). Food preparation methods do not eliminate MeHg (Morgan et al., 1997), which limits the ability of subsistence fishers to avoid contaminant exposure. Furthermore, these communities are often located near point sources of pollution, such as FUD sites (Scrudato et al., 2012; von Hippel et al., 2016).

Sivuqaq (St. Lawrence Island), Alaska is a remote island located in the northern Bering Sea, approximately 200 km off the west coast of mainland Alaska (Fig. 1). The island's proximity to Russia (formerly USSR) during the Cold War made it a strategic location for U.S. military defense sites. Two radar surveillance stations were installed on Sivuqaq as part of an early warning system to detect USSR aircraft, including a White Alice Communications Station on the Northeast Cape. The Northeast Cape FUD site was in operation from 1954 to 1972, until technological advances rendered it obsolete and it was abandoned (Carpenter et al., 2005). High levels of persistent organic pollutants and toxic metals continue to leach from the Northeast Cape FUD site despite large-scale remediation that occurred in the early 2000s (Scrudato et al., 2012; von Hippel et al., 2018; Welfinger-Smith et al., 2011). Scrudato et al. (2012) reported a six-fold increase in Hg concentrations in soils sampled near the Northeast Cape FUD site as compared to nearby background sites, as well as elevated concentrations of chlorinated contaminants such as PCBs, which suggests that fish and wildlife in this area may also be contaminated with these contaminants.

Nearly all residents of Sivuqaq have familial ties to Siberia and identify themselves as Siberian Yupik or Sivuqaq Yupik. They have a subsistence culture that relies heavily on the harvest of marine mammals and fish (AKDFG, 2006; Welfinger-Smith et al., 2011). Savoonga is the closest extant village to the Northeast Cape FUD site. In a 2009 survey of subsistence harvests in Savoonga, 82% of households reported harvesting a variety of fish species, including fish caught in both marine and freshwater habitats (Tahbone and Trigg, 2010). Although the original residents of Northeast Cape were displaced and relocated to Savoonga when the military site was constructed, many residents of Savoonga still maintain hunting and fishing camps in proximity to the FUD site. Carpenter et al. (2005) found that residents with camps at Northeast Cape had higher levels of PCBs in their blood serum than did residents of Gambell, a village on the western side of the island. This finding validated concerns of the Northeast Cape families and suggests that people who continue to subsist at Northeast Cape continue to be exposed to higher levels of military-associated contaminants, including Hg and PCBs, through consumption of fish caught near the FUD site.

The Suqitughneq (Suqi) River runs north through the Northeast Cape FUD site from the Kinipaghulghat Mountains to the Bering Sea. Several native fishes inhabit the Suqi River, including the salmonid Dolly Varden (*Salvelinus malma*), which is a subsistence food source for the local people. Residents report that the Suqi River was once among the most productive salmonid streams on the island, but that fuel spills and other contaminants from military operations at Northeast Cape greatly diminished the fish populations and degraded their habitats (Miller et al., 2013). Residents report that these fish populations have not recovered.

von Hippel et al. (2018) showed that resident fish species collected downstream of the Northeast Cape FUD site in the Suqi River are negatively impacted by military contaminants, including PCBs. Effects on fish included endocrine disruption and altered gene expression. These results raise the question of whether local contamination also poses a health risk to residents who consume fish caught in the Suqi River. Given the findings of Scrudato et al. (2012) that Hg and PCBs are primary military contaminants of concern in the Suqi River watershed, and the findings of von Hippel et al. (2018) that highly chlorinated PCBs occur at high concentrations in small fish in the Suqi River (ninespine stickleback [*Pungitius pungitius*] and Alaska blackfish [*Dallia pectoralis*]), we decided to focus on Hg and PCBs in Dolly Varden as representative contaminants in subsistence fish.

We quantified total Hg and PCB concentration in skin and muscle tissue of Dolly Varden collected from the Suqi River to determine if concentrations were elevated in fish living near the Northeast Cape FUD site and if those concentrations exceeded the U.S. Environmental Protection Agency (EPA) advisory limits for safe consumption. Because both MeHg and PCBs bioaccumulate and biomagnify, we also analyzed stable isotope ratios of carbon and nitrogen to examine associations between dietary carbon source (e.g., freshwater vs. marine), trophic level, site variability in isotope ratios, and contaminant concentration. We hypothesized that Dolly Varden collected from sites receiving inflow from the Northeast Cape FUD site would have significantly elevated total Hg and PCB concentrations and that the stable isotope ratios would account for some of the variation of both contaminants. Additionally, we sex-genotyped all fish to learn how variation in contamination relates to sex.

METHODS

Fish collection

We collected Dolly Varden from the Suqi River in June-July of 2012, 2013, and 2015 using unbaited 0.32 and 0.64 cm wire-mesh minnow traps (n=41 total fish). We collected fish from eight long-term monitoring sites (von Hippel et al., 2018) along the Suqi River (Fig. 2) and one reference site on the Tapisaggak (Tapi) River, located in an adjacent watershed 5 km to the east of the Northeast Cape FUD site. All fish were euthanized in the field with an overdose of pH-neutral fish anesthetic MS-222 (University of Alaska Anchorage IACUC #159870-20 and

#439949-1). Samples were stored on ice in the field and transferred to -80°C in the laboratory until analysis. We photographed each fish with a unique identifier and measured the standard length prior to dissecting a ~2 x 4 x 1 cm section of skin and muscle tissue, representing typical tissues consumed by residents, for the quantification of total Hg, PCBs, and stable isotope ratios. We also sampled a fin clip (2 x 2 mm) for sex genotyping. Dolly Varden were not captured at every site each year, which prevented us from comparing Hg and PCB concentrations across all sampling years.

Sex genotyping

Dolly Varden were sexed by multiplex PCR genotyping of the male-specific Y-chromosome gene *sdY* and the *18S* gene (present in both males and females) following the protocol described in Yano et al. (2013). We ran genomic DNA extractions on 15 mg of fin tissue for each fish using the Qiagen DNeasy Blood & Tissue kit. We quantified DNA concentrations using a ThermoFisher Nanodrop 1000 Spectrophotometer. PCR amplification of the *sdY* and *18S* genes was performed using 0.4 µM of each *sdY* primer, 0.2 µM of each *18s* primer, 50-100 ng of DNA, 1.5 units of TaKaRa Taq DNA polymerase (TaKara Bio), 1.5 mM $MgCl_2$, and 2.5 µl of 10X PCR buffer per 25 µl reaction. PCR products were electrophoresed on 2% agarose gels and imaged using an Alpha Innotech FluorChem SP imager.

Mercury analysis

We quantified total Hg concentrations in samples containing both skin and muscle tissue from 41 Dolly Varden samples. We extracted and freeze-dried each section (~2 g wet weight; ww) of skin and muscle tissue from frozen whole-body fish. We used a subsample of 0.25-0.50 g dried tissue for each homogenate sample and digested them using an open acid digestion protocol outlined by Mohammed et al. (2017). We added 1.5 ml hydrochloric acid (HCl; Fisher Chemical), 1 ml of 5% potassium permanganate in 0.1% HCl ($KMnO_4$; Fisher Chemical), 500 µl hydrogen peroxide (H_2O_2; Medivators Inc.), and 7 ml deionized (DI) water to each sample and let them sit overnight at room temperature. After approximately 12 hours, we capped samples and placed them in a hot water bath at 85°C for 2 hours. Following digestion, we filtered samples using a vacuum filtration apparatus to remove residual tissue debris from each sample. We then added hydroxylamine hydrochloride ($NH_2OH \bullet HCl$; Medivators Inc.) to each sample to neutralize the $KMnO_4$ added during the digestion step and diluted each sample to 15 ml with DI water.

MeHg typically accounts for >90% of bioaccumulated Hg in fish, with the highest MeHg concentrations found in muscle tissue (Bloom, 1992). Previous work on Dolly Varden collected from the Yukon Territory, Canada showed that MeHg accounted for over 91% of the total bioaccumulated Hg (Tran et al., 2016). Because of this, we measured total Hg concentration as a proxy for MeHg concentration. We analyzed samples for total Hg concentrations on a Perkin Elmer FIMS-100 cold-vapor atomic absorption analyzer with an attached auto-sampling unit using argon gas. Tin (II) chloride dihydrate ($SnCl_2$; Fisher Chemical) was used as the reducing agent and 3% (v/v) HCl as the carrier solution. For quality assurance and quality control, each analysis contained a 10 ppb certified tuna fish flesh homogenate reference sample (International Atomic Energy Agency [IAEA] 436), a 1 ppb spiked Dolly Varden sample, an extraction blank, and an analysis blank that we prepared along with target samples. Analysis blanks and standards contained all digestion reagents to control for the sample matrix. Additionally, we re-analyzed a random subset of five samples to determine reproducibility. Calibration standards for total Hg analysis were made from 1000 µg/ml Hg standard stock (PerkinElmer, catalog #N9300174) and serially diluted to 10 ppb, 5 ppb, and 0.5 ppb concentrations for instrument calibration. A certified standard (10 ppm Hg; Inorganic Ventures, catalog #MSHG-10PPM-125ML) was diluted to 1 ppb and run as an unknown sample for quality control. Analytical extraction efficiencies averaged 98 ± 0.04% (n=4) for certified reference material and 96 ± 0.07% (n=4) for spiked recoveries.

We compared total Hg concentrations to the EPA‘s unlimited consumption screening level for subsistence fishers consuming Hg-contaminated fish (0.049 µg/g in fish; USEPA, 2000). The EPA recommends using this threshold in either dry or wet weight depending on which measurement more accurately reflects the method of fish preparation. Sivuqaq residents prepare fish in a variety of ways, including dried and half dried (fish are frozen and later eaten with seal oil), boiled, fried, and baked. For this reason, we report the EPA unlimited consumption guideline in both dry weight (dw; 0.049 µg/g) and the wet weight equivalent (0.049 µg/g ww = 0.2 µg/g dw). We considered the EPA’s unlimited screening level to be more appropriate for Sivuqaq than the less conservative EPA Fish Tissue Residue Criterion for MeHg (0.3 Hg µg/g ww; USEPA, 2001) because Dolly Varden are a subsistence food for residents and the consumption rate for subsistence fishers more accurately reflects the fish consumption rate of rural Alaskan communities.

PCB analysis

We analyzed total PCB concentrations in freeze-dried skin and muscle tissue from the same 41 individual Dolly Varden analyzed for Hg using methods described in Zheng et al. (2020). Approximately 2 g of fresh tissue was freeze dried, pre-treated with diatomaceous earth, and spiked with surrogate standards (PCB-14, -65, and -166) before extraction using a Dionex Accelerated Solvent Extractor 350 with hexane and acetone (1:1, v/v) at 90ºC and 1500 psi over three static cycles. We separated 10% of the extract to determine lipid content by gravimetric analysis. The remaining 90% of extract was reduced to 1 mL by rapid evaporation using nitrogen gas and cleaned on a multilayer silica column containing glass wool, 2 cm of neutral silica, 5 cm of acid silica, 2 cm of neutral silica, and 1 cm of sodium sulfate. We eluted PCBs with 40 mL hexane and dichloromethane (1:1, v/v) and then concentrated, solvent exchanged to hexane, reduced again to 1 mL with nitrogen gas, and spiked with internal standards (PCB-30 and -204). We analyzed samples using an Agilent 7890 gas chromatograph (GC) equipped with an electron capture detector and a DB-5MS fused silica capillary column (60 m × 0.32 mm × 0.25 µm). We used helium as the carrier gas and held the flow rate constant at 4.3 mL/min. The GC thermocycler was set to the following sequence: 100°C for 1 min, 1°C /min to 240°C, 10°C /min to 280°C, and held for 20 min. We set the injector (splitless mode) and the electron capture detector temperatures to 250°C and 350°C, respectively. Six procedural blank and six matrix spike recovery samples were included in the extraction of all fish samples. All PCB concentrations were blank corrected and method detection limits (MDLs) were set at three times the standard deviation of the target analyte levels detected in the procedural blanks. For the compounds not detected in the blanks, MDLs were based on a signal-to-noise ratio of three. The average absolute recovery for the spiked samples (mean ± standard error) was 85 ± 1.7%. The mean (with standard errors) recoveries of the surrogate standards were 84 ± 3.8%, 84 ± 3.1% and 86 ± 4.1% for PCB-14, -65 and -166, respectively. We assumed equal contributions for co-eluted PCB congeners and assigned equal parts of the total PCB concentration for each congener (e.g., PCB 4 and PCB 10 each received half of the reported PCB concentration).

Stable isotope analysis

We analyzed stable isotopes to determine if total Hg and PCB concentrations were associated with either nitrogen isotope ratios (reflecting trophic level) or carbon isotope ratios (reflecting dietary source of carbon). We collected approximately 1 mg of freeze-dried skin and muscle tissue from each fish analyzed for contaminants and homogenized tissue in a tissue mill with 3.2 mm steel beads. We sealed each sample into a tin capsule and analyzed for $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C/N at the Colorado Plateau Stable Isotope Laboratory. We ran samples on a ThermoScientific Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo-Electron Corp., Bremen, Germany) configured through a Finnigan CONFLO III coupled to a Carlo Erba NC2100 elemental analyzer (CE Instruments, Milan, Italy). All instrumentation was calibrated using a suite of standards from the IAEA, including drift and linearity standards (peach leaves; SRM 1547), isotope standards (IAEA CH6 & IAEA CH7 for $\delta^{13}C$ and IAEA N1 & IAEA N2 for $\delta^{15}N$), elemental standards (including acetanilide, cystine, and methionine), and secondary check standards (including NIST pine needles and NIST apple leaves). Nine replicates of the internal laboratory working standard (peach leaves; SRM 1547) were interspersed every 11 fish samples to ensure measurement reproducibility ($\delta^{13}C = -26.15 \pm 0.04‰$, $\delta^{15}N = 1.88 \pm 0.04‰$). External isotope reference standard reproducibility for carbon and nitrogen was below ± 0.2‰.

Statistical analysis

We conducted all statistical analyses using R statistical computing software, R version 4.1.0 (2009-2021 RStudio, Inc.). We used an alpha level of 0.05 to determine significance for all statistical tests. Due to small sample sizes, we grouped collection sites according to their location relative to the FUD site. Sites 2, 6, and 7 are located downstream of the Northeast Cape FUD site and were pooled as downstream sites (n=6 fish; Fig. 2). Sites 13 and 14 are located upstream of the FUD site and were pooled as upstream sites (n=10 fish; Fig. 2). Sites 10, 11, and 12 receive direct input from the Northeast Cape FUD site and were pooled as input sites (n=21 fish; Fig. 2). Samples collected from the Tapi River were designated as the reference site (n=4 fish). We ran a linear mixed model (results not shown) to account for the potential random effect of pooled collection sites at each location; however, we found no differences by site and removed site as a variable from our model. Furthermore, the mixed effects model and an ANOVA model produced similar findings for modelling Hg and PCB concentrations.

We established whether sampling location was a significant factor in predicting total Hg (per dw) and PCB concentrations (per ww) using ANOVA models. Total Hg and total PCB concentrations were natural log transformed for ANOVAs and Pearson product-moment correlations to correct for abnormal data distributions and heteroskedasticity. We confirmed ANOVA results with the non-parametric Kruskal-Wallis and Dunn's multiple comparisons tests to account for small sample sizes and non-normal distributions of our data. We did not use a mixed effect model because the random effects of fish sex and sampling location were not significant (results not shown). We employed a one-way ANOVA to model total Hg concentration because sampling location was the only significant variable for the model. We employed a two-way ANOVA to model total PCB concentrations because both sampling location and sex were significant predictors. We did not length standardize total Hg concentrations because all fish were of the same species and the Pearson product-moment correlation between total Hg and standard length was not significant (r=0.03, n=41, p=0.843). We corrected for pairwise comparisons of total Hg and PCB concentrations for both year and location using the Tukey method. We analyzed sex differences using Mann-Whitney U non-parametric tests to account for non-normality and small sample sizes. We were unable to compare year-to-year differences in Hg and PCB concentrations because we did not capture fish at each site for all sampling years. Only input sites had fish sampled from all three years. We used raw data for non-parametric analyses. We analyzed $\delta^{15}N$ and $\delta^{13}C$ isotope ratios separately against both total Hg and PCB concentration and standard length to determine relationships using Pearson product-moment correlations. To compare our results to those in the literature, we converted Hg dry weight concentrations to wet weight using the equation from Canham et al. (2021) and assuming a mean fish moisture content of 76% as reported by Eagles-Smith and Ackerman (2014) (see Table S1, which provides both ww and dw values for Hg and both ww and lipid weight (lw) values for PCBs).

RESULTS

Fish samples and model selection

The concentrations of Hg and PCBs in Dolly Varden suggest that the Northeast Cape FUD site remained a major source of contamination during the study period. Of the 41 Dolly Varden caught, 23 were male and 18 were female. Mean total Hg concentration (± standard error) in females (0.217 ± 0.033 µg/g dw) was significantly higher than in males (0.133 ± 0.022 µg/g dw) across all samples (Mann-Whitney U test, p=0.030); however, this is likely an artifact of the disproportionate number of female fish caught at input sites (15 females versus 6 males). PCB concentration did not differ by sex. We found the highest mean total Hg concentrations in fish collected at input sites receiving water flow directly from the Northeast Cape FUD site (0.224 ± 0.03 µg/g dw; Table 1 and Fig. 3). Fish collected at sites upstream of the FUD site had the highest mean total PCB concentrations (31.7 ± 5.33 ng/g ww; Table 1 and Fig. 4). Congener-specific concentrations of PCBs are presented in Table S2. Dolly Varden samples had a mean lipid content of 2.16%.

We found that standard length was not a significant predictor of total Hg or PCB concentration. Dolly Varden standard length ranged between 81.5 - 178.0 mm, with a mean of 120.5 ± 3.5 mm, and was comparable among sampling locations and years. Because standard length did not correlate with either total Hg concentration (Pearson correlation, $r=0.03$, $n = 41$, $p = 0.843$) or total PCB concentration (Pearson correlation, $r=-0.03$, $n = 41$, $p = 0.876$), we excluded standard length from the models (Suppl. Fig. 1). Similarly, likelihood ratio tests indicated that the interactions between standard length, location, and year were not significant.

Total Hg concentration

Model selection tests indicated that sampling location was the only significant covariate in modelling Hg concentration. Total Hg concentrations in Dolly Varden differed significantly across sampling locations (ANOVA, $F_{3,37}=5.56$, $p=0.003$; Kruskal-Wallis $\chi^2=12.34$, $p=0.006$). Total Hg concentrations in fish collected at input sites were 4.3-fold higher than in fish from reference sites (ANOVA, $p=0.015$; Dunn's test, $p=0.018$) and 1.7-fold higher than in fish from upstream sites (ANOVA, $p=0.014$; Dunn's test, $p=0.040$; Fig. 3).

Total PCB concentration

Model selection tests indicated that sampling location and sex were the only significant covariates in modelling PCB concentration. A two-way ANOVA model with location and sex as fixed factors best explained the variance in PCB concentration across samples. Total PCB concentrations in Dolly Varden differed significantly among sampling locations and sex (ANOVA $F_{4,36}=4.192$, $p=0.007$; Fig. 4). Fish collected at input sites had significantly higher total

PCB concentrations than did reference fish (2.0-fold higher; ANOVA, p=0.043) and downstream fish (1.6-fold higher; ANOVA, p=0.043), but did not differ from upstream fish. Total PCB concentration did not significantly correlate with total Hg concentration (Pearson, r=-0.21, n=41, p=0.178). Dolly Varden collected at Suqi River sites tended to have heavier PCBs than those collected at the Tapi River reference site (Fig. 5). *Hepta*- and *octa*- chlorinated congeners were significantly higher in Suqi River Dolly Varden than in Tapi River Dolly Varden (Kruskal-Wallis, χ^2=7.48, p=0.006 and χ^2=5.78, p=0.016, respectively).

Stable isotope signatures

Given that both Hg and PCBs bioaccumulate and biomagnify, we hypothesized that nitrogen and carbon stable isotope ratios would correlate with total Hg and PCB concentrations, but the relationships were not as expected. Nitrogen stable isotope ratios ranged from 5.5‰ to 11.4‰, spanning nearly two trophic levels (based on a trophic fractionation of 3.4‰; Post, 2002), with a mean of 8.66 ± 0.18‰. $\delta^{15}N$ did not vary by site and did not correlate with either total PCB concentration or standard length. We found a significant negative relationship between total Hg concentration and $\delta^{15}N$ when input sites were included (Pearson correlation, r=-0.31, n=41, p=0.049). This relationship was slightly stronger when input sites were excluded from the analysis (Pearson correlation, r=-0.36, n=20, p=0.116; Fig. 6); however, it was no longer significant, likely due to low statistical power. The power to detect a significant effect across all samples and at a linear correlation coefficient of 0.3 was 49%. The power fell to 26% when input sites were removed from the analysis.

Carbon stable isotope ratios ranged from -34.0‰ to -22.1‰, with a mean of -29.3 ± 0.4‰. We found a significant positive relationship between $\delta^{13}C$ and $\delta^{15}N$ (Pearson correlation, r=0.51, n=41, p<0.001; Fig. S2). $\delta^{13}C$ values differed significantly by site (ANOVA, $F_{3,37}$=8.18,

$p<0.001$; Kruskal-Wallis, $\chi^2=14.87$, $p=0.002$). Fish collected at input sites had significantly lower $\delta^{13}C$ values than did fish collected at reference or upstream sites (ANOVA, $p<0.001$ and $p=0.047$; Dunn's test, $p=0.005$ and $p=0.034$, respectively). We found significant negative relationships between $\delta^{13}C$ and both total Hg (Pearson correlation, $r=-0.35$, $n=41$, $p=0.026$) and total PCB concentration (Pearson correlation, $r=-0.36$, $n=41$, $p=0.021$) when input sites were included in the analysis (Fig. 7). However, these trends did not hold when fish collected at input sites were removed from the analysis (Pearson correlation, $r=-0.11$, $n=20$, $p=0.634$ and $r=-0.32$, $n=20$, $p=0.162$, respectively).

Contaminant values in relation to EPA health screening levels

We found that 89% of Dolly Varden collected from the Suqi River had total Hg concentrations in muscle and skin that exceeded the EPA screening level of 0.049 µg/g dw. This screening level applies to unlimited consumption of Hg-contaminated fish by subsistence fishers using a drying preparation method such as smoked, dried or half-dried fish (USEPA, 2000). Of these fish with high Hg concentrations, 64% were collected from input sites receiving water flow directly from the Northeast Cape FUD site (Fig. 3). All fish collected at input sites exceeded the 0.049 µg/g dw EPA threshold. For fish prepared without drying (e.g., raw, baked, fried, or boiled), the EPA recommends using the threshold as a wet weight value rather than a dry weight value (0.049 µg/g ww = 0.2 µg/g dw). At this threshold, 35% of Dolly Varden collected from the Suqi River exceeded the EPA screening level for safe consumption of Hg-contaminated fish, of which 77% were collected from input sites (Fig. 3). Nearly half (48%) of Dolly Varden from input sites exceeded this threshold, while none of the fish collected from the Tapi River reference site exceeded this threshold. If the less conservative EPA Fish Tissue Residue Criterion for MeHg (0.3 Hg µg/g ww; USEPA, 2001) is employed, then none of the fish in the Suqi River exceeded the threshold. However, the 0.3 Hg µg/g ww criterion is based on a consumption rate of 17.5 g of fish per day, whereas rural communities in Western Alaska consume an average of 183 g (6.5 oz) of fish per day, about 10 times as much (Polissar and Neradilek, 2019). Therefore, the more conservative unlimited consumption screening level is more appropriate for the Sivuqaq community.

All the Dolly Varden sampled in this study exceeded the EPA's guideline for unrestricted (128 oz/month) consumption of PCB-contaminated fish (cancer risk for human consumption of fish; 0.0015 µg/g [ppm] ww; Fig. 4; USEPA, 2000). Of the fish caught from the Suqi River, 73% exceeded the EPA safe consumption limit for 24 oz/month (cancer risk for human consumption of fish; 0.016 µg/g ww; Fig. 4) and 11% of fish surpassed the EPA safe consumption limit for 8 oz/month (cancer risk for human consumption of fish; 0.047 µg/g ww).

DISCUSSION

FUD sites as a source of pollution

Despite large-scale remediation at the Northeast Cape FUD site completed in 2014 (ADEC, 2019), contamination at the site may continue to pose a health risk to Sivuqaq residents who conduct subsistence activities there. Mean total PCB concentrations presented in this study were substantially higher than previously reported by Welfinger-Smith et al. (2011) in Dolly Varden collected by subsistence hunters on Sivuqaq (0.00255 ± 0.00199 µg/g ww) and by Zheng et al. (2020) in ninespine stickleback collected from Troutman Lake on the opposite side of the island (0.01 µg/g ww), indicating that Dolly Varden have higher PCB concentrations at Northeast Cape than elsewhere on the island. Our results are consistent with those reported by von Hippel et al. (2018) for ninespine stickleback and Alaska blackfish collected at input and downstream sites in the Suqi River. Dolly Varden in the current study had higher PCB concentrations at upstream sites than did stickleback or blackfish, likely because Dolly Varden are stronger swimmers that are better able to swim between input and upstream sites.

PCB congener profiles can distinguish between point sources of pollution and accumulation via global distillation (Hong et al., 2012; Muir et al., 2000; von Hippel et al., 2018). Heavier PCB classes are less volatile and tend to remain relatively close to the pollution source whereas lighter weight PCBs undergo long-range transport and accumulate far from their sources of emission (Wania and Mackay, 1993). As a result, Arctic environments without point sources of pollution have a higher abundance of volatile PCBs (*tri*-, *tetra*-, and *penta*-chlorinated congeners) and relatively low levels of heavier PCBs. We found that fish collected at Suqi River sites had heavier PCBs than did fish collected at the Tapi River reference site, especially for classes with six or more chlorine atoms (Fig. 5). These results are consistent with von Hippel et al. (2018), who found that both stickleback and blackfish collected at sites downstream of the FUD site had significantly higher concentrations of heavier PCBs than did fish collected from upstream sites. These contaminant signatures indicate that the FUD site remains a point source of PCB pollution. These results imply that the consumption of fish caught at Northeast Cape, particularly near the FUD site, may contribute to disproportionately high exposure to PCBs for the Sivuqaq community.

Collectively, our data suggest that the Northeast Cape FUD site is a point source for both Hg and PCB contamination and that contaminants originating from the FUD site are being incorporated into the local food web. Our Hg findings are consistent with Scrudato et al. (2012), who analyzed sediment cores and found that the FUD site at Northeast Cape is the primary source of Hg in the watershed. PCB congener profiles in this study and in von Hippel et al.

(2018) indicate that the Northeast Cape FUD site is a point source of PCB contamination. Furthermore, the FUD site appears to contribute to elevated serum levels of PCBs (Carpenter et al., 2005) and hexachlorobenzene (Byrne et al., 2015) in Savoonga residents who hunt and fish at Northeast Cape. Regardless of which conservative screening level is used, the FUD site continues to pose a risk for consumption of Suqi River fish due to exposure to Hg and PCBs.

More generally, these results point to the need to assess local sources of pollution in the Arctic, and not just atmospheric deposition due to global distillation, and to adequately remediate contaminated sites. FUD sites were often abandoned by the military without adequate containment of contaminants, leading to levels of local pollution that far exceed the background levels due to atmospheric deposition. Furthermore, FUD sites, including those on Sivuqaq (Zheng et al., 2020), are often a source of numerous other pollutants, including emerging contaminants. In that light, the Hg and PCBs should be viewed as indicator pollutants of a broader problem. Additionally, the Arctic is warming at approximately twice the global average (Richter-Menge et al., 2019), which exacerbates the problem as POPs formerly sequestered in ice and permafrost re-enter food webs and the human diet.

Risks to wildlife

In addition to the risk posed to residents by elevated concentrations of Hg and PCBs, the FUD site also presents a risk to piscivorous wildlife. Dietary exposure to MeHg has been linked to reduced survival, growth, gonadal development, and spawning success in fish (Drevnick and Sandheinrich, 2003; Friedmann et al., 1996; Hammerschmidt et al., 2002). We found that 30% and 49% of Dolly Varden from the Suqi River exceeded MeHg dietary thresholds associated with biochemical (0.06 µg/g ww) and reproductive (0.04 µg/g ww) impairment in predatory fishes, respectively (Depew et al., 2012). None of the Dolly Varden exceeded MeHg thresholds for altered behavior (0.5 µg/g ww) or growth (1.44 µg/g ww) in fishes, or reproductive impairment for avian piscivores (0.16 µg/g ww; Fushman et al., 2017). For PCBs, we found that 89% of Dolly Varden from the Suqi River exceeded the lower limit of dietary threshold values associated with adverse effects in marine mammals (0.01 µg/g ww) (Kannan et al., 2000). However, PCB concentrations in Dolly Varden did not exceed Environment Canada's prey tissue residue guideline of 0.05 µg/g ww for wildlife consumers of fish (Alava et al., 2012). PCB levels were below thresholds for fish survival, growth, and reproduction (Berninger and Tillitt, 2019).

Life history variation and contaminant modeling

A growing body of research highlights the need to incorporate life history variables to accurately assess contaminant distributions and dynamics in food webs (Burke et al., 2020; Swanson and Kidd, 2010; Thomas et al., 2016). The focus of this study was to evaluate total Hg and PCB concentrations relevant to human consumption; however, investigation of life history differences of Dolly Varden may provide insights into both the movement of contaminants in this system and consumption risks. Specifically, differences in total Hg and PCB concentrations may be further explained by age, growth rates, and life history differences between anadromous and resident freshwater ecotypes (Howland et al., 2001). We would expect older Dolly Varden to have higher Hg and PCB values due to bioaccumulation. Because the FUD site is a point source of Hg and PCB pollution, we would expect resident freshwater fish to have higher concentrations than do anadromous fish, which complete most of their growth at sea (Rikardsen et al., 2006; Rikardsen et al., 2000). Anadromous fish are often more enriched in $\delta^{13}C$ than freshwater fish, so $\delta^{13}C$ signatures can be used to differentiate between freshwater and anadromous ecotypes (Guiry et al., 2020; Robson et al., 2016; Ruokonen et al., 2019). Although we did not differentiate between Dolly Varden ecotypes in this study, our $\delta^{13}C$ values are consistent with isotopic signatures of resident freshwater Dolly Varden (Hart et al., 2015; Tran et al., 2016). Tran et al. (2016) found that resident freshwater Dolly Varden had a mean muscle $\delta^{13}C$ value of -31.8‰, whereas anadromous Dolly Varden had a mean $\delta^{13}C$ value of -22.7‰. Studies on other northern fishes report similar findings. For example, Ruokonen et al. (2019) showed that $\delta^{13}C$ signatures in scales allowed the differentiation between resident freshwater and anadromous brown trout (means, -23‰ and -18‰, respectively). Guiry et al. (2020) found that $\delta^{13}C$ signatures in scale collagen could differentiate between resident freshwater *Oncorhynchus nerka* (kokanee; mean, -24‰) and anadromous *O. nerka* (sockeye salmon; mean, -16‰).

Stable isotope signatures of nitrogen also provide valuable ecological information relevant to contaminant modeling. For example, resident freshwater fish often have lower $\delta^{15}N$ isotopic signatures than do anadromous fish due to the fact that marine foods are enriched in heavier nitrogen isotopes (France, 1995), although these trends are not as strong as with $\delta^{13}C$ (McCarthy and Waldron, 2000; Ruokonen et al., 2019). Our $\delta^{15}N$ values are also consistent with isotopic signatures of resident freshwater Dolly Varden (Tran et al., 2016). Tran et al. (2016) reported that resident freshwater Dolly Varden had a significantly lower $\delta^{15}N$ signature than did anadromous Dolly Varden (means, 9.2‰ and 15.0‰, respectively). Future studies at Northeast Cape should distinguish between anadromous and resident freshwater Dolly Varden. This can be achieved in several ways, such as via analysis of strontium in otoliths (Campana, 1999; Hart et al., 2015; Kennedy et al., 2002). Such analyses may show that anadromous Dolly Varden harvested from the Suqi River are safe to eat, in which case the focus of public outreach could include education on the identification of anadromous Dolly Varden or mapping of areas of the Suqi River with the greatest proportion of anadromous Dolly Varden.

We observed a significant negative slope between $\delta^{13}C$ and both total Hg concentration and total PCB concentration, but those effects disappeared when fish collected at input sites were excluded from the analyses. Several possibilities warrant further investigation. These patterns may be an artifact of elevated total Hg and PCBs at the input sites and not due to established bioaccumulation and biomagnification patterns (Kidd et al., 2011; Lavoie et al., 2013), even though the $\delta^{15}N$ values spanned nearly two trophic levels (using a trophic fractionation value of 3.4‰; Post, 2002). Alternatively, differences in $\delta^{13}C$ values may be driven by life history. Individual fish on the right side of the graphs on Fig. 6 may be anadromous whereas those on the left side may be resident freshwater. We found that the correlation between $\delta^{15}N$ and total Hg increased when fish collected at input sites were excluded, suggesting that the lack of significance when these fish were excluded is likely due to a reduction in power to detect the correlation. In contrast, input sites appeared to drive the negative relationship between total Hg and $\delta^{13}C$ values.

Health disparities

The traditional knowledge of Sivuqaq residents speaks to a history of health disparities associated with the Northeast Cape FUD site. Elders have observed that cancers, reproductive disorders, and thyroid disease are more prevalent among people who engage in subsistence activities at Northeast Cape (Carpenter et al., 2005; Miller et al., 2013). In 2011, the tribal government of Savoonga requested a Public Health Assessment and Health Consultation by the Agency for Toxic Substances and Disease Registry (ATSDR) to assess health disparities associated with the Northeast Cape FUD site. The ATSDR assessment (USATSDR, 2017), released in 2017, concluded that contaminant levels in fish from the Suqi River do not pose a health risk to local residents. This conclusion was based on a 2001 sampling project conducted by the U.S. Army Corps of Engineers in which eight Dolly Varden collected from the estuary of the Suqi River were analyzed for PCBs (USATSDR, 2006). Given the high variability in PCB concentrations among fish in the Suqi River found in this study and in von Hippel et al. (2018), a sample size of eight individuals is insufficient for a reliable assessment. Furthermore, the Army Corps sampling occurred before the major remediation efforts at Northeast Cape. Remediation activities often release contaminants during the process, which can lead to an increase in contaminant concentration for some time after remediation (Scrudato et al., 2012; Voie et al., 2002). Dolly Varden used in the ATSDR health assessment were collected from the estuary (about 2.4 km downstream of the FUD site) instead of at input sites. Estuarine environments receive tidal influx that disperses and dilutes contaminants and are thus not comparable to upstream contaminated sites. To our knowledge, the ATSDR did not examine PCB profiles to differentiate potential sources of PCBs in Dolly Varden. Additionally, the study on which the ATSDR based their assessment did not differentiate between anadromous and resident freshwater Dolly Varden. Resident freshwater fish accumulate contaminants throughout their lifespan in the local habitat, whereas anadromous fish complete most growth in the ocean and are thus unlikely to reflect local freshwater pollution sources.

Collectively, our results contradict the findings reported in the ATSDR assessment. The ATSDR reported mean concentrations of Aroclor 1254 of 0.014 μg/g and Aroclor 1260 of 0.00096 μg/g, which is less than half of the mean total PCBs (0.029 μg/g) that we measured in Dolly Varden collected at input sites. PCB profiles in this study and in von Hippel et al. (2018) show that fish collected near the Northeast Cape FUD site have significantly heavier PCBs than do fish from reference sites across three fish species. Because heavier PCB classes are less volatile and do not reach higher latitudes through global distillation, heavily chlorinated PCBs in

Suqi River fish are likely a result of FUD site contamination. In summary, the ATSDR concluded that the FUD site does not pose a health risk to Sivuqaq residents (USATSDR, 2017). However, our results do not support this conclusion and highlight the need for robust health assessments based on relevant data to accurately assess risk. In particular, an assessment of human health consequences for consumption of Suqi River fish should include: 1) a large sample size of fish collected from sites spanning from the headwaters to the estuary across multiple years, 2) an assessment of life history for individual fish to differentiate between resident freshwater and anadromous, and 3) analysis of all pollutants known to be associated with the Northeast Cape FUD site and consideration of additive and/or synergistic effects.

Limitations

Small sample sizes limited our statistical power to detect significant differences. We found a significant negative correlation between δ^{15}N and total Hg when input sites were included; however, it was no longer significant when input sites were removed from the analysis, despite a stronger correlation coefficient. Similarly, the relationship between total PCB concentration and δ^{13}C was no longer significant when input sites were excluded from analysis. Future research should increase the sample size for more robust statistical analyses. However, we trapped only a small number of Dolly Varden despite considerable effort, which likely reflects a population that has not yet recovered from the effects of FUD site pollution.

As mobile animals, Dolly Varden may swim between input sites and either upstream or downstream sites, and we are not able to determine what fraction of their time they developed at different sites. Nevertheless, we found elevated Hg and PCB concentrations in Dolly Varden collected at input sites, suggesting that the signal of contamination originating at the FUD site is sufficiently strong to be apparent despite fish movement. Future research could overcome this limitation by caging fish in different locations such that all exposure would have occurred at that location.

We measured total Hg rather than MeHg in fish muscle tissue, and MeHg is the form of primary health concern. However, MeHg typically accounts for over 90% of measured total Hg in fish (Bloom, 1992), including in Dolly Varden (Tran et al., 2015, 2016). Additionally, the EPA recommends measuring total Hg as a proxy for MeHg when comparing fish Hg concentrations to their unlimited consumption screening levels (USEPA, 2000).

CONCLUSION

Dolly Varden are an important subsistence food source for Sivuqaq residents and fish caught in the Suqi River may pose health risks due to contaminants originating at the Northeast Cape FUD site. Northeast Cape was a vital community and gathering place for traditional foods prior to the construction of the military site during the Cold War. Residents of Sivuqaq want to safely re-establish the community at Northeast Cape, which necessitates long-term robust health assessments, as well as suitable remediation and monitoring of remaining contamination originating from the FUD site. Furthermore, additional remediation of the Suqi River would facilitate the re-establishment of healthy populations of other subsistence fish species, such as salmon, which will further enhance community rebuilding. A vigorous process of assessment, remediation, and monitoring should be accomplished under Tribal supervision and agreement to ensure sovereignty of the data and relevance of the process to Tribal priorities, such as decisions on siting of the restored community.

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REFERENCES

ADEC, 2019. Northeast Cape and Gambell Formerly Used Defense Sites. Alaska Department of Environmental Conservation. https://dec.alaska.gov/spar/csp/sites/st-lawrence/.

AKDFG, 2006. Community subsistence information system: Savoonga. Alaska Department of Fish and Game Subsistence Division.

Alava, J.J., Ross, P.S., Lachmuth, C., Ford, J.K., Hickie, B.E., Gobas, F.A., 2012. Habitat-based PCB environmental quality criteria for the protection of endangered killer whales (*Orcinus orca*). Environ Sci Technol 46, 12655-12663.

AMAP, 2011. AMAP assessment 2011: mercury in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xiv + 193 pp.

Ampleman, M.D., Martinez, A., DeWall, J., Rawn, D.F.K., Hornbuckle, K.C., Thorne, P.S., 2015. Inhalation and dietary exposure to PCBs in urban and rural cohorts via congener-specific measurements. Environ Sci Technol 49, 1156-1164.

Aronson, K.J., Miller, A.B., Woolcott, C.G., Sterns, E.E., McCready, D.R., Lickley, L.A., Fish, E.B., Hiraki, G.Y., Holloway, C., Ross, T., Hanna, W.M., SenGupta, S.K., Weber, J.P., 2000. Breast adipose tissue concentrations of polychlorinated biphenyls and other organochlorines and breast cancer risk. Cancer Epidemiol Biomarkers Prev 9, 55-63.

Berninger, J.P., Tillitt, D.E., 2019. Polychlorinated biphenyl tissue-concentration thresholds for survival, growth, and reproduction in fish. Environ Toxicol Chem 38, 712-736.

Bloom, N.S., 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can J Fish Aquat Sci 49, 1010-1017.

Burger, J., Gochfeld, M., Jeitner, C., Burke, S., Stamm, T., Snigaroff, R., Snigaroff, D., Patrick, R., Weston, J., 2007. Mercury levels and potential risk from subsistence foods from the Aleutians. Sci Total Environ 384, 93-105.

Burke, S.M., Zimmerman, C.E., Laske, S.M., Koch, J.C., Derry, A.M., Guernon, S., Branfireun, B.A., Swanson, H.K., 2020. Fish growth rates and lake sulphate explain variation in mercury levels in ninespine stickleback (*Pungitius pungitius*) on the Arctic Coastal Plain of Alaska. Sci Total Environ 743, 140564.

Burreau, S., Zebühr, Y., Broman, D., Ishaq, R., 2004. Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) studied in pike (*Esox lucius*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from the Baltic Sea. Chemosphere 55, 1043-1052.

Byrne, S., Miller, P.K., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2015. Persistent organochlorine pesticide exposure related to a formerly used defense site on St. Lawrence Island, Alaska: data from sentinel fish and human sera. J Toxicol Environ Health Part A 78, 976-992.

Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 188, 263-297.

Canham, R., González-Prieto, A.M., Elliott, J.E., 2021. Mercury exposure and toxicological consequences in fish and fish-eating wildlife from anthropogenic activity in Latin America. Integr Environ Assess Manag 17, 13-26.

Carpenter, D.O., DeCaprio, A.P., O'Hehir, D., Akhtar, F., Johnson, G., Scrudato, R.J., Apatiki, L., Kava, J., Gologergen, J., Miller, P.K., Eckstein, L., 2005. Polychlorinated biphenyls in serum of the Siberian Yupik people from St. Lawrence Island, Alaska. Int J Circumpolar Health 64, 322-335.

Clarkson, T.W., Magos, L., Myers, G.J., 2003. The toxicology of mercury: current exposures and clinical manifestations. N Engl J Med 349, 1731-1737.

Crinnion, W.J., 2011. Polychlorinated biphenyls: persistent pollutants with immunological, neurological, and endocrinological consequences. Altern Med Rev 16, 5-13.

Depew, D.C., Basu, N., Burgess, N.M., Campbell, L.M., Devlin, E.W., Drevnick, P.E., Hammerschmidt, C.R., Murphy, C.A., Sandheinrich, M.B., Wiener, J.G., 2012. Toxicity of dietary methylmercury to fish: derivation of ecologically meaningful threshold concentrations. Environ Toxicol Chem 31, 1536-1547.

Dewailly, E., Nantel, A., Weber, J.P., Meyer, F., 1989. High levels of PCBs in breast milk of Inuit women from arctic Quebec. Bull Environ Contam Toxicol 43, 641-646.

Drevnick, P.E., Sandheinrich, M.B., 2003. Effects of dietary methylmercury on reproductive endocrinology of fathead minnows. Environ Sci Technol 37, 4390-4396.

Eagles-Smith, C.A., Ackerman, J.T., 2014. Mercury bioaccumulation in estuarine wetland fishes: evaluating habitats and risk to coastal wildlife. Environ Pollut 193, 147-155.

France, R., 1995. Stable nitrogen isotopes in fish: literature synthesis on the influence of ecotonal coupling. Estuar Coast Shelf Sci 41, 737-742.

Friedmann, A.S., Watzin, M.C., Brinck-Johnsen, T., Leiter, J.C., 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquat Toxicol 35, 265-278.

Fuchsman, P.C., Brown, L.E., Henning, M.H., Bock, M.J., Magar, V.S., 2017. Toxicity reference values for methylmercury effects on avian reproduction: critical review and analysis. Environ Toxicol Chem 36, 294-319.

Guallar, E., Sanz-Gallardo, M.I., van't Veer, P., Bode, P., Aro, A., Gómez-Aracena, J., Kark, J.D., Riemersma, R.A., Martín-Moreno, J.M., Kok, F.J., 2002. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med 347, 1747-1754.

Guiry, E., Royle, T.C.A., Matson, R.G., Ward, H., Weir, T., Waber, N., Brown, T.J., Hunt, B.P.V., Price, M.H.H., Finney, B.P., Kaeriyama, M., Qin, Y., Yang, D.Y., Szpak, P., 2020. Differentiating salmonid migratory ecotypes through stable isotope analysis of collagen: archaeological and ecological applications. PLoS One 15, e0232180.

Hall, B.D., Bodaly, R.A., Fudge, R.J.P., Rudd, J.W.M., Rosenberg, D.M., 1997. Food as the dominant pathway of methylmercury uptake by fish. Water Air Soil Pollut 100, 13-24.

Hammerschmidt, C.R., Sandheinrich, M.B., Wiener, J.G., Rada, R.G., 2002. Effects of dietary methylmercury on reproduction of fathead minnows. Environ Sci Technol 36, 877-883.

Harada, M., 1995. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 25, 1-24.

Harley, J., Lieske, C., Bhojwani, S., Castellini, J.M., López, J.A., O'Hara, T.M., 2015. Mercury and methylmercury distribution in tissues of sculpins from the Bering Sea. Polar Biol 38, 1535-1543.

Hart, L.M., Bond, M.H., May-McNally, S.L., Miller, J.A., Quinn, T.P., 2015. Use of otolith microchemistry and stable isotopes to investigate the ecology and anadromous migrations of northern Dolly Varden from the Egegik River, Bristol Bay, Alaska. Environ Biol Fishes 98, 1633-1643.

Hong, Q., Wang, Y., Luo, X., Chen, S., Chen, J., Cai, M., Cai, M., Mai, B., 2012. Occurrence of polychlorinated biphenyls (PCBs) together with sediment properties in the surface sediments of the Bering Sea, Chukchi Sea and Canada Basin. Chemosphere 88, 1340-1345.

Howland, K.L., Tonn, W.M., Babaluk, J.A., Tallman, R.F., 2001. Identification of freshwater and anadromous inconnu in the Mackenzie River system by analysis of otolith strontium. Trans Am Fish Soc 130, 725-741.

Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. Hum Ecol Risk Assess 6, 181-201.

Kennedy, B.P., Klaue, A., Blum, J.D., Folt, C.L., Nislow, K.H., 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Can J Fish Aquat Sci 59, 925-929.

Kidd, K., Clayden, M., Jardine, T., 2011. Bioaccumulation and biomagnification of mercury through food webs. In Environmental Chemistry and Toxicology of Mercury (eds. G. Liu, Y. Cai, and N. O'Driscoll). pp. 453-499.

Klaverkamp, J.F., Turner, M.A., Harrison, S.E., Hesslein, R.H., 1983. Fates of metal radiotracers added to a whole lake: accumulation in slimy sculpin (*Cottus cognatus*) and white sucker (*Catostomus commersoni*). Sci Total Environ 28, 119-128.

Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L.M., 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environ Sci Technol 47, 13385-13394.

McCarthy, I.D., Waldron, S., 2000. Identifying migratory *Salmo trutta* using carbon and nitrogen stable isotope ratios. Rapid Commun Mass Spectrom 14, 1325-1331.

Miller, P.K., Waghiyi, V., Welfinger-Smith, G., Byrne, S.C., Kava, J., Gologergen, J., Eckstein, L., Scrudato, R., Chiarenzelli, J., Carpenter, D.O., Seguinot-Medina, S., 2013. Community-based participatory research projects and policy engagement to protect environmental health on St. Lawrence Island, Alaska. Int J Circumpolar Health 72, 21656.

Mohammed, E., Mohammed, T., Mohammed, A., 2017. Optimization of an acid digestion procedure for the determination of Hg, As, Sb, Pb and Cd in fish muscle tissue. MethodsX 4, 513-523.

Morgan, J.N., Berry, M.R., Graves, R.L., 1997. Effects of commonly used cooking practices on total mercury concentration in fish and their impact on exposure assessments. J Expo Anal Environ Epidemiol 7, 119-133.

