RESPONSES OF YOUNG-OF-THE-YEAR BLUEFISH, *POMATOMUS SALTATRIX*, TO CONTAMINANTS FROM AN URBAN ESTUARY

by

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ABSTRACT OF THE DISSERTATION

Responses of young-of-the-year bluefish, *Pomatomus saltatrix*, exposed to contaminants from an urban estuary

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Certain populations of young-of-the-year (YOY) bluefish, *Pomatomus saltatrix*, reside in contaminated estuaries of the mid-Atlantic bight during periods of rapid growth and development. YOY bluefish from the Tuckerton, NJ area of Great Bay (TK) were fed daily in a laboratory with common prey fish, menhaden and mummichog, from two sites: TK (reference) or Hackensack River (HR) (contaminated). Bluefish were also collected from the HR and TK site for analysis. HR-fed and field-caught bluefish and HR prey fish and stomach contents contained significantly elevated concentrations of PCBs, DDTs, and mercury. HR bluefish had reduced growth, feeding, and activity. tPCB and tDDT concentrations in prey in the stomachs of HR bluefish were higher than those in the field-caught specimens. Prey with higher body burdens may become slower and easier to capture. If bluefish are preferentially foraging on such prey, greater amounts of contaminants can be trophically transferred.

PCB congeners accumulated at different concentrations creating PCB fingerprints which correlated with feeding ecology of the fish. PCB fingerprints in the HR-fed bluefish were nearly identical to each other and closest to that of the mummichog, their sole prey during the last month of the feeding experiment. PCB fingerprints of field-caught bluefish were similar to menhaden, the dominant prey in HR field bluefish stomachs. In contaminated marine systems PCB fingerprints can be utilized to establish trophic levels and possibly prey preference in individual fish.

In addition to altered behavior and growth, the HR-fed and field bluefish had significantly enlarged, irregular thyroid follicles, lined with thickened epithelial cells compared to the TK fish. The mean concentration of dopamine metabolites and the dopaminergic activity levels were significantly lower in HR field fish than in TK field. In contrast the mean concentrations of dopamine and serotonin and their metabolites and norepinephrine were significantly greater in the HR-fed bluefish compared the TK-fed. Overall the exposed fish displayed neurological and hormonal disruptions that may be responsible for their altered behavior and growth. In conclusion, the altered growth, feeding, activity and physiology of YOY bluefish exposed to these contaminated regimes may have detrimental effects on migration fitness and recruitment success.

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INTRODUCTION

Bluefish

The bluefish, *Pomatomus saltatrix*, is a monotypic species in the family Pomatomidae, related to the jacks and pompanos. Adult bluefish are found in a variety of habitats, usually in response to food availability and spawning cues. Adults have few predators, mainly mako sharks and swordfish, and can live 12 years and weigh up to 10 kg (Fahay 1999).

Their life history is similar to many coastal pelagic species. Most adult populations undertake extensive coastal migrations regulated by water temperatures, traveling in large loosely aggregated groups of like-size fish (Salerno et al. 2001). They migrate to off shore spawning grounds and release large numbers of eggs. The eggs and larvae are transported inshore with the warm-water current to juvenile nursery habitats; the eggs hatch in about 48 hours and the yolk is absorbed in another 24-48 hours. Pelagic juveniles form from larvae at about 18 to 25 days and 10-12 mm total length (Juanes et al. 1996).

The population off the east coast of North America has been studied for over a century, but there are still many unresolved questions. Adults spend the winters off the coast of Florida and begin a northerly migration in the spring, moving in and out of bays and sounds (Hare and Cowan 1996). Bluefish off the east coast of North America spawn over the outer continental shelf and shelf break (Hare and Cowan 1996, Miskiewicz et al. 1996). Evidence suggests that there are two distinct spawning events on the northeast

continental shelf resulting in spring and summer cohorts of larvae (Kendall and Walford 1979). Most bluefish mature at age two and have high fecundity. Females can produce 900,000 to 4,500,000 eggs. The distribution of eggs is related to temperature and salinity and can vary from year to year. Bluefish larvae have been found offshore between Cape Cod, MA and Palm Beach, Florida. After spring spawn, as pelagic juveniles grow and some are able to swim, they are carried inshore by the Gulf Stream from where they were hatched in the South Atlantic Bight (West Palm Beach, FL to Cape Hatteras NC) to estuaries along the Middle Atlantic Bight (Cape Hatteras, NC to Cape Cod, MA) (Hare and Cowen 1996). Spring spawned snappers (juveniles) arrive in the estuaries at about 60-76 days old and 55 mm total length around late May to mid-June (Nyman and Conover 1988, McBride and Conover 1991). Summer spawns either stay in coastal nursery areas or move to Mid Atlantic Bight estuaries in mid to late August at about 33-47 days old and 46 mm long (Kendall and Walford 1979, McBride and Conover 1991, Able and Fahay 1998). Summer-spawned individuals arriving in August do not attain as large a size at emigration (Nyman and Conover 1987, McBride and Conover 1991, Munch and Conover 2000, Able et al. 2003). Spring-spawned cohorts dominate the adult bluefish population probably due to a size advantage at the onset of winter migration (McBride and Conover 1991).

Some recent evidence indicates continual spawning over a protracted period without two distinct cohorts, but instead separate recruitment periods (Smith et al. 1994, Juanes et al. 1996, Miskiewicz et al. 1996). Studies of spatial and temporal distribution of eggs and larvae challenge the common view of two separate spawning periods along eastern coast of US (Muelbert and Sinque 1996). Success in the movement of larvae

from coastal waters to estuarine nursery areas is linked to offshore circulation patterns and cross shelf exchange. The driving forces are the changing flow patterns of the warm water Gulf Stream and the presence of onshore currents, which is advantageous for recruitment to nursery areas and routinely change during the spawning season (Miskiewicz et al. 1996). The Slope Sea (the region between the Gulf Stream and the Middle Atlantic Bight shelf edge) also presents an obstacle for larvae spawned in the lower Middle Atlantic Bight and may inhibit recruitment (Juanes et al. 1996). These factors, splitting the recruitment, may create a picture of what appears to be two separate spawning cohorts.

The physical oceanography of the South Atlantic Bight and the Middle Atlantic Bight are very different and could affect the transport of eggs and larvae. In the South Atlantic Bight, the Gulf Stream, a western boundary current, flows along the shelf and has a major influence on shelf circulation. Then at Cape Hatteras the Gulf Stream veers away from the shelf to approximately 100-300km off shore of the middle and northern Middle Atlantic Bight shelf edge. This alteration seriously decreases the direct influence on the shelf water flow, which generally moves along-shelf towards the southwest in the Middle Atlantic Bight (Juanes et al. 1996). At the southern end of the Middle Atlantic Bight, shelf waters are drawn into the Gulf Stream. Successful recruitment of larvae and juveniles to inshore nursery grounds may be largely dependent on the timing of the breakdown of the surface shelf/slope temperature front, their ability to be transported across the Slope Sea, and summertime winds (Hare and Cowen 1996, Juanes et al. 1996). The habitat condition of the estuary that they recruit into as juveniles could strongly effect their fitness at the end of the summer.

The prey abundance and quality strongly influences YOY bluefish size at the end of their growing season (Juanes and Conover 1994, Buckel and Conover 1997, Buckel et al. 1999a). An ontogenetic shift in diet after transition from oceanic to coastal habitats occurs from primarily copepods to fish and some invertebrates and an increase in growth rate is observed with this shift in feeding behavior (Mcbride and Conover 1991, Juanes and Conover 1994). Bluefish are opportunistic feeders, notorious for being voracious predators with swallow rates of up to 25% their body weight per day, in preparation from southward migration (Juanes and Conover 1994, Buckel et al 1999b, Juanes et al. 2001). Exponential growth is evident during larval and pelagic juvenile stages and linear growth of juveniles takes place once entering the estuaries and inshore habitats (Juanes et al. 1996).

Small bluefish have been found to eat a diet split almost equally between fish and invertebrates and as they get older they eat mainly fish. They consume larger and more diverse prey as they grow (Buckel and Conover 1997). Faster growth is achieved on diets high in fish (Buckel et al. 1999b). Bluefish survival may be tied to body size prior to autumn migration (Munch and Conover 2000). Concentrations of both adults and juveniles are greatest from North Carolina to Cape Cod in late summer when the water temperature is at a preferred range of 19-22 C. They remain there until water temperatures drop below 13°-15°C (Salerno et al. 2001). In autumn, YOY bluefish at about 180 mm total length begin to migrate out of the bays and move south along the coast. Superior size achieved during the estuary growth phase may be vital to successful migration and over wintering periods (Juanes and Conover 1994, Able et al 2003).

Stock Status

Bluefish, *Pomatomus saltatrix* are harvested by valuable and popular recreational and commercial fisheries in several areas around the world and populations have shown dramatic fluctuations in abundance. Consistently ranking in the top ten species harvested by weight by recreational marine anglers in the Mid-Atlantic, landings average 22,000 metric tons per year from 1974-2001. Recruitment has been declining since 1989, and substantial reduction in catch over the previous decade (the average catch 1996-1998 was only 24% of 1981-1988 averages) triggered concerns about stock sustainability; however, the cause of the decline is still unknown (Lewis 2002, ASMFC 2003). Possible causes include overfishing, declining habitat quality and reproductive success, and shifts in feeding ecology (MAFMC 1998). Recent stock assessments have reported increases since 1999 and that the bluefish stock has been classified as rebuilt and is no longer considered overfished. Nevertheless, ongoing research into population dynamics is important to provide information for improved future management of this ecologically important species (Shepard 2006, Balsiger 2008).

Mid-Atlantic Bight Estuaries

Estuaries along the mid-Atlantic Bight (MAB) are considered essential fish habitat for young of the year bluefish (Fahay et al. 1999, NOAA 2006). Their health at this life stage is an important factor determining the stock size of fish (Gartland et al. 2006). Survivorship of YOY bluefish may be highly dependent on which estuary they end up in during larval transport. Many MAB estuaries contain varying degrees of contamination including PCBs, PAHs and metals, ranging from relatively pristine (Great

Bay) to heavily impacted (Newark Bay). A national survey of fish and shellfish from U.S. estuaries ranked PCBs as the most prevalent toxicant nationwide and in the Northeast followed, by mercury, PAHs, and DDT (Harvey et al. 2008). While the use of PCBs and DDTs has been banned for decades as persistent, bioaccumulative and toxic pollutants the importance of research into their impacts on marine organisms also persists. Anthropogenic sources of contaminants along the Mid-Atlantic can lead to elevated body burdens of contaminants in the YOY that inhabit those estuaries. (O'Connor et al. 1982, Varanasi and Stein 1991, Kennish and Ruppel 1998, Weis and Ashley 2007).

PCBs, organic pesticides, mercury and other heavy metals and contaminants may be contributing to the population reductions by affecting the health of the YOY in the estuaries and adversely affecting their migratory ability and recruitment into the adult breeding population. Unlike resident estuarine fishes such as mummichogs, migratory bluefish are very unlikely to be able to evolve tolerance to contaminants (Weis 2001). Because of their early life history exposure in estuaries that may be contaminated, their high lipid content, and their piscivory, bluefish can acquire high levels of contaminants, including PCBs and methylmercury that biomagnify up the food chain (O'Connor et al. 1982, O'Neill and Kieley 1992).

The months that YOY bluefish live in polluted estuaries are a critical period of rapid growth and development. Continual exposure to these high levels of contaminants via the water, sediments and food can result in increasing deleterious effects. It is important to understand how habitat quality and sublethal effects of contaminants

influence the ability of YOY to reach an adequate size and condition necessary to survive their southward winter migration and recruit to the breeding population.

Contaminants

PCBs

Environmental stressors such as contaminants can have a severe impact on fish. Polychlorinated biphenyls, PCBs, are major environmental problems because they are bioaccumulative, persistent and toxic. They were initially used in transformers and other electrical equipment and were later used for lubricants and plasticizers. The manufacturing of PCBs was stopped because of evidence found that they caused harmful effects and concentrated in the environment because they do not readily degrade. They concentrate mainly in soil and sediment because they are hydrophobic. Once they enter the food chain they are lipophilic and are not easily metabolized. They can enter fish through water via gills, sediment and particularly through contaminated prey (Feldman and Titus 2001).

The log K_{ow} values of PCB congeners range from 4.5 to 8 and in aquatic organisms dietary uptake has been shown to be the main source of bioaccumulation for chemicals (such as PCBs) with octanol-water partition coefficients log (K_{ow}) greater than 5-6 (Gobas et al. 1999). The analysis of pumpkinseed sunfish in a controlled laboratory experiment displayed an increase of 5 times the accumulated PCB levels in fish with trophic exposure to contaminated prey from the Upper Hudson River compared to those fed clean prey (Feldman and Titus 2001). The exposed fish also displayed a chlorine

shift to a greater proportion of the more chlorinated congeners than in fish with only aqueous exposure. Different congeners may accumulate more in different species of animals and trophic levels (Feldman and Titus 2001). More highly chlorinated congeners more concentrated in older larger specimens maybe because they are eliminated more slowly.

Generally bioaccumulation factors increase with chlorine content from the trichlorobiphenyls up through the hexachlorobiphenyls and then generally decrease with higher chlorine content of hepta- and octachlorobiphenyls (EPA 1999). Less chlorinated PCBs don't bioconcentrate as efficiently and are more readily metabolized and excreted (Giesy and Kannan, 2002). For instance bichlorobiphenyls display an approximately 450-fold decrease in the tendency to bioaccumulation in fish compared with tri- and tetrachlorinated PCBs (Magnusson et al. 2006). Many factors of the species and the chemicals can affect the final level of bioaccumulation including uptake and depuration rate, feeding ecology, extent of pollutant metabolism, animal growth rate, size and longevity (Deshpande et al 2000). In addition, depuration rate of PCBs has been found to decrease with increased hydrophobicity of the compound (Magnusson et al. 2006). PCB's tend to biomagnify up the food chain and can bioaccumulate in fish to levels hundreds of thousand times higher than found in the water (Rasmussen et al. 1990).

PCBs are carcinogenic in animals and may cause cancer in exposed adults and many other serious health effects in both humans and animals including damage to the nervous system of developing fetus, the immune system, endocrine system, and the reproductive system (Eriksson and Fredriksson 1996). Neurodevelopmental and

cognitive impairments in young children have been reported to be caused from prenatal exposure to elevated PCBs (Roegge et al 2004). PCBs are associated with a wide array of toxic effects on fish including liver damage, impairment of osmoregulation, reduction of immune functions, reproductive dysfunctions, impairment of sexual maturation, endocrine disruption, developmental disturbances, apoptosis, ATPase inhibition, and mortality. Postnatal exposure to rodents has been shown to affect subsequent behaviors (Eriksson and Fredriksson 1996). Neurodevelopmental and cognitive impairments in young children have been reported to be caused from prenatal exposure to elevated PCBs (Jacobson et al 1992). These developments of impairments appear to be more likely when the PCBs are highly chlorinated congeners (Stewart et al. 2000).

Most of the acute toxic effects reported in fish are related to commercial PCBs mixtures and to coplanar PCBs, while relatively few findings exist concerning effects of non-coplanar PCBs. While in the past, most studies addressed acute toxicity of pollutants, recently a growing concern has become the sublethal effects of exposure to contaminants and the overall impact on the ecosystem and the populations. Recent developments have brought to light non-dioxin-like effects of PCBs, therefore it is pertinent to examine critically the effective doses at which both coplanar and non-coplanar PCBs could elicit effects in animals (Giesy and Kannan 2002). Previous studies support the hypothesis that non-coplanar congeners may be responsible for the neurobehavioral effects of PCBs, but most of these effects were reported in mammals including humans (Shain and Seegal 1991). Unfortunately a limited amount of information is available on behavioral toxic potencies of PCBs on aquatic organisms, fish in particular. Despite the fact that the production of PCBs has been banned they are still

prevalent in the ecosystem and more importantly they are mixing with various other highly toxic pollutants such as mercury.

Mercury

Mercury has been well known as an environmental pollutant since the 1950's. It is a common aquatic and marine pollutant which bioaccumulates in fish through trophic transfer, water and sediment (Grippo 2003). Unlike organic contaminants (PCBs and dioxins) which concentrate in the skin and fat, mercury concentrates in the muscle tissue of fish. As a result mercury is not filleted or cooked out of fish and can pose a serious threat to human's consuming the contaminated fish.

The main source of mercury to most aquatic ecosystems is deposition from the atmosphere, via rainfall, in addition to some isolated cases of known point sources. There are many natural and anthropogenic sources of mercury to the environment. Volcanoes, natural mercury deposits, and volatilization from the ocean are some of the natural sources. Coal combustion, chlorine alkali processing, waste incineration, and metal processing, make up the bulk of the anthropogenic sources. It has been estimated that the amount of mercury has been doubled or tripled in the atmosphere by human activities resulting in 1.5% per year increase in atmospheric burden (USGS 1997).

Mercury is deposited from the atmosphere in its inorganic form and then may be converted to an organic form, methylmercury, by a metabolic process of bacteria in aquatic sediments. This conversion to methylmercury is important because methylmercury is much more toxic than inorganic mercury and takes much longer to be

eliminated by other organisms. Methylmercury-containing bacteria may be consumed or the bacteria may release the methylmercury to the water where it is quickly adsorb to plankton in which it will also be consumed up the food chain and bioacculmulated or possibly biomagnified in the larger organisms (USGS 1997).

Mercury exposure can disrupt neurodevelopment, immune function, reproduction and behavior (Weis el al. 2001, Grippo and Heath 2003, Newman and Unger 2003, Scott and Sloman 2004). Exposure to mercury has been linked numerous behavioral impairments in fish including; poor predator avoidance in golden shiner and mummichogs, impaired prey capture and feeding behavior of fat head minnows and mummichogs and altered swimming activity of mummichogs (Tsai et al. 1995, Zhou et al. 2001, Grippo and Heath, 2003, Webber and Haines 2003, Weis et al 2003).

Behavioral ecotoxicology

The field of behavioral ecotoxicology is slowly expanding and fish behavior studies are becoming more common. Behavior is essential to a species survival in their environment. It is the product of the interaction of an organism with its external environment and can be used as an indicator of environmental stressors (Little and Finger 1990). Subtle changes in behavior, whether food acquisition, activity, predator avoidance, or reproduction and inappropriate behavioral responses to environmental and physological stimuli due to toxic effects of aquatic contaminants can have severe implications for survival. Behavioral reactions to contaminants can occur if physiological and biochemical processes are directly impaired. Exposure to pollutants affects the behavior of the individual organism at observable levels and at lower levels of

biological organization (e.g. neurotransmitters), and in turn influences conditions at population and community level. Fish are immersed in their physical and chemical environment, and therefore, in continuous interactions with potential environmental pollutants. If the contaminants are detectable by sense organs they can lead to preference-avoidance behavior that can significantly influence the level or duration of exposure, i.e. fish may move away, which could influence their habitat selection and/or spawning sites. Pollutants may alter a wide variety of fish behaviors, including sexual and reproductive behavior, swimming performance, feeding, respiratory behavior, learning, schooling behavior, avoidance/preference behavior, chemosensory communication and social behavior (Little and Finger 1990, Weis 2001, Weis 2003, Zala and Penn 2004).

Since behavior is the outcome of many complex developmental and physiological processes, it can provide a more inclusive measure than just one or a few biochemical or physiological parameters (Zala and Penn 2004, Scott and Sloman 2004). Overall, the threshold concentration levels for behavioral effects of contanimants are found to be 0.1 – 5.0 % of LC50 concentrations and behavioral changes often occurred 75% earlier than onset of mortality (Little and Finger 1990). Behavior may be a more useful indicator or biomarker and it is noninvasive, inexpensive, and potentially more powerful. Behaviors that normally occur in wild should be investigated, and the organisms should be exposed to contaminants and mixtures of contaminants that they are likely to encounter in nature.

Swimming activity

It has been shown that the swimming performance of fish is affected by a range of chemical stressors, can be utilized as an indicator of sublethal toxicity in fish and have implications for survival. The average toxicant concentration induces changes of swimming behavior at concentrations less than 16% of the concentrations that cause mortality and these changes can often be measured before other effects such as reduction of growth (Little and Finger 1990).

Several aspects of swimming behavior may be change simultaneously. Swimming capacity is the measure of orientation to water flow, and the physical capacity to swim against it. Swim activity is the frequency and duration of movements, speed and distance traveled during movement, frequency and angle of turns, position in the water column and form and pattern of swimming (Little and Finger 1990). A 28 day exposure to rainbow trout (*Oncorhynchus myhiss*) to dioxin reduced frequency of activity, changed posture of swimming and affected their positioning in water (Little and Finger 1990).

Xenobiotics can alter swimming movements such as changes in water column position (surfacing or resting on bottom), swimming posture (head up swimming), body movements or swimming patterns (frequent turns or spiralling). These characteristics can often be measured fairly easily and successfully. Accurate and consistent descriptions of subtle changes in patterns of movement require more complex set ups, such as high resolution photodetector chambers and computer-interfaced video systems that can quantify changes in detailed swimming behavior. Hyperactivity or hypoactivity can be measured as the frequency of entries and exits through a divided partition or number of

grid lines passed. These can disrupt feeding with a reduced search area and increase vulnerability to predation by making them more conspicuous.

Locomotion is important for such activities as feeding, predator avoidance, competitive interactions, schooling and migration behavior and therefore disruption could have many ecological consequences. Reducing the activity (and the costs that go along with it) can be an effective strategy if exposure is temporary. However, a lowered activity over longer-term exposures is likely to lead to an impaired performance of reduced feeding, mating activity, etc. and may be deleterious at the organism, population and commutity level (Schmidt et al. 2004).

Feeding

Reduced feeding is a common response to exposure to sublethal quantities of various contaminants (Little et al. 1990). Normal prey capture and predator avoidance behavior is vital for individual growth and survival and consequently to preserve the population size, size-structure, and age-structure. The reduced feeding may be linked to impaired growth and population declines. Successful feeding is influenced by motivation (frequency of strikes), search effectiveness, orientation to prey, strikes and misses, and appropriate coordination, and prey handling time (Buckel, et al. 1999, Weis 2001, Weis 2003). In some studies, frequency of strikes have been found to be less sensitive to contaminants than the actual prey capture suggesting that coordination was more effected that motivation (Little 2000). On the other hand, Brown et al. (1987) found that PCP

(pentachlorophenol) treated fish made fewer strikes, indicating decreased motivation to feed. Sandheinrich and Atchison (1989) observed an increase in prey handling time by toxic exposure. Particular toxicants may have different mechanisms of neurobehavioral toxicity.

