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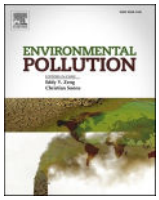

Differential gene expression and developmental pathologies associated with persistent organic pollutants in sentinel fish in Troutman Lake, Sivuqaq, Alaska☆

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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 vertebrate development. 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 stickleback exceeded the U.S. Environmental Protection Agency'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. We found that ninespine stickleback from Troutman Lake exhibited suppressed gonadal development compared to threespine stickleback (*Gasterosteus aculeatus*) studied elsewhere. Troutman Lake stickleback also displayed two distinct hepatic phenotypes, one with lipid accumulation and one with glycogen-type vacuolation. We compared the transcriptomic profiles of these liver phenotypes using RNA sequencing and found significant upregulation of genes involved in ribosomal and metabolic pathways in the lipid accumulation group. Additionally, stickleback displaying liver lipid accumulation had significantly fewer thyroid follicles than the vacuolated phenotype. 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.

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1. 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 pose 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 home to 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 explains 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).

Both legacy and emerging POPs contribute to arctic contamination. 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). 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 to 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), stickleback contaminant profiles 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 used for recreating and as a source of drinking water by Gambell residents (USATSDR, 2020).

The people of Sivuqaq have expressed concern about health risks posed by exposure to POPs (Miller et al., 2013; USATSDR, 2020). 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 certain 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. Additionally, 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 confirmed that exposure to POPs, whether they originated at hotspots of pollution such as FUD sites or via atmospheric deposition, could lead to the adverse health outcomes.

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 its 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 fish living downstream of Sivuqaq FUD sites originated primarily at those sites, indicating that these FUD sites remain point 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

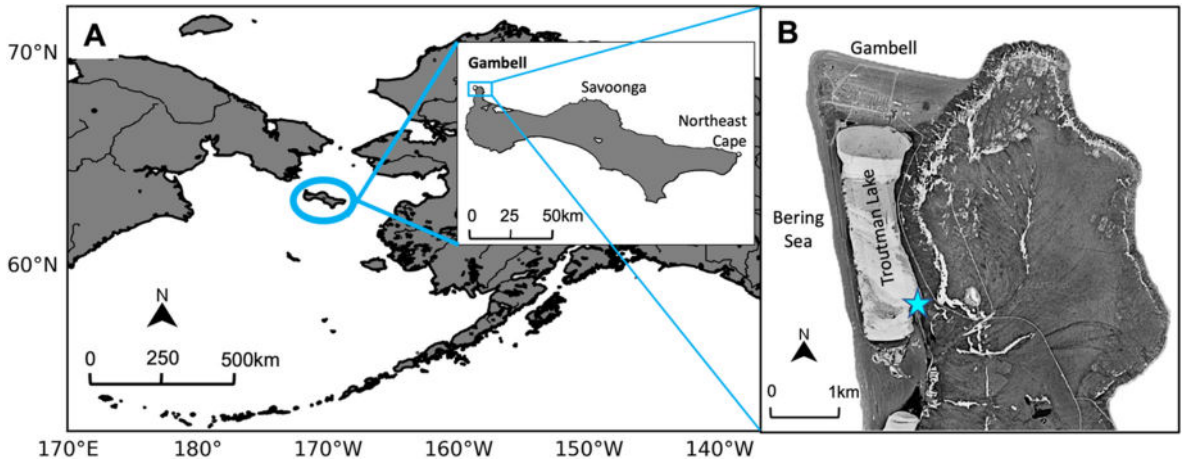

Fig. 1. Location of **A)** the Alaska Native Village of Gambell on Sivuqaq, Alaska and **B)** sampling location in Troutman Lake.

exposure and tissue-specific mechanisms of toxicity and pathologies.

At the request of the Sivuqaq community, we examined 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. 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. 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 wild 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.

2. Materials & methods

2.1. Fish collection

We collected adult ninespine stickleback from a single location in Troutman Lake during their breeding season 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 pH-neutral MS-222 fish anesthetic. We dissected fish in the field for both genetic and histological studies. For histology, we dissected half of the liver, the thyroid region, and one gonad from each fish and fixed these tissues in either Dietrich's solution (2015 samples) or 10% buffered formalin (2018 samples). For gene expression analysis, we used fish collected in 2018 and we placed half of the liver in PTFE vials containing RNAlater (the other half was used for histology). For endocrine analyses, whole fish samples were frozen at −20 °C at the field site and then transferred to −80 °C in the lab. We stored samples at −80 °C for genetic analyses and at room temperature for histological analyses. 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 University IACUC (# 17–003).