Muir, D., Riget, F., Cleemann, M., Skaare, J., Kleivane, L., Nakata, H., Dietz, R., Severinsen, T., Tanabe, S., 2000. Circumpolar trends of PCBs and organochlorine pesticides in the arctic marine environment inferred from levels in ringed seals. Environ Sci Technol 34, 2431-2438.

Müllerová, D., Kopecký, J., 2007. White adipose tissue: storage and effector site for environmental pollutants. Physiol Res 56, 375-382.

Oken, E., Wright, R.O., Kleinman, K.P., Bellinger, D., Amarasiriwardena, C.J., Hu, H., Rich-Edwards, J.W., Gillman, M.W., 2005. Maternal fish consumption, hair mercury, and infant cognition in a U.S. cohort. Environ Health Perspect 113, 1376-1380.

Peng, X., Liu, F., Wang, W.X., 2016. Organ-specific accumulation, transportation, and elimination of methylmercury and inorganic mercury in a low Hg accumulating fish. Environ Toxicol Chem 35, 2074-2083.

Polissar, N., Neradilek, M., 2019. Alaska statewide and regional estimates of consumption rates in rural communities for salmon, halibut, herring, non-marine fish, and marine invertebrates. United States Environmental Protection Agency. EPA Contract EP-C-14-016.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703-718.

Richter-Menge, J., Druckenmiller, M., Jeffries, M., 2019. Arctic Report Card. https://www.arctic.noaa.gov/Report-Card.

Rikardsen, A.H., Amundsen, P.-A., Knudsen, R., Sandring, S., 2006. Seasonal marine feeding and body condition of sea trout (*Salmo trutta*) at its northern distribution. ICES J Mar Sci 63, 466-475.

Rikardsen, A.H., Amundsen, P.A., Bjørn, P.A., Johansen, M., 2000. Comparison of growth, diet and food consumption of sea-run and lake-dwelling arctic charr. J Fish Biol 57, 1172-1188.

Robson, H.K., Andersen, S.H., Clarke, L., Craig, O.E., Gron, K.J., Jones, A.K.G., Karsten, P., Milner, N., Price, T.D., Ritchie, K., Zabilska-Kunek, M., Heron, C., 2016. Carbon and nitrogen stable isotope values in freshwater, brackish and marine fish bone collagen from Mesolithic and Neolithic sites in central and northern Europe. Environ Archaeol 21, 105-118.

Ruokonen, T.J., Kiljunen, M., Erkinaro, J., Orell, P., Sivonen, O., Vestola, E., Jones, R.I., 2019. Migration strategies of brown trout (*Salmo trutta*) in a subarctic river system as revealed by stable isotope analysis. Ecol Freshw Fish 28, 53-61.

Safe, S.H., 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24, 87-149.

Schell, L.M., Gallo, M.V., Deane, G.D., Nelder, K.R., DeCaprio, A.P., Jacobs, A., 2014. Relationships of polychlorinated biphenyls and dichlorodiphenyldichloroethylene (p,p'-DDE) with testosterone levels in adolescent males. Environ Health Perspect 122, 304-309.

Scheringer, M., Salzmann, M., Stroebe, M., Wegmann, F., Fenner, K., Hungerbuhler, K., 2004. Long-range transport and global fractionation of POPs: insights from multimedia modeling studies. Environ Pollut 128, 177-188.

Scrudato, R., Chiarenzelli, J., Miller, P.K., Alexander, J.C., Arnason, J., Zamzow, K., Zweifel, K., Gologergen, J., Kava, J., Waghiyi, V., Carpenter, D., 2012. Contaminants at arctic formerly used defense sites. J Local Glob Health Sci 2, 1-12.

Swanson, H.K., Kidd, K.A., 2010. Mercury concentrations in arctic food fishes reflect the presence of anadromous arctic charr (*Salvelinus alpinus*), species, and life history. Environ Sci Technol 44, 3286-3292.

Tahbone, S.T., Trigg, E.W., 2010. 2009 comprehensive subsistence harvest survey, Savoonga, Alaska. Native Village of Savoonga, Kawerak, Inc., North Pacific Research Board, National Science Foundation.

Thomas, S.M., Kiljunen, M., Malinen, T., Eloranta, A.P., Amundsen, P.-A., Lodenius, M., Kahilainen, K.K., 2016. Food-web structure and mercury dynamics in a large subarctic lake following multiple species introductions. Freshw Biol 61, 500-517.

Tran, L., Reist, J.D., Power, M., 2015. Total mercury concentrations in anadromous northern Dolly Varden from the northwestern Canadian Arctic: a historical baseline study. Sci Total Environ 509-510, 154-164.

Tran, L., Reist, J.D., Power, M., 2016. Northern Dolly Varden charr total mercury concentrations: variation by life-history type. Hydrobiologia 783, 159-175.

Trudel, M., Rasmussen, J.B., 1997. Modeling the elimination of mercury by fish. Environ Sci Technol 31, 1716-1722.

USATSDR, 2006. Health Consultation: Polyaromatic Hydrocarbons and Polychlorinated Biphenyls in Fish from the Suqitughneq River. United States Agency for Toxic Substances and Disease Registry.

USATSDR, 2017. Health Consultation: Northeast Cape Formerly Used Defense Site. United States Agency for Toxic Substances and Disease Registry.

USDOI, 2016. Report to Congress: Hazardous Substance Contamination of Alaska Native Claim Settlement Act Lands in Alaska. United States Department of the Interior Bureau of Land Management.

USEPA, 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. United States Environmental Protection Agency Office of Water.

USEPA, 2001. Water Quality Criterion for the Protection of Human Health—Methylmercury. United States Environmental Protection Agency Office of Science and Technology.

USEPA, 2014. Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations. United States Environmental Protection Agency National Health and Nutrition Examination Survey.

Van Oostdam, J., Donaldson, S.G., Feeley, M., Arnold, D., Ayotte, P., Bondy, G., Chan, L., Dewaily, É., Furgal, C.M., Kuhnlein, H., Loring, E., Muckle, G., Myles, E., Receveur, O., Tracy, B., Gill, U., Kalhok, S., 2005. Human health implications of environmental contaminants in Arctic Canada: a review. Sci Total Environ 351-352, 165-246.

Voie, O.A., Johnsen, A., Rossland, H.K., 2002. Why biota still accumulate high levels of PCB after removal of PCB contaminated sediments in a Norwegian fjord. Chemosphere 46, 1367-1372.

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: health implications for indigenous residents. Environ Pollut 234, 279-287.

von Hippel, F.A., Trammell, E.J., Merila, J., Sanders, M.B., Schwarz, T., Postlethwait, J.H., Titus, T.A., Buck, C.L., Katsiadaki, I., 2016. The ninespine stickleback as a model organism in arctic ecotoxicology. Evol Ecol Res 17, 487-504.s

Wada, H., Cristol, D.A., McNabb, F.A., Hopkins, W.A., 2009. Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. Environ Sci Technol 43, 6031-6038.

Wang, Q., Kim, D., Dionysiou, D.D., Sorial, G.A., Timberlake, D., 2004. Sources and remediation for mercury contamination in aquatic systems: a literature review. Environ Pollut 131, 323-336.

Wania, F., Mackay, D., 1993. Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. Ambio 22, 10-18.

Wassermann, M., Wassermann, D., Cucos, S., Miller, H.J., 1979. World PCBs map: storage and effects in man and his biologic environment in the 1970s. Ann N Y Acad Sci 320, 69-124.

Welfinger-Smith, G., Minholz, J.L., Byrne, S., Waghiyi, V., Gologergen, J., Kava, J., Apatiki, M., Ungott, E., Miller, P.K., Arnason, J.G., Carpenter, D.O., 2011. Organochlorine and metal contaminants in traditional foods from St. Lawrence Island, Alaska. J Toxicol Environ Health A 74, 1195-1214.

Williams, P., Cravez, P., 2018. Environmental justice: challenges of contaminated site cleanup in rural Alaska. Alaska Justice Forum 35.

Wurgler, F.E., Kramers, P.G., 1992. Environmental effects of genotoxins (eco-genotoxicology). Mutagenesis 7, 321-327.

Yano, A., Nicol, B., Jouanno, E., Quillet, E., Fostier, A., Guyomard, R., Guiguen, Y., 2013. The sexually dimorphic on the Y-chromosome gene (sdY) is a conserved male-specific Y-chromosome sequence in many salmonids. Evol Appl 6, 486-496.

Zheng, G., Miller, P., von Hippel, F.A., Buck, C.L., Carpenter, D.O., Salamova, A., 2020. Legacy and emerging semi-volatile organic compounds in sentinel fish from an arctic formerly used defense site in Alaska. Environ Pollut 259, 113872.

LIST OF TABLES

Table 1. Mean total mercury (Hg) and mean total polychlorinated biphenyl (PCB) concentrations in Dolly Varden (± 1 standard error) collected from four locations over three years at the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. These include sites downstream of the formerly used defense (FUD) site, input sites adjacent to the FUD site, sites upstream of the FUD site, and a reference site in an adjacent watershed.

Location	**Mean [total Hg] µg/g dw**	**Mean [total PCBs] µg/g ww**
Reference	0.052 ± 0.012	0.014 ± 0.003
Downstream	0.127 ± 0.012	0.018 ± 0.004
Input	0.224 ± 0.029	0.029 ± 0.004
Upstream	0.129 ± 0.041	0.032 ± 0.005

LIST OF FIGURES

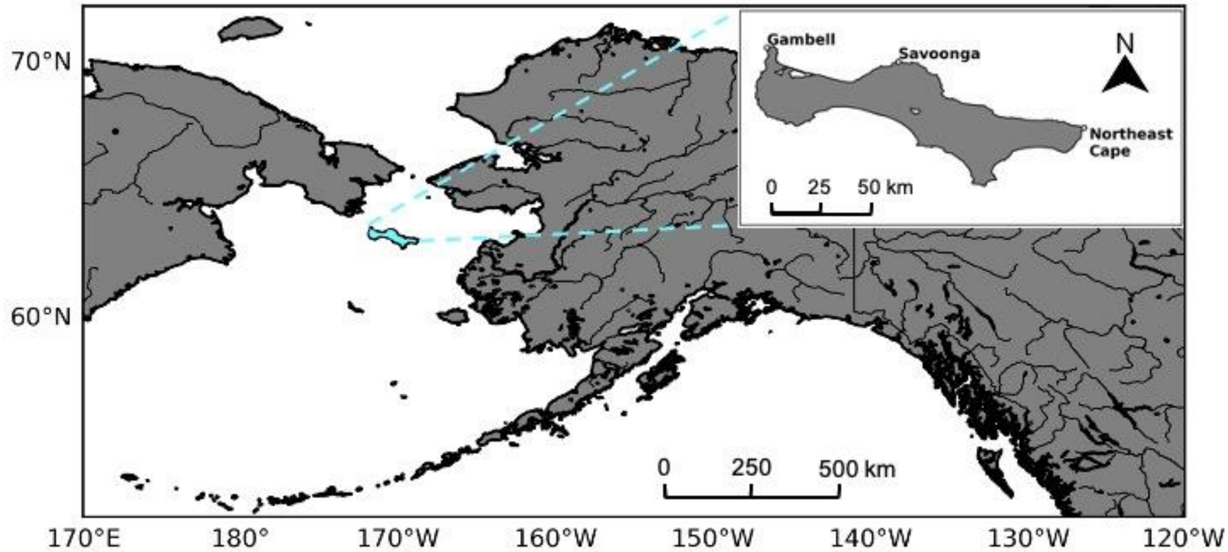


Figure 1. Location of Sivuqaq (St. Lawrence Island), Alaska, and Northeast Cape.

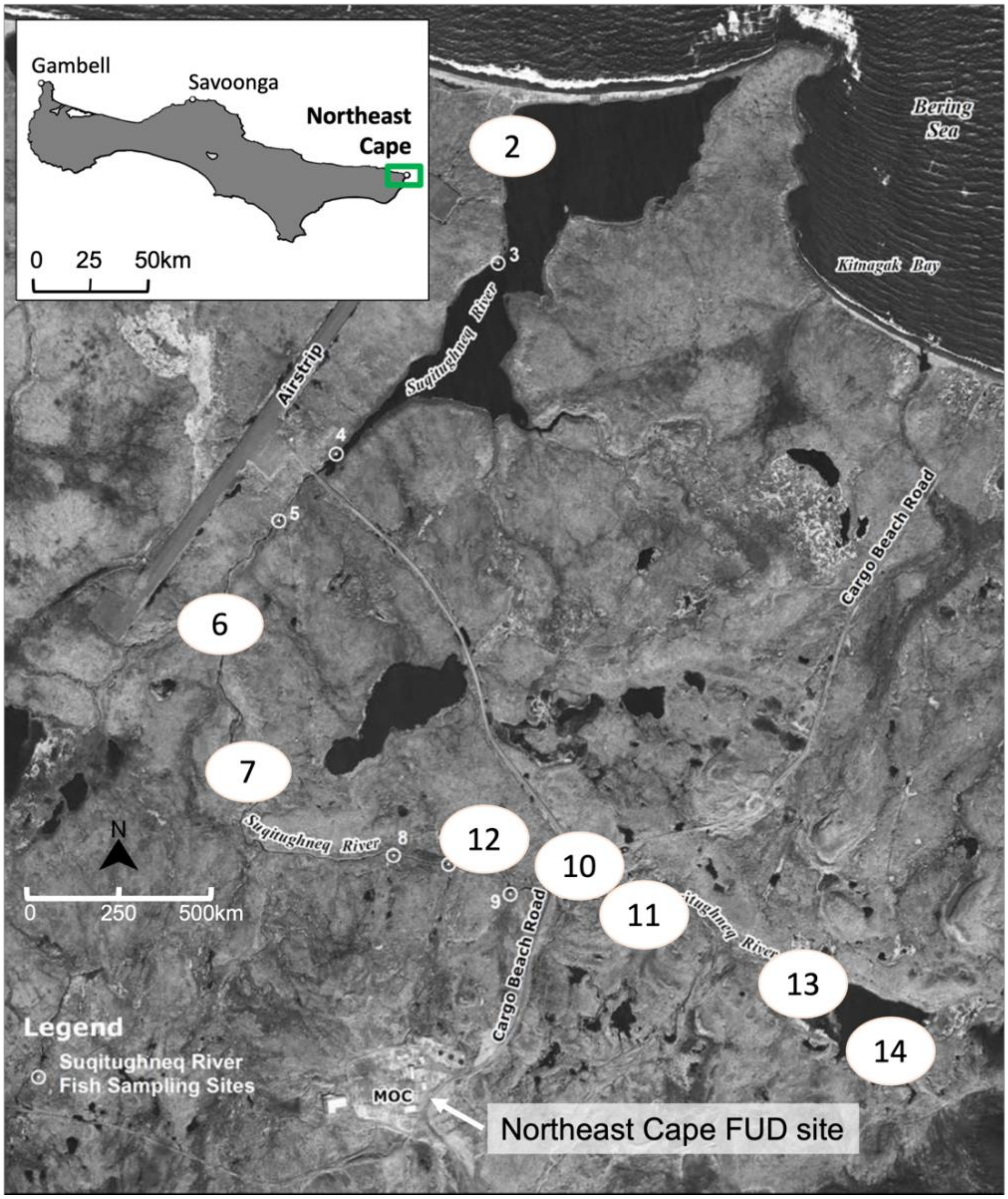


Figure 2. Sampling site locations along the Suqitughneq (Suqi) River on the Northeast Cape of Sivuqaq (St. Lawrence Island), Alaska. Site numbers are long-term monitoring sites. Sites 2, 6, and 7 are designated as downstream sites because they are located downstream of the formerly used defense (FUD) site. Sites 10, 11, and 12 are designated as input sites because they receive direct input from the FUD site. Sites 13 and 14 are designated as upstream sites due to their location upstream of the FUD site. Fish were also collected from the Tapisaggak (Tapi) River in an adjacent watershed (not pictured), which serves as a reference site.

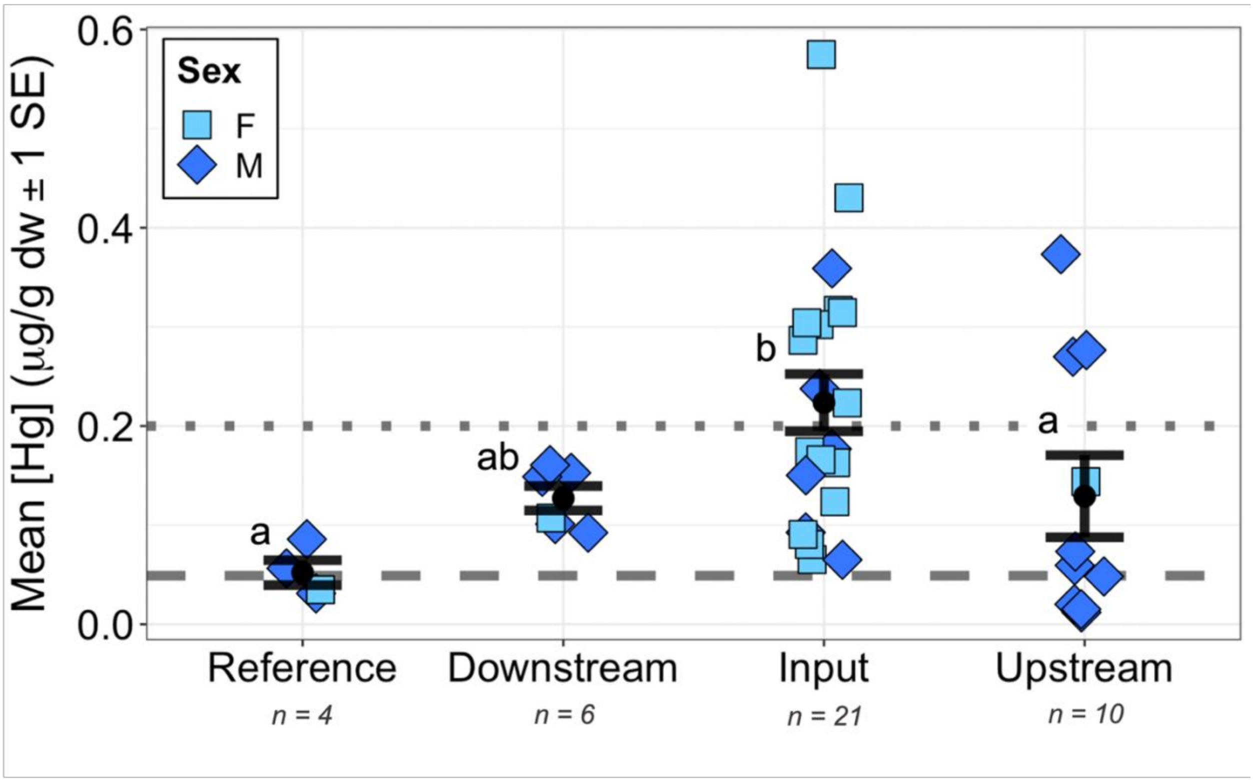


Figure 3. Total mercury (Hg) concentration in Dolly Varden collected from four locations over three years at the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. These include sites downstream of the formerly used defense (FUD) site, input sites adjacent to the FUD site, sites upstream of the FUD site, and a reference site in an adjacent watershed. Locations with different letters significantly differ in total Hg concentration ($p<0.05$). A one-way ANOVA suggests that fish collected at input sites had significantly higher total Hg concentration than did fish collected at reference and upstream sites ($p=0.015$ and $p=0.014$, respectively). The dashed horizontal line indicates the U.S. EPA screening level of 0.049 µg/g dw for unlimited fish consumption for subsistence fishers using a drying cooking method. The dotted horizontal line indicates the U.S. EPA screening level of 0.2 µg/g dw for unlimited fish consumption for subsistence fishers using a non-drying cooking method.

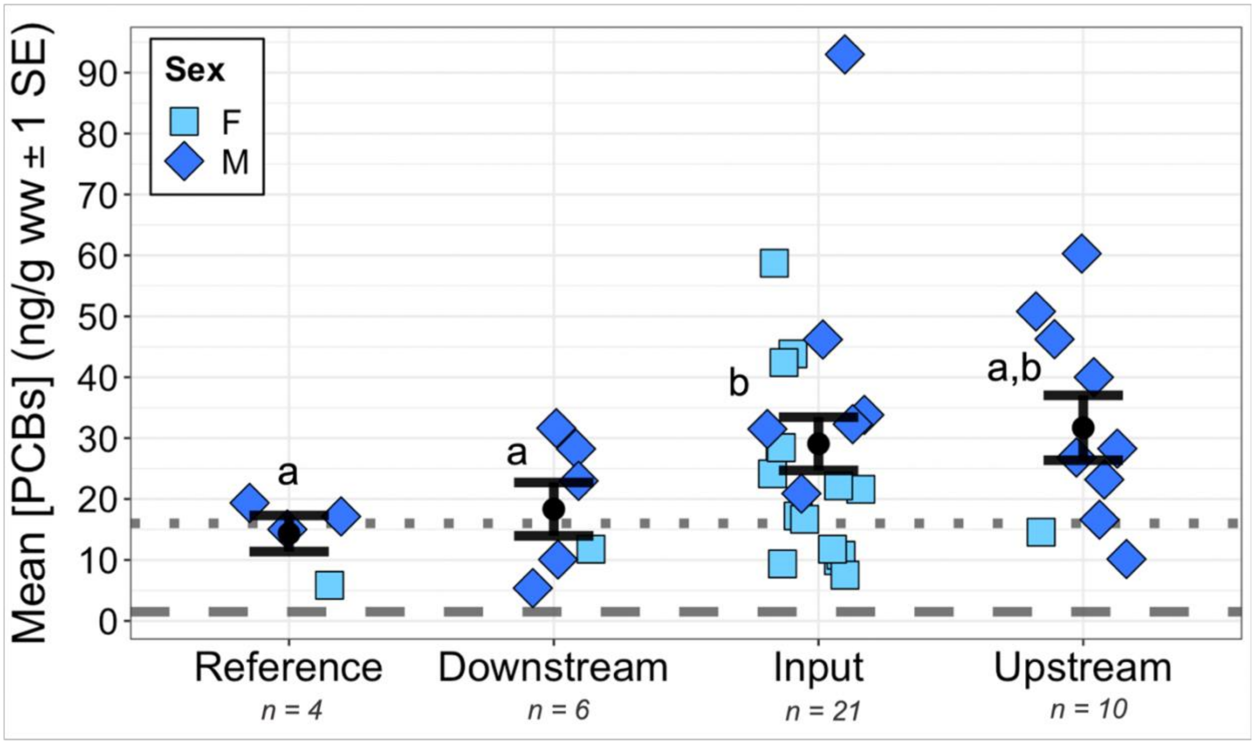


Figure 4. Total PCB concentration in Dolly Varden collected from four locations over three years at the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. Locations with different letters significantly differ in total PCB concentration ($p<0.05$). A two-way ANOVA with location and sex as fixed factors suggests that fish collected at input sites had significantly higher total PCB concentration than did fish collected at reference and downstream sites ($p=0.043$ for both comparisons). The dashed horizontal line indicates the U.S. EPA screening level for unlimited fish consumption for PCBs (cancer risk for human consumption; 0.0015 µg/g ww). The dotted horizontal line indicates the U.S. EPA screening level for three servings (8oz) of fish per month (cancer risk for human consumption; 0.016 µg/g ww).

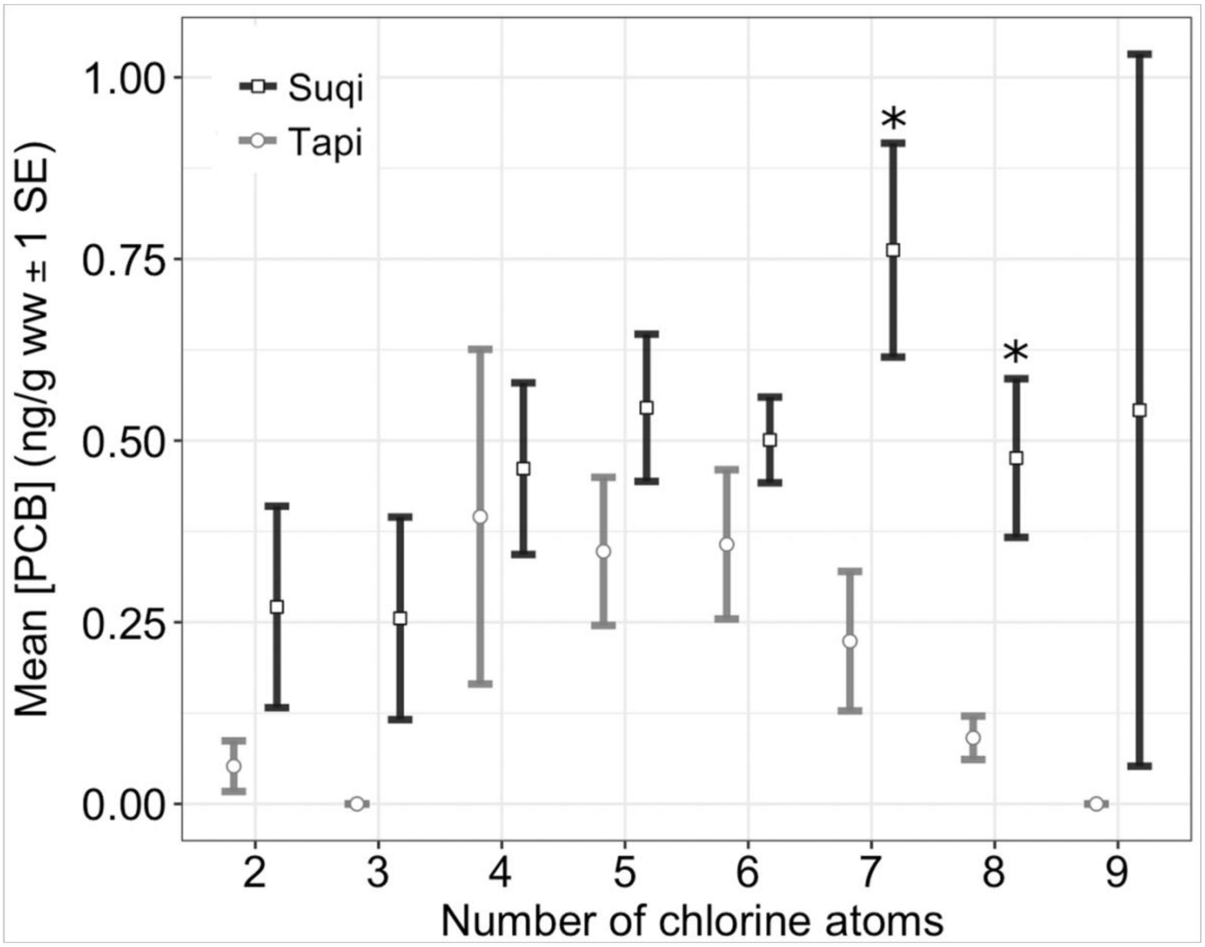


Figure 5. Differences in PCB concentration, based on the number of chlorine atoms present on the biphenyl ring, in Dolly Varden collected from two rivers on the Northeast Cape of Sivuqaq (St. Lawrence Island), Alaska. The Suqitughneq (Suqi) River (black square; n=37 fish) receives inflow from the Northeast Cape formerly used defense (FUD) site. The Tapisaggak (Tapi) River (gray circle; n=4) is located in an adjacent watershed and serves as a reference site. Lightly- to intermediately- chlorinated PCBs (those with 2-6 chlorine atoms) did not differ significantly between Suqi River and Tapi River Dolly Varden, though the means were always lower in the Tapi River. *Hepta*- and *octa*- chlorinated congeners were significantly higher in Suqi River Dolly Varden than in Tapi River Dolly Varden (Kruskal-Wallis χ^2=7.48, p=0.006 and χ^2=5.78, p=0.016, respectively), suggesting that the Northeast Cape FUD site is a point source of PCBs.

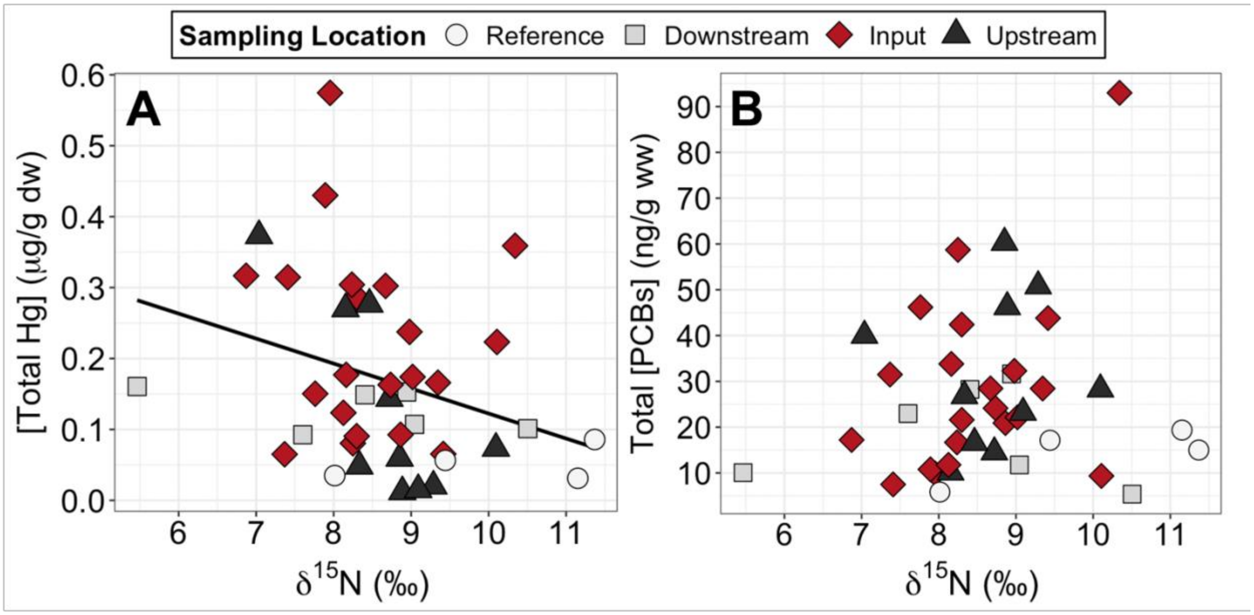


Figure 6. δ^{15}N stable isotope signature plotted against A) total Hg concentration and B) total PCB concentration in Dolly Varden collected from the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. Color and shape indicate sampling location: sites downstream of the formerly used defense (FUD) site (medium gray square), input sites adjacent to the FUD site (red diamond), upstream sites (black triangle), and reference sites (light gray circle). We found a significant negative relationship between δ^{15}N and total Hg concentration when input sites were included (Pearson correlation; r=-0.31, n=41, p=0.049). This relationship was stronger when input sites were removed from the analysis, but no longer significant (Pearson correlation; r=-0.36, n=20, p=0.116), likely due to the loss of statistical power. We did not find a significant correlation between δ^{15}N and total PCB concentration.

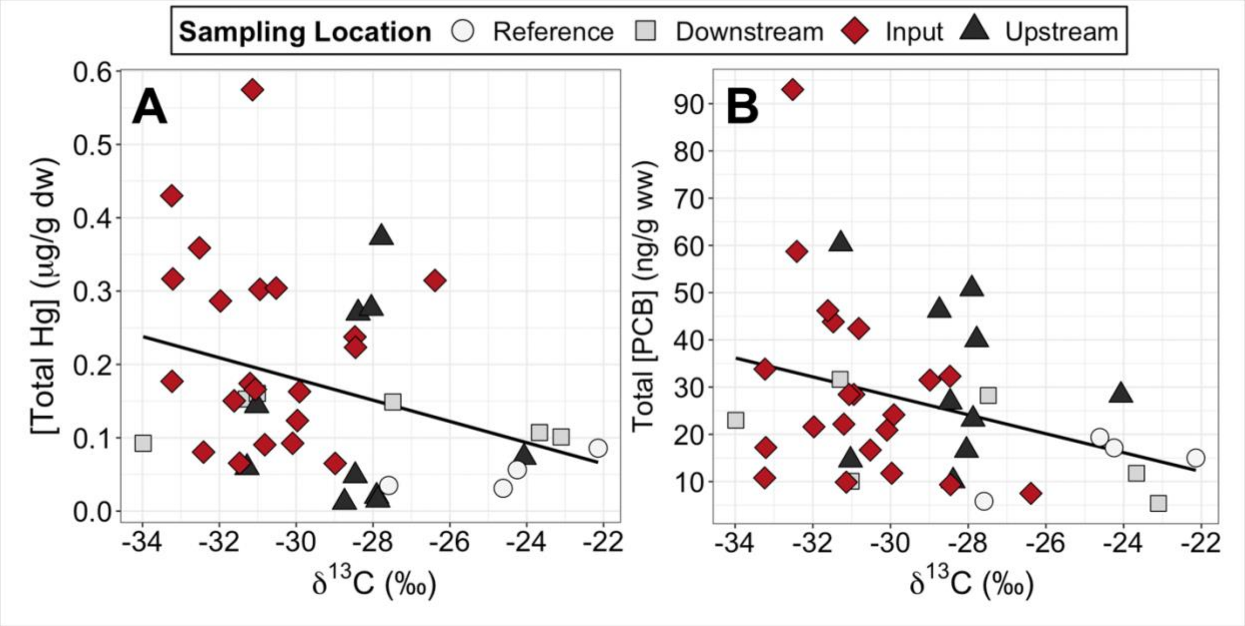


Figure 7. $\delta^{13}C$ stable isotope signature plotted against A) total Hg concentration and B) total PCB concentration in Dolly Varden collected from the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. Color and shape indicate sampling location: sites downstream of the formerly used defense (FUD) site (medium gray square), input sites adjacent to the FUD site (red diamond), upstream sites (black triangle), and reference sites (light gray circle). We found significant negative relationships between $\delta^{13}C$ and both total Hg concentration (Pearson correlation, r=-0.35, n=41, p=0.026) and total PCB concentration (r=-0.36, n=41, p=0.021) when input sites were included. However, this trend did not hold when input sites were removed from the analysis (Hg: r=-0.11, n= 20, p=0.634; PCB: Pearson r=-0.32, n=20, p=0.162).

LIST OF SUPPLEMENTARY MATERIALS

Supplemental Table 1. Total Hg and total PCB concentrations in Dolly Varden collected over three years in the Suqi and Tapi Rivers at the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. Total Hg concentrations are reported in both dry weight (dw) and wet weight (ww). Total PCB concentrations are reported in both ww and lipid weight (lw).

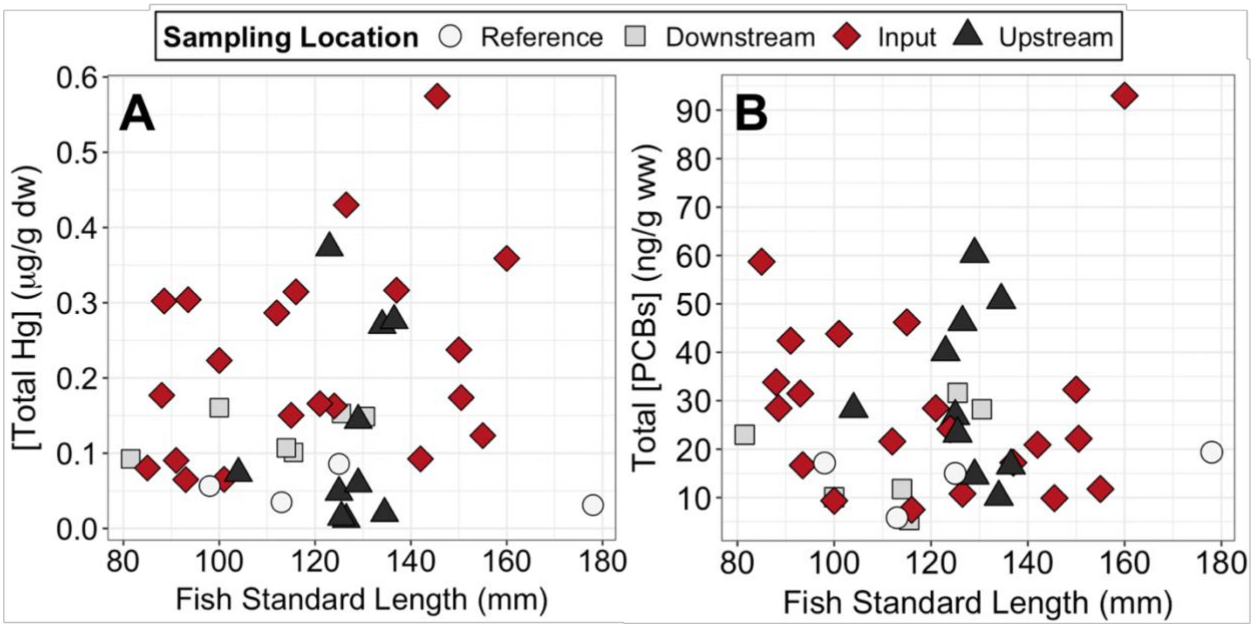
Fish Sample Number	Site Number	Year	[Total Hg] µg/g dw	[Total Hg] µg/g ww *	[Total PCBs] µg/g ww	[Total PCBs] µg/g lw
1	2	2015	0.101	0.024	0.005	1.721
2	6	2015	0.153	0.037	0.032	2.451
3	6	2015	0.149	0.036	0.028	2.372
4	7	2015	0.107	0.026	0.012	0.588
5	7	2015	0.161	0.039	0.010	0.530
6	10	2015	0.317	0.076	0.017	0.501
7	10	2015	0.302	0.073	0.028	0.854
8	11	2015	0.575	0.138	0.010	0.844
9	11	2015	0.430	0.103	0.011	1.528
10	11	2015	0.315	0.075	0.008	0.366
11	11	2015	0.287	0.069	0.022	0.628
12	11	2015	0.304	0.073	0.017	1.161
13	12	2015	0.177	0.042	0.034	1.282
14	13	2015	0.373	0.090	0.040	2.967
15	13	2015	0.048	0.012	0.027	1.059
16	13	2015	0.059	0.014	0.060	3.042
17	13	2015	0.012	0.003	0.046	1.825
18	13	2015	0.144	0.035	0.015	2.036
19	14	2015	0.270	0.065	0.010	0.562
20	14	2015	0.020	0.005	0.051	4.106
21	7	2013	0.092	0.022	0.023	0.709
22	11	2013	0.163	0.039	0.024	1.397
23	11	2013	0.092	0.022	0.021	1.332
24	11	2013	0.065	0.016	0.044	0.891
25	11	2013	0.174	0.042	0.022	0.621
26	11	2013	0.080	0.019	0.059	4.236
27	11	2013	0.166	0.040	0.028	2.178
28	11	2013	0.090	0.022	0.042	2.098
29	11	2013	0.065	0.016	0.031	0.854
30	13	2013	0.015	0.004	0.023	1.392

31	13	2013	0.073	0.018	0.028	1.899
32	13	2013	0.276	0.066	0.017	1.511
33	11	2012	0.359	0.086	0.093	4.324
34	11	2012	0.150	0.036	0.046	0.810
35	11	2012	0.123	0.030	0.012	0.734
36	11	2012	0.238	0.057	0.032	1.486
37	11	2012	0.223	0.054	0.009	0.693
38	Tapi	2012	0.086	0.021	0.015	0.474
39	Tapi	2012	0.031	0.007	0.019	0.801
40	Tapi	2012	0.035	0.008	0.006	0.207
41	Tapi	2012	0.056	0.014	0.017	0.690

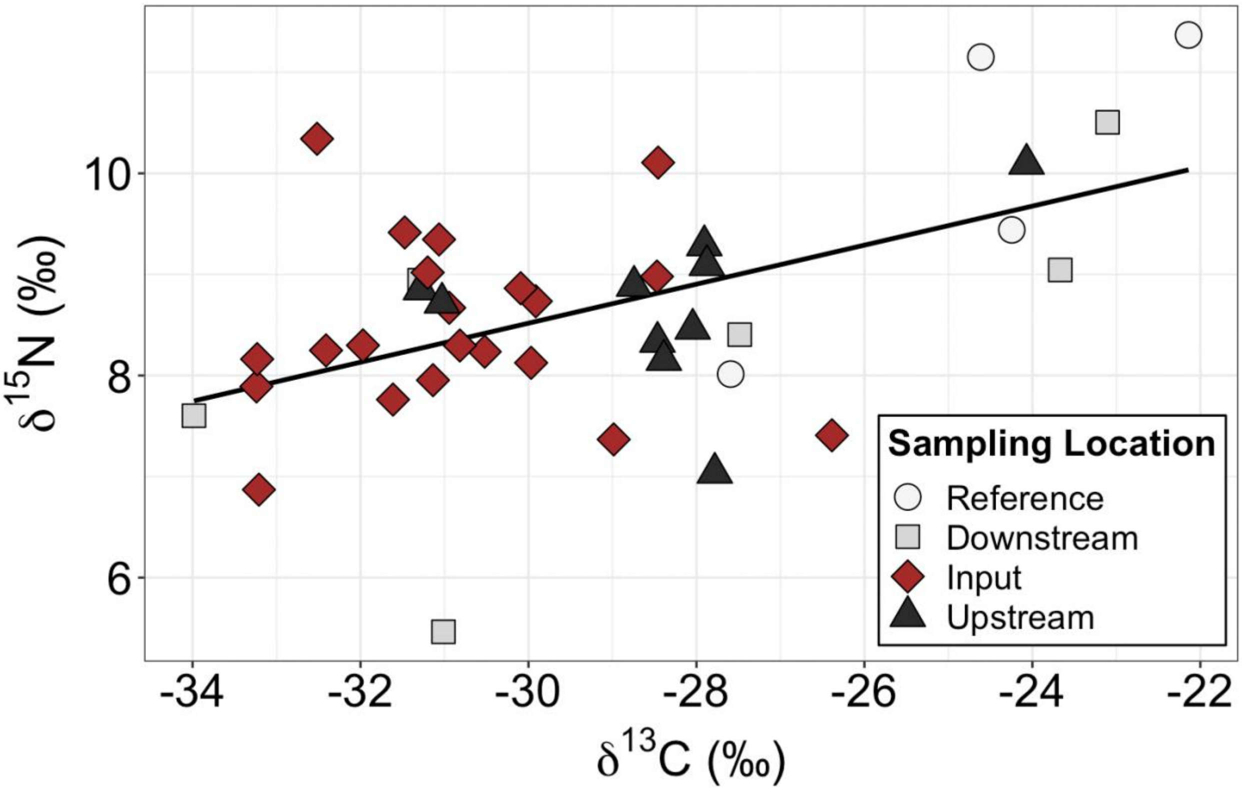
* We converted Hg dry weights to wet weight using the equation reported in Canham et al. (2021) and assuming a mean fish moisture content of 76% (Eagles-Smith and Ackerman, 2014). Wet weight = Dry weight × [1−(76%/100)]

Supplemental Table 2. PCB congener concentrations in Dolly Varden collected from over three years in the Suqi and Tapi Rivers at the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. PCB concentrations are reported in both wet weight (ww) and lipid weight (lw).