Predator Avoidance

Another consideration is the vulnerability of prey to predation, hindered avoidance and predator evasion or the ability to escape from predators. The impact of anthropogenic stressors on the avoidance behavior of aquatic organisms has been observed and demonstrated in laboratory experiments of prey exposed to contaminants (Kraus and Kraus 1986, Smith and Weis 1997, Weis et al. 2000, Webber and Haines 2003, Scott and Sloman 2004). Prey exposed to contaminants may fail to detect predators, have a poor fast start performance, reduced stamina, inability to school, altered activity patterns, increased conspicuousness (hyperactive) all leading to increased predator vulnerability (Mesa et al. 1994, Scott and Sloman 2004). Impaired predator avoidance by contaminated prey can have major ecological implications and result in considerable transfer of contaminants through the food web. Changes in predator/prey interactions can lead to changes in populations and community dynamics.

Mummichogs from the Newark Bay tested in laboratory studies displayed behavioral problems related to contaminants including reduced activity and ability to capture grass shrimp (*Palaemonetes pugio*) prey, and poor avoidance of predation

compared to individuals from cleaner estuaries (Smith and Weis 1997, Weis et al. 2001). A poorer diet in the field consisting of less live food and more nutrient deficient detritus than that of fish from cleaner areas may be responsible for their slow growth and reduced life span. Their reduced ability to avoid predation may also be partly responsible for their shorter life span.

Endocrine Disruption and Thyroid Hormones

Endocrine disrupting chemicals (EDCs) have been found to affect sexual and reproductive behavior of fish such as females displaying male behavior or males displaying more aggressive courtship. DDE has resulted in changes in courtship behavior and body coloration (Scott and Sloman 2004). Chemosensory communication is integral to sexual and social behavior. Hormones influence the development and expression of scent producing glands and olfaction. Salmon response to priming pheromones released by females was shown to be inhibited when exposed to low doses of pesticides (Moore and Waring 2001). Aggression and dominance are also under hormone control and have been shown to be altered in fish by EDCs (Zala and Penn 2004).

PCBs, DDT and methylmercury are known endocrine disrupting chemicals in fish and mammals. While most attention is paid to the disruption of the reproductive system, the thyroid system can also be altered, which could have severe implications on the hormone levels, behavior, growth, neurodevelopment and life cycle of the organism. Unlike other vertebrates, which possess discrete, vascularized thyroid glands, most

teleost fish lack this glandular form and instead possess unencapulsated thyroid follicles scattered throughout the connective tissue of the lower jaw.

Xenobiotics may alter hormone synthesis a number a ways, via binding to receptor sites, altering transport of the hormones in the blood, or altering enzymes, deiodinases, that metabolize the thyroid hormones (Zhou et al. 1999a, Weis 2001). Endocrine disrupting chemicals act by binding to blood proteins that are involved in the transport of thyroid hormones, which reduces the thyroid hormone carrying capacity of the blood. TSH is secreted from the pituitary gland and stimulates the thyroid gland to secrete thyroxine or T4, which is the precursor or prohormone to tri-iodothyronine, or T3, the biologically active hormone in teleost fishes (Leatherland 1994).

The inhibition or impairment of the thyroid gland's ability to synthesize T4 will lead to a drop in T4 blood levels after the thryoglobulin reserves are depleted (Zhou et. 1999a). The system often responds by the pituitary secreting increased levels, hypersecretion, of TSH, which results in the enlargement, budding, and irregularity of the thyroid gland, commonly known as a goiter. These goiters are common in vertebrates that have a deficiency of iodine, which is needed to synthesize T4. Marine teleost fish are unlikely to have iodine deficiency, but salminoids from contaminated environments in the Great lakes have been found to have goiters (Leatherland 1994). Contaminants have been shown to affect thyroid follicles; however, Leatherland 1994 were not able to induce goiters in the laboratory in these fish, therefore, it is unclear whether xenobiotics or other factors are the cause of the goiters in the fish. On the other hand, goiters have been induced in birds and mammals simply by feeding them contaminated fish. These

differences may be due to the higher thyroid hormone binding and turnover rates in mammals and birds, than ecotoderms. Also the ability of fish to store these lipophilic xenobiotics in their lipids and keep them out of their blood, may make them less susceptible. Other studies have shown exposure to contaminants in the laboratory and in the field, can alter thyroid hormones and histology in fish (Zhou et al. 1999a). Mummichogs from the contaminated Piles Creek near Newark Bay NJ were found to have irregularly shaped and greatly expanded thyroid follicles or goiters compared to those from the reference site, Tuckerton in southern New Jersey (Zhou et al. 1999a).

Neurobehavioral development is contingent on proper thyroid hormone synthesis. Altered levels of thyroid hormones have been shown to alter levels of neurotransmitters in mammals which in turn can affect behavior including spontaneous activity and feeding. Decreased thyroid hormone production, or hypothyroidism could lead to reduced activity or feeding and increased thyroid hormone synthesis or hyperthyroidism, could lead to hyperthyroidism which could make the fish less conspicuous and more vulnerable to predation. These and additional direct and indirect alterations of other functions (ie. metabolism) and systems could reduce the fitness of the fish and the likelihood of survival.

Neurotransmitters

There is evidence that neurotoxic induced changes lead to behavioral changes. At the biochemical level exposure to various contaminants have be shown to alter the release or synthesis of neurotransmitters. The effects of xenobiotics and influence on different

neurotoxic mechanisms are dependent on the particular toxicant, exposure time, dosage and species (Zhou et al. 1999b). The biogenic monoamines, norepinephrine (NE), serotonin (5-HT) and dopamine (DA) and its metabolite L-DOPA are most often studied because of their involvement in various behaviors including locomotion, condition responses and feeding (Grippo 2003). PCBs have been shown to alter levels of neurotransmitters, particularly dopamine, which is associated with altered behavior.

Shain et al. 1991 reviewed PCBs as neurotoxicants in laboratory rodents and nonhuman primates. Locomotory activity and dopamine function have been found to be the primary effects in rodents, while a decrease in dopamine function and cognitive function are the most common reactions in nonhuman primates. Interactions among individual PCB congeners were examined, and a mixture of three non-coplanar PCBs, including PCB 28 was more potent in reducing brain dopamine content than the equal amounts of each congener in tissue cultures of nonhuman primate brain (Shain et al. 1991). Exposure to relatively low concentrations also significantly altered brain dopamine content in rats, increasing them during the first three days and decreasing them after longer period of exposure. This study highlights the importance of exposure time and the mixtures and types of PCB congeners.

Shain et al. (1991) suggest that the neurotoxicity of PCBs may occur by a different mechanism than the hepatotoxicity or immunotoxicity, and that the "ortho" PCB congeners may be more likely to be neurotoxicants. In their study, 43 individual congeners were tested and they determined the concentration of each that reduced the cell dopamine level by 50% (EC50). 2'2'dichlorobiphenyl, an ortho substituted noncoplanar congener was found to be the most potent at EC50=65*u*M. They concluded that it is

possible that the sites that control dopamine content in the cell have a preference for ortho or ortho-. para-substituted congeners resulting in the neurotoxicity of these PCB congeners (Shain et al. 1991).

Fingerman and Russell (1980) examined the levels of these neurotransmitters in gulf killifish, *Fundulus grandis,* to determine if they are altered by the exposure to PCB Aroclor 1242 and if this correlates with observed effects on locomotor activity. They found that exposure to PCB Aroclor mixture 1242 for 24 hours significantly decreased levels of DA and NE and led to increased levels of swimming activity. Locomotor activity is a good indicator of neurotoxicity because it reflects the fuctional status of the nervous system and is a necessary behavior for the fishes survival (Fingerman and Russell 1980).

The neurotransmitters dopamine (DA) and serotonin (5-HT) and their metabolites, DOPAC, homovanillic acid (HI/A), and 5-hydroxy-indolacetic acid (5-HIAA) in cerebellums and medullas were analyzed in mummichogs from the polluted site, Piles Creek, Linden NJ. These fish were found to have significantly lower concentrations of 5-HT and its metabolite 5-HIAA in their medullas, but not in their cerebellums compared to a reference population (Smith et al. 1995). The contaminated fish displayed reduced motivation and feeding success. The rate of prey capture and the frequency of attack were significantly higher in the reference site fish. This might be related to the reduced levels of serotonin and a reduction in activity and aggressive behavior caused by exposure to contaminants (Smith et al. 1995). Mercury was considered as a neurotoxicant in this study, but there are other contaminants, including PCBs which may

contribute to neurotoxicity in this site. It is very possible that PCBs, mercury and other contaminants are working together to have an additive or even synergistic effect of the neurology and behavior of aquatic organisms residing in contaminated, industrialized water bodies.

Mercury widely pollutes aquatic ecosystems and is toxic to aquatic animals by neurotoxic effects mainly in the brain and kidney (Tsai 1995) Serotonin concentrations were found to decrease in the hypothalamus of tilapia exposed to mercury, leading to a loss of motor control. (Tsai 1995). Exposure to mercury has also been linked to changes in neurochemical alterations to dopamine and serotonin levels in larval mummichogs (Zhou et al. 1999b).

A study of a mixture of methylmercury and PCBs was found to reduced dopamine levels in rat brains more than either chemical did alone (Bemis and Seegal 1999). Concentrations of dopamine (DA) were determined from the tissue of striatal punches obtained from adult rat brain that were exposed to either PCBs only, MeHg only, or a combination of the two. They used a 1:1 mixture of Aroclors 1254 and 1260 at concentrations of 10, 20, 40, 100, or 200 ppm and MeHg at concentrations of 1, 4, 10, 14, 20, or 40 µM. Exposure to PCBs only reduced tissue DA and exposure to MeHg had no significant effect. Interestingly when striatal punches were simultaneously exposed to PCBs and MeHg significantly greater decreases in tissue DA concentrations were found than those caused by PCBs alone. The authors hypothesized that this manifestation of synergism between these two toxicants may be due to a “common site of action (i.e.,

toxicant-induced increases in intracellular calcium and changes in second messenger systems) that influences DA function" (Bemis and Seegal 1999).

PCBs and methylmercury may have additive or interactive adverse effects on nervous system function. High doses of MeHg have been shown to cause a motor deficit by targeting the cerebellum and damaging balance and coordination (Roegge et al. 2004). Roegge et al. (2004) exposed female Long-Evans rats to MeHg, PCBs, PCBs and MeHg beginning 4 weeks prior to breeding, through pregnancy, and continuing 16 days postnatal. Combined PCB and MeHg exposure caused significant impairments in balance and coordination when performing a motor experiment requiring them to cross a rotating rod. Neither chemical alone caused a significant increase in the number of impairments. Two possible explanations were given to the differences in toxicity. The first is that PCBs and MeHg have independent mechanisms of toxicity, and the combination of the two chemical's effects results in the rotating-rod deficit. Second, PCBs and MeHg participate in the same mechanism of toxicity and this is therefore doubled. These studies exemplify the need to address additive, synergistic, and antagonistic effects when studying behavioral and neurotoxical effects of pollutants. Small concentrations of these xenobiotics may not present sublethal behavioral or physiological changes on their own, but could cause detrimental alterations when combined together.

Trophic transfer

YOY bluefish may accumulate elevated levels of contaminants primarily from their food by living in contaminated estuaries. Fish consumption by humans has been

restricted in areas of New Jersey, especially from Newark Bay, by advisories because of risk of health of effects from PCBs.

Trophic transfer is considered the most important role in the uptake of contaminants for animals on the higher levels of the food chain. YOY bluefish prey includes smaller forage fish such as mummichugs, (*Fundulus heteroclitus*), silversides (*Menidia menidia*), menhaden (*Brevoortia tyrannus*), as well as shrimp (*Crangon spp*. and *Palaemonetes spp*). The level of PCBs, DDTs, mercury and other contaminants in some prey species may be higher than in others, and can therefore transfer more to the bluefish. It is possible that the accumulation of toxicants in YOY bluefish may cause behavioral abnormalities. This may include impairment of their predatory ability and may also affect their growth and ability to migrate and recruit into the adult population.

Prey with higher lipid content such as menhaden (*Brevoortia tyrannus*) and killifish (*Fundulus heteroclitus* and *F. majalis*) may be more contaminated with lipophilic contaminants, resulting in an increased bioaccumulation of these contaminants through trophic transfer (Kennish and Ruppel 1998, Monosson et al. 2003). In addition, bluefish have been found to contain a much higher concentration of lipids than other species from the estuaries (Deshpande et al. 2002). The YOY bluefish are likely to be affected more by bioaccumulation of high levels of contaminants than adults since earlier life history stages tend to be more vulnerable as they experience various developmental stages. These elevated levels of lipids in the contaminated bluefish may impact their condition and have detrimental effects on their winter migration success and survival. Lipid utilization during YOY winter migration may result in PCBs, DDTs and methylmercury

deposited into storage lipids during the summer to be redistributed into sensitive organs such as liver and brain (Boon and Duinker 1985a, Jørgensen et al. 1999).

It is important to learn if the trophic transfer of chemicals like PCBs and mercury found in Newark Bay/Hackensack River can cause sublethal effects in YOY bluefish, influencing their ability to grow. The research examined in this dissertation examines whether contaminants such as PCBs, DDTs and mercury might be affecting YOY bluefish behavior, growth and physiology and contributing to the noticeable decline in bluefish landings. Poor health and condition of YOY at the end of the summer may cause population reductions by affecting their migratory ability, overwinter survival and recruitment into the adult breeding population.

This study examined numerous hypotheses:

1) Bluefish and prey fish from a Newark Bay estuarine tributary, Hackensack River (HR) would have elevated PCBs, pesticide and mercury concentrations compared to those from a relatively undisturbed reference site, a Great Bay estuary in Tuckerton, NJ (TK).

2) Given that bluefish are considered voracious predators most of the bluefish caught during daylight hours would have fish in their stomachs.

3) TK bluefish fed diets of contaminated fish from Hackensack in laboratory experimental conditions would bioaccumulate significantly greater levels of PCBs, pesticides and mercury than those fed diets of prey fish from Tuckerton in the lab.

4) TK bluefish fed diets of contaminated fish from Hackensack would show altered growth and behavior, including feeding and activity.

5) Bluefish from Hackensack and those fed contaminated prey from Hackensack would display alterations in thyroid status and neurotransmitter concentrations.

6) PCB congener specific fingerprints of bluefish would be similar to the PCB fingerprints of the prey fish they are feeding on.

In the following chapters the methodology and the outcome of the hypothesis tests are presented. Hypotheses three and four are addressed in a four month controlled laboratory feeding exposure experiment. Results of the behavior studies and contaminant analysis of the laboratory experiment are presented in Chapter 1. Chapter 2 examines hypotheses one and two and correlates the laboratory results with observation of growth, feeding and contaminant body burdens in the field collected TK and HR bluefish. Chapter 3 investigates the physiological and biochemical changes from contaminant exposure, hypothesis five, to explain mechanisms underlying the changes observed in the behavior and growth of the HR bluefish. In this chapter alterations in thyroid histology and neurotransmitter analysis are described and correlations between the thyroid status, neurochemistry and behavior impairments are discussed. Finally, Chapter four addresses hypothesis six and provides a statistical analysis of the congener specific PCB fingerprints in the bluefish, prey and stomach content analyzed in the previous chapters. In a technique similar to the use of stable isotopes or fatty acid fingerprints, the PCB fingerprints are compared to gain better understanding of the feeding ecology of the YOY

bluefish and the role that prey selection may play on the bioaccumulation rate and sublet effects of a predator residing in a contaminated habitat.

Together these chapters employed laboratory and field studies to provide better insight into the condition, behavior, physiology and foraging ecology of young-of-the-year bluefish residing in polluted habitats such as the Hackensack River. The Fishery Management Plan for bluefish reported in 2002 that future research needed to include the study of contaminants on their survival, and include studies on predator/prey relationships (Lewis 2002). It is important to assess how characteristics of a habitat might affect the quality of the YOY, creating a linkage between habitat quality and year class strength.

REFERENCES

Able, K.W., and M.P. Fahay. 1998. The first year in the life of estuarine fishes in the Middle Atlantic Bight. New Brunswick: Rutgers University Press.

Able, K.W., M. Burlas, and D. Byrne. 2003. Use of ocean and estuarine habitats by young-of-the-year bluefish (*Pomatomus saltatrix*) in the New York Bight. Fishery Bulletin 101:201-214.

ASMFC. 2003. Population Assessment and the Short-term Stock Projections of the Bluefish Population. A Report to the Atlantic States Marine Fisheries Commission and the Mid-Atlantic Fishery Management Council Monitoring Committee. Atlantic States Marine Fisheries Commission.

Balsiger, J. W. 2008. 2008 Report to Congress, Status of U.S. Fisheries. NMFS (National Marine Fisheries Service) http://www.nmfs.noaa.gov/sfa/statusoffisheries/booklet_status_of_us_ fisheries08.pdf. Accessed 10 October 2009. Bemis, J. C. and R. F. Seegall. 1999. Polychlorinated biphenyls and methylmercury act synergistically to reduce rat brain dopamine content in vitro Environmental Health Perspectives 107:879-885.

Boon, J. P. and J. C. Duinker. 1985. Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (*Solea solea*) in relation to concentrations in water and to lipid metabolism under conditions of starvation. Aquatic Toxicology 7:119-134.

Brown, S. B., R. E. Evans, L. Vandenbyllardt, K. W. Finnson, V. P. Palace, A. S. Kane, A. Y. Yarechewski, and D. C. G. Muir. 2004. Altered thyroid status in lake trout (Salvelinus namaycush) exposed to co-planar 3,3', 4,4', 5-pentachorobiphenyl. Aquatic Toxicology 67:75-85.

Buckel, J.A. and D.O. Conover 1997. Movements, feeding periods, and daily ration of piscivorous young-of-the-year bluefish, *Pomatomous saltatrix,* in the Hudson River estuary. Fishery Bulletin 95:665-679

Buckel, J.A., D.O. Conover, N.D. Steinberg, and K.A. McKown. 1999a. Impact of age-0 bluefish (*Pomatomus saltatrix*) predation on age-0 fishes in the Hudson River estuary: evidence for density-dependent loss of juvenile striped bass (*Morone saxatilis)*. Canadian Journal of Fisheries and Aquatic Sciences 56:275-287.

Buckel, J.A., M.J. Fogarty, and D.O Conover. 1999b. Foraging habits of bluefish, *Pomatomus saltatrix,*on the US east coast continental shelf. Fishery Bulletin 97:758-775.

Deshpande, A. D., A. F. J. Draxler, V. S. Zdanowicz, M. E. Schrock, A. J. Paulson, T. W. Finneran, B. L. Sharack, K. Corbo, L. Arlen, E. A. Leimburg, B. W. Dockum, R. A. Pikanowski, B. May, and L. B. Rosman. 2000. Contaminant levels in muscle of four species of recreational fish from the New York Bight Apex. NOAA Technical Memorandum NMFS-NE-157, NOAA, Woods Hole, MA.

Deshpande, A. D., R. J. Huggett, and R. A. Halbrook. 2002. Polycyclic aromatic hydrocarbon metabolites in the bile of a territorial benthic fish, oyster toadfish (*Opsanus tau*) from the Elizabeth River, Virginia. Archives of Environmental Contamination and Toxicology 42:43-52.

EPA. U.S. Environmental Protection Agency. 1999. Polychlorinated biphenyls (PCBs) update: Impact on fish advisories. Office of Water 4305. EPA-823-F-99-019.

Eriksson, P., A. Fredriksson. 1996. Developmental neurotoxicity of four ortho-substituted PCBs in neonatal mouse. Environmental Toxicology and Pharmacology 1: 155-165.

Fahay, M.P., P.L Berrien, D.L. Johnson, and W.W. Morse. 1999. Essential fish habitat source document: Bluefish, *Pomatomas saltatrix*, life history and habitat characteristics. NOAA Tech. Mem. NMFS-NE-144.

Feldman, R. S. and J. E. Titus 2001. Polychlorinated biphenyl accumulation differs among pumpkinseed sunfish during experimental field exposure:the role of invertebrate prey. Aquatic Toxicology 51:389-404.

Fingerman, S. W. and L. C. Russel. 1980. Effects of the polychlorinated biphenyl Aroclor 1242 on locomotor activity and on the neurotransmitters dopamine and norepinephrine in the brain of the Gulf killifish, Fundulus grandis. Bulletin of Environmental Contaminants and Toxicology 25:267.

Gartland, J., R.J. Latour, A.D. Halvorson, and H.M. Austin. 2006. Diet composition of young-of-the-year bluefish in the lower Chesapeake Bay and the coastal ocean of Virginia. Transactions of the American Fisheries Society 135:371-378.

Giesy, J. P. and K. Kannan. 2002. Dioxin-like and non-dioxinlike effects of polychlorinated biphenyls: Implications for risk assessment. Lake Reservoir Research Management 7:139-181.

Gobas, F. A., J. B. Wilcockson, R. W. Russell, and G. D. Haffner. 1999. Mechanisms of biomagnification in fish under laboratory and field conditions. Environmental Science and Technology 33:133-141.

Grippo, M.A., and A.G. Heath. 2003. The effect of mercury on the feeding behavior of fathead minnows (*Pimphales promelas*). Ecotoxicology Environmental Safety 55:187-198.

Hare, J.A., and R.K Cowen. 1997. Size, growth development and survival of the planktonic larvae of *Pomatomus saltatrix* (Pisces: Pomatomidae). Ecology 78:2415-2431.

Jacobson, J. L., S. W. Jacobson, R. J. Padgett, G. A. Brumitt, and R. L. Billings. 1992. Effects of prenatal PCB exposure on cognitive processing efficiency and sustained attention. Developmental Psychology 28:297-306.

Jørgensen, E. H., B. E. Bye, and M. Jobling. 1999. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (*Salvelinus alpinus*). Aquatic Toxicology 44:233-244.

Juanes, F., and D.O. Conover. 1994. Rapid growth, high feeding rates, and early piscivory in young-of-the-year bluefish (*Pomatomus saltatrix*). Canadian Journal of Fisheries and Aquatic Sciences 51:1752-1761.

Juanes, F., Hare, J.A., Miskiewicz, A.G., 1996. Comparing early life history strategies of Pomatomus saltatrix: A global approach. Marine Freshwater Research 47: 365-379.

Juanes, F., J.A. Buckel and F.S. Scharf. 2001. Predatory behaviour and selectivity of a primary : comparison of fish and non-fish prey. Marine Ecology Progress Series 217: 157-165.

Kendall, A. W. J. and L. A. Walford. 1979. Sources and distribution of bluefish,*Pomatomus saltatrix*, larvae and juveniles off the east coast of the United States. Fishery Bulletin 77: 213-227.

Kennish, M. J. and B. E. Ruppel. 1998. Organochlorine contamination in selected estuarine and coastal marine finfish and shellfish of New Jersey. Water Air Soil Pollution 101:123-136.

Kraus, M. L. and D. B. Kraus. 1986. Differences in effects of mercury on predator avoidance in two populations of grass shrimp, Palaemonetes pugio. Marine Environmental Research 18:277-289.Lewis, M. 2002. 2002 review of the Atlantic States Marine Fisheries Commission fishery management plan for bluefish.*in* W. Atlantic States Marine Fisheries Commission, DC, editor.

Leatherland, J. F. 1994. Reflections on the thyroidology of fishes from molecules to humankind. Guelph Ichthyology Review 2:1-67.

