INVESTIGATING THE USE OF ATLANTIC SILVERSIDE (*MENIDIA MENIDIA*) AS A BIOMONITOR FOR POLYCYCLIC AROMATIC HYDROCARBON (PAH) POLLUTION IN THE SAINT JOHN HARBOUR

by

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science with Honours in Biology

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THE UNIVERSITY OF NEW BRUNSWICK

SAINT JOHN

April, 2022

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ABSTRACT

The Saint John Harbour (SJH) in Saint John, New Brunswick, is exposed to polycyclic aromatic hydrocarbons (PAHs) from several sources, and these contaminants can have significant adverse effects on wildlife. Biomonitors are species that accumulate and respond to contaminants, and can provide an indication of pollutant bioavailability to wildlife. The objective of this project was to determine whether the Atlantic silverside (*Menidia menidia*) is a good biomonitor for PAH pollution in the SJH. Sediment and fish were collected from four sites in the SJH, and PAH concentrations in sediment and fish tissue, fish condition indices, and hepatic ethoxyresorufin-O-deethylase (EROD) activity were measured. It was found that PAHs did not accumulate in detectable levels in fish tissue. Contrary to our predictions, we found that fish condition indices and hepatic EROD were not related to sediment PAH levels. Fish collected at Hazen Creek exhibited higher hepatic EROD activity than fish collected from the three other sites, despite Hazen Creek having the lowest sediment PAH concentrations. The high levels of EROD activity observed at this site could have been due to physiochemical factors, biological factors, or the presence of other contaminants.

DEDICATION

I would like to dedicate this to my family and friends, who have supported me throughout every step of this process.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Anne Crémazy, and the members of the Crémazy lab for their guidance and support throughout this project. I would also like to thank Angella Mercer and Allyson Boss for their long days and late nights spent in the lab. Finally, thank you to ACAP Saint John for making this project possible.

STATEMENT OF RESEARCH CONTRIBUTION

Sediment was collected by ACAP Saint John and analyzed by the Research and Productivity Council in Fredericton between 2018 and 2021. Fish were collected by ACAP Saint John, the Crémazy lab, and I in September 2021. I conducted the fish processing, tissue subsampling, tissue homogenization and centrifugation, BCA assay, EROD assay, and data and statistical analyses with the help of Angella Mercer and members of the Crémazy lab between September 2021 and February 2022. Fluorescence readings for the EROD assay were conducted by Allyson Boss in January 2022.

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List of Symbols, Nomenclature or Abbreviations

7-ER	7-Ethoxyresorufin
ACAP	Atlantic Coastal Action Program
AhR	Aryl hydrocarbon receptor
ANOVA	Analysis of variance
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
CCME	Canadian Council of Ministers of the Environment
CYP	Cytochrome P450
DMSO	Dimethyl sulfoxide
EROD	Ethoxyresorufin-O-deethylase
HC	Hazen Creek
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGB	HEPES grinding buffer
IH	Inner Harbour
ISQG	Interim sediment quality guideline
K	Fulton's condition factor
KCl	Potassium chloride
KOH	Potassium hydroxide
LSI	Liver somatic index
MS-222	Tricaine mesylate
NADPH	Nicotinamide adenine dinucleotide phosphate
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzodioxins
PCDF	Polychlorinated dibenzofurans
RPC	Research and Productivity Council
SC	Spar Cove
SJH	Saint John Harbour
TCB	Tin Can Beach
TOC	Total organic carbon

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that are composed of two or more fused aromatic rings (Menzie et al., 1992). They are ubiquitous in the environment and are formed primarily by the incomplete combustion of organic material, such as oil (Maliszewska-Kordybach, 1999). While PAHs can arise from natural events such as forest fires and volcanic eruptions, anthropogenic activities, such as petroleum processing, are the primary sources of PAHs in the environment (Maliszewska-Kordybach, 1999). The aquatic environment is particularly at risk of PAH pollution from oil spills, polluted soil runoff, deposition of atmospheric particles, and industrial and municipal effluents (Wick et al., 2011). In the aquatic environment, the sediment compartment is the ultimate PAH sink (Wolska et al., 2012), making benthic organisms and benthic-feeding organisms particularly at risk of exposure via the water and their diet (Meador et al., 1995). Due to their hydrophobic nature, PAHs have been found to accumulate in biological tissues (Meador et al., 1995), where they can cause adverse effects (Maliszewska-Kordybach, 1999). Their main toxic pathway is via biotransformation into toxic metabolites, which can then covalently bind to DNA molecules to form DNA adducts (Varanasi & Stein, 1991), increasing the risk of mutations and cancer (Tuvikene, 1995). In fish, PAH exposure can negatively affect growth and development (Alves et al., 2017; Gundersen et al., 1996), cardiac function (Edmunds et al., 2015; Incardona et al., 2004), immune function (Reynaud et al., 2004), and reproduction (Monteiro et al., 2000).