2.2. PCB analysis

Total PCB concentrations were analyzed in three composite ninespine stickleback samples, representing a total of 30 adult fish (10 fish per composite, ~0.5–2.5 g/fish). Samples were prepared and analyzed alongside samples published in von Hippel et al. (2018). PCB quantification was run by Axys Analytical Services Ltd. (Sidney, British Columbia, Canada) using EPA Method 1668 A/C (Axys Method MLA-010 Rev 11). 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.

2.3. 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). While each fish was sectioned for liver, thyroid, and gonadal histology, laboratory errors during sectioning limited comparisons of individual histomorphologies among the three organs. For example, only ten fish were analyzed for all three organs in 2015.

2.3.1. 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 sections were captured using a Leica DM6 B microscope (Leica Microsystems, Wetzlar, Germany) and Leica Application Suite (LASX) software at both 100× and 400×. We selected areas for analysis that appeared homogenous; we did not analyze sections that appeared torn or distorted because 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 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). Teleost liver morphology differs from that in mammals (Akiyoshi and Inoue, 2004). In stickleback, hepatocytes are organized into tubular cordons separated by sinusoids. We considered deviation from this pattern as disorganized. Although H&E staining does not confirm the composition of vacuoles or cellular inclusions, morphology can be used to infer composition (Wolf and Wheeler, 2018).

2.3.2. 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 100× to count the total number of thyroid follicles. At 400×, 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). 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. We also examined sections for the presence of lipids around thyroid follicles. Lipids were identified as white, unstained, and circular structures as described by Gardell et al. (2017).

2.3.3. 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).

2.4. RNA sequencing

Troutman Lake stickleback displayed two distinct liver phenotypes (Fig. 2). We employed RNA-seq analysis to examine gene expression differences associated with these liver phenotypes in ten male fish collected in 2018. We compared five males that exhibited liver lipid droplet accumulation (Fig. 2A) with five males that exhibited glycogen-type vacuolation of hepatocytes (Fig. 2B). 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.

2.5. Thyroid hormone quantification

We used different hormone extraction protocols for fish collected in 2015 (n = 39) and 2018 (n = 40). 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 12 × 75 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 12 × 75 mm borosilicate glass tube. After drying in a ThermoSavant SpeedVac Concentrator (model SDP121P; Thermo Fisher Scientific, Waltham, MA, USA) at 35 °C, tubes were stored at −80 °C. The day before samples were assayed, they were resuspended with 0.5 mL of assay buffer (*X*065, Arbor Assays), shaken for 1 h, and then stored at 4 °C overnight. Methanol extracts were diluted 1:4 for

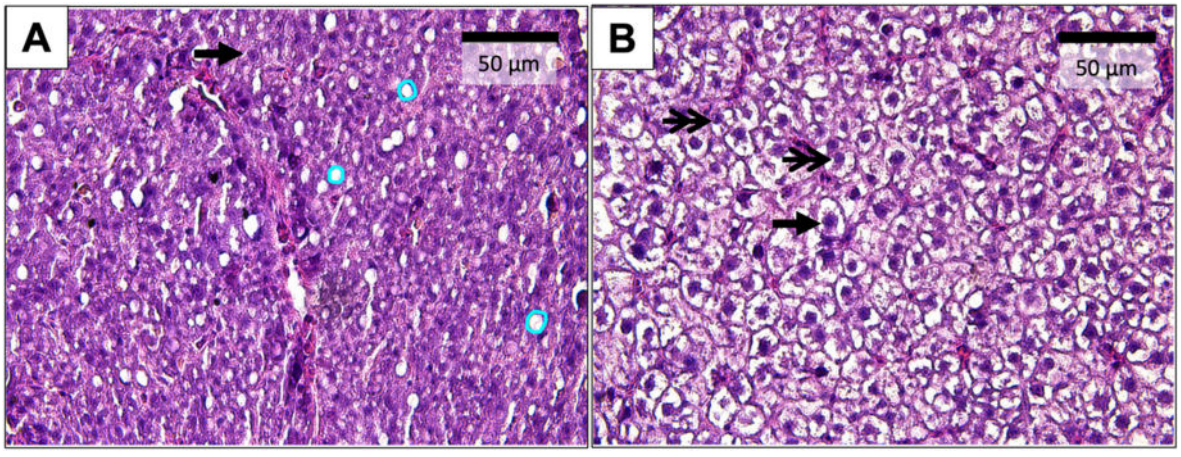

Fig. 2. Histological images of liver tissue from two male ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake in Gambell, Alaska. We found large variation in lipid accumulation and hepatocyte size across samples. Troutman Lake stickleback displayed two distinct liver phenotypes: (**A**) lipid droplet accumulation, and (**B**) increased glycogen-type hepatocyte vacuolation. Single arrows denote hypertrophied nuclei. Double arrows denote displaced nuclei. Blue circles denote lipid droplets. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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 are 45.4 pg/mL, 1.04 ng/mL, and 46.6 pg/mL, respectively.

