Parasite Populations and Polycyclic Aromatic Hydrocarbons (PAHs) in Biota from the Sydney Tar Ponds, Cape Breton, Nova Scotia, Canada: Investigation of Potential Long-term Biomonitors

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A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
the Degree of Masters of Science in Applied Science

September, 2009 Halifax, Nova Scotia

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Date: September 24, 2009

Your file Votre référence
ISBN: 978-0-494-55996-3
Our file Notre référence
ISBN: 978-0-494-55996-3

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Abstract

Historical industrialization around the Sydney Tar Ponds resulted in contaminated water and sediment. This study determined baseline levels of polycyclic aromatic hydrocarbons (PAHs) in sediments and biota from the Tar Ponds and explored the use of a diversity of resident organisms as potential biomonitors of remediation. European green crab (*Carcinus maenas*) and grass shrimp (*Palaemonetes* spp.) were found to accumulate a greater number and concentration of PAHs than American eel (*Anguilla rostrata*) and mummichog (*Fundulus heteroclitus*). Of the biota sampled, *Carcinus maenas* are suggested to be the best biomonitor. The diminished parasite levels of *F. heteroclitus*, found in the Tar Ponds, are also an effective biomonitor. As remediation of the Tar Ponds proceeds, it is proposed that the levels of parasites will increase to a healthy level.

Date: September 25, 2009

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LIST OF ABBREVIATIONS AND SYMBOLS USED

ANOVA	Analysis of varience
Å	Angstrom
BC	Black carbon
BSAF	Biota-sediment accumulation factor
CCME	Canadian Council of Ministers of the Environment
CYP1A	Cytochrome P-450 monooxygenase (CYP1A) system
df	Degrees of freedom
Dry wt	Dry weight
EPA	Environmental Protection Agency, United States
ERL	Toxic effects response low
ERM	Toxic effects response median
F	F-statistic
FUNUID	*Fundulus* spp. (uncertain whether it was *F. heteroclitus*, *F. diaphanus* or a *F. heteroclitus* x *F. diaphanus* hybrid)
FUNHET	*Fundulus heteroclitus*
GC/MS	Gas chromatography/ mass spectrometry
GPC	Gel Permeation Chromatography
K	Condition factor
K_{oc}	Organic carbon/water partition coefficient
K_{ow}	*n*-Octanol/water partition coefficient
L3	Third larval stage
MANOVA	Multivariate analysis of variance
MLE	Maximum likelihood estimation

n	Number of observations (i.e. *F. heteroclitus* n = 3 means 3 individual *F. heteroclitus* were analyzed separately, or three were pooled and analyzed as a group
n.d.	Not detected
p	Probability
PAH	Polycyclic aromatic hydrocarbon, also known as polynuclear aromatic hydrocarbon
PCB	Polychlorinated biphenyl
r^2	Linear regression coefficient of determination
RPC	Research and Productivity Council, Canada
Std. dev.	Standard deviation
Std. err.	Standard error
STPA	Sydney Tar Ponds Agency
ΣPAH	Total polycyclic aromatic hydrocarbons (based on 18 PAHs)
UID	Unidentified
Wet wt	Wet weight

GLOSSARY

		Page
Abundance:	in parasitology, it is the number of individual parasites of a particular species found in one fish whether or not the host is infected (Bush *et al.*, 1997).	36
Acanthocephalans:	(Phylum: Acanthocephala) are endoparasites commonly known as thorny-head worms and are found as adults in its host's digestive system. Their life cycle requires at least two hosts, such as an arthropod (or insect) and a fish (Roberts and Janovy, 2000).	39
Aging:	in sediments, this is the phenomenon when the bioavailability of organic contaminants in soils and sediments decreases over time and there is no net change of the contaminant concentration (Lu *et al.*, 2006; Semple *et al.*, 2003; White *et al.*, 1999).	29
Bioremediation:	is the process by which contaminants are degraded by biological means, such as microorganisms (Atlas and Bartha, 1998; Newman and Unger, 2003).	29
Black carbon (BC):	is also referred to as soot or charcoal particles and is a type of carbonaceous material generally formed from incomplete combustion of biomass and fossil fuels (Cornelissen and Gustafsson, 2004, 2005; Cretney and Yunker, 2000)	29
Bioavailability:	is the fraction of the total contaminant concentration for uptake into an organism (Newman and Unger, 2003).	27
Catabolism:	is the microbial metabolism or breakdown of a compound, which often produces energy for other microbial activities (Atlas and Bartha, 1998).	24
Cestodes:	(Phylum: Platyhelminthes) are endoparasites commonly known as tapeworms and are found as adults in the digestive system of its host. Their life cycle can be quite complex and typically involves a series of intermediate hosts and a definitive (or final) host (Roberts and Janovy, 2000).	40
Condition Factor (K):	is a value used to compare the health of an organisms, where a higher K value corresponds to an healthier organism. K is calculated by the following equation (Moyle and Cech, 2004): $K = 100 \text{ X } [\text{total body weight (g)}/(\text{total length in cm})^3]$	53

Cytochrome P-450 monooxygenase (CYP1A) system:	is a series of metabolic steps that decrease the hydrophobicity of contaminants by the attachment of hydroxyl (-OH) groups, which increases the organisms' ability to excrete contaminants from its body (Newman and Unger, 2003).	31
Definitive host:	this is the host where the parasite develops from a larval stage into an adult and reaches sexual maturity (Roberts and Janovy, 2000).	40
Ectoparasites:	are parasites, such as monogenes and parasitic copepods, which live on the gills, fins, and/or surface of the organism (Roberts and Janovy, 2000).	38
Encyst:	this is the process by which a larval parasite forms protective coating around its self, which may allow the parasite to enter a resting stage (Roberts and Janovy, 2000)	83
Endoparasites:	are parasites, such as acanthocephalans, cestodes, trematodes, and nematodes, which live in or on the internal organs of the organism (Roberts and Janovy, 2000).	38
Free-living stage:	a parasite larval form which is not found in a host. These larval forms are often encysted on vegetation or coated to protect the parasite from the environment (Roberts and Janovy, 2000).	37
Fugacity (*f*):	is a partial pressure (Pa) measurement of the leaving or escaping tendency of a compound from a particular phase (Mackay, 2004).	28
Fugacity capacity (Z):	is a measurement of the ability of an organism to bioaccumulate a contaminant, which is related to the lipid content of the organism and is inversely related to fugacity of the organism (Klosterhaus *et al.*, 2002; Mackay, 2004; Russell *et al.*, 1999).	28
Intermediate hosts:	is an organism required for the development of the larval parasite (Roberts and Janovy, 2000).	37
Interstitial water:	is water between and around sediment particles and is often higher in organic contaminant concentration compared to the concentration in the water column (Lu *et al.*, 2004; Mitra and Dickhut, 1999).	26

Lipophilic:	is a characterisitic of many organic contaminants, such as PAHs and PCBs, which tend to accumulate in the lipids (or fat) of organisms (Horton *et al.*, 2002; vanLoon and Duffy, 2000).	24
Monogenes:	(Phylum: Platyhelminthes) are ectoparasites with highly adapted holdfasts for attachment to a specific host. Their life cycle typically only include one host (Roberts and Janovy, 2000).	39
Mean intensity:	in parasitology, it is the mean total number of parasites per infected fish from one site (Bush *et al.*, 1997).	52
Nematodes:	(Phylum: Nematoda) are endoparasites with a digestive system. Their life cycle requires four moults to reach sexual maturity (Roberts and Janovy, 2000).	40
Non-point sources:	release contaminants which cannot be traced to one particular location. Long range transport of PAHs from highly contaminated sites via atmospheric circulation is a non-point source (Roche *et al.*, 2002; Zhang *et al.*, 2008).	25
Parasitic copepods:	(Phylum: Arthropoda) are ectoparasites commonly found on the gills of fish. Their life cycle has eight free living larval stages and one host (Roberts and Janovy, 2000)	39
Paratenic host:	this is a host which bridges an ecological barrier and is not needed in parasite development. For example, paratenic hosts assist in transmitting the host from a lower to higher level in a food chain to increase the likelihood a parasite being consumed by either an intermediate or definitive host (Roberts and Janovy, 2000).	212
Parts per billion (ppb):	is a measurement unit of trace contaminants; it is synonymous with the units ng/g, µg/kg and µg/L. 1000ppb = 1ppm	--
Parts per million (ppm):	is a measurement unit of trace contaminants; it is synonymous with the units µg/g, mg/kg and mg/L. 1000ppb = 1ppm	--
Prevalence:	in parasitology, it is the percentage of fish infected with at least one parasite divided by the number of fish examined (Bush *et al.*, 1997).	36

Point sources:	release contaminants which originate from one location. Examples of point sources of PAH are industrial sites such as coke ovens or aluminum smelters (Avci *et al.*, 2005; Mitra *et al.*, 2002; Secco *et al.*, 2005).	25
***n*-Octanol/water partition coefficient (K_{ow}):**	is the concentration of a chemical in octanol (often n-octanol) divided by the concentration of the same chemical in water at equilibrium and is a measure of the chemical partitioning from water into organisms (vanLoon and Duffy, 2000).	24
Organic carbon/water partition coefficient (K_{oc}):	is the concentration of a chemical sorbed in or to sediment divided by the concentration of the same chemical in water at equilibrium and is used to describe the partitioning of organic chemicals between water and sediment (vanLoon and Duffy, 2000).	25
Sedimentary organic carbon:	is carbonaceous material which has not undergone combustion (Jonker and Koelmans, 2002).	29
Toxic effects response low (ERL):	is a toxicological value which predicts that biota which live in environments with lower contaminant concentrations will rarely exhibit toxic effects (Wade *et al.*, 2008).	32
Toxic effects response median (ERM):	is a toxicological value which predicts that biota which live in environments with higher contaminant concentrations will often exhibit toxic effects (Wade *et al.*, 2008).	33
Type 1 Biomonitoring:	traces the change in species composition in an ecosystem (Walker *et al.*, 2001).	35
Type 2 Biomonitoring:	traces the change in the chemical concentration in biota by either measuring the concentration in the organism or its dietary items (Walker *et al.*, 2001).	35
Type 3 Biomonitoring:	traces the toxicological effects induced, on or in the organism, by the contaminant (Walker *et al.*, 2001)	35
Type 4 Biomonitoring:	traces the development of organismal genetic resistance as a biomonitor (Walker *et al.*, 2001)	35

ACKNOWLEDGEMENTS

This is the section where you pretty much acknowledge everyone you ever met. There have been numerous people who have moulded and guided me throughout my post-secondary education. I wish I could give all of them the credit they deserve, but that would be a thesis by its self. Firstly, I must acknowledge my parents, Dr. Fred Rockwell and Barbara Yee, and brother, Adrian Rockwell, for guiding and encouraging me throughout my life. I can remember my father instilling a wonder of nature in me by our many nature hikes and collecting insects in lakes. My mother has provided me needed perspective, and her words of, "If it was easy, everyone would have a Bachelor of Science," has carried me through many weary times in my undergraduate and graduate studies. Then there is my brother, who is just plain fantastic.

During my undergraduate days at Malaspina University-College (now called Vancouver Island University), I was tutored and inspired by many mentors. I owe my passion for discovery and environmental chemistry to Dr. Erik Krogh and Dr. Chris Gill. Thank-you for providing me with a strong chemistry background and allowing me the needed opportunities to develop my analytical skills. Who knew my summer experience of gutting and boiling fish in concentrated acids would lead to this! I remember my first introduction to the world of parasitology in a first year biology course. Thank-you, Dr. Tim Goater, for allowing me to catch the "parasitology bug", so to speak. After that one course my disgust turned to a fascination of parasite goodness!

After my undergraduate days, I decided I wanted to continue my education. Thank-you Dr. K. Martha M. Jones, one of my co-supervisors, for taking a chance on someone you only knew through e-mail. This Master of Science (M.Sc.) opportunity has become the perfect mixture of ecotoxicology and parasitology. Thank-you for your

countless hours of editing, encouragement, and advice. During my summers at Cape Breton University, I was privileged to work with Sylvia Dove, Jared Tomie, Cheryl Wall, and Justin Yurchesyn. Thank-you for all your assistance.

At Saint Mary's University, I have many colleagues and friends, which added greatly to the M.Sc. experience. Of these people, I would especially like to acknowledge Sara Collins, Dale Jefferson, and Jennifer Lau. Also, I am grateful for Dr. Ron Russell, my other co-supervisor, who has also spent many hours editing my thesis. Thank-you for your ecotoxicological and statistical expertise; because of you, I leave graduate school still with a portion of sanity. Thanks are also due to the members of my committee; Dr. Bill Jones and Dr. Adam Piorko for offering their time.

One of the few things which are certain in life – and especially graduate studies – is change. Parasitology became an unplanned staple in my thesis. Dr. David Cone, another member of my committee, has played a vital role in my thesis. Thank-you for a little bench space in your laboratory and the time you spent helping me identify my countless parasite specimens. One of the neatest – and grossest – discoveries of my necropsies was the first citing of the swimbladder nematode *Anguillicoloides crassus* in American eels (*Anguilla rostrata*) from Canadian estuaries. I would like to thank Dr. David Marcogliese and Dr. Frank Moravec for confirming the identification of this exotic invasive parasite.

Funding for this research was provided by several organizations including Sydney Tar Ponds Agency (STPA) research grant awarded to Dr. Martha Jones and research grants to Dr. Bill Jones and Dr. Ron Russell. Thanks are due to Cape Breton University and Saint Mary's University for funding and logistical support throughout my graduate studies.

My graduate studies were a rewarding – yet sometimes trying – experience. Over the last two years my eternal best friend, Graeme Thompson, has been one of my few constants through it all. Graeme has spent hours hearing about my successes and failures and frustrations. Even though my graduate studies are coming to an end, I am comforted by the knowledge that this will continue on. IVAU.

This thesis is dedicated to those who dare to dream,

especially my grandmother, Yvonne Rockwell.

1. INTRODUCTION

1.1 Sydney Tar Ponds

The Sydney Tar Ponds is one of Canada's most contaminated sites (AMEC, 2005; Tay *et al.*, 2003), which is located in Sydney, Cape Breton, Nova Scotia. The Sydney Tar Ponds are composed of four parts: the North Tar Pond, the South Tar Pond, Coke Ovens Brook Connector, and the former Coke Ovens site (Figure 1). The North and South Tar Ponds are part of Muggah Creek, which empties into the South Arm of the Sydney Harbour (Figure 1).

The Sydney Tar Ponds support a wide array of species. Terrestrial habitats surrounding the Tar Ponds support red fox (*Vulpes vulpes*), muskrat (*Ondatra zibethicus*), coyote (*Canis latrans*), and white tailed deer (*Odocoileus virginianus*) (AMEC, 2005). Many invertebrate and fish species, such as European green crabs (*Carcinus maenas*), blue mussels (*Mytilus edulis*), mummichogs (*Fundulus heteroclitus*), sticklebacks (*Gasterosteus aculeatus*, *Gasterosteus wheatlandi*, and *Apeltes quadracus*), American eels (*Anguilla rostrata*), tomcod (*Microgadus tomcod*), and brown bullheads (*Ameiurus nebulosus*) reside in the Sydney Tar Ponds or at the mouth of the North Pond where it empties into the Sydney Harbour (AMEC, 2005; Jones, 2007).

Biological surveys on the Sydney Harbour are based mainly on fishery surveys (Vandermeulen, 1989). Several fish species, such as cod, mackerel, winter flounder (*Pseudopleuronectes americanus*), hake (*Urophycis tenuis*), and cunner (*Tautoglobrus acesperus*) have been caught there (Vandermeulen, 1989). Shrimp, mussels (*Mytilus edulis* and *Modiolus modiolus*) and lobster (*Homarus americanus*) are also found in the Sydney Harbour (Ernst *et al.*, 1999; Vandermeulen, 1989). Studies have indicated that

pollution released from the Sydney Tar Ponds into the Sydney Harbour has negatively impacted biota in the Sydney Harbour (Ernst *et al.*, 1999; JWEL-IT, 1996*a*; Tay *et al.*, 2003; Vandermeulen, 1989). The South Arm of the Sydney Harbour receives direct input from Muggah Creek; it has a depauperate benthic community, which is dominated by polychaetes and sea anemones (Vandermeulen, 1989). The North Arm of the Sydney Harbour does not have direct input from Muggah Creek and has been noted to have a healthier benthic community (Vandermeulen, 1989).

Pollution in the Sydney Tar Ponds resulted from 100 years of unregulated industrial activities. Industries that developed around the Tar Ponds included coke and steel production, rail yards, and dump sites (AMEC, 2005). It is thought that the coking ovens and the steel plant released contamination, which migrated into the South and then North Tar Pond of Muggah Creek (JWEL-IT, 1996*a*, *b*). Contaminants were then released from the North Tar Pond into the South Arm of the Sydney Harbour (JWEL-IT, 1996*a*, *b*; Matheson *et al.*, 1983; Sirota *et al.*, 1983, 1984). This has resulted in high levels of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals in biota, sediment, and water from Muggah Creek and Sydney Harbour (AMEC, 2005; Acres, 1984; Ernst *et al.*, 1999; Furimsky, 2002; Jones, 2007; JWEL-IT, 1996*a*, *b*; Vandermeulen, 1989). A brief description of PCBs and metals in the Sydney Tar Ponds will be provided. The rest of this thesis will focus on PAHs in the Sydney Tar Ponds.

PCBs are a foreign organic, environmental contaminant, which are not naturally found in the environment (Ceccarini and Giannarelli, 2006). PCBs primarily bioaccumulate in the lipids of organisms (Christensen *et al.*, 2005; Maruya and Lee, 1998; Tanabe *et al.*, 2004; Tay *et al.*, 2003). PCBs may act as endocrine disruptors and

environmental estrogens (Ceccarini and Giannarelli, 2006; Ross *et al.*, 1996). They may alter an organism's neurobehavior, reproduction, and development (Ceccarini and Giannarelli, 2006). Many different PCB congeners have been found in sediment and biota from the Sydney Tar Ponds (Jones, 2007; JWEL-IT, 1996*b*).

Metals are inorganic contaminants, which can be toxic to organisms above critical concentrations. JWEL-IT (1996*b*) found copper, lead, nickel, and zinc from all Sydney Harbour sediment cores to be higher than Canadian Council of Ministers of the Environment (CCME) Marine Sediment Guidelines (MacDonald *et al.*, 1992). They also found chromium and manganese in some Sydney Harbour sediment cores to be higher than CCME Marine Sediment Guidelines (MacDonald *et al.*, 1992). High levels of cadmium, lead, mercury, and zinc have been found in Sydney Tar Pond sediments (Vandermeulen, 1989).

1.2 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs), also referred to as polynuclear aromatic hydrocarbons, are a class of organic compounds which are a combination of at least two aromatic rings with at least one common carbon-carbon bond (Figure 2). PAHs have been present in the environment since the formation of the earth (Wakeham *et al.*, 1980*a*, *b*). PAHs are ubiquitous and can be formed from natural and anthropogenic activities. Naturally formed PAHs may occur from forest fires, microbial biosynthesis, and weathering of petroleum seeps or ancient sediments (Wakeham *et al.*, 1980*a*, *b*). The environmental concentration of PAHs has dramatically increased since the industrial revolution (LaFlamme and Hites, 1978; Wakeham, 1980*a*; Yunker *et al.*, 1999).

Anthropogenic PAHs are generally formed by incomplete combustion of organic matter (vanLoon and Duffy, 2000). Incomplete combustion is the result of impurities in organic matter, low temperature, and limited concentration of oxygen (vanLoon and Duffy, 2000). PAHs may be formed from industrial activities (coke ovens and alumininum smelters), incineration, and vehicle engines (Bieri *et al.*, 1986; Connell, 1997; McGowin, 2006).

1.2.1 Physiochemical Properties of PAHs

The smaller PAHs (e.g. naphthalene) are volatile, more prone to microbial catabolism, and less lipophilic, compared to larger PAHs (e.g. benzo[*a*]pyrene) (Atlas and Bartha, 1998; Bamforth and Singleton, 2005; vanLoon and Duffy, 2000). Microbial **catabolism** is the metabolism or breakdown of a compound, which often produces energy for other microbial activities (Atlas and Bartha, 1998). **Lipophilic** refers to the tendency of a contaminant to enter the lipid or fat of an organism (vanLoon and Duffy, 2000). Less lipophilic contaminants have a lower tendency to enter and to accumulate in organismal lipid. Lipophilicity is often related to the **hydrophobicity**, which is the tendency of a contaminant to enter water. A decrease in lipophilicity often corresponds with a decrease in hydrophicity, which is a decrease in tendency of a contaminant to enter water (vanLoon and Duffy, 2000).

Hydrophobicity is often described by the ***n*-octanol/water partition coefficient** ($\boldsymbol{K_{ow}}$). K_{ow} is the concentration of a chemical in octanol (often *n*-octanol) divided by the concentration of the same chemical in water at equilibrium and is a measure of the chemical partitioning from water into organisms (vanLoon and Duffy, 2000). A higher K_{ow} value reflects a greater hydrophobic nature of a contaminant compared to a

contaminant with a lower K_{ow}. The **organic carbon/water partition coefficient (K_{oc})** is the concentration of a chemical sorbed in or to sediment divided by the concentration of the same chemical in water at equilibrium and is used to describe the partitioning of organic chemicals between water and sediment (vanLoon and Duffy, 2000). K_{oc} can be approximated by the K_{ow}. The log K_{ow} and log K_{oc} of PAHs typically range from 2-7 and 3-6 respectively (Table 1).

1.2.2 PAHs in the Environment

PAHs enter the environment from point or non-point sources. **Point sources** release contaminants which originate from one location. Examples of point sources of PAH are industrial sites such as coke ovens or aluminum smelters (Avci *et al.*, 2005; Mitra *et al.*, 2002; Secco *et al.*, 2005). **Non-point sources** release contaminants which cannot be traced to one particular location. Long range transport of PAHs from highly contaminated sites via atmospheric circulation is a non-point source (Roche *et al.*, 2002; Zhang *et al.*, 2008). Contaminated run-off water from streets and fields into water bodies and subsequent transport by moving water is another possible non-point source (Brezonik and Stadelmann, 2002; Schiff and Bay, 2003; Tsihrintzis and Hamid, 1997).

In aquatic environments PAHs are often associated with organic matter (Chin and Gschwend, 1992; Lu *et al.*, 2003; Socha and Carpenter, 1987). PAHs have low water solubility (Table 1). In relatively pristine aquatic environments, the concentration of each PAH not associated with sediment in the water column is generally less than 5 μg/kg (Barbee *et al.*, 2008). In contaminated aquatic environments, the concentrations of freely dissolved individual PAHs are generally less than 1000 μg/kg or 1 mg/kg (Readman *et al.*, 1982).

Due to their hydrophobic nature, PAHs are generally associated with sediments (Gustafsson *et al.*, 1997; Khim *et al.*, 2001; Kraaij *et al.*, 2002) or dissolved organic matter in the water column (Döring and Marschner, 1998; Haitzer *et al.*, 1998; Landrum *et al.*, 1985). PAHs may also be found in **interstitial water**, which is water between and around sediment particles (Lu *et al.*, 2004; Mitra and Dickhut, 1999). The concentration of PAHs associated with sediments is greater than PAHs in interstitial water, which are in turn greater than concentrations in the water column (Gao *et al.*, 1998; Hyun *et al.*, 2007; Maskaoui *et al.*, 2002). The concentrations of PAHs in sediments distant from contaminated sites are generally below 1mg/kg (Barbee *et al.*, 2008; Djomo *et al.*, 1996; Kim *et al.*, 2008; Krauss *et al.*, 2000: Tables 2 and 3). Sediments from contaminated sites vary in their degree of contamination (Cornelissen *et al.*, 2008; Johnson-Restrepo *et al.*, 2008; Khim *et al.*, 2001: Tables 4 and 5). Differences in PAH concentration among contaminated sites are due to the concentration of PAHs and the rate of elimination of PAHs from that particular environment.

Once PAHs have been introduced into the environment, several processes may occur. PAHs may be degraded by microbial catabolism (Dabestani and Ivanov, 1999). Also, PAHs may be degraded abiotically by photolysis, which is a process where light breaks chemical bonds (Dabestani and Ivanov, 1999). PAHs may volatilize into air and be removed from the immediate area by atmospheric transport (Fernández *et al.*, 2003; Simpson *et al.*, 1996). PAHs may leach from one environmental compartment to another (Reid *et al.*, 2000). In the terrestrial environment PAHs may leach from the soil compartment into the groundwater compartment. PAHs may be sequestered in and/or on organic matter (Gustafsson *et al.*, 1997; Haitzer *et al.*, 1998; Kraaij *et al.*, 2002). Non-

metabolizable or slowly metabolizing PAHs may bioaccumulate in biota (DeLeon, 1988; Meador *et al.*, 1995).

1.2.3 PAHs in Biota

The concentration of PAHs in biota is highly variable among sampling sites from different parts of the world. In remote areas the concentration of specific PAHs in biota are generally below 1 μg/kg and do not exceed 1000 μg/kg or 1 mg/kg (Pancirov and Brown, 1977; Vives *et al.*, 2004: Table 6). Concentrations of PAHs in biota from contaminated areas are highly variable (Table 7). In both remote and contaminated sites, the concentrations of PAHs are generally higher in liver and hepatopancreas tissues than in muscle or other biota tissues (Hellou and Warren, 1997; Hellou *et al.*, 1994).

The uptake and bioaccumulation of PAHs in biota depends on a combination of biotic and abiotic factors, which varies between species and environments (Bender *et al.*, 1988; Hellou *et al.*, 1994). PAHs may be taken up from the water column or interstitial water (Leady *et al.*, 1999). Also, PAHs may be ingested by feeding on sediments or prey items (Forbes *et al.*, 1998; Hickey *et al.*, 1995; Lee *et al.*, 1976). The concentration of PAHs in biota depends on many factors including: the rate of PAH uptake and elimination (Bender *et al.*, 1988), the organism's ability to metabolize the PAHs (Gewurtz *et al.*, 2000), the physiochemical properties of the compound (Baumard *et al.*, 1998; Krauss *et al.*, 2000), and the bioavailability of the PAHs (Landrum *et al.*, 1992; McCarthy and Jimenez, 1985). **Bioavailability** is the fraction of the total contaminant concentration available for uptake into an organism (Newman and Unger, 2003).

The uptake and bioaccumulation of PAHs depends on the fugacity of the organism in relation to its surrounding environment (Klosterhaus *et al.*, 2002; Mackay, 2004;

Russell *et al.*, 1999). **Fugacity** (***f***) is a measurement of the leaving or escaping tendency of a compound from a particular phase. The **fugacity capacity** (**Z**) of an organism to bioaccumulate hydrophobic contaminants is related to the lipid content of the organism and is inversely related to the fugacity of the organism (Klosterhaus *et al.*, 2002; Mackay, 2004; Russell *et al.*, 1999). Organisms with higher lipid content will have a lower fugacity and a higher fugacity capacity compared to organisms with lower lipid content. If the fugacity capacity of the organism is greater than the environment, there will be a net movement of the contaminant into the organism.

1.3 Bioaccumulation and Bioavailability of PAHs

The presence of PAHs or other contaminants in the environment does not necessarily mean that the contaminant will be toxic to or bioaccumulate in biota (Ehlers and Luthy, 2003, Erickson *et al.*, 1993; Reid *et al.*, 2000). The toxicological effects and bioaccumulation of the contaminant is related to the fraction of the contaminant which is available to biota; this is the bioavailable fraction, also called the bioavailability, of the contaminant. Many biological and chemical techniques have been developed to assess bioavailability (Kelsey *et al.*, 1997; Krauss and Wilcke, 2001; Kukkonen *et al.*, 2004; Nilsson *et al.*, 2006; Reid *et al.*, 2000). The contaminant bioavailability is influenced by a combination of many abiotic (Kraaij *et al.*, 2001; Lamoureux and Brownawell, 1999; Zeng *et al.*, 2003) and biotic factors (Nakata *et al.*, 2006; Thomann *et al.*, 1992; Van Hoof *et al.*, 2001).

Studies have shown the bioavailability of organic contaminants to be influenced by the type of organic carbon in or on the sediment (Accardi-dey and Gschwend, 2002;

Chin and Gschwend, 1992; Döring and Marschner, 1998; Gustafsson *et al.* 1997). **Black carbon** (**BC**) is a type of carbonaceous material formed from incomplete combustion of biomass and fossil fuels. BC is also referred to as soot or charcoal particles. Due to its aromatic and condensed structure, BC is able to strongly sorb organic contaminants (Cornelissen and Gustafsson, 2005; Rust *et al.*, 2004). Jonker and Koelmans (2002) found that the sorption of PAHs to BC to be 1000 times stronger than PAHs to **sedimentary organic carbon**, which is carbonaceaous material which has not undergone combustion. It is thought that the strong sorption of organic contaminants to BC may account for the limited bioavailability and decreased effectiveness in the bioremediation of organic contaminants (Cornelissen and Gustafsson, 2004, 2005; Cretney and Yunker, 2000). **Bioremediation** is the process by which contaminants are degraded by biological means, such as microorganisms (Atlas and Bartha, 1998).

Aging or weathering of sediments has also been found to limit the bioaccumulation and bioavailability of PAHs (Lu *et al.*, 2006; Semple *et al.*, 2003; White *et al.*, 1999). **Aging** in sediments is the phenomenon when the bioavailability of organic contaminants in soils and sediments decreases over time and there is no net change of the contaminant concentration (Lu *et al.*, 2006; Semple *et al.*, 2003; White *et al.*, 1999). This results in reduction of exposure and toxicity of contaminants over time, but does not completely eliminate the threat of the contaminant in the environment (Alexander, 1995, 2000; Hatzinger and Alexander, 1995). This was demonstrated by Alexander and Alexander (1999) where they exposed *Pseudomonas patida*, a bacterium, to PAH-spiked soil. After seven days, the rate of mutations induced by PAHs in *P. patida* had decreased by 72% compared to the initial rate of mutations. They concluded that the genotoxicity of PAH declines with increased aging of the soil.

There are differences in the bioavailability of organic contaminants within ecosystems. The hydrophobicity, size, and structure of an organic contaminant may influence the contaminant bioavailability (Burkhard *et al.*, 2004; Kukkonen *et al.*, 2005; Schuler *et al.*, 2003). Studies have found a negative correlation between hydrophobicity and bioaccumulation of contaminants (Maruya and Lee, 1998). Kannen *et al.* (1998*a*) found bioaccumulation of PCBs dependent on hydrophobicity and molecular size. Studies have suggested that molecular size of organic contaminants may restrict or prevent membrane permeability (Kannen *et al.*, 1998*a*, *b*; Ma *et al.*, 1998). Opperhulzen *et al.* (1985) suggested that PAHs with widths over 9.5Å could not penetrate the cell membrane, thus preventing the accumulation of PAHs in fishes.

Biotic factors may also play a role in the variation in bioaccumulation and bioavailability of organic contaminants. Organismal behaviour influences contaminant bioaccumulation (Forbes *et al.*, 1998; Millward *et al.*, 2001; Noblet *et al.*, 2003). Ingestion of sediments is considered to be a major source of organic contaminants to biota (Croce *et al.*, 2005; Loonen *et al.*, 1997). Some organisms selectively feed on sediment particles with higher total organic carbon (Boese *et al.*, 1996; Lee *et al.*, 1990; Krauss and Wilcke, 2001). Since the concentration of organic contaminants is often related to organic carbon concentration, the selective consumption of these particles may increase an organism's exposure to contaminants.

The presence of other organisms may also affect bioaccumulation and bioavailability. Ciarelli *et al.* (1999) found a linear relationship between uptake of the PAH fluoranthene in mussels (*Mytilus edulis*) and the density of amphipods (crustacean: *Corophium volutator*) in the sediment. They found that amphipod activity increased the total suspended sediments in the water column. This resulted in increased suspended

particles and associated fluoranthene in mussels filter feeding. In a later study by Ciarelli *et al.* (2000), they found the bioaccumulation of fluoranthene to be greater in polychaetes (*Neris virens*) in sediments with more amphipods compared to sediments with fewer or no amphipods.

1.4 Toxicological Properties of PAHs

The uptake of PAHs causes a diversity of physiological responses in organisms (Bacanskas *et al.*, 2004; Bard *et al.*, 2002; Burchiel and Luster, 2001; Mann *et al.*, 1999). The resultant physiological responses can be harmful to the organism's health and/or reproductive success (Avci *et al.*, 2005; Bain, 2002; Bláha *et al.*, 2002; Incardona *et al.*, *in press*). PAHs are generally not directly toxic to organisms (Barbee *et al.*, 2008; Dabestabi and Ivanov, 1999; McGowin, 2006). However, after PAHs react with organismal enzymes, the resultant PAH metabolites become potential mutagens or carcinogens. The **cytochrome P-450 monooxygenase (CYP1A) system**, also called the mixed-function oxidase (MFO) system, is found in both invertebrates (Livingston *et al.*, 1990; Walker *et al.*, 2001) and vertebrates (Fragoso *et al.*, 2006; Shailaja and D'Silva, 2003). The CYP1A system functions by adding hydroxyl groups to PAHs. The addition of hydroxyl groups increases water solubility of PAHs, thus allowing an efficient excretion of the PAH metabolite (Newman and Unger, 2003; Walker *et al.*, 2001). In many contaminated environments, the activity of CYP1A systems is often increased (Hansson *et al.*, 2006; Sturve *et al.*, 2006; Wassenberg *et al.*, 2002).

Despite the benefits of CYP1A, the reactions result in an intermediate epoxide functional group on the PAH (Dabestani and Ivanov, 1999: Figure 3). The epoxide is

highly reactive with DNA and hemoglobin. The initial toxicological responses are formation of liver and skin tumours (Arcand-Hoy and Metcalfe, 1999; Pinkney and Harshbarger, 2006; Pinkney *et al.*, 2004). Over time, interactions between the epoxide and the tissues may result in lung, bladder, and/or skin cancer and high PAH concentrations may result in immunosuppression (McGowin, 2006; Newman and Unger, 2003; Rose *et al.*, 2001). In some highly contaminated areas, organisms have a suppressed expression of CYP1A (Arzuaga and Elskus, 2002; Meyer *et al.*, 2002; Nacci *et al.*, 1999), which varies between species (Anulacion *et al.*, 1998; Vuorinen *et al.*, 2006; Wirgin *et al.*, 1996). Nacci *et al.* (2002) suggested that the decrease in levels of metabolizing enzymes, such as CYP1A, may be an adaptation to prevent DNA damage and cancer.

Liver damage has been found in a diversity of organisms from contaminated sites (Harshbarger and Clark, 1990; Marty *et al.*, 2003; Stine *et al.*, 2004; Vogelbein *et al.*, 1990, 1999). Myers *et al.* (1998) found that fishes exposed to sediment with total PAH concentration greater than 500-1000µg/kg (0.5-1mg/kg) would develop liver lesions. PAH exposure may also result in fin erosion, change in diet and eating habits, no weight gain, and/or cloudy eyes (Hargis *et al.*, 1984)

The toxicological effects of PAHs generally occur after the organism has been exposed to or has bioaccumulated a threshold concentration. The value may change depending on the health of the organism and the environmental conditions (Weis, 2002; Weis and Weis, 1989). "Toxic effects response low" and "toxic effects response median" are threshold values to aid in predicting the onset of PAH toxic effects (Wade *et al.*, 2008: Table 8). Biota which live in or near sediments with PAH concentrations below the **toxic effects response low** (**ERL**) will rarely exhibit toxic effects. Biota which live in or near

sediments with PAH concentrations above the **toxic effects response median (ERM)** will often exhibit toxic effects (Wade *et al.*, 2008).

The CCME (2002) developed interim sediment quality guidelines (Table 9). In marine sediments individual PAH concentrations should be <0.2 mg/kg dry weight (wt). Also, marine sediments with individual PAH concentrations < 1.5 mg/kg will result in over 50% of organisms experiencing toxicological effects. For example, 0.763 mg/kg dry wt of benzo[*a*]pyrene in marine sediments results in 78% of organisms experiencing toxicological effects (CMME, 2002).

