2018

Healthy fish, healthy people: how fish can inform our understanding of effects of metabolism disrupting compound exposure on wildlife and human health

BOSTON UNIVERSITY

SCHOOL OF PUBLIC HEALTH

Dissertation

HEALTHY FISH, HEALTHY PEOPLE:
HOW FISH CAN INFORM OUR UNDERSTANDING OF EFFECTS OF METABOLISM DISRUPTING COMPOUND EXPOSURE ON WILDLIFE AND HUMAN HEALTH

by

KATHRYN ANNA CRAWFORD

B.S., University of Vermont, 2005
M.S., University of Vermont, 2007

Submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

2018

Approved by

First Reader

Wendy J. Heiger-Bernays, Ph.D.
Clinical Professor of Environmental Health

Second Reader

Jennifer J. Schlezinger, Ph.D.
Associate Professor of Environmental Health

Third Reader

Diane E. Nacci, Ph.D.
Research Biologist
Office of Research and Development
U.S. Environmental Protection Agency

Fourth Reader

Mark E. Hahn, Ph.D.
Senior Scientist, Biology Department
Woods Hole Oceanographic Institution

Outside Reader

Alicia R. Timme-Laragy, Ph.D.
Assistant Professor of Environmental Health Science
University of Massachusetts – Amherst

DEDICATION

This dissertation is dedicated to my parents, Lloyd and Suzanne, who have provided me with endless love and encouragement to follow my dreams, my brother Nick, who is a constant source of inspiration for a life fully lived, and Annie, my dear friend and domestic partner who has been a part of this journey since Day 1.

ACKNOWLEDGMENTS

First, to my primary advisor, Wendy Heiger-Bernays: you have been a true mentor. As an advisor, you have trained me to be a scientist, to artfully tell a story with data, and to balance scientific integrity with advocacy. As a mentor, you have taught me lessons extending far beyond the technicalities of science. You have patiently encouraged self-confidence, you have candidly shared what it means to be a woman in science, and you have shown me the value of always finding time to visit the mountains. Thank you for pushing me beyond limits I thought possible. Your mentorship has made me a stronger and better person.

To my committee, Jennifer Schlezinger, Diane Nacci and Mark Hahn: I am grateful to have had the opportunity to work with each of you so closely while completing my doctoral research. Observing your approaches to science – notably, where they overlap and differ – has been invaluable to my own development as a scientist. I cannot thank you enough for welcoming me into your labs, generously sharing expertise and resources throughout. Jennifer, I am forever in awe of your fierce determination. Your voracious appetite for science has shown me the true meaning of "having the fire in your belly." Diane, your elegance in the art of scientific storytelling and true Excel craftsmanship are lessons I will hold onto for years to come. A special thanks for taking care to ensure that ecology, a field dear to my heart, remained central to my dissertation. Mark, your methodical approach to research leaves no stone unturned and has instilled in me an utmost appreciation for detail, which I will carry with me in my own scientific career.

To Mike McClean: Thank you for your constant advocacy and unwavering faith in me. Your willingness to take me on as an advisee when I entered the BUSPH Environmental Health doctoral program, your compassionate ear and sound advice in moments of stress, and your guidance as I transition to the next phase of my career have made my success in the Environmental Health doctoral program at BUSPH possible. More broadly, I thank you for your ongoing commitment to the BUSPH Environmental Health doctoral program.

To my science Big Brothers, Bryan Clark and Neel Aluru: I could not have completed this research without your scientific insights, mentorship, and friendship. You have taught me the ins and outs of killifish, and you have tirelessly responded to questions about fish physiology, toxicology, molecular biology, career development, and the list goes on. Bryan, thank you for your countless hours helping to launch and maintain the studies presented herein. Thank you, too, for gently and patiently teaching me the value of friendly scientific "arguments". Neel, I credit much of this dissertation to the months I shared an office with you while completing a research rotation at the Woods Hole Oceanographic Institution (WHOI). You opened my eyes to the world of molecular toxicology and sparked my interest in devising a research project centered on killifish. Thank you for your quiet encouragement over the years since.

To all members of the Hahn and Nacci Labs: Thank you for your "behind the scenes" assistance at every turn. Sibel Karchner and Diana Franks, your patient, generous sharing

of technical expertise has allowed me to reinvent myself as a molecular toxicologist. Your warmth has made me feel welcome at WHOI and within the tight-knit killifish world. Ian Kirby, Joe Bishop and Ashley Bertrand, your work to maintain happy and healthy killifish, and your dissection wizardry made this research feasible.

To the Department of Environmental Health community of faculty, staff and students: My tenure at BUSPH would neither have been as academically rigorous, nor as rich in personal connections without each of you. I am grateful to have had the opportunity to work alongside you and look forward to remaining colleagues in the years to come. A special thank you to Tom Webster, Dave Sherr, Birgit Claus-Henn, Patricia Fabian and Kevin Lane for providing scientific input at periods throughout my dissertation research. Thank you, too, to Madeline Scammell and Birgit for your leadership of the doctoral program, and to Bobbie White and Jon Levy for captaining the Department of Environmental Health "ship" along a course of excellence. To Lindsay Underhill, my rock throughout this program and a forever friend, I would not be here today without you.

To my family and friends: I cannot thank you enough for standing by my side over the past many years. You have celebrated with me in moments of triumph and lifted my spirits in moments of despair. Your steadfast love and support mean the world to me. I am indebted to my parents, Lloyd and Suzanne, and my brother, Nick, for their encouragement and support throughout my life, and their belief in my ability to embark upon this journey and see it through. A special shout out to Annie Laws, my friend since

our very first day of class at BUSPH and my domestic partner. You have seen the good, bad and ugly of my course through this program. Your strength, humor and equanimity have provided invaluable stability over the years.

In many ways my dissertation is a new story about a place that has been the subject of so many stories before mine. I am grateful to New Bedford Harbor, members of the surrounding communities and its wildlife inhabitants for persevering, and for allowing scientists to continue studying their home. I am personally thankful to have been granted the trust and permission of scientists whose life's work has been dedicated to understanding the ecology and public health of the region to join them in their research efforts. By extension, I was afforded rapport and trust with communities in the region, which allowed this dissertation to be possible. And last, but not least, I am thankful to the many killifish who inadvertently dedicated their lives to science.

HEALTHY FISH, HEALTHY PEOPLE:

HOW FISH CAN INFORM OUR UNDERSTANDING OF

EFFECTS OF METABOLISM DISRUPTING COMPOUND EXPOSURE

ON WILDLIFE AND HUMAN HEALTH

KATHRYN ANNA CRAWFORD

Boston University School of Public Health, 2018

Major Professor: Wendy J. Heiger-Bernays, Clinical Professor of Environmental Health

ABSTRACT

Biologists have traditionally studied adverse health effects of contaminants on wildlife, whereas public health researchers have independently studied effects of the same chemicals on humans. This siloed approach limits maximal progress towards understanding and managing pollution if relevant findings are not translated between fields. A new threat to human health, and potentially ecological health, is metabolic disruption. Metabolism disrupting chemicals (MDCs) are environmental chemicals that can act at systemic and molecular levels across the lifespan to interfere with normal adipose tissue development, lipid storage in the liver, and alter whole-body energetics. Using fish from New Bedford Harbor (NBH), Massachusetts, a marine Superfund site, this research demonstrates the benefit of using a holistic approach to examine exposures to and effects of contaminants in urban waterways. The overall goals of this dissertation were to investigate trends in polychlorinated biphenyl (PCB) contamination in NBH and to test the hypothesis that PCB and/or organotin exposure has resulted in metabolic

disruption in Atlantic killifish (*Fundulus heteroclitus*) resident in New Bedford Harbor. First, trends in PCBs in seafood harvested throughout NBH since 2003 were characterized over time and space. PCBs declined in shellfish, but not finfish, over time. My risk assessment shows that human health risks associated with seafood consumption have decreased, but safe levels in seafood are not likely to be reached by the end of NBH sediment remediation, in the early 2020s. PCBs and tributyltin (TBT), a pollutant also commonly found in commercial harbors because of its use as an antifouling agent in marine paints, act as MDCs by distinct mechanisms. Dioxin-like PCBs act through the aryl hydrocarbon receptor. TBT acts through nuclear receptors, particularly PPARγ and RXR. In the second aim, using historical sediments and current sediments, I document the presence of tin in NBH above background levels. Biological investigations show that adult killifish two generations removed from NBH have more adipose tissue and higher levels of liver triglycerides than killifish from an uncontaminated location. Initial analyses show that changes in PPAR signaling may be particularly important in male killifish. In the third aim, I demonstrate that killifish embryos are minimally responsive to changes in PPARγ-regulated gene expression when treated with TBT or mammalian agonists. However, embryonic TBT exposure interferes with caudal fin development, likely through RXR activation and a reduction in bone formation signaling. Overall, these findings demonstrate metabolic disruption is occurring in a fish species resident to a highly polluted harbor and support the use of sentinel species not only for addressing potential human exposures but also potential adverse human health effects.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. Introduction

New Bedford Harbor

New Bedford Harbor (NBH) is a tidal estuary located in southeastern Massachusetts (**Figure 1.1**), at the mouth of the Acushnet River. NBH is bordered by the city of New Bedford and the towns of Fairhaven and Acushnet, which together are home to approximately 100,000 people. NBH is also home to a thriving ecosystem, including mollusks, fish and shore birds. The harbor and its surrounding region was a commercial epicenter for the northeastern United States during the industrial and manufacturing revolutions. Today, NBH remains a globally significant marine fishing port with the highest grossing fisheries landings in the United States (van Voorhees, 2016). Yet, the region's industrial history has left a legacy of environmental pollution that continues to impact human and wildlife residents, alike.

Chemical Contamination

The use of polychlorinated biphenyls (PCBs) in capacitor manufacturing adjacent to NBH during the mid-1900s resulted in severe, widespread PCB contamination following uncontrolled discharge of wastes directly into the harbor and indirectly through sewer systems (Pesch et al., 2011). PCB contamination was first detected in NBH sediment, water, and biota in 1976 when the US Environmental Protection Agency (US EPA) conducted a survey of PCBs in New England (Santos, 1978). PCB concentrations were measured as high as 100,000 milligrams per kilogram dry-weight (mg/kg dw) in

sediments (Weaver, 1982), 53 mg/kg wet-weight (ww) in shellfish, and 22 mg/kg ww in finfish fillets (Santos, 1978) . These findings, in conjunction with subsequent investigations, prompted the establishment of a seafood advisory (**Figure 1.2**) for fish and shellfish harvested from NBH and led to the harbor's designation as a federal Superfund Site in 1983. Removal of contaminated sediments from NBH has been ongoing since the 1980s. Remediation is expected to be complete by the early 2020s, when a projected total of more than 900,000 cubic yards of sediment will have been removed (Dave Lederer, US EPA, personal communication).

New Bedford Harbor is also contaminated with metals. Copper, chromium, zinc, and lead are elevated in NBH sediments from former ship building, whaling and metal plating industries (reviewed in Pesch et al., 2011). Tin in the harbor's sediments (Crawford et al., manuscript in preparation) likely originates, at least in part, from organotin-based antifouling paints used on the harbor's large fleet of commercial fishing and shipping boats, as in other harbors around the world (Antizar-Ladislao, 2008). While PCB contamination has driven regulatory and remedial initiatives in NBH, metal contaminants are also addressed during sediment removal.

This dissertation focuses on the impact of PCBs and the organotin tributyltin (TBT) on fish biology and potential risks to people from consumption of contaminated fish. PCBs and TBT are associated with myriad adverse health effects in humans and wildlife, including disruption of lipid homeostasis and metabolic health (e.g., Baker et al., 2013;

Gadupudi, Gourronc, Ludewig, Robertson, & Klingelhutz, 2015; Grün & Blumberg, 2006; Heindel et al., 2017), which is described in greater detail below. Due to their toxicity and persistence in wildlife and humans, PCBs were banned from production and most commercial uses in the United States in 1979, and TBT was phased out of marine paints between 2003 and 2008 worldwide. However, PCBs and TBT are recalcitrant to environmental degradation and both persist in aquatic environments, bound to organic carbon in sediments and stored in lipid-rich tissues in biota (e.g., Clark, Clark, Paterson, Mackay, & Norstrom, 1988; Kay et al., 2005; Nie, Lan, Wei, & Yang, 2005; Oliver & Niimi, 1988).

Wildlife and Human Exposures

Wildlife are exposed to persistent organic pollutants (POPs) such as PCBs and TBT through the food chain or direct contact with contaminated sediments and water. Exposure patterns vary dramatically between species in accordance with life history, diet, trophic position, and ecological niche. POPs also bioaccumulate in aquatic environments, biomagnifying in higher trophic organisms (Nie et al., 2005; Kay et al., 2005; Oliver et al., 1988; Clark et al., 1988). Non-migratory fish species, such as the Atlantic killifish (*Fundulus heteroclitus*, described in greater detail in the following sections) are endemic to coastal salt marshes along the East Coast of the US, including NBH, and are useful for understanding localized effects of pollution for two primary reasons. First, their POP body burdens reflect conditions in NBH as evidenced by elevated PCB levels in killifish tissue (e.g., Black, Gutjahr-Gobell, Pruell, Bergen, & McElroy, 1998; Nacci et al.,

unpublished data). To the best of my knowledge organotins have not been measured in NBH killifish. Second, teleost fishes, such as zebrafish, medaka, and killifish are useful model organisms for studying molecular mechanisms of toxicity because they share many physiological similarities with other vertebrates, including humans. Killifish living in urban waterways may also provide information about health effects caused by chronic exposure to pollutants found in coastal regions of the US, which may be relevant for humans living in nearby urban areas.

Both PCBs and TBT are measurable in human blood and tissue including liver (e.g., Xue, Liu, Zartarian, Geller, & Schultz, 2014; reviewed in Antizar-Ladislao, 2008). Human exposure to PCBs is primarily through consumption of contaminated food products, particularly those high in fats such as dairy products, eggs, animal fats, fish, and other wildlife (CDC, 2009). Research has also revealed that the indoor environment presents a significant source of PCB exposure in the general US population because of their use in building materials, such as caulks and paints (Herrick, McClean, Meeker, Baxter, & Weymouth, 2004; Herrick, Stewart, & Allen, 2016; Rudel, Seryak, & Brody, 2008). Additionally, recent research has identified airborne PCBs as a potential source of human exposure in areas with high environmental PCB levels, such as the communities surrounding NBH (Ampleman, Martinez, DeWall, Rawn, Hornbuckle, & Thorne, 2015; Martinez, Hadnott, Awad, Herkert, Tomsho, Basra, Scammell, Heiger-Bernays, & Hornbuckle, 2017). Ingestion of contaminated food, including seafood, and drinking water also serve as primary pathways of human exposure to TBT (reviewed in Antizar-

Ladislao, 2008), although there has been less study of human exposure to TBT than PCBs. Organotins were formerly used an antifouling agent in marine paints (Omae, 2003). They are used in hypoallergenic bedding (Intertek, 2010; US EPA, 1998) and as a fungicide on crops (US EPA, 2001). Despite the long-standing seafood advisory, consumption of NBH-caught seafood continues (Basra, Komal; Fabian, Patricia M; Scammell, Basra, Fabian, & Scammell, 2017), particularly among vulnerable populations living in surrounding communities, who may also be high-frequency fish consumers (Schwartz, Jacobson, Fein, Jacobson, & Price, 1983; von Stackelberg, Li, & Sunderland, 2017).

Fish: Making the Case for a Holistic Approach to Environmental Health

Initiatives such as One Health and the Planetary Health Alliance, were born from the need to bridge the gap between environmental science and public health, thereby reducing the historically siloed approach to research and policy in each discipline. Research efforts centered at this intersection combine expertise from complementary scientific fields to enhance the knowledge base informing research, while reducing the potential for duplicated research efforts across disciplines. Teleost fishes serve as important laboratory animal models for studying the etiology and treatment of human diseases, as well as diseases afflicting wild animal populations. Studying fish in NBH can inform both our understanding of how contaminants in the harbor affect wildlife and how humans may be exposed to these contaminants through seafood consumption (**Figure 1.3**). My dissertation research uses NBH killifish to highlight the importance of a

transdisciplinary approach that holistically assesses impacts of pollutants on ecological and human communities. This research explores the effects of POPs on lipid homeostasis and metabolic health in fish, as well as potential for human exposure to POPs through fish consumption in a vulnerable sub-population. The following sections and chapters of this dissertation are set in the context of NBH and investigate connections between POPs and metabolic disruption utilizing fish to inform human POP exposure and as toxicological models.

Obesity and Metabolic Syndrome

The global prevalence of obesity, defined as a condition in which a person's body mass index (BMI) greater than or equal to 30, has increased markedly since the 1970s. In the United States, adult obesity rose from 14.5% in the 1976-1980 National Health and Nutritional Examination Survey (NHANES) to 37.7% in the 2013-2014 NHANES (Flegal, Carroll, Ogden, & Johnson, 2002; Flegal, Kruszon-Moran, Carroll, Fryar, & Ogden, 2016). Similar trends are seen around the world (Stevens, Singh, Lu, Danaei, Lin, Finucane, Bahalim, McIntire, Gutierrez, Cowan, Paciorek, Farzadfar, Riley, & Ezzati, 2012), making the public health implications of obesity a global concern. Obesity significantly increases the risk for developing metabolic syndrome, which is the co-occurrence of several conditions including high blood pressure, high blood sugar, excess central adiposity, and dyslipidemia, as well as increasing the risk of heart disease, stroke, and diabetes (Mayo, 2015; Morse, Gulati, & Reisin, 2010). Metabolic syndrome occurs in approximately 5% of normal weight individuals, 22% of overweight individuals and

60% of obese individuals in the US (Park, Zhu, Palaniappan, Heshka, Carnethon, & Heymsfield, 2003).

At the organismal level, metabolic homeostasis is the balance of energy intake, utilization and storage. Maintenance of metabolic homeostasis involves many organs (e.g., liver, muscle, adipose tissue, pancreas, gastrointestinal tract, brain, and immune cells), which are integrated through a complex network of physical and chemical signaling pathways (e.g., neuronal signals, insulin, glucose, adipokines, hepatokines, fatty acids, and cytokines), as reviewed in Lempradl et al. (2015). These signaling pathways govern the transition between catabolic and anabolic states in cells. In the specific context of lipid homeostasis, these processes involve either the breakdown of triglycerides via the tricarboxylic acid cycle (TCA) and ß-oxidation (catabolism) or the synthesis of triglycerides from fatty acids (anabolism).

Adipose tissue and the liver play essential roles in lipid homeostasis. Adipose tissue is an endocrine organ, secreting adipokines and responding to signaling molecules (e.g., insulin) produced by other metabolically-relevant organs. It is also the primary storage site for excess energy in the form of triglycerides, which are later consumed as needed to meet energy demands during fasting, including between meals. The liver is an endocrine organ as well, secreting hepatokines and responding to other organs' signaling molecules (e.g., insulin). The liver converts glucose to glycogen and fatty acids for short-term energy use or transport to storage depots, such as muscle or adipose tissue.

Obesity reflects a fundamental disruption to energy homeostasis. Obesity is associated with rapid expansion of adipose tissue, which outpaces the growth of associated vasculature, leading to hypoxia in adipose tissue (Sun, Kusminski, & Scherer, 2011). Hypoxia, in turn, induces inflammation and fibrosis in adipose tissue, limiting further expansion and diminishing the ability of adipose tissue to store additional lipids (Halberg, Khan, Trujillo, Wernstedt-Asterholm, Attie, Sherwani, Wang, Landskroner-Eiger, Dineen, Magalang, Brekken, & Scherer, 2009; Khan, Muise, Iyengar, Wang, Chandalia, Abate, Zhang, Bonaldo, Chua, & Scherer, 2009; Sun, 2011; Sun, Tordjman, Clément, & Scherer, 2013). The inability of adipose tissue to remove fatty acids from the blood leads to lipotoxicity, the ectopic accumulation of lipid in non-adipose tissue, such as the liver (reviewed in Engin, 2017; Samuel & Shulman, 2012). Excess lipid storage in hepatocytes is known as non-alcoholic fatty liver disease (NAFLD) in the absence of co-occurring alcoholism (Brunt, Wong, Nobili, Day, Sookoian, Maher, Bugianesi, Sirlin, Neuschwander-Tetri, & Rinella, 2015; Parker, 2018). Metabolic syndrome is a primary risk factor for NAFLD, which is strongly associated with obesity, insulin resistance, and type 2 diabetes mellitus (reviewed in Arab, Arrese, & Trauner, 2018). NAFLD can also progress from fibrosis to cirrhosis to liver failure or liver cancer in some patients (e.g., Brunt et al., 2015).

Key Molecular Regulators of Lipid Homeostasis

Regulation of lipid homeostasis involves multiple ligand-activated transcription factors that control the expression of genes central to the biosynthesis, transport, and catabolism

of lipids in metabolically active tissue throughout the body (reviewed in Lempradl et al., 2015). Four transcription factors that can be activated by endogenous and exogenous compounds, and are relevant to this dissertation include: (1) retinoid X receptor (RXR), the heterodimeric partner for several nuclear receptors critical to maintaining lipid homeostasis (reviewed in Evans & Mangelsdorf, 2014; Lempradl et al., 2015; Shulman & Mangelsdorf, 2005); (2) peroxisome proliferator activated receptor gamma (PPARγ), which forms a heterodimer with RXR and is an essential regulator of adipocyte formation and function (P Tontonoz, Hu, Graves, Budavari, & Spiegelman, 1994); (3) PPARα, which also forms a heterodimer with RXR and regulates ß-oxidation (Kersten, Desvergne, & Wahli, 2000); and (4) the aryl hydrocarbon receptor (AHR), most widely known for its involvement in xenobiotic metabolism (Nebert, 2017). Each of these transcription factors are discussed in detail below.

Retinoid X Receptors, RXRs

The retinoid X receptors (alpha, beta, and delta) are nuclear receptors that can either homodimerize or act as heterodimeric partners to a host of other nuclear receptors (reviewed in Evans and Manglesdorf, 2014). RXRs form permissive heterodimers with PPARs, liver X receptor (LXR), farsenoid X receptor (FXR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR). By definition, a permissive heterodimer is one whose transcriptional activity is activated by ligand binding to either RXR or its partner. RXR forms non-permissive heterodimers with thyroid receptor (TR), vitamin D receptor (VDR), and retinoic acid receptor (RAR), such that RXR ligands are not

sufficient to initiate transcription. RXR is critical to maintaining lipid homeostasis through heterodimerization with PPARs (fatty acids, described below), LXR (cholesterol), and FXRs (bile acid) (reviewed in Evans & Mangelsdorf, 2014; Lempradl et al., 2015; Shulman & Mangelsdorf, 2005). RXR is activated by endogenous ligands, including 9-*cis*-retinoic acid and 9-*cis*-13,14-dihydroretinoic acid (de Lera, Krezel, & Rühl, 2016; Heyman, Mangelsdorf, Dyck, Stein, Eichele, Evans, & Thaller, 1992), as well as environmental pollutants, such as TBT (A. H. Baker, Watt, Huang, Gerstenfeld, & Schlezinger, 2015; Grün et al., 2006; le Maire et al., 2009; Watt et al., 2018). *In silico* research also suggests that some phthalates, including diethyhexyl phthalate (DEHP), can activate RXR (Sarath Josh, 2014).

Peroxisome Proliferator-Activated Receptor Gamma, PPARγ

PPARγ is a member of the PPAR family of nuclear receptors central to differentiation of white and brown adipocytes from mesenchymal stromal cells (adipogenesis), adipocyte function, insulin sensitivity, triglyceride and metabolic homeostasis, immune response, and bone homeostasis (e.g., Tontonoz et al., 1994). Mammals have two alternatively spliced PPARγ isoforms, PPARγ1 and PPARγ2 (reviewed in Janani & Kumari, 2015; Mueller et al., 2002), whereas to the best of my knowledge, teleost fishes have only one PPARγ isoform. In mammals, PPARγ1 is highly expressed in adipose tissue, as well as heart, skeletal muscle, and liver (Gavrilova et al., 2003; Mukherjee, Jow, Croston, & Paterniti, 1997; Tontonoz et al., 1994). PPARγ2 is solely and highly expressed in adipose tissue and is essential for adipose formation (Wang, Mullican, DiSpirito, Peed, & Lazar,

2013). PPARγ activation by either endogenous or exogenous ligands requires dimerization with RXR and controls triglyceride uptake and storage (Tontonoz and Spiegelman, 2008; Nolte et al., 1998). Many endogenous compounds and pharmaceuticals are known to activate PPARγ, including unsaturated fatty acids, eicosanoids (e.g., prostaglandin PGJ2), and thiazolidinedione drugs (e.g., rosiglitazone) (Grygiel-Górniak, 2014). Several classes of environmental pollutants are also known to activate PPARγ, including TBT (Grün, 2006, 2006), phthalates (Feige, Gelman, Rossi, Zoete, Métivier, Tudor, Anghel, Grosdidier, Lathion, Engelborghs, Michielin, Wahli, & Desvergne, 2007; Hurst & Waxman, 2003), and brominated (Springer, Dere, Hall, McDonnell, Roberts, Butt, Stapleton, Watkins, McClean, Webster, Schlezinger, & Boekelheide, 2012) and alternative (Pillai, Fang, Beglov, Kozakov, Vajda, Stapleton, Webster, & Schlezinger, 2014) flame retardants, all of which are found ubiquitously in the environment. Generally, studies evaluating the capacity for chemicals to activate PPARγ do not distinguish between the two isoforms. Activation of PPARγ leads to transcription of target genes such as perilipin (PLIN), which regulates mobilization of fatty acids from lipid droplets in adipocytes; and fatty acid binding protein 4 (FABP4), which is a carrier protein for fatty acids, eicosanoids, and retinoids that plays a key role in their uptake, transport and metabolism (Arimura, Horiba, Imagawa, Shimizu, & Sato, 2004; Tontonoz and Spiegelman, 2008; Wu et al., 2010; Rosen and MacDougald, 2006).

Peroxisome Proliferator-Activated Receptor Alpha, PPARα

Like PPARγ, PPARα is a nuclear receptor that heterodimerizes with RXR and is integral

to lipid homeostasis. However, PPARα is principally involved in fatty acid transport and ß-oxidation (e.g., van Raalte, Li, Pritchard, & Wasan, 2004), thereby working to balance the lipid sequestering actions of PPARγ. In mammals, PPARα is mostly highly expressed in cells with high peroxisomal and mitochondrial ß-oxidation, including in the liver, heart, brown adipose tissue, kidney, skeletal muscle, and throughout the gastrointestinal tract (e.g., Braissant, Foufelle, Scotto, Dauça, & Wahli, 1996; van Raalte et al., 2004). PPARα is activated during periods of energy deprivation, including between meals, to stimulate fatty acid uptake, utilization, and catabolism, and is necessary for ketogenesis (e.g., van Raalte et al., 2004; Braissant et al., 1996). PPARα can be activated by endogenous (e.g., unsaturated and saturated fatty acids, eicosanoids) or exogenous (e.g., fibrate and nonsteroidal anti-inflammatory drugs, phthalate ester plasticizers, per- and polyfluoroalkyl substances) ligands. Activation induces transcription of genes such as peroxisomal acyl-coenzyme A oxidase 1 (ACOX1), the enzyme that initiates fatty acid ß-oxidation, and carnitine palmitoyl transferase 1 (CPT1), which catalyzes translocation of acyl Co-A esters across mitochondrial membrane, the rate-limiting step in ß-oxidation (van Raalte et al., 2004). Simultaneously, PPARα blocks transcription of genes important to *de novo* fatty acid synthesis, including fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC; van Raalte et al., 2004).

Aryl Hydrocarbon Receptor, AHR

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor implicated in metabolic dysregulation (Arsenescu, Arsenescu, King, Swanson, & Cassis, 2008; N. A.

Baker, 2013; N. A. Baker, Shoemaker, English, Larian, Sunkara, Morris, Walker, Yiannikouris, & Cassis, 2015; Kerley-Hamilton, Trask, Ridley, Dufour, Ringelberg, Nurinova, Wong, Moodie, Shipman, Moore, Korc, Shworak, & Tomlinson, 2012; La Merrill, Kuruvilla, Pomp, Birnbaum, & Threadgill, 2009). *In vitro*, AHR is essential to regulating glucose tolerance, lipid accumulation in adipocytes, inflammation in adipose tissue and insulin sensitivity (Arsenescu, 2008; N. A. Baker, 2013, 2015; La Merrill, 2009). AHR is activated by both endogenous and exogenous ligands. Dioxin-like compounds, including polychlorinated dibenzo-p-dioxin (PCDDs), coplanar polychlorinated biphenyls (PCBs) and some polycyclic aromatic hydrocarbons (PAHs), are the best understood AHR ligands, the most potent agonist being 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Endogenous ligands have remained more elusive, but tryptophan metabolites, kynurenine and xanthurenic acid, and others have been identified and are reviewed by Denison et al. (2011) and Opitz et al. (2011). Classically, AHR is known for regulating the expression of Phase I and Phase II enzymes involved in xenobiotic metabolism and for its contribution to TCDD toxicity (e.g., Denison & Heath-Pagliuso, 1998; Michael S Denison & Nagy, 2003; Mimura & Fujii-Kuriyama, 2003). CYP1A, a transcriptional gene target of AHR, is regarded as the canonical molecular marker of AHR pathway activation by exogenous ligands. One way AHR may regulate energy balance is through its capacity to down regulate PPARγ (discussed in detail below).

Metabolism Disrupting Compounds

One conclusion from obesity-relevant research is that the Western diet (fat- and carbohydrate-rich (Cordain, Eaton, Sebastian, Mann, Lindeberg, Watkins, O'Keefe, & Brand-Miller, 2005)) and lack of exercise cannot entirely explain the global obesity epidemic. The environmental "obesogen" hypothesis, was first proposed in the early 2000s in a review article by Grun and Blumberg (2006) that highlighted research linking exposure to endocrine disrupting compounds and impaired energy regulation, adipogenesis and obesity. The environmental obesogen hypothesis has evolved to include a wide range of environmental pollutants that impact many facets of metabolic syndrome (reviewed in Heindel et al., 2015). As such, the term environmental "obesogen" has been supplanted by "metabolism disrupting compound" to reflect the current state of the science on the multiple ways environmental pollutants can interfere with energy regulation (J. J. J. J. Heindel, 2015).

Metabolism disrupting compounds (MDCs) are structurally diverse, act through a variety of molecular pathways, and include pollutants common in aquatic environments such as TBT, PCBs, phthalates, per- and poly-fluoroalkyl substances (PFAS), and flame retardants (Arsenescu et al., 2008; A. H. Baker et al., 2015; N. A. Baker et al., 2013; Grün et al., 2006; Grün & Blumberg, 2006; le Maire et al., 2009; Pillai et al., 2014; Springer et al., 2012). Metabolic disruption by environmental MDCs has been documented in humans (e.g., La Merrill & Birnbaum, 2011; Silverstone et al., 2012) and animal models, including biomedically-relevant species, such as zebrafish (*Danio rerio*)

(e.g., N. A. Baker et al., 2013; Grün et al., 2006; M et al., 2010; Seth, Stemple, & Barroso, 2013; Tingaud-Sequeira, Ouadah, & Babin, 2011). The two MDCs of particular relevance to NBH and this dissertation are TBT and PCBs.

Tributyltin, TBT

The original environmental obesogen identified was TBT. Early studies in mammalian models demonstrated that TBT could induce adipogenesis *in vitro* and *in vivo* through activation of PPARγ and RXR (Grün, 2006; Kanayama, Kobayashi, Mamiya, Nakanishi, & Nishikawa, 2005). TBT exposure also induced expression of lipogenic PPARγ-RXR target genes in adipose tissue and liver in mice (Grun et al., 2006). More recent studies have corroborated the finding that TBT exposures induce adipogenesis (e.g., Pereira-Fernandes et al., 2013; Watt & Schlezinger, 2015) and both hypertrophy and hyperplasia of adipocytes (reviewed in Heindel et al., 2017). In addition, studies have verified alterations to transcriptional profiles in adipocytes (e.g., Kim, Li, Monti, & Schlezinger, 2018) and liver (e.g., Wang, Zhang, & Pan, 2017; Zhang, Sun, Kong, Yang, & Guan, 2016).

The literature linking TBT to metabolic outcomes has expanded to investigate its role beyond increasing adiposity. First, TBT induces systemic metabolic disruption. For example, TBT increases hepatic triglycerides (e.g., Lyssimachou et al., 2015; Wang et al., 2017) and NAFLD (Zuo, Chen, Wu, Zhang, Su, Chen, & Wang, 2011). TBT also induces apoptosis in pancreatic ß-cells, impairing glucose and insulin homeostasis (C.-F. Huang,

Yang, Tsai, Wu, Liu, & Lan, 2018). Furthermore, TBT exposure has been found to disrupt hypothalamic-pituitary control of metabolic homeostasis in animal models. Specifically, TBT has been shown to increase weight gain by impairing hypothalamic-pituitary signaling (e.g., hyperinsulinemia, hyperleptinemia and hypoadiponectinemia; Sena et al., 2017) and altering feeding behavior, leading to increased food intake (J. Zhang, 2016). Second, effects of TBT on lipid homeostasis in adipose and liver appear to be transgenerational (Chamorro-García et al., 2013; Chamorro-Garcia et al., 2017; reviewed by Heindel, vom Saal, Blumberg, Bovolin, Calamandrei, Ceresini, Cohn, Bilbo, et al., 2015), and exposures during prenatal and early life represent significant threats to metabolic dysregulation later in life, given the sensitivity of animals to hormone disruption during development (reviewed by (J. J. Heindel & Schug, 2013).

In spite of these recent advances in our understanding of TBT-induced metabolic disruption, biochemical mechanisms underlying metabolic disruption are still not fully understood. Although epidemiologic literature exploring links between human TBT exposure and metabolic outcomes is limited, evidence suggests that metabolic disruption occurs in humans, too. Rantakaokko et al., (2014) found positive associations between placental TBT concentrations and increased weight gain in newborns. Given the weight of evidence of TBT-induced metabolic disruption in a variety of vertebrate models and the fact that tin-based compounds are commonly measured in human urine, blood, and liver (Lehmler, Gadogbe, Liu, & Bao, 2018; reviewed in Antizar-Ladislao et al., 2008), further studies are warranted to more fully characterize ongoing human exposures.

Polychlorinated Biphenyls, PCBs

PCBs are a collection of 209 individual chemical congeners, which can be classified by their toxicological mechanisms of action into two groups: dioxin- and non-dioxin-like PCBs. Both categories of PCBs have been shown to disrupt metabolism. The dioxin-like PCB77 has been found to increase adipocyte differentiation at low concentrations, yet suppress adipogenesis at higher levels *in vitro* (Arsenescu et al., 2008). PCB77 has also been associated with adipocyte hypertrophy and hyperplasia (reviewed in Heindel et al., 2017). In contrast, PCB126 has been shown to suppress adipogenesis in a human preadipocyte cell line (G. Gadupudi, 2015). In both cases, the effect of the dioxin-like PCB was attenuated by inactivation of the AHR, confirming the role of the AHR in dioxin-like PCB-mediated effects on adipogenesis. PCB126 has also been shown to cause inflammation in adipocytes (Gourronc, Robertson, & Klingelhutz, 2018), which impairs adipocyte function and is a key step in the pathway to developing metabolic syndrome, particularly type 2 diabetes mellitus. Impaired pancreatic ß cell development has also been discovered following early life exposure to PCB126 (Timme-Laragy, Sant, Rousseau, & diIorio, 2015). Furthermore, impaired glucose and insulin sensitivity is associated with dioxin-like PCBs acting through the AHR (N. A. Baker, 2013, 2015). PCB126 has been shown to disrupt hepatic gluconeogenesis and fatty acid oxidation at the transcriptional level and to increase liver triglyceride levels *in vivo* (Gadupudi, Klaren, Olivier, Klingelhutz, & Robertson, 2016). From the perspective of whole-body metabolic homeostasis, PCB77 has been shown to increased weight gain in mice

(Arscenescu et al., 2008), yet dioxin-like PCBs cause wasting syndrome at higher concentrations (Safe, 1994).

Non-dioxin-like PCBs have traditionally been considered to be less acutely toxic than dioxin-like PCBs and have received considerably less research attention with respect to metabolic health. Nevertheless, non-dioxin-like PCBs also appear to disrupt metabolic homeostasis. PCB153 has been shown to increase lipid accumulation and leptin expression *in vitro* at concentrations similar to those measured in human adipose tissue (Ferrante, Amero, Santoro, Monnolo, Simeoli, Di Guida, Mattace Raso, & Meli, 2014). PCB153 also increased blood glucose and insulin intolerance, inhibited insulin-dependent uptake of glucose and lipid accumulation in liver and adipose tissue, and increased inflammation *in vivo* (Wu et al., 2017).

Both dioxin- and non-dioxin-like PCBs are associated with metabolic syndrome in epidemiologic studies (Clair, Pinkston, Rai, Pavuk, Dutton, Brock, Prough, Falkner, McClain, & Cave, 2018; Ruzzin, Lee, Carpenter, & Jacobs, 2012; Sergeev & Carpenter, 2011; Silverstone, 2012; Thayer, Heindel, Bucher, & Gallo, 2012). For example, PCB exposure in a human population living in close proximity to a former PCB manufacturing facility has been associated with elevated rates of obesity, diabetes, dyslipidemia (elevated levels of plasma cholesterol and/or triglycerides), and hepatic steatosis (e.g., Clair et al., 2018; Silverstone et al., 2012). Collectively, toxicological and epidemiologic evidence for PCB-induced metabolic disruption is compelling. Yet, the precise

mechanisms by which PCBs impact metabolic homeostasis are, at times, contradictory, and need further study. Understanding mechanisms of PCB exposure is particularly important since there is a growing body of literature surrounding volatile PCBs (e.g., Carpenter, 2015; Martinez et al., 2017). Volatile PCBs include both dioxin-like and non-dioxin-like congeners and present a new set of exposure scenarios that have previously been overlooked but have the potential to greatly impact people living and working in close proximity to PCB-contaminated buildings and locations.

Cross-talk Between AHR and PPARγ

The process by which proteins from different molecular signaling cascades interact with one another is referred to as cross-talk (Vert & Chory, 2011). Evidence for cross-talk between AHR and PPARγ pathways exists in mammalian models, with AHR activation generally being associated with the suppression of PPARγ activity and adipogenesis, adipose tissue mass, adipose inflammation and glucose tolerance (e.g., N. A. Baker et al., 2015). Early evidence for the inhibitory effects of dioxin-like compounds on PPARγ came from *in vitro* studies in which 3T3-L1 mouse preadipocytes exposed to TCDD in the presence of PPARγ ligands did not differentiate into mature adipocytes (S Shimba, Todoroki, Aoyagi, & Tezuka, 1998). Shimba et al. (2001) also found that AHR activation led to reduced adipocyte gene expression and morphological differentiation, yet PPARγ activation with thiazolidinedione drugs restored the ability of AHR-overexpressing cells to differentiate. Arsenescu et al. (2008) found a positive association between AHR activation and adipogenesis and PPARγ expression at low doses of exposure of 3T3-L1

cells to coplanar PCB77 and TCDD (Arsenescu et al., 2008). AHR suppressed adipogenesis and induced weight gain at higher concentrations of PCB77 and TCDD (Arsenescu et al., 2008). Taken together, these results support the possibility of a non-monotonic response to AHR-PPARγ interactions, the likes of which may be consistent with other environmental MDCs and endocrine disrupting compounds (e.g., Thayer et al., 2012; Vandenberg et al., 2012).

Several possible mechanisms have been postulated for interaction between AHR and PPARγ, the simplest being direct transcriptional control of PPARγ expression by AHR. Using a human preadipocyte cell line, Gadupudi et al. (2015) observed a reduction in adipogenesis following exposure to PCB126, especially when cells were exposed to PCB126 as preadipocytes rather than during adipogenesis. This result suggests that AHR activation inhibits PPARγ activity. Using mouse embryonic fibroblasts, Alexander et al. (1998) demonstrated that AHR activation by TCDD before or during hormone-induced adipocyte differentiation resulted in the suppression of PPARγ, adipogenesis and triglyceride synthesis. Based on a review of epidemiologic evidence for links between dioxin exposure and diabetes, Remillard and Bunce (2002) posited that AHR activation antagonizes PPARγ functions, leading to diabetogenesis.

Other possible mechanisms for AHR interaction with PPARγ are indirect. Müllerová and Kopecký (2007) postulate that dioxin-like compounds acting through the AHR may interfere with the RXR. Long-term, low-dose exposure to dioxin is known to reduce

hepatic retinyl ester levels and species-related reduction/excess of retinoic acid (RA) in extrahepatic tissue (Nacci, S. Jayaraman, J. Specker, Jayaraman, & Specker, 2001; Nilsson & Håkansson, 2002). RXRβ knock-out mice have been reported to show no retinoid disruption following TCDD exposure relative to wild-type mice (Hoegberg, Schmidt, Fletcher, Nilsson, Trossvik, Gerlienke Schuur, Brouwer, Nau, Ghyselinck, Chambon, & Håkansson, 2005). TCDD may alter the ratio of cis- and trans-retinoic acid, modulating the transcriptional pathway of RXR, including its ability to heterodimerize with PPARγ (Müllerová, 2007). Alternately, NRF2 signaling may play a role in the relationship between AHR activation and PPARγ-induced adipogenesis. Shin et al. (2007) found that NRF2 regulates AHR expression, altering its signaling cascade, reducing target gene (e.g., CYP1A) expression, and inhibiting adipogenesis in mouse embryonic fibroblasts. Another proposed mechanism for the suppressive effect of AHR on PPARγ, adipogenesis, and metabolic homeostasis is through a convoluted mechanism involving the inflammatory response (Baker et al., 2015; Kim et al., 2012).

What Teleost Fish can Teach us About Metabolic Disruption

Teleost fish are relevant to the study of metabolic disruption from three primary perspectives: (1) the rising prominence of zebrafish in biomedical research, (2) the importance of energy balance to the aquaculture industry, and (3) the significance of fish as both sentinel species and a source of persistent organic pollutants (POPs) exposures for humans.

Zebrafish (*Danio rerio*), a teleost fish, have emerged as an important vertebrate model in biomedical research, including research on metabolic disease (Holtta-Vuori, 2010; Seth, 2013; Tingaud-Sequeira, 2011) and NAFLD (Asaoka, Terai, Sakaida, & Nishina, 2013; Huff, da Silveira, Carnevali, Renaud, & Hardiman, 2018; Schlegel, 2012; J. Zhang, 2016). Zebrafish are amenable to studying metabolic diseases because of the high degree of similarity between zebrafish adipose tissue and mammalian white adipose tissue. Additionally, molecular regulation of energy balance and hypothalamic circuitry is largely conserved across vertebrates, making zebrafish findings relevant to humans (e.g., Schlegel, 2012; Seth et al., 2013). The recent sequencing of the zebrafish genome has also accelerated their adoption as a biomedical model (Seth et al., 2013). A growing body of literature using zebrafish in lipid- and metabolic-related research has focused on a wide range of topics including: adipose tissue distribution, MDC- and diet-induced obesity, type II diabetes, adipokine signaling, early life exposure and transgenerational implications for obesity, and *in vivo* high throughput screening for endocrine disrupting chemicals (e.g., Flynn, Trent, & Rawls, 2009; Ibabe, Bilbao, & Cajaraville, 2005; Imrie & Sadler, 2010; Minchin et al., 2015; Minchin & Rawls, 2017; Oka et al., 2010; Ortiz-Villanueva et al., 2017; Riu et al., 2014; Sant et al., 2016; Timme-Laragy et al., 2015; Tingaud-Sequeira et al., 2011).

Energy balance is also an active area of research in teleost species that are important in the aquaculture industry, such as salmon and tilapia. Metabolic physiology is integral to optimal growth, quality and commercial value. Disruption to whole-body lipid

homeostasis can signify inefficient use of feed resources, cause adverse health outcomes for fish, and decrease the nutritional quality of the fish for humans (Bolla, Nicolaisen, & Amin, 2011; Craig, MacKenzie, Jones, & Gatlin, 2000; Lu, Xu, Li, Liu, Wang, & Zhang, 2013; Nanton, Lall, Ross, & McNiven, 2003; Sargent, Bell, McEvoy, Tocher, & Estevez, 1999). Thus, much research on teleost metabolism exists and may be leveraged for use in comparative biology applications (Wafer, Tandon, & Minchin, 2017).

Given the high degree of commonality between teleost fishes, the rapidly expanding body of literature on metabolic-related research in zebrafish and commercially important aquaculture, teleost fish offer a great deal to a comparative biology approach to understanding etiology of metabolic diseases in humans. Studying complex diseases, such as metabolic syndrome and NAFLD, through the lens of comparative biology improves our understanding of the molecular mechanisms driving adverse health outcomes in humans because we gain a more holistic context for taxa-specific nuances, which enhances our ability to translate *in vitro* and *in vivo* findings to human health. Furthermore, teleost fish living in urban waterways can inform our understanding of health effects associated with exposure to ubiquitous aquatic contaminants. Adverse health effects seen in fishes may provide context for local effects of pollution on other vertebrates, including humans. Measuring levels of contaminants in fishes can also inform our understanding of pollutant bioaccumulation and human exposures to POPs through seafood consumption. This may be particularly relevant for sensitive sub-

populations, such as children, pregnant women, and other vulnerable members of a community.

Atlantic killifish (*Fundulus heteroclitus*) as a Toxicological Model

Killifish inhabit estuaries and salt marshes along the eastern US, including NBH. Killifish are both an ecological sentinel species and a vertebrate model amenable to mechanistic toxicology studies (Burnett, Bain, Baldwin, Callard, Cohen, Di Giulio, Evans, Gómez-Chiarri, Hahn, Hoover, Karchner, Katoh, Maclatchy, Marshall, Meyer, Nacci, Oleksiak, Rees, Singer, Stegeman, Towle, Van Veld, Vogelbein, Whitehead, Winn, & Crawford, 2007). This species has been studied extensively for its response to Superfund chemicals, most notably PCBs and PAHs acting through AHR (e.g., Nacci, Champlin, & Jayaraman, 2010; Oleksiak et al., 2011; Proestou, Flight, Champlin, & Nacci, 2014; Reid et al., 2016). The small home range of killifish has led to the evolution of genetically distinct populations of fish that have developed adaptations to their local environment (e.g., Nacci et al., 2010). Specifically, killifish living in NBH demonstrate dramatic heritable resistance, or evolved tolerance, to AHR pathway activation (e.g., Bello, Franks, Stegeman, & Hahn, 2001; D. Nacci et al., 1999; D. E. Nacci et al., 2010; Reid et al., 2016). AHR pathway activation in killifish is commonly evaluated by measuring 7-ethoxyresorufin-O-deethylase (EROD) activity, *cyp1a* mRNA expression and the prevalence of cardiac teratogenesis. NBH killifish exhibit diminished AHR responsiveness with each of these endpoints. Furthermore, tolerance to AHR-induced toxicity has proven to be heritable, persisting in killifish held in uncontaminated

laboratory conditions for two or more generations (D. E. Nacci, 2010).

Although this wild killifish population with diminished AHR pathway responsiveness is not a true selectively-targeted AHR knock-down/-out, comparisons between NBH killifish and those from uncontaminated reference populations (i.e. with normal sensitivity to AHR pathway activation) serve as valuable models for understanding the direct and indirect effects of AHR pathway variation *in vivo*. Research to understand adverse effects of AHR-related adaptations on killifish fitness is of stated interest to specialists in the field (e.g., The Hahn Lab, WHOI; The Nacci Lab, US EPA AED). It is important to note that NBH is contaminated with a wide range of other environmental pollutants, such as metals. Killifish living in the harbor may have evolved adaptation to compounds other than those acting through the AHR. Therefore, studies comparing physiological responses to chemical exposures in NBH killifish to those from reference locations are useful.

One particularly interesting opportunity for expanding the use of killifish as a toxicological model is the arena of environmental MDCs, which is unexplored in this species. While studying NBH killifish, researchers have noticed differences in adipose tissue mass and liver lipid content, common metrics of lipid homeostasis, between killifish in NBH and those from reference sites, including Scorton Creek (SC) in Sandwich, Massachusetts. It is possible that the presence of MDCs in NBH contributes to altered lipid homeostasis in local killifish populations. Previously, reports of

discrepancies in lipid content between killifish populations were anecdotal, observed in field-caught animals living in very different environments.

Chemical contamination in NBH is an important distinction between NBH and SC, but the estuaries are distinct in other ways as well. NBH is a low-energy estuary, characterized by minimal wave action and vegetative growth in the intertidal zone (Jackson, Nordstrom, Eliot, & Masselink, 2002) and a robust microbenthic community (Wildish & Kristmanson, 1979). This environment affords greater food availability for killifish, and the increased turbidity provides protection against predation by shore birds and larger fish (James-Pirri, Raposa, & Catena, 2001; Kneib, 1986; McMahon, Johnson, & Ambrose, 2005). In contrast, SC is a high-energy estuary, characterized by clear, fast-moving water and scoured substrate. By extension, SC killifish are more vulnerable to predation and likely have less food availability.

While ecological differences between NBH and SC are critical to understanding differences in physiology of killifish living in each location, controlled laboratory experiments offer a means to evaluate the possibility that MDCs are contributing to the anecdotal variability in killifish size and lipid levels between the two sites. As discussed above, both the PPARγ and AHR ligand-activated transcription pathways are important to the maintenance of metabolic homeostasis, and there is evidence that these pathways interact. Given differences in AHR responsiveness between NBH and SC killifish populations, we hypothesize that MDCs may impact the two populations differently

(**Figure 1.4**). AHR ligands in NBH are both abundant and well-characterized, with PCBs being the drivers for risk assessments associated with ongoing Superfund remedial activities in the harbor. MDCs acting through PPARγ have been less well characterized in the harbor, but their presence is suspected given the ubiquity of these compounds in the environment and the region's long history as a working harbor, where TBT-based marine paint was undoubtedly used. With the recent sequencing of the killifish genome and an increasing number of publications of transcriptomic analyses on this species, killifish are well poised to serve jointly as a biomedical and environmentally relevant model in mechanistic toxicology, including for our purposes of evaluating environmental MDC toxicity.