Fish Sample Number:	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Fish 13	Fish 14	Fish 15	Fish 16	Fish 17	Fish 18	Fish 19	Fish 20	Fish 21	Fish 22	Fish 23	Fish 24	Fish 25	Fish 26	Fish 27	Fish 28	Fish 29	Fish 30	Fish 31	Fish 32	Fish 33	Fish 34	Fish 35	Fish 36	Fish 37	Fish 38	Fish 39	Fish 40	Fish 41
Site Number:	2	6	6	7	7	10	10	11	11	11	11	11	12	13	13	13	13	13	14	14	7	11	11	11	11	11	11	11	11	13	13	13	11	11	11	11	11	Tapi	Tapi	Tapi	Tapi
Year:	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2015	2013	2013	2013	2013	2013	2013	2013	2013	2013	2013	2013	2013	2012	2012	2012	2012	2012	2012	2012	2012	2012
PCB Congener (ww; ng/g)																																									
4+10																												0.11													
5+8						0.21	0.34							0.15			0.53							0.41		1.66		0.40			0.55							0.38		0.76	
6																								0.36																	
7+9																																									
11																																									
12																																									
13																					3.66			4.17	2.25		1.46	3.41				0.97									
16																																									
17+15																																									
18																																									
19																																									
22																																									
26																																									
28																																									
31																																									
32	0.11							0.02	0.06					0.03				0.10																							
33																					2.27	1.81	2.22	3.64	1.45	5.16	2.02	3.63	3.66	2.15	3.14	1.40									
41+71																																									
42																																									
45																																									
47		0.19	0.17	0.67	0.58			0.13		0.36	0.17	0.08	0.90	0.32	0.88	0.53			0.33	0.48	0.70	0.47	0.49	1.00	0.48	1.28	0.59	0.96	0.76	0.44	0.70	0.31		1.46	0.50	1.40		1.04	0.58		0.38
48																																									
49	0.40	0.41	0.37			0.39	0.64	0.10		0.33			0.93	0.60	0.63	0.97	0.70		0.79	0.79	1.02	0.55	0.54	1.22	0.41	1.22	0.32	1.01	0.94	0.64	0.86	0.34	0.96	0.84	0.52	1.45	0.26	0.73	0.42	0.28	
52								0.17		0.56	0.63		0.64	0.31	0.92		0.47				1.50	1.18	0.90	1.16	0.58	1.92	1.04	1.45	2.01	0.85	1.78	0.75	0.55	0.83	0.47	1.67			0.63		
53																0.02																		0.07	0.02						
56+60				0.68	0.59	0.81	1.33	0.08				0.28	0.99	0.77	0.92	2.06				1.37	0.78	0.59	1.06	1.42	0.63	1.67	0.70	1.42	1.59	0.66	0.78	0.56	1.24	0.77					0.65		
64	1.88	2.76	2.47	4.16	3.57	3.07	5.06	2.61	2.06	3.87	3.57	3.11	3.74	2.85	5.69	2.16	1.93	2.04	4.50	3.99													8.43	5.25	3.51	9.65	5.59	5.10	3.02	4.16	4.53
66						0.12	0.21						0.17								0.56	0.54	0.49	0.80	0.46	1.68	0.55	0.76	0.98	0.60	0.76	0.43									
70+76																																							0.49		
74								0.01				0.05																													
77		0.23	0.21	1.21	1.04									0.76	1.80	1.43				0.77	0.81	0.29	0.74	0.60			0.63	1.65	1.78	0.10			0.68								
81																																									
83																																									
84+92											0.53		0.73	0.40		0.87		0.54				0.43	0.50	0.46	0.65	0.90			0.60	0.28	0.65	0.46		0.82					0.67		0.52
85		0.29	0.26					0.20	0.54			0.20	0.60	0.66	0.54	1.05	0.46	0.43		1.02	0.32	0.33	0.44	0.76	0.30	1.23	0.25	0.72	0.73	0.31	0.31	0.33	0.63	0.60	0.33			0.38	0.34		0.74
87		0.70	0.63			0.39	0.64	0.10			0.47	0.35	0.95	1.09	0.90	1.77	0.72	0.72		1.40	0.40	0.54	0.42	0.83	0.54	1.32	0.39	1.14	0.65	0.35	0.54	0.44	1.19	0.78	0.52			0.22	0.21		
89														0.76		1.84	0.80			1.28	0.67	0.69	0.60	1.08	0.58	1.35	0.56	1.14	0.91	0.40	0.50	0.35							0.82		0.89
91																																									
95	0.20	0.72	0.65	0.45	0.39	0.49	0.81	0.24		0.34	0.60	0.51	1.25	0.89	0.55	1.55	0.80	0.36	0.65	1.11	0.80	0.94	0.58	1.34	0.99	1.90	0.63	1.31	1.07	0.76	1.03	0.52	1.16	1.25	0.52	1.22	0.47	0.55	0.60	0.31	0.49
97		0.43	0.39	0.55	0.47						0.59		0.70	0.85	0.64	1.67	0.37	0.49		1.31	0.48	0.50	0.38	1.07	0.63	1.12	0.38	1.09	0.64	0.43	0.55	0.24	0.76	0.55	0.28	0.80		0.39	0.30		
99																																									
100																																									
101		2.11	1.89			1.54	2.54	0.38			2.90	1.34	3.17	2.65	1.75	4.02	2.61	1.39		3.78	1.59	2.79	1.63	2.71	2.18	4.49	1.92	2.85	2.29	1.78	2.40	1.44	5.86	3.70		3.17			2.23		1.42
105+132+153	0.33	4.56	4.07	0.78	0.67	2.59	4.28	1.26	1.72	0.44	2.64	2.37	4.08	3.31	2.20	2.77	5.53	1.74	0.88	3.53	1.59	2.97	2.12	4.01	2.09	7.37	3.60	4.35	2.85	2.65	2.99	1.67	13.08	6.34	1.07	2.61	0.82	2.40	2.88	0.33	3.29
110	0.29	0.70	0.62	0.49	0.42	0.39	0.65	0.27	0.49		0.52	0.50	1.66	1.13	0.62	1.94	0.75	0.81		1.81	0.49	0.77	0.49	0.91	0.76	1.83	0.57	0.92	0.54	0.23	0.73	0.45	1.40	1.45	0.35	0.90			0.28		
114																0.03							0.03			0.04							0.68								
118	0.12	0.33	0.30	0.29	0.25	0.30	0.50	0.14	0.28		0.36	0.22	0.75	0.53	0.35	0.84	0.41	0.34		0.67	0.25	0.29	0.31	0.34	0.36	0.86	0.26	0.40	0.35	0.19	0.37	0.22	0.80	0.59	0.18	0.42		0.40	0.50		0.70
119																																									
123+149		2.24	1.99	0.32	0.27	0.92	1.57	0.54	0.61	0.34	1.01	0.98	1.66	1.42	0.84	1.30	2.59	0.50	0.62	1.31	0.71	1.28	0.86	2.00	0.88	3.03	1.53	2.31	1.13	1.57	1.23	0.89	5.25	3.18	0.55	1.25	0.35	0.43	0.48		0.55
126	0.28													3.61		1.94	10.61			1.55																					
128		0.28	0.25			0.24	0.40	0.04			0.22	0.13	0.33	0.17	0.14		0.32	0.19			0.07	0.11	0.10	0.63	0.11	0.39	0.13	0.41	0.20	0.11	0.17	0.09	2.11	0.98				0.37	0.23		0.21
131		0.44	0.39	0.10	0.09	0.10	0.17	0.19	0.30	0.09	0.33	0.31	0.49	0.60	0.76	0.27	0.19	1.12	0.08	0.23	0.23	0.25	0.31	0.19	0.56	0.39	0.11	0.19	0.16	0.09	0.50	0.29	0.40	0.35	0.22	0.98	0.18		0.10		
135+144		0.53	0.47			0.22	0.37	0.07			0.28	0.23	0.36	0.37	0.20	0.48	0.53	0.15		0.37	0.17	0.25	0.13	0.36	0.24	0.62	0.31	0.53	0.34	0.30	0.15	0.15	0.90	0.50		0.53			0.10		
156	0.08	0.15	0.13	0.13	0.11	0.10	0.17	0.05	0.11		0.15	0.09	0.15	0.23	0.08	0.67	0.06			0.48		0.09	0.13	0.16	0.09	0.27	0.12	0.17	0.12	0.08	0.09	0.07	0.62	0.26					0.06		
163+138	0.15	3.55	3.17	0.50	0.43	1.63	2.69	0.88	1.24	0.34	1.72	1.58	2.81	2.21	1.34	1.58	4.13	1.08	0.59	2.08	0.96	1.82	1.42	2.78	1.28	4.65	2.38	2.71	1.82	1.79	1.91	1.14	9.78	4.38	0.69	1.34	0.65	1.60	1.68		2.12
167													0.18	0.57		1.80	0.19			1.50		0.09				0.39	0.10		0.09	0.07	0.08			0.24							
169								0.15		0.50	0.34		0.16	0.25	0.29	0.47	0.19			0.13	0.27	0.18	0.34	0.51	0.27	0.62	0.18	0.44	0.42	0.24	0.39	0.18	0.81	0.22	0.20	0.64			0.23		
170+190	0.22	2.13	1.90	0.13	0.11	0.76	1.25	0.40	0.57		0.96	0.88	1.23	1.33	1.17	0.93	2.43	0.49	0.26	1.24	0.44	0.77	0.59	1.75	0.68	2.12	1.42	1.23	1.07	0.95	0.98	0.61	7.39	1.89	0.41	0.87	0.27	0.41	0.41		0.25
172		0.35	0.31			0.17	0.28	0.08	0.18		0.18	0.13	0.28	0.15	0.17		0.28	0.13		4.39	0.09	0.16	0.15	0.29	0.11	0.40	0.24	0.25	0.17	0.18	0.18	0.09	1.15	0.41	0.08						
174	0.11	1.56	1.39	0.24	0.21	0.45	0.74	0.27	0.43		0.70	0.54	0.80	1.09	0.60	1.18	1.94	0.29	0.19	1.46	0.35	0.67	0.49	1.23	0.48	1.63	1.14	1.25	0.63	1.15	0.69	0.49	4.41	1.54	0.24	0.77	0.20		0.24		
180	0.05	4.29	3.83	0.61	0.53	1.58	2.60	0.83	1.17	0.17	1.64	1.66	2.68	1.99	1.71	1.00	3.80	1.00	0.63	1.84	0.76	1.78	1.32	3.08	1.06	4.65	3.27	2.73	1.68	2.31	1.91	0.99	14.67	4.63	0.66	1.34	0.56	0.60	0.61		0.81
194		0.96	0.86	0.19	0.16	0.27	0.45	0.16	0.28		0.48	0.31	0.55	0.44	0.41		0.98	0.28	0.14	0.96	0.41	0.29	0.28	0.72	0.26	0.81	0.52	0.43	0.28	0.43	0.37	0.20	3.05	0.75	0.17	0.48			0.27		0.10
199	0.67	0.09	0.08	0.24	0.20			0.05				0.17	0.04	1.23		4.15	0.10			2.76	0.11		0.13	0.29	0.08	0.17	0.11	0.12	0.15	0.12	0.16	0.06	0.36	0.21					0.09		
201		0.69	0.61			0.21	0.35	0.23	0.27	0.16	0.39	0.39	0.47	0.51	0.44	0.36	0.93	0.21	0.15	0.51	0.37	0.41	0.38	0.85	0.42	0.92	0.48	0.55	0.61	0.50	0.45	0.28	2.64	0.75	0.18	0.45			0.10		
202+171	0.49	0.77	0.69			0.26	0.43	0.17	0.35		0.22	0.28	0.33	1.17	0.28	2.82	0.88	0.15	0.13	1.95	0.13	0.25	0.23	0.52	0.18	0.65	0.38	0.37	0.28	0.38	0.30	0.16	2.07	0.61	0.09	0.33			0.15		0.16
205														0.84		2.90				0.95																					
206		0.16	0.15					0.03	0.13					3.75		12.94			0.21	3.79		0.08	0.10	0.18	0.12		0.10			0.09	0.08	0.06									
207																																									
TOTAL (ww)	**5.38**	**31.63**	**28.24**	**11.76**	**10.07**	**17.21**	**28.46**	**9.88**	**10.79**	**7.50**	**21.60**	**16.68**	**33.81**	**40.01**	**26.82**	**60.30**	**46.24**	**14.55**	**10.15**	**50.80**	**22.98**	**24.14**	**20.90**	**43.81**	**22.16**	**58.72**	**28.44**	**42.41**	**31.49**	**23.20**	**28.28**	**16.61**	**93.00**	**46.19**	**11.77**	**32.30**	**9.35**	**15.01**	**19.36**	**5.84**	**17.14**
PCB Congener (lw; ng/g)																																									
4+10																												5													
5+8						6	10							11			21							8		120		20			37							12		27	
6																								7																	
7+9																																									
11																																									
12																																									
13																					113			85	63		112	169				88									
16																																									
17+15																																									
18																																									
19																																									
22																																									
26																																									
28																																									
31																																									
32	34							1	9					2				14																							
33																					70	105	142	74	41	372	155	180	99	129	211	127									
41+71																																									
42																																									
45																																									
47		15	14	34	30			11		17	5	5	34	24	35	27			18	39	22	27	31	20	14	93	45	47	21	27	47	28		26	31	64		33	24		15
48																																									
49	128	32	31			11	19	9		16			35	45	25	49	28		44	64	32	32	34	25	12	88	25	50	25	39	58	31	44	15	32	67	19	23	17	10	
52								15		27	18		24	23	36		18				46	68	57	24	16	138	80	72	55	51	120	68	26	15	29	77			26		
53																1																		1	1						
56+60				34	31	23	40	7				20	38	57	36	104				110	24	34	68	29	18	120	54	70	43	39	52	51	57	13					27		
64	602	214	207	208	188	89	152	223	291	189	104	217	142	212	225	109	76	286	249	323													392	92	219	444	414	161	125	147	182
66						4	6						6								17	31	32	16	13	121	42	38	26	36	51	39									
70+76																																							20		
74								1				3																													
77		18	17	60	55									56	71	72				62	25	17	47	12			48	81	48	6			32								
81																																									
83																																									
84+92											15		28	30		44		76				25	32	9	18	65			16	17	44	42		14					28		21
85		22	22					17	76			14	23	49	21	53	18	61		82	10	19	28	15	8	89	19	36	20	19	21	30	29	11	21			12	14		30
87		55	53			11	19	9			14	24	36	81	35	89	28	100		113	12	31	27	17	15	95	30	57	18	21	36	40	55	14	32			7	8		
89														56		93	31			103	21	40	38	22	16	97	43	56	25	24	33	32							34		36
91																																									
95	64	56	54	23	20	14	24	21		16	17	35	48	66	22	78	32	50	36	90	25	54	37	27	28	137	48	65	29	46	69	47	54	22	33	56	35	17	25	11	20
97		34	32	27	25						17		27	63	25	84	15	69		106	15	29	24	22	18	81	29	54	17	26	37	22	35	10	18	37		12	12		
99																																									
100																																									
101		164	158			45	76	33			84	93	120	196	69	203	103	195		305	49	161	104	55	61	324	147	141	62	107	161	131	273	65		146			92		57
105+132+153	107	353	342	39	35	76	128	108	244	21	77	165	155	245	87	140	218	243	49	285	49	172	135	82	59	531	276	215	77	159	200	151	608	111	67	120	61	76	119	12	133
110	91	54	52	24	22	11	19	23	70		15	35	63	84	25	98	30	113		146	15	45	31	19	21	132	43	45	15	14	49	41	65	25	22	42			12		
114																1							2			3							32								
118	40	26	25	15	13	9	15	12	39		10	15	29	39	14	43	16	47		54	8	17	20	7	10	62	20	20	9	12	25	20	37	10	11	19		13	21		28
119																																									
123+149		174	167	16	14	27	47	46	87	17	29	68	63	106	33	65	102	70	34	106	22	74	55	41	25	219	117	114	31	94	83	81	244	56	34	57	26	14	20		22
126	89													268		98	419			125																					
128		22	21			7	12	3			6	9	13	13	6		13	26			2	6	6	13	3	28	10	20	5	7	12	8	98	17				12	10		8
131		34	33	5	5	3	5	17	42	4	10	22	19	44	30	14	8	157	4	19	7	14	20	4	16	28	9	10	4	5	33	26	19	6	14	45	13		4		
135+144		41	40			7	11	6			8	16	14	28	8	24	21	22		30	5	14	8	7	7	45	23	26	9	18	10	14	42	9		24			4		
156	25	12	11	7	6	3	5	5	15		4	6	6	17	3	34	3			39		5	8	3	3	19	9	8	3	5	6	6	29	5					2		
163+138	46	275	266	25	23	47	81	75	176	16	50	110	107	164	53	80	163	151	33	168	29	105	90	57	36	335	183	134	49	107	128	103	455	77	43	62	48	50	70		85
167													7	43		91	7			122		5				28	8		2	4	5			4							
169								13		24	10		6	18	11	24	8			11	8	10	22	10	8	44	14	22	11	14	26	17	38	4	13	30			10		
170+190	71	165	160	7	6	22	37	34	81		28	61	47	99	46	47	96	69	15	101	14	45	38	36	19	153	109	61	29	57	66	56	344	33	26	40	20	13	17		10
172		27	26			5	8	7	25		5	9	11	11	7		11	18		355	3	9	9	6	3	29	18	12	5	11	12	8	54	7	5						
174	36	121	117	12	11	13	22	23	61		20	38	30	81	24	60	77	40	10	118	11	39	31	25	13	118	88	62	17	69	46	44	205	27	15	36	15		10		
180	15	332	322	31	28	46	78	71	166	8	48	116	102	148	67	51	150	140	35	149	23	103	84	63	30	336	251	135	46	139	128	90	682	81	41	62	41	19	25		33
194		75	72	9	9	8	13	14	39		14	22	21	33	16		39	40	8	77	13	17	18	15	7	58	39	21	7	26	25	18	142	13	10	22			11		4
199	215	7	7	12	11			4				11	2	91		210	4			223	3		8	6	2	12	8	6	4	7	11	5	17	4					4		
201		53	51			6	10	20	39	8	11	27	18	38	17	18	37	29	8	41	11	24	24	17	12	67	37	27	17	30	30	26	123	13	11	21			4		
202+171	156	60	58			8	13	15	49		6	20	13	86	11	142	35	21	7	158	4	14	15	11	5	47	29	18	7	23	20	15	96	11	6	15			6		6
205														62		146				77																					
206		13	12					3	18					278		653			12	306		5	7	4	3		8			5	5	6									
207																																									
TOTAL (lw)	**1721**	**2451**	**2372**	**588**	**530**	**501**	**854**	**844**	**1528**	**366**	**628**	**1161**	**1282**	**2967**	**1059**	**3042**	**1825**	**2036**	**562**	**4106**	**709**	**1397**	**1332**	**891**	**621**	**4236**	**2178**	**2098**	**854**	**1392**	**1899**	**1511**	**4324**	**810**	**734**	**1486**	**693**	**474**	**801**	**207**	**690**



Supplemental Figure 1. Fish standard length plotted against A) total Hg concentration and B) total PCB concentration in Dolly Varden collected from the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. Color and shape indicate sampling location: sites downstream of the formerly used defense (FUD) site (medium gray square), input sites adjacent to the FUD site (red diamond), upstream sites (black triangle), and reference sites (light gray circle). Fish standard length did not correlate with either total Hg concentration (Pearson correlation; r=0.03, n=41, p=0.843) or total PCB concentration (Pearson correlation; r=-0.03, n=41, p=0.876), and thus we excluded standard length from the models.



Supplemental Figure 2. $\delta^{13}C$ stable isotope signature plotted against $\delta^{15}N$ stable isotope signature in Dolly Varden collected from the Northeast Cape on Sivuqaq (St. Lawrence Island), Alaska. Color and shape indicate sampling location: sites downstream of the formerly used defense (FUD) site (medium gray square), input sites adjacent to the FUD site (red diamond), upstream sites (black triangle), and reference sites (light gray circle). We found a significant positive relationship between $\delta^{13}C$ and $\delta^{15}N$ when input sites were included (Pearson correlation; r=0.51, n=41, p<0.001) as well as when we removed input sites the analysis (Pearson correlation; r=0.69, n=20, p<0.001).

CHAPTER 3: Transcriptomic and developmental effects of persistent organic pollutants in sentinel fishes collected near an arctic formerly used defense site

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KEYWORDS

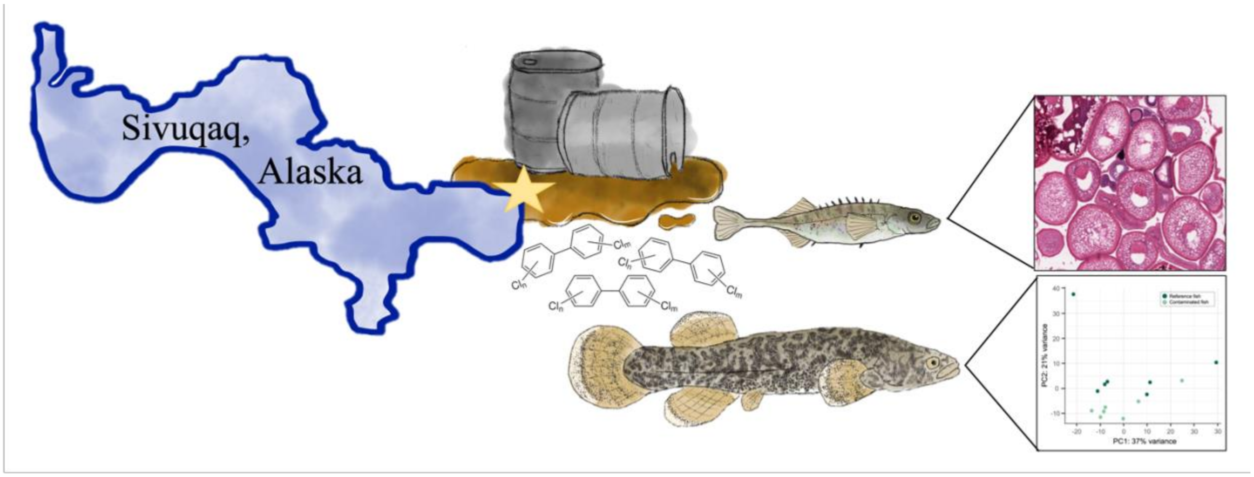
Alaska, Endocrine-disrupting compounds, FUD site, Military contamination, POPs, Ninespine stickleback, Sivuqaq (St. Lawrence Island), Yupik

HIGHLIGHTS

- Residents of Sivuqaq, Alaska live a subsistence lifestyle including at Northeast Cape (NEC)

- Pollution from the NEC formerly used defense (FUD) site negatively affects native fishes
- Fish exposed to FUD site pollution overexpressed genes involved in ribosomal and FoxO pathways
- Thyroid follicle counts were higher in fish exposed to NEC FUD site pollution

GRAPHICAL ABSTRACT



ABSTRACT

Alaska contains over 600 formerly used defense (FUD) sites, many of which serve as point sources of pollution to arctic environments. These sites are often co-located with rural communities that live a subsistence lifestyle and depend on lipid-rich foods that bioaccumulate and biomagnify persistent organic pollutants (POPs). Many POPs are carcinogenic and endocrine disrupting compounds that are associated with various adverse health outcomes in humans. As a result, elevated exposure to POPs from point sources of pollution may contribute to disproportionate incidence of disease in rural communities. We investigated the health implications of POP exposure in sentinel fishes collected near the Northeast Cape FUD site on Sivuqaq (St. Lawrence Island), Alaska. Sivuqaq residents are predominantly Siberian Yupik and rely heavily on subsistence foods. At the request of the Sivuqaq community, we examined differential gene expression and developmental pathologies associated with exposure to POPs originating at the Northeast Cape FUD site. We found that Alaska blackfish (*Dallia pectoralis*) from contaminated sites overexpressed genes involved in ribosomal and FoxO signaling pathways compared to blackfish from reference sites. We found that ninespine stickleback (*Pungitius pungitius*) from contaminated sites exhibited thyroid follicle hyperplasia. Despite previous research reporting transcriptomic and endocrine differences in stickleback from contaminated versus reference sites, we did not find significant differences in kidney or gonadal histomorphological measurements. Although the present study was constrained by small sample sizes, our results demonstrate that contaminant input from the Northeast Cape FUD site alters gene expression and thyroid development in native fishes. Future work is needed to elucidate developmental pathologies associated with exposure to FUD site contamination at Northeast Cape.

INTRODUCTION

Point sources of pollution are a growing concern for arctic communities. The Arctic contains thousands of contaminated sites that leach persistent organic pollutants (POPs) into the local environment and may contribute to health disparities for Arctic Indigenous Peoples (Miller et al., 2013; von Hippel et al., 2016). Once in the environment, POPs bioaccumulate and biomagnify in the lipid-rich arctic food webs leading to elevated levels of POPs in subsistence foods (Welfinger-Smith et al., 2011). Arctic Indigenous Peoples often rely on long-lived, high trophic level animals for subsistence foods and may be exposed to high levels of POPs through their diet. Indeed, Arctic Indigenous Peoples are among the most highly exposed populations to some POPs (Blais, 2005; Dewailly et al., 1989).

Alaska played an important role in the United States (U.S.) defense plan during World War II (WWII) and the Cold War (Denfeld, 1994). The U.S. military installed hundreds of defense sites across Alaska, including the Distant Early Warning Line, Ballistic Missile Early Warning System, and White Alice Communications Systems (Winkler, 1997). Many of these sites were co-located with villages that provided access to remote areas of strategic importance. However, as radar technology advanced in the 1960s and 1970s, the U.S. military shifted defensive strategies to satellite communication and radar surveillance stations were abandoned. Building materials, wastes, debris, and barrels containing toxic substances from military activity were often left on site without proper containment, resulting in a legacy of environmental contamination (Sepez et al., 2007). Alaska contains over 600 formerly used defense (FUD) sites, many of which continue to serve as point sources of pollution to arctic environments (USDOI, 2016; USEPA, 2004; von Hippel et al., 2016).

The most expensive remediation effort of an Alaskan FUD site to date occurred in the early 2000s on the Northeast Cape of Sivuqaq (St. Lawrence Island), Alaska. Sivuqaq is located in the Bering Sea approximately 200 km west of mainland Alaska and 75 km southeast of the Chukotka Peninsula of Russia (Fig. 1). The U.S. Army Corps of Engineers spent over 100 million dollars to remediate the Northeast Cape FUD site, which served as a White Alice radar surveillance station from 1952-1972 (Miller et al., 2013). However, studies conducted at Northeast Cape both during and after remediation activities reported elevated levels of POPs in fish tissues (Byrne et al., 2015; Byrne et al., 2017; Jordan-Ward et al., 2022; von Hippel et al., 2018) and blood sera of Sivuqaq residents (Byrne et al., 2015; Byrne et al., 2017; Carpenter et al., 2005). Prior to military occupation, Northeast Cape was a village of predominantly Siberian Yupik residents, but the residents of Northeast Cape were relocated to the village of Savoonga during the construction of the defense site (Miller et al., 2013). Although residents no longer reside year-round at Northeast Cape, many residents of Savoonga frequent traditional subsistence hunting and fishing camps at Northeast Cape. Sivuqaq residents are interested in re-establishing the community at Northeast Cape and want to ensure that remaining contamination at the FUD site does not pose a health risk.

The Sivuqaq community is concerned about the health implications posed by exposure to FUD site pollution and report that residents who engage in subsistence activities at Northeast Cape have higher incidence of certain cancers, thyroid disease, and reproductive disorders (Carpenter et al., 2005; Miller et al., 2013). In 2011, the tribal government of Savoonga requested that the Agency for Toxic Substances and Disease Registry (ATSDR) assess health risks posed by the Northeast Cape FUD site. The ATSDR assessment concluded that contaminant levels in fish do not pose a health risk to local residents (USATSDR, 2017). However, the ATSDR conclusion was based on insufficient data for a reliable assessment (Jordan-Ward et al., 2022) and contradicts the results of multiple studies conducted at Northeast Cape (Carpenter et al., 2005; Scrudato et al., 2012; von Hippel et al., 2018). For example, Jordan-Ward et al. (2022) found that both polychlorinated biphenyls (PCBs) and mercury (Hg) concentrations in a subsistence fish collected near the Northeast Cape FUD site exceeded the Environmental Protection Agency’s guidelines for safe consumption (USEPA, 2000, 2014). Furthermore, Carpenter et al. (2005) found that people who subsist at Northeast Cape have elevated concentrations of PCBs in their blood serum. PCBs are carcinogenic and endocrine disrupting compounds that are associated with various adverse health outcomes in humans, including those reported by Sivuqaq residents (Ayotte et al., 2003; Schell et al., 2014; WHO, 2016). High levels of Hg in humans have been linked to low birth weights, cognitive deficiencies, and reproductive impairment (Clarkson et al., 2003; Guallar et al., 2002; Harada, 1995; Oken et al., 2005). Therefore, the Northeast Cape FUD site may negatively affect the health of Sivuqaq residents who consume subsistence foods collected near the Northeast Cape FUD site, despite conclusions reported by the ATSDR.

Extensive research at Northeast Cape shows that the FUD site remains a significant point source of POP and toxic metal pollution (Byrne et al., 2015; Carpenter et al., 2005; Jordan-Ward et al., 2022; Scrudato et al., 2012; von Hippel et al., 2018). Scrudato et al. (2012) found that concentrations of PCBs, dichlorodiphenyldichloroethylene (DDE), Hg, and mirex in sediment cores and plants were elevated at the Northeast Cape FUD site, indicating that this FUD site remains a point source of pollution. These findings led to research by von Hippel et al. (2018) that found elevated concentrations of PCBs in ninespine stickleback (*Pungitius pungitius*; hereafter referred to as stickleback) and Alaska blackfish (*Dallia pectoralis*) collected near the FUD site. Furthermore, von Hippel et al. (2018) found that stickleback from contaminated sites had altered gene expression and thyroid hormone levels, as well as male expression of vitellogenin, which is a biomarker for exposure to estrogenic contaminants. Collectively, these studies demonstrate that exposure to POPs originating at the Northeast Cape FUD site negatively affects health endpoints in native fish species.

The current study utilizes Alaska blackfish and stickleback to examine changes in gene expression and tissue-specific development, respectively, in fish exposed to FUD site contamination. Teleost fishes are useful models for studying the effects of contaminant exposure on human health because they are often exposed to similar contaminant mixtures and provide relevant biomarkers (Tierney et al., 2014; von Hippel et al., 2016). Indeed, stickleback on Sivuqaq share similar contaminant profiles to those observed in blood sera of Sivuqaq residents (Byrne et al., 2015; Byrne et al., 2017). We collected blackfish and stickleback from long-term monitoring sites along the Suqitughneq (Suqi) River and a reference site in the Tapisaggak (Tapi) River on the Northeast Cape (von Hippel et al., 2018). The present study expanded on previous findings in stickleback to elucidate if transcriptomic effects are consistent across sentinel fish species and to test if altered gene expression and endocrine disruption in stickleback lead to developmental pathologies. Because von Hippel et al. (2018) found that gene expression was a sensitive measure in the stickleback, we examined differential gene expression in blackfish to identify transcriptional differences driven by exposure to FUD site contamination. We hypothesized that blackfish from contaminated sites would have decreased expression of genes involved in DNA repair and cell signaling, consistent with results reported in stickleback by von Hippel et al. (2018). We also evaluated histomorphological differences of thyroid, gonad, and kidney tissues in stickleback from contaminated versus reference sites. We hypothesized that stickleback from contaminated sites would exhibit developmental pathologies consistent with altered gene expression and endocrine function in response to POP exposure.

MATERIALS & METHODS

Fish collection and processing

We collected ninespine stickleback and Alaska blackfish from the Tapi River (reference site) and from 12 long-term monitoring sites along the Suqi River (von Hippel et al., 2018) in June and July of 2013 and 2015. The Suqi River flows north to the Bering Sea and receives direct inflow from streamlets draining the Northeast Cape FUD site (Fig. 1 A&B). Sites receiving inflow from the Northeast Cape FUD site (Sites 10-12) and downstream sites (Sites 2-9) are considered contaminated sites. Sites 13 and 14 are located upstream of the Northeast Cape FUD site and serve as reference sites because they do not receive direct contamination from the FUD site. We captured both stickleback and blackfish using unbaited 0.32 and 0.64 cm wire-mesh minnow traps. We euthanized fish with an overdose of MS-222 (fish anesthetic) and randomly selected blackfish for transcriptomic analysis and stickleback for histological analyses (transcriptomic analysis of stickleback had already been completed, von Hippel et al., 2018). Blackfish selected for transcriptomic analysis were placed in tubes containing RNA later. In 2013, fish selected for histological analyses were preserved in the field by placing carcasses into Dietrich's solution, and organs were removed and processed in the laboratory. In 2015, organs were dissected in the field and placed into Dietrich's solution, and the remaining carcasses were frozen. All research protocols were approved by the University of Alaska Anchorage Institutional Animal Care and Use Committee (IACUC; #159870-20 and #439949-1) and the University of Oregon IACUC (#13-12R4).

Alaska blackfish RNA sequencing

We compared gene expression in liver tissue from seven female blackfish collected in 2015 at a highly contaminated site downstream of the Northeast Cape FUD site in the Suqi River (Site 12; Fig. 1B) to seven female blackfish collected at a reference site upstream of the FUD site (Site 13; Fig. 1B). We selected these sites based on PCB concentrations in blackfish reported by von Hippel et al. (2018), which showed that blackfish caught at Site 12 had significantly higher PCB concentrations than did blackfish caught at Site 13. Furthermore, Site 12 receives direct input from a streamlet running through the former barrel storage area and main operations center of the Northeast Cape FUD site.

We extracted total RNA from liver tissue with RiboPure (Invitrogen, Massachusetts, USA) and then purified mRNA using the Dynabeads mRNA Purification Kit (Ambion, Texas, USA). We prepared RNA libraries with the NEXTflex Rapid Directional qRNA-Seq kit (BIOO, Barcelona, Spain) according to the manufacturer's protocol and sequenced on an Illumina HiSeq 4000 (Illumina, San Diego, California) to generate paired-end 150 nucleotide (nt) reads. Reads were processed with the Dupligänger pipeline (Sydes, 2019) to remove unique molecular identifiers (UMI), trim adapters (Martin, 2011), and perform quality trimming with Trimmomatic (Bolger et al., 2014), requiring an average Phred score of 20 across a sliding window of 5 nt and a minimum read length of 50 nt. Reads were aligned to the *Dallia pectoralis* genome DPEC1.1 annotation with STAR (Dobin et al., 2013). After quality trimming, we extracted and sorted unique alignments with SAMtools (Li et al., 2009), and removed PCR duplicates with Dupligänger. We generated feature counts with HTSeq (Anders et al., 2015) in strict mode and performed differential expression analysis with DESeq2 (Love et al., 2014), requiring a FDR<0.1. Differentially expressed genes were plotted by principal component analysis (PCA) to compare contaminated versus reference fish and check for outliers (Fig. 2).

To identify zebrafish (*Danio rerio*) orthologs of blackfish genes, we used CRB-BLAST (Aubry et al., 2014) to compare them to zebrafish proteins. Human orthologs of these zebrafish genes were then exported from Ensembl version 102 (Yates et al., 2020). We performed functional enrichment on the human orthologs of differentially expressed genes using PANTHER (Mi et al., 2013).

Ninespine stickleback histology

Histological processing was conducted at the University of Oregon histology core facility. Kidney, gonad, and thyroid tissues were dehydrated and embedded into paraffin blocks.

These blocks were sectioned horizontally into 5 μm sections using a microtome. Each section was mounted on a slide and stained with hematoxylin and eosin (H&E).

Thyroid histomorphology

We analyzed the anterior portion of fixed stickleback collected in 2013 (n=48) and 2015 (n=42) from long-term monitoring sites along the Suqi and Tapi Rivers. Because thyroid follicles are dispersed within the thoracic region of most teleosts (Chanet and Meunier, 2014; Geven et al., 2007), we only analyzed sections that included the brachial arteries and skeletal muscles in the thoracic region to ensure consistency in sectioning location across samples. We followed methods for thyroid histological analysis outlined by Petersen et al. (2015) and Gardell et al. (2017). Briefly, we imaged two thyroid sections per individual fish at 100x and 400x magnification using a Leica DM6 B microscope (Leica Microsystems, Wetzlar, Germany). We selected histological sections for analysis based on the presence and quality of thyroid follicles, and the amount of visible colloid (with preference given to sections displaying more visible colloid). We used images captured at 100x magnification to count the total number of thyroid follicles and then randomly selected five follicles from each section for further analysis. For each of the five selected follicles, we measured follicle area, colloid area, and thyrocyte height as described by Petersen et al. (2015) and Gardell et al. (2017). We quantified all measurements using an Intuos touch pad (Wacom, Vancouver, WA) and Image J (NIH) software. The data presented in this study reflect a mean value for the two sections analyzed per fish.

Kidney histomorphology

We analyzed kidney histomorphology in stickleback collected in 2013 (n=44; kidneys were not sampled in 2015) following methods described by Petersen et al. (2015). Briefly, we imaged two tail sections (sectioned transversally) per individual fish at 2.5x and 20x magnification on a Leica DM4500B imaging microscope using Leica Application System (LAS) software (V.4.3). We only analyzed sections containing a complete and intact kidney, with clear glomeruli and kidney tubules. Sections containing ripped tissue were not analyzed. For each section, we measured the number of glomeruli, height of kidney epithelial cells, and areas of the kidney, renal tubule and glomerulus.

Gonad histomorphology

We analyzed the gonads of female and male stickleback collected in 2015 (no gonads were analyzed in 2013 due to sectioning errors). We imaged two sections per fish to reduce the potential of artifacts introduced during the sectioning process. Several tissue samples were ripped during the sectioning process, which decreased our sample size to 13 females and 16 males. We only imaged and analyzed sections containing complete and intact gonads. Sections that met selection criteria were imaged using a Leica Aperio CS2 slide scanner using Leica ImageScope software. We analyzed sections using ImageJ (Schneider et al., 2012) for gonadal endpoints described by Sokołowska and Kulczykowska (2006) and Furin et al. (2015). For females, we measured the area of the ovary, counted the number of oocytes, and staged each oocyte using criteria described by Furin et al. (2015) and detailed in Sokołowska and Kulczykowska (2006). Briefly, we staged each oocyte as early (stage 1), intermediate (stage 2), late (stage 3), mature (stage 4), or regressed (Furin et al., 2015; Sokołowska and Kulczykowska, 2006). The stages of male testicular lobules were largely homogeneous and therefore we staged each testis as early (stage 1), intermediate (stage 2), or late (stage 3) as described in Furin et al. (2015).

Statistical analyses

We ran all statistical analyses for histological comparisons in R version 4.1.2 (2009-2021 RStudio, Inc.). Due to small sample sizes, we grouped collection sites along the Suqi River according to their location relative to the Northeast Cape FUD site. Sites receiving inflow from the FUD site and downstream sites (Sites 2-12; Fig. 1) were pooled together as downstream (contaminated) sites. Sites located upstream of the Northeast Cape FUD site (Sites 13 and 14; Fig. 1) were pooled together as reference sites. We ran a linear mixed model to test for potential random effects of pooled collection sites, but we found no significant effects and thus removed site as a variable in our models.

We established whether sampling location was a significant factor in explaining differences in thyroid histomorphological endpoints using two-way analysis of variance (ANOVA) models. We natural log transformed thyroid response variables to account for non-normal distributions and heteroskedasticity. Model selection criteria revealed that both sampling location (upstream vs. downstream) and year were significant predictors of thyroid follicle number; therefore, we employed a two-way ANOVA to model thyroid follicle number.

ANOVA model selection criteria indicated that sex was the only significant variable accounting for variance in kidney tubule area and kidney epithelial cell height. We therefore employed one-way ANOVAs to model these response variables. Shapiro-Wilks tests indicated that raw data were non-normally distributed, and thus we natural log transformed both kidney response variables. We checked the ANOVA results against non-parametric Mann-Whitney *U* tests and found that they were comparable. Thus, we only report statistical results for the one-way ANOVAs. Although sampling location was not a significant variable in models of kidney tubule area and epithelial cell height, we employed a two-way ANOVA with sex and sampling location (upstream vs. downstream) as fixed factors to examine sex differences within each sampling location (Fig. 4). Due to small sample sizes, we examined differences in gonad histomorphology using only non-parametric Mann-Whitney U tests.

For all ANOVA models, we tested normality using Shapiro-Wilks tests and corrected for multiple pairwise comparisons using Bonferroni and Tukey p-value adjustment methods. All Shapiro-Wilks normality tests on log-transformed data indicated that our models met normality assumptions. We determined significance at an alpha level of 0.1 for RNA-seq analysis and an alpha level of 0.05 for all other statistical analyses.

RESULTS

RNA sequencing

We found 430 differentially expressed genes ($p<0.1$) between female blackfish collected downstream compared to upstream of the Northeast Cape FUD site. PANTHER software (Mi et al., 2013) provided gene ontology (GO) clustering and functional relationships of differentially expressed genes. We found that genes involved in the structural constituent of ribosome (GO:0003735) were upregulated 4.4-fold in blackfish collected downstream of the FUD site (FDR=0.007). The basigin interaction (R-HSA-210991; 9.3-fold upregulated; FDR=0.033), amino acid transport across the plasma membrane (R-HSA-352230; 8.8-fold upregulated; FDR=0.013), and eukaryotic translation elongation (R-HSA-156842; 7.0-fold upregulated; FDR<0.001) were the most enriched reactome pathways in blackfish collected from the contaminated site. Genes involved in cellular responses to stress (R-HSA-2262752; 2.5-fold upregulated; FDR=0.003) and FOXO-mediated transcription (R-HSA-9614085; 6.0-fold upregulated; FDR=0.012) were also enriched in blackfish from the contaminated site.

Thyroid histology

We found that the number of thyroid follicles in stickleback differed significantly by sampling location and year (Fig. 3). Stickleback collected in 2013 had similar thyroid follicle counts across all sampling locations, but stickleback collected in 2015 from contaminated sites (n=22) had 1.6-fold more thyroid follicles than did stickleback from upstream sites (n=20; ANOVA, p=0.006). Thyroid follicle counts did not differ between stickleback collected from the Tapi River reference site and contaminated sites. Stickleback caught from the Tapi River (n=9) and contaminated sites in 2013 (n=31) had significantly more thyroid follicles than did those caught from upstream sites in 2015 (n=8; ANOVA, p=0.001 and p<0.001, respectively; Fig. 3). Conversely, stickleback caught from contaminated sites in 2015 had significantly fewer thyroid follicles than did upstream fish caught in 2013 (ANOVA, p<0.002; Fig. 3). Thyroid follicle counts significantly differed within upstream sites, with stickleback in 2013 having 3-fold more thyroid follicles than those in 2015 (ANOVA, p<0.001; Fig. 3). Despite finding differences in thyroid follicle number, we did not find differences between mean follicle area, colloid area, or thyrocyte height. We did not find a statistically significant difference between male and female stickleback for any thyroid histomorphology.

Kidney histology

We did not find differences in kidney histomorphology across sampling locations, but we did find that kidney tubule area and epithelial cell heights significantly differed by sex. Mean kidney epithelial cell height was 2.1-fold longer in males than females across all sites (ANOVA, p=0.002). This finding was consistent within each sampling location (ANOVA, p<0.001 for all comparisons; Fig. 4A). Similarly, male stickleback had 1.7-fold larger tubules than females (ANOVA, p<0.001; Fig. 4B). We did not find a significant difference in tubule area between males and females at the Tapi River site, but we did find differences at downstream and upstream Suqi sites, with males having significantly larger tubules than females (ANOVA, p=0.045 for both comparisons; Fig. 4B). We found that the total kidney area, number of glomeruli, and mean area of glomeruli were similar across sampling locations and sex.

Gonad histology

We did not observe any statistically significant differences in gonadal measurements across sampling locations for either male or female stickleback. Although not significant, stickleback downstream of the FUD site tended to have smaller ovaries than did stickleback collected upstream of the FUD site (Mann-Whitney *U*, p=0.138). Similarly, downstream stickleback tended to have a smaller proportion of mature oocytes compared to upstream stickleback (Mann-Whitney *U*, p=0.313). The lack of significant differences is likely due to low statistical power. The power to detect a significant medium effect (d=0.5; Cohen, 1988) using a two-sided t-test with unequal sample sizes was only 15% and 13% for males and females, respectively.

DISCUSSION

Differentially expressed genes

Alaska blackfish females from the downstream contaminated site transcriptionally differed from blackfish females caught at the upstream reference site. Blackfish from the contaminated site overexpressed genes involved in ribosomal structure and function pathways, indicating that these fish are under conditions of cellular stress. Exposure to xenobiotic contaminants can cause ribosomal stress and accumulation of ribosomal proteins within the cytoplasm (Zhou et al., 2012; Zhou et al., 2015). Previous studies have documented enrichment of genes associated with ribosomal pathways in response to PCB exposure (Aluru et al., 2018; Padhi et al., 2008). For example, Aluru et al. (2018) found that zebrafish exposed to PCB-126 upregulated genes involved in the structural constituent of ribosome pathway. We also found this pathway enriched in blackfish from the contaminated site (GO:0003735, 4.4-fold upregulated, FDR=0.007). Furthermore, ribosomal stress induces the p53 pathway, which initiates cell cycle arrest and apoptosis (Harris and Levine, 2005; Jin and Levine, 2001). These results are consistent with findings reported by von Hippel et al. (2018) in stickleback and suggest that exposure to FUD site pollution causes a transcriptional response related to disruption of cellular homeostasis and altered DNA replication and cell cycle processes in blackfish.

The PANTHER analysis revealed enrichment of genes related to the forkhead box O (FoxO) signaling pathway in blackfish living downstream of the Northeast Cape FUD site. The FoxO signaling pathway comprises a network of genes that serve a variety of biological functions, including regulation of cell death, DNA repair, and tumor suppression (Myatt and Lam, 2007; Vurusaner et al., 2012). Indeed, we found that the gene *bcl2l11* (*bim*), which encodes for a protein that induces apoptosis, was upregulated 1.8-fold in blackfish from contaminated sites (padj=0.027). *bcl2l11* can induce accumulation of cellular reactive oxygen species (ROS) (Hagenbuchner et al., 2012). These results suggest that blackfish exposed to FUD site contamination are responding to cellular stress and may have decreased capability to respond to cellular damage caused by xenobiotics due to perturbations in normal cell cycle and tumor suppression genes and the induction of ROS.

Transcriptional results in the present study contrast certain results in ninespine stickleback collected from the same long-term monitoring sites along the Suqi River. von Hippel et al. (2018) examined differential gene expression in female stickleback from highly contaminated sites downstream of the Northeast Cape FUD site compared to stickleback from reference sites upstream of the FUD site. This study found that genes involved in cell cycle and cell division pathways were downregulated in female stickleback exposed to Northeast Cape FUD site pollution, whereas we found that genes important in cell cycle regulation pathways were upregulated in female blackfish. Additionally, von Hippel et al. (2018) reported upregulation of liver glycogen phosphorylase (*pygl*), cytochrome p450 (*cyp1a1*) and the fish ortholog for ATP binding cassette subfamily B (*abcb4*). Our expression data did not show differential expression for either *pygl* or *cyp1a1* in blackfish, but we did find upregulation of *abcb4* (2.5-fold upregulated, padj=0.087). The fish gene *abcb4* can facilitate multixenobiotic resistance (MXR) to toxic contaminants in several species (Jackson and Kennedy, 2017; Kurelec, 1992; Smital et al., 2000) by increasing transport of exogenous compounds and reducing xenobiotic uptake (Fischer et al., 2013; Jackson and Kennedy, 2017; Smital et al., 2000). For example, elevated expression of *abcb4* decreased uptake of toxic substances in zebrafish embryos and protected against contaminant toxicity (Fischer et al., 2013). Therefore, upregulation of *abcb4* could indicate that blackfish are mounting a transcriptional response to mitigate the effects of FUD site pollution. Several possibilities warrant further investigation to understand the discrepancies between blackfish and stickleback transcriptomic responses at Northeast Cape. von Hippel et al. (2018) showed that PCB concentrations in both stickleback and blackfish are elevated downstream of the FUD site; however, blackfish could be more resistant to POP toxicity than stickleback and better able to mount a robust transcriptional response to POP exposure. Alternatively, blackfish could be more sensitive to certain PCB congeners, such as ortho-substituted PCB congeners, which are induce expression of genes involved in FoxO signaling and insulin resistance pathways in zebrafish (Aluru et al., 2019).

Thyroid histology

We did not find histological effects in stickleback collected downstream of the FUD site despite previously reported differential gene expression, thyroid hormone disruption, and induction of vitellogenin in these fish (von Hippel et al., 2018). The only thyroid histomorphology that varied across sampling sites was the number of thyroid follicles, with stickleback from contaminated sites having significantly more thyroid follicles than stickleback from upstream sites in 2015 (Fig. 1). This is consistent with von Hippel et al. (2018), who found that stickleback collected at some sites downstream of the Northeast Cape FUD site had significantly lower concentrations of thyroxine (T_4), indicating hypothyroid conditions. Several studies have established that the Northeast Cape FUD site remains a point source of PCBs (Jordan-Ward et al., 2022; Scrudato et al., 2012; von Hippel et al., 2018), and that PCB concentrations are elevated in several native fish species, including stickleback, blackfish, and Dolly Varden (*Salvelinus malma*) caught near the FUD site (Jordan-Ward et al., 2022; von Hippel et al., 2018). All three species collected downstream of the FUD site had elevated concentrations of highly chlorinated PCB congeners compared to fish collected upstream, indicating that the high PCB concentrations are due to point sources of PCB pollution (Jordan-Ward et al., 2022; von Hippel et al., 2018). Because PCBs cause hypothyroidism in a variety of vertebrates, including humans (Brown et al., 2004; Schell et al., 2008), exposure to Northeast Cape FUD site pollution may potentiate the risk for thyroid disease in local fishes and residents who consume fish caught near the FUD site. Exposure to PCBs originating at the FUD site may contribute to the elevated number of thyroid follicles observed in stickleback collected from contaminated sites in 2015, although stickleback from contaminated sites did not differ from those collected from the Tapi River. The Tapi River, however, is not an ideal reference site as it too was exposed to some military waste disposal (Byrne et al., 2015). Thyroid follicle hyperplasia is associated with hypothyroidism in fish (Deal and Volkoff, 2020; Raine et al., 2001; Sharma et al., 2016). Collectively, the results from the present study and from von Hippel et al. (2018) suggest that PCB contamination originating at the Northeast Cape FUD site drives hypothyroid conditions in stickleback living downstream of the FUD site.

Thyroid measurements in stickleback collected in 2013 may be biased due to the lack of stickleback collected from Site 12 in that year, which may explain the lack of significant differences in thyroid traits between upstream and downstream fish for samples collected that year. Site 12 is the most polluted site in the Suqi River due to confluence of streamlets flowing from former dump sites at the FUD site. PCB concentrations in multiple fish species are elevated at sites receiving inflow from the Northeast Cape FUD site, and this is especially so for site 12 (Jordan-Ward et al., 2022; von Hippel et al., 2018).

Kidney histology

Stickleback did not differ in kidney histomorphology across sampling locations, indicating that the Northeast Cape FUD site did not significantly affect kidney morphology in these fish during the study period. However, we found that male stickleback had significantly longer kidney epithelial cells and larger kidney tubules than did female stickleback across sampling locations (Fig. 4 A&B). These results are consistent with the life history of male stickleback during the breeding season. The males of stickleback species, including the ninespine stickleback, produce the glue protein spiggin in their kidneys during the breeding season, which is used in nest construction (Jakobsson et al., 1999; Kawahara and Nishida, 2006; Ostlund-Nilsson et al., 2007). The production of spiggin causes kidney hypertrophy (Borg et al., 1993) and likely explains the sex differences observed in this study.

Gonad histology

Exposure to xenobiotic estrogens disrupts normal hormonal signaling and delays gonadal maturation in fish (Berg et al., 2016; Meier et al., 2011), and induces estrogen-mediated responses. For example, von Hippel et al. (2018) found that exposure to FUD site contamination induced vitellogenin production in male stickleback collected from the Suqi River. We hypothesized that stickleback collected at contaminated sites in the Suqi River would exhibit suppressed oocyte and testicular maturation as a result of exposure to xenoestrogens. Although we found that female stickleback from contaminated downstream sites trended toward smaller ovaries and fewer mature oocytes compared to upstream stickleback, these differences were not statistically significant. Indeed, we did not observe any significant differences in gonadal measurements across sampling locations for either male or female stickleback. This may be due to small sample sizes that reduced our statistical power to detect a median effect to 15% and 13% for males and females, respectively. Future studies should obtain sample sizes of at least 64 samples per sampling location to obtain a power of 80%.

More generally, our ability to detect significant differences in stickleback histomorphologies is limited by small sample sizes and the lack of stickleback samples from Site 12 in 2013. As a result, our histological results are inconclusive as to the effects of FUD site contamination on tissue development in stickleback living downstream of the FUD site. Future studies would benefit from larger sample sizes at each sampling site.