The Saint John Harbour (SJH) is exposed to PAHs from several sources, including an oil refinery, sea traffic, and creosote. ACAP Saint John, a local non-government organization, has been monitoring these contaminants as part of the Coastal Environmental Baseline Program,

which is a Fisheries and Ocean Canada initiative focused on industrial ports across Canada. PAH levels in sediments of the SJH have been measured since 2018, and ACAP has found some levels to be above the Canadian disposal at sea limit (2.5 mg/kg dry weight) in various sites (Reinhart, 2021). Notably, ACAP found mean sediment PAH levels to be above this limit at Spar Cove (21.9 ± 35.3 mg/kg), Tin Can Beach (3.85 ± 2.39 mg/kg), and Marsh Creek (59.8 ± 66.7 mg/kg) (Reinhart, 2021). While knowledge of sediment or water PAH levels is valuable, it provides limited information on the potential for bioaccumulation and toxicity in local wildlife. To provide an indication of contaminant bioavailability and effects, environmental monitoring programs often measure biological accumulation and markers of exposure and toxicity in biomonitors (Schwacke et al., 2013).

Biomonitors are species that accumulate contaminants without significant adverse effects, and can be used to assess environmental risks of contaminants (Beeby, 2001). A good biomonitoring species is abundant, easy to collect, has a small home range, and has a measurable and consistent response to the contaminant of interest (Gerhardt, 2002). Atlantic silverside (*Menidia menidia*) possess several of these characteristics, as described by Doyle et al. (2011) in an earlier SJH pollution study. This small annual fish (mean weight = 2.59 ± 0.11 g, mean length = 8.17 ± 0.08 cm) is abundant, easy to catch, and have a wide geographic distribution that extends from the Gulf of St. Lawrence to the northern coast of Florida (Doyle et al., 2011). Atlantic silverside migrate from continental shelf waters during the winter (November–April) to avoid the low temperatures of shallow inshore waters (Schultz et al., 1998) and return to the shore zone in the spring to spawn (Doyle et al., 2011) once during their year-long lifespan (Conover et al., 2005). During their onshore period (May–October), they remain locally resident, maintaining a relatively small range (Doyle et al., 2011). Atlantic silverside consume mainly

benthic and epibenthic organisms such as crustaceans (Doyle et al., 2011), so they may be expected to accumulate sediment PAHs through their diet. Finally, fish have been shown to be more responsive than invertebrates to the presence of PAHs in the aquatic environment, due to their sensitive aryl hydrocarbon receptors (AhR; (Hahn, 2001). For all of these reasons, Atlantic silverside may have the potential to become a biomonitor for PAH pollution in the SJH.

When assessing exposure of aquatic organisms to PAHs, various approaches have historically been used, such as PAH bioaccumulation in tissues and the use of biomarkers (McDonald et al., 1995). Fish have the capacity to metabolize PAHs, so tissue PAH concentrations may be low despite continuous environmental exposure (Rodrigues et al., 2013). Biomarkers are measurable changes at biochemical, cellular, or physiological levels following exposure to contaminants (Rodrigues et al., 2013), and are generally better suited to assess PAH exposure and effects in fish than tissue concentrations (Whyte et al., 2000). A popular biomarker of PAH exposure is the activity of ethoxyresorufin-O-deethylase (EROD) in fish livers. EROD is a member of the CYP1A subfamily of the cytochrome P450 (CYP) family of xenobiotic-metabolizing enzymes (Whyte et al., 2000). CYPs oxidize PAHs into water-soluble intermediates that are more easily excreted (Shimada & Fujii-Kuriyama, 2004), and they are induced following the binding of PAHs to the AhR (Whyte et al., 2000). The EROD assay monitors EROD induction and is a widely used biomarker of exposure to PAHs (Petrulis et al., 2001).

The objective of this project was to determine whether Atlantic silverside are a good biomonitoring species to assess PAH pollution in the SJH. To do so, we analyzed sediment and fish that were collected from four sites across the SJH. Sites were chosen to represent a wide range of PAH contamination levels. We measured PAH concentrations in sediment and fish

tissue, fish condition indices (Fulton's condition factor condition (K) and liver somatic index (LSI)), as well as EROD activity in fish livers. Because Atlantic silverside are abundant, easy to collect, have a small home range, and are responsive to PAHs, we hypothesized that Atlantic silverside are good biomonitors to evaluate PAH pollution in the SJH. Thus, we predicted that PAH tissue accumulation and hepatic EROD activity would increase with increasing sediment PAH concentration in the SJH.

2. Materials and Methods

2.1 Fish and sediment collection

Atlantic silverside (mean weight = 2.59 ± 0.11 g, mean length = 8.17 ± 0.08 cm) were collected over the span of two weeks in September 2021 at four locations across the SJH: Hazen Creek (HC), Inner Harbour (IH), Spar Cove (SC), and Tin Can Beach (TCB; Figure 1). At each location, 40 fish were collected using a beach seine. The seine was pulled parallel to the shore at low tide for three minutes. The collected fish were transferred to a cooler containing water collected at the site that was aerated with a bubbler. The fish were then immediately transported back to the University of New Brunswick Saint John where they were euthanized using 500 mg/L MS-222 (Syndel Laboratories Ltd.) in a 1.5 L container of seawater. To expedite fish processing, five fish were euthanized at a time. Immediately after being euthanized, the fish were quickly blotted dry, weighed, and measured for total length. The livers of 10 fish from each site were quickly dissected, weighed, placed in cryovials, and flash frozen in liquid nitrogen. These livers were kept in a -80°C freezer until EROD analysis could be performed. The remaining 30 fish collected from each site were stored in a -20°C freezer until they could be transported for tissue PAH analysis.