1.5 Effects of PAHs in the Sydney Tar Ponds

The harmful effects of the contamination in the Sydney Tar Ponds became apparent when lobsters (*Homarus americanus*) collected from the adjacent Sydney Harbour were highly contaminated with PAHs and PCBs (Prouse, 1994; Sirota *et al.*, 1983). This resulted in the closure of the Sydney Harbour lobster fishery (Prouse, 1994; Sirota *et al.*, 1983). Researchers have linked the high PAH and PCB concentrations in *H. americanus* tissue with an influx of pollutants from the Sydney Tar Ponds (Tay *et al.*, 2003).

Past research on the Sydney Tar Ponds had an abiotic focus. Studies examined the type, concentrations, and distribution of contaminants (AMEC, 2005; Furimsky, 2002). There are limited studies investigating the magnitude of contaminants in biota from the Sydney Tar Ponds and the surrounding areas (AMEC, 2005; Ernst *et al.*, 1999; Jones, 2007; Hale, 2004). There are fewer studies which address the toxicological effects

of contamination on the organisms which live in or around the Sydney Tar Ponds (Paetzold *et al.*, 2009; Tay *et al.*, 2003; Vandermeulen and Mossman, 1996).

Little research has focused on the bioaccumulation of PAHs in fishes or invertebrates from the Sydney Tar Ponds. Preliminary ecotoxicology analyses by J. Hellou and M. Jones for the presence of 85 PAHs found high levels of PAHs in three mummichogs (*Fundulus heteroclitus*) (J. Hellou and M. Jones, unpublished data). Among the *F. heteroclitus* analyzed from the Tar Ponds, there was high variability in the PAH concentrations. In the reference *F. heteroclitus* the PAH concentrations were low or below detection limits. The five PAHs detected at the highest concentrations in the Tar Pond *F. heteroclitus* included compounds such as: acenaphthene, anthracene, fluorene, fluoranthene, and phenanthrene. Jones (2007) suggested that more ecotoxicological research is needed prior to commencement of the remediation project in the Tar Ponds.

1.6 Assessment of Ecosystem Health

Aquatic organisms are often used as biological monitors and have provided qualitative and quantitative information on the levels of contaminant emissions (Namieśnik, 2001). For effective biomonitoring, the organism must be carefully selected (Walker *et al.*, 2001). The species should be easy to locate and collect at statistically relevant levels to compare between the contaminated and reference sites. It should have measurable and reproducible responses to the contaminant. Finally, the results should be reproducible (Walker *et al.* 2001).

There are many different types of biomonitoring studies. Some biomonitoring studies require the species to be tolerant of adverse environmental conditions and/or to

bioaccumulate the contaminant. Organisms used to assess environmental quality include: bivalves (Devier *et al.*, 2005; Gewurtz *et al.*, 2003), crabs (Eickhoff *et al.*, 2003; Ikonomou *et al.*, 2002), fishes (Baršienė *et al.*, 2006; Hansson *et al.*, 2006; Oliveira Ribeiro *et al.*, 2005; Said and El Agroudy, 2006), parasites (Marcogliese and Cone, 1997*a*, 1998; Turcekova *et al.*, 2002; Williams *et al.*, 1992), plants (Hale, 2004), and animals (Ross *et al.*, 2004).

Biomonitoring studies are very diverse. The various types of biomonitoring can be broadly grouped into four categories (Walker *et al.*, 2001). **Type 1 biomonitoring** traces the change in species composition in an ecosystem (Iliopoulou-Georgudaki *et al.*, 2003; Külköylüoğlu, 2004). **Type 2 biomonitoring** traces the change of chemical concentration in biota (Levinton *et al.*, 2006; Vuorinen *et al.*, 2006; Yunker *et al.*, 2002). It does not necessarily require the analysis of the species of concern. For example, for the monitoring of protected or endangered species, their dietary items could be analyzed to approximate the potential for contaminant bioaccumulation (Moriarty, 1999). **Type 3 biomonitoring** traces the toxicological effects induced, on or in the organism, by the contaminant (Bright and Ellis, 1990; Simms and Ross, 2000; Weis *et al.*, 2003). **Type 4 biomonitoring** traces the development of genetic resistance as a biomonitor (Minier *et al.*, 1999).

1.7 Parasites as Indicators of Ecosystem Health

Many studies have demonstrated that parasites can be used in assessing ecosystem health (Cone *et al.*, 1993; Hanzelova, 1992; Landsberg *et al.*, 1998; MacKenzie, 1999; Marcogliese, 2001; Thompson *et al.*, 2005). Parasites can be used in Type 1 (Cone *et al.*, 1993; Sanchez-Ramirez *et al.*, 2007; Sasal *et al.*, 2007) and Type 2 biomonitoring (Eira *et al.*, 2005; Howell, 1983; Sures, 2001; Sures and Siddall, 2001; Sures *et al.*, 1999; Sures *et al.*, 2003). The utilization of parasite biomonitors has been explored for both terrestrial and aquatic hosts such as: birds (Barus *et al.*, 2000), cattle (Sures *et al.*, 1998), fishes (Brotheridge *et al.*, 1998; Landsberg *et al.*, 1998), harbour porpoises (Szefer *et al.*, 1998), and rabbits (Eira *et al.*, 2005). The majority of studies on the potential use of parasites as biomonitors have focused on sites contaminated with inorganics (Gheorgiu *et al.*, 2006; Hernandez *et al.*, 2007; Pascual and Abolto, 2005; Schludermann *et al.*, 2003). Few studies have investigated the use of parasites as biomonitors in sites contaminated with PAHs (Diamant *et al.*, 1999; Faulkner and Lochmiller, 2000; Khan, 2003), or the effect of these contaminants on parasite communities in host populations *in situ* (Schmalz Jr. *et al.*, 2002). Even fewer published studies have examined parasite communities in hosts residing in such highly contaminated environments as the Sydney Tar Ponds.

1.7.1 Using Parasites in Type 1 Biomonitoring

Type 1 biomonitoring traces the change in parasite prevalence, abundance, and/or communities in a host population (Cone *et al.*, 1993; Sanchez-Ramirez *et al.*, 2007; Sasal *et al.*, 2007). **Prevalence** is the number of hosts infected with at least one parasite divided by the number of fish examined and multiplied by 100 (Bush *et al.*, 1997). **Abundance** is the quantity of a particular parasite species found in one host regardless of

whether or not the host is infected (Bush *et al.*, 1997). In a stressed ecosystem, the changes in prevalence and abundance of parasite infection will vary based on a variety of factors. Parasites vary in their life cycles. Some parasites have a complex life cycle, which is when several hosts, called **intermediate hosts**, are required for parasite development. If one or more of the intermediate hosts are not present in the ecosystem, the parasite will not be transmitted and overtime will be removed from the ecosystem (Hechinger *et al.*, 2007; Hudson *et al.*, 2006; Marcogliese, 2005; Whitney *et al.*, 2007: Figure 4). Many parasites utilize intermediate hosts which are sensitive to changes in the environment (Bellas and Thor, 2007; Holcombe *et al.*, 1987; Külköylüoğlu, 2004; Raisuddin *et al.*, 2007). In contaminated environments these intermediate hosts may have decreased population levels or be extinct from that ecosystem. The result may be a decreased level of parasite infection or complete removal of that parasite from the ecosystem (Cone *et al.*, 1993; Koprivnikar *et al.*, 2002; Whitney et al., 2007). Parasite life cycles may include a **free-living stage**, which is a larval parasite not found in a host. Environmental contaminants generally decrease the survival of free-living parasite stages (Cross *et al.*, 2005; Pietrock and Marcogliese, 2003; Pietrock *et al.*, 2002; Reddy *et al.*, 2004). Many free-living stages have a protective coating, but studies have found it ineffective in protecting parasites from contaminant toxicity (Pietrock and Marcogliese, 2003).

In contaminated areas metals or organic contaminants may accumulate in parasite tissues (Sures *et al.*, 1999; Sures and Siddall, 2003). Highly tolerant parasite species may be more prevalent in contaminated environments compared to less tolerant species which would succumb to the toxic effects of the accumulated contaminants (Bhuthimethee *et al.*, 2005; Cone *et al.*, 1993). Parasites vary in their physiology and thus in their abilities to

bioaccumulate contaminants; acanthocephalans and cestodes have been found to accumulate metals at a higher level compared to nematodes (Sures *et al.*, 1998). Nematodes are examples of parasites with a lower bioaccumulation efficiency. This may allow nematodes to live in hosts residing in highly contaminated environments where parasites with high bioaccumulation efficiency, similar to acanthocephalans and cestodes, could not. In contaminated environments, parasites with high tolerance to contaminants and low bioaccumulation efficiency may be favoured.

Some studies have found that increased pollution results in increased parasitism (Brotheridge *et al.*, 1998; Gendron *et al.*, 2003; Khan, 2003). This may possibly be caused by: an exclusion of parasite predators, a decrease in the host's resistance, or an optimization of the intermediate host's habitat (Möller, 1987). Other studies have found pollution to have an antagonistic effect on parasitism (Bhuthimethee *et al.*, 2005; Pietrock and Marcogliese, 2003; Whitney *et al.*, 2007). In theory, as a contaminated site is remediated, the parasite populations should be restored to similar prevalences and abundances as reference sites (Huspeni and Lafferty, 2004; Marcogliese and Cone; 1997*a*).

1.7.2 Parasite Fauna Used in Biomonitoring

Parasites are extremely diverse and many different groups have been evaluated for their utilization in biomonitoring (Eira *et al.*, 2005; Pascual and Abollo, 2005; Sanchez-Ramirez *et al.*, 2007; Sasal *et al.*, 2007; Sures *et al.*, 2003; Szefer *et al.*, 1998). Monogenes and parasitic copepods are **ectoparasites**, which live on the gills, fins, or surface of the fish. Acanthocephalans, cestodes, trematodes, and nematodes are **endoparasites**, which live in or on the internal organs of the fish. Brief descriptions of

each major group of ectoparasites and endoparasites are included here, but additional information on parasites found in/on Nova Scotia fishes is in Appendix A.

Monogenes (Phylum: Platyhelminthes) are parasites with high host- and site-specificity, which implies they will only attach to a few types of hosts and in a narrow range of locations on the host (Pechenik, 2000; Roberts and Janovy, 2000). At the posterior end of monogenes is a holdfast of hooks or suckers, which are structured specifically to attach to a particular location on its host. Typically, they have only one host in their lifecycle. Sexually mature monogenes release eggs into the environment, which then hatch to release a free-living, ciliated larval phase. The larval monogene finds a host and attaches itself using its holdfast (Pechenik, 2000; Roberts and Janovy, 2000).

Parasitic copepods (Phylum: Arthropoda) are commonly found on the gills of North American fishes (Bere, 1930; Kabata, 1979; Wilson, 1788). Parasitic copepods have adaptive antennas modified into sharp claws to attach to fish gill filaments (Bere, 1936; Kabata, 1979). Their life cycle has eight free-living larval stages after which they attach themselves to a fish gill filament (Roberts and Janovy, 2000).

Acanthocephalans (Phylum: Acanthocephala) are commonly known as thorny-headed worms. Mature acanthocephalans have an unsegmented, elongated body, with a thorny-head, called a proboscis, which is used as a holdfast in its host's digestive system. Proboscides variation is used in the identification of acanthocephalan species (Margolis and Arthur, 1979). Acanthocephalans have no digestive system and rely on the absorption of nutrients from their host (Roberts and Janovy, 2000). Acanthocephalan life cycles require at least two hosts. The definitive host releases fertilized eggs into the water. The eggs are ingested by an arthropod, such as an insect or crustacean, where the

eggs hatch and develop. The arthropod is then ingested by the **definitive host**, where the parasite reaches sexual maturity (Roberts and Janovy, 2000).

Cestodes (Phylum: Platyhelminthes) are commonly know as tape worms. They lack a digestive system and feed by absorbing nutrients from digestive juices in the intestine of vertebrates. Cestodes are a segmented flatworm and have a holdfast, called a scolex, composed of suckers, grooves, hooks, spines, and/or tentacles (Roberts and Janovy, 2000). Cestode life cycles are highly variable and may be extremely complex (Roberts and Janovy, 2000). The definitive host releases fertilized eggs, which are then generally ingested by an arthropod. The intermediate host is then ingested by another intermediate host or the definitive host (Pechenik, 2000). The number of intermediate hosts depends on the cestode species.

Nematodes (Phylum: Nematoda) are commonly known as roundworms (Roberts and Janovy, 2000). Nematodes are elongated, tapered at both ends, and have a protective covering called a cuticle. Nematode development requires the moulting of the cuticle layer four times to reach maturity and a series of intermediate hosts. Unlike acanthocephalans and cestodes, nematodes have a digestive system (Roberts and Janovy, 2000).

1.8 Potential Biomonitors for the Sydney Tar Ponds

The Sydney Tar Ponds are currently in the initial stages of remediation. Over a number of years the Sydney Tar Ponds and the surrounding contaminated areas will be remediated by an *in situ* solidification and stabilization process, which isolates the contaminants on site (W. Kaiser, personal communication). Stabilization and solidification was first utilized in the 1970s and is currently a commonly utilized remediation technology (Conner and Hoeffner, 1998*a*). Solidification reduces the permeability of the contaminant from the soil, by the injection of liquid substances, like cement, into the sediment to form a solid block (Conner and Hoeffner, 1998*a*). Solidification prevents the movement of water through the contaminated sediment, which decreases the movement of contaminants into the surrounding environment (Conner and Hoeffner, 1998*b*; Mulligan *et al.*, 2001; Oosterhoudt *et al.*, 2004). Stabilization reduces the leachability of the contaminant through the formation of chemical bonds to chemically immobilize and/or reduce the solubility of the contaminant (Conner and Hoeffner, 1998*b*; Mulligan *et al.*, 2001; Oosterhoudt *et al.*, 2004).

In order to track and evaluate the effectiveness of the remediation, baseline values of the degree of contamination need to be established. Many studies have determined the concentration of inorganic and organic contaminants in the soil (AMEC, 2005; Furimsky, 2002; JWEL-IT, 1996*a*, *b*; Vandermeulen, 1989). Little research has been conducted to determine the concentrations of any contaminants in biota that live in or around the Tar Ponds (AMEC, 2005; Ernst *et al.*, 1999; Hale, 2004; Jones, 2007).

Many invertebrate and vertebrate species reside in the Sydney Tar Ponds (AMEC, 2005; Jones, 2007). This study focused on grass shrimp (*Palaemonetes* spp.), European green crab (*Carcinus maenas*), American eels (*Anguilla rostrata*), and mummichog

(*Fundulus heteroclitus*). *Palaemonetes* spp. occur in freshwater and marine habitats; they are commonly found in estuaries near submerged vegetation (Pechenik, 2000; Squires, 1990). Detritus and diatoms have been found in their stomachs (Squires, 1990). They are prey for larger crustaceans and fishes.

Carcinus maeanas is an invasive species found in shallow marine and estuarine habitats (Naczk *et al.*, 2004; Pechenik, 2000). They are thought to have been introduced to the North American Atlantic coast in the mid-1800s and spread to Nova Scotia around the 1950s. *Carcinus maenas* has a high reproductive output, is tolerant of a wide range of salinity and temperature, and thrives in high densities; these qualities allow *C. maeanas* to invade a diversity of aquatic habitats (Naczk *et al.*, 2004). They are aggressive omnivores and gut content analyses have found bivalves, snails, annelids, crustaceans, and algae (Naczk *et al.*, 2004).

Anguilla rostrata is a catadromous fish, which means that sexually mature individuals migrate from fresh water to salt water for spawning. Within a year, larval *A. rostrata* migrate into Canadian waters from the Sargasso Sea where the adults congregate to spawn (Scott and Crossman, 1973; Scott and Scott, 1988). The main food source for young *A. rostrata* is plankton (Scott and Scott, 1988). Once the young eels reach Canadian waters, they metamorphose into juvenile eels, and begin to feed on a diversity of fishes and invertebrates. In estuaries and freshwater, *A. rostrata* spend the day buried in the sediments and feed actively in the evening (Scott and Crossman, 1973). They are generally solitary fish, with a restricted home range (Barker, 1997; Smith and Tighe, 2002). *Anguilla rostrata* overwinter buried in sediments (Scott and Scott, 1988). The females can grow about 1m in length, while the males generally do not grow larger than 0.6 m (Scott and Scott, 1988). Larval *A. rostrata*, elvers, are prey for other fishes and

older *A. rostrata* (Scott and Scott, 1988). Adult *A. rostrata* are rarely prey, but great black-backed gulls (*Larus marinus*) and some diving birds, such as double-crested cormorants (*Phalacrocorax auritus*), have been documented to eat them in Nova Scotian waters (M. Jones, personal communication; Scott and Crossman, 1973; Scott and Scott, 1988).

Fundulus heteroclitus is tolerant to a wide range of salinities, dissolved oxygen, and turbidity (Scott and Crossman, 1973; Scott and Scott, 1988). They are commonly found in salt marshes and estuaries, but can also be found in freshwater (Scott and Crossman, 1973). *Fundulus heteroclitus* are found in shoreline vegetative areas and are highly tolerant to environmental change (Able, 2002). They are not known to undertake migrations (Scott and Scott, 1988). Fundulus heteroclitus have limited home ranges and have high site fidelity (Lotrich, 1995; Paetzold *et al.*, 2009; Skinner *et al.*, 2005; Sweeney *et al.*, 1998). In Canadian waters, *F. heteroclitus* generally do not exceed 13cm (Scott and Crossman, 1973). During the breeding season, mature males and females can be distinguished by colouration patterns. Males have a dark appearance with thin, silvery vertical bars. Females have a silvery appearance with thin, black vertical bars (Scott and Crossman, 1973). Their heads are adapted for surface feeding, but they are opportunistic, omnivorous feeders. They feed on: small crustaceans, polychaetes, insect larvae, vegetation, and small fishes (Scott and Crossman, 1973; Scott and Scott, 1988). Stomach analyses have found them to ingest detritus, but this is suggested to be from accidental ingestion (Able, 2002).

Anguilla rostrata and shrimp (*Palaemonetes pugio*, *Pandalus borealis*) have previously been evaluated for their uptake, toxicology and elimination of organic contaminants (Oliveira Ribeiro *et al.*, 2005; Dillon, 1981, 1982; Hellou *et al.*, 1997).

Many studies have focused on the bioaccumulation of organic contaminations in crabs (Ikonomou *et al.*, 2002; Pancirov and Brown, 1977; Rouleau *et al.*, 1999; Yunker and Cretney, 2000). Studies have demonstrated crabs to be effective biomonitors (Burkhard *et al.*, 1997; Hale, 1988; Hellou *et al.*, 1994; Mothershead *et al.*, 1991; Yunker and Cretney, 2000). *Fundulus heteroclitus* has been examined for its potential use as a Type 2 biomonitor (Couillard and Nellis, 1999).

The parasites living in and on *F. heteroclitus* and *A. rostrata* may be used in Type 1 biomonitoring. *Fundulus heteroclitus* are hosts to a diversity of parasites. Past studies have found parasites in or on the eyes, gills, intestine, liver, skin, and stomach of *F. heteroclitus* (Dillon, 1966; Harris and Vogelbein, 2006; Hawley, 1998; Marcogliese, 1995).

Parasite populations have been extensively studied for *Anguilla* spp. (Aguilar *et al.*, 2005; Gollock *et al.*, 2004; Graynoth and Taylor, 2004; Kennedy *et al.*, 1998; Sures *et al.*, 2003; Rodriguez *et al.*, 2005), but there are limited studies on *A. rostrata* (Barker and Cone, 2000; Marcogliese and Cone, 1998). Despite the limited studies, the parasites of *A. rostrata* have been found to be useful biomonitors of ecosystem stress (Cone *et al.*, 1993; Marcogliese and Cone, 1997*a*). There is a possibility that parasites in these fish hosts may be used as biomonitors.

1.9 Objectives of this Study

This study evaluated biota for their potential use in Type 1 or 2 biomonitoring of the Sydney Tar Ponds remediation. This study measured PAH concentrations in grass shrimp (*Palaemonetes* spp.), European green crab (*C. maenas*), American eel (*A. rostrata*), mummichog (*F. heteroclitus*), and sediment of the Sydney Tar Ponds and reference estuaries. These PAH concentrations will provide baseline data for the long-term monitoring of the effectiveness of the remediation project on the biota and sediment of the Sydney Tar Ponds. Also, this study compared the parasite communities in *A. rostrata* and *F. heteroclitus* from Sydney Tar Ponds to reference sites. The goal of the parasitological component of this study was to determine the prevalence and abundance of various parasite species in the fishes from Sydney Tar Ponds and to investigate the potential use of parasites as part of a biomonitoring protocol for the Sydney Tar Ponds.

2. METHODS

2.1 Field Collection

Biota and sediment samples were collected from the North and South Tar Ponds, Sydney, Cape Breton, Nova Scotia, and two Cape Breton reference sites: Mira River and River Ryan (Figure 5). The approximate locations of biota and sediment Tar Pond collections are illustrated in Figure 6. Sampling localities within the reference sites were selected based on such physical parameters as topography, tidal regime, and salinity. Aquatic biota were collected during the summer of 2006 from these sites using minnow traps baited with white bread and eel pots baited with sardines. Fishes were anesthetised using clove oil in accordance with animal care guidelines of both Cape Breton University and Saint Mary's University Animal Care Committees. Biota were frozen and stored in a freezer at -20°C until chemical analysis. The invertebrate and vertebrate species collected for chemical analysis included: grass shrimp (*Palaemonetes* spp.), European green crab (*C. maenas*), and mummichog (*F. heteroclitus*) from the Sydney Tar Ponds, Mira River, and River Ryan (Tables 10-11). American eel (*A. rostrata*) were collected from the Tar Ponds and Mira River, but not River Ryan.

Sediment samples were collected from the North and South Tar Ponds, Mira River, and River Ryan during July 2007. Prior to collection, all glassware was rinsed several times with hexane to remove organic contaminants from the inner surface. Sediment samples were collected in triplicate using a Petite Ponar grab and then frozen at -20°C within 24 hours of collection until extraction and instrumental chemical analysis.

Anguilla rostrata and *F. heteroclitus* were collected for parasitological analysis in June-August 2007. *Anguilla rostrata* were collected from Sydney Harbour (n = 5), Mira

River (n = 10), and the North Tar Pond (n = 6) using eel pots baited with sardines. Sydney Harbour was used as a reference site for parasite analysis, because *A. rostrata* were not caught in River Ryan (Figure 5). *Anguilla rostrata* were sampled where the Wentworth Park duck pond empties into Sydney Harbour. *F. heteroclitus* were collected from River Ryan (n = 62), Mira River (n = 60), and North Tar Pond (n = 49) (Table 12). *Fundulus* spp. were collected from River Ryan (n = 7), Mira River (n = 4), and the North Tar Pond (n = 4) (Table 12); these fishes could not be identified to the species taxonomical level. *Fundulus* spp. displayed morphological characteristics of both *F. heteroclitus* and *F. diaphanus*. Other studies have found *Fundulus* hybrids in Nova Scotia (Chávez and Turgeon, 2007; Fritz and Garside, 1974). In the reference sites, *Fundulus heteroclitus* and *Fundulus* spp. were collected using minnow traps baited with white bread or a beach seine. In the North Tar Pond, *Fundulus heteroclitus* and *Fundulus* spp. were only collected using minnow traps baited with white bread. After initial external parasitological analysis (see 2.3 Parasitological Analysis section for details), fishes were anesthetised with clove oil and frozen (-20°C) within 12 hours of collection.

2.2 PAHs Analysis

Biota and sediment samples were analyzed by the Research and Productivity Council (RPC), Fredericton, New Brunswick. Analyses were based on U.S. Environmental Protection Agency (EPA) Method 3540C (US EPA, 1996a) and 3510 (US EPA, 1996b) for PAH extraction from sediment and biota samples respectively and EPA method 8270C for gas chromatography/mass spectrometry (GC/MS) analysis (US EPA, 1996c).

Five grams of sediment were ground by mortar and pestle in 10g of anhydrous sodium sulfate and placed in an extraction thimble of a Soxhlet extractor. One ml of surrogate solution consisting of 2-fluorbiphenyl and *p*-terphenyl-d_{14} was added to the sediment and sodium sulfate mixture. Boiling chips and 300mL of acetone:hexane (1:1) extraction solution was placed in a 500mL round bottom flask and attached to the Soxhlet extractor. The mixture was refluxed in the Soxhlet extractor for 16 to 24 hours at 4 to 6 cycles/hour. The solution was removed from the round bottom flask and placed in a 1L separatory funnel with 300mL double distilled water and mixed for 1-2 minutes to partition the extract. The hexane solution was removed from the separatory funnel and dried by passing through a short column (35 cm X 2.1 cm ID) filled with anhydrous sodium sulfate. The column was washed with 100-125ml of hexane. The resulting eluate was concentrated to 10mL on a rotary evaporator. Sample fractionation and cleanup was performed by Gel Permeation Chromatography (GPC) and the final volume was adjusted to 10ml using hexane.

Biological data were collected prior to sending biota samples for chemical analyses (Table 10-11). The sex, length, and weight were recorded for each organism. **Carapace length** of *C. maenas* was recorded, which is the longest width across the carapace, or shell. Standard and total lengths were recorded for most *F. heteroclitus*. If the caudal (tail) fin was damaged, only the standard length was recorded. Total length was recorded for *A. rostrata*. The **standard length** is the distance between the tip of the snout to the posterior end of the vertebral column. The **total length** is the distance between the tip of the snout to the anterior end of the tail. A 5g wet weight tissue sample was submitted for PAH analysis,. Several organisms of the same species were pooled where individuals weighed less than 5g (Table 10-11).

Tissues were homogenized to a free flowing power with a mortar and pestle. Following homogenization, the biota samples were saponified with 20 mL 6N ethanolic potassium hydroxide for 18 hours at 40°C. One mL of surrogate standard was added to the saponified sample. The saponified sample was placed in a 1L separatory funnel and extracted with 60 mL of hexane. The hexane solvent was collected after the mixture was allowed to settle for at least 30 min. As with the sediment PAH extraction, the biota hexane extract was dried on a sodium sulfate column, concentrated by rotary evaporator, and finally cleanup and fractionation via GPC.

Both the hexane extracts from the sediment and biota samples were analyzed by an Agilent gas chromatograph coupled with a mass spectrometer (GC/MS). Sample analysis was accomplished by GC/MS on a 30m X 0.32mm I.D., 1μm film thickness fused silica DB-5 column. Ultra high purity helium, supplied at a constant flow of 2 mL/min, was used as a carrier gas. On column injection was programmed: 50°C initial temperature ramped to 270°C at 100°C/min with with a 1μl injection volume. Oven temperature was programmed 50°C for 1 min ramped at 25°C/min to 270°C.

Organic carbon content of Sydney Tar Pond sediment samples was determined by a Leco combustion analyzer. Organic carbon content was not determined for Mira River or River Ryan. Lipid content of biota samples was determined gravimetrically. A known amount of the biota hexane extract was weighed and then heated. After heating, the extract was reweighed. By comparing the differences in weight before and after heating, the percent of lipid was determined. The raw data from PAH sediment and biota concentrations, biota lipid analysis, and organic carbon are in Appendix B.

For quality assurance and quality control (QA/QC) samples were analyzed in batches not exceeding fifteen. Reagent blanks, duplicates, and spiked blanks were each

run at least once per preparation batch. Surrogate standards included 2-fluorobiphenyl and p-terphenyl-d_{14}. Detection limits for PAH analysis were 0.1 to 0.01 mg/kg.

2.3 Parasitological Analysis

An initial external parasitological analysis was conducted prior to anesthetising the fish. The fins and skin were examined for the presence and abundance of parasitic crustaceans, such as sea lice (*Argulus* spp.). *Argulus* spp., a brachiurid, were removed and preserved in formalin for future reference and identification. After anesthetising the fish, the standard and total lengths, weight, and sex were recorded for each *F. heteroclitus* and *Fundulus* spp. Only total length and weight were recorded for *A. rostrata.*

Fundulus heteroclitus and *A. rostrata* were thawed prior to necropsy. The gills were removed from the branchial chamber and the gill arches were separated to examine the gill filaments for the presence and number of parasitic crustaceans, monogenes, and trematode metacercaria. All ectoparasites, except *Argulus* spp., were preserved in 95% ethanol. Parasites were preserved in ethanol to allow for potential genetic work.

The musculature, body cavity, gonads, and viscera, which includes the stomach, intestine, liver, heart, and spleen, were thoroughly examined for macroparasites using a dissecting microscope. For *A. rostrata*, the swim bladder was also examined. The location, abundance, and general group of each parasite were recorded for each individual fish. The genus of each group of parasite was determined with microscopic examination. Endoparasites were preserved in 95% ethanol.

Fundulus spp. were necropsied as dicussed for *F. heteroclitus*. Only a few unidentified *Fundulus* spp. (i.e. potential hybrids) were collected from River Ryan (n =

6), Mira River (n = 4), and the North Tar Pond (n = 4). Because of the uncertainty if the *Fundulus* spp. were hybrids, *F. heteroclitus*, or *F. diaphanus*, these fishes were not included in data analysis. The raw data from the *Fundulus* spp., *F. heteroclitus*, and *A. rostrata* necropsies are in Appendix C and D, respectively.

Glycerine mounts of a subsample of each type of parasite were prepared for taxonomic identification. The mounts were prepared by placing several drops of water on a slide, and ethanol-preserved or freshly removed parasites were placed in the water. Several drops of 95% glycerine were placed on top, and the parasite was covered with a cover slip. Slides were left for at least 24 hours prior to microscopic examination.

2.4 Data Analysis

The Ryan-Joiner method was used to assess normality in the Tar Pond sediment PAH data, which was subsequently found to be normally distributed (Iha *et al.*, 2009; Quijón *et al.*, 2008; Robinson *et al.*, 2007). A two way analysis of variance (ANOVA) was performed on the Tar Pond sediment data only. The control sites were not included in the ANOVA due to the high proportion of non-detects (observations below the detection limit). There were only seven and six detects of PAHs (n = 51 PAH measurements/site) respectively in Mira River and Ryan River. There were 87% non-detects in the control sites. Non-detects were not observed in Tar Ponds sediment PAH data.

Biota PAH values were highly variable and were often left-censored data, which is data below detection limits. These nondetection values prevented the calculation of descriptive statistics, such as the mean and standard error. Various methods were

investigated to allow the calculation of descriptive statistics. The Kaplan-Meier or product limit estimator method was the most appropriate analytical method for these data due to the small sample size and non-normal distribution (Helsel, 2006; Helsel and Hirsch, 2002). This method is normally used for right-censored, which is data that exceeds the detection limits; thus, the data were inverted to transform the left-censored data set to right-censored. The Kaplan-Meier method was performed on the transformed data using Minitab version 15. The total PAH concentration (ΣPAH) was calculated based on the sum of the 18 PAHs analyzed.

Studies typically utilize lipid-adjusted biota PAH values in comparing PAH concentrations among different species (Brunson *et al.*, 1998; Galloway *et al.*, 2004; Hickey *et al.*, 1995; Landrum *et al.*, 2007; Moermond *et al.*, 2007). Generally, organisms with higher lipid content will have a higher capacity to bioaccumulate organic contaminants compared to organisms with lower lipid content (Di Toro *et al.*, 1991; Klosterhaus *et al.*, 2002, Mackay, 2004; Russell *et al.*, 1999). Thus, the utilization of lipid-adjusted values accounts for the variation in organismal bioaccumulation capacity. Initially, the wet weight PAH values were converted to lipid-adjusted PAH concentrations (see Appendix B). Kruskal-Wallis tests showed no significant difference between wet weight and lipid-adjusted biota PAH concentrations (Table 16). The use of lipid-adjusted PAH concentrations instead of wet weight PAH values did not provide any advantage, thus, wet weight PAH concentrations were utilized in comparison among biota due to their inherent simplicity.

Many of the PAHs were not detected in the fishes; thus, six PAHs which were detected in *C. maenas*, *F. heteroclitus*, *A. rostrata*, and *Palaemonetes* spp. were compared among the biota. Acenaphthalene, fluorene, fluoranthene, and phenanthrene were

normally distributed. Naphthalene and pyrene were not normally distributed. Concentrations of naphthalene and pyrene were naturally log transformed to increase normality. A multivariate analysis of variance (MANOVA) was performed. If the Hotelling-Lawley test indicated a significant difference among the biota, individual univariate F tests were completed. A Levene's test was used to test of homogeneity of variances.

For the fish parasitological data the abundance, prevalence, and mean intensity for *A. rostrata* and *F. heteroclitus* were calculated for each site. Abundance is the number of individuals of a particular species of parasite found in one fish (Bush *et al.*, 1997). Prevalence is the percent of fish infected with at least one parasite divided by the number of fish examined (Bush *et al.*, 1997). **Mean intensity** is the mean total number of parasites per infected fish from one site (Bush *et al.*, 1997). Also, the number of parasite species per *F. heteroclitus* was compared among the sites. There were too few samples of *A. rostrata* collected and necropsied to draw any conclusions about differences in parasite assemblages among study sites.

A **condition factor (K)**, or index of plumpness, was calculated to compare the health of *F. heteroclitus* among sites. A higher K value corresponds to a healthier organism than an organism with a lower K value. K is calculated by the following equation (Moyle and Cech, 2004):

$$K = \quad 100 \text{ X } [\text{total body weight (g)}/(\text{total length in cm})^3]$$

A Kruskal-Wallis was performed to determine if there were differences among the condition factors. If significant results were discovered, Dunn's method was used for pairwise multiple comparisons.

3. RESULTS

3.1 PAHs in Sediments

The majority of the eighteen PAHs analyzed were below detection limits in the two reference sites, Mira River and River Ryan (< 0.01 mg/kg dry wt; Table 13). In Mira River sediment samples, fluoranthene and pyrene were both detected at mean concentrations of 0.02 mg/kg dry wt. In River Ryan sediment samples, anthracene was detected at a mean concentration of 0.01 mg/kg dry wt. In one of the sediment samples from River Ryan, chrysene/triphenylene was detected at 0.08 mg/kg dry wt.

All eighteen PAHs analyzed were detected above detection limits in both the North and South Tar Ponds (Table 13). The range of PAH concentrations varied between the Tar Ponds. In the North Tar Pond, acenaphthylene had the lowest mean concentration of 0.5 mg/kg dry wt, while benz[*a*]anthracene had the highest mean concentrations of 4.6 mg/kg dry wt. In the South Tar Pond, acenaphthylene had the lowest mean concentration (2.0 mg/kg dry wet) while fluoranthene had the lowest concentration of 187 mg/kg dry wt.

PAH concentrations were significantly different between the North and South Tar Ponds (ANOVA: Table 14). There were no significant differences ($p = 0.655$) among PAHs. Additionally, there was no interaction between site and PAH ($p = 0.772$) at the Tar Ponds. Higher concentrations of all eighteen PAHs were detected in South Tar Pond sediments than in the North Tar Pond (Figure 7).

Generally, the South Tar Pond had higher organic carbon concentrations compared to the North Tar Pond (Appendix B: Table B2). The range of organic carbon concentrations varied between the Tar Ponds. In the North Tar Pond, the concentration

varied between 2.3 and 7.6% weight. In the South Tar Pond, the concentration varied between 6.7 and 52.9% weight. The organic carbon concentration of 52.9% is an extremely high value compared to the other organic carbon concentrations (Appendix B: Table B2). It is uncertain what caused the high organic carbon concentration. It is likely that there was a concentration of organic industrial waste, such as an aromatic compound, in that sediment sample which would have caused a spike in the organic carbon concentration.

3.2 PAHs in Biota

The majority of biota samples analyzed from Mira River and River Ryan, had PAH concentrations below detection limits (<0.05 mg/kg wet wt: Appendix B). All PAH concentrations from control sites were below detection limits in *C. maenas* (n = 3 per site), *Palaemonetes* spp. (n = 2 and 1, for Mira River and River Ryan, respectively) and *A. rostrata* (n = 2 from Mira River only). All PAH concentrations in *F. heteroclitus* from the Mira River (n = 3) were below detection limits, while one *F. heteroclitus* sample from River Ryan had traces of pyrene and benz[*a*]anthracene both at mean concentrations of 0.04 mg/kg wet wt (Appendix B: B10).