2.6. 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. Additionally, stickleback are small fish and endocrine analyses required the entire fish sample, which prevented us from comparing hormone concentrations to histological and transcriptomic data within individuals.

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 RStudio, Inc.).

3. Results

3.1. 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% (Supplemental Table 2).

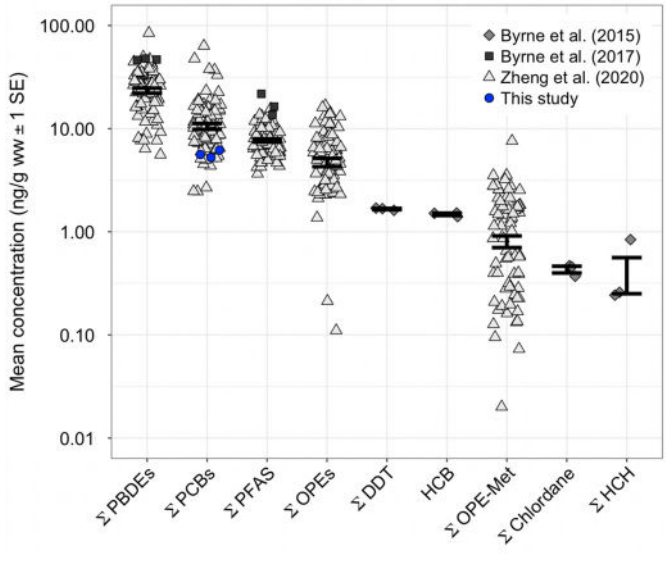

Fig. 3. Contaminant chemistry profiles in ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake on Sivuqaq, Alaska across four studies. Contaminant classes are arranged from highest to lowest concentration based on the mean value.

3.2. Liver histology

We analyzed the livers of 33 stickleback (18 males and 15 females) for histological abnormalities. 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 and those with glycogen-type vacuolation of hepatocytes (Fig. 2). Of the 33 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 samples had fewer fish exhibiting deformed cellular shapes and disorganized cordons than the 2015 samples, but more fish with lipid droplet accumulation (53% in 2018 vs 19% in 2015). Of the fish with lipid droplet accumulation, 67% were males.

3.3. Thyroid histology

In 2015, male and female stickleback did not differ in follicle area (mean ± SE: 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 (mean ± SE; 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 tissue surrounding thyroid follicles in both male and female stickleback (Fig. 5).

The mean number of thyroid follicles (±SE) 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, $p = 0.030$). No other thyroid histomorphology differed between liver phenotypes.

3.4. 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; Fig. 6). 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. 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).

3.5. Transcriptional profiling

We used RNA-seq to compare gene expression profiles between male stickleback exhibiting liver lipid droplet accumulation and those exhibiting increased hepatocyte vacuolation (Fig. 2). Principal

Table 1
Number (and percent) of ninespine stickleback exhibiting histological abnormalities associated with liver pathology.

Year	Sex	*Number of fish exhibiting each histological abnormality*				
		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%)