CONCLUSIONS

The Arctic is a repository for POPs that bioaccumulate and biomagnify in lipid-rich food webs due both to global distillation and to local hotpots of pollution. Because many arctic communities rely heavily on high-trophic level subsistence foods, Arctic Indigenous Peoples may be disproportionately exposed to high levels of POPs through their diet. We found that exposure to FUD site pollution significantly altered gene expression in Alaska blackfish and thyroid histomorphology in ninespine stickleback, despite these fish being low-trophic level organisms. Although neither blackfish nor stickleback are a subsistence food source on Sivuqaq, elevated PCB concentrations and observed biological effects in these fishes indicate that higher trophic level organisms that live near or consume foods caught at the Northeast Cape FUD site, including humans, may experience negative health effects. Additionally, subsistence fish living downstream of the Northeast Cape FUD site are also contaminated with PCBs and other POPs (Jordan-Ward et al., 2022). Collectively, results from the present study and previous studies suggest that the Northeast FUD site poses health risks to local wildlife and Sivuqaq residents despite large-scale remediation efforts. Further remediation of the Northeast Cape FUD site is needed in order for the Sivuqaq community to safely re-establish the village at Northeast Cape and should be conducted under tribal supervision and agreement.

ACKNOWLEDGEMENTS

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REFERENCES

Aluru, N., Karchner, S.I., Krick, K.S., Zhu, W., Liu, J., 2018. Role of DNA methylation in altered gene expression patterns in adult zebrafish (*Danio rerio*) exposed to 3, 3', 4, 4', 5-pentachlorobiphenyl (PCB 126). Environ Epigenet 4, dvy005.

Aluru, N., Krick, K.S., McDonald, A.M., Karchner, S.I., 2019. Developmental exposure to PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl) alters circadian rhythms and the expression of clock and metabolic genes. Toxicol Sci 173, 41-52.

Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq- a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166-169.

Aubry, S., Kelly, S., Kümpers, B.M.C., Smith-Unna, R.D., Hibberd, J.M., 2014. Deep evolutionary comparison of gene expression identifies parallel recruitment of trans-factors in two independent origins of C4 photosynthesis. PLoS Genet 10, e1004365.

Ayotte, P., Muckle, G., Jacobson, J.L., Jacobson, S.W., Dewailly, E., 2003. Assessment of pre- and postnatal exposure to polychlorinated biphenyls: lessons from the Inuit Cohort Study. Environ Health Perspect 111, 1253-1258.

Berg, V., Kraugerud, M., Nourizadeh-Lillabadi, R., Olsvik, P.A., Skare, J.U., Alestrom, P., Ropstad, E., Zimmer, K.E., Lyche, J.L., 2016. Endocrine effects of real-life mixtures of persistent organic pollutants (POP) in experimental models and wild fish. J Toxicol Environ Health 79, 538-548.

Blais, J.M., 2005. Biogeochemistry of persistent bioaccumulative toxicants: processes affecting the transport of contaminants to remote areas. Can J Fish Aquat Sci 62, 236-243.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114-2120.

Borg, B., Antonopoulou, E., Andersson, E., Carlberg, T., Mayer, I., 1993. Effectiveness of several androgens in stimulating kidney hypertrophy, a secondary sexual character, in castrated male three-spined sticklebacks, *Gasterosteus aculeatus*. Can J Zool 71, 2327-2329.

Brown, S.B., Adams, B.A., Cyr, D.G., Eales, J.G., 2004. Contaminant effects on the teleost fish thyroid. Environ Toxicol Chem 23, 1680-1701.

Byrne, S., Miller, P.K., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2015. Persistent organochlorine pesticide exposure related to a formerly used defense site on St. Lawrence Island, Alaska: data from sentinel fish and human sera. J Toxicol Environ Health A 78, 976-992.

Byrne, S., Seguinot-Medina, S., Miller, P.K., Waghiyi, V., von Hippel, F.A., Buck, C.L., Carpenter, D.O., 2017. Exposure to polybrominated diphenyl ethers and perfluoroalkyl substances in a remote population of Alaska Natives. Environ Pollut 231, 387-395.

Carpenter, D.O., DeCaprio, A.P., O'Hehir, D., Akhtar, F., Johnson, G., Scrudato, R.J., Apatiki, L., Kava, J., Gologergen, J., Miller, P.K., Eckstein, L., 2005. Polychlorinated biphenyls in serum of the Siberian Yupik people from St. Lawrence Island, Alaska. Int J Circumpolar Health 64, 322-335.

Chanet, B., Meunier, F.J., 2014. The anatomy of the thyroid gland among "fishes": phylogenetic implications for the Vertebrata. Cybium 38, 90–116.

Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences (2nd edition). Routledge. https://doi.org/10.4324/9780203771587.

Deal, C.K., Volkoff, H., 2020. The role of the thyroid axis in fish. Front Endocrinol 11, 596585.

Denfeld, D.C., 1994. The Cold War in Alaska: a management plan for cultural resources, 1994-1999. US Army Corps of Engineers, Alaska District.

Dewailly, E., Nantel, A., Weber, J.P., Meyer, F., 1989. High levels of PCBs in breast milk of Inuit women from arctic Quebec. Bull Environ Contam Toxicol 43, 641-646.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15-21.

Fischer, S., Klüver, N., Burkhardt-Medicke, K., Pietsch, M., Schmidt, A.M., Wellner, P., Schirmer, K., Luckenbach, T., 2013. Abcb4 acts as multixenobiotic transporter and active barrier against chemical uptake in zebrafish (*Danio rerio*) embryos. BMC Biol 11, 69.

Furin, C.G., von Hippel, F.A., Postlethwait, J.H., Buck, C.L., Cresko, W.A., O'Hara, T.M., 2015. Developmental timing of sodium perchlorate exposure alters angiogenesis, thyroid follicle proliferation and sexual maturation in stickleback. Gen Comp Endocrinol 219, 24-35.

Gardell, A.M., von Hippel, F.A., Adams, E.M., Dillon, D.M., Petersen, A.M., Postlethwait, J.H., Cresko, W.A., Buck, C.L., 2017. Exogenous iodide ameliorates perchlorate-induced thyroid phenotypes in threespine stickleback. Gen Comp Endocrinol 243, 60-69.

Geven, E.J., Nguyen, N.K., van den Boogaart, M., Spanings, F.A., Flik, G., Klaren, P.H., 2007. Comparative thyroidology: thyroid gland location and iodothyronine dynamics in Mozambique tilapia (*Oreochromis mossambicus* Peters) and common carp (*Cyprinus carpio* L.). J Exp Biol 210, 4005-4015.

Hagenbuchner, J., Kuznetsov, A., Hermann, M., Hausott, B., Obexer, P., Ausserlechner, M.J., 2012. FOXO3-induced reactive oxygen species are regulated by BCL2L11 (Bim) and SESN3. J Cell Sci 125, 1191-1203.

Harris, S.L., Levine, A.J., 2005. The p53 pathway: positive and negative feedback loops. Oncogene 24, 2899-2908.

Jackson, J.S., Kennedy, C.J., 2017. Regulation of hepatic abcb4 and cyp3a65 gene expression and multidrug/multixenobiotic resistance (MDR/MXR) functional activity in the model teleost, *Danio rerio* (zebrafish). Comp Biochem Physiol C Toxicol Pharmacol 200, 34-41.

Jakobsson, S., Borg, B., Haux, C., Hyllner, S.J., 1999. An 11-ketotestosterone induced kidney-secreted protein: the nest building glue from male three-spined stickleback, *Gasterosteus aculeatus*. Fish Physiol Biochem 20, 79-85.

Jin, S., Levine, A.J., 2001. The p53 functional circuit. J Cell Sci 114, 4139-4140.

Jordan-Ward, R., von Hippel, F.A., Zheng, G., Salamova, A., Dillon, D., Gologergen, J., Immingan, T., Dominguez, E., Miller, P., Carpenter, D., Postlethwait, J.H., Byrne, S., Buck, C.L., 2022. Elevated mercury and PCB concentrations in Dolly Varden (*Salvelinus malma*) collected near a formerly used defense site on Sivuqaq, Alaska. Sci Total Environ 826, 154067.

Kawahara, R., Nishida, M., 2006. Multiple occurrences of spiggin genes in sticklebacks. Gene 373, 58-66.

Kurelec, B., 1992. The multixenobiotic resistance mechanism in aquatic organisms. Crit Rev Toxicol 22, 23-43.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., Subgroup, G.P.D.P., 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078-2079.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15, 550.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet 17, 3.

Meier, S., Morton, H.C., Andersson, E., Geffen, A.J., Taranger, G.L., Larsen, M., Petersen, M., Djurhuus, R., Klungsøyr, J., Svardal, A., 2011. Low-dose exposure to alkylphenols adversely affects the sexual development of Atlantic cod (*Gadus morhua*): acceleration of the onset of puberty and delayed seasonal gonad development in mature female cod. Aquat Toxicol 105, 136-150.

Mi, H., Muruganujan, A., Thomas, P.D., 2013. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res 41, D377-386.

Miller, P.K., Waghiyi, V., Welfinger-Smith, G., Byrne, S.C., Kava, J., Gologergen, J., Eckstein, L., Scrudato, R., Chiarenzelli, J., Carpenter, D.O., Seguinot-Medina, S., 2013. Community-based participatory research projects and policy engagement to protect environmental health on St. Lawrence Island, Alaska. Int J Circumpolar Health 72, 21656.

Myatt, S.S., Lam, E.W.F., 2007. The emerging roles of forkhead box (Fox) proteins in cancer. Nat Rev Cancer 7, 847-859.

Ostlund-Nilsson, S., Mayer, I., Huntingford, F.A., 2007. The biology of the threespine stickleback. Integr Comp Biol 47, 900-901.

Padhi, B.K., Pelletier, G., Williams, A., Berndt-Weis, L., Yauk, C., Bowers, W.J., Chu, I., 2008. Gene expression profiling in rat cerebellum following in utero and lactational exposure to mixtures of methylmercury, polychlorinated biphenyls and organochlorine pesticides. Toxicol Lett 176, 93-103.

Petersen, A.M., Dillon, D., Bernhardt, R.R., Torunsky, R., Postlethwait, J.H., von Hippel, F.A., Loren Buck, C., Cresko, W.A., 2015. Perchlorate disrupts embryonic androgen synthesis and reproductive development in threespine stickleback without changing whole-body levels of thyroid hormone. Gen Comp Endocrinol 210, 130-144.

Raine, J.C., Takemura, A., Leatherland, J.F., 2001. Assessment of thyroid function in adult medaka (*Oryzias latipes*) and juvenile rainbow trout (*Oncorhynchus mykiss*) using immunostaining methods. J Exp Zool 290, 366-378.

Schell, L.M., Gallo, M.V., Deane, G.D., Nelder, K.R., DeCaprio, A.P., Jacobs, A., 2014. Relationships of polychlorinated biphenyls and dichlorodiphenyldichloroethylene (p,p'-DDE) with testosterone levels in adolescent males. Environ Health Perspect 122, 304-309.

Schell, L.M., Gallo, M.V., Denham, M., Ravenscroft, J., DeCaprio, A.P., Carpenter, D.O., 2008. Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p,p'-DDE, and other toxicants in Akwesasne Mohawk youth. Environ Health Perspect 116, 806-813.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9, 671-675.

Scrudato, R., Chiarenzelli, J., Miller, P.K., Alexander, J.C., Arnason, J., Zamzow, K., Zweifel, K., Gologergen, J., Kava, J., Waghiyi, V., Carpenter, D., 2012. Contaminants at arctic formerly used defense sites. J Local Glob Health Sci 2, 1-12.

Sepez, J., Package, C., Malcolm, P.E., Poole, A., 2007. Unalaska, Alaska: memory and denial in the globalization of the Aleutian landscape. Polar Geogr 30, 193-209.

Sharma, P., Grabowski, T.B., Patiño, R., 2016. Thyroid endocrine disruption and external body morphology of zebrafish. Gen Comp Endocrinol 226, 42-49.

Smital, T., Sauerborn, R., Pivčević, B., Krča, S., Kurelec, B., 2000. Interspecies differences in P-glycoprotein mediated activity of multixenobiotic resistance mechanism in several marine and freshwater invertebrates. Comp Biochem Physiol C Toxicol Endocrinol 126, 175-186.

Sokołowska, E., Kulczykowska, E., 2006. Annual reproductive cycle in two free living populations of three-spined stickleback (*Gasterosteus aculeatus L.*): patterns of ovarian and testicular development. Oceanologia 48.

Sydes, J., 2019. Dupligänger is a reference-based, UMI-aware, 5'-trimming-aware PCR duplicate removal pipeline. (Version 0.98) Zenodo.

Tierney, K.B., Farrell, A.P., Brauner, C.J., 2014. Organic Chemical Toxicology of Fishes. Fish Physiology, Academic Press 33, 1-580.

USATSDR, 2017. Health Consultation: Northeast Cape Formerly Used Defense Site. United States Agency for Toxic Substances and Disease Registry.

USDOI, 2016. Report to Congress: Hazardous Substance Contamination of Alaska Native Claim Settlement Act Lands in Alaska. United States Department of the Interior Bureau of Land Management.

USEPA, 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. United States Environmental Protection Agency Office of Water.

USEPA, 2004. Making Environmental Progress, Improving Local Communities: Accomplishments of the EPA Region 10 Superfund Program. United States Environmental Protection Agency Superfund Program.

USEPA, 2014. Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations. United States Environmental Protection Agency National Health and Nutrition Examination Survey.

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: health implications for indigenous residents. Environ Pollut 234, 279-287.

von Hippel, F.A., Trammell, E.J., Merila, J., Sanders, M.B., Schwarz, T., Postlethwait, J.H., Titus, T.A., Buck, C.L., Katsiadaki, I., 2016. The ninespine stickleback as a model organism in arctic ecotoxicology. Evol Ecol Res 17, 487-504.

Vurusaner, B., Poli, G., Basaga, H., 2012. Tumor suppressor genes and ROS: complex networks of interactions. Free Radic Biol Med 52, 7-18.

Welfinger-Smith, G., Minholz, J.L., Byrne, S., Waghiyi, V., Gologergen, J., Kava, J., Apatiki, M., Ungott, E., Miller, P.K., Arnason, J.G., Carpenter, D.O., 2011. Organochlorine and metal contaminants in traditional foods from St. Lawrence Island, Alaska. J Toxicol Environ Health A 74, 1195-1214.

WHO, 2016. Polychlorinated Biphenyls and Polybrominated Biphenyls. World Health Organization, Lyon, France.

Winkler, D.F., 1997. Searching the skies: the legacy of the United States Cold War defense radar program. Library of Congress.

Yates, A.D., Achuthan, P., Akanni, W., Allen, J., Allen, J., Alvarez-Jarreta, J., Amode, M.R., Armean, I.M., Azov, A.G., Bennett, R., Bhai, J., Billis, K., Boddu, S., Marugán, J.C., Cummins, C., Davidson, C., Dodiya, K., Fatima, R., Gall, A., Giron, C.G., Gil, L., Grego, T., Haggerty, L., Haskell, E., Hourlier, T., Izuogu, O.G., Janacek, S.H., Juettemann, T., Kay, M., Lavidas, I., Le, T., Lemos, D., Martinez, J.G., Maurel, T., McDowall, M., McMahon, A., Mohanan, S., Moore, B., Nuhn, M., Oheh, D.N., Parker, A., Parton, A., Patricio, M., Sakthivel, M.P., Abdul Salam, A.I., Schmitt, B.M., Schuilenburg, H., Sheppard, D., Sycheva, M., Szuba, M., Taylor, K., Thormann, A., Threadgold, G., Vullo, A., Walts, B., Winterbottom, A., Zadissa, A., Chakiachvili, M., Flint, B., Frankish, A., Hunt, S.E., G, I.I., Kostadima, M., Langridge, N., Loveland, J.E., Martin, F.J., Morales, J., Mudge, J.M., Muffato, M., Perry, E., Ruffier, M., Trevanion, S.J., Cunningham, F., Howe, K.L., Zerbino, D.R., Flicek, P., 2020. Ensembl 2020. Nucleic Acids Res 48, D682-d688.

Zhou, X., Liao, J.-M., Liao, W.-J., Lu, H., 2012. Scission of the p53-MDM2 loop by ribosomal proteins. Genes Cancer 3, 298-310.

Zhou, X., Liao, W.-J., Liao, J.-M., Liao, P., Lu, H., 2015. Ribosomal proteins: functions beyond the ribosome. J Mol Cell Biol 7, 92-104.

LIST OF FIGURES

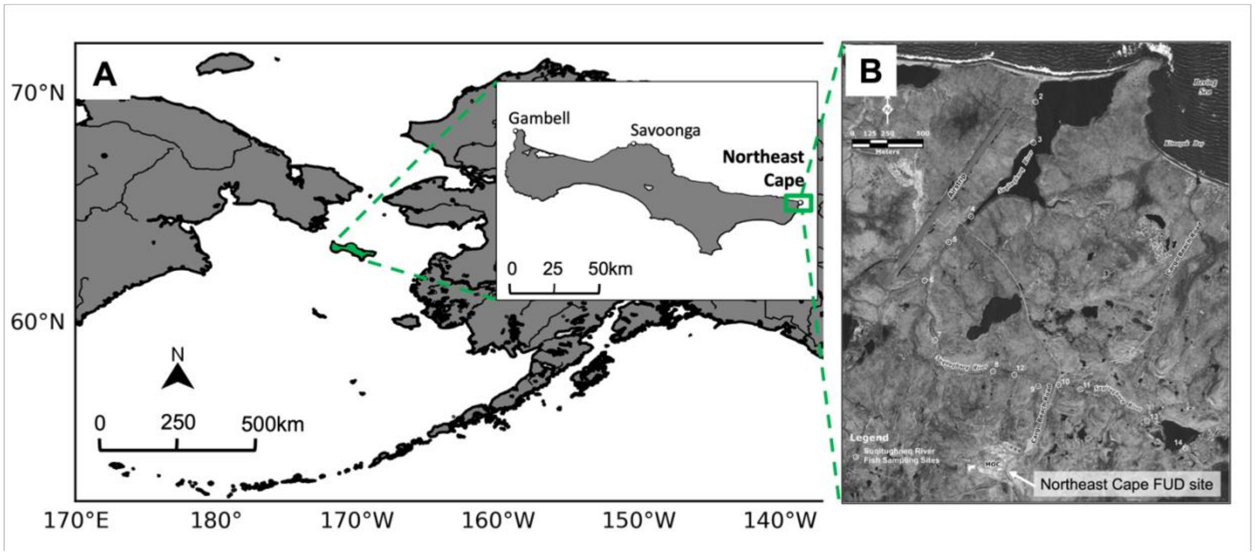

Figure 1. Location of Sivuqaq (St. Lawrence Island), Alaska (**A**), and sampling locations near the Northeast Cape formerly used defense (FUD) site (**B**). We collected ninespine stickleback (*Pungitius pungitius*) and Alaska blackfish (*Dallia pectoralis*) from long-term monitoring sites along the Suqitughneq River (**B**) and a reference site in the Tapisaggak River in an adjacent watershed (not pictured).

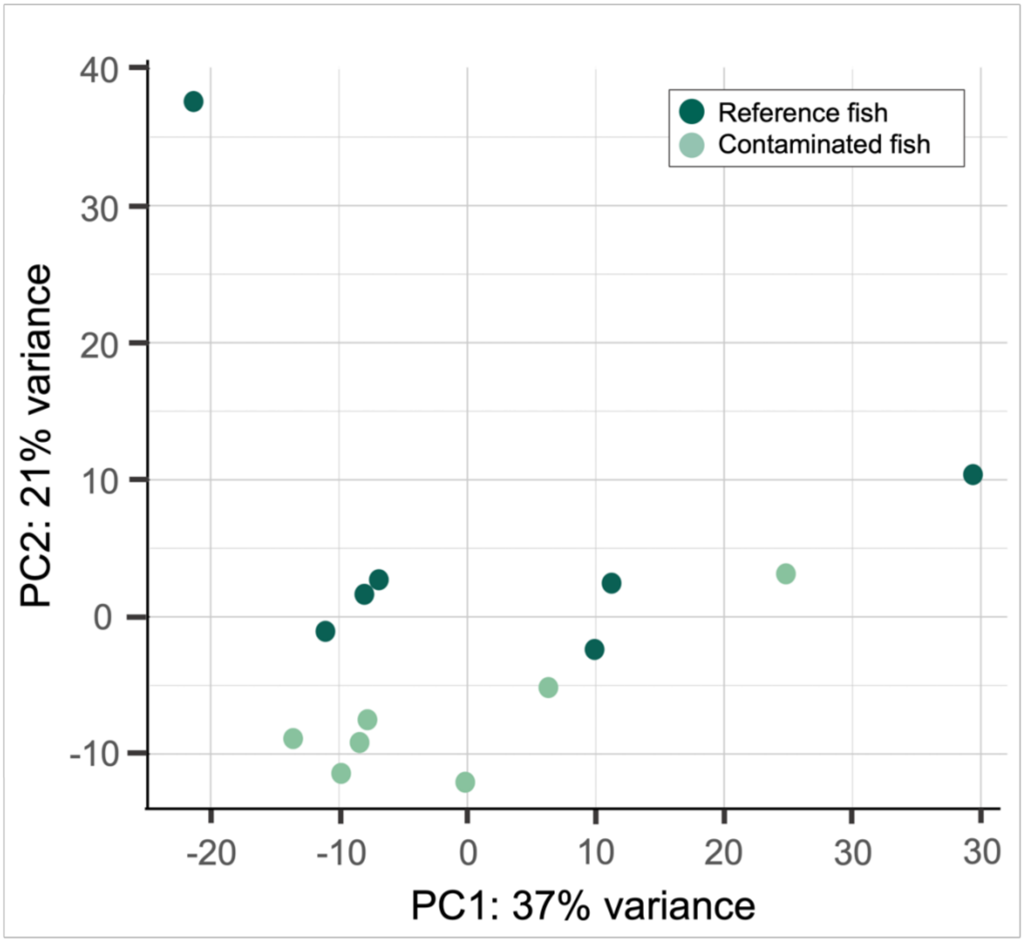

Figure 2. Principal component analysis of differential gene expression in Alaska blackfish (*Dallia pectoralis*) collected from a contaminated site downstream of the formerly used defense (FUD) site and an upstream reference site on Sivuqaq (St. Lawrence Island), Alaska.

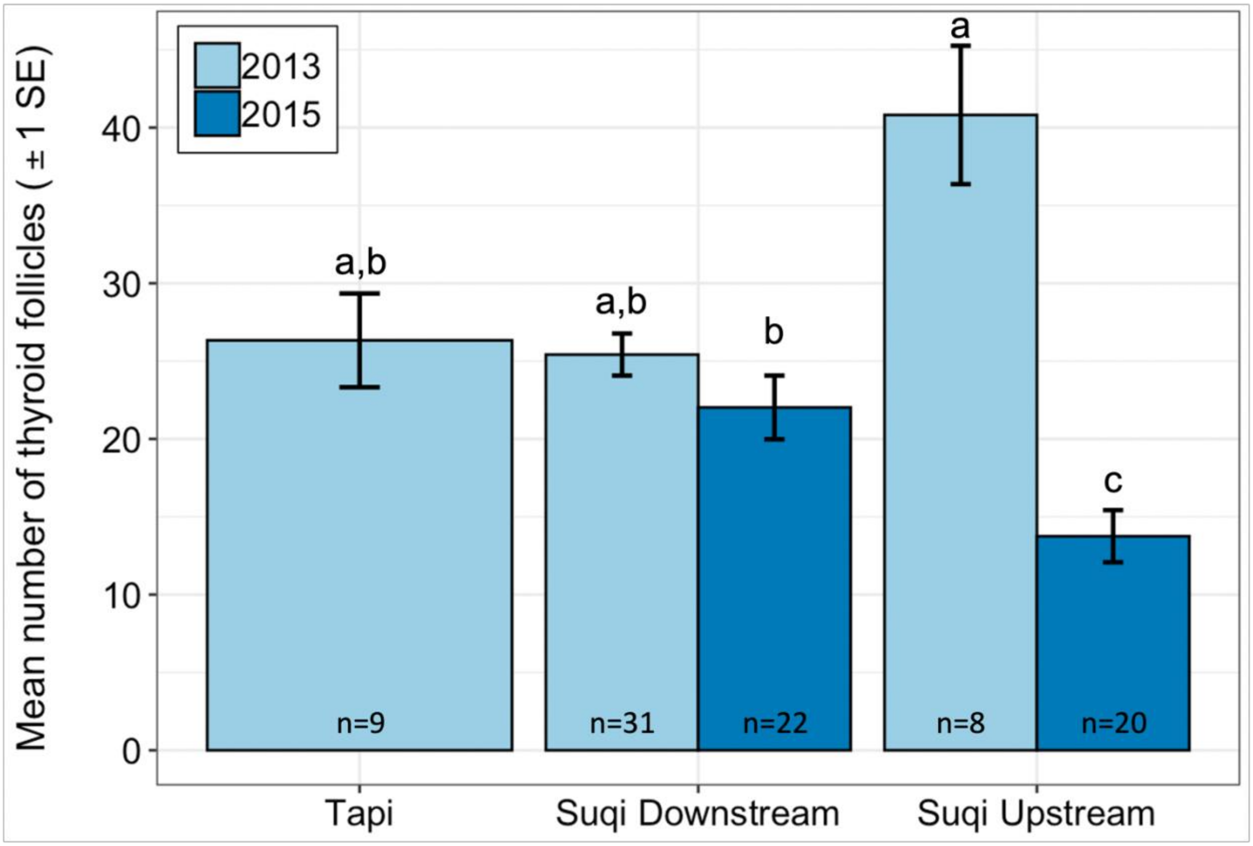

Figure 3. Mean number of thyroid follicles (± standard error) in ninespine stickleback (*Pungitius pungitius*) collected from the Suqitughneq (Suqi) River and a reference site in the Tapisaggak (Tapi) River on Sivuqaq, Alaska. Sites downstream of the Northeast Cape formerly used defense (FUD) site are contaminated and those upstream of the FUD site serve as a reference. Stickleback from contaminated sites were not statistically different from fish collected from the Tapi River but differed from upstream reference fish. Stickleback caught downstream of the FUD site in both 2013 and 2015 had significantly more thyroid follicles than did upstream fish caught in 2015 (ANOVA, p<0.001 and p=0.006, respectively). Downstream fish caught in 2015, but not 2013, had significantly fewer thyroid follicles than did upstream fish collected in 2013 (p=0.002). Fish collected at upstream sites in 2015 had significantly fewer thyroid follicles than did fish collected at upstream sites in 2013 (ANOVA, p<0.001) and at the Tapi River reference site (ANOVA, p=0.001). Significance values were Bonferroni corrected for multiple comparisons.

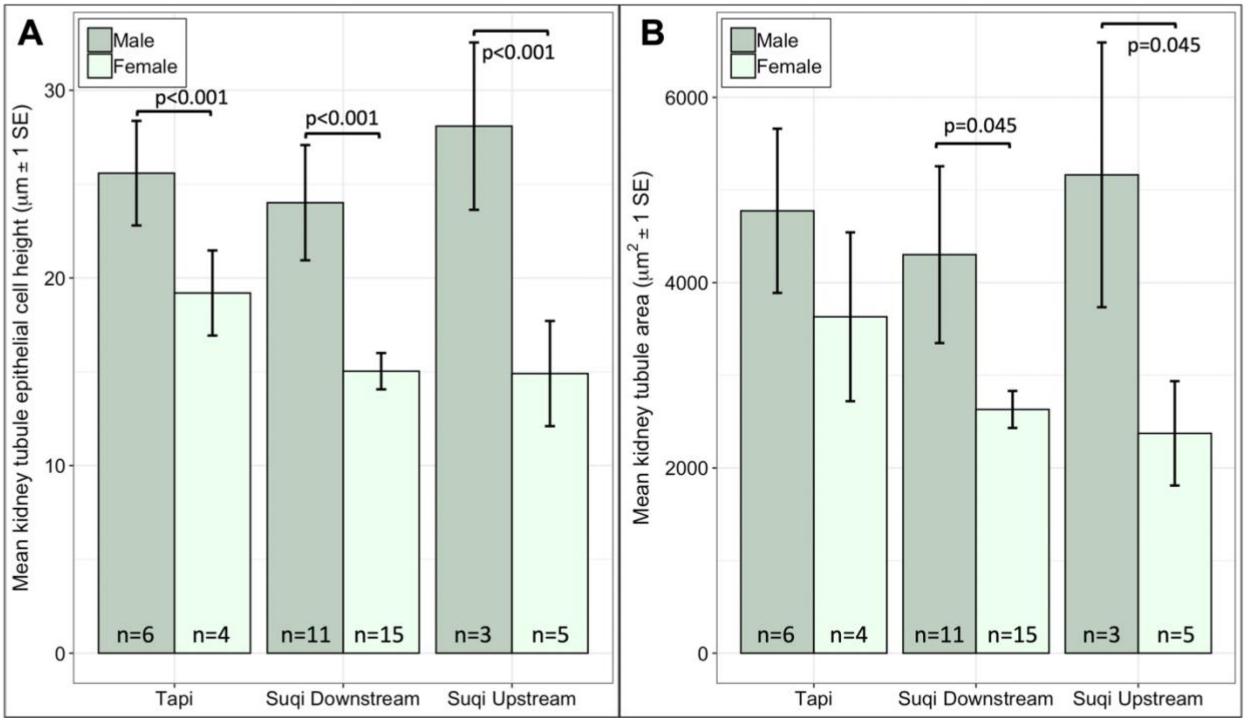

Figure 4. Mean (± standard error) of (**A**) kidney tubule epithelial cell height (KEH; μm) and (**B**) kidney tubule area (μm^2) in ninespine stickleback (*Pungitius pungitius*) collected from the Suqitughneq (Suqi) River and a reference site in the Tapisaggak (Tapi) River on Sivuqaq, Alaska. Sites downstream of the Northeast Cape formerly used defense (FUD) site are contaminated and those upstream of the FUD site serve as a reference. We did not find significant differences among sites but found that female stickleback within each site had shorter KEHs than did males (**A**). Similarly, female stickleback collected from the Suqi River had significantly smaller tubules than did males (**B**). Significance values were Bonferroni corrected for multiple comparisons.

CHAPTER 4: Differential gene expression and developmental pathologies associated with persistent organic pollutants in sentinel fish in Troutman Lake, Sivuqaq, Alaska

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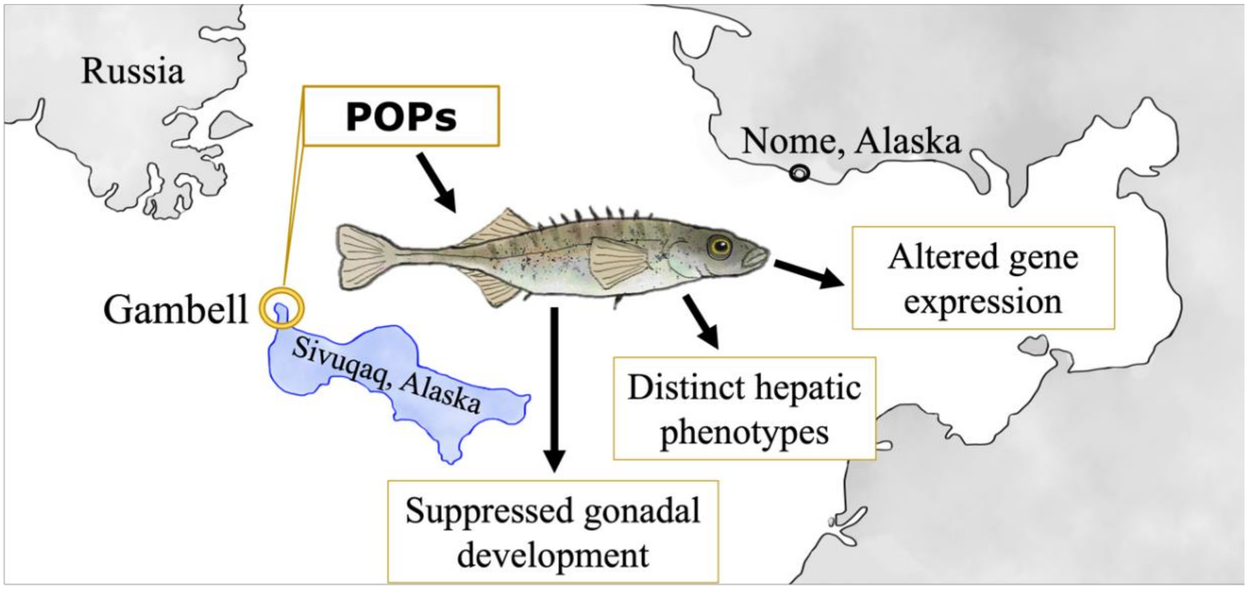
KEYWORDS

Arctic, Endocrine-disrupting compounds, FUD site, Histology, Liver, Military contamination, PBDEs, PCBs, PFAS, RNA sequencing, Thyroid, Yupik

HIGHLIGHTS

- Arctic communities are exposed to POPs from global distillation and local hotspots
- Troutman Lake, adjacent to the Yupik village Gambell, has elevated POPs
- The stickleback is an environmental sentinel of POP exposure and health effects
- Stickleback exhibited suppressed gonadal maturation and 2 distinct liver phenotypes
- Liver lipid phenotype associated with upregulation of ribosomal and metabolic genes

GRAPHICAL ABSTRACT



ABSTRACT

Persistent organic pollutants (POPs) are lipophilic compounds that bioaccumulate in animals and biomagnify within food webs. Many POPs are endocrine disrupting compounds that impact development of numerous tissues in vertebrates, including people. POPs accumulate in the Arctic via global distillation and thereby impact high trophic level vertebrates as well as people who live a subsistence lifestyle. The Arctic also contains thousands of point sources of pollution, such as formerly used defense (FUD) sites. Sivuqaq (St. Lawrence Island), Alaska was used by the U.S. military during the Cold War and FUD sites on the island remain point sources of POP contamination. We examined the effects of POP exposure on ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake in the village of Gambell as a model for human exposure and disease. During the Cold War, Troutman Lake was used as a dump site by the U.S. military. We found that PCB concentrations in ninespine stickleback exceeded the EPA's guideline for unlimited consumption despite these fish being low trophic level organisms. We examined effects at three levels of biological organization: gene expression, endocrinology, and histomorphology. To our knowledge, this is the first study to examine the histomorphology of the ninespine stickleback. We examined variability in concentrations of thyroid hormones and cortisol to guide future research on endocrine disruption. Ninespine stickleback from Troutman Lake exhibited suppressed gonadal development compared to threespine stickleback (*Gasterosteus aculeatus*) studied elsewhere. We found two distinct hepatic phenotypes in Troutman Lake ninespine stickleback, one exhibiting lipid droplet accumulation and one exhibiting glycogen-type vacuolation. We compared the transcriptomic profiles of these phenotypes using RNA sequencing and found significant differences in ribosomal and metabolic pathways. Additionally, the lipid accumulation group had significantly fewer thyroid follicles than the vacuolated group. These results will guide future health studies for Sivuqaq residents. More generally, our study and previous work highlight health concerns for people and wildlife due to pollution hotspots in the Arctic, and the need for health-protective remediation.

INTRODUCTION

Persistent organic pollutants (POPs) are highly stable synthetic compounds that persist in the environment (Kelly et al., 2007). POPs are a grave concern for Arctic Indigenous communities (Hoover et al., 2012) because the Arctic acts as a hemispheric sink of globally distilled pollutants transported from lower latitudes (Mackay and Wania, 1995; Rigét et al., 2010; Wania, 2003). Once POPs enter the Arctic, low temperatures and low intensity sunlight further slow their degradation (Scheringer et al., 2004), which makes them available for long-term incorporation into biological systems (Pacyna et al., 2015). POPs bioaccumulate and biomagnify in lipid-rich arctic food webs, which poses health risks to Arctic communities that depend on subsistence foods (Gobas et al., 1993; Kelly et al., 2007; Suk et al., 2004). The Arctic is the home of many Indigenous peoples who rely on traditional subsistence diets that include lipid-rich foods such as fish and marine mammals (Welfinger-Smith et al., 2011). As a result, these communities are often chronically exposed to POPs through their diet (Van Oostdam et al., 2005). Additionally, the Arctic contains thousands of formerly used defense (FUD) sites dating from World War II and the Cold War, many of which are located near villages (USDOI, 2016; von Hippel et al., 2016). FUD sites can be significant sources of POPs and contribute to disproportionately high levels of exposure in people and wildlife in certain areas (Byrne et al., 2018b; Hoover et al., 2012). The combination of global distillation and local hotspots of pollution explain why the Arctic contains some of the most highly POP-contaminated animals and people in the world (AMAP, 1998, 2015; von Hippel et al., 2016).

The Arctic is contaminated by both legacy and emerging POPs. Legacy POPs include banned or restricted chemicals such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine (OC) pesticides. Emerging POPs include chemicals that are still increasing in levels of environmental contamination, such as organophosphate esters (OPEs) and per- and polyfluorinated alkyl substances (PFAS). The widespread use of these compounds has contributed to pervasive contamination of the global environment and concerns for adverse health effects associated with high or chronic exposures (Dewailly et al., 1989; Hoover et al., 2012; Lohmann et al., 2007; Wania and MacKay, 1996). Because many POPs are endocrine disruptors and neurotoxicants, chronic exposures present an important public health concern for people of the Arctic (Faass et al., 2013; Linares et al., 2015; Sonne et al., 2017).

Sivuqaq (St. Lawrence Island), Alaska is the largest island in the Bering Sea and is located approximately 200 km off the west coast of mainland Alaska (Fig. 1). The United States military installed two radar surveillance stations on the island during the Cold War, including one in the Yupik village of Gambell (FUD site property #F10AK0696; USACE, 2008). The main base camp for the Gambell defense site was built on the northern coast of Troutman Lake directly adjacent to Gambell (USATSDR, 2020). Former military use of Gambell occurred within and around the village, extending from the Bering Sea on the west to the top of Sevuokuk Mountain on the east (USATSDR, 2020). The Gambell FUD site covers approximately 7 km^2 and includes areas around Troutman Lake that were used as disposal sites during military operations from 1948-1965 (USACE, 2008; USATSDR, 2020). Cleanup activities at the FUD site included the removal of over 29 tons of hazardous and non-hazardous wastes, such as transformer debris containing PCBs (USATSDR, 2020). Although subsequent PCB sampling and analysis showed that concentrations were below the EPA cleanup criteria (USACE, 2008), contaminant profiles of stickleback suggest a remaining point source of pollution and indicate that military contamination continues to impact local food webs (Zheng et al., 2020). Troutman Lake is still used for recreating and as a source of drinking water by Gambell residents (USATSDR, 2020).

The people of Sivuqaq report health disparities, including high incidence of cancers and developmental disorders, and express concern about health risks posed by exposure to POPs originating from local FUD sites and atmospheric deposition (Miller et al., 2013; USATSDR, 2020). In particular, residents articulate deep concern about learning and developmental problems among their children that may be associated with elevated exposure to POPs (e.g., PCBs and PBDEs; Miller et al., 2013). These concerns led to multiple studies that found that PBDEs are ubiquitous in dust collected from Sivuqaq households and that Sivuqaq residents have elevated concentrations of PCBs (Carpenter et al., 2005), OC pesticides (Byrne et al., 2015), PBDEs (Byrne et al., 2017), and PFAS (Byrne et al., 2018b) in their blood sera. Research elsewhere has demonstrated that exposure to these contaminants is associated with numerous adverse health outcomes, including disrupted neurodevelopment, cancers, reproductive impairment, and thyroid disorders (Bonefeld-Jorgensen et al., 2001; Bonefeld-Jorgensen et al., 2011; Singh et al., 2014; Yu et al., 2015). Indeed, blood sera levels of PBDEs and PFAS in Sivuqaq residents were significantly associated with thyroid hormone concentrations (Byrne et al., 2018a; Byrne et al., 2018b). These studies confirm that exposure to POPs, whether they originate at hotspots of pollution such as FUD sites or via atmospheric deposition, could lead to the adverse health outcomes reported by the people of Sivuqaq.

Teleost fishes are useful models for studies of contaminant exposures and effects on human health because they can elucidate mechanisms of toxicity and provide relevant biomarkers (Tierney et al., 2014). This study utilized the ninespine stickleback (*Pungitius pungitius*), a useful model organism in arctic ecotoxicology due to their ubiquity in the Arctic, including in contaminated sites; hardiness; and availability of biomarkers (von Hippel et al., 2016). The ninespine stickleback is an excellent proxy for human exposure to contaminants on

Sivuqaq because contaminant profiles in these fish closely mirror those found in blood sera of Sivuqaq residents (Byrne et al., 2015; Byrne et al., 2017). Furthermore, von Hippel et al. (2018), Zheng et al. (2020), and Jordan-Ward et al. (2022) found that contaminants that accumulate in Sivuqaq fish originate primarily at FUD sites, indicating that these FUD sites remain significant sources of POP pollution. Because ninespine stickleback are exposed to many of the same contaminants as Sivuqaq residents, organ-specific analyses in stickleback may elucidate human health effects of local contaminant exposure and tissue-specific mechanisms of toxicity and pathologies.

At the request of the Sivuqaq community, we examined the effects of POP exposure on ninespine stickleback collected from Troutman Lake as a model for human exposure and disease. We examined effects at three levels of biological organization: gene expression, endocrinology, and histomorphology. We focused on histomorphologies of gonad, liver, and thyroid because these organs are targets of disruption by many POPs (Gore et al., 2015). We analyzed thyroid hormone levels because the POPs that are elevated in Troutman Lake stickleback disrupt thyroid function (Oulhote et al., 2016; Yu et al., 2015). We hypothesized that thyroid hormone levels and thyroid morphologies would be consistent with hypothyroidism in many fish, but that variation would occur and be associated with individual vulnerability or susceptibility to POP exposure. During histological analysis of liver tissue, we noticed that ninespine stickleback displayed two distinct phenotypes; some fish displayed glycogen-type hepatocellular vacuolation while others displayed accumulation of hepatic lipid droplets. Liver serves important metabolic functions (Mitra and Metcalf, 2012) and is often the primary organ involved in the biotransformation of contaminants in fishes (Brusle and Anadon, 1996). Therefore, we hypothesized that fish with lipid accumulation in the liver may be more sensitive to obesogenic contaminants. We sequenced mRNA of liver tissue to examine transcriptomic differences between the two phenotypes.

The present study is limited by a lack of a nearby reference population. Troutman Lake and Nayvaghaq Lake are the only lakes close to Gambell, and both were used as disposal sites by the military during operation of the Gambell defense site (USACE, 2008; USATSDR, 2020). Therefore, our study focuses on variability of biological endpoints within the Troutman Lake stickleback population. To our knowledge, this is the first study to examine histology of the ninespine stickleback. Therefore, we rely on comparisons to histomorphologies of the threespine stickleback (*Gasterosteus aculeatus*) and other fishes. Together, our results describe the transcriptomic, endocrinological, and histomorphological characteristics of Troutman Lake ninespine stickleback exposed to FUD site pollution to address concerns regarding human health and the environment.

MATERIALS & METHODS

Fish collection

We collected adult ninespine stickleback from Troutman Lake in the village of Gambell in late June of 2015 and early July of 2018 (Fig. 1). We trapped stickleback using unbaited 0.32 cm and 0.64 cm wire-mesh minnow traps and euthanized fish with an overdose of MS-222 fish anesthetic. We dissected fish in the field for both genetic and histological studies. For histology, we placed tissues in either Dietrich’s solution (2015 samples) or 10% buffered formalin (2018 samples). For gene expression analysis, we placed tissues in PTFE vials containing RNAlater. We stored samples for genetic analyses at -80°C and at room temperature for histological analysis. Sex of each fish was noted in the field and confirmed with histological analysis of the gonads. All research protocols were approved by the University of Alaska Anchorage Institutional Animal Care and Use Committee (IACUC; #439949-1), the University of Oregon IACUC (#13-12R4), and the Northern Arizona IACUC (# 17-003).

PCB analysis

Total PCB concentrations were analyzed in three composite ninespine stickleback samples, representing a total of 30 adult fish. Ninespine stickleback are small fish and composite samples were required to obtain sufficient mass for congener-specific analysis. Therefore, we were not able to compare PCB concentration to histological endpoints or transcriptomic data within an individual. Ten fish were homogenized per sample (~10 g) and prepared and analyzed for PCBs as described by von Hippel et al. (2018). PCB quantification was run by Axys Analytical Services Ltd. (Sidney, British Columbia, Canada) using EPA Method 1668A/C (Axys Method MLA-010 Rev 11).

Histology

Tissue samples from stickleback collected in 2015 were processed at the University of Oregon histology core facility and tissue samples collected in 2018 were processed at the Northern Arizona University histology core facility. In both cases, tissue samples were dehydrated and embedded in paraffin blocks. These were sectioned horizontally into 5 µm sections using a microtome. Each section was then stained with hematoxylin and eosin (H&E).

Liver histomorphology

We analyzed histomorphologies of liver tissue from stickleback collected in 2015 (n=16; 4 males and 12 females) and 2018 (n=17; 14 males and 3 females). Photomicrographs of liver tissue sections were captured using a Leica DM6 B microscope (Leica Microsystems, Wetzlar, Germany) and Leica Application Suite (LASX) software at both 100x and 400x. We selected areas for analysis that appeared homogenous; we did not analyze sections that appeared torn or distorted as a result of the sectioning process, or if the areas contained lots of vasculature. As described by Minicozzi et al. (2019), we analyzed liver tissue for presence or absence of morphological characteristics associated with liver pathology. This included the spectrum of phenotypes associated with non-alcoholic fatty liver disease, such as nuclear displacement and deformation, cellular deformation, disorganized hepatic cordons, and hepatocyte vacuolation (Minicozzi et al., 2019; Wolf and Wheeler, 2018).

Thyroid histomorphology

We analyzed histomorphologies of thyroid tissue from stickleback collected in 2015 (n = 29; 7 males and 22 females) and 2018 (n = 28; 8 males and 20 females) with the same equipment and software as described above for liver. In stickleback, the brachial arteries and skeletal muscles in the thoracic region create a diamond shape, which served as reference points for consistent sectioning, imaging, and analysis of thyroid follicles. We did not find these reference points in 2018 samples due to the location of sectioning. Teleosts do not have thyroid follicles contained in a thyroid gland, but rather the follicles are dispersed within the thoracic region, with most located in the mid-thoracic area (Chanet and Meunier, 2014; Geven et al., 2007). Therefore, sections taken closer to the gill region (as with the 2018 samples) result in fewer thyroid follicles than sections taken in the mid-thoracic region (as with the 2015 samples). Because of these discrepancies, we restricted our analysis to within-year variation for thyroid histomorphologies and present 2018 data in Supplemental Table 1 only.

We selected two thyroid sections for each fish based on the presence and quality of thyroid follicles, with preference given to those with more colloid per follicle. We used histopictographs captured at 100x to count the total number of thyroid follicles. At 400x, we numbered and randomly selected five follicles using a random number generator (www.random.org). For each of the five selected follicles, we measured follicle area, colloid area, and thyrocyte height at the four cardinal points of the follicle image, as described by Petersen et al. (2015) and Gardell et al. (2017). Lipids were identified as white, unstained, and circular structures as described by Gardell et al. (2017). We quantified all measurements using an Intuos touch pad (Wacom, Vancouver, WA) and Image J (NIH) software. Reported values represent the mean of each endpoint for an individual fish.