Sediment was collected by ACAP Saint John a total of 13 times at the four sites used in this study between March and November of 2018 to 2021 (2018 n = 1 / site, 2019 and 2020 n = 3 / site, 2021 n = 6 / site) (Reinhart, 2021). At each site, a plastic corer was used to collect the top 5 cm of sediment at low tide. Sediment samples were then placed in glass jars and frozen at -4°C until they could be transported for PAH analysis.

Figure 1: Map of sampling locations in the SJH.

2.2 Determination of PAH concentrations

Fish were randomly selected for tissue PAH analysis, and eight composite samples containing 3 or 4 fish were prepared for each site. All PAH analyses (sediment and fish tissue) were done by the Research Productivity Council (RPC) lab in Fredericton, New Brunswick. Samples were assessed for naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene/triphenylene, benzo(b+j)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, benzo(g,h,i)perylene, and dibenz(a,h)anthracene. Both the sediment and fish tissue samples were analyzed after extraction using gas chromatography mass spectrometry following

US EPA methods 8270C, with a detection limit of 0.05 mg/kg (wet weight) for fish tissue samples, and 0.01 mg/kg (dry weight) for sediment samples. Samples were also analyzed for percent moisture, and sediment PAH levels were reported on a dry weight basis. For quality control, the surrogates 2-fluorobiphenyl and p-terphenyl-d14 were added to each sample and blank.

2.3 Hepatic EROD assay

2.3.1 Liver homogenization

The targeted liver weight for use in the EROD assay was ~25 mg wet weight. Liver samples were transferred from the -80ºC freezer to a metal mortar containing liquid nitrogen and quickly grinded. Then, ~25 mg (6.60-62.0 mg) of ground liver tissue was transferred to a bullet tube. These samples were then kept on ice at all times. A repeater pipette was used to add cold HEPES grinding buffer (HGB) to the bullet tubes. This solution was prepared by adding 2.79 g of KCl (Fisher Chemical) and 1.30 g of HEPES (Fisher Chemical) to 250 mL of milliQ water in a clean 500 mL glass bottle. The pH of the solution was adjusted to ~7.5 using 50% KOH while the solution was cold. The volume of HGB added to the liver samples depended on their weight: a total of 500 µL, 750 µL, 1000 µL and 1250 µL of HGB were added to livers weighing 1-29 mg, 30-39 mg, 40-49 mg, and 50-59 mg wet weight, respectively. The first portion of HGB (250 µL) of HGB was added to homogenize the liver tissue with a motor-driven tissue grinder, then the rest of the HGB was added to the homogenized sample. The bullet tubes were briefly vortexed, then centrifuged at 2ºC for 20 minutes at 9000 g. A micropipette was then used to collect the supernatants and dispense into cryovials kept on ice. A micropipette was used to transfer 33 µL of supernatant to a labelled bullet tube, which was then diluted with 66 µL of milliQ water for

immediate use in a protein assay. The remaining supernatants were immediately placed in a -80°C freezer for use in the EROD assay approximately one week later.

2.3.2 Determination of protein concentration

A protein assay was conducted on the diluted liver homogenates using the bicinchoninic acid (BCA) method, immediately after centrifugation. An ampoule containing 2000 µg/mL bovine serum albumin (BSA) standard (Thermo Scientific) was opened and dispensed into bullet tubes with milliQ water to prepare protein standards at concentrations of 0 µg/mL, 25 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, 750 µg/mL, 1000 µg/mL, and 1500 µg/mL. Precisely 25 µL of each standard and of each homogenate (kept on ice at all times) were dispensed in triplicate into individual wells of a clear polystyrene 96-well plate. Then, 200 µL of working reagent was added to each well. This working reagent was prepared by mixing 18 mL of BCA reagent A (sodium carbonate, sodium bicarbonate, BCA, and sodium tartrate in 0.1 M sodium hydroxide; Thermo Scientific) with 0.36 mL of BCA reagent B (4% cupric sulfate; Thermo Scientific) in a clean glass beaker. The plate was covered and incubated at 37°C for 30 minutes, then absorbance was measured at 562 nm using a spectrophotometer (BioTek Epoch 2 Microplate Spectrophotometer, EPOCH2NS).

2.3.3 Determination of EROD activity

EROD activity (pmol/min/mg) was measured as the rate of resorufin production (measured by a change in fluorescence over time) per mg of total liver protein (measured with the BCA assay). For standard preparation, a resorufin stock solution A was first prepared by adding 50 mg of resorufin (Biotium) to 10 mL of DMSO (VWR Chemicals) in a 20 mL glass scintillation vial. A resorufin stock solution B was then prepared on the day of use by diluting 25

μL of resorufin stock solution A in 4.795 mL of DMSO in a separate 20 mL glass scintillation vial. The latter solution was then wrapped in tin foil and kept at room temperature until it was used in the EROD assay. Resorufin stock solution B was dispensed into 1.5 mL bullet tubes with milliQ water to prepare resorufin standards at concentrations of 0 μg/mL, 0.3125 μg/mL, 0.625 μg/mL, 1.25 μg/mL, 2.5 μg/mL, and 5 μg/mL.