Study Aims

PCB contamination of NBH is declining but remains significant. Thus, both the human communities around the harbor and the wildlife inhabitants living in the harbor will continue to be exposed to PCBs. Diet remains the primary pathway for exposure to PCBs and TBT, notably seafood consumption. Dioxin-like PCBs and TBT are implicated in metabolic disruption in humans and toxicological animal models. Through this dissertation research, I investigated the role of PCBs and TBT in explaining altered lipid homeostasis in NBH killifish. Both the AHR and PPARγ ligand-activated transcription pathways are important to the maintenance of metabolic homeostasis, and there is evidence that the pathways interact. Given differences in AHR responsiveness between NBH and SC killifish populations, I hypothesized that MDCs may impact the populations

differently. The first aim was to quantify spatial and temporal trends in PCB concentrations in seafood species caught and consumed in NBH. The second aim was to understand the effects of exposure to PCBs and TBT on lipid homeostasis in adult NBH killifish. The third aim was to identify molecular mechanisms involved with altered lipid homeostasis in NBH killifish. Lipid homeostasis in killifish following exposure to MDC has not been previously explored. Thus, I establish killifish as a novel, ecological and human-relevant vertebrate model for studying the metabolism-disrupting effects of environmental toxicants *in vivo*.

Figures

Figure 1.1. Locations of killifish populations used in research described herein (A). Offspring of killifish collected from New Bedford Harbor (B), a marine Superfund site located in southeastern Massachusetts, and a reference estuary, Scorton Creek (SC), in Sandwich, Massachusetts (A), were used in this study to evaluate metrics of lipid homeostasis and their responsiveness to environmental metabolism disrupting compound (MDC) exposure.

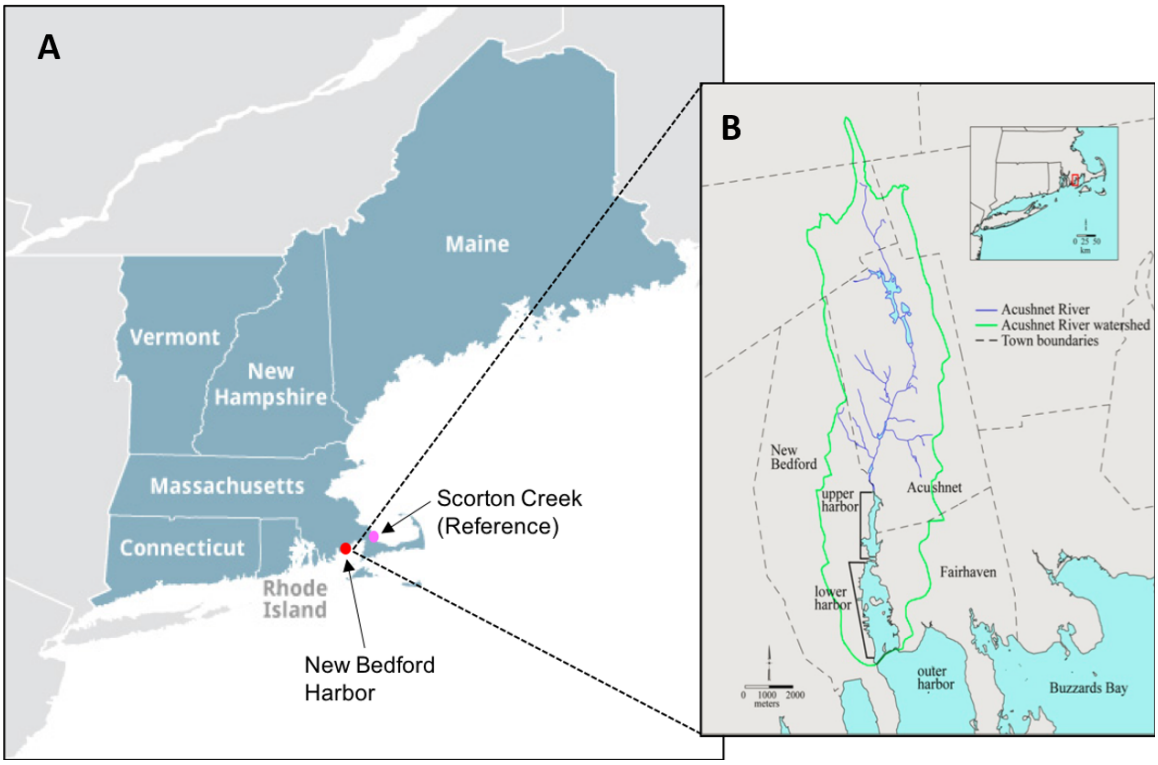


Figure 1.2. Map of New Bedford Harbor (NBH), MA, including delineations of the three seafood management Areas (1, 2 and 3). The NBH seafood management Areas were established in 1979 (left) and seafood consumption advisories have subsequently been updated to reflect additional data on PCB levels in NBH seafood tissue (right). Details of the NBH seafood advisory is available on the US EPA's NBH Technical Documents website (https://www.epa.gov/new-bedford-harbor/fish-consumption-regulations-and-recommendations).

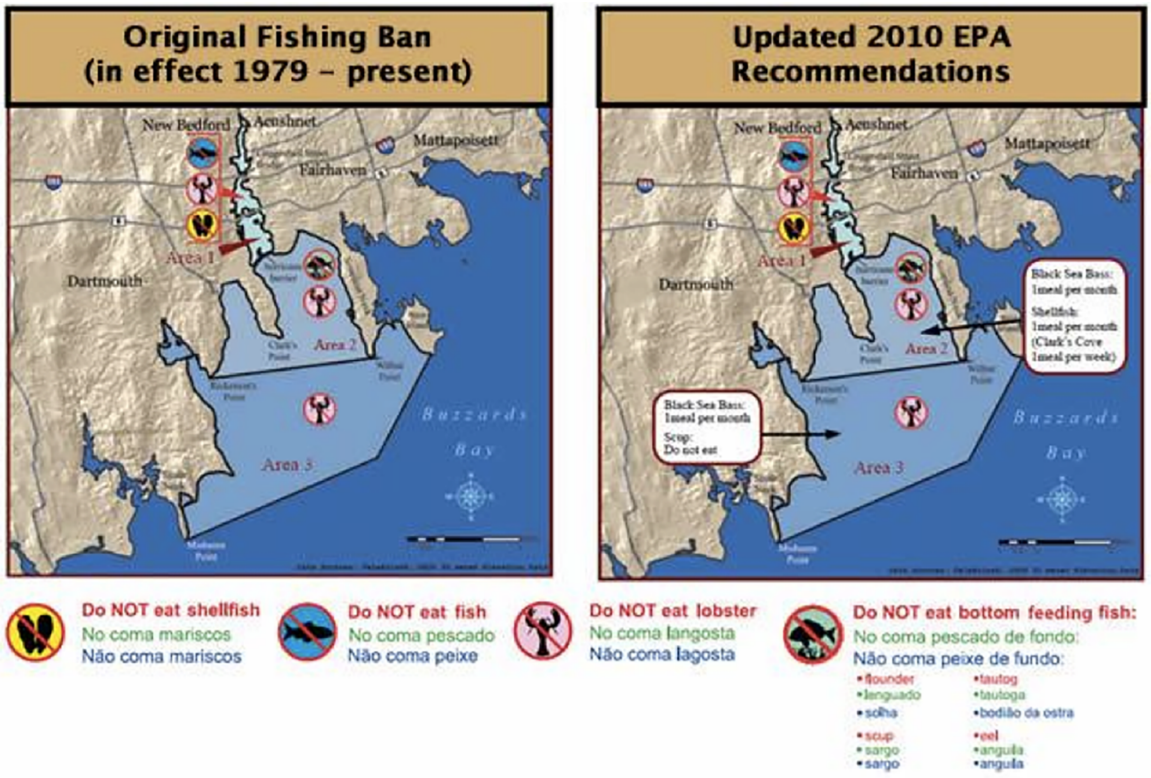


Figure 1.3. Schematic depicting how fish can inform our understanding of how humans are exposed to metabolism disrupting compounds (MDCs) in the environment, and the molecular mechanisms through which these compounds elicit adverse health effects in humans and wildlife, alike. Specifically, this body of research evaluates polychlorinated biphenyl (PCB) levels in seafood harvested from New Bedford Harbor and potentially consumed. It also investigates the molecular actions of dioxin-like PCBs and the organotin, tributyltin (TBT) on lipid homeostasis in the ecological sentinel species, Atlantic killifish (*Fundulus heteroclitus*).

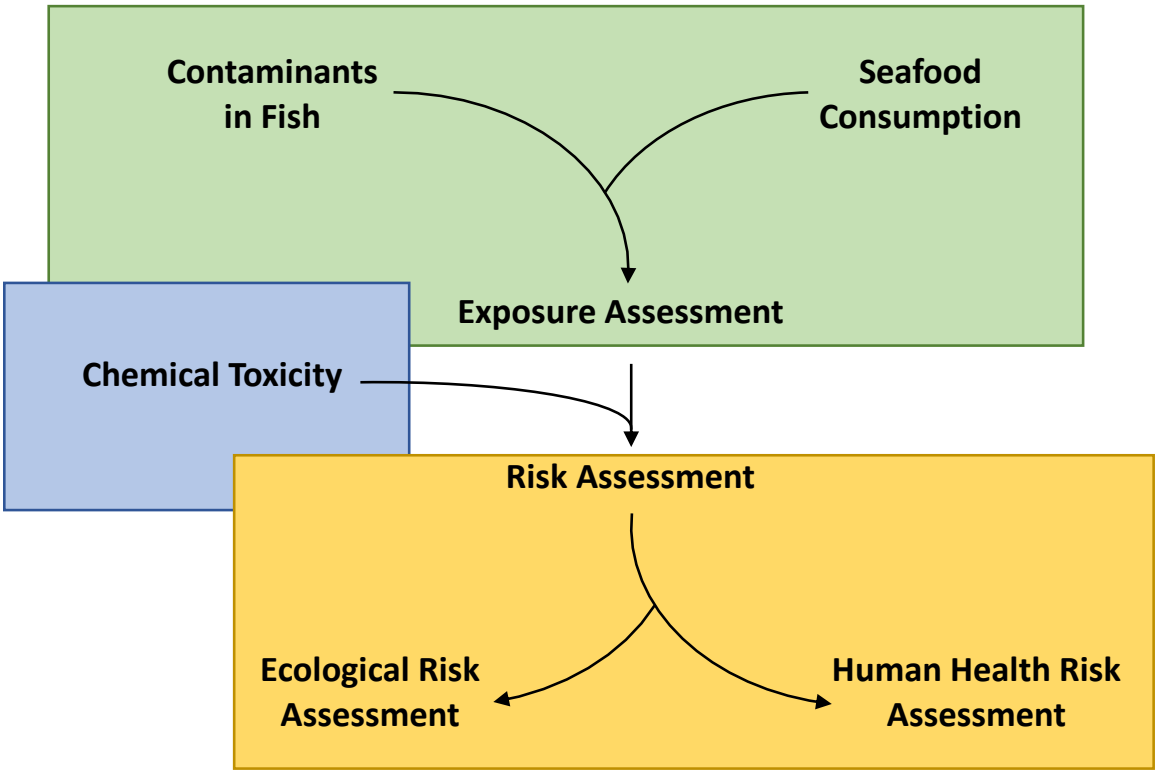
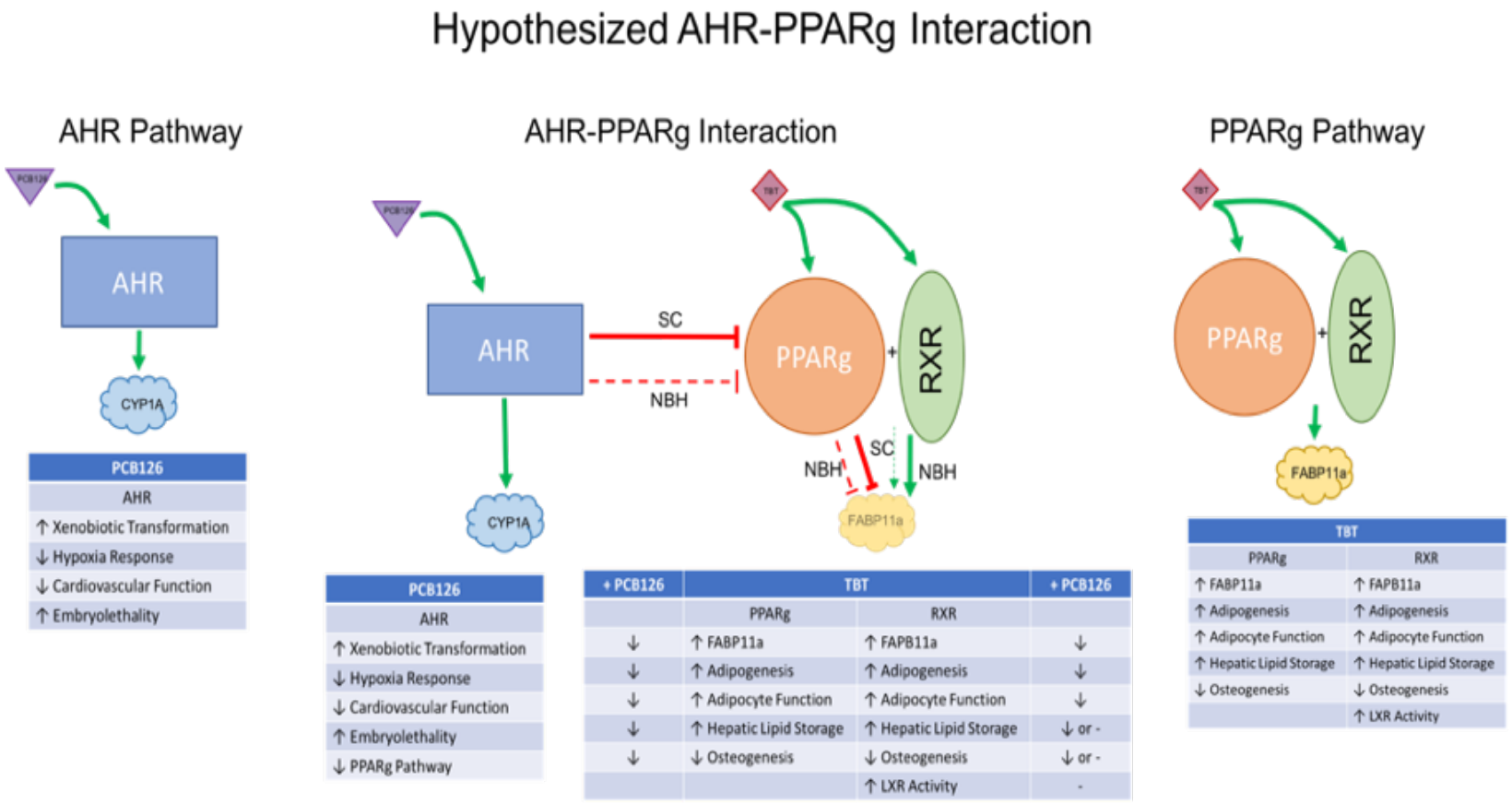


Figure 1.4. Schematic showing hypothesized interaction between AHR and PPARγ pathways in reference (Scorton Creek, SC) and tolerant (New Bedford Harbor, NBH) killifish populations. Solid green lines indicate activation; dashed green lines indicate unknown, but suspected, activation; solid red lines indicate inhibition; and dashed red lines indicate unknown, but suspected, inhibition.



CHAPTER 2: Predictions of polychlorinated biphenyl concentrations in seafood based on long-term monitoring and remediation in New Bedford Harbor, Massachusetts

Abstract

Electrical manufacturing near New Bedford Harbor, MA in the mid-1900s led to severe PCB contamination of the harbor and ultimately the harbor's designation as a Superfund Site. Restrictions on the harvest of seafood from NBH have been in effect since 1979. Efforts to reduce the overall mass of PCBs in NBH by dredging PCB-contaminated sediments have been ongoing since the 1980s. One goal of dredging is to reduce PCB concentrations in NBH seafood, monitored annually by MassDEP and MDMF. We used PCB concentrations in quahogs (2003-2016) and scup (2003-2014) to evaluate PCB distribution across seafood management areas. Seafood PCB concentrations were used to evaluate improvements in environmental quality by examining total PCBs and patterns of PCB congeners within homolog groups, and by assessing human cancer risk from seafood consumption at four time points (past: 1980; present: 2012-2016; future: 2025 and 2035). PCB concentrations in quahogs generally declined with increased time and distance from the PCB source, as does the cancer risk associated with their consumption. PCB concentrations in scup follow similar spatial patterns, but show high annual variability. We conclude that quahogs are a reliable proxy for *in-situ* conditions, environmental quality, and human health risk.

Introduction

The New Bedford Harbor (NBH) region of Massachusetts was a commercial epicenter for the northeastern United States during the industrial and manufacturing revolutions. Today, NBH remains a globally significant marine fishing port (van Voorhees, 2016). The uncontrolled discharge of polychlorinated biphenyls (PCBs) from capacitor manufacturing operations into the harbor during the mid 1930s to the late 1970s resulted in severe PCB contamination, which was first detected in the mid-1970s (100,000 milligrams per kilogram dry-weight (mg/kg dw; Weaver, 1982) in sediments, 53 mg/kg wet-weight (ww) in shellfish, and 22 mg/kg ww in finfish fillets) (Santos, 1978). The contamination led to the harbor's designation as a federal Superfund Site in 1983. Early investigations revealed a gradient of decreasing sediment PCB concentrations with increased distance from the land-based sources and resulted in designation of three management areas (Areas, **Figure 1.2**). Area 1, containing the original "hot spot" (PCBs > 4,000 mg/kg dw), has undergone dredging since the late-1980s to remove PCB-contaminated sediments; Area 2, delineated by a hurricane barrier, has undergone navigational dredging; and Area 3 extends into Buzzards Bay.

Consumption of seafood caught in Area 1 is prohibited and restrictions become less stringent in Areas 2 and 3 (**Figure 1.2**). Despite these restrictions, consumption of NBH-caught seafood continues (Basra, Komal; Fabian, Patricia M; Scammell, 2017), including amongst high frequency fish consumers and vulnerable populations living in surrounding communities, which may co-occur in certain demographics (von Stackelberg, 2017). The

U.S. Environmental Protection Agency (US EPA) developed a site-specific threshold of 0.02 mg/kg ww for PCBs in consumable NBH seafood based on health risk analyses, accounting for high-frequency seafood consumption by the population around NBH (US EPA, 1998). Because biota PCB concentrations are strongly influenced by sediment PCB concentrations (Kay, 2005; Nie, 2005; Oliver, 1988), one goal of ongoing sediment dredging in NBH is to reduce the human health risk of PCB exposure from consumable seafood (US EPA, 1998). Sediment cleanup goals established for NBH range between 1 and 50 mg/kg dw, depending on location within the harbor (US EPA, 1998).

To monitor remediation effectiveness, seafood species caught in NBH and consumed (e.g., quahog, or hard-shell clam, *Mercenaria mercenaria*, and scup, *Stenotomus chrysops*) are sampled annually by the MA Department of Environmental Protection (MassDEP) and Division of Marine Fisheries (MDMF; MassDEP, 2002, 2015). Aroclors and 136 PCB congeners have been analyzed in the edible portion of seafood species since 2003 with the goals of characterizing temporal changes in PCB levels and evaluating differences in PCB concentrations between seafood in the three Areas. A number of studies have quantified temporal changes in PCB concentrations in wildlife (Nacci et al., 2016; Scheider, Cox, Hayton, Hitchin, & Vaillancourt, 1998; Szlinder-Richert, Barska, Mazerski, & Usydus, 2009), but evaluating analogous trends in NBH is unique considering the harbor has undergone extensive remediation (projected to ultimately total 900,000 cubic yards of sediment). This study is the first to examine temporal and spatial trends of PCB concentrations in NBH quahogs and scup and to contextualize the trends

using a health risk assessment to assess the progress of harbor clean-up efforts.

Methods

Detailed sampling procedures, analytical methods for lipid and PCB analysis, and data validation summaries are found in annual NBH seafood monitoring reports (MassDEP, 2002, 2015).

Seafood Collection and Data Sources

Quahogs and scup were collected by MDMF annually throughout the three NBH Areas since 2003 (**Figure 1.2**). All specimens were of legally harvestable size and collected during legal harvest seasons. Quahogs were collected pre-spawn to capture peak lipid concentration and to provide conservative estimates of PCBs in the edible tissue. Due to site conditions and spatial variability, organisms were not found nor sampled at every location each year. Beginning in 2015, scup will be collected once every five years, whereas quahog collection will continue on an annual basis. Collection and organism summary statistics are provided in **Table A1**.

We compiled historic data on PCB concentrations in seafood collected from 1976-1979 (Kolek, Andrew; Ceurvels, 1981; Nisbet & Reynolds, 1984; Santos, 1978). We aggregated data for quahogs with four shellfish species and for scup with seven bottom-feeding finfish species (**Table A2**). Shellfish are stationary and in intimate contact with sediment. Therefore, shellfish may closely reflect sediment PCBs over a small spatial

scale. Finfish are mobile over a larger spatial area, in some instances migrating seasonally between coastal and off-shore waters. Finally, physiological differences between these groups might influence how they process PCBs to which they are exposed (Borgå, Fisk, Hoekstra, & Muir, 2004).

PCB Analysis

Quahog composite samples consisted of approximately 12 individual whole, shelled organisms per location. Scup composites were comprised of edible, skin-on fillets from roughly five individual fish per location. Composite samples were extracted by US EPA Method 3570. Extracts were analyzed for lipid content, Aroclors and 136 PCB congeners based on US EPA Methods 680 and 8270D. Included in this suite of congeners are all non-*ortho* and mono-*ortho* substituted PCBs. Lipid content was reported as a weight-based percentage. PCB analytes were reported as mg/kg ww). Analytes detected outside the analytical calibration limits were reported as estimated ("J") values. Analytes not detected above the sample quantitation limit (SQL) were reported as one half the SQL (half-SQL) in an effort to avoid underestimating PCB concentrations in seafood tissue.

Data Analyses

Wet-weight PCB concentrations of individual congeners (PCB_{WW}) were lipid-normalized (PCB_{LN}). Total PCBs were calculated as the sum of individual congeners, including J-values and half-SQL, for both wet-weight ($\sum PCB_{WW}$) and lipid-normalized ($\sum PCB_{LN}$) concentrations. PCB_{LN} of individual congeners were grouped by homolog to evaluate

congener patterns. The SQL for 73 congeners measured in quahogs decreased significantly (alpha=0.05) from 2003-2016, whereas only seven congener-specific SQLs declined in scup from 2003-2014. Although changes in SQL over time may influence temporal trends in PCBs levels, particularly in less contaminated regions of the harbor, half-SQL values were included to provide conservative estimates of PCB concentrations.

Health Risk Assessment

We evaluated the health risk of consuming NBH-caught seafood for three time periods: (1) Past: 1980, prior to the harbor's Superfund designation; (2) Present: the three most recent years each species was sampled by MassDEP ("present") and 2015; and (3) Future: 2025 and 2035, after forecasted completion of sediment remediation. Exposure Point Concentrations (EPCs), the PCB concentrations in seafood that people are expected to consume, were calculated for $\sum PCB_{WW,}$ consistent with PCB EPCs used in US EPA risk calculations. EPCs were calculated for quahogs and scup at each time point, in each Area. EPCs for 1980, 2015, 2025 and 2035 were estimated by linear regression equations describing the temporal change in log-transformed $\sum PCB_{WW}$ (quahogs: 2003-2016; scup: 2003-2014). "Present" EPCs were calculated using the most recent empirical $\sum PCB_{WW}$ data for each species (quahogs: 2014-2016; scup: 2012-2014), selecting the 95% upper confidence limit (UCL) on the mean.(Singh, Anita; Maichle, 2017) Using US EPA exposure and toxicity assumptions from the NBH seafood advisory (**Table A3**),(Lombardo, 2015) we calculated excess lifetime cancer risk (ELCR) from consuming one (Central Tendency Exposure, CTE) or four (Reasonable Maximum Exposure, RME)

meals of NBH quahogs and scup per month. An 8-ounce serving was used for adult seafood meals, whereas children's seafood meals were assumed be 4-ounces. Cancer risk was evaluated due to the availability of an EPA-derived cancer slope factor for total PCBs and completeness of $\sum PCB_{WW}$ data. Non-cancer risk was not evaluated due to limited data on Aroclor 1254 concentrations in NBH seafood tissue, which to compare to the EPA-derived oral reference dose (RfD).

Statistical Analyses

All statistical analyses were performed using Microsoft R Open 3.3.2. ANOVA and t-tests were used to evaluate differences in mean seafood PCB levels between Areas. Linear regression was used to evaluate changes in log-transformed PCB concentrations over time. Statistical significance was evaluated using alpha=0.05.

Results and Discussion

PCB congener detection, concentrations and patterns

Summary statistics show that $\sum PCB_{LN}$ in the three Areas ranged from 7.55-1,480 mg/kg in quahogs (2003-2016) and from 2.96-250 mg/kg in scup (2003-2014) (**Table A1**). The frequency of congener detection above the SQL ranged from 0-97% in quahog and 0-100% in scup, with 16 and 48 congeners detected in at least 90% of quahog and scup samples, respectively. Three congeners (PCBs 76, 184, 205) were not detected in any sample for either species. Dioxin-like PCBs comprise up to 10.8% of $\sum PCB_{LN}$ in

quahogs and up to 20.3% of $\sum PCB_{LN}$ in scup (**Figure A1**).

The distributions of PCB_{LN} of congeners within each homolog show that quahogs are dominated by tri- to hexa-chlorobiphenyls (CBs), with the highest mean congener concentration measured in tri-CBs (**Figure 2.2a**). In contrast, scup are dominated by tetra- to hexa-CBs, with highest mean congener concentrations in penta- and hexa-CBs (**Figure 2.2b**). Comparing homolog patterns to those of Aroclors can indicate the PCB source, and can characterize degradation and bioaccumulation patterns in the environment. NBH PCB contamination resulted primarily from release of Aroclors-1242 and -1016, and to a lesser extent 1254 (Santos, 1978). The homolog pattern in quahogs from Areas 1 and 2 most closely resembles weathered Aroclors-1242 and -1016 (**Table A4**), characterized by the disproportionate loss of lighter, more volatile congeners over time. Since bivalves are stationary and have negligible capacity for xenobiotic biotransformation (Kania-Korwel & Lehmler, 2016), the PCB congener pattern found in quahogs is considered a proxy for *in-situ* conditions.

The abundance of penta- and hexa-CBs in NBH scup (**Table A4**) can either be explained by Aroclor-1254 discharge into NBH (Santos, 1978) or the tendency for highly chlorinated congeners to bioaccumulate in aquatic environments (Borgå, 2004), biomagnifying in higher trophic organisms (Kay, 2005; Nie, 2005; Oliver, 1988), like scup. The presence of hydroxylated PCB metabolites in scup (R. D. White, Shea, & Stegeman, 1997) suggests that scup physiology also may contribute to the relative

absence of di-, tri- and tetra-CBs since less chlorinated congeners are generally most amenable to biotransformation. Overall, the difference in homolog signatures between quahogs and scup likely reflects their respective ecological niches, trophic positions, and biotransformation capacity.

Spatial and temporal patterns of PCBs in NBH seafood

Spatially, $\sum PCB_{LN}$ in quahogs decreased with increased distance from the PCB source (Area 1 > Area 2 > Area 3, ANOVA $p \leq 0.0001$) when data were pooled by Area across all years (**Figure 2.3a**). The same pattern was observed in scup (**Figure 2.3b**; Area 2 > Area 3, T-test $p \leq 0.0001$). Trends for dioxin-like and non-dioxin-like $\sum PCB_{LN}$ were similar (**Figure A1**). These findings are consistent with spatial PCB gradients previously demonstrated in other NBH shellfish(Santos, 1978) and finfish (Deshpande, Dockum, Cleary, Farrington, & Wieczorek, 2013; Santos, 1978).

Temporally, $\sum PCB_{LN}$ in quahogs declined significantly from 2003-2016 (Area 1: -3.2% mg/kg/year, p=0.0039; Area 2: -3.1% mg/kg/year, p=0.0006; Area 3: -2.1% mg/kg/year, p=0.0017) (**Figure 2.3a, Figure A2a**). However, significant declines in $\sum PCB_{LN}$ were not observed in scup (**Figure 2.3b, Figure A2b**). PCB concentrations in stationary shellfish reflect anticipated changes in *in-situ* PCB levels over time, following extensive sediment remediation, whereas mobile finfish do not.

Temporal declines in PCB_{LN} in quahogs are significant for dominant homolog groups

(**Figure 2.3c**, **Table A5**). The absence of significant declines in other homologs is likely attributable to lower congener detection frequencies and a higher prevalence of non-detect congeners substituted with half-SQL. PCB_{LN} in scup declined significantly in tetra-CBs in Area 2 and tetra- to septa-CBs in Area 3 (**Figure 2.3d**, **Table A5**). Together, these data show that PCB concentrations in NBH seafood decrease with time and distance from the PCB source. Declining trends in PCB concentrations observed in NBH seafood since 2003, particularly quahogs in Area 1, suggest that sediment remediation is associated with improvements in environmental quality.

To investigate PCB trends in NBH seafood over a longer period of time, we aggregated available historic data from NBH shellfish and bottom-dwelling finfish collected between 1976-1979 (**Table A2)**. PCB concentrations in NBH seafood were reported as Aroclor-1254 (mg/kg ww). The highest concentration of Aroclor-1254 measured in quahogs in the late-1970s was 4.0 mg/kg in Area 1 (Santos, 1978); whereas concentrations as high as 53 mg/kg were reported in soft-shell clams collected in Area 1 (Kolek, Andrew; Ceurvels, 1981). The spatial gradient in PCB concentration reported above also was evident among NBH shellfish collected during the late-1970s (mean Aroclor-1254 in Area 1 > Area 2 > Area 3). The highest Aroclor-1254 concentration reported in scup was 11.4 mg/kg in Area 2 (Kolek, Andrew; Ceurvels, 1981; Santos, 1978). In bottom-dwelling finfish, the highest Aroclor-1254 concentration was 22 mg/kg in winter flounder in Area 1 (Santos, 1978). While PCB concentrations in scup did not follow the spatial gradient observed with shellfish, PCB concentrations in aggregated bottom-dwelling

finfish were consistent with the spatial pattern of PCBs in quahogs (mean Aroclor-1254 in Area 1 > Area 2 > Area 3). In comparison, the maximum Aroclor-1254 measured between 2003-2013 was 2.40 mg/kg in quahogs in Area 1 and 3.65 mg/kg in scup in Area 2.

Comparative risk assessment for fish consumption: past (1976-1979), present (2012-2016) and future (2025 and 2035)

To analyze the human health impact of decreasing PCB concentrations in NBH seafood, we calculated the cancer risk from consuming NBH-caught seafood during three time periods (past, present and future), using $\sum PCB_{WW}$ measured in NBH quahogs and scup since 2003. EPCs and the associated log-transformed $\sum PCB_{WW}$ linear regression parameters used to estimate PCB concentrations in NBH seafood in 1980, 2015, 2025 and 2035 are shown in **Table A2** and **Figure A3** for all Areas. We calculated the cancer risk associated with consuming one (CTE) or four (RME) meals of quahogs or scup per month (**Figure 2.4**, **Table A6**) relative to the risk criterion of 1E-4 used by US EPA for NBH. Temporal and spatial trends in cancer risk for quahogs and scup mimic the directionality of $\sum PCB_{WW}$ changes over time due to the linearity of models used to derive EPCs and cancer risk. We show that in Area 2 $\sum PCB_{WW}$ in quahogs is declining at a rate of -3.4% mg/kg/yr (2003-2016), associated with CTE cancer risk reductions for adults from a risk level of 2E-4 in 1980, to 1E-5 (2015), 6E-6 (2025), and 3E-6 (2035) (**Figure 2.4a**, **Table A6**). The "present" cancer risk for adults is 2E-5. By contrast, $\sum PCB_{WW}$ in

Area 2 scup increased by 0.28% mg/kg/yr (2003-2014), resulting in CTE cancer risk increases for adults from a risk level of 6E-5 in 1980, to risk levels of 8E-5 (2015), 9E-5 (2025) and 9E-5 (2035) (**Figure 2.4b**, **Table A6**). The "present" cancer risk for adults is 2E-4.

Our 2015 and "present" risk calculations in both quahogs and scup are congruent with existing Massachusetts Department of Public Health and US EPA fish consumption advisories for all Areas (US EPA, 2017) (**Figure 1.2**), despite differences in EPCs. US EPA calculated $\sum PCB_{WW}$ substituting "zero" concentration for non-detect congeners, whereas we substituted half-SQL. US EPA also calculated EPCs using $\sum PCB_{WW}$ data for quahogs and scup measured over a longer period of time. Risk estimates for historic and future time points rely on the assumption that trends in $\sum PCB_{WW}$ since 2003 can be generalized to describe the pattern of change in PCB concentrations between 1980 and 2035. We expect the rate of PCB removal from NBH during periods of active sediment remediation will differ from that of natural attenuation. Trends in $\sum PCB_{WW}$ from 2003-2016 may overestimate or underestimate the rate of decline occurring from 1980-2035. The pace of PCB sediment remediation is expected to increase with fund availability.

In summary, we show significant declines in $\sum PCB_{LN}$ concentrations in quahogs over time and with increasing distance from the PCB source in Area 1. Temporal declines in PCB concentrations also are evident in human cancer risk reductions from consumption of NBH quahogs between 1980 and 2035. Temporal trends in PCB concentrations in scup

are less conclusive. Therefore, changes in cancer risk related to scup consumption are less predictive of temporal trends in NBH PCBs. Quahogs are expected to more accurately reflect environmental quality because they are stationary and exhibit lower capacity for xenobiotic metabolism (Farrington, Goldberg, Risebrough, Martin, & Bowen, 1983), and decreases in quahog PCB concentrations are consistent with extensive sediment remediation by the US EPA. While we recommend that annual PCB congener and Aroclor monitoring in NBH seafood continue for all species to ensure the NBH seafood database is adequately robust to evaluate trends in seafood PCB concentrations as remediation continues, we recognize financial limitations exist. Thus, annual quahog sampling should be prioritized given their usefulness for understanding environmental conditions. Comprehensive evaluation of remediation efficacy also should include NBH-wide PCB fate and transport modeling, integrating available PCB sediment and air data with seafood PCB data.

Figures

Figure 2.2. Distribution of ∑PCB_{LN} (log mg/kg ww) concentrations measured in quahogs (a) and scup (b) grouped by number of chlorines (homolog) for all years in each Area. Data are reported as mean ± interquartile range (IQR) of individual congeners within each homolog for 38-80 samples collected in a given area between 2003-2016 for quahogs and 2003-2014 for scup. Outliers are depicted.

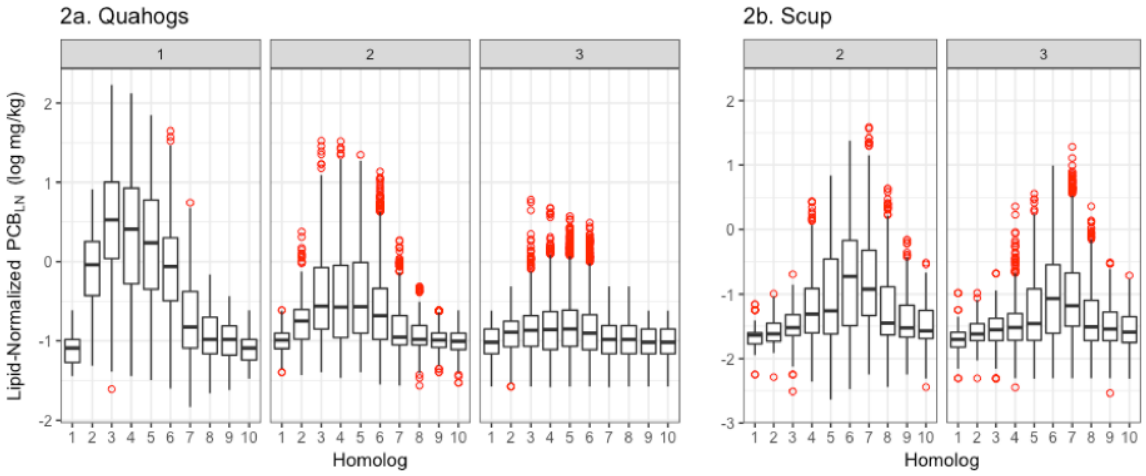


Figure 2.3. Changes in $\sum PCB_{LN}$ and PCB_{LN} (log mg/kg ww) over time in quahogs (a, c) and scup (b, d) in each Area. $\sum PCB_{LN}$ (a, b) are reported as the annual mean of the 1-7 (quahogs) and five (scup) composite samples collected in a given Area, in a given year. PCB_{LN} (c, d) show the annual mean concentration of PCB congeners within each homolog in quahogs and scup in a given year and Area. Included in $\sum PCB_{LN}$ are all non-*ortho* (PCBs 77, 81, 126, 169) and mono-*ortho* (PCBs 105, 114, 118, 123, 156, 157, 167, 189) substituted PCBs, as well as all National Oceanographic and Atmospheric Administration (NOAA) and World Health Organization (WHO) congeners. Complete data presented in Figure S2 and Table S5.

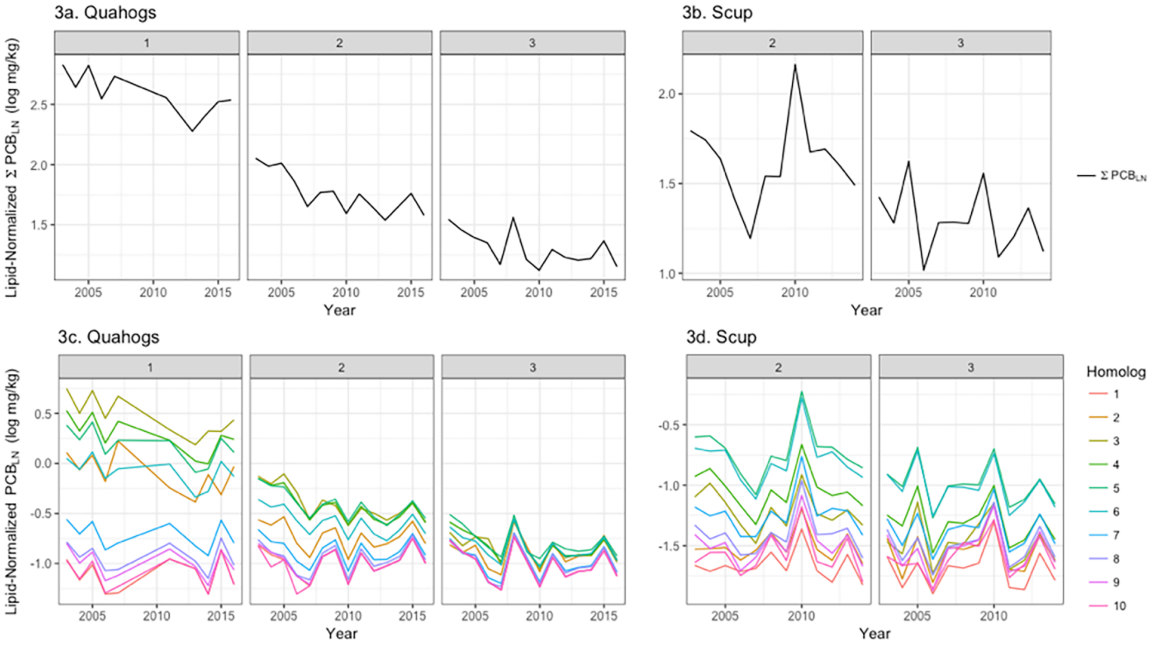
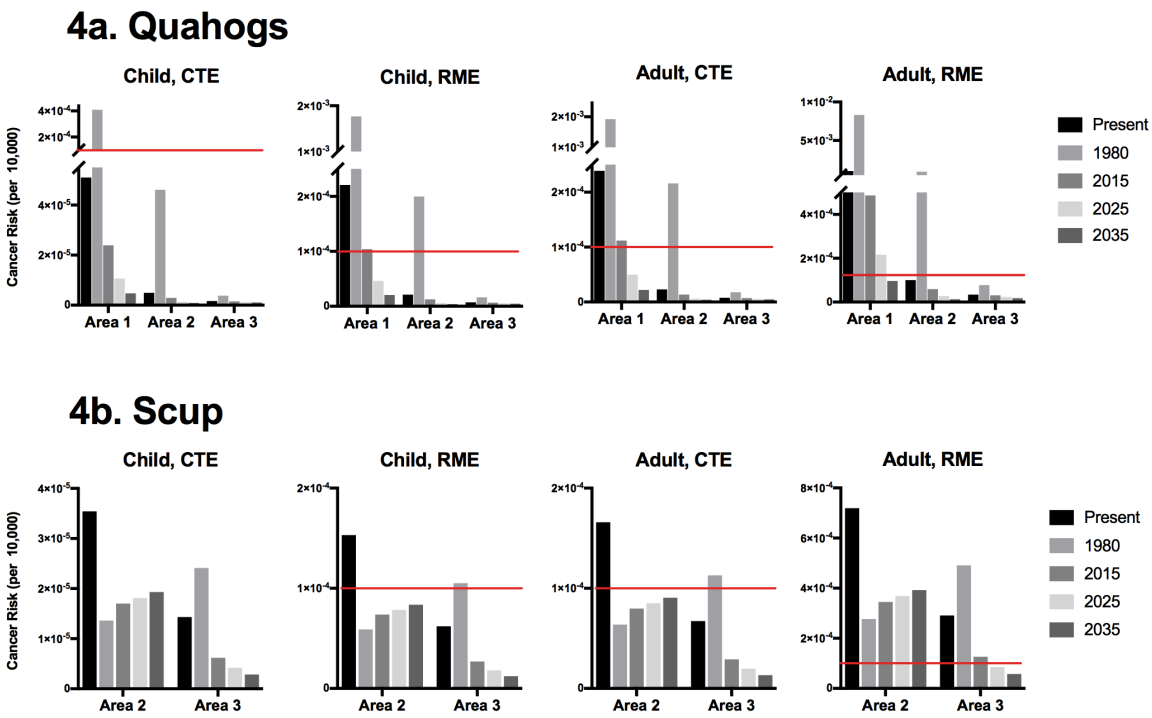


Figure 2.4. Summary of human cancer risk estimates for PCB exposures associated with the consumption of quahogs (a) and scup (b) harvested from NBH for young children (1-6 years) and adults (16-70 years). Cancer risk was calculated using US EPA exposure and toxicity assumptions in Table S3, the Exposure Point Concentrations (EPCs) for ∑PCB$_{WW}$ (mg/kg ww) shown in Table S6, and the oral cancer slope factor for total PCBs (2.0 (mg/kg/day)$^{-1}$).(IRIS, 1989) **Cancer risk was calculated for consumption of one (Central Tendency Exposure, CTE) or four (Reasonable Maximum Exposure, RME) meals of NBH quahogs and scup per month. All cancer risk estimates were evaluated relative to the health risk criterion of 1 E-4 (indicated by the red line) used by US EPA for NBH. A complete summary of cancer risk estimates for NBH quahogs and scup included in Table S7.**



CHAPTER 3: Altered lipid homeostasis in a PCB-resistant Atlantic killifish (*Fundulus heteroclitus*) population from New Bedford Harbor

Abstract

Sentinel species such as the Atlantic killifish (*Fundulus heteroclitus*) living in urban waterways can be used as toxicological models to understand impacts of environmental metabolism disrupting compound (MDC) exposure on both wildlife and humans. Exposure to MDCs is associated with increased risk of metabolic syndrome, including impaired lipid and glucose homeostasis, adipogenesis, appetite and satiety, and basal metabolism. MDCs are ubiquitous in the environment, including in aquatic environments. New Bedford Harbor (NBH), Massachusetts is polluted with polychlorinated biphenyls (PCBs), and, as we show for the first time, tin (Sn). PCBs and organotins are ligands for two receptor systems known to regulate lipid homeostasis, the aryl hydrocarbon receptor (AHR) and the peroxisome proliferator-activated receptors (PPARs), respectively. In the current study, we compared lipid homeostasis in laboratory-reared killifish from NBH (F2) and a reference location (Scorton Creek (SC), Massachusetts; F1 and F2) to evaluate how adaptation to local conditions may influence responses to MDCs. Adult killifish from each population were exposed to two polychlorinated biphenyls: 3,3',4,4',5-pentachlorobiphenyl (PCB126, dioxin-like) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153, non-dioxin-like), tributyltin (TBT, a PPARγ ligand) by a single intraperitoneal injection and analyzed after a 3 days exposure. Lipid homeostasis was evaluated phenotypically by measuring liver triglycerides and organosomatic indices, and at the

molecular level by measuring the expression of *pparγ* and *pparα* and a target gene for each receptor. Acute MDC exposure did not affect phenotypic outcomes. However, overall NBH killifish had higher liver triglycerides and adiposomatic indices than SC killifish. Both season and population were significant predictors of the lipid phenotype. Acute MDC expression altered hepatic gene expression only in male killifish from SC. PCB126 exposure induced *cyp1a* and *pparγ*, whereas PCB153 exposure induced *pparα*. TBT exposure did not induce *ppar*-pathways. Comparisons of lipid homeostasis in two killifish populations extends our understanding of how MDCs act on fish and provides a basis to infer adaptive benefits of these differences in the wild.

Introduction

The dramatic rise in obesity worldwide has drawn considerable clinical and research attention. One conclusion from obesity-related research is that diet and exercise cannot entirely explain the global obesity epidemic. This discovery has led to the identification of environmental metabolism disrupting compounds (MDCs), a class of compounds that alter metabolic homeostasis, including lipid and glucose homeostasis, adipogenesis, appetite and satiety, and basal metabolism (e.g., (Grün, 2006; Grün & Blumberg, 2009; J. J. Heindel, 2017). MDCs, also referred to as "obesogens", are structurally diverse and act through multiple molecular pathways. Among them are pollutants common in aquatic environments such as organotins (e.g., tributyltin, TBT; (Grün, 2006; J. J. Heindel, 2017) and dioxin- and non-dioxin-like polychlorinated biphenyls (PCBs; Baker et al., 2013; Gadupudi et al., 2015; Wahlang et al., 2013).

Three ligand-activated transcription factors play particularly important roles in metabolic homeostasis and can be activated by environmental metabolic disrupting chemicals: peroxisome proliferator activated receptor gamma (PPARγ), an essential regulator of adipogenesis (Tontonoz et al., 1994); PPARα, which regulates ß-oxidation (Kersten, 2000); and the aryl hydrocarbon receptor (AHR) (Nebert, 2017), most widely known for its involvement in xenobiotic metabolism. Activation of the PPARγ pathway induces adipogenesis, hypertrophy and hyperplasia of adipocytes, and lipogenic gene expression in adipose tissue and liver (e.g., Grün et al., 2006; Heindel et al., 2017; S. M. Kim et al., 2018; Pereira-Fernandes et al., 2013; Y. Wang et al., 2017; Watt & Schlezinger, 2015).

PPARα pathway activation stimulates fatty acid transport and β-oxidation during periods of energy deprivation, including between meals (van Raalte, 2004), countering lipid sequestering effects of PPARγ. AHR is essential for regulating lipid accumulation and inflammation in adipocytes, glucose tolerance, insulin sensitivity (Arsenescu, 2008; N. A. Baker, 2013, 2015; Kerley-Hamilton, 2012; La Merrill, 2009), and proper β cell development (Timme-Laragy, 2015). The potential for the AHR to suppress PPARγ gene expression and activity has been implicated in rodent models (e.g., Gadupudi et al., 2015), but remains poorly understood.

Ligands for both AHR (dioxin-like PCBs) and PPARγ (TBT) are found in urban waterways, near major population centers in the United States. New Bedford Harbor (NBH) is a marine Superfund site, located in southeastern Massachusetts (**Figure 1.1).** PCBs were used in electronics manufacturing near NBH, and their discharge into the environment has led to widespread, severe contamination throughout the harbor. Organotins were used as an antifouling agent in marine boat paints and NBH has long served as a major fishing and shipping port in the region, though tin has not previously been measured in NBH.

NBH is home to a number of wildlife populations, including the Atlantic killifish (*Fundulus heteroclitus*), which is both an ecological sentinel and a vertebrate model amenable to mechanistic toxicology studies (Burnett, 2007). Seasonal climate variability results in dramatic changes in feeding behavior, body composition and energetics. During

the summer months, energy resources are allocated to growth and reproduction, whereas in the fall and winter, energy storage and maintenance become the respective priorities (e.g., Berg & Bremset, 1998; Kandemir, Polat, & Turk, 2007; Sheridan, 1994). Killifish breed by broadcast spawning with semi-lunar periodicity during the summer months (late May through August in New England) and have an approximately two-year generation time. Killifish have been studied extensively for their response to industrial chemicals, most notably PCBs and polycyclic aromatic hydrocarbons (PAHs) acting through the AHR (e.g., Nacci et al., 2010; Oleksiak et al., 2011; Reid et al., 2016). The small home ranges of killifish have led to the evolution of genetically distinct populations along the US eastern seaboard. Killifish living in polluted estuaries, like NBH, demonstrate dramatic heritable resistance, also referred to as evolved tolerance, to AHR pathway activation (e.g., (Bello, 2001; Joel N. Meyer & Di Giulio, 2003; D. Nacci, 1999; D. E. Nacci, 2010; Reid, 2016) and have emerged as an important example of convergent evolution (Reid, 2016; Andrew Whitehead, Pilcher, Champlin, & Nacci, 2012).

In this study, we sought to examine liver lipid homeostasis in NBH killifish in comparison to reference killifish from Scorton Creek (SC), Massachusetts. We tested the hypothesis that exposure to select environmental MDCs (e.g., PCBs and organotins) would increase liver triglyceride levels and perturb molecular signaling related to lipid homeostasis across seasons. We also tested the hypothesis that SC and NBH killifish would respond differently to MDC exposure due to population-specific exposure histories and adaptations. This study is the first to report Sn in NBH sediments, which may be

associated with the historic use of organotin compounds in marine paints in the harbor.