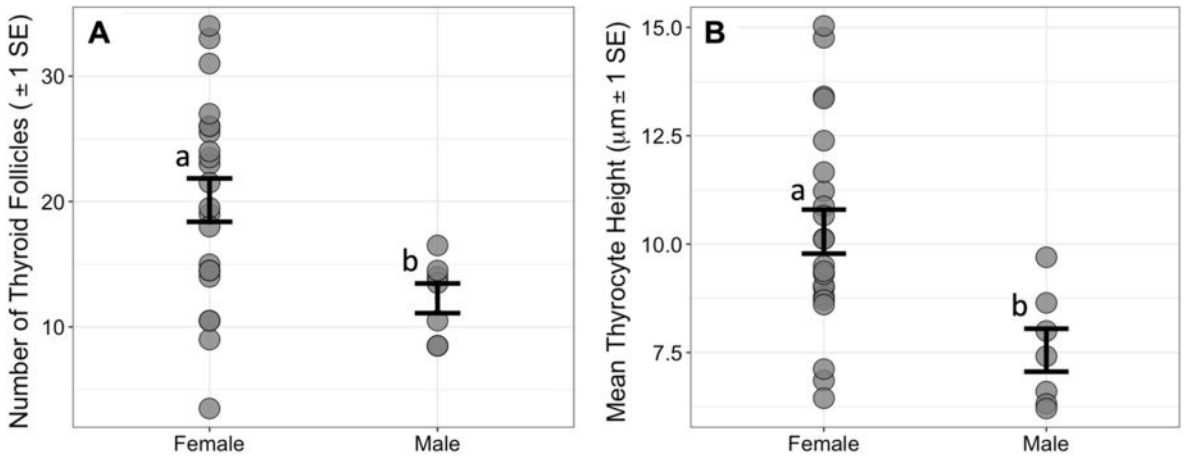

Fig. 4. Number of thyroid follicles (**A**) and mean thyrocyte height (**B**) by sex in ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake on Sivuqaq, Alaska in 2015. Female stickleback (n = 22) had significantly more thyroid follicles and longer thyrocytes than did male stickleback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

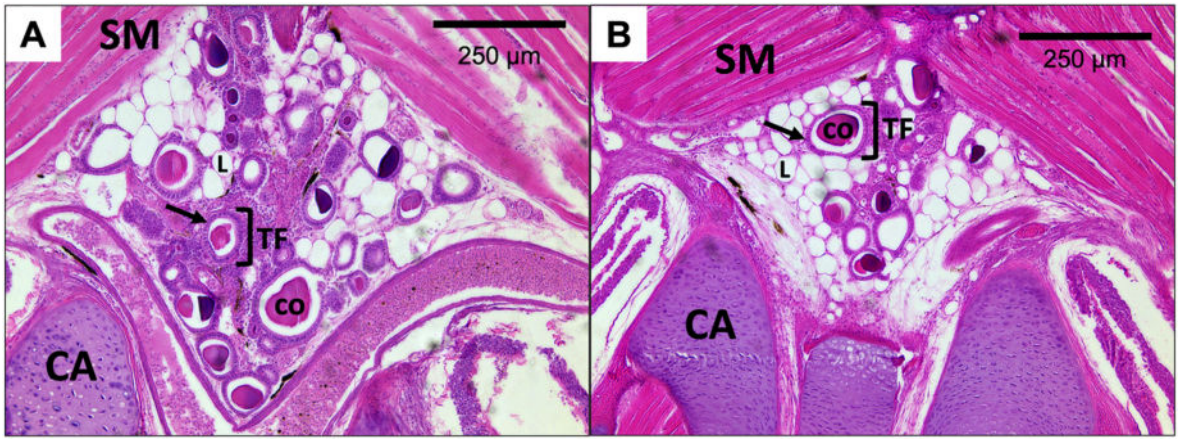

Fig. 5. Thyroid morphologies in a female (**A**) and a male (**B**) ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake on Sivuqaq, Alaska in 2015. These images show the disbursement of thyroid follicles at 10× magnification. Arrows indicate thyrocytes. CA = cartilage, CO = colloid, L = lipid, SM = skeletal muscle, TF = thyroid follicle.

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:0044,237, 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 in these fish were the endoplasmic reticulum chaperone complex pathway (GO:0034,663, 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:0042,254) 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).

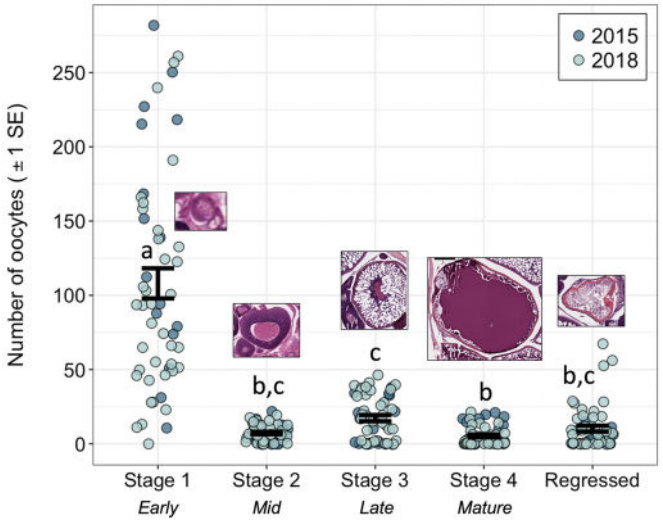

Fig. 6. Oocytes staged in 53 ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake on Sivuqaq, Alaska. Female stickleback had significantly more early-stage oocytes than mid-stage, late-stage, or mature oocytes ($p < 0.0001$ for all comparisons). Females also had significantly more late-stage oocytes than mature oocytes ($p = 0.005$).