On the day of analysis, homogenates were thawed on ice, then briefly vortexed. A micropipette was used to add 50 μL of vortexed sample in triplicate to the appropriate wells of a black 96-well polystyrene plate with a clear bottom (Thermo Scientific). A micropipette was then used to add 10 μL of vortexed resorufin standards to the appropriate wells in quadruple. Using a repeater pipette, 40 μL of cold HEPES Buffer (6.5 g of HEPES in 250 mL milliQ water, adjusted to pH~7.5 using 50% KOH) was added to the wells. Then 50 μL of 7-ethoxyresorufin/HEPES buffer (7-ER/HB) (0.22mg/mL 7-ER (Sigma-Aldrich), added to HB for final concentration of ~0.0024 mg/ml) was added to the wells. The plate was covered with aluminum foil and incubated at ambient temperature for ten minutes. Three minutes prior to the end of the incubation period, 20 mg/mL NADPH solution was prepared by adding 1250 μL of milliQ water to 25 mg of NADPH (EDM Millipore Corp.) in an amber vial. Then, 10 μL of the solution was quickly added to all the wells using a repeater pipette. The plate was immediately read at 530 nm excitation and 590 nm emission, with a read every minute for 13 minutes and a shake 2 seconds prior to each reading, using a spectrofluorometer (Gemini EM dual-scanning microplate spectrofluorometer, Molecular Devices).

2.4 Data analysis

Condition factor (K = 100 x body weight/body length^3) and liver somatic index (LSI = 100 x liver weight/body weight) were calculated for each fish.

To calculate EROD activity (pmol/min/mg), the mean fluorescence (in RFU) of each resorufin standard over 13 minutes was calculated. The mean fluorescence of the standards (in RFU) was plotted against the quantity of resorufin in each of the standards (in pmol), and the slope of this standard curve (S_{cal}, in RFU/pmol) was obtained by linear regression. The change in fluorescence for each sample (in RFU) over 13 minutes was plotted against time (in min), and the slope of this sample curve (S_{sample}, in RFUs/min) was obtained by linear regression. The slopes of the triplicate measurements were averaged for each sample. EROD activity in each sample well (measured as the rate of resorufin production, in pmol/min), was then calculated by dividing the mean slope of each sample by the standard curve slope (EROD activity = S_{sample}/S_{cal}). The final EROD activity in each fish liver (in pmol/min/mg) was then obtained by normalizing this EROD activity level (pmol/min) by the protein content (in mg) in each sample well. This latter content was obtained by multiplying the protein concentration obtained for the same sample in the BCA assay (in mg/ml) by the sample well volume (i.e., 0.05 mL).

Statistical analyses were completed using GraphPad Prism (version 9.2.0 (283)). Outliers were removed using the ROUT (robust regression and outlier removal) method (Motulsky & Brown, 2006), and a one-way analysis of variance (ANOVA) was used to determine if there were significant differences ($p < 0.05$) in K, LSI, sediment PAH concentrations, and hepatic EROD activities between SJH sites. If there were significant differences between sites, Tukey's post-hoc tests were used to determine which sites differed from each other.

3. Results

3.1 PAH concentrations

Average total PAH concentration in sediments differed across sites in the SJH (Figure 2). The average total PAH sediment concentrations (mg/kg ± SE) were highest in Spar Cove (4.86 ± 1.34 mg/kg) and Tin Can Beach (4.51 ± 0.664 mg/kg), followed by Inner Harbour (0.591 ± 0.143 mg/kg), then Hazen Creek (0.0592 ± 0.0213 mg/kg). There was no significant difference in mean sediment PAH concentration between Hazen Creek and Inner Harbour, or between Spar Cove and Tin Can Beach. The most abundant PAH across all samples was fluoranthene (26.0 ± 26.6%), followed by phenanthrene (15.1 ± 12.1%), benzo[b,j]fluoranthene (9.22 ± 4.84%), benz[a]anthracene (8.80 ± 5.89%), chrysene + triphenylene (7.47 ± 5.05%), benzo[a]pyrene (7.27 ± 3.94%), anthracene (4.30 ± 3.75%), benzo[e]pyrene (4.28 ± 2.38%), indeno[1,2,3-cd]pyrene (4.24 ± 2.77%), benzo(g,h,i)perylene-D12 (3.82 ± 2.35%), benzo[k]fluoranthene (3.46 ± 2.28%), fluorene (1.84 ± 2.48%), acenaphthene (1.31 ± 2.60%), dibenz[a,h]anthracene (1.01 ± 0.83%), naphthalene (1.00 ± 1.22%), acenaphthylene (0.55 ± 0.43%), and pyrene (0.38 ± 20.5%).

Despite the high sediment PAH levels observed in certain sites, total PAH concentrations in fish were below the detection limit (0.05 mg/kg wet weight) at all sites, for all measured fish (10 fish/site).