Methods

Total Tin (Sn) Analysis in NBH Sediments

Sediment samples collected from within NBH and outside it, in Buzzards Bay, Massachusetts between the years of 1989 and 2018 were analyzed for total tin (Sn). A map of sediment sample locations is provided in **Figure 3.1a**. The 1989 archived sediment sample serves as a reference to describe background Sn levels in local sediments and geologic material. The 1989 sample was collected outside NBH, in Buzzards Bay, and beyond the area known to be significantly impacted by PCB contamination in NBH. Additionally, the 1980s was a period of high global use of organotins as antifouling agents in marine paints (Law, Bolam, James, Barry, Deaville, Reid, Penrose, & Jepson, 2012; Omae, 2003; Sonak, Pangam, Giriyan, & Hawaldar, 2009), suggesting that the 1989 sample from Buzzards Bay reflects global organotin use and serves as a conservative reference sample for evaluating local contributions of tin to total tin levels in NBH. The samples from 2005 and 2012 were selected to provide collocated sampling locations with the 2018 samples. Sediment cores were collected in 1989, 2005 and 2012 by divers and were sectioned in two-centimeter (cm) increments. Sediment sections were dried and archived. 2018 samples were collected from dredged sediments removed from NBH as part of ongoing PCB sediment remediation and dried in an identical manner to archived samples. Aliquots of archived and 2018 samples were analyzed for Sn by Chemical Solutions, Ltd. (Harrisburg, PA) using hydrofluoric acid

extraction followed by inductively coupled plasma mass spectrometry (ICP-MS) with a practical quantitation limit (PQL) of 0.5 µg/g (dry weight, dw).

Animal Care and Selection

All activities were performed in accordance with U.S. Environmental Protection Agency Atlantic Ecology Division Laboratory animal care practices and every effort was made to minimize pain and distress. Adult, reproductively active *F. heteroclitus* (F0) were collected from New Bedford Harbor (NBH, PCB-contaminated) and Scorton Creek (SC, reference) using baited traps as described in Nacci et al. (2002). Killifish were held in approximately 300 or 600 L flow-through aquaria receiving 5µM filtered, ambient temperature seawater from Narragansett Bay, Rhode Island. Variable light:dark cycles represented ambient conditions throughout the year. Killifish were fed *ad libidum*, as described in Nacci et al. (2002). F0 killifish spawned with semi-lunar periodicity throughout summer months, during which time embryos (F1) were harvested and reared to reproductive maturity (1-2 years) under the conditions described above. F2 embryos were then harvested from F1 adults and raised to reproductive maturity in the same manner.

Experiments were designed to evaluate the role of dioxin-like and non-dioxin-like PCBs and TBT on measures of lipid homeostasis: liver triglycerides, adipose tissue mass and gene expression related to lipid homeostasis in liver. Weight-matched killifish from NBH and SC (**Table 3.1**) were selected for use in four replicate intraperitoneal (IP) injection

exposure experiments, referred to herein by their date: October 2015, March and October 2016 and May 2017. Given seasonal changes in resource allocation in killifish, two experimental replicates were conducted in the fall and two in the spring to capture periods of high and low lipid reserves. At least two weeks prior to each experiment, water in the flow-through aquaria was warmed to and held at 23°C, the approximate ambient summer temperature in Narragansett Bay. NBH F2 adults were used to ensure complete depuration of persistent organic pollutants (POPs) from NBH and to evaluate heritable effects of adaptation to NBH contaminants. Since SC fish represent the reference site, maternal transfer of POPs is not of concern. F2 killifish from SC were selected when possible (October 2016 and May 2017). When F2 size matching between NBH and SC animals was not possible, F1 killifish from SC were utilized (October 2015 and March 2016).

Chemical Exposure by Intraperitoneal Injection

Killifish from each population were divided among tanks, with 6-10 individuals per tank and equal number of males and females, depending on the experimental replicate (N=10, 5 males and 5 females in October 2015; N=6, 3 males and 3 females in all others). Killifish were distributed to achieve similar mean weight across tanks (**Table 3.1**: mean (SD): October 2015: SC – 6.12 (0.99), NBH – 6.48 (0.96); March 2016: SC – 7.35 (2.46), NBH – 7.14 (1.96); October 2016: SC – 3.44(0.39), NBH – 3.47 (0.49); May 2017: SC – 4.55 (0.53), NBH – 4.72 (0.67)) and were allowed to acclimate to these tanks for one week prior to IP injection. In each experimental replicate, all killifish administered a

given chemical treatment were housed in one tank per population. On the seventh day, killifish were anesthetized in tricaine methanesulfonate (MS222) and administered chemicals by IP injection.

3,3′,4,4′,5-pentachlorobiphenyl (PCB126) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153) used in the first experimental replicate (October 2015) were purchased from AccuStandard (New Haven, CT, USA). PCB126 and PCB153 used in all other experimental replicates were purchased from Ultra Scientific (North Kingstown, RI). Tributyltin chloride (TBT) was purchased from Sigma Aldrich (St. Louis, MO, USA). S26948, a non-thiazolidinedione (TZD) selective PPARγ agonist and antidiabetic pharmaceutical agent, was purchased from Tocris Bioscience (Minneapolis, MN, USA). Chemicals were suspended in DMSO or acetone. Exposures consisted of vehicle (0.03% DMSO or acetone), PCB126 (29 ng/g), PCB153 (2,120 ng/g), TBT (10 µg/g), or S26948 (100 µg/g) in corn oil, by IP injection (1 ug corn oil solution per g fish). PCB doses were selected with the goal of achieving concentrations comparable to those measured in NBH wild-caught, undepurated killifish livers (Black, Gutjahr-Gobell, Pruell, Bergen, Mills, & McElroy, 1998). TBT and S26948 doses were selected based on EC_{50} concentrations noted in other studies (e.g., Carmona et al., 2007; Grün, 2014). Chemical exposures differed between the experimental replicates and are summarized in **Table 3.2**. In October 2016 and May 2017, naïve (uninjected) killifish from the same cohort were sampled to confirm that outcome measures of interest were not altered relative to vehicle-treated animals. Three days after IP injections, killifish were euthanized in MS222.

Animal weight (grams, wet weight, ww) and standard length were measured for all animals except those euthanized in October 2015. Liver, adipose tissue, gonad and brain were resected and weighed (g, ww).

Liver Triglyceride Analysis

Liver triglycerides (mg TAG per g liver) were measured as described in Norris et al. (2003). Briefly, an approximately 45 mg (ww) piece of liver was digested in ethanolic potassium hydroxide. Following neutralization with magnesium chloride, glycerol was measured spectrophometrically at 540 nm using Free Glycerol Reagent (Sigma Aldrich). Triglyceride concentration was calculated from a triolein standard curve included on the same plate.

Gene Expression Analysis

Total RNA was extracted from an approximately 30 mg (ww) piece of liver using RNA STAT-60™ (Tel-Test Inc. Friendswood, TX, USA). Genomic DNA was removed using the Aurum™ Total RNA Mini Kit (BioRad). cDNA was prepared from total RNA using the GoScript™ Reverse Transcription System (Promega), using equal parts random and Oligo $(dT)_{15}$ primers. qPCR reactions were performed using iTaq Universal SYBR® Green Supermix (BioRad) with 400nM forward and reverse primers. Primer sequences are shown in **Table 3.3**. Thermocycling parameters for all genes were 95°C for three minutes to activate iTaq DNA polymerase, followed by 40 cycles of melting at 95°C for

15 seconds and annealing at 66°C for 60 seconds. A manually-selected threshold in the linear phase of the amplification curve was used to determine threshold cycle (C_T) values to standardize across multiple plates.

All gene expression data were normalized using the Pfaffl method (Pfaffl, 2001), using primer-specific amplification efficiency and the expression of constitutively expressed (housekeeping) genes, and control samples (liver from naïve, uninjected killifish of the same cohort as those used for chemical exposures). Three housekeeping genes were evaluated for stability across population, sex and chemical treatment. Variability was greatest in *β-actin* (overall mean (standard deviation): 1.157 (0.798)), with differences between populations (SC > NBH) and sexes (male > female). Expression of *ef1α* was less variable (0.874 (0.317)) with no difference between populations, though expression differed slightly between sexes (female > male). *Rn18s* was most stably expressed (0.997 (0.172)) with no discernable pattern of variability between populations, sexes or chemical treatment groups. Thus, all gene expression data presented herein are normalized to *rn18s* and reported as a relative expression ratio.

Data and Statistical Analyses

All statistical analyses were performed using Microsoft R Open 3.3.2, including ggplot2, lmerTest and lmtest packages. Condition factor (CF) was calculated using killifish weight (ww) and standard length at euthanasia. Hepatosomatic (HSI), adiposomatic (ASI) and gonadosomatic (GSI) indices were calculated using resected organ and killifish weight

(ww) at euthanasia. Killifish characteristics, including phenotypic parameters are summarized in **Table 3.1** for each experimental replicate.

A comparative approach was taken to statistically evaluate phenotypic outcomes (TAG, ASI and HSI) across all experimental replicates. ANOVA and t-tests were used to compare means between populations, experimental replicates, sexes and chemical treatment groups. To better account for factors contributing to each phenotypic outcome and seasonal oscillations in killifish lipid reserves, we also used multiple linear regression and linear mixed effects models. This was possible due to a relatively large overall sample size (N=249) compared to many toxicological studies. We included fixed effects for population, sex, fish weight, season and chemical treatment as covariates. ASI was also considered in models to evaluate liver TAG to account for potential confounding. Based on *a priori* knowledge of killifish physiology, season was considered a random effect and all linear mixed effects models. To improve statistical power, the parsimonious multiple linear regression and linear mixed effects models for each phenotypic outcome were selected and identified herein as "fully adjusted." Intra-class correlation coefficients (ICCs) were calculated to evaluate within group correlations. Statistical significance was evaluated at $\alpha = 0.05$ for all analyses.

Gene expression was evaluated using ANOVA and t-tests to evaluate differences in mean relative expression between populations, sexes and treatment groups for killifish in the May 2017 IP injection study. No differences were found in the expression of

housekeeping or target genes between naïve (uninjected) and control (DMSO) killifish livers. As a result, the naïve treatment group was eliminated from the dataset for statistical analyses. All data points, including possible outliers, were used in analyses due to the small sample size (N=3) when data were fully parsed by population, sex and chemical treatment.

Results

This study quantified concentrations of tin in NBH and Buzzards Bay and evaluated the effects of adult exposures to MDCs on lipid homeostasis in killifish at the phenotypic and molecular levels. Total tin was measured in sediment samples collected between 1989 and 2018 to determine how concentrations in NBH compared to a reference location in Buzzards Bay. SC and NBH F2 killifish were exposed TBT, PCB126 and PCB153 by IP injection. Phenotypic measures of lipid homeostasis, including adiposomatic index and liver triglyceride concentration were compared across population, season and chemical treatment to identify important predictors of lipid phenotype in killifish. Expression of PPARγ, PPARα and AHR pathway genes were characterized in killifish liver to explore molecular effects of MDC exposure on lipid homeostasis.

Total Sn in New Bedford Harbor

NBH is well known for its contamination by PCBs, yet other contaminants, including metals such as tin, that have not previously been analyzed likely exist in the harbor given prominent industries present in the region since the industrial revolution. Given that NBH

has been and continues to be an active commercial harbor with significant boat traffic, we hypothesized that organotin contamination is present. To test this hypothesis, total Sn was measured in historic (archived) and current (2018) NBH sediments to document the presence of Sn in the harbor (**Figure 3.1b**). The reference level of Sn in Buzzards Bay as measured in archived sediment cores was 2.2 µg/g. Tin concentrations inside the NBH hurricane barrier ranged from 11 to 38 µg/g in archived sediment samples and from 3.2 to 21 µg/g in current sediment. In all cases, sediment samples from within NBH had higher Sn concentrations than those reported in the Buzzards Bay reference sample. In archived sediment samples collected near the former Aerovox facility (a major source of PCB contamination in NBH) and the northern side of Pope's Island (**Figure 3.1a**), Sn levels declined sharply between 2005 and 2012. Tin levels in 2018 dredged sediment near the northern side of Pope's Island were slightly lower than those measured in the same vicinity in 2012. Tin concentrations increased slightly between 2005 and 2012 along the southern side of Pope's Island. Tin levels increased more significantly in the same time period near the hurricane barrier.

Fish Characteristics

Condition factor and GSI were calculated for killifish in all but the first experiment, in which standard length and resected organ weights were not recorded at euthanization. Killifish weight, CF and GSI is summarized in **Table 3.1** for each experiment. Killifish weight (g, ww) and CF were consistent for each sex, across populations, within all experimental replicates. However, due to size class limitations in F1 SC killifish available

to be paired with F2 NBH animals in March 2016, weight and CF were significantly higher in females than males (two-way ANOVA: $p_{weight} = 0.0332$; $p_{CF} = 0.0170$) within each population. Weight-matching was maintained for males and females across the two populations. There were no differences in killifish weight or CF between control and chemical treatment groups.

GSI was higher in females than males, especially during spring experiments (March 2016 and May 2017; **Table 3.1**) as has been shown previously (Fernández-Delgado, 1989; Greytak, et al., 2005). GSI was lower in NBH killifish relative to those from SC, which is also consistent with earlier studies (Greytak, 2005). Killifish weight was positively associated with GSI in linear regression models. GSI did not differ between treatment groups in either multiple linear or mixed effects regression models.

Population and Season, but not Acute MDC Exposure, Determine Lipid Phenotypes

The liver is a significant storage site for lipids in many fish (reviewed in Nanton et al., 2003; Sheridan, 1988, 1994), and thus we began by analyzing liver lipids (triglycerides, TAG). Liver TAG was not affected by either acute PCB or TBT treatment (**Figure 3.2a**). However, there appeared to be the potential for seasonally- and population-dependent effects. Thus, we took advantage of the unusually large sample size to explore the effect of multiple factors (e.g., season, population, sex, treatment) using several approaches to analyze liver TAG data: two-way ANOVA, multiple linear regression and linear mixed effects models. Results from both adjusted and unadjusted models are contained in **Table**

3.4. No differences in liver TAG were observed between males and females, as has been reported in studies of other fish species (e.g., Craig et al., 2000; Scott, Fåhræus-Van Ree, & Parrish, 2002). Liver TAG showed marked seasonal variability (**Figure 3.2b**) with the fall levels being significantly higher than spring for both NBH and SC killifish. Killifish from NBH consistently had higher TAG levels than SC individuals. We also evaluated HSI by ANOVA and linear regression modeling techniques. In agreement with liver TAG analyses, HSI was higher in NBH killifish relative to SC animals (**Figure 3.3**) and to a significant degree after adjusting for other covariates (**Table 3.4**). HSI was significantly higher in female killifish relative to males, as reported in Pait & Nelson (2009), and was higher in the fall than the spring after adjusting for other covariates (**Table 3.4**). Weight and ASI were significantly positively associated with HSI (**Table 3.4**).

Adipose tissue and gonads can also be sites of lipid accumulation in fish. The distribution of ASI in each experiment is shown in **Figure 3.4a**. ASI was significantly higher in NBH killifish compared to SC killifish (**Table 3.4**). This pattern persisted regardless of the whether SC fish were from the F1 or F2 generations. Females had higher ASI than males, after controlling for other covariates (**Table 3.4**). Season was not a significant predictor of ASI and as a result, linear mixed effects models using season as a mixed effect were not used to analyze ASI. Season does, however, affect ASI in SC killifish differently than NBH animals. In the spring, ASI in NBH killifish was significantly higher than in SC killifish. This difference diminishes over the course of the summer, such that ASI was

comparable between the populations in the fall. Killifish weight and chemical treatment were not significant predictors of ASI. In addition to these findings, ASI was also significantly positively associated with liver TAG. The distribution of GSI in each experiment is shown in **Figure 3.4b**. GSI was higher in SC killifish compared to NBH killifish (**Table 3.4**). After controlling for additional covariates, GSI was significantly positively associated with killifish weight and was significantly higher in females than males (**Table 3.4**) as has been documented previously (e.g., Fernández-Delgado, 1989; Greytak et al., 2005).

Over all, these results support anecdotal field and laboratory observations that suggest greater lipid storage in NBH killifish relative to SC individuals (Greytak, 2005).

Population and Sex are Important Mediators of MDC-Induced Changes in Gene Expression

Expression of *cyp1a* is a biomarker of AHR pathway activation that differentiates sensitive and tolerant killifish populations (e.g., Bello et al., 2001; Oleksiak et al., 2011; Whitehead, Clark, Reid, Hahn, & Nacci, 2017). There was no difference in basal *cyp1a* expression between control SC and NBH killifish (**Figure 3.5a**). PCB126 significantly induced *cyp1a* expression in male SC killifish (**Figure 3.5b**; $p = 0.0009$, two-way ANOVA, Dunnett's). Relative expression of *cyp1a* in SC animals administered PCB126 was much greater in male than female animals ($p = 0.1045$, t-test). The same was true in NBH killifish ($p = 0.0026$, t-test), though *cyp1a* expression was also higher in male

controls compared to female controls in this population.

Members of the PPAR nuclear receptor family play important roles in maintaining lipid homeostasis in vertebrates. PPARγ regulates adipocyte differentiation and lipid storage. mRNA expression of *pparγ* did not differ between control (DMSO-treated) killifish from SC and NBH (**Figure 3.6a**). Unexpectedly, *pparγ* was significantly upregulated in male SC killifish treated with PCB126 (**Figure 3.6b**; p = 0.0208, ANOVA, Dunnett's). In contrast, significant upregulation of *pparγ* did not occur in female SC killifish or NBH killifish. The same pattern was observed when *pparγ* expression in PCB126-treated NBH killifish embryos were compared with those from a reference killifish population in an existing RNAseq dataset (Reid et al., 2016, Table S3). Fatty acid binding proteins (FABPs) are a class of transport proteins for fatty acids and lipophilic substances. Using a sequence homology approach we identified *fabp1b* in killifish from the zebrafish orthologue known to be under selective transcriptional control of *pparγ* (Laprairie, Denovan-Wright, & Wright, 2016). *fabp1b* expression tended to be higher in control killifish from SC compared to those from NBH, but the difference was not statistically significant (**Figure 3.6c**; p = 0.1079, t-test). The pattern of *fabp1b* expression in the livers of the chemical-treated animals was consistent with *pparγ* expression: *fabp1b* expression was highest in SC males exposed to PCB126 (**Figure 3.6d**). However, despite the large increase in *fabp1b* expression among PCB126-treated SC killifish, the change was not statistically significant (p = 0.308, ANOVA, Dunnett's) due to high variability and small sample size (N=3). S26948, a mammalian PPARγ-selective synthetic ligand, has not

previously been studied in fish to the best of our knowledge. It does not appear to activate the *pparγ* pathway in killifish under the current exposure scenario.

On the other hand, PPARα regulates liberation of fatty acids and their use as an energy source. Therefore, we also measured mRNA expression of *pparα* and its target gene, *cpt1b* (Y. Liu, Wang, Wei, Zhang, Xu, & Dai, 2008). Basal expression of both genes in DMSO-treated animals is equivalent in both populations (**Figures 3.6e** and **3.6g**). No significant changes in *pparα* or *cpt1b* expression were observed following any chemical exposure in SC or NBH killifish. However, PCB153 caused slight increases in both *pparα* or *cpt1b* expression in SC male killifish, suggesting a possible effect of PCB153 on the pathway.

In summary, SC killifish are more responsive to MDC exposure at the transcriptional level than NBH killifish. Furthermore, male killifish are more susceptible to transcriptional activation of nuclear receptor pathways by MDCs than female killifish. This pattern was observed in genes involved with the AHR, PPARγ, and PPARα pathways. PCB126 significantly upregulated *cyp1a* and *pparγ* expression in male SC killifish. Small sample size and high inter-individual variability made it difficult to detect changes in the expression of other genes following exposure to other MDCs.

Discussion

With increasing recognition of the role MDCs may play in the epidemic of obesity worldwide, we sought to characterize the effects of exposures to MDCs on lipid homeostasis in an ecological sentinel species, Atlantic killifish, which is likely exposed to multiple MDCs in its home habitat. We tested the hypothesis that killifish whose parents or grandparents were from NBH would exhibit altered lipid homeostasis (e.g., increased adiposity and hepatic triglycerides, changes in expression of genes involved in lipid homeostasis) compared to killifish from SC, a reference location, because of heritable adaptations to persistent organic pollutants in NBH. We evaluated SC and NBH killifish under uncontaminated common garden conditions to evaluate whether the observation of elevated levels of adipose tissue in field-caught NBH killifish was better explained as a response to a resource-rich or complexly contaminated environment. We also exposed laboratory-reared SC and NBH killifish to known and suspected MDCs with the expectation that NBH killifish might display tolerance by mechanisms related or unrelated to those already identified.

Evidence of Lipid Disruption in NBH Killifish: A Cost of Adaptation?

Both ASI and liver TAG were elevated in NBH relative to SC killifish, with the greatest differences occurring in the spring. The differences we observed in ASI and liver TAG between populations corroborates field observations that F0 NBH killifish have more abdominal adipose tissue than SC individuals, which was the original motivation for this

research study. Our results show that the liver is an important and dynamic lipid storage depot in killifish. Mean (range) liver TAG comprised 7.0% (0.18-15%) of overall liver mass in SC and 11% (3.0-20%) in NBH killifish. This is consistent with literature demonstrating that teleost fish tend to store more lipids in their liver (*ca* 10-20%, up to 67% in teleost species such as cod; reviewed in Sheridan, 1988, 1994; haddock: Nanton et al., 2003; red drum: Craig et al., 2000; tilapia: reviewed in Sheridan, 1988) than do mammals (*ca* 3%; e.g., Nam et al., 2015). The relative apportionment of lipids to adipose tissue, muscle, and liver is species-specific (Sheridan, 1988). Despite the fact that the liver plays a more significant role in lipid storage in teleosts than mammals under normal conditions (reviewed in Lowe, O'Rahilly, & Rochford, 2011), several studies have shown that excess liver lipids can have deleterious effects on fish health, akin to those seen in mammals (e.g., Bolla et al., 2011; Lu et al., 2013; Nanton et al., 2003).

Population-level differences in metrics of lipid homeostasis in F1 and F2 killifish held under "common garden" laboratory conditions are noteworthy. These variations suggest that there are intergenerationally persistent differences in lipid homeostasis between killifish populations in NBH and SC. Killifish are a well-known vertebrate example of evolved tolerance of environmental pollutants (reviewed in (Andrew Whitehead, 2017). These adaptations are driven by genetic mechanisms through selective pressure on the AHR signaling pathway (Reid, 2016) and enable killifish to survive in highly polluted estuaries. Conventional scientific theory of evolution and adaptation to environmental pollutants suggests that fitness costs ensue since the original, and presumably optimal,

phenotype has been altered (Coustau, Chevillon, & ffrench-Constant, 2000; reviewed in Whitehead et al., 2017). However, examples of such costs are less common than would be expected in literature on resistance to pesticides (reviewed in ffrench-Constant & Bass, 2017) or antibiotics (Andersson & Hughes, 2010). Similarly, studies exploring fitness costs in killifish have also identified fewer unequivocal examples of fitness costs than would be expected (reviewed in Whitehead et al., 2017). A few examples that have been identified in some pollution-adapted killifish populations (but not including NBH) include altered bioenergetics (Jayasundara, Fernando, Osterberg, Cammen, Schultz, & Di Giulio, 2017; Lindberg, Jayasundara, Kozal, Leuthner, & Di Giulio, 2017) and increased susceptibility to hypoxia (Joel N. Meyer, 2003), oxidative stress (Harbeitner, Hahn, & Timme-Laragy, 2013), and infectious disease (Frederick, Van Veld, & Rice, 2007).

Differences in lipid homeostasis between NBH and reference killifish populations likely have underpinnings in adaptation to their respective environments. Relative differences in ASI and liver TAG are consistent across generations F0, F1, and F2. As discussed above, excess liver TAG is associated with adverse health outcomes in fish, which is also well documented in humans and mammalian toxicological models. Additionally, greater levels of lipid accumulation in storage depots such as adipose tissue and liver could be associated with changes in resource allocation to somatic growth, reproduction, and maintenance (e.g., Martin, Heintz, Danner, & Nisbet, 2017; Roff, 1983), although our studies were not designed to explore questions of energy budgeting. However, killifish in other studies have shown higher HSI, condition factor, fecundity and successful

reproduction (e.g., Greytak et al., 2005; Nacci, S. Jayaraman, J. Specker et al., 2001), suggesting that a fitness cost associated with changes in lipid resource apportionment are likely subtle, involving molecular mechanisms, site-specific environmental conditions and the adaptive phenotype.

While chemical contamination in NBH is an obvious distinction between the ecological conditions in NBH and SC, there are multiple other differences between the two locations. NBH is a low-energy estuary, characterized by low non-storm (<0.25 m) and storm (<0.5 m) wave height, finer sediment and vegetative growth in the intertidal zone (Jackson, 2002), and a more robust microbenthic community (Wildish, 1979). The water is more turbid, reducing risk of predation on killifish by shore birds and larger fish (Kneib, 1986), and there is greater food availability for scavengers such as killifish, which feed predominantly on salt marsh detritus, copepods, diatoms, polycheates, and insects (James-Pirri, 2001; Kneib, 1986; McMahon, 2005). In contrast, SC is a high energy-estuary, characterized by clear, fast-moving water and scoured substrate. By extension, SC killifish may be more vulnerable to predation and likely have less food availability. Mammalian toxicology and epidemiologic literature have shown that both malnutrition and overnutrition during developmentally sensitive windows are associated with metabolic syndrome, including liver steatosis (Brumbaugh & Friedman, 2014; Burgio, Lopomo, & Migliore, 2015; El Hajj, Schneider, Lehnen, & Haaf, 2014; Sookoian, Gianotti, Burgueño, & Pirola, 2013; N. Wang, Chen, Ning, Li, Han, Zhu, Chen, Xia, Jiang, Wang, Wang, Jensen, & Lu, 2016), although multiple complex

mechanisms are likely responsible for the connection between nutritional status during development and metabolic syndrome later in life. Therefore, heritable responses to ecological conditions between SC and NBH could explain the differences in lipid homeostasis we observed in laboratory-raised killifish of F1 and F2 generations.

Laboratory-raised killifish were held in filtered ambient water from Narragansett Bay except during our actual experimental periods, when water was artificially warmed to 23°C. Natural fluctuations in water temperature in the bay caused F1 and F2 killifish to exhibit natural seasonal variability in body bioenergetics and feeding behavior. Our data clearly demonstrate the importance of season as a predictor of lipid homeostasis in SC and NBH killifish. Mean (range) liver triglyceride levels in the spring were 4.2% (0.56 - 12.6%) in SC killifish and 9.1% (3.0 - 20.1%) in NBH killifish. Liver triglyceride levels were higher in the fall in both populations (SC: 9.6% (1.8 - 15.3%); NBH: 12.6% (6.2 - 18.1%). This pattern is consistent with seasonal variability in liver triglycerides reported in other fish species (e.g., Kandimer & Polat, 2007, Berg & Bremset, 2005) and can be explained by seasonal changes in feeding behavior and metabolic demands (Sheridan, 1994).

ASI was also significantly positively associated with TAG, suggesting that after controlling for population, season and chemical treatment, killifish with greater lipid levels store these energy reserves in multiple depots.

The PPAR group of nuclear receptors is important to maintaining lipid homeostasis. We hypothesized that either basal expression of the PPARs or their target genes would show differences between populations. The similarity of basal expression of these genes between the two populations suggests that heritable differences in lipid homeostasis observed at the phenotypic level may not involve these nuclear receptor pathways. An important caveat, though, is the need for more extensive evaluation of hepatic gene expression because here we evaluated mRNA expression for each nuclear receptor and a single target gene. While PPAR signaling is largely conserved across vertebrates, taxa-specific differences in PPAR pathway genes are known to exist among teleost fishes (e.g., Laprairie et al., 2016). Furthermore, PPARγ has distinct differences in ligand specificity between fish and mammals (He, Liu, Chen, Ning, Qin, Li, Zhang, & Du, 2015; Leaver, Boukouvala, Antonopoulou, Diez, Favre-Krey, Ezaz, Bautista, Tocher, & Krey, 2005; Wafer, 2017). Since PPAR pathways have not previously been investigated in killifish, gene expression analyses should be expanded to include additional genes using targeted qPCR or transcriptomic approaches.

Effects of MDCs on Regulators of Lipid Homeostasis

Lipid homeostasis was not expected to be significantly affected by administration of MDCs for three days, and therefore we also assessed gene expression as a potentially more sensitive measure of exposure. We measured both basal expression of *pparγ*, *fabp1b*, *pparα*, and *cpt1b*, as well as the expression of these genes following killifish exposure to MDCs. We hypothesized that the populations would respond differently to

MDC exposure. PCB126 significantly upregulated *cyp1a* expression in the livers of SC killifish but not those from NBH, consistent with previous studies (e.g., Bello et al., 2001; Oleksiak et al., 2011; reviewed in Whitehead et al., 2017). *Cyp1a* was much more strongly induced by PCB126 in male killifish than females from each population. Greater responsiveness of male fish to AHR pathway induction has been shown previously, both in killifish (Meyer, Nacci, & Di Giulio, 2002) and other fish species (e.g., flounder: Elskus, Pruell, & Stegeman, 1992) and may be attributed to an inhibitory relationship between the estrogen receptor (ER) and AHR pathways under normal, endogenous conditions (Gräns, Wassmur, & Celander, 2010; Navas & Segner, 2001). However, previous research on AHR-ER crosstalk in fish has shown that AHR pathway activation by an exogenous ligand overwhelms baseline negative control by the ER in complex patterns of reciprocal inhibition (Gräns, 2010; Navas, 2001; Yan, Lu, & He, 2012).

Alternatively, the differences in *cyp1a* expression may have been due to the sex differences in chemical distribution following IP injection that were also found, resulting in sex-specific biologically effective concentrations in the liver. PCB126 and PCB153 levels measured in livers of IP injected animals from the October 2015 study presented herein were much greater in males than females (PCB126 (mean, ng/g dry weight): SC females = 16.3, SC males = 30.5, NBH females = 15.1, NBH males = 12.1; PCB153: SC females = 1814.9, SC males = 4458.0, NBH females = 470.5, NBH males = 4920.4; Nacci et al., unpublished data). These differences in PCB levels in killifish liver could possibly be due to sex-specific patterns in the partitioning of lipophilic contaminants

between liver and reproductive organs. Additionally, transcriptomic analyses of the forebrains from these same animals revealed differential expression of many more genes in male animals than female animals relative to controls (Goldstone et al., manuscript in preparation). This transcriptional response was most pronounced in SC killifish, but was also observed in NBH individuals. For instance, the lack of significant *cyp1a* induction in female SC killifish may be explained by a lower biologically effective dose in their liver relative to males. This may also partially explain *pparγ* and *fabp1b* responsiveness in male, but not female, SC killifish following PCB126 exposure. Therefore, sex-related patterns in gene expression presented herein may be related to differential exposure.

The most striking observation of PPAR pathway activation was the sex-specificity of response to PCB126 and PCB153. Both PPARγ and PPARα pathways were more responsive to PCBs in male compared to female killifish, regardless of the population. Sex-specific differences in lipid homeostasis and adiposity are known in humans and mammalian animal models at the phenotypic (e.g., Montague, Prins, Sanders, Digby, & O'Rahilly, 1997; Varlamov, Bethea, & Roberts, 2015; Yessoufou & Wahli, 2010) and transcriptional (e.g., Yessoufou and Wahli, 2010; Leuenberger et al., 2009; Lane et al., 2003; Li et al., 2001) levels. Negative crosstalk between ERß and PPARγ has emerged as a possible mechanism to explain sex specificity (Benz, Bloch, Wardat, Böhm, Maurer, Mahmoodzadeh, Wiedmer, Spranger, Foryst-Ludwig, & Kintscher, 2012; Foryst-Ludwig, Clemenz, Hohmann, Hartge, Sprang, Frost, Krikov, Bhanot, Barros, Morani, Gustafsson, Unger, & Kintscher, 2008; X. Wang & Kilgore, 2002). Studies showing sex-specificity to

PPARγ pathway activation in rodent models corroborate our findings, with greater responsiveness in males (Lane, MacLennan, Daood, Hsu, Janke, Pham, Puri, & Watchko, 2003; Li, Brown, Silvestre, Willson, Palinski, & Glass, 2000). Similar sex-specificity has been shown in PPARα pathway responsiveness, with evidence of estrogen-induced inhibition leading to suppression of genes critical for fatty acid beta-oxidation and lipid catabolism (reviewed in Yoon, 2010). Taken together, sexually dimorphic effects of PCB exposure on PPAR pathways are consistent with prior literature. This study was conducted before the official start of the killifish spawning season in an effort to avoid the most dramatic sexually dimorphic effects of reproductive hormones and lipid homeostasis. However, gonad resection revealed preliminary egg development in some females, indicating that the timing of our study did not fully precede spawning. Because of this, we recommend that gene expression for PPARγ and PPARα pathways be measured in another experimental replicate (either from October or March). We also recommend that additional PPARγ and PPARα target genes be measured to confirm that signaling through these pathways functions analogously to other teleost and mammalian animal models.

Induction of PPAR pathways by PCBs was unexpected as neither dioxin-like nor non-dioxin-like PCBs are generally considered agonists for PPARγ or PPARα (e.g., Taxvig et al., 2012). In fact, AHR activation has been shown to negatively regulate the PPARγ pathway through crosstalk mechanisms that are not well understood (e.g., Gadupudi et al., 2015). Nevertheless, *pparγ* was significantly induced by PCB126 in SC male killifish.

The mRNA expression of a *pparγ* target gene, *fabp1b*, was also increased in SC male killifish following PCB126 exposure, but high inter-individual variability and a small sample size confounded our ability to detect statistical significance.

These findings are unexpected, but not unprecedented. Arsenescu et al. (2008) found that low doses of dioxin-like PCB77 induced *pparγ* expression and promoted adipogenesis in 3T3-L1 murine preadipocytes. Recent in silico docking studies of human PPARα, PPARβ/δ and PPARγ have also shown good binding affinity for dioxin-like PCBs and overlapping amino acid residues (40-58%) with synthetic agonists, suggesting the possibility of direct activation of PPAR pathways by dioxin-like compounds (Sérée et al., 2004; Sheikh et al., 2016; Villard et al., 2011). Killifish PPARγ has 76% sequence identity with human PPARγ (NCBI genome BLAST), making it plausible that PCB126 could directly activate PPARγ. The PPARγ pathway has not previously been studied in killifish, so there is no existing literature to which to compare our results. However, data from Reid, et al. (2016) do demonstrate that PCB126 induced *pparγ* in embryos from sensitive killifish populations and to a much lower extent in tolerant populations, including NBH. Since independently evolved adaptation to dioxin-like PCBs in multiple tolerant killifish populations has been linked to selective pressure on the AHR pathway (Reid, 2016), our results may reflect interactions between the AHR and PPAR pathways.

Expression of *pparα* and its target, *cpt1b*, were slightly elevated following PCB153 exposure in SC males, but again, not significantly. Again, this finding is not entirely

unique, as Wahlang et al. (2016) demonstrated that Aroclor 1260 induced expression of *pparα* and *cpt1α*, a related CPT1 isoform in mice. Aroclors are, by definition, mixtures of individual PCB congeners. Induction of the PPARα pathway by Aroclor 1260 is particularly relevant to our findings since PCB153 is the second most prevalent congener in Aroclor 1260, comprising approximately 10% of the overall mixture (IARC, 2016). However, transcriptomic analyses of liver from cod exposed to PCB153 have shown enrichment of *de novo* lipid biosynthesis pathways genes, including *acly*, *acaca* and *fasn* (Yadetie, Karlsen, Eide, Hogstrand, & Goksøyr, 2014). This suggests the relationship between non-dioxin-like PCBs and lipid homeostasis is complex and involves molecular mechanisms beyond PPARs. The slight induction of *pparα* by PCB126 in NBH males may be explained by direct activation of the pathway as discussed above.

Surprisingly, TBT exposure did not result in changes in lipid homeostasis and PPARγ pathway activation in our study, as has been shown in other teleost fish (reviewed in Capitão, Lyssimachou, Castro, & Santos, 2017). TBT is capable of binding and activating both PPARγ and RXR in mammalian and fish models (Baker et al., 2015; Grün & Blumberg, 2006; Ouadah-Boussouf & Babin, 2016), yet has been shown to act most strongly through RXR (Baker et al., 2015; Balaguer, Delfosse, Grimaldi, & Bourguet, 2017). One possible explanation for the lack of TBT responsiveness in our study is the use of IP injection exposure. Other studies reporting TBT effects on adiposity and PPARγ pathways in teleosts have used water-borne (e.g., zebrafish: Capitão et al., 2017; Ouadah-Boussouf & Babin, 2016) and dietary (e.g., salmon: Meador, Sommers, Cooper, &

Yanagida, 2011; Pavlikova, Kortner, & Arukwe, 2010) exposures, which may explain differences between our results and previously published literature.

A few studies using teleosts for PPARγ-related mechanistic toxicology research have reported that potent pharmaceutical ligands, such as rosiglitazone, did not activate PPARγ as in mammalian models (G. Liu, Moon, Metcalfe, Lee, & Trudeau, 2005; Riu, 2014). However, subsequent studies have shown rosiglitazone to be efficacious at activating the PPARγ-pathway in multiple fish species (e.g., rainbow trout: Sánchez-Gurmaches, Cruz-Garcia, Gutiérrez, & Navarro, 2010; zebrafish: Ouadah-Boussouf & Babin, 2016; Tingaud-Sequeira et al., 2011). Given uncertainty about teleost responsiveness to rosiglitazone as a positive control for PPARγ pathway activation, we selected S26948, a non-thiazolidinedione PPARγ-selective pharmaceutical compound. However, this compound has not previously been used in PPARγ-related toxicological investigations in teleosts. We also did not observe PPARγ pathway activation in killifish exposed to S26948 during embryonic development (Crawford et al., in preparation).

Applying Epidemiologic Methods to Wildlife Population Studies

Comparison of statistical approaches (ANOVA, multiple linear regression and linear mixed effects models) highlights the utility of using more sophisticated statistical methods to evaluate toxicological data in order to accommodate complex data structures and covariate relationships. ANOVA tests are used to illuminate unadjusted, overall differences in group (population, chemical treatment) means, assuming the groups have

normally distributed residuals and equal variances. Post hoc tests such as Dunnett's are then used to test pairwise comparisons and identify differences between specific groups (e.g., a chemical treatment versus a control). Regression models test linear relationships between outcome and predictor variables, yielding estimates of the magnitude of association between each predictor and the outcome. Multiple linear regression attempts to explain the relationship between predictors and outcome by fitting a linear equation to the observed data, and assumes the residuals are normally distributed, constant and independent (not correlated). Linear mixed effects models are an extension of these traditional linear models, and are frequently used when there is potential non-independence in experimental units due to hierarchical structures in the data generating process. Both types of models are robust despite modest deviations from these assumptions (Everitt & Hothorn, 2010; Wooldridge, 2006).

In this study, we ran linear mixed effects models to address clustering by experimental replicate and season. However, little difference in model performance was noted between parsimonious multiple linear regression and linear mixed effects models. In fact, multiple linear regression models slightly outperformed linear mixed effects models when comparing Akaike information criterion (AIC), which measures both goodness of fit and model simplicity using a log-likelihood ratio test.

Regression modeling approaches allowed us to understand associations between GSI, HSI, ASI and liver TAG and covariates such as seasonality, killifish weight and sex that

were not accounted for with two-way ANOVA comparisons of means. As has been argued previously (Fox, 1991; Schmidt, Schmidtke, Kohl, Wilhelm, Schiemann, van der Voet, & Steinberg, 2016), regression modeling techniques are important and underutilized statistical tools in toxicology research and should be incorporated into analysis plans when sample sizes are adequate. Additionally, analysis of our data by ANOVA alone would have resulted in spurious interpretations of chemical treatments. For example, the two-way ANOVA evaluating liver TAG across populations and chemical treatments found that PCB126 significantly increased liver TAG. Given that *pparγ* and *fabp1b* were induced by PCB126 in gene expression analyses from our May 2017 experimental replicate, we would have concluded that PCB126 may be increasing liver TAG by way of PPARγ pathway activation. However, after controlling for other covariates, such as killifish weight, population, season and ASI, we see that the effects of PCB126 on liver TAG are attenuated. Thus, linear regression approaches allowed us to capture more sophisticated relationships between important predictors of liver TAG.

Conclusions

Killifish living in NBH are known to be exposed to PCBs. Exposure to other pollutants such as organotins, is likely given elevated levels of Sn measured in NBH sediments relative to sediments from nearby areas. Consistently throughout the year, but most strikingly in the spring, laboratory-reared killifish from the NBH population had higher ASI and liver TAG than similarly raised killifish from SC. These differences occurred after a period of suppressed caloric consumption during the winter, and reflect

differences in lipid storage. This finding is consistent with field observations and suggests that ecological and/or toxicological histories have influenced lipid homeostasis in these killifish populations. Basal expression of *pparγ* and *pparα* and their respective target genes, *fabp1b* and *cpt1b*, in DMSO-treated killifish does not differ between SC and NBH. Killifish were generally unresponsive to activation of the PPARγ or PPARα pathways by the classic environmental MDC, TBT, or the PPARγ-specific synthetic compound, S26948. As expected, induction of AHR pathway by PCB126 was significant only in SC, but not NBH, killifish. Surprisingly, exposure to PCB126 significantly increased liver *pparγ* pathway gene expression in male SC killifish. While it is known that the AHR pathway in NBH killifish is resistant to induction by dioxin-like PCBs, this study has provided novel information that NBH killifish are resistant to short term treatment with MDCs. Multiple linear regression models revealed significant associations between organosomatic indices and liver TAG and covariates, such as season, sex, and weight, which would have been overlooked with basic comparisons of means approaches. This highlights the necessity of using statistical tools such as regression modeling techniques to understand complex associations between outcomes and covariates in toxicological studies.

Figures and Tables

Figure 3.1a. Sediment samples were collected from NBH and Buzzards Bay between 1989 and 2018 and analyzed for total tin (Sn). The 1989 archived sediment sample was selected to serve as both a geographic and temporal reference, as it is outside NBH and is believed to be minimally affected by legacy pollutants from human activity in and around NBH. Archived samples from 2005 and 2012 were selected to provide collocated sampling sites with the 2018 samples. Sediment cores were collected in 1989, 2005 and 2012 and were sectioned in two-inch increments. Sediment sections were then dried and archived. 2018 samples were collected from dredged sediments removed from NBH as part of ongoing sediment remediation and dried in an identical manner to archived samples. Abbreviations: D – dredged sediments; HB – hurricane barrier.

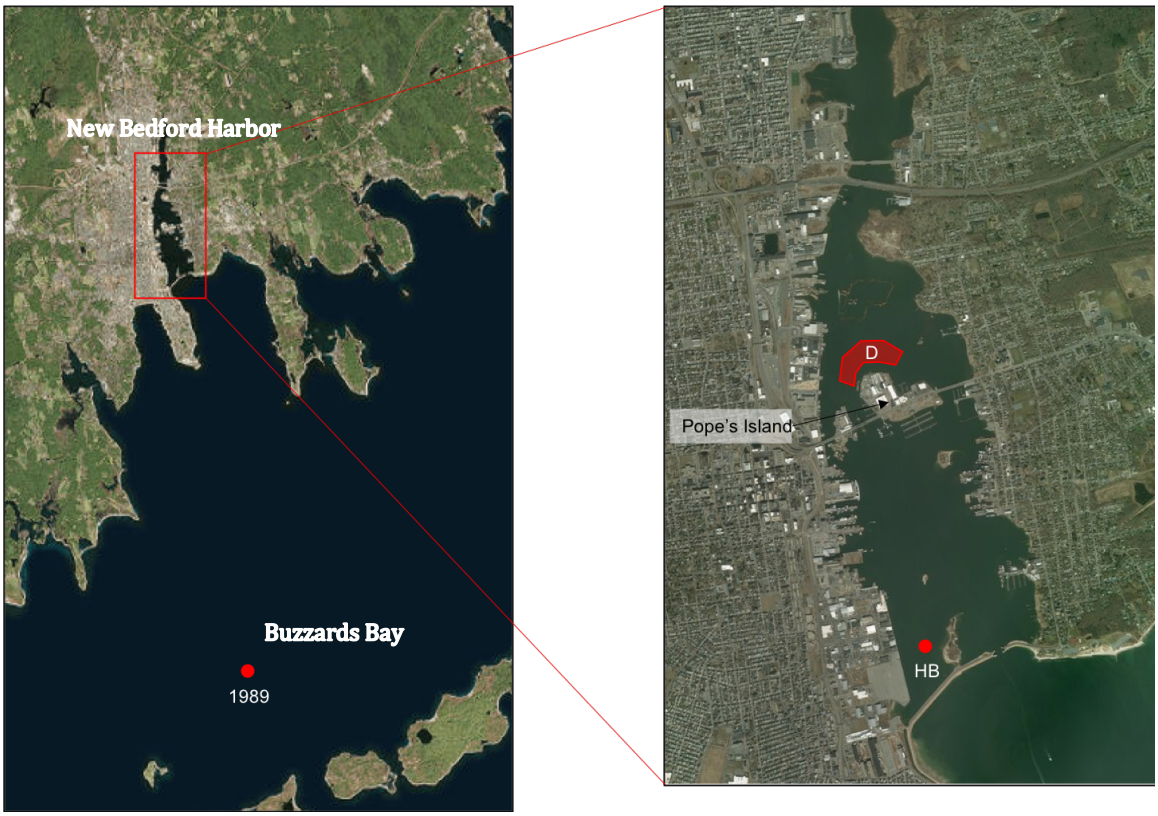


Figure 3.1b. Total tin concentrations in NBH and Buzzards Bay sediments. Aliquots (1 g, dw) of archived (1989, 2005, and 2012) and 2018 samples were analyzed for total Sn by Chemical Solutions, Ltd. (Harrisburg, PA, USA) using a hydrofluoric acid extraction, followed by inductively coupled plasma mass spectrometry (ICP-MS) with a practical quantitation limit (PQL) of 0.5 µg/g. Total Sn concentrations are lowest in Buzzards Bay (1989). Concentrations decline over time near the Aerovox facility and in samples located north of Pope's Island. No temporal change was observed in samples located south of Pope's Island. Tin concentrations increased at the Hurricane Barrier over time

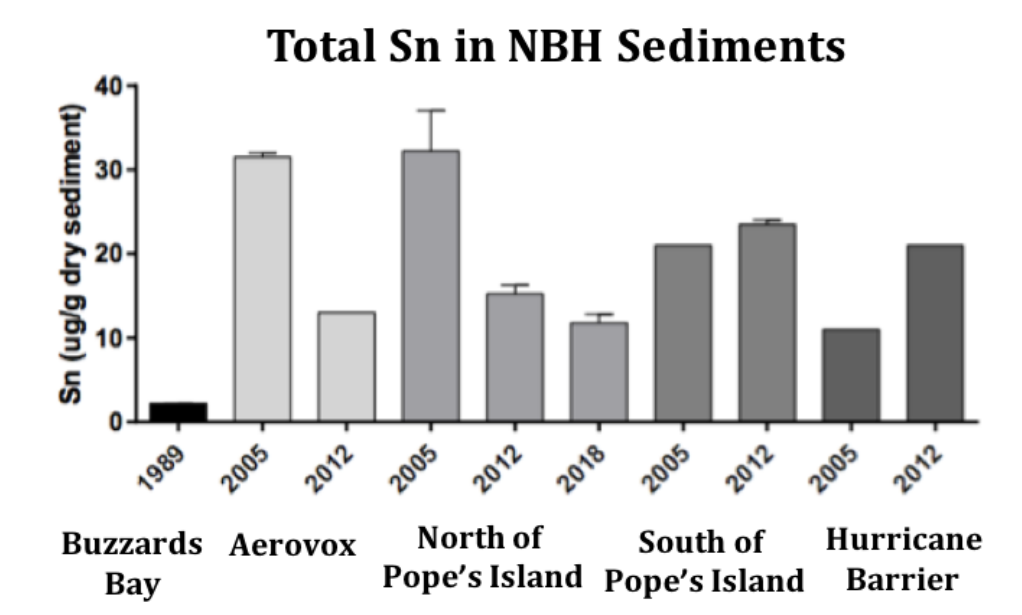


Figure 3.2a. The effects of MDC exposure on liver triglycerides (TAG) were evaluated following administration of environmental MDCs by intraperitoneal (IP) injection for a three-day duration. Liver TAG was pooled across spring (March 2016 and May 2017) and fall (October 2015 and October 2016) experimental replicates, with N ≥ 6 per chemical exposure for each population. Liver TAG was compared across chemical exposure, population and season using three-way ANOVA and Dunnett's post-hoc comparisons to determine differences in liver TAG between chemical exposure and the vehicle-only control for each population. Statistical significance was evaluated at α = 0.05. Liver TAG was not affected by MDC exposure under this exposure scenario. Red circles indicate outliers (points falling more than 1.5 times the interquartile range below or above the first or third interquartile range, respectively).

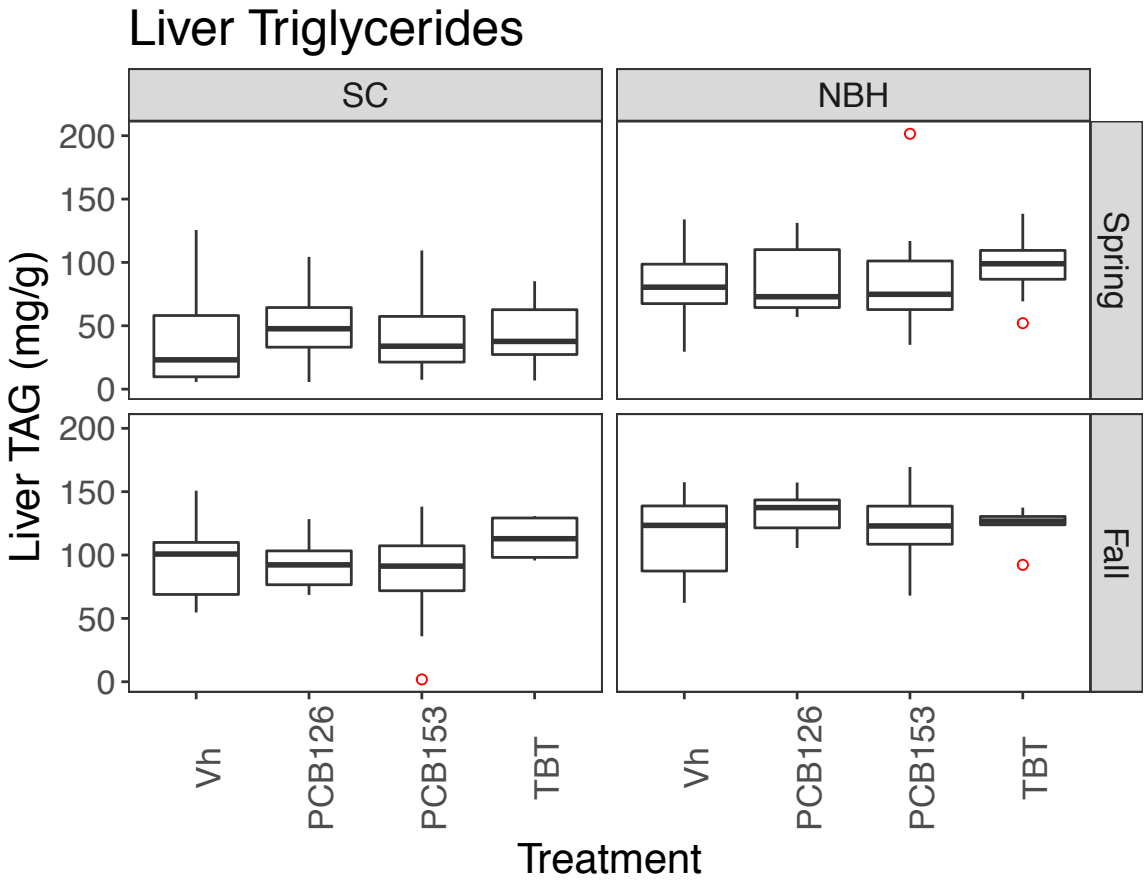


Figure 3.2b. Seasonal effects of liver triglycerides (TAG) were measured in weight-matched F2 killifish from NBH (red) and F1 or F2 killifish from SC (blue). Four experimental replicates were conducted, with two in the fall (October 2015 and October 2016) and two in the spring (March 2016 and May 2017). SC F1 killifish were used in October 2015 and March 2016 experiments, and SC F2 killifish were used during October 2016 and May 2017 experiments. Each experimental replicate included at least N=30 killifish per population, with equal numbers of males and females. Liver TAG was measured spectrophotometrically at 540nm, following an ethanolic potassium hydroxide extraction. Liver TAG was compared across seasons and populations using two-way ANOVA and Dunnett's post-hoc comparisons to determine differences in liver TAG between populations in each experimental replicate. Statistical significance was evaluated at $\alpha = 0.05$. Liver TAG varied seasonally, with higher levels in the fall than in the spring. The seasonal variability in liver TAG was greater in SC than NBH killifish, suggesting SC killifish deplete a greater amount of their hepatic energy stores over the course of the winter than do NBH killifish.