PAHs were detected in thirteen of the fifteen biota samples analyzed from the Sydney Tar Ponds (Appendix B). One *A. rostrata* and one *F. heteroclitus* had PAH concentrations below detection limits (<0.10 and <0.05 mg/kg wet wt respectively). The composition and concentration of PAHs varied among specimens from each species analyzed and among species collected from the Sydney Tar Ponds.

PAHs were detected in all of the *C. maenas* analysed from the Tar Ponds (n = 3 non-pooled samples). ΣPAHs varied from 0.5 to 5.0 mg/kg wet wt in *C. maenas* (Table 15). The type of individual PAHs were not detected with any consistency in *C. maenas* (Appendix B: Table B4). In all *C. maenas* samples, phenanthrene, fluoranthene, and pyrene were detected. The concentration of phenanthrene, fluoranthene, and pyrene varied from 0.06 - 1.06, 0.06 - 0.99, and 0.06 - 0.82 mg/kg wet wt respectively among samples. The only PAH not detected in any of the *C. maenas* samples was dibenzo[*a*,*h*]anthracene (Appendix B: Table B4).

PAHs were detected in all of the *Palaemonetes* spp. samples analysed from the Tar Ponds (3 pooled samples). ΣPAHs varied from 1.8 to 3.1 mg/kg wet wt in *Palaemonetes* spp. (Table 15). The number of PAHs detected in *Palaeomonetes* spp. were not detected with any consistency (Appendix B: Table B6), similar to *C. maenas* sampled from the Tar Ponds. Unlike the *C. maenas* samples, only eight or nine of the PAHs were detected in *Palaemonetes* spp. above detection limits. The PAHs with the highest mean concentrations were fluoranthene, pyrene, and phenanthrene at 0.77, 0.47, and 0.24 mg/kg wet wt respectively.

From the five *A. rostrata* samples from the Tar Ponds, one sample had PAH concentrations below detection limits (Appendix B: Table B8). The ΣPAHs concentrations were highly variable with ΣPAHs ranging from not detected to 2.3 mg/kg wet weight (Table 15). Similar to the other biota analyzed, PAHs were not detected consistently in the five *A. rostrata* samples. Acenaphthene, fluoranthene, naphthalene, and phenanthrene were the only PAHs detected in three of the five *A. rostrata* samples. Of the PAHs detected in *A. rostrata* the PAHs with the highest mean concentrations were

fluoranthene, phenanthrene, and naphthalene at 0.23, 0.24, and 0.22 mg/kg wet wt respectively (Appendix B: Table B8).

PAHs were detected in all of the *F. heteroclitus* samples from the Tar Ponds (2 non-pooled; 2 pooled samples) except one (Appendix B: Table B10). ΣPAHs ranged from not detected to 0.7 mg/kg wet wt (Table 15). In the *F. heteroclitus* samples where PAHs were detected (three of the four samples) only phenanthrene and fluoranthene were detected constantly at concentrations ranging from 0.08 – 0.15 and 0.06 – 0.19 mg/kg wet wt respectively (Appendix B: Table B10). Of the seven PAHs detected in *F. heteroclitus*, the PAHs with the highest mean concentrations were phenanthrene and fluoranthene at 0.10 and 0.09 mg/kg wet wt. The other five PAHs detected in *F. heteroclitus* had mean concentrations ranging fom 0.04 to 0.06 mg/kg wet wt. Like the other biota samples, there was variability in the concentrations of the PAHs among the *F. heteroclitus* sampled (Appendix B).

There was also variability in the composition and concentration of PAH among species collected from the Tar Ponds (Figure 8). Acenaphthene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene were detected in *A. rostrata*, *C. maenas*, *Palaemonetes* spp., and *F. heteroclitus*. *Anguilla rostrata* accumulated only the above six PAHs. *Fundulus heteroclitus* accumulated one additional PAH: anthracene. *Palaemonetes* spp. accumulated the above six PAHs along with anthracene and chrysene/triphenylene. *Carcinus maenas* accumulated all of the PAHs analyzed except dibenz[*a*,*h*]anthracene; thus, *C. maenas* accumulated the greatest number of PAHs compared to the other biota analyzed.

The concentration of PAHs appears to be generally higher in *C. maenas* and *Palaemonetes* spp. compared to *A. rostrata* and *F. heteroclitus* (Figure 8). Only

fluoranthene and pyrene were significantly different among the six PAHs which were found in all biota (MANOVA: Table 16). *Palaemonetes* spp. compared to both *A. rostrata* and *F. heteroclitus* had significantly different fluoranthene concentrations (Tukey test: Table 17). *Palaemonetes* spp. and *F. heteroclitus* had significantly different pyrene concentrations (Tukey test: Table 17). All other pair-wise comparisons were not significant ($p > 0.05$). Homogeneity of variances was confirmed by Levene's test for all PAHs ($p > 0.05$).

3.3 Parasitological Analysis

In total, nine parasite genera were found in or on *F. heteroclitus* (n = 171). *Fundulus heteroclitus* from the Sydney Tar Ponds had the lowest prevalence and abundance of parasites compared to River Ryan and Mira River (Tables 18-19). River Ryan had the highest diversity of parasite genera (Tables 18-19). Several ectoparasites were found on the gills and skin of *F. heteroclitus* from River Ryan and Mira River. Parasitic crustaceans, *Argulus* spp., were found on the gills and skin. Larval trematodes, echinostome metacercariae, and another parasitic copepod, *Ergasilus manicatus*, were found on the gills of *F. heteroclitus* from River Ryan and Mira River. The monogene, *Salsuginus* sp., were found on the gills of *F. heteroclitus* from all three sites.

The only ectoparasite found on *F. heteroclitus* from the Tar Ponds was *Salsuginus* sp. with a prevalence of 8.2% (Table 18). *Salsuginus* sp. had a higher prevalence on *F. heteroclitus* from Mira River and River Ryan of 76.7% and 59.7% respectively. On the gills of *F. heteroclitus* from River Ryan *Salsuginus* sp. were the most prevalent, while echinostome metacercariae were the least prevalent ectoparasite (Table 18). On the gills

of *F. heteroclitus* from Mira River echinostome metacerariae were the most prevalent and *Argulus* sp. was the least prevalent ectoparasite (Table 18).

Endoparasites were found in a diversity of tissues and organs within *F. heteroclitus*, such as the connective tissue, gonads, heart, liver, and spleen (Appendix C). *Fundulus heteroclitus* from the Tar Ponds had the lowest prevalence and abundance of endoparasites compared to the other sites (Table 19). An unidentifiable acanthocephalan and third larval stage (L3) ascarid nematode were the most prevalent endoparasites in *F. heteroclitus* from the Tar Ponds. An unidentifiable cestode and trematode were the least prevalent endoparasite in *F. heteroclitus* from the Tar Ponds. For *F. heteroclitus* from the Mira River, acanthocephalans, *Neoechinochynchus* sp., were the most prevalent endoparasite, while unidentifiable metacerariae and the trematode, *Hamalometron pallidum*, were the least prevalent. Unlike the Mira River results, the unidentifiable metacerariae in *F. heteroclitus* from River Ryan were the most prevalent and abundant endoparasite. The cestode, *Proteocephalus* sp., was the least prevalent endoparasite in *F. heteroclitus* from River Ryan.

Parasite populations and community compositions varied among sites. In over 75% of *F. heteroclitus* from the Tar Ponds there were no parasites (Figure 9). A maximum of three parasite species were observed in 2% of *F. heteroclitus* from the Tar Ponds. In contrast, on average there were two parasite species per individual *F. heteroclitus* from River Ryan (Figure 9); and less than 5% of *F. heteroclitus* from River Ryan had no parasites. *Fundulus heteroclitus* from Mira River had at least two, to a maximum of six, parasite species per fish. On average each Mira River *F. heteroclitus* had three to four parasite species per fish (Figure 9).

With respect to fish health, the condition factors (K) for *F. heteroclitus* varied among the sites. *Fundulus heteroclitus* from the Mira River had the highest K values (1.35 +/- 0.02 std.err.), followed by River Ryan (1.33 +/-0.03), and Tar Ponds (1.22 +/- 0.02). There were significant differences in K among the sites (Kruskal-Wallis, $H = 27.1$, $df = 2$, and $p < 0.05$). Mira River and River Ryan were not significantly different from each other; however, both reference sites were significantly higher than the Tar Ponds.

In total, only three parasite genera could be identified in or on *A. rostrata* ($n = 21$). Parasitic copepods, L3 nematodes, acanthocephalans, and another type of nematode were found (Table 20). A monogene, *Pseudodactylogyurus anguillae*, and an unidentifiable parasitic copepod were found on the gills. There were three unidentifiable endoparasites. An unidentifiable acanthocephalan was found in the intestine and stomach. An L3 nematode and an unidentifiable nematode were found in the connective tissue and swim bladder respectively. The endoparasite genera which could be identified in the digestive tract and swimbladder were cestode, *Bothriocephalus* sp., and nematode, *Anguillicoloides crassus*, respectively.

The parasite populations and communities within *A. rostrata* differed among sites (Appendix D), as observed with *F. heteroclitus*. There were only three parasite species identified in the Tar Ponds; however, in Mira River and Sydney Harbour there were seven and three parasite species identified, respectively. Thus, *A. rostrata* from the Mira River had the greatest parasite species diversity.

4. DISCUSSION

4.1 PAHs in Sediments

PAHs in sediments from the reference sites exhibited trace levels (< 0.05 dry wt mg/kg) of three PAHs: anthracene, fluoranthene, and pyrene (Table 13). The PAH concentrations in Mira River and River Ryan were comparable to PAH concentrations found in reference sites used in other studies around the world (Barbee *et al.*, 2008; Djomo *et al.*, 1996; Kim *et al.*, 2008; Krauss *et al.*, 2000). For example, Kim *et al.* (2008) and Barbee *et al.* (2008) evaluated PAH concentrations in various remote lakes around the world. Both of theses studies found the concentration of PAHs to be below 1 mg/kg dry wt. Thus, the level of PAHs in Mira River and River Ryan are similar to PAH concentrations in other reference sites.

The geography of the Tar Ponds may account for the South Tar Pond being more contaminated with PAHs than the North Tar Pond. Since the North Tar Pond is further away from industrial activities, one would expect the PAH concentration to be lower. Water currents move sediments through the Coke Ovens Brook Connector, into the South Tar Pond, and finally into the North Tar Pond. As sediments move to lower tidal energy sections of the Tar Ponds, particles could settle out of the water column and onto the sediment bed. The net result of this action would be fewer contaminated particles moving into the North Pond compared to the South Pond. Since PAHs are generally sorbed to sediment particles (Arfi and Bouvy, 1995; Kukkonen and Landrum, 1995; Talley *et al.*, 2002), this would result in lower PAH concentrations in the North Pond compared to the South Pond.

There is little research on the differential in PAH concentrations between the North and South Tar Ponds. Querbach (2002) analyzed the distribution of contaminants in sediments from the Sydney Harbour North and South Arm. She collected sediment cores from varying distances from where the North Tar Pond emptied into the harbour. Sediment cores collected at 0.00, 0.57, and 0.92 km from the mouth of Muggah Creek had PAH concentrations of 353.6, 208.3, and 95.0 mg/kg respectively. Sediment cores collected at 0.00, 0.57, and 0.92 km from the mouth of Muggah Creek had PCB concentrations of 7.1, 3.3, and 1.5 mg/kg respectively. In general, there was a decrease in PAH and PCB sediment concentrations with an increased distance from Muggah Creek. JWEL-IT*b* (1996) also collected sediment samples from the Sydney Harbour. PAHs were detected at all sampling stations in the Harbour, but PAH concentration was the highest in the South Arm. They also found that the PAH concentration decreased with an increased distance from Muggah Creek. Although these studies were conducted in the Harbour, it demonstrates a general pattern that sediment PAH concentration decreases with increased distance from contamination.

A similar pattern has been found in other sites. Upon examining the sediments around Ulsan Bay, Korea, Khim *et al.* (2001) found the concentrations of PCBs and PAHs to decrease further away from industrial activities. Simpson *et al.* (1996) and Bieri *et al.* (1986) also found that levels of PAH decreased with increased distances from industrial activities. Since the North Tar Pond is further away from industrial activity, the South Tar Pond should be higher in contaminations.

The sediment PAH concentrations in the Sydney Tar Ponds were higher than other contaminated sites (Bieri *et al.*, 1986; Leite *et al.*, 2008; Voparil *et al.*, 2004). Elizabeth River, Virginia, USA, had a similar history to the Sydney Tar Ponds. Both Elizabeth

River and the Tar Ponds experienced many years of unregulated dumping and many shoreline industrial activities (AMEC, 2005; Huggett *et al.*, 1984; JWEL-IT, 1996*a*, *b*; Mitra *et al.*, 1999; Mulvey *et al.*, 2002; Walker and Dickhut, 2001). In sediment samples from Elizabeth River, PAH concentrations varied between 6-42 mg/kg dry wt for benzo[*e*]pyrene and fluoranthene respectively (Bieri *et al.*, 1986: Table 5). The North Tar Pond sediment concentrations were similar to or lower than Elizabeth River sediment concentrations. Yet the South Tar Pond sediment concentrations were higher than Elizabeth River sediment concentrations. Benzo[*e*]pyrene and fluoranthene concentrations were 41 and 187 mg/kg dry wt respectively in the South Tar Pond, and 6 and 42 mg/kg dry wt respectively in Elizabeth River sediments (Bieri *et al.*, 1986).

The PAH concernations in the Tar Ponds are well beyond the CCME guidelines for marine sediments (Table 9; Figure 7). Acenaphthylene had the lowest mean concentration in both the North (0.5 mg/kg dry wt) and South (2.0 mg/kg dry wt) Tar Ponds. The acenaphthylene CCME interm marine sediment guideline is 0.00587 mg/kg dry wt; thus, the acenaphthylene levels in both the Tar Ponds are about 100 fold higher than CCME guidelines (CCME, 2002). According to CCME (2002), 51% of biota exposed to 0.128 mg/kg dry wt acenaphthylene concentrations will exhibit adverse toxicological effects. Acenaphthylene sediment concentrations of 0.64 mg/kg will often cause biota which live in or near sediments to exhibit toxicological effects (CCME, 2002; Wade *et al.*, 2008; Tables 8-9). Thus, the sediment concentrations are potentially toxic to biota, which live in or near the Tar Pond sediments.

High variability (i.e. standard errors) was observed among samples from each respective site. All sediment samples were collected within 1m of each other. Despite the samples being sampled from the same locality, there was high variability. Also,

ANOVA revealed no significant differences among the PAHs ($p = 0.655$). Both these factors suggest that the concentrations of PAHs were not homogeneously distributed throughout the Tar Ponds. Previous studies have demonstrated heterogenous distribution of PCBs in the Tar Ponds (AMEC, 2005) and the Sydney Harbour (JWEL-IT*b*, 1996). The precise mechanism of the spatial heterogeneity is unknown.

Numerous studies have documented seasonal activities causing heterogeneity in PAH sediment concentrations (Bierman, 1990; Liang *et al.*, 2007; Moermond *et al.*, 2005). Maruya *et al.* (1997) observed heterogeneity in sediment PAH concentrations. Heterogeneity was attributed to variation in black carbon content of the sediment particles. PAHs sorbed to the black carbon, and during the rainy season particles washed into the marsh by surface runoff. During the dry season, winds and tidal activity resuspended and transported particles through the system. Particles settled out onto the sediment bed when they entered lower tidal energy portions of the marsh. The Tar Ponds do not have a rainy and dry season. In the Tar Ponds, PAHs may partition to the black carbon and be suspended in the water column. The particles would be transported though the Tar Ponds to low energy areas of the estuary; thus, in low energy portions of the Tar Ponds, there would be higher levels of PAHs compared to higher energy portions of the Tar Ponds.

Unregulated dumping would be the most likely source of heterogeneity. For over 100 years industries were established around the Muggah Creek estuary (AMEC, 2005). At the time there were few waste mangament guidelines, and these industries released many of their wastes into the Muggah Creek. Also, material dump sites were established along Muggah Creek (AMEC, 2005). The unregulated dumping may have resulted in

pockets of contamination where the contaminats were released into the environment, thus, causing heterogeneity in the Tar Ponds.

4.2 PAHs in Biota

The mean PAH concentrations in biota from the two reference sites were below 0.05 mg/kg. In relatively uncontaminated environments, like Mira River and River Ryan, studies have found biota from these sites to have PAH concentrations typically less than 0.01 mg/kg (Pancirov and Brown, 1977; Vives *et al.*, 2004: Table 6). The concentrations of PAHs in biota from the Tar Ponds were much higher compared to biota from other contaminated sites in other studies (Eickhoff *et al.*, 2003*a*; Lima *et al.*, 2008; Nakata *et al.*, 2003: Table 7). Typically the concentration of organic contaminants is higher in the hepatopancreas of crabs and muscle tissue of fishes, respectively (Fernandes *et al.*, 2007; Hale, 1988; Hellou *et al.*, 1994). Eickhoff *et al.* (2003*a*) found the concentration of PAHs to be less than 5 µg/kg (0.005mg/kg) wet wt in the hepatopancreas of Dungeness crab (*Cancer magister*) from an aluminum contaminated site in Kitimat Arm, British Columbia. Anthracene, fluoranthrene, and chrysene were the PAHs with the highest concentrations at 2.09, 4.29, and 2.95 µg/kg wet wt respectively (Eickhoff *et al.*, 2003*a*). Lima *et al.* (2008) analyzed the PAH concentrations in fish (shanny, *Lipophrys pholis*) muscle from a contaminated site in northwest Europe. The tissue PAH concentrations were below 6 µg/kg wet wt. Phenanthrene and fluoranthene were at highest concentrations of 3.3 and 6.0 ppb wet wt respectively (Lima *et al.*, 2008).

In this study, whole samples, not specific organs, were evaluated for PAHs. *Carcinus maenas* samples from the Tar Ponds had mean anthracene and fluoranthene

concentrations of 80 and 490 μg/kg wet wt respectively (Appendix B: Table B4). *Fundulus heteroclitus* from the Tar Ponds had mean phenanthrene and fluoranthene concentrations of 100 and 90 μg/kg wet wt respectively (Appendix B: Table B10). *Anguilla rostrata* had mean phenanthrene and fluoranthene concentrations of 240 and 230 μg/kg wet wt respectively (Appendix B: Table B18). Thus, the biota from the Tar Ponds is extremely contaminated compared to other contaminated sites. Also, the analysis of whole samples would most likely dilute concentrations of accumulated PAHs in the hepatopancreas and muscle. Yet, the concentration of PAHs of the whole organisms from the Tar Ponds were over 1000 times higher than the concentration of organ and tissue PAHs found by Eickhoff *et al.* (2003*a*) and Lima *et al.* (2008).

4.3 Differential Bioaccumulation of PAHs in Invertebrates and Vertebrates

Carcinus maenas and *Palaemonetes* spp. from the Sydney Tar Ponds accumulated a greater range and concentration of PAHs than *A. rostrata* and *F. heteroclitus* (Table 15; Figure 8). *Carcinus maenas* accumulated all of the PAHs analyzed, but dibenz[*a*,*h*]anthracene was not detected in *C. maenas*. *Palaemonetes* spp. accumulated the same six PAHs accumulated by *A. rostrata* and *F. heteroclitus*, as well as anthracene and chrysene/triphenylene. Invertebrates accumulated greater concentrations of PAHs in their tissues than vertebrates. *Carcinus maenas* and *Palaemonetes* spp. accumulated 2.6 and 2.4 mg/kg wet wt of ΣPAHs, respectively, while *F. heteroclitus* and *A. rostrata* accumulated 0.7 and 1.3 mg/kg wet wt of ΣPAHs, respectively (Table 15).

Nakata *et al.* (2003) measured greater PAH concentrations in Japanese mud crab (*Macrophthalmus japonicus*) and other lower trophic organisms than in coastal fishes,

squid, and finless porpoises. The authors suggested this to be due to crabs directly ingesting sediment. The differences in bioaccumulation may also be due to association with interstitial water and differing biotransformational abilities among taxa.

4.3.1 Role of Ingesting Sediment

Carcinus maenas is strongly associated with sediment and may ingest sediments during feeding. *Carcinus maenas* from the Tar Ponds were observed with thick patches of tar attached to their abdomens, indicating burrowing behaviours (M. Jones, personal communication). The association of *C. maenas* with the sediment could increase the probability of sediment ingestion with food. *Carcinus maenas* prey on a variety of species such as algae, bivalves, juvenile crustaceans, and juvenile fishes (Cohen *et al.*, 1995). *Carcinus maenas* are known to ingest bryozoa, hydrozoa, nemertea, nematode, oligichaeta, photonida, polychaeta, and turbelaria (Cohen *et al.*, 1995). These organisms are found in sediments. The ingestion of these organisms may also result in the accidental ingestion of sediment.

The ingestion of contaminated sediments or prey items is considered to be a key route in the accumulation of organic contaminants in biota (Forbes *et al.*, 1998; Sormunen *et al.*, 2008; Thomann *et al.*, 1992; Voparil *et al.*, 2004). The digestive process of many organisms involves secretion of surfactants into the digestive lumen (Bock and Mayer, 1999; Rubas and Grass, 1991; Zimmer, 1997). Surfactants are both hydrophobic and hydrophilic in nature allowing the hydrophilic portion to interact with the digestive juices, while the hydrophobic portion interacts with the lipids (Horton *et al.*, 2002). The advantage of surfactants in the absorption of lipids is that surfactants aid in the absorption of organic contaminants associated with lipids. Surfactants are believed to increase the

solubility of PAH by forming micelles around the PAH (Mayer *et al.*, 1996). *Carcinus maenas* may increase their exposure to PAHs by ingesting sediments while foraging for benthic infauna (e.g. nemerteans, nematodes, platyhelminthes, oligochaetes, polychaetes, etc.) (Cohen *et al.*, 1995).

Ciarelli *et al.* (2000) and Croce *et al.* (2005) found that increased ingestion of contaminated sediment particles increases organic contaminant burden in organisms. Ciarelli *et al.* (1999) studied the effect of amphipod activity on the bioaccumulation of fluoranthene in mussels. These authors found that increased density of amphipods, (*Corophium volutator*) in the sediment resulted in increased uptake of fluoranthene in mussels. *Corophium volutator* activity also increased total suspended sediments in the water column resulting in increased suspended particles, and associated fluoranthene, entering the mussels during filter feeding. The burrowing behaviour of *C. maenas* may also result in sediments being suspended in the water column.

On numerous occasions, the Tar Ponds can be extremely turbid (M. Jones, personal communication). It has been suggested that the shallow nature of the Tar Ponds increased the tendency of wind currents to suspend sediments in the water column (M. Jones, personal communication). The suspended sediments in the water column of the Tar Ponds may result in sediment-associated organisms, such as *C. maenas* and *Palaemonetes* spp., having a higher exposure to PAHs, since PAHs are often sorped to sediment particles (Gewurtz *et al.*, 2000). Organisms which are not as closely associated with the sediment, such as *F. heteroclitus* and *A. rostrata,* would most likely not show similarly elevated PAH accumulation with sediment suspension. A similar phenomenon was found by Maruya *et al.* (2001) in a study on various fishes. The small, bottom dwelling finfish had higher levels of toxaphene, an organochlorine pesticide, compared to

larger predatory fish which are not as closely associated with sediment. Thus, degree of sediment association is important in evaluating bioaccumulation of PAHs.

4.3.2 Role of Interstitial Water

Based on K_{oc}, organic contaminants should partition between the sediment and interstitial water. Through the partitioning there will be higher concentrations of PAHs in interstitial water compared to water column (Maskaoui *et al.*, 2002; McGroddy *et al.*, 1995). Interstitial water is an important source of contaminants in the accumulation and toxicology of PAHs (Cornelissen *et al.*, 2006; Kosian *et al.*, 1998; Sverdrup *et al.*, 2002). Hawthorne *et al.* (2007) found sediments with lower total PAH concentrations to be more toxic to amphipods than sediments with higher total PAH concentrations. By exposing the amphipods to extracted interstitial water, they found that concentration of total PAH in interstitial water was positively associated with toxic effects. Thus Hawthorne *et al.* (2007) found sediment PAH concentration to not relate with toxic effects. Instead, they found interstitial water PAH concentration to positively correlate with toxic effects.

Gewurtz *et al.* (2000) compared the levels of PAHs and PCBs in various organisms in Lake Erie. Mayflies (*Hexagenia* spp.) accumulated the greatest concentrations of PAHs and PCBs. The authors suggested this was due to *Hexagenia* spp. ingesting sediment and detritus, and inhabiting sediments. Mussels ranked with the second highest PAH and PCB loading. Mussels are filter-feeders; Gewurtz *et al.* (2000) suggested that they accumulated PAHs by filtering suspended sediment. Mussels are strongly associated with sediments and interstitial water; thus, the movement of interstitial water across gills may increase the bioaccumulation of PAHs. A similar

mechanism may have augmented PAH accumulation in *C. maenas* and *Palaemonetes* spp. in this study. Both *C. maenas* and *Palaemonetes* spp. were more highly associated with sediments than fishes, *A. rostrata* and *F. heteroclitus*. Thus, *C. maenas* and *Palaemonetes* spp. experienced greater exposure to interstitial water than the fishes.

Lu *et al.* (2004) found phenanthrene uptake from interstitial water was the major contributor to PAH accumulation in oligochaetes. The authors suggested that ingested sediment contributed less than 20% of total phenanthrene uptake. The importance of interstitial water in PAH accumulation is dependant on hydrophobicity. Lu *et al.* (2004) observed that benzo[*a*]pyrene, a PAH with a higher hydrophobicity than phenanthrene, was accumulated only from ingested sediment by oligoachetes. The authors suggested that interstitial water contributed less than 5% for benzo[*a*]pyrene. A similar pattern may exist in this study. For the less hydrophobic PAHs like fluoranthene, pyrene, and chrysene/triphenylene, the levels of the respective PAHs were similar in *C. maenas* and *Palaemonetes* spp. Yet for the more hydrophobic PAHs like benz[*a*]anthracene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene, *C. maenas* had greater concentrations of these PAHs than *Palaemonetes* spp. PAHs with a log K_{ow} greater than 5.80 were not detected in *Palaemonetes* spp. Similar to findings by Lu *et al.* (2004), the hydrophobicity of the PAH may determine the importance of interstitial water in the uptake of PAHs in biota. The PAHs with a log K_{ow} less than 5.80 may be taken up by *C. maenas* and *Palaemonetes* spp. via interstitial water. The uptake of PAHs with log K_{ow} greater than 5.80 may be caused by another mechanism. The burrowing behaviour of *C. maenas* could cause ingestion of sediments. Since *Palaemonetes* spp. are more pelagic than *C. maenas, Palaemonetes* spp. are less likely to ingest sediments. PAHs with log K_{ow} greater than 5.80 may be taken up by ingestion of sediment instead of interstitial water.

4.3.3 Role of Varying Biotransformation Abilities

Differences in bioaccumulation of PAHs among *C. maenas* and *Palaemonetes* spp. and fishes, *A. rostrata* and *F. heteroclitus*, may also be due to differences in biotransformation abilities. Invertebrates are able to metabolize PAHs and other organic contaminants (Burkhard *et al.*, 1997; Eickhoff *et al.*, 2003*a*, *b*; Jorgensen *et al.*, 2008; Lee *et al.*, 1976; Watson *et al.*, 2004), but not as quickly as fishes and other vertebrates (Eickhoff *et al.*, 2003*a*; Gewurtz *et al.*, 2000). Erickhoff *et al.* (2003*a*) detected PAHs in Dungeness crab (*Cancer magister*) tissues, but previous analysis found only traces of PAH in the ground fish from the same area. Similar results were found between invertebrates and vertebrates in this study. *Carcinus maenas* and *Palaemonetes* spp. had high PAH concentrations while only traces of selected PAHs were detected in *A. rostrata* and *F. heteroclitus*.

The rate and importance of biotransformation varies between trophic levels (Corsolini *et al.*, 2007; Thomann and Komlos, 1999; Wan *et al.*, 2008). Baumard *et al.* (1998) collected and analyzed the concentration of PAHs in a diversity of marine species. The importance of biotransformation in the bioaccumulation of PAHs was low in mussels. In fishes, biotransformation was more important compared to mussels, in the type and concentration of PAHs accumulated. Other studies found bioaccumulation of organic contaminants variable between trophic levels (Burkhard, 2003; Froese *et al.*, 1998; Veltman *et al.*, 2005).

4.4 Factors in Depressed Bioaccumlation

With the high sediment PAH levels, one would expect a high bioaccumulation of PAHs. The lower than expected bioaccumulation of PAHs may also result from aging of sediments, decreased affinity for lipid relative to sediment, and/or another sorptive phase (Bervoets *et al.*, 2005; Corneliseen and Gustafsson, 2005; Krauss *et al.*, 2000; Sundelin *et al.*, 2004). Aging results over a period of time when the organic contaminant is sorbed onto or into the organic matter of the sediment (Alexander, 2000; Kraaij *et al.*, 2001; Reid *et al.*, 2000). When the contaminant is released into the environment, it is thought that the contaminant is quickly adsorped to the sediment through hydrogen bonding and/or van der Waals forces (Semple *et al.*, 2003). Over a period of time (weeks to months), the contaminant may move into the organic matter of the sediment and/or form stronger bonds such as covalent bonds with the organic matter (Semple *et al.*, 2003). Through these stronger interactions, the bioavailability and toxicity of the organic contaminant decreases (Hatzinger and Alexander, 1995; Kraaij *et al.*, 2001; White *et al.*, 1999).

Erickson *et al.* (1993) studied the microbial community during the bioremediation of a manufactured gas plant site. They found that the PAHs in the site were not metabolized by the microorganisms. The PAHs in the soil did not appear to be toxic to the microbial communities. If PAHs were spiked into the soil, there was a rapid decrease in PAH concentration. The spiked sediments had not formed strong interactions with the organic matrix of the sediment; thus, were bioavailable for microbial uptake (Erickson *et al.*, 1993; Semple *et al.*, 2003). Similar results were found by Kraaij *et al.* (2001) where a portion of sediment previously contaminated by PAHs was was spiked with PAHs. Kraaij *et al.* (2001) found PAH bioaccumulation for amphipods was significantly higher for spiked sediments compared to aged soil.

PAHs have been deposited in the Tar Ponds over the past century, so it is plausible that aging has occurred. The operation of the coke ovens and the production of steel ceased in 1988 and 2000, respectively (AMEC, 2005). The extent of the aging process occurring in the Sydney Tar Ponds is unknown. However, the extent of aging is a time-dependent process; thus there will be a decrease in bioavailability of contaminant.

The low bioaccumulation may also be due to decreased affinity for biota lipid relative to sediment, and/or another sorptive phase (Kukkonen *et al.*, 2005; Lu *et al.*, 2006; Maruya *et al.*, 1997; Moermond *et al.*, 2005). Bervoets *et al.* (2005) found high variation in the uptake of trace metals, PCBs, and pesticides in mussels. The authors suggested this to be due to mussel physiology and differential partitioning between mussel tissues and sediment.

Black carbon has a high affinity for many organic contaminants (Cornelissen *et al.*, 2004*a*, *b*; ten Hulscher *et al.*, 2003). The decrease or variability of the uptake of contaminants has been attributed to the presence of black carbon (Cornelissen and Gustafsson, 2005; Cretney and Yunker, 2000; Hauck *et al.*, 2007). It was suggested that black carbon has influenced the uptake of contaminants for a diversity of species (Cretney and Yunker, 2000; Lamoureux and Brownawell, 1999; Thorsen *et al.*, 2004). The presence of black carbon in sediment may increase sediment capacity to sorb PAHs, which would decrease the fugacity, the leaving tendency, of the PAH (Rust *et al.*, 2004).

Black carbon is formed by incomplete combustion of fossil fuels such as coal (Cornelissen and Gustafsson, 2005; Mitra *et al.*, 2002). Due to the past industrial activities around the Tar Ponds, it is plausible that black carbon was introduced into the Tar Ponds. Studies have indicated that black carbon is ubiquitous and accounts for 1-15% of total organic carbon in soils and sediments (Accardi-Dey and Gschwend, 2002;

Gustafsson and Gschwend, 1998; Middelburg *et al.*, 1999). Black carbon may become associated with PAHs through industrial activities. The black carbon with the sorbed PAH would then be introduced into the Tar Ponds. PAHs formed from fossil fuels and associated with black carbon have lower bioavailability, thus depressed bioaccumlation of PAHs, compared to PAHs associated with other types of carbons (Jonker and Koelmans, 2002; Kukonen *et al.*, 2005; Rust *et al.*, 2004; Thorsen *et al.*, 2004).

4.5 Variation in the Bioaccumulation of PAHs Within a Species

There was great variation in the bioaccumulation of PAHs in biota from the Tar Ponds. The variation in PAH accumulation in biota from the Tar Ponds may be related to abiotic and/or biotic factors. Some organisms have a greater potential to accumulate contaminants than other organisms (Lu *et al.*, 2006; Maruya *et al.*, 2001; Veltman *et al.*, 2005). Contaminant concentration alone is not an indicator of bioaccumulation, and species differences should be considered. Schuler *et al.* (2003) examined the uptake of benzo[*a*]pyrene and hexachlorobiphenyl from aged sediments into freshwater invertebrates. Schuler *et al.* (2003) found the decrease in bioavailability of contaminants to vary among species. The variation in the bioaccumulation of PAH in Tar Pond biota may be due to differences in behaviour, physiology, and combination of uptake routes.

Individual organisms may vary in their food selection. Organisms which ingest particles or live in or around environments comprised of much organic matter may be exposed to greater concentrations of PAHs. Forbes *et al.* (1998) studied polychaete behaviour and found that worms select particles high in organic matter, which resulted in greater PAH exposure compared to polychaetes which selected low organic matter

sediments. Forbes *et al.* (1998) also suggested that polychaete behaviour may influence exposure. They suggested that the modification of the environment by polychaete worms (burrowing and irrigating the sediment) may influence the diffusion and movement of contaminants in and out of the sediments, thus, increasing polychaete exposure to PAHs. Leppänen and Kukkonen (2004) studied the effects of intraspecific differences in feeding behaviour among oligochaetes. They found that individuals which ingested sediment particles accumulated greater concentrations of polybrominated diphenylethers, a flame retardant, than oligochaetes which did not ingest sediment particles.

The variation in the Tar Pond biota PAH concentrations may be caused by individual differences in biotransformation. Many organisms are able to biotransform PAHs and other harmful organic contaminants (Corsolini *et al.*, 2007; Drouillard *et al.*, 2007; Tomruk and Guven, 2008). Biotransformation of organic contaminants is individual- and species-specific (Anulacion *et al.*, 1998; Moisey *et al.*, 2001; Wirgin *et al.*, 1996). Leadley *et al.* (1999) exposed brown bullheads (*Ameiurus nebulosus*) to hydrocarbon contaminated sediments. Brown bullheads were selected for similarities based on size, feeding status, and exposure history. Despite the selection criteria, there was variability in biotransformation of hydrocarbons in bullheads. Vandermeulen and Mossman (1996) collected winter flounder (*Pleuronectes americanus*) from the Sydney Harbour, NS, Canada. Similar to Leadley *et al.* (1999), Vandermeulen and Mossman (1996) found high variability in biotransformation activity in winter flounder. Only 20-40% of the variability in biotransformation activity could be explained by differences in location, sex, maturity, and season. Other studies also indicate that there are complex, confounded interactions which affect the bioaccumulation of organic contaminants (Baumard *et al.*, 1998; Bustnes *et al.*, 2008). The varying effects of growth rates and

aging, differences in individual organismal food preference, and differences in individual ability to biotransform PAHs may all result in high variability in contaminant accumulation. It is uncertain which of these factors, if any of them, influenced the uptake or elimination of PAHs in Tar Ponds biota. Additional research is required to investigate which of these factors are responsible for the observed variability.