LeBlanc, L.A., J.A. Buckel, D.O. Conover, and L.M. Brownawell. 2006. Tests of bioaccumulation models for polychlorinated biphenyl compounds: a study of young-of-the-year bluefish in the Hudson River estuary, USA. Environmental Toxicology and Chemistry 25:2067-2076.

Lewis, M. 2002. 2002 review of the Atlantic States Marine Fisheries Commission fishery management plan for bluefish (*Pomatomus saltatrix*). In: Atlantic States Marine Fisheries Commission, W., DCs (Ed.). 10 pp.

Little, E.E., R.D. Archeski, B.A. Flerov, and V.I. Kozlovskaya. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. Archives of Environmental Contamination and Toxicology 19:380-385.

Little, E. E., and S. E. Finger. 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environmental Toxicology and Chemistry 9:13-19.

Magnusson, K., R. Ekelund, R. Grabie, and P. A. Bergqvist. 2006. Bioaccumulation of PCB congeners in marine benthic infauna Marine Environmental Research 61:379-395.

MAFMC. Amendment I to the Bluefish Management Plan *in* D. MAFMC (Mid-Atlantic Fishery Management Council), Delaware, editor.

McBride, R. S. and D. O. Conover. 1991. Recruitment of young-of-the-year bluefish, *Pomatomas saltatrix* to the New York Bight: variation in abundance and growth of spring-and summer-spawned cohorts. Marine Ecology Progress Series 78:205-216.

Mesa, M. G., T. P. Gasomski, and J. H. Peterson. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. Journal of Fish Biology 45 Supplement A:81-96.

Miskiewicz, A. G., B. D. Bruce, and P. Dixon. 1996. Distribution of tailor (Pomatomus saltatrix) larvae along the coast of New South Wales, Australia. Marine and Freshwater Research 47:331-336.

Monosson, E., J. T. F. Ashley, A. E. McElroy, D. Woltering, and A. A. Elskus. 2003. PCB congener distributions in muscle, liver and gonad of *Fundulus heteroclitus* from the lower Hudson River Estuary and Newark Bay. Chemosphere 52:777-787.

Moore, A. and C. P. Waring. 2001. The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon. Aquatic Toxicology 52:1-12.

Muelbert, J. H. and C. Sinque. 1996. Distribution of bluefish (Pomatomus saltatrix) larvae along the Continental Shelf off southern Brazil. Marine and Freshwater Research 47:311-314.

Munch, S. B. and D.O. Conover. 2000. Recruitment dynamics of bluefish, *Pomatomus saltatrix*, from Cape Hatteras to Cape Cod, 1973-1999. ICES Journal of Marine Science 57:393-402.

NOAA Technical Memorandum NMFS-NE-198. 2006. *Essential Fish Habitat Source Document:* Bluefish, *Pomatomus saltatrix*, Life History and Habitat Characteristics *Second Edition.* U. S. Department of Commerce, Northeast Fisheries Science Center Woods Hole, Massachusetts.

Nyman, R. M. and D. O. Conover. 1988. The relationship between spawning season and the recruitment of young-of-the-year bluefish, Pomatomus saltatrix, to New York. Fishery Bulletin 86:237-250.

O'Connor, J. M., J. B. Klotz, and T. J. Kneip. 1982. Sources, sinks and distribution of organic contaminations in the New York Bight ecosystem. Estuarine Research Federation, Columbia, SC.

O'Neill, H. J. and K. M. Kieley. 1992. Polynuclear aromatic hydrocarbons: Atlantic region data summary and review. Technical report 92-01, Conservation and Protection Atlantic Region, Moneton, New Brunswick.

Rasmussen, J. B., D.J. Rowan, D. R. Lean and J. H. Carey. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Canadian Journal of Fisheries and Aquatic Sciences 47:2030-2038.

Varanasi, U. and J. E. Stein. 1991. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environmental Health Perspectives 90:93-100.

Roegge, C. S., V. C. Wang, B. E. Powers, A. Y. Klintsova, S. Vilareal, W. T. Greenough, and S. L. Schantz. 2004. Motor impairment in rats exposed to PCBs and methylmercury during early development. Toxicological Sciences 77:315-324.

Salerno, D. J., J. Burnett, and R. M. Ibara. 2001. Age, growth, maturity and spatial distribution of bluefish, Pomatomus saltatrix, (Linnaeus), off the Northeast coast of the United States, 1985-96. Journal of Northwest Atlantic Fisheries and Science 29:31-39.

Sandheinrich, M.B., and G.J. Atchison. 1989. Sublethal copper effects on bluegill, *Lepomos macrochirus*, foraging behavior. Canandian Journal of Fisheries and Aquatic Sciences 46:1977-1984.

Schmidt, K., C. E. W. Steinburg, S. Pflugmacher, and G. Staaks. 2004. Xenobiotic substances such as PCB mixtures (Arochlor 1254) and TBT can influence swimming behavior and biotransformation activity (GST) of carp (Cyprinus carpio). Environmental Toxicology 19:460-470.

Scott, G. R. and K. A. Sloman. 2004. The effects of environmental pollutants on complex fish behavior: integrating behavioral and physiological indicators of toxicity Aquatic Toxicology 68:369-392.

Shain, W., B. Bush, and R. Seegal. 1991. Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. Toxicology and Applied Pharmacology 111:33-42.

Smith, W., P. Berrien, and T. Potthoff. 1994. Spawning Patterns of Bluefish, Pomatomus-Saltatrix, in the Northeast Continental-Shelf Ecosystem. Bulletin of Marine Science 54:8-16.

Smith, G., A. Khan, J. S. Weis, and P. Weis. 1995. Behavior and brain chemistry correlates in mummichogs (Fundulus heteroclitus) from polluted and unpolluted environments. Marine Environmental Research 39:329-333.

Smith, G. M. and J. S. Weis. 1997. Predator/prey interactions in Fundulus heteroclitus: effects of living in a polluted environment. Journal of Experimental Marine Biology and Ecology 209:75-87.

Stewart, P., J. Reihman, E. Londy, T. Darvill, and J. Pagano. 2000. Prenatal PCB exposure and postnatal behavioral assessment scale (NBAS) performance. Neurotoxicology and Teratology 22:21-29.

Tsai, C., T. Jang, and L. Wang. 1995. Effects of mercury on serotonin concentration in the brain of tilapia, Oreochromis mossambicus. Neuroscience Letters 184:208-211.

USGS. 1997. Mercury Contamination of Aquatic Ecosystems. http://wi.water.usgs.gov/pubs/FS-216-95/

Varanasi, U., and J. E. Stein. 1991. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environmental Health Perspectives 90:93-100.

Webber, H. M. and T. A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (Notemigonus crysoleucas). Environmental Toxicology and Chemistry 22:1556-1561.

Weis, J. S., G. Smith, T. Zhou, and C. Bass. 2000. Predator/prey interactions: a link between the individual level and both higher and lower level effects of toxicants in the aquatic ecosystems. Journal of Aquatic Ecosystem Stress and Recovery 7:145-153.

Weis, J. S., G. Smith, T. Zhou, C. Bass, and P. Weis. 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. BioScience 51:209-217.

Weis, J. S., T. Zhou, J. Skurnick, and P. Weis. 2003. Evaluating prey capture by larval mummichogs, (Fundulus heterclitus) as a potential biomarker for contaminants. Marine Environmental Research 55:27-38.

Weis, P. and J. T. F. Ashley. 2007. Contaminants in fish of the Hackensack Meadowlands, New Jersey: size, sex, and seasonal relationships as related to health risks. Archives of Environmental Contaminant Toxicology 52:80-89.

CHAPTER 1

The effect of contaminated prey on feeding, activity and growth of young-of-the-year bluefish, *Pomatomus saltatrix* in the laboratory

Introduction

Bluefish, *Pomatomus saltatrix*, are harvested by recreational and commercial fisheries in several areas around the world and populations have shown dramatic fluctuations in abundance. Landings and recruitment declined substantially during the 1980s and 1990s, but the cause of the decline has not been clearly identified (Lewis 2002, ASMFC 2003). Recent stock assessments have reported increases since 1999 and that the bluefish stock has been rebuilt and is no longer considered overfished; however, ongoing research into population dynamics is important (Shepard 2006, Balsiger 2008). Hypotheses being investigated include over-fishing, declining habitat quality and reproductive success, and shifts in feeding ecology (Shepherd and Packer 2006). The present study examined a measure of habitat quality through determining the impact of contaminated prey on bluefish behavior.

Adult bluefish spend the winters off the coast of Florida and migrate north in the spring, when spawning occurs off the mid-Atlantic coast (Kendall and Walford 1979, Hare and Cowen 1997). As pelagic juveniles grow, they are carried inshore by the Gulf Stream into estuaries along the Middle Atlantic Bight (Hare and Cowen 1997, Able and Fahay 1998). The juveniles arrive in the estuaries at about 60-76 days old and 60 mm

total length in late May to mid-June (Nyman and Conover 1988, McBride and Conover 1991, Juanes et al. 1996). Poor age-one recruitment has been determined as a cause of the past decline of the abundance of the Atlantic bluefish stock (Conover et al. 2003, Gartland et al. 2006). Survivorship of YOY bluefish may be dependent on which estuary they reside in during the summer. Many estuaries contain contaminants including polychlorinated biphenyls (PCBs), pesticides, PAHs, dioxins and metals. Fish and their prey from contaminated estuaries accumulate detectable levels of contaminants in their tissues (O'Connor et al. 1982, Varanasi and Stein 1991, Kennish and Ruppel 1998, Deshpande et al. 2002a, Williams 2006).

PCBs, mercury, pesticides and other contaminants may contribute to population reductions by affecting the health and behavior of the YOY, their migratory ability, and recruitment into the adult breeding population. The summer months, when they live in polluted estuaries, are a critical period of rapid growth and development in which they consume 20-30% of their body weight per day (Juanes and Conover 1994b). Continual exposure to high levels of contaminants via the water, sediments, and especially food can result in deleterious effects. Methylmercury, PCBs and organic pesticides, in particular, are major environmental problems because they are bioaccumulative, persistent and toxic (Newman and Unger 2003). In addition, because bluefish contain high concentrations of lipids, they are prone to bioaccumulate high concentrations of lipophilic contaminants such as PCBs (Deshpande et al. 2002a). The YOY bluefish are likely to be affected more by contaminants than adults because earlier life history stages tend to be more vulnerable (Newman and Unger 2003). Also, unlike resident estuarine fishes such as mummichogs,

migratory bluefish are very unlikely to be able to evolve a genetic tolerance to the contaminants (Weis et al. 2001, Weis 2002).

Exposure to contaminants during early life stages can lead to sublethal effects, including altered behavior and growth. Behavior can be used as an indicator of environmental stressors (Little and Finger 1990), and subtle changes in food acquisition, swimming activity, predator avoidance, or reproductive behavior, can have major implications for survival.

We hypothesize that the accumulation of toxicants in YOY bluefish may cause behavioral abnormalities, including impairment of their predatory ability and motor functions that could affect their growth and ability to migrate and recruit into the adult population. The main objective of this study was to examine in a laboratory experiment whether the trophic transfer of chemicals (PCBs, mercury, pesticides) in the Hackensack River ecosystem would result in the bioaccumulation of these contaminants and cause sublethal effects including altered behavior and growth.

Material and methods

Study location

Two locations in New Jersey, the Tuckerton area of Great Bay (TK) and the Hackensack River (HR), were selected as study sites for their known differences in habitat quality (Figure 1.1). The Hackensack River empties into Newark Bay and is surrounded by a highly industrialized and urbanized region near New York City. It

encompasses numerous sewage treatment plants and sanitary landfills, and it has a long history of degraded water quality and sediment contamination. Although conditions have been improving recently, the water and sediments remain polluted with PCBs, dioxins, metals and other contaminants (Konsevick and Bragin 2007). A 2001-2003 survey in the Hackensack River compared sediment concentrations to an earlier survey from 1986-1987 (Konsevick and Bragin 2007), and found that while many metal concentrations have decreased, they remain high and most are still above the effects range low (ER-L), the concentration at which adverse biological effects were found 10% of the time (Long et al. 1995). Most importantly, the average HR sediment mercury concentration level was 3.55 $\mu g\ g^{-1}$, well above 0.71 $\mu g\ g^{-1}$, the effects range median (ER-M), which is the median concentration found in the literature to adversely affect 50% of species. In addition, total sediment PCB concentrations of 734 ng g^{-1} were reported, which is likely to biomagnify up the food chain and is greater than the predicted sediment effect threshold (sediment concentration expected to lead to adverse sublethal effects on salmonids) reported by Meador (2000). In the 1980s, the State of New Jersey issued fisheries advisories and banned all commercial fishing and crabbing in the Hackensack River (Hauge et al. 1990, Weis and Ashley 2007). Weis and Ashley (2007) stressed that white perch (*Morone americana*) from the Hackensack River should not be considered edible because many exceeded Hg and PCB guidelines for "one meal per month" and some exceeded the US FDA "no consumption at all" level (USFDA 1993, USEPA 1999a, b, 2000, 2001).

Tuckerton, NJ, our reference site, it is the home of the Jacques Cousteau National Estuary Research Reserve where metal concentrations in the water and sediment and

organisms are much lower. Khan et al. (1989) reported low sediment levels of contaminants such as mercury, Hg (0.05 μg g^{-1}), cadmium (0.13 μg g^{-1}) and copper (12.9 μg g^{-1}). We know of no published data on PCB or pesticide concentrations at this site.

All TK fish collections for this study were at the beach or in the lagoons near Graveling Point (N 39˚ 32.4' / W 74˚ 23.2') or from the dock and shore of the Rutgers Marine Field station which is approximately 7 km southeast of Graveling Point. HR fish samples were collected at the same study sites that were sampled in the New Jersey Meadowlands Commission survey (T3 (N 40˚ 45.1'/W 74˚ 05.0'), S2, and S3) (Konsevick and Bragin 2007). Fish were collected during daylight hours at various tidal periods. Spring-cohort bluefish were determined to be those with total lengths of >160mm. This was estimated from data reported in previous studies (McBride and Conover 1991, Wiedenmann and Essington 2006).

Stomach Content Analysis

In order to determine dominant prey items, stomach content was analyzed in 27 field-caught spring –cohort YOY bluefish from TK and 27 HR bluefish collected with a 60 m seine net from September 14-29, 2003. The fish were sacrificed and their stomach contents were removed; the fish and stomach contents were weighed, placed immediately on dry ice and then stored in a -80° C freezer. Some bluefish regurgitated upon capture and this material was collected in glass containers, labeled and placed immediately on dry ice.

Stomach content of each fish was identified under a dissecting microscope. Scales were primarily used to determine the prey species when it was not obvious by physical features due to digestion or small pieces. Identified pieces of fish and invertebrates were counted, weighed and placed in separate glass containers for each species. Unidentified fish were grouped together and unidentified invertebrates were grouped together. For the purpose of this study only the average percent weight was calculated for each prey species found in the stomachs of the bluefish in order to determine which species may be contributing the most to the trophic transfer of contaminants.

Sample collection and laboratory exposure by feeding

From July 16 – 22, 2004, 66 YOY bluefish measuring approximately 115 mm in length were collected with a 30 m seine net from TK (Graveling Point) for the laboratory experiments. They were transferred into a bay-water filled cooler and then placed into an aerated 3.54 m^3 tank filled with ambient seawater for transport to the James J. Howard Marine Sciences Laboratory, Sandy Hook, NJ. Six of these fish ("T-zero") were immediately placed in a -80˚C freezer for chemical analysis.

Four circular tanks, 1.83 m diameter, 1.22 m deep, were installed in a seawater lab with a continuous flow of ambient seawater from Sandy Hook Bay. Water temperature was 19-23˚C, salinity was 21-26, and the photoperiod was 14 hr light/10 hr dark. Each fish was measured and placed in one of the four tanks. In order to minimize

contact, fish were transferred from the transport tank with a small net and placed in a 150 mm long x 10 mm acrylic box that had a ruler mounted on the bottom and was tared on a scale. This enabled weight and length measurements to be taken with minimal handling. Each tank received 15 fish of similar length and weight.

Bluefish were exposed to contamination by feeding them contaminated prey. From June - November 2004, mummichog (*Fundulus heteroclitus*) and menhaden (*Brevoortia tyrannus*) were collected from HR and from TK to be fed to the TK bluefish in the two experimental and two reference tanks, respectively. Ten mummichogs and ten menhaden from TK and HR were arbitrarily selected and put in methylene chloride-cleaned glass containers, placed immediately on dry ice and then stored in a -80° C freezer for chemical analysis. Most prey fish were immediately frozen and later defrosted as needed for feeding. Some were kept alive in 115 liter coolers with air stones for <24 hrs before being fed live to the bluefish approximately once a week. The bluefish were fed to satiation daily from July 16 – November 20, 2004; the experimental groups (HR-fed) were TK bluefish fed menhaden and mummichogs from HR, and the reference groups (TK-fed) were TK bluefish fed menhaden and mummichogs from TK.

Behavior Trials

Consumption

On September 14, 2004, after two months of feeding on respective prey items, quantitative feeding trials were begun in the holding tanks. Rations of frozen-thawed

mummichogs from HR or TK were dropped into the appropriate tanks. The ration was based on the number of bluefish remaining in the tank. One whole mummichog (approximately 2 grams) was given per every two bluefish. Using a stopwatch, the time to consume the entire ration was recorded. If the entire ration was not consumed, this was recorded. The bluefish were given one minute to feed before the remaining mummichogs were counted. Remaining heads or tails were also counted. Additional rations were then offered until each tank of fish was fed to satiation (at least five rations were offered to each tank for each trial). The total number of mummichogs consumed was recorded and the number consumed per bluefish was calculated for each tank. A total of 16 trials were conducted over a period of one month, from September 14 – October 13, 2004, three to four times per week.

Spontaneous Swimming Activity

Spontaneous activity was measured by calculating the undisturbed swimming speed of small groups of fish. Swimming speed trials commenced on October 17, 2004. Two 1800 l (2.4 x 0.8 x 0.8 m) rectangular tanks equipped with plexiglass viewing windows along the entire tank length were set up in a seawater lab with a continuous flow of ambient seawater from Sandy Hook Bay. Six equal areas (i.e., 0.4 m) of the tanks were delineated by marking vertical bars on the back wall of the tank with black permanent marker. A video camera, television monitor and VCR were set up for each tank so that the entire length of each tank could be recorded simultaneously. Three fish at a time were transferred into the observation tank and after 12 hours of acclimation were recorded during undisturbed 15-minute periods in the morning, afternoon and

evening over the five days. Eight groups of three HR-fed fish and eight groups of three TK-fed bluefish were videotaped. The tapes were analyzed for spontaneous swimming activity.

During the first five minutes of each observation period, the time spent swimming irregularly was recorded and a percentage was calculated. Irregular swimming behavior was assigned when the fish were not swimming together in a tight school circling the entire tank: if they were huddling in corners, swimming vertically, not moving, swimming in small circles, or if there was a gap of two or more of the tank sections between them. Normal swimming behavior was considered to be occurring when the three fish were swimming together (schooling) approximately one to two body lengths apart (Juanes and Conover 1994b, Stehlik 2009). In a "school", fish would circle the length of the tank, swimming along the back wall and then circling back across the front glass.

In addition, during each 15-minute period, the time that it took the lead fish to swim between the center two sections of the tank (0.813 m) was recorded 10 times. The swimming rate was recorded only when the fish were swimming along the back of the tank (not the glass front) and it was easy to see if they were swimming in a straight path and at what point they crossed the lines. These times were averaged and the swimming rate in m s^{-1} was calculated for each group of three fish. The bluefish were sacrificed and length and weight were measured. The mean length of the three fish was calculated and used to calculate the swimming rate for each group of fish in body lengths s^{-1}.

Contaminant Analysis

PCB and Pesticide

Five whole menhaden and five mummichogs from HR and TK were analyzed for PCBs and pesticides. Average sample wet weights of HR mummichog and menhaden were 4.63 ± 0.47 g and 4.73 ± 1.02 g, respectively. Average sample wet weights of TK mummichog and menhaden were 4.02 ± 0.46 g and 5.05 ± 0.074 g, respectively.

Ten whole TK-fed bluefish (average weight 120.65 ± 5.54 g) and ten whole HR-fed bluefish (average wet weight 97.46 ± 5.34 g), i.e., five fish from each of the four holding tanks, were analyzed for PCBs and pesticides. Four whole bluefish caught in July from TK ("T-zero") (average wet weight 16.57 ± 6.92 g) were also analyzed for

PCB and pesticide analysis was performed by following the guidelines of Krahn et al.(1988), USEPA (1993), Sloan et al. (1993) and Deshpande et al. (2000). Briefly, tissue samples were chopped, freeze-dried, homogenized with a blender or mortar and pestle and sodium sulfate and extracted with methylene chloride using an automated Soxhlet extraction apparatus (Organomation, Berlin, Massachusetts). Solvents and reagents were all analytical grade or GC grade (Fisher Scientific). Florisil/silica/alumina glass column chromatography was performed and twenty percent (volume) of the extract was dried and weighed for gravimetric lipid determination. The sample was further purified using a Phenomenex Envirosep-ABC size-exclusion column on HP1050 HPLC, and fractions of the samples containing target analytes were collected using a Foxy Fraction Collector. The sample was screened using an Agilent 5890 GC with electron

capture detection (GC/ECD), diluted or concentrated as needed and finally analyzed for 31 PCB congeners and 24 pesticide compounds listed in Appendix 1 (Deshpande et al. 2000, Deshpande et al. 2002b). Primary calibration mixture (PCB Calibration Check Solution C-CCSEC: AccuStandard, New Haven, Connecticut + chlorinated pesticides SRM 2261: NIST, Gaithersburg, Maryland) and supplementary calibration mixture (PCBs SRM 2274 + chlorinated pesticides SRM 2275; NIST, Gaithersburg, Maryland) were used in the internal standard method to quantify the target analyte peaks.

The "Aroclor-based" (Aroclor is a trademark for naming mixtures of chlorinated biphenyls and polypenyls) total PCB value was calculated by taking the sum of the concentrations of 18 specific PCB congeners and multiplying that value by 2.0 (Appendix 1, Table A.1) (Deshpande et al. 2002a). This value represents an estimate of the total sum of all PCB congeners and was used for comparison of the samples. Congeners BZ-8 and BZ-101 (BZ represents the Ballschmiter-Zell nomenclature system for a designated PCB congener) were not included in the Aroclor estimate because they were found to co-elute with other analytes.

Mercury Analysis

A 2 g wet weight section of muscle tissue from each bluefish (ten HR-fed and ten TK-fed), and a 2 g wet weight sample of each of five homogenized whole HR and TK menhaden and mummichogs were analyzed for total mercury following the procedure of Weis and Ashley (2007). In brief, dried tissues were HNO_3 -mineralized in a MARS-5 programmed microwave digester and analyzed for total mercury concentrations using

cold vapor atomic absorption spectrophotometry (CVAAS) with a Baccharach MAS-50D instrument. All reagents were certified for mercury analysis (Fisher Scientific).