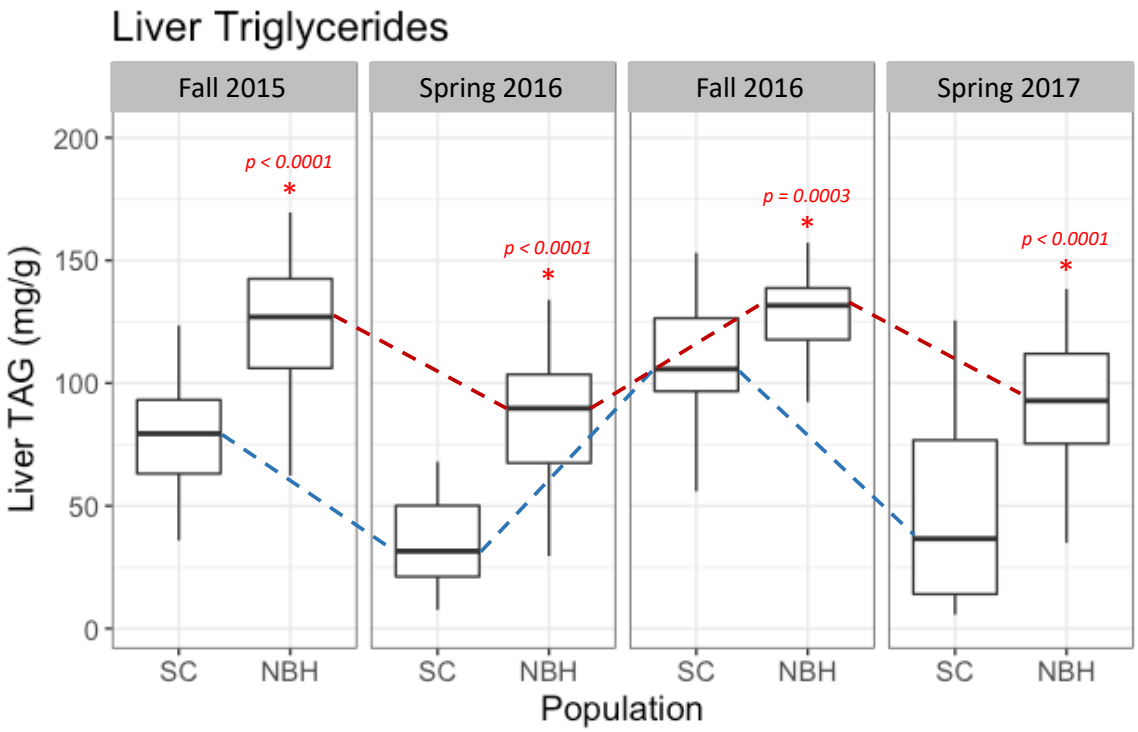


Figure 3.3. Hepatosomatic index (HSI) was calculated for SC and NBH killifish in three out of four experimental replicates. Organ weights were not measured during the October 2015 experiment, so HSI is not available for that study. HSI was compared across seasons and population using two-way ANOVA and Dunnett's post-hoc comparisons to determine differences between populations in each experimental replicate. Statistical significance was evaluated at α = 0.05. HSI varied (although not significantly) by population and season, but was not altered by MDC exposure under this exposure scenario (data not shown). Outliers are denoted as red circles (as defined in Figure 3.2a).

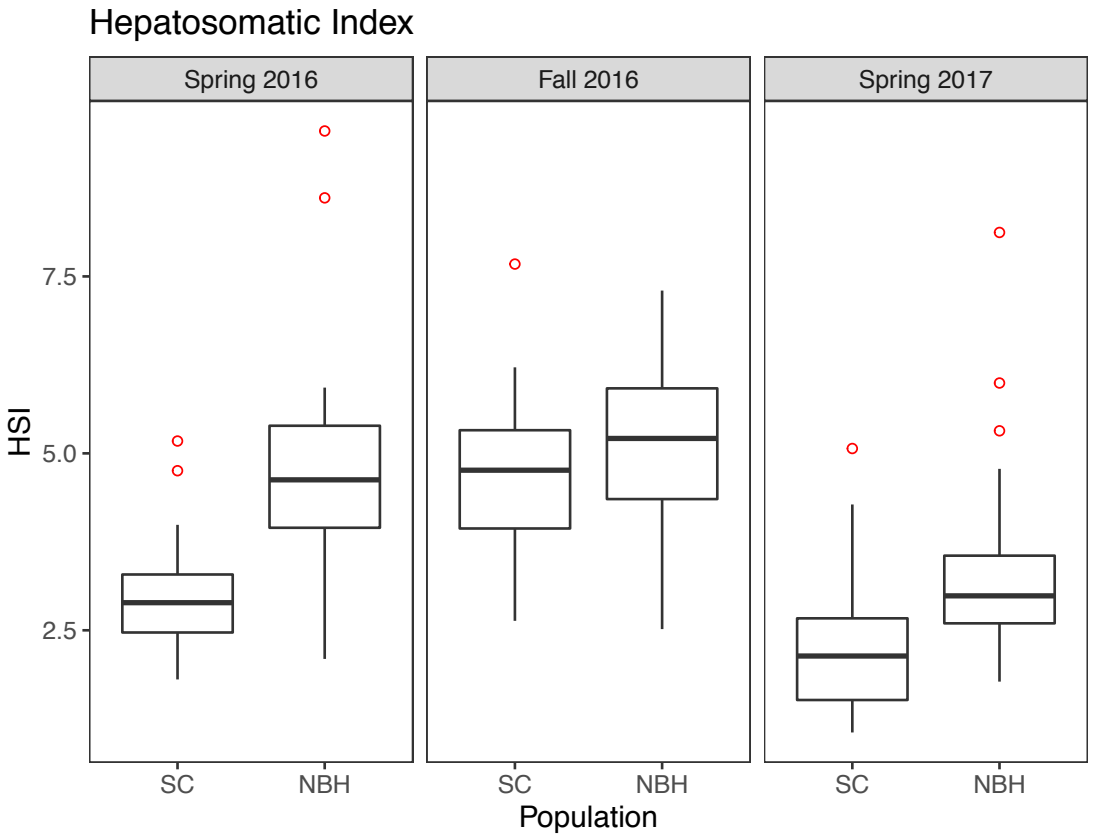


Figure 3.4a. Adiposomatic index (ASI) was calculated for SC and NBH killifish in three out of four experimental replicates (March 2016, October 2016 and May 2017). Organ weights were not measured during the October 2015 experiment, so ASI is not available for that study. ASI was compared across seasons and population using two-way ANOVA and Dunnett's post-hoc comparisons to determine differences between populations in each experimental replicate. Statistical significance was evaluated at $\alpha = 0.05$. ASI varied (although not significantly) by population and season, but was not altered by MDC exposure under this exposure scenario (data not shown). Outliers are denoted as red circles (as defined in Figure 3.2a).

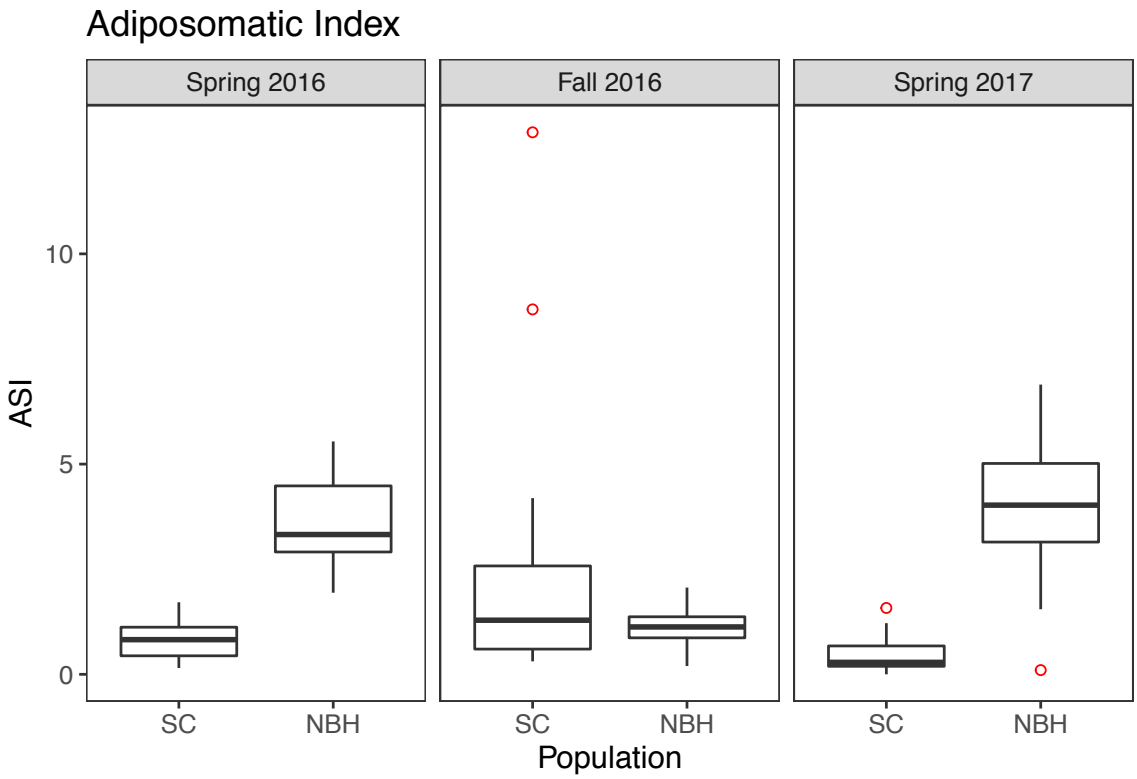


Figure 3.4b. Gonadosomatic index (GSI) was calculated for SC and NBH killifish in three out of four experimental replicates. Organ weights were not measured during the October 2015 experiment, so GSI is not available for that study. GSI was compared across sex, seasons and population using ANOVA and Dunnett's post-hoc comparisons to determine differences between populations in each experimental replicate. Statistical significance was evaluated at α = 0.05. GSI varied (although not significantly) by sex, season and population, but was not altered by MDC exposure under this exposure scenario (data not shown). Outliers are denoted as red circles (as defined in Figure 3.2a).

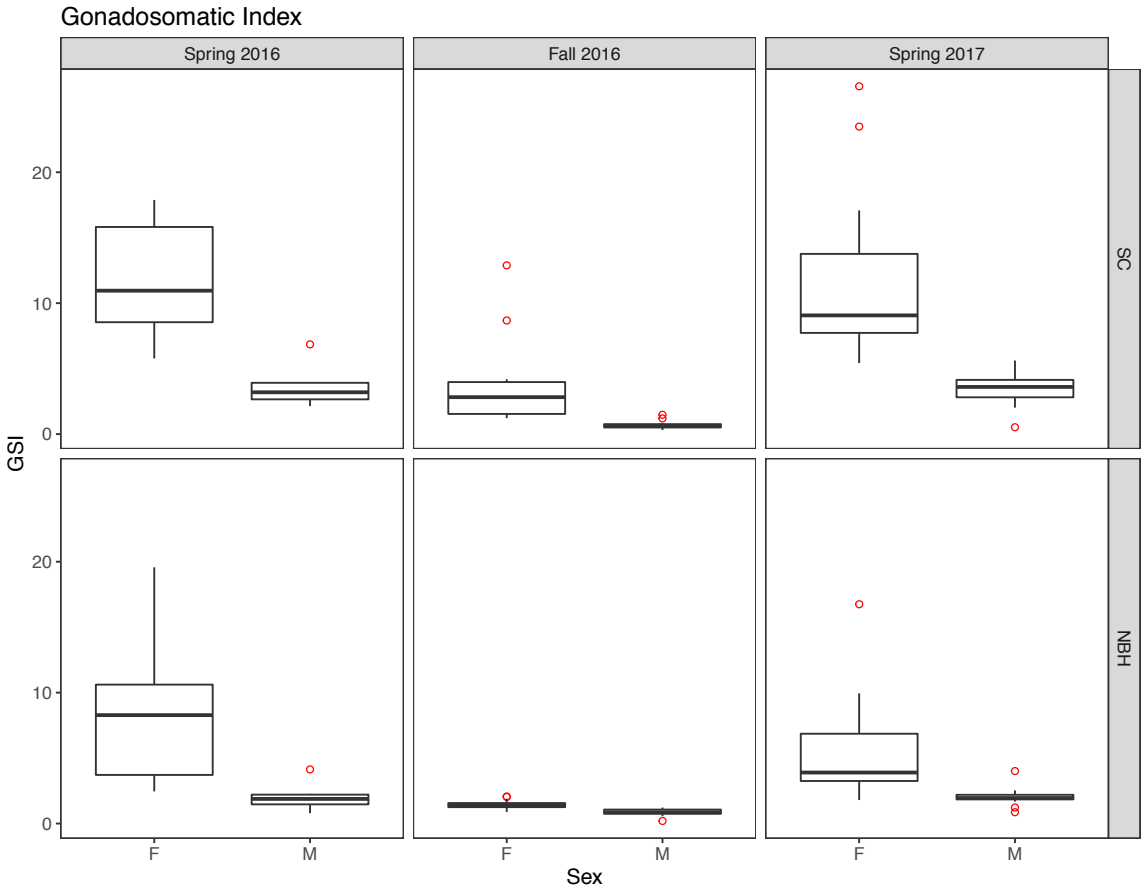


Figure 3.5a-b. Relative expression of *cyp1a* was measured by qPCR in livers of F2 killifish from both SC and NBH exposed to MDCs by IP injection in May 2017. *Cyp1a* was measured as a marker of AHR pathway activation. A manually-selected threshold was selected to standardize threshold cycle (C_T) across multiple plates. Relative expression was calculated by normalizing all C_T values by the Pfaffl method to naïve liver samples (uninjected killifish from both SC and NBH), and the constitutively expressed housekeeping gene *rn18s*. Gene expression is reported as a ratio relative to naïve, untreated control liver samples from SC and NBH killifish. Statistical significance was evaluated at α = 0.05. A) Relative *cyp1a* expression in vehicle-treated killifish from SC and NBH, pooled across sexes (N=6 per population). No significant difference in basal *cyp1a* expression was found between SC and NBH killifish. B) Relative *cyp1a* expression in chemical-treated killifish from SC and NBH, parsed by population and sex for each chemical treatment (N=3). PCB126 significantly induced *cyp1a* expression in SC killifish (p = 0.0009, two-way ANOVA, Dunnett's), but not in NBH animals. *Cyp1a* in SC animals administered PCB126 was much greater in male than female animals (p = 0.1045, t-test).

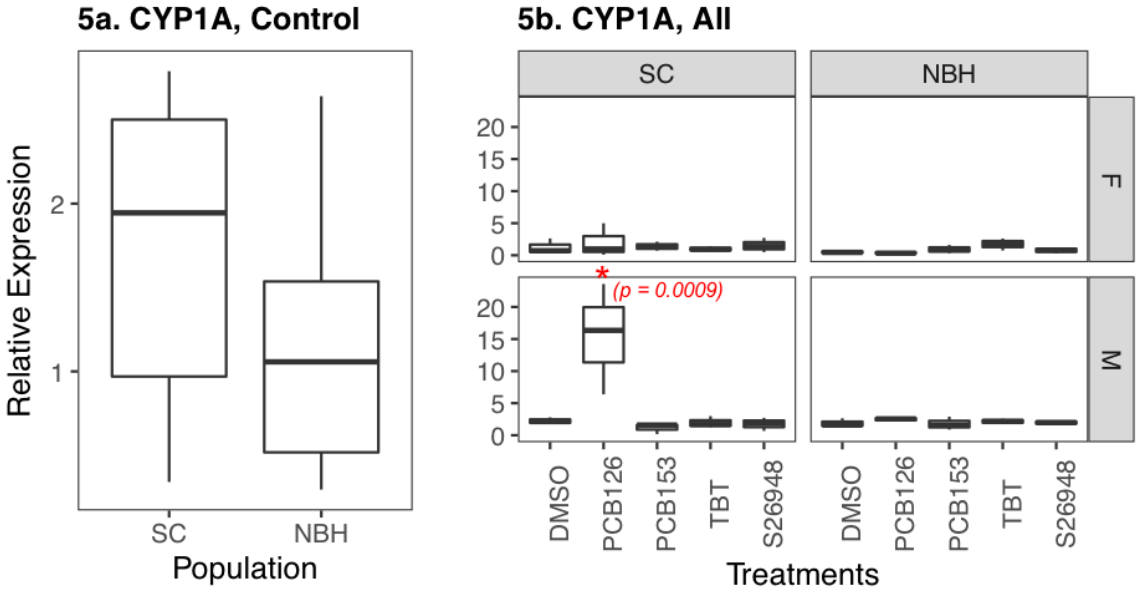


Figure 3.6a-h. Relative gene expression of *pparγ* and its target gene, *fabp1b*, and *pparα* and its target, *cpt1b*. Gene expression was quantified by qPCR in livers of F2 killifish from SC and NBH exposed to MDCs by IP injection in May 2017. Gene-specific, manually-selected thresholds were selected to standardize threshold cycle (C_T) for a given gene across multiple plates. Relative expression was calculated as described in Figure 3.5. Gene expression is reported as a ratio relative to naïve, untreated control liver samples from SC and NBH killifish. Statistical significance was evaluated at α = 0.05. A, C, E, G) Relative expression in vehicle-treated killifish pooled across sexes (N=6 per population) did not differ significantly between SC and NBH. B, D, F, H) Relative expression in chemical-treated killifish from SC and NBH, parsed by population and sex for each chemical treatment (N=3). B) *Pparγ* was significantly upregulated in male killifish treated with PCB126 from SC (p = 0.0208, ANOVA, Dunnett's), but NBH. No other statistically significant changes in gene expression were identified following MDC exposure.

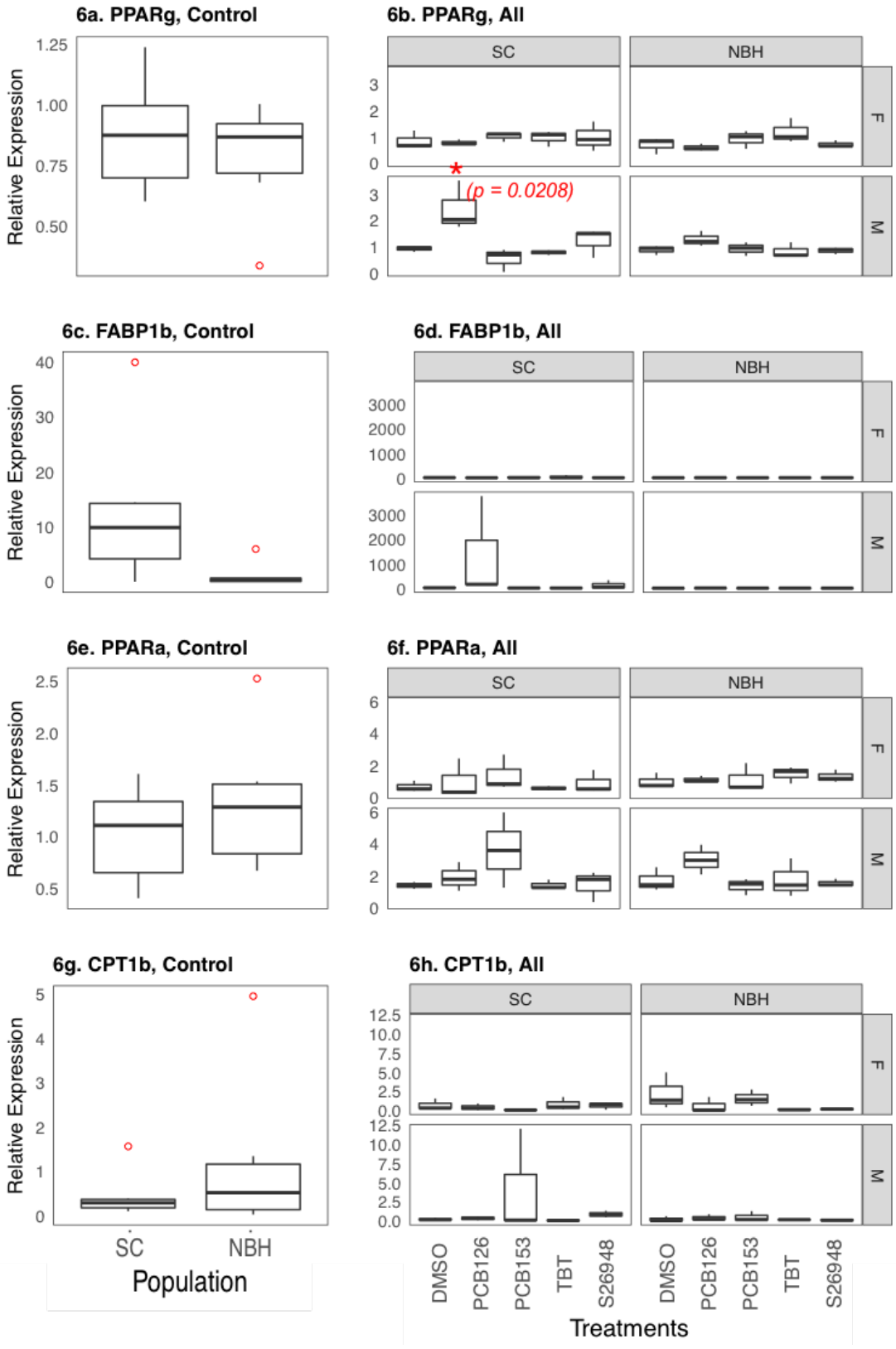

6a. PPARg, Control
6b. PPARg, All
6c. FABP1b, Control
6d. FABP1b, All
6e. PPARa, Control
6f. PPARa, All
6g. CPT1b, Control
6h. CPT1b, All
Relative Expression
SC
NBH
F
M
(p = 0.0208)
Population
DMSO
PCB126
PCB153
TBT
S26948
Treatments

Table 3.1. Descriptive statistics for phenotypic outcomes: killifish weight at euthanasia; condition factor; gonadosomatic, hepatosomatic and adiposomatic indices; and liver triglycerides. Killifish standard length and organ weights were not recorded at the time of euthanization during the October 2015 study so condition factor and organ indices were not calculated for that experimental replicate. Since no statistically significant differences were detected between chemical treatments, summary statistics presented herein represent all killifish within an experimental replicate. All killifish and organ weights are reported as grams, wet weight (g, ww).

Date	Gen	Pop	Sex	N	Weight (g)		Condition Factor (CF)		Gonadosomatic Index		Hepatosomatic Index		Adiposomatic Index		Triglycerides (mg TAG/g	
					Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Oct 2015	**F1**	**SC**	**Overall**	**27**	**6.12 (0.99)**	**4.61 - 8.9**									**78.01 (25.87)**	**1.82 - 123.49**
			F	14	5.84 (1.08)	4.61 - 8.9									78.29 (23.57)	35.86 - 123.49
			M	13	6.43 (0.81)	5.33 - 7.93									77.71 (29.13)	1.82 - 113.73
	F2	**NBH**	**Overall**	**30**	**6.48 (0.96)**	**5.16 - 7.96**									**122.04 (27.5)**	**62.26 - 169.51**
			F	15	6.46 (1.08)	5.17 - 7.96									126.45 (24.95)	80.55 - 169.51
			M	15	6.5 (0.86)	5.16 - 7.79									117.62 (30.04)	62.26 - 152.82
Mar 2016	**F1**	**SC**	**Overall**	**24**	**7.35 (2.46)**	**4.36 - 14.11**	**2.11 (0.34)**	**1.64 - 3**	**7.77 (5.18)**	**2.14 - 17.89**	**3.09 (0.82)**	**1.81 - 5.17**	**0.78 (0.48)**	**0.08 - 1.71**	**34.25 (17.24)**	**7.6 - 68**
			F	12	9.15 (2.08)	6.39 - 14.11	2.26 (0.22)	1.83 - 2.57	11.83 (4.28)	5.78 - 17.89	3.44 (0.85)	2.34 - 5.17	0.79 (0.5)	0.08 - 1.69	29.88 (17.81)	7.6 - 55.77
			M	12	5.55 (1.14)	4.36 - 7.47	1.95 (0.37)	1.64 - 3	3.72 (1.41)	2.14 - 6.85	2.73 (0.63)	1.81 - 3.68	0.78 (0.49)	0.2 - 1.71	38.62 (16.21)	17.45 - 68
	F2	**NBH**	**Overall**	**24**	**7.14 (1.96)**	**4.34 - 10.55**	**2.16 (0.24)**	**1.67 - 2.7**	**4.76 (4.69)**	**0.79 - 19.57**	**4.79 (1.64)**	**2.1 - 9.55**	**3.4 (1.13)**	**1.68 - 5.54**	**86.98 (25.47)**	**29.53 - 133.94**
			F	12	7.4 (1.61)	4.6 - 10.08	2.23 (0.2)	1.89 - 2.59	7.49 (5.39)	1.37 - 19.57	4.71 (0.9)	2.9 - 5.93	3.56 (1.17)	1.68 - 5.15	79.36 (28.54)	29.53 - 133.94
			M	12	6.87 (2.29)	4.34 - 10.55	2.1 (0.26)	1.67 - 2.7	2.02 (0.83)	0.79 - 4.13	4.86 (2.15)	2.1 - 9.55	3.23 (1.11)	1.76 - 5.54	94.61 (20.36)	60.63 - 129.11
Oct 2016	**F2**	**SC**	**Overall**	**36**	**3.44 (0.39)**	**2.61 - 4.15**	**2.01 (0.23)**	**1.62 - 2.67**	**2.21 (2.7)**	**0.31 - 12.89**	**4.66 (1.12)**	**2.63 - 7.67**	**2.21 (2.7)**	**0.31 - 12.89**	**109.07 (23.71)**	**55.89 - 153.12**
			F	18	3.48 (0.36)	2.92 - 4.13	2.01 (0.23)	1.69 - 2.49	3.73 (3.17)	1.21 - 12.89	4.79 (1.2)	2.68 - 7.67	3.73 (3.17)	1.21 - 12.89	111.22 (24.29)	59.41 - 150.74
			M	18	3.39 (0.43)	2.61 - 4.15	2 (0.23)	1.62 - 2.67	0.7 (0.32)	0.31 - 1.47	4.52 (1.05)	2.63 - 6.22	0.7 (0.32)	0.31 - 1.47	106.93 (23.62)	55.89 - 153.12
	F2	**NBH**	**Overall**	**36**	**3.47 (0.49)**	**2.38 - 4.4**	**1.92 (0.15)**	**1.64 - 2.24**	**1.13 (0.62)**	**0.2 - 3.8**	**5.01 (1.32)**	**2 - 7.3**	**1.13 (0.62)**	**0.2 - 3.8**	**128.51 (18.81)**	**84.43 - 180.62**
			F	18	3.61 (0.47)	2.76 - 4.4	1.93 (0.17)	1.68 - 2.24	1.45 (0.71)	0.7 - 3.8	5.61 (0.99)	3.59 - 7.3	1.45 (0.71)	0.7 - 3.8	128.39 (23.62)	84.43 - 180.62
			M	18	3.32 (0.47)	2.38 - 3.98	1.9 (0.13)	1.64 - 2.14	0.81 (0.27)	0.2 - 1.22	4.41 (1.36)	2 - 7.29	0.81 (0.27)	0.2 - 1.22	128.63 (13.07)	106.66 - 150.55
May 2017	**F2**	**SC**	**Overall**	**36**	**4.55 (0.53)**	**3.35 - 5.77**	**2.03 (0.33)**	**1.48 - 3.35**	**7.82 (6.23)**	**0.52 - 26.56**	**2.37 (1.02)**	**0.84 - 5.07**	**0.47 (0.38)**	**0 - 1.58**	**47.89 (35.7)**	**5.63 - 125.56**
			F	18	4.8 (0.48)	3.99 - 5.77	2.09 (0.37)	1.66 - 3.35	11.91 (6.54)	3.89 - 26.56	2.94 (1.03)	1.45 - 5.07	0.53 (0.41)	0 - 1.58	43.3 (35.12)	5.63 - 125.56
			M	18	4.3 (0.47)	3.35 - 5.26	1.96 (0.27)	1.48 - 2.54	3.73 (1.3)	0.52 - 5.62	1.81 (0.63)	0.84 - 3.61	0.41 (0.35)	0 - 1.14	52.49 (36.68)	5.66 - 109.43
	F2	**NBH**	**Overall**	**36**	**4.72 (0.67)**	**3.53 - 6.07**	**1.91 (0.24)**	**1.6 - 2.64**	**3.71 (3.16)**	**0.86 - 16.75**	**3.4 (1.21)**	**1.77 - 8.12**	**4.12 (1.42)**	**0.1 - 6.89**	**94.21 (31)**	**34.95 - 201.53**
			F	18	4.82 (0.76)	3.65 - 6.07	1.93 (0.25)	1.6 - 2.64	5.48 (3.67)	1.79 - 16.75	3.79 (1.53)	1.77 - 8.12	3.87 (1.57)	0.1 - 6.89	99.1 (37.7)	34.95 - 201.53
			M	18	4.61 (0.56)	3.53 - 5.63	1.9 (0.24)	1.62 - 2.64	1.94 (0.68)	0.86 - 4.01	3.02 (0.63)	2.09 - 4.23	4.37 (1.25)	2.68 - 6.68	89.32 (22.51)	51.97 - 126.88

Table 3.2. Summary of outcome measures measured during intraperitoneal (IP) injections, which were conducted during four experimental replicates (October 2015, March 2016, October 2016, and May 2017) using weight-matched F1 and F2 killifish from SC and NBH. Sample size, chemical exposures, and outcome variables measured in experimental replicate are summarized.

Variables		Experimental Replicates			
		Oct. 2015	Mar. 2016	Oct. 2016	May 2017
Total Number of Fish		60	48	72	72
Sample Size (per Population, Treatment, Sex)		5	3	3	3
Population/	NBH	F2	F2	F2	F2
Generation	SC	F1	F1	F2	F2
Treatments	Naive			✓	✓
	Acetone (2,120 ug/kg)	✓			
	DMSO (2,120 ug/kg)		✓	✓	✓
	PCB126 (29 ug/kg)	✓		✓	✓
	PCB153 (2,120 ug/kg)	✓	✓	✓	✓
	TBT (10 ug/kg)		✓	✓	✓
	S26948 (100 ug/kg)		✓	✓	✓
Fish Morphometry	Weight (g)	✓	✓	✓	✓
	Length (mm)		✓	✓	✓
	Condition Factor		✓	✓	✓
Organ Weight (g), Index	Liver, Hepatosomatic (HSI)		✓	✓	✓
	Adipose, Adiposomatic (ASI)		✓	✓	✓
	Gonad, Gonadosomatic (GSI)		✓	✓	✓
Triglycerides	(mg TAG/g liver)	✓	✓	✓	✓
Gene Expression (*Cyp1A, Pparg, Fabp1b, Ppara, Cpt1b*)					✓

Table 3.3. Primer information, including sequence, product size, primer efficiency, and the original citation for previously published primers. B-actin, β-actin; rn18S, 18S ribosomal RNA; ef1a, elongation factor 1 alpha; cyp1a, cytochrome P450 1A; pparg, peroxisome proliferator-activated receptor gamma; fabp1b, fatty acid binding protein 1b; ppara, peroxisome proliferator-activated receptor alpha; cpt1b, carnitine palmitoyltransferase 1b. These primers were validated to evaluate transcript specificity (visual inspection of the melt curve) and amplification efficiency. All novel primers were designed to overlap exon junctions to prevent potential amplification of contaminating genomic DNA.

Gene	Forward (5' --> 3')	Reverse (5' --> 3')	BP	Tm (°C)	Citation
B-actin	TGGAGAAGAGCTACGAGCTCC	CCGCAGGACTCCATTCCGAG	114	66	Oleksiak et al., 2011
rn18S	TGGTTAATTCCGATAACGAACGA	CGCCACTTGTCCCTCTAAGAA	97	65	Patel et al., 2006
ef1a	GGGAAAGGGCTCCTTCAAGT	ACGCTCGGCCTTCAGCTT	55	60	Bears et al., 2006
cyp1a	CTTTCACAATCCCACACTGCTC	GGTCTTTCCAGAGCTCTGGG	125	66	Oleksiak et al., 2011
pparg	GGAAAGAGATGGAGACGCACAACC	CCGACGCATCGTCTGGGAACTTGG	102	66	Karchner, unpublished
fabp1b	AGCCTTTCATGAAGGCCCTTGGTC	GTGGTGACCGTCACCTTGAAGTCG	115	66	Karchner, unpublished
ppara	CGGCCATCATCTGCTGTGGGGACC	GGGGAAGAGGAACGCGTCGTCTGG	134	66	Karchner, unpublished
cpt1b	CCTCTTCTGCCTTTACATAGTGTCC	AGCTTCCACGGCTCTGAAAGCACC	84	66	Karchner, unpublished

Table 3.4. Effect estimates and standard errors for phenotypic outcomes (GSI, HSI, ASI and TAG). ANOVA, multiple linear regression and linear mixed effect regression model were compared while evaluating associations between population, sex, season and chemical treatment and phenotypic outcome measures. This exercise was made possible by a relatively large sample size (N=249) compared to many toxicological studies. Parsimonious multiple linear regression and linear mixed effects models for each phenotypic outcome were selected and identified herein as "adjusted." Statistical significance was evaluated at $\alpha = 0.05$ for all analyses and bold values indicate instances where statistical significance was achieved ($p \leq 0.05$). For categorical covariates, the reference group is included in parentheses (e.g., SC, Vh, Spring and F). Log likelihood ratio tests were used to compare model fitness (AICs) for regression modeling techniques for each phenotypic outcome.

	TAG			**HSI**			**ASI**		**GSI**		
	2-Way ANOVA	Adjusted Multiple Linear Regression	Adjusted Mixed Effects Linear Regression	2-Way ANOVA	Adjusted Multiple Linear Regression	Adjusted Mixed Effects Linear Regression	2-Way ANOVA	Adjusted Multiple Linear Regression	2-Way ANOVA	Adjusted Multiple Linear Regression	Adjusted Mixed Effects Linear Regression
Population (SC)	**39.3 (4.66)**	**32.0 (4.64)**	**32.1 (4.64)**	**1.04 (0.228)**	**0.849 (0.209)**	**0.851 (0.209)**	**1.67 (0.285)**	**1.671 (0.281)**	**-2.80 (0.734)**	**-2.787 (0.525)**	**-2.787 (0.525)**
Treatment (Vh)											
PCB126	**15.1 (7.12)**	-0.450 (7.28)	-0.435 (7.28)	0.332 (0.387)	0.192 (0.327)	0.192 (0.327)	-0.0260 (0.486)	-0.0260 (0.480)	0.322 (1.25)	1.42 (0.909)	1.42 (0.909)
PCB153	1.20 (6.67)	0.796 (6.43)	0.799 (6.43)	0.395 (0.347)	0.397 (0.289)	0.397 (0.289)	-0.0690 (0.435)	-0.0690 (0.429)	-0.247 (1.12)	-0.268 (0.803)	-0.269 (0.803)
TBT	-2.55 (7.50)	5.01 (6.45)	5.03 (6.45)	0.405 (0.347)	0.00463 (0.292)	0.00300 (0.292)	0.0494 (0.435)	0.0494 (0.429)	-0.266 (1.12)	-0.333 (0.803)	-0.336 (0.803)
S26948	1.68 (7.50)	-0.0119 (6.44)	-0.0452 (6.44)	-0.0517 (0.349)	0.335 (0.292)	0.336 (0.292)	0.452 (0.435)	0.452 (0.429)	0.019 (1.12)	0.213 (0.805)	0.220 (0.805)
Season (Spring)		**40.9 (5.37)**	*mixed effect*		**1.83 (0.244)**	*mixed effect*				-3.12 (0.672)	*mixed effect*
Weight		**-4.29 (1.32)**	**-4.39 (1.32)**		**0.154 (0.0613)**	**0.149 (0.0611)**				**0.549 (0.169)**	**0.570 (0.168)**
Sex (F)					**-0.498 (0.198)**	**-0.502 (0.198)**		**-0.659 (0.281)**		**-4.43 (0.540)**	**-4.41 (0.540)**
ASI		**3.12 (1.17)**	**3.10 (1.17)**		**0.115 (0.0540)**	**0.114 (0.0538)**					
AIC		2248.0	2148.9		553.37	565.64				899.2	906.89
Log-likelihood p-value		$p \leq 0.0001$			$p = 0.0002$					$p \leq 0.0001$	

CHAPTER 4: Effects of early life exposure to environmental PPARγ-RXR and AHR pathway activators on development and gene expression in Atlantic killifish (*Fundulus heteroclitus*)

Abstract

Vertebrates, including humans and animal models, that are exposed to environmental metabolism disrupting compounds (MDCs) early in life are at increased risk of developing metabolic syndrome. Tributyltin (TBT) and dioxin-like polychlorinated biphenyls (PCBs, e.g., 3,3',4,4',5-Pentachlorobiphenyl, PCB126) are two MDCs frequently found in urban waterways, including New Bedford Harbor (NBH), an estuarine Superfund site located in Massachusetts. Peroxisome proliferator-activated receptor gamma (PPARγ) and retinoid-X-receptor (RXR), are activated by TBT, and the aryl hydrocarbon receptor (AHR) is activated by PCB126. Both pathways are independently involved with adipose and bone homeostasis, and also interact with one another at the molecular level. We evaluated the effects of MDC exposure in embryonic Atlantic killifish (*Fundulus heteroclitus*), an ecological sentinel species and a toxicological model that has been extensively studied in NBH, to test the hypothesis that AHR activation by PCB126 killifish would affect PPARγ pathway responsiveness following TBT exposure. F2 killifish embryos from NBH and a clean reference site (Scorton Creek (SC), MA) were exposed to TBT (5, 50, and 100 nM) and PCB126 (61 pM) individually and in combination from 1-7 days post-fertilization (dpf) and analyzed at 10 dpf. As expected, PCB126 increased expression of the AHR target gene *cyp1a* to a

greater extent in PCB-sensitive killifish from SC than in PCB-tolerant killifish from NBH. Surprisingly, PCB126 induced *pparγ* expression in male SC killifish. To corroborate this finding, we reviewed existing data on the expression of *pparγ* in control and PCB126-treated embryos from four other pairs of sensitive/tolerant killifish populations and found the same relationship. Expression of *pparγ* and its transcriptional targets, fatty acid binding proteins 11a and 1b (*fabp11a* and *fabp1b*) were not affected by TBT exposure in killifish from either population. However, we observed both NBH and SC killifish embryos exposed to TBT (50 and 100 nM) displayed caudal fin deformities at 10 dpf, and the phenotype was not altered by co-exposure to PCB126. *Osx/sp7*, a gene necessary for bone formation, and *col2a1b,* a gene involved in cartilage formation, were significantly suppressed in killifish exposed to TBT at 100 nM. This deformity was recapitulated by exposure to an RXR-specific ligand, LG100268, but not a PPARγ-specific ligand, S26948. Collectively, these data show that the AHR, PPARγ and RXR pathways are responsive to environmental MDCs in ways that have implications for lipid homeostasis and skeletal development.

Introduction

Global trends in obesity prevalence have risen sharply since the 1980s. Because obesity is a risk factor for the development of metabolic syndrome, the co-occurrence of several conditions including high blood pressure, high blood sugar, excess central adiposity, and dyslipidemia, increasing the risk of heart disease, stroke and diabetes (Mayo, 2015; Morse, 2010), it has drawn considerable clinical and research attention. Diet, exercise and genetics are important predictors of obesity, but research has also identified a class of endocrine disrupting compounds, termed metabolism disrupting compounds (MDCs), which are capable of interfering with metabolic and lipid homeostasis (e.g., Heindel et al., 2017). MDCs comprise a structurally diverse class of common environmental pollutants, which act through a variety of mechanisms (e.g., Casals-Casas & Desvergne, 2011; Heindel et al., 2017). Among these compounds are polychlorinated biphenyls (PCBs) and organotins (e.g., tributyltin, TBT) that are persistent organic pollutants found ubiquitously in the environment (e.g., Capitão et al., 2017; Heindel et al., 2017; Le Magueresse-Battistoni, Labaronne, Vidal, & Naville, 2017). Because the function of metabolically active organs and molecular mechanisms governing lipid homeostasis are largely conserved across vertebrates, using comparative biology to study MDCs can improve our understanding of how these compounds elicit aberrant effects on lipid homeostasis across widely different taxa (Bodofsky, Koitz, & Wightman, 2017; Zhao, Zhang, Giesy, & Hu, 2015). Furthermore, taxa-specific nuances in lipid homeostasis are increasingly well documented, strengthening the capacity to extrapolate findings across species (e.g., Capitão et al., 2017; Michel, 2018).

Many adverse health outcomes that manifest in adulthood are known to have origins in exposure to environmental conditions during developmentally sensitive windows of time, such as during fetal development (e.g., Barker & Osmond, 1986; Currie, Zivin, Mullins, & Neidell, 2014). Early life exposures to MDCs can reprogram molecular machinery, leading to disease susceptibility later in life and the potential to also elicit effects in offspring (transgenerational effects; e.g., Chamorro-García et al., 2013; Jirtle & Skinner, 2007; Schug, Janesick, Blumberg, & Heindel, 2011). Early life exposures to both TBT and dioxin-like compounds are associated with transgenerational effects on lipid homeostasis (e.g., Chamorro-Garcia et al., 2017; Chamorro-García et al., 2013; D. N. Meyer, Baker, & Baker, 2018). TBT induces adipogenesis, impairs mature adipocyte function and disrupts lipid homeostasis in other metabolically active organs such as the liver, where it induces hepatic steatosis (e.g., Grün et al., 2006; J. Zhang et al., 2016). In contrast, PCB126 suppresses adipogenesis (Gadupudi et al., 2015; Gourronc et al., 2018). PCB126 also decreases gluconeogenesis and peroxisomal fatty acid oxidation and induces hepatic steatosis (Gadupudi, Klaren, et al., 2016; Gadupudi, Klingelhutz, & Robertson, 2016).

Three ligand-activated transcription factors that play important roles in lipid homeostasis and can be inappropriately activated by environmental MDCs are (1) peroxisome proliferator activated receptor gamma (PPARγ), the essential regulator adipocyte differentiation from mesenchymal stromal cells (adipogenesis) and lipid storage; (2) retinoid X receptor (RXR), the heterodimeric partner of several nuclear receptors,

including PPARγ; and (3) aryl hydrocarbon receptor (AHR), most widely known for its involvement in xenobiotic metabolism (Evans, 2014; J. J. Heindel, 2017; Janani, 2015; Le Magueresse-Battistoni, 2017; Nebert, 2017). TBT activates both PPARγ and RXR (Baker et al., 2015; Grün et al., 2006), whereas dioxin-like PCBs, including PCB126 (a representative dioxin-like PCB), act through the AHR (e.g., White & Birnbaum, 2009; L. Zhang et al., 2015).

There is strong evidence that TBT and PCB126 co-occur in urban waterways near major population centers in the United States and each compound can impact lipid homeostasis. Moreover, studies have suggested that AHR interacts with PPARγ through poorly understood crosstalk (e.g., Gadupudi et al., 2015; Huang, Zuo, Zhang, & Wang, 2015). AHR activation has been shown to negatively regulate PPARγ by suppressing TBT-mediated adipogenesis in human preadipocytes (Gadupudi et al., 2015). However, TBT exposure has also been found to rescue AHR-mediated effects such as oxidative stress following benzo(a)pyrene exposures in zebrafish embryos (Huang et al., 2015).

New Bedford Harbor (NBH) is a marine Superfund site located in southeastern Massachusetts (**Figure 1.1**). PCBs were used in electronics manufacturing near NBH and discharged in to the environment, leading to widespread, severe contamination throughout the harbor. Organotins were used as an antifouling agent in marine paints, and NBH has long served as a major fishing and shipping port in the region. Therefore, total tin (Sn) in NBH sediments was recently measured (**Chapter 3**). NBH is home to a

number of wildlife populations, including the Atlantic killifish ("killifish," *Fundulus heteroclitus*). Killifish are found in estuarine environments along the east coast of North America and have been studied extensively for their response to contaminants, most notably PCBs and polycyclic aromatic hydrocarbons (PAHs) acting through the AHR (e.g., (D. Nacci, 1999; D. E. Nacci, 2010; Oleksiak, 2011; Proestou, 2014). The small home ranges of killifish have led to the evolution of genetically distinct populations, which have developed adaptations to their local environment (e.g., (Joel N. Meyer, 2003; D. Nacci, 1999; D. E. Nacci, 2010; Reid, 2016) and have emerged as an important example of convergent evolution (Reid, 2016; Andrew Whitehead, 2012). For instance, killifish living in NBH demonstrate dramatic heritable resistance, or evolved tolerance, to AHR pathway activation (e.g., (Bello, 2001; D. Nacci, 1999; D. E. Nacci, 2010; Reid, 2016).

Evolution of heritable alterations to AHR signaling in NBH killifish as compared to those in SC, offers an intriguing opportunity to study molecular crosstalk between AHR and other transcriptional pathways. Because early life exposures to environmental pollutants are important to cellular programming and resulting adverse physiological effects, killifish embryos are often used in toxicological investigations. In this study, we evaluated the use of killifish as a novel, ecologically and human-relevant vertebrate model for studying the metabolism disrupting effects of early life exposure to both AHR and PPARγ ligands *in vivo*. Specifically, we hypothesized that differential AHR sensitivity in NBH and SC killifish would affect PPARγ pathway responsiveness

following TBT exposure (**Figure 1.4**). To this end, we also explored the effects of co-exposures to TBT and PCB126 to interrogate how these responses change following simultaneous activations of both transcriptional pathways. Finally, we investigated the role of these compounds on skeletal development at phenotypic and transcriptional levels. Given that pollutants rarely exist independently in aquatic environments, studies such as this one, are needed to understand the more-subtle effects of chemicals exposure to environmental pollution.

Methods

Materials

Dimethyl sulfoxide (DMSO) was purchased from American Bioanalytical (Natick, MA, USA). 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) was purchased from Ultra Scientific (North Kingstown, RI, USA). Tributyltin chloride (TBT) and LG100268, a pan-RXR agonist were purchased from Sigma Aldrich (St. Louis, MO, USA). S26948, a non-thiazolidinedione (TZD) selective PPARγ agonist and antidiabetic pharmaceutical agent, was purchased from Tocris Bioscience (Minneapolis, MN, USA). Stocks were prepared by dissolving PCB126, TBT, S26948, and LG100268 in DMSO. All other reagents were purchased from Thermo Scientific (Suwanee, GA) unless otherwise noted.

Animal Care and Breeding

Reproductively active, adult killifish (F0) were collected from New Bedford Harbor (NBH, PCB-contaminated) and Scorton Creek (SC, reference) using baited traps and held

in common garden laboratory conditions as described in Nacci et al. (2002) . Killifish were maintained in flow-through aquaria receiving filtered ambient seawater from Narragansett Bay, RI and fed *ad libidum*. F0 killifish and their offspring (F1) spawned with semi-lunar periodicity throughout the summer months (late May through August in New England) and were reared to reproductive maturity (1-2 years per generation) under the conditions described above. Non-invasive techniques (breeding baskets and manual spawning) were used to collect F2 eggs from F1 females during seasonal, semi-lunar spawning cycles between May 2016 and August 2017. Basket eggs were fertilized *in situ* by male killifish. Manually-spawned eggs were fertilized *in vitro* by expressing sperm from males directly into a glass dish containing the eggs. Fertilized embryos were held at 23°C for 24 hours and were then screened microscopically to remove unfertilized or improperly developing embryos.

Chemical Exposures

Exposures were conducted at 23°C under 14:10 hour light:dark regimen. All exposures occurred in 20-mL glass scintillation vials beginning at 24 hours post fertilization (hpf). Each vial contained one embryo in 10mL of exposure solution. Exposure solutions were made in 5µM filtered ambient seawater. Embryos remained in exposure solution until 7 days post fertilization (dpf), at which point they were transferred to individual wells of a 12-well plate containing filter paper moistened with filtered water. Embryo phenotype was screened microscopically at 10 dpf (further detail provided in Section 2.3).