4.6 Potential Type 2 Biomonitor for the Sydney Tar Ponds

Type 2 biomonitors trace the change of chemical concentrations in biota (Levinton *et al.*, 2006; Vuorinen *et al.*, 2006; Yunker *et al.*, 2002). *Carcinus maenas* is the most suitable Type 2 biomonitor of the four potential species assessed. *Carcinus maenas* have a high reproductive output (Naczk *et al.*, 2004); indicating potentially large populations of *C. maenas* in the Tar Ponds. Secondly. *C. maenas* can tolerate adverse environmental conditions (Naczk *et al.*, 2004). The tolerance to adverse conditions allows this species to successfully inhabit and flourish at degraded sites. Finally, *C. maenas* are easily collected and identified.

Carcinus maenas has measurable and reproducible responses to the contaminant. This study documented greater accumulation of PAHs in *C. maenas* than in *Palaemonetes* spp., *F. heteroclitus*, or *A. rostrata.* The goal of Tar Ponds remediation efforts is to decrease the level of contamination in the biota, sediment, and water. By selecting a biomonitor with the highest initial PAH concentration, one is able to trace the decrease in PAH concentrations for a longer period of time. Also, *C. maenas* bioaccumulated 16-17 different types of PAHs, while the other species assessed did not bioaccumulate as great a range of PAHs. This may be due to *C. maenas* having a lower ability to metabolize

PAHs and a higher fugacity capacity compared to *F. heteroclitus* and *A. rostrata.* Thus, *C. maenas* allows the biomonitoring of a greater range of PAHs compared to other species.

In this study, *C. maenas* were not assessed for their ability to trace changes in environmental contaminant concentrations. An effective Type 2 biomonitor should accumulate the contaminant at concentrations which correlate to the environmental (i.e. water and/or sediment) contaminant concentrations. Thus, as the concentration of PAHs increases in the environment, PAHs increase to a corresponding degree in *C. maenas*. This relationship should be further investigated before *C. maenas* are used as biomonitors.

Although this relationship was not investigated for *C. maenas* in this other crab species have accumulated contaminants at concentrations which correlate with its environment (Baumard et al., 1998; Hale, 1988; Humason and Gadbois, 1982; Ikonomou *et al.*, 2002; Mothershead et al., 1991; Pancirov and Brown, 1977). Eickhoff *et al.* (2003*a*) evaluated the accumulation of PAHs in Dungeness crabs (*Cancer magister*) downstream from an aluminum smelter. The concentration of PAHs in the hepatopancreas and muscle tissues correlated with the environmental PAH concentrations. Another study using *C. magister* also demonstrated *C. magister* can be used as a Type 2 biomonitor. They found a decrease in the concentration of polychlorinated dibenzo-*p*-dioxin in the hepatopancreas of *C. magister* as the site was remediated (Yunker and Cretney 2000). Thus, these studies demonstrate that the levels of organic contaminants in the environment can be reflected in crab tissue concentrations.

4.7 Fish Parasite Populations and Communities

This is one of the first parasitological surveys of *F. heteroclitus* and *A. rostrata* from Cape Breton estuaries. A number of studies have been conducted on the parasites of fundulids and *A. rostrata* sampled from Nova Scotia (Barker, 1997; Barker and Cone, 2000; Fantham and Porter, 1948; Fantham *et al.*, 1940; Gowanloch, 1927; Hawley, 1998; Marcogliese, 1995; Wiles, 1975). All of the parasite species observed in *F. heteroclitus* were previously observed in *F. heteroclitus* throughout the Atlantic coast of North America (Harris and Vogelbein, 2006: see Appendix A).

A recent parasitological survey of *F. heteroclitus* from Lawrencetown Lake, Nova Scotia found that they all were infected with the monogene, *Gyrodactylus* sp., on the skin and fins (Hawley 1998). Also, over 50% of *F. heteroclitus* were infected with *Argulus funduli* and/or *Ergasilus funduli*. The present study did not examine *F. heteroclitus* for *Gyrodactylus* spp., but the monogene, *Salsuginus* sp. was found on the gills of *F. heteroclitus* from all sites. *Argulus* sp. and *E. manicatus* were found on the gills, but at prevalences less than 50% of what Hawley (1998) observed. In River Ryan, *Argulus* sp. and *E. manicatus* were found at 9.7% and 14.5% prevalence respectively. In Mira River, *Argulus* sp. and *E. manicatus* were found at 25.0% and 73.3% prevalence respectively. Parasitic copepods were not observed on *F. heteroclitus* from the Tar Ponds.

Hawley (1998) also found a high diversity of endoparasites in *F. heteroclitus* from Lawrencetown Lake. In 76% of the *F. heteroclitus* metacercaria were found in the fish viscera, which consist of the digestive system, excretory system, and associated tissues. Acanthocephalans (*Acanthocephalus* sp. and *Neochinorhynchus* sp.), cestodes (*Proteocephalus* sp.), and unknown nematodes were also found in the viscera of *F. heteroclitus* (Hawley, 1998). Similar parasites were found in *F. heteroclitus* from Cape

Breton, but at different prevalences than reported by Hawley (1998). *Fundulus heteroclitus* from River Ryan and Mira River were infected with *Neochinorhynchus* sp. at 33.9% and 70.0% prevalence respectively. Similar to prevalence observed by Hawley (1998), this study found 66.1% of *F. heteroclitus* from River Ryan were infected with metaceraceriae in the viscera. In Mira River only 5% of *F. heteroclitus* had metaceraceriae in the viscera. Only River Ryan *F. heteroclitus* were infected with *Proteocephalus* sp. In all three sites, L3 ascarid nematodes were found in connective tissues, but at varying prevalences (Table 19).

Parasite populations have been extensively studied in *Anguilla* spp. (Aguilar *et al.*, 2005; Graynoth and Taylor, 2004; Gollock *et al.*, 2004; Sures *et al.*, 2003; Rodriguez *et al.*, 2005), but there are limited studies on *A. rostrata* (Barker and Cone, 2000; Marcogliese and Cone, 1998). *Anguilla rostrata* accumulate copepods, cestodes, trematodes, monogenes, and nematodes on and/or in the gills, intestine, stomach, and swimbladder (Barker, 1997; Barker and Cone, 2000; Gollock *et al.*, 2004). Barker (1997) found a diversity of trematodes in the intestine, but *Paraquimperia tenerrima* had the highest prevalence. Cestodes and nematodes also were found in the intestine. Gills were infected with the monogene, *Pseudodactylogrus anguillae*, and the copepod, *Ergasilus celestis*. Like Barker (1997), this study observed *P. anguillae* on the gills. *Paraquimperia tenerrima* were not found in the intestine, but *Bothriocephulus* sp., a cestode was found in the digestive system. Unlike Barker (1997), nematodes were not found in the intestine, but L3 nematodes were found on connective tissues throughout *A. rostrata.*

The exotic swimbladder nematode, *Anguillicoloides crassus*, was found in the swimbladders of *A. rostrata. Anguillicoloides crassus* is an exotic species which has

been found in *Anguilla* spp.from Asia, Europe, and North America (Barse *et al.*, 2001; Evans and Matthews, 1999; Fries *et al.*, 1996; Kirk, 2003; Moser *et al.*, 2001; Peters and Hartmann, 1986). Although *A. crassus* has been found in the United States, this is the first identification of *A. crassus* in Canadian waters (Rockwell *et al.*, 2009).

4.8 Parasitism and Host Stress

It is well established that contaminants increase the susceptibility of an organism to diseases and parasitism (Khan and Thulin, 1991; Lafferty and Kuris, 1999; Rapport *et al.*, 1998; Vethaak and Rheinallt, 1992). The high concentration of PAHs, along with PCBs and metals, in the Sydney Tar Ponds, is a likely source of stress on *F. heteroclitus*. *Fundulus heteroclitus* from the Tar Ponds were thinner and had less food in their stomachs compared to fishes from the reference sites. This is reinforced by *F. heteroclitus* from the Tar Ponds having a significantly lower calculated K value than *F. heteroclitus* from River Ryan and Mira River. The lower K values *F. heteroclitus* indicate a lower body condition compared to the reference sites. These characteristics are indicative of stressful environments (Weis, 2002; Weis and Weis, 1989; Weis *et al.*, 2003). Yet among the sites sampled, the Tar Ponds had the lowest parasite prevalence, abundance, and species richness. The decrease in parasite levels in contaminated sites has been found in other studies (Bhuthimethee *et al.*, 2005; Diamant *et al.*, 1999; Macrogliese and Cone, 1997). Faulkner and Lochmiller (2000) studied the trematode communities in hispid cotton rat (*Sigmodon hispidus*) living near an oil refinery waste site. The cestode, *Schizotaenia sigmodontis*, had a two-fold higher abundance in *S. hispidus* from reference sites compared to *S. hispidus* from the waste site. Also, *S.*

hispidus from the reference site had a greater diversity of trematode species richness compared to *S. hispidus* from the waste site.

In contaminated environments changes in parasite populations and communities can be related to where the parasite lives on its host. MacKenzie (1999) suggested that in contaminated environments ectoparasites will increase in infection levels, while endoparasites will decrease in infection levels. There have been studies which follow these suggested trends (Cone *et al.*, 1993; Faulkner and Lochmiller, 2000; Khan *et al.*, 1994; Marcogliese *et al.*, 1998). Other studies do not follow these suggested trends (Brotheridge *et al.*, 1998; Diamant *et al.*, 1999; Hernandez *et al.*, 2007; Pettersen *et al.*, 2006). There are confounding effects, which make it difficult to predict the result of increased contamination on parasite fauna (Esch *et al.*, 1975; Lafferty and Kuris, 1999; Lafferty and Holt, 2003; Morley *et al.*, 2003).

Also, the parasite levels may depend on the level of contamination in the surrounding environment. Sanchez-Ramirez *et al.* (2007) found the gill monogene (*Cichlidogyrus sclerosus*) abundance to be higher on Nile tilapia (*Oreochromis nicloticus*) exposed to low and moderately high polluted sediment. *Cichlidogyrus sclerosus* abundance decreased when the *O. nicloticus* was exposed to higher pollutant concentration. Similar to some of the sediments used by Sanchez-Ramirez *et al.* (2007), the Sydney Tar Ponds are an extremely contaminated site (JWEL-IT, 1996*a*, *b*; Vandermeulen, 1989). The high level of inorganic and organic contaminants in the biota, sediment, and water in the Tar Ponds could prevent the accumulation and survival of both ectoparasites and endoparasites.

4.9 Parasite Local Extinction

The Sydney Tar Ponds is a depauperate ecosystem. There are few published studies on the fauna found in or around the Tar Ponds. It is established that sediment and water from the Tar Ponds are highly contaminated with PAHs, PCBs, and metals (AMEC, 2005; JWEL-IT, 1996*a*, *b*; Vandermeulen, 1989). This could possibly result in a hindrance to parasite health and survival. The contamination levels may prevent the survival of parasite free-living larval stages and/or the intermediate hosts required for parasite development.

4.9.1 Parasite Free-living Larval Stages and Contamination

Free-living stages are involved in many parasite lifecycles. These larval stages often have a protective coating to survive in the environment. Cestodes, trematodes, nematodes, and acanthocephalans release eggs from their respective hosts with a protective coating. Some trematodes have metacercariae, a larval trematode stage that **encyst**, form cysts, on vegetation. Nematodes have a thick waxy coating called a cuticle. Pietrock and Marcogliese (2003) reasoned that although these protective coatings may provide protection from the environment, the larval stages still must often rely on limited energy reserves and unpredictable environmental conditions. Past studies have found abiotic factors such as temperature, pH, salinity, and light to decrease the survival of a diversity of free-living stages (Heinonen *et al.*, 1999; Pietrock and Marcogliese, 2003).

Contaminants have toxic effects on free-living larval stages. Both inorganic (Cross *et al.*, 2001; Morley *et al.*, 2001; Wolmarans *et al.*, 1988) and organic (Guttowa and Boniecka, 1975; Kuntz and Stirewalt, 1946; Okafor and Igbinosa, 1988) contaminants influence the survival and infectivity of free-living parasites. Also, it is suggested that protective coatings offer little protection to anthropogenic contaminants

(Pietrock and Marcogliese, 2003); although Reddy *et al.* (2004) did find encysted metacercariae to be protected from the lethal effects of copper from copper sulfate ($CuSO_4$). They investigated the effects of copper, on trematode larval stages of *Echinostoma caproni* and *Echinostroma trivolvis* and their snail host *Biomphalaria glabrata*. They found that the concentrations of copper used to kill *B. glabrata* (1 hour in 0.001% $CuSO_4$) killed both the cercariae and excysted metacercariae, but not the encysted metacercariae. Excysted metacercariae are larval parasites which have broken out of the protective cyst coating. Authors suggested that the cyst wall protected the encysted metacercariae.

Although the copper dose which Reddy *et al.* (2004) applied did not cause acute effects to encysted metacercariae, the copper may have caused chronic effects which may not be easily measurable. For example the copper may affect the parasite development. The copper may decreases the parasite's health, which may hinder the parasite's ability to transform into later larval developmental stages or produce viable eggs. Koprivnikar *et al.* (2006*a*) investigated the effects of the herbicide, atrazine, on the cercariae of four species of trematodes. Between species there was varying cercariae sensitivity to atrazine. Atrazine was found to decrease the longevity and ability to infect larval amphibians, thus demonstrating that the long-term effects of contaminants must be considered.

Contaminants may reduce the survival of the free-living stage. Pietrock *et al.* (2002) found that heavy metal concentrations affected the health of free-living stages of trematodes. Cercariae of the trematode *Diplostomum* sp. were exposed to cadmium concentrations ranging between 0.2 and 200 µg/l (Pietrock *et al.* 2002). Cadmium concentrations greater than 20 µg/l resulted in a change in the rate of cercariae mortality

and a reduced cercariae survival time. The authors suggested that the effects of contaminants on free-living stages, like cercariae, may be due to two reasons. Firstly, contaminants may reduce the time for the free-living stage to find an acceptable host to infect. Secondly, contaminants may decrease the ability of the free-living stage to transform in the next stage of the life cycle. Reddy *et al.* (2004) found contaminants to interfere with the ability of cercariae to infect a second intermediate host.

Contaminants may also decrease the motility of free-living stages. Cross *et al.* (2005) exposed the gastropod *Littorina littorea* infected with trematode *Cryptocotyle lingua* to heavy metal contaminated water. The *C. lingua* cercariae released from *L. littorea* exposed to contaminated water had a slower swimming rate compared to cercariae released from *L. littorea* not exposed to contaminated water. Authors also observed that cercariae from contaminated water swam in less direct routes and had decreased life spans. Authors suggested that contaminants affected the development of the cercariae anatomy used in swimming.

The Sydney Tar Ponds has often been referred to as Canada's most contaminated site. The high levels of PAHs, PCBs, and metals may be lethal to many, if not all, free-living larval stages. Although no studies have investigated the effects of these high contaminant levels, there are studies which have found lower levels of contaminants to be toxic to free-living parasites (Cross *et al.*, 2001; Pietrock *et al.* 2002; Reddy *et al.*, 2004). Thus, it is quite likely free-living stages of parasites would not be able to survive in the Tar Ponds.

4.9.2 Parasite Intermediate Hosts and Contamination

The lack of parasites in the Tar Ponds may also be due to the lack of intermediate hosts. The abundance and presence of potential parasite hosts can influence the prevalence and abundance of parasite species (Hechinger and Lafferty, 2005; Huspeni and Lafferty, 2004; Whitney *et al.*, 2007). Johnson and Chase (2004) investigated the link between the abundance of *Planorbella* spp. and the level of amphibian parasitic infection. From 27 ponds in Michigan, they found that higher densities of *Planorbella* spp. correlated with increased abundance of *R. ondatrae* in amphibians. Thus, as the levels of the intermediate host increased the level of parasitic infection in amphibian populations increased. In a study of the blood fluke, *Schistosoma haematobium*, Stauffer *et al.* (1997) also found that increases in snail hosts caused increases in parasitic infection.

Möller (1987) suggested that increased parasite levels may be due to an increased suitability of the environment for the host. Both Stauffer *et al.* (1997) and Johnson and Chase (2004) concluded that changes in the environment increased the suitability of the environment for the host. Stauffer *et al.* (1997) found that over-fishing led to an increase in habitat range for the snail, an intermediate host of the blood fluke *Schistosoma haematobium*. The decreased competition between the fish and snail resulted in an increased abundance of the snail. Stauffer *et al.* (1997) suggested the increased level of snails resulted in an increase of parasitic infection prevalence among school children.

Eutrophication is another type of environmental contamination, which may increase the suitability of an environment for parasite hosts. Eutrophication is a process where runoff water from agriculture and urbanization enters water bodies (Andersen *et al.*, 2006; Ryther and Dunstan, 1971). The runoff water is often high in nutrients, such as nitrate and phosphate, which can increase the amount of potential parasite hosts (Boesch

et al., 2001; Boström *et al.*, 2002; Lafferty and Kuris, 2005; Valtonen *et al.*, 1997; Verdonschot, 2006). Johnson and Chase (2004) found eutrophication increased the biomass of the snail, *Planorbella* spp., in Michigan ponds. Johnson and Chase (2004) suggested that the increased levels of nutrients, caused by eutrophication, would decrease the mortality of infected snails. The longer life span of snails could result in a longer period for the snail to release more cercariae into the environment. Another result from eutrophication may be the environment being able to support a higher density of snails, which would also result in possibly more cercariae in the environment to infect the amphibians.

Both Johnson and Chase (2004) and Stauffer *et al.* (1997) demonstrate that alterations in the levels of intermediate hosts will affect the distribution of parasites in an ecosystem. If increased intermediate host levels cause an increase in parasite levels, then decreased intermediate host levels should cause decreases in parasite levels. Cone *et al.* (1993) examined the parasite communities of *A. rostrata* from Nova Scotia. They found *A. rostrata* from acidic sites (pH 4.5 – 5.0) had a lower species diversity compared to *A. rostrata* from limed, more alkaline, sites (pH 6.0 – 7.0). The parasites with sensitive intermediate hosts or free-living larval stages were absent from acidic rivers. Field studies found a decline and elimination of these intermediate hosts in the acidic rivers compared to the other sites. The parasites with acid-tolerant intermediate hosts were found in all the sites studied.

Whitney *et al.* (2007) investigated how the loss of the endangered bird, the Light-footed clapper rail (*Rallus longirostris levipes*), would affect trematode communities in a California wetland. They found *R. levipes* to be an intermediate host to four trematode species. It was suggested that the removal of *R. levipes* from the ecosystem may decrease

the abundance of these trematodes and possibly alter the parasite communities of many other organisms in that ecosystem.

In three Ontario lakes, the prevalence of eight species of a myxozoan parasite *Myxobolus* spp. were evaluated (Koprivnikar *et al.*, 2002). The species of oligochaete in each lake was also noted. Although the importance of a particular oligochaete species for *Myxobolus* spp. development is unclear, Koprivnikar *et al.* (2002) found that the prevalence of certain oligochaetes corresponded with the absence or presence of certain *Myxobolus* spp. Thus, Cone *et al.* (1993), Koprivnikar *et al.* (2002), and Whitney *et al.* (2007) demonstrated that decreases in parasite hosts result in a corresponding decrease in parasite prevalence and abundance.

A similar phenomenon may have occurred in the Tar Ponds. Unlike the reference sites, the *F. heteroclitus* from the Tar Ponds had a very depauperate parasite assemblage. The majority of the *F. heteroclitus* from Mira River and River Ryan were infected with parasites which utilized crustaceans and gastropods as intermediate hosts. Copepods, ostracods, and snails are intermediate hosts for *Proteocephalus* spp., *Neoechinorhynchus* spp., and *Homalometron pallidum* life cycles respectively (see Appendix A for more details). These organisms have been found to be sensitive to environmental contaminant concentrations (Bellas and Thor, 2007; Holcombe *et al.*, 1987; Raisuddin *et al.*, 2007).

DiPinto *et al.* (1993) examined the effects of PCB Aroclor 1254 on the reproductive output of copepods (*Microarthridion littorale*). Copulating pairs of *M. littorale* were exposed to PCB sediment concentrations as high as 83 mg/kg. The number of larval copepods and nauplii produced were reduced from exposure to PCB contaminated sediment. Low sediment PCB concentrations of 4 mg/kg negatively affected copepod reproduction.

Barata *et al.* (2005) evaluated the acute toxicity of PAHs on adult copepods (*Oithona davisae*). After 48 hours exposure to 56.1 and 0.8 μmol/L of naphthalene and pyrene respectively, the survival of the *O. davisae* was affected. Also, Barata *et al.* (2005) observed that *O. davisae* exposed to a mixture of PAHs demonstrated additive toxic effects. These studies demonstrate that low concentrations of PAHs and PCBs are harmful to copepods. The additive effects of these harmful organic contaminants could possibly mean that environments contaminated with a diversity of organic contaminants and/or trace levels of organic contaminants could be lethal to copepods. The separate and additive concentrations of organic contaminants in the Sydney Tar Ponds well exceed the toxic PAH and PCB values observed to affect copepods (AMEC, 2005; Barata *et al.*, 2005; DiPinto *et al.*, 1993; JWEL-IT, 1996*a*, *b*; Vandermeulen, 1989). Also, the total concentrations of PAHs and/or PCBs would be extremely toxic, if not unbearable, for copepods to live.

Ostracods are potential intermediate hosts for *Neoechinorhynchus* sp., which have been found in *Fundulus heteroclitus* (Dickson and Threlfall, 1975; Hopp, 1954; Marcogliese, 1995; Walkey, 1967; Ward, 1940). In this study, *Neoechinorhynchus* spp. were found in *F. heteroclitus* from Mira River and River Ryan, but a limited number were found in *F. heteroclitus* from the Sydney Tar Ponds. The sensitivity of ostracods to environmental changes, such as the introduction of environmental contaminants, has been documented. In sites with lower water quality, Külköylüoğlu (2004, 2005) found the diversity of ostracods to decrease. Külköylüoğlu (2005) found the number of ostracods to decrease about 50% compared to non-impacted sites. In sites with lower water quality, ostracods which were able to tolerate large ranges of environmental predominated (Külköylüoğlu 2004, 2005).

No zooplankton or benthic invertebrate surveys have been conducted in the Sydney Tar Ponds. It is unknown if any or some of these intermediate hosts are present in the Tar Ponds. It is possible that the high concentrations of PAHs, PCBs, and metals hinder the development and survival of copepods, ostracods, and snails, which are contaminant-sensitive intermediate hosts (Bellas and Thor, 2007; Holcombe *et al.*, 1987; Raisuddin *et al.*, 2007). The high levels of contamination in the Sydney Tar Ponds pose a high risk in conducting such biological surveys. Despite the lack of planktonic and sediment-infauna data, the high level of contamination and lack of parasites in *F. heteroclitus* indicate that the presence of these intermediate hosts is quite unlikely.

4.10 Sydney Tar Pond Parasite Biomonitors

The Sydney Tar Ponds are under the initial stages of remediation (W. Kaiser, personal communication). The old city dump was capped, which terminated the leakage of contaminated groundwater into the former coke ovens sites; thus, this prevented more contamination entering the Tar Ponds. The Coke Ovens Brook was rerouted, which prevented contaminants from moving from the coke ovens sites into the South and subsequently the North Tar Pond. The solidification and stabilization process of the Tar Ponds is scheduled to commence in 2009. Through the solidification and stabilization process the PAH, PCB, and metal contaminant levels are expected to decrease (W. Kaiser, personal comunciation). As the contamination levels decrease, the environment will hopefully become more hospitable for these sensitive intermediate hosts such as copepods and snails. The return of crustaceans and gastropods to the former Tar Ponds

will most likely result in an increased prevalence and abundance of parasites with complex life cycles.

Parasite communities can recolonize and recover in remediated areas (Cone *et al.*, 1993; Huspeni and Lafferty, 2004; Marcogliese and Cone, 1997*a*). As discussed earlier, Cone *et al.* (1993) found *A. rostrata* from acidic sites lacked parasites which had sensitive intermediate hosts. In a continuation of the study by Cone *et al.* (1993), Marcogliese and Cone (1997*a*) found that as the pH of the acidic site increased, the parasite species diversity increased. This study suggests that as contaminated sites are remediated that parasite communities can recolonize and recover (Marcogliese and Cone, 1997*a*).

Parasites are effective and reliable biomonitors (Lafferty, 1997; MacKenzie *et al.*, 1995; Marcogliese and Cone, 1997*a*; Poulin, 1992; Sasal *et al.*, 2007; Sures, 2004). Landsberg *et al.* (1998) and Sures *et al.* (1997) suggested that parasites are more sensitive biomonitors than their fish or crustacean hosts. Also, parasites may be an even more sensitive biomonitor of contaminants compared to other invertebrates often used as indicator species. Reddy *et al.* (2004) found cercariae and metacercariae to be less tolerant to copper than its snail host.

Marcogliese and Cone (1997*a*) suggested several reasons why macroparasites are effective biomonitors. Firstly, parasites are easy to sample by collecting and necropsying host organs. Secondly, the collection and necropsy of hosts is inexpensive compared to other types of biomonitoring protocols. Thirdly, the identification of the basic parasite groups requires little training. Finally, parasites generally have a shorter life span compared to their hosts. Any changes in the contaminant levels will be reflected quicker in the host's parasite community than the types of hosts in the environment.

4.11 Predictions for Levels of Parasitism for Remediated Sydney Tar Ponds

After remediation it is unclear which parasite species will be in theTar Ponds. It is quite possible that in the future, the parasite species found in Mira River and Ryan River will be noted in *F. heteroclitus* from the former Tar Ponds. Before and after a salt marsh restoration project, Huspeni and Lafferty (2004) measured the prevalence and abundance of larval trematodes in the California horn snail (*Cerithidea californica*). Before restoration, the salt marsh and control sites had 12% and 28% respective mean trematode prevalence and 4.5 and 7 respective trematode species. After restoration, the salt marsh had 43% mean trematode prevalence and 9 trematode species, while at the control site the trematode community structure was unchanged. After restoration, the trematode communities at the salt marsh and control site were similar. The authors linked the return of trematodes to be caused to the return of birds and other vertebrates to the salt marshes (Huspeni and Lafferty 2004). Also, the length of time required for parasites to recolonize the former Tar Ponds in not known. Annual collection of *Fundulus* spp. will allow tracking of changes in parasite levels.

The re-introduction of the intermediate hosts and parasites may possibly lead to high initial levels of parasitism in the former Tar Ponds. *Fundulus heteroclitus* surviving the remediation process would have been historically chronically exposed to extremely toxic levels of inorganic and organic contaminants. Since contamination has been found to alter the development, immunity, and health of organisms (Grinwis *et al.*, 1998, 2000; Lafferty and Holt, 2003; Sandland and Carmosini, 2006), the *F. heteroclitus* surviving the remediation of the Tar Ponds may have a higher susceptibility to parasitism. Also, the effects of chronic contaminant exposure may be passed onto future generations. Nacci *et al.* (1999, 2002) studied the long-term effects of organic environmental contamination on

F. heteroclitus. The parent *F. heteroclitus* were chronically exposed to highly contaminated sediments. In response to the chronic toxic effects, the parent *F. heteroclitus* had a suppressed CYP1A system. The first generation of lab-reared *F. heteroclitus* had similar levels of a suppressed CYP1A system compared to the parent *F. heteroclitus*. The second generation of *F. heteroclitus* still had a suppressed CYP1A system, but the CYP1A activity levels were higher compared to the parent *F. heteroclitus*. The suppressed CYP1A system is thought to decrease the formation of tumours and/or cancer of the skin and liver often associated with organism exposure to PAH and/or PCB contaminated sediment (Arcand-Hoy and Metcalfe, 1999; Arzuaga and Elskus, 2002; Meyer *et al.*, 2002; Pinkney and Harshbarger, 2006; Rose *et al.*, 2001). The long-term effects of a suppressed CYP1A system are unknown. Also, there are probably other effects from chronic exposure to high levels of organic and inorganic contaminants, which may decrease the health of future *F. heteroclitus* generations; thus leaving them prone to parasitic infection.

Contamination often leaves fishes and other organisms more prone to parasitism (Christin *et al.*, 2003; Khan and Thulin, 1991; Rapport *et al.*, 1998; Taylor *et al.*, 1999; Vethaak and Rheinallt, 1992). In a study on Atlantic cod (*Gadus morhua*) and longhorn sculpins (*Myoxocephalus octodecemspinosus*), Khan (1990) found fishes chronically exposed to petroleum hydrocarbons had higher levels of ciliated parasites on their gills. *Gadus morhua* and *M. octodecemspinosus* had 88% and 95% of their gills infected with 102.3 (+/-3.4) and 19.0 (+/-0.9) parasites per infected fish (+/- standard error), respectively. *Gadus morhua* and *M. octodecemspinosus* from the control site had 9% and 48% of their gills infected with 0.9 (+/-0.1) and 1.1 (+/-0.3) parasites per infected fish (+/- standard error) respectively. Khan (1990) suggested contaminant stress to cause the

higher prevalence and mean intensity. Thus, it is possible that after remediation of the Sydney Tar Ponds, there could be a high level of parasitic infection for *F. heteroclitus* in the Tar Ponds compared to Mira River and River Ryan.

4.12 Future Parasitological Work

This study provided background levels of parasitism in the Sydney Tar Ponds. Yet for optimal use of parasite biomonitors, an understanding of the influences on the ecosystem, host ecology, and parasite lifecycles is needed (Schludermann *et al.*, 2003; Sasal *et al.*, 2007; Siddall *et al.*, 1994). Because of the highly toxic contaminant concentrations, there is little knowledge on the structure of the ecosystem in the Sydney Tar Ponds. Until the Tar Ponds are remediated, only limited biological surveys may be conducted safely. Few macroparasitological studies have been conducted in Atlantic Canada and even fewer have been conducted in Cape Breton. This limits the understanding of the exact intermediate hosts utilized in the transmission of parasites in Cape Breton estuaries. Through the necropsy of crustaceans and gastropods, the needed intermediate hosts in parasites of *F. heteroclitus* in Cape Breton may be identified. This will increase our understanding of these parasites' transmission patterns. By the re-appearance of a particular parasite species in Tar Ponds fishes post-remediation, these surveys may allow better inference of species re-introduced into the ecosystem (Marcogliese, 2005; Marcogliese and Cone, 1997*b*; Thompson *et al*, 2005)

4.13 Concluding Statements

1. The South Tar Pond is higher in sediment bound PAHs than the North Tar Ponds.

2. In both the North and South Tar Ponds the PAH sediment concentrations is above the CCME sediment guidelines. According to previous studies and CCME guidelines, the high PAH levels are at levels expected to be toxic to biota.

3. The level of PAHs in Tar Pond sediments and biota is extremely high compared to other contaminated sites.

4. In the Tar Ponds, *C. maenas* and *Palaemonetes* spp. accumulated a greater range and concentration of PAHs compared to *A. rostrata* and *F. heteroclitus*.

5. Of the biota studied *C. maenas* is the best biomonitor due to bioaccumulating the highest concentration and greatest diversity of PAHs. Also, *C. maenas* bioaccumlate PAHs at concentrations that reflect the environmental concentrations.

6. *Fundulus heteroclitus* and *A. rostrata* had lower prevalences and abundance of ectoparasites and endoparasites in the Tar Ponds compared to fishes from reference sites. The high level of contaminants may hinder parasite health and survival. Also, the high levels of contaminants may prevent the survival of parasite free-living larval stages and/or the intermediate hosts required for parasite development.

7. The predicted effects of Tar Ponds remediation on parasite ecology is unclear. Parasite species which are found in other parts of Cape Breton, such as in the Mira River and River Ryan, will most likely become established in the remediated Tar Ponds. Also, it is thought that as the contaminant levels decrease the level of parasitism will likely increase in fishes.

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TABLES

Table 1: **Physiochemical properties of polycyclic aromatic hydrocarbons (PAHs) examined in Sydney Tar Ponds biota and sediment samples** (Mackay *et al.*, 1992; McGowin, 2006).

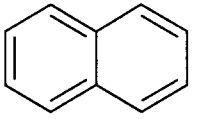
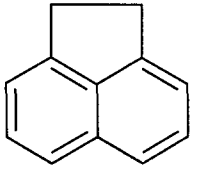
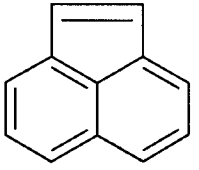
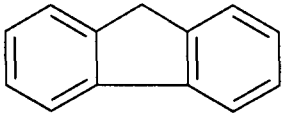
PAH	Structure	Molecular Weight (g/mol)	Temperature at 25°C		
			Water Solubility (g/m^3)	Log K_{ow}	Log K_{oc}
Naphthalene		128	31	3.37	3.11
Acenaphthalene		152	38	3.92	3.79
Acenaphthylene		154	16	4.00	3.83
Fluorene		166	1.9	4.18	4.15

Table 1 (continued)

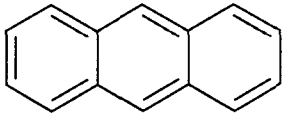
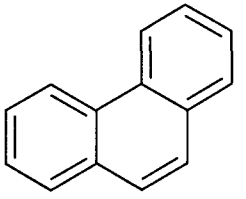
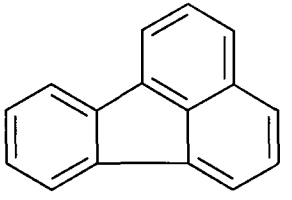
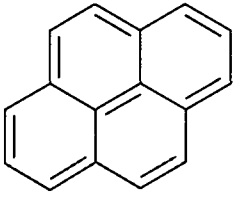
PAH	Structure	Molecular Weight (g/mol)	Temperature at 25°C		
			Water Solubility (g/m^3)	Log K_{ow}	Log K_{oc}
Anthracene		178	0.045	4.45	4.41
Phenanthrene		178	1.1	4.57	4.22
Flouranthene		202	0.26	5.22	4.74
Pyrene		202	0.13	5.18	4.82

Table 1 (continued)

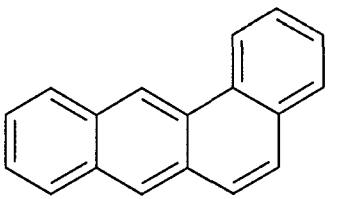
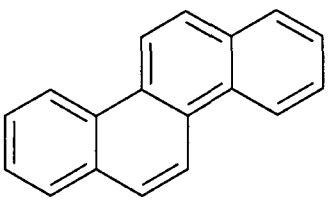
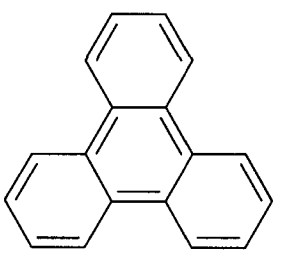
PAH	Structure	Molecular Weight (g/mol)	Temperature at 25°C		
			Water Solubility (g/m^3)	Log K_{ow}	Log K_{oc}
Benz[*a*]anthracene		228	0.011	5.91	5.66
Chrysene		228	0.002	5.37	5.14
Triphenylene		228	0.043	5.45	4.0

Table 1 (continued)

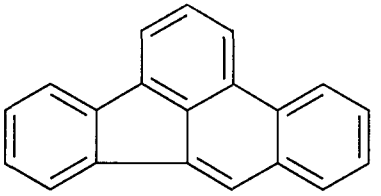
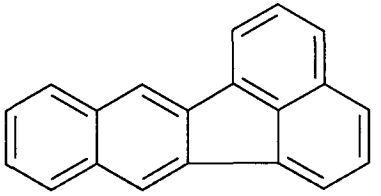
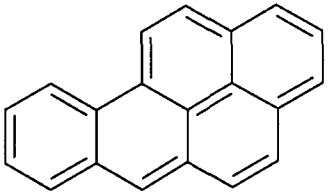
PAH	Structure	Molecular Weight (g/mol)	Temperature at 25°C		
			Water Solubility (g/m^3)	Log K_{ow}	Log K_{oc}
Benzo[*b*]fluoranthene		252	0.0015	5.80	5.89
Benzo[*k*]fluoranthene		252	0.0008	6.00	5.89
Benzo[*a*]pyrene		252	0.0038	6.04	5.71

Table 1 (continued)

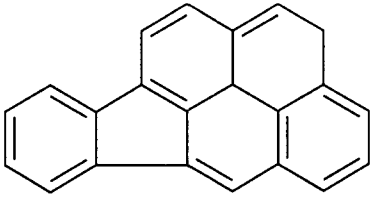
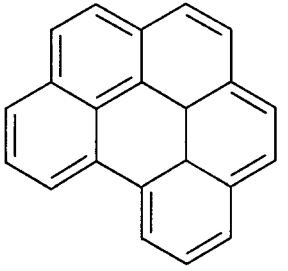
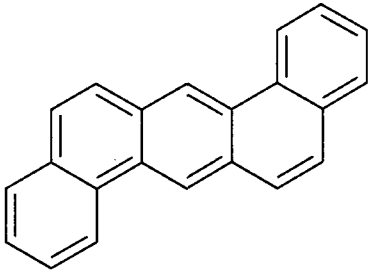
PAH	Structure	Molecular Weight (g/mol)	Temperature at 25°C		
			Water Solubility (g/m^3)	Log K_{ow}	Log K_{oc}
Indeno[1,2,3-*cd*]pyrene		276	0.00019	6.58	6.14
Benzo[*g*,*h*,*i*]perylene		276	0.00026	6.50	6.2
Dibenz[*a*,*h*]anthracene		278	0.0006	6.75	5.97

Table 2: **Range of total polycyclic aromatic hydrocarbons (ΣPAH) concentrations from uncontaminated sites in other studies** (Krauss *et al.*, 2000). ΣPAH is the sum of twenty PAHs.