We 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 (*pparα*) 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 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 3 A (*CYP3A*) gene (Saad et al., 2016) that helps metabolize 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.

3.6. Endocrinology

Ninespine stickleback exhibited high variability in thyroid hormone concentration (Fig. 7). 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 analyzed on 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, $p < 0.001$; 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. 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.

4. Discussion

4.1. 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. 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

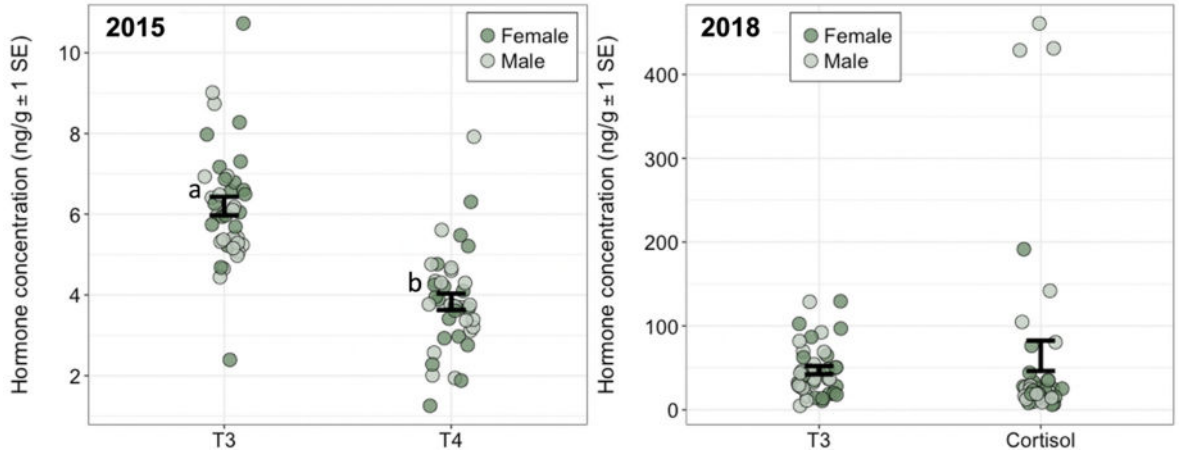

Fig. 7. Thyroid hormone concentrations in ninespine stickleback (*Pungitius pungitius*) collected from Troutman Lake on Sivuqaq, Alaska. T_3 concentrations were significantly higher than T_4 concentrations in 2015 (left graph; Mann-Whitney *U* test, $n = 39$, $p < 0.0001$). Three male stickleback had abnormally high concentrations of cortisol in 2018 ($n = 40$).

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 transport 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 from previous studies suggest that PCB contamination of Troutman Lake is due primarily to a local point source of pollution.

4.2. Liver histology

The molecular basis of the two liver phenotypes that we observed and the differences between sampling years 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. 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 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 OC pesticides and PCBs in trahira (*Hoplias malabaricus*). In the present study, vacuolated livers in stickleback 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.

4.3. 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 characterized 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. Additionally, both male and female stickleback displayed lipid accumulation phenotypes around thyroid follicles (Fig. 5) similar to those observed in threespine stickleback exposed to perchlorate (Gardell et al., 2017).

4.4. 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 at peak breeding season but found that Troutman Lake ninespine stickleback had far fewer mature oocytes and testes than expected.

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.

4.5. 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 with the lipid phenotype, supporting the hypothesis that these individuals 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 (Grun and Blumberg, 2006; Heindel et al., 2017; Maqbool et al., 2016; Yang et al., 2017). Obesogenic compounds disrupt endocrine function of oxidative stress and nuclear receptor pathways (Grun 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 *pparα* 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.

4.6. 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).

4.7. 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. 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; Grun 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 endocrine-disrupting compounds. 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.

5. Conclusions

The contaminant profiles of ninespine stickleback on Sivuqaq closely mirror those of residents' blood sera (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 exemplifies 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 nearly four times the global average (Rantanen et al., 2022). 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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All authors contributed to final draft preparation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122765.

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