Gonad histomorphology

We analyzed histomorphologies of gonads from stickleback collected in 2015 (n = 22; 6 males and 16 females) and 2018 (n = 61; 24 males and 37 females). Gonads were imaged using a Leica Aperio CS2 slide scanner and Leica ImageScope software. Only sections containing complete and full gonads were imaged. Two sections of each gonad were imaged and analyzed per individual fish to reduce the potential of artifacts introduced during the sectioning process. After imaging, we imported histopictographs into ImageJ (Schneider et al., 2012) and identified oocyte and testis stages using biomarkers detailed in Sokołowska and Kulczykowska (2006) and Furin et al. (2015). For female stickleback, we used ovary sections that contained the most oocytes. Because ninespine stickleback have asynchronous oocyte maturation (Tyler and Sumpter, 1996), the ovaries contain oocytes at multiple stages. We staged each oocyte visible in the ovary histopictographs as either early (stage 1), intermediate (stage 2), late (stage 3), mature (stage 4), or regressed (Furin et al., 2015; Sokołowska and Kulczykowska, 2006). The stages of male testicular lobules were largely homogeneous and therefore we staged each testis as either early (stage 1), intermediate (stage 2), or late (stage 3) as described in Furin et al. (2015).

RNA sequencing

Stickleback collected from Troutman Lake displayed two distinct liver phenotypes (Fig. 2). We employed RNA-seq analysis to examine gene expression differences associated with these liver phenotypes in ten fish. We compared five stickleback that exhibited liver lipid droplet accumulation (Fig. 2A&B) with five stickleback that exhibited glycogen-type vacuolation of hepatocytes (Fig. 2C&D). We extracted total RNA from liver using the RiboPure RNA Purification kit (Invitrogen) and enriched for mRNA using the Dynabeads mRNA Purification Kit (Ambion). We prepared RNA-seq libraries using the NEXTflex Rapid Directional qRNA-Seq kit (BIOO) and sequenced libraries on an Illumina HiSeq 4000 to generate paired-end 150 nucleotide reads.

We employed the Dupligänger (Sydes, 2019) pipeline to process nucleotide reads and remove unique molecular identifiers (UMI). We then removed adapters using Cutadapt (Martin, 2011) and quality trimmed reads with Trimmomatic (Bolger et al., 2014), requiring an average Phred score of 20 across a sliding window of 5 nucleotides and a minimum read length of 50 nucleotides. Reads were then aligned to the ninespine stickleback genome version NSP_V7 with NCBI RefSeq annotation GCF_902500615.1 (Varadharajan et al., 2019) using STAR (Dobin et al., 2013). We used SAMtools (Li et al., 2009) to filter and sort unique alignments, and Dupligänger to remove PCR duplicates. Feature counts were identified with HTSeq (Anders et al., 2015) in strict mode and differential expression analysis was performed with DESeq2 (Love et al., 2014). One sample was excluded from the analysis because it was identified as a clear outlier in PCA plots and heatmaps (Fig. S1). Genes were considered differentially expressed (DE) with an adjusted p-value (padj) of < 0.1.

Zebrafish (*Danio rerio*) orthologs of ninespine stickleback transcripts were assigned with CRB-BLAST (Aubry et al., 2014). Human orthologs of zebrafish genes were exported from Ensembl version 102 (Yates et al., 2019). Functional enrichment was performed on the human orthologs of differentially expressed genes using the PANTHER Classification System (Mi et al., 2013). A more stringent adjusted p-value of <0.01 was used for PANTHER analyses to provide gene ontology (GO) enrichment scores and to identify significantly upregulated or downregulated biological process pathways for the most enriched genes.

Thyroid hormone quantification

We used different hormone extraction protocols for fish collected in 2015 and 2018. In 2015, we homogenized whole-body stickleback and extracted thyroxine (T_4) and triiodothyronine (T_3) using barbital as described by Gardell et al. (2015), Petersen et al. (2015), and von Hippel et al. (2018). We diluted barbital extracts 1:4 with assay buffer prior to analysis of T_4 and T_3.

In 2018, we freeze-dried and powdered whole stickleback prior to analysis and extracted hormones using methanol. We added 4 mL 100% HPLC methanol to each homogenate sample in a 12x75 mm borosilicate glass tube. Tubes were shaken overnight on a multi-tube vortexer (Glas-Col Large Capacity Mixer, speed set on 65; Glas-Col, Terre Haute, IN, USA), centrifuged for 15 min at 1056 g, and the supernatant was collected into a new 12x75 mm borosilicate glass tube. After drying in a ThermoSavant SpeedVac Concentrator (model SDP121P; Thermo Fisher Scientific, Waltham, MA, USA) at 35ºC, tubes were stored at -80ºC. The day before samples were assayed, they were resuspended with 0.5 mL of assay buffer (X065, Arbor Assays), shaken for 1 h, and then stored at 4ºC overnight. Methanol extracts were diluted 1:4 for cortisol assays and run undiluted for T_3.

We quantified hormones for both 2015 and 2018 samples using commercially available ELISA kits (thyroxine EIA, K050-H1; triiodothyronine EIA, K056-H1; cortisol EIA, K003-H1; Arbor Assays, Ann Arbor, MI). We validated all kits for use with barbital extracts (2015 samples) and methanol extracts (2018 samples) using tests of parallelism and accuracy. We followed the manufacturer's assay protocols with no modifications for all kits. All samples and standards were run in duplicate with an internal control reference standard. All samples fell below the upper limit of the standard curve and the coefficient of variation between duplicates was <10%, and therefore we did not re-run any samples. All internal controls deviated less than 10% from the expected value. The manufacturer's reported limit of detection for cortisol, T_4 and T_3 and are 45.4 pg/mL, 1.04 ng/mL, and 46.6 pg/mL, respectively. Because stickleback are small fish, endocrine analyses required the entire fish sample, which prevented us from comparing hormone concentrations to histological and transcriptomic data within individuals.

Statistical analyses

Because all stickleback in this study were collected from the same area in Troutman Lake, the bulk of our results are descriptive statistics to provide means and variation observed in the population. Measurements are reported as mean ± SE. For comparisons among sex and sampling years, we employed non-parametric statistical methods (Kruskal-Wallis and Mann-Whitney *U* tests) due to non-normal data distributions and heteroskedasticity. Statistical analyses were restricted to within-year differences for thyroid measurements due to disparities in methodology between sampling years. For females, we report statistics on data pooled for both years because the number of oocytes did not differ between years (Mann-Whitney *U* test, p=0.44). All statistical analyses were conducted using R statistical computing software, R version 4.1.0 (2009-2021 Studio, Inc.).

RESULTS

Contaminant concentrations

We measured PCB concentrations in three stickleback composite samples representing a total of 30 fish and compared them to contaminant profiles in stickleback collected from Troutman Lake in previous studies (Fig. 3; Byrne et al., 2015; Byrne et al., 2018b; Zheng et al., 2020). Total PCB concentrations measured in this study were 6.18, 5.61, and 5.25 ng/g ww. These concentrations are within the range reported by Zheng et al. (2020) in stickleback collected from Troutman Lake in 2018 (Fig. 3). PCB congener profiles revealed that *hexa*-chlorinated and *hepta*-chlorinated congeners contributed 55% to the mean total PCB concentration in Troutman Lake stickleback, while *tri*-chlorinated congeners contributed 3% (Table S2).

Liver histology

We analyzed the livers of 33 ninespine stickleback (18 males and 15 females) for histopathologies consistent with non-alcoholic fatty liver disease. We observed large variation in lipid accumulation and hepatocyte size across livers, but generally found that fish displayed one of two hepatic phenotypes: those with increased lipid droplets (Fig. 2A&B) and those with glycogen-type vacuolation of hepatocytes (Fig. 2C&D). Of the 33 ninespine stickleback analyzed, we found that 70% displayed nuclear displacement, 48% displayed cellular deformation, 82% displayed nuclear hypertrophy, and 48% displayed disorganized cordons (Table 1). The 2018 sample had fewer fish exhibiting deformed cellular shapes and disorganized cordons than the 2015 sample, but more fish with lipid droplet accumulation (53% in 2018 vs 19% in 2015). Of the fish with lipid droplet accumulation, 67% were males. Both male and female stickleback displayed the vacuolated hepatocyte liver phenotype.

Thyroid histology

We sampled 29 ninespine stickleback for thyroid histology in 2015 (7 males and 22 females) and 28 ninespine in 2018 (8 males and 20 females). Histological sections from 2018 did not capture the same biological landmarks (brachial arteries and skeletal muscle) as the 2015 samples and are therefore presented in Supplemental Table 1 only. In 2015, male and female stickleback did not differ in follicle area (3492 ± 47 μm^2) or colloid area (1013 ± 25 μm^2). Follicle and colloid areas were highly correlated (Pearson's product-moment, $r = 0.85$, $n = 29$, $p<0.0001$). Male stickleback had significantly fewer thyroid follicles per section (12 ± 1) than did female stickleback (20 ± 1; Mann-Whitney U test, $p=0.01$; Fig. 4A). Additionally, male stickleback had significantly shorter thyrocytes than did female stickleback (Mann-Whitney U test, $p=0.004$; Fig. 4B). We observed lipid accumulation in surrounding thyroid follicles in both male and female stickleback (Fig. 5).

The mean number of thyroid follicles significantly differed by liver phenotype. Individual stickleback displaying hepatic lipid accumulation had almost half as many thyroid follicles (9 ± 3) than did stickleback with the vacuolated phenotype (19 ± 2; Mann-Whitney U test, $p=0.030$). Of the thyroid data with matching liver data, male and female fish had similar means as found in the entire data set, but sex differences were no longer significant, likely due to small sample sizes. No other thyroid histomorphology differed between liver phenotypes.

Gonad histology

Female stickleback had significantly more early-stage oocytes than they did either mid, late, mature, or regressed stages (Dunn's test, $n=53$, $p<0.0001$ for all comparisons; Figs. 6&7). Late-stage oocytes (vacuoles occupied all areas of cytoplasm) were present at significantly greater numbers than were mature oocytes (egg yolk filled most of oocyte as a homogenous mass; Dunn's test p=0.005). We found that mature oocytes comprised only 9% of the total number of oocytes. Similarly, the majority of male stickleback in this study exhibited early-stage testicular lobules (57%), while only 6% exhibited mature testicular lobules (Fig. 7). Of the gonad and matching liver data, neither female nor male gonad endpoints significantly differed between liver phenotypes, but sample sizes were small (females: n=6 vacuolated phenotype and n=2 lipid droplet phenotype; males: n=5 vacuolated phenotype, n=6 lipid droplet phenotype).

Transcriptional profiling

We used RNA-seq to compare gene expression profiles between male stickleback exhibiting liver lipid droplet accumulation (Fig. 2A&B) and those exhibiting increased hepatocyte vacuolation (Fig. 2C&D). Principal component analysis of RNA-seq reads showed that these phenotypes were transcriptionally distinct (Fig. S1). The principal component analysis identified one individual from the hepatocytic vacuolation group as an extreme outlier (Fig. S1A). We investigated the transcriptional profile in this fish and found that the sample was contaminated with intestinal tissue (Fig. S2); it was therefore removed from further analyses.

Analysis of the RNA-seq reads identified 4818 differentially expressed genes (padj<0.1) between the two hepatic phenotypes. We used a more stringent adjusted p-value of 0.01 (2329 genes) for gene input into the PANTHER Classification System to gain a better understanding of pathways associated with the most differentially expressed genes. We found that genes involved in metabolic and biosynthetic processes, including cellular metabolic processes (GO:0044237, fold-enrichment=3.11, FDR<0.0001) and cellular response to stress (R-HSA-2262752, fold-enrichment=1.75, FDR<0.0001), were the most enriched biological pathways in fish displaying the liver lipid droplets. We found that the most enriched cellular pathways were the endoplasmic reticulum chaperone complex pathway (GO:0034663, fold-enrichment score=5.48, FDR=0.035) and the oligosaccharyltransferase complex pathway (GO:0008250, fold-enrichment score=5.22, FDR=0.009). Genes involved in structural constituents of the ribosome (GO:0003735) and ribosome biogenesis (GO:0042254) were also enriched in fish with liver lipid accumulation (fold-enrichment=3.19, FDR<0.001 and fold-enrichment=1.89, FDR<0.005, respectively).

We examined the expression of several genes involved in enriched pathways and pathways of interest to better understand the transcriptional differences between stickleback in the two liver groups. We found that expression of the thyroid hormone receptor isoform ß (*THRB*) gene was downregulated 2.3-fold in stickleback displaying hepatic lipid accumulation compared to fish with the vacuolated phenotype (padj<0.001). Similarly, the type II iodothyronine deiodinase (*dio2*) gene, which catalyzes the conversion of T_4 to T_3, was 4-fold downregulated in stickleback displaying the liver lipid phenotype (padj=0.003). We found that expression of peroxisome proliferator-activated receptor α (*ppar*α) was significantly higher in stickleback displaying the lipid accumulation phenotype (fold-change=2.68, p=0.001). The fish gene *abcb4*, a paralog of the mammalian P-glycoprotein gene *ABCB1*, encodes for a protein that helps transport phospholipids across hepatocyte membranes and was significantly upregulated in Troutman Lake stickleback displaying the vacuolated phenotype (2.3-fold upregulated, padj<0.001) (Jackson and Kennedy, 2017). We also found that the fish gene *cyp3a65*, an ortholog of the human Phase I metabolic enzyme P450 3A (*CYP3A*) gene (Saad et al., 2016) that facilitates metabolic degradation of xenobiotic compounds (Jackson and Kennedy, 2017), was upregulated 2.9-fold in fish with vacuolated livers (padj=0.002). We did not find significant differences in expression of vitellogenin genes between stickleback displaying different liver phenotypes.

Endocrinology

Ninespine stickleback exhibited high variability in thyroid hormone concentration (Fig. 8). T_4, T_3, and cortisol concentrations did not differ significantly by sex (Mann-Whitney U test, $p>0.1$ for all tests) and subsequent statistics were used to analyze pooled data ($n=39$ for 2015 and $n=40$ for 2018). T_3 concentrations were significantly higher than T_4 concentrations in 2015 (Mann-Whitney U test, $p<0.001$; Fig. 8A; note that we did not measure T_4 in 2018). Three male stickleback in 2018 had abnormally high cortisol levels compared to the other samples (Fig. 8B). We did not compare T_3 concentrations between years because different analytical methods were used. Despite finding sex differences in thyroid morphology, we did not find significant sex differences in the concentrations of T_4, T_3, or cortisol.

DISCUSSION

Contaminant concentrations

Ninespine stickleback from Troutman Lake were analyzed for several classes of contaminants: PCBs (this study; Zheng et al., 2020), PBDEs (Byrne et al., 2017; Zheng et al., 2020), PFAS (Byrne et al., 2017; Zheng et al., 2020), OC pesticides (Byrne et al., 2015), and OPEs and their metabolites (Zheng et al., 2020), all of which negatively impact human health and the environment (Faass et al., 2013; Linares et al., 2015; Sonne et al., 2017). Of these contaminants, total PBDEs were detected at the highest concentrations, followed by PCBs and PFAS (Fig. 3; Byrne et al., 2015; Byrne et al., 2017; Zheng et al., 2020). Total PBDE concentrations were comparable to the range observed in pilot whales from the Faroe Islands (Byrne et al., 2017; Rotander et al., 2012). Although stickleback are not a subsistence food source for Sivuqaq residents, they serve as an important prey species for piscivorous birds (Cairns et al., 1991). Gambell hosts a variety of seabirds, including a large rookery on Sevuokuk

Mountain to the east of the village that contains populations of glaucous and glaucous-winged gulls (*Larus hyperboreus* and *Larus glaucescens*, respectively), pelagic cormorants (*Phalacrocorax pelagicus*), and several species of auklets (Sealy et al., 1971; Welfinger-Smith et al., 2011). These birds and their eggs are important subsistence food sources for Gambell residents (Welfinger-Smith et al., 2011).

PCB concentrations in Troutman Lake stickleback exceeded (by 3.8-fold) the EPA's guideline for unlimited fish consumption (cancer risk for human consumption; 1.5 ng/g ww) (USEPA, 2000). Stickleback are relatively short-lived, low-trophic level fish that feed on invertebrates and are not expected to have elevated concentrations of highly chlorinated PCB congeners in remote parts of Alaska that lack point sources of pollution. However, we found that concentrations of *hexa*-chlorinated and *hepta*-chlorinated congeners contributed the most to the total PCB concentration in Troutman Lake stickleback. Atmospheric transportation and deposition of PCBs results in surface concentrations predominant in *tri*-chlorinated congeners (44-96% of total PCBs) in the Bering Sea, which surrounds Sivuqaq (Hong et al., 2012). Conversely, heavier PCB congeners are less volatile and do not readily undergo long-range atmospheric transport. Thus, our data and those reported by Zheng et al. (2020) suggest that PCB contamination of Troutman Lake is due primarily to a local source of pollution.

Liver histology

The molecular basis of the two liver phenotypes that we observed warrants additional investigation, along with analysis of whether one of the phenotypes is associated with vulnerability to POP exposure while the other is associated with resilience. Both liver phenotypes observed in this study appeared abnormal compared to threespine stickleback from a laboratory control group (Minicozzi et al., 2019) and other wild fishes (Feist et al., 2015).

Because the liver is the primary site of xenobiotic metabolism, it is often a target of POP toxicity (Deierlein et al., 2017; La Merrill et al., 2019; Safe, 1994). Many xenobiotic contaminants are known to increase hepatic lipid accumulation in fishes (Li et al., 2019; Maradonna et al., 2015). For example, Li et al. (2019) found that PCB exposure caused lipid accumulation in zebrafish by disrupting genes related to lipogenesis and lipid catabolism. In addition to lipid accumulation, metabolic responses to environmental pollution can increase energy demands and lead to depleted glycogen stores in the liver (Anderson et al., 2003; Hugla and Thomé, 1999). Indeed, fish exposed to PCBs (Anderson et al., 2003) and toxic metals (Javed and Usmani, 2013) exhibited depleted liver glycogen levels. Carbohydrates are stored as glycogen in the liver and provide a rapid source of glucose under low blood glucose conditions (Li et al., 2022). Exposure to environmental pollution is often associated with hepatic glycogen depletion, possibly through perturbations of biochemical activities, such as disruptions to glycogenolysis and/or increased energy demands for contaminant detoxification (De Coen and Janssen, 2003; Hugla and Thomé, 1999; Peplow and Edmonds, 2005; Rochman et al., 2013). These studies suggest that Troutman Lake stickleback with lipid droplet accumulation are more sensitive to obesogenic contaminants because they exhibited more lipid droplets and less glycogen than did stickleback with vacuolated livers.

Nevertheless, increased hepatocyte vacuolation in response to contaminant exposure also occurs, especially for contaminants that act as xenoestrogens (Madureira et al., 2015; Miranda et al., 2008; Tarn et al., 1983; Xu et al., 2017). Troutman Lake stickleback have elevated concentrations of PFAS, including PFOA and PFOS (Byrne et al., 2017; Zheng et al., 2020), which are positively correlated with hepatocyte vacuolation (Giari et al., 2015; Wolf et al., 2008; Xu et al., 2017). Certain PCBs are also associated with glycogen accumulation in fish. For example, Miranda et al. (2008) found that increased liver glycogen content served as a biomarker of elevated exposure to chlorinated pesticides and PCBs in trahira (*Hoplias malabaricus*). In the present study, ninespine stickleback with vacuolated livers appeared similar in morphology to livers in dourado (*Salminus franciscanus*) exposed to toxic metal contamination in the Paraopeba River of Brazil (Savassi et al., 2020) and to barfin plaice (*Liopsetta pinnifasciata*) exposed to pollution in Amursky Bay, Japan (Shved et al., 2011). Although previous studies found that vacuolation differed by sex (Shved et al., 2011; Wolf and Wheeler, 2018), both male and female stickleback in the present study displayed this phenotype. Because the present study lacks a suitable reference group and ninespine stickleback histology has not been well characterized, additional field and laboratory studies are needed to elucidate the effects of FUD site pollution on the liver. Teleosts differ widely in the amount of neutral lipids stored in hepatocytes (Akiyoshi and Inoue, 2004), and the utility of histological studies of ninespine stickleback in contaminated sites will be enhanced when their development in clean water has been well characterized.

Thyroid histology

We compared thyroid follicle count and liver phenotype within individual stickleback and found that stickleback with liver lipid accumulation had fewer thyroid follicles than did stickleback with vacuolated livers. Similarly, male stickleback had fewer thyroid follicles than did female stickleback across all samples. However, because normal thyroid histomorphology is not well documented in the ninespine stickleback, we cannot determine the direction of change in the number of thyroid follicles for Troutman Lake stickleback. Laboratory studies in threespine stickleback used as untreated control fish revealed a mean of ~20 thyroid follicles per section (Furin et al., 2015), which is similar to the means of female ninespine stickleback and fish with vacuolated livers in the present study. If these fish represent typical thyroid follicle counts in ninespine stickleback, then male ninespine stickleback and those with lipid accumulation in the current study exhibited thyroid follicle hypoplasia, which is associated with hyperthyroid conditions (Deal and Volkoff, 2020; Raine et al., 2001; Sharma et al., 2016). Conversely, female ninespine stickleback and those with vacuolated livers could be hypothyroid. Indeed, increased thyrocyte height observed in female ninespine stickleback may result from elevated thyroid stimulating hormone (TSH), indicating hypothyroid conditions in fish (Deal and Volkoff, 2020). Although the present study cannot determine thyroid condition in Troutman Lake stickleback, our results suggest that POP exposure may affect male and female stickleback differently, and that liver phenotype is associated with changes in thyroid condition.

Gonad histology

Both male and female stickleback from Troutman Lake exhibited suppressed gonadal maturation compared to patterns in wild female ninespine stickleback (Sokolowska and Krzysztof, 2002) and both sexes of threespine stickleback (Sokołowska and Kulczykowska, 2006) at peak breading season. Sokołowska and Kulczykowska (2006) detailed the annual reproductive cycle of two wild threespine stickleback populations and found that over 80% of oocytes were mature in females and about 60-100% of testes were mature in males during the spawning period. Because threespine and ninespine stickleback share similar reproductive life history traits (Baker et al., 1998; Heins et al., 2003; Heins et al., 1999), we expected to find similar maturity levels in Troutman Lake ninespine stickleback collected in June and July, but instead found that Troutman Lake stickleback exhibited far fewer mature oocytes and testes compared to wild threespine stickleback populations during these months.

Suppressed ovarian and testicular maturation in Troutman Lake stickleback could be caused by chronic exposure to endocrine disrupting compounds. Because both female and male fish depend on steroid hormones for proper gonadal development (Delbes et al., 2022), exposure to xenobiotic estrogens may disrupt normal hormonal signaling and delay gonadal maturation (Berg et al., 2016; Meier et al., 2011). For example, exposure to alkylphenols, which elicit estrogenic effects, delayed oocyte development and maturation in Atlantic cod (*Gadus morhua*) (Meier et al., 2007; Meier et al., 2011). Similarly, exposure to wastewater effluent containing endocrine disrupting compounds suppresses follicular development in various fish species (Douxfils et al., 2007; Jobling et al., 2002). The contaminants present in Troutman Lake stickleback are endocrine disruptors that modulate activity of the hypothalamic-pituitary-gonadal and the hypothalamic-pituitary-thyroid axes. PCBs and PBDEs can elicit both estrogenic and anti-estrogenic effects and disrupt normal reproductive systems in many animals, including humans (Allen et al., 2016; Jansen et al., 1993; Li et al., 2013; Petro et al., 2012). For example, Kraugerud et al. (2012) found that female burbot (*Lota lota*) exposed to POPs, including PCBs, PBDEs, and DDT, had significantly lower counts of late-stage ovarian follicles. Although the lack of a suitable reference population limits our ability to ascertain the cause of gonadal immaturity in Troutman Lake stickleback, our findings are consistent with previous contaminant exposure studies in fishes that resulted in suppressed maturation of gonads (Horri et al., 2018; Vasseur and Cossu-Leguille, 2006). As a result, chronic exposure to pollutants may impair reproductive processes in Troutman Lake stickleback; however, additional research is required to characterize both the timing of peak breeding in Troutman Lake and the normal pathway of gonad maturation in uncontaminated ninespine stickleback.

Transcriptional profiling

Omics techniques provide insights into perturbed genetic pathways in wild fishes exposed to environmental pollution. Our results comparing transcriptional profiles of two liver phenotypes in stickleback collected from Troutman Lake demonstrate significant differences in expression of genes involved in ribosomal and metabolic pathways. Overexpression of ribosomal genes often occurs under conditions of cellular stress and may indicate modification of key metabolic pathways, including protein biosynthesis (Spriggs et al., 2010; Zheng et al., 2018). Indeed, we found that genes associated with ribosome biogenesis were enriched in Troutman Lake stickleback with lipid accumulation in their livers. Ribosomal biogenesis requires significant cellular energy (Pelava et al., 2016; Zhou et al., 2015) and could contribute to depleted glycogen levels in stickleback exhibiting the lipid phenotype, supporting the hypothesis that these stickleback are more sensitive to environmental pollution. Similarly, we found enrichment of genes associated with endoplasmic reticulum complexes and pathways in the lipid accumulation group. Exposure to PCBs induces metabolic disorders by altering lipid and carbohydrate metabolism (Aluru et al., 2019; Mesnier et al., 2015) and causes ultrastructural changes to both the smooth and rough endoplasmic reticulum (Gallant et al., 2000; Hugla et al., 1996; Klaunig et al., 1979). For example, Hinton et al. (1978) found that PCB-induced fatty liver in rats was likely facilitated by disturbed transport of lipoproteins from the endoplasmic reticulum. Lipid droplets observed in Troutman Lake stickleback could accumulate through similar mechanisms.

Many POPs act as obesogenic compounds by influencing metabolic processes, including lipid metabolism (Grün and Blumberg, 2006; Heindel et al., 2017; Maqbool et al., 2016; Yang et al., 2017). Obesogenic compounds primarily disrupt endocrine function of oxidative stress and nuclear receptor pathways (Grün and Blumberg, 2006; Heindel et al., 2017; Hong et al., 2015;

Lee et al., 2016; Maqbool et al., 2016; Mazeaud et al., 1977). Results from our functional annotation of RNA-seq reads indicate that gene sets involved in these pathways are upregulated in stickleback with hepatic lipid accumulation relative to those with vacuolation, including cellular metabolic processes and cellular response to stress. Cellular metabolic processes include genes involved in lipid metabolic and catabolic processes, such as genes that encode for PPAR proteins. PPARs increase uptake of fatty acids in cells and regulate transcription of genes involved in lipoprotein metabolism (Montaigne et al., 2021). Over-expression of PPARα may initiate liver lipid accumulation in response to contaminant exposure (Huff et al., 2018; Li et al., 2019). PFOA and PFOS act as agonists for PPARα and modulate expression in multiple organisms (Krøvel et al., 2008; Takacs and Abbott, 2006). We found that expression of *ppar*α was significantly higher in stickleback displaying the lipid accumulation phenotype, which suggests that these fish may be more sensitive to obesogenic contaminants. However, we did not find significant differences in other PPAR isoforms, particularly PPARγ, as found in other studies (Dépatie et al., 2020; Li et al., 2019; Reinling et al., 2017). Additionally, morphological characteristics associated with non-alcoholic fatty liver disease and liver steatosis (e.g., nuclear displacement) were less frequent in stickleback displaying lipid droplet accumulation, indicating that lipid accumulation or transcriptional changes may protect against hepatotoxicity by sequestering POPs and preventing POP effects in some Troutman Lake stickleback (Lee et al., 2017).

ATP-binding cassette (ABC) transporters confer multixenobiotic resistance (MXR) to toxic contaminants in several species (Jackson and Kennedy, 2017; Kurelec, 1992; Smital et al., 2000). Upregulation of P-glycoprotein family genes, specifically *ABCB1* in mammals and *abcb4* in zebrafish, facilitate MXR in wild populations exposed to pollutants by increasing transport of exogenous compounds and reducing xenobiotic uptake (Fischer et al., 2013; Jackson and Kennedy, 2017; Smital et al., 2000). For example, Fischer et al. (2013) found that elevated expression of *abcb4* was negatively associated with uptake of toxic compounds in zebrafish embryos and provided protection against contaminant toxicity. Transcriptional regulation of *abcb4* often works in concert with *CYP3A* genes to increase excretion of xenobiotic contaminants (Jackson and Kennedy, 2017; Perloff et al., 2001). Several *CYP3A* genes, including *cyp3a65* in zebrafish, metabolize xenobiotic contaminants and are upregulated in response to exposure to xenobiotic substances (Chang et al., 2013; Kubota et al., 2014). For example, Jackson and Kennedy (2017) found that transcriptional regulation of both *abcb4* and *cyp3a65* mediated MXR in zebrafish. Both *abcb4* and *cyp3a65* were significantly upregulated in Troutman Lake stickleback displaying the vacuolated phenotype. These transcriptional differences support the hypothesis that stickleback with vacuolated livers are more resistant to environmental pollution than stickleback displaying the lipid droplet phenotype.

Many POPs that are elevated in Troutman Lake stickleback, including PCBs and PFAS, induce estrogenic effects and increase expression of vitellogenin genes in male fish (Gao et al., 2013; Nomiyama et al., 2010; Sumpter and Jobling, 1995; von Hippel et al., 2018). Because males do not secrete vitellogenin under normal conditions, vitellogenin serves as a biomarker of xenobiotic estrogens (Hansen et al., 1998), including in ninespine stickleback (von Hippel et al., 2016). We examined transcriptional differences in genes involved in the production of vitellogenin to test the hypothesis that Troutman Lake stickleback exhibiting hepatic lipid accumulation are more sensitive to estrogenic contaminants and to examine upstream mechanisms of observed suppression of gonadal maturity. However, we did not find significant differences in expression of vitellogenin genes between stickleback displaying different liver phenotypes. Several possibilities warrant further investigation. Stickleback collected from Troutman Lake may experience similar transcriptional effects of estrogen pathways and are thus not transcriptionally different in these pathways. Additionally, fish in both liver groups may experience estrogenic effects, but differ in their sensitivity and response to contaminant mixtures. Overall, we do not have enough data to disentangle the role of endocrine-disrupting POPs on liver histomorphology within Troutman Lake stickleback.

Endocrinology

Thyroid hormones play an important role in lipid metabolism and energy homeostasis (Liu and Brent, 2010; Sinha et al., 2014), and perturbations in circulating T_4 and T_3 may contribute to the observed liver phenotypes in Troutman Lake stickleback. Specifically, hypothyroidism is associated with increased fat accumulation and non-alcoholic fatty liver disease (Ludwig et al., 2015; Sinha et al., 2018). While the present study lacks a reference population to determine if Troutman Lake stickleback exhibit biomarkers for hypothyroidism, we found that expression of *THRB* was downregulated in stickleback displaying hepatic lipid accumulation compared to fish with the vacuolated phenotype (padj<0.001). *THRB* helps regulate cholesterol metabolism (Gullberg et al., 2002), and mutant mice with *THRB* knockdown exhibited excessive lipid accumulation in the liver (Araki et al., 2009). Additionally, we found that the *dio2* gene was downregulated in stickleback displaying the liver lipid phenotype (padj=0.003). Some PCB congeners suppress *dio2* expression (Liu et al., 2014), while several PBDE congeners (e.g., BDE-71, BDE-153, and BDE-209) increase *dio2* expression (Noyes et al., 2011; Yu et al., 2010). As such, exposure to local sources of POPs may affect thyroid hormone homeostasis and contribute to metabolic disruption in Troutman Lake stickleback (Liu and Brent, 2010; Warner and Mittag, 2012).

Similarly, suppressed gonadal maturation observed in Troutman Lake stickleback could also result from perturbations to the hypothalamic-pituitary-thyroid axis. POP toxicity may elicit indirect effects through crosstalk mechanisms between thyroid and reproductive systems (Kuiper et al., 2008; Li et al., 2014; Yu et al., 2015). Thyroid hormone activation by *dio2* is necessary for normal embryonic development (Walpita et al., 2009) and successful reproduction in zebrafish (Houbrechts et al., 2019). Therefore, downregulation of *dio2* in Troutman Lake stickleback displaying the liver lipid phenotype may contribute to developmental delays.

PCBs and PBDEs are structurally similar to thyroid hormones and elicit a decrease in circulating T_4 levels (Fisher et al., 2005; Lema et al., 2008; Tomy et al., 2004; Turyk et al., 2007). In humans, different PBDE congeners can elicit different effects on circulating thyroid hormones (Byrne et al., 2018a; Turyk et al., 2008). On Sivuqaq, BDE-153 concentrations in blood sera of Gambell residents were negatively associated with circulating T_3 concentrations while *penta*-BDE congeners were positively associated with T_3 concentrations (Byrne et al., 2018b). Oulhote et al. (2016) found that elevated plasma levels of total PBDEs in Canadian women were associated with a higher prevalence of hypothyroidism. Most animal studies report that estrogenic PBDEs and PCBs induce hypothyroid conditions (Brown et al., 2004; Hallgren et al., 2001; Miller et al., 2010). Proper thyroid function is critical for the health of the developing brain (Porterfield, 1994; Zoeller et al., 2002), and POP-mediated fluctuations in thyroid hormone levels at critical windows of susceptibility may have lasting health consequences, especially for cognitive development in children (Gilbert and Lasley, 2013; Henrichs et al., 2013).

Limitations

The life history of Troutman Lake stickleback has not been investigated; however, Troutman Lake does not have an outlet to the Bering Sea, except for periodic storm surges that break over the storm berm (USATSDR, 2020). Therefore, stickleback live year-round in Troutman Lake and are exposed to environmental contaminants throughout their lifetime. We collected stickleback from a single location (Fig. 1), and it is unlikely that differences observed in this study are explained by life history differences, such as resident freshwater versus anadromous ecotypes. Therefore, we hypothesized that transcriptomic and phenotypic differences in Troutman Lake stickleback result from differences in sensitivity to contaminant exposure. Because many POPs are obesogenic and increase liver lipid droplets in fish (Li et al., 2019; Pfohl et al., 2021), we hypothesized that stickleback displaying liver lipid accumulation were more sensitive to obesogenic effects. Although this study alone cannot attribute the observed variation in transcriptional profiles or the presence of histopathologies to contaminant exposure, our results are consistent with the findings in other fish species exposed to the same contaminants that are elevated in Troutman Lake (Brown et al., 2004; Grün and Blumberg, 2006; Yu et al., 2015). Furthermore, our transcriptomic results indicate that ninespine stickleback displaying liver lipid accumulation are transcriptionally distinct and suggest that these fish are more sensitive to obesogenic chemicals. Future research on ninespine stickleback histomorphology is required to better elucidate directional changes in tissue-specific responses to environmental contamination.

Most research on histomorphologies in the *Gasterosteidae* family, which includes the ninespine stickleback, has been conducted on threespine stickleback. To our knowledge, this is the first study to examine histomorphologies in ninespine stickleback. Therefore, we inferred normal phenotypes from research in threespine stickleback. These two species share many life history and associated morphological traits (Copp et al., 1998; Herczeg et al., 2010), and thus we hypothesize that ninespine stickleback also share many of the same histological characteristics.

However, future research should characterize normal histomorphology and seasonal variation in reproductive traits in ninespine stickleback, especially given the increasing utility of these fish in arctic ecotoxicology (von Hippel et al., 2016).

Understanding the impact of contaminant exposure in wild fish populations is challenging amid the complexities of contaminant mixtures and the potential for non-additive effects. Ninespine stickleback in Troutman Lake are exposed to a diverse mixture of contaminants (Fig. 3) that interfere with endocrine function in many ways, which restricts our ability to ascertain the underlying mechanisms driving transcriptome differences and histopathology.

CONCLUSIONS

The contaminant profiles of ninespine stickleback on Sivuqaq closely mirror that of the blood serum of residents (Byrne et al., 2015; Byrne et al., 2017), making them a suitable model organism for human health effects of contaminant exposure on the island. The current study and previous work (von Hippel et al., 2018) also show that ninespine stickleback on Sivuqaq display health outcomes that are relevant for the health concerns of island residents, including differential expression of genes associated with cancer, cellular metabolism, and developmental effects. Future work should further develop the ninespine stickleback as a One Health model for people throughout the Arctic, given that local hotspots of pollution occur in all arctic countries and are often located in or adjacent to Indigenous communities (von Hippel et al., 2016). The widespread distribution of the ninespine stickleback in the Arctic, including in freshwater, brackish water, and marine habitats, along with its ability to survive in contaminated sites and the availability of biomarkers of contaminant exposure, provide an opportunity to expand its utility to study diverse problems in pollution science (von Hippel et al., 2016). Furthermore, the current study exemplified individual variation in responses to contaminants and highlights the need for precision medicine approaches.

The harvest and consumption of traditional foods is central to the nutritional, cultural, and economic health of Arctic Indigenous Peoples. However, subsistence diets may contribute to elevated exposure to POPs (Welfinger-Smith et al., 2011). For example, concentrations of PBDEs found in the blood of Yupik people of the Yukon-Kuskokwim Delta region of Alaska are the highest known human PBDE concentrations in the circumpolar Arctic (Wilson et al., 2014). Health disparities due to disproportionate exposure to pollutants are exacerbated by the rapid pace and magnitude of climate change in the Arctic, which is warming at more than twice the global average (AMAP, 2015). The combination of a warming climate and increased mobilization of POPs previously sequestered in ice and permafrost are expected to increase contamination of the Arctic and result in large-scale ecological and human health consequences (Mckinney et al., 2015; Serreze and Barry, 2011).

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REFERENCES

Akiyoshi, H., Inoue, A., 2004. Comparative histological study of teleost livers in relation to phylogeny. Zoolog Sci 21, 841-850.

Allen, J.G., Gale, S., Zoeller, R.T., Spengler, J.D., Birnbaum, L., McNeely, E., 2016. PBDE flame retardants, thyroid disease, and menopausal status in U.S. women. Environ Health 15, 60.

Aluru, N., Krick, K.S., McDonald, A.M., Karchner, S.I., 2019. Developmental exposure to PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl) alters circadian rhythms and the expression of clock and metabolic genes. Toxicol Sci 173, 41-52.

AMAP, 1998. AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xii+859 pp.

AMAP, 2015. Summary for Policy-makers: Arctic Pollution Issues 2015. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. 12 pp.

Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq-a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166-169.

Anderson, M.J., Cacela, D., Beltman, D., Teh, S.J., Okihiro, M.S., Hinton, D.E., Denslow, N., Zelikoff, J.T., 2003. Biochemical and toxicopathic biomarkers assessed in smallmouth bass recovered from a polychlorinated biphenyl-contaminated river. Biomarkers 8, 371-393.

Araki, O., Ying, H., Zhu, X.G., Willingham, M.C., Cheng, S.Y., 2009. Distinct dysregulation of lipid metabolism by unliganded thyroid hormone receptor isoforms. Mol Endocrinol 23, 308-315.

Aubry, S., Kelly, S., Kümpers, B.M.C., Smith-Unna, R.D., Hibberd, J.M., 2014. Deep evolutionary comparison of gene expression identifies parallel recruitment of trans-factors in two independent origins of C4 photosynthesis. PLoS Genet 10, e1004365.

Baker, J.A., Foster, S.A., Heins, D.C., Bell, M.A., King, R.W., 1998. Variation in female life-history traits among Alaskan populations of the threespine stickleback, *Gasterosteus aculeatus L*. (Pisces: *Gasterosteidae*). Biol J Linn Soc Lond 63, 141-159.

Berg, V., Kraugerud, M., Nourizadeh-Lillabadi, R., Olsvik, P.A., Skare, J.U., Alestrom, P., Ropstad, E., Zimmer, K.E., Lyche, J.L., 2016. Endocrine effects of real-life mixtures of persistent organic pollutants (POP) in experimental models and wild fish. J Toxicol Environ Health 79, 538-548.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114-2120.

Bonefeld-Jorgensen, E.C., Andersen, H.R., Rasmussen, T.H., Vinggaard, A.M., 2001. Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 158, 141-153.

Bonefeld-Jorgensen, E.C., Long, M., Bossi, R., Ayotte, P., Asmund, G., Krüger, T., Ghisari, M., Mulvad, G., Kern, P., Nzulumiki, P., Dewailly, E., 2011. Perfluorinated compounds are related to breast cancer risk in greenlandic inuit: a case control study. Environ Health 10, 88.

Brown, S.B., Adams, B.A., Cyr, D.G., Eales, J.G., 2004. Contaminant effects on the teleost fish thyroid. Environ Toxicol Chem 23, 1680-1701.

Brusle, J., Anadon, G.G., 1996. The structure and function of fish liver. Fish Morphology, CRC Press., 77-93.

Byrne, S., Miller, P.K., Seguinot-Medina, S., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2018a. Associations between serum polybrominated diphenyl ethers and thyroid hormones in a cross sectional study of a remote Alaska Native population. Sci Rep 8, 2198.

Byrne, S., Miller, P.K., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2015. Persistent organochlorine pesticide exposure related to a formerly used defense site on St. Lawrence Island, Alaska: data from sentinel fish and human sera. J Toxicol Environ Health Part A 78, 976-992.

Byrne, S., Seguinot-Medina, S., Miller, P.K., Waghiyi, V., von Hippel, F.A., Buck, C.L., Carpenter, D.O., 2017. Exposure to polybrominated diphenyl ethers and perfluoroalkyl substances in a remote population of Alaska Natives. Environ Pollut 231, 387-395.

Byrne, S.C., Miller, P.K., Seguinot-Medina, S., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2018b. Exposure to perfluoroalkyl substances and associations with serum thyroid hormones in a remote population of Alaska Natives. Environ Res 166, 537-543.

Cairns, D., Chapdelaine, G., Montevecchi, W., 1991. Prey exploitation by seabirds in the Gulf of St. Lawrence. Can J Fish Aquat Sci 113, 277-291.

Carpenter, D.O., DeCaprio, A.P., O'Hehir, D., Akhtar, F., Johnson, G., Scrudato, R.J., Apatiki, L., Kava, J., Gologergen, J., Miller, P.K., Eckstein, L., 2005. Polychlorinated biphenyls in serum of the Siberian Yupik people from St. Lawrence Island, Alaska. Int J Circumpolar Health 64, 322-335.

Chanet, B., Meunier, F.J., 2014. The anatomy of the thyroid gland among “fishes”: phylogenetic implications for the Vertebrata. Cybium 38, 90–116.

Chang, C.-T., Chung, H.-Y., Su, H.-T., Tseng, H.-P., Tzou, W.-S., Hu, C.-H., 2013. Regulation of zebrafish CYP3A65 transcription by AHR2. Toxicol Appl Pharmacol 270, 174-184.

Copp, G., Edmonds-Brown, V., Cottey, R., 1998. Behavioural interactions and microhabitat use of stream-dwelling sticklebacks *Gasterosteus aculateus* and *Pungitius pungitius* in the laboratory and field. Folia Zool 47, 275-286.

De Coen, W.M., Janssen, C.R., 2003. The missing biomarker link: relationships between effects on the cellular energy allocation biomarker of toxicant-stressed *Daphnia magna* and corresponding population characteristics. Environ Toxicol Chem 22, 1632-1641.

Deal, C.K., Volkoff, H., 2020. The role of the thyroid axis in fish. Front Endocrinol 11.

Deierlein, A.L., Rock, S., Park, S., 2017. Persistent endocrine-disrupting chemicals and fatty liver disease. Curr Environ Health Rep 4, 439-449.

Delbes, G., Blázquez, M., Fernandino, J.I., Grigorova, P., Hales, B.F., Metcalfe, C., Navarro-Martín, L., Parent, L., Robaire, B., Rwigemera, A., Van Der Kraak, G., Wade, M., Marlatt, V., 2022. Effects of endocrine disrupting chemicals on gonad development: mechanistic insights from fish and mammals. Environ Res 204, 112040.

Dépatie, C., Houde, M., Verreault, J., 2020. Environmental exposure of northern pike to a primary wastewater effluent: impact on the lipidomic profile and lipid metabolism. Aquat Toxicol 221, 105421.

Dewailly, E., Nantel, A., Weber, J.P., Meyer, F., 1989. High levels of PCBs in breast milk of Inuit women from arctic Quebec. Bull Environ Contam Toxicol 43, 641-646.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15-21.

Douxfils, J., Mandiki, R., Silvestre, F., Bertrand, A., Leroy, D., Jean-Pierre, T., Patrick, K., 2007. Do sewage treatment plant discharges substantially impair fish reproduction in polluted rivers? Sci Total Environ 372, 497-514.

Faass, O., Ceccatelli, R., Schlumpf, M., Lichtensteiger, W., 2013. Developmental effects of perinatal exposure to PBDE and PCB on gene expression in sexually dimorphic rat brain regions and female sexual behavior. Gen Comp Endocrinol 188, 232-241.

Feist, S.W., Stentiford, G.D., Kent, M.L., Ribeiro Santos, A., Lorance, P., 2015. Histopathological assessment of liver and gonad pathology in continental slope fish from the northeast Atlantic Ocean. Mar Environ Res 106, 42-50.

Fischer, S., Klüver, N., Burkhardt-Medicke, K., Pietsch, M., Schmidt, A.-M., Wellner, P., Schirmer, K., Luckenbach, T., 2013. Abcb4 acts as multixenobiotic transporter and active barrier against chemical uptake in zebrafish (*Danio rerio*) embryos. BMC Biol 11, 69.

Fisher, J.W., Campbell, J., Muralidhara, S., Bruckner, J.V., Ferguson, D., Mumtaz, M., Harmon, B., Hedge, J.M., Crofton, K.M., Kim, H., Almekinder, T.L., 2005. Effect of PCB 126 on hepatic metabolism of thyroxine and perturbations in the hypothalamic-pituitary-thyroid axis in the rat. Toxicol Sci 90, 87-95.

Furin, C.G., von Hippel, F.A., Postlethwait, J.H., Buck, C.L., Cresko, W.A., O'Hara, T.M., 2015. Developmental timing of sodium perchlorate exposure alters angiogenesis, thyroid follicle proliferation and sexual maturation in stickleback. Gen Comp Endocrinol 219, 24-35.

Gallant, T.L., Singh, A., Chu, I., 2000. PCB 118 induces ultrastructural alterations in the rat liver. Toxicology 145, 127-134.

Gao, Y., Li, X., Guo, L.-H., 2013. Assessment of estrogenic activity of perfluoroalkyl acids based on ligand-induced conformation state of human estrogen receptor. Environ Sci Technol 47, 634-641.

Gardell, A.M., Dillon, D.M., Smayda, L.C., von Hippel, F.A., Cresko, W.A., Postlethwait, J.H., Buck, C.L., 2015. Perchlorate exposure does not modulate temporal variation of whole-body thyroid and androgen hormone content in threespine stickleback. Gen Comp Endocrinol 219, 45-52.

Gardell, A.M., von Hippel, F.A., Adams, E.M., Dillon, D.M., Petersen, A.M., Postlethwait, J.H., Cresko, W.A., Buck, C.L., 2017. Exogenous iodide ameliorates perchlorate-induced thyroid phenotypes in threespine stickleback. Gen Comp Endocrinol 243, 60-69.

Geven, E.J., Nguyen, N.K., van den Boogaart, M., Spanings, F.A., Flik, G., Klaren, P.H., 2007. Comparative thyroidology: thyroid gland location and iodothyronine dynamics in Mozambique tilapia (*Oreochromis mossambicus* Peters) and common carp (*Cyprinus carpio* L.). J Exp Biol 210, 4005-4015.

Giari, L., Guerranti, C., Perra, G., Lanzoni, M., Fano, E.A., Castaldelli, G., 2015. Occurrence of perfluorooctanesulfonate and perfluorooctanoic acid and histopathology in eels from north Italian waters. Chemosphere 118, 117-123.

Gilbert, M.E., Lasley, S.M., 2013. Developmental thyroid hormone insufficiency and brain development: a role for brain-derived neurotrophic factor (BDNF)? Neuroscience 239, 253-270.

Gobas, F.A.P.C., Zhang, X., Wells, R., 1993. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environ Sci Technol 27, 2855-2863.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. Executive summary to EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocrine Reviews 36, 593-602.

Grün, F., Blumberg, B., 2006. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinology 147, s50-s55.