Quality Assurance

For PCB and pesticide quality assurance, each batch of samples included a method blank, three method surrogate internal standards added prior to extraction, HPLC purification and GC analysis, and a CARP-2 certified reference material - CRM (NRC, Canada). Analysis of method blanks produced no detection or very low detection of all analytes, verifying that the equipment and glassware used were clean. Each sample type and batch analyzed had quantitative mean recoveries for the surrogate internal standards; mean percent recovery was 68% ± 38. A CARP-2 sample (NRC, Canada) was run with each of the four batches and the majority of the absolute value of the z scores for the target analytes were 2 or below. Overall, the results of the quality control data provided assurance that the determinations of the concentrations of PCB and pesticide analytes for the bluefish and prey samples were accurate.

Each Hg sample reading was adjusted by the percent error found from the values of the CRM used (DORM-2, NRC, Canada). The mean detection level (MDL) was three times the standard deviation found from the average of all method blanks. The average of all five method blanks was 0.0034 ± 0.0009 µg, and 3 X the SD = 0.0027 µg. This was then divided by the average weight of all of the samples which was 0.27g. Therefore, the MDL was set at 0.0100 µg/g and any sample with a value less than this was reported as <MDL and assigned a value of ½ the MDL or 0.005 µg/g for the purpose of statistical analyses.

Statistical Analysis

All statistical analyses were performed on Minitab13.1 software program employing a significance level of $p < 0.05$. Means and standard errors are presented in the text, tables and figures.

Length and Weight

Differences of initial lengths and weights of 15 fish in each of the four holding tanks were compared utilizing a one-way ANOVA followed by pairwise comparisons using Fisher's Least Significant Difference (LSD). Differences of initial and final lengths and weights of the HR-fed and TK-fed bluefish were tested via one-way ANOVA.

Consumption

The number of mummichogs consumed per bluefish for the 16 feeding trials was compared for HR-fed and TK-fed bluefish. The consumption rate of each ration (first three rations) for TK-fed and HR-fed fish was compared using a one-way ANOVA followed by pairwise comparison using Fisher's Least Significant Difference (LSD). Only the consumption rates of the first three rations were analyzed because for the majority of the trials ration 4 and ration 5 were not entirely consumed (especially for the HR-fed) and therefore a time was not obtained.

The HR-fed bluefish were significantly smaller than the TK-fed bluefish at the end of the four months of feeding exposure; therefore it was important to determine if the difference in size was a factor in feeding differences. Since the length of the fish was only measured at the beginning and end of the four month exposure period (to minimize

handling and stress) the average length for the TK-fed fish and the HR-fed fish for each trial date was calculated using a TK-fed or HR-fed linear growth curve. The number of mummichogs consumed per bluefish for the 16 feeding trials was compared for HR-fed and TK-fed bluefish with an ANCOVA with the calculated length at the date of each trial as a covariate to adjust for size differences. The consumption rate for each ration (first three rations) for TK-fed and HR-fed fish was also analyzed with an ANCOVA with the calculated length as a covariate.

The percentage of trials in which the entire ration was consumed was calculated for HR and TK for each ration (ration 1 - ration 5). A regression analysis was performed comparing the number of mummichogs consumed for both the HR-fed bluefish and the TK-fed bluefish over the sixteen trials.

Spontaneous Swimming Activity

The average spontaneous swimming speed in body lengths s^{-1} of HR-fed versus TK-fed bluefish was compared with a one-way ANOVA. The average spontaneous swimming speed in m s^{-1} of the HR-fed and TK-fed bluefish was compared with an ANCOVA utilizing the average length of each group of three fish as the covariate.

Contaminants – PCBs, DDTs, and Hg

The mean Aroclor PCB concentrations and the sum of concentrations of the pesticide DDT and its metabolites (total DDTs) are presented and compared statistically for each group of fish (bluefish and prey). Total DDT concentration was used for the data analysis of pesticides because these were the pesticides found at highest

concentration in the bluefish and prey, ranging from 40-70% of total pesticides. A report with more detailed chemistry results of the specific PCB congeners and pesticides will follow.

The Aroclor PCB concentrations of HR-fed and TK-fed bluefish were compared utilizing a one-way ANOVA. PCB concentrations of the TK and HR prey (mummichog and menhaden) were compared with a one-way ANOVA followed by pairwise comparisons using Fisher's Least Significant Difference (LSD). These same statistical tests were performed for total DDTs and mercury concentrations.

MANOVAs comparing the individual PCB congener concentrations of the HR-fed to the TK-fed bluefish were performed. The concentrations of ortho-substituted congeners in the HR-fed and TK-fed bluefish were compared via a MANOVA. An additional MANOVA compared the coplanar and dioxin-like congeners in the HR-fed and TK-fed bluefish. (Ortho-substituted congeners contain two or more chlorines in the ortho (2,2' or 6,6') position. Coplanar congeners are mono-ortho or non-ortho. Dioxin-like congeners contain at least 4 chlorine substitutes, chlorines in both para positions (4,4'), at least 2 chlorines at the meta positions (3,3' or 5,5') and are coplanar. Each individual congener concentration in the HR-fed and TK-fed bluefish were compared via a one-way ANOVA.

Results

Stomach Content Analysis

Stomach content analysis revealed that menhaden made up the greatest proportion of weight in the diet of the 27 HR bluefish, followed by mummichog, striped killifish (*Fundulus majalis*) and silverside (*Menidia menidia*). Menhaden also comprised the greatest percent weight for the 27 TK bluefish, followed by silverside, mummichog and striped killifish (Figure 1.2). Invertebrates, including amphipods and grass shrimp, were also found, but their weight was less than 1% of the entire diet. Because menhaden and mummichog were dominant species in the stomach content for both populations, the use of these two species as the prey in the laboratory feeding experiments was justified.

Bluefish - Size

The mean initial length ($F=0.76$, $p=0.53$) and weight ($F=0.47$, $p=0.76$) of the 15 fish in each of the four tanks were not significantly different. The mean length and weight of the HR-fed and TK-fed bluefish at the start of the feeding exposure experiment were not significantly different (Table 1.1). At the end of four months the length and weight of the TK-fed fish were significantly greater than those of the HR-fed fish (Table 1.1). On average, the TK-fed bluefish displayed a 10.5% greater length and a 14% greater weight than the HR-fed bluefish.

Behavior

Consumption

Prior to the initiation of the quantitative feeding experiments we noted a decrease in feeding in the HR-fed bluefish. They were leaving a substantial amount of food on the bottom of the tank and not feeding as aggressively as they had been the first two months. There had been minor mortality in the tanks because of cannibalism, transfer stress, and jumping out during the first week of holding resulting in 13 bluefish remaining in tank 1, 12 in tank 2, 12 in tank 3, and 14 in tank 4. These values were used to calculate the average number of mummichogs eaten per bluefish. The quantitative feeding trials revealed that TK-fed bluefish consumed significantly more mummichogs than HR-fed bluefish after two months of exposure, even after adjusting for differences in length (Table 1.1). In addition, the number of mummichogs consumed by the HR-fed bluefish decreased linearly ($F=17.47$; $p=0.001$) over the 4 week feeding trials (Figure 1.3). There was not a significant linear trend in mummichog consumption by the TK-fed ($F=1.21$, $p=0.291$) (Figure 1.3).

When the entire ration was eaten, the average consumption rate of the TK-fed fish was up to five times faster than HR-fed fish, and significantly faster for each ration (Figure 1.4). The TK-fed fish showed only a slight decrease in the amount and rate of consumption after two full rations and continued to feed readily for up to five rations. However, HR-fed fish showed a significant decrease in the amount and rate of consumption after consuming only one ration, and were subsequently less likely to

consume entire rations (Figure 1.5). Differences in length were not a factor in the differences in consumption (Table 1.1).

Spontaneous Swimming Activity

The average spontaneous swimming rate of the TK-fed bluefish was significantly greater than that of the HR-fed bluefish when calculated in both m s^{-1} and body lengths sec^{-1} (Table 1.1). Differences in length were not a factor in the differences in swimming speed (Table 1.1). The HR-fed fish often huddled in corners, not always swimming together or schooling, and often did not swim in the steady circular pattern typical of the TK-fed fish and of bluefish in general. Occasionally, an individual would dart out from the school, but when this occurred HR-fed fish had difficulty regrouping whereas the TK-fed fish would catch up and continue schooling with little disruption. In addition, the HR-fed fish often became disorganized when turning at the end of the tank; swimming in small circles or huddling in the corner. Typically, if one fish swam irregularly the other two would also be disrupted. Often one fish would begin swimming and the other two would stay in the corner, then the first fish would turn around to rejoin the others and the other two would begin to swim; the first fish would pass them and lethargically turn around to catch the "school". On other occasions, one fish would hover in mid-water in the tank and the other two fish would swim around it in small circles or they would pause each time they approached it, and sometimes circled around or turned to go the other way. Overall, the HR-fed bluefish exhibited irregular swimming patterns 31% of the time while the TK-fed bluefish exhibited irregular swimming only 9.4% of the time.

Contaminants

PCB Concentrations

HR-fed bluefish accumulated high PCB levels (2183 ± 79 ng g^{-1}), above the US FDA guideline of 2000 ng g^{-1}, averaging seven times greater than those fed TK prey (359 ± 17 ng g^{-1}), seven times greater than the HR prey (358 ± 106 ng g^{-1}) and fifteen times greater than the time-zero bluefish (142 ± 25 ng g^{-1}) (Figure 1.6 and 1.7).

There were no significant PCB differences between mummichog and menhaden within either site (Figure 1.7), but the total Aroclor PCB concentrations of the HR menhaden and mummichog were significantly greater than those from TK. The HR prey fish PCB levels were 10 -14 fold greater than TK prey (33 ± 3.6 ng g^{-1}) (Figure 1.6 and 1.7). The PCB concentrations of TK-fed bluefish were ten-fold greater than TK-prey and significantly greater than the TK–zero bluefish, indicating that TK prey transferred small amounts of PCBs to the bluefish over 4 months (Figure 1.6 and 1.7, Table 1.2).

HR-fed bluefish had significantly greater concentrations of individual ortho-substituted PCB congeners ($p<0.0001$) and coplanar and dioxin-like congeners ($p<0.0001$) compared to the TK-fed fish (Figure 1.8). One-way ANOVAs of each congener revealed that all congeners were significantly greater in the HR-fed ($p < 0.0001$) except PCB 18 ($p = 0.063$) and PCB 126 ($p = 0.088$). PCB 77 and PCB 110 were co-eluting peaks in the GC/ECD analysis; therefore, the results are presented as the sum of the two concentrations. The sum of PCB 77 and 110 was significantly greater ($p<0.0001$) in the HR-fed bluefish.

Pesticides

The patterns of results for total DDTs were similar to those of PCBs, but at much lower concentrations (Figure 1.9, Table 1.2). However, HR menhaden (209 ± 16.2 ng g^{-1}) DDT concentrations were significantly greater than HR mummichog (50.66 ± 16.16 ng g^{-1}) which were greater but not significantly different from the TK prey (9.64 ± 2.30 ng g^{-1}) (Figure 1.9). DDT concentrations of HR-fed bluefish (260.9 ± 16.2 ng g^{-1}) were two-fold greater than TK-fed (119.95 ± 8.42 ng g^{-1}) and 10-fold greater than TK-zero (26.8 ± 3.7 ng g^{-1}) bluefish. The TK-fed bluefish had DDT concentrations five-fold that of the T-zero bluefish and ten-fold that of the TK prey (Figure 1.9). Additional chlorinated pesticides that were present in HR-fed bluefish included endosulfan I (90.3 ± 4.9 ng g^{-1}), dieldrin (54.3 ± 2.7 ng g^{-1}), and trans- and cis-nonachlor (38.2 ± 41.8 ng g^{-1}, 47.5 ± 2.8 ng g^{-1}).

Mercury

HR-fed bluefish contained total mercury concentrations significantly greater than TK-fed bluefish (1.52 ± 0.08 µg g^{-1} vs. 0.45 ± 0.018µg g^{-1}) (Figure 1.10, Table 1.2). Total mercury concentrations of HR bluefish were 2-5 fold greater than the HR prey (0.38 ± 0.058 µg g^{-1}) (Figure 1.10). The concentration of mercury in the TK prey (0.012 ± 0.0021 µg g^{-1}) was significantly lower than the HR prey and below the mean detection level (MDL) in all fish except for one mummichog. However, all TK-fed bluefish had detectable levels, revealing uptake and biomagnification.

Discussion

Contaminant Concentrations

Overall, the results of the analysis are consistent with our hypothesis that YOY bluefish feeding on prey fish from a contaminated estuary would bioaccumulate high levels of contaminants, including PCBs, pesticides and mercury. Prey fish from the contaminated HR had levels of PCBs, DDTs, and Hg that were significantly higher than those from the relatively clean estuary (TK), and these contaminants were shown to biomagnify up the food web to YOY bluefish which had elevated PCBs, Hg and DDTs. PCB concentrations of the HR-fed bluefish were three-fold greater than that in YOY bluefish from the Hudson River (Williams 2006). Eight of the HR-fed bluefish exceeded the US FDA action level of 2000 ng g^{-1} for PCBs. Meador (2000) reported that a tissue concentration of 140 ng g^{-1} would be expected to have adverse effects such as decreased growth and altered physiology in juvenile salmonids. This level is well below the body burdens in the HR-fed bluefish. It is also lower than the body burden of the HR-prey and TK-fed bluefish, indicating possible adverse effects even in the reference fish.

The HR-fed bluefish accumulated elevated concentrations of certain dioxin-like and coplanar PCB congeners and ortho-substituted PCB congeners, many of which have been linked to behavioral disruption, although congener-specific studies are limited in fish. The dioxin-like and coplanar congeners would bind to the Ah receptor, possibly leading to neuroendocrine and behavior disruptions. The ortho-substituted congeners are more likely to have neurotoxic effects, altering neurotransmitter concentrations and behavior (Seegal et al. 1991, Simon et al. 2007). The HR-fed fish contain high

concentrations of PCB 118 and 28 which are dioxin-like coplanar congeners that have been shown to alter behavior and affect thyroid status (Holene et al. 1995, Eriksson and Fredriksson, 1996, Osius et al. 1999). The ortho-substituted PCB 52 and 153 present in the HR-fed tissue have been correlated with altered behavior and neurotransmitter concentrations (Bowman et al. 1981, Eriksson and Fredriksson 1996, Holene et al. 1998, Mariussen et al. 1999, Fischer et al. 2008). Additional ortho-substituted congeners found in high concentrations, PCB 95, 44 and 52, were assigned high neurotoxic equivalency potency values in a study by Simon et al. (2007). The sum of the co-eluting congeners, PCB 77 and PCB 110 was the highest concentration in the HR-fed fish. PCB 77 is one of the most potent dioxin-like congeners and PCB 110 is one of the most potent neurotoxins (Simon et al. 2007). Therefore, elevated concentrations of either of these congeners may be associated with detrimental behavioral changes.

DDT concentrations were much lower and below the FDA and EPA action levels (5000 and 1000 ng g-1, respectively) and the protective level of 600 ng g^{-1} concentrations determined by Becker et al (2005). However, goldfish exposed to low concentrations of DDT did experience altered activity and schooling behavior (Weis and Weis, 1974). The total DDT body burden, acting additively or synergistically with the other toxic chlorinated pesticides (including dieldrin and endosulfan II) and the high PCBs and Hg body burdens could result in ecologically significant disruptions in behavior and growth.

Mercury analysis revealed all HR-fed bluefish were well above the US FDA "no consumption level" (1µg g^{-1}). Beckvar et al. (2005) calculated a whole body mercury concentration of 0.2 µg g^{-1} as a protective level in juvenile and adult fish based on a

review of studies of sublethal endpoints. This concentration is well below the concentration of Hg in the exposed and reference bluefish and the HR-prey fish.

The average mercury concentration of TK-fed bluefish was close to the EPA guideline for one meal per month (0.47 μg g^{-1}) and six out of ten fish had concentrations greater than 0.45 μg g^{-1}. It is likely that most of the mercury in the bluefish is methylmercury because it appears to be biomagnifying from the prey concentrations (Bloom 1992, Newman and Unger 2003). The contaminants, especially mercury, biomagnified in the TK-fed bluefish to a much greater degree than in the HR-fed bluefish. PCBs and DDTs accumulated in TK-fed bluefish at concentrations 10 times that of their prey, while the PCB and DDT concentrations in the HR-fed bluefish were only seven and two-fold that of their prey, respectively. Mercury in the TK-fed bluefish was 37 times that of their prey, while HR bluefish were only four-fold that of the prey. The bluefish were fed the same prey species (menhaden and mummichog) in this study indicating that the difference in biomagnification is not a product of different diets. Further studies examining the high biomagnification rates and surprisingly elevated concentrations of Hg in the TK-fed fish would be important. Despite the fact that the TK-fed bluefish were feeding on prey from relatively "pristine" waters, mercury biomagnified to concentrations that are too high for unrestricted human consumption and may be at levels that could have sublethal effects on the organisms themselves.

Bluefish Behavior and Size

The high contaminant concentrations in the HR-fed bluefish were associated with significant alterations in behavior and size. After four months of contaminant exposure, length, weight, feeding, and spontaneous locomotion were significantly lower in bluefish fed contaminated prey.

Successful feeding is influenced by motivation (frequency of strikes), search effectiveness, selection, orientation , coordination, capture success and handling time (Scharf et al. 1998, Buckel et al. 1999a, Buckel et al. 1999b, Weis et al. 2001, Weis et al. 2003). Fathead minnows exposed to high concentrations of mercury had impaired foraging efficiency, increased pause time and decreased capture speed (Grippo and Heath 2003b). In some studies, frequency of strikes was more sensitive to contaminants than actual prey capture, suggesting that motivation was more affected than coordination as Brown and Johansen (1987) found with pentachlorophenol-treated fish. Similarly, exposure to a contaminated diet led to a decreased motivation to feed in YOY bluefish.

The decrease in feeding is the likely cause of the decrease in overall growth of the HR fed bluefish. An alternative argument can be raised that the smaller size of the contaminated fish caused the reduced feeding; however, this is unlikely because the difference in size was much less than the difference in feeding in both time to eat the ration and the amount of food consumed. Our analysis revealed significant differences in both food consumption and swimming speed even after adjusting statistically for differences in length. Furthermore, the consumption by the HR-fed bluefish continued to decline during the feeding trials indicating increasing effects of the contaminants. The

TK-fed bluefish also had a slight decline in feeding towards the end of the month long feeding experiment which is likely due to the decreasing water temperatures, but it was not significant and still much higher than the HR-fed bluefish.

While the reduced feeding during the experiment was probably the cause of, rather than the result of the smaller size, this may be the beginning of a downward trend. As the fish continue to eat less and growth decreases, their smaller size will ultimately lead to altered prey selection and a further decline in both food consumption and swimming speed.

HR-fed bluefish exhibited both slower and irregular locomotion compared to the TK-fed reference fish and YOY bluefish reported in other studies. The HR-fed swimming speed falls below the range of 1 to 2 body lengths s^{-1} reported by Juanes and Conover (1994b) of YOY bluefish from Great South Bay, NY. Stehlik (2009) observed YOY bluefish from Sandy Hook Bay over a nine month period and found that during the same month (October) of the present study the Sandy Hook bluefish swam at a rate of 1.65 body lengths s^{-1} which is well above the swimming speed of the HR-fed bluefish. The exposed fish in the present study are swimming at half the speed as the reference fish and bluefish from other estuaries. The reduced activity may be a compensation for the reduced feeding as a means to conserve energy and also suggests possible deleterious physiological effects. Locomotion is important for feeding, predator avoidance, competitive interactions, schooling and migration; therefore reduction in swimming could have ecological consequences (Schmidt et al. 2004).

In addition to swimming slower, the HR-fed bluefish often broke from their school and took time to regroup. Irregular swimming behavior of one HR-fed fish instigated confused and disrupted swimming patterns of the other two fish, which could result in a number of disadvantages. Schooling creates hydrodynamic efficiency and the HR-fed fish would be unlikely to hold precise positioning and take advantage of the energy efficiency of the school (Moyle and Cech 1988). Disrupted schooling may also impact food finding efficiency, mate location during spawning and enhanced migration accuracy. Most importantly, schooling reduces the risk of predation by providing safety in numbers (Moyle and Cech 1988, Helfman et al 1997). A bluefish straying from the group would be a likely target. It would be interesting to investigate if the presence of contaminated fish may disrupt the schooling pattern of the "healthy" fish resulting in increased vulnerability of the entire population.

The laboratory results correlate with observations from the field. HR field-caught bluefish had elevated concentrations of PCBs, DDTs, and Hg compared to the TK field-caught bluefish (Chapter 2). HR field-caught bluefish collected in late September/early October were significantly smaller than the TK field-caught bluefish at that time. The smaller size of the HR YOY bluefish suggests that reduced feeding also occurs in the field. A relatively low percentage of the HR bluefish contained food in their guts when compared to percentages reported in numerous other studies of YOY bluefish in the Mid-Atlantic Bight regions (Juanes et al. 1993, Juanes and Conover 1994a, Buckel and Conover 1997, Buckel et al. 1999, Buckel and McKown 2002; Gartland et al. 2006, Chapter 2). If, in addition to reduced feeding, the field fish also exhibit irregular swimming behavior, poor schooling ability, and slower movement, this

could make them more conspicuous, less protected and less able to escape predators. Behavioral experiments on YOY HR bluefish caught in the fall prior to migration would be an important next step to determine to what degree altered behavior is occurring in the wild population. Bluefish migrating out of the urban estuaries could be slower and smaller and therefore potentially more vulnerable to predation and starvation compared to bluefish from cleaner estuaries.

REFERENCES

Able, K.W., and M.P. Fahay. 1998. The first year in the life of estuarine fishes in the Middle Atlantic Bight. New Brunswick: Rutgers University Press.

Able, K.W., M. Burlas, and D. Byrne. 2003. Use of ocean and estuarine habitats by young-of-the-year bluefish (*Pomatomus saltatrix*) in the New York Bight. Fishery Bulletin 101:201-214.

ASMFC. 2003. Population Assessment and the Short-term Stock Projections of the Bluefish Population. A Report to the Atlantic States Marine Fisheries Commission and the Mid-Atlantic Fishery Management Council Monitoring Committee. Atlantic States Marine Fisheries Commission. 22 pp.

Beckvar, N., T.M. Dillon, and L.B. Read. 2005. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects threshold. Environmental Toxicology and Chemistry 24:2094-2105.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Canadian Journal of Marine Science 49:1010-1017.

Bowman, R. E., M. P. Heironimus, and D. A. Barsotti. 1981. Locomotor hyperactivity in PCB-exposed rhesus monkeys. NeuroToxicology 2**:** 251–268.

Brown J.A. and P.H. Johansen. 1987. Impairment of early feeding behavior of largemouth bass by pentachlorophenol exposure: a preliminary assessment. Transactions of the American Fisheries Society 116:71-78.