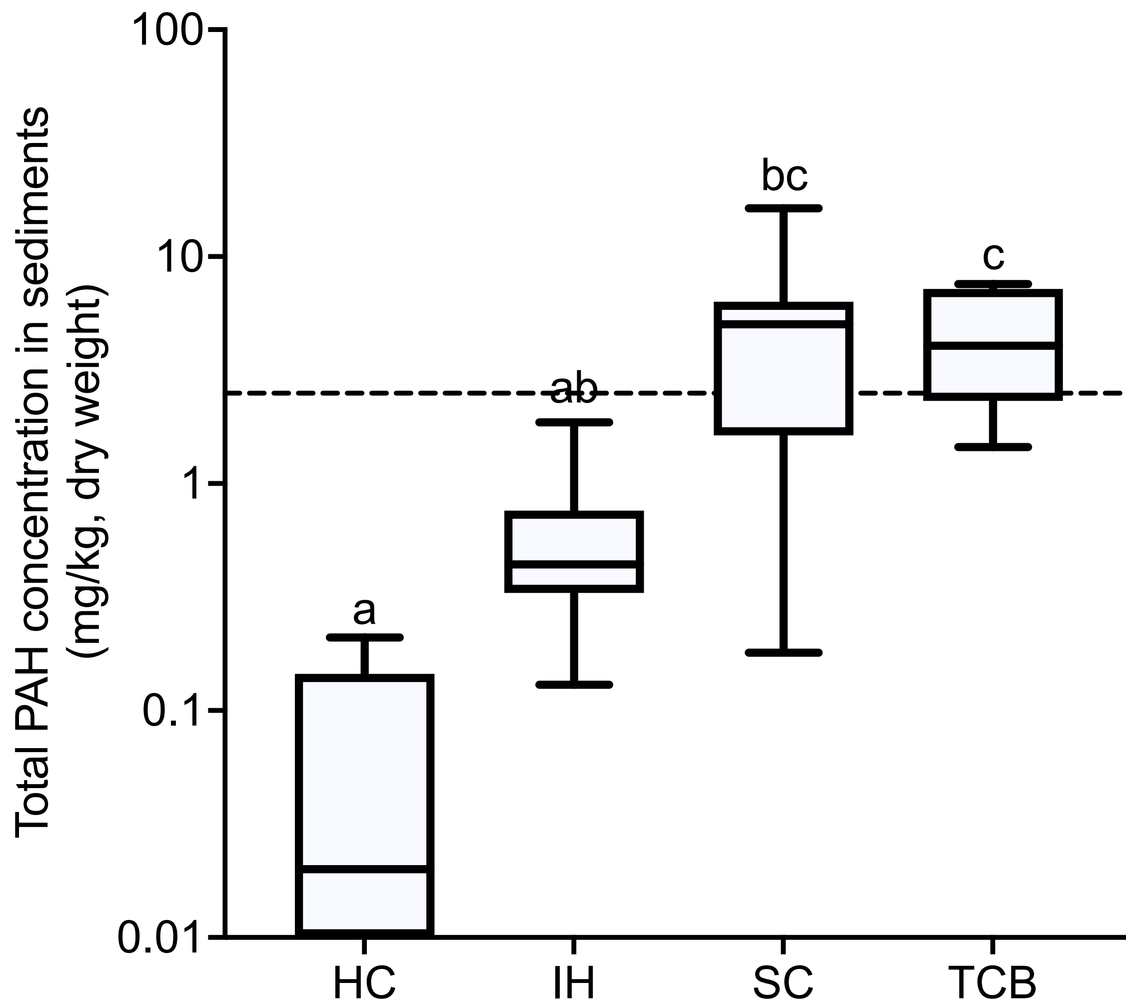

Figure 2. Total sediment PAH concentrations at four sites in the Saint John Harbour (n = 13 for each site except Spar Cove, where n = 12) from collections made between March and November from 2018 to 2021. Letters indicate significant difference between sites ($p < 0.05$, one-way ANOVA with Tukey's test). The y-axis is log-transformed. Dashed line represents the Canadian disposal at sea guideline (2.5 mg/kg).

3.2 Fish condition

The average condition factor (± SE) of fish collected at Hazen Creek, Inner Harbour, Spar Cove, and Tin Can Beach was found to be 0.465 ± 0.00613, 0.431 ± 0.00470, 0.469 ± 0.00781, and 0.412 ± 0.00591, respectively (Figure 3a). Fish at Inner Harbour and Tin Can Beach had significantly lower K than fish collected at Hazen Creek and Spar Cove ($p < 0.05$) (Figure 3a).

Average LSI (% ± SE) of fish collected at Hazen Creek, Inner Harbour, Spar Cove, and Tin Can Beach was found to be 2.83 ± 0.363%, 1.88 ± 0.180%, 3.20 ± 0.390%, and 2.25 ± 0.301%, respectively (Figure 3b). Fish from Spar Cove had a significantly lower LSI (2x lower) than the fish from Inner Harbour ($p < 0.05$) (Figure 3b).