Individual Chemical Exposures

Individual chemical exposures were conducted over two replicate experiments, each containing 20 embryos per treatment (total N=40 per chemical treatment per population). Treatment groups were as follows: naïve (untreated), vehicle control (0.01% DMSO), TBT (Low: 5 nM, Medium: 50 nM and High: 100 nM), S26948 (1 μM), and LG100268 (200 nM). TBT concentrations were selected to establish a dose-response relationship around the EC_{50} in fish and mammals (~50 nM). To the best of our knowledge, S26948 and LG100268 have not been studied in teleost fish so *in vitro* EC_{50} concentrations for each synthetic compound served as the basis for our exposure selection (S26948: 8.83 nM in COS-7 cells transfected with human PPARγ, Carmona et al., 2007; LG100268: 2 nM in COS-7 cells transfected with human RXRα, Grün et al., 2006). Given the six-day static exposure and assuming that these compounds have a short biological half-life, concentrations one to two orders of magnitude higher than the published EC_{50} were selected for S26948 and LG100268. Due to limited F2 SC embryo availability during the 2017 breeding season, LG100268 exposures were only conducted on NBH embryos.

Co-Chemical Exposures

Co-chemical exposures were conducted over three replicate experiments containing at least 20 embryos per treatment (total $N \geq 60$ per chemical treatment per population). Treatment groups were as follows: naïve (untreated), vehicle control (0.02% DMSO), TBT (Medium: 50 nM and High: 100 nM), PCB126 (61 pM), and TBT + PCB126 (Medium TBT + PCB126 and High TBT + PCB126). Medium and High TBT doses were selected for use in co-exposures because of their ability to consistently induce caudal fin

malformation visible during 10 dpf phenotype screening (further detail in Section 2.3). The PCB126 concentration was selected based on prior studies showing activation of the AHR pathway in SC, but not NBH, killifish embryos with low prevalence of cardiac teratogenicity (Nacci et al., 2010; Nacci et al., unpublished data). This concentration was selected to ensure that the AHR-mediated response to PCB126 did not overwhelm the effects of TBT exposure in co-exposure treatment groups.

Phenotypic Screening and Embryo Pooling

At 10 dpf, embryos were examined using a Nikon SMZ1500 stereomicroscope to identify TBT- and PCB126-mediated teratogenesis. TBT-mediated teratogenesis presented as caudal fin malformation, specifically stunted fin ray development and reduced overall fin length. The severity of caudal fin deformity was scored as 0 (normal), 1 (mild deformity – slightly shortened caudal fin), 2 (moderate deformity – shortened caudal fin, less discernable fin rays), and 3 (severe deformity – extremely short caudal fin, poorly defined or indiscernible fin rays; **Figure 4.1**). Some individuals scored as 3 appeared to have overall shorter tails; however, this was difficult to quantify in an intact embryo, and therefore was not considered during phenotypic scoring. PCB126 exposure caused cardiac teratogenesis, as has been studied extensively (e.g., Clark, Matson, Jung, & Di Giulio, 2010; Whitehead, Triant, Champlin & Nacci, 2010). The prevalence of cardiac teratogenesis was low and was scored as presence/absence (0 = normal, 1 = deformed), using previously described characteristics of cardiac malformation (Matson, Clark, Jenny, Fleming, Hahn, & Di Giulio, 2008).

Following phenotypic screening, two pools of embryos (N=5 embryos per pool) were flash frozen for subsequent gene expression analysis. Embryos with phenotypes not representative of the rest of the treatment group (e.g., gross maldevelopment following Low TBT exposure) were excluded from pools. Individual chemical exposure experiments consisted of N=4 pools per chemical treatment, whereas co-chemical exposures experiments consisted of N=6 pools per chemical treatment.

RNA Extraction and qPCR

Total mRNA was extracted from pooled embryos using RNA STAT-60™ (Tel-Test Inc. Friendswood, TX, USA). Genomic DNA was removed using the Aurum™ Total RNA Mini Kit (BioRad). cDNA was prepared from total mRNA using the GoScript™ Reverse Transcription System (Promega), using equal parts random and Oligo (dT)15 primers. qPCR reactions were performed using iTaq Universal SYBR® Green Supermix (BioRad) with 400nM forward and reverse primers. Primer sequences are shown in **Table 4.1**. Thermocycling parameters for all genes were 95°C for three minutes to activate iTaq DNA polymerase, followed by 40 cycles of melting at 95°C for 15 seconds and annealing at 66°C for 60 seconds. A manually-selected threshold in the linear phase of the amplification curve was used to determine
threshold cycle (C_T) values to standardize across multiple plates.

All gene expression data were normalized with the Pfaffl method (Pfaffl, 2001), using primer-specific amplification efficiency and the expression of constitutively expressed

(housekeeping) genes and naïve (untreated) pooled embryo samples from both SC and NBH. Three housekeeping genes were evaluated for stability across populations and experimental replicates. Variability was greatest in *β-actin* (overall mean (standard deviation): 1.33 (0.996)), with differences between populations (SC > NBH) and experimental replicates. Expression of *ef1α* was less variable (0.982 (0.498)), again with SC > NBH. *rn18s* was most stably expressed (1.06 (0.248)), with no discernable pattern of variability between populations, experimental replicates or chemical treatments. Thus, all gene expression data presented herein are normalized to *rn18S* in a consistent set of naïve embryos from both SC and NBH and reported as a relative expression ratio.

Statistical Analysis

All exposures were repeated over the course of at least two experimental replicates. Fifteen to thirty embryos, depending on embryo availability in a particular spawning cycle, were included in each treatment group during each experimental replicate. Embryo mortality never exceeded 20% in control treatments. Power calculations were conducted using G*Power (Faul et al., 2007). LG100268 experiments were only conducted in NBH embryos due to limited F2 SC embryo availability. Given the consistency in both population's susceptibility to caudal fin deformity following TBT exposure, we believe the responsiveness of NBH embryos to LG100268 can be extrapolated to SC embryos. Therefore, we pooled caudal fin deformity scores for embryos exposed to therapeutic compounds across populations.

All other statistical analyses were performed using Microsoft R Open 3.3.2. Gene expression and phenotypic data were analyzed separately for individual and co-chemical exposures. Gene expression was evaluated using ANOVA and t-tests to evaluate differences in mean relative expression between populations and treatment groups. All data points, including possible outliers (points falling more than 1.5 times the interquartile range below or above the first or third interquartile range, respectively), were used in analyses due to the small sample size ($N \leq 6$). Mean caudal fin deformity score and standard error were calculated for each treatment group and chemical exposure groups were compared to DMSO-exposed controls (ANOVA, Dunnett's post-hoc comparison). Statistical significance was evaluated at $\alpha = 0.05$ for all analyses.

Results

This study evaluated the effects of early life exposures to MDCs on molecular markers of lipid homeostasis and caudal fin development in killifish. SC and NBH F2 killifish embryos were exposed to TBT and PCB126 through water-borne exposures following gastrulation (1 dpf) through 7 dpf. At 10 dpf, embryos were screened for phenotypic abnormalities and then mRNA was isolated for gene expression analysis by qPCR. Findings were compared across chemical treatments and populations to evaluate whether differential responsiveness of the AHR pathway to endogenous ligands in SC and NBH killifish can inform our understanding of molecular crosstalk between the PPARγ-RXR and AHR pathways.

PPARγ Pathway Responsiveness

To determine whether chemical exposures caused changes in PPARγ pathway activation between populations or chemical treatments, we analyzed the expression of *pparγ*, itself, as well as two transcriptional target genes previously studied in teleost fish, *fabp11a* and *fabp1b*. *Fabp11a* is the widely accepted ortholog of mammalian FABP4, a commonly used marker of the PPARγ-RXR pathway and mature adipocytes (Flynn, 2009; Imrie, 2010). However, *fabp1b* has also been shown to be a selective target of *pparγ* in zebrafish (Laprairie, 2016), therefore, we used the killifish ortholog of this gene as a second *pparγ* gene target. Basal mRNA expression of *pparγ*, *fabp11a*, and *fabp1b* (**Figure 4.2a-c**) did not differ between SC and NBH embryos. Additionally, TBT had no effect on the expression of these genes (**Figures 4.2d-f**). S26948, a non-TZD PPARγ agonist, also did not significantly alter the expression of PPARγ pathway genes (**Figure 4.2g-i**).

Co-Exposure to PPARγ-RXR and AHR Agonists

Co-exposures to TBT High and PCB126 ("TBT High + PCB126") were designed to evaluate potential crosstalk between PPARγ-RXR and AHR pathways. SC embryos are regarded as having normally-functioning AHR pathways. Comparatively, NBH embryos demonstrate reduced sensitivity to PCB126 due to selective pressure on the AHR pathway from pollutant exposure in NBH and subsequent heritable adaptations (e.g., Reid et al., 2016). An independent set of embryos from those used in the dose-response study were used in these experiments.

First, we measured relative expression of *cyp1a*, a canonical molecular marker of AHR pathway activation, to confirm activation of this transcription factor by PCB126 exposure. Basal *cyp1a* expression was not different between SC and NBH embryos ($p = 0.672$, t-test; **Figure 4.3a**). PCB126 significantly induced *cyp1a* in SC, but not NBH embryos alone (**Figure 4.3b**; $p = 0.0074$, ANOVA, Dunnett's). *Cyp1a* was also induced in SC embryos when exposed in combination with TBT High ($p < 0.001$, ANOVA, Dunnett's). Surprisingly, *cyp1a* was induced by the combination of PCB126 and TBT High in NBH embryos (**Figure 4.3b**; $p = 0.0241$, ANOVA, Dunnett's).

Next, we evaluated the expression of *pparγ* and *fabp1b* as markers of PPARγ pathway activation by TBT High alone and in combination with PCB126. Again, basal mRNA expression of both genes was similar between populations (**Figure 4.4a-b**). In SC embryos, *pparγ* expression was significantly increased by co-exposure to TBT High + PCB126 ($p = 0.0177$, one-way ANOVA, Dunnett's) compared to DMSO-treated embryos (**Figure 4.4c**). PCB126 alone and in combination with TBT High increased *pparγ* expression slightly in NBH embryos, although the change in expression relative to control embryos was not significant and NBH embryos were generally less responsive to chemical treatment than those from SC. Review of data from existing transcriptomic analyses of pooled embryos from four pairs of AHR-sensitive and -tolerant killifish populations, revealed the same pattern of significant *pparγ* induction of sensitive, but not tolerant, populations following PCB126 exposure (**Figure 4.5**, adapted from Reid et al., 2016; Whitehead et al., 2017). The pattern of *fabp1b* expression in SC embryos in our

study was similar to *pparγ* expression in the same samples (**Figure 4.4d**), but no statistically significant changes in mRNA expression were measured. In NBH embryos, *fabp1b* was significantly suppressed following TBT High exposure ($p = 0.0332$, one-way ANOVA, Dunnett's) with the same trend apparent in embryos exposed to TBT High + PCB126.

Caudal Fin Deformity

TBT exposure during early embryonic development and organo-differentiation caused teratogenesis characterized by impaired caudal fin development (see representative images in **Figure 4.2**). The severity and prevalence of caudal fin deformities increased in a dose-dependent manner (**Figure 4.6**; two-way ANOVA, Tukey post-hoc comparison by treatment). Embryos exposed to TBT Low (5 nM) did not exhibit caudal fin teratogenesis above the background level in control embryos (> 95% normal caudal fin phenotype in both populations for each treatment). Mild to moderate caudal fin deformities were observed with exposure to TBT Medium (50 nM) and moderate to severe deformities were observed with TBT High (100 nM), significantly increasing the mean deformity score relative to controls. The mean deformity score did not differ significantly between SC and NBH embryos (**Figure 4.6**; two-way ANOVA: $p = 0.435$).

The same pattern of caudal fin deformity was observed when SC and NBH embryos were co-exposed to TBT and PCB126 (**Figure 4.7**). PCB126 did not independently induce caudal fin deformities. Since PCB126 is well-known to cause other teratogenic effects

(e.g., cardiac malformations in sensitive but not tolerant killifish populations), we screened embryos exposed to PCB126 alone and in combination with TBT for the presence or absence of cardiac abnormalities. The prevalence of cardiac malformation in SC embryos was 3.4% and 11.9% in control or PCB-treated groups, respectively. By contrast, cardiac malformations were only observed in 1.4% and 4.2% of NBH embryos in control and PCB treatments, respectively. The prevalence of cardiac teratogenicity in each population was unchanged when embryos were co-exposed to TBT and PCB126 compared to those exposed to PCB126 alone.

Molecular Basis for Caudal Fin Deformity

The caudal fin and caudal bone complex are recognized as skeletal regions particularly sensitive to disruption during teleost development (Fernández, Hontoria, Ortiz-Delgado, Kotzamanis, Estévez, Zambonino-Infante, & Gisbert, 2008; Fernández, Pimentel, Ortiz-Delgado, Hontoria, Sarasquete, Estévez, Zambonino-Infante, & Gisbert, 2009; Haga, Suzuki, & Takeuchi, 2002). In an effort to understand the molecular mechanism causing TBT-mediated caudal fin deformities, we measured the relative expression of genes known to be markers of osteoblasts (*osx/sp7*) and chondrocytes (*col2a1a* and *col2a1b*). No differences in basal expression of these genes were found between populations (**Figure 4.8a-c**). *Osx/sp7* was significantly suppressed following exposure to TBT High ($p = 0.0108$, one-way ANOVA, Dunnett's; **Figure 4.8d**). Similar expression patterns were seen in SC embryos for *col2a1a* (**Figure 4.8e**) and *col2a1b* (**Figure 4.8f**), with *col2a1b* being significantly suppressed by TBT High ($p = 0.0386$, one-way ANOVA,

Dunnett's). While mean *osx/sp7* expression tended to be lower across all TBT treatments in NBH embryos relative to controls, the suppression was not statistically significant. No discernable treatment-related pattern of *col2a1a* and *col2a1b* was observed in NBH embryos.

Using an independent set of embryos, we examined effects on gene expression following TBT and PCB126 co-exposures. Again, expression of *osx/sp7*, *col2a1a*, and *col2a1b* did not differ between controls from NBH and SC (**Figure 4.9a-c**). While PCB126 significantly increased *cyp1a* and *pparγ* mRNA expression in these embryos (see **Figures 4.3** and **4.4**), we did not observe significant effects on *osx/sp7*, *col2a1a* and *col2a1b* expression in SC or NBH embryos. Curiously, TBT High treatment in co-chemical exposure experiments did not replicate trends seen in *osx/sp7*, *col2a1a* and *col2a1b* expression in individual chemical experiments that suggested suppression of bone and cartilage formation. This is likely due to high variability in mRNA expression between samples and small sample size (discussed below).

Since TBT is known to bind and activate both PPARγ and RXR, we also evaluated caudal fin development in F2 killifish embryos exposed to selective synthetic ligands for each receptor (S26948 and LG100268, respectively) to determine which receptor is involved with caudal fin deformities. S26948 exposure did not produce caudal fin deformities in embryos. However, LG100268 induced deformities in killifish embryos similar to those observed following TBT exposure (**Figure 4.10**).

Discussion

We sought to determine whether MDCs commonly found in urban waterways affect molecular markers of lipid homeostasis in SC and NBH killifish during embryogenesis. We used comparisons between the two populations, which have different AHR pathway responsiveness, and co-chemical exposures to evaluate whether molecular crosstalk between PPARγ and AHR may be involved with differences in lipid homeostasis observed in F1 and F2 adult killifish from the two populations. While clear differences in responsiveness to MDCs between SC and NBH were not observed, this study furthered our understanding of mechanisms by which MDCs affect developing fish, including caudal fin teratogenesis following TBT exposure. The small sample sizes ($N \geq 6$) in our studies and high inter-individual variability in gene expression inherent in killifish confounded our ability to characterize effects of environmental MDC exposure on molecular pathways governing lipid homeostasis and skeletal formation. A discussion of our findings and inter-sample variability in pooled killifish embryos is provided in the following sections.

Lack of Response of the PPARγ Pathway

Overall, the PPARγ pathway in killifish was largely unresponsive to TBT exposure in the 1-10 dpf embryo, evidenced by a lack of induction of either *pparγ* itself and or its target genes (*fabp11a*, and *fabp1b*). This was surprising because TBT is widely known to activate PPARγ and RXR in a variety of vertebrate models, including other teleost fishes (e.g., Baker et al., 2015; Capitão et al., 2017; Grün et al., 2006). However, adipose tissue development has not been chronicled in embryonic killifish and 10 dpf embryos may not

have many, if any, differentiated adipocytes. Zebrafish begin to develop adipose tissue around 10-15 dpf, about 6-9 days post-hatching (Imrie, 2010; Minchin & Rawls, 2011), whereas killifish do not hatch until approximately 15 dpf. Thus, the developmental timeframe for killifish is appreciably slower than that of zebrafish. Furthermore, we evaluated gene expression in pools of whole, homogenized embryos, so the molecular signal from a limited number of adipocytes may have been overwhelmed by noise from other cell types present in the embryos at 10 dpf. Therefore, the basal expression of PPARγ pathway genes may not be strong enough to detect differences between populations or changes caused by chemical exposures. Evaluating gene expression at a later developmental stage (e.g., 28 dpf) may improve the chances of detecting differences in adipocyte-related gene expression following chemical exposure.

We also exposed killifish embryos to the synthetic PPARγ agonist, S26948, which also did not elicit changes in the expression of *pparγ*, *fabp11a*, or *fabp1b*. The lack of responsiveness of killifish from both populations to S26948 exposure might be attributed to any of several factors: (1) adipose tissue is not well developed at 10 dpf, as discussed above, (2) this compound is not a ligand for killifish *pparγ*, (3) the compound was metabolized during the course of the six-day static exposure and three-day depuration prior to embryo preservation, and/or (4) the compound is unstable in seawater. To the best of our knowledge, S26948 has not been tested as a PPARγ ligand in teleost fish. We selected this synthetic ligand rather than the classic PPARγ positive control, rosiglitazone, given debate about the responsiveness of teleost PPARγ to rosiglitazone

(Liu et al., 2005; Ouadah-Boussouf & Babin, 2016; Riu et al., 2014; Sánchez-Gurmaches et al., 2010; Tingaud-Sequeira et al., 2011). The lack of teleost PPARγ activation by known mammalian PPARγ agonists, such as rosiglitazone, may be explained by poor conservation of key amino acid residues in the PPARγ ligand binding of fishes known to be important to be important for ligand binding in mammalian PPARγ (e.g., Leaver et al., 2005). While timing of adipose tissue development is a concern, we have also shown that S26948 did not activate the PPARγ pathway in killifish liver following a three-day exposure by intraperitoneal injection (**Chapter 3**). Synthetic ligands tend to have short biological half-lives by design, and how this may impact the biologically effective dose in killifish embryos following a six-day, static, water-borne exposure, and three-day depuration period is unknown. Similarly, the stability of S26948 in seawater is unknown, although no evidence of instability (e.g., precipitate) was observed during experiments.

Fin Teratogenicity

While these studies were not designed to examine the effect of TBT on skeletal development, TBT-induced caudal fin deformities are not necessarily surprising. In mammalian models, bone has been identified as a direct target TBT-mediated toxicity *in vitro* (A. H. Baker, 2015; Shoucri, Martinez, Abreo, Hung, Moosova, Shioda, & Blumberg, 2017; Watt, 2018, 2015; Yonezawa, Hasegawa, Ahn, Cha, Teruya, Hagiwara, Nagai, & Woo, 2007) and in vivo (Kirchner, Kieu, Chow, Casey, & Blumberg, 2010; Tsukamoto, Mizuta, Fujimoto, Ohte, Osawa, Miyamoto, Yoneyama, Murata, Machiya, Jimi, Kokabu, & Katagiri, 2015). TBT suppresses osteogenesis in mouse primary bone

marrow cultures (Watt et al., 2015; Baker et al., 2015), yet appears to have independent effects on osteoblasts and osteoclasts, which work in concert to maintain constant bone remodeling (Watt et al., 2018). Further, early life exposures to also cause axial skeleton and fin abnormalities in other fish and amphibian species, which are often characterized by bent or shortened spines or tails (medaka, *Oryzias latipes*: Bentivegna & Piatkowski, 1998; Hano et al., 2007; mahi mahi, *Coryphaena hippurus*: Adema-Hannes & Shenker, 2008; false kelpfish, *Sebastiscus marmoratus*: Zhang et al., 2011; West African clawed frog, *Xenopus tropicalis*: Guo et al., 2010).

TBT causes adverse effects on a variety of cell types. Liu et al. (2012) show that TBT inhibits the development of dorsal and ventral fins in *Xenopus tropicalis*, suggesting that both neural crest and ventral mesoderm progenitor cells are affected by TBT exposure during early embryogenesis. Osteocytes, chondrocytes, and adipocytes differentiate from common mesenchymal progenitors in fish, as they do in mammals (Paul & Crump, 2016). The complex regulatory processes governing differentiation of these multipotent cells shifts from favoring bone and cartilage formation, to favoring fat formation following activation of the PPARγ-RXR pathway (e.g., Kawai & Rosen, 2010), which may, in turn, be regulated by the AHR pathway through nuclear receptor crosstalk. Given the role of TBT and PCB126 in activating the PPARγ-RXR and AHR pathways, respectively, we hypothesize that TBT may be targeting the shared precursor, multipotent mesenchymal stromal cells to elicit adverse effects on fat, bone and cartilage formation. Specifically, we hypothesized that exposure to TBT alone would increase the expression

of genes related to adipogenesis and suppress genes related to osteogenesis and chondrogenesis.

Several mechanisms by which TBT alters skeletogenesis have been proposed. TBT is well-known to bind and activate RXRs (e.g., Baker et al., 2015; le Marie et al., 2009), which serve as heterodimeric partners for other nuclear receptors controlling growth and development. Zang et al. (2011) showed evidence of increased apoptosis in curved regions of *Sebastiscus marmoratus* tails and speculated that this was an RXRα-mediated response to TBT exposure (Bentivegna & Piatkowski, 1998). Guo et al. (2010) proposed that TBT-activation of RXR interferes with retinoic acid (RA) or thyroid signaling to cause skeletal and ocular deformities, respectively. RA affects expression of genes (e.g., *hox*) critical to patterning along the vertebral axis and, ultimately, limb development (Haga et al., 2011). However, RAR/RXR is a non-permissive heterodimer, and TBT is not known to bind or activate RAR (e.g., A. H. Baker et al., 2015). Our data also suggest that a RXR-mediated mechanism, and likely an RXR-RXR homodimer mediated mechanism, is responsible for the stunting of caudal fin development observed following TBT exposure. First, TBT and LG100268, a RXR-specific agonist, both induced caudal fin deformities, whereas the PPARγ-specific agonist, S26948 did not. Second, TBT and LG100268 have been shown to modulate gene expression through RXR alone and to suppress osteogenesis and osteoclastogenesis in a similar manner (A. H. Baker, 2015; Watt, 2018).

A limited number of killifish exposed to TBT *in ovo* were raised for approximately 12 months after hatching. Deformity of the caudal peduncle remained visible in one-year-old fish (data not shown). In laboratory conditions, devoid of predators and where food is reliable and plentiful, TBT exposure and caudal fin deformities did not affect killifish survival. However, such deformities could have significant impacts on the survival of killifish and other species affected by TBT exposure in the wild, where skeletal malformation could impact an individual's ability to feed or avoid predation. As such, further studies should be done to characterize environmental exposures related to skeletal malformation in laboratory and wild populations. These findings should be considered by ecological risk assessors when considering sensitive endpoints for environmental toxicant exposure, particularly in aquatic environments.

AHR agonists, including PCB126, have also been shown to induce axial skeletal deformities in fishes (e.g., medaka, *Oryzias latipes*: Watson et al., 2017; zebrafish, *Danio rerio*: Baker, Peterson, & Heideman, 2013; and killifish: Black et al., 1998; Wassenberg & Giulio, 2004). However, we did not observe caudal fin malformation in embryos exposed to PCB126 alone. This may be due to differences in molecular and cellular processes giving rise to dermal bone (e.g., fin rays) and endoskeletal bone (e.g., vertebral body) (Apschner, Schulte-Merker, & Witten, 2011; Witten, Harris, Huysseune, & Winkler, 2017). Another potential factor is the exceptionally low dose of PCB126 (61 pM) selected for this study in order to prevent AHR-mediated effects and cardiac teratogenesis from dominating co-exposures with TBT. This PCB126 concentration is at

least an order of magnitude lower than other studies (e.g., 150 and 300 pM, Watson et al., 2017). Although Black et al. (1998) reported spinal curvatures in killifish exposed to PCBs in NBH, the harbor concentrations of PCB126 are likely to be higher than that used in our study.

We were intrigued by the fact that the TBT-induced caudal fin deformities were similar between both populations. Generally, other phenotypic abnormalities caused by environmental contaminants acting through the AHR have been shown to affect populations of killifish from uncontaminated environments more severely than killifish from heavily polluted environments (e.g., cardiac malformations in embryonic killifish following PCB126 exposure; e.g., Clark, Bone, & Di Giulio, 2014; Clark, Matson, Jung, & Di Giulio, 2010a; D. E. Nacci et al., 2010; Whitehead et al., 2010). Therefore, we would also have expected an AHR-mediated response to differ between the two populations. However, similarity in caudal fin deformities between SC and NBH embryos following embryonic MDC exposure, regardless of whether embryos were exposed to TBT alone or in combination with PCB126, provides further evidence that the RXR pathway is not influenced by resistance to AHR pathway activation. Our findings also suggest that indirect regulation by AHR (e.g., through crosstalk with other nuclear receptors) is not likely controlling skeletal development in killifish embryos since, again, we would have expected the response to differ between the two populations.

Evidence of Crosstalk Between PPARγ and AHR Pathways

An important component of our original study design was to evaluate whether AHR pathway activation negatively regulates the PPARγ pathway through molecular crosstalk with PPARγ. NBH killifish have evolved heritable resistance to environmental AHR agonists, such as dioxin-like PCBs, which is often measured by recalcitrance to the induction of AHR target genes, notably CYP1A (e.g., Nacci et al., 2010; Bello et al., 2001; Reid et al., 2016). We measured *cyp1a* expression as a positive control to confirm successful AHR pathway activation and anticipated population differences in AHR responsiveness (SC >> NBH). SC embryo responsiveness to AHR pathway activation by PCB126 is exactly as would be expected: significantly elevated above background levels, but relatively modest given the low PCB126 dose selected for use in this study, which confirmed successful water-borne exposure to MDCs in our study. *Cyp1a* expression was also induced in NBH embryos, but only 10-15 times that of controls (as opposed to 20-30 times controls in SC embryos). *Cyp1a* induction in NBH embryos is a surprising occurrence at the low PCB126 concentration utilized in our study, but one that has been previously documented. Clark et al. (2014) show that resistance to AHR ligand-induced cardiac teratogenesis is heritable through the F2 generation, whereas *cyp1a* responsiveness to PCB126 was higher in F2 and F1 killifish embryos relative to controls. Slight induction of *cyp1a* was also seen in Oleksiak et al. (2011). Nevertheless, killifish from NBH show reduced sensitivity to PCB126 exposure, as would be expected based on prior research (D. Nacci, 1999; D. E. Nacci, 2010).

Another noteworthy finding from our study about AHR pathway activation in killifish is that *cyp1a* expression was highest in both SC and NBH embryos in the TBT High + PCB126 treatment group. This suggests that TBT may potentiate AHR pathway activation when co-exposures occur. While previous studies have shown TBT to inhibit *cyp1a*, low levels of TBT exposure appear to enhance PCB126 induction of *cyp1a in vitro* and in both rodents and fish (reviewed in Oshima et al., 2009). The ability of TBT to magnify the effects of PCB126 has significant implications in aquatic environments in particular, since PCBs and organotins are legacy pollutants common to aquatic environments impacted by industrial and commercial land use and often co-occur.

We hypothesized that PCB126 exposure, alone and in combination with TBT, would counteract endogenous and TBT-induced effects on *pparγ* and its target, *fabp1b*, in SC but not NBH killifish. Specifically, we expected to find that the expression of *pparγ* and *fabp1b* in SC embryos would be reduced in PCB126 treatment groups relative to TBT-only treatment groups, whereas expression of these genes would remain unchanged in NBH embryos. Instead, *pparγ* and *fabp1b* in SC embryos were increased in PCB126 and co-chemical exposure groups, relative to TBT-only treatments. *Pparγ* expression was significantly increased in SC embryos exposed to TBT High + PCB126. This was the opposite of what has been shown *in vitro*, where PCB126 has reduced expression of *pparγ* and its target genes (e.g., Gadupudi et al., 2015), and where AHR knockdown in adipocytes increased adipose tissue mass (N. A. Baker, 2015). However, analysis of the effect of PCB126 exposure across multiple AHR-sensitive and -tolerant killifish

populations confirms our finding that SC embryos are more sensitive to *pparγ* induction by PCB126 (Reid, 2016). Additionally, a similar pattern of *pparγ* induction was seen in livers of adult killifish from SC (**Chapter 3**).

Variability

On the whole, gene expression in pooled embryo samples across experimental replicates was highly variable. Given our small sample sizes (N=4 or 6 per chemical treatment per population), it was extremely difficult to detect significant differences and identify true trends in the expression of the PPARγ-pathway, osteogenic and chondrogenic genes between chemical treatments. Killifish are well known to have high genetic diversity (e.g., McMillan, Bagley, Jackson, & Nacci, 2006; Reid et al., 2016). Even individuals from the F2 generation are effectively an outbred, wild population, which naturally implies greater variability between individuals than in populations of inbred or genetically identical animals, such as zebrafish or mice.

Intriguingly, relative expression of each gene for embryo pools from a given replicate experiment (two pools of N=5 embryos per pool, per experiment) tended to be clustered together compared with other embryo pools in the same treatment group from other experiments. This suggests that the specific characteristics of embryos from a given spawning cycle are more similar to one another than they are to embryos from other cycles. Similar batch-to-batch variability has been characterized in other teleost fish species, including a meta-analysis of fathead minnow (*Pimephales promelas*) and

zebrafish (*Danio rerio*), where temporally proximate batches were found to have greater transcriptomic similarity (Wang et al., 2014).

We pooled 10 dpf embryos for gene expression analyses in an effort to minimize inter-individual variability and to maintain the project at a pilot study scale. However, high variability in gene expression means our experiments were under powered to detect significant differences between treatment groups. For instance, we used the variance measured in our TBT range-finding study to calculate the achieved power for each gene (assuming an α error probability = 0.05 for four groups: DMSO and TBT Low, Medium, and High). Achieved power for *pparγ*, *fabp11a*, and *fabp1b* was 0.106, 0.133 and 0.262, respectively. Sample sizes required to detect statistically significant differences between treatment groups were 360, 252, and 112 respectively (assuming power (1-β error probability) = 0.8; α error probability = 0.05), whereas our sample size was 36 in for this study, lower by an order of magnitude. By comparison, our co-chemical exposure experiments had higher variance and additional treatment groups, meaning the achieved power was lower and the required sample size to detect significant differences would be greater.

Conclusion

In conclusion, gene expression associated with the PPARγ pathway (*pparγ*, *fabp11a*, and *fabp1b*) was not affected by TBT exposure in 10 dpf embryos for either population of killifish. While embryonic systems are extremely useful models to explore biological

mechanisms, developmental competence, or maturity, of some systems may limit responsiveness in comparison to later life stages. Unexpectedly, PCB126 induced *pparγ* expression in 10 dpf embryos, an effect that was most pronounced in SC embryos as compared to those from NBH. This finding is corroborated in previous studies in multiple pairs of AHR-sensitive and -tolerant killifish populations, as well as by our research on PCB126 exposures in adult killifish. TBT caused caudal fin deformities, characterized by reduced fin ray and axial skeleton development in both SC and NBH embryos at 10 dpf. These effects were observed regardless of whether embryos were exposed to TBT alone or in combination with PCB126. A likely mechanism of action is activation of RXR. In light of these findings, we believe killifish serve as a useful model to study early life exposures to MDCs and bone-disrupting chemicals. However, experiments should be performed in older larvae in order to study effects of MDCs on lipid homeostasis endpoints. Given the high degree of inter-individual variability in killifish embryos and their tendency to vary by batches (i.e., specific spawning cycles), large sample sizes would be needed to detect small changes in gene expression associated with MDC exposure under the current exposure regime.

Figures and Tables

Figure 4.1. Representative images of caudal fin development. Normal caudal fin development at 10 dpf (left) is characterized by well-defined fin rays and a body plan that wraps fully over the embryo's face and heart. Severe caudal fin deformities induced by TBT Medium and High (50 and 100 nM, respectively) exposure were characterized by poorly defined fin rays, a stunted caudal fin and often a body plan that did not fully wrap over the face and heart.

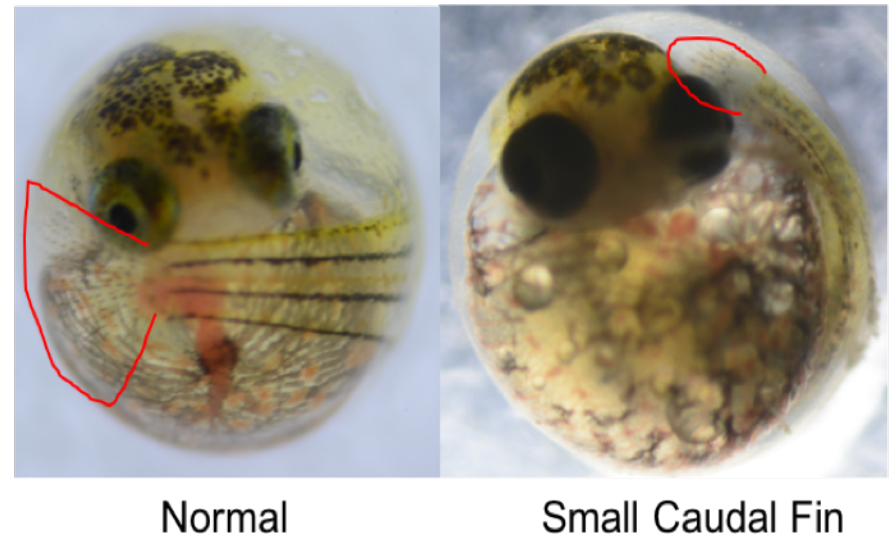


Figure 4.2a-i. The relative expression of PPARγ pathway was measured by qPCR in pooled embryonic killifish (N=5 embryos per pool; N=4 pools per chemical treatment, per population from two experimental replicates referred to as "ELA") from SC and NBH to evaluate the potential for MDCs to interfere with lipid homeostasis. Specifically, we measured *pparγ*, an essential regulator of adipocyte differentiation, function ,and lipid sequestration, as well as two transcriptional targets, *fabp11a* and *fabp1b*. All threshold cycle (C_T) values were normalized using the Pfaffl method to naïve samples (pools of untreated SC and NBH embryos) and the constitutively expressed housekeeping gene rn18s. Gene expression is reported as a relative expression ratio in relation to a consistent group of naïve embryos from both SC and NBH. Relative expression of *pparγ* (A), *fabp11a* (B), and *fabp1b* (C) compared between vehicle-treated embryos from SC and NBH. Relative expression of *pparγ* (D), *fabp11a* (E), and *fabp1b* (F) in SC and NBH embryos exposed to TBT Low, Medium, and High treatments. Finally, we present the relative expression of *pparγ* (G), *fabp11a* (H), and *fabp1b* (I) in SC and NBH embryos exposed to S26948, a selective PPARγ therapeutic ligand. Changes in gene expression were evaluated using two-way ANOVA and t-tests and statistical significance was evaluated at $a = 0.05$. No significant changes in expression were identified for *pparγ*, *fabp11a*, or *fabp1b* relative to controls following chemical exposure.

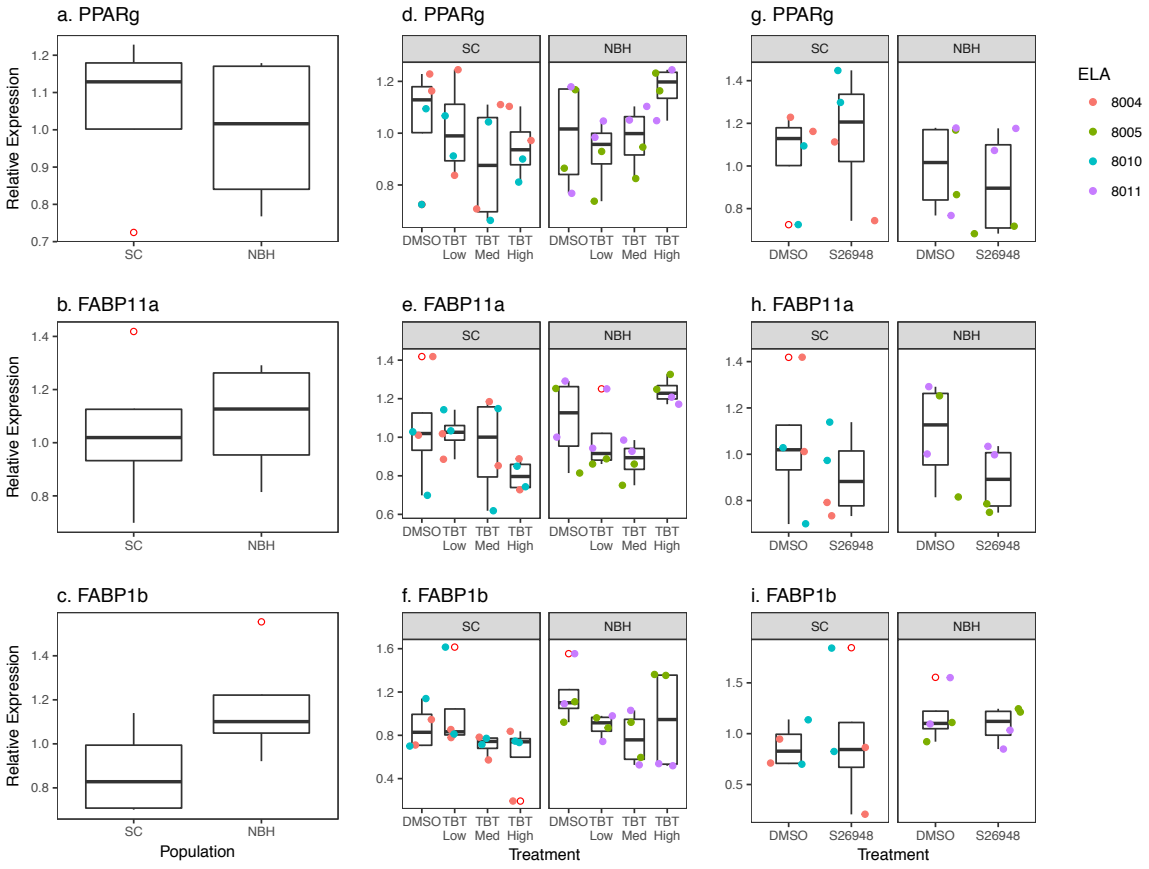


Figure 4.3a-b. The relative expression of cyp1a was measured by qPCR in pooled embryonic killifish (N=5 embryos per pool; N=6 pools per chemical treatment, per population from three experimental replicates referred to as "ELA") from SC and NBH to evaluate AHR pathway activation. A manually-selected threshold was utilized to standardize critical thresholds (CT) across multiple plates. All threshold cycle (C_T) values were normalized using the Pfaffl method to naïve samples (pools of untreated SC and NBH embryos) and the constitutively expressed housekeeping gene rn18s. Gene expression is reported as a relative expression ratio in relation to a consistent group of naïve embryos from both SC and NBH. Statistically significant ($p \leq 0.05$) differences in gene expression between a chemical treatment group and the population-specific control are noted with a red asterisk (*). (A) Basal *cyp1a* expression measured in DMSO-treated embryos was not different between SC and NBH ($p = 0.672$, t-test). (B) PCB126 (61 pM) significantly induced *cyp1a* in SC embryos ($p = 0.0074$, ANOVA, Dunnett's). *Cyp1a* was also induced in SC embryos when exposed in combination with TBT High (100 nM; $p < 0.001$, ANOVA, Dunnett's). Surprisingly, *cyp1a* was induced by PCB126 in NBH embryos (PCB126: $p = 0.0736$; TBT High + PCB126: $p = 0.0241$, ANOVA, Dunnett's).

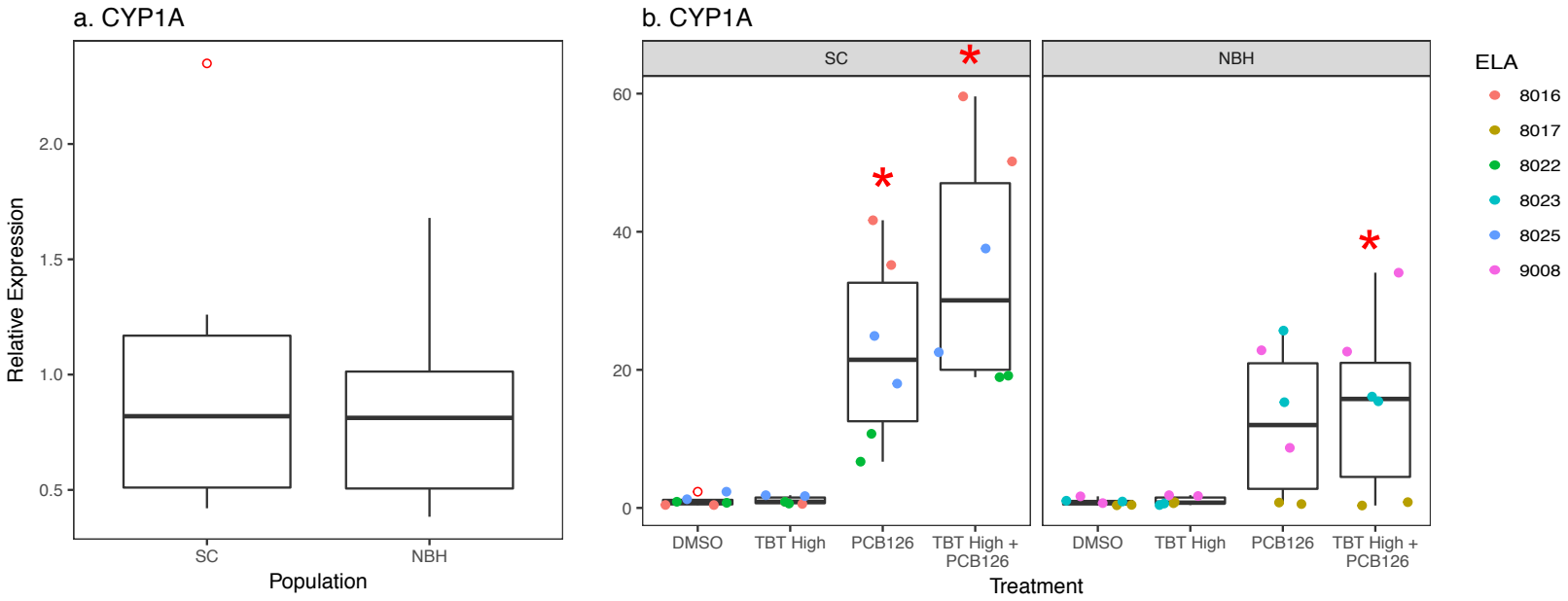


Figure 4.4a-d. The relative expression of *pparγ* and *fabp1b* were measured by qPCR in pooled embryonic killifish (N=5 embryos per pool; N=6 pools per chemical treatment, per population from three experimental replicates referred to as "ELA") from SC and NBH to evaluate the potential for MDCs to interfere with lipid homeostasis following co-exposure to PPARγ (TBT) and AHR (PCB126) agonists A manually-selected threshold was utilized to standardize critical thresholds (CT) across multiple plates. All threshold cycle (C_T) values were normalized using the Pfaffl method to naïve samples (pools of untreated SC and NBH embryos) and the constitutively expressed housekeeping gene rn18s. Gene expression is reported as a relative expression ratio in relation to a consistent group of naïve embryos from both SC and NBH. Basal expression of *pparγ* (A) and *fabp1b* (B) did not differ between SC and NBH. Relative expression of *pparγ* (C) was significantly increased in SC embryos by co-exposure to TBT High + PCB126 compared to DMSO-treated embryos (p = 0.0177, one-way ANOVA, Dunnett's). In NBH embryos, *fabp1b* (D) was significantly suppressed following TBT High exposure (p = 0.0332, one-way ANOVA, Dunnett's) with the same trend apparent in embryos exposed to TBT High + PCB126.

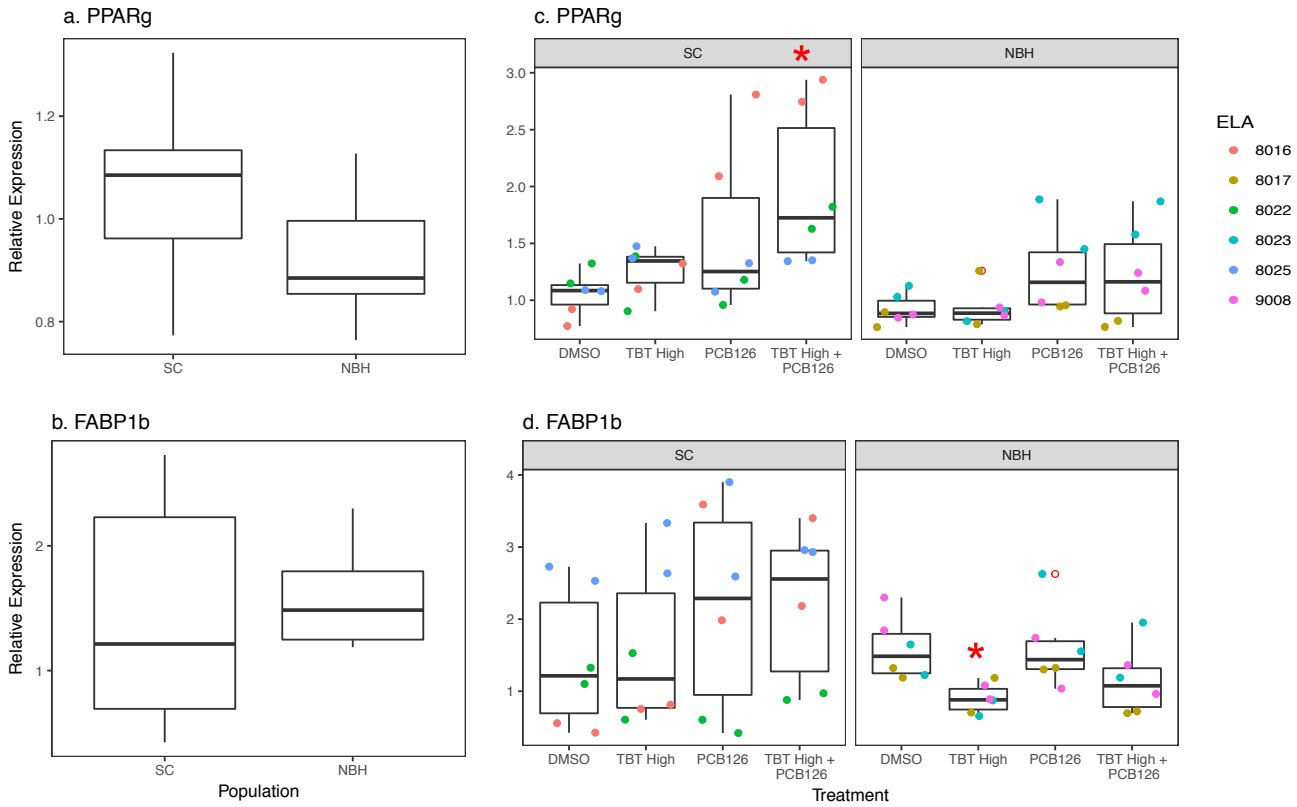


Figure 4.5. Embryos from multiple killifish populations known to have evolved tolerance to environmental contaminants acting through the AHR pathway show reduced susceptibility to PCB126-induced *pparγ* expression compared to embryos from sensitive populations (Figures adapted with permission from Whitehead et al., 2017 (A), Reid et al., 2016 (B)). (A) Pairs of killifish populations sensitive (open circles) and tolerant (closed circles) to AHR pathway activation by PCB126 are color coded. Sensitive populations were collected from Block Island (BI), Flax Pond (FP), Sandy Hook (SH), and Kings Creek (KC), whereas tolerant populations were collected from New Bedford Harbor (NBH), Bridgeport (BP), Newark (NWK), and Elizabeth River (ER). (B) Relative mRNA expression data presented in Reid et al. (2016, Table S3) was used to calculate the change in *pparγ* expression between vehicle- and PCB126 (200 ng/L)-exposed killifish embryos from each population. Again, paired sensitive (open bars) and tolerant (paired bars) populations are color-coded. Statistical analysis presented in Reid et al. (2016) identifies a significant interaction of PCB126-exposure by population for *pparγ* expression across all population comparisons (Table S3, p = 0.0008).

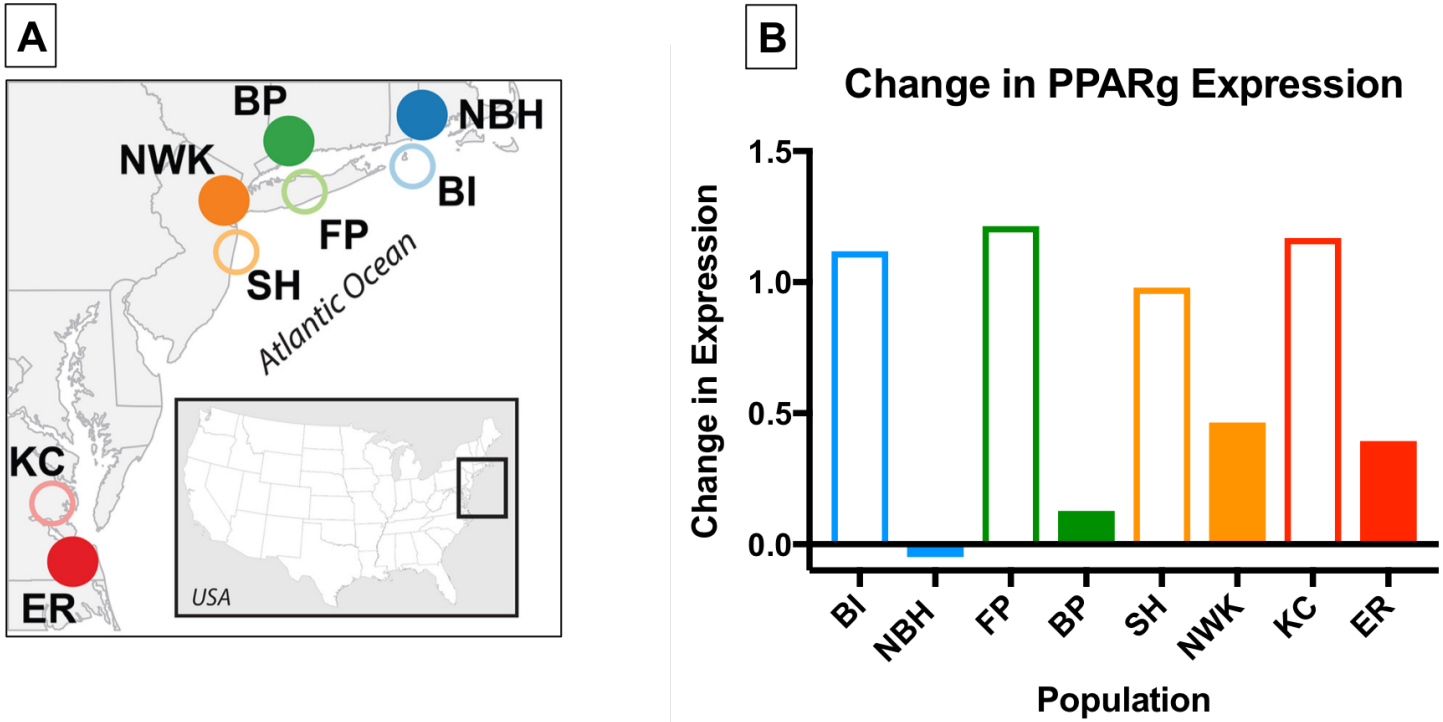


Figure 4.6. Mean (standard error, SEM) caudal fin deformity score for 10 dpf embryos following exposure to DMSO (0.01 %) and TBT at Low (5 nM), Medium (50 nM) and High (100 nM) concentrations. Deformity score ranges from 0 to 3, with 0 = normal and 3 = severe deformity. Total N≥50, screened from two replicate experiments. The severity and prevalence of caudal fin deformities increased in a dose-dependent manner (two-way ANOVA, Tukey post-hoc comparison by treatment). The mean deformity score did not differ significantly between SC and NBH embryos (two-way ANOVA: $p = 0.435$). Statistically significant ($p \leq 0.05$) differences in gene expression between a chemical treatment group are noted with a red asterisk (*).