Buckel, J.A. and D.O. Conover 1997. Movements, feeding periods, and daily ration of piscivorous young-of-the-year bluefish, *Pomatomous saltatrix*, in the Hudson River estuary. Fishery Bulletin 95:665-679

Buckel, J.A., D.O. Conover, N.D. Steinberg, and K.A. McKown. 1999a. Impact of age-0 bluefish (*Pomatomus saltatrix*) predation on age-0 fishes in the Hudson River estuary: evidence for density-dependent loss of juvenile striped bass (*Morone saxatilis)*. Canadian Journal of Fisheries and Aquatic Sciences 56:275-287.

Buckel, J.A., M.J. Fogarty, and D.O Conover. 1999b. Foraging habits of bluefish, *Pomatomus saltatrix,*on the US east coast continental shelf. Fishery Bulletin 97:758-775.

Buckel, J.A., and K.A. McKown. 2002. Competition between juvenile striped bass and bluefish:resource partitioning and growth rate. Marine Ecology Progress Series 234:191-204.

Candelmo, A., Deshpande, A., Dockum, B, Samson, J., Weis, P, Weis, J. 2008. The effect of contaminated exposure on feeding and growth of young-of-the-year bluefish, *Pomatomus saltatrix* in the field. in prep.

Conover, D. O., T. Gilmore, and S. B. Munch. 2003. Estimating the relative contribution of spring- and summer- spawned cohorts to the Atlantic coast bluefish stock. Transactions of the American Fisheries Society 132:1117-1124.

Deshpande, A.D., A.F.J. Draxler, V.S. Zdanowicz, M.E. Schrock, A.J. Paulson, T.W. Finneran, B.L. Sharack, K. Corbo, L. Arlen, E.A. Leimburg, B.W. Dockum, R.A. Pikanowski, B. May, and L.B. Rosman. 2000. Contaminant levels in muscle of four species of recreational fish from the New York Bight Apex. NOAA Tech. Mem. NMFS-NE-157.

Deshpande, A.D., A.F.J. Draxler, V.S. Zdanowicz, M.E. Schrock, and A.J. Paulson. 2002a. Contaminant levels in the muscle of four species of fish important to the recreational fishery of the New York Bight Apex. Marine Pollution Bulletin 44:164-171.

Deshpande, A.D., R.J. Huggett, and R.A. Halbrook. 2002b. Polycyclic aromatic hydrocarbon metabolites in the bile of a territorial benthic fish, oyster toadfish *(Opsanus tau)* from the Elizabeth River, Virginia. Archives of Environmental Contamination and Toxicololgy 42:43-52.

Eriksson, P., A. Fredriksson. 1996. Developmental neurotoxicity of four ortho-substituted PCBs in neonatal mouse. Environmental Toxicology and Pharmacology 1**:** 155-165.

Fahay, M.P., P.L Berrien, D.L. Johnson, and W.W. Morse. 1999. Essential fish habitat source document: Bluefish, *Pomatomas saltatrix,* life history and habitat characteristics. NOAA Tech. Mem. NMFS-NE-144.

Fischer, C., A. Fredriksson, and P. Eriksson. 2008. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244:157-165.

Gartland, J., R.J. Latour, A.D. Halvorson, and H.M. Austin. 2006. Diet composition of young-of-the-year bluefish in the lower Chesapeake Bay and the coastal ocean of Virginia. Transactions of the American Fisheries Society 135:371-378.

Grippo, M.A., and A.G. Heath. 2003. The effect of mercury on the feeding behavior of fathead minnows (*Pimphales promelas*). Ecotoxicology Environmental Safety 55:187-198.

Hare, J.A., and R.K Cowen. 1997. Size, growth development and survival of the planktonic larvae of *Pomatomus saltatrix* (Pisces: Pomatomidae). Ecology 78:2415-2431.

Hauge, P., J. Bukowski, P. Morton, M. Boriek, J. McClain, and G. Casey. 1990. Polychlorinated biphenyls (PCBs), chlordane, and DDTs in selected fish and shellfish from New Jersey waters, 1986–1987:Results from New Jerseys Toxics in Biota Monitoring Program.In: NJ Dept of Environ Protection, T.s (Ed.). http://www.state.nj.us/dep/dsr/pcb86-87.pdf. Accessed 10 May 2008.

Helfman, G.S., B.B. Collette, and D.E. Douglas. 1997. Diversity of Fishes. Malden: Blackwell Science.

Holene E, I. Nafstad, J.U. Skaare, T. Sagvolden 1998. Behavioral hyperactivity in rats following postnatal exposure to sub-toxic doses of polychlorinated biphenyl congeners 153 and 126. Behavioral Brain Research 94: 213–224.

Hurst, T.P., and D. O. Conover. 1998. Winter mortality of young-of-the year Hudson River striped bass (Morone saxatilis): size-dependent patterns and effects on recruitment. Canandian Journal of Fisheries and Aquatic Science 55:1122-1130.

Juanes, F., R.E.Marks, K.A. McKown, and D.O. Conover. 1993. Predation by age-0 bluefish on age-0 anadromous fishes in the Hudson River estuaries. Transactions of the American Fisheries Society 122:348-356.

Juanes, F., and D.O. Conover. 1994a. Rapid growth, high feeding rates, and early piscivory in young-of-the-year bluefish (*Pomatomus saltatrix*). Canadian Journal of Fisheries and Aquatic Sciences 51:1752-1761.

Juanes, F., and D.O. Conover. 1994b. Piscivory and prey size selection in young-of-the-year bluefish: predator preference or size-dependent capture success? Marine Ecology Progress Series 114:59-69.

Juanes, F., Hare, J.A., Miskiewicz, A.G., 1996. Comparing early life history strategies of Pomatomus saltatrix: A global approach. Marine Freshwater Research 47**:** 365-379.

Kasumyan, A.O. 2001. Effects of chemical pollutants on foraging behavior and sensitivity of fish to food stimuli. Journal of Ichthyology 41:76-87.

Kendall, A. W. J. and L. A. Walford. 1979. Sources and distribution of bluefish, *Pomatomus saltatrix*, larvae and juveniles off the east coast of the United States. Fishery Bulletin 77: 213-227.

Kennish, M. J., and B. E. Ruppel. 1998. Organochlorine contamination in selected estuarine and coastal marine finfish and shellfish of New Jersey. Water Air Soil Pollution 101:123-136.

Khan, A.T., J.S. Weis, and L. D'Andrea. 1989. Bioaccumulation of four heavy metals in two populations of grass shrimp, *Palaamonetes pugio*. Bulletin of Environmental Contaminants and Toxicololgy 42:339-343.

Konsevick, E., and A. B. Bragin. 2007. Chemical characteristics of sediment collected as part of the fishery resource inventory of the lower Hackensack River within the Hackensack Meadowlands District: A comparative study 2001-2003 & 1987-1988.

Krahn, M.M., C.A. Wigren, R.W. Pearce, L.K. Moore, R.G. Bogar, W.D. MacLeod, S.L. Chan, and D.W. Brown. 1988. Standard analytical procedures of the NOAA National Analytical Facility, 1988: new HPLC cleanup and revised extraction procedures for organic contaminants. NOAA Tech Memo. NMFS FNWC-153; 52pp

LeBlanc, L.A., J.A. Buckel, D.O. Conover, and L.M. Brownawell. 2006. Tests of bioaccumulation models for polychlorinated biphenyl compounds: a study of young-of-the-year bluefish in the Hudson River estuary, USA. Environmental Toxicology and Chemistry 25:2067-2076.

Lewis, M. 2002. 2002 review of the Atlantic States Marine Fisheries Commission fishery management plan for bluefish (*Pomatomus saltatrix*). In: Atlantic States Marine Fisheries Commission, W., DCs (Ed.). 10 pp.

Little, E.E., R.D. Archeski, B.A. Flerov, and V.I. Kozlovskaya. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. Archives of Environmental Contamination and Toxicology 19:380-385.

Little, E. E., and S. E. Finger. 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environmental Toxicology and Chemistry 9:13-19.

Long, E. R., D. D. MacDonald, S. L. Smith, and F. D. Calder. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management 19:81-97.

Meador, J. 2000. An analysis in support of tissue and sediment based threshold concentrations of polychlorinated biphenyls (PCBs) to protect juvenile salmonids listed by the Endangered Species Act. National Oceanic and Atmospheric Administration Report.

McBride, R.S., and D.O. Conover. 1991. Recruitment of young-of-the-year bluefish *Pomatomus saltatrix* to the New York Bight: variation in abundance and growth of spring- and summer-spawned cohorts. Marine Ecology Progress Series 78:205-216.

Moyle, P.B., J.J Cech Jr. 1988. Fishes, an introduction to ichthyology. Engelwood Cliffs: Prentice Hall.

Newman, M.C. and M.A. Unger. 2003. Fundamentals of ecotoxicology. Lewis Publishers Washington D.C.

NOAA (National Oceanic and Atmospheric Administration). 1989. A summary of data on tissue contamination from the first three years (1986-1988) of the Mussel Watch Program. NOAA Tech. Memo. NOS OMA 49.154 pp.

Balsiger, J. W. 2008. 2008 Report to Congress, Status of U.S. Fisheries. NMFS (National Marine Fisheries Service) http://www.nmfs.noaa.gov/sfa/statusoffisheries/booklet_status_of_us_ fisheries08.pdf. Accessed 10 October 2009.

Nyman, R.M., and D.O. Conover, D.O. 1988. The relationship between spawning season and the recruitment of young-of-the-year bluefish, Pomatomus saltatrix, to New York. Fishery Bulletin 86**:** 237-250.

O'Connor, J. M., J. B. Klotz, and T. J. Kneip. 1982. Sources, sinks and distribution of organic contaminations in the New York Bight ecosystem. Estuarine Research Federation, Columbia, SC.

Osius, N. W. Karmaus, H. Kruse, and J. Witten 1999. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. Environmental Health Perspectectives 107: 843–849.

Sandheinrich, M.B., and G.J. Atchison. 1989. Sublethal copper effects on bluegill, *Lepomos macrochirus*, foraging behavior. Canandian Journal of Fisheries and Aquatic Sciences 46:1977-1984.

Scharf, F. S., J. A. Buckel, F. Juanes, and D. O. Conover. 1998. Predation by juvenile piscivorous bluefish (Pomatomus saltatrix): the influence of prey to predator size ratio and prey type on predator capture success and prey profitability. Canadian Journal of Fisheries and Aquatic Sciences 55:1695-1703.

Scharf, F.S., J.A. Buckel, and F. Juanes. 2002. Size-dependent vulnerability of juvenile anchovy *Anchoa mitchilli* to bluefish predation: Does large body size always provide refuge? Marine Ecololgy Progress Series 233:241-252.

Schmidt, K., C. E. W. Steinburg, S. Pflugmacher, and G. Staaks. 2004. Xenobiotic substances such as PCB mixtures (Arochlor 1254) and TBT can influence swimming behavior and biotransformation activity (GST) of carp (Cyprinus carpio). Environmental Toxicology 19:460-470.

Shepherd, G. R., and D. B. Packer. 2006. Essential fish habitat source document: Bluefish, Pomatomas saltatrix, life history and habitat characteristics. 2nd edition. NOAA Tech. Mem. NMFS-NE-144.

Simon, T., J. K. Britt, and R. C. James. 2007. Development of a neurotoxic equivalence scheme of relative potency for assessing the risk of PCB mixtures. Regulatory Toxicology and Pharmacology 48:148-170.

Slater, J.J., T.E. Lankford, and J.A. Buckel. 2007. Overwintering ability of young-of-the-year bluefish Pomatomus saltatrix: effect of ration and cohort of origin on survival. Marine Ecololgy Progress Series 339**:** 259-269.

Sloan, C.A., N.G. Adams, R.W. Pearce, D.W. Brown, and S.L. Chan. 1993. Northwest Fisheries Science Center organic analytical procedures. In; Launestein, G.G.; Cantillo, A.Y. , eds. Sampling and analytical methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects, 1984-1992. Vol. 4. Comprehensive descriptions of trace organic analytical method. NOAA Tech. Memo. NOS ORCA 71:182 pp.

Stehlik, L.L. 2009. Effects of seasonal change on activity rhythms and swimming behavior of age-0 bluefish (*Pomatomus saltatrix*) and a description of gliding behavior. FisheryBulletin 107:1-12.

USEPA. 1993. Laboratory methods manual estuaries. EPA Doc. 600/4-91/024 Available from: EPA Office of Research and Development, Cincinnati, OH. 289 pp.

USEPA. 1999a. Guidance for assessing chemical contaminant data for use in fish advisories. Vol. 2. Risk assessment and fish consumption limits, 3rd ed. EPA 823-B-99-008. US EPA, Office of Water, Washington, DC.

USEPA. 1999b. Mercury update: impact on fish advisories. EPA-823-F-99-019. US EPA, Office of Water, Washington, DC.

USEPA. 2000. Guidance for assessing chemical contamination data for use in fish advisories. Vol. 2. Risk assessment and fish consumption limits, 3rd ed. EPA 823-B-00-008. Office of Water, Washington, DC.

USEPA. 2001. Mercury update: Impact on fish advisories. EPA-823-F-01-011. US EPA, Office of Water, Washington, DC.

USFDA. 1993. National Shellfish Sanitation Program. Guide for the control of molluscan shellfish. US Food and Drug Administration,Washington, DC.

Varanasi, U., and J. E. Stein. 1991. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environmental Health Perspectives 90:93-100.

Weidenmann, J., and T.E. Essington. 2006. Density-dependent overwinter survival in young- of-year bluefish (*Pomatomus saltatrix*)? A new approach for assessing stage-structural survival. Canandian Journal of Fisheries and Aquatic Sciences 63:1934-1943.

Weis, J. S. 2002. Tolerance to environmental contaminants in the mummichog, *Fundulus heteroclitus*. Human and Ecological Risk Assessment 8:933-953.

Weis, J. S., G. Smith, T. Zhou, C. Bass, and P. Weis. 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. BioScience 51:209-217.

Weis, J. S., T. Zhou, J. Skurnick, and P. Weis. 2003. Evaluating prey capture by larval mummichogs, (Fundulus heterclitus) as a potential biomarker for contaminants. Marine Environmental Research 55:27-38.

Weis, P., and J. T. F. Ashley. 2007. Contaminants in fish of the Hackensack Meadowlands, New Jersey: size, sex, and seasonal relationships as related to health risks. Archives of Environmental Contamination andToxicology 52:80-89.

Weis, P., and J. Weis. 1974. DDT causes changes in activity and schooling behavior in goldfish. Environmental Research 7:68-74.

Williams, S. D. 2006. Bioaccumulation patterns of organochlorines in young-of-the-year bluefish (Pomatomus saltatrix) in the Hudson River Estuary. Florida Agricultural and Mechanical University, Tallahasee, Florida.

Table 1.1 – Differences in size, appetite and swimming speed of TK-fed and HR-fed bluefish

	TK-fed	HR-fed	F value	p value
Size				
Initial Length (mm)	114.93±1.29	115.67±1.59	0.13	0.712[a]
Initial Weight (g)	15.20±0.51	14.88±0.86	0.11	0.745[a]
Final Length (mm)	227.32 ± 3.60*	217.42 ± 2.60	6.62	0.016[a]
Final Weight (g)	120.06 ± 4.89*	106.92 ± 3.63	5.91	0.019[a]
Swimming speed				
m s^{-1}	0.37 ± 0.09 *	0.19 ± 0.06	328.28	<0.0001 [b]
Body lengths s^{-1}	1.65 ± 0.038 *	0.87 ±0.022	335.34	<0.0001[a]
Consumption				
# mummichogs consumed per fish	2.74 ± 0.14 *	1.80 ±0.14	39.39	<0.0001 [b]

[a] ANOVA results

[b] ANCOVA results (length as a covariate)

Significantly greater values marked with an asterisk *

Table 1.2 Statistical differences between contaminant concentrations in bluefish and their prey

ANOVA

Source of variance	df	F value	p value
Total PCB (HR vs TK)			
Lab HR-fed, Lab TK-fed & Field-TK-0 bluefish*	2	371.11	<0.0001
HR &TK menhaden & HR&TK mummichog*	3	7.28	0.002
Total DDTs (HR vs TK)			
Lab bluefish (HR-fed, TK-fed & TK-0)*	2	65.74	<0.0001
HR &TK menhaden & HR&TK mummichog*	3	14.90	<0.0001
Total Mercury (HR vs TK)			
Lab HR-fed & TK-fed bluefish*	1	258.09	<0.0001
HR &TK menhaden & HR&TK mummichog*	3	41.76	<0.0001

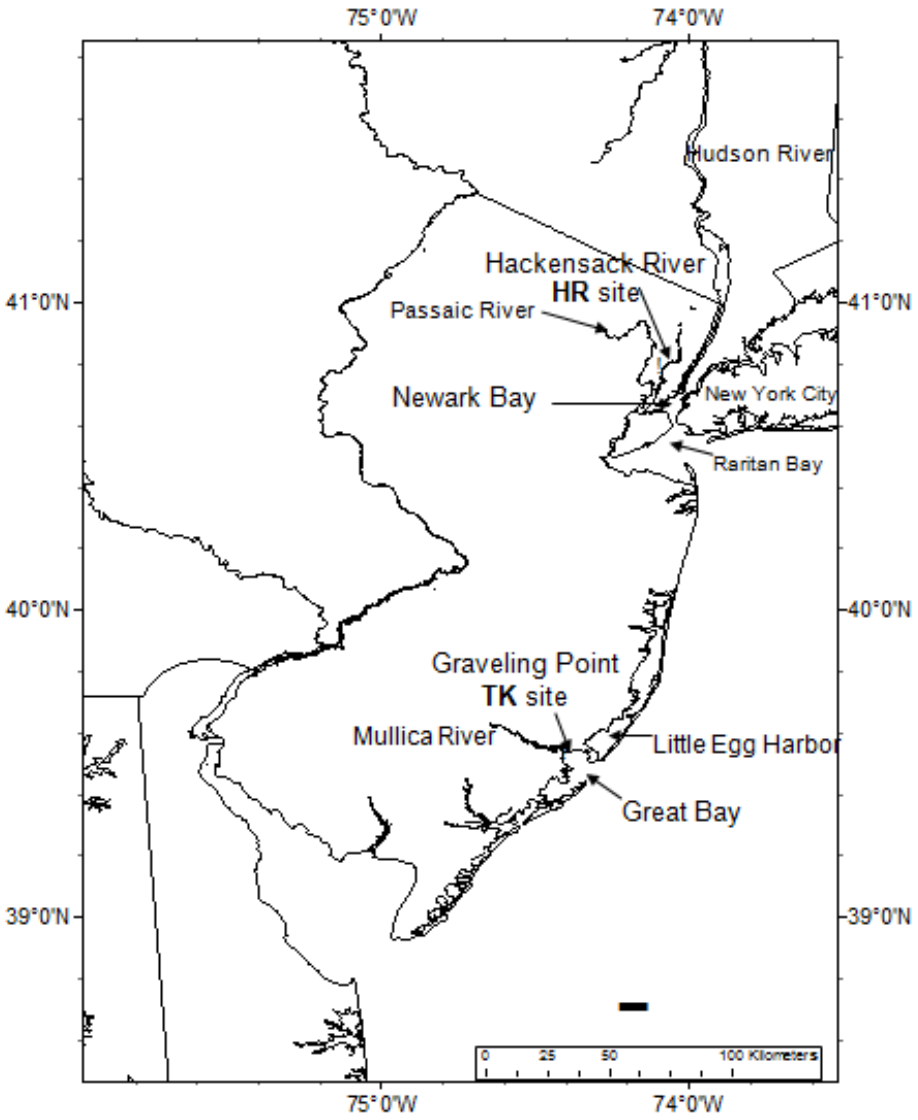

Figure 1.1 Map of study sites in New Jersey. Tuckerton, NJ area of Great Bay, Graveling point site labeled (TK site) and the Hackensack River, Newark Bay site labeled (HR site).

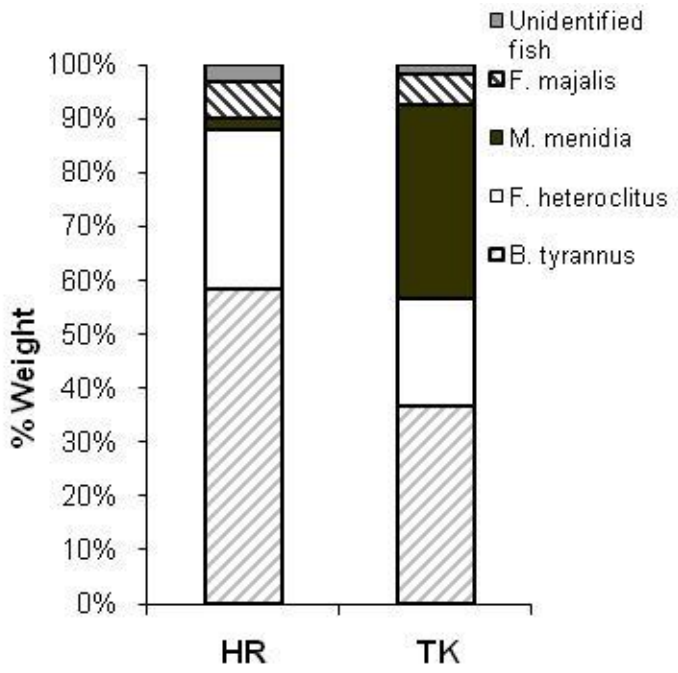

Figure 1.2 Stomach content of field caught HR (n=25) and TK (n=27) bluefish. Percent by weight of prey fish species found in gut.