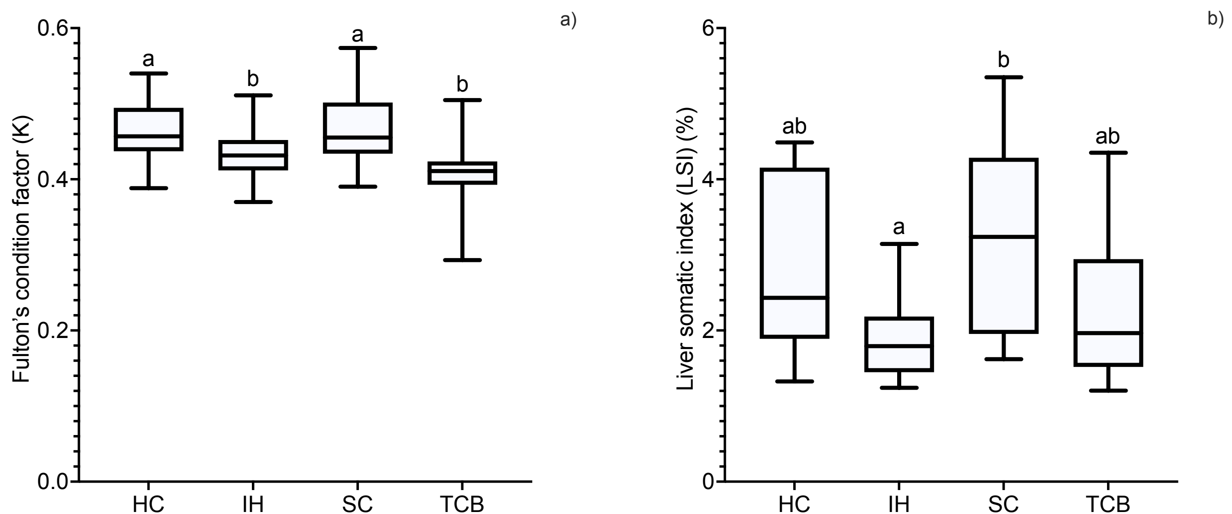

Figure 3. a) Condition factor (K) and **b)** liver somatic index (LSI) of Atlantic silverside collected in various sites of the Saint John Harbour (n = 30 fish / site for K and n = 10 fish / site for LSI). Letters indicate significant difference between sites ($p < 0.05$, one-way ANOVA with Tukey's test).

3.3 Hepatic EROD activity

Hepatic EROD activity (pmol/min/mg) in fish collected at Hazen Creek, Inner Harbour, Spar Cove, and Tin Can Beach was found to be 0.187 ± 0.0402 pmol/min/mg, 0.0605 ± 0.00682 pmol/min/mg, 0.0994 ± 0.0173 pmol/min/mg, and 0.0879 ± 0.0144 pmol/min/mg, respectively (Figure 4). Fish at Hazen Creek exhibited the highest hepatic EROD activity, which was significantly different from that of fish collected at Inner Harbour (3 times higher) and at Tin Can Beach (2x higher) (Figure 4).

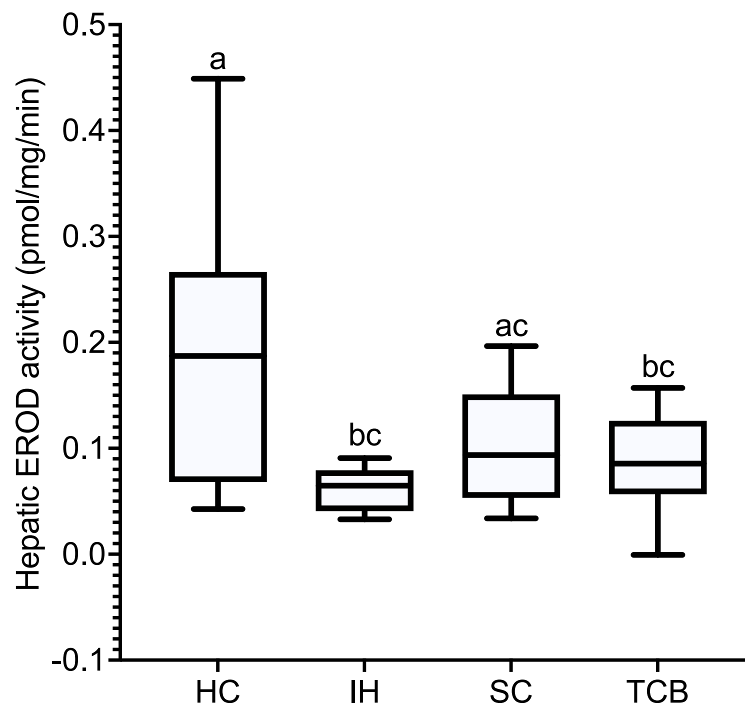

Figure 4. Hepatic EROD activity (pmol/mg/min) of Atlantic silverside collected in various sites of the Saint John Harbour (n = 10 fish / site). Letters indicate significant difference between sites ($p < 0.05$, one-way ANOVA with Tukey's test).

4. Discussion

A wide gradient of total sediment PAH concentrations was observed across the sampled sites, with an ~80-fold difference between the least contaminated site (Hazen Creek) and the most contaminated sites (Spar Cove and Tin Can Beach). Sediment PAH levels exceeded the Canadian disposal at sea limit (2.5 mg/kg dry weight) at Spar Cove and Tin Can Beach by about 2-fold. Possible sources of PAH pollution near Spar Cove and Tin Can Beach include a nearby oil refinery and pulp and paper mill, marine and road traffic, heated residence and businesses, and creosote-coated railway ties. The sediment PAH levels observed in this project are similar to those measured in other Canadian harbours. For example, a 2018 study in Nova Scotia harbours found sediment PAH concentrations between 0.037 and 337 mg/kg dry weight (Davis et al., 2018). Bolton et al. (2004) measured sediment PAH levels in the Vancouver harbour ranging from 4.3 to 11 mg/kg (dry weight).

Despite high sediment PAH concentrations at some sites, the PAH concentrations in fish tissues were below the 0.05 mg/kg (wet weight) detection limit at all sites. PAHs have been found to bioaccumulate in fish tissue after chronic exposure (Hellou et al., 1994), and bioaccumulation factors (BAF = PAH concentration in organism/PAH concentration in sediment) ranging from 0.3 to 49 were found in a 2018 study assessing PAH bioaccumulation in fish collected from an area of Paranagua Bay with port activity (Froehner et al., 2018). In this project, based on the detection limit of the PAH analysis in fish tissues, the BAF was < 2.5 for all fish at all sites.