Soil or Sediment Source	**Range of ΣPAH** mg/kg
Grassland near running water	0.4 - 7.2
Forest	0.5 - 19.6
House garden	0.7 - 17.7

Table 3: **Concentrations of selected polycyclic aromatic hydrocarbons (PAHs) from uncontaminated sediments in other studies**. Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

Location	PAH Concentration (mg/kg dry wt)								Reference
	NAP	**ANY**	**ANA**	**FLU**	**PHA**	**FLA**	**PYR**	**BAA**	
Freshwater Lake, United States	--	--	--	--	--	--	--	0.1-0.2	Barbee *et al.*, 2008
Remote Lake, Korea	0.083	0.0276	0.0718	0.0814	0.422	0.620	0.605	0.221	Kim *et al.*, 2008
Adore River, France	--	--	--	--	0.00176	0.00106	0.0007	0.00031	Djomo *et al.*, 1996
	CHR	**BBF**	**BKF**	**BEY**	**BAY**	**INP**	**BGP**	**DAN**	
Freshwater Lake, United States	0.1-0.2	--	0.0-0.1	--	0.0-0.1	0.1	--	0.0	Barbee *et al.*, 2008
Remote Lake, Korea	0.273	0.341	0.0872	0.194	0.347	0.222	0.029	0.217	Kim *et al.*, 2008
Adore River, France	0.00058	--	0.0002	0.00046	--	--	--	--	Djomo *et al.*, 1996

Table 4: **Concentrations of total Environmental Protection Agency (EPA) polycyclic aromatic hydrocarbons (ΣPAH) from contaminated sediments in other studies**. ΣPAH is the sum of sixteen EPA Priority PAHs.

Location	**ΣPAH Concentration** mg/kg dry wet	**Reference**
Ulsan Bay, Korea	0.405	Khim *et al.*, 2001
Oslo Harbour, Norway	17	Cornelissen *et al.*, 2008
Northern Columbia	2.090	Johnson-Restrepo *et al.*, 2008

Table 5: **Concentrations of selected polycyclic aromatic hydrocarbons (PAHs) from contaminated sediments in other studies**. Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

Location	PAH Concentration (mg/kg dry wt)								Reference
	NAP	**ANY**	**ANA**	**FLU**	**PHA**	**FLA**	**PYR**	**BAA**	
Elizabeth River, USA	--	--	--	--	25	42	28	11	Bieri *et al.*, 1986
Mystic Channel, Boston, USA	--	--	18.2	--	120	146	74.2	37.6	Voparil *et al.*, 2004
Canguiri River, (Light urban area), Brazil	--	.00072	--	0.0121	0.0062	0.0149	0.0129	0.0151	Leite *et al.*, 2008
Iguccu River (Heavy urban area), Brazil	0.2567	.0183	0.0029	0.0625	.0636	0.0992	0.1047	0.0101	Leite *et al.*, 2008
	CHR	**BBF**	**BKF**	**BEY**	**BAY**	**INP**	**BGP**	**DAN**	
Elizabeth River, USA	19	--	--	6	9	--	--	--	Bieri *et al.*, 1986
Mystic Channel, Boston, USA	50.9	36.2	16.8	--	43	18.2	24.4	2.5	Voparil *et al.*, 2004
Canguiri River (Light urban area), Brazil	0.0086	0.0024		--	0.0187	0.0082	0.0222	0.0249	Leite *et al.*, 2008
Iguccu River (Heavy urban area), Brazil	0.0134	0.0136		--	0.0345	0.1643	0.3902	0.4665	Leite *et al.*, 2008

Table 6: **Concentration of polycyclic aromatic hydrocarbons (PAHs) in biota from remote areas in other studies.** Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

Location	Species	Polycylcic Aromatic Hydrocarbon (ng/g wet weight) NAP	ANY	ANA	FLU	PHA	ANT	FLA	PYR	BAA	Reference
Texas, USA											
Clean pond	Shrimp	--	--	--	--	--	--	--	<0.3	<0.2	Pancirov and Brown, 1977
European Lakes											
Away from industry	Various fish liver (57)	--	--	--	2.2	12	0.9	2.4	2.0	0.20	Vives *et al.*, 2004
Ontario, Canada											
Remote Lake	Lake Trout	--	--	--	--	--	--	--	<0.3	<0.5	Pancirov and Brown, 1977

Table 6 (continued): Concentration of polycyclic aromatic hydrocarbons (PAHs) in biota from remote areas in other studies. Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

Location	Species	Polycylcic Aromatic Hydrocarbon (ng/g wet weight) CHR	BBF	BKF	BEY	BAY	INP	BGP	DAN	Reference
Texas, USA										
Clean pond	Shrimp	--	--	--	--	<1	--	--	--	Pancirov and Brown, 1977
European Lakes										
Away from industry	Various fish liver (57)	1.0	0.47	0.28	--	0.33	0.03	0.07	0.05	Vives *et al.*, 2004
Ontario, Canada										
Remote Lake	Lake Trout	--	--	--	--	<1	--	--	--	Pancirov and Brown, 1977

Table 7: **Concentration of polycyclic aromatic hydrocarbons (PAHs) in biota from contaminated areas in other studies.** Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

Location	Species, organ (number)	Polycylcic Aromatic Hydrocarbon (ng/g wet weight)									Reference
		NAP	ANY	ANA	FLU	PHA	ANT	FLA	PYR	BAA	
West coast, Canada N. British Columbia	Crab hepatopancreas (142) (*Cancer magister*)	--	--	4.5	--	5	2.09	4.29	2.27	0.88	Eickhoff *et al.*, 2003
Ariake Sea, Japan Kyushu Island	Crab, whole (3) (*Macrophthalmus japonicus*)	--	--	0.05	--	--	--	--	--	0.35	Nakata *et al.*, 2003
Ariake Sea, Japan Kyushu Island	Clam tissue (3) (*Cyclina sinensis*)	--	--	0.03	--	--	--	--	--	0.62	Nakata *et al.*, 2003
Ariake Sea, Japan Kyushu Island	Lugworm, whole (2)	--	--	0.41	--	--	--	--	--	1.7	Nakata *et al.*, 2003

Table 7 (continued): Concentration of polycyclic aromatic hydrocarbons (PAHs) in biota from contaminated areas in other studies. Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

Location	Species, organ (number)	Polycylcic Aromatic Hydrocarbon (ng/g wet weight)									Reference
		NAP	ANY	ANA	FLU	PHA	ANT	FLA	PYR	BAA	
Marsh/Estuary USA											
Savannah, Georgia	Oyster tissue (9) (*Crassostrea virginica*)	0.2	--	0.3	0.4	2.1	0.12	5	19	0.5	Kumar *et al.*, 2008
Ariake Sea, Japan											
Kyushu Island	Oyster tissue (3) (*Crassostrea gigas*)	--	--	<0.02	--	--	--	--	--	0.69	Nakata *et al.*, 2003
Portuguese coast											
	Fish, shanny muscle (93) (*Lipophrys pholis*)	--	0.6	--	1.4	3.3	0.2	6.0	5.6	0.8	Lima *et al.*, 2008

Table 7 (continued): Concentration of polycyclic aromatic hydrocarbons (PAHs) in biota from contaminated areas in other studies. Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

Location	Species, organ (number)	Polycylcic Aromatic Hydrocarbon (ng/g wet weight)								Reference
		CHR	BBF	BKF	BEY	BAY	INP	BGP	DAN	
West coast, Canada N. British Columbia	Crab hepatopancreas (142) (*Cancer magister*)	2.95	0.89		--	0.41	--	--	--	Eickhoff *et al.*, 2003
Ariake Sea, Japan Kyushu Island	Crab, whole (3) (*Macrophthalmus japonicus*)	0.21	0.48	0.11	--	0.30	<0.20	--	--	Nakata *et al.*, 2003
Ariake Sea, Japan Kyushu Island	Clam tissue (3) (*Cyclina sinensis*)	0.26	0.55	0.23	--	0.25	<0.20	--	--	Nakata *et al.*, 2003
Ariake Sea, Japan Kyushu Island	Lugworm, whole (2)	0.67	1.9	0.51	--	0.82	<0.20	--	--	Nakata *et al.*, 2003

Table 7 (continued): Concentration of polycyclic aromatic hydrocarbons (PAHs) in biota from contaminated areas in other studies. Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

Location	Species, organ (number)	Polycylcic Aromatic Hydrocarbon (ng/g wet weight)								Reference
		CHR	BBF	BKF	BEY	BAY	INP	BGP	DAN	
Marsh/Estuary USA										
Savannah, Georgia	Oyster tissue (9) (*Crassostrea virginica*)	0.3	0.7	1.7	--	11	36	<0.01	1.3	Kumar *et al.*, 2008
Ariake Sea, Japan										
Kyushu Island	Oyster tissue (3) (*Crassostrea gigas*)	0.27	0.74	0.26	--	0.22	<0.20	--	--	Nakata *et al.*, 2003
Portuguese coast										
	Fish, shanny muscle (93) (*Lipophrys pholis*)	1.6	1.4	0.7	1.8	0.6	0.8	1.0	0.6	Lima *et al.*, 2008

Table 8: **Sediment toxic effects range low (ERL) and toxic effects range median (ERM) guidelines for selected polycyclic aromatic hydrocarbons (PAHs)** (Wade *et al.*, 2008). Benthic biota which live in or near sediments below the effects range low (ERL) will rarely exhibit toxicological effects. Benthic biota which live in or near sediment over the effects range median (ERM) will often exhibit toxicological effects.

Polycyclic Aromatic Hydrocarbon (PAH)	Concentration (µg/kg)	
	Effects range low (ERL)	Effects range median (ERM)
Low Molecular Weight PAHs		
Acenaphthene	16	500
Acenaphthylene	44	640
Anthracene	85.3	1100
Fluorene	19	540
Naphthalene	160	2100
High Molecular Weight PAHs		
Benz[*a*]anthracene	261	1600
Benzo[*a*]pyrene	430	1600
Chrysene	384	2800
Dibenzo[*a*,*h*]anthracene	63.4	260
Fluoranthene	600	5100
Pyrene	665	2600

Table 9: **Canadian marine sediment quality guidelines for selected polycyclic aromatic hydrocarbons (PAHs)** (CCME, 2002). Abbreviations: Interim sediment quality guidelines (ISQC); probable effect levels (PEL); incidence of adverse organismal effects at the PEL (IPEL).

PAH	ISQC (µg/kg dry wt)	PEL (µg/kg dry wt)	IPEL (%)
Acenaphthene	6.71	88.9	57
Acenaphthylene	5.87	128	51
Anthracene	46.9	245	75
Benz[*a*]anthracene	74.8	693	78
Benzo[*a*]pyrene	88.8	763	71
Chrysene	108	846	72
Dibenz[*a*,*h*]anthracene	6.22	135	65
Fluoranthene	113	1494	80
Fluorene	21.2	144	70
Naphthalene	34.6	391	71
Phenanthrene	86.7	544	78
Pyrene	153	1398	83

Table 10: Biological data for two Cape Breton reference sites, Mira River and River Ryan, that were analyzed for polycyclic aromatic hydrocarbons (PAHs). Total length was measured for *Anguilla rostrata* and *Fundulus heteroclitus* except for several *F. heteroclitus* samples where the standard length (SL) was measured. Carapace width was measured for *Carcinus maenas*.

Sample Location	Species	Sample	Individual Samples			Pooled Samples			
			Sex (F/M)	Weight (g)	Length (mm)	n	Sex (F/M)	Weight (g)	Length (mm)
Mira Ryan	***A. rostrata***	A	--	19.69	260	--	--	--	--
	(n = 2)	B	--	15.29	228	--	--	--	--
	C. maenas	A	M	5.42	30.7	--	--	--	--
	(n = 3)	B	M	5.51	32.6	--	--	--	--
		C	--	--	--	3	F	1.62	24.1
							F	1.43	18.4
							M	1.55	19.1
	F. heteroclitus	A	F	7.73	83.3	--	--	--	--
	(n = 3)	B	--	--	--	3	M	3.12	67.3
							F	2.60	61.4
							F	1.73	47.5 (SL)
		C	--	--	--	3	M	0.95	40.4 (SL)
							F	3.48	54.1(SL)
							M	3.08	64.2
	Palaemonetes spp.	A	--	--	--	15	--	5.15	--
	(n = 2)	B	--	--	--	16	--	5.31	--

Table 10 (continued): Biological data for samples from two Cape Breton reference sites, Mira River and River Ryan, that were analyzed for polycyclic aromatic hydrocarbons (PAHs). Total length was measured for *Anguilla rostrata* and *Fundulus heteroclitus* except for several *F. heteroclitus* samples where the standard length (SL) was measured. Carapace width was measured for *Carcinus maenas*.

Sample Location	Species	Sample	Individual Samples			Pooled Samples			
			Sex (F/M)	Weight (g)	Length (mm)	n	Sex (F/M)	Weight (g)	Length (mm)
River Ryan	***C. maenas***	A	M	41.64	62.5	--	--	--	--
	(n = 3)	B	M	39.61	55.8	--	--	--	--
		C	F	5.70	29.0	--	--	--	--
	F. heteroclitus	A	F	5.09	70.5	--	--	--	--
	(n = 3)	B	--	--	--	2	M	3.40	63.0
							M	2.44	51.9
		C	--	--	--	4	F	1.34	59.0
							M	1.50	50.8
							M	1.52	47.1
							F	1.57	58.4
	***Palaemonetes* spp.** (n = 1)	A	--	--	--	20	--	6.11	--

Table 11: Biological data for the North and South Tar Ponds that were analyzed for polycyclic aromatic hydrocarbons (PAHs). Total length was measured for *Anguilla rostrata* and *Fundulus heteroclitus*. The distance across the carapace length was measured for *Carcinus maenas*.

Sample Location	Species	Sample	Individual Samples			Pooled Samples			
			Sex (F/M)	Weight (g)	Length (mm)	n	Sex (F/M)	Weight (g)	Length (mm)
Sydney Tar Ponds	***A. rostrata*** (n = 5)	A	--	20.07	243	--	--	--	--
		B	--	186.23	500	--	--	--	--
		C	--	14.96	243	--	--	--	--
		D	--	31.15	183	--	--	--	--
		E	--	83.73	355	--	--	--	--
	C. maenas (n = 3)	A	F	7.15	31.3	--	--	--	--
		B	M	9.81	36.8	--	--	--	--
		C	M	8.21	36.3	--	--	--	--
	F. heteroclitus (n = 4)	A	F	10.96	94.1	--	--	--	--
		B	F	7.16	84.4	--	--	--	--
		C	--	--	--	2	M	5.01	78.2
							M	1.31	53.2
		D	--	--	--	3	M	2.56	61.4
							M	1.42	56.0
							M	1.25	53.7
	***Palaemonetes* spp.** (n = 3)	A	--	--	--	5	--	5.67	--
		B	--	--	--	8	--	5.69	--
		C	--	--	--	9	--	5.25	--

Table 12: **Numbers of female and male *Fundulus heteroclitus* and unidentified *Fundulus* spp. from the Tar Ponds and reference estuaries used for parasitological analysis.**

Sample Site	Month	n	*Fundulus* spp.	Sex	
				Females	Males
North Tar Pond	**June**	**12**	3 *Fundulus* sp.	3	0
			9 *F. heteroclitus*	5	4
	July	**17**	0 *Fundulus* sp.	0	0
			17 *F. heteroclitus*	4	13
	August	**24**	1 *Fundulus* sp.	1	0
			23 *F. heteroclitus*	13	10
	TOTAL	**53**	4 *Fundulus* sp.	4	0
			49 *F. heteroclitus*	22	27
Mira River	**June**	**32**	3 *Fundulus* sp.	3	0
			29 *F. heteroclitus*	20	9
	July	**16**	1 *Fundulus* sp.	1	0
			15 *F. heteroclitus*	9	6
	August	**16**	0 *Fundulus* sp.	0	0
			16 *F. heteroclitus*	11	5
	TOTAL	**64**	4 *Fundulus* sp.	4	0
			60 *F. heteroclitus*	40	20
River Ryan	**June**	**28**	4 *Fundulus* sp.	3	1
			24 *F. heteroclitus*	12	12
	July	**20**	2 *Fundulus* sp.	2	0
			18 *F. heteroclitus*	10	8
	August	**21**	1 *Fundulus* sp.	1	0
			20 *F. heteroclitus*	9	11
	TOTAL	**69**	7 *Fundulus* sp.	6	1
			62 *F. heteroclitus*	31	31
ALL SITES	**Total**	**186**	15 *Fundulus* sp.	14	1
			171 *F. heteroclitus*	95	78

Table 13: **Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in sediments from two Cape Breton reference sites, Mira River and River Ryan, and the Sydney Tar Ponds, Nova Scotia.**

Selected PAH	Mean Concentration (mg/kg dry wt)			
	Reference Sites		North Tar Pond	South Tar Pond
	Mira River	River Ryan	Mean (+/- Standard error)	Mean (+/- Standard error)
Naphthalene	<0.01	<0.01	1.7 (+/- 0.2)	11 (+/- 2)
Acenaphthylene	<0.01	<0.01	0.5 (+/- 0.1)	2.1 (+/- 0.4)
Acenaphthene	<0.01	<0.01	0.12 (+/- 0.02)	23 (+/- 4)
Fluorene	<0.01	<0.01	0.7 (+/- 0.1)	21 (+/- 4)
Phenanthrene	<0.01	<0.01	3.5 (+/- 0.5)	80 (+/- 13)
Anthracene	<0.01	0.01	1.6 (+/- 0.2)	27 (+/- 5)
Fluoranthene	0.02	<0.01	8 (+/- 1)	271 (+/- 45)
Pyrene	0.02	<0.01	6 (+/- 1)	211 (+/- 35)
Benz[*a*]anthracene	<0.01	<0.01	4.6 (+/- 0.8)	78 (+/- 13)
Chrysene/Triphenylene	<0.01	<0.01	3.8 (+/- 0.6)	83 (+/- 14)
Benzo[*b*]fluoranthene	<0.01	<0.01	5 (+/- 1)	67 (+/- 11)
Benzo[*k*]fluoranthene	<0.01	<0.01	5 (+/- 1)	67 (+/- 11)
Benzo[*e*]pyrene	<0.01	<0.01	5 (+/- 1)	50 (+/- 8)
Benzo[*a*]pyrene	<0.01	<0.01	7 (+/- 1)	54 (+/- 9)
Indenopyrene	<0.01	<0.01	5 (+/- 1)	42 (+/- 7)
Benzo[*g,h,i*]perylene	<0.01	<0.01	5 (+/- 1)	36 (+/- 6)
Dibenz[*a,h*]anthracene	<0.01	<0.01	1.2 (+/- 0.3)	12 (+/- 2)

Table 14: **Two way analysis of variance (ANOVA) results for dry weight polycyclic aromatic hydrocarbon (PAH) concentrations between North and South Tar Pond sediments.**

	Degrees of Freedom (df)	Mean-Square (MS)	F-ratio (F)	Probability (p)
PAH	16	3.80	0.823	0.655
Site	1	5.55	1.20	0.001
PAH*Site	16	3.28	0.712	0.772
Error	68	4.61		

Table 15: Total polycyclic aromatic hydrocarbons (ΣPAHs) in biota from the Sydney Tar Ponds.

Std. Error is the standard error.

Species	n	Total Polycyclic Aromatic Hydrocarbons (mg/kg wet wt) Sample A	B	C	D	E	Mean	Std. Error
Carcinus maenas	*3*	2.4	5.0	0.5	--	--	2.6	1.3
Palaemonetes spp.	*3*	3.1	2.3	1.8	--	--	2.4	0.4
Fundulus heteroclitus	*4*	0.8	0.4	1.0	0.5	--	0.7	0.1
Anguilla rostrata	*5*	0.7	0.9	2.3	1.0	1.4	1.3	0.3

Table 16: Kruskal-Wallis comparison of wet weight and lipid adjusted PAH concentrations (mg/kg) in biota.

	Wet Wt.		Lipid Adj.	
PAH	**H**	**p**	**H**	**p**
Acenaphthene	0.55	0.9	1.11	0.8
Fluorene	0.62	0.9	0.67	0.9
Phenanthrene	2.87	0.4	2.97	0.4
Fluoranthene	6.45	0.09	7.16	0.07
Naphthalene	1.89	0.6	1.73	0.6
Pyrene	7.99	0.05	7.75	0.05

Table 17: One way multivariate analysis of variance (MANOVA) results for wet weight concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in Tar Pond biota.

Univariate F-Tests

PAH	Degrees of Freedom (df)	Mean-Square (MS)	F-ratio (F)	Probability (p)
Acenaphthene	3	0.0010	0.188	0.902
Error	10	0.0055		
Fluorene	3	0.0003	0.177	0.910
Error	10	0.0015		
Phenanthrene	3	0.0829	1.205	0.358
Error	10	0.0688		
Fluoranthene	3	0.3091	5.025	0.022
Error	10	0.0615		
Naphthalene*	3	0.4882	0.669	0.590
Error	10	0.7294		
Pyrene*	3	3.9453	4.520	0.030
Error	10	0.8729		

***These PAHs were natural logarithm transformed.**

Multuvariate Test Statistic

Statistic	Value	F-Statistic	df	p
Hotelling-Lawley Trace	15.5	3.16	18, 11	0.028

Table 18: Tukey test pairwise comparison probability values from testing for significance between biota from the Sydney Tar Ponds. Biota analyzed: *Carcinus maenas*, *Anguilla rostrata*, *Fundulus heteroclitus*, and *Palaemonetes* spp.

Tukey (df = 10)	Pairwise Comparison Probabilities	
	Fluoranthene	**Pyrene**
C. maenas X *A. rostrata*	0.31	0.31
C. maenas X *F. heteroclitus*	0.30	0.12
C. maenas X *Palaemonetes* spp.	0.55	0.91
A. rostrata X *F. heteroclitus*	0.99	0.78
A. rostrata X *Palaemonetes* spp.	0.03	0.10
F. heteroclitus X *Palaemonetes* spp.	0.04	0.04

Table 19: **Summary of external parasites found on *Fundulus heteroclitus*.** The standard error was calculated for the abundance and mean intensity.

Site		Parasite Species			
		Argulus spp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Tar Ponds					
(n = 49)	Prevalence	0	0	8.2	0
	Range	0	0	0 - 6	0
	Abundance	0	0	0.2 (+/- 0.1)	0
	Mean Intensity	0	0	3 (+/- 1)	0
River Ryan					
(n = 62)	Prevalence	9.7	4.8	59.7	14.5
	Range	0 - 2	0 - 2	0 - 15	0 - 4
	Abundance	0.18 (+/- 0.08)	0.06 (+/- 0.04)	2.8 (+/- 0.5)	0.24 (+/-0.09)
	Mean Intensity	1.8 (+/-0.5)	1.2 (+/-0.3)	4.8 (+/-0.7)	1.7 (+/-0.4)
Mira River					
(n = 60)	Prevalence	25.0	98.3	76.7	73.3
	Range	0 - 6	0 - 868	0 - 44	0 - 34
	Abundance	0.4 (+/- 0.1)	76 (+/- 19)	5 (+/- 1)	8 (+/- 1)
	Mean Intensity	1.5 (+/-0.3)	78 (+/- 19)	7 (+/- 1)	10 (+/- 2)

Table 20: **Summary of endoparasites found in *Fundulus heteroclitus*.** The standard error was calculated for the abundance and mean intensity.

Site		Parasite Species				
		Neoechinochynchus sp.	L3 Ascarid nematode	unidentified metacercaria	*Homalometron pallidum*	*Proteocephalus* sp.
Tar Ponds						
(n = 49)	Prevalence	8.2*	8.2	0	4.1*	4.1*
	Range	0 – 1	0 - 1	0	0 - 2	0 - 1
	Abundance	0.08 (+/- 0.04)	0.08 (+/- 0.04)	0	0.06 (+/- 0.04)	0.04 (+/- 0.03)
	Mean Intensity	1.0 (+/- 0.0)	1.0 (+/- 0.0)	0	1.5 (+/- 0.5)	1.0 (+/- 0.0)
River Ryan						
(n = 62)	Prevalence	33.9	12.9	66.1	14.5	3.2
	Range	0 – 17	0 - 2	0 – 12	0 - 4	0 - 3
	Abundance	0.9 (+/- 0.4)	0.16 (+/- 0.06)	2.6 (+/- 0.4)	0.2 (+/-0.1)	0.06 (+/- 0.05)
	Mean Intensity	2.8 (+/-0.9)	1.3 (+/-0.2)	3.9 (+/-0.5)	1.7 (+/-0.4)	2 (+/-1)
Mira River						
(n = 60)	Prevalence	70.0	13.3	5.0	6.7	0
	Range	0 – 23	0 - 2	0 - 3	0 - 2	0
	Abundance	3.1 (+/-0.6)	0.2 (+/- 0.1)	0.1 (+/- 0.1)	0.10 (+/- 0.05)	0
	Mean Intensity	4.4 (+/- 0.7)	1.3 (+/- 0.2)	2.0 (+/- 0.6)	1.5 (+/- 0.4)	0

Table 21: **Summary of parasites found in/on *Anguilla rostrata*.** The standard error was calculated for the abundance and mean intensity.

Site		Gill		Connective Tissue	Intestine, Stomach
		UID Parastic Copepod	*Pseudodactylogyurus anguillae*	L3 Nematode	UID Acanthocephalan*
North Tar Pond					
(n = 6)	Prevalence:	0	16.7	50.0	0
	Range:	0	0 - 3	0 - 4	0
	Abundance:	0	0.5	1.0	0
	Mean Intensity:	0	3	2.0 (+/- 1)	0
Mira River					
(n = 10)	Prevalence:	10.0	20.0	10.0	20.0
	Range:	0 – 3	0 - 3	0 - 6	0 - 35
	Abundance:	0.3 (+/- 0.3)	0.4 (+/- 0.3)	0.6 (+/- 0.6)	4 (+/- 3)
	Mean Intensity:	3	2 (+/- 1)	6	19 (+/- 16)
Sydney Harbour					
(n = 5)	Prevalence:	0	0	20.0	0
	Range:	0	0	0 - 2	0
	Abundance:	0	0	0.4 (+/- 0.4)	0
	Mean Intensity:	0	0	2	0

Table 21 (continued): Summary of parasites found in/on *Anguilla rostrata*.

Site		Intestine, Stomach	Swim Bladder	
		Bothriocephulus sp.	*Anguillicoloides crassus*	UID Nematode**
North Tar Pond				
(n = 6)	Prevalence:	16.7	0	0
	Range:	0 - 3	0	0
	Abundance:	0.5	0	0
	Mean Intensity:	3	0	0
Mira River				
(n = 10)	Prevalence:	20.0	60.0	10.0
	Range:	0 - 8	0 - 7	0 - 1
	Abundance:	0.9 (+/- 0.8)	1.6 (+/- 0.7)	0.1 (+/- 0.1)
	Mean Intensity:	5 (+/- 4)	2.7 (+/-0.9)	1.0 (+/- 0.4)
Sydney Harbour				
(n = 5)	Prevalence:	20.0	20.0	0
	Range:	0 - 1	0 - 11	0
	Abundance:	0.2 (+/-0.2)	2.2 (+/- 2.2)	0
	Mean Intensity:	1	11	0

The exact identification of several of the parasites are unidentified (UID). *UID acanthocephalan may be either *Echinorhynchus* sp. or *Acanthocephulus* sp. **UID nematode may be *Danikonema* sp. Futher staining of specimens is required for identification.

FIGURES

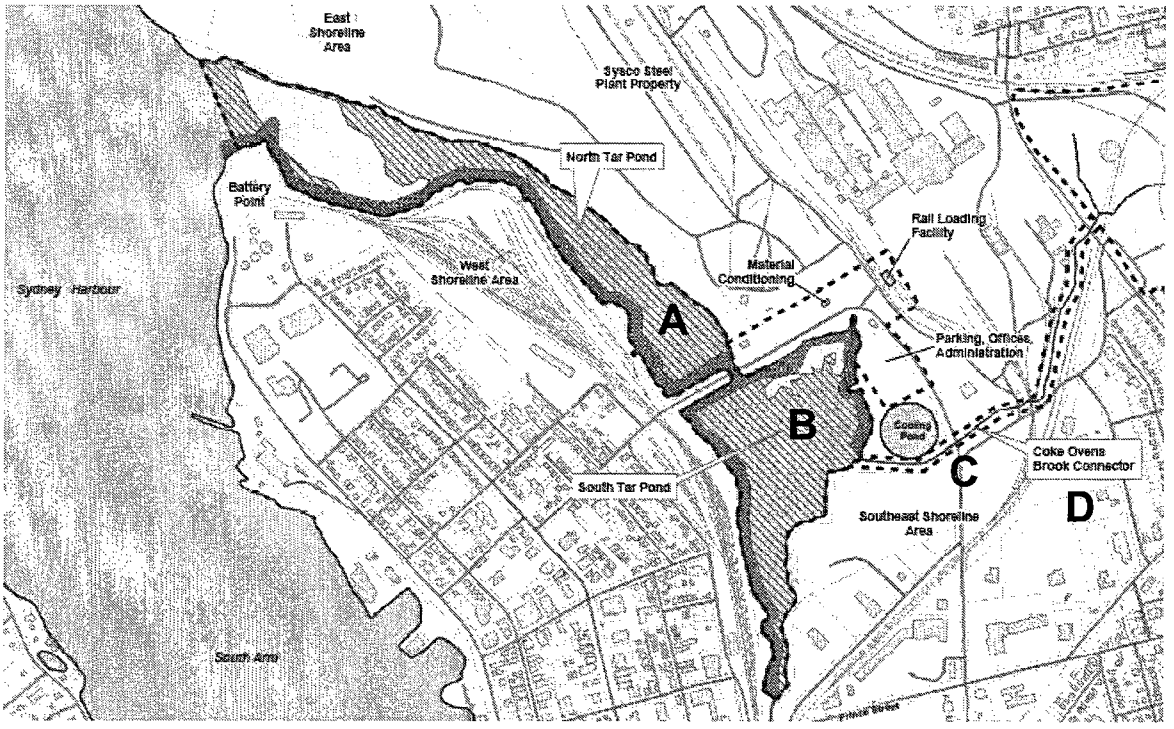


Figure 1: **Map of the Sydney Tar Ponds, Nova Scotia, Canada** (AMEC, 2005). The Sydney Tar Ponds are composed of four parts: the North Tar Pond (A), the South Tar Pond (B), the former Coke Ovens site (C), and Cove Ovens Brook Connector (D).

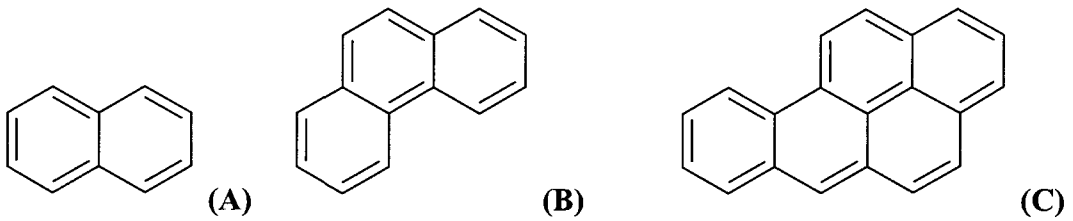


Figure 2: **Structures of selected polycyclic aromatic hydrocarbons (PAHs).**

(A) Naphthalene, (B) Phenanthrene, and (C) Benzo[*a*]pyrene.

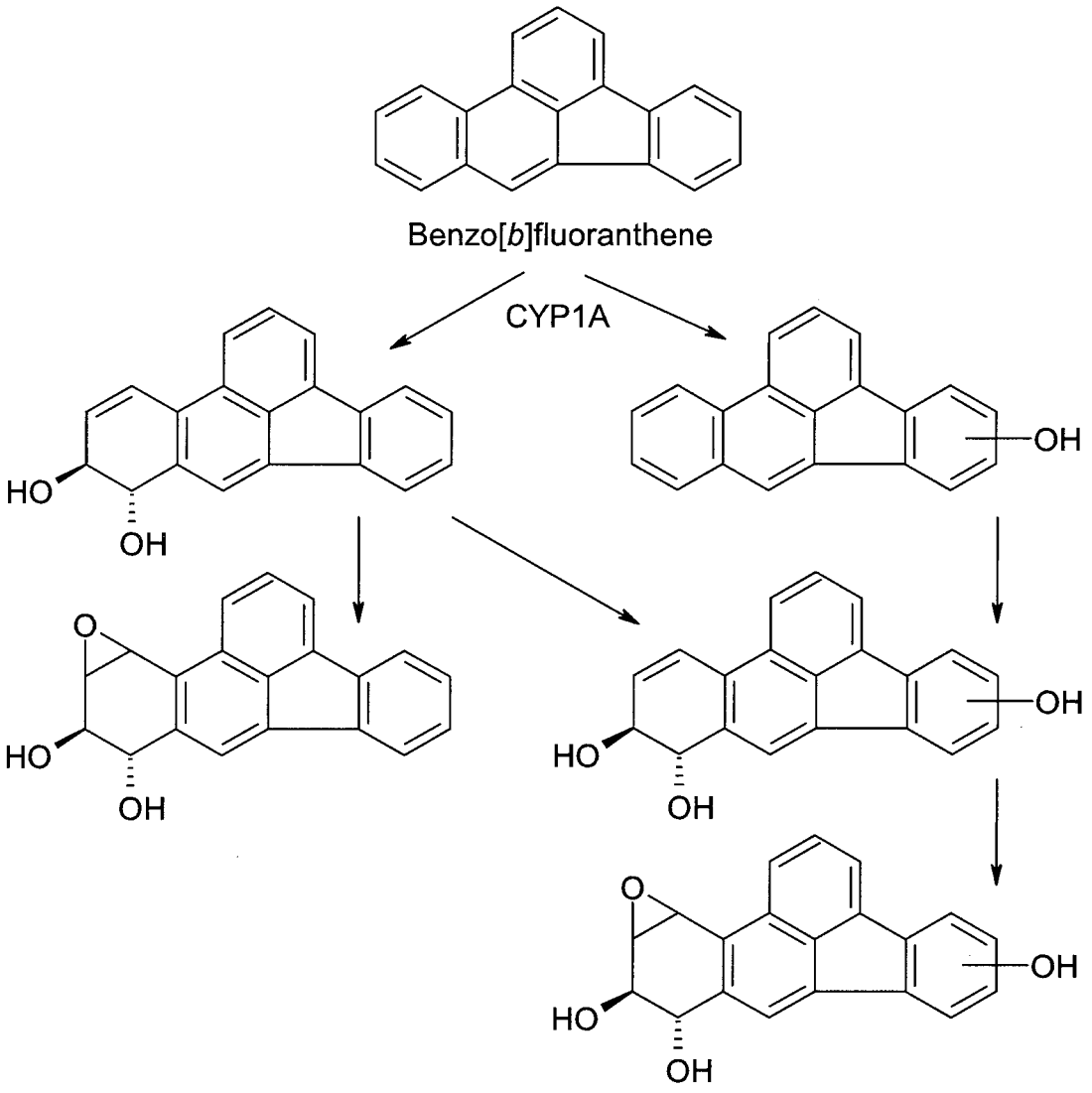


Figure 3: **The formation of epoxides through cytochrome P-450 (CYP1A) metabolism of a selected polycyclic aromatic hydrocarbon, benzo[*b*]fluoranthene** (from Dabestani and Ivanov, 1999).