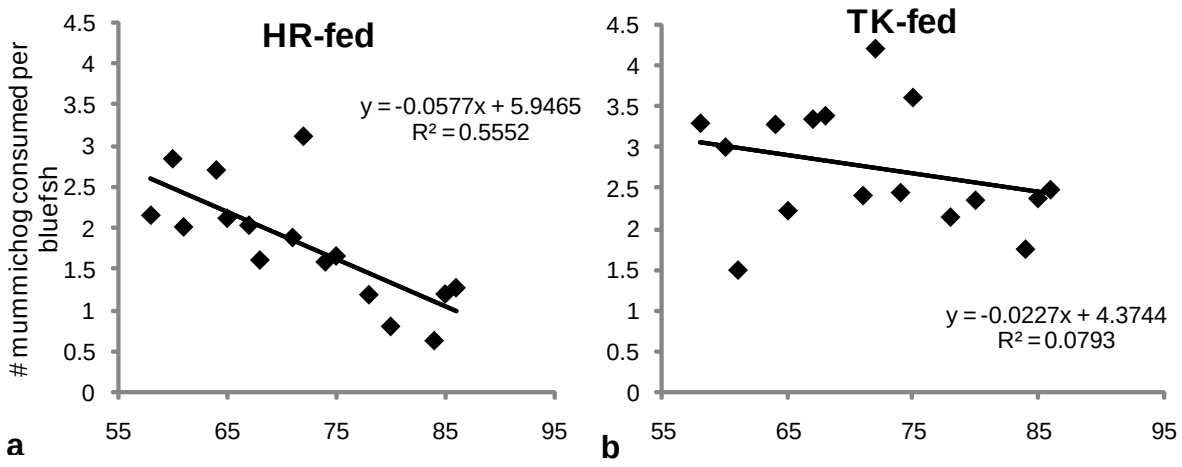

Figure 1.3 Mean number of mummichogs consumed per bluefish for each trial from September 14, 2004 – October 13, 2004. **a.** HR-fed bluefish regression analysis ($F=17.47$, $p=0.001$) **b.** TK-fed bluefish regression analysis ($F=1.21$, $p=0.291$)

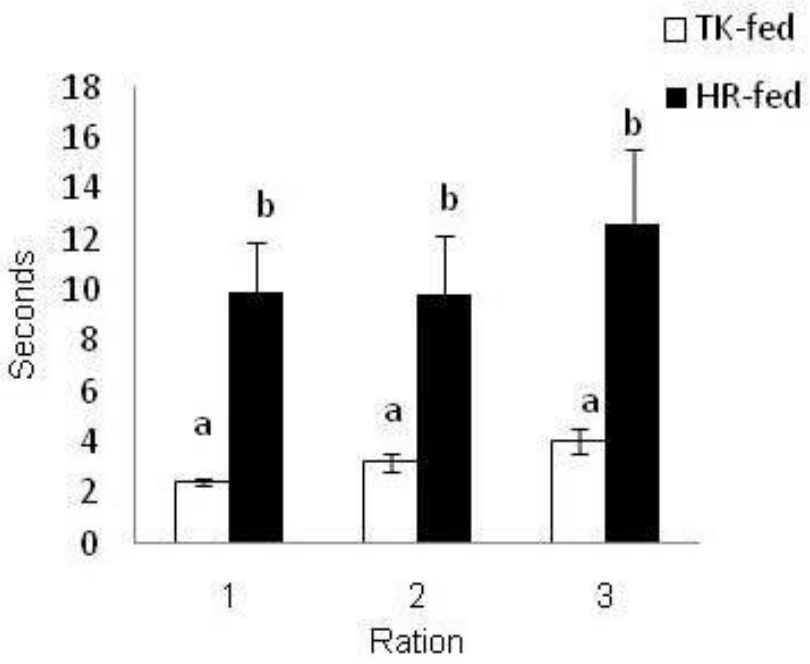

Figure 1.4 Bluefish consumption rate. Each bar represents the mean time taken (s) to consume the entire ration (ration 1-3) of mummichogs for the TK-fed and HR-fed bluefish. The mean ± S.E. of 16 timed feeding trials. The bars with different letters are significantly different (ANOVA, F=8.53, $p < 0.0001$; pairwise comparison with Fisher's LSD).

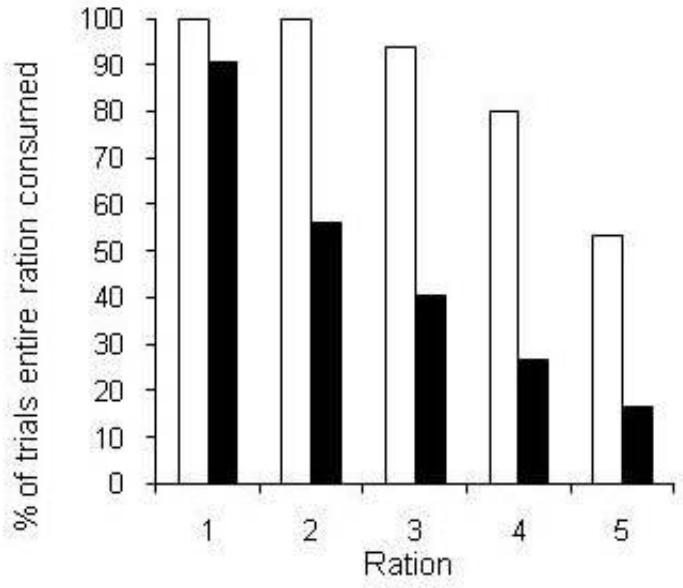

Figure 1.5 % of feeding trials entire ration consumed. The percent of feeding trials in which the entire ration was consumed by bluefish within one minute for each of the five rations offered. Black bars are HR-fed and white bars are TK-fed bluefish.

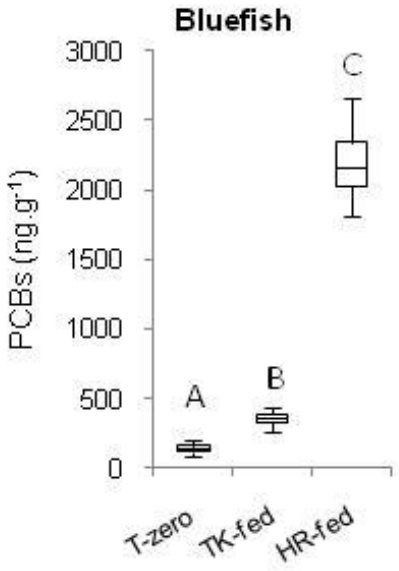

Figure 1.6 tPCB concentrations (ng g^{-1}) of laboratory bluefish. Tuckerton fed bluefish (TK-fed) and Hackensack fed bluefish (HR-fed), Tuckerton initial field-caught (July) bluefish (T-zero). Groups with different letters are significantly different (ANOVA, $P < 0.0001$; pairwise comparison with Fisher's LSD).

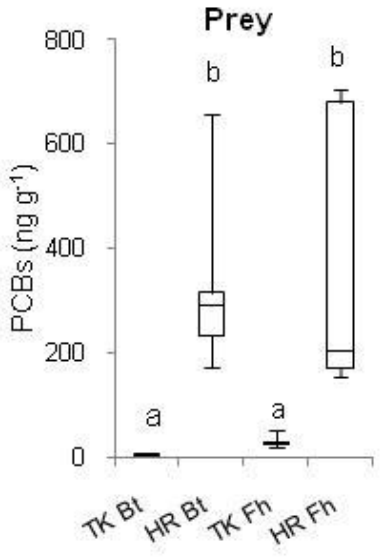

Figure 1.7 tPCB concentrations (ng g^{-1}) of HR & TK field-caught prey. Menhaden *Brevoortia tyrannus* (Bt) and mummichog *Fundulus heteroclitus* (Fh). Groups with different letters are significantly different (ANOVA, $P < 0.0001$; pairwise comparison with Fisher's LSD).

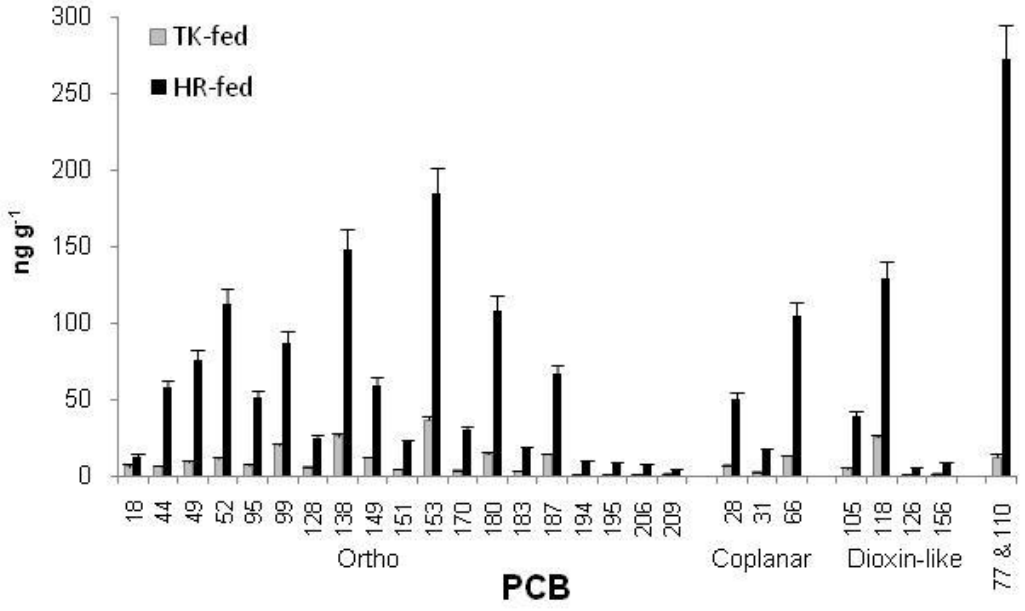

Figure 1.8 Mean concentration (ng g^{-1}) of individual PCB congeners in HR-fed & TK-fed bluefish. The congeners are separated into ortho-substituted, coplanar and dioxin-like. The sum of PCB 77 and 110 is presented because they co-elute.

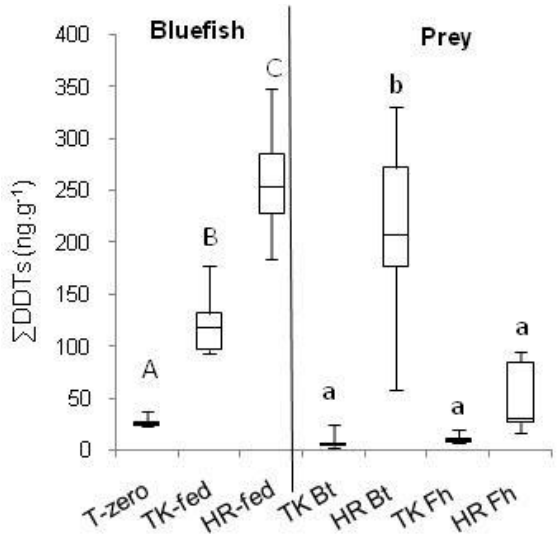

Figure 1.9 tDDT concentrations (ng g^{-1}) of laboratory bluefish & prey. TK-fed, HR-fed, and T-zero bluefish & prey HR & TK field-caught menhaden (Bt) and mummichog (Fh). Groups with different letters are significantly different (ANOVA, $P < 0.0001$; pairwise comparison with Fisher's LSD).

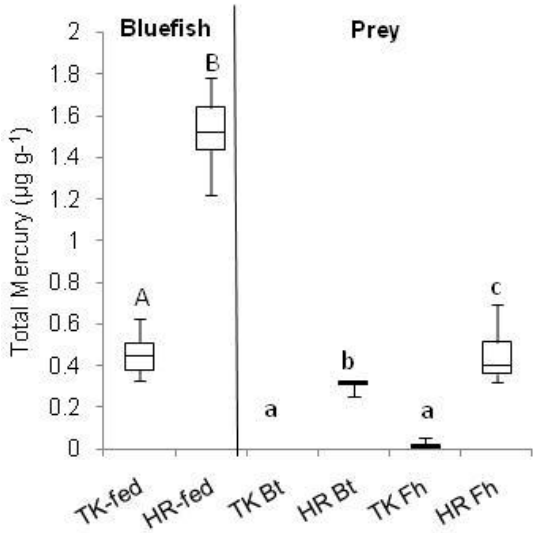

Figure 1.10 Total mercury (µg g^{-1}) in laboratory bluefish & prey. HR-fed and TK-fed bluefish and HR and TK field-caught menhaden (Bt) and mummichog (Fh). Groups with different letters are significantly different (ANOVA, $P < 0.0001$; pairwise comparison with Fisher's LSD).

CHAPTER 2

Uptake of contaminants and the effects of exposure in the young-of-the-year bluefish, *Pomatomus saltatrix* collected from a contaminated estuary, Hackensack River, NJ

Introduction

Estuaries along the Mid-Atlantic Bight (MAB) have been identified by the National Marine Fisheries Service as the essential fish habitats for young-of-the-year (YOY) bluefish (Fahay et al. 1999). The young fish at about 50-mm length enter the Mid-Atlantic estuaries in early June and utilize them as a nursery and feeding ground (Able et al. 2003). An ontogenetic shift in diet from primarily copepods to fish and some invertebrates occurs after transition from oceanic to coastal habitats, and an exponential increase in growth rate is associated with this shift in feeding behavior (McBride and Conover 1991, Juanes and Conover 1994).

Several MAB estuaries are contaminated with PCBs, pesticides, and metals (Kennish and Ruppel 1998, Ashley 2003, Monosson et al. 2003; Weis et al. 2005, Weis and Ashley 2007, Ashley et al. 2009). YOY bluefish utilizing polluted MAB estuaries during periods of rapid growth and development of vital organs are chronically exposed to contaminants in the water column, sediments and food. Bluefish consumption by humans has recently been restricted in seven East Coast states including New Jersey because of risk of adverse health effects from PCBs and other bioaccumulative toxicants. Pregnant women and children are advised to avoid consuming large bluefish caught off

the coast and the rest of the population are advised to restrict consumption to four meals per year.

Trophic transfer is considered to be the most important route in the uptake of contaminants for animals at higher levels of the food chain such as bluefish (Thomman et al. 1991). Bluefish are opportunistic feeders, and notorious for being voracious predators. They consume up to 25% of their body weight per day in preparation for southward migration in the fall (Juanes and Conover 1994a, Buckel et al 1999a, Juanes et al. 2001). Prey abundance and quality strongly influence bluefish size at the end of their growing season (Juanes and Conover 1994b, Buckel and Conover 1997, Buckel et al. 1999b), and their survival may be linked to body size prior to autumn migration (Munch and Conover 2000). Small bluefish eat a diet split almost equally between fish and invertebrates and as they get older they eat primarily fish (Buckel et al 1998 and 1999a). YOY bluefish prey includes, but is not limited to smaller forage fish such as mummichogs, (*Fundulus heteroclitus*), striped killifish, (*Fundulus majalis*), silversides (*Menidia menidia*), menhaden (*Brevoortia tyrannus*) and bay anchovy (*Anchoa mitchilli),* juvenile striped bass (*Morone saxatilis*) as well as shrimp (*Crangon spp*. and *Palaemonetes spp*) (Buckel and Conover 1997, Buckel and McKown 2002). Diets high in fish lead to faster growth (Buckel et al. 1999b), but may also lead to higher exposure to contaminants if the fish utilized the contaminated habitats.

The level of PCBs, mercury and other contaminants in some prey species may be higher than in others, and can therefore transfer more contaminants to the bluefish. Prey with higher lipid content such as menhaden and mummichogs may be more contaminated

with lipophilic PCBs, resulting in an increased bioaccumulation of these contaminants through trophic transfer (Kennish and Ruppel 1998, Monosson et al. 2003). In a previous study, these prey fish made up the largest percentage of stomach contents of YOY bluefish collected from the Hackensack River (Chapter 1). In addition, the adult bluefish have been found to contain a much higher concentration of lipids than other species in the New York Bight, making them even more vulnerable to accumulation of lipophilic toxicants (Deshpande et al. 2002).

This paper will focus on three particularly noxious contaminants polychlorinated biphenyls (PCBs), DDTs and mercury. PCBs and organic pesticides including DDTs are major environmental concerns because they are bioaccumulative, persistent and toxic (Newman and Unger 2003). They concentrate mainly in the soil and the sediments because they are hydrophobic. Once they enter the food chain they bind to lipids and are not easily metabolized. Contaminants in the water column can enter the fish via the exchange across the gill membrane. Contaminants in the sediments can be transferred to fish by direct exposure, although predominently through the consumption of contaminated prey (Feldman and Titus 2001). PCBs and DDTs tend to biomagnify up the food chain and can bioaccumulate in fish to the levels hundreds of thousand times higher than those found in the water column. Analysis of aquatic organisms from the lakes in Ontario reported a 3.5 fold increase in PCBs at each trophic level and concentrations in lake trout flesh increased with length of food chain, lipid content and proximity to urban areas (Rasmussen et al. 1990). PCBs and organic pesticides, including DDT, are associated with a wide array of deleterious effects on fish including liver damage and impairment of osmoregulation, immune functions, reproduction,

maturation, endocrine function, and development (Newman and Unger 2003). Despite the fact that the production and use of PCBs and DDTs has been banned for decades, they remain in the ecosystem and may be working synergistically with other highly toxic pollutants such as mercury (Kennish and Ruppel 1998, Beckvar et al. 2005).

Mercury has been well known as an environmental pollutant since the 1950s. It is a commonly occurring freshwater, estuarine, and marine pollutant, particularly in the form of methylmercury, which bioaccumulates in fish through trophic transfer, and via gill and transdermal exchanges from water and sediments (Grippo and Heath 2003, Newman and Unger 2003). Exposure to mercury has been linked to impairment of development, immune function, reproduction and behavior (Weis el al. 2001, Grippo and Heath 2003, Newman and Unger 2003, Scott and Sloman 2004). It is associated with poor predator avoidance in golden shiner and mummichogs, impaired prey capture and feeding behavior of fathead minnows and mummichogs and altered swimming activity of mummichogs (Tsai et al. 1995, Zhou et al. 2001, Grippo and Heath 2003, Webber and Haines 2003, Weis et al 2003).

Given that bluefish are an important recreational and commercial as well as an ecologically important species along the U.S, East Coast and that the stocks have been fluctuating generally in a downward trend over the past two decades, contaminant analyses of field-collected fish from these two different estuaries can provide much needed information on the impact of habitat quality on the condition of YOY bluefish. Such an analysis is particularly important because bluefish condition at the end of the summer may significantly impact their migratory competence and recruitment success

into adult populations. Therefore, the objective of the study was to compare the size and contaminant concentrations of YOY bluefish from two different MAB estuaries with different degrees of contamination histories.

Study Location

Two estuarine locations in New Jersey were examined in this study. The reference estuary was in the area surrounding the Tuckerton Marine Field Station on Great Bay at the mouth of the Mullica River (TK) (Figure 1.1). All TK fish collections for this study were at the beach or in the lagoons near Graveling Point (N 39˚ 32.4' / W 74˚ 23.2') or from the dock and shore of the Rutgers Marine Field station. The contaminated site was located in the Hackensack River-Newark Bay Estuary (HR) (Figure 1.1). HR fish samples were collected at the same study sites in the Hackensack River that were sampled in a survey conducted by the New Jersey Meadowlands Commission ((N 40˚ 45.1'/W 74˚ 05.0'); T3, T1, S2, and S3) (Konsevick and Bragin 2007). Fish from each study site were collected during daylight hours at various tidal periods.

The Hackensack River runs approximately 72 km through New York and New Jersey and empties into the Newark Bay. The watershed is surrounded by a highly urbanized region, including suburban areas outside of New York City. The River flows through and drains the New Jersey Meadowlands estuarine habitat. Two centuries of industrial pollution have left the water and sediment severely polluted and while conditions have improved over the past few decades, urban runoff, sewage treatment plant discharges, sewer overflows and sanitary landfills continue to impair the habitat

quality (Kiviat and Macdonald 2004, Marshall 2004). Studies have found that fish contain elevated body burdens of PCB, mercury and other metals (Hauge et al. 1990, Weis and Ashley 2007) and the state of New Jersey had banned all commercial fishing and crabbing in the Hackensack River. White perch (*Morone americana*) from the Hackensack River contained Hg and PCB levels that exceed the guidelines for "one meal per month" and some were above the US FDA "no consumption at all" level (USFDA 1993, USEPA 1999a, b, 2000, 2001).

Sediment concentrations of mercury in the Hackensack River have been found to be well above the effects range median (ER-M), which is the median concentration found in the literature to adversely affect 50% of species, and PCB sediment concentrations were greater than the sediment effect threshold (SET) which is expected to lead to tissue concentrations that would result in adverse sublethal effects in salmonids (Meador 2000, (Konsevick and Bragin 2007). The Mullica River-Great Bay estuary is a productive estuarine system comprised of 87 square km of salt marsh and 56 square km of shallow estuarine waters. The watershed is comprised of a number of wildlife reserves and refuges including the New Jersey Pinelands National Reserve, the Edwin B. Forsy National Wildlife Refuge, the Great Bay Wildlife Management Area, and the Jacques Cousteau National Research Reserve. It is one of the least anthropogenically disturbed estuaries on the U.S. East Coast (NOAA 2004).

Material and Methods

Field collections

YOY spring-cohort bluefish from the Tuckerton sites were collected with either a 60 m seine net, a gill net or hook and line. YOY spring cohort bluefish from the HR sites were collected using a 60m seine net and a bottom otter trawl from sites T1, T3, S2, S3 in the Hackensack River. Spring-cohort YOY bluefish were determined to be those with total lengths >160mm based on data reported in previous studies (McBride and Conover 1991, Weidenmann and Essington 2006). Bluefish less than 160mm down to 120 mm were caught from each site during field collections and were considered to be summer spawned bluefish and were released. All samples were placed on dry ice in the field and were stored at -80°C for PCB, pesticide and mercury analysis. The specific sample collections and the analyses they were utilized for from each year follow:

2002 Collection

Eight bluefish were collected from HR on July 24, 2002 and ten bluefish were collected from TK on July 26, 2002 and the total length and weight of each fish was measured.

From September 24 - October 2, 2002 42 bluefish were collected from TK, their total length and wet weight was measured and the fillets of ten of these fish were dissected for PCB and pesticide analysis. From September 19 - October 1, 2002 37 bluefish were collected from HR, their total length and wet weight was measured and the fillets of ten of these fish were dissected for PCB and pesticide analysis.

2003 Collection

From September 14 - 28, 2003 35 bluefish were collected from TK and from September 22-29, 2003 46 bluefish were collected from HR, their total length and wet weight was measure and their stomach content was removed, weighed and identified. The fillets of ten of these HR fish were dissected for PCB and pesticide analysis. A 2-gram section of the muscle tissue of ten of the HR and ten of the TK bluefish was set aside for mercury analysis.

Six mummichog and menhaden collected from the stomachs of the 2003 TK bluefish were analyzed for PCB and pesticide analysis. Six mummichog and ten menhaden collected from the stomachs of the 2003 HR bluefish were analyzed for PCB and pesticide analysis.

Ten mummichog and menhaden were collected from each site (TK and HR) at the same time and location as the bluefish were collected in 2003. Five mummichog and menhaden from each were used for PCB and pesticide analysis and five mummichog and menhaden were used for mercury analysis.

2006 Collection

From September 12-22, 2006 26 bluefish were collected from HR and their stomach content was removed, weighed and identified.

2007 Collection

From September 17 to October 11, 2007 95 bluefish were collected from HR and their stomach content was removed, weighed and identified. Ten menhanden from the 2007 HR bluefish stomachs were analyzed for PCB and pesticides. Ten menhaden were collected in the field during the same time and location as the bluefish and were analyzed for PCB and pesticides.

Stomach content analysis

The bluefish were sacrificed, their stomachs were dissected and weighed, the presence or absence of food in the stomach was recorded and the bluefish and stomachs were placed immediately on dry ice and then stored in a -80° C freezer until analysis. Some bluefish regurgitated stomach content upon capture. These contents were collected in methylene chloride-washed glass containers, labeled, and placed immediately on dry ice.