Gullberg, H., Rudling, M., Saltó, C., Forrest, D., Angelin, B., Vennström, B.r., 2002. Requirement for thyroid hormone receptor β in T3 regulation of cholesterol metabolism in mice. Mol Endocrinol 16, 1767-1777.

Hallgren, S., Sinjari, T., Håkansson, H., Darnerud, P.O., 2001. Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. Arch Toxicol 75, 200-208.

Hansen, P.D., Dizer, H., Hock, B., Marx, A., Sherry, J., McMaster, M., Blaise, C., 1998. Vitellogenin – a biomarker for endocrine disruptors. Trends Analyt Chem 17, 448-451.

Heindel, J.J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M.A., Nadal, A., Palanza, P., Panzica, G., Sargis, R., Vandenberg, L.N., vom Saal, F., 2017. Metabolism disrupting chemicals and metabolic disorders. Reprod Toxicol 68, 3-33.

Heins, D., Johnson, J., Baker, J.A., 2003. Reproductive ecology of the nine-spined stickleback from south-central Alaska. J Fish Biol 63, 1131-1143.

Heins, D.C., Singer, S.S., Baker, J.A., 1999. Virulence of the cestode *Schistocephalus solidus* and reproduction in infected threespine stickleback, *Gasterosteus aculeatus*. Can J Zool 77, 1967-1974.

Henrichs, J., Ghassabian, A., Peeters, R.P., Tiemeier, H., 2013. Maternal hypothyroxinemia and effects on cognitive functioning in childhood: how and why? Clin Endocrinol 79, 152-162.

Herczeg, G., Turtiainen, M., Merila, J., 2010. Morphological divergence of North-European nine-spined sticklebacks (*Pungitius pungitius*): signatures of parallel evolution. Biol J Linn Soc Lond 101, 403-416.

Hinton, D.E., Glaumann, H., Trump, B.F., 1978. Studies on the cellular toxicity of polychlorinated biphenyls (PCBs). Virchows Archiv B 27, 279-306.

Hong, M.Y., Lumibao, J., Mistry, P., Saleh, R., Hoh, E., 2015. Fish oil contaminated with persistent organic pollutants reduces antioxidant capacity and induces oxidative stress without affecting its capacity to lower lipid concentrations and systemic inflammation in rats. J Nutr 145, 939-944.

Hong, Q., Wang, Y., Luo, X., Chen, S., Chen, J., Cai, M., Cai, M., Mai, B., 2012. Occurrence of polychlorinated biphenyls (PCBs) together with sediment properties in the surface sediments of the Bering Sea, Chukchi Sea and Canada Basin. Chemosphere 88, 1340-1345.

Hoover, E., Cook, K., Plain, R., Sanchez, K., Waghiyi, V., Miller, P., Dufault, R., Sislin, C., Carpenter, D.O., 2012. Indigenous peoples of North America: environmental exposures and reproductive justice. Environ Health Perspect 120, 1645.

Horri, K., Alfonso, S., Cousin, X., Munschy, C., Loizeau, V., Aroua, S., Bégout, M.-L., Ernande, B., 2018. Fish life-history traits are affected after chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs. Sci Total Environ 610-611, 531-545.

Houbrechts, A.M., Van houcke, J., Darras, V.M., 2019. Disruption of deiodinase type 2 in zebrafish disturbs male and female reproduction. Journal of Endocrinology 241, 111-123.

Huff, M., da Silveira, W.A., Carnevali, O., Renaud, L., Hardiman, G., 2018. Systems analysis of the liver transcriptome in adult male zebrafish exposed to the plasticizer (2-ethylhexyl) phthalate (DEHP). Sci Rep 8, 2118.

Hugla, J.L., Goffinet, G., Kremers, P., Dubois, M., Lambert, V., Stouvenakers, N., Thome, J.P., 1996. Ultrastructural modifications in cultured fetal quail hepatocytes exposed to pesticides and PCBs. Ecotoxicol Environ Safety 34, 145-155.

Hugla, J.L., Thomé, J.P., 1999. Effects of polychlorinated biphenyls on liver ultrastructure, hepatic monooxygenases, and reproductive success in the barbel. Ecotoxicol Environ Safety 42, 265-273.

Jackson, J.S., Kennedy, C.J., 2017. Regulation of hepatic abcb4 and cyp3a65 gene expression and multidrug/multixenobiotic resistance (MDR/MXR) functional activity in the model teleost, *Danio rerio* (zebrafish). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 200, 34-41.

Jansen, H.T., Cooke, P.S., Porcelli, J., Liu, T.-C., Hansen, L.G., 1993. Estrogenic and antiestrogenic actions of PCBs in the female rat: In vitro and in vivo studies. Reprod Toxicol 7, 237-248.

Javed, M., Usmani, N., 2013. Assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated rivulet water and their effect on glycogen metabolism and histology of *Mastacembelus armatus*. SpringerPlus 2, 390.

Jobling, S., Beresford, N., Nolan, M., Rodgers-Gray, T., Brighty, G.C., Sumpter, J.P., Tyler, C.R., 2002. Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. Biol Reprod 66, 272-281.

Jordan-Ward, R., von Hippel, F.A., Zheng, G., Salamova, A., Dillon, D., Gologergen, J., Immingan, T., Dominguez, E., Miller, P., Carpenter, D., Postlethwait, J.H., Byrne, S., Buck, C.L., 2022. Elevated mercury and PCB concentrations in Dolly Varden (*Salvelinus malma*) collected near a formerly used defense site on Sivuqaq, Alaska. Sci Total Environ 826, 154067.

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Morin, A.E., Gobas, F.A., 2007. Food web-specific biomagnification of persistent organic pollutants. Science 317, 236-239.

Klaunig, J.E., Lipsky, M.M., Trump, B.F., Hinton, D.E., 1979. Biochemical and ultrastructural changes in teleost liver following subacute exposure to PCB. J Environ Pathol Toxicol 2, 953-963.

Kraugerud, M., Doughty, R.W., Lyche, J.L., Berg, V., Tremoen, N.H., Alestrøm, P., Aleksandersen, M., Ropstad, E., 2012. Natural mixtures of persistent organic pollutants (POPs) suppress ovarian follicle development, liver vitellogenin immunostaining and hepatocyte proliferation in female zebrafish (*Danio rerio*). Aquat Toxicol 116-117, 16-23.

Krøvel, A.V., Søfteland, L., Torstensen, B., Olsvik, P.A., 2008. Transcriptional effects of PFOS in isolated hepatocytes from Atlantic salmon *Salmo salar* L. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 148, 14-22.

Kubota, A., Goldstone, J.V., Lemaire, B., Takata, M., Woodin, B.R., Stegeman, J.J., 2014. Role of pregnane X receptor and aryl hydrocarbon receptor in transcriptional regulation of pxr, CYP2, and CYP3 genes in developing zebrafish. Toxicol Sci 143, 398-407.

Kuiper, R.V., Vethaak, A.D., Cantón, R.o.F., Anselmo, H., Dubbeldam, M., van den Brandhof, E.-J., Leonards, P.E.G., Wester, P.W., den Berg, M.v., 2008. Toxicity of analytically cleaned pentabromodiphenylether after prolonged exposure in estuarine European flounder (*Platichthys flesus*), and partial life-cycle exposure in fresh water zebrafish (*Danio rerio*). Chemosphere 73, 195-202.

Kurelec, B., 1992. The multixenobiotic resistance mechanism in aquatic organisms. Crit Rev Toxicol 22, 23-43.

La Merrill, M.A., Johnson, C.L., Smith, M.T., Kandula, N.R., Macherone, A., Pennell, K.D., Kanaya, A.M., 2019. Exposure to persistent organic pollutants (POPs) and their relationship to hepatic fat and insulin insensitivity among Asian Indian immigrants in the United States. Environ Sci Technol 53, 13906-13918.

Lee, M.-C., Puthumana, J., Lee, S.-H., Kang, H.-M., Park, J.C., Jeong, C.-B., Han, J., Hwang, D.-S., Seo, J.S., Park, H.G., Om, A.-S., Lee, J.-S., 2016. BDE-47 induces oxidative stress, activates MAPK signaling pathway, and elevates de novo lipogenesis in the copepod *Paracyclopina nana*. Aquat Toxicol 181, 104-112.

Lee, Y.M., Kim, K.S., Jacobs, D.R., Jr., Lee, D.H., 2017. Persistent organic pollutants in adipose tissue should be considered in obesity research. Obes Rev 18, 129-139.

Lema, S.C., Dickey, J.T., Schultz, I.R., Swanson, P., 2008. Dietary exposure to 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) alters thyroid status and thyroid hormone-regulated gene transcription in the pituitary and brain. Environ Health Perspect 116, 1694-1699.

Li, D.-L., Huang, Y.-J., Gao, S., Chen, L.-Q., Zhang, M.-L., Du, Z.-Y., 2019. Sex-specific alterations of lipid metabolism in zebrafish exposed to polychlorinated biphenyls. Chemosphere 221, 768-777.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., Proc, G.P.D., 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078-2079.

Li, W., Zhu, L., Zha, J., Wang, Z., 2014. Effects of decabromodiphenyl ether (BDE-209) on mRNA transcription of thyroid hormone pathway and spermatogenesis associated genes in Chinese rare minnow (*Gobiocypris rarus*). Environ Toxicol 29, 1-9.

Li, X., Gao, Y., Guo, L.-H., Jiang, G., 2013. Structure-dependent activities of hydroxylated polybrominated diphenyl ethers on human estrogen receptor. Toxicology 309, 15-22.

Li, X., Han, T., Zheng, S., Wu, G., 2022. Hepatic glucose metabolism and its disorders in fish. Adv Exp Med Biol 1354, 207-236.

Linares, V., Belles, M., Domingo, J.L., 2015. Human exposure to PBDE and critical evaluation of health hazards. Arch Toxicol 89, 335-356.

Liu, C., Ha, M., Li, L., Yang, K., 2014. PCB153 and p,p'-DDE disorder thyroid hormones via thyroglobulin, deiodinase 2, transthyretin, hepatic enzymes and receptors. Environmental science and pollution research international 21, 11361-11369.

Liu, Y.-Y., Brent, G.A., 2010. Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation. Trends in Endocrinology & Metabolism 21, 166-173.

Lohmann, R., Breivik, K., Dachs, J., Muir, D., 2007. Global fate of POPs: current and future research directions. Environ Pollut 150, 150-165.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15.

Ludwig, U., Holzner, D., Denzer, C., Greinert, A., Haenle, M.M., Oeztuerk, S., Koenig, W., Boehm, B.O., Mason, R.A., Kratzer, W., Graeter, T., 2015. Subclinical and clinical hypothyroidism and non-alcoholic fatty liver disease: a cross-sectional study of a random population sample aged 18 to 65 years. BMC Endocr Disord 15, 41.

Mackay, D., Wania, F., 1995. Transport of contaminants to the Arctic: partitioning, processes and models. Sci Total Environ 160-161, 25-38.

Madureira, T.V., Malhão, F., Pinheiro, I., Lopes, C., Ferreira, N., Urbatzka, R., Castro, L.F.C., Rocha, E., 2015. Estrogenic and anti-estrogenic influences in cultured brown trout hepatocytes: focus on the expression of some estrogen and peroxisomal related genes and linked phenotypic anchors. Aquat Toxicol 169, 133-142.

Maqbool, F., Mostafalou, S., Bahadar, H., Abdollahi, M., 2016. Review of endocrine disorders associated with environmental toxicants and possible involved mechanisms. Life Sci 145, 265-273.

Maradonna, F., Nozzi, V., Santangeli, S., Traversi, I., Gallo, P., Fattore, E., Mita, D.G., Mandich, A., Carnevali, O., 2015. Xenobiotic-contaminated diets affect hepatic lipid metabolism: implications for liver steatosis in *Sparus aurata* juveniles. Aquat Toxicol 167, 257-264.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. 2011 17, 3.

Mazeaud, M.M., Mazeaud, F., Donaldson, E.M., 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans Am Fish Soc 106, 201-212.

Mckinney, M.A., Pedro, S., Dietz, R., Sonne, C., Fisk, A.T., Roy, D., Jenssen, B.M., Letcher, R.J., 2015. A review of ecological impacts of global climate change on persistent organic pollutant and mercury pathways and exposures in arctic marine ecosystems. Current Zoology 61, 617-628.

Meier, S., Andersen, T.E., Norberg, B., Thorsen, A., Taranger, G.L., Kjesbu, O.S., Dale, R., Morton, H.C., Klungsøyr, J., Svardal, A., 2007. Effects of alkylphenols on the reproductive system of Atlantic cod (*Gadus morhua*). Aquat Toxicol 81, 207-218.

Meier, S., Morton, H.C., Andersson, E., Geffen, A.J., Taranger, G.L., Larsen, M., Petersen, M., Djurhuus, R., Klungsøyr, J., Svardal, A., 2011. Low-dose exposure to alkylphenols adversely affects the sexual development of Atlantic cod (*Gadus morhua*): acceleration of the onset of puberty and delayed seasonal gonad development in mature female cod. Aquat Toxicol 105, 136-150.

Mesnier, A., Champion, S., Louis, L., Sauzet, C., May, P., Portugal, H., Benbrahim, K., Abraldes, J., Alessi, M.C., Amiot-Carlin, M.J., Peiretti, F., Piccerelle, P., Nalbone, G., Villard, P.H., 2015. The transcriptional effects of PCB118 and PCB153 on the liver, adipose tissue, muscle and colon of mice: highlighting of glut4 and lipin1 as main target genes for PCB induced metabolic disorders. PLoS One 10, e0128847.

Mi, H.Y., Muruganujan, A., Thomas, P.D., 2013. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res 41, D377-D386.

Miller, P.K., Waghiyi, V., Welfinger-Smith, G., Byrne, S.C., Kava, J., Gologergen, J., Eckstein, L., Scrudato, R., Chiarenzelli, J., Carpenter, D.O., Seguinot-Medina, S., 2013. Community-based participatory research projects and policy engagement to protect environmental health on St. Lawrence Island, Alaska. Int J Circumpolar Health 72, 21656.

Miller, V.M., Kahnke, T., Neu, N., Sanchez-Morrissey, S.R., Brosch, K., Kelsey, K., Seegal, R.F., 2010. Developmental PCB exposure induces hypothyroxinemia and sex-specific effects on cerebellum glial protein levels in rats. Int J Dev Neurosci 28, 553-560.

Minicozzi, M.R., von Hippel, F.A., Furin, C.G., Buck, C.L., 2019. Sodium perchlorate induces non-alcoholic fatty liver disease in developing stickleback. Environ Pollut 251, 390-399.

Miranda, A.L., Roche, H., Randi, M.A.F., Menezes, M.L., Ribeiro, C.A.O., 2008. Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish *Hoplias malabaricus*: histopathological, physiological, and immunological findings. Environ Int 34, 939-949.

Mitra, V., Metcalf, J., 2012. Metabolic functions of the liver. Anaesth Intensive Care 13, 54-55.

Montaigne, D., Butruille, L., Staels, B., 2021. PPAR control of metabolism and cardiovascular functions. Nat Rev Cardiol 18, 809-823.

Nomiyama, K., Nomura, Y., Takahashi, T., Uchiyama, Y., Arizono, K., Shinohara, R., 2010. Hydroxylated polychlorinated biphenyls (OH-PCBs) induce vitellogenin through estrogenic activity in primary-cultured hepatocytes of the *Xenopus laevis*. Chemosphere 78, 800-806.

Noyes, P.D., Hinton, D.E., Stapleton, H.M., 2011. Accumulation and debromination of decabromodiphenyl ether (BDE-209) in juvenile fathead minnows (*Pimephales promelas*) induces thyroid disruption and liver alterations. Toxicol Sci 122, 265-274.

Oulhote, Y., Chevrier, J., Bouchard, M.F., 2016. Exposure to polybrominated diphenyl ethers (PBDEs) and hypothyroidism in Canadian women. J Clin Endocrinol Metab 101, 590-598.

Pacyna, J.M., Cousins, I.T., Halsall, C., Rautio, A., Pawlak, J., Pacyna, E.G., Sundseth, K., Wilson, S., Munthe, J., 2015. Impacts on human health in the Arctic owing to climate-induced changes in contaminant cycling – the EU ArcRisk project policy outcome. Environ Sci Policy 50, 200-213.

Pelava, A., Schneider, C., Watkins, Nicholas J., 2016. The importance of ribosome production, and the 5S RNP–MDM2 pathway, in health and disease. Biochemical Society Transactions 44, 1086-1090.

Peplow, D., Edmonds, R., 2005. The effects of mine waste contamination at multiple levels of biological organization. Ecological Engineering 24, 101-119.

Perloff, M.D., von Moltke, L.L., Störmer, E., Shader, R.I., Greenblatt, D.J., 2001. Saint John's wort: an in vitro analysis of P-glycoprotein induction due to extended exposure. Br J Pharmacol 134, 1601-1608.

Petersen, A.M., Dillon, D., Bernhardt, R.R., Torunsky, R., Postlethwait, J.H., von Hippel, F.A., Loren Buck, C., Cresko, W.A., 2015. Perchlorate disrupts embryonic androgen synthesis and reproductive development in threespine stickleback without changing whole-body levels of thyroid hormone. Gen Comp Endocrinol 210, 130-144.

Petro, E.M., Leroy, J.L., Covaci, A., Fransen, E., De Neubourg, D., Dirtu, A.C., De Pauw, I., Bols, P.E., 2012. Endocrine-disrupting chemicals in human follicular fluid impair *in vitro* oocyte developmental competence. Hum Reprod 27, 1025-1033.

Pfohl, M., Marques, E., Auclair, A., Barlock, B., Jamwal, R., Goedken, M., Akhlaghi, F., Slitt, A.L., 2021. An ‘omics approach to unraveling the paradoxical effect of diet on perfluorooctanesulfonic acid (PFOS) and perfluorononanoic acid (PFNA)-induced hepatic steatosis. Toxicol Sci 180, 277-294.

Porterfield, S.P., 1994. Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ Health Perspect 102, 125-130.

Raine, J.C., Takemura, A., Leatherland, J.F., 2001. Assessment of thyroid function in adult medaka (*Oryzias latipes*) and juvenile rainbow trout (*Oncorhynchus mykiss*) using immunostaining methods. J Exp Zool 290, 366-378.

Reinling, J., Houde, M., Verreault, J., 2017. Environmental exposure to a major urban wastewater effluent: effects on the energy metabolism of northern pike. Aquat Toxicol 191, 131-140.

Rigét, F., Bignert, A., Braune, B., Stow, J., Wilson, S., 2010. Temporal trends of legacy POPs in Arctic biota, an update. Sci Total Environ 408, 2874-2884.

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci Rep 3, 3263.

Rotander, A., van Bavel, B., Polder, A., Rigét, F., Auðunsson, G.A., Gabrielsen, G.W., Víkingsson, G., Bloch, D., Dam, M., 2012. Polybrominated diphenyl ethers (PBDEs) in marine mammals from Arctic and North Atlantic regions, 1986-2009. Environ Int 40, 102-109.

Saad, M., Cavanaugh, K., Verbueken, E., Pype, C., Casteleyn, C., Van Ginneken, C., Van Cruchten, S., 2016. Xenobiotic metabolism in the zebrafish: a review of the spatiotemporal distribution, modulation and activity of Cytochrome P450 families 1 to 3. J Toxicol Sci 41, 1-11.

Safe, S.H., 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24, 87-149.

Savassi, L.A., Paschoalini, A.L., Arantes, F.P., Rizzo, E., Bazzoli, N., 2020. Heavy metal contamination in a highly consumed Brazilian fish: immunohistochemical and histopathological assessments. Environ Monit Assess 192, 542.

Scheringer, M., Salzmann, M., Stroebe, M., Wegmann, F., Fenner, K., Hungerbuhler, K., 2004. Long-range transport and global fractionation of POPs: insights from multimedia modeling studies. Environ Pollut 128, 177-188.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9, 671-675.

Sealy, S.G., Bédard, J., Udvardy, M.D.F., Fay, F.H., 1971. New records and zoogeographical notes on the birds of St. Lawrence Island, Bering Sea. Condor 73, 322-336.

Serreze, M.C., Barry, R.G., 2011. Processes and impacts of Arctic amplification: a research synthesis. Glob Planet Change 77, 85-96.

Sharma, P., Grabowski, T.B., Patiño, R., 2016. Thyroid endocrine disruption and external body morphology of Zebrafish. Gen Comp Endocrinol 226, 42-49.

Shved, N., Kumeiko, V., Syasina, I., 2011. Enzyme-linked immunosorbent assay (ELISA) measurement of vitellogenin in plasma and liver histopathology in barfin plaice *Liopsetta pinnifasciata* from Amursky Bay, Sea of Japan. Fish Physiol Biochem 37, 781-799.

Singh, K., Bjerregaard, P., Chan, H.M., 2014. Association between environmental contaminants and health outcomes in indigenous populations of the Circumpolar North. Int J Circumpolar Health 73, 25808-25808.

Sinha, R.A., Singh, B.K., Yen, P.M., 2014. Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. Trends in Endocrinology & Metabolism 25, 538-545.

Sinha, R.A., Singh, B.K., Yen, P.M., 2018. Direct effects of thyroid hormones on hepatic lipid metabolism. Nat Rev Endocrinol 14, 259-269.

Smital, T., Sauerborn, R., Pivčević, B., Krča, S., Kurelec, B., 2000. Interspecies differences in P-glycoprotein mediated activity of multixenobiotic resistance mechanism in several marine and freshwater invertebrates. Comp Biochem Physiol C Toxicol Endocrinol 126, 175-186.

Sokolowska, E., Krzysztof, S.E., 2002. Reproductive cycle and the related spatial and temporal distribution of the ninespine stickleback (*Pungitius pungitius L.*) in Puck Bay. Oceanologia 44, 475–490.

Sokołowska, E., Kulczykowska, E., 2006. Annual reproductive cycle in two free living populations of three-spined stickleback (*Gasterosteus aculeatus L.*): patterns of ovarian and testicular development. Oceanologia 48.

Sonne, C., Torjesen, P.A., Fuglei, E., Muir, D.C.G., Jenssen, B.M., Jørgensen, E.H., Dietz, R., Ahlstrøm, Ø., 2017. Exposure to persistent organic pollutants reduces testosterone concentrations and affects sperm viability and morphology during the mating peak period in a controlled experiment on farmed arctic foxes (*Vulpes lagopus*). Environ Sci Technol 51, 4673-4680.

Spriggs, K.A., Bushell, M., Willis, A.E., 2010. Translational regulation of gene expression during conditions of cell stress. Mol Cell 40, 228-237.

Suk, W.A., Avakian, M.D., Carpenter, D., Groopman, J.D., Scammell, M., Wild, C.P., 2004. Human exposure monitoring and evaluation in the Arctic: the importance of understanding exposures to the development of public health policy. Environ Health Perspect 112, 113-120.

Sumpter, J.P., Jobling, S., 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ Health Perspect 103 Suppl 7, 173-178.

Sydes, J., 2019. Dupligänger is a reference-based, UMI-aware, 5'-trimming-aware PCR duplicate removal pipeline., 0.98 ed. Zenodo.

Takacs, M.L., Abbott, B.D., 2006. Activation of mouse and human peroxisome proliferator–activated receptors (α, β/δ, γ) by perfluorooctanoic acid and perfluorooctane sulfonate. Toxicol Sci 95, 108-117.

Tarn, P.P.L., Bun Ng, T., Woo, N.Y.S., 1983. Effects of oestradiol-17β and testosterone on the histology of pituitary, liver, ovary and skin of previtellogenic *Epinephelus akaara* (Teleostei, *Serranidae*). Cell Tissue Res 231, 579-592.

Tierney, K.B., Farrell, A.P., Brauner, C.J., 2014. Organic chemical toxicology of fishes. Fish Physiol 33.

Tomy, G.T., Palace, V.P., Halldorson, T., Braekevelt, E., Danell, R., Wautier, K., Evans, B., Brinkworth, L., Fisk, A.T., 2004. Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Salvelinus namaycush*). Environ Sci Technol 38, 1496-1504.

Turyk, M.E., Anderson, H.A., Persky, V.W., 2007. Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and DDE in adults. Environ Health Perspect 115, 1197-1203.

Turyk, M.E., Persky, V.W., Imm, P., Knobeloch, L., Chatterton, R., Anderson, H.A., 2008. Hormone disruption by PBDEs in adult male sport fish consumers. Environ Health Perspect 116, 1635-1641.

Tyler, C.R., Sumpter, J.P., 1996. Oocyte growth and development in teleosts. Rev Fish Biol Fish 6, 287-318.

USACE, 2008. Project Closeout Report: Hazardous, Toxic, and Radioactive Waste (HTRW) Gambell FUDS, St. Lawrence Island, Alaska. United States Army Corps of Engineers.

USATSDR, 2020. Evaluation of Environmental Exposures at the Gambell Formerly Used Defense Site Agency for Toxic Substances & Disease Registry.

USDOI, 2016. Report to Congress: Hazardous Substance Contamination of Alaska Native Claim Settlement Act Lands in Alaska. United States Department of the Interior Bureau of Land Management.

USEPA, 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. United States Environmental Protection Agency Office of Water.

Van Oostdam, J., Donaldson, S.G., Feeley, M., Arnold, D., Ayotte, P., Bondy, G., Chan, L., Dewaily, É., Furgal, C.M., Kuhnlein, H., Loring, E., Muckle, G., Myles, E., Receveur, O., Tracy, B., Gill, U., Kalhok, S., 2005. Human health implications of environmental contaminants in Arctic Canada: a review. Sci Total Environ 351-352, 165-246.

Varadharajan, S., Rastas, P., Loytyynoja, A., Matschiner, M., Calboli, F.C.F., Guo, B.C., Nederbragt, A.J., Jakobsen, K.S., Merila, J., 2019. A High-Quality Assembly of the Nine-Spined Stickleback (Pungitius pungitius) Genome. Genome Biol Evol 11, 3291-3308.

Vasseur, P., Cossu-Leguille, C., 2006. Linking molecular interactions to consequent effects of persistent organic pollutants (POPs) upon populations. Chemosphere 62, 1033-1042.

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: health implications for indigenous residents. Environ Pollut 234, 279-287.

von Hippel, F.A., Trammell, E.J., Merila, J., Sanders, M.B., Schwarz, T., Postlethwait, J.H., Titus, T.A., Buck, C.L., Katsiadaki, I., 2016. The ninespine stickleback as a model organism in arctic ecotoxicology. Evol Ecol Res 17, 487-504.

Walpita, C.N., Crawford, A.D., Janssens, E.D.R., Van der Geyten, S., Darras, V.M., 2009. Type 2 iodothyronine deiodinase is essential for thyroid hormone-dependent embryonic development and pigmentation in zebrafish. Endocrinology 150, 530-539.

Wania, F., 2003. Assessing the potential of persistent organic chemicals for long-range transport and accumulation in polar regions. Environ Sci Technol 37, 1344-1351.

Wania, F., MacKay, D., 1996. Peer reviewed: tracking the distribution of persistent organic pollutants. Environ Sci Technol 30, 390-396.

Warner, A., Mittag, J., 2012. Thyroid hormone and the central control of homeostasis. Journal of Molecular Endocrinology 49, R29-R35.

Welfinger-Smith, G., Minholz, J.L., Byrne, S., Waghiyi, V., Gologergen, J., Kava, J., Apatiki, M., Ungott, E., Miller, P.K., Arnason, J.G., Carpenter, D.O., 2011. Organochlorine and metal contaminants in traditional foods from St. Lawrence Island, Alaska. J Toxicol Environ Health A 74, 1195-1214.

Wilson, S., Hung, H., Katsoyiannis, A., Kong, D., van Oostdam, J., Riget, F., Bignert, A., 2014. Trends in Stockholm Convention persistent organic pollutants (POPs) in arctic air, human media and biota. Arctic Monitoring and Assessment Programme (AMAP).

Wolf, D.C., Moore, T., Abbott, B.D., Rosen, M.B., Das, K.P., Zehr, R.D., Lindstrom, A.B., Strynar, M.J., Lau, C., 2008. Comparative hepatic effects of perfluorooctanoic acid and WY 14,643 in PPAR-α knockout and wild-type mice. Toxicol Pathol 36, 632-639.

Wolf, J.C., Wheeler, J.R., 2018. A critical review of histopathological findings associated with endocrine and non-endocrine hepatic toxicity in fish models. Aquat Toxicol 197, 60-78.

Xu, C., Jiang, Z.-Y., Liu, Q., Liu, H., Gu, A., 2017. Estrogen receptor beta mediates hepatotoxicity induced by perfluorooctane sulfonate in mouse. Environ Sci Pollut Res Int 24, 13414-13423.

Yang, C., Kong, A.P.S., Cai, Z., Chung, A.C.K., 2017. Persistent organic pollutants as risk factors for obesity and diabetes. Curr Diab Rep 17, 132.

Yates, A.D., Achuthan, P., Akanni, W., Allen, J., Allen, J., Alvarez-Jarreta, J., Amode, M.R., Armean, I.M., Azov, A.G., Bennett, R., Bhai, J., Billis, K., Boddu, S., Marugán, J.C., Cummins, C., Davidson, C., Dodiya, K., Fatima, R., Gall, A., Giron, C.G., Gil, L., Grego, T., Haggerty, L., Haskell, E., Hourlier, T., Izuogu, O.G., Janacek, S.H., Juettemann, T., Kay, M., Lavidas, I., Le, T., Lemos, D., Martinez, J.G., Maurel, T., McDowall, M., McMahon, A., Mohanan, S., Moore, B., Nuhn, M., Oheh, D.N., Parker, A., Parton, A., Patricio, M., Sakthivel, M.P., Abdul Salam, A.I., Schmitt, B.M., Schuilenburg, H., Sheppard, D., Sycheva, M., Szuba, M., Taylor, K., Thormann, A., Threadgold, G., Vullo, A., Walts, B., Winterbottom, A., Zadissa, A., Chakiachvili, M., Flint, B., Frankish, A., Hunt, S.E., IIsley, G., Kostadima, M., Langridge, N., Loveland, J.E., Martin, F.J., Morales, J., Mudge, J.M., Muffato, M., Perry, E., Ruffier, M., Trevanion, S.J., Cunningham, F., Howe, K.L., Zerbino, D.R., Flicek, P., 2019. Ensembl 2020. Nucleic Acids Res 48, D682-D688.

Yu, L., Deng, J., Shi, X., Liu, C., Yu, K., Zhou, B., 2010. Exposure to DE-71 alters thyroid hormone levels and gene transcription in the hypothalamic–pituitary–thyroid axis of zebrafish larvae. Aquat Toxicol 97, 226-233.

Yu, L., Han, Z., Liu, C., 2015. A review on the effects of PBDEs on thyroid and reproduction systems in fish. Gen Comp Endocrinol 219, 64-73.

Zheng, G., Miller, P., von Hippel, F.A., Buck, C.L., Carpenter, D.O., Salamova, A., 2020. Legacy and emerging semi-volatile organic compounds in sentinel fish from an arctic formerly used defense site in Alaska. Environ Pollut 259, 113872.

Zheng, M., Lu, J., Zhao, D., 2018. Toxicity and transcriptome sequencing (RNA-seq) analyses of adult zebrafish in response to exposure carboxymethyl cellulose stabilized iron sulfide nanoparticles. Sci Rep 8, 8083.

Zhou, X., Liao, W.-J., Liao, J.-M., Liao, P., Lu, H., 2015. Ribosomal proteins: functions beyond the ribosome. Journal of Molecular Cell Biology 7, 92-104.

Zoeller, T.R., Dowling, A.L.S., Herzig, C.T.A., Iannacone, E.A., Gauger, K.J., Bansal, R., 2002. Thyroid hormone, brain development, and the environment. Environ Health Perspect 110, 355-361.

LIST OF TABLES

Table 1. Number (and percent) of ninespine stickleback exhibiting histological abnormalities associated with non-alcoholic fatty liver disease.

		Number of fish exhibiting each histological abnormality				
Year	**Sex**	**Displaced nuclei**	**Deformed cellular shape**	**Hypertrophied nuclei**	**Disorganized cordons**	**Lipid phenotype**
2015	Males (4 total)	3 (75%)	3 (75%)	3 (75%)	3 (75%)	1 (25%)
2015	Females (12 total)	9 (75%)	7 (58%)	10 (83%)	7 (58%)	2 (17%)
2018	Males (14 total)	10 (71%)	5 (36%)	12 (86%)	6 (43%)	7 (50%)
2018	Females (3 total)	1 (33%)	1 (33%)	2 (67%)	0 (0%)	2 (67%)

LIST OF FIGURES

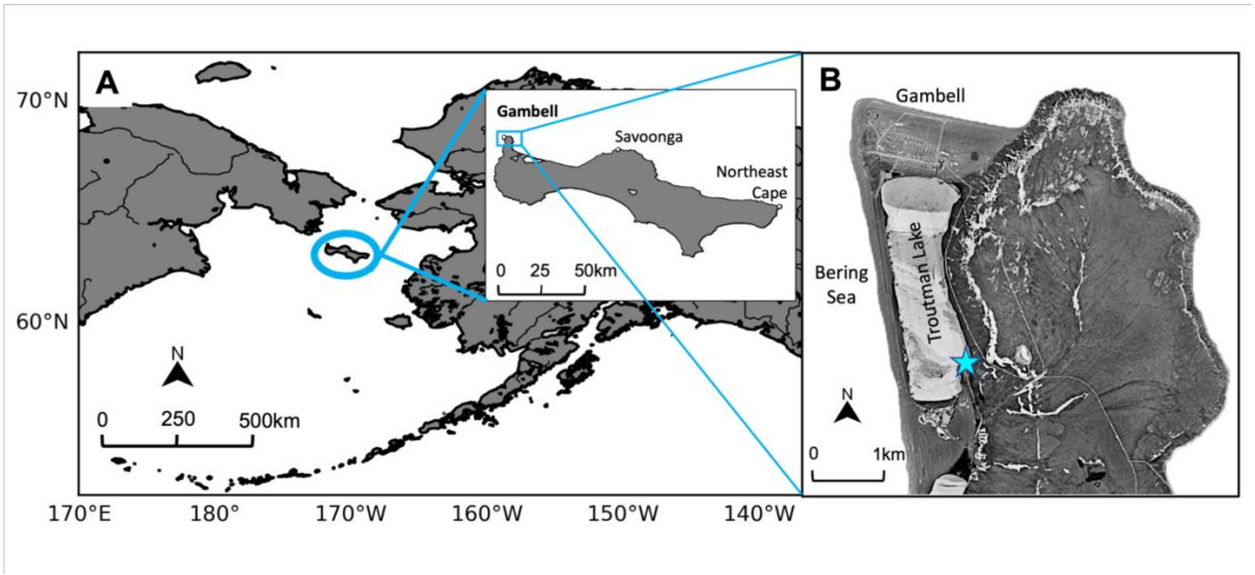


Figure 1. Location of **A)** the Alaska Native Village of Gambell on Sivuqaq, Alaska and **B)** sampling location in Troutman Lake.

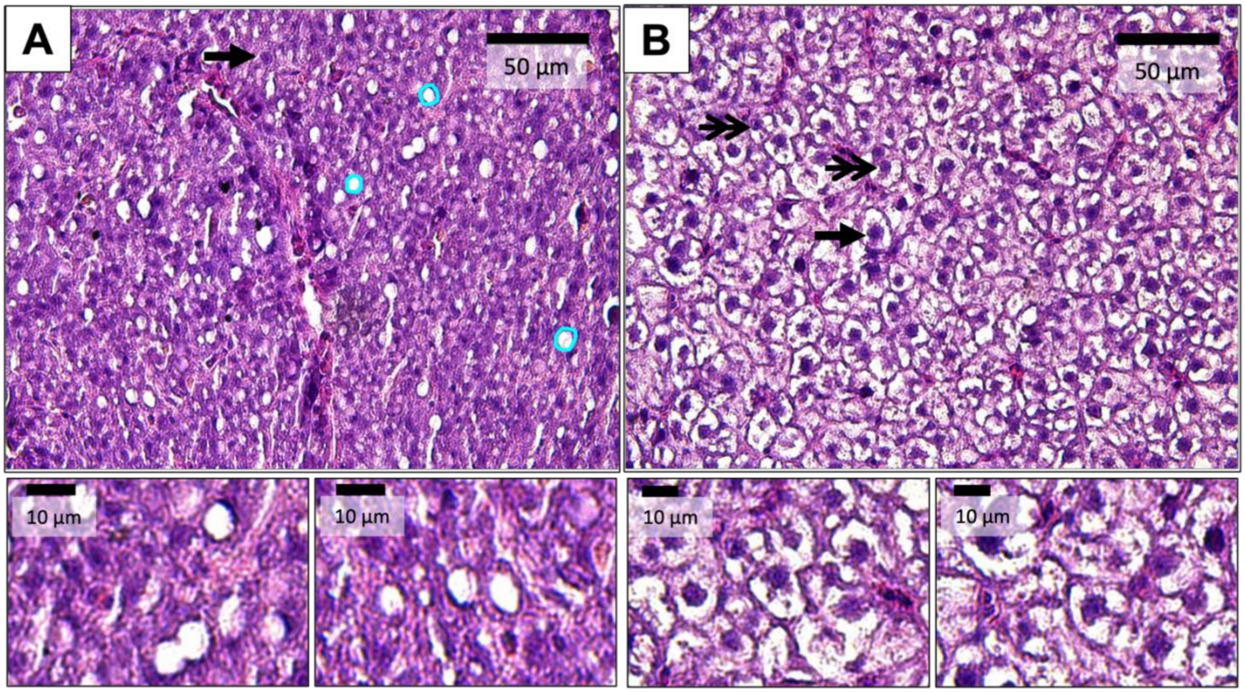


Figure 2. Histological images of liver tissue from male ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake in Gambell, Alaska. We found large variation in lipid accumulation and hepatocyte size across samples. Troutman Lake stickleback displayed two distinct liver phenotypes: (**A**) lipid droplet accumulation in a male stickleback; (**B**) increased glycogen-type hepatocyte vacuolation in a male. Stickleback samples for RNA sequencing (RNA-seq) were grouped by phenotype. Single arrows denote hypertrophied nuclei. Double arrows denote displaced nuclei. Blue circles denote lipid droplets.

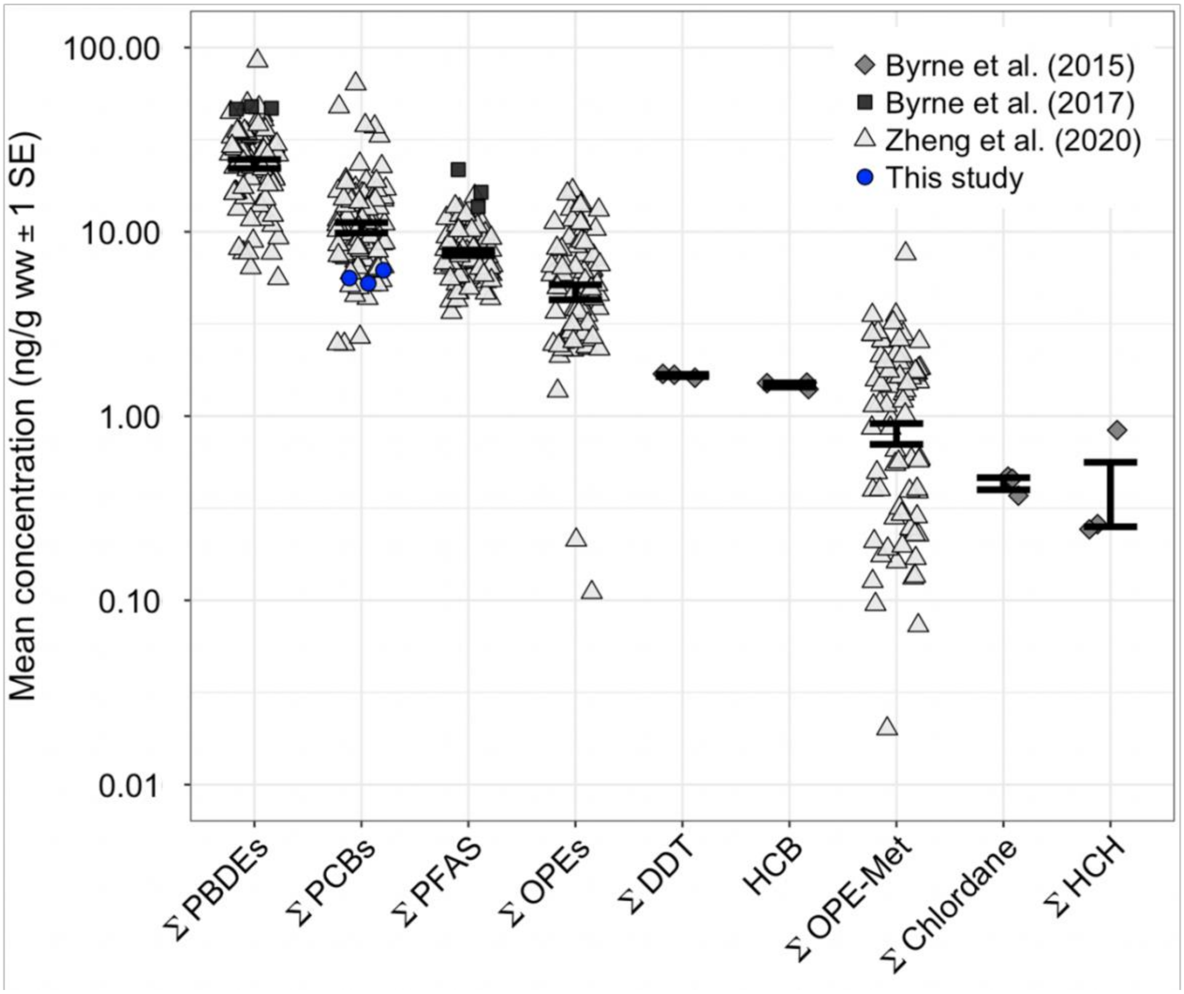


Figure 3. Contaminant chemistry profiles in ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake on Sivuqaq, Alaska across four studies. Contaminant classes are arranged from highest to lowest concentration based on the mean value.

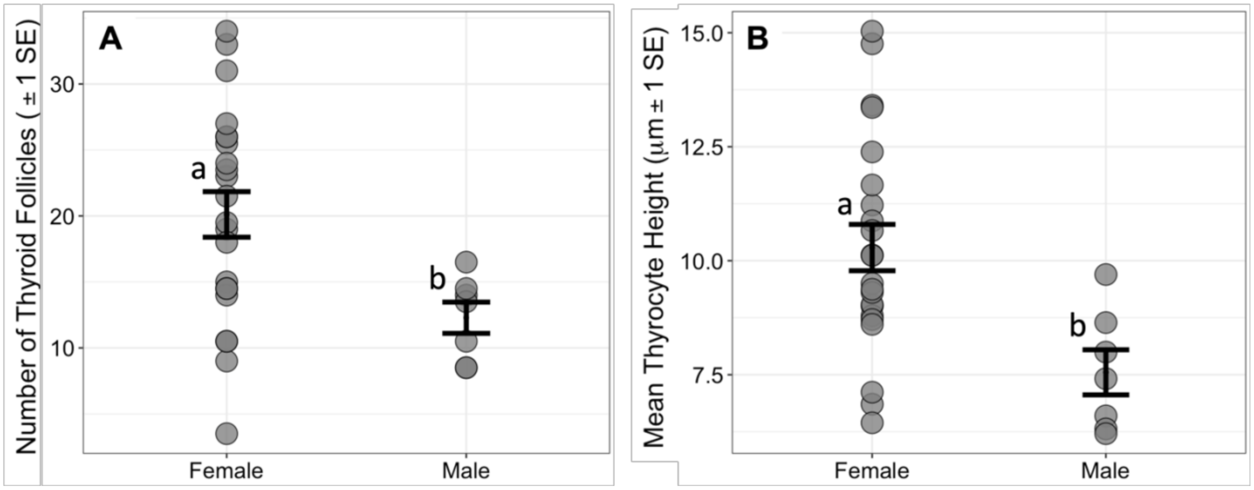


Figure 4. Number of thyroid follicles (**A**) and mean thyrocyte height (**B**) by sex in ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake in Gambell, Alaska in 2015. Female stickleback (n= 22) had significantly more thyroid follicles and longer thyrocytes than did male stickleback (n=7; Mann-Whitney *U* tests: p=0.01 and p=0.004, respectively).

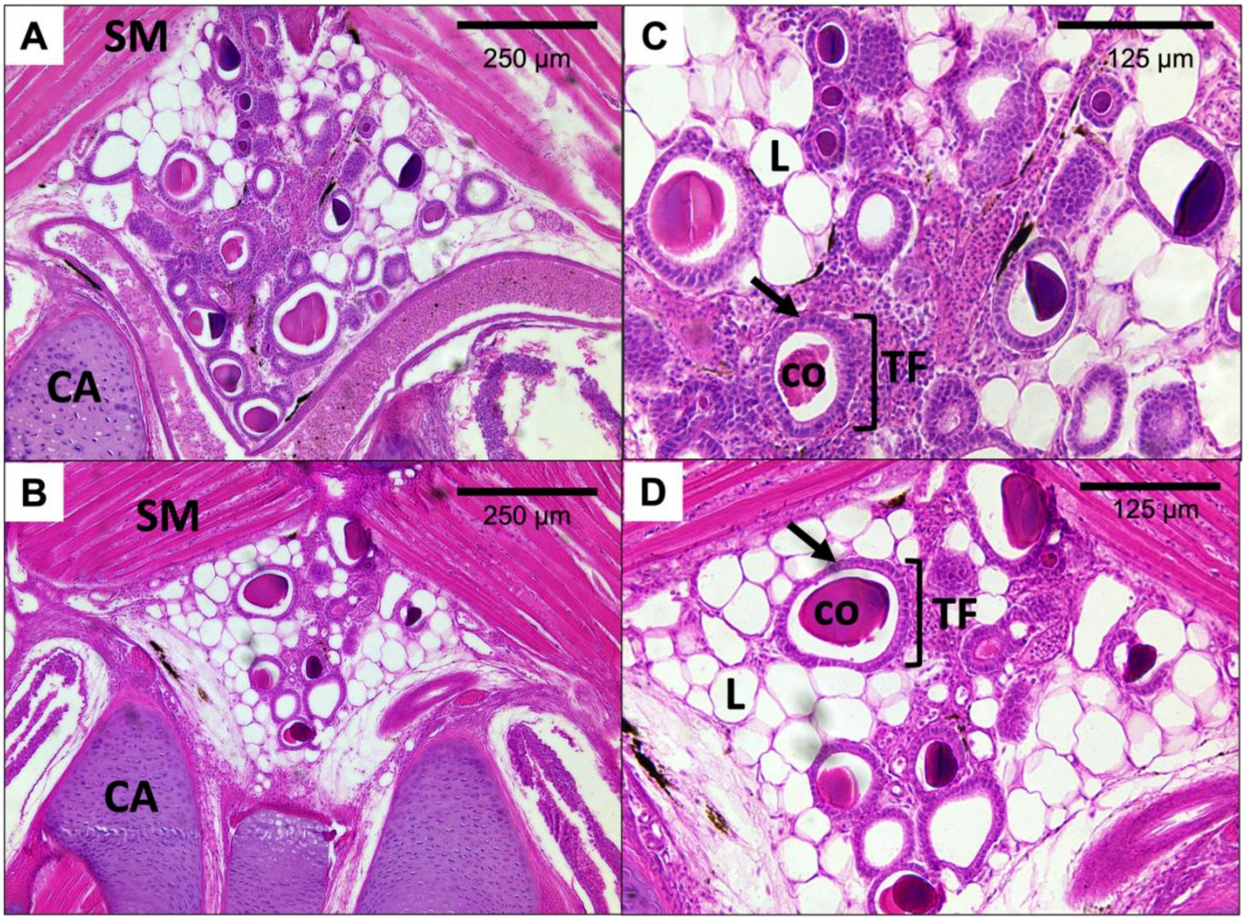


Figure 5. Thyroid morphologies in a female (**A** and **C**) and a male (**B** and **D)** ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake in Gambell, Alaska. A & B show the disbursement of thyroid follicles at 10X magnification and C & D show a subsection of the image at 20X magnification. Both male and female stickleback displayed lipid accumulation phenotypes similar to those observed in threespine stickleback (*Gasteroteus acculeatus*) exposed to perchlorate (Gardell et al., 2017). Arrows indicate thyrocytes. CA = cartilage, CO = colloid, L = lipid, SM = skeletal muscle, TF = thyroid follicle.