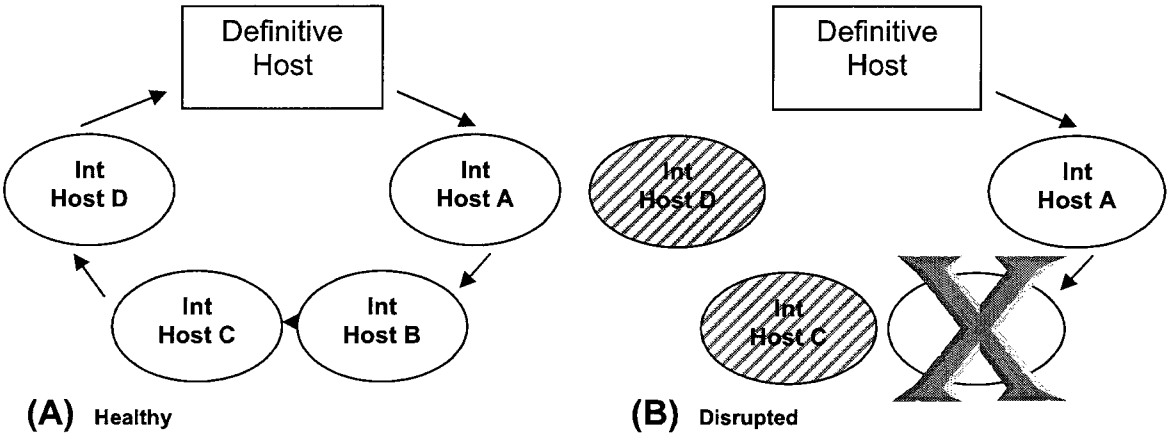


Figure 4: **Schematic of a hypothetical, complex parasite life cycle**. Int is an abbreviation for intermediate. Arrows indicate the transmission of parasite between hosts. **(A)** In a healthy ecosystem, all hosts required for the development of the parasite are present. The parasite is transmitted between hosts and is sexually mature in the definitive host. **(B)** In a disrupted ecosystem, where one of the intermediate hosts is removed, the parasite will not be transmitted through its life cycle. The infective definitive host releases the immature parasite into the environment. The immature parasite infects and develops in intermediate host A. Since intermediate host B is removed from the ecosystem, the immature parasite cannot infect or develop in intermediate hosts C or D or reach maturity in the definitive host. Thus, the lifecycle and development of the parasite is halted in the disrupted ecosystem.

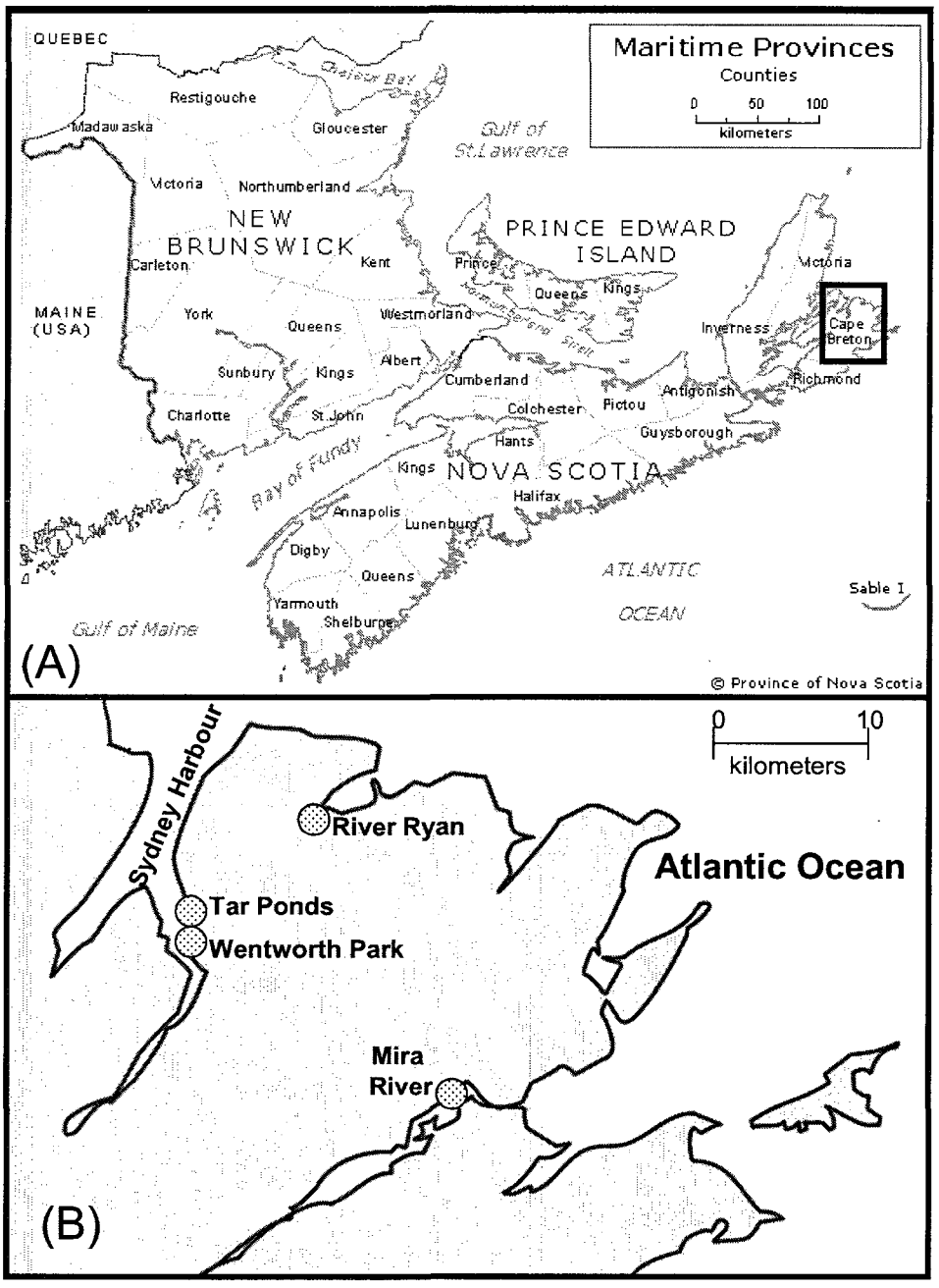


Figure 5: Locations of study sites. (A) Samples were collected from Cape Breton, Nova Scotia (http://www.gov.ns.ca/snsmr/freemaps/ Accessed March 6, 2009). (B) Locations of the four sampling sites: River Ryan, Sydney Tar Ponds, Wentworth Park, and Mira River.

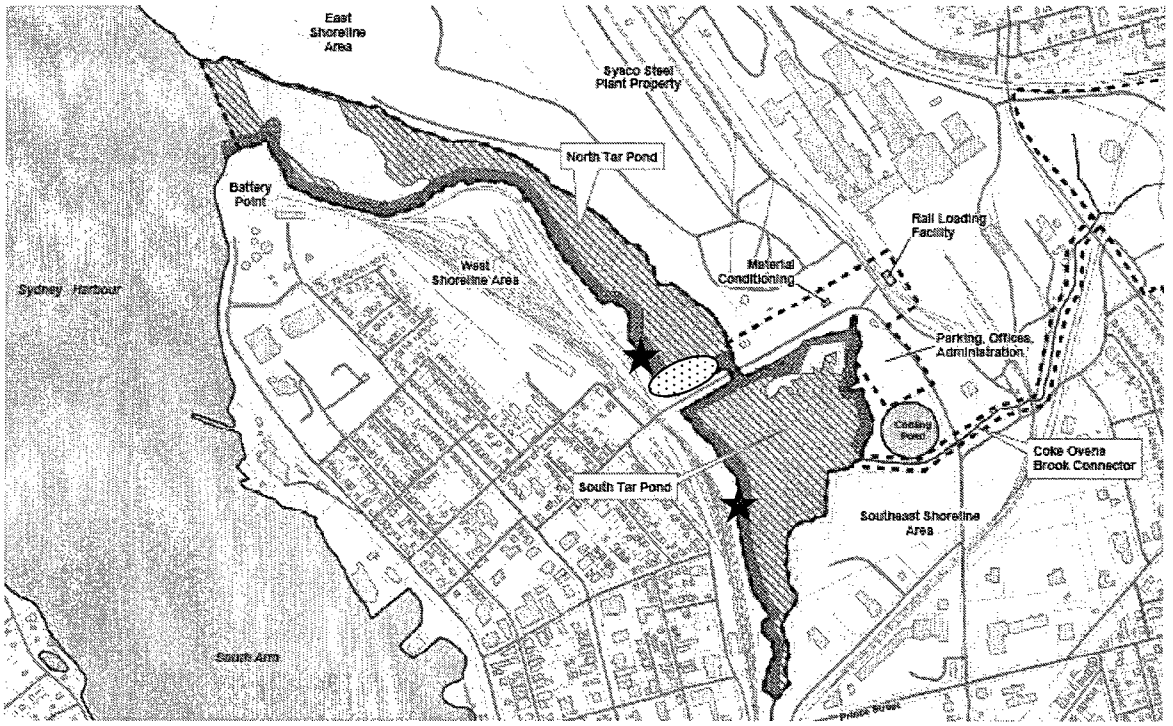


Figure 6: **Approximate locations of biota and sediment sampling in the Sydney Tar Ponds, Nova Scotia**. Map adapted from AMEC (2006). The dotted ellipse indicates biota sampling location at the Ferry Street Bridge. Black stars indicate sediment sampling locations.

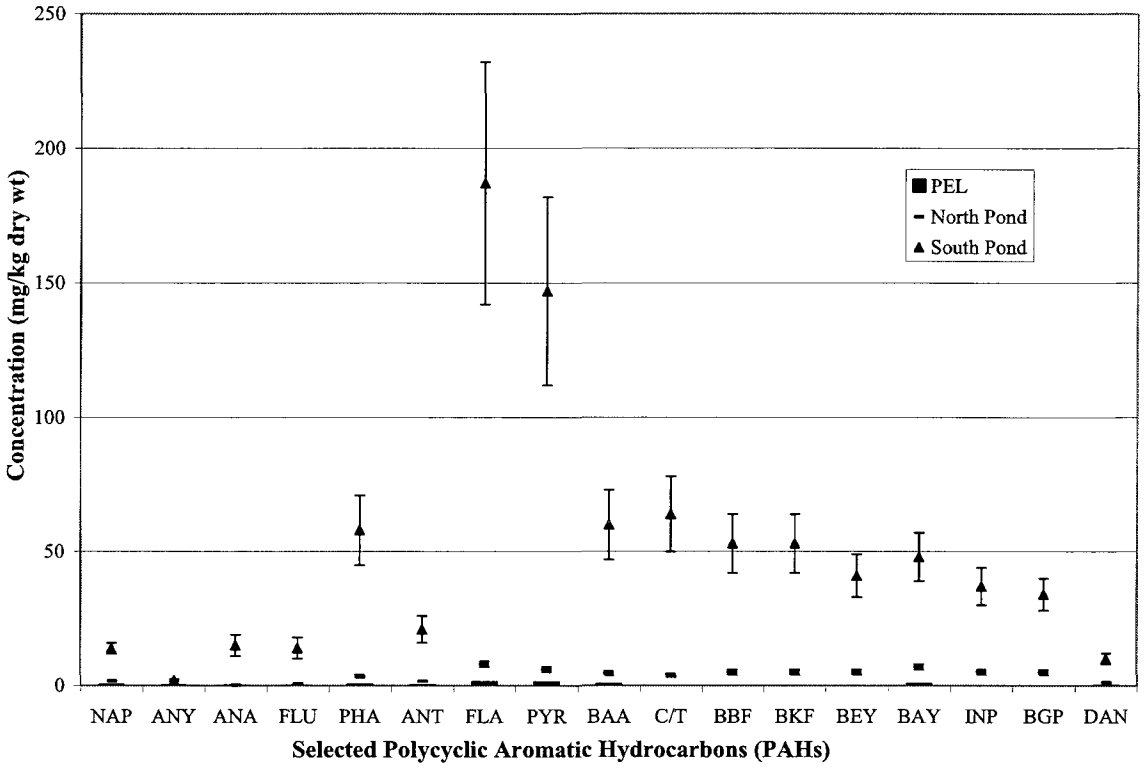


Figure 7: **Average concentrations of selected polycyclic aromatic hydrocarbons from the North (n = 3) and South (n = 3) Tar Ponds sediment.** Error bars represent the standard error. PEL is the probable effects level where at a particular concentration biota near contaminated sediment will experience toxicological effects (CCME, 2002). Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; C/T, chrysene/triphenylene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

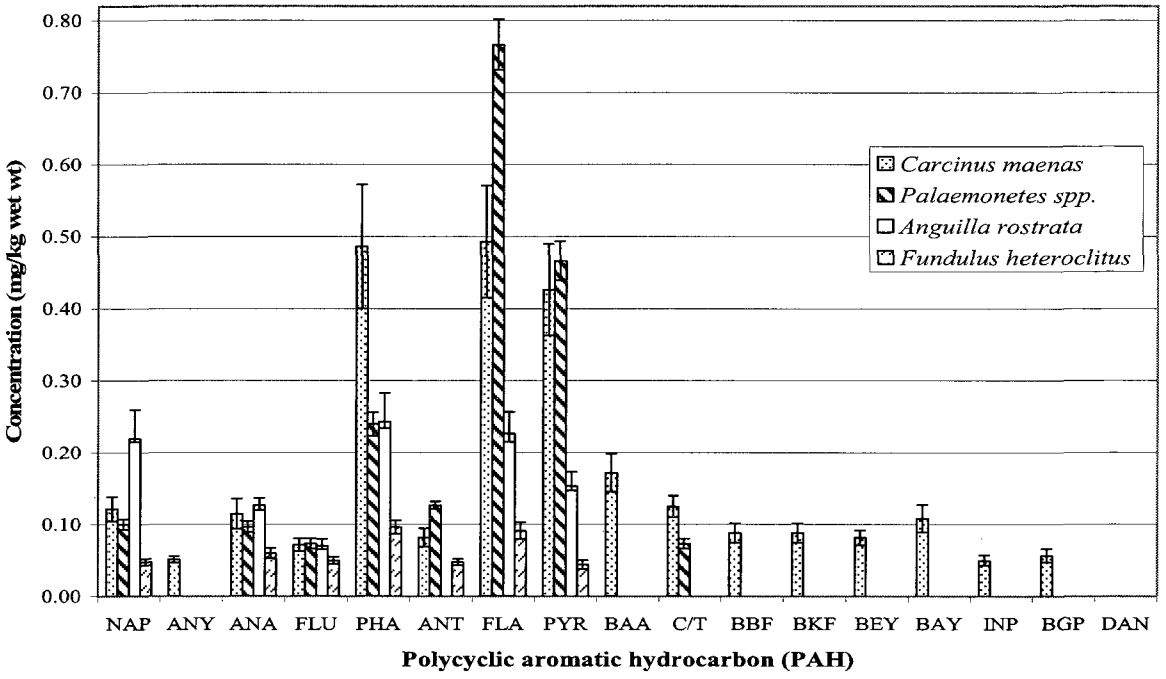


Figure 8: **Average concentrations of selected polycyclic aromatic hydrocarbons in Sydney Tar Pond biota**. Error bars represent the standard error. Compound identification: NAP, naphthalene; ANY, acenaphthylene; ANA, acenaphthene; FLU, fluorene; PHA, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; C/T, chrysene/triphenylene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BEY, benzo[*e*]pyrene; BAY, benzo[*a*]pyrene; INP, indenopyrene; BGP, benzo[*g,h,i*]perylene; DAN, dibenz[*a,h*]anthracene.

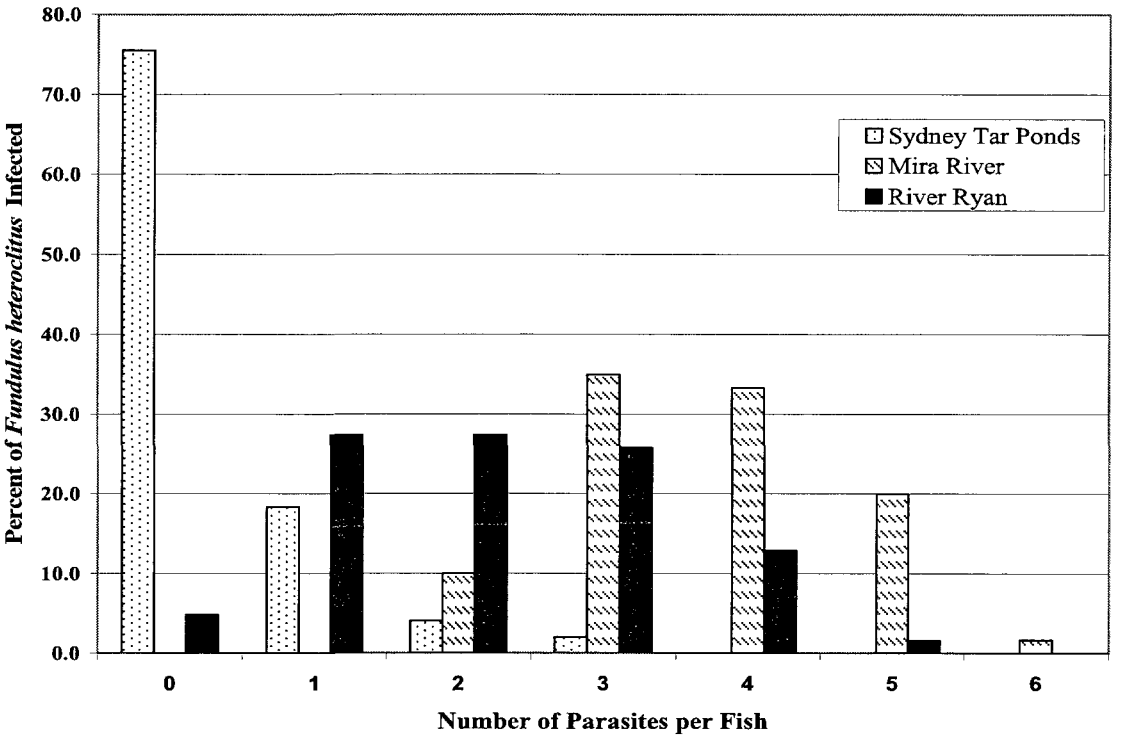


Figure 9: **The distribution of parasites in/on *Fundulus heteroclitus* from two Cape Breton reference sites, Mira River and River Ryan, and the Sydney Tar Ponds, Nova Scotia.**

APPENDIX

Appendix A: Ecology of Selected Parasites of *Fundulus heteroclitus* from Nova Scotia, Canada.

Outline:

A1. *Neoechinorhynchus* spp. (Acanthocephalan)

A2. *Proteocephalus* spp. (Cestode)

A3. *Argulus* spp. (Parasitic Crustacean)

A4. *Ergasilus manicatus* (Parasitic Crustacean)

A5. *Homalometron pallidum* (Trematode)

A1. *Neoechinorhynchus* spp. (Acanthocephalan)

Species previously found in *Fundulus heteroclitus* (mummichog) from the East coast of North America:

adult stage	*Neoechinorhynchus rutili* (Newfoundland, Canada, Dickson and Threlfall, 1975)
cystacanth	*N. cylindratus* (Nova Scotia, Canada, Marcogliese, 1995), *N. rostratum* (Maine, USA, Manter, 1926; Massachusetts, USA, Amin and Bullock, 1998)

Characteristics:

Neoechinorhynchus spp. are generally 1-2cm long (Amin and Bullock, 1998; Crompton, 1970; Marcogliese, 1995; Roberts and Janovy Jr., 2000). Males tend to be smaller than females of the same species. Their body is unsegmented with a holdfast, the proboscis, at the anterior end. The proboscis allows the adult acanthocephalan to attach its self to the intestine of its host. Larval acanthocephalans are typically found in the liver of its host (Amin and Bullock, 1998; Crompton, 1970; Marcogliese, 1995; Roberts and Janovy Jr., 2000).

Lifecycle:

Fertilized eggs are released with the feces from the definitive host (Lassiere and Crompton, 1988; Hopp, 1954; Schmidt, 1985). An egg is ingested by an intermediate host, which is typically an ostracod crustacean. The ingested egg hatches to release an acanthor, a larval acanthocephalan. The acanthor develops into an acanthella, the second

larval stage. After this point in the life cycle two different life histories may occur. Firstly, the ostracod may be consumed by a fish. The acanthella will penetrate the gut of the fish and develop into a cystacanth, the third larval stage. Even though parasite development occurs in this fish it is not considered an intermediate host. The fish is not necessary for the development of the parasite; therefore, the fish is considered a **paratenic host**. This fish is consumed by another fish, wherein the cystacanth develops into the adult stage. Secondly, in the ostracod the acanthella may develop into a cystacanth. The cystacanth is then consumed by a fish. In this case, the acanthocephalan reaches sexual maturity in the first fish infected (Lassiere and Crompton, 1988; Hopp, 1954; Schmidt, 1985).

Potential Hosts:

The ostracod species *Cypria globula*, *Cypria maculate*, and *Cypridopsis vidua*, are established intermediate hosts in this acanthocephalan life cycle (Hopp, 1954; Walkey, 1967; Ward, 1940). A diversity of paratenic hosts may be utilized in transmission. Ward (1940) studied the lifecycle of *Neoechinorhynchus cylindratus*. She found the infected ostracods were ingested by the bluegill sunfish, *Lepomis macrochirus*, which is in turn consumed by final hosts such as piscivorous basses (*Micropterus* spp.). Since bass and other piscivorous fishes generally do not prey on ostracods, and are more likely to prey on bluegill sunfish, the bluegill sunfish is considered a paratenic host in this lifecycle. In the lifecycle of *N. emydis*, snails may act as a paratenic host. Hopp (1954) found that snails, *Campeloma rufum*, may ingest infected ostracods. Map turtles, *Graptemys geographica*, consume a large amount of snails in their diet, which infected turtles with *N. emydis*. Lassiere and Crompton (1988) found adult *N. rutili* in brown trout, *Salmo trutta*, and

three-spined sticklebacks, *Gasterosteus aculeatus*. They observed that uninfected brown trout which fed on infected three-spined sticklebacks would become infected. Therefore, sticklebacks may act as a vector to infect other fish species.

A2. *Proteocephalus* spp. (Cestode)

Species previously found in *F. heteroclitus* from the East coast of North America:

immature *Proteocephalus* sp. (Virginia, USA, Harris and Vogelbein, 2006; Newfoundland, Canada, Dickinson and Threlfall, 1975)

Proteocephalus macrocephalus (Nova Scotia, Canada, Marcogliese, 1995)

Characteristics:

Like other cestodes, *Proteocephalus* spp. are segmented with both male and female reproductive organs in each segment called a proglottid (Roberts and Janovy Jr., 2000). Cestodes attach to their hosts by an attachment organ called a scolex. *Proteocephalus* spp. are often characterized by a four suckered scolex (Roberts and Janovy Jr., 2000).

Lifecycle:

The release of cestode eggs from the definitive host and into the aquatic environment may occur in a number of ways (Mackiewicz, 1988; Scholz, 1999). Firstly, the eggs may be directly released into the intestine of the fish, and the eggs are excreted with the fish waste. Secondly, a fragment of the cestode may be released into the aquatic environment. Thirdly, *Proteocephalus* spp. may protrude part of their body from the fish's anus and expel eggs directly into the water. Once the eggs are released into the water, the eggs increase in volume; this allows the eggs to float on the water surface, which increases the likelihood of egg ingestion by pelagic copepods, the

intermediate host. After an egg has been ingested the oncosphere, the first larval stage, is rapidly released from the egg. The oncosphere penetrates the gut and into the body cavity of the copepod. It is there that the oncosphere develops into a metacestode (also known as a plerocercoid). The infected copepod is ingested by a fish. The fish may be a paratenic or definitive host. If the fish is a paratenic host, *Proteocephalus* spp. will not grow or reach sexual maturity. If the fish is a definitive host, *Proteocephalus* spp. will grow and develop to sexual maturity in the intestine (Mackiewicz, 1988; Scholz, 1999).

Potential Hosts:

The majority of known intermediate hosts of *Proteocephalus* spp. belong to the order Copepoda (families Diaptomidae and Cyclopidae; Scholz, 1999). These are pelagic copepods, which feed primarily on free-floating, surface phytoplankton (Pechenik, 2000). Willemse (1968) suggested that organisms from the order Copepoda are attracted to the free-floating eggs of *Proteocephalus* spp. Small fish may act as paratenic hosts. *Proteocephalus* spp have been found to reach high abundances in small prey fish. The definitive host, a larger fish, were then exposed to high concentrations of *Proteocephalus* spp. from consuming the paratenic host (Scholz, 1999).

A3. *Argulus* spp. (Parasitic Crustacean)

Species previously found on *Fundulus heteroclitus* from the east coast of North America:

Argulus funduli	New Brunswick, Canada, Bere, 1930; Rhode Island, USA, Mulkana, 1966
Argulus megalops	Massachusetts, USA, Wilson, 1904

Characteristics:

Argulus spp. are commonly known as sea lice. In healthy ecosystems, *Argulus* spp. are often found at low prevalences (Pickering and Willoughby, 1977). In highly enclosed areas, such as aquaculture farms, *Argulus* spp. may reach high prevalences. *Argulus* spp. require the blood of its fish host for subsistence. Unlike many other parasite species, *Argulus* spp. are able to unattach from one host and attach to another host. Thus *Argulus* spp. may feed on many different fish hosts. Their feeding produces ulcerated blood lesions, which can leave the fish open to fungal and/or bacterial infections (Pickering and Willoughby, 1977).

Lifecycle:

The male and female adult *Argulus* spp. may mate on the fish host, in the water column, or on an aquatic substrate (Bower-Shore, 1940; Hakalahti et al., 2004). The female lays eggs on substrata. All of the larval nauplius stages occur within the egg. After the *Argulus* spp completes the various nauplius development stages, a juvenile *Argulus* spp emerges. This larval stage is a smaller version of the adult *Argulus* spp. The

juvenile *Argulus* spp. seeks out a suitable fish host for feeding and maturity to adult stage (Bower-Shore, 1940; Hakalahti et al., 2004).

Potential Hosts:

Argulus spp. are not very host-specific (Pasternak et al., 2000). They can infect a diversity of fishes. On a study of fish parasites in Lake Huron, Bangham (1955) found *Argulus* spp. on lake herring (Leucichthys *A. artedi*) and trout perch (*Percopsis omiscomaycus*).

A4. *Ergasilus manicatus* (Parasitic Crustacean)

Species previously found on *Fundulus heteroclitus* from the east coast of North America:

Ergasilus manicatus	Massachusetts, USA, Roberts, 1970; Virgina, USA, Zwerner and Lawler, 1972; Maryland, USA, Barse, 1998
Ergasilus funduli	Virgina, USA, Harris and Vogelbein, 2006

Characteristics:

Ergasilus spp. are found on the gills of many freshwater and marine fishes. The females have modified antennae which allow them to strongly attach to a gill filament of the fish. Females are larger than males, but are the only sex found on fish gills. Males are found in the water column and have not been reported to parasitize fishes (Kabata, 1979; Roberts and Janovy, 2000; Wilson, 1911).

Lifecycle:

Ergasilus spp. metamorphose only during the free-living stages of the life cycle (Roberts and Janovy, 2000; Wilson, 1911). It is thought that the females are fertilized by the males in an early free-living larval stage of the life cycle. The spermatophores from the male are stored in order to fertilize all the eggs the female produces throughout her adult life. All of the eggs in the female's egg sacs hatch at the same time. The nauplii leave the egg sacs and over time undergo a series of moults to form three naupliar and five copepodid free-living larval stages (Roberts and Janovy, 2000; Wilson, 1911).

Potential Hosts:

Ergasilus spp. are found on a diversity of fishes. Bere (1930) found *E. manicalus* on whitebait (*Menidia notata*), rainbow smelt (*Osmerus mordax*) and *E. centrachidarum* on Atlantic tomcod (*Microgadus tomcod*). Bere (1936) found *Ergasilus lizae* on mummichog (*F. heteroclitus*) and striped mullet (*Mugil cephalus*). Mueller (1937) found *Ergasilus* spp. on largemouth bass (*Aplites salmonidae*), bullhead (*Ameiurus* spp.), and forktail catfish (*Ictalurus* spp.).

A5. *Homalometron pallidum* (Trematode)

Species previously found on *Fundulus heteroclitus* from the east coast of North America:

adult stage — Canadian Atlantic, Stafford, 1904; Maine, USA, Manther, 1926; Nova Scotia, Canada, Fantham and Porter, 1948; Newfoundland, Canada, Dickinson and Threlfall, 1975

Characteristics:

This trematode was first found in the intestine of *F. heteroclitus* (Stunkard, 1964). It is found in the intestines of fishes from marine, brackish, and freshwater. In *Fundulus* spp. sometimes high levels of larval *H. pallidum* was observed, but generally not more than two or three adult trematodes developed (Stunkard, 1964).

Lifecycle:

The life cycle of *H. pallidum* was described by Stunkard (1964). Eggs are released from the definitive host. From the egg a free-swimming larval stage, called the miracidium emerges. The miracidium penetrates a snail, the first intermediate host. In the snail, the miracidium metamorphoses into a sporocyst. Inside the sporocyst, the embryos develop into rediae. Each rediae may then produce one or more daughter rediae. The development of rediae and daughter rediae allows the development of many cercariae, free-living larval trematodes, by the process of asexual reproduction. Cercariae are released from the snail. Cercariae seek out and penetrate the second intermediate host, which is often a fish, where the parasite encysts as a metacercariae. The definitive

host feeds on either the second intermediate host or a paratenic host and the metacercariae matures into the adult stage.

Potential Hosts:

Like most trematodes, the first intermediate host is a snail such as *Hydrobia minuta* (Roberts and Janovy, 2000; Stunkard, 1964). Stunkard (1964) found encysted metacercarial *H. pallidum* stages in *Gemma gemma* and *H. minuta*. He also found that small polychaete worms could act as paratenic hosts and have encysted metacercarial *H. pallidum* stages as well. Adult *H. pallidum* can be found in mummichog (*F. heteroclitus*) and other fish species such as northern kingfish (*Menticirrhus saxatilis*), *Morone americana*, white perch (*Pseudopleuronectes americanus*), tautog (*Tautoga onitis*), silver perch (*Bairdiella chrysura*), and spot (*Leiostomus xanthurus*) (Cribb and Bray, 1999; Linton, 1940; Manter, 1931).

Appendix B: Raw data from Polycyclic Aromatic Hydrocarbons (PAHs) Analysis in Sediment and Biota

Outline:

B1. Raw data for sediments

Table B1: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) from Mira River and River Ryan.

Table B2: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) from the North and South Tar Ponds, Nova Scotia.

B2. Raw data for biota

Table B3: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in European green crab (*Carcinus maenas*) from Mira River and River Ryan.

Table B4: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in European green crab (*Carcinus maenas*) from the Sydney Tar Ponds.

Table B5: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in grass shrimp (*Palaemonetes* species) from Mira River and River Ryan.

Table B6: Calculated concentrations of selected polycyclic aromatic

Table B13: Calculated lipid adjusted concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in mummichog (*Fundulus heteroclitus*) from River Ryan and the Sydney Tar Ponds.

B1. Raw data for sediments

Table B1: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) from Mira River and River Ryan. Concentration of not detectable (n.d.) is a PAH concentration below 0.01 mg/kg dry wt.

Selected PAH		Mira River Concentration (dry wt mg/kg)				PAH	River Ryan Concentration (dry wt mg/kg)			
		A	B	C	Mean		A	B	C	Mean
Naphthalene	NAP	n.d.	n.d.	n.d.	**n.d.**	**NAP**	n.d.	n.d.	n.d.	**n.d.**
Acenaphthylene	ANY	n.d.	n.d.	n.d.	**n.d.**	**ANY**	n.d.	n.d.	n.d.	**n.d.**
Acenaphthene	ANA	n.d.	n.d.	n.d.	**n.d.**	**ANA**	n.d.	n.d.	n.d.	**n.d.**
Fluorene	FLU	n.d.	n.d.	n.d.	**n.d.**	**FLU**	n.d.	n.d.	n.d.	**n.d.**
Phenanthrene	PHA	n.d.	n.d.	n.d.	**n.d.**	**PHA**	n.d.	n.d.	n.d.	**n.d.**
Anthracene	ANT	n.d.	n.d.	n.d.	**n.d.**	**ANT**	0.01	n.d.	0.02	**0.01**
Fluoranthene	FLA	0.01	0.03	0.02	**0.02**	**FLA**	n.d.	n.d.	0.02	**n.d.**
Pyrene	PYR	n.d.	0.03	0.01	**0.02**	**PYR**	n.d.	n.d.	0.01	**n.d.**
Benz(*a*)anthracene	BAA	n.d.	0.01	n.d.	**n.d.**	**BAA**	n.d.	n.d.	0.02	**n.d.**
Chrysene/Triphenylene	C/T	n.d.	0.01	n.d.	**n.d.**	**C/T**	n.d.	n.d.	0.08	
Benzo(*b*)fluoranthene	BBF	n.d.	n.d.	n.d.	**n.d.**	**BBF**	n.d.	n.d.	n.d.	**n.d.**
Benzo(*k*)fluoranthene	BKF	n.d.	n.d.	n.d.	**n.d.**	**BKF**	n.d.	n.d.	n.d.	**n.d.**
Benzo(*e*)pyrene	BEY	n.d.	n.d.	n.d.	**n.d.**	**BEY**	n.d.	n.d.	n.d.	**n.d.**
Benzo(*a*)pyrene	BAY	n.d.	n.d.	n.d.	**n.d.**	**BAY**	n.d.	n.d.	n.d.	**n.d.**
Indenopyrene	INP	n.d.	n.d.	n.d.	**n.d.**	**INP**	n.d.	n.d.	n.d.	**n.d.**
Benzo(*g,h,i*)perylene	BGP	n.d.	n.d.	n.d.	**n.d.**	**BGP**	n.d.	n.d.	n.d.	**n.d.**
Dibenz(*a,h*)anthracene	DAN	n.d.	n.d.	n.d.	**n.d.**	**DAN**	n.d.	n.d.	n.d.	**n.d.**

Table B2: **Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) from the North and South Tar Ponds, Nova Scotia.**

Selected PAH		North Tar Pond Concentration (mg/kg dry wt)					PAH	South Tar Pond Concentration (mg/kg dry wt)				
		A	B	C	Mean	Std. Error		A	B	C	Mean	Std. Error
Naphthalene	NAP	3.1	1.6	0.41	**1.7**	0.2	**NAP**	26	7.6	7.8	**13.8**	2
Acenaphthylene	ANY	1.2	0.13	0.05	**0.5**	0.1	**ANY**	4.5	0.89	0.72	**2.0**	0.4
Acenaphthene	ANA	0.27	0.07	0.03	**0.12**	0.02	**ANA**	41	1.0	1.5	**15**	4
Fluorene	FLU	1.6	0.22	0.2	**0.7**	0.1	**FLU**	39	1.5	2.4	**14**	4
Phenanthrene	PHA	7.1	1.3	2.2	**3.5**	0.5	**PHA**	150	9.1	15	**58**	13
Anthracene	ANT	3.2	0.65	0.86	**1.6**	0.2	**ANT**	52	4.3	5.3	**21**	5
Fluoranthene	FLA	17	2.5	4.1	**8**	1	**FLA**	500	24	37	**187**	45
Pyrene	PYR	13	1.9	3.2	**6**	1	**PYR**	390	20	31	**147**	35
Benz(*a*)anthracene	BAA	9.8	1.5	2.5	**4.6**	0.8	**BAA**	150	14	16	**60**	13
Chrysene/Triphenylene	C/T	8.0	1.3	2.1	**3.8**	0.6	**C/T**	160	14	17	**64**	14
Benzo(*b*)fluoranthene	BBF	11	1.3	1.8	**5**	1	**BBF**	130	14	15	**53**	11
Benzo(*k*)fluoranthene	BKF	11	1.3	1.8	**5**	1	**BKF**	130	14	15	**53**	11
Benzo(*e*)pyrene	BEY	13	1.6	1.1	**5**	1	**BEY**	99	13	12	**41**	8
Benzo(*a*)pyrene	BAY	17	1.5	1.9	**7**	1	**BAY**	110	17	17	**48**	9
Indenopyrene	INP	14	1.3	1.1	**5**	1	**INP**	85	14	12	**37**	7
Benzo(*g,h,i*)perylene	BGP	12	1.4	1.2	**5**	1	**BGP**	75	15	12	**34**	6
Dibenz(*a,h*)anthracene	DAN	3.0	0.31	0.38	**1.2**	0.3	**DAN**	23	3.3	2.6	**10**	2
Organic Carbon Concentration %		7.6	2.3	3.1	**4.3**	0.5		52.9	9.9	6.7	**23**	4

B2. Raw data for biota

Table B3: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in European green crab (*Carcinus maenas*) from Mira River and River Ryan.