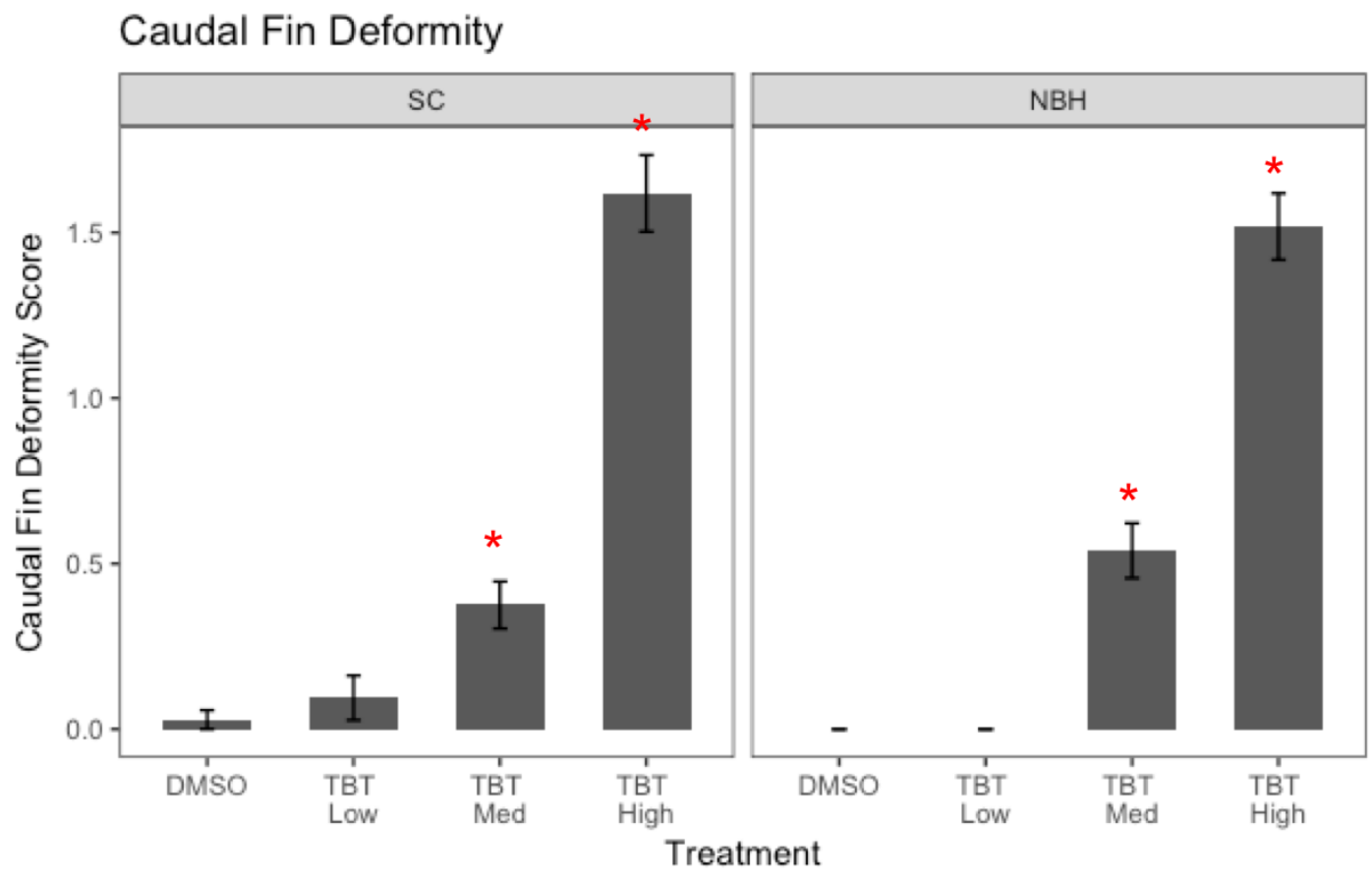


Figure 4.7. Mean (standard error, SEM) caudal fin deformity score for 10 dpf embryos following exposure to DMSO (0.01 %) and TBT at Medium (50 nM) and High (100 nM) concentrations alone and in combination with PCB126 (61 pM). PCB126 activates the AHR pathway, which, although known to cause axial skeleton deformities, does not appear to be the primary driver behind caudal fin deformities observed in this study. Deformity score ranges from 0 to 3, with 0 = normal and 3 = severe deformity. Total N≥50, screened during from three replicate experiments.

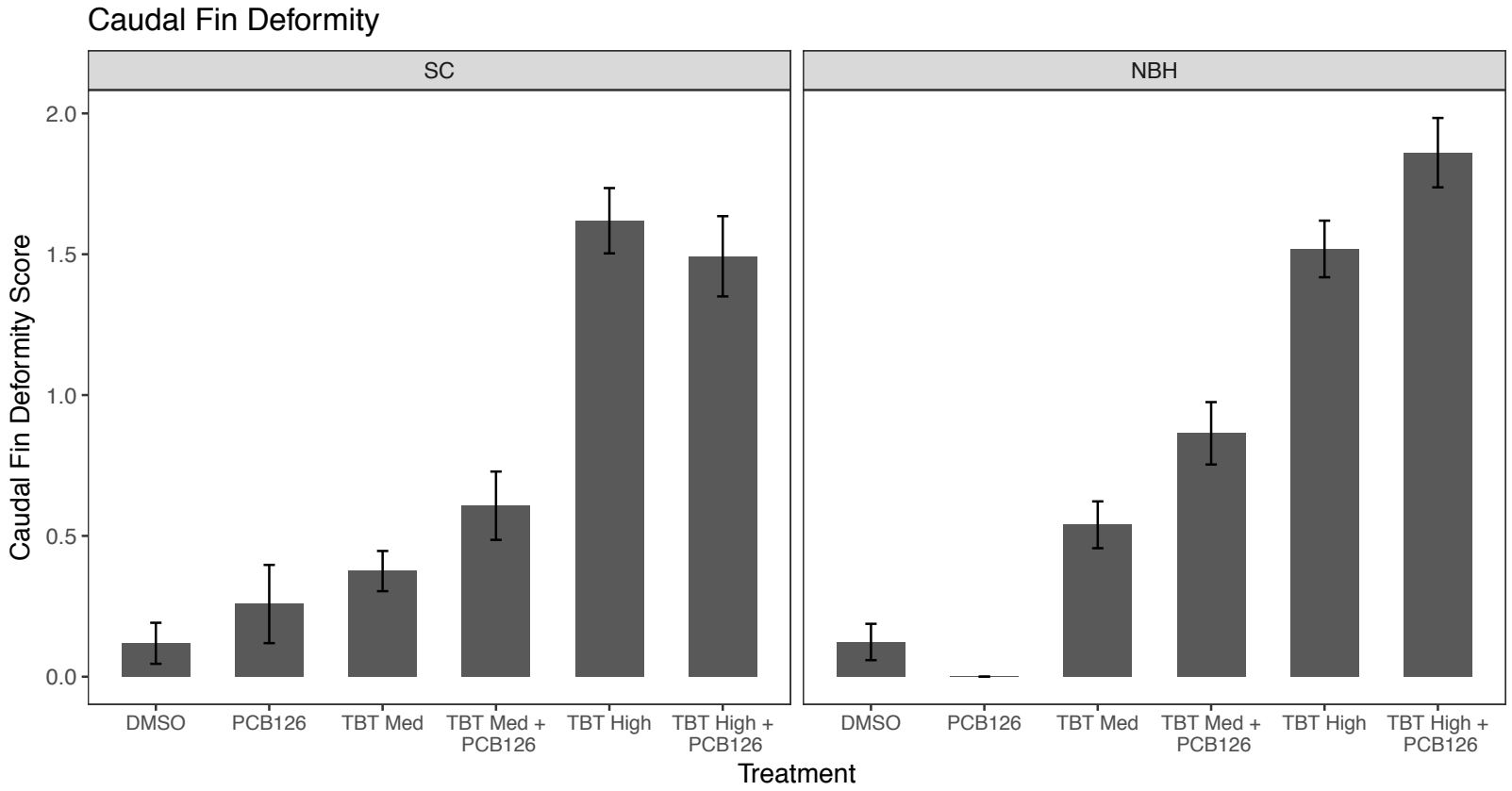


Figure 4.8a-f. To evaluate whether TBT-induced caudal fin deformities were associated with molecular markers of osteogenesis and chondrogenesis, we measured the relative expression of osx/sp7, col2a1a, and col2a1b by qPCR in pooled embryonic killifish (N=5 embryos per pool; N=4 pools per chemical treatment, per population from two experimental replicates referred to as "ELA") from SC and NBH. Specifically, osx/sp7 is a marker of osteogenesis, whereas col2a1a and col2a1b are markers of chondrogenesis. All threshold cycle (C_T) values were normalized using the Pfaffl method to naïve samples (pools of untreated SC and NBH embryos) and the constitutively expressed housekeeping gene rn18s. Gene expression is reported as a relative expression ratio. Relative expression of osx/sp7 (A), col2a1a (B), and col2a1b (C) was compared between vehicle-treated embryos from SC and NBH. Relative expression osx/sp7 (D), col2a1a (E), and col2a1b (F) was also measured in embryos exposed to TBT at Low (5 nM), Medium (50 nM), and High (100 nM) concentrations. Statistically significant ($p \leq 0.05$) differences in gene expression between a chemical treatment group and the population-specific control are noted with a red asterisk (*). *Osx/sp7* was significantly suppressed in SC embryos in the highest TBT treatment (p = 0.0108, one-way ANOVA, Dunnett's). Similar expression patterns were seen in SC embryos for *col2a1a* and *col2a1b*, with *col2a1b* being significantly suppressed at the highest TBT dose (p = 0.0386, one-way ANOVA, Dunnett's). While mean *osx/sp7* expression tended to be lower across all TBT treatments in NBH embryos relative to controls, the suppression was not statistically significant. No discernable treatment-related pattern of *col2a1a* and *col2a1b* was observed in NBH embryos.

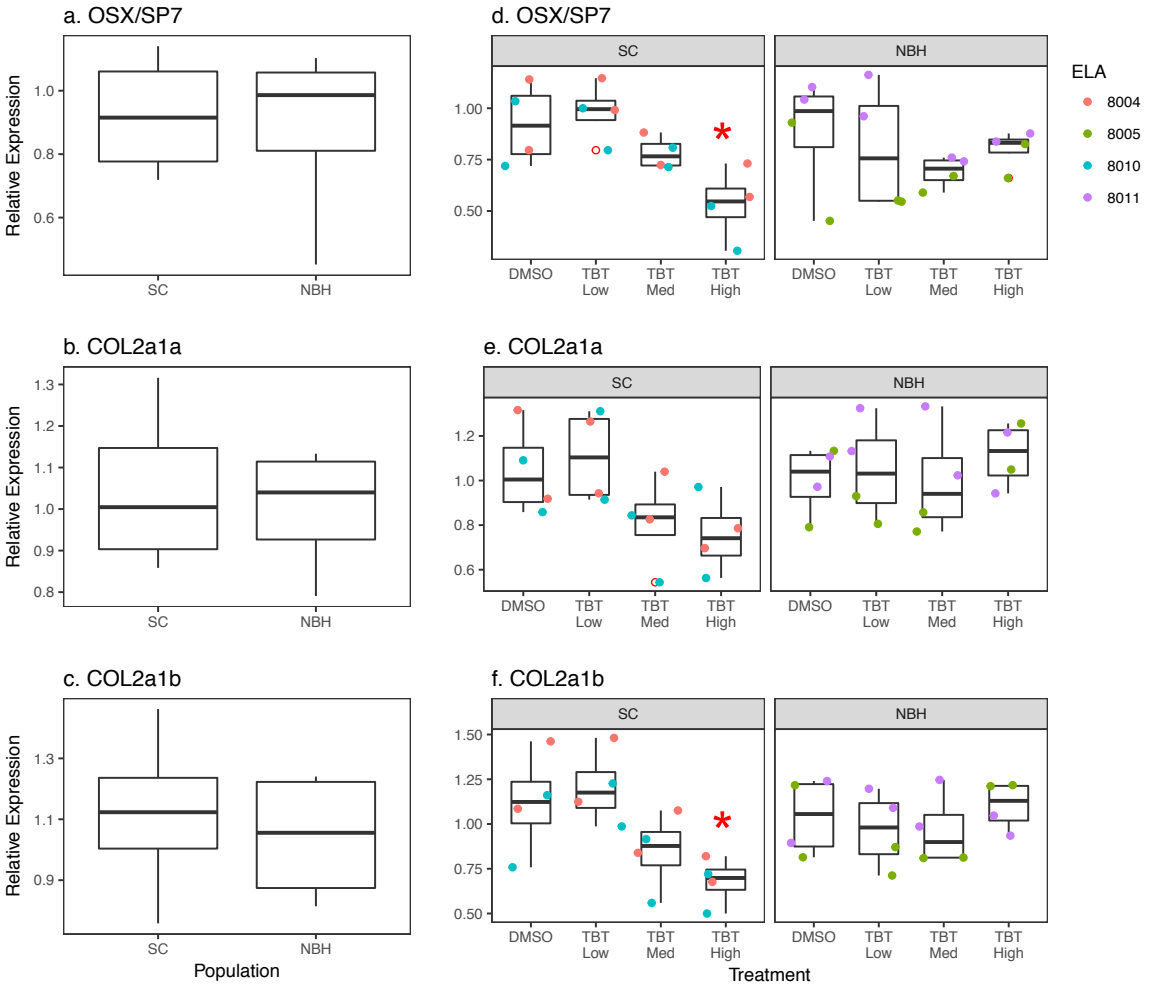

a. OSX/SP7
b. COL2a1a
c. COL2a1b
d. OSX/SP7
e. COL2a1a
f. COL2a1b
Relative Expression
SC
NBH
Population
Treatment
DMSO
TBT Low
TBT Med
TBT High
ELA
8004
8005
8010
8011
*

Figure 4.9a-f. To evaluate whether co-exposure to TBT High (100 nM) and PCB126 (61 pM) changed molecular markers of osteogenesis and chondrogenesis relative to control (0.01% DMSO) or exposure to either chemical individually, we measured the relative expression of osx/sp7, col2a1a, and col2a1b by qPCR in pooled embryonic killifish (N=5 embryos per pool; N=6 pools per chemical treatment, per population from three experimental replicates referred to as "ELA") from SC and NBH. Specifically, osx/sp7 is a marker of osteogenesis, whereas col2a1a and col2a1b are markers of chondrogenesis. A manually-selected threshold was utilized to standardize threshold cycle (C_T) across multiple plates. All critical CT values were normalized using the Pfaffl method to naïve samples (pools of untreated SC and NBH embryos) and the constitutively expressed housekeeping gene rn18s. Gene expression is reported as a relative expression ratio. Relative expression of osx/sp7 (A), col2a1a (B), and col2a1b (C) was compared between vehicle-treated embryos from SC and NBH. Relative expression of osx/sp7 (D), col2a1a (E), and col2a1b (F) was also measured in embryos exposed to TBT and PCB126 alone and in combination. No significant changes in mRNA expression were observed for any gene, in any exposure group, for either population.

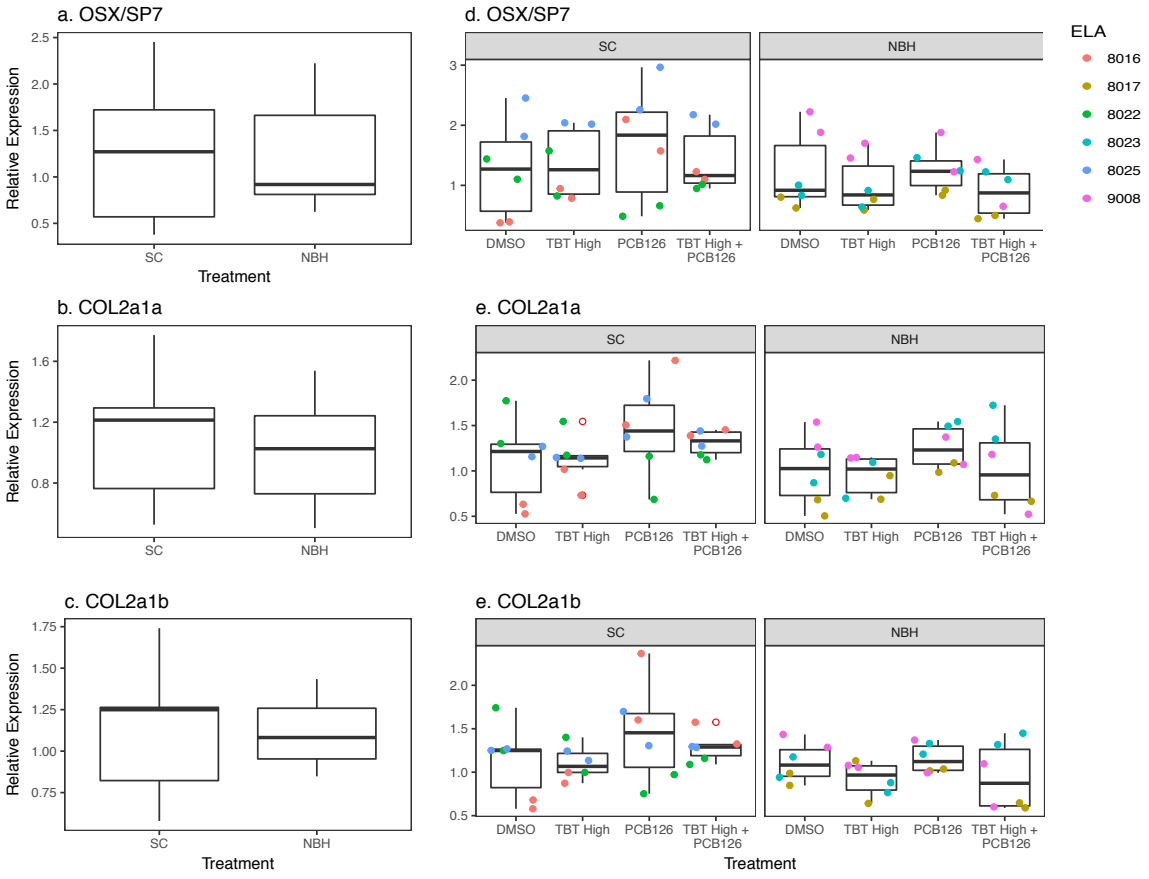


Figure 4.10. Mean (standard error, SEM) caudal fin deformity score for 10 dpf embryos following exposure to DMSO (0.01 %), S26948 (1 µM), and LG100268 (200 nM). Both S26948 and LG100268 are synthetic pharmaceutical compounds, which are selective agonists for PPARγ and RXR, respectively. Since TBT activates both PPARγ and RXR, these compounds were selected to investigate whether caudal fin deformities caused by TBT were a result of its activation of PPARγ, RXR, or both. DMSO and S26948 exposures were conducted in both SC and NBH embryos, whereas only NBH embryos were exposed to LG100268 due to limited SC embryo availability. Given similarity in TBT-induced caudal fin deformities across populations (and the absence of caudal fin deformities following S26948 exposure), we pooled phenotype score across populations. Total embryos per treatment group N≥30, screened from two replicate experiments.

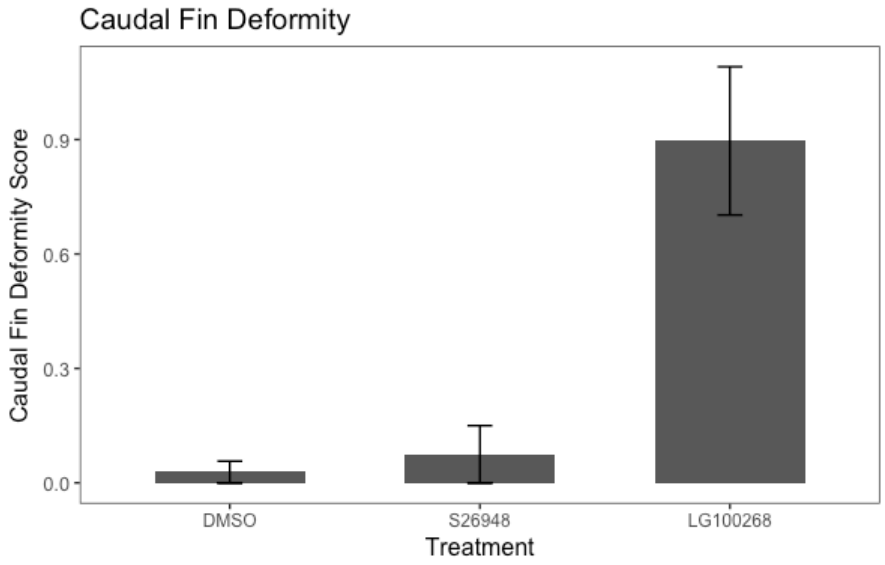


Table 4.1. Primer information, including sequence, product size, primer efficiency, annealing temperature, and the original citation for previously published primers. All novel primers were designed to overlap exon junctions to prevent potential amplification of contaminating genomic DNA Bactin, β-actin; rn18S, 18S ribosomal RNA; ef1a, elongation factor 1 alpha; cyp1a, cytochrome P450 1A; pparg, peroxisome proliferator-activated receptor gamma; fabp11a and fabp1b, fatty acid binding proteins 11a and 1b; osx/sp7, osterix (or Sp7 Transcription Factor); col2a1a and col2a1b, collagen, type II, alpha 1a and 1b.

Gene	Forward (5' --> 3')	Reverse (5' --> 3')	BP	Tm (°C)	Citation
B-actin	TGGAGAAGAGCTACGAGCTCC	CCGCAGGACTCCATTCCGAG	114	66	Oleksiak et al., 2011
rn18S	TGGTTAATTCCGATAACGAACGA	CGCCACTTGTCCCTCTAAGAA	97	65	Patel et al., 2006
EF1a	GGGAAAGGGCTCCTTCAAGT	ACGCTCGGCCTTCAGCTT	55	60	Bears et al., 2006
CYP1A	CTTTCACAATCCCACACTGCTC	GGTCTTTCCAGAGCTCTGGG	125	66	Oleksiak et al., 2011
PPARg	GGAAAGAGATGGAGACGCACAACC	CCGACGCATCGTCTGGGAACTTGG	102	66	Karchner, unpublished
FABP11a	GGCAAGCTTATTCAGAAACAGAGC	CCACGTCGCCCATTACGCATTTCGC	103	66	Karchner, unpublished
FABP1b	AGCCTTTCATGAAGGCCCTTGGTC	GTGGTGACCGTCACCTTGAAGTCG	115	66	Karchner, unpublished
COL2a1a	GGTGAGACCTGCGTCTACCCAAGC	CCATCCTGAGCATAGCTGAAGTGG	137	66	Karchner, unpublished
COL2a1b	GGGAGTCCTGCGTGAACCCCAAGC	CTGTCGTCACCATAACTGAAGTGG	136	66	Karchner, unpublished

CHAPTER 5: Conclusion

Overall, the aim of this work was to demonstrate how a holistic research approach to environmental health questions can inform both human and ecological risk of exposure to pollutants (**Figure 1.3**). Specifically, this research was conducted in the context of pollutants in New Bedford Harbor. I evaluated change in human exposure to PCBs through seafood consumption over time, and used an endemic sentinel species of fish (Atlantic killifish, *Fundulus heteroclitus*) as a toxicological model to study how activation of AHR and PPARγ pathways are affected by pollutants known to disrupt lipid homeostasis in vertebrates.

In Chapter 2, I collated and used data on PCB concentrations in NBH finfish and shellfish collected annually by MassDEP and US EPA to quantify spatial patterns and temporal trends of PCB concentrations in seafood from the harbor. PCB concentrations declined with distance from the original source of PCBs. As anticipated, PCB concentrations also declined over time in quahogs, commensurate with sediment dredging to reduce the overall PCB mass in NBH, whereas PCB concentrations in scup showed less change over time. Cancer risks posed to humans consuming NBH-harvested seafood followed trends in PCB concentrations measured in finfish and shellfish tissue. Ongoing monitoring of PCB levels in seafood is imperative to track progress of efforts to restore the environmental quality of NBH, and to ensure that the seafood consumption advisory for the harbor adequately reflects current conditions.

In Chapter 3, I presented the first data showing total Sn in NBH sediments, which occurred at concentrations above background levels in the region. Given the harbor's history as a working port, I believe that organotin-based marine paints commonly used during the latter half of the 20th century may have contributed to total Sn concentrations in NBH. Organotins, including TBT, should be measured in NBH sediment and fish to evaluate whether wildlife living in the harbor may be exposed to elevated levels of Sn.

In Chapter 3, I also provided a novel characterization of lipid homoeostasis in F2 adult killifish (i.e., killifish from a generation never directly exposed to or carrying a body burden of PCBs) of both sexes. F2 killifish from NBH, as well as a reference location, Scorton Creek (SC), Massachusetts, were exposed to TBT, PCB126, and PCB153 by IP injection for three days and lipid homeostasis was evaluated at the phenotypic (adiposomatic index and liver triglycerides) and molecular (AHR, PPARγ, and PPARα gene expression). NBH killifish had higher adiposomatic indices and liver triglyceride than those from SC. Prominent seasonal variability in liver triglyceride levels was identified in killifish from both populations. Furthermore, NBH killifish were less responsive to natural seasonal variability in adipose tissue and liver lipid levels. Together, these findings support the conclusion that alterations to lipid homeostasis in killifish from a heavily polluted, high-energy environment are heritable. Acute MDC exposure did not affect phenotypic measures of lipid homeostasis in either population, but did modulate PPAR-dependent gene expression pathways in SC male (but not female) killifish. To better understand the role of MDCs on lipid homeostasis in killifish, studies using longer-

term exposures, either through diet or repeated IP injection, are warranted. Additional metrics of lipid homeostasis, such as characterizing the lipid profile in killifish blood and liver, would provide a more complete picture of whole-body lipid homeostasis. Transcriptomic analysis of mRNA from killifish liver and adipose tissue would offer better resolution to evaluate molecular differences between SC and NBH killifish, which may explain the differences in lipid content we observed between the two populations. These studies should also be repeated using other populations of AHR-sensitive and -tolerant killifish to determine whether these effects are seen in killifish living in other polluted waterways.

In Chapter 4, I investigated effects of the effects of exposure to TBT and PCB126, alone and in combination, on embryonic F2 killifish from NBH and SC to evaluate the potential for molecular cross-talk between PPARγ and AHR transcriptional pathways. The data showed that the PPARγ pathway was largely recalcitrant to MDC exposure in 10-day old killifish embryos, suggesting that PPARγ signaling did not play a biologically significant role at this point in their embryological development. I also inferred that adipose tissue may not have developed by this time. Unexpectedly, PCB126 induced *pparγ* mRNA expression in SC, but less so in NBH, killifish embryos. This finding was corroborated both by my research in **Chapter 3**, as well as by an independent study of other pairs of AHR-sensitive and -tolerant killifish populations. TBT caused caudal fin deformities in a dose-dependent manner, which appeared to be induced by inappropriate activation of the RXR pathway by TBT. Gene expression analyses suggested that both osteogenesis and

chondrogenesis were impacted by TBT exposure, perhaps through effects on a shared precursor cell. With findings from my research in mind, additional studies should be performed to characterize adipose tissue, bone and cartilage development in killifish throughout embryogenesis and larval life stages. *In vivo* imaging and transcriptomic analyses of individual, as opposed to pooled, embryos would greatly enhance the ability to identify effects of TBT and PCBs on lipid homeostasis and skeletal development at phenotypic and molecular levels.

Collectively, this work provides an extensive investigation into the effect of marine contaminants on metabolic disruption and the PPAR pathways in an important toxicological model, *F. heteroclitus*. It also brings data on PCB concentrations in NBH seafood to the scientific literature.

Limitations

Limitations specific to each study are discussed in the respective chapters. Overall, this work was designed to draw connections between human and ecological exposure to environmental pollutants. I recognize that taking this perspective necessarily involves assumptions, which have some inherent uncertainties. For example, to interpret altered lipid homeostasis in NBH killifish in the context of human health, I surmise that alterations in lipid homeostasis caused by chronic MDC exposure can be extrapolated to other species. One way to think about the connection between killifish physiology and human health is through the lens of comparative biology. For example, teleost fish and

mammals share many of the same nuclear receptor pathways that govern lipid homeostasis and white adipocyte function is comparable across taxa. However, warm-blooded animals have distinct adipose physiology, including brown adipose tissue, that enables their thermoregulation. In light of these differentiators, literature carefully documenting commonalities and differences in lipid homeostasis of zebrafish and mammals can be leveraged to draw conclusions about how findings in killifish relate to mammalian physiology.

This research is the first significant investigation of metabolic disruption and PPAR pathways in killifish, which is both a strength and limitation. In order to contextualize the findings, I drew heavily upon literature conducted in vitro and in other toxicological models (e.g., zebrafish, medaka, mice, rats). Generally, my findings concur with previous studies in other vertebrate species. For example, evidence of disruption to lipid homeostasis in adult F2 killifish from NBH is consistent with reports of transgenerational changes (e.g., those changes for which exposures in one generation can lead to persistent effects in subsequent generations) caused by exposure to environmental MDCs in other toxicological models. However, little is known about basal differences in measures of lipid homeostasis between geographically distinct killifish populations living in uncontaminated locations. Further experimentation is needed so that my findings can be better interpreted in the context of natural variability in lipid homeostasis between killifish populations from both non-polluted/polluted and energy-rich/poor environments.

This research is the first to examine adipose and, by serendipity, bone development in killifish. Therefore, I needed to rely upon knowledge of zebrafish development to interpret the results. Zebrafish develop much more rapidly than killifish and do not display discernable adipose tissue until 10 to 30 days post-fertilization. Therefore, the lack of PPARγ responsiveness to the well-known environmental PPARγ ligand, TBT, is more likely a function of the timing of embryo development in my study than the fact that killifish are not affected by TBT. Similarly, activation of the PPARγ-RXR pathway by TBT is associated with suppression of osteogenesis and impaired bone remodeling in vitro and in vivo due to altered fate of mesenchymal stromal cells. While I expect that this may be a mechanism for caudal fin deformities observed in TBT-exposed embryos, a more strongly powered study is needed to confirm the trend in reduced osterix and collagen gene expression. However, the precise timing of bone and cartilage formation in killifish has not been previously characterized, which required that I compare my findings to those seen in other fish and amphibious models. These examples highlight the need for more research on lipid homeostasis in adult killifish and adipose and bone development in embryonic killifish, ideally at later developmental stages than 10 dpf.

An important negative result of this research was the lack of effect of S26948, a novel PPARγ ligand, on lipid homeostasis or caudal fin development. I tested S26948 to address the current lack of confirmed PPARγ-specific agonist in fish species. Rosiglitazone, a thiazolidinedione, is commonly used in mammalian studies exploring PPARγ activation by environmental and other therapeutic ligands. However, because

there is debate over its efficacy in piscine models, I selected S26948, a non-thiazolidinedione-based agonist as a potential positive control. Like previous mammalian PPARγ ligands, S26948 appeared to be non-efficacious in killifish. While the lack of a true positive control in these studies presents a challenge because TBT elicits toxicological effects through multiple molecular mechanisms (e.g., lipid and bone disruption by activation of both PPARγ and RXR, and reproductive effects associated with aromatase inhibition), the results add to the growing database of ligand structures that are not compatible with PPARγ activation in fish and may open new avenues for investigation.

Public Health and Ecological Implications

There is growing awareness in a variety of scientific disciplines, including environmental and ecological science, public health, veterinary science, medicine and economics, that a holistic research approach is required to address pressing environmental issues facing humanity in the 21st century. As a result, several initiatives such as One Health and the Planetary Health Alliance have emerged to improve the integration of environmental and human health research efforts. Interdisciplinary research efforts strengthen research efforts that are mutually beneficial to environmental and human health sciences. The research studies presented herein illuminate the role that fish can play in bridging the gap between how scientists and policy makers consider contaminants in aquatic environments from the perspective of their effects on wildlife and humans.

This research was conducted in the context of New Bedford Harbor, Massachusetts, where centuries of industry have left lasting environmental impacts on the region, including both its human and wildlife inhabitants. Residents of surrounding communities, regulators, public policy makers, and scientific researchers are in a perpetual negotiation for how best to: protect human and wildlife health to the greatest extent possible despite contamination in the region, remediate affected portions of the harbor, study the effects of pollution on wildlife and humans, and do all of this within the context of available financial and scientific resources. People living in communities surrounding the harbor have varying degrees of awareness of the presence of and health effects associated with contaminants in the region. My analysis of PCB levels in finfish and shellfish have implications for communicating risks of consuming locally-harvested seafood from NBH to members of surrounding communities. In particular, my research is relevant for those who may be subsistence fishing from the harbor. I urge regulators and policy makers to improve efforts to communicate the health benefits and risks associated with seafood consumption, specifically in the context of disadvantaged members of communities surrounding NBH. Furthermore, I challenge myself and other members of the scientific research community to play an active role in these efforts by both engaging in research translation efforts. For instance, those of us with expertise in these areas should meet with regulators and policy makers to ensure that the results of this research are incorporated into messaging about fish consumption advisories, and with community members who may be directly affected by our research.

The results of my research establish killifish as an important toxicological model for studying MDCs in aquatic environments. The effects of MDCs on metabolic homeostasis in wildlife is less well studied than in humans and biomedically-relevant animal models, and even in these cases is not well understood. However, cellular metabolism and systemic metabolic homeostasis is fundamental to all life and perturbations to metabolic health have the potential to adversely impact wildlife at the individual and population level. Given that killifish live in urban waterways near major US population centers, their exposures to contaminants and the ensuing physiological effects reflect the broader risks from environmentally-relevant, chronic exposure to pollutants. Thus, research in killifish can inform the quest for better human health. My research on lipid homeostasis in killifish can also be extrapolated to commercially important seafood species, such as tilapia. Impaired lipid homeostasis caused by contaminants in the environment or their feed could impact nutritionally important lipids for humans (e.g., omega-3 fatty acids), impacting how to balance public health messaging about the risks and benefits of seafood consumption. Furthermore, optimal metabolic health of the fish is central to economic viability in the aquaculture industry. Disruptions to metabolic health could lead to unnecessary expenses in animal feed or veterinary care, or a reduction in revenue generated from degraded fish quality. Understanding how environmental pollutants impact lipid homeostasis could inform improvements to aquaculture practices.

In summary, further investment in interdisciplinary environmental health research is needed to bridge the gap between ecological and human health disciplines. Sentinel

species, such as killifish, have a critical role to play in bridging this gap. Initiatives such as One Health and the Planetary Health Alliance offer a narrative for scientists to contextualize their research. Regulatory and policy efforts must prioritize a holistic approach to addressing environmental health issues. For instance, human and ecological risk assessments should be conducted in an integrated fashion, and communication of public policies should convey environmental and public health implications in tandem for more effective advocacy and better communication to non-technical audiences. This body of research is a step in the right direction.

APPENDIX

Table A1: Summary statistics for quahogs and scup describing sampling frequency, composition of composite samples, and the lipid content, $\sum PCB_{WW}$ and $\sum PCB_{LN}$ in seafood samples by NBH seafood management Area and year. Abbreviations: GM – geometric mean; SD – standard deviation; mg/kg ww – milligrams PCBs per gram seafood tissue on a wet weight basis; na – not available; ns – not sampled.

Species	Area	Year	2003	2004	2005	2006	2007	2008	2009
Quahog (*Mercenaria mercenaria*)	1	No. Locations Sampled (Sampled/Total)	5/5	5/5	5/5	5/5	2/5	0/5	0/5
		No. Individuals per Composite Sample (Mean (Min, Max))	14 (13,15)	15 (13,19)	12 (12,12)	12 (12,12)	12 (12,12)	na	na
		Body Lipids, % (GM (Mean, SD))	0.21 (0.22, 0.08)	0.37 (0.37, 0.08)	0.24 (0.24, 0.04)	0.46 (0.47, 0.10)	0.51 (0.51, 0.07)	na	na
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	1.42 (1.58, 0.82)	1.60 (2.28, 2.25)	1.61 (1.88, 1.25)	1.61 (1.93, 1.32)	2.75 (3.64, 3.38)	na	na
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	676 (716, 289)	439 (564, 441)	667 (778, 474)	351 (409, 259)	541 (675, 570)	na	na
	2	No. Locations Sampled (Sampled/Total)	5/9	5/9	5/9	5/9	6/9	6/9	6/9
		No. Individuals per Composite Sample (Mean (Min, Max))	13 (12,14)	13 (7,15)	12 (12,12)	12 (12,12)	12 (12,12)	12 (12,12)	12 (12,12)
		Body Lipids, % (GM (Mean, SD))	0.15 (0.16, 0.06)	0.20 (0.23, 0.13)	0.21 (0.22, 0.05)	0.30 (0.31, 0.09)	0.39 (0.40, 0.11)	0.20 (0.21, 0.06)	0.16 (0.17, 0.05)
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	0.17 (0.28, 0.35)	0.19 (0.28, 0.29)	0.22 (0.34, 0.31)	0.22 (0.28, 0.20)	0.17 (0.24, 0.25)	0.12 (0.14, 0.11)	0.10 (0.12, 0.10)
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	113 (152, 145)	97 (136, 120)	103 (149, 116)	73 (85, 51)	45 (54, 39)	59 (71, 45)	60 (67, 37)
	3	No. Locations Sampled (Sampled/Total)	5/9	5/9	5/9	5/9	5/9	4/9	4/9
		No. Individuals per Composite Sample (Mean (Min, Max))	15 (12,19)	17 (13,20)	12 (12,12)	12 (12,12)	12 (12,12)	12 (12,12)	12 (12,12)
		Body Lipids, % (GM (Mean, SD))	0.14 (0.15, 0.06)	0.19 (0.19, 0.05)	0.21 (0.22, 0.05)	0.34 (0.34, 0.05)	0.42 (0.45, 0.21)	0.12 (0.13, 0.04)	0.22 (0.22, 0.03)
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	0.05 (0.05, 0.02)	0.05 (0.06, 0.01)	0.05 (0.06, 0.05)	0.08 (0.11, 0.09)	0.06 (0.07, 0.03)	0.05 (0.05, 0)	0.04 (0.04, 0)
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	35.12 (35.57, 6.46)	28.64 (29.83, 9.13)	24.77 (29.21, 21.89)	22.46 (31.73, 26.08)	14.79 (16, 7.76)	36.47 (37.14, 7.77)	16.34 (16.49, 2.59)
Scup (*Stenotomus chrysops*)	2	No. Locations Sampled (Sampled/Total)	5/5	5/5	5/5	5/5	5/5	5/5	5/5
		No. Individuals per Composite Sample (Mean (Min, Max))	3 (3,3)	5 (5,5)	5 (5,5)	5 (5,5)	5 (4,5)	5 (5,5)	5 (5,5)
		Muscle Lipids, % (GM (Mean, SD))	1.09 (1.11, 0.28)	1.2 (1.35, 0.67)	1.11 (1.15, 0.3)	1.29 (1.57, 1.31)	1.13 (1.19, 0.46)	0.81 (0.82, 0.2)	1.16 (1.17, 0.13)
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	0.67 (0.78, 0.4)	0.66 (0.8, 0.55)	0.48 (0.55, 0.31)	0.33 (0.39, 0.24)	0.18 (0.21, 0.13)	0.28 (0.34, 0.25)	0.4 (0.45, 0.23)
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	62.16 (70.11, 37.29)	55.3 (57.9, 19.94)	43.38 (46.93, 19.56)	25.23 (33.55, 27.88)	15.64 (18.58, 11.15)	34.74 (41.97, 28.29)	34.59 (37.68, 16.36)
	3	No. Locations Sampled (Sampled/Total)	5/5	5/5	5/5	5/5	5/5	5/5	5/5
		No. Individuals per Composite Sample (Mean (Min, Max))	3 (3,3)	5 (5,5)	5 (5,5)	5 (5,5)	5 (5,5)	5 (5,5)	5 (5,5)
		Muscle Lipids, % (GM (Mean, SD))	0.91 (0.92, 0.14)	1.64 (1.64, 0.09)	1.07 (1.15, 0.55)	1.82 (1.92, 0.74)	1 (1.02, 0.21)	1.1 (1.13, 0.24)	0.98 (1.05, 0.41)
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	0.24 (0.26, 0.11)	0.31 (0.32, 0.05)	0.45 (0.57, 0.46)	0.19 (0.2, 0.08)	0.19 (0.22, 0.12)	0.21 (0.22, 0.08)	0.19 (0.2, 0.07)
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	26.6 (27.52, 8.68)	19.07 (19.33, 3.62)	42.07 (45.99, 19.28)	10.4 (10.55, 1.85)	19.16 (21.35, 11.2)	19.26 (19.77, 4.88)	18.98 (22.27, 12.03)

Species	Area	Year	2010	2011	2012	2013	2014	2015	2016
Quahog (*Mercenaria mercenaria*)	1	No. Locations Sampled (Sampled/Total)	0/5	4/5	0/5	5/5	5/5	3/5	1/5
		No. Individuals per Composite Sample (Mean (Min, Max))	na	14 (13,14)	na	13 (13,13)	13 (13,13)	12 (12,12)	12
		Body Lipids, % (GM (Mean, SD))	na	0.20 (0.2, 0.05)	na	0.27 (0.30, 0.13)	0.49 (0.49, 0.02)	0.16 (0.17, 0.07)	0.38
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	na	0.72 (0.80, 0.41)	na	0.52 (0.59, 0.31)	1.25 (1.35, 0.65)	0.55 (0.57, 0.20)	1.31
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	na	359 (412, 238)	na	189 (269, 233)	256 (279, 147)	333 (341, 90)	345
	2	No. Locations Sampled (Sampled/Total)	6/9	7/9	6/9	9/9	9/9	6/9	6/9
		No. Individuals per Composite Sample (Mean (Min, Max))	12 (12,13)	13 (13,16)	12 (12,13)	13 (13,13)	13 (13,13)	12 (12,12)	12 (12,12)
		Body Lipids, % (GM (Mean, SD))	0.36 (0.37, 0.09)	0.18 (0.19, 0.06)	0.26 (0.27, 0.07)	0.26 (0.26, 0.04)	0.22 (0.23, 0.07)	0.13 (0.13, 0.05)	0.22 (0.22, 0.02)
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	0.14 (0.18, 0.14)	0.10 (0.13, 0.12)	0.12 (0.14, 0.11)	0.09 (0.11, 0.08)	0.10 (0.12, 0.10)	0.07 (0.08, 0.05)	0.08 (0.10, 0.07)
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	39 (48, 38)	57 (74, 57)	45 (50, 29)	35 (391, 24)	45 (53, 38)	58 (74, 60)	38 (44, 31)
	3	No. Locations Sampled (Sampled/Total)	4/9	4/9	4/9	9/9	9/9	4/9	4/9
		No. Individuals per Composite Sample (Mean (Min, Max))	13 (12,13)	13 (12,14)	14 (9,23)	13 (13,13)	13 (13,13)	12 (12,12)	12 (12,12)
		Body Lipids, % (GM (Mean, SD))	0.38 (0.41, 0.17)	0.2 (0.21, 0.09)	0.3 (0.31, 0.09)	0.29 (0.31, 0.11)	0.26 (0.28, 0.1)	0.17 (0.20, 0.12)	0.30 (0.3, 0.06)
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	0.05 (0.05, 0.01)	0.04 (0.04, 0.01)	0.05 (0.05, 0.01)	0.05 (0.05, 0.01)	0.04 (0.04, 0.01)	0.04 (0.04, 0.01)	0.04 (0.04, 0.01)
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	13.26 (13.47, 2.67)	19.72 (20.33, 5.59)	16.96 (17.35, 4.36)	16.07 (16.86, 6.23)	16.56 (17.98, 9.37)	23.31 (24.47, 8.67)	14.18 (14.29, 1.98)
Scup (*Stenotomus chrysops*)	2	No. Locations Sampled (Sampled/Total)	5/5	5/5	5/5	5/5	5/5	ns	ns
		No. Individuals per Composite Sample (Mean (Min, Max))	5 (5,5)	5 (4,5)	5 (5,5)	5 (5,5)	5 (5,5)	ns	ns
		Muscle Lipids, % (GM (Mean, SD))	0.53 (0.58, 0.3)	1.14 (1.16, 0.24)	1.67 (1.8, 0.77)	0.9 (0.91, 0.14)	1.57 (1.59, 0.24)	ns	ns
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	0.77 (0.83, 0.35)	0.54 (0.62, 0.38)	0.82 (1.06, 0.8)	0.36 (0.43, 0.26)	0.49 (0.51, 0.18)	ns	ns
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	145.57 (163.36, 76.83)	47.34 (56.1, 39.8)	49.25 (53.69, 22.88)	39.64 (48.45, 29.24)	30.94 (31.71, 7.48)	ns	ns
	3	No. Locations Sampled (Sampled/Total)	5/5	5/5	5/5	5/5	5/5	ns	ns
		No. Individuals per Composite Sample (Mean (Min, Max))	5 (5,5)	5 (5,6)	5 (5,6)	5 (4,5)	5 (4,5)	ns	ns
		Muscle Lipids, % (GM (Mean, SD))	0.46 (0.49, 0.18)	1.58 (1.88, 1.45)	1.61 (1.64, 0.38)	0.87 (0.89, 0.24)	1.42 (1.45, 0.33)	ns	ns
		$\sum PCB_{WW}$, mg/kg ww (GM (Mean, SD))	0.17 (0.18, 0.07)	0.19 (0.23, 0.14)	0.25 (0.28, 0.12)	0.2 (0.33, 0.41)	0.19 (0.21, 0.09)	ns	ns
		$\sum PCB_{LN}$, mg/kg ww (GM (Mean, SD))	36.06 (39.57, 19.85)	12.31 (16.37, 11.72)	15.86 (17.01, 7.74)	23.13 (32.53, 33.64)	13.24 (15.33, 8.65)	ns	ns

Table A2. Historic concentrations of Aroclor 1254 (mg/kg ww*) in shellfish and bottom-dwelling finfish harvested from NBH between 1976 and 1979 (data compiled from: Santos, 1978; Kolek & Ceurvels, 1981; and Nisbet & Reynolds, 1984). Instances where Statistic = "N/A" signify that only one Aroclor 1254 measurement was reported for a given species. *Note: Every effort was made to verify whether historic reports presented Aroclor 1254 as mg/kg ww or dw; data presented herein are assumed to be ww.

Shellfish, Bivalves		Statistic	Area 1	Area 2	Area 3	Overall
Species						
Mussles	*Mytilus edulis*	N/A	3.7	--	--	--
Oyster	*Crassostrea virginica*	N/A	15.8	--	--	--
Quahog	*Mercinaria mercinaria*	Min	1.6	0.2	0.3	0.2
		Mean	2.6	1.0	0.4	1.1
		Median	2.1	0.7	0.4	0.7
		Max	4.0	3.3	0.6	4.0
		N	3	12	5	20
Soft-shelled clam	*Mya arenaria*	Min	14.6	--	--	14.6
		Mean	29.7	--	--	29.7
		Median	22.0	--	--	22.0
		Max	53.0	--	--	53.0
		N	7	--	--	7
Shellfish, Bivalves	All Species	Min	1.6	0.2	0.3	0.2
		Mean	21.0	1.0	0.4	8.6
		Median	21.0	0.7	0.4	1.3
		Max	53.0	3.3	0.6	53.0
		N	11	12	5	29

Finfish, Bottom-dwelling		Statistic	Area 1	Area 2	Area 3	Overall
Species						
Black sea bass	*Centropristis striata*	N/A	--	--	0.4	--
Fourpsot flounder	*Hippoglossina oblonga*	N/A	--	--	0.8	--
Scup	*Stenotomus chrysops*	Min	2.3	6.1	0.0	0.0
		Mean	4.2	9.6	0.4	4.8
		Median	4.2	11.4	0.0	4.2
		Max	6.1	11.4	1.3	11.4
		N	2	3	3	8
Summer flounder	*Paralichthys dentatus*	Min	2.1	0.2	0.3	0.2
		Mean	6.1	5.1	2.2	4.5
		Median	6.1	7.1	1.2	4.0
		Max	10.0	7.9	4.0	10.0
		N	2	3	2	7
Tautog	*Tautoga onitis*	Min	--	0.1	0.1	0.1
		Mean	--	1.3	0.7	1.0
		Median	--	1.2	0.7	0.9
		Max	--	4.6	1.1	4.6
		N	--	9	7	16
Window pane flounder	*Scophthalmus aquosus*	Min	5.4	--	3.1	3.1
		Mean	9.5	--	3.1	6.9
		Median	8.8	--	3.1	5.4
		Max	14.3	--	3.1	22.0
		N	3	--	2	5
Winter flounder	*Pseudopleuronectes americanus*	Min	6.0	0.0	0.2	0.0
		Mean	10.4	5.6	3.9	5.6
		Median	8.1	5.6	1.0	3.9
		Max	22.0	11.0	20.0	22.0
		N	7	5	19	31
Finfish, Bottom-dwelling	All Species	Min	2.1	0.0	0.0	0.0
		Mean	8.7	4.2	2.6	4.3
		Median	7.9	2.6	0.9	1.6
		Max	22.0	11.4	20.0	22.0
		N	14	20	35	69

Table A3. Exposure and dose-response values used by US EPA to evaluate human cancer risk from consuming NBH-harvested seafood (adapted from US EPA, 2015: Third Five-Year Review Report, Appendix D (Risk Assessment Updates)).