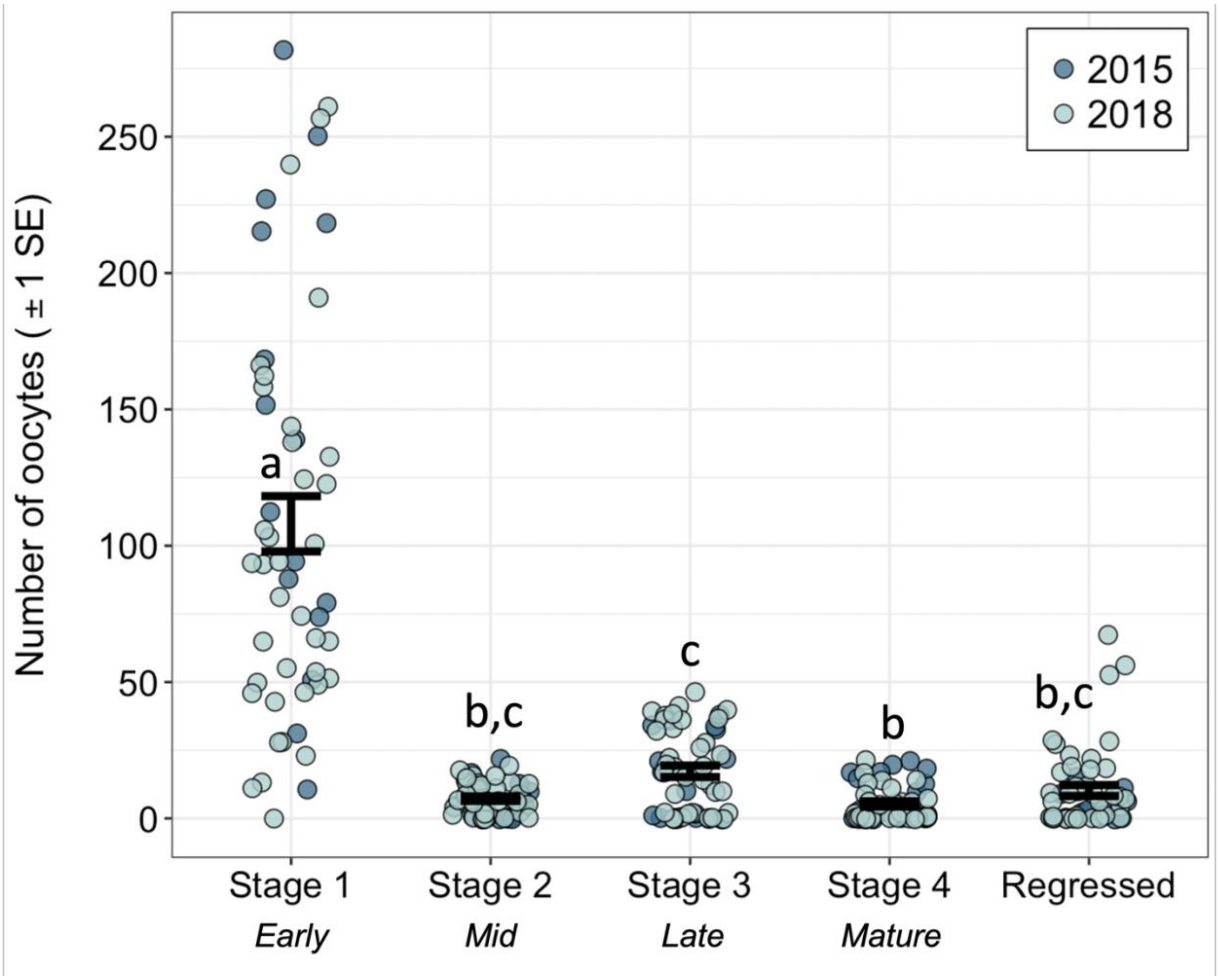


Figure 6. Oocytes staged in 53 ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake in Gambell, Alaska. Oocyte stage was determined using criteria outlined by Furin et al. (2015) and histological markers described by Sokolowska and Kulczykowska (2006). We tested significance using Kruskal-Wallis rank sum tests to account for violations in normality and heteroskedasticity. Pairwise comparisons using Dunn's test indicated that female stickleback had significantly more early-stage oocytes than mid-stage, late-stage, or mature oocytes ($p<0.0001$ for all comparisons). Females also had significantly more late-stage oocytes than mature oocytes ($p=0.005$). We pooled data across sampling years because 2015 and 2018 oocyte counts did not differ significantly (Mann-Whitney U test, $p=0.44$).

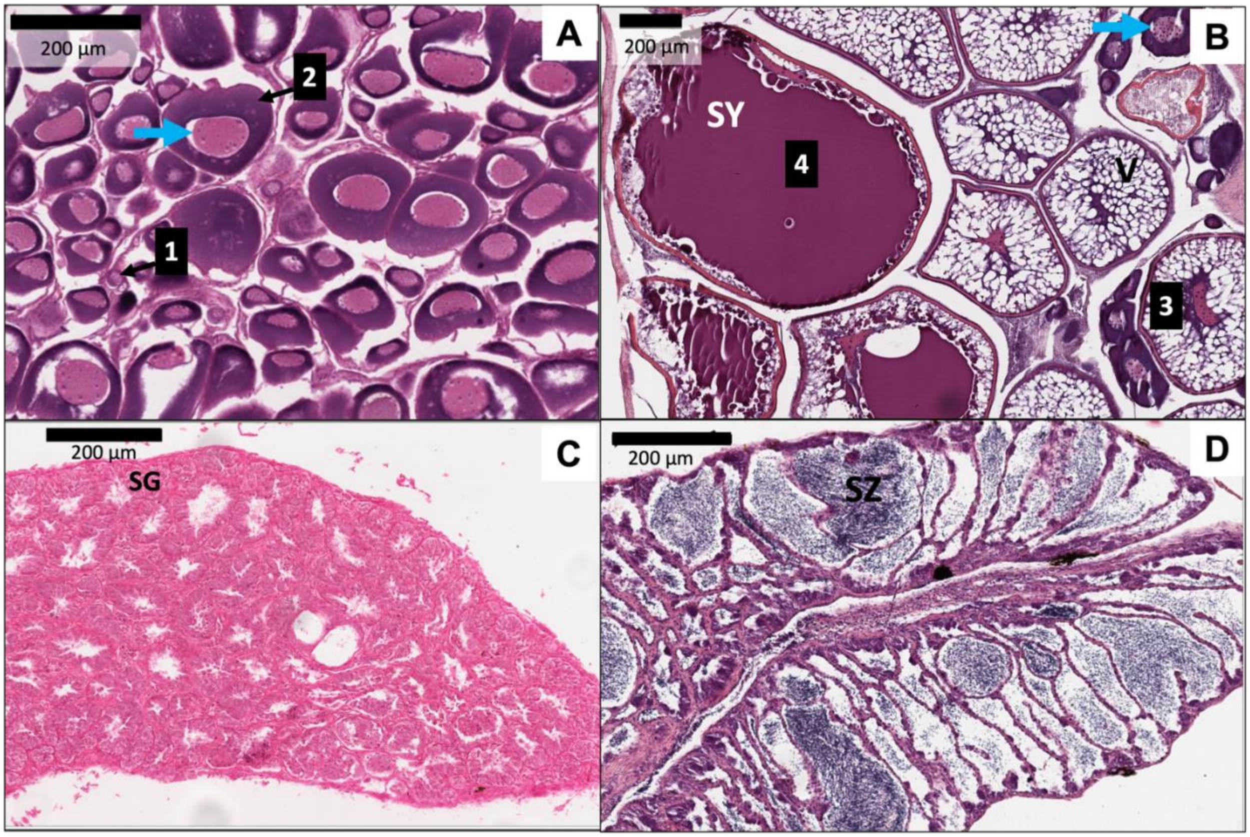


Figure 7. Histological images of ovaries (**A** & **B**) and testes (**C** & **D**) in ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake in Gambell, Alaska. Fish were collected at peak breading season (July). We found a large variation in testis and oocyte maturity level. **(A)** An ovary with early-stage oocytes only. **(B)** An ovary with late-stage and mature oocytes. Blue arrows in **(A)** and **(B)** denote nuclei and numbers indicate stage of oocyte: early (1), intermediate (2), late (3) and mature (4). **(C)** An early-stage testis. **(D)** A mature testis. SG = spermatogonia. SY = secondary yolk. SZ = spermatozoa. V = vacuoles.

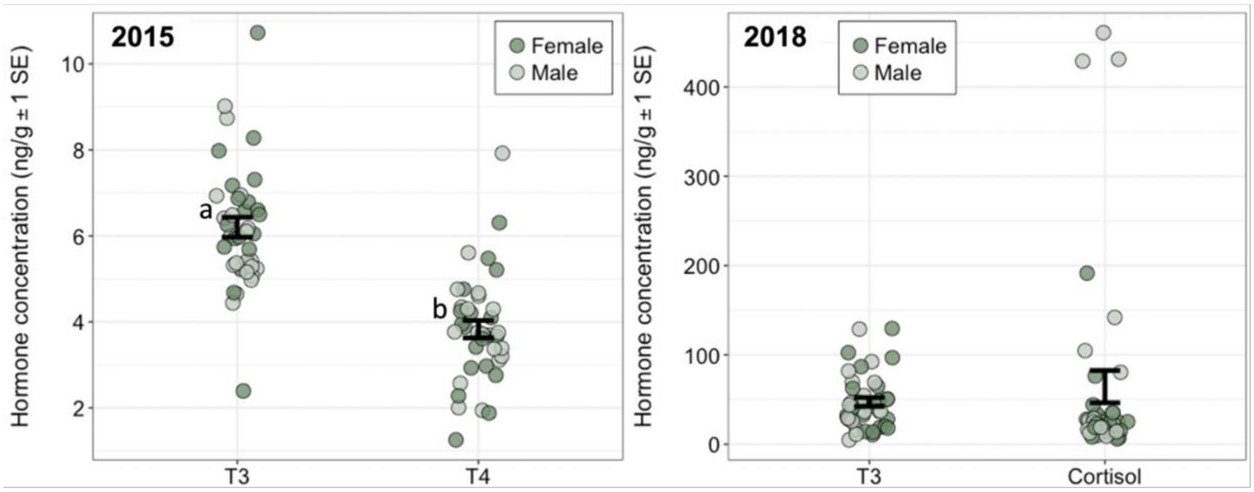
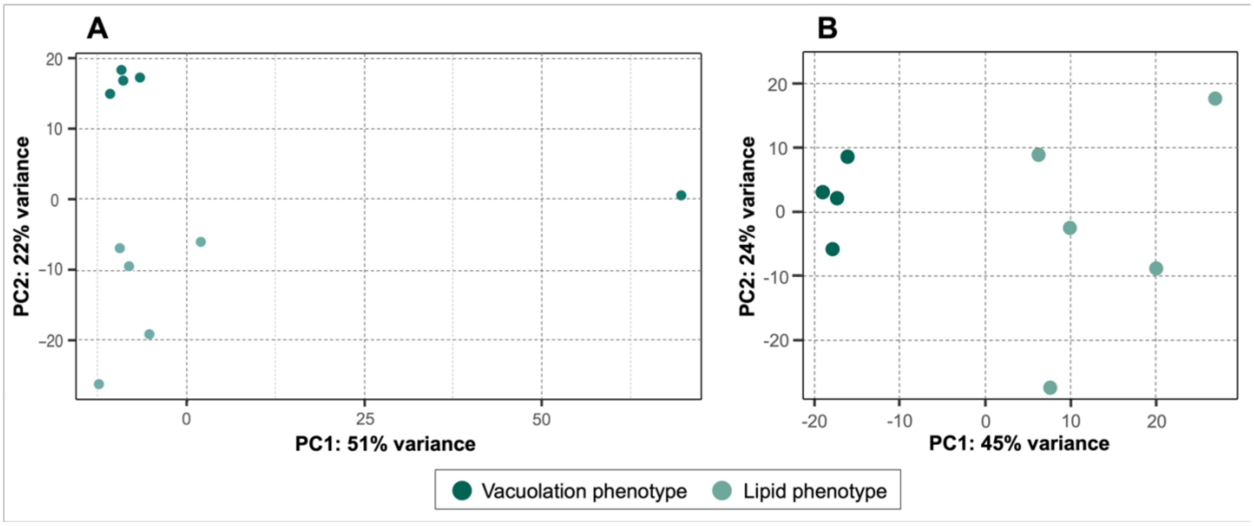
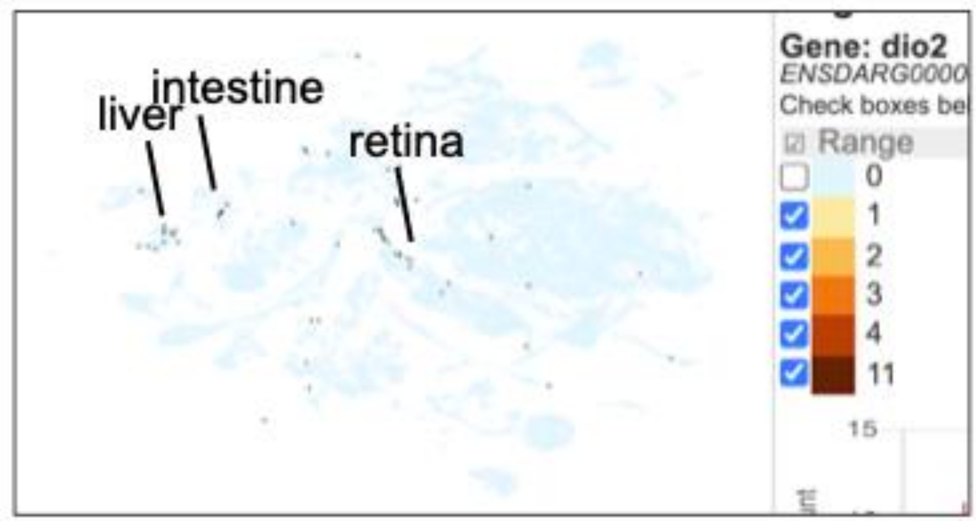


Figure 8. Thyroid hormone concentrations in ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake in Gambell, Alaska. T_3 concentrations were significantly higher than T_4 concentrations in 2015 (left graph; Mann-Whitney U test, n=39, p<0.0001). Three male stickleback had abnormally high concentrations of cortisol in 2018 (n=40). We restricted statistical analyses for thyroid measurements to within-year differences; we did not examine between-year differences due to disparities in methodology and analytes between sampling years.

LIST OF SUPPLEMENTARY MATERIALS



Supplemental Figure 1. Principal component analysis of transcriptional reads from RNA sequencing on liver tissue in male ninespine stickleback (*Pungitius pungitius*) displaying lipid accumulation versus liver glycogen vacuolation. Stickleback were collected from Troutman Lake on Sivuqaq, Alaska. **A)** All fish included in RNAseq analysis, including the outlier to the far right. **B)** All fish excluding the outlier. The outlier was not included in bioinformatic analyses.



Supplemental Figure 2. RNA sequencing gene expression results in liver tissue of the ninespine stickleback (*Pungitius pungitius*) outlier. This individual was removed from further analysis because gene mapping showed that the sample was contaminated with intestinal tissue.

CHAPTER 5: Formerly used defense sites on Unalaska Island, Alaska: mapping a legacy of environmental pollution

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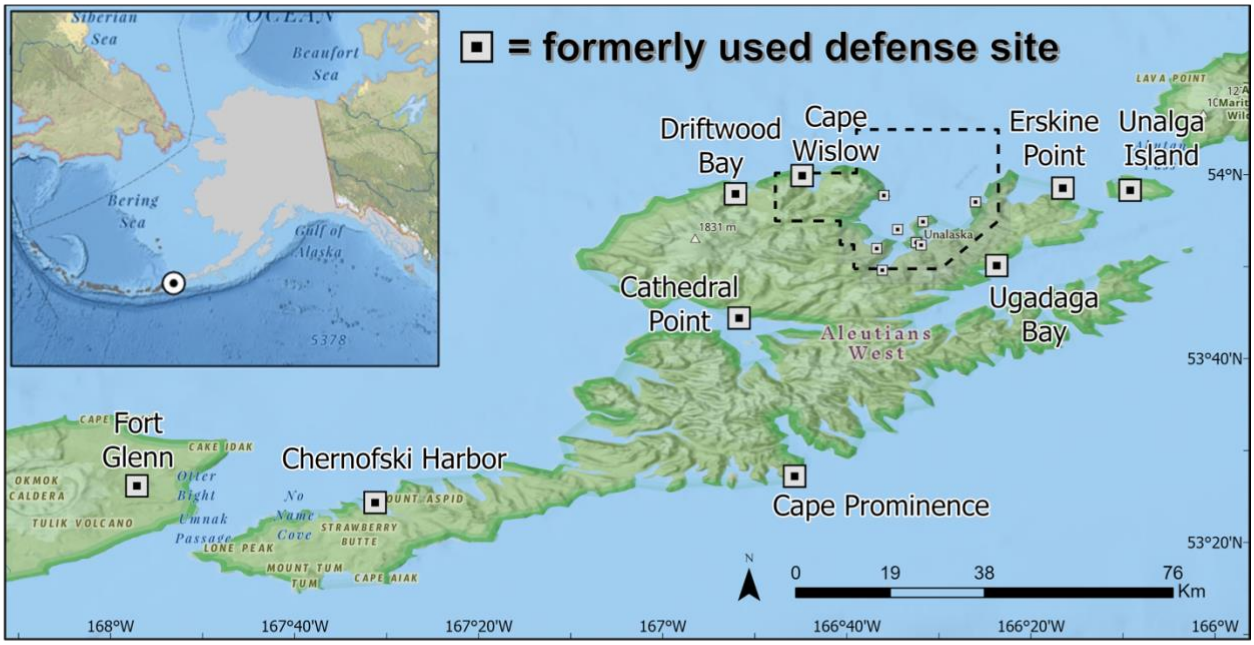
KEYWORDS

Aleutian Islands, Army Corps of Engineers, Environmental justice, FUD site, Military, Persistent organic pollutants

HIGHLIGHTS

- Unalaska Island in the Aleutians contains 16 formerly used defense (FUD) sites
- The Army Corps implemented varying cleanup criteria at Unalaska FUD sites
- Contamination remains above state cleanup levels despite remediation at many sites
- Contaminant distributions suggest that the City of Unalaska is a pollution hotspot
- FUD sites may continue to pose a health risk for local communities and wildlife

GRAPHICAL ABSTRACT



ABSTRACT

Unalaska Island, Alaska served as a base of operations for the U.S. military during the Aleutian Islands Campaign of World War II (WWII). The U.S. military installed major bases on Unalaska and nearby islands, many of which were built adjacent to Unangan (Aleut) communities. These defense sites were largely abandoned at the end of WWII, though some continued to operate during the Cold War. The military used many toxic compounds in their operations and left a legacy of pollution that may pose health risks to residents and local wildlife. We mined and synthesized data from military remediation reports conducted by the U.S. Army Corps of Engineers (Army Corps) at 18 formerly used defense (FUD) sites on Unalaska Island and nearby islands. We focused on 22 contaminants and mapped distributions across FUD sites to identify hotspots of pollution. To our knowledge, this is the first study to map contaminant distributions originating from military activity across Unalaska. Concentrations of toxic metals and persistent organic pollutants (POPs) exceeded cleanup thresholds set by the Alaska Department of Environmental Conservation at nine sites that were remediated. One site was listed as closed, but we were unable to locate sampling data for the site. The remaining eight sites were characterized by the Army Corps as “no Department of Defense action indicated”. Contamination was most severe at the Amaknak FUD site, which is located adjacent to the City of Unalaska. Our results indicate that Unalaska FUD sites remain a potential health threat to residents and local wildlife and highlight the need for future remediation and monitoring efforts on Unalaska.

INTRODUCTION

Alaska held strategic importance to the United States (U.S.) during World War II (WWII) and the Cold War (Roucek, 1983). Located at the nexus of east and west, Alaska provided tactical defense positions to the U.S military. Unalaska Island, located in the Fox Islands of the Aleutian Archipelago (Fig. 1), served as a base of operations by the U.S. military during the Aleutian Islands Campaign of WWII. The Dutch Harbor Naval Operating Base on Unalaska was the westernmost major naval base in Alaska at the onset of WWII and supported extensive facilities, including barracks, underground storage tanks, munitions storage facilities, and a powerplant. In June 1942, Japanese forces launched an aerial attack on Dutch Harbor and Fort Mears, and occupied the islands of Attu and Kiska from 1942 to 1943 (Rourke, 1997; USNPS, 2020). In response to these attacks, the U.S. military launched the Aleutian Islands Campaign and rapidly expanded its military presence in the archipelago, including the installation and expansion of defense sites on Unalaska. Because many strategically important locations were in remote regions of Alaska, the military often constructed defense sites adjacent to communities with existing infrastructure (Scrudato et al., 2012; von Hippel et al., 2016). The Dutch Harbor military base was built adjacent to the town of Unalaska (Fig. 1) and expanded across western Unalaska and Amaknak Island to include positions at Ulakta Head (Fort Schwatka), Eider Point (Fort Learnard), Constantine Point (Fort Brumback), Hill 400, Morris Cove, Constantine Bay, Kaletka Bay, English Bay, Agamgik Bay, Zharaoff Point, Ugadaga Bay, Uniktali Bay, Erskine Point, Nateekin Bay and Hog Island (Klein et al., 1987; USACE, 2003, 2017b). As a result, Dutch Harbor became one of the largest U.S. bases developed during WWII (Klein et al., 1987).

The Unangan (Aleut) people of the Aleutian Islands suffered greatly during the war. Those living on islands occupied by the Japanese were placed in prisoner of war camps in Japan, where about 50% perished (Sepez et al., 2007). In July 1942, the U.S. relocated 881 Unangan residents from their traditional lands to camps in southeast Alaska; non-native civilians were not forced to evacuate (Klein et al., 1987; Madden, 1992; Mason, 2010; Sepez et al., 2007; USNLM Online). Harsh conditions, food shortages, and inadequate housing and health care at the camps led to the death of about one in ten people during the forced exile (Klein et al., 1987; Madden, 1992; Mason, 2010). Many elders did not survive the internment, which devastated Unangan culture (Kalytiak, 2016; Klein et al., 1987; Madden, 1992; Sepez et al., 2007). Despite the defeat of Japanese forces at Attu and Kiska Islands in 1943, Unangan people were kept at internment camps until 1945 and 1946 (Gable, 1980). The U.S. did not return the people of Biorka, Kashega, and Makushin to their traditional villages, but instead relocated them to other communities, including Unalaska (Klein et al., 1987; Mason, 2010). Unangan residents of Unalaska found that many of their traditional homes and lands had been looted and damaged during their absence (Kirkland and Coffin, 1981).

Military sites on Unalaska were mostly abandoned following WWII, leaving behind a legacy of pollution. Many of the buildings and much of the materials used during operations were left on site, including munitions, lumber, paint, electrical wiring, insulation, transformers, and tanks and drums containing hazardous substances (e.g., brake fluid, fuels, anti-freeze, polychlorinated biphenyls [PCBs], and asbestos) (Carpenter et al., 2005; Sepez et al., 2007; USNPS, 2015).

Concerns about environmental contamination and impacts to human health elsewhere in the U.S. prompted the federal government to enact environmental legislation, including the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA; also referred to as Superfund) of 1980 and the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Lubbert and Chu, 2007). Section 211 of SARA created the Defense Environmental Restoration Program for formerly used defense (FUD) sites and granted the Department of Defense (DoD) authority to oversee cleanup activities at FUD sites (Lubbert and Chu, 2007; USACE, 2021a).

Military activity in Alaska during WWII and the Cold War resulted in ~600 FUD sites (USDOI, 2016; USEPA, 2004), with over 500 FUD sites listed as high-priority sites for remediation (Williams and Cravez, 2018). The Army Corps of Engineers (Army Corps) found that over 250 FUD sites in Alaska contain hazardous, toxic, and/or radioactive waste (USACE, 2015). However, many of these sites were not prioritized for remediation because of the difficulty and cost associated with remediating remote sites and the relatively small human populations impacted by contamination (Williams and Cravez, 2018). As of 2021, 80% of the FUD sites in Alaska listed in the Army Corps Defense Environmental Restoration Program did not have cleanup projects (USACE, 2021b), including sites identified as containing hazardous waste (USACE, 2015). Furthermore, sites that undergo environmental investigation and remediation are often deemed safe for human use based on insufficient sample sizes and budget limitations that prevent comprehensive risk analysis and cleanup measures (Jordan-Ward et al., 2022; von Hippel et al., 2018).

Approximately 4,254 residents live year-round on Unalaska Island, with thousands of additional people employed by the fishing industry residing on the island during the summer months (2020 Census; City of Unalaska, 2017). The International Port of Dutch Harbor is the largest fishing port by volume of seafood in the U.S. and provides over 318 million kg of fish and shellfish annually (City of Unalaska, 2021; McKenney, 2021). Moreover, the productive marine environment supports a robust ecosystem that includes endangered and threatened species, including Steller sea lions (*Eumetopias jubatus*), northern sea otters (*Enhydra lutris kenyoni*), and humpback whales (*Megaptera novaeangliae*) (AKDFG, 2022; Brewer, 2022). Millions of seabirds breed during the summer months in the Aleutian Islands (Brewer, 2022; Byrd, 2005). Inland animals include the red fox (*Vulpes vulpes*) and the endemic Unalaska collard lemming (*Dicrostonyx groenlandicus unalascensis*) (Gotthardt and McClory, 2005). Many local animals serve as key subsistence food sources for Unalaska residents (RIDOLFI Inc., 2020; USACE, 1999).

Following the enactment of CERCLA and SARA, the DoD and Army Corps began evaluating FUD sites on Unalaska for remediation. Remediation projects on Unalaska and Amaknak Island began in 1984 and were still underway during the current study (USACE, 2020a). This study synthesized data from Army Corps remediation reports to identify hotspots of pollution associated with FUD sites on Unalaska and nearby islands. We focused on areas important to the Qawalangin Tribe of Unalaska and FUD sites within the City of Unalaska

(RIDOLFI Inc., 2020). The Indigenous communities of Unalaska neither participated in land-use decisions during construction and operation of these sites nor profited from military activities on their traditional lands, yet they suffered the consequences of forced evacuation and environmental contamination from military use. We hope that this study inspires future contaminant modeling and restoration projects at Unalaska FUD sites to better protect local communities and wildlife.

METHODS

Data collection and analysis

We identified 18 FUD sites on Unalaska and nearby islands using the Army Corps FUD site portal and Alaska Department of Environmental Conservation (ADEC) Contaminated Sites Database (Table 1; Fig. 1) (ADEC, 2021; RIDOLFI Inc., 2020; USACE, 2021b). We collected remediation reports from ADEC records, including site characterization, site inspection, remedial action, and site closure reports conducted by the Army Corps. Each FUD site was divided into subsites mirroring those listed in the reports. For example, the Amaknak FUD site was divided into 12 subsites, including Building 551, Captain's Bay, and Pyramid Valley (USACE, 2017b). Because our goal was to identify current hotspots of pollution that may negatively impact the Unalaska community and wildlife, we only extracted data from the most recent reports for each subsite to best reflect existing pollution levels. The data in this study reflect soil concentrations of each contaminant (mg/kg) because soil was the most sampled medium in Army Corps FUD site reports.

We did not extract every contaminant tested at each FUD site; instead, we focused on contaminants known to elicit adverse health effects in humans and wildlife (Table 2).

Contaminants of concern included petroleum hydrocarbons, persistent organic pollutants (POPs), and toxic metals and metalloids. In total, we extracted data for 22 contaminants across the study sites (Table 2). FUD sites or subsites within a FUD site that had been partially or fully remediated and had no exceedances were removed from the analysis, but we included those that had at least one contaminant concentration exceeding ADEC cleanup levels (Table 2). For sites included in the analysis, we extracted all reported concentrations for each contaminant, even if samples had concentrations below the cleanup level. This allowed us to spatially identify the hotspots for contaminants of concern (Fig. 2). For example, Chernofski Harbor had a lead (Pb) concentration of 6,300 mg/kg at the Project Area 05 subsite, far exceeding the ADEC cleanup threshold of 400 mg/kg (USACE, 2018). To visualize how Pb concentrations were distributed over the Chernofski Harbor FUD site area, we extracted Pb concentrations for all other subsites, including Project Area 09 that reported Pb concentrations well below the cleanup level (e.g., 2.74 mg/kg) (USACE, 2018). About half of the extracted samples contained contaminant concentrations below the ADEC cleanup value.

Mapping contaminant distributions

GPS coordinates for each sampling location were collected from FUD site reports when available and estimated from site maps when GPS documentation was either insufficient or unavailable. Several FUD site reports listed coordinates using the U.S. state plane coordinate system (northing and easting coordinates). We converted these coordinates to decimal degrees (latitude and longitude degrees) using Google Earth online coordinate conversion software with Alaska Zone 10 (code 5010) and U.S. Survey Feet parameters (Earth Point, 2021). All maps were created using ArcGIS Pro (version 2.9.0), which was also used to perform all spatial analyses. To identify site locations, we created a minimum bounding polygon tool using the samples associated with a site. To further examine the footprint of contamination, we created a hexagon grid using the general tessellation tool at two scales. The entire study area (2,043-2,076 km^2) was represented by a 5 km scale and the City of Unalaska (408-415 km^2) by a 1 km scale (inset maps in Figs. 2-4). This spatial scale allowed samples to fall within a single hexagon at both scales.

We compared contaminant concentrations to the ADEC Method Two cleanup levels for the migration to groundwater exposure pathway (Table 2) (City of Unalaska, 2021), reflecting screening levels used by the Army Corps at many Unalaska FUD sites. Although cleanup levels vary across FUD sites, we elected to compare all contaminant concentrations to ADEC screening levels as a point of reference. Thus, our data do not indicate the risk of exposure to contaminants but allow us to examine how FUD site pollution varies across the landscape. For Pb and PCBs, we used the ADEC human health cleanup value for areas receiving annual precipitation of more than 40 inches (102 cm; Unalaska receives about 152 cm of precipitation annually) (City of Unalaska, 2021). For benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, dibenzo[a,h]anthracene, and 1,2,4-trimethylbenzene, we used the screening levels used at the Chernofski Harbor FUD site, which were more conservative than the ADEC Method Two cleanup levels (USACE, 2018). All cleanup values used in this study reflect values used in multiple FUD site reports; however, these cleanup levels do not necessarily reflect risk posed by a contaminant at a specific FUD site due to varied local conditions such as soil and water chemistry.

We designed a standard scoring system based on ADEC cleanup thresholds to compare contaminant concentrations across FUD sites at the same scale (Table 2). This enabled us to visualize areas with elevated levels of contaminants. We evaluated contaminants based on their concentration relative to the ADEC cleanup levels, which we termed the exceedance score. We applied an exceedance score from 0 to 3 for each contaminant sample: a sample received a score of zero if the concentration was not applicable (i.e., unavailable, not extracted due to low values, or not detected), a score of 1 if the concentration was below the ADEC cleanup level, a score of 2 if the contaminant concentration was above the ADEC cleanup level and below 10X the cleanup level, and a score of 3 if the concentration was more than 10X higher than the ADEC cleanup level (Table 2).

To understand cumulative and average exceedances among contaminants within a specific area, we also calculated cumulative and mean exceedance scores. The cumulative score for a sample was measured as the sum of the exceedance scores for each contaminant measured in that area. Cumulative scores (Fig. 3) across the study area represent the sum of the exceedance scores for each sample within the boundary of a hexagon. The cumulative score depends on the number of contaminants analyzed within each area. For example, the maximum cumulative score is 3 in an area where only one contaminant was analyzed, whereas the maximum cumulative score is 15 in an area where five contaminants were analyzed. Because the cumulative score is biased by the number of contaminants tested, we also report a mean exceedance score for each area (Fig. 4). Mean exceedance scores were calculated by dividing each cumulative score by the number of contaminants tested in the area, thus providing a mean exceedance score for each hexagonal area (Fig. 4).

RESULTS & DISCUSSION

Contaminant concentrations at Unalaska FUD sites remain above ADEC cleanup levels despite remediation efforts. We found that only nine of the 18 FUD sites on Unalaska and nearby islands had past and/or active remediation projects and on-site sampling data collected by the Army Corps (Table 1). One FUD site (Unalaska Defense Site) was listed as closed, but we were unable to locate sampling data for the site. The remaining eight FUD sites had no planned remediation projects. Each of the nine FUD sites with sampling data contained at least one contaminant that exceeded the ADEC cleanup criteria (Table 2), with varying cumulative scores (Fig. 3). We found that contaminant concentrations across all sampled FUD sites were typically between 0 to 10-fold higher than the ADEC cleanup criteria (Fig. 4). Of the samples that exceeded the cleanup criteria, 24% had concentrations over 10-fold higher than the cleanup criteria, although this varied by the contaminant tested (Table 2). Contamination originating from FUD sites was most severe within the boundary of the City of Unalaska (Fig. 3) despite extensive remediation efforts at Dutch Harbor, including the removal of over 200 underground storage tanks and treatment of 42,000 tons of petroleum-contaminated soil (USACE, 2020a).

FUD site reports varied in the stage of remediation, cleanup criteria thresholds, and number of analytes tested. We found that cleanup criteria thresholds were not standardized across Unalaska FUD sites. For example, cleanup thresholds for benzo[a,h]anthrocene ranged from 0.27-5.5 mg/kg (USACE, 2003, 2018, 2020d). We found that diesel range organics (DRO) was the most common contaminant analyzed at Unalaska FUD sites, and DRO concentrations were consistently over 10-fold higher than ADEC cleanup thresholds (Fig. 2). Other contaminants, including dichlorodiphenyltrichloroethane (DDT) and pyrene, were only tested at one FUD site (Chernofski Harbor and Ugadaga Bay, respectively). DDT was used at Alaska military bases during WWII and may continue to leach into local environments (Hogan et al., 2006). Indeed Anthony et al. (2007) found elevated dichlorodiphenyldichloroethylene (DDE; a metabolite of DDT) concentrations in bald eagle (*Haliaeetus leucocephalus*) eggs collected near former military sites on several Aleutian Islands, including at Dutch Harbor on Unalaska. To our knowledge, the Army Corps did not measure DDT metabolites, including DDE, at any of the Unalaska FUD sites.

We found that cleanup at several FUD sites was determined complete through a process with changing guidelines and inconsistencies between state and federal procedures. For example, the Army Corps concluded that no further action was necessary at several sites despite reporting contaminants that exceeded the cleanup criteria used at that site (USACE, 2007, 2020d). Several drum sites at the Fort Learnard FUD site were not recommended for further action because the extent of contamination, albeit in exceedance of the site-specific cleanup criteria, was of small volume (USACE, 2007). Similarly, the Army Corps did not recommend further remediation of an above-ground storage tank at the Hog Island FUD site even though Pb and arsenic (As) levels exceeded cleanup criteria (USACE, 2020d). We also found that cleanup decisions at some sites were based on results that were biased low and/or where quality control measures had failed (USACE, 2017b, 2018, 2020b, c). Furthermore, site recommendations at the Unalga Island and Chernofski Harbor FUD sites were based on analytical data collected on equipment that had limits of quantification higher than the cleanup criteria. Additionally, PCB concentrations at most Unalaska FUD sites were tested as Aroclor mixtures, which likely underestimate the true concentration present in the environment and preclude the analysis of congener-specific effects that may be important to remediation efforts. These issues point to the need for consistent application of cleanup criteria that meet the most stringent applicable state or federal guidelines for the protection of human health and the environment.

Many Army Corps reports attributed elevated toxic metal(loid) concentrations to naturally occurring sources and not to FUD site contamination. We found that As concentrations were consistently over 10-fold higher than the ADEC cleanup threshold of 0.2 mg/kg (Fig. 2). While the Aleutian Islands do have elevated metal and metalloid concentrations from natural sources (Gough et al., 1988; Perryman et al., 2020), we found that the Army Corps did not consistently assess background levels and that this precluded analysis of the contribution of FUD sites to local metal(loid) pollution. FUD sites as a source of metal(loid)s can be significant and was at times downplayed. For example, the Army Corps concluded that high As concentrations at the Chernofski Harbor FUD site were consistent with background levels despite a 16.5-fold increase in the maximum As concentration at the FUD site (163 mg/kg and 9.86 mg/kg, respectively) (USACE, 2018). Similarly, maximum As concentrations at the Driftwood Bay FUD site were 11-fold higher than background levels (84.4 mg/kg and 7.38 mg/kg, respectively) (USACE, 2009, 2017a). Despite these discrepancies, the Army Corps concluded that elevated As concentrations were likely due to natural sources.

Eight of the 18 FUD sites (44%) had no active or planned cleanup projects, despite reported debris present from military activity dating back to WWII (Table 1) (RIDOLFI Inc., 2020). This is consistent with Williams and Cravez (2018), who reported that 33% of all Alaska FUD sites did not have cleanup projects. The Army Corps designates sites as “No DOD action indicated, Category I” (NDAI) if no evidence of hazardous materials or contamination from former military activity is observed or reported (USGAO, 2002). Once an NDAI determination is made, the site is no longer monitored for potential hazards related to former military use (USGAO, 2002). However, in 2002, the U.S. General Accounting Office (GAO) conducted a study on FUD site NDAI determination and found that the Army Corps’ process for determining if a site meets NDAI criteria was insufficient (USGAO, 2002). In particular, the Army Corps failed to identify all potential hazards and did not establish sufficient evidence of safety for 38%

of FUD sites listed as not needing remediation across the U.S. Furthermore, the GAO found that the Army Corps failed to conduct required site visits for 18% of NDAI sites and failed to notify current site owners of NDAI determination for 72% of sites. For FUD sites that were remediated, the GAO found that the Army Corps did not adequately conduct the required 5-year reviews or re-evaluate FUD sites for emerging contaminants once their cleanup process was complete (USGAO, 2009). Therefore, FUD sites remediated by the Army Corps may still pose a risk to human health and the environment.

The GAO determined that the Cape Wislow FUD site (listed as Cape Winslow in the report) on Unalaska was not sufficiently characterized and data were lacking on potential hazards to provide a sound basis for NDAI determination (USGAO, 2002). Located on the northeast coast of Unalaska, Cape Wislow served as an aircraft warning system during WWII (USDOI, 1991) and included a 518 m tramway, housing and utility structures, and a 7.6 m steel radar tower with a 15.2 m antenna (Klein et al., 1987). Although the site was abandoned in 1947, significant debris from military infrastructure and materials were left at the site (Klein et al., 1987). Potential contaminants of concern at Cape Wislow include toxic metals from electronics and PCBs from degraded storage tanks (RIDOLFI Inc., 2020). The local community is concerned about the impacts of pollution on sockeye salmon (*Oncorhynchus nerka*) in Wislow (Reese) Bay and McLees Lake, which are less than a kilometer from the Cape Wislow FUD site (RIDOLFI Inc., 2020). Sockeye salmon harvested from McLees Lake comprised 48-89% of Unalaska subsistence harvests between 2012 and 2016 (Lipka and Fox, 2017). Despite the importance of McLees Lake as a subsistence fishery, the Army Corps did not plan to evaluate potential risks posed by contamination at the Cape Wislow FUD site (USGAO, 2002). To date, the Cape Wislow FUD site remains an NDAI site, which prevents the Army Corps from investigating potential health risks posed by the site.

Alaska FUD sites may continue to pose a risk to human health and the environment. For example, research at FUD sites on Sivuqaq (St. Lawrence Island), Alaska shows that toxic military pollution persists even after extensive remediation efforts, including the most expensive remediation project to date at an Alaska FUD site (ADEC, 2019; Miller et al., 2013; von Hippel et al., 2018). Although cleanup was determined complete for the Northeast Cape FUD site on Sivuqaq, contamination originating from the FUD site continues to drive elevated concentrations of organochlorine pesticides, mercury (Hg), and PCBs in local soils and wildlife, and remains a concern for Sivuqaq residents (Carpenter et al., 2005; Jordan-Ward et al., 2022; Scrudato et al., 2012; von Hippel et al., 2018). On-site investigators at the Amaknak FUD site on Unalaska noted the presence of fuel odors, stressed vegetation, and soil staining, yet sample results were below the ADEC cleanup criteria and no further action was recommended (USACE, 2003). Results from the present study and from previous work (Adams et al., 2019; Jordan-Ward et al., 2022; von Hippel et al., 2018) demonstrate that current cleanup thresholds may not be protective for communities that rely heavily on subsistence foods and thus have increased exposure to contaminants in their environment. Because many FUD sites in Alaska are co-located with rural communities, contaminated sites may exacerbate risks to human health (von Hippel et al., 2016; Williams and Cravez, 2018). The Army Corps did not evaluate contaminant concentrations in subsistence foods at Unalaska FUD sites, which would inform important exposure pathways to Unalaska residents. Future remediation projects would benefit from more stringent cleanup thresholds at FUD sites adjacent to subsistence communities. For example, the Environmental

Protection Agency (EPA) has stricter regulatory thresholds for subsistence fishers consuming contaminated fish due to increased exposure rates (USEPA, 2000).

Contamination originating at Unalaska FUD sites may continue to pose health risks to local wildlife despite remediation efforts. Although we found that PCB concentrations fell below regulatory thresholds at most Unalaska FUD sites (Fig. 2), these sites may still act as point sources of PCB contamination (Adams et al., 2019; Anthony et al., 2007; Miles et al., 2009). Anthony et al. (2007) found that bald eagle eggs collected at Dutch Harbor had elevated total PCB concentrations compared to eggs collected at reference sites. Similarly, Adams et al. (2019) found elevated PCB concentrations in blue mussels (*Mytilus edulis*) and threespine stickleback (*Gasterosteus aculeatus*) collected from Unalaska FUD sites compared to those collected at non-military sites. Furthermore, Adams et al. (2019) found that PCB concentrations in mussels collected from Building 551 and Delta Western (subsites within the Amaknak FUD site) exceeded the EPA's guideline for safe consumption of fish contaminated with PCBs (cancer risk for unrestricted human consumption). These findings are consistent with previous work in the western Aleutian Islands that found that FUD sites contributed to higher total PCB and DDE concentrations in both fishes (Miles et al., 2009) and seabirds (Ricca et al., 2008). In 2011, the Army Corps issued a cleanup complete determination for Delta Western, even though remaining contamination was 10-fold higher than the ADEC cleanup criteria (ADEC, 2011). Collectively, these results demonstrate that Alaska FUD sites continue to pollute local environments despite remediation efforts and may remain a potential health threat to residents and local wildlife.

Contaminant distributions across Unalaska FUD sites show that the City of Unalaska remains a hotspot of pollution (Figs. 3 and 4). Many Unalaska residents rely on subsistence foods harvested from traditional lands, including those co-located with FUD sites (Adams et al., 2019; Lipka and Fox, 2017; USACE, 1999). From 1997-1998, approximately 888 metric tons of subsistence foods were harvested within 24 km downstream of the Ugadaga Bay FUD site (USACE, 1999). This site contains several contaminants that exceed cleanup criteria, including As, chromium (Cr), Pb, PCBs, benzo[a]anthracene, benzo[a]pyrene, benzo[a]fluoranthene, dibenzo[a,h]anthracene, and naphthalene (USACE, 1999). Similarly, several stream systems flow through the Amaknak FUD site to Dutch Harbor, Captains Bay, and Unalaska Bay and could provide a pathway for migration and incorporation of contaminants into food webs downstream of the FUD site (Lemke and Vanderpool, 1995). As a result, residents who consume subsistence foods collected downstream of the Amaknak FUD site may be exposed to military contaminants, such as PCBs, through their diet (Adams et al., 2019).

Collectively, the results from the present study and from previous work suggest that FUD sites in the Bering Sea region continue to pollute local environments and that remediation of these sites may not sufficiently protect communities and wildlife adjacent to them (Figs. 2-4). Our analysis was limited to available data from military reports and data quality standards used at each site. We were not able to evaluate contaminant levels for sites with NDAI status, even though they may also pose a health risk to residents and local wildlife (Adams et al., 2019; RIDOLFI Inc., 2020). Nonetheless, we demonstrated that several FUD sites, including those adjacent to and within the City of Unalaska, remain hotspots of environmental contamination despite remediation efforts (Figs. 2-4). While cleanup efforts are active at many of these sites, criteria for cessation of cleanup have changed over time and vary among sites. These problems could be addressed through a revision of federal guidelines such that the Army Corps is empowered to apply consistent and protective measures at FUD sites throughout the United States.

CONCLUSIONS

Military installations on Unalaska Island left behind a legacy of pollution that may continue to impact residents and the local environment. Contaminant concentrations remain above state cleanup thresholds at many sites despite remediation efforts. Contaminant distributions suggest that the City of Unalaska remains a hotspot of pollution. Additionally, cleanup thresholds to not account for the high consumption levels of local foods by subsistence communities.

The present study and previous research exemplify the need for more robust evaluation and remediation of contaminants at FUD sites. At present, FUD sites are managed independently without consideration of the cumulative effects on the environment. Similarly, cleanup levels are set for each contaminant and do not take into account potential synergistic effects of contaminant mixtures. Our analysis of Unalaska FUD sites also highlights the importance of continued efforts to involve community members in the evaluation and remediation of FUD sites. The Unangan people of Unalaska were not included in decisions on military site selection or activities that took place adjacent to their community during WWII and the Cold War. Such lack of local control over land use and the subsequent pollution of those lands is a common issue in communities throughout the Arctic. Disproportionate exposure to carcinogenic and endocrine disrupting compounds remains an environmental justice issue in the Arctic and may contribute to health disparities (AMAP, 2015). Collectively, our results suggest that future remediation projects should: 1) conduct comprehensive testing for both legacy and emerging contaminants at FUD sites to better evaluate risk, 2) assess cleanup thresholds to ensure that they are protective for rural communities, which may require changes to federal guidelines, and 3) involve Tribal government and local residents in decision-making processes and site prioritization.

The Aleutian Archipelago provides vital habitat for globally significant wildlife populations and supports some of the largest fisheries in the world. In addition to the FUD sites examined in the present study, the Army Corps lists another 41 FUD sites located within the Aleutian Archipelago (59 total) and 29 within the Bering Sea region (Supp. Table 1). Future research could mine data from the remaining FUD sites in the Aleutian and Bering Sea region to identify additional hotspots of contamination. This, in turn, would inform conservation programs and actions to improve the health of rural communities.

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REFERENCES

2020 Census, United States Census Bureau https://www.census.gov (accessed 12 February 2022).

Adams, E.M., von Hippel, F.A., Hungate, B.A., Buck, C.L., 2019. Polychlorinated biphenyl (PCB) contamination of subsistence species on Unalaska Island in the Aleutian Archipelago. Heliyon 5, e02989.

ADEC, 2011. Site Report: Delta Western Tank Farm, Dutch Harbor, Alaska. https://dec.alaska.gov/Applications/SPAR/PublicMVC/CSP/SiteReport/25576 (accessed 12 February 2022).

ADEC, 2019. Northeast Cape and Gambell Formerly Used Defense Sites. Alaska Department of Environmental Conservation. https://dec.alaska.gov/spar/csp/sites/st-lawrence/ (accessed 02 January 2022).

ADEC, 2021. Contaminatd Sites Search. Alaska Department of Environmental Conservation, Division of Spill Prevention and Response. https://dec.alaska.gov/Applications/SPAR/PublicMVC/CSP/Search (accessed 15 November 2021).

AKDFG, 2022. Federal Special Status Species. Alaska Department of Fish and Game. http://www.adfg.alaska.gov/index.cfm?adfg=specialstatus.fedendangered (accessed 30 January 2022).