Stomach content analysis was performed under a high-efficiency particle air laminar-flow hood. Dissecting implements and containers were cleaned with ultrapure 10% nitric acid, double deionized water, methanol and methylene chloride. Stomach contents of each specimen were identified under a dissecting microscope. Identified pieces of fish were placed in separate methylene chloride-washed glass containers and stored at -80°C until chemical analysis was performed. The percentage of bluefish with food in their stomachs was calculated for each site.

PCB and pesticide analysis

PCBs and pesticides were analyzed in twenty two fillets of HR (2002 and 2003) bluefish (average weight 40.73 ± 2.69 g) and ten fillets of 2002 TK bluefish (average weight 50.53 ± 4.61 g). PCB and pesticide analysis was performed on stomach content samples of whole prey or those with a significant identifiable portion of the body and organs remaining. The average sample weight of six mummichog and menhaden from 2003 TK bluefish stomachs was 5.06 ± 0.57 g and 6.38 ± 0.79 g, respectively. The average sample weight of six mummichog and ten menhaden from 2003 HR bluefish stomachs was 3.97 ± 0.79 g and 4.45 ± 0.51 g, respectively. The average sample weight of five 2003 TK field collected mummichog and menhaden was 4.02 ± 0.46 g and 5.05 ± 0.074 g, respectively. The average sample weight of five 2003 HR field collected mummichog and menhaden was 4.63 ± 0.47 g and 4.89 ± 0.35 g, respectively. The average sample weight of ten menhaden from 2007 HR bluefish stomachs and ten 2007 HR menhaden collected from the field was 4.17 ± 0.50 g and 4.72 ± 0.36 g, respectively.

PCB and pesticide analysis was performed according to the guidelines of Krahn et al. (1988),USEPA (1993), Sloan et al. (1993) and Deshpande et al. (2000). Briefly, tissue samples were freeze-dried, homogenized with a blender or mortar and pestle and sodium sulfate and then extracted with methylene chloride using an automated Soxhlet extraction apparatus (Organomation, Berlin Massachusetts). Florisil/silica/alumina glass column chromatography was performed and the sample was then further purified using a Phenomenex Envirosep-ABC size-exclusion column on a HP1050 HPLC. The sample was finally analyzed using an Agilent 5890 GC with electron capture detection for 31

PCB congeners and 24 pesticide compounds listed in Appendix 1, Table A.1 (Deshpande et al. 2000, Deshpande et al. 2002b). A primary calibration mixture (PCB Calibration Check Solution C-CCSEC: AccuStandard, New Haven, Connecticut + chlorinated pesticides SRM 2261: NIST, Gaithersburg, Maryland) and a supplementary calibration mixture (PCBs SRM 2274 + chlorinated pesticides SRM 2275; NIST, Gaithersburg, Maryland) were used in the internal standard method to quantify the target analyte peaks. The menhaden from the stomach contents of bluefish collected in 2007 and the menhaden collected in the field during the same time were analyzed with a revised procedure. The samples were not freeze dried, but were dried chemically with diatomaceous earth also referred to as hydromatrix (Dionex). The dried samples were extracted with methylene chloride using a Dionex ASE 300 Accelerated Solvent Extractor. The remainder of the cleanup and analysis procedures remained the same.

The "Aroclor-based" total PCB value (tPCB) was calculated by taking the sum of the concentrations of 18 specific PCB congeners and multiplying that value by 2.0 (Table A.1) (Deshpande et al. 2002a). This value represents an estimate of the sum of all PCB congeners and it can be used for comparison of historical data on PCB analysis.

Mercury Analysis

A 2 g section of muscle tissue of each of the ten 2003 HR and 10 TK bluefish and a 2 g wet weight sample of each of five homogenized whole 2003 HR and TK menhaden and mummichog was analyzed for total mercury following the procedure of Weis and

Ashley (2007). Dried tissues were HNO_3 -mineralized in a MARS-5 programmed microwave digester and analyzed for total mercury concentrations by cold vapor atomic absorption spectrophotometry (CVAAS) with a Baccharach MAS-50D instrument.

Quality Assurance

For PCB and pesticide quality assurance, each batch of samples included a method blank, three method surrogate internal standards added prior to extraction, HPLC purification and GC analysis, and a CARP-2 certified reference material - CRM - (NRC, Canada). Analysis of method blanks produced no detection or very low detection of all analytes, demonstrating cleanliness of glassware and equipment. Each sample type and batch analyzed had quantitative mean recoveries for the surrogate internal standards; mean percent recovery was 68% ± 38. A CARP-2 sample (NRC, Canada) was run with each of the four batches and the majority of the absolute value of the z-scores for the target analytes were 2 or below. Overall, the results of the quality control data provided confidence that the concentrations of PCB and pesticide analytes for the bluefish and prey samples were accurate.

Each mercury sample reading was adjusted by the percent error found from the values of the CRM used (DORM-2, NRC, Canada). The mean detection level (MDL) was three times the standard deviation found from the average of all method blanks divided by the average sample weight. The MDL was set at 0.0100 $\mu g\ g^{-1}$ and any sample with a

value less than that this was reported as <MDL and assigned a value of one-half the MDL or 0.005 μg g^{-1} for the purpose of statistical analyses.

Statistical analysis

The mean Aroclor PCB estimate, tPCB, and sum of the concentration of the pesticide DDT and its metabolites (total DDTs) is reported for each group of fish. For the purpose of brevity specific congener concentrations and pesticides other than DDTs will only be reported for the TK and HR bluefish not the prey. The total DDTs (tDDT) was used for the data analysis of pesticides because they were found at highest concentration in the bluefish and prey, ranging from 40-70% of total pesticides. Means and standard errors are presented in the text and on the graphs. All statistical analyses were performed on Minitab13.1 software program with a significance level of $p<0.05$.

Bluefish

A MANOVA compared the length and weight of the HR versus the TK bluefish collected in July 2002. The length (mm) of the bluefish collected in the fall in different years (2002 versus 2003) was compared for each site via a one-way ANOVA. Weight was also compared between years (2002 and 2003) for each via a one-way ANOVA. A MANOVA compared the length and weight of fall 2002/2003 HR versus 2002/2003 TK bluefish. Differences of length and weight between 2002/2003 HR and TK bluefish were also tested separately via a one-way ANOVA.

The tPCB concentrations between HR and TK field bluefish were compared using a one-way ANOVA. This test was also used to analyze differences in tDDT and Hg concentrations between TK and HR bluefish. MANOVAs comparing the individual PCB congener concentrations of the HR to the TK bluefish were performed. A MANOVA was used to compare the concentrations of ortho-substituted congeners in the HR and TK bluefish. Additional MANOVAs compared the coplanar and dioxin-like congeners in the HR and TK bluefish. Ortho-substituted congeners contain two or more chlorines in the ortho (2,2' or 6,6') position. Coplanar congeners are mono-ortho or non-ortho. Dioxin-like congeners contain at least 4 chlorine substitutes, chlorines in both para positions (4,4'), at least 2 chlorines at the meta positions (3,3' or 5,5') and are coplanar. Each individual congener concentration in the HR and TK bluefish were compared via a one-way ANOVA.

Prey from field and bluefish stomachs

tPCB and tDDT concentrations of mummichog and menhaden from bluefish stomachs were compared between sites (TK and HR) for each species and contaminant using a one-way ANOVA, followed by a pairwise comparisons using Fisher's LSD. To compare concentrations of PCBs and DDT in prey from stomachs versus prey from field, five MANOVAs were performed. These five tests included comparisons of HR 2003 menhaden from field and stomachs, TK 2003 menhaden from field and stomachs, HR 2003 mummichog from field and stomachs, TK 2003 mummichog from field and stomachs, and HR 2007 menhaden from field and stomachs. A one-way ANOVA was

also performed comparing the tPCB and tDDT concentrations separately for each of the above groups.

Results

Size of bluefish

July 2002 Collection

The length and weight of the bluefish collected in July 2002 from the HR and TK were not significantly different from one another (Table 2.1). HR bluefish were 111.3±6.7 mm and 18.2±4.2 g, and TK bluefish were 115.2±3.9mm and 17.5±1.8 g.

September 2002/2003 Collection

The size of the HR fish caught mostly in September of 2002 and 2003 were not statistically different. This is also true for the TK bluefish caught in September of 2002 and 2003 (Table 2.1). The average weight and length of September 2002/2003 HR bluefish (104.9 ± 3.5g and 199.1 ± 1.8mm) was significantly less than that of September 2002/2003 TK fish (151.5 ± 8.2g and 227.5 ± 3.5mm) (Table 2.1). HR bluefish were between 160-240 mm long and most frequently between 180-210 mm (Figure 2.1). TK bluefish were between 170-280 mm long, most frequently between 210-240 mm (Figure 2.1). The TK bluefish were 14.9% longer and 44.4% heavier than the HR bluefish.

Stomach content

In 2003, 74.3% of the 35 TK bluefish and 52.2% of the 46 HR bluefish had food in their stomachs. In 2006, only 23.1% of the 26 HR bluefish and in 2007 only 20.0% of the 95 HR bluefish had food in their stomachs. On average, only 29.3% of the 167 HR bluefish caught had stomach contents. As no corresponding sampling was conducted in Tuckerton, reference data are not available for 2003, 2006 and 2007.

Contaminants

PCBs

Six of the HR bluefish exceeded the US FDA tolerance level of 2000 ng g^{-1} for PCBs in edible tissues for human consumption. The fall collected HR 2002/2003 bluefish tPCB (1483.7 ± 143.5 ng g^{-1}) were significantly greater (six-fold) than the 2002 TK fish (242.2 ± 29.4 ng g^{-1}) (Figure 2.2, Table 2.2).

tPCBs of menhaden and mummichog from bluefish stomachs were averaged to calculate a mean body burden of the stomach content. tPCB of the HR bluefish was 2-3 fold greater than their 2003 stomach content (599.0 ± 87.0 ng g^{-1}). tPCB of the TK bluefish was six-fold greater than their stomach content (43.0 ± 4.6 ng g^{-1}) indicating greater biomagnification than in the HR fish.

Results of the MANOVAs revealed that HR bluefish had significantly greater concentrations of ortho-substituted PCB congeners ($F=15.43$, $p<0.0001$), coplanar ($F=32.1$; $p<0.0001$), and dioxin-like congeners ($F=15.24$; $p<0.0001$) compared to the TK fish (Figure 2.3). One-way ANOVAs of each congener revealed that all congeners were

significantly greater in the HR except PCB 195 (p = 0.473), PCB 206 (p = 0.591) and PCB 209 (p=0.112). PCB 77 and PCB 110 were co-eluting peaks in the GC/ECD analysis; therefore, the results are presented as the sum of the two concentrations. The sum of PCB 77 and 110 was significantly greater (p<0.0001) in the HR bluefish.

tPCB of the 2003 HR menhaden and mummichog in the bluefish stomach were significantly greater (10 -14 fold) than those from TK (Figure 2.4, Table 2.2). In 2003, tPCB in mummichog from HR stomachs (648.9±55.6 ng g-1) were greater than the field-caught mummichog (382.2±126.3 ng g^{-1}), but not statistically significant (Figure 2.5, Table 2.3). This was also true for the 2003 menhaden from HR stomachs (578.2±122.4 ng g^{-1}) compared to the field-caught menhaden (333.4±84.6 ng g^{-1}) (Figure 2.5, Table 2.3). On the other hand, in 2007, tPCB in menhaden from HR bluefish stomachs (1145.8±83.0 ng g^{-1}) was significantly greater than the field-caught menhaden (844.1±63.5 ng g^{-1}) (Figure 2.5, Table 2.3). Despite the limited data, this observation raises the concern that the more contaminated individuals might have been susceptible to increased predation by the bluefish, potentially leading to increased exposure of bluefish to the harmful contaminants.

tDDTs

tDDT HR bluefish (209.0±18.6 ng g^{-1}) were significantly greater (2-3 fold) than TK (70.6±4.9 ng g^{-1}) (Figure 2.5, Table 2.2). tDDTs in menhaden and mummichog from stomachs were averaged to calculate a mean body burden of the stomach content. tDDT

of HR bluefish were two-fold greater than that of their 2003 stomach content (113.8±12.9 ng g^{-1}). tDDT of TK bluefish was nine-fold greater than their stomach content (8.1±0.9 ng g^{-1}) again indicating greater biomagnification in the TK bluefish. Elevated concentrations of other chlorinated pesticides present in HR-fed bluefish included endosulfan I (62.8 ± 7.7 ng g^{-1}), dieldrin (39.6 ± 5.6 ng g^{-1}), and trans- and cis-nonachlor (22.1 ± 2.4 ng g^{-1} and 25.9 ± 2.9 ng g^{-1}).

tDDT in the 2003 HR menhaden and mummichog from stomachs were significantly greater (10 -12 fold) than those from TK (Figure 2.6, Table 2.2). In 2003, tDDT in mummichog from HR stomachs (114.5±8.8 ng g^{-1}) was significantly greater than field-caught mummichog (50.7±16.2 ng g^{-1}) (Figure 2.7, Table 2.3), similar to the PCBs described above. However, tDDT in menhaden from 2003 HR bluefish stomach content (113.5±18.2 ng g^{-1}) was not significantly different from field-caught menhaden (149.1±25.7 ng g^{-1}) (Figure 2.7, Table 2.3). tDDT in 2007 menhaden from HR bluefish stomachs (230.7±16.2 ng g^{-1}) was significantly greater than the field-caught menhaden (168.9±15.1 ng g^{-1}) (Figure 2.7, Table 2.3), again suggesting impaired predator avoidance in the more highly contaminated prey.

PCBs and DDTs MANOVA

A MANOVA comparing both tPCB and tDDT of the 2003 HR menhaden stomach content versus field caught prey revealed significant differences between stomach content and field caught prey. This was true also for the 2003 HR mummichog

from field and stomachs and the 2007 HR menhaden from field and stomachs (Table 2.3). There were no differences found between TK menhaden from field and from stomachs or TK mummichog from field and from stomachs (Table 2.3).

Mercury

Mercury concentrations of HR field-caught bluefish (1.01±0.08 μg g^{-1}) were significantly greater than TK field-caught bluefish (0.47±0.087 μg g^{-1}) (Figure 2.2, Table 2.2). The HR field bluefish were all above the US EPA 0.47 μg g^{-1} one meal per month level and four were above US FDA "no consumption level" (1μg g^{-1}). Also, high concentrations (greater than the 0.47 μg g^{-1} meal per month level) of total mercury were unexpectedly found in three TK bluefish and one was above the 1 μg g^{-1} level. The mean mercury concentrations of HR bluefish was 2-3 times that of their prey (0.38±0.041 μg g^{-1}) and the mean mercury of the TK bluefish was 45 times that of the TK prey (0.012±0.0021 μg g^{-1}).

Discussion

YOY bluefish residing in the contaminated Hackensack River estuary have elevated tissue residues of PCBs, DDTs and mercury, appear to be smaller in size and feed less than those from the reference site. The smaller size of fish from HR collected in the fall could be a combined result of reduced feeding and sublethal physiological effects of contaminant exposure. HR bluefish had reduced food in their stomachs. In 2003 52% of the HR bluefish had food in their stomachs compared to 74% of the TK bluefish. Examination of a total of 167 HR bluefish over three years revealed that only 29.3%

contained food in their stomachs. This is considerably lower than TK and the percentages of 63-92% reported in numerous other studies of YOY bluefish in the Mid-Atlantic Bight region (Juanes and Conover 1994, Buckel and Conover 1997, Buckel et al. 1999b, Buckel and McKown 2002, Gartland et al. 2006). Gartland et al. (2006) found 81.9 % YOY bluefish with full stomachs in the Chesapeake Bay. Buckel et al. (1999b) examined fish from Chesapeake Bay to Southern New England in the fall and which contained 63-86% full stomachs. Buckel & McKown (2002) reported 81-92% full stomachs in YOY bluefish from western Long Island Sound and Staten Island. Juanes and Conover (1994b) found 80% or greater full stomachs in Long Island Sound bluefish.

YOY bluefish are considered voracious, feeding continuously over the entire diel cycle, with rapid gastric evacuation rates that allow them to feed up to four times per day (Juanes and Conover 1994b, Buckel and Conover 1996, Buckel et al. 1999b, Buckel and McKown 2002) and with unusually high growth rates during their first year (McBride and Conover 1991, Juanes and Conover 1994a). However, this does not appear to be the situation in the HR population. Neuman et al. (2004) reported 54% empty stomachs of the bluefish collected from July to October in Mill Creek and Doctor Creek, tributaries of the Hackensack River, and the gut fullness indices was significantly higher in July than in the following months and was very low in October. This is contradictory to what would be expected as bluefish are expected to eat voraciously prior to fall migration.

It is also possible that the feeding grounds of the Hackensack River may be inferior to other sites such as TK. Many factors may influence predatory success in the habitat, including vegetation, dissolved oxygen levels, salinity, turbidity, depth, and prey

population abundance and density. The reduced food in the stomachs of the HR bluefish could be a result of reduced availability of food, but numerous pieces of evidence suggest otherwise. During our sampling numerous potential prey species were simultaneously collected along with the bluefish, including juvenile menhaden, mummichog, bay anchovy, silverside and juvenile white perch. Wetland restoration and mitigation efforts have been performed in this region by the New Jersey Meadowlands Commission and a Fisheries Resource Survey conducted by the Meadowlands Environmental Research Institute of the New Jersey Meadowlands Commission from 2001-2003 revealed an overall improvement in the fish community of the Hackensack River including an increase in biomass, abundance and diversity of species indicating increased community stability compared to a 1987-1988 survey (Bragin et al. 2005). Neuman et al. (2004) also concluded that marsh creeks of the Hackensack River estuary provide adequate feeding habitat for the estuarine predators. These studies indicate that there is adequate food for the YOY bluefish; thus, their empty stomachs are more likely due to altered feeding behavior. In addition, Chapter 1 revealed that YOY bluefish from TK, maintained in the laboratory on a diet of prey from HR for four months also had reduced feeding (impaired motivation to feed despite they were often the same ration of prey the TK tank fish were offered), activity and growth compared to those fed prey from TK. The high percentage of empty stomachs and smaller size of the bluefish from HR provides evidences that these fish may also be experiencing reduced motivation to feed and slower swimming speed making them inefficient predators and resulting in reduced growth.

It may be argued that the size difference of the bluefish may be due to differences in recruitment dates in the two estuaries. Thus, recruitment of spring-spawned YOY

bluefish may start earlier into the southern Great Bay estuaries than into the more northern Hackensack site, and therefore the southern fish would have more time to grow. There are presently no studies examining the recruitment dates of YOY bluefish into the Hackensack River estuary; however, there are numerous studies of the more northern Hudson River estuary. YOY bluefish have been sampled in the Hudson River estuaries as early as late May to mid-June (McBride and Conover 1991; Buckel et al. 1998; Williams 2006). Similarly, YOY spring-spawned bluefish have been sampled in the Great Bay southern New Jersey estuaries early to mid June (Able et al 2003). From the limited data, recruitment date varies from year to year, but location does not appear to be a major factor. Any differences in the growth rates may thus be attributable to the differences in the condition of the two habitats.

The small sampling of bluefish in July 2002 from HR and TK revealed that both the length and weight of the fish from the two sites were not significantly different. Although limited the data suggests that the fish are still in the same size range mid-summer and the differences in growth are occurring during the latter part of their residence in the estuaries as they are continuously exposed to the contaminants. This observation suggests a chronic effect in which the bluefish we sampled from the Hackensack River may have a latency period of about two months for the adverse effects to become evident. This possibility is also supported by the results of our laboratory exposure study in which bluefish were fed HR prey from July to November and their reduced feeding became evident in September and continued to decline through November at which point they were also found to be smaller than the reference fish (Chapter 1).

Finally, it is possible that migration may start earlier in the Hackensack River due to the early onset of colder waters and the larger individuals may migrate out sooner, but again there is limited research on this topic and the surface temperatures during fall collections in 2002 and 2003 ranged from 21-24˚C in both Hackensack and Tuckerton. More research on the recruitment patterns of the YOY bluefish along the Mid Atlantic Bight would be beneficial. Also, periodic length and weight measurements of samples of fish throughout their residency in the estuaries would provide an understanding of whether the sublethal effects and differences in size between the HR and TK bluefish are persisting over time. Notwithstanding the uncertainty of the recruitment timing, the evidence of frequent empty stomachs and smaller size of the HR bluefish compared to the TK bluefish is notable. TK bluefish were 14.9% longer and weighed 44.4% more than the HR bluefish, which may be attributed to their reduced feeding. Exposure to high concentrations of contaminants in the HR, particularly the endocrine disrupting contaminants PCBs, DDTs, and methylmercury, may also alter the hormonal concentrations including those of thyroid and growth hormones and lead to reduced growth and altered behavior (Power et al. 2001, Clotfelther et al. 2004, Baldigo et al. 2006).

Contaminant Concentrations

The tPCB concentrations were extremely elevated in the HR bluefish and their prey. The body burdens of the HR bluefish, the HR prey fish and stomach content and even the TK bluefish were well above the EPA advisory level for unrestricted human consumption (50 ng g^{-1}), the 1 in 100 cancer risk level set for other wildlife consuming

the fish (110 ng g^{-1}) and the tissue residue concentration (140 ng g^{-1}) reported to have adverse effects on juvenile salmonids (Newell 1987, Pellettieri et al. 1996, Meador 2000).

Many of the PCB congeners found in elevated concentrations in the HR bluefish have been linked to behavioral disruption, although there are few congener-specific studies in fish. The dioxin-like and coplanar congeners are known to bind to the Ah receptor, possibly leading to neuroendocrine and behavior disruptions (Osius et al. 1999). The ortho-substituted congeners are considered neurotoxins and may alter neurotransmitter concentrations and behavior (Seegal et al. 1991, Simon et al. 2007, Fischer et al. 2008). Elevated concentrations of the dioxin-like congeners, PCB 66 and 28, and the coplanar PCB 118 which are reported to alter behavior and thyroid status were present in the HR fish tissue (Holene et al. 1995, Eriksson and Fredriksson, 1996, Osius et al. 1999). In addition, the ortho-substituted PCBs 44, 52 and 153 have been correlated with altered behavior and neurotransmitter concentrations and have high neurotoxic equivalency potency values (Bowman et al. 1981, Eriksson and Fredriksson 1996, Holene et al. 1998, Mariussen et al. 1999, Simon et al 2007, Fischer et al. 2008). The sum of the co-eluting congeners, PCB 77 and PCB 110 was the highest concentration in the HR fish. Further gas chromatography - mass spectrometric analysis has revealed that PCB 110 is likely contributing to most of the tissue concentration. PCB 110 is established as one of the most potent neurotoxins therefore, the elevated concentrations of this congener as seen in the HR bluefish may be associated with detrimental neurological and behavioral changes (Simon et al. 2007).