Receptor	Age (yrs)	Exposure Condition	IR	FI	EF	ED	BW	AT-c	SF
Adult	16-70	CTE	0.227	1	12	55	70	25550	2.0
		RME	0.227	1	52	55	70	25550	2.0
Young Child	1-6	CTE	0.114	1	12	5	15	25550	2.0
		RME	0.114	1	52	5	15	25550	2.0

CTE = Central Tendancy Exposure

RME = Reasonable Maximum Exposure

IR = Ingestion Rate (kg/meal)

FI = Fraction Ingestion from site (assumed to be 1, unitless)

ED = Exposure Duration (years)

EF = Exposure Frequency (meals/year)

BW = Body Weight (kg)

AT-c = Averaging Time, cancer (days, 70 years * 365 days/yr = 25,550 days)

SF= Oral cancer slope factor $((mg/kg/day)^{-1})$

Table A4. Homologs as a percent of $\sum PCB_{LN}$ by weight in NBH quahogs and scup, and Aroclors (Frame et al., 1996) discharged to NBH. Homolog groups are denoted by number of chlorines (e.g., Homolog 2 represents di-chlorobiphenyls). The contribution of each homolog to $\sum PCB_{LN}$ and Aroclor mixtures increases as cell shading changes from green to red. ND denotes homologs not detected in an Aroclor mixture.

Homolog	Quahogs			Scup		Aroclors		
	Area 1	Area 2	Area 3	Area 2	Area 3	1016	1242	1254
1	0.1	0.5	1	0.1	0.2	0.7	0.8	ND
2	1.6	2.3	4.3	0.6	1.1	17.5	15	0.2
3	23.7	14.4	10.4	3.6	2.9	54.7	44.9	1.3
4	34.8	27.3	20.8	16	11.8	22.1	20.9	10.3
5	25.7	28.4	25	36.5	34.3	5.1	18.9	59.1
6	12.1	18.3	21.4	35.5	39.1	ND	0.3	26.8
7	1.7	5.5	10.1	6.1	8	ND	ND	2.7
8	0.3	2.3	4.9	1.2	1.9	ND	ND	0
9	0.1	0.7	1.5	0.3	0.5	ND	ND	0
10	0	0.2	0.5	0.1	0.1	ND	ND	ND

Table A5. Linear regression parameters describing temporal changes in PCB_{LN} (log mg/kg ww) by homolog group in each Area for quahogs (2003-2016) and scup (2003-2014). Homolog groups are denoted by number of chlorines (e.g., Homolog 2 represents di-chlorobiphenyls). Red boxes denote statistical significance for the slope of PCB_{LN} at alpha=0.05. Scup were sampled only in Areas 2 and 3; ns - not sampled. Trends in log-transformed PCB_{LN} are displayed graphically in Figures 3c-d.

Area	Homolog	Quahogs			Scup		
		Intercept	Slope	Slope p-value	Intercept	Slope	Slope p-value
1	1	-6.9394	0.0029	0.5583	ns	ns	ns
	2	57.3261	-0.0286	<0.0001	ns	ns	ns
	3	71.4826	-0.0354	<0.0001	ns	ns	ns
	4	60.6364	-0.0301	<0.0001	ns	ns	ns
	5	49.5989	-0.0246	<0.0001	ns	ns	ns
	6	35.7921	-0.0179	0.0005	ns	ns	ns
	7	18.4730	-0.0096	0.0280	ns	ns	ns
	8	8.4288	-0.0047	0.2040	ns	ns	ns
	9	14.2987	-0.0076	0.1408	ns	ns	ns
	10	-4.5477	0.0017	0.8202	ns	ns	ns
2	1	-6.2741	0.0026	0.5003	2.8015	-0.0022	0.6509
	2	15.6480	-0.0082	0.0165	-3.5056	0.0010	0.9106
	3	57.6683	-0.0289	<0.0001	-3.7878	0.0011	0.7685
	4	52.5674	-0.0264	<0.0001	23.9180	-0.0125	0.0212
	5	46.0575	-0.0231	<0.0001	15.2019	-0.0081	0.1593
	6	37.6348	-0.0190	<0.0001	5.1993	-0.0029	0.6416
	7	8.7383	-0.0048	0.0103	3.1276	-0.0019	0.7393
	8	-5.9781	0.0025	0.2099	-6.0217	0.0024	0.6373
	9	-3.1947	0.0011	0.7267	-3.6478	0.0011	0.8113
	10	-18.1095	0.0085	0.1736	-2.4265	0.0005	0.9449
3	1	21.3005	-0.0111	0.0171	-3.2995	0.0008	0.8868
	2	0.4421	-0.0007	0.8310	-18.3097	0.0083	0.3422
	3	28.2092	-0.0145	<0.0001	-7.0123	0.0027	0.5123
	4	39.0097	-0.0198	<0.0001	13.7936	-0.0076	0.0626
	5	38.2639	-0.0194	<0.0001	22.1024	-0.0117	0.0086
	6	25.7069	-0.0132	<0.0001	25.8190	-0.0134	0.0151
	7	17.8268	-0.0094	<0.0001	28.8851	-0.0149	0.0051
	8	15.0244	-0.0080	0.0008	1.3155	-0.0013	0.7657
	9	21.4718	-0.0112	0.0031	-4.2087	0.0014	0.7443
	10	21.3005	-0.0111	0.0951	-8.0544	0.0032	0.6373

Table A6. Linear regression parameters and Exposure Point Concentrations (EPCs) for $\sum PCB_{WW}$ (log mg/kg ww) in NBH quahogs and scup. Trends in log-transformed $\sum PCB_{WW}$ are shown graphically in Figure S3. EPCs for the "Present" time period represent the 95% upper confidence limit (UCL) on the mean of the three most recent years for which data are available for quahogs (2014-2016) and scup (2012-2014). All other EPCs are estimates of the geometric mean $\sum PCB_{WW}$ derived from the linear equations described herein.

	Parameter/ Time	Quahogs			Scup	
		Area 1	Area 2	Area 3	Area 2	Area 3
Linear Regression Parameters	Slope	-0.0352	-0.0343	-0.0115	0.0028	-0.0169
	Intercept	70.80	68.07	21.80	-5.88	33.29
	R^2	0.2338	0.1546	0.0699	0.0009	0.0589
Exposure Point Concentrations	Present	1.4290	0.1380	0.0467	0.9910	0.4010
	1980	11.4604	1.2922	0.1061	0.3810	0.6760
	2015	0.6706	0.0813	0.0420	0.4759	0.1732
	2025	0.2980	0.0369	0.0322	0.5071	0.1173
	2035	0.1324	0.0167	0.0247	0.5404	0.0795

Table A7: Human cancer risk calculations for PCB exposures associated with the consumption of quahogs and scup harvested from NBH. Lifetime average daily dose (LADD) was calculated for young children (1-6 years) and adults (16-70 years) using the exposure and dose-response values in Table A3 and the Exposure Point Concentrations (EPCs) for $\sum PCB_{WW}$ shown in Table A6. Excess Lifetime Cancer Risk (ELCR) was calculated using the oral cancer slope factor for total PCBs (2.0 $(mg/kg/day)^{-1}$; US EPA IRIS, 1989). Exposure scenarios represent the consumption of one (Central Tendency Exposure, CTE) or four (Reasonable Maximum Exposure, RME) meals of NBH quahogs and scup per month. Green values indicate acceptable risk levels (ELCR ≤ 1E-4), whereas those in red are unacceptable (ELCR > 1E-4).

Species	Date	Exposure Scenario	Area 1				Area 2				Area 3			
			Young Child		Adult		Young Child		Adult		Young Child		Adult	
			LADD	ELCR	LADD	ELCR	LADD	ELCR	LADD	ELCR	LADD	ELCR	LADD	ELCR
Quahogs	Present	CTE	2.55E-05	5.10E-05	1.20E-04	2.39E-04	2.46E-06	4.93E-06	1.16E-05	2.31E-05	8.33E-07	1.67E-06	3.91E-06	7.82E-06
	(2014-2016)	RME	1.11E-04	2.21E-04	5.19E-04	1.04E-03	1.07E-05	2.13E-05	5.01E-05	1.00E-04	3.61E-06	7.22E-06	1.70E-05	3.39E-05
	1980	CTE	2.05E-04	4.09E-04	9.60E-04	1.92E-03	2.31E-05	4.61E-05	1.08E-04	2.16E-04	1.89E-06	3.79E-06	8.89E-06	1.78E-05
		RME	8.86E-04	1.77E-03	4.16E-03	8.32E-03	9.99E-05	2.00E-04	4.69E-04	9.38E-04	8.21E-06	1.64E-05	3.85E-05	7.71E-05
	2015	CTE	1.20E-05	2.39E-05	5.62E-05	1.12E-04	1.45E-06	2.90E-06	6.81E-06	1.36E-05	7.50E-07	1.50E-06	3.52E-06	7.04E-06
		RME	5.19E-05	1.04E-04	2.43E-04	4.87E-04	6.29E-06	1.26E-05	2.95E-05	5.90E-05	3.25E-06	6.50E-06	1.52E-05	3.05E-05
	2025	CTE	5.32E-06	1.06E-05	2.50E-05	4.99E-05	6.58E-07	1.32E-06	3.09E-06	6.18E-06	5.75E-07	1.15E-06	2.70E-06	5.40E-06
		RME	2.30E-05	4.61E-05	1.08E-04	2.16E-04	2.85E-06	5.70E-06	1.34E-05	2.68E-05	2.49E-06	4.99E-06	1.17E-05	2.34E-05
	2035	CTE	2.36E-06	4.73E-06	1.11E-05	2.22E-05	2.99E-07	5.97E-07	1.40E-06	2.80E-06	4.41E-07	8.83E-07	2.07E-06	4.14E-06
		RME	1.02E-05	2.05E-05	4.81E-05	9.62E-05	1.29E-06	2.59E-06	6.07E-06	1.21E-05	1.91E-06	3.83E-06	8.98E-06	1.80E-05
Scup	Present	CTE	--	--	--	--	1.77E-05	3.54E-05	8.30E-05	1.66E-04	7.16E-06	1.43E-05	3.36E-05	6.72E-05
	(2012-2014)	RME	--	--	--	--	7.66E-05	1.53E-04	3.60E-04	7.19E-04	3.10E-05	6.20E-05	1.46E-04	2.91E-04
	1980	CTE	--	--	--	--	6.80E-06	1.36E-05	3.19E-05	6.38E-05	1.21E-05	2.41E-05	5.66E-05	1.13E-04
		RME	--	--	--	--	2.95E-05	5.89E-05	1.38E-04	2.77E-04	5.23E-05	1.05E-04	2.45E-04	4.91E-04
	2015	CTE	--	--	--	--	8.49E-06	1.70E-05	3.99E-05	7.97E-05	3.09E-06	6.18E-06	1.45E-05	2.90E-05
		RME	--	--	--	--	3.68E-05	7.36E-05	1.73E-04	3.45E-04	1.34E-05	2.68E-05	6.29E-05	1.26E-04
	2025	CTE	--	--	--	--	9.05E-06	1.81E-05	4.25E-05	8.50E-05	2.09E-06	4.19E-06	9.83E-06	1.97E-05
		RME	--	--	--	--	3.92E-05	7.84E-05	1.84E-04	3.68E-04	9.07E-06	1.81E-05	4.26E-05	8.52E-05
	2035	CTE	--	--	--	--	9.64E-06	1.93E-05	4.53E-05	9.05E-05	1.42E-06	2.84E-06	6.66E-06	1.33E-05
		RME	--	--	--	--	4.18E-05	8.36E-05	1.96E-04	3.92E-04	6.15E-06	1.23E-05	2.89E-05	5.77E-05

Figure A1: Concentrations in dioxin-like and non-dioxin like ∑PCB_{LN} (log mg/kg ww) in quahogs (a) and scup (b) in each seafood management Area over time. Dioxin-like congeners (n=12) include all non-*ortho* (PCBs 77, 81, 126 and 169) and mono-*ortho* (PCBs 105, 114, 118, 123, 156, 157, 167 and 189) substituted PCBs capable of causing dioxin-like toxicity. All other congeners (n=124) are considered non-dioxin-like. Data are reported as annual means of ∑PCB_{LN} for dioxin- and non-dioxin-like congeners measured in quahogs from each Area (n= 1 to 7 composite samples/Area/year), and in scup (n=5 composite samples/Area/year).

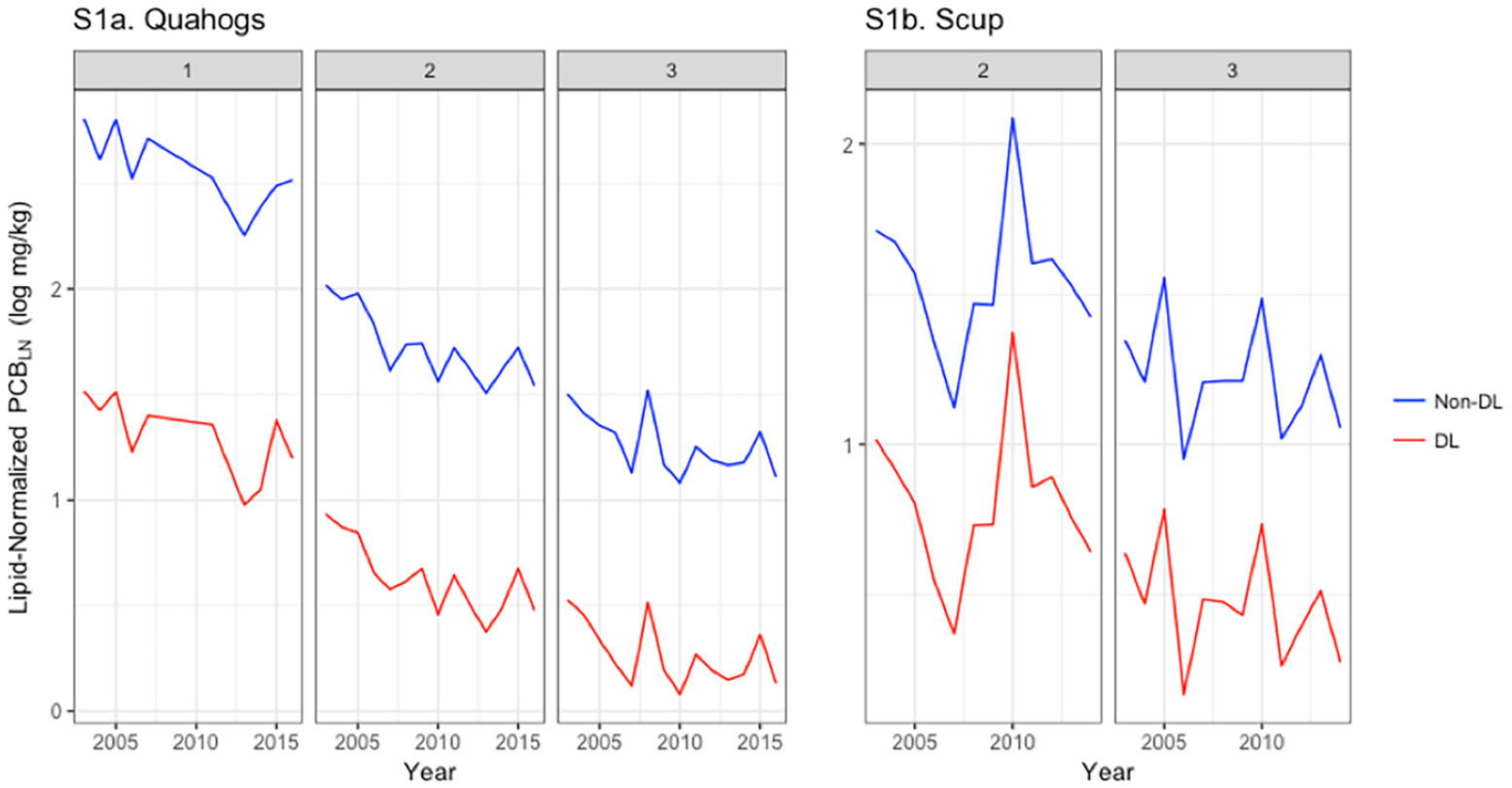


Figure A2: Linear regression analysis of changes in $\sum PCB_{LN}$ (log mg/kg ww) over time in each NBH seafood management Area in quahogs (a) and scup (b). Corresponding annual mean $\sum PCB_{LN}$ is presented in Figure 3a-b. N=1 to 7 composite samples/Area/year for quahogs and N=5 composite samples/Area/year for scup.

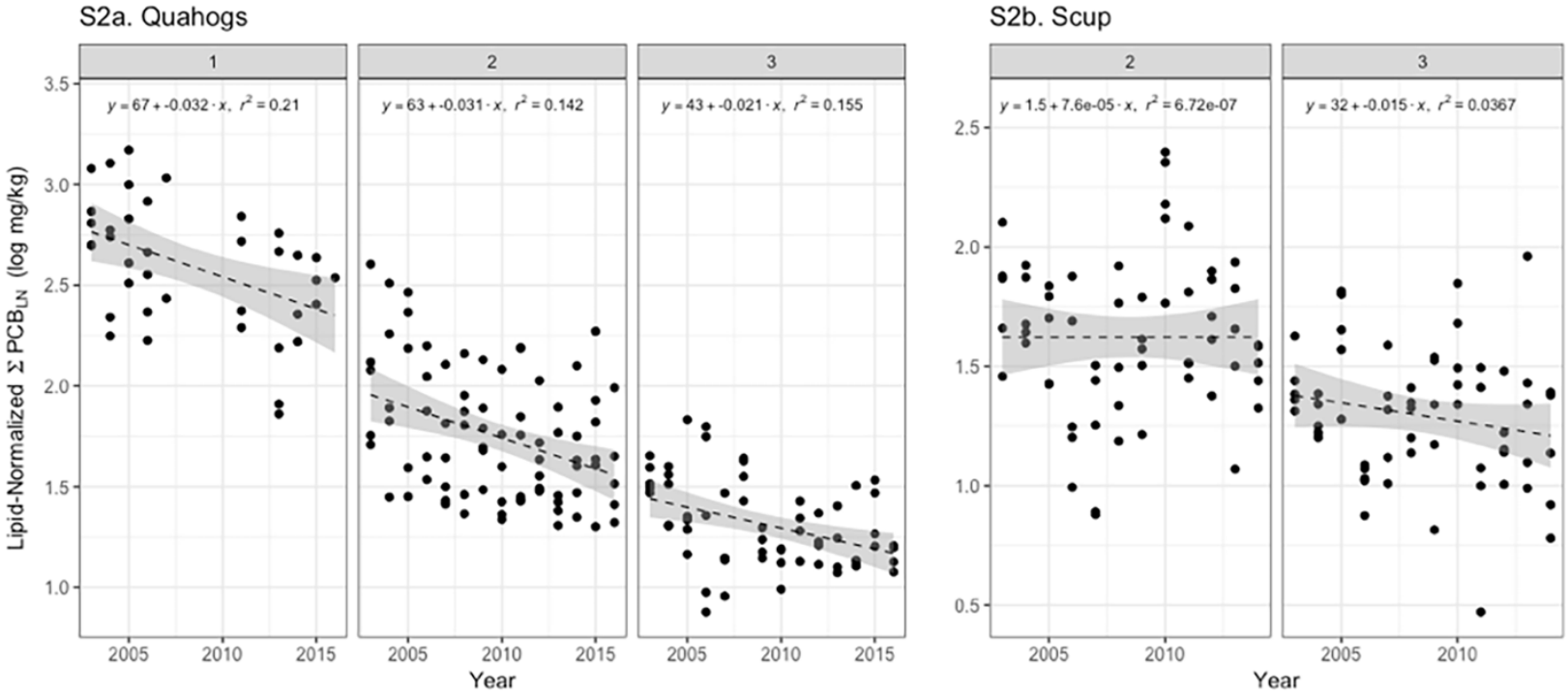


Figure A3. Linear regression analysis for changes in $\sum PCB_{WW}$ ((log mg/kg ww); linear regression parameters for $\sum PCB_{WW}$ (log mg/kg ww)) are presented in Table S6, and were used to estimate $\sum PCB_{WW}$ (mg/kg ww) concentrations in NBH quahogs (a) and scup (b) in 1980, 2015, 2025 and 2035. PCB estimates (mg/kg ww) at these four time points were used as Exposure Point Concentrations (EPCs) to evaluate changes in human cancer risk from PCB exposure associated with consuming NBH-harvested seafood over time. N=1 to 7 composite samples/Area/year for quahogs and N=5 composite samples/Area/year for scup.

BIBLIOGRAPHY

Alexander, D., Ganem, L., Fernandez-Salguero, P., Gonzalez, F., & Jefcoate, C. (1998). Aryl-hydrocarbon receptor is an inhibitory regulator of lipid synthesis and of commitment to adipogenesis. *Journal of Cell Science*, *111*(22), 3311–3322.

Ampleman, M. D., Martinez, A., DeWall, J., Rawn, D. F. K., Hornbuckle, K. C., & Thorne, P. S. (2015). Inhalation and Dietary Exposure to PCBs in Urban and Rural Cohorts via Congener-Specific Measurements. *Environmental Science & Technology*, *49*(2), 1156–1164. https://doi.org/10.1021/es5048039

Andersson, D. I., & Hughes, D. (2010). Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature Reviews. Microbiology*, *8*(4), 260–271. https://doi.org/10.1038/nrmicro2319

Antizar-Ladislao, B. (2008). Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environment International*, *34*(2), 292–308. https://doi.org/10.1016/j.envint.2007.09.005

Apschner, A., Schulte-Merker, S., & Witten, P. E. (2011). Not All Bones are Created Equal – Using Zebrafish and Other Teleost Species in Osteogenesis Research. *Methods in Cell Biology*, *105*, 239–255. https://doi.org/10.1016/B978-0-12-381320-6.00010-2

Arab, J. P., Arrese, M., & Trauner, M. (2018). Recent Insights into the Pathogenesis of Nonalcoholic Fatty Liver Disease. *Annual Review of Pathology: Mechanisms of Disease*, *13*(1), 321–350. https://doi.org/10.1146/annurev-pathol-020117-043617

Arimura, N., Horiba, T., Imagawa, M., Shimizu, M., & Sato, R. (2004). The Peroxisome Proliferator-activated Receptor γ Regulates Expression of the Perilipin Gene in Adipocytes. *Journal of Biological Chemistry*, *279*(11), 10070–10076. https://doi.org/10.1074/jbc.M308522200

Arsenescu, V., Arsenescu, R. I., King, V., Swanson, H., & Cassis, L. A. (2008). Polychlorinated biphenyl-77 induces adipocyte differentiation and proinflammatory adipokines and promotes obesity and atherosclerosis. *Environmental Health Perspectives*, *116*(6), 761–768. https://doi.org/10.1289/ehp.10554

Asaoka, Y., Terai, S., Sakaida, I., & Nishina, H. (2013). The expanding role of fish models in understanding non-alcoholic fatty liver disease. *Disease Models & Mechanisms*, *6*(4), 905–914. https://doi.org/10.1242/dmm.011981

Baker, A. H., Watt, J., Huang, C. K., Gerstenfeld, L. C., & Schlezinger, J. J. (2015). Tributyltin Engages Multiple Nuclear Receptor Pathways and Suppresses Osteogenesis in Bone Marrow Multipotent Stromal Cells. *Chemical Research in*

Toxicology, 28(6), 1156–1166. https://doi.org/10.1021/tx500433r

Baker, N. A., Karounos, M., English, V., Fang, J., Wei, Y., Stromberg, A., … Cassis, L. A. (2013). Coplanar polychlorinated biphenyls impair glucose homeostasis in lean C57BL/6 mice and mitigate beneficial effects of weight loss on glucose homeostasis in obese mice. *Environmental Health Perspectives*, *121*(1), 105–110. https://doi.org/10.1289/ehp.1205421

Baker, N. A., Shoemaker, R., English, V., Larian, N., Sunkara, M., Morris, A. J., … Cassis, L. A. (2015). Effects of Adipocyte Aryl Hydrocarbon Receptor Deficiency on PCB-Induced Disruption of Glucose Homeostasis in Lean and Obese Mice. *Environmental Health Perspectives*, *123*(10), 944–950. https://doi.org/10.1289/ehp.1408594

Baker, T. R., Peterson, R. E., & Heideman, W. (2013). Early dioxin exposure causes toxic effects in adult zebrafish. *Toxicological Sciences : An Official Journal of the Society of Toxicology*, *135*(1), 241–250. https://doi.org/10.1093/toxsci/kft144

Balaguer, P., Delfosse, V., Grimaldi, M., & Bourguet, W. (2017). Structural and functional evidences for the interactions between nuclear hormone receptors and endocrine disruptors at low doses. *Comptes Rendus Biologies*, *340*(9–10), 414–420. https://doi.org/10.1016/J.CRVI.2017.08.002

Barker, D. J., & Osmond, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet, 1*(8489), 1077–1081.

Basra, K.; Fabian, P. M; Scammell, M. K. (2017). Consumption of contaminated seafood in an environmental justice community: A qualitative and spatial analysis of fishing controls. *Environmental Justice*, *11*(1), env.2017.0010. https://doi.org/10.1089/env.2017.0010

Bello, S. M., Franks, D. G., Stegeman, J. J., & Hahn, M. E. (2001). Acquired Resistance to Ah Receptor Agonists in a Population of Atlantic Killifish (Fundulus heteroclitus) Inhabiting a Marine Superfund Site: In Vivo and in Vitro Studies on the Inducibility of Xenobiotic Metabolizing Enzymes. *Toxicological Sciences*, *60*(1), 77–91. https://doi.org/10.1093/toxsci/60.1.77

Benz, V., Bloch, M., Wardat, S., Böhm, C., Maurer, L., Mahmoodzadeh, S., … Kintscher, U. (2012). Sexual dimorphic regulation of body weight dynamics and adipose tissue lipolysis. *PloS One*, *7*(5), e37794. https://doi.org/10.1371/journal.pone.0037794

Berg, O. K., & Bremset, G. (1998). Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. *Journal of Fish Biology*, *52*(6), 1272–1288. https://doi.org/10.1111/j.1095-8649.1998.tb00971.x

Black, D. E., Gutjahr-Gobell, R., Pruell, R. J., Bergen, B., & McElroy, A. E. (1998). Effects of a mixture of non- ortho - and mono- ortho -polychlorinated biphenyls on reproduction in Fundulus heteroclitus (Linnaeus). *Environmental Toxicology and Chemistry*, *17*(7), 1396–1404. https://doi.org/10.1002/etc.5620170725

Black, D. E., Gutjahr-Gobell, R., Pruell, R. J., Bergen, B., Mills, L., & McElroy, A. E. (1998). Reproduction and polychlorinated biphenyls in *Fundulus heteroclitus* (Linnaeus) from New Bedford Harbor, Massachusetts, USA. *Environmental Toxicology and Chemistry*, *17*(7), 1405–1414. https://doi.org/10.1002/etc.5620170726

Bodofsky, S., Koitz, F., & Wightman, B. (2017). Conserved and exapted functions of nuclear receptors in animal development. *Nuclear Receptor Research*, *4,* 101305. https://doi.org/10.11131/2017/101305

Bolla, S., Nicolaisen, O., & Amin, A. (2011). Liver alterations induced by long term feeding on commercial diets in Atlantic halibut (Hippoglossus hippoglossus L.) females. Histological and biochemical aspects. *Aquaculture*, *312*(1–4), 117–125. https://doi.org/10.1016/J.AQUACULTURE.2010.12.019

Borgå, K., Fisk, A. T., Hoekstra, P. E., & Muir, D. C. G. (2004). Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in Arctic marine food webs. *Environmental Toxicology and Chemistry*, *23*(10), 2367–2385.

Braissant, O., Foufelle, F., Scotto, C., Dauça, M., & Wahli, W. (1996). Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology*, *137*(1), 354–366. https://doi.org/10.1210/endo.137.1.8536636

Brumbaugh, D. E., & Friedman, J. E. (2014). Developmental origins of nonalcoholic fatty liver disease. *Pediatric Research*, *75*(1–2), 140–147. https://doi.org/10.1038/pr.2013.193

Brunt, E. M., Wong, V. W.-S., Nobili, V., Day, C. P., Sookoian, S., Maher, J. J., … Rinella, M. E. (2015). Nonalcoholic fatty liver disease. *Nature Reviews Disease Primers*, *1*, 15080. https://doi.org/10.1038/nrdp.2015.80

Burgio, E., Lopomo, A., & Migliore, L. (2015). Obesity and diabetes: from genetics to epigenetics. *Molecular Biology Reports*, *42*(4), 799–818. https://doi.org/10.1007/s11033-014-3751-z

Burnett, K. G., Bain, L. J., Baldwin, W. S., Callard, G. V, Cohen, S., Di Giulio, R. T., … Crawford, D. L. (2007). Fundulus as the premier teleost model in environmental biology: opportunities for new insights using genomics. *Comparative Biochemistry*

and Physiology. Part D, Genomics & Proteomics, *2*(4), 257–286. https://doi.org/10.1016/j.cbd.2007.09.001

Capitão, A., Lyssimachou, A., Castro, L. F. C., & Santos, M. M. (2017). Obesogens in the aquatic environment: an evolutionary and toxicological perspective. *Environment International*, *106*, 153–169. https://doi.org/10.1016/j.envint.2017.06.003

Carmona, M. C., Louche, K., Lefebvre, B., Pilon, A., Hennuyer, N., Audinot-Bouchez, V., … Consortium of the French Ministry of Research and Technology. (2007). S 26948: a New Specific Peroxisome Proliferator Activated Receptor Modulator With Potent Antidiabetes and Antiatherogenic Effects. *Diabetes*, *56*(11), 2797–2808. https://doi.org/10.2337/db06-1734

Carpenter, D. O. (2015). Exposure to and health effects of volatile PCBs. *Reviews on Environmental Health*, *30*(2), 81–92. https://doi.org/10.1515/reveh-2014-0074

Casals-Casas, C., & Desvergne, B. (2011). Endocrine disruptors: from endocrine to metabolic disruption. *Annual Review of Physiology*, *73*, 135–162. https://doi.org/10.1146/annurev-physiol-012110-142200

Centers for Disease Control and Prevention. (2009). *Fourth National Report on Human Exposure to Environmental Chemicals*. https://www.cdc.gov/exposurereport/index.html

Chamorro-Garcia, R., Diaz-Castillo, C., Shoucri, B. M., Käch, H., Leavitt, R., Shioda, T., & Blumberg, B. (2017). Ancestral prenatal obesogen exposure results in a transgenerational thrifty phenotype in mice. *Nature Communications*, *8*(1), 2012. https://doi.org/10.1038/s41467-017-01944-z

Chamorro-García, R., Sahu, M., Abbey, R. J., Laude, J., Pham, N., & Blumberg, B. (2013). Transgenerational Inheritance of Increased Fat Depot Size, Stem Cell Reprogramming, and Hepatic Steatosis Elicited by Prenatal Exposure to the Obesogen Tributyltin in Mice. *Environmental Health Perspectives*, *121*(3), 359–366. https://doi.org/10.1289/ehp.1205701

Clair, H. B., Pinkston, C. M., Rai, S. N., Pavuk, M., Dutton, N. D., Brock, G. N., … Cave, M. C. (2018). Liver Disease in a Residential Cohort With Elevated Polychlorinated Biphenyl Exposures. *Toxicological Sciences*. https://doi.org/10.1093/toxsci/kfy076

Clark, B. W., Bone, A. J., & Di Giulio, R. T. (2014). Resistance to teratogenesis by F1 and F2 embryos of PAH-adapted Fundulus heteroclitus is strongly inherited despite reduced recalcitrance of the AHR pathway. *Environmental Science and Pollution Research International*, *21*(24), 13898–13908. https://doi.org/10.1007/s11356-013-

2446-7

Clark, B. W., Matson, C. W., Jung, D., & Di Giulio, R. T. (2010). AHR2 mediates cardiac teratogenesis of polycyclic aromatic hydrocarbons and PCB-126 in Atlantic killifish (Fundulus heteroclitus). *Aquatic Toxicology (Amsterdam, Netherlands)*, *99*(2), 232–240. https://doi.org/10.1016/j.aquatox.2010.05.004

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., … Brand-Miller, J. (2005). Origins and evolution of the Western diet: health implications for the 21st century. *The American Journal of Clinical Nutrition*, *81*(2), 341–354. https://doi.org/10.1093/ajcn.81.2.341

Coustau, Chevillon, & ffrench-Constant. (2000). Resistance to xenobiotics and parasites: can we count the cost? *Trends in Ecology & Evolution*, *15*(9), 378–383.

Craig, S. R., MacKenzie, D. S., Jones, G., & Gatlin, D. M. (2000). Seasonal changes in the reproductive condition and body composition of free-ranging red drum, Sciaenops ocellatus. *Aquaculture*, *190*(1–2), 89–102. https://doi.org/10.1016/S0044-8486(00)00386-0

Currie, J., Zivin, J. G., Mullins, J., & Neidell, M. (2014). What Do We Know About Short- and Long-Term Effects of Early-Life Exposure to Pollution? *Annual Review of Resource Economics*, *6*(1), 217–247. https://doi.org/10.1146/annurev-resource-100913-012610

de Lera, Á. R., Krezel, W., & Rühl, R. (2016). An Endogenous Mammalian Retinoid X Receptor Ligand, At Last! *ChemMedChem*, *11*(10), 1027–1037. https://doi.org/10.1002/cmdc.201600105

Denison, M. S., & Heath-Pagliuso, S. (1998). The Ah receptor: a regulator of the biochemical and toxicological actions of structurally diverse chemicals. *Bulletin of Environmental Contamination and Toxicology*, *61*(5), 557–568.

Denison, M. S., & Nagy, S. R. (2003). Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annual Review of Pharmacology and Toxicology*, *43*, 309–334. https://doi.org/10.1146/annurev.pharmtox.43.100901.135828

Denison, M. S., Soshilov, A. A., He, G., DeGroot, D. E., & Zhao, B. (2011). Exactly the Same but Different: Promiscuity and Diversity in the Molecular Mechanisms of Action of the Aryl Hydrocarbon (Dioxin) Receptor. *Toxicological Sciences*, *124*(1), 1–22. https://doi.org/10.1093/toxsci/kfr218

Deshpande, A. D., Dockum, B. W., Cleary, T., Farrington, C., & Wieczorek, D. (2013). Bioaccumulation of polychlorinated biphenyls and organochlorine pesticides in

young-of-the-year bluefish (Pomatomus saltatrix) in the vicinity of a Superfund Site in New Bedford Harbor, Massachusetts, and in the adjacent waters. *Marine Pollution Bulletin*, *72*(1), 146–164. https://doi.org/10.1016/j.marpolbul.2013.04.008

El Hajj, N., Schneider, E., Lehnen, H., & Haaf, T. (2014). Epigenetics and life-long consequences of an adverse nutritional and diabetic intrauterine environment. *Reproduction (Cambridge, England)*, *148*(6), R111-20. https://doi.org/10.1530/REP-14-0334

Elskus, A. A., Pruell, R., & Stegeman, J. J. (1992). Endogenously-mediated, pretranslational suppression of cytochrome P4501A in PCB-contaminated flounder. *Marine Environmental Research*, *34*(1–4), 97–101. https://doi.org/10.1016/0141-1136(92)90090-9

Engin, A. B. (2017). What Is Lipotoxicity? (pp. 197–220). Springer, Cham. https://doi.org/10.1007/978-3-319-48382-5_8

U.S. Environmental Protection Agency. (1998). *Technical Data Sheet: Ultra-Fresh*DM-50*.

Evans, R. M., & Mangelsdorf, D. J. (2014). Nuclear Receptors, RXR, and the Big Bang. *Cell*, *157*(1), 255–266. https://doi.org/10.1016/j.cell.2014.03.012

Everitt, B. S., & Hothorn, T. (2010). *A Handbook of Statistical Analyses Using R*. Boca Raton, FL: CRC Press.

Farrington, J. W., Goldberg, E. D., Risebrough, R. W., Martin, J. H., & Bowen, V. T. (1983). U.S. "Mussel Watch" 1976-1978: an overview of the trace-metal, DDE, PCB, hydrocarbon and artificial radionuclide data. *Environmental Science & Technology*, *17*(8), 490–496. https://doi.org/10.1021/es00114a010

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*, 175–191.

Feige, J. N., Gelman, L., Rossi, D., Zoete, V., Métivier, R., Tudor, C., … Desvergne, B. (2007). The endocrine disruptor monoethyl-hexyl-phthalate is a selective peroxisome proliferator-activated receptor gamma modulator that promotes adipogenesis. *The Journal of Biological Chemistry*, *282*(26), 19152–19166. https://doi.org/10.1074/jbc.M702724200

Fernández-Delgado, C. (1989). Life-history patterns of the salt-marsh killifish Fundulus heteroclitus (L.) introduced in the estuary of the guadalquivir river (South West Spain). *Estuarine, Coastal and Shelf Science*, *29*(6), 573–582. https://doi.org/10.1016/0272-7714(89)90011-5

Fernández, I., Hontoria, F., Ortiz-Delgado, J. B., Kotzamanis, Y., Estévez, A., Zambonino-Infante, J. L., & Gisbert, E. (2008). Larval performance and skeletal deformities in farmed gilthead sea bream (Sparus aurata) fed with graded levels of Vitamin A enriched rotifers (Brachionus plicatilis). *Aquaculture*, *283*(1–4), 102–115. https://doi.org/10.1016/J.AQUACULTURE.2008.06.037

Fernández, I., Pimentel, M. S., Ortiz-Delgado, J. B., Hontoria, F., Sarasquete, C., Estévez, A., … Gisbert, E. (2009). Effect of dietary vitamin A on Senegalese sole (Solea senegalensis) skeletogenesis and larval quality. *Aquaculture*, *295*(3–4), 250–265. https://doi.org/10.1016/J.AQUACULTURE.2009.06.046

Ferrante, M. C., Amero, P., Santoro, A., Monnolo, A., Simeoli, R., Di Guida, F., … Meli, R. (2014). Polychlorinated biphenyls (PCB 101, PCB 153 and PCB 180) alter leptin signaling and lipid metabolism in differentiated 3T3-L1 adipocytes. *Toxicology and Applied Pharmacology*, *279*(3), 401–408. https://doi.org/10.1016/j.taap.2014.06.016

Ffrench-Constant, R. H., & Bass, C. (2017). Does resistance really carry a fitness cost? *Current Opinion in Insect Science*, *21*, 39–46. https://doi.org/10.1016/j.cois.2017.04.011

Flegal, K. M., Carroll, M. D., Ogden, C. L., & Johnson, C. L. (2002). Prevalence and trends in obesity among US adults, 1999-2000. *JAMA: The Journal of the American Medical Association*, *288*(14), 1723–1727. https://doi.org/10.1001/jama.288.14.1723

Flegal, K. M., Kruszon-Moran, D., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2016). Trends in Obesity Among Adults in the United States, 2005 to 2014. *JAMA: The Journal of the American Medical Association*, *315*(21), 2284. https://doi.org/10.1001/jama.2016.6458

iFlynn, E. J., Trent, C. M., & Rawls, J. F. (2009). Ontogeny and nutritional control of adipogenesis in zebrafish (Danio rerio). *Journal of Lipid Research*, *50*(8), 1641–1652. https://doi.org/10.1194/jlr.M800590-JLR200

Foryst-Ludwig, A., Clemenz, M., Hohmann, S., Hartge, M., Sprang, C., Frost, N., … Kintscher, U. (2008). Metabolic Actions of Estrogen Receptor Beta (ERβ) are Mediated by a Negative Cross-Talk with PPARγ. *PLoS Genetics*, *4*(6), e1000108. https://doi.org/10.1371/journal.pgen.1000108

Fox, G. A. (1991). Practical causal inference for ecoepidemiologists. *Journal of Toxicology and Environmental Health*, *33*(4), 359–373. https://doi.org/10.1080/15287399109531535

Frederick, L. A., Van Veld, P. A., & Rice, C. D. (2007). Bioindicators of Immune Function in Creosote-adapted Estuarine Killifish, *Fundulus heteroclitus*. *Journal of Toxicology and Environmental Health, Part A*, *70*(17), 1433–1442.

https://doi.org/10.1080/15287390701382910

Gadupudi, G., Gourronc, F. A., Ludewig, G., Robertson, L. W., & Klingelhutz, A. J. (2015). PCB126 inhibits adipogenesis of human preadipocytes. *Toxicology in Vitro*, *29*(1), 132–141. https://doi.org/10.1016/j.tiv.2014.09.015

Gadupudi, G. S., Klaren, W. D., Olivier, A. K., Klingelhutz, A. J., & Robertson, L. W. (2016). PCB126-Induced Disruption in Gluconeogenesis and Fatty Acid Oxidation Precedes Fatty Liver in Male Rats. *Toxicological Sciences*, *149*(1), 98–110. https://doi.org/10.1093/toxsci/kfv215

Gavrilova, O., Haluzik, M., Matsusue, K., Cutson, J. J., Johnson, L., Dietz, K. R., … Reitman, M. L. (2003). Liver Peroxisome Proliferator-activated Receptor γ Contributes to Hepatic Steatosis, Triglyceride Clearance, and Regulation of Body Fat Mass. *Journal of Biological Chemistry*, *278*(36), 34268–34276. https://doi.org/10.1074/jbc.M300043200

Gourronc, F. A., Robertson, L. W., & Klingelhutz, A. J. (2018). A delayed proinflammatory response of human preadipocytes to PCB126 is dependent on the aryl hydrocarbon receptor. *Environmental Science and Pollution Research*, *25*(17), 16481–16492. https://doi.org/10.1007/s11356-017-9676-z

Gräns, J., Wassmur, B., & Celander, M. C. (2010). One-way inhibiting cross-talk between arylhydrocarbon receptor (AhR) and estrogen receptor (ER) signaling in primary cultures of rainbow trout hepatocytes. *Aquatic Toxicology*, *100*(3), 263–270. https://doi.org/10.1016/J.AQUATOX.2010.07.024

Greytak, S. R., Champlin, D., & Callard, G. V. (2005). Isolation and characterization of two cytochrome P450 aromatase forms in killifish (Fundulus heteroclitus): Differential expression in fish from polluted and unpolluted environments. *Aquatic Toxicology*, *71*(4), 371–389. https://doi.org/10.1016/J.AQUATOX.2004.12.007

Grün, F. (2014). The Obesogen Tributyltin. *Vitamins & Hormones, 94*, 277–325. https://doi.org/10.1016/B978-0-12-800095-3.00011-0

Grün, F., & Blumberg, B. (2006). Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology*, *147*(6 Suppl), S50-55. https://doi.org/10.1210/en.2005-1129

Grün, F., & Blumberg, B. (2009). Endocrine disrupters as obesogens. *Molecular and Cellular Endocrinology*, *304*(1–2), 19–29. https://doi.org/10.1016/j.mce.2009.02.018

Grün, F., Watanabe, H., Zamanian, Z., Maeda, L., Arima, K., Cubacha, R., … Blumberg, B. (2006). Endocrine-disrupting organotin compounds are potent inducers of

adipogenesis in vertebrates. *Molecular Endocrinology*, *20*(9), 2141–2155. https://doi.org/10.1210/me.2005-0367

Grygiel-Górniak, B. (2014). Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications--a review. *Nutrition Journal*, *13*(1), 17. https://doi.org/10.1186/1475-2891-13-17

Haga, Y., Suzuki, T., & Takeuchi, T. (2002). Retinoic Acid Isomers Produce Malformations in Postembryonic Development of the Japanese Flounder, Paralichthys olivaceus. *Zoological Science*, *19*(10), 1105–1112. https://doi.org/10.2108/zsj.19.1105

Halberg, N., Khan, T., Trujillo, M. E., Wernstedt-Asterholm, I., Attie, A. D., Sherwani, S., … Scherer, P. E. (2009). Hypoxia-Inducible Factor 1 Induces Fibrosis and Insulin Resistance in White Adipose Tissue. *Molecular and Cellular Biology*, *29*(16), 4467–4483. https://doi.org/10.1128/MCB.00192-09

Harbeitner, R. C., Hahn, M. E., & Timme-Laragy, A. R. (2013). Differential sensitivity to pro-oxidant exposure in two populations of killifish (Fundulus heteroclitus). *Ecotoxicology*, *22*(2), 387–401. https://doi.org/10.1007/s10646-012-1033-x

He, A.-Y., Liu, C.-Z., Chen, L.-Q., Ning, L.-J., Qin, J.-G., Li, J.-M., … Du, Z.-Y. (2015). Molecular characterization, transcriptional activity and nutritional regulation of peroxisome proliferator activated receptor gamma in Nile tilapia (Oreochromis niloticus). *General and Comparative Endocrinology*, *223*, 139–147. https://doi.org/10.1016/J.YGCEN.2015.05.008

Heindel, J. J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M. A., … vom Saal, F. (2017). Metabolism disrupting chemicals and metabolic disorders. *Reproductive Toxicology*, *68*, 3–33. https://doi.org/10.1016/j.reprotox.2016.10.001

Heindel, J. J., vom Saal, F. S., Blumberg, B., Bovolin, P., Calamandrei, G., Ceresini, G., … Bilbo, S. (2015). Parma consensus statement on metabolic disruptors. *Environmental Health*, *14*(1), 54. https://doi.org/10.1186/s12940-015-0042-7

Heindel, J. J., & Schug, T. T. (2013). The Perfect Storm for Obesity. *Obesity*, *21*(6), 1079–1080. https://doi.org/10.1002/oby.20222

Herrick, R. F., McClean, M. D., Meeker, J. D., Baxter, L. K., & Weymouth, G. A. (2004). An unrecognized source of PCB contamination in schools and other buildings. *Environmental Health Perspectives*, *112*(10), 1051–1053. https://doi.org/10.1289/EHP.6912

Herrick, R. F., Stewart, J. H., & Allen, J. G. (2016). Review of PCBs in US schools: a brief history, an estimate of the number of impacted schools, and an approach for

evaluating indoor air samples. *Environmental Science and Pollution Research*, *23*(3), 1975–1985. https://doi.org/10.1007/s11356-015-4574-8

Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M., & Thaller, C. (1992). 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. *Cell*, *68*(2), 397–406. https://doi.org/10.1016/0092-8674(92)90479-V

Hoegberg, P., Schmidt, C. K., Fletcher, N., Nilsson, C. B., Trossvik, C., Gerlienke Schuur, A., … Håkansson, H. (2005). Retinoid status and responsiveness to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking retinoid binding protein or retinoid receptor forms. *Chemico-Biological Interactions*, *156*(1), 25–39. https://doi.org/10.1016/j.cbi.2005.06.006

Holtta-Vuori, M., Salo, V., Nyberg, L., Brackmann, C., Enejder, A., Panula, PE, I., … Ikonen, E. (2010). Zebrafish: gaining popularity in lipid research. *The Biochemical Journal*, *429*(2), 235–242. https://doi.org/10.1042/BJ20100293

Huang, C.-F., Yang, C.-Y., Tsai, J.-R., Wu, C.-T., Liu, S.-H., & Lan, K.-C. (2018). Low-dose tributyltin exposure induces an oxidative stress-triggered JNK-related pancreatic β-cell apoptosis and a reversible hypoinsulinemic hyperglycemia in mice. *Scientific Reports*, *8*(1), 5734. https://doi.org/10.1038/s41598-018-24076-w

Huang, L., Zuo, Z., Zhang, Y., & Wang, C. (2015). Toxicogenomic analysis in the combined effect of tributyltin and benzo[a]pyrene on the development of zebrafish embryos. *Aquatic Toxicology*, *158*, 157–164. https://doi.org/10.1016/J.AQUATOX.2014.10.024

Huff, M., da Silveira, W. A., Carnevali, O., Renaud, L., & Hardiman, G. (2018). Systems Analysis of the Liver Transcriptome in Adult Male Zebrafish Exposed to the Plasticizer (2-Ethylhexyl) Phthalate (DEHP). *Scientific Reports*, *8*(1), 2118. https://doi.org/10.1038/s41598-018-20266-8

Hurst, C. H., & Waxman, D. J. (2003). Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. *Toxicological Sciences*, *74*(2), 297–308. https://doi.org/10.1093/toxsci/kfg145

Ibabe, A., Bilbao, E., & Cajaraville, M. P. (2005). Expression of peroxisome proliferator-activated receptors in zebrafish (Danio rerio) depending on gender and developmental stage. *Histochemistry and Cell Biology*, *123*(1), 75–87. https://doi.org/10.1007/s00418-004-0737-2

Imrie, D., & Sadler, K. C. (2010). White adipose tissue development in zebrafish is regulated by both developmental time and fish size. *Developmental Dynamics*, *239*(11), 3013–3023. https://doi.org/10.1002/dvdy.22443

Intertek. (2010). *EU Organotin Restrictions Extended to Consumer Goods*.

Integrated Risk Information System. (1989). *Polychlorinated biphenyls (PCBs) (CASRN 1336-36-3)*.

Jackson, N. L., Nordstrom, K. F., Eliot, I., & Masselink, G. (2002). 'Low energy' sandy beaches in marine and estuarine environments: A review. *Geomorphology*, *48*(1–3), 147–162. https://doi.org/10.1016/S0169-555X(02)00179-4

James-Pirri, M. ., Raposa, K. ., & Catena, J. . (2001). Diet Composition of Mummichogs, Fundulus heteroclitus, from Restoring and Unrestricted Regions of a New England (U.S.A.) Salt Marsh. *Estuarine, Coastal and Shelf Science*, *53*(2), 205–213. https://doi.org/10.1006/ECSS.2001.0807

Janani, C., & Ranjitha Kumari, B. D. (2015). PPAR gamma gene – A review. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *9*(1), 46–50. https://doi.org/10.1016/J.DSX.2014.09.015

Jayasundara, N., Fernando, P. W., Osterberg, J. S., Cammen, K. M., Schultz, T. F., & Di Giulio, R. T. (2017). Cost of Tolerance: Physiological Consequences of Evolved Resistance to Inhabit a Polluted Environment in Teleost Fish Fundulus heteroclitus. *Environmental Science & Technology*, *51*(15), 8763–8772. https://doi.org/10.1021/acs.est.7b01913

Kanayama, T., Kobayashi, N., Mamiya, S., Nakanishi, T., & Nishikawa, J. (2005). Organotin compounds promote adipocyte differentiation as agonists of the peroxisome proliferator-activated receptor gamma/retinoid X receptor pathway. *Molecular Pharmacology*, *67*(3), 766–774. https://doi.org/10.1124/mol.104.008409

Kandemir, Ş., Polat, N. (2007). Monthly variation of total lipid and total fatty acid in Muscle. *Turkish Journal of Fisheries and Aquatic Sciences*, *7*, 27–31.