AMAP, 2015. Summary for Policy-makers: Arctic Pollution Issues 2015. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. 12 pp.

Anthony, R.G., Miles, A.K., Ricca, M.A., Estes, J.A., 2007. Environmental contaminants in bald eagle eggs from the Aleutian Archipelago. Environ Toxicol Chem 26, 1843-1855.

Brewer, R., 2022. The City of Unalaska: Wildlife. City of Unalaska. https://www.ci.unalaska.ak.us/community/page/wildlife (accessed 30 January 2022).

Byrd, W., 2005. Distribution patterns and population trends of breeding seabirds in the Aleutian Islands. Fish Oceanogr 14, 139-159.

Carpenter, D.O., DeCaprio, A.P., O'Hehir, D., Akhtar, F., Johnson, G., Scrudato, R.J., Apatiki, L., Kava, J., Gologergen, J., Miller, P.K., Eckstein, L., 2005. Polychlorinated biphenyls in serum of the Siberian Yupik people from St. Lawrence Island, Alaska. Int J Circumpolar Health 64, 322-335.

City of Unalaska, 2017. Unalaska Community Profile. City of Unalaska. https://www.ci.unalaska.ak.us/sites/default/files/fileattachments/City%20Manager%26%23039%3Bs%20Office/page/963/community_profile_2017_03_27.pdf (accessed 30 January 2022).

City of Unalaska, 2021. The City of Unalaska. https://www.ci.unalaska.ak.us (accessed 07 January 2022).

Earth Point, 2021. State Plane Coordinate System - Convert, View on Google Earth. Tools for Google Earth. https://www.earthpoint.us/stateplane.aspx (accessed 18 November 2021).

Gable, D., 1980. The Aleuts: forgotten casualties of Japanese invasion, American internment. Fish and Wildlife News, 6-7.

Gotthardt, T.A., McClory, J.G., 2005. Northern Collard Lemmng and Alaska Subspecies. Alaska Department of Fish and Game. https://www.adfg.alaska.gov/static/species/speciesinfo/_aknhp/Collared_lemming.pdf (accessed 18 February 2022).

Gough, L.P., Severson, R.C., Shacklette, H.T., 1988. Element Concentrations in Soils and Other Surficial Materials of Alaska U.S. Department of the Interior, U.S. Geological Survey.

Hogan, M., Christopherson, S., Rothe, A., 2006. Formerly used defense sites in the Norton Sound region: location, history of use, contaminants present, and status of clean-up efforts. Alaska Community Action on Toxics.

Jordan-Ward, R., von Hippel, F.A., Zheng, G., Salamova, A., Dillon, D., Gologergen, J., Immingan, T., Dominguez, E., Miller, P., Carpenter, D., Postlethwait, J.H., Byrne, S., Buck, C.L., 2022. Elevated mercury and PCB concentrations in Dolly Varden (*Salvelinus malma*) collected near a formerly used defense site on Sivuqaq, Alaska. Sci Total Environ 826, 154067.

Kalytiak, T., 2016. 'I want to strengthen the fabric of Unangan lives'. University of Alaska Anchorage. June 29, 2016.

Kirkland, J.C., Coffin, D.F., 1981. The relocation and internment of the Aleuts during World War II. Aleutian-Pribilof Islands Association, Inc.

Klein, J.L., Nolan, J.L., Warren-Findlay, J., Brenner, W.A., Gillespie, R.E., Vetter, J., 1987. World War II in Alaska: A Historic and Resources Management Plan. United States Army Corps of Engineers.

Lemke, K.J., Vanderpool, A.M., 1995. Overview of Environmental and Hydrogeologic Conditions at Dutch Harbor, Alaska. Open-File Report 95-411. U.S. Geological Survey.

Lipka, C.G., Fox, E.K.C., 2017. McLees Lake Salmon Escapement Monitoring Report, 2012-2017. Alaska Department of Fish and Game, Fishery Management Report No. 17-49, Anchorage.

Lubbert, R., Chu, T., 2007. Challenges to cleaning up formerly used defense sites in the twenty-first century. Fed Facil Environ J 11, 5-18.

Madden, R., 1992. The forgotten people: the relocation and internment of Aleuts during World War II. Am Indian Cult Res J 16, 55-76.

Mason, R., 2010. You can’t go home again: processes of displacement and emplacement in the “lost villages” of the Aleutian Islands. Alsk J Anthropol 8, 17-29.

McKenney, H., 2021. Dutch Harbor is nation’s top fishing port for 23rd straight year. Alaska Public Media. May 27, 2021.

Miles, A.K., Ricca, M.A., Anthony, R.G., Estes, J.A., 2009. Organochlorine contaminants in fishes from coastal waters west of Amukta Pass, Aleutian Islands, Alaska, USA. Environ Toxicol Chem 28, 1643-1654.

Miller, P.K., Waghiyi, V., Welfinger-Smith, G., Byrne, S.C., Kava, J., Gologergen, J., Eckstein, L., Scrudato, R., Chiarenzelli, J., Carpenter, D.O., Seguinot-Medina, S., 2013. Community-based participatory research projects and policy engagement to protect environmental health on St. Lawrence Island, Alaska. Int J Circumpolar Health 72, 21656.

Perryman, C.R., Wirsing, J., Bennett, K.A., Brennick, O., Perry, A.L., Williamson, N., Ernakovich, J.G., 2020. Heavy metals in the Arctic: distribution and enrichment of five metals in Alaskan soils. PLoS One 15, e0233297.

Ricca, M.A., Keith Miles, A., Anthony, R.G., 2008. Sources of organochlorine contaminants and mercury in seabirds from the Aleutian Archipelago of Alaska: inferences from spatial and trophic variation. Sci Total Environ 406, 308-323.

RIDOLFI Inc., 2020. Strategic project implementation plan, Unalaska, Alaska Native American Lands Environmental Mitigation Program. Prepared by RIDOLFI Inc. for the Qawalangin Tribe of Unalaska.

Roucek, J.S., 1983. The geopolitics of the Arctic. Am J Econ Sociol 42, 463-471.

Rourke, N.E., 1997. War Comes to Alaska: The Dutch Harbor Attack, June 3-4, 1942. Burd Street Press.

Scrudato, R., Chiarenzelli, J., Miller, P.K., Alexander, J.C., Arnason, J., Zamzow, K., Zweifel, K., Gologergen, J., Kava, J., Waghiyi, V., Carpenter, D., 2012. Contaminants at arctic formerly used defense sites. J Local Glob Health Sci 2, 1-12.

Sepez, J., Package, C., Malcolm, P.E., Poole, A., 2007. Unalaska, Alaska: memory and denial in the globalization of the Aleutian landscape. Polar Geogr 30, 193-209.

USACE, 1999. Ugadaga Bay Fire Control Station Draft Preliminary Asessment Report United States Army Corps of Engineers. Superfund Technical Assistance and Response Team. TDD: 97-02-0010. Contract No. 68-W6-008. .

USACE, 2003. 2001-2002 Islandwide SI/RI/IRA Report: Amaknak and Unalaska Islands, Alaska. United States Army Corps of Engineers. Prepared by Jacobs Engineering Group Inc. Contract No. DACA85-95-D-0018, Task Order No. 02.

USACE, 2007. Final Characterization Report, Fort Learnard, Unlaska Island, Alaska United States Army Corps of Engineers. Prepared by Jacobs Engineering Group Inc. Contract No. DACA 85-95-D-0018 Task Order No. 23.

USACE, 2009. Final Site Characterization Report: Driftwood Bay Radio Relay Station, Alaska. United States Army Corps of Engineers.

USACE, 2015. Formerly Used Defense Sites per State - Alaska. United States Army Corps of Engineers.

USACE, 2017a. Final 2015 - 2016 Remedial Action Report for Remedy Implementation at the Former Driftwood Bay Radio Relay Station, Alaska. United States Army Corps of Engineers. Prepared by MWH Americas Inc., Contract No.: FA8903-09-D-8556 Task Order No.: 008.

USACE, 2017b. Final Dutch Harbor Limited Removal Action Report: Amaknak and Unalaska Islands, Alaska. United States Army Corps of Engineers. Prepared by Ahtna Engineering Services, LLC. Contract No. W911KB-14-D-0005 Task Order No. 0004. .

USACE, 2018. Final Site Investigation at the Chernofski Harbor - Mutton Cove formerly used defense site. United States Army Corps of Engineers. Prepared By Environmental Management, Inc. Contract No.: W911KB-17-C-0015.

USACE, 2020a. Amaknak Formerly Used Defense Site, Environmental Update. United States Army Corps of Engineers.

USACE, 2020b. Draft Removal Action Report, Cape Prominence Formerly Used Defense Site Unalaska Island, Alaska. U.S. Army Corps of Engineers. Prepared by Bristol Environmental Remediation Services, LLC. Contract No. W911KB-14-D-0006, Task Order No. W911KB19F0014.

USACE, 2020c. Final CON/HTRW Removal Action, Formerly Used Defense Site F10AK001502, Unalga Island, Alaska. United States Army Corps of Engineers. Prepared by Ahtna Engineering Services, LLC. Contract No. W911KB-14-D-0005, Project W911KB18F0118.

USACE, 2020d. Final Demolition and Remedial Investigation Report Former Radio Range Facility: Hog Island, Alaska. United States Army Corps of Engineers. Prepared by Brice Environmental Services Corporation. Contract Number 697DCK-19-C-00153.

USACE, 2021a. Formerly Used Defense Site Projects. United States Army Corps of Engineers https://www.hnc.usace.army.mil/Media/Fact-Sheets/Fact-Sheet-Article-View/Article/482110/formerly-used-defense-sites/ (accessed 18 November 2021).

USACE, 2021b. FUDS Portal. United States Army Corps of Engineers. https://www.usace.army.mil/Missions/Environmental/Formerly-Used-Defense-Sites/FUDS-Inventory/ (accessed 15 November 2021).

USDOI, 1991. World War II in the Aleutians. U.S. Department of the Interior, National Park Service.

USDOI, 2016. Report to Congress: Hazardous Substance Contamination of Alaska Native Claim Settlement Act Lands in Alaska. United States Department of the Interior Bureau of Land Management.

USEPA, 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. United States Environmental Protection Agency Office of Water.

USEPA, 2004. Making Environmental Progress, Improving Local Communities: Accomplishments of the EPA Region 10 Superfund Program. United States Environmental Protection Agency Superfund Program.

USGAO, 2002. Environmental Contamination: Corps Needs to Reassess its Determinations that Many Former Defense Sites Do Not Need Cleanup. United States General Accounting Office, Report to Congressional Requesters. GAO-02-658.

USGAO, 2009. The U.S. Army Corps of Engineers Needs to Improve its Process for Reviewing Completed Cleanup Remedies to Ensure Continued Protection. United States General Accounting Office, Report to the Committee on Armed Services, House of Representatives. GAO-10-46.

USNLM Online, 1942: Unangan evacuated, interned during WWII., U.S. National Library of Medicine, National Institutes of Health.

USNPS, 2015. Aleutian Islands World War II. United States National Park Service. https://www.nps.gov/aleu/planyourvisit/safety.htm (accessed 13 February 2022).

USNPS, 2020. World War II in Alaska. United States National Park Service. https://www.nps.gov/articles/world-war-ii-in-alaska.htm (accessed 18 February 2022).

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: health implications for indigenous residents. Environ Pollut 234, 279-287.

von Hippel, F.A., Trammell, E.J., Merila, J., Sanders, M.B., Schwarz, T., Postlethwait, J.H., Titus, T.A., Buck, C.L., Katsiadaki, I., 2016. The ninespine stickleback as a model organism in arctic ecotoxicology. Evol Ecol Res 17, 487-504.

Williams, P., Cravez, P., 2018. Environmental justice: challenges of contaminated site cleanup in rural Alaska. Alaska Justice Forum 35, 1-5.

LIST OF TABLES

Table 1. List of formerly used defense (FUD) sites analyzed in this study. ACS = Alaska Communication System. NDAI = No Department of Defense Action Indicated.

FUD Site Property Name	FUD Site ID	Project Status	Number of Samples
Amaknak	F10AK0841	Active	83
Cape Prominence Aircraft Warning System	F10AK0806	Active	20
Cape Wislow Aircraft Warning System	F10AK0019	NDAI	0
Captain's Bay Steamship Northwestern	F10AK0397	NDAI	0
Cathedral Point	F10AK0339	NDAI	0
Chernofski Harbor Supply & Storage	F10AK0013	Active	32
Constantine Point End Base Station	F10AK0295	NDAI	0
Driftwood Bay Radio Relay Station	F10AK0016	Closed	71
Erskine Point Fire Control Station	F10AK0296	NDAI	0
Fort Glenn	F10AK0298	Active	58
Fort Learnard	F10AK0017	Active	23
Hog Island Defense Site	F10AK0609	Closed	19
Ugadaga Bay Station	F10AK0241	Active	10
Unalaska ACS Communication Station	F10AK0610	NDAI	0
Unalaska ACS Site	F10AK0611	NDAI	0
Unalaska ACS Transmitter and Receiver Site	F10AK0612	NDAI	0
Unalaska Defense Site	F10AK0014	Closed	0
Unalga Island Naval Radio Station	F10AK0015	Active	28

Table 2. List of contaminants and Alaska Department of Environmental Conservation (ADEC) contaminant screening levels extracted from Army Corps of Engineers formerly used defense (FUD) site remediation reports on Unalaska Island and nearby islands in the Aleutian Archipelago, Alaska. Percentages reflect the number of samples that were 10-fold higher than the ADEC cleanup criteria out of the total number of samples exceeding the cleanup criteria. NA = no exceedances.

Contaminant	**Cleanup criteria for soil (mg/kg)**	**Percent of samples over 10-fold higher than the cleanup criteria**
Diesel Range Organics (DRO)	230	56%
Residual Range Organics (RRO)	9700	19%
Arsenic	0.2	74%
Chromium	0.089	100%
Barium	1600	NA
Copper	370	50%
Mercury	0.36	0%
Lead	400	31%
Zinc	2490	NA
PCBs (many tested as Aroclors)	1	50%
DDT	5.1	0%
Benzo[a]anthracene	0.28	9%
Benzo[a]pyrene	0.17	15%
Benzo[b]fluoranthene	1.7	0%
Dibenzo[a,h]anthracene	0.17	0%
1,3,5-Trimethylbenzene	0.66	0%
1,2,4-Trimethylbenzene	0.16	0%
Naphthalene	0.038	30%
Pyrene	87	NA
Trichloroethylene	0.011	0%
1,2-Dibromoethane	0.00024	NA
Chloroform	0.0071	0%

LIST OF FIGURES

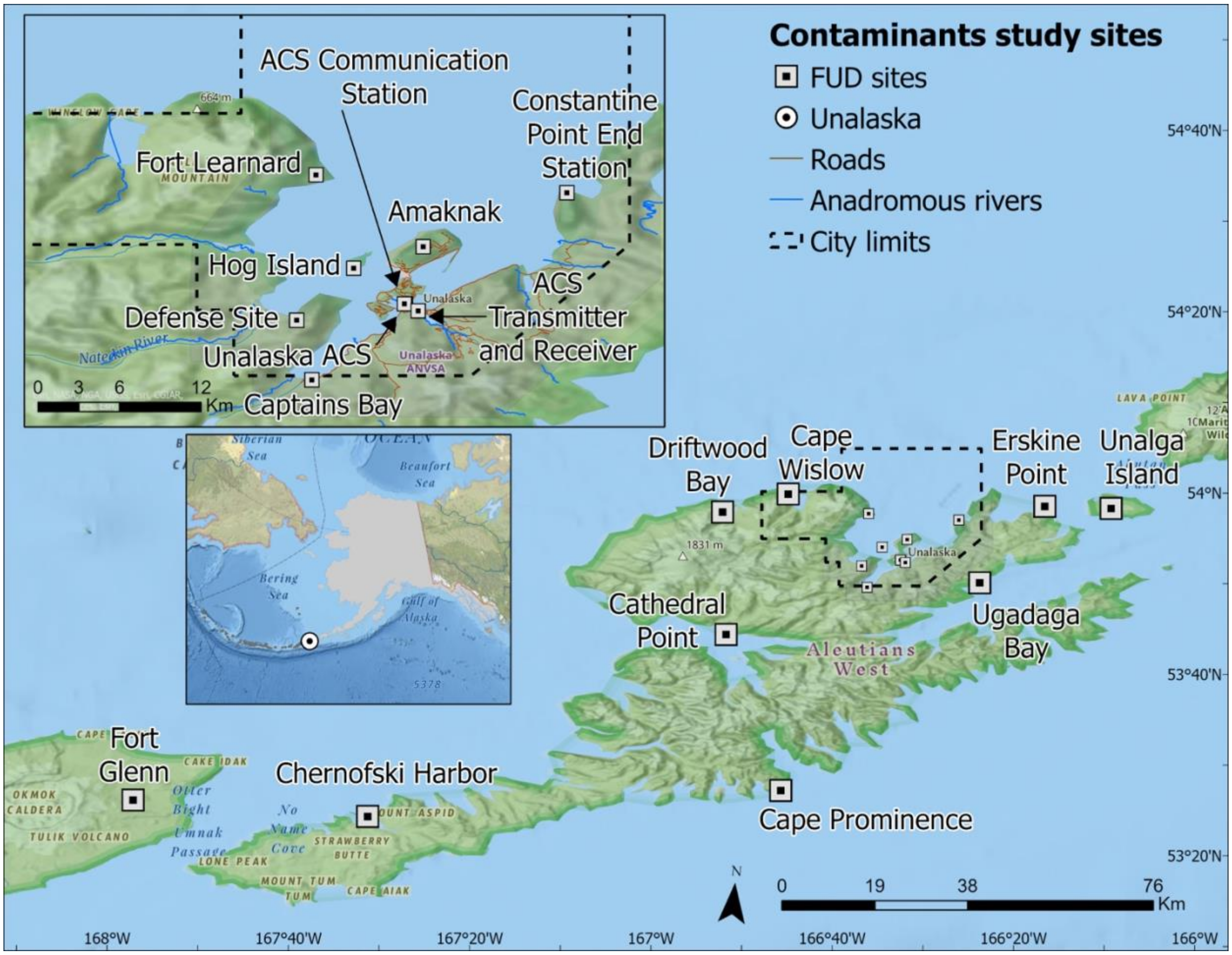

Figure 1. Map of formerly used defense (FUD) sites on Unalaska Island, Alaska. The dashed boundary indicates the City of Unalaska.

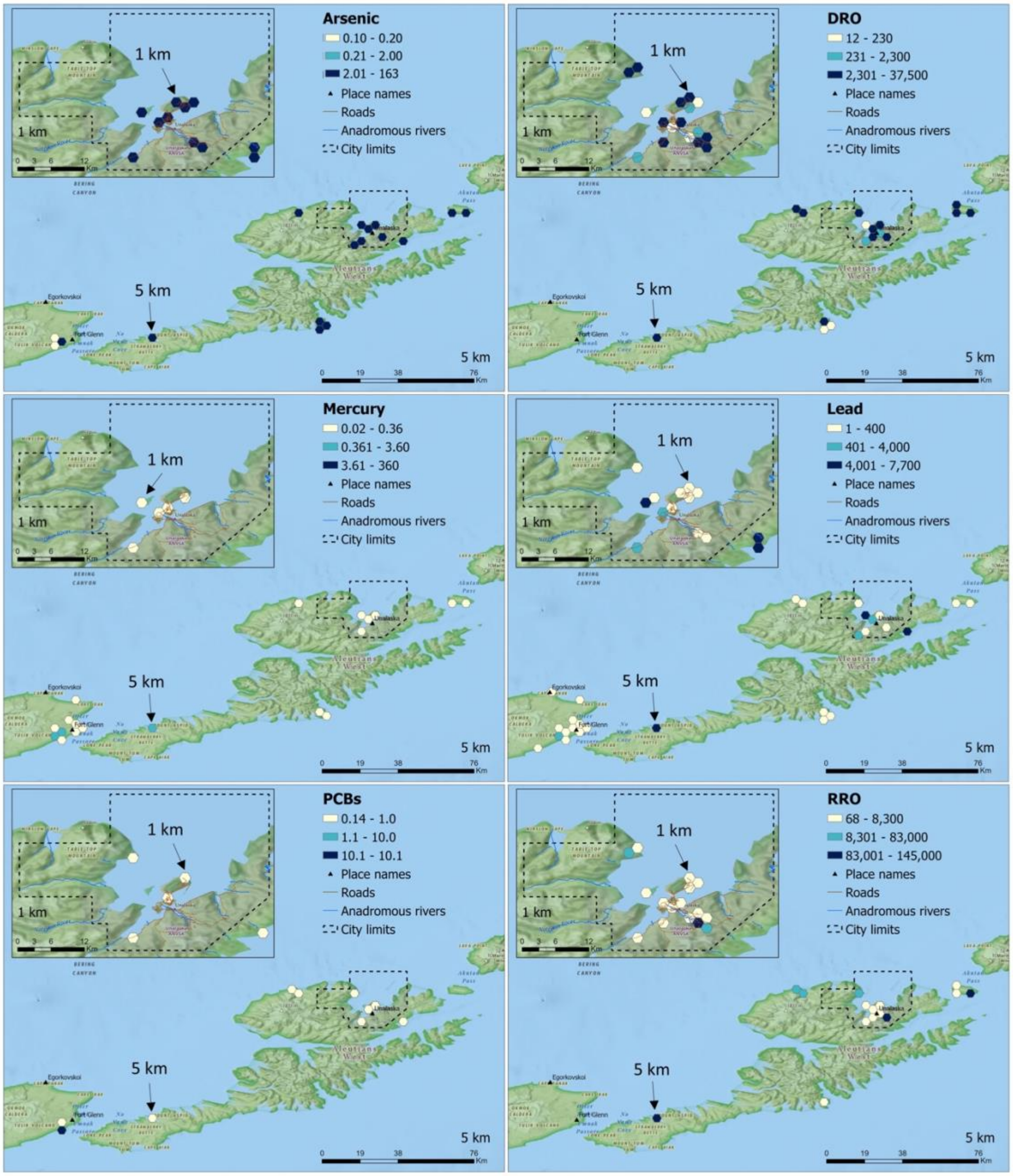

Figure 2. Distributions of six contaminants originating from formerly used defense (FUD) sites on Unalaska Island, Alaska and nearby islands. Contaminant concentrations were extracted from Army Corps of Engineers remediation reports and compared with the Alaska Department of Environmental Conservation (ADEC) Method Two cleanup levels for soils. Color denotes the contaminant exceedance level based on the maximum concentration within a hexagonal area; beige = not applicable or below ADEC cleanup level, medium blue = 0-10X higher than ADEC cleanup level, navy = over 10X higher than ADEC cleanup level. Hexagons in the main map of Unalaska denote 5km^2 areas and those in the inset map of the City of Unalaska denote 1km^2 areas.

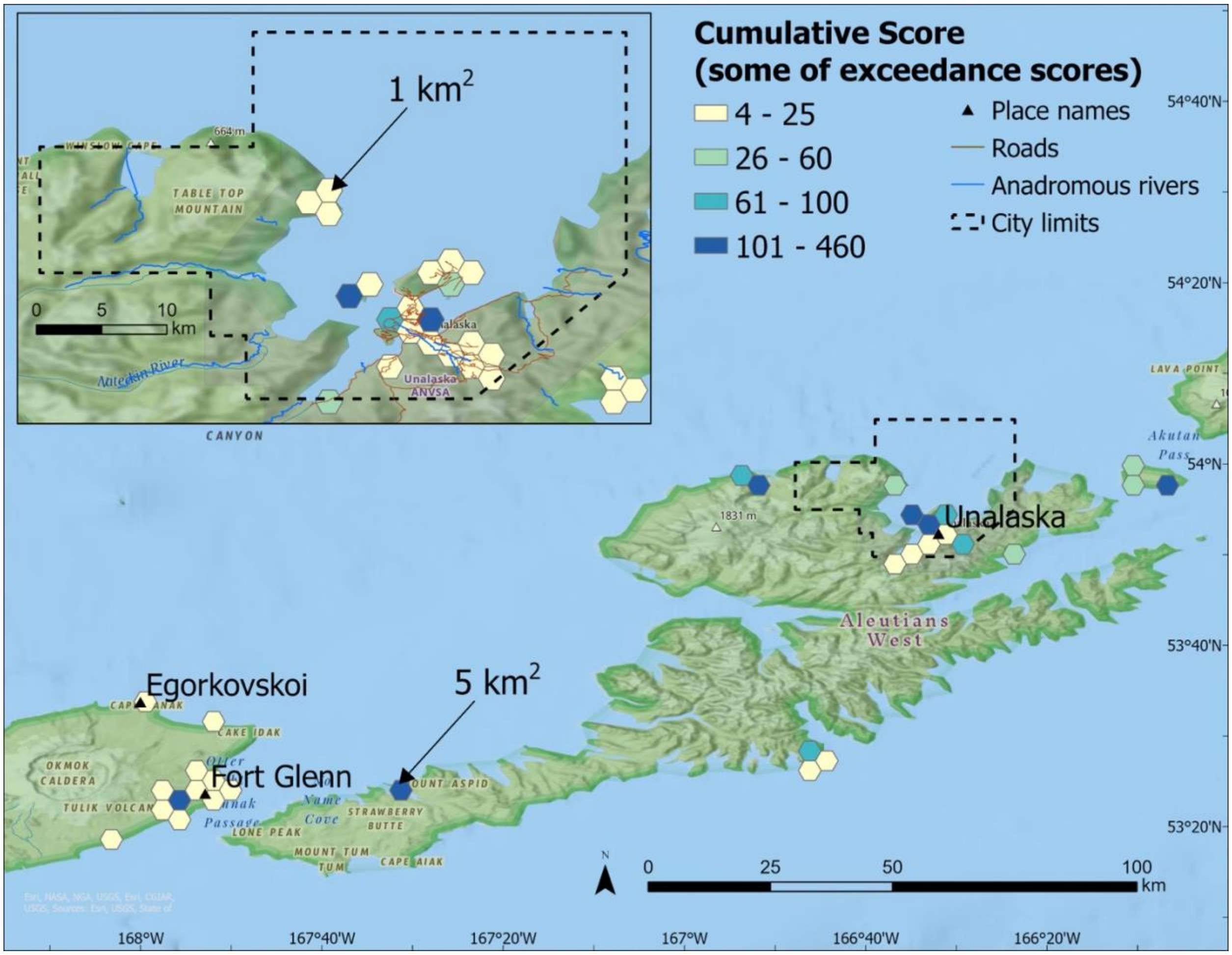

Figure 3. Cumulative score of contamination originating from formerly used defense (FUD) sites on Unalaska Island, Alaska and nearby islands. Contaminant concentrations were extracted from Army Corps of Engineers FUD site cleanup reports and compared with Alaska Department of Conservation (ADEC) Method Two cleanup criteria for soils. Exceedance scores were calculated on a scale from 0-3 for each soil sample, and then summed over all samples in each hexagonal area to generate the cumulative score. A sample received an exceedance score of 0 if the contaminant was not detected or analyzed, a score of 1 if the contaminant concentration was below the ADEC cleanup level, a score of 2 if the contaminant concentration was 0-10X higher than the ADEC cleanup level, and a score of 3 if the contaminant concentration was more than 10X higher than the ADEC cleanup level. Cumulative scores reflect the total exceedance score for each hexagonal area. Hexagons in the main map of Unalaska denote 5km^2 areas and those in the inset map of the City of Unalaska denote 1km^2 areas.

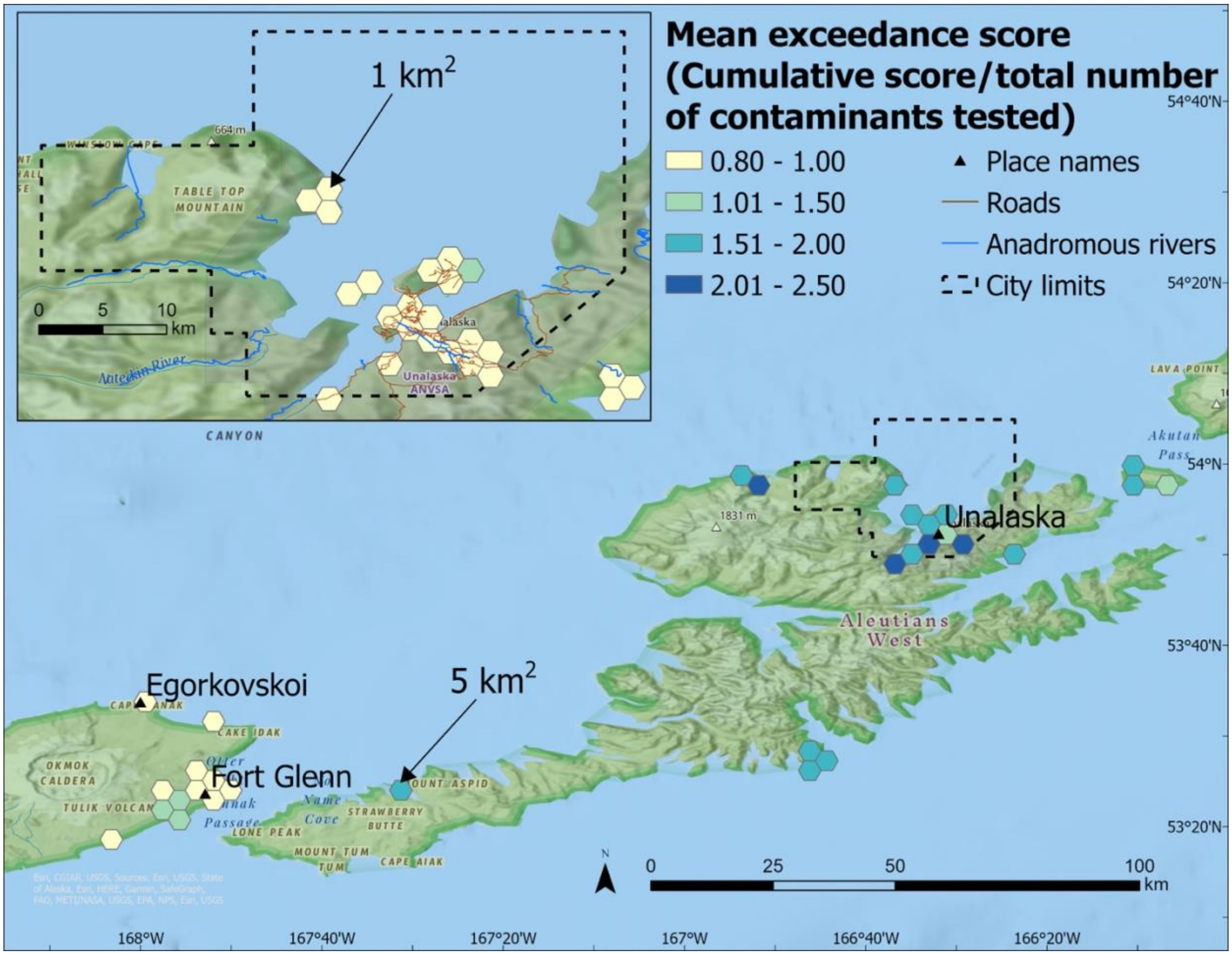

Figure 4. Mean exceedance score of contamination originating from formerly used defense (FUD) sites on Unalaska Island, Alaska and nearby islands. Contaminant concentrations were extracted from Army Corps of Engineers FUD site cleanup reports and compared with Alaska Department of Conservation (ADEC) Method Two cleanup criteria for soils. Mean exceedance score for each hexagonal area is indicated by color: beige = below state cleanup level, light green = low exceedance of the state cleanup levels, teal = medium exceedance of the state cleanup levels, navy = high exceedance of the state cleanup levels. Hexagons in the main map of Unalaska denote 5km^2 areas and those in the inset map of the City of Unalaska denote 1km^2 areas.

LIST OF SUPPLEMENTARY MATERIALS

Supplemental Table 1. List of additional formerly used defense (FUD) sites in the Aleutian (#1-41) and Bering Sea (#42-70) region.

	Location	FUDS Property Name	FUD Site ID
1	Aleutians East Borough	Caton Island	F10AK0029
2	Aleutians East Borough	Sanak Island Army Aircraft Warning System Station	F10AK0204
3	Tigalda Island, Aleutians East Borough	Tigalda Island	F10AK0376
4	Akutan Island, Aleutians East Borough	Akutan	F10AK0018
5	Akutan Island, Aleutians East Borough	Akutan Naval RS	F10AK1028
6	Aleutians West Census Area	Chuginadak Weather Station	F10AK1039
7	Atka Island, Aleutians West Census Area	Atka Cape Kudugnax	F10AK1063
8	Atka Island, Aleutians West Census Area	Atka Air Force Auxiliary Field	F10AK0851
9	Koniuji Island, Aleutians West Census Area	Koniuji Island	F10AK1078
10	Kasatochi Island, Aleutians West Census Area	Kasatochi Island	F10AK1070
11	Aleutians West Census Area	Fenimore Rock	F10AK1077
12	Tagalak Island, Aleutians West Census Area	Tagalak Island	F10AK1071
13	Chugul Island, Aleutians West Census Area	Chugul Island	F10AK1076
14	Ulak Island, Aleutians West Census Area	Ulak Island	F10AK1069
15	Great Sitkin Island, Aleutians West Census Area	Great Sitkin Island	F10AK0114
16	Little Tanaga Island, Aleutians West Census Area	Little Tanaga Island	F10AK1075
17	Kagalaska Island, Aleutians West Census Area	Kagalaska Island	F10AK1074
18	Adak Island, Aleutians West Census Area	Davis Air Force Base	F10AK0434
19	Adak Island, Aleutians West Census Area	Cape Yakak Radio Station	F10AK0040
20	Kanaga Island, Aleutians West Census Area	Kanaga Island	F10AK0130

21	Tanaga Island, Aleutians West Census Area	Tanaga Island	F10AK0228
22	Ilak Island, Aleutians West Census Area	Ilak Island	F10AK1072
23	Ogliuga Island, Aleutians West Census Area	Ogliuga Island	F10AK0180
24	Yuulax / Ulak Island, Aleutians West Census Area	Yuulax (Ulak) Island	F10AK1073
25	Semisopochnoi Island, Aleutians West Census Area	Semisopochnoi Island	F10AK0208
26	Amchitka Island, Aleutians West Census Area	Amchitka Air Force Auxiliary Field	F10AK0858
27	Kiska Island, Aleutians West Census Area	Kiska Island Garrison (B & L)	F10AK0137
28	Buldir Island, Aleutians West Census Area	Buldir Island	F10AK0026
29	Izki and Alaid Islands, Aleutians West Census Area	Nizki-Alaid Islands	F10AK0176
30	Agattu Island, Aleutians West Census Area	Agattu Island	F10AK0025
31	Attu Island, Aleutians West Census Area	Attu Island Military Sites	F10AK0055
32	AK Peninsula, Aleutians East Borough	Port Moller	F10AK0028
33	AK Peninsula, Aleutians East Borough	Sand Point Naval Auxiliary Air Facility	F10AK0386
34	Chernabura Island, Aleutians East Borough	Chernabura Island Navy	F10AK0430
35	AK Peninsula, Aleutians East Borough	King Cove Naval Facility	F10AK1034
36	Deer Island, Aleutians East Borough	Deer Island Aircraft Warning System Station	F10AK0314
37	AK Peninsula, Aleutians East Borough	Cold Bay Pavlof Unit	F10AK0466
38	AK Peninsula, Aleutians East Borough	Cold Bay - Fort Randall	F10AK0845
39	Amak Island, Aleutians East Borough	Amak Island	F10AK0310
40	AK Peninsula, Aleutians East Borough	Scotch Cap	F10AK0031
41	AK Peninsula, Aleutians East Borough	Cape Sarichef	F10AK0502
42	Bethel Census Area	Platinum Battalion	F10AK0187
43	St. Paul Island, Aleutians West Census Area	St Paul/St George	F10AK0861

44	St. Paul Island, Aleutians West Census Area	St. Paul Naval Radio Station	F10AK1042
45	Bethel Census Area	Nunivak Weather Station	F10AK1049
46	St. Matthew Island, Bethel Census Area	St. Matthew Loran Station	F10AK1036
47	St. Lawrence Island, Nome Census Area	Gambell	F10AK0696
48	St. Lawrence Island, Nome Census Area	Northeast Cape (St Lawrence Island)	F10AK0969
49	Nome Census Area	Fort St Michael	F10AK0307
50	Nome Census Area	Unalakleet Air Force Station	F10AK0036
51	Nome Census Area	Unalakleet NGS	F10AK0783
52	Nome Census Area	South River Recreation Site	F10AK0352
53	Nome Census Area	Unalakleet Recreation Fish Camp	F10AK0597
54	Nome Census Area	North River Radio Relay Site	F10AK0037
55	Nome Census Area	Unalakleet Recreation Annex	F10AK0596
56	King Island, Nome Census Area	King Island National Guard Site	F10AK0447
57	Nome Census Area	Ungalik River Airstrip Drum Site	F10AK1065
58	Nome Census Area	Koyuk Weather Station	F10AK1043
59	Nome Census Area	Moses Point Garrison	F10AK0162
60	Nome Census Area	Air Force Cache #09	F10AK0408
61	Nome Census Area	Cape Nome Alaska Communications System	F10AK0293
62	Nome Census Area	Nome Area Defense Region	F10AK0052
63	Nome Census Area	Anvil Mountain Aircraft Warning System Station	F10AK1018
64	Nome Census Area	Cape Rodney	F10AK0294
65	Nome Census Area	Sledge Island	F10AK0614
66	Nome Census Area	Feather River	F10AK1008
67	Nome Census Area	Port Clarence Loran Station	F10AK0843
68	Nome Census Area	Point Spencer	F10AK0812
69	Nome Census Area	Teller Supply	F10AK0231
70	Nome Census Area	Cape Prince Wales Aircraft Warning System Station	F10AK0074

CHAPTER 6: Conclusions

The Arctic is a repository of persistent organic pollutants (POPs) and serves as an important indicator region for assessing the effects of POP exposure. POPs accumulate in the Arctic from atmospheric deposition of globally distilled POPs transported from lower latitudes, and from local point sources of pollution. Located at the nexus of east and west, the Alaskan Arctic was strategically important during World War II and the Cold War. Thousands of defense sites were constructed across the region, many of which were built adjacent to rural communities. After the Cold War, most of these defense sites were abandoned, resulting in a legacy of pollution that continues to pose a health risk to local communities and wildlife living near these sites (Adams et al., 2019; Jordan-Ward et al., 2022; Scrudato et al., 2012; von Hippel et al., 2018). Disproportionate exposure to anthropogenic contaminants is a growing concern for Arctic Indigenous Peoples (AMAP, 2015; Miller et al., 2013), and may contribute to health disparities, including increased risk of cancer (Hoover et al., 2012).

The purpose of my dissertation research was to advance our understanding of the impacts of formerly used defense (FUD) site contamination on sentinel fishes and local communities in the Bering Sea region of Alaska. Specifically, my research investigated contaminant concentrations and health effects in sentinel fishes exposed to FUD site contamination on Sivuqaq (St. Lawrence Island), Alaska, and assessed contaminant distributions and FUD site remediation on Unalaska Island and nearby islands in the Aleutian Archipelago. My work integrated concepts and methodologies spanning multiple disciplines, including ecotoxicology, analytical chemistry, endocrinology, genomics, geographic information systems, and statistics. I utilized three native fish species that serve as environmental sentinels of POP exposure and health effects to test if FUD sites serve as point sources of POPs and if exposure to FUD site pollution causes adverse effects at multiple levels of biological organization.

My dissertation research demonstrated that Sivuqaq FUD sites remain a significant source of POP pollution and pose a health risk to residents that frequently consume fish caught near these sites. My research examined mercury (Hg) concentrations in Dolly Varden (*Salvelinus malma*) and polychlorinated biphenyl (PCB) concentrations in several fish species caught near Sivuqaq FUD sites. I found that Dolly Varden exposed to effluent from the Northeast Cape FUD site had significantly higher Hg and PCB concentrations than did fish from the reference site, indicating that this FUD site remains contaminated with PCB and Hg pollution. Similarly, I found elevated concentrations of PCBs in ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake, which was used as a disposal site for metallic debris and drums during military operations at the Gambell defense site (USACE, 2008; USATSDR, 2020). PCB congener profiles in both Dolly Varden and ninespine stickleback showed elevated concentrations of heavier PCBs. Because heavier PCBs are less volatile and do not readily undergo long-range transport, the presence of these congeners in Sivuqaq fishes suggest local sources of pollution in addition to accumulation via atmospheric deposition. Furthermore, PCB concentrations in both Dolly Varden and ninespine stickleback exposed to FUD site contamination exceeded the Environmental Protection Agency’s (EPA’s) guidelines for unlimited consumption (cancer risk for human consumption) of PCB-contaminated fish. Hg concentrations in Dolly Varden caught from contaminated sites also exceeded the EPA’s guideline for unlimited consumption of Hg-contaminated fish. Because Dolly Varden serve as a subsistence food source for Sivuqaq residents, and because both PCBs and Hg are known to elicit negative health effects in humans (Clarkson et al., 2003; Wada et al., 2009), my results indicate that the Northeast Cape FUD site poses a health risk to Sivuqaq residents who consume fish caught at Northeast Cape. Collectively, my research demonstrated that FUD sites on Sivuqaq remain significant point sources of POP pollution and that concentrations of PCBs and Hg in Dolly Varden pose a health risk to Sivuqaq residents.

My dissertation research expanded on previous work on Sivuqaq to elucidate the tissue-specific effects and pathologies associated with FUD site contaminant exposure. I examined effects at three levels of biological organization and found that both ninespine stickleback and Alaska blackfish (*Dallia pectoralis*) exposed to FUD site contamination at the Gambell and Northeast Cape FUD site, respectively, had differentially expressed genes related to ribosomal and metabolic functions. I also found that genes known to confer multixenobiotic resistance (MXR) to toxic substances (Jackson and Kennedy, 2017; Kurelec, 1992; Smital et al., 2000) were upregulated in both ninespine stickleback and Alaska blackfish exposed to FUD site pollution. While my endocrine results were inconclusive, I found histological evidence of suppressed gonadal maturation in ninespine stickleback collected from Troutman Lake and thyroid follicle hyperplasia in ninespine stickleback collected from Northeast Cape. Collectively, these results and results from previous research suggest that POP pollution originating at Sivuqaq FUD sites drives hypothyroid conditions and impairs reproductive pathways in local fishes.

My research on Unalaska Island and nearby islands aimed to identify hotspots of FUD site contamination and evaluate remediation of FUD sites in this region. I found that concentrations of toxic metals and POPs exceeded state cleanup thresholds set by the Alaska Department of Environmental Conservation at eight FUD sites that were remediated by the Army Corps of Engineers. I found that contamination was most severe at FUD sites located adjacent to the City of Unalaska. Furthermore, I found that cleanup at several FUD sites was determined complete without sufficient evidence of potential risks to residents who depend on subsistence foods. These results indicate that Unalaska FUD sites remain a health threat to residents and local wildlife and are consistent with my findings on Sivuqaq. The largest remediation effort at an Alaska FUD site to date occurred at the Northeast Cape FUD site on Sivuqaq, and in 2017, the Agency for Toxic Substances and Disease Registry determined that the Northeast Cape FUD site did not pose a health risk to Sivuqaq residents. My results contradict the findings reported by the ATSDR assessment and instead show that the Northeast Cape FUD site remains a significant source of POPs and a health risk for Sivuqaq residents. Similarly, the Army Corps of Engineers determined that cleanup at the Gambell FUD site was complete (USACE, 2008), yet I found that PCB congener profiles in ninespine stickleback collected from Troutman Lake indicate that the Gambell FUD site remains a point source of PCB pollution. Thus, my research demonstrates that current remediation processes at Alaska FUD sites are insufficient to protect human health and the environment.

Collectively, the results of my dissertation research exemplify the importance of Alaska FUD sites as point sources of pollution in the Bering Sea region and highlight the health risks that FUD sites pose to wildlife and rural communities subsisting near them. These problems are exacerbated by the rapid pace and magnitude of climate change in the Arctic, which is warming at more than twice the global average (AMAP, 2015). Given that the mobilization of POPs and the rates of global distillation and deposition in the Arctic are expected to accelerate, my findings suggest large-scale ecological consequences and increasing health risks for people inhabiting the Arctic. Future remediation projects at Alaska FUD sites would benefit from a more vigorous process of assessment, remediation, and monitoring to ensure the safety of human health and the environment. Future remediation projects should include more stringent cleanup thresholds at FUD sites located adjacent to subsistence communities. Moreover, all remediation activities should be accomplished with community input, supervision, and agreement.

REFERENCES

Adams, E.M., von Hippel, F.A., Hungate, B.A., Buck, C.L., 2019. Polychlorinated biphenyl (PCB) contamination of subsistence species on Unalaska Island in the Aleutian Archipelago. Heliyon 5, e02989.

AMAP, 2015. Summary for Policy-makers: Arctic Pollution Issues 2015. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. 12 pp.

Clarkson, T.W., Magos, L., Myers, G.J., 2003. The toxicology of mercury: current exposures and clinical manifestations. N Engl J Med 349, 1731-1737.

Hoover, E., Cook, K., Plain, R., Sanchez, K., Waghiyi, V., Miller, P., Dufault, R., Sislin, C., Carpenter, D.O., 2012. Indigenous peoples of North America: environmental exposures and reproductive justice. Environ Health Perspect 120, 1645.

Jackson, J.S., Kennedy, C.J., 2017. Regulation of hepatic abcb4 and cyp3a65 gene expression and multidrug/multixenobiotic resistance (MDR/MXR) functional activity in the model teleost, *Danio rerio* (zebrafish). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 200, 34-41.

Jordan-Ward, R., von Hippel, F.A., Zheng, G., Salamova, A., Dillon, D., Gologergen, J., Immingan, T., Dominguez, E., Miller, P., Carpenter, D., Postlethwait, J.H., Byrne, S., Buck, C.L., 2022. Elevated mercury and PCB concentrations in Dolly Varden (*Salvelinus malma*) collected near a formerly used defense site on Sivuqaq, Alaska. Sci Total Environ 826, 154067.

Kurelec, B., 1992. The multixenobiotic resistance mechanism in aquatic organisms. Crit Rev Toxicol 22, 23-43.

Miller, P.K., Waghiyi, V., Welfinger-Smith, G., Byrne, S.C., Kava, J., Gologergen, J., Eckstein, L., Scrudato, R., Chiarenzelli, J., Carpenter, D.O., Seguinot-Medina, S., 2013. Community-based participatory research projects and policy engagement to protect environmental health on St. Lawrence Island, Alaska. Int J Circumpolar Health 72, 21656.

Scrudato, R., Chiarenzelli, J., Miller, P.K., Alexander, J.C., Arnason, J., Zamzow, K., Zweifel, K., Gologergen, J., Kava, J., Waghiyi, V., Carpenter, D., 2012. Contaminants at arctic formerly used defense sites. J Local Glob Health Sci 2, 1-12.

Smital, T., Sauerborn, R., Pivčević, B., Krča, S., Kurelec, B., 2000. Interspecies differences in P-glycoprotein mediated activity of multixenobiotic resistance mechanism in several marine and freshwater invertebrates. Comp Biochem Physiol C Toxicol Endocrinol 126, 175-186.

USACE, 2008. Project Closeout Report: Hazardous, Toxic, and Radioactive Waste (HTRW) Gambell FUDS, St. Lawrence Island, Alaska. United States Army Corps of Engineers.

USATSDR, 2020. Evaluation of Environmental Exposures at the Gambell Formerly Used Defense Site Agency for Toxic Substances & Disease Registry.

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: health implications for indigenous residents. Environ Pollut 234, 279-287.

Wada, H., Cristol, D.A., McNabb, F.A., Hopkins, W.A., 2009. Suppressed adrenocortical responses and thyroid hormone levels in birds near a mercury-contaminated river. Environ Sci Technol 43, 6031-6038.