PAH levels in fish tissues may not be reflective of environmental PAH levels for various reasons (van der Oost et al., 2003). Bioavailability of sediment PAHs to aquatic organisms depends partially on the presence of organic carbon, as sorption of PAHs to the organic carbon

fraction of the sediment reduces bioavailability (Ghosh et al., 2003). Sediment PAH levels are known to increase with increasing sediment total organic carbon (TOC) in the SJH (Van Geest et al., 2015). Sediment PAH levels have also been found to be proportional to sediment TOC in a previous study of an area with port activity (Froehner et al., 2018). The TOC levels of sediment from the inner SJH are low, ranging from 0.66-0.88% (Van Geest et al., 2015), compared to 0.09-8.8% TOC in sediment from Sydney Harbour, Nova Scotia (Walker et al., 2015). Furthermore, a 2015 study measured TOC in sediment from sites in the SJH area (McNamara Point, Saints Rest Beach, Black Beach, and the Digby Ferry Terminal) and found the lowest TOC levels at McNamara Point (also known as Hazen Creek; Power, 2015). Thus, it does not seem that low PAH bioaccumulation in Atlantic silverside can be attributed to low bioavailability associated with high sediment TOC levels. The bioavailability of PAHs also depends on their molecular weight, with low molecular weight PAHs being more available for biological uptake due to their tendency to remain in the water column (Vagi et al., 2021). The most abundant type of PAH in the SJH sediment is fluoranthene, a high molecular weight PAH (Canadian Council of Ministers of the Environment, 1999). This PAH was present at levels above those recommended by Canadian Council of Ministers of the Environment (CCME) interim sediment quality guidelines (ISQGs) (Canadian Council of Ministers of the Environment, 1999), and could be contributing to low PAH bioavailability at the sites assessed in this project. Tissue accumulation of PAHs is also affected by the metabolism and lipid content of the organism. One potential reason for the low PAH bioaccumulation seen in this study is the ability of fish to metabolize PAHs, leading to low tissue concentrations of parent PAHs (Meador et al., 1995). Additionally, species-specific factors, such as lipid content, can also affect tissue PAH accumulation, with greater accumulation occurring as lipid content increases (Soclo et al., 2008).

The lipid content of Atlantic silverside typically ranges from 7.70–12.4% (Schauer & Simpson, 1978), which is lower than that of sobaity sea bream (Hossain et al., 2019), similar to that of round sardinella (Rebah et al., 2010), and higher than that of pikeperch (Uysal & Aksoylar, 2005) and bluefish (Stormer & Juanes, 2018). While PAH metabolism may be contributing to low bioaccumulation in the SJH, it does not appear that low PAH accumulation in fish tissues can be attributed to low lipid content. Because whole-fish measurements were not reflective of environmental PAH levels in this study, measurement of PAH levels in lipid-rich tissues such as the liver and bile may better reflect environmental levels, as PAHs tend to accumulate in these tissues (Logan, 2007).

Fulton's condition factor (K) is used as an indicator of fish wellbeing, with heavier fish of a given length considered to be in better health (Bolger & Connolly, 1989). The K value was found to be similar across all sites (mean = 0.444, min = 0.412, max = 0.469), only increasing by 1.14 times from the lowest-K site (Tin Can Beach) to the highest-K site (Spar Cove). Liver somatic index (LSI) is a measure of the relative weight of the liver, and is often used as an indication of energy status in fish (Chellappa et al., 1995). LSI was also similar across sites (mean = 2.54%, min = 1.88%, max = 3.20%), increasing by 1.70 times from the lowest-LSI site (Inner Harbour) to the highest-LSI site (Spar Cove). These K and LSI values are similar to those observed in a previous study of Atlantic silverside in the SJH, which measured K values between 0.44 and 0.56 and LSI values between 1.60% and 4.80% (Doyle et al., 2011). The trend observed in our study aligns with what was observed in the above-mentioned study, which found K to be highest at Hazen Creek but did not find LSI to increase alongside K (Doyle et al., 2011). Low food availability is associated with decreased condition (Froese, 2006) and LSI (Heidinger & Crawford, 1977) in fish. Given the relationship between food availability and K and LSI, high

food availability at Spar Cove could be responsible for the high condition and LSI observed in fish collected at this site. Additionally, elevated levels of *E.Coli* at Spar Cove and Hazen Creek indicate the likely presence of municipal sewage inputs (Reinhart, 2021), and Hazen Creek is known to be near an outflow from wastewater treatment plant (Power, 2015). Elevated nutrient inputs could explain the elevated K and LSI observed at these sites. Similarly, Power (2015) found that Sand Shrimp (*Crangon septemspinosa*) from McNamara Point (Hazen Creek) were generally longer and heavier than those from other Harbour sites, suggesting a nutrient enrichment effect. Fulton's condition factor and LSI do not appear to be good indicators of PAH pollution in the SJH, and may be more indicative of food availability and nutrient inputs at each site.