Kania-Korwel, I., & Lehmler, H.-J. (2016). Toxicokinetics of chiral polychlorinated biphenyls across different species - A review. *Environmental Science and Pollution Research*, *23*(3), 2058–2080. https://doi.org/10.1007/s11356-015-4383-0

Kawai, M., & Rosen, C. J. (2010). PPARγ: a circadian transcription factor in adipogenesis and osteogenesis. *Nature Reviews Endocrinology*, *6*(11), 629–636. https://doi.org/10.1038/nrendo.2010.155

Kay, D. P., Blankenship, A. L., Coady, K. K., Neigh, A. M., Zwiernik, M. J., Millsap, S. D., … Giesy, J. P. (2005). Differential accumulation of polychlorinated biphenyl congeners in the aquatic food web at the Kalamazoo River Superfund Site, Michigan. *Environmental Science & Technology, 39*(16), 5964–5974.

Kerley-Hamilton, J. S., Trask, H. W., Ridley, C. J. A., Dufour, E., Ringelberg, C. S., Nurinova, N., … Tomlinson, C. R. (2012) Obesity is mediated by differential aryl hydrocarbon receptor signaling in mice fed a Western diet. *Environmental Health Perspectives*, *120*(9), 1252–1259. https://doi.org/10.1289/ehp.1205003

Kersten, S., Desvergne, B., & Wahli, W. (2000). Roles of PPARs in health and disease. *Nature*, *405*(6785), 421–424. https://doi.org/10.1038/35013000

Khan, T., Muise, E. S., Iyengar, P., Wang, Z. V, Chandalia, M., Abate, N., … Scherer, P. E. (2009). Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. *Molecular and Cellular Biology*, *29*(6), 1575–1591. https://doi.org/10.1128/MCB.01300-08

Kim, M. J., Pelloux, V., Guyot, E., Tordjman, J., Bui, L.-C., Chevallier, A., … Barouki, R. (2012). Inflammatory pathway genes belong to major targets of persistent organic pollutants in adipose cells. *Environmental Health Perspectives*, *120*(4), 508–514. https://doi.org/10.1289/ehp.1104282

Kim, S. M., Li, A., Monti, S., & Schlezinger, J. J. (2018). Tributyltin induces a transcriptional response without a brite adipocyte signature in adipocyte models. *BioRxiv*, 328203. https://doi.org/10.1101/328203

Kirchner, S., Kieu, T., Chow, C., Casey, S., & Blumberg, B. (2010). Prenatal exposure to the environmental obesogen tributyltin predisposes multipotent stem cells to become adipocytes. *Molecular Endocrinology (Baltimore, Md.)*, *24*(3), 526–539. https://doi.org/10.1210/me.2009-0261

Kneib, R. T. (1986). The role of Fundulus heteroclitus in salt marsh trophic dynamics. *American Zoologist, 26*(1), 259–269.

Kolek, Andrew; Ceurvels, R. (1981). *Polychlorinated biphenyl (PCB) analyses of marine organisms in the New Bedford area, 1976-1980.* Boston: Commonwealth of Massachusetts, Division of Marine Fisheries. https://www.biodiversitylibrary.org/bibliography/62745#/summary

La Merrill, M., & Birnbaum, L. S. (2011). Childhood obesity and environmental chemicals. *The Mount Sinai Journal of Medicine, New York*, *78*(1), 22–48. https://doi.org/10.1002/msj.20229

La Merrill, M., Kuruvilla, B. S., Pomp, D., Birnbaum, L. S., & Threadgill, D. W. (2009). Dietary fat alters body composition, mammary development, and cytochrome p450 induction after maternal TCDD exposure in DBA/2J mice with low-responsive aryl hydrocarbon receptors. *Environmental Health Perspectives*, *117*(9), 1414–1419. https://doi.org/10.1289/ehp.0800530

Lane, R. H., MacLennan, N. K., Daood, M. J., Hsu, J. L., Janke, S. M., Pham, T. D., … Watchko, J. F. (2003). IUGR Alters Postnatal Rat Skeletal Muscle Peroxisome Proliferator-Activated Receptor-γ Coactivator-1 Gene Expression in a Fiber Specific Manner. *Pediatric Research*, *53*(6), 994–1000. https://doi.org/10.1203/01.PDR.0000064583.40495.51

Laprairie, R. B., Denovan-Wright, E. M., & Wright, J. M. (2016). Subfunctionalization of peroxisome proliferator response elements accounts for retention of duplicated fabp1 genes in zebrafish. *BMC Evolutionary Biology*, *16*(1), 147. https://doi.org/10.1186/s12862-016-0717-x

Law, R. J., Bolam, T., James, D., Barry, J., Deaville, R., Reid, R. J., … Jepson, P. D. (2012). Butyltin compounds in liver of harbour porpoises (Phocoena phocoena) from the UK prior to and following the ban on the use of tributyltin in antifouling paints (1992–2005 & 2009). *Marine Pollution Bulletin*, *64*(11), 2576–2580. https://doi.org/10.1016/J.MARPOLBUL.2012.07.014

Le Magueresse-Battistoni, B., Labaronne, E., Vidal, H., & Naville, D. (2017). Endocrine disrupting chemicals in mixture and obesity, diabetes and related metabolic disorders. *World Journal of Biological Chemistry*, *8*(2), 108–119. https://doi.org/10.4331/wjbc.v8.i2.108

le Maire, A., Grimaldi, M., Roecklin, D., Dagnino, S., Vivat-Hannah, V., Balaguer, P., & Bourguet, W. (2009). Activation of RXR-PPAR heterodimers by organotin environmental endocrine disruptors. *EMBO Reports*, *10*(4), 367–373. https://doi.org/10.1038/embor.2009.8

Leaver, M. J., Boukouvala, E., Antonopoulou, E., Diez, A., Favre-Krey, L., Ezaz, M. T., … Krey, G. (2005). Three Peroxisome Proliferator-Activated Receptor Isotypes from Each of Two Species of Marine Fish. *Endocrinology*, *146*(7), 3150–3162. https://doi.org/10.1210/en.2004-1638

Lehmler, H.-J., Gadogbe, M., Liu, B., & Bao, W. (2018). Environmental tin exposure in a nationally representative sample of U.S. adults and children: The National Health and Nutrition Examination Survey 2011–2014. *Environmental Pollution*, *240*, 599–606. https://doi.org/10.1016/J.ENVPOL.2018.05.019

Lempradl, A., Pospisilik, J. A., & Penninger, J. M. (2015). Exploring the emerging complexity in transcriptional regulation of energy homeostasis. *Nature Reviews Genetics*, *16*(11), 665–681. https://doi.org/10.1038/nrg3941

Li, A. C., Brown, K. K., Silvestre, M. J., Willson, T. M., Palinski, W., & Glass, C. K. (2000). Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *The Journal of Clinical Investigation*, *106*(4), 523–531. https://doi.org/10.1172/JCI10370

Lindberg, C. D., Jayasundara, N., Kozal, J. S., Leuthner, T. C., & Di Giulio, R. T. (2017). Resistance to polycyclic aromatic hydrocarbon toxicity and associated bioenergetic consequences in a population of Fundulus heteroclitus. *Ecotoxicology*, *26*(3), 435–448. https://doi.org/10.1007/s10646-017-1775-6

Liu, G., Moon, T. W., Metcalfe, C. D., Lee, L. E. J., & Trudeau, V. L. (2005). A teleost in vitro reporter gene assay to screen for agonists of the peroxisome proliferator-activated receptors. *Environmental Toxicology and Chemistry*, *24*(9), 2260–2266.

Liu, J., Cao, Q., Yuan, J., Zhang, X., Yu, L., & Shi, H. (2012). Histological observation on unique phenotypes of malformation induced in Xenopus tropicalis larvae by tributyltin. *Journal of Environmental Sciences*, *24*(2), 195–202. https://doi.org/10.1016/S1001-0742(11)60759-6

Liu, Y., Wang, J., Wei, Y., Zhang, H., Xu, M., & Dai, J. (2008). Induction of time-dependent oxidative stress and related transcriptional effects of perfluorododecanoic acid in zebrafish liver. *Aquatic Toxicology*, *89*(4), 242–250. https://doi.org/10.1016/J.AQUATOX.2008.07.009

Lombardo, G. (2015). *Third five-year review report for the New Bedford Harbor Superfund site, Bristol County, Massachusetts; Appendix D: Risk Assessment Updates*. Boston, MA. https://doi.org/SDMS DocID 583507

Lowe, C. E., O'Rahilly, S., & Rochford, J. J. (2011). Adipogenesis at a glance. *Journal of Cell Science*, *124*(16), 2681–2686. https://doi.org/10.1242/jcs.079699

Lu, K.-L., Xu, W.-N., Li, X.-F., Liu, W.-B., Wang, L.-N., & Zhang, C.-N. (2013). Hepatic triacylglycerol secretion, lipid transport and tissue lipid uptake in blunt snout bream (Megalobrama amblycephala) fed high-fat diet. *Aquaculture*, *408–409*, 160–168. https://doi.org/10.1016/J.AQUACULTURE.2013.06.003

Lyssimachou, A., Santos, J. G., André, A., Soares, J., Lima, D., Guimarães, L., … Santos, M. M. (2015). The Mammalian "Obesogen" Tributyltin Targets Hepatic Triglyceride Accumulation and the Transcriptional Regulation of Lipid Metabolism in the Liver and Brain of Zebrafish. *PLoS One*, *10*(12), e0143911. https://doi.org/10.1371/journal.pone.0143911

Martin, B. T., Heintz, R., Danner, E. M., & Nisbet, R. M. (2017). Integrating lipid storage into general representations of fish energetics. *Journal of Animal Ecology*, *86*(4), 812–825. https://doi.org/10.1111/1365-2656.12667

Martinez, A., Hadnott, B. N., Awad, A. M., Herkert, N. J., Tomsho, K., Basra, K., … Hornbuckle, K. C. (2017). Release of Airborne Polychlorinated Biphenyls from New Bedford Harbor Results in Elevated Concentrations in the Surrounding Air. *Environmental Science & Technology Letters*, *4*(4), 127–131.

https://doi.org/10.1021/acs.estlett.7b00047

MassDEP. (2002). *Seafood Monitoring and Field Sampling Work Plan, New Bedford Harbor Superfund Site, Massachusetts Department of Environmental Protection*.

MassDEP. (2015). *Seafood Monitoring and Field Sampling Work Plan, New Bedford Harbor Superfund Site, Massachusetts Department of Environmental Protection*.

Matson, C. W., Clark, B. W., Jenny, M. J., Fleming, C. R., Hahn, M. E., & Di Giulio, R. T. (2008). Development of the morpholino gene knockdown technique in Fundulus heteroclitus: A tool for studying molecular mechanisms in an established environmental model. *Aquatic Toxicology*, *87*(4), 289–295. https://doi.org/10.1016/J.AQUATOX.2008.02.010

Mayo, C. (2015). Metabolic Syndrome. Retrieved from http://www.mayoclinic.org/diseases-conditions/metabolic-syndrome/basics/definition/con-20027243

McMahon, K. W., Johnson, B. J., & Ambrose, W. G. (2005). Diet and movement of the killifish,Fundulus heteroclitus, in a Maine salt marsh assessed using gut contents and stable isotope analyses. *Estuaries*, *28*(6), 966–973. https://doi.org/10.1007/BF02696024

McMillan, A. M., Bagley, M. J., Jackson, S. A., & Nacci, D. E. (2006). Genetic diversity and structure of an estuarine fish (Fundulus heteroclitus) indigenous to sites associated with a highly contaminated urban harbor. *Ecotoxicology*, *15*(6), 539–548. https://doi.org/10.1007/s10646-006-0090-4

Meador, J. P., Sommers, F. C., Cooper, K. A., & Yanagida, G. (2011). Tributyltin and the obesogen metabolic syndrome in a salmonid. *Environmental Research*, *111*(1), 50–56. https://doi.org/10.1016/j.envres.2010.11.012

Meyer, J. N., & Di Giulio, R. T. (2003). Heritable adaptation and fitness costs in killifish (Fundulus heteroclitus) inhabiting a polluted estuary. *Ecological Applications*, *13*(2), 490–503. https://doi.org/10.1890/1051-0761(2003)013[0490:HAAFCI]2.0.CO;2

Meyer, J. N., Nacci, D. E., & Di Giulio, R. T. (2002). Cytochrome P4501A (CYP1A) in Killifish (Fundulus heteroclitus): Heritability of Altered Expression and Relationship to Survival in Contaminated Sediments. *Toxicological Sciences*, *68*(1), 69–81. https://doi.org/10.1093/toxsci/68.1.69

Michel, M. (2018). Editorial: Comparative Studies of Energy Homeostasis in Vertebrates. *Frontiers in Endocrinology*, *9*, 291. https://doi.org/10.3389/fendo.2018.00291

Mimura, J., & Fujii-Kuriyama, Y. (2003). Functional role of AhR in the expression of toxic effects by TCDD. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1619*(3), 263–268. https://doi.org/10.1016/S0304-4165(02)00485-3

Minchin, J. E. N., Dahlman, I., Harvey, C. J., Mejhert, N., Singh, M. K., Epstein, J. A., … Rawls, J. F. (2015). Plexin D1 determines body fat distribution by regulating the type V collagen microenvironment in visceral adipose tissue. *Proceedings of the National Academy of Sciences*, *112*(14), 201416412. https://doi.org/10.1073/pnas.1416412112

Minchin, J. E. N., & Rawls, J. F. (2011). In vivo analysis of white adipose tissue in zebrafish. *Methods in Cell Biology*, *105*, 63–86. https://doi.org/10.1016/B978-0-12-381320-6.00003-5

Minchin, J. E. N., & Rawls, J. F. (2017). A classification system for zebrafish adipose tissues. *Disease Models & Mechanisms*, *10*(6).

Montague, C. T., Prins, J. B., Sanders, L., Digby, J. E., & O'Rahilly, S. (1997). Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes*, *46*(3), 342–347. https://doi.org/10.2337/DIAB.46.3.342

Morse, S. A., Gulati, R., & Reisin, E. (2010). The obesity paradox and cardiovascular disease. *Current Hypertension Reports*, *12*(2), 120–126. https://doi.org/10.1007/s11906-010-0099-1

Mueller, E., Drori, S., Aiyer, A., Yie, J., Sarraf, P., Chen, H., … Spiegelman, B. M. (2002). Genetic analysis of adipogenesis through peroxisome proliferator-activated receptor gamma isoforms. *The Journal of Biological Chemistry*, *277*(44), 41925–41930. https://doi.org/10.1074/jbc.M206950200

Mukherjee, R., Jow, L., Croston, G. E., & Paterniti, J. R. (1997). Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARgamma2 versus PPARgamma1 and activation with retinoid X receptor agonists and antagonists. *The Journal of Biological Chemistry*, *272*(12), 8071–8076. https://doi.org/10.1074/JBC.272.12.8071

Müllerová, D., & Kopecký, J. (2007). White adipose tissue: storage and effector site for environmental pollutants. *Physiological Research / Academia Scientiarum Bohemoslovaca*, *56*(4), 375–381.

Nacci, S. Jayaraman, J. Specker, D., Jayaraman, S., & Specker, J. (2001). Stored Retinoids in Populations of the Estuarine Fish Fundulus heteroclitus Indigenous to PCB-Contaminated and Reference Sites. *Archives of Environmental Contamination and Toxicology*, *40*(4), 511–518. https://doi.org/10.1007/s002440010204

Nacci, D., Coiro, L., Champlin, D., Jayaraman, S., McKinney, R., Gleason, T. R., … Cooper, K. R. (1999). Adaptations of wild populations of the estuarine fish Fundulus heteroclitus to persistent environmental contaminants. *Marine Biology*, *134*(1), 9–17. https://doi.org/10.1007/s002270050520

Nacci, D. E., Champlin, D., Coiro, L., McKinney, R., & Jayaraman, S. (2002). Predicting the occurrence of genetic adaptation to dioxinlike compounds in populations of the estuarine fish *Fundulus heteroclitus*. *Environmental Toxicology and Chemistry*, *21*(7), 1525–1532. https://doi.org/10.1002/etc.5620210726

Nacci, D. E., Champlin, D., & Jayaraman, S. (2010). Adaptation of the Estuarine Fish Fundulus heteroclitus (Atlantic Killifish) to Polychlorinated Biphenyls (PCBs). *Estuaries and Coasts*, *33*(4), 853–864. https://doi.org/10.1007/s12237-009-9257-6

Nacci, D., Hahn, M. E., Karchner, S. I., Jayaraman, S., Mostello, C., Miller, K. M., … Nisbet, I. (2016). Integrating monitoring and genetic methods to infer historical risks of PCB and DDE to Common and Roseate Terns nesting near the New Bedford Harbor Superfund site (Massachusetts, USA). *Environmental Science & Technology, 50*(18), 10226–10235. https://doi.org/10.1021/acs.est.6b02108

Nam, M., Choi, M.-S., Jung, S., Jung, Y., Choi, J.-Y., Ryu, D. H., & Hwang, G.-S. (2015). Lipidomic Profiling of Liver Tissue from Obesity-Prone and Obesity-Resistant Mice Fed a High Fat Diet. *Scientific Reports*, *5*(1), 16984. https://doi.org/10.1038/srep16984

Nanton, D. A., Lall, S. P., Ross, N. W., & McNiven, M. A. (2003). Effect of dietary lipid level on fatty acid β-oxidation and lipid composition in various tissues of haddock, Melanogrammus aeglefinus L. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *135*(1), 95–108. https://doi.org/10.1016/S1096-4959(03)00052-6

Navas, J. M., & Segner, H. (2001). Estrogen-mediated suppression of cytochrome P4501A (CYP1A) expression in rainbow trout hepatocytes: role of estrogen receptor. *Chemico-Biological Interactions*, *138*(3), 285–298. https://doi.org/10.1016/S0009-2797(01)00280-0

Nebert, D. W. (2017). Aryl hydrocarbon receptor (AHR): “pioneer member” of the basic-helix/loop/helix per - Arnt - sim (bHLH/PAS) family of “sensors” of foreign and endogenous signals. *Progress in Lipid Research*, *67*, 38–57. https://doi.org/10.1016/j.plipres.2017.06.001

Nie, X., Lan, C., Wei, T., & Yang, Y. (2005). Distribution of polychlorinated biphenyls in the water, sediment and fish from the Pearl River estuary, China. *Marine Pollution Bulletin*, *50*(5), 537–546.

Nilsson, C. B., & Håkansson, H. (2002). The retinoid signaling system - A target in dioxin toxicity. *Critical Reviews in Toxicology*, *32*(3), 211–232. https://doi.org/10.1080/20024091064228

Nisbet, I. C. T., & Reynolds, L. M. (1984). Organochlorine residues in common terns and associated estuarine organisms, Massachusetts, USA, 1971–81. *Marine Environmental Research*, *11*(1), 33–66. https://doi.org/10.1016/0141-1136(84)90009-6

Nolte, R. T., Wisely, G. B., Westin, S., Cobb, J. E., Lambert, M. H., Kurokawa, R., … Milburn, M. V. (1998). Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature*, *395*(6698), 137–143. https://doi.org/10.1038/25931

Norris, A. W., Chen, L., Fisher, S. J., Szanto, I., Ristow, M., Jozsi, A. C., … Kahn, C. R. (2003). Muscle-specific PPARγ-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones. *Journal of Clinical Investigation*, *112*(4), 608–618. https://doi.org/10.1172/JCI17305

Oka, T., Nishimura, Y., Zang, L., Hirano, M., Shimada, Y., Wang, Z., … Tanaka, T. (2010). Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. *BMC Physiology*, *10*(1), 21. https://doi.org/10.1186/1472-6793-10-21

Oleksiak, M. F., Karchner, S. I., Jenny, M. J., Franks, D. G., Welch, D. B. M., & Hahn, M. E. (2011). Transcriptomic assessment of resistance to effects of an aryl hydrocarbon receptor (AHR) agonist in embryos of Atlantic killifish (Fundulus heteroclitus) from a marine Superfund site. *BMC Genomics*, *12*(1), 263. https://doi.org/10.1186/1471-2164-12-263

Oliver, B. G., & Niimi, A. J. (1988). Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environmental Science & Technology*, *22*(4), 388–397. https://doi.org/10.1021/es00169a005

Omae, I. (2003). Organotin antifouling paints and their alternatives. *Applied Organometallic Chemistry*, *17*(2), 81–105. https://doi.org/10.1002/aoc.396

Opitz, C. A., Litzenburger, U. M., Sahm, F., Ott, M., Tritschler, I., Trump, S., … Platten, M. (2011). An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature*, *478*(7368), 197–203. https://doi.org/10.1038/nature10491

Ortiz-Villanueva, E., Navarro-Martín, L., Jaumot, J., Benavente, F., Sanz-Nebot, V., Piña, B., & Tauler, R. (2017). Metabolic disruption of zebrafish (Danio rerio) embryos by bisphenol A. An integrated metabolomic and transcriptomic approach.

Environmental Pollution, *231*(Pt 1), 22–36. https://doi.org/10.1016/j.envpol.2017.07.095

Oshima, Y., Nakayama, K., Takeshi, H., Kim, S. G., Shimasaki, Y., Kang, I. J., & Honjo, T. (2009). Toxic Interactions Between Tributyltin and Polychlorinated Biphenyls in Aquatic Organisms. In *Ecotoxicology of Antifouling Biocides* (pp. 195–205). Tokyo: Springer Japan. https://doi.org/10.1007/978-4-431-85709-9_11

Ouadah-Boussouf, N., & Babin, P. J. (2016). Pharmacological evaluation of the mechanisms involved in increased adiposity in zebrafish triggered by the environmental contaminant tributyltin. *Toxicology and Applied Pharmacology*, *294*, 32–42. https://doi.org/10.1016/j.taap.2016.01.014

Pait, A. S., & Nelson, J. O. (2009). A survey of indicators for reproductive endocrine disruption in Fundulus heteroclitus (killifish) at selected sites in the Chesapeake Bay. *Marine Environmental Research*, *68*(4), 170–177. https://doi.org/10.1016/j.marenvres.2009.06.006

Park, Y.-W., Zhu, S., Palaniappan, L., Heshka, S., Carnethon, M. R., & Heymsfield, S. B. (2003). The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. *Archives of Internal Medicine*, *163*(4), 427–436.

Parker, R. (2018). The role of adipose tissue in fatty liver diseases. *Liver Research*. https://doi.org/10.1016/J.LIVRES.2018.02.002

Pavlikova, N., Kortner, T. M., & Arukwe, A. (2010). Peroxisome proliferator-activated receptors, estrogenic responses and biotransformation system in the liver of salmon exposed to tributyltin and second messenger activator. *Aquatic Toxicology*, *99*(2), 176–185. https://doi.org/10.1016/j.aquatox.2010.04.014

Pereira-Fernandes, A., Demaegdt, H., Vandermeiren, K., Hectors, T. L. M., Jorens, P. G., Blust, R., & Vanparys, C. (2013). Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. *PLoS One*, *8*(10), e77481. https://doi.org/10.1371/journal.pone.0077481

Pesch, C. E., Voyer, R. A., Latimer, J. S., Copeland, J., Morrison, G., & McGovern, D. (2011). *Imprint of the Past: Ecological History of New Bedford Harbor*. Narragansett, RI.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*(9), e45.

Pillai, H. K., Fang, M., Beglov, D., Kozakov, D., Vajda, S., Stapleton, H. M., …

Schlezinger, J. J. (2014). Ligand binding and activation of PPARγ by Firemaster® 550: effects on adipogenesis and osteogenesis in vitro. *Environmental Health Perspectives*, *122*(11), 1225–1232. https://doi.org/10.1289/ehp.1408111

Proestou, D. A., Flight, P., Champlin, D., & Nacci, D. (2014). Targeted approach to identify genetic loci associated with evolved dioxin tolerance in Atlantic killifish (Fundulus heteroclitus). *BMC Evolutionary Biology*, *14*(1), 7. https://doi.org/10.1186/1471-2148-14-7

Rantakokko, P., Main, K. M., Wohlfart-Veje, C., Kiviranta, H., Airaksinen, R., Vartiainen, T., … Virtanen, H. E. (2014). Association of placenta organotin concentrations with growth and ponderal index in 110 newborn boys from Finland during the first 18 months of life: a cohort study. *Environmental Health*, *13*(1), 45. https://doi.org/10.1186/1476-069X-13-45

Reid, N. M., Proestou, D. A., Clark, B. W., Warren, W. C., Colbourne, J. K., Shaw, J. R., … Whitehead, A. (2016). The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. *Science*, *354*(6317), 1305–1308. https://doi.org/10.1126/science.aah4993

Remillard, R. B. J., & Bunce, N. J. (2002). Linking dioxins to diabetes: epidemiology and biologic plausibility. *Environmental Health Perspectives*, *110*(9), 853–858.

Riu, A., McCollum, C. W., Pinto, C. L., Grimaldi, M., Hillenweck, A., Perdu, E., … Gustafsson, J.-A. (2014). Halogenated bisphenol-A analogs act as obesogens in zebrafish larvae (Danio rerio). *Toxicological Sciences*, *139*(1), 48–58. https://doi.org/10.1093/toxsci/kfu036

Roff, D. A. (1983). An Allocation Model of Growth and Reproduction in Fish. *Canadian Journal of Fisheries and Aquatic Sciences*, *40*(9), 1395–1404. https://doi.org/10.1139/f83-161

Rosen, E. D., & MacDougald, O. A. (2006). Adipocyte differentiation from the inside out. *Nature Reviews. Molecular Cell Biology*, *7*(12), 885–896. https://doi.org/10.1038/nrm2066

Rudel, R. A., Seryak, L. M., & Brody, J. G. (2008). PCB-containing wood floor finish is a likely source of elevated PCBs in residents' blood, household air and dust: A case study of exposure. *Environmental Health*, *7*(1), 2. https://doi.org/10.1186/1476-069X-7-2

Ruzzin, J., Lee, D.-H., Carpenter, D. O., & Jacobs, D. R. (2012). Reconsidering metabolic diseases: the impacts of persistent organic pollutants. *Atherosclerosis*, *224*(1), 1–3. https://doi.org/10.1016/j.atherosclerosis.2012.02.039

Safe, S. H. (1994). Polychlorinated Biphenyls (PCBs): Environmental Impact, Biochemical and Toxic Responses, and Implications for Risk Assessment. *Critical Reviews in Toxicology*, *24*(2), 87–149. https://doi.org/10.3109/10408449409049308

Samuel, V. T., & Shulman, G. I. (2012). Mechanisms for Insulin Resistance: Common Threads and Missing Links. *Cell*, *148*(5), 852–871. https://doi.org/10.1016/j.cell.2012.02.017

Sánchez-Gurmaches, J., Cruz-Garcia, L., Gutiérrez, J., & Navarro, I. (2010). Endocrine control of oleic acid and glucose metabolism in rainbow trout (Oncorhynchus mykiss) muscle cells in culture. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *299*(2), R562–R572. https://doi.org/10.1152/ajpregu.00696.2009

Sant, K., Jacobs, H., Xu, J., Borofski, K., Moss, L., Moss, J., & Timme-Laragy, A. (2016). Assessment of Toxicological Perturbations and Variants of Pancreatic Islet Development in the Zebrafish Model. *Toxics*, *4*(3), 20. https://doi.org/10.3390/toxics4030020

Santos, S. (1978). *Polychlorinated biphenyls in New England*. Boston, MA. https://doi.org/SDMS DocID 222009

Sarath Josh, M. K., Pradeep, S., Vijayalekshmi Amma, K. S., Balachandran, S., Abdul Jaleel, U. C., Doble, M., … Benjamin, S. (2014). Phthalates efficiently bind to human peroxisome proliferator activated receptor and retinoid X receptor α, β, γ subtypes: An *in silico* approach. *Journal of Applied Toxicology*, *34*(7), 754–765. https://doi.org/10.1002/jat.2902

Sargent, J., Bell, G., McEvoy, L., Tocher, D., & Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, *177*(1–4), 191–199. https://doi.org/10.1016/S0044-8486(99)00083-6

Scheider, W. A., Cox, C., Hayton, A., Hitchin, G., & Vaillancourt, A. (1998). Current Status and Temporal Trends in Concentrations of Persistent Toxic Substances in Sport Fish and Juvenile Forage Fish in the Canadian Waters of the Great Lakes. *Environmental Monitoring and Assessment*, *53*(1), 57–76. https://doi.org/10.1023/A:1005929332152

Schlegel, A. (2012). Studying non-alcoholic fatty liver disease with zebrafish: A confluence of optics, genetics, and physiology. *Cellular and Molecular Life Sciences : CMLS*, *69*(23), 3953–3961. https://doi.org/10.1007/s00018-012-1037-y

Schmidt, K., Schmidtke, J., Kohl, C., Wilhelm, R., Schiemann, J., van der Voet, H., & Steinberg, P. (2016). Enhancing the interpretation of statistical P values in toxicology studies: implementation of linear mixed models (LMMs) and

standardized effect sizes (SESs). *Archives of Toxicology*, *90*(3), 731–751. https://doi.org/10.1007/s00204-015-1487-8

Schwartz, P. M., Jacobson, S. W., Fein, G., Jacobson, J. L., & Price, H. A. (1983). Lake Michigan fish consumption as a source of polychlorinated biphenyls in human cord serum, maternal serum, and milk. *American Journal of Public Health*, *73*(3), 293–296. https://doi.org/10.2105/AJPH.73.3.293

Scott, K. D., Fåhræus-Van Ree, G. E., & Parrish, C. C. (2002). Sex Differences in Hepatic Lipids of Toxaphene-Exposed Juvenile Yellowtail Flounder (Pleuronectes ferrugineus Storer). *Ecotoxicology and Environmental Safety*, *51*(3), 168–176. https://doi.org/10.1006/EESA.2001.2135

Sena, G. C., Freitas-Lima, L. C., Merlo, E., Podratz, P. L., de Araújo, J. F. P., Brandão, P. A. A., … Graceli, J. B. (2017). Environmental obesogen tributyltin chloride leads to abnormal hypothalamic-pituitary-gonadal axis function by disruption in kisspeptin/leptin signaling in female rats. *Toxicology and Applied Pharmacology*, *319*, 22–38. https://doi.org/10.1016/j.taap.2017.01.021

Sérée, E., Villard, P.-H., Pascussi, J.-M., Pineau, T., Maurel, P., Nguyen, Q. B., … Barra, Y. (2004). Evidence for a new human CYP1A1 regulation pathway involving PPAR-α and 2 PPRE sites. *Gastroenterology*, *127*(5), 1436–1445. https://doi.org/10.1053/j.gastro.2004.08.023

Sergeev, A. V, & Carpenter, D. O. (2011). Increase in metabolic syndrome-related hospitalizations in relation to environmental sources of persistent organic pollutants. *International Journal of Environmental Research and Public Health*, *8*(3), 762–776. https://doi.org/10.3390/ijerph8030762

Seth, A., Stemple, D. L., & Barroso, I. (2013). The emerging use of zebrafish to model metabolic disease. *Disease Models & Mechanisms*, *6*(5), 1080–1088. https://doi.org/10.1242/dmm.011346

Sheikh, I. A., Khweek, A. A., & Beg, M. A. (2016). Peroxisome Proliferator-activated Receptors as Potential Targets for Carcinogenic Activity of Polychlorinated Biphenyls: A Computational Perspective. *Anticancer Research*, *36*(11), 6117–6124. https://doi.org/10.21873/anticanres.11202

Sheridan, M. A. (1988). Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *90*(4), 679–690. https://doi.org/10.1016/0305-0491(88)90322-7

Sheridan, M. A. (1994). Regulation of lipid metabolism in poikilothermic vertebrates. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*,

107(4), 495–508. https://doi.org/10.1016/0305-0491(94)90176-7

Shimba, S., Todoroki, K., Aoyagi, T., & Tezuka, M. (1998). Depletion of arylhydrocarbon receptor during adipose differentiation in 3T3-L1 cells. *Biochemical and Biophysical Research Communications*, *249*(1), 131–137. https://doi.org/10.1006/bbrc.1998.9100

Shimba, S., Wada, T., & Tezuka, M. (2001). Arylhydrocarbon receptor (AhR) is involved in negative regulation of adipose differentiation in 3T3-L1 cells: AhR inhibits adipose differentiation independently of dioxin. *Journal of Cell Science*, *114*(15), 2809–2817.

Shin, S., Wakabayashi, N., Misra, V., Biswal, S., Lee, G. H., Agoston, E. S., … Kensler, T. W. (2007). NRF2 modulates aryl hydrocarbon receptor signaling: influence on adipogenesis. *Molecular and Cellular Biology*, *27*(20), 7188–7197. https://doi.org/10.1128/MCB.00915-07

Shoucri, B. M., Martinez, E. S., Abreo, T. J., Hung, V. T., Moosova, Z., Shioda, T., & Blumberg, B. (2017). Retinoid X Receptor Activation Alters the Chromatin Landscape To Commit Mesenchymal Stem Cells to the Adipose Lineage. *Endocrinology*, *158*(10), 3109–3125. https://doi.org/10.1210/en.2017-00348

Shulman, A. I., & Mangelsdorf, D. J. (2005). Retinoid X Receptor Heterodimers in the Metabolic Syndrome. *New England Journal of Medicine*, *353*(6), 604–615. https://doi.org/10.1056/NEJMra043590

Silverstone, A. E., Rosenbaum, P. F., Weinstock, R. S., Bartell, S. M., Foushee, H. R., Shelton, C., & Pavuk, M. (2012). Polychlorinated Biphenyl (PCB) Exposure and Diabetes: Results from the Anniston Community Health Survey. *Environmental Health Perspectives*, *120*(5), 727–732. https://doi.org/10.1289/ehp.1104247

Singh, Anita; Maichle, R. (2017). ProUCL Version 5.1 User Guide Statistical Software for Environmental Applications for Data Sets with and without Nondetect Observations. US EPA, Office of Research and Development.

Sonak, S., Pangam, P., Giriyan, A., & Hawaldar, K. (2009). Implications of the ban on organotins for protection of global coastal and marine ecology. *Journal of Environmental Management*, *90*, S96–S108. https://doi.org/10.1016/j.jenvman.2008.08.017

Sookoian, S., Gianotti, T. F., Burgueño, A. L., & Pirola, C. J. (2013). Fetal metabolic programming and epigenetic modifications: a systems biology approach. *Pediatric Research*, *73*(4–2), 531–542. https://doi.org/10.1038/pr.2013.2

Springer, C., Dere, E., Hall, S. J., McDonnell, E. V, Roberts, S. C., Butt, C. M., …

Boekelheide, K. (2012). Rodent thyroid, liver, and fetal testis toxicity of the monoester metabolite of bis-(2-ethylhexyl) tetrabromophthalate (tbph), a novel brominated flame retardant present in indoor dust. *Environmental Health Perspectives*, *120*(12), 1711–1719. https://doi.org/10.1289/ehp.1204932

Stevens, G. A., Singh, G. M., Lu, Y., Danaei, G., Lin, J. K., Finucane, M. M., … Ezzati, M. (2012). National, regional, and global trends in adult overweight and obesity prevalences. *Population Health Metrics*, *10*(1), 22. https://doi.org/10.1186/1478-7954-10-22

Sun, K., Kusminski, C. M., & Scherer, P. E. (2011). Adipose tissue remodeling and obesity. *The Journal of Clinical Investigation*, *121*(6), 2094–2101. https://doi.org/10.1172/JCI45887

Sun, K., Tordjman, J., Clément, K., & Scherer, P. E. (2013). Fibrosis and adipose tissue dysfunction. *Cell Metabolism*, *18*(4), 470–477. https://doi.org/10.1016/j.cmet.2013.06.016

Szlinder-Richert, J., Barska, I., Mazerski, J., & Usydus, Z. (2009). PCBs in fish from the southern Baltic Sea: Levels, bioaccumulation features, and temporal trends during the period from 1997 to 2006. *Marine Pollution Bulletin*, *58*(1), 85–92. https://doi.org/10.1016/j.marpolbul.2008.08.021

Taxvig, C., Dreisig, K., Boberg, J., Nellemann, C., Schelde, A. B., Pedersen, D., … Vinggaard, A. M. (2012). Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARγ activation. *Molecular and Cellular Endocrinology*, *361*(1–2), 106–115. https://doi.org/10.1016/J.MCE.2012.03.021

Thayer, K. A., Heindel, J. J., Bucher, J. R., & Gallo, M. A. (2012). Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. *Environmental Health Perspectives*, *120*(6), 779–789. https://doi.org/10.1289/ehp.1104597

Timme-Laragy, A. R., Sant, K. E., Rousseau, M. E., & diIorio, P. J. (2015). Deviant development of pancreatic beta cells from embryonic exposure to PCB-126 in zebrafish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *178*, 25–32. https://doi.org/10.1016/J.CBPC.2015.08.012

Tingaud-Sequeira, A., Ouadah, N., & Babin, P. J. (2011). Zebrafish obesogenic test: a tool for screening molecules that target adiposity. *The Journal of Lipid Research*, *52*(9), 1765–1772. https://doi.org/10.1194/jlr.D017012

Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., & Spiegelman, B. M. (1994). mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes &*

Development, *8*(10), 1224–1234.

Tontonoz, P., & Spiegelman, B. M. (2008). Fat and beyond: the diverse biology of PPARgamma. *Annual Review of Biochemistry*, *77*, 289–312. https://doi.org/10.1146/annurev.biochem.77.061307.091829

Tsukamoto, S., Mizuta, T., Fujimoto, M., Ohte, S., Osawa, K., Miyamoto, A., … Katagiri, T. (2015). Smad9 is a new type of transcriptional regulator in bone morphogenetic protein signaling. *Scientific Reports*, *4*(1), 7596. https://doi.org/10.1038/srep07596

US EPA. (2001). *Fentin hydroxide - Chemical Details | Chemical Search | Pesticides | US EPA*.

US EPA REG 01. (1998). *Declaration for the Record of Decision: New Bedford Harbor Superfund Site Upper and Lower Harbor Operable Unit*. https://doi.org/SDMS DocID 38206

US EPA REG 01. (2017). New Bedford Harbor Fish Consumption Regulations and Recommendations. Retrieved August 3, 2017, from https://www.epa.gov/new-bedford-harbor/fish-consumption-regulations-and-recommendations

van Raalte, D. H., Li, M., Pritchard, P. H., & Wasan, K. M. (2004). Peroxisome proliferator-activated receptor (PPAR)-alpha: a pharmacological target with a promising future. *Pharmaceutical Research*, *21*(9), 1531–1538.

van Voorhees, D. (2016). *Fisheries of the United States 2015. Current Fishery Statistics No. 2015*. Silver Spring, MD.

Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs, D. R., Lee, D.-H., … Myers, J. P. (2012). Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine Reviews*, *33*(3), 378–455. https://doi.org/10.1210/er.2011-1050

Varlamov, O., Bethea, C. L., & Roberts, C. T. (2015). Sex-Specific Differences in Lipid and Glucose Metabolism. *Frontiers in Endocrinology*, *5*, 241. https://doi.org/10.3389/fendo.2014.00241

Vert, G., & Chory, J. (2011). Crosstalk in cellular signaling: background noise or the real thing? *Developmental Cell*, *21*(6), 985–991. https://doi.org/10.1016/j.devcel.2011.11.006

Villard, P.-H., Barlesi, F., Armand, M., Dao, T.-M.-A., Pascussi, J.-M., Fouchier, F., … Seree, E. (2011). CYP1A1 Induction in the Colon by Serum: Involvement of the PPARα Pathway and Evidence for a New Specific Human PPREα Site. *PLoS One*,

6(1), e14629. https://doi.org/10.1371/journal.pone.0014629

von Stackelberg, K., Li, M., & Sunderland, E. (2017). Results of a national survey of high-frequency fish consumers in the United States. *Environmental Research*, *158*, 126–136. https://doi.org/10.1016/j.envres.2017.05.042

Wafer, R., Tandon, P., & Minchin, J. E. N. (2017). The Role of Peroxisome Proliferator-Activated Receptor Gamma (PPARG) in Adipogenesis: Applying Knowledge from the Fish Aquaculture Industry to Biomedical Research. *Frontiers in Endocrinology*, *8*, 102. https://doi.org/10.3389/fendo.2017.00102

Wahlang, B., Falkner, K. C., Gregory, B., Ansert, D., Young, D., Conklin, D. J., … Cave, M. (2013). Polychlorinated biphenyl 153 is a diet-dependent obesogen that worsens nonalcoholic fatty liver disease in male C57BL6/J mice. *The Journal of Nutritional Biochemistry*, *24*(9), 1587–1595. https://doi.org/10.1016/j.jnutbio.2013.01.009

Wahlang, B., Prough, R. A., Falkner, K. C., Hardesty, J. E., Song, M., Clair, H. B., … Cave, M. C. (2016). Polychlorinated Biphenyl-Xenobiotic Nuclear Receptor Interactions Regulate Energy Metabolism, Behavior, and Inflammation in Non-alcoholic-Steatohepatitis. *Toxicological Sciences*, *149*(2), 396–410. https://doi.org/10.1093/toxsci/kfv250

Wang, F., Mullican, S. E., DiSpirito, J. R., Peed, L. C., & Lazar, M. A. (2013). Lipoatrophy and severe metabolic disturbance in mice with fat-specific deletion of PPAR. *Proceedings of the National Academy of Sciences*, *110*(46), 18656–18661. https://doi.org/10.1073/pnas.1314863110

Wang, N., Chen, Y., Ning, Z., Li, Q., Han, B., Zhu, C., … Lu, Y. (2016). Exposure to Famine in Early Life and Nonalcoholic Fatty Liver Disease in Adulthood. *The Journal of Clinical Endocrinology & Metabolism*, *101*(5), 2218–2225. https://doi.org/10.1210/jc.2016-1076

Wang, R.-L., Bencic, D. C., Garcia-Reyero, N., Perkins, E. J., Villeneuve, D. L., Ankley, G. T., & Biales, A. D. (2014). Natural Variation in Fish Transcriptomes: Comparative Analysis of the Fathead Minnow (Pimephales promelas) and Zebrafish (Danio rerio). *PLoS One*, *9*(12), e114178. https://doi.org/10.1371/journal.pone.0114178

Wang, X., & Kilgore, M. W. (2002). Signal cross-talk between estrogen receptor alpha and beta and the peroxisome proliferator-activated receptor gamma1 in MDA-MB-231 and MCF-7 breast cancer cells. *Molecular and Cellular Endocrinology*, *194*(1–2), 123–133. https://doi.org/10.1016/S0303-7207(02)00154-5

Wang, Y., Zhang, J., & Pan, M. (2017). Tributyltin targets hepatic transcriptional regulation of lipid metabolism in mice. *Toxicological & Environmental Chemistry*,

99(3), 492–504. https://doi.org/10.1080/02772248.2016.1196207

Wassenberg, D. M., & Giulio, R. T. D. (2004). Teratogenesis in Fundulus heteroclitus embryos exposed to a creosote-contaminated sediment extract and CYP1A inhibitors. *Marine Environmental Research*, *58*(2–5), 163–168. https://doi.org/10.1016/J.MARENVRES.2004.03.012

Watt, J., Baker, A. H., Meeks, B., Pajevic, P. D., Morgan, E. F., Gerstenfeld, L. C., & Schlezinger, J. J. (2018). Tributyltin induces distinct effects on cortical and trabecular bone in female C57Bl/6J mice. *Journal of Cellular Physiology*, *233*(9), 7007–7021. https://doi.org/10.1002/jcp.26495

Watt, J., & Schlezinger, J. J. (2015). Structurally-diverse, PPARγ-activating environmental toxicants induce adipogenesis and suppress osteogenesis in bone marrow mesenchymal stromal cells. *Toxicology*, *331*, 66–77. https://doi.org/10.1016/j.tox.2015.03.006

Weaver, G. (1982). *PCB Pollution in the New Bedford, Massachusetts Area: A status report*. *Massachuserts Coastal Zone Management*. https://doi.org/SDMS DocID 62224

White, R. D., Shea, D., & Stegeman, J. J. (1997). Metabolism of the Aryl Hydrocarbon Receptor Agonist 3,3′,4,4′-Tetrachlorobiphenyl by the Marine Fish Scup (Stenotomus chrysops) In Vivo and In Vitro. *Drug Metabolism and Disposition*, *25*(5).

White, S. S., & Birnbaum, L. S. (2009). An Overview of the Effects of Dioxins and Dioxin-Like Compounds on Vertebrates, as Documented in Human and Ecological Epidemiology. *Journal of Environmental Science and Health, Part C*, *27*(4), 197–211. https://doi.org/10.1080/10590500903310047

Whitehead, A., Clark, B. W., Reid, N. M., Hahn, M. E., & Nacci, D. (2017). When evolution is the solution to pollution: Key principles, and lessons from rapid repeated adaptation of killifish (*Fundulus heteroclitus*) populations. *Evolutionary Applications*, *10*(8), 762–783. https://doi.org/10.1111/eva.12470

Whitehead, A., Pilcher, W., Champlin, D., & Nacci, D. (2012). Common mechanism underlies repeated evolution of extreme pollution tolerance. *Proceedings. Biological Sciences*, *279*(1728), 427–433. https://doi.org/10.1098/rspb.2011.0847

Whitehead, A., Triant, D. A., Champlin, D., Nacci, D. E., & von Deimling, A. (2010). Comparative transcriptomics implicates mechanisms of evolved pollution tolerance in a killifish population. *Molecular Ecology*, *19*(23), 5186–5203. https://doi.org/10.1111/j.1365-294X.2010.04829.x

Wildish, D. J., & Kristmanson, D. D. (1979). Tidal Energy and Sublittoral Macrobenthic Animals in Estuaries. *Journal of the Fisheries Research Board of Canada*, *36*(10), 1197–1206. https://doi.org/10.1139/f79-173

Witten, P. E., Harris, M. P., Huysseune, A., & Winkler, C. (2017). Small teleost fish provide new insights into human skeletal diseases. In *Methods in Cell Biology* (Vol. 138, pp. 321–346). https://doi.org/10.1016/bs.mcb.2016.09.001

Wooldridge, J. M. (2006). *Introductory Econometrics: A Modern Approach*. Mason, OH: South-Western College Publishing.

Wu, C. W., Chu, E. S. H., Lam, C. N. Y., Cheng, A. S. L., Lee, C. W., Wong, V. W. S., … Yu, J. (2010). PPARgamma is essential for protection against nonalcoholic steatohepatitis. *Gene Therapy*, *17*(6), 790–798. https://doi.org/10.1038/gt.2010.41

Wu, H., Yu, W., Meng, F., Mi, J., Peng, J., Liu, J., … Wang, X. (2017). Polychlorinated biphenyls-153 induces metabolic dysfunction through activation of ROS/NF-κB signaling via downregulation of HNF1b. *Redox Biology*, *12*, 300–310. https://doi.org/10.1016/J.REDOX.2017.02.026

Xue, J., Liu, S. V, Zartarian, V. G., Geller, A. M., & Schultz, B. D. (2014). Analysis of NHANES measured blood PCBs in the general US population and application of SHEDS model to identify key exposure factors. *Journal of Exposure Science & Environmental Epidemiology*, *24*(6), 615–621. https://doi.org/10.1038/jes.2013.91

Yadetie, F., Karlsen, O. A., Eide, M., Hogstrand, C., & Goksøyr, A. (2014). Liver transcriptome analysis of Atlantic cod (Gadus morhua) exposed to PCB 153 indicates effects on cell cycle regulation and lipid metabolism. *BMC Genomics*, *15*(1), 481. https://doi.org/10.1186/1471-2164-15-481

Yan, Z., Lu, G., & He, J. (2012). Reciprocal inhibiting interactive mechanism between the estrogen receptor and aryl hydrocarbon receptor signaling pathways in goldfish (Carassius auratus) exposed to 17β-estradiol and benzo[a]pyrene. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *156*(1), 17–23. https://doi.org/10.1016/J.CBPC.2012.03.001

Yessoufou, A., & Wahli, W. (2010). Multifaceted roles of peroxisome proliferator-activated receptors (PPARs) at the cellular and whole organism levels. *Swiss Medical Weekly*. https://doi.org/10.4414/smw.2010.13071

Yonezawa, T., Hasegawa, S., Ahn, J.-Y., Cha, B.-Y., Teruya, T., Hagiwara, H., … Woo, J.-T. (2007). Tributyltin and triphenyltin inhibit osteoclast differentiation through a retinoic acid receptor-dependent signaling pathway. *Biochemical and Biophysical Research Communications*, *355*(1), 10–15. https://doi.org/10.1016/J.BBRC.2006.12.237

Yoon, M. (2010). PPARα in Obesity: Sex Difference and Estrogen Involvement. *PPAR Research*, *2010*. https://doi.org/10.1155/2010/584296

Zhang, J., Sun, P., Kong, T., Yang, F., & Guan, W. (2016). Tributyltin promoted hepatic steatosis in zebrafish (Danio rerio) and the molecular pathogenesis involved. *Aquatic Toxicology*, *170*, 208–215. https://doi.org/10.1016/J.AQUATOX.2015.11.028

Zhang, L., Hatzakis, E., Nichols, R. G., Hao, R., Correll, J., Smith, P. B., … Patterson, A. D. (2015). Metabolomics Reveals that Aryl Hydrocarbon Receptor Activation by Environmental Chemicals Induces Systemic Metabolic Dysfunction in Mice. *Environmental Science & Technology*, *49*(13), 8067–8077. https://doi.org/10.1021/acs.est.5b01389

Zhao, Y., Zhang, K., Giesy, J. P., & Hu, J. (2015). Families of Nuclear Receptors in Vertebrate Models: Characteristic and Comparative Toxicological Perspective. *Scientific Reports*, *5*(1), 8554. https://doi.org/10.1038/srep08554

Zuo, Z., Chen, S., Wu, T., Zhang, J., Su, Y., Chen, Y., & Wang, C. (2011). Tributyltin causes obesity and hepatic steatosis in male mice. *Environmental Toxicology*, *26*(1), 79–85. https://doi.org/10.1002/tox.20531

CURRICULUM VITAE