Selected PAH		Mira River (n = 3) Concentration (mg/kg wet wt)				PAH	River Ryan (n = 3) Concentration (mg/kg wet wt)			
		A	B	C	Mean		A	B	C	Mean
Naphthalene	NAP	<0.05	<0.05	<0.05	**<0.05**	**NAP**	<0.05	<0.05	<0.05	**<0.05**
Acenaphthylene	ANY	<0.05	<0.05	<0.05	**<0.05**	**ANY**	<0.05	<0.05	<0.05	**<0.05**
Acenaphthene	ANA	<0.05	<0.05	<0.05	**<0.05**	**ANA**	<0.05	<0.05	<0.05	**<0.05**
Fluorene	FLU	<0.05	<0.05	<0.05	**<0.05**	**FLU**	<0.05	<0.05	<0.05	**<0.05**
Phenanthrene	PHA	<0.05	<0.05	<0.05	**<0.05**	**PHA**	<0.05	<0.05	<0.05	**<0.05**
Anthracene	ANT	<0.05	<0.05	<0.05	**<0.05**	**ANT**	<0.05	<0.05	<0.05	**<0.05**
Fluoranthene	FLA	<0.05	<0.05	<0.05	**<0.05**	**FLA**	<0.05	<0.05	<0.05	**<0.05**
Pyrene	PYR	<0.05	<0.05	<0.05	**<0.05**	**PYR**	<0.05	<0.05	<0.05	**<0.05**
Benz[*a*]anthracene	BAA	<0.05	<0.05	<0.05	**<0.05**	**BAA**	<0.05	<0.05	<0.05	**<0.05**
Chrysene/Triphenylene	C/T	<0.05	<0.05	<0.05	**<0.05**	**C/T**	<0.05	<0.05	<0.05	**<0.05**
Benzo[*b*]fluoranthene	BBF	<0.05	<0.05	<0.05	**<0.05**	**BBF**	<0.05	<0.05	<0.05	**<0.05**
Benzo[*k*]fluoranthene	BKF	<0.05	<0.05	<0.05	**<0.05**	**BKF**	<0.05	<0.05	<0.05	**<0.05**
Benzo[*e*]pyrene	BEY	<0.05	<0.05	<0.05	**<0.05**	**BEY**	<0.05	<0.05	<0.05	**<0.05**
Benzo[*a*]pyrene	BAY	<0.05	<0.05	<0.05	**<0.05**	**BAY**	<0.05	<0.05	<0.05	**<0.05**
Indenopyrene	INP	<0.05	<0.05	<0.05	**<0.05**	**INP**	<0.05	<0.05	<0.05	**<0.05**
Benzo[*g,h,i*]perylene	BGP	<0.05	<0.05	<0.05	**<0.05**	**BGP**	<0.05	<0.05	<0.05	**<0.05**
Dibenz[*a,h*]anthracene	DAN	<0.05	<0.05	<0.05	**<0.05**	**DAN**	<0.05	<0.05	<0.05	**<0.05**

Table B4: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in European green crab (*Carcinus maenas*) from the Sydney Tar Ponds. For PAHs with concentrations <0.05 mg/kg wet wt half the detection limit (0.025 mg/kg wet wt) was used to calculate mean PAH concentrations.

Selected PAH		Sydney Tar Ponds (n = 3) Concentration (mg/kg wet wt)					
		A	B	C	Mean	Std. Dev.	Std. Error
Naphthalene	NAP	0.23	0.11	< 0.05	**0.12**	0.10	0.02
Acenaphthylene	ANY	0.05	0.08	< 0.05	**0.05**	0.03	0.00
Acenaphthene	ANA	0.26	0.06	< 0.05	**0.12**	0.13	0.02
Fluorene	FLU	0.06	0.13	< 0.05	**0.07**	0.05	0.01
Phenanthrene	PHA	0.34	1.06	0.06	**0.49**	0.52	0.09
Anthracene	ANT	0.05	0.17	<0.05	**0.08**	0.08	0.01
Fluoranthene	FLA	0.43	0.99	0.06	**0.49**	0.47	0.08
Pyrene	PYR	0.4	0.82	0.06	**0.43**	0.38	0.06
Benz[*a*]anthracene	BAA	0.15	0.34	< 0.05	**0.17**	0.16	0.03
Chrysene/Triphenylene	C/T	0.15	0.2	< 0.05	**0.13**	0.09	0.02
Benzo[*b*]fluoranthene	BBF	0.06	0.18	< 0.05	**0.09**	0.08	0.01
Benzo[*k*]fluoranthene	BKF	0.06	0.18	< 0.05	**0.09**	0.08	0.01
Benzo[*e*]pyrene	BEY	0.07	0.15	< 0.05	**0.08**	0.06	0.01
Benzo[*a*]pyrene	BAY	0.06	0.24	< 0.05	**0.11**	0.12	0.02
Indenopyrene	INP	< 0.05	0.1	< 0.05	**0.05**	0.04	0.01
Benzo[*g,h,i*]perylene	BGP	< 0.05	0.12	< 0.05	**0.06**	0.05	0.01
Dibenz[*a,h*]anthracene	DAN	< 0.05	< 0.05	< 0.05	**<0.05**	--	--

Table B5: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in grass shrimp (*Palaemonetes* species) from Mira River and River Ryan.

Selected PAH		Mira River (n = 2) Concentration (mg/kg wet wt)			PAH	River Ryan (n = 1) Concentration (mg/kg wet wt)
		A	B	Mean		A
Naphthalene	NAP	<0.10	<0.10	**<0.10**	**NAP**	**<0.10**
Acenaphthylene	ANY	<0.10	<0.10	**<0.10**	**ANY**	**<0.10**
Acenaphthene	ANA	<0.10	<0.10	**<0.10**	**ANA**	**<0.10**
Fluorene	FLU	<0.10	<0.10	**<0.10**	**FLU**	**<0.10**
Phenanthrene	PHA	<0.10	<0.10	**<0.10**	**PHA**	**<0.10**
Anthracene	ANT	<0.10	<0.10	**<0.10**	**ANT**	**<0.10**
Fluoranthene	FLA	<0.10	<0.10	**<0.10**	**FLA**	**<0.10**
Pyrene	PYR	<0.10	<0.10	**<0.10**	**PYR**	**<0.10**
Benz[*a*]anthracene	BAA	<0.10	<0.10	**<0.10**	**BAA**	**<0.10**
Chrysene/Triphenylene	C/T	<0.10	<0.10	**<0.10**	**C/T**	**<0.10**
Benzo[*b*]fluoranthene	BBF	<0.10	<0.10	**<0.10**	**BBF**	**<0.10**
Benzo[*k*]fluoranthene	BKF	<0.10	<0.10	**<0.10**	**BKF**	**<0.10**
Benzo[*e*]pyrene	BEY	<0.10	<0.10	**<0.10**	**BEY**	**<0.10**
Benzo[*a*]pyrene	BAY	<0.10	<0.10	**<0.10**	**BAY**	**<0.10**
Indenopyrene	INP	<0.10	<0.10	**<0.10**	**INP**	**<0.10**
Benzo[*g,h,i*]perylene	BGP	<0.10	<0.10	**<0.10**	**BGP**	**<0.10**
Dibenz[*a,h*]anthracene	DAN	<0.10	<0.10	**<0.10**	**DAN**	**<0.10**

Table B6: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in grass shrimp (*Palaemonetes* species) from the Sydney Tar Ponds. For PAHs with concentrations <0.05 mg/kg wet wt half the detection limit (0.025 mg/kg wet wt) was used to calculate mean PAH concentrations.

Selected PAH		Sydney Tar Ponds (n = 3) Concentration (mg/kg wet wt)					
		A	B	C	Mean	Std. Dev.	Std. Error
Naphthalene	NAP	0.12	0.13	0.05	**0.10**	0.04	0.01
Acenaphthylene	ANY	<0.10	<0.10	<0.10	**<0.10**	--	--
Acenaphthene	ANA	0.14	0.1	0.05	**0.10**	0.05	0.01
Fluorene	FLU	0.12	0.05	0.05	**0.07**	0.04	0.01
Phenanthrene	PHA	0.35	0.21	0.16	**0.24**	0.10	0.02
Anthracene	ANT	0.16	0.12	0.1	**0.13**	0.03	0.01
Fluoranthene	FLA	0.98	0.76	0.56	**0.77**	0.21	0.04
Pyrene	PYR	0.64	0.44	0.32	**0.47**	0.16	0.03
Benz[*a*]anthracene	BAA	<0.10	<0.10	<0.10	**<0.10**	--	--
Chrysene/Triphenylene	C/T	0.12	0.05	0.05	**0.07**	0.04	0.01
Benzo[*b*]fluoranthene	BBF	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*k*]fluoranthene	BKF	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*e*]pyrene	BEY	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*a*]pyrene	BAY	<0.10	<0.10	<0.10	**<0.10**	--	--
Indenopyrene	INP	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*g,h,i*]perylene	BGP	<0.10	<0.10	<0.10	**<0.10**	--	--
Dibenz[*a,h*]anthracene	DAN	<0.10	<0.10	<0.10	**<0.10**	--	--

Table B7: **Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in American eel (*Anguilla rostrata*) from Mira River.**

Selected PAH		Mira River (n = 2) Concentration (mg/kg wet wt)		
		A	B	Mean
Naphthalene	NAP	<0.05	<0.05	**<0.05**
Acenaphthylene	ANY	<0.05	<0.05	**<0.05**
Acenaphthene	ANA	<0.05	<0.05	**<0.05**
Fluorene	FLU	<0.05	<0.05	**<0.05**
Phenanthrene	PHA	<0.05	<0.05	**<0.05**
Anthracene	ANT	<0.05	<0.05	**<0.05**
Fluoranthene	FLA	<0.05	<0.05	**<0.05**
Pyrene	PYR	<0.05	<0.05	**<0.05**
Benz[*a*]anthracene	BAA	<0.05	<0.05	**<0.05**
Chrysene/Triphenylene	C/T	<0.05	<0.05	**<0.05**
Benzo[*b*]fluoranthene	BBF	<0.05	<0.05	**<0.05**
Benzo[*k*]fluoranthene	BKF	<0.05	<0.05	**<0.05**
Benzo[*e*]pyrene	BEY	<0.05	<0.05	**<0.05**
Benzo[*a*]pyrene	BAY	<0.05	<0.05	**<0.05**
Indenopyrene	INP	<0.05	<0.05	**<0.05**
Benzo[*g,h,i*]perylene	BGP	<0.05	<0.05	**<0.05**
Dibenz[*a,h*]anthracene	DAN	<0.05	<0.05	**<0.05**

Table B8: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in American eel (*Anguilla rostrata*) from the Sydney Tar Ponds. For PAHs with concentrations < 0.05 or < 0.10 mg/kg wet wt half the detection limit (0.025 or 0.05 mg/kg wet wt respectively) was used to calculate mean PAH concentrations.

Selected PAH		Sydney Tar Ponds (n = 5) Concentration (mg/kg wet wt)							
		A	B	C	D	E	Mean	Std. Dev.	Std. Error
Naphthalene	NAP	0.15	<0.10	0.47	0.14	<0.10	**0.22**	0.22	0.04
Acenaphthylene	ANY	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Acenaphthene	ANA	0.09	<0.10	0.2	0.13	<0.10	**0.13**	0.08	0.01
Fluorene	FLU	< 0.05	<0.10	0.11	<0.10	<0.10	**0.07**	0.03	0.01
Phenanthrene	PHA	0.08	<0.10	0.48	<0.10	0.2	**0.24**	0.22	0.04
Anthracene	ANT	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Fluoranthene	FLA	0.08	<0.10	0.33	<0.10	0.3	**0.23**	0.15	0.03
Pyrene	PYR	< 0.05	<0.10	0.18	<0.10	0.23	**0.15**	0.09	0.02
Benz[*a*]anthracene	BAA	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Chrysene/Triphenylene	C/T	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*b*]fluoranthene	BBF	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*k*]fluoranthene	BKF	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*e*]pyrene	BEY	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*a*]pyrene	BAY	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Indenopyrene	INP	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Benzo[*g,h,i*]perylene	BGP	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--
Dibenz[*a,h*]anthracene	DAN	< 0.05	<0.10	<0.10	<0.10	<0.10	**<0.10**	--	--

Table B9: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in mummichog (*Fundulus heteroclitus*) from Mira River and River Ryan. For PAHs with concentrations <0.05 mg/kg wet wt half the detection limit (0.025 mg/kg wet wt) was used to calculate mean PAH concentrations.

Selected PAH		Mira River (n = 3) Concentration (mg/kg wet wt)				PAH	River Ryan (n = 3) Concentration (mg/kg wet wt)			
		A	B	C	Mean		A	B	C	Mean
Naphthalene	NAP	<0.05	<0.05	<0.05	**<0.05**	**NAP**	<0.05	<0.05	<0.05	**<0.05**
Acenaphthylene	ANY	<0.05	<0.05	<0.05	**<0.05**	**ANY**	<0.05	<0.05	<0.05	**<0.05**
Acenaphthene	ANA	<0.05	<0.05	<0.05	**<0.05**	**ANA**	<0.05	<0.05	<0.05	**<0.05**
Fluorene	FLU	<0.05	<0.05	<0.05	**<0.05**	**FLU**	<0.05	<0.05	<0.05	**<0.05**
Phenanthrene	PHA	<0.05	<0.05	<0.05	**<0.05**	**PHA**	<0.05	<0.05	<0.05	**<0.05**
Anthracene	ANT	<0.05	<0.05	<0.05	**<0.05**	**ANT**	<0.05	<0.05	<0.05	**<0.05**
Fluoranthene	FLA	<0.05	<0.05	<0.05	**<0.05**	**FLA**	<0.05	<0.05	<0.05	**<0.05**
Pyrene	PYR	<0.05	<0.05	<0.05	**<0.05**	**PYR**	0.07	<0.05	<0.05	**0.04**
Benz(a)anthracene	BAA	<0.05	<0.05	<0.05	**<0.05**	**BAA**	0.08	<0.05	<0.05	**0.04**
Chrysene/Triphenylene	C/T	<0.05	<0.05	<0.05	**<0.05**	**C/T**	<0.05	<0.05	<0.05	**<0.05**
Benzo(b)fluoranthene	BBF	<0.05	<0.05	<0.05	**<0.05**	**BBF**	<0.05	<0.05	<0.05	**<0.05**
Benzo(k)fluoranthene	BKF	<0.05	<0.05	<0.05	**<0.05**	**BKF**	<0.05	<0.05	<0.05	**<0.05**
Benzo(e)pyrene	BEY	<0.05	<0.05	<0.05	**<0.05**	**BEY**	<0.05	<0.05	<0.05	**<0.05**
Benzo(a)pyrene	BAY	<0.05	<0.05	<0.05	**<0.05**	**BAY**	<0.05	<0.05	<0.05	**<0.05**
Indenopyrene	INP	<0.05	<0.05	<0.05	**<0.05**	**INP**	<0.05	<0.05	<0.05	**<0.05**
Benzo(g,h,i)perylene	BGP	<0.05	<0.05	<0.05	**<0.05**	**BGP**	<0.05	<0.05	<0.05	**<0.05**
Dibenz(a,h)anthracene	DAN	<0.05	<0.05	<0.05	**<0.05**	**DAN**	<0.05	<0.05	<0.05	**<0.05**

Table B10: Calculated concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in mummichog (*Fundulus heteroclitus*) from the Sydney Tar Ponds. Concentration of not detectable (n.d.) is a PAH concentration <0.05 mg/kg wet wt.

Selected PAH		Sydney Tar Ponds (n = 4) Concentration (mg/kg wet wt)						
		A	B	C	D	Mean	Std. Dev.	Std. Error
Naphthalene	NAP	0.07	< 0.05	0.07	< 0.05	**0.05**	0.03	0.004
Acenaphthylene	ANY	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--
Acenaphthene	ANA	0.1	< 0.05	0.09	< 0.05	**0.06**	0.04	0.007
Fluorene	FLU	0.08	< 0.05	0.07	< 0.05	**0.05**	0.03	0.005
Phenanthrene	PHA	0.15	< 0.05	0.13	0.08	**0.10**	0.06	0.009
Anthracene	ANT	0.07	< 0.05	0.07	< 0.05	**0.05**	0.03	0.004
Fluoranthene	FLA	0.09	< 0.05	0.19	0.06	**0.09**	0.07	0.012
Pyrene	PYR	< 0.05	< 0.05	0.1	< 0.05	**0.04**	0.04	0.006
Benz(a)anthracene	BAA	< 0.05	< 0.05	0.05	< 0.05	**<0.05**	--	--
Chrysene/Triphenylene	C/T	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--
Benzo(b)fluoranthene	BBF	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--
Benzo(k)fluoranthene	BKF	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--
Benzo(e)pyrene	BEY	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--
Benzo(a)pyrene	BAY	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--
Indenopyrene	INP	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--
Benzo(g,h,i)perylene	BGP	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--
Dibenz(a,h)anthracene	DAN	< 0.05	< 0.05	< 0.05	< 0.05	**<0.05**	--	--

B3. Lipid analysis of biota

Table B11: Lipid content of biota analyzed for polycyclic aromatic hydrocarbons (PAHs). Detection limits were <0.05 g lipid/ 100g sample.

		Lipid Content Concentration (g lipid/ 100g sample)			
	Sample	*Carcinus maenas*	*Palaemonetes* spp.	*Anguilla rostrata*	*Fundulus heteroclitus*
Mira River	**A**	0.62	2.08	6.10	3.10
	B	1.16	1.94	2.11	2.00
	C	1.28	--	--	1.73
River Ryan	**A**	1.75	2.19	--	2.55
	B	1.12	--	--	2.69
	C	1.9	--	--	2.74
Tar Ponds	**A**	2.15	2.87	3.59	2.19
	B	0.62	2.6	1.85	2.43
	C	<0.05	2.48	2.76	1.96
	D	--	--	3.89	1.68
	E	--	--	2.13	--

B4. Lipid adjusted biota concentrations

Table B12: Calculated lipid adjusted concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in European green crab (*Carcinus maenas*) and American eel (*Anguilla rostrata*) from the Sydney Tar Ponds. Concentration of not detectable (n.d.) is a PAH concentration below 0.05 mg/kg wet wt.

Selected PAH		Concentration (mg/kg lipid tissue) European Green Crab (n = 3)						American Eel (n =1)
		1	2	3	Mean	Std. Dev.	Std. Error	
Naphthalene	NAP	10.7	17.7	n.d.	**9.5**	8.9	1.5	**4.2**
Acenaphthylene	ANY	2.3	12.9	n.d.	**5.1**	6.9	1.1	**n.d.**
Acenaphthene	ANA	12.1	9.7	n.d.	**7.3**	6.4	1.1	**2.5**
Fluorene	FLU	2.8	21.0	n.d.	**7.9**	11.4	1.9	**n.d.**
Phenanthrene	PHA	15.8	171.0	120.0	**102.3**	79.1	13.2	**2.2**
Anthracene	ANT	2.3	27.4	n.d.	**9.9**	15.2	2.5	**n.d.**
Fluoranthene	FLA	20.0	159.7	120.0	**99.9**	72.0	12.0	**2.2**
Pyrene	PYR	18.6	132.3	120.0	**90.3**	62.4	10.4	**n.d.**
Benz(a)anthracene	BAA	7.0	54.8	n.d.	**20.6**	29.9	5.0	**n.d.**
Chrysene/Triphenylene	C/T	7.0	32.3	n.d.	**13.1**	17.0	2.8	**n.d.**
Benzo(b)fluoranthene	BBF	2.8	29.0	n.d.	**10.6**	16.0	2.7	**n.d.**
Benzo(k)fluoranthene	BKF	2.8	29.0	n.d.	**10.6**	16.0	2.7	**n.d.**
Benzo(e)pyrene	BEY	3.3	24.2	n.d.	**9.2**	13.1	2.2	**n.d.**
Benzo(a)pyrene	BAY	2.8	38.7	n.d.	**13.8**	21.6	3.6	**n.d.**
Indenopyrene	INP	n.d.	16.1	n.d.	**5.4**	9.3	1.6	**n.d.**
Benzo(g,h,i)perylene	BGP	n.d.	19.4	n.d.	**6.5**	11.2	1.9	**n.d.**
Dibenz(a,h)anthracene	DAN	n.d.	n.d.	n.d.	**n.d.**	--	--	**n.d.**

Table B13: Calculated lipid adjusted concentrations of selected polycyclic aromatic hydrocarbons (PAHs) in mummichog (*Fundulus heteroclitus*) from River Ryan and the Sydney Tar Ponds. Concentration of not detectable (n.d.) is a PAH concentration below 0.05 mg/kg wet wt.

Selected PAH		Concentration (mg/kg lipid tissue)											
		River Ryan (n = 3)				PAH	Sydney Tar Ponds (n = 4)						
		1	2	3	Mean		1	2	3	4	Mean	Std. Dev.	Std. Error
Naphthalene	NAP	n.d.	n.d.	n.d.	**n.d.**	**NAP**	0.03	n.d.	3.6	n.d.	**0.9**	1.8	0.9
Acenaphthylene	ANY	n.d.	n.d.	n.d.	**n.d.**	**ANY**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--
Acenaphthene	ANA	n.d.	n.d.	n.d.	**n.d.**	**ANA**	0.05	n.d.	4.6	n.d.	**1.2**	2.3	1.1
Fluorene	FLU	n.d.	n.d.	n.d.	**n.d.**	**FLU**	0.04	n.d.	3.6	n.d.	**0.9**	1.8	0.9
Phenanthrene	PHA	n.d.	n.d.	n.d.	**n.d.**	**PHA**	0.07	n.d.	6.6	4.8	**2.9**	3.4	1.7
Anthracene	ANT	n.d.	n.d.	n.d.	**n.d.**	**ANT**	0.03	n.d.	3.6	n.d.	**0.9**	1.8	0.9
Fluoranthene	FLA	n.d.	n.d.	n.d.	**n.d.**	**FLA**	0.04	n.d.	9.7	3.6	**3.3**	4.6	2.3
Pyrene	PYR	2.7	n.d.	n.d.	**0.9**	**PYR**	n.d.	n.d.	5.1	n.d.	**1.3**	2.6	1.3
Benz(a)anthracene	BAA	3.1	n.d.	n.d.	**1.1**	**BAA**	n.d.	n.d.	2.6	n.d.	**0.6**	1.3	0.6
Chrysene/Triphenylene	C/T	n.d.	n.d.	n.d.	**n.d.**	**C/T**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--
Benzo(b)fluoranthene	BBF	n.d.	n.d.	n.d.	**n.d.**	**BBF**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--
Benzo(k)fluoranthene	BKF	n.d.	n.d.	n.d.	**n.d.**	**BKF**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--
Benzo(e)pyrene	BEY	n.d.	n.d.	n.d.	**n.d.**	**BEY**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--
Benzo(a)pyrene	BAY	n.d.	n.d.	n.d.	**n.d.**	**BAY**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--
Indenopyrene	INP	n.d.	n.d.	n.d.	**n.d.**	**INP**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--
Benzo(g,h,i)perylene	BGP	n.d.	n.d.	n.d.	**n.d.**	**BGP**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--
Dibenz(a,h)anthracene	DAN	n.d.	n.d.	n.d.	**n.d.**	**DAN**	n.d.	n.d.	n.d.	n.d.	**n.d.**	--	--

Appendix C: Raw data from *Fundulus* spp.

Parasitological Analysis

Outline:

C1. Biological data of *Fundulus* spp. used in parasitological analysis

Table C1: Biological data of *Fundulus* spp. (n = 53) from Sydney Tar Ponds used in parasitological analysis.

Table C2: Biological data of *Fundulus* spp. (n = 69) from River Ryan used in parasitological analysis.

Table C3: Biological data of *Fundulus* spp. (n = 64) from Mira River used in parasitological analysis.

C2. Raw data of parasites found in *Fundulus* spp.

Table C4: Parasites found in *Fundulus* spp (n = 53) from the Sydney Tar Ponds.

Table C5: Ectoparasites found in *Fundulus* spp. (n = 69) from River Ryan.

Table C6: Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 69) from River Ryan.

Table C7: Ectoparasites found in *Fundulus* spp. (n = 64) from Mira River.

Table C8: Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 64) from Mira River.

C1. Biological data of *Fundulus* spp. used in parasitological analysis

Table C1: Biological data of *Fundulus* spp. (n = 53) from Sydney Tar Ponds used in parasitological analysis. TL, total length; SL, standard length; FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Sex (M/F)	TL (mm)	SL (mm)	Weight (g)	Plump (mm)	Species
SYPO01	F	84.29	68.89	6.97	14.79	FUNHET
SYPO02	M	64.5	53.51	2.76	11.24	FUNHET
SYPO03	F	51.2	42.26	1.8	8.56	FUNUID
SYPO04	F	49.36	41.59	1.81	9.71	FUNUID
SYPO05	M	41.27	33.88	0.87	7.32	FUNHET
SYPO06	M	51.04	40.71	1.72	9.92	FUNHET
SYPO07	F	51.66	42.8	1.86	8.45	FUNUID
SYPO08	F	89.51	70.97	8.3	13.67	FUNHET
SYPO09	M	54.01	45.27	1.78	9.56	FUNHET
SYPO10	F	51.85	42.85	1.87	8.62	FUNHET
SYPO11	F	49.29	41.11	1.62	7.82	FUNHET
SYPO12	F	50.76	43.79	1.68	7.98	FUNHET
SYPO51	F	56.6	45.23	2	9.75	FUNHET
SYPO52	F	51.87	44.63	1.8	8.55	FUNHET
SYPO100	M	59.94	50.23	2.30	9.90	FUNHET
SYPO101	M	51.87	43.76	1.83	9.70	FUNHET
SYPO102	M	55.65	46.04	2.17	9.61	FUNHET
SYPO103	M	47.1	38.84	1.22	7.96	FUNHET
SYPO104	M	51.24	42.42	1.51	8.40	FUNHET
SYPO105	F	61.53	52.2	3.55	10.59	FUNHET
SYPO106	M	43.7	36.17	0.90	6.91	FUNHET
SYPO107	F	95.32	79.5	12.09	18.20	FUNHET
SYPO108	M	61.93	51.71	2.60	10.79	FUNHET
SYPO109	M	53.98	43.07	1.51	9.48	FUNHET
SYPO110	M	50.1	42.74	1.49	8.38	FUNHET
SYPO111	M	55.15	47.55	1.97	9.65	FUNHET
SYPO112	M	39.42	33.86	0.66	5.81	FUNHET
SYPO113	M	54.11	45.44	1.86	9.04	FUNHET
SYPO114	M	45.84	37.13	1.13	5.34	FUNHET
SYPO150	F	72.48	62.24	4.95	11.69	FUNHET
SYPO151	F	74.01	61.80	5.25	11.06	FUNHET
SYPO152	M	63.68	52.69	3.34	11.35	FUNHET
SYPO153	M	72.92	61.98	5.03	12.34	FUNHET

Table C1 (continued): Biological data of *Fundulus* spp. (n = 53) from Sydney Tar Ponds used in parasitological analysis. TL, total length; SL, standard length; FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Sex (M/F)	TL (mm)	SL (mm)	Weight (g)	Plump (mm)	Species
SYPO154	F	66.66	55.69	3.62	10.56	FUNHET
SYPO155	F	56.94	47.45	2.06	8.82	FUNHET
SYPO156	M	51.89	43.36	1.71	8.31	FUNHET
SYPO157	F	53.92	45.43	1.87	7.86	FUNHET
SYPO158	F	46.53	38.23	1.31	7.52	FUNUID
SYPO159	M	53.74	45.14	1.99	9.24	FUNHET
SYPO160	M	55.78	48.67	2.21	9.14	FUNHET
SYPO161	F	53.15	42.46	1.66	7.71	FUNHET
SYPO162	M	50.14	41.47	1.38	7.40	FUNHET
SYPO163	M	50.53	43.43	1.62	8.09	FUNHET
SYPO164	F	51.07	44.18	1.57	7.52	FUNHET
SYPO165	F	52.11	45.37	1.87	8.56	FUNHET
SYPO166	F	51.00	44.26	1.60	7.34	FUNHET
SYPO167	M	42.29	36.98	0.89	6.32	FUNHET
SYPO168	M	45.03	38.32	1.06	6.87	FUNHET
SYPO169	F	51.02	44.02	1.79	7.92	FUNHET
SYPO170	F	45.06	37.87	1.2	6.89	FUNHET
SYPO171	M	45.94	39.05	1.28	7.65	FUNHET
SYPO172	F	44.85	37.61	1.09	7.31	FUNHET
SYPO173	F	46.05	38.82	1.21	7.55	FUNHET

Table C2: Biological data of *Fundulus* spp. (n = 69) from River Ryan used in parasitological analysis. TL, total length; SL, standard length; FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Sex (M/F)	TL (mm)	SL (mm)	Weight (g)	Plump (mm)	Species
RIRY01	M	70.9	57.92	4.98	14.3	FUNHET
RIRY02	M	63.37	50.97	3.2	11.95	FUNHET
RIRY03	M	67.07	55.57	3.45	10.95	FUNHET
RIRY04	M	43.18	34.94	0.87	8.11	FUNHET
RIRY05	M	80.06	67.17	6.96	15.17	FUNHET
RIRY07	M	66.17	54.99	4.03	12.61	FUNHET
RIRY08	F	49.08	38.01	1.48	8.05	FUNHET
RIRY10	F	34.45	28.27	0.41	4.99	FUNHET
RIRY11	F	45.44	38.11	1.07	6.94	FUNHET
RIRY12	M	40.83	33.43	0.77	6.43	FUNHET
RIRY13	F	88.46	74.09	10.49	16.22	FUNHET
RIRY14	M	46.62	38.01	1.1	7.56	FUNHET
RIRY15	F	41.99	34.96	0.8	6.41	FUNHET
RIRY16	F	75.21	64.45	6.28	14.08	FUNHET
RIRY17	F	50.3	41.14	1.65	8.68	FUNHET
RIRY18	F	80.76	68.25	6.71	13.93	FUNHET
RIRY19	F	95.18	81.16	13.05	19.09	FUNHET
RIRY20	M	71.64	59.88	4.3	12.54	FUNHET
RIRY21	F	46.87	39.4	1.16	7.64	FUNHET
RIRY22	M	42.19	33.62	0.82	7.25	FUNHET
RIRY23	M	91.56	76.3	10.19	18.13	FUNHET
RIRY24	F	44.85	36.77	0.99	7.56	FUNHET
RIRY25	F	50.98	41.05	1.63	9.23	FUNHET
RIRY26	M	38.8	32.49	1.74	6.54	FUNHET
RIRY27	F	41.98	34.82	0.74	6.62	FUNUID
RIRY28	F	82.67	68.52	7.74	15.57	FUNUID
RIRY29	F	66.07	54.49	3.38	12.24	FUNUID
RIRY30	M	39.82	31.4	0.68	6.21	FUNUID
RIRY50	F	49.7	41.82	1.49	8.11	FUNUID
RIRY51	F	103.32	89.82	18.41	20.82	FUNHET
RIRY52	F	49.63	40.33	1.43	7.13	FUNHET
RIRY53	M	48.64	40.25	1.49	7.88	FUNHET
RIRY54	M	52.17	42.78	1.99	9.13	FUNHET
RIRY55	M	50.02	41	1.55	8.8	FUNHET
RIRY56	F	55.51	45.63	2.2	9.31	FUNHET
RIRY57	F	59.47	50.3	3.16	10.1	FUNHET
RIRY58	M	75.42	61.53	5.69	13.9	FUNHET

Table C2 (continued): Biological data of *Fundulus* spp. (n = 69) from River Ryan used in parasitological analysis. TL, total length; SL, standard length; FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Sex (M/F)	TL (mm)	SL (mm)	Weight (g)	Plump (mm)	Species
RIRY59	M	80.3	64.5	6.48	20.71	FUNHET
RIRY60	F	57.4	47.8	2.91	11.9	FUNHET
RIRY61	F	51.7	43.5	1.7	8.6	FUNHET
RIRY62	F	56	47.5	2.59	12.3	FUNHET
RIRY63	F	50.8	43	1.35	7.5	FUNUID
RIRY64	M	55	46.5	2.56	10.5	FUNHET
RIRY65	F	61.3	50.9	2.87	11.5	FUNHET
RIRY66	F	53	45.1	2.38	10.5	FUNHET
RIRY67	F	52.1	46.9	1.98	10.4	FUNHET
RIRY68	M	49.1	40.9	1.45	7.1	FUNHET
RIRY69	M	50.8	44	2.1	10.1	FUNHET
RIRY100	F	69.78	58.39	5.08	11.3	FUNHET
RIRY101	M	61.04	51.78	3.08	11.22	FUNHET
RIRY102	F	65.11	55.12	3.327	10.13	FUNUID
RIRY103	F	73.9	61.72	5.26	12.71	FUNHET
RIRY104	M	73.62	61.74	5.59	12.67	FUNHET
RIRY105	M	63.21	52.26	3.34	10.37	FUNHET
RIRY106	M	70.8	57.46	4.8	13.01	FUNHET
RIRY107	M	74.0	60.51	4.62	14.87	FUNHET
RIRY108	M	42.0	35.28	0.89	7.70	FUNHET
RIRY109	F	68.58	57.83	4.04	11.28	FUNHET
RIRY110	F	59.59	49.79	2.75	10.03	FUNHET
RIRY111	M	71.68	57.99	4.77	12.35	FUNHET
RIRY112	F	61.85	50.62	3.23	10.08	FUNHET
RIRY113	M	44.5	37.12	1.05	6.89	FUNHET
RIRY114	M	61.35	50.96	3.14	10.36	FUNHET
RIRY115	M	56.3	45.7	2.48	9.48	FUNHET
RIRY116	F	64.01	53.78	3.39	10.46	FUNHET
RIRY117	M	57.79	48.28	2.66	10.42	FUNHET
RIRY118	F	69.89	58.57	4.28	11.06	FUNHET
RIRY119	F	73.86	62.70	5.77	11.93	FUNHET
RIRY120	F	46.34	37.72	1.18	7.90	FUNHET

Table C3: Biological data of *Fundulus* spp. (n = 64) from Mira River used in parasitological analysis. TL, total length; SL, standard length; FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Sex (M/F)	TL (mm)	SL (mm)	Weight (g)	Plump (mm)	Species
MIRI01	F	46.86	37.77	1.48	7.7	FUNUID
MIRI03	M	58.77	47.42	2.62	10.63	FUNHET
MIRI04	F	47.01	38.25	1.5	8.94	FUNHET
MIRI05	F	74.95	62.32	6.13	14.44	FUNHET
MIRI07	F	52.45	43.34	1.9	9.2	FUNUID
MIRI08	F	64.28	52.3	4.18	12.92	FUNHET
MIRI09	M	49.99	41.46	1.63	8.92	FUNHET
MIRI10	M	51.7	41.43	1.8	9.57	FUNHET
MIRI11	F	--	65.06	6.69	14.42	FUNHET
MIRI12	F	57.66	45.86	2.66	9.36	FUNUID
MIRI13	M	51.83	41.37	1.59	8.03	FUNHET
MIRI14	M	68.79	56.49	4.43	13.53	FUNHET
MIRI15	F	50.47	41.01	1.5	8.15	FUNHET
MIRI16	F	57.53	46.11	2.43	9.6	FUNHET
MIRI17	F	--	42.84	1.65	9.36	FUNHET
MIRI18	F	55.76	46.75	2.41	9.53	FUNHET
MIRI19	F	--	64.61	6.33	14.11	FUNHET
MIRI20	M	73.33	60.69	5.17	14.32	FUNHET
MIRI21	F	51.31	43.2	1.87	9.4	FUNHET
MIRI22	F	53.73	45.48	2.03	9.11	FUNHET
MIRI23	F	47.47	37.78	1.18	7.68	FUNHET
MIRI24	F	55.36	45.36	2.29	10.19	FUNHET
MIRI26	M	53	42.39	1.62	8.52	FUNHET
MIRI27	F	59.18	48.8	2.94	11.16	FUNHET
MIRI28	M	68.79	58.87	4.82	12.99	FUNHET
MIRI29	F	60.79	50.04	3.04	11.21	FUNHET
MIRI30	F	54.96	44.61	2.21	10.41	FUNHET
MIRI31	F	56.03	45.68	2.5	6.11	FUNHET
MIRI32	F	63.88	51.75	3.57	10.47	FUNHET
MIRI33	M	57.1	45.37	2.54	10.71	FUNHET
MIRI34	F	62.53	49.96	3.71	12.58	FUNHET
MIRI35	F	53.02	43.87	1.92	9.46	FUNHET
MIRI50	F	62.46	52.14	3.19	11.37	FUNHET
MIRI51	F	67.29	55.6	4.57	12.61	FUNHET
MIRI52	F	106.1	87.91	20.36	21.85	FUNHET
MIRI53	F	56.11	45.89	2.11	9.19	FUNUID
MIRI54	M	53.18	43.7	1.95	9.18	FUNHET

Table C3 (continued): Biological data of *Fundulus* spp. (n = 64) from Mira River used in parasitological analysis. TL, total length; SL, standard length; FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Sex (M/F)	TL (mm)	SL (mm)	Weight (g)	Plump (mm)	Species
MIRI55	M	66.73	52.82	3.71	12	FUNHET
MIRI56	F	61.73	52.45	3.2	10.79	FUNHET
MIRI57	F	64.8	51.82	3.48	10.89	FUNHET
MIRI58	F	54.58	43.19	1.76	8.73	FUNHET
MIRI59	M	52.15	42.67	1.64	8.24	FUNHET
MIRI60	M	55.93	45.07	2.55	10.83	FUNHET
MIRI61	F	56.33	46.83	2.47	9.42	FUNHET
MIRI62	F	53.58	44.28	2.12	9.18	FUNHET
MIRI63	M	59.59	50.24	2.69	10.22	FUNHET
MIRI64	F	66.38	53.76	4.07	11.32	FUNHET
MIRI65	M	54.14	44.69	2.01	8.97	FUNHET
MIRI100	F	72.95	61.43	5.39	11.78	FUNHET
MIRI101	M	93.99	78.58	11.43	17.21	FUNHET
MIRI102	F	101.13	84.55	14.08	18.41	FUNHET
MIRI103	F	91.43	77.44	11.781	18.11	FUNHET
MIRI104	F	102.02	85.82	16.5	18.85	FUNHET
MIRI105	M	65.53	54.92	3.5	10.27	FUNHET
MIRI106	M	100.7	85.28	15.18	19.49	FUNHET
MIRI107	M	73.22	61.23	4.94	12.76	FUNHET
MIRI108	F	76.8	63.77	6.07	12.82	FUNHET
MIRI109	F	37.06	30.82	0.59	5.56	FUNHET
MIRI110	F	95.28	82.45	13.57	18.37	FUNHET
MIRI111	F	79.09	64.88	6.51	13.59	FUNHET
MIRI112	F	83.74	70.07	7.83	14.27	FUNHET
MIRI113	F	84.66	71.46	9.48	15.56	FUNHET
MIRI115	F	77.58	64.56	6.03	12.85	FUNHET
MIRI117	M	66.41	55.06	3.812	12.62	FUNHET

C2. Raw data of parasites found in *Fundulus* spp.