Hepatic EROD activity was highest at Hazen Creek (0.1870 ± 0.0402 pmol/min/mg) and lowest at Inner Harbour (0.0605 ± 0.0068 pmol/min/mg). These levels are similar to those measured previously in Atlantic silverside from the SJH (Doyle et al., 2011). At similar sediment PAH levels, higher EROD activities have been measured in flounder (Richardson et al., 2001), Mediterranean rainbow wrasse (Fasulo et al., 2010), and gilt-head bream (Morales-Caselles et al., 2007). However, species of fish vary in basal EROD activity, as well as EROD activity following contaminant exposure (Whyte et al., 2000), and there is little data available on EROD activity in Atlantic silverside across pollution gradients. While EROD activity has been found to be correlated with PAH levels in muscle tissue of fish (Westernhagen et al., 1999), we could not assess this relationship in our study as tissue PAH levels were below the detection limit at all sites. In this study, hepatic EROD activity was not related to PAH sediment levels, as Hazen Creek had the lowest PAH sediment level but the highest EROD activity.

There are a number of factors that can affect EROD activity and could explain why the highest EROD activity was observed at Hazen Creek, despite this site having the lowest sediment PAH concentration. Heavy metals are known to affect EROD activity in fish. Cadmium has been shown to enhance EROD activity whereas copper has been found to have an inhibitory effect on EROD activity (Whyte et al., 2000). This conflicts with what was observed in this study, as Hazen Creek had the highest levels of EROD activity despite being previously found to have similar levels of cadmium but high levels of copper when compared to other sites further away from industrial activities (Doyle et al., 2011). Water temperature also has a significant effect on EROD activity. While contaminant uptake and release into tissues is greater at higher temperatures, temperature compensation mechanisms of poikilotherms at low temperatures may result in increased enzyme activities at lower temperatures (Whyte et al., 2000). ACAP Saint John has found similar mean water temperatures between 2018 and 2020 at the sites assessed in this project, with the greatest mean temperature at Spar Cove (15.6^{o}C) and the lowest mean temperature at Tin Can Beach (13.1^{o}C) (Reinhart, 2021). Studies have found significant variation in CYP1A1 activity across wider temperature ranges than those seen in this project (Fragoso et al., 2006; Lyons et al., 2011; Sleiderink et al., 1995), and it is unlikely that such a small change in temperature could produce such significant variation in EROD activity among sites. Finally, EROD is induced by binding of compounds to the AhR (Whyte et al., 2000). In addition to PAHs, the AhR also binds halogenated aromatic hydrocarbons (HAHs) (Hankinson, 1995) such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). PCBs are released into the environment from a number of sources, including industrial effluents and transformer oil leaks (Wolska et al., 2012). While PCB production was banned in the United States in 1979 (US EPA, 2015) they are extremely

persistent and can remain present in the environment for decades (Boyle & Highland, 1979). A 2001 study found sediment PCB concentrations below 0.10 mg/kg at Saints Rest and Hazen Creek, and a 2015 study found mean dry weight sediment PCB levels in the SJH to be 8.2 ± 0.89 µg/kg at inner harbour sites and 3.8 ± 0.5 µg/kg at outer harbour sites (Van Geest et al., 2015). While these levels do not exceed Canadian disposal at sea guidelines for PCBs (100 µg/kg dry weight) (Van Geest et al., 2015), PCBs and PAHs have been found to have similar potencies as AhR agonists in fish (Barron et al., 2004) and PCBs may serve as EROD inducers at the sites assessed in this project. PCDDs and PCDFs arise in the environment mainly as by-products of industrial processes (Srogi, 2008), such as paper product bleaching, as well as coal and gasoline combustion (Whyte et al., 2000). They are persistent in the environment and tend to bioaccumulate (Srogi, 2008). Additionally, PCDDs and PCDFs have been found to induce EROD activity in fish (Whyte et al., 2000). While industrial activity in the SJH area may serve as a source of PCDD and PCDF pollution, there is little data on the presence of these contaminants in the SJH. Due to the presence of an oil refinery and a natural gas-fueled generating station located near Hazen Creek, it is plausible that HAH pollution may be contributing to the unexpectedly high levels of EROD activity observed at that site. EROD activity was not found to be a good biomarker of PAH exposure in this study, and other biomarkers, such as biliary PAH metabolites (Wang et al., 2008), may serve as better indicators of PAH pollution in the SJH.

5. Conclusion

The objective of this project was to determine whether Atlantic silverside are a good biomonitoring species to assess PAH pollution in the SJH. It was hypothesized that Atlantic silverside are good biomonitors to evaluate PAH pollution in the SJH, and it was predicted that PAH tissue accumulation and hepatic EROD activity would increase with increasing sediment PAH concentration. PAH tissue accumulation was below the detection limit at all sites, indicating that whole-body PAH accumulation is not a reliable indicator of PAH exposure in Atlantic silverside. Hepatic EROD activity in Atlantic silverside did not increase with increasing sediment PAH concentration, suggesting that this measurement does not provide a good indication of PAH exposure. The results of our study suggest that EROD activity in Atlantic silverside is not a good biomarker to evaluate PAH pollution in the SJH. However, other biomarkers in this fish (e.g., biliary PAH metabolites), may serve as a more reliable indicator of PAH pollution in the SJH. Additionally, PAH concentrations in the liver may yield better bioaccumulation assessments than whole-body PAH concentrations. The high level of EROD activity at Hazen Creek could be due to the presence of other contaminants, such as dioxins and metals. In the future, measurements of PAH levels in other tissues, as well as measurements of other contaminants at the sites assessed in this project should be taken.

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