Table C4: Parasites found in *Fundulus* spp (n = 53) from the Sydney Tar Ponds. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp; UID, unidentified; L3, third larval stage.

	Organ(s):	**Gills**	**Intestine, Liver**	**Connective tissue**	**Intestine, Liver**	**Intestine**		
	Parasite Species:	*Salsuginus* sp.	UID acanthocephalan	L3 Ascarid nematode	UID adult digene	UID cestode	**TOTAL**	
Fish ID	**Fish Species**						**# parasites**	**# species**
SYPO01	FUNHET	0	0	0	0	0	**0**	**0**
SYPO02	FUNHET	0	0	0	0	0	**0**	**0**
SYPO03	FUNUID	0	0	0	0	0	**0**	**0**
SYPO04	FUNUID	0	0	0	0	0	**0**	**0**
SYPO05	FUNHET	0	0	0	0	0	**0**	**0**
SYPO06	FUNHET	0	0	0	0	0	**0**	**0**
SYPO07	FUNUID	0	0	0	0	0	**0**	**0**
SYPO08	FUNHET	0	0	1	0	0	**1**	**1**
SYPO09	FUNHET	0	0	0	0	0	**0**	**0**
SYPO10	FUNHET	0	0	0	0	0	**0**	**0**
SYPO11	FUNHET	0	0	0	0	0	**0**	**0**
SYPO12	FUNHET	0	0	0	0	0	**0**	**0**
SYPO51	FUNHET	0	0	0	0	0	**0**	**0**
SYPO52	FUNHET	0	0	0	0	0	**0**	**0**
SYPO100	FUNHET	0	1	0	0	1	**2**	**2**
SYPO101	FUNHET	0	0	0	0	0	**0**	**0**
SYPO102	FUNHET	0	0	0	0	0	**0**	**0**
SYPO103	FUNHET	0	1	0	0	0	**1**	**1**

Table C4 (continued): Parasites found in *Fundulus* spp (n = 53) from the Sydney Tar Ponds. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp; UID, unidentified; L3, third larval stage.

	Organ(s):	**Gills**	**Intestine, Liver**	**Connective tissue**	**Intestine, Liver**	**Intestine**		
	Parasite Species:	*Salsuginus* sp.	UID acanthocephalan	L3 Ascarid nematode	UID adult digene	UID cestode	**TOTAL**	
Fish ID	**Fish Species**						**# parasites**	**# species**
SYPO104	FUNHET	6	1	1	0	0	**8**	**3**
SYPO105	FUNHET	0	0	1	0	0	**1**	**1**
SYPO106	FUNHET	0	0	0	0	0	**0**	**0**
SYPO107	FUNHET	0	1	0	0	1	**2**	**2**
SYPO108	FUNHET	0	0	0	0	0	**0**	**0**
SYPO109	FUNHET	1	0	0	0	0	**1**	**1**
SYPO110	FUNHET	0	0	0	0	0	**0**	**0**
SYPO111	FUNHET	0	0	0	0	0	**0**	**0**
SYPO112	FUNHET	0	0	1	0	0	**1**	**1**
SYPO113	FUNHET	0	0	0	0	0	**0**	**0**
SYPO114	FUNHET	0	0	0	0	0	**0**	**0**
SYPO150	FUNHET	0	0	0	2	0	**2**	**1**
SYPO151	FUNHET	0	0	0	0	0	**0**	**0**
SYPO152	FUNHET	0	0	0	0	0	**0**	**0**
SYPO153	FUNHET	0	0	0	0	0	**0**	**0**
SYPO154	FUNHET	0	0	0	0	0	**0**	**0**
SYPO155	FUNHET	0	0	0	0	0	**0**	**0**
SYPO156	FUNHET	0	0	0	0	0	**0**	**0**
SYPO157	FUNHET	0	0	0	0	0	**0**	**0**
SYPO158	FUNUID	0	0	0	0	0	**0**	**0**

Table C4 (continued): Parasites found in *Fundulus* spp (n = 53) from the Sydney Tar Ponds. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp; UID, unidentified; L3, third larval stage.

	Organ(s):	**Gills**	**Intestine, Liver**	**Connective tissue**	**Intestine, Liver**	**Intestine**		
	Parasite Species:	*Salsuginus* sp.	UID acanthocephalan	L3 Ascarid nematode	UID adult digene	UID cestode	**TOTAL**	
Fish ID	**Fish Species**						**# parasites**	**# species**
SYPO159	FUNHET	0	0	0	0	0	**0**	**0**
SYPO160	FUNHET	0	0	0	0	0	**0**	**0**
SYPO161	FUNHET	0	0	0	0	0	**0**	**0**
SYPO162	FUNHET	0	0	0	0	0	**0**	**0**
SYPO163	FUNHET	0	0	0	0	0	**0**	**0**
SYPO164	FUNHET	0	0	0	0	0	**0**	**0**
SYPO165	FUNHET	0	0	0	0	0	**0**	**0**
SYPO166	FUNHET	0	0	0	0	0	**0**	**0**
SYPO167	FUNHET	0	0	0	1	0	**1**	**1**
SYPO168	FUNHET	0	0	0	0	0	**0**	**0**
SYPO169	FUNHET	3	0	0	0	0	**3**	**1**
SYPO170	FUNHET	0	0	0	0	0	**0**	**0**
SYPO171	FUNHET	0	0	0	0	0	**0**	**0**
SYPO172	FUNHET	0	0	0	0	0	**0**	**0**
SYPO173	FUNHET	1	0	0	0	0	**1**	**1**

Table C5: External parasites found on *Fundulus* spp. (n = 69) from River Ryan. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

	Organ(s):	**Gills, Skin**		**Gill**	
	Parasite Species:	*Argulus* sp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Fish ID	**Fish Species:**				
RIRY01	FUNHET	2	0	0	0
RIRY02	FUNHET	0	0	0	0
RIRY03	FUNHET	0	0	0	0
RIRY04	FUNHET	0	0	1	0
RIRY05	FUNHET	0	0	2	0
RIRY07	FUNHET	0	0	0	0
RIRY08	FUNHET	0	0	0	0
RIRY10	FUNHET	0	0	0	0
RIRY11	FUNHET	0	0	0	0
RIRY12	FUNHET	0	0	1	0
RIRY13	FUNHET	0	0	0	0
RIRY14	FUNHET	2	0	2	0
RIRY15	FUNHET	0	1	0	0
RIRY16	FUNHET	0	0	1	1
RIRY17	FUNHET	0	0	0	0
RIRY18	FUNHET	0	0	0	0
RIRY19	FUNHET	0	0	1	0
RIRY20	FUNHET	0	0	0	0
RIRY21	FUNHET	0	1	0	0
RIRY22	FUNHET	0	0	0	0

Table C5 (continued): External parasites found on *Fundulus* spp. (n = 69) from River Ryan. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

	Organ(s):	**Gills, Skin**		**Gill**	
	Parasite Species:	*Argulus* sp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Fish ID	**Fish Species:**				
RIRY23	FUNHET	0	0	0	0
RIRY24	FUNHET	0	0	0	0
RIRY25	FUNHET	0	0	0	0
RIRY26	FUNHET	1	0	0	0
RIRY27	FUNUID	0	0	1	0
RIRY28	FUNUID	0	0	0	0
RIRY29	FUNUID	0	0	0	0
RIRY30	FUNUID	1	0	0	0
RIRY50	FUNUID	0	0	0	0
RIRY51	FUNHET	0	0	2	1
RIRY52	FUNHET	0	0	1	0
RIRY53	FUNHET	0	0	1	0
RIRY54	FUNHET	0	0	1	0
RIRY55	FUNHET	0	0	2	0
RIRY56	FUNHET	0	0	0	0
RIRY57	FUNHET	0	0	0	0
RIRY58	FUNHET	1	0	2	0
RIRY59	FUNHET	0	0	5	1
RIRY60	FUNHET	0	0	4	0
RIRY61	FUNHET	0	0	1	0

Table C5 (continued): External parasites found on *Fundulus* spp. (n = 69) from River Ryan. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

	Organ(s):	**Gills, Skin**		**Gill**	
	Parasite Species:	*Argulus* sp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Fish ID	**Fish Species:**				
RIRY62	FUNHET	0	0	0	0
RIRY63	FUNUID	0	0	0	0
RIRY64	FUNHET	0	0	4	0
RIRY65	FUNHET	0	0	2	0
RIRY66	FUNHET	0	0	0	0
RIRY67	FUNHET	0	0	0	0
RIRY68	FUNHET	4	0	5	0
RIRY69	FUNHET	0	0	0	0
RIRY100	FUNHET	1	0	2	0
RIRY101	FUNHET	0	0	12	1
RIRY102	FUNUID	0	0	1	0
RIRY103	FUNHET	0	0	1	0
RIRY104	FUNHET	0	0	6	4
RIRY105	FUNHET	0	0	8	0
RIRY106	FUNHET	0	0	2	0
RIRY107	FUNHET	0	0	9	1
RIRY108	FUNHET	0	0	11	0
RIRY109	FUNHET	0	0	6	0
RIRY110	FUNHET	0	0	11	0
RIRY111	FUNHET	0	0	5	0

Table C5 (continued): External parasites found on *Fundulus* spp. (n = 69) from River Ryan. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

	Organ(s):	**Gills, Skin**		**Gill**	
	Parasite Species:	*Argulus* sp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Fish ID	**Fish Species:**				
RIRY112	FUNHET	0	0	15	2
RIRY113	FUNHET	0	0	3	0
RIRY114	FUNHET	0	0	7	0
RIRY115	FUNHET	0	2	0	1
RIRY116	FUNHET	0	0	11	0
RIRY117	FUNHET	0	0	14	0
RIRY118	FUNHET	0	0	9	3
RIRY119	FUNHET	0	0	2	0
RIRY120	FUNHET	0	0	4	0

Table C6: Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 69) from River Ryan. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Organ(s): Parasite Species: Fish Species	Intestine, Liver *Neoechinochynchus* sp.	Connective Tissue L3 Ascarid nematode	Heart unidentified metacercaria	Intestine *Homalometron pallidum*	Intestine *Proteocephalus* sp.	TOTAL # parasites	TOTAL # species
RIRY01	FUNHET	0	0	0	0	0	**2**	**1**
RIRY02	FUNHET	0	0	9	0	0	**9**	**1**
RIRY03	FUNHET	0	0	1	0	1	**2**	**2**
RIRY04	FUNHET	0	0	6	0	0	**7**	**2**
RIRY05	FUNHET	1	0	0	0	0	**3**	**2**
RIRY07	FUNHET	0	0	0	0	0	**0**	**0**
RIRY08	FUNHET	0	0	2	0	0	**2**	**1**
RIRY10	FUNHET	0	0	0	0	0	**0**	**0**
RIRY11	FUNHET	0	0	0	0	0	**0**	**0**
RIRY12	FUNHET	0	0	1	0	0	**2**	**2**
RIRY13	FUNHET	1	0	0	0	0	**1**	**1**
RIRY14	FUNHET	1	0	5	0	0	**10**	**4**
RIRY15	FUNHET	0	0	0	0	0	**1**	**1**
RIRY16	FUNHET	0	0	0	0	0	**2**	**2**
RIRY17	FUNHET	1	0	12	0	0	**13**	**2**
RIRY18	FUNHET	17	0	3	1	0	**21**	**3**
RIRY19	FUNHET	3	0	0	0	3	**7**	**3**
RIRY20	FUNHET	1	0	0	0	0	**1**	**1**
RIRY21	FUNHET	1	0	0	0	0	**2**	**2**
RIRY22	FUNHET	0	0	1	1	0	**2**	**2**
RIRY23	FUNHET	0	0	6	0	0	**6**	**1**
RIRY24	FUNHET	0	0	1	0	0	**1**	**1**

Table C6 (continued): Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 69) from River Ryan.

FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Organ(s): Parasite Species: Fish Species	Intestine, Liver *Neoechinochynchus* sp.	Connective Tissue L3 Ascarid nematode	Heart unidentified metacercaria	Intestine *Homalometron pallidum*	Intestine *Proteocephalus* sp.	TOTAL # parasites	TOTAL # species
RIRY25	FUNHET	3	0	0	0	0	**3**	**1**
RIRY26	FUNHET	0	0	1	0	0	**2**	**2**
RIRY27	FUNUID	0	0	1	0	0	**2**	**2**
RIRY28	FUNUID	0	0	0	0	0	**0**	**0**
RIRY29	FUNUID	0	0	0	2	0	**2**	**1**
RIRY30	FUNUID	0	0	4	1	0	**6**	**3**
RIRY50	FUNUID	0	0	1	2	0	**3**	**2**
RIRY51	FUNHET	0	0	6	0	0	**9**	**3**
RIRY52	FUNHET	0	0	0	0	0	**1**	**1**
RIRY53	FUNHET	0	0	5	1	0	**7**	**3**
RIRY54	FUNHET	0	0	3	0	0	**4**	**2**
RIRY55	FUNHET	0	0	0	0	0	**2**	**1**
RIRY56	FUNHET	0	0	6	0	0	**6**	**1**
RIRY57	FUNHET	0	0	11	0	0	**11**	**1**
RIRY58	FUNHET	0	1	4	0	0	**8**	**4**
RIRY59	FUNHET	0	0	1	0	0	**7**	**3**
RIRY60	FUNHET	6	0	1	0	0	**11**	**3**
RIRY61	FUNHET	0	0	8	0	0	**9**	**2**
RIRY62	FUNHET	1	1	1	0	0	**3**	**3**
RIRY63	FUNUID	0	0	3	0	0	**3**	**1**
RIRY64	FUNHET	0	0	0	0	0	**4**	**1**
RIRY65	FUNHET	1	0	1	0	0	**4**	**3**

Table C6 (continued): Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 69) from River Ryan.

FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Organ(s): Parasite Species: Fish Species	Intestine, Liver *Neoechinochynchus* sp.	Connective Tissue L3 Ascarid nematode	Heart unidentified metacercaria	Intestine *Homalometron pallidum*	Intestine *Proteocephalus* sp.	TOTAL # parasites	TOTAL # species
RIRY66	FUNHET	0	0	0	1	0	**1**	**1**
RIRY67	FUNHET	1	0	1	0	0	**2**	**2**
RIRY68	FUNHET	0	0	5	0	0	**14**	**3**
RIRY69	FUNHET	0	0	1	1	0	**2**	**2**
RIRY100	FUNHET	0	0	9	0	0	**12**	**3**
RIRY101	FUNHET	0	0	4	0	0	**17**	**3**
RIRY102	FUNUID	0	0	0	0	0	**1**	**1**
RIRY103	FUNHET	0	0	0	0	0	**1**	**1**
RIRY104	FUNHET	0	0	5	0	0	**15**	**3**
RIRY105	FUNHET	2	2	6	0	0	**18**	**4**
RIRY106	FUNHET	0	0	8	0	0	**10**	**2**
RIRY107	FUNHET	1	0	0	0	0	**11**	**3**
RIRY108	FUNHET	0	0	1	0	0	**12**	**2**
RIRY109	FUNHET	0	2	10	0	0	**18**	**3**
RIRY110	FUNHET	1	1	1	0	0	**14**	**4**
RIRY111	FUNHET	0	1	0	1	0	**7**	**3**
RIRY112	FUNHET	1	0	3	0	0	**21**	**4**
RIRY113	FUNHET	0	0	0	4	0	**7**	**2**
RIRY114	FUNHET	1	1	1	0	0	**10**	**4**
RIRY115	FUNHET	0	0	3	0	0	**6**	**3**
RIRY116	FUNHET	1	1	1	4	0	**18**	**5**
RIRY117	FUNHET	12	0	2	1	0	**29**	**4**

Table C6 (continued): Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 69) from River Ryan.

FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

Fish ID	Organ(s): Parasite Species: Fish Species	Intestine, Liver *Neoechinochynchus* sp.	Connective Tissue L3 Ascarid nematode	Heart unidentified metacercaria	Intestine *Homalometron pallidum*	Intestine *Proteocephalus* sp.	TOTAL # parasites	TOTAL # species
RIRY118	FUNHET	1	0	1	0	0	**14**	**4**
RIRY119	FUNHET	0	0	0	0	0	**2**	**1**
RIRY120	FUNHET	0	0	1	0	0	**5**	**2**

Table C7: External parasites found on *Fundulus* spp. (n = 64) from Mira River. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

	Organ(s):	**Gills, Skin**		**Gill**	
	Parasite Species:	*Argulus* sp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Fish ID	**Fish Species:**				
MIRI01	FUNUID	0	10	3	3
MIRI03	FUNHET	0	16	2	0
MIRI04	FUNHET	1	7	1	0
MIRI05	FUNHET	1	26	3	2
MIRI07	FUNUID	0	6	0	0
MIRI08	FUNHET	0	8	0	1
MIRI09	FUNHET	0	0	0	1
MIRI10	FUNHET	0	12	1	0
MIRI11	FUNHET	0	42	0	2
MIRI12	FUNUID	0	23	4	1
MIRI13	FUNHET	0	27	0	0
MIRI14	FUNHET	0	117	2	2
MIRI15	FUNHET	1	14	2	0
MIRI16	FUNHET	0	12	1	2
MIRI17	FUNHET	2	8	1	0
MIRI18	FUNHET	1	2	3	0
MIRI19	FUNHET	0	301	0	1
MIRI20	FUNHET	1	89	0	15
MIRI21	FUNHET	0	13	0	0
MIRI22	FUNHET	0	8	5	1
MIRI23	FUNHET	1	10	3	0

Table C7 (continued): External parasites found on *Fundulus* spp. (n = 64) from Mira River. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

	Organ(s):	**Gills, Skin**		**Gill**	
	Parasite Species:	*Argulus* sp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Fish ID	**Fish Species:**				
MIRI24	FUNHET	0	4	4	0
MIRI26	FUNHET	0	27	1	0
MIRI27	FUNHET	0	25	4	0
MIRI28	FUNHET	0	150	0	3
MIRI29	FUNHET	2	55	1	3
MIRI30	FUNHET	0	12	2	1
MIRI31	FUNHET	0	15	4	0
MIRI32	FUNHET	0	95	0	3
MIRI33	FUNHET	0	10	2	1
MIRI34	FUNHET	0	23	0	2
MIRI35	FUNHET	0	35	5	6
MIRI50	FUNHET	0	15	6	7
MIRI51	FUNHET	0	14	1	18
MIRI52	FUNHET	0	153	2	10
MIRI53	FUNUID	0	79	0	3
MIRI54	FUNHET	0	13	6	2
MIRI55	FUNHET	1	20	17	15
MIRI56	FUNHET	0	37	8	5
MIRI57	FUNHET	1	21	5	11
MIRI58	FUNHET	0	17	5	2
MIRI59	FUNHET	0	11	11	6

Table C7 (continued): External parasites found on *Fundulus* spp. (n = 64) from Mira River. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

	Organ(s):	**Gills, Skin**		**Gill**	
	Parasite Species:	*Argulus* sp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Fish ID	**Fish Species:**				
MIRI60	FUNHET	0	34	3	4
MIRI61	FUNHET	0	294	2	11
MIRI62	FUNHET	0	23	16	3
MIRI63	FUNHET	1	29	4	12
MIRI64	FUNHET	1	20	19	20
MIRI65	FUNHET	1	27	3	11
MIRI100	FUNHET	0	51	6	33
MIRI101	FUNHET	0	169	0	45
MIRI102	FUNHET	0	538	6	4
MIRI103	FUNHET	0	179	2	32
MIRI104	FUNHET	6	868	0	0
MIRI105	FUNHET	1	32	15	13
MIRI106	FUNHET	0	73	0	34
MIRI107	FUNHET	0	15	44	20
MIRI108	FUNHET	0	58	22	31
MIRI109	FUNHET	0	7	8	1
MIRI110	FUNHET	0	448	0	14
MIRI111	FUNHET	0	43	9	0
MIRI112	FUNHET	0	34	26	12
MIRI113	FUNHET	0	67	14	19
MIRI115	FUNHET	0	30	13	0

Table C7 (continued): External parasites found on *Fundulus* spp. (n = 64) from Mira River. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp.

	Organ(s):	**Gills, Skin**		**Gill**	
	Parasite Species:	*Argulus* sp.	Echinostome metacercaria	*Salsuginus* sp.	*Ergasilus manicatus*
Fish ID	**Fish Species:**				
MIRI117	FUNHET	0	69	4	18

Table C8: Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 64) from Mira River. FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp; L3, third larval stage.

Fish ID	Organ(s): Parasite Species: Fish Species	Liver, spleen *Neoechinochynchus* sp.	Connective Tissue L3 Ascarid nematode	Heart unidentified metacercaria	Intestine *Homalometron pallidum*	TOTAL # parasites	TOTAL # species
MIRI01	FUNUID	8	0	0	0	**24**	**4**
MIRI03	FUNHET	4	0	0	0	**22**	**3**
MIRI04	FUNHET	6	0	0	0	**15**	**4**
MIRI05	FUNHET	1	0	0	0	**33**	**5**
MIRI07	FUNUID	0	0	0	0	**6**	**1**
MIRI08	FUNHET	1	0	0	0	**10**	**3**
MIRI09	FUNHET	2	0	0	0	**3**	**2**
MIRI10	FUNHET	1	0	0	0	**14**	**3**
MIRI11	FUNHET	1	0	0	0	**45**	**3**
MIRI12	FUNUID	0	0	0	0	**28**	**3**
MIRI13	FUNHET	0	0	2	0	**29**	**2**
MIRI14	FUNHET	6	0	0	0	**127**	**4**
MIRI15	FUNHET	6	1	0	0	**24**	**5**
MIRI16	FUNHET	0	0	0	0	**15**	**3**
MIRI17	FUNHET	12	0	0	0	**23**	**4**
MIRI18	FUNHET	2	0	0	0	**8**	**4**
MIRI19	FUNHET	1	0	0	0	**303**	**3**
MIRI20	FUNHET	2	0	0	0	**107**	**4**
MIRI21	FUNHET	11	0	0	0	**24**	**2**
MIRI22	FUNHET	0	0	0	0	**14**	**3**

Table C8 (continued): Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 64) from Mira River.

FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp; L3, third larval stage.

Fish ID	Organ(s): Parasite Species: Fish Species	Liver, spleen *Neoechinochynchus* sp.	Connective Tissue L3 Ascarid nematode	Heart unidentified metacercaria	Intestine *Homalometron pallidum*	TOTAL # parasites	TOTAL # species
MIRI23	FUNHET	2	0	0	0	**16**	**4**
MIRI24	FUNHET	0	0	0	0	**8**	**2**
MIRI26	FUNHET	3	0	1	0	**32**	**4**
MIRI27	FUNHET	6	0	0	0	**35**	**3**
MIRI28	FUNHET	23	0	0	0	**176**	**3**
MIRI29	FUNHET	1	0	0	0	**62**	**5**
MIRI30	FUNHET	0	0	0	0	**15**	**3**
MIRI31	FUNHET	0	0	0	0	**19**	**2**
MIRI32	FUNHET	4	0	0	0	**102**	**3**
MIRI33	FUNHET	0	0	0	0	**13**	**3**
MIRI34	FUNHET	2	0	0	0	**27**	**3**
MIRI35	FUNHET	1	0	0	0	**47**	**4**
MIRI50	FUNHET	0	0	0	0	**28**	**3**
MIRI51	FUNHET	0	1	0	0	**34**	**4**
MIRI52	FUNHET	0	0	0	0	**165**	**3**
MIRI53	FUNUID	9	0	0	0	**91**	**3**
MIRI54	FUNHET	11	0	0	0	**32**	**4**
MIRI55	FUNHET	0	0	0	0	**53**	**4**
MIRI56	FUNHET	0	0	0	0	**50**	**3**
MIRI57	FUNHET	1	0	0	0	**39**	**5**

Table C8 (continued): Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 64) from Mira River.

FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp; L3, third larval stage.

Fish ID	Organ(s): Parasite Species: Fish Species	Liver, spleen *Neoechinochynchus* sp.	Connective Tissue L3 Ascarid nematode	Heart unidentified metacercaria	Intestine *Homalometron pallidum*	TOTAL # parasites	TOTAL # species
MIRI58	FUNHET	4	0	0	0	**28**	**4**
MIRI59	FUNHET	9	0	0	0	**37**	**4**
MIRI60	FUNHET	10	0	0	0	**51**	**4**
MIRI61	FUNHET	0	0	3	1	**311**	**5**
MIRI62	FUNHET	0	0	0	0	**42**	**3**
MIRI63	FUNHET	2	0	0	0	**48**	**5**
MIRI64	FUNHET	5	0	0	0	**65**	**5**
MIRI65	FUNHET	1	0	0	0	**43**	**5**
MIRI100	FUNHET	0	0	0	0	**90**	**3**
MIRI101	FUNHET	9	2	0	0	**225**	**4**
MIRI102	FUNHET	16	1	0	0	**565**	**5**
MIRI103	FUNHET	1	0	0	0	**214**	**4**
MIRI104	FUNHET	1	2	0	0	**877**	**4**
MIRI105	FUNHET	1	1	0	0	**63**	**6**
MIRI106	FUNHET	3	0	0	0	**110**	**3**
MIRI107	FUNHET	2	0	0	1	**82**	**5**
MIRI108	FUNHET	1	1	0	0	**113**	**5**
MIRI109	FUNHET	0	0	0	2	**18**	**4**
MIRI110	FUNHET	4	0	0	0	**466**	**3**
MIRI111	FUNHET	0	0	0	0	**52**	**2**

Table C8 (continued): Endoparasites and the total number of parasites found in *Fundulus* spp. (n = 64) from Mira River.

FUNHET; *Fundulus heteroclitus*; FUNUID, *Fundulus* spp; L3, third larval stage.

Fish ID	Organ(s): Parasite Species: Fish Species	Liver, spleen *Neoechinochynchus* sp.	Connective Tissue L3 Ascarid nematode	Heart unidentified metacercaria	Intestine *Homalometron pallidum*	TOTAL # parasites	TOTAL # species
MIRI112	FUNHET	1	0	0	0	**73**	**4**
MIRI113	FUNHET	2	0	0	2	**104**	**5**
MIRI115	FUNHET	0	1	0	0	**44**	**3**
MIRI117	FUNHET	1	0	0	0	**92**	**4**

Appendix D: Raw Data from American eel (*Anguilla rostrata*) Parasitological Analysis

Outline:

D1. Biological data of American eel (*Anguilla rostrata*) used in parasitological analysis

Table D1: Biological data of American eel (*Anguilla rostrata*) used in parasitological analysis and the total number of parasites found in *A. rostrata.*

D2. Raw data of Parasites Found in American eel (*Anguilla rostrata*)

Table D2: Ectoparasites found on *Anguilla rostrata* from the North Tar Pond, Mira River, and Sydney Harbour.

Table D3: Endoparasites found in *Anguilla rostrata* from the North Tar Pond, Mira River, and Sydney Harbour.

D1. Biological data of American eel (*Anguilla rostrata*) used in parasitological analysis

Table D1: Biological data of American eel (*Anguilla rostrata*) used in parasitological analysis and the total number of parasites found in *A. rostrata*.

Site	Fish ID	Total Length (mm)	Weight (g)	Plump (mm)	TOTAL # parasites	TOTAL # species
North Tar Pond (n = 6)	SYPO13	395	94.59	19	**4**	**1**
	SYPO14	316	38.46	11.6	**0**	**0**
	SYPO15	--	--	--	**6**	**2**
	SYPOXX	605	--	--	**1**	**1**
	SYPO17	--	--	--	**1**	**1**
	SYPO50	532	261.3	35.5	**0**	**0**
Mira River (n = 10)	MIRI36	--	76.63	14.5	**4**	**2**
	MIRI 37	459	149.67	19.7	**2**	**1**
	MIRI38	388	100.25	19.8	**1**	**1**
	MIRI39	319	43.71	12.9	**3**	**1**
	MIRI66	344	62.54	14.8	**3**	**2**
	MIRI101	317	51.95	18.46	**7**	**1**
	MIRI102	260	26.18	13.99	**4**	**2**
	MIRI103	322	45.07	18.57	**52**	**4**
	MIRI104	255	23.17	14.52	**0**	**0**
	MIRIXX	--	--	--	**1**	**1**
Sydney Harbour (n = 5)	WEPA01	43.8	84.97	20.12	**0**	**0**
	WEPA02	48.4	129.98	23.73	**0**	**0**
	WEPA03	38.4	72.36	21.08	**13**	**2**
	WEPA04	48.1	161.83	27.22	**1**	**1**
	WEPA05	37.4	69.05	19.70	**0**	**0**

D2. Raw data of parasites found in American eel (*Anguilla rostrata*)

Table D2: Ectoparasites found on *Anguilla rostrata* from the North Tar Pond, Mira River, and Sydney Harbour. UID: unidentified

	Organ:	**Gill**	**Gill**
	Parasite Species:	UID Parastic Copepod	*Pseudodactylogyurus anguillae*
Site	**Fish ID**		
North Tar Pond (n = 6)	SYPO13	0	0
	SYPO14	0	0
	SYPO15	0	3
	SYPOXX	0	0
	SYPO17	0	0
	SYPO50	0	0
Mira River (n = 10)	MIRI36	0	0
	MIRI 37	0	0
	MIRI38	0	0
	MIRI39	0	0
	MIRI66	0	0
	MIRI101	0	0
	MIRI102	3	1
	MIRI103	0	3
	MIRI104	0	0
	MIRIXX	0	0
Sydney Harbour (n = 5)	WEPA01	0	0
	WEPA02	0	0
	WEPA03	0	0
	WEPA04	0	0
	WEPA05	0	0

Table D3: Endoparasites found in *Anguilla rostrata* from the North Tar Pond, Mira River, and Sydney Harbour. UID: unidentified; L3, third larval stage.

	Tissue/ Organ:	**Connective Tissue**	**Intestine, Stomach**		**Swim Bladder**	
	Parasite Species:	L3 Nematode	UID Acanthocephalan*	*Bothocephulus* sp.	*Anguillicoloides crassus*	UID Nematode**
Site	**Fish ID**					
North Tar Pond (n = 6)	SYPO13	4	0	0	0	0
	SYPO14	0	0	0	0	0
	SYPO15	0	0	3	0	0
	SYPOXX	1	0	0	0	0
	SYPO17	1	0	0	0	0
	SYPO50	0	0	0	0	0
Mira River (n = 10)	MIRI36	0	3	0	1	0
	MIRI 37	0	0	0	2	0
	MIRI38	0	0	1	0	0
	MIRI39	0	0	0	3	0
	MIRI66	0	0	0	2	1
	MIRI101	0	0	0	7	0
	MIRI102	0	0	0	0	0
	MIRI103	6	35	8	0	0
	MIRI104	0	0	0	0	0
	MIRIXX	0	0	0	1	0

Table D3 (continued): Endoparasites found in *Anguilla rostrata* from the North Tar Pond, Mira River, and Sydney Harbour.

UID: unidentified; L3, third larval stage.

	Tissue/ Organ:	**Connective Tissue**	**Intestine, Stomach**		**Swim Bladder**	
	Parasite Species:	L3 Nematode	UID Acanthocephalan*	*Bothocephulus* sp.	*Anguillicoloides crassus*	UID Nematode**
Site	**Fish ID**					
Sydney Harbour (n = 5)	WEPA01	0	0	0	0	0
	WEPA02	0	0	0	0	0
	WEPA03	2	0	0	11	0
	WEPA04	0	0	1	0	0
	WEPA05	0	0	0	0	0

The exact identification of several of the parasites are unknown. *The UID acanthocephalan may be either *Echinorhynchus* sp. or *Acanthocephlus* sp. **The UID nematode may be *Danikonema* sp. Further staining of specimens is required for identification.