Mercury concentrations were also highly elevated in the HR bluefish, prey, and surprisingly, even in some of the TK bluefish. HR bluefish and prey and TK bluefish all have body burdens of Hg well above the sublethal effects level (0.2 µg g^{-1}) that was determined to be a protective level in juvenile and adult fish based on a review of studies of sublethal endpoints (Beckvar et al. 2005). tDDT concentrations in all samples were below the FDA and EPA action levels (5000 and 1000 ng g^{-1}) and the protective level of 600 ng g^{-1} body concentrations determined by Beckvar et al 2005. However, 270 ng g^{-1} tDDT was determined to be the 1 in 100 cancer risk criteria for other wildlife consuming the fish (Newell 1987) which is within the range of the concentrations found in the HR bluefish. At these concentrations of toxicants it is highly likely that the fish will be experiencing behavioral and physiological effects.

Interestingly, the TK bluefish appear to be biomagnifying the contaminants to a greater degree than the HR bluefish. On average, the PCBs and DDTs were accumulated in TK bluefish at concentrations six and nine-fold that found in their stomach content, respectively. On the other hand, the HR fish biomagnified PCBs and DDTs at only two to three fold that of their stomach content. Mercury biomagnification is also greater in the TK bluefish. While mercury concentrations from the stomach content was not obtained the mercury concentration in the TK bluefish was 45 times that of the prey and the HR bluefish are only 2 to 3 fold that of the prey. Results from Chapter 1 also revealed a greater degree of biomagnification of PCBs, DDTs and Hg in the TK-prey fed bluefish compared to the HR-prey fed bluefish. The HR-fed and TK-fed laboratory bluefish were all fed the same prey species (menhaden and mummichog), therefore the difference seen in the laboratory fish was not due to differences in diets (Chapter 1). This

suggests that differences in biomagnification in the HR and TK bluefish from the field are not sole products of differences in diets. Despite the fact that the TK fish were caught in relatively "pristine" waters, their PCB and mercury concentrations are biomagnifying at levels considered too high for unrestricted human consumption and may also be causing sublethal effects on the organisms themselves. Lesser biomagnification factor in the HK fish may be resulting from the mobilization of a greater portion of contaminant body burden, a topic which is beyond the scope of present discussion.

Another noteworthy finding is the differences between the body burdens of the prey fish collected in the field versus from the bluefish stomachs. Overall the HR prey collected from bluefish stomachs had elevated concentrations of PCBs and DDTs compared to those collected in the field. The observation that these fish with higher contaminant concentrations had been eaten suggests that the fish with elevated body burdens may be easier to capture due to the debilitating neurotoxic and behavioral effects. The higher predation of contaminated prey could also lead to greater trophic transfer of contaminants to the bluefish and other predators (Weis and Weis 1974). In turn, the predators experiencing behavioral impairment including impaired prey capture ability due to contaminant exposure may preferentially feed on the slower prey fish, with the additional consequence of even greater contaminant burdens. The impact of anthropogenic stressors on predator avoidance behavior of aquatic organisms has been observed and demonstrated repeatedly in laboratory experiments of prey exposed to contaminants (Kraus and Kraus 1986, Smith and Weis 1997, Weis et al. 2000, Webber and Haines 2003, Scott and Sloman 2004). Prey exposed to contaminants may fail to detect predators, have a poor fast start performance, reduced stamina, inability to school,

altered activity patterns and increased conspicuousness (hyperactivity), all leading to increased vulnerability to predation (Mesa et al. 1994, Scott and Sloman 2004). It has been shown in the laboratory that mummichogs with higher body burdens of mercury have reduced activity and reduced predator avoidance (Smith and Weis 1997, Weis et al. 2001), and therefore they may be transferring greater quantities of toxicants to the predators. The current data appear to be a field validation of those observations. Going up one trophic level, the HR bluefish have empty stomachs and reduced size suggesting impaired feeding behavior. In addition, the HR-fed bluefish from the previous laboratory study had slower swimming speed and irregular and impaired schooling (Chapter 1), which would also make them more vulnerable to predation once they move away from the protection of the estuary during migration and would lead to further trophic transfer and bioaccumulation in the top predators like the mako sharks (Deshpande et al. in preparation). Future experiments assessing the prey selection and capture ability of the HR bluefish and the predator avoidance behavior of the bluefish and their prey would be beneficial to better understanding the community dynamics in this estuary. HR bluefish may be preferentially feeding in areas of higher contaminants and slower prey, which could lead to increased accumulation of contaminants. The use of mean contaminant levels in random samples of prey collected from the field to study exposure through a food web may be an underestimate of what the actual exposure via trophic transfer is to the predators. Future work should include a sampling of prey from the predator stomach to assure an accurate assessment of the prey levels.

The reduced feeding and growth of the HR bluefish may make them more vulnerable to predation and starvation and have a substantial impact on their migratory

competence and overwinter survival. While this was a site specific study it is possible that YOY bluefish residing in other urban estuaries along the Mid-Atlantic bight during their first summer may be experiencing similar behavioral and growth impairments. Size-dependent winter mortality can play a key role in year class strength and overall stock size; in general, larger individuals are more likely to survive their first winter (Conover 1992, Hurst and Conover 1998, Schultz et al. 1998, Lankford and Targett 2001, Slater et al. 2007). In addition, during YOY winter migration and their prolonged period of reduced feeding, lipid utilization may cause PCBs and methylmercury stored in lipids during the summer to be redistributed into sensitive organs such as liver and brain and lead to further detrimental effects, as reported in other fish (Boon and Duinker 1985a, Jørgensen et al. 1999). Increased over-winter mortality of contaminated individuals may be associated with decreased contribution of this population to the stock size. Weidenmann and Essington (2006) concluded that YOY bluefish overwinter survival may be highly density dependent and they argued that catch per unit effort (CPUE) resulting from fall surveys of YOY bluefish may not be accurate measurements of cohort strength. The inclusion of the contaminated HR bluefish in CPUE surveys may also produce misleading inflated stock estimates because these fish may be much less likely to survive overwinter (Hurst and Conover 1998, Slater et al. 2007, Weidenmann and Essington 2006). Future research focusing on the behavior and the fitness of the YOY bluefish during the overwintering period is necessary to better understand the impacts of contaminant exposure on their survivorship.

In conclusion, contaminant exposure and bioaccumulation of elevated concentration of PCBs, DDTs, and Hg was found to be associated with reduced feeding

and growth. HR specimens also appear to be feeding more frequently on more contaminated prey fish leading to an increased trophic transfer. Exposure may be decreasing survivorship and year class strength in populations that utilize this estuary and other contaminated estuaries as nurseries. Clearly more work is needed examining the alterations to the community dynamics and population structure of YOY bluefish, their prey and predators utilizing urbanized estuarine ecosystems along the Mid Atlantic Bight.

REFERENCES

Able, K.W., M. Burlas, and D. Byrne. 2003. Use of ocean and estuarine habitats by young-of-the-year bluefish (*Pomatomus saltatrix*) in the New York Bight. Fishery Bulletin 101:201-214.

Ashley, J.T.F., Webster, M.L., Horwitz, R.J., Velinsky, D.J., Baker, J.E., 2009. Polychlorinated biphenyls in sediment and biota from the Delaware River Estuary. Proceedings of Academy of Natural Scieces Philadephia 158:89-105.

Ashley, J.T.F., Horwitz, R., Steinbacher, J.C., Ruppel, B., 2003. A comparison of congeneric PCB patterns in American eels and striped bass from the Hudson and Delaware River estuaries. Marine Pollution Bulletin 46:1294-1308.

Amrhein, J.F., C.A. Stow, C. Wible. 1999. Whole-fish versus filet polychlorinated-biphenyl concentrations: an analysis using classification and regression tree models. Environmental Toxicology and Chemistry 18:1817-1823.

Baldigo, B.P., Sloan, R.J., Smith, S.B., Denslow, N.D., Blazer, V.S., Gross, T.S., 2006. Polychlorinated biphenyls, mercury, and potential endocrine disruption in fish from the Hudson River, New York, USA. Aquatic Sciences 68: 206-228.

Beckvar, N., T.M. Dillon, and L.B. Read. 2005. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects threshold. Environmental Toxicology and Chemistry 24:2094-2105.

Boon, J. P. and J. C. Duinker. 1985. Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (*Solea solea*) in relation to concentrations in water and to lipid metabolism under conditions of starvation. Aquatic Toxicology 7:119-134.

Bowman, R. E., M. P. Heironimus, and D. A. Barsotti. 1981. Locomotor hyperactivity in PCB-exposed rhesus monkeys. NeuroToxicology 2: 251–268.

Bragin, B., J. Misuik, C. A. Woolcott, K. R. Barrett, and R. Jusino-Atresino. 2005. A fishery resource inventory of the lower Hackensack River within the Hackensack meadowlands district: a comparative study 2001-2003 vs 1987-1988. New Jersey Meadowlands Commission Meadowlands Environmental Research Institute.

Buckel, J. A. and D. O. Conover. 1996. Gastric evacuation rates of piscivorous young-of-the-year bluefish. Transactions of the American Fisheries Society 125:591-599.

Buckel, J.A. and D.O. Conover 1997. Movements, feeding periods, and daily ration of piscivorous young-of-the-year bluefish, *Pomatomas saltatrix*, in the Hudson River estuary. Fishery Bulletin 95:665-679

Buckel, J.A., B.H. Letcher, and D.O. Conover. 1998. Effects of delayed onset of piscivory on the size of Age-0 bluefish. Transactions of the American Fisheries Society 127:576-587.

Buckel, J. A., M. J. Fogarty, and D. O. Conover. 1999a. Foraging habits of bluefish, Pomatomus saltatrix, on the US east coast continental shelf. Fishery Bulletin 97:758-775.

Buckel, J.A., D.O. Conover, N.D. Steinberg, and K.A. McKown. 1999b. Impact of age-0 bluefish (*Pomatomus saltatrix*) predation on age-0 fishes in the Hudson River estuary: evidence for density-dependent loss of juvenile striped bass (*Morone saxatilis)*. Canadian Journal of Fisheries and Aquatic Sciences 56:275-287.

Buckel, J. A. and K. A. McKown. 2002. Competition between juvenile striped bass and bluefish: resource partitioning and growth rate. Marine Ecology-Progress Series 234:191-204.

Clotfelther, E.D., Bell, A.M., Levering, K.R., 2004. The role of animal behaviour in the study of endocrine-disrupting chemicals. Animal Behavior 68: 665-676.

Conover, D.O. 1992. Seasonality and the scheduling of life history at different latitudes. Journal of Fish Biology 41 (Suppl. B): 161-178.

Deshpande, A. D., A. F. J. Draxler, V. S. Zdanowicz, M. E. Schrock, A. J. Paulson, T. W. Finneran, B. L. Sharack, K. Corbo, L. Arlen, E. A. Leimburg, B. W. Dockum, R. A. Pikanowski, B. May, and L. B. Rosman. 2000. Contaminant levels in muscle of four species of recreational fish from the New York Bight Apex. NOAA Technical Memorandum NMFS-NE-157, NOAA, Woods Hole, MA.

Deshpande, A. D., A. F. J. Draxler, V. S. Zdanowicz, M. E. Schrock, and A. J. Paulson. 2002a. Contaminant levels in the muscle of four species of fish important to the recreational fishery of the New York Bight Apex. Marine Pollution Bulletin 44:164-171.

Deshpande, A. D., R. J. Huggett, and R. A. Halbrook. 2002b. Polycyclic aromatic hydrocarbon metabolites in the bile of a territorial benthic fish, oyster toadfish (*Opsanus tau*) from the Elizabeth River, Virginia. Archives of Environmental Contamination and Toxicology 42:43-52.

Eriksson, P., A. Fredriksson. 1996. Developmental neurotoxicity of four ortho-substituted PCBs in neonatal mouse. Environmental Toxicology and Pharmacology 1: 155-165.

Fahay, M.P., P.L Berrien, D.L. Johnson, and W.W. Morse. 1999. Essential fish habitat source document: Bluefish, *Pomatomas saltatrix*, life history and habitat characteristics. NOAA Tech. Mem. NMFS-NE-144.

Feldman, R. S. and J. E. Titus 2001. Polychlorinated biphenyl accumulation differs among pumpkinseed sunfish during experimental field exposure:the role of invertebrate prey. Aquatic Toxicology 51:389-404.

Fischer, C., A. Fredriksson, and P. Eriksson. 2008. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244:157-165.

Gartland, J., R.J. Latour, A.D. Halvorson, and H.M. Austin. 2006. Diet composition of young-of-the-year bluefish in the lower Chesapeake Bay and the coastal ocean of Virginia. Transactions of American Fishery Society 135:371-378.

Grippo, M.A., and A.G. Heath. 2003. The effect of mercury on the feeding behavior of fathead minnows (*Pimphales promelas*). Ecotoxicology Environmental Safety 55:187-198.

Hauge, P., J. Bukowski, P. Morton, M. Boriek, J. McClain, and G. Casey. 1990. Polychlorinated biphenyls (PCBs), chlordane, and DDTs in selected fish and shellfish from New Jersey waters, 1986–1987:Results from New Jerseys Toxics in Biota Monitoring Program..*in* T. NJ Dept of Environ Protection, editor. Available from http://www.state.nj.us/dep/dsr/pcb86-87.pdf.

Holene E, I. Nafstad, J.U. Skaare, T. Sagvolden 1998. Behavioral hyperactivity in rats following postnatal exposure to sub-toxic doses of polychlorinated biphenyl congeners 153 and 126. Behavioral Brain Research 94: 213–224.

Hurst, T.P., and D. O. Conover. 1998. Winter mortality of young-of-the year Hudson River striped bass (Morone saxatilis): size-dependent patterns and effects on recruitment. Canandian Journal of Fisheries and Aquatic Science 55:1122-1130.

Jørgensen, E. H., B. E. Bye, and M. Jobling. 1999. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (*Salvelinus alpinus*). Aquatic Toxicology 44:233-244.

Juanes, F., J.A. Buckel and F.S. Scharf. 2001. Predatory behaviour and selectivity of a primary : comparison of fish and non-fish prey. Marine Ecology Progress Series 217: 157-165.

Juanes, F., Hare, J.A., Miskiewicz, A.G., 1996. Comparing early life history strategies of Pomatomus saltatrix: A global approach. Marine Freshwater Research 47: 365-379.

Juanes, F. and D. O. Conover. 1994a. Rapid Growth, High Feeding Rates, and Early Piscivory in Young-of-the-Year Bluefish (Pomatomus Saltatrix). Canadian Journal of Fisheries and Aquatic Sciences 51:1752-1761.

Juanes, F., and D.O. Conover. 1994b. Piscivory and prey size selection in young-of-the-year bluefish: predator preference or size-dependent capture success? Marine Ecology Progress Series 114:59-69.

Kennish, M.J., and B.E. Ruppel. 1998. Organochlorine contamination in selected estuarine and coastal marine finfish and shellfish of New Jersey. Water Air and Soil Pollution 101:123-136.

Kiviat, E. and K. MacDonald. 2004. Biodiversity patterns and conservation in the Hackensack Meadowlands, New Jersey. Urban Habitats 2:28-62.

Konsevick, E. and A. B. Bragin. 2007. Chemical characteristics of sediment collected as part of the fishery resource inventory of the lower Hackensack River within the Hackensack Meadowlands District: A comparative study 2001-2003 & 1987-1988.

Krahn, M.M., C.A. Wigren, R.W. Pearce, L.K. Moore, R.G. Bogar, W.D. MacLeod, S.L. Chan, and D.W. Brown. 1988. Standard analytical procedures of the NOAA National Analytical Facility, 1988: new HPLC cleanup and revised extraction procedures for organic contaminants. NOAA Tech Memo. NMFS FNWC-153; 52pp

Kraus, M. L. and D. B. Kraus. 1986. Differences in effects of mercury on predator avoidance in two populations of grass shrimp, Palaemonetes pugio. Marine Environmental Research 18:277-289.

Lankford, T.E., Targett, T.E., 2001. Physiological performance of young-of-the-year Atlantic croakers from different Atlantic coast estuaries: Implications for stock structure. Transactions of the American Fisheries Society 130: 367-375.

Marshall S. 2004. The Meadowlands before the Commission:Three centuries of human use and alteration of the Newark and Hackensack Meadows. Urban Habitats 2:4-27.

Mariussen, E., Fonnum, F., 2006. Neurochemical targets and behavioral effects of organohalogen compounds: An update. Critical Reviews in Toxicology 36: 253-289.

Meador, J. 2000. An analysis in support of tissue and sediment based threshold concentrations of polychlorinated biphenyls (PCBs) to protect juvenile salmonids listed by the Endangered Species Act. National Oceanic and Atmospheric Administration Report.

Mesa, M. G., T. P. Gasomski, and J. H. Peterson. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. Journal of Fish Biology 45 Supplement A:81-96.

McBride, R.S., and D.O. Conover. 1991. Recruitment of young-of-the-year bluefish *Pomatomus saltatrix* to the New York Bight: variation in abundance and growth of spring- and summer-spawned cohorts. Marine Ecology Progress Series 78:205-216.

Monosson, E., J. T. F. Ashley, A. E. McElroy, D. Woltering, and A. A. Elskus. 2003. PCB congener distributions in muscle, liver and gonad of *Fundulus heteroclitus* from the lower Hudson River Estuary and Newark Bay. Chemosphere 52:777-787.

Munch, S. B. and D.O. Conover. 2000. Recruitment dynamics of bluefish, *Pomatomus saltatrix*, from Cape Hatteras to Cape Cod, 1973-1999. ICES Journal of Marine Science 57:393-402.

Neuman, M.J., G. Ruess, and W. Able. 2004. Species composition and food habits of dominant fish predators in salt marshes of an urbanized estuary, the Hackensack Meadowlands, New Jersey. Urban Habitats 2:62-82.

Newell, A.J., D.W. Johnson, and L.K. Allen. 1987. Niagara River biota contamination project:fish flesh criteria for piscivorous wildlife. Technical Report 87-3 Division of Fish and Wildlife Bureau of Environmental Protection.

Newman, M.C. and M.A. Unger. 2003. Fundamentals of ecotoxicology. Lewis Publishers Washington D.C.

NOAA (National Oceanic and Atmospheric Administration). 1989. A summary of data on tissue contamination from the first three years (1986-1988) of the Mussel Watch Program. NOAA Tech. Memo. NOS OMA 49.154 pp.

NOAA, National Estuarine Research Reserve Jacques Cousteau Reserve, New Jersey 2004. Accessed June 21, 2009. http://nerrs.noaa.gov/JacquesCousteau/welcome.html M. B. Osius, N. W.

Karmaus, H. Kruse, and J. Witten 1999. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. Environmental Health Perspectectives 107: 843–849.

Pellettieri, W. H. Hallenbeck, G. R. Brenniman, M. Cailas, and M. Clark. 1996. PCB intake from sport fishing along the Northern Illinois shore of Lake Michigan. Bulletin of Environmental Contamination and Toxicololgy 57:766-770

Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Bjornsson, B.T., Einarsdottir, I.E., Canario, A.V.M., Sweeney, G.E., 2001. Thyroid hormones in growth and development of fish. Comparative Biochemistry and Physiology C, Comparative Pharmacology and Toxicology 130: 447-459.

Rasmussen, J. B., D.J. Rowan, D. R. Lean and J. H. Carey. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Canadian Journal of Fisheries and Aquatic Sciences 47:2030-2038.

Relyea, R., Hoverman, J., 2006. Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecology Letters. 9:1157-1171.

Schultz, E.T., D.O. Conover, and A. Ehtisham. 1998. The dead of winter:size-dependent variation and genetic differences in seasonal mortality among Atlantic silversides (Atherinidae: *Menidia menidia*) from di€erent latitudes. Canadian Journal of Fisheries and Aquatic Sciences 55:1149-1157.

Scott, G. R. and K. A. Sloman. 2004. The effects of environmental pollutants on complex fish behavior: integrating behavioral and physiological indicators of toxicity Aquatic Toxicology 68:369-392.

Seegal, R.F., Bush, B., Brosch, K.O., 1991. Subchronic exposure of the adult rat to Aroclor 1254 yields regionally-specific changes in central dopaminergic function. NeuroToxicology. 12:55-66.

Simon, T., J. K. Britt, and R. C. James. 2007. Development of a neurotoxic equivalence scheme of relative potency for assessing the risk of PCB mixtures. Regulatory Toxicology and Pharmacology 48:148-170.

Slater, J.J., T.E. Lankford, and J.A. Buckel. 2007. Overwintering ability of young-of-the-year bluefish Pomatomus saltatrix: effect of ration and cohort of origin on survival. Marine Ecology Progress Series 339: 259-269.

Sloan, C.A., N.G. Adams, R.W. Pearce, D.W. Brown, and S.L. Chan. 1993. Northwest Fisheries Science Center organic analytical procedures. In; Launestein, G.G.;

Cantillo, A.Y. , eds. Sampling and analytical methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects, 1984-1992. Vol. 4. Comprehensive descriptions of trace organic analytical method. NOAA Tech. Memo. NOS ORCA 71:182 pp.

Smith, G. M. and J. S. Weis. 1997. Predator/prey interactions in Fundulus heteroclitus: effects of living in a polluted environment. Journal of Experimental Marine Biology and Ecology 209:75-87.

Thomann, R.V., Mueller, J.A., Winfield, R.P., Huang, C.-R., 1991. Model of Fate and Accumulation of PCB Homologues in Hudson Estuary. Journal of Environmental Engineering. 117: 161-178.

Tsai, C. T. Jang, and L. Wang. 1995. Effects of mercury on serotonin concentration in the brain of tilapia, *Oreochromis mossambicus*. Neurosci. Let. 184:208-211.

USEPA. 1993. Laboratory methods manual estuaries. EPA Doc. 600/4-91/024 Available from: EPA Office of Research and Development, Cincinnati, OH. 289 pp.

USEPA. 1999a. Guidance for assessing chemical contaminant data for use in fish advisories. Vol. 2. Risk assessment and fish consumption limits, 3rd ed. EPA 823-B-99-008. US EPA, Office of Water, Washington, DC.

USEPA. 1999b. Mercury update: impact on fish advisories. EPA-823-F-99-019. US EPA, Office of Water, Washington, DC.

USEPA. 2000. Guidance for assessing chemical contamination data for use in fish advisories. Vol. 2. Risk assessment and fish consumption limits, 3rd ed. EPA 823-B-00-008. Office of Water, Washington, DC.

USEPA. 2001. Mercury update: Impact on fish advisories. EPA-823-F-01-011. US EPA, Office of Water, Washington, DC.

USFDA. 1993. National Shellfish Sanitation Program. Guide for the control of molluscan shellfish. US Food and Drug Administration,Washington, DC

Webber, H. M. and T. A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (Notemigonus crysoleucas). Environmental Toxicology and Chemistry 22:1556-1561.

Weidenmann, J., and T.E. Essington. 2006. Density-dependent overwinter survival in young- of-year bluefish (*Pomatomus saltatrix*)? A new approach for assessing stage-structural survival. Canadian Journal of Fisheries and Aquatic Sciences. 63:1934-1943.

Weis, P., and J. Weis. 1974. DDT causes changes in activity and schooling behavior in goldfish. Environmental Research 7:68-74.

Weis, J. S., G. Smith, T. Zhou, and C. Bass. 2000. Predator/prey interactions: a link between the individual level and both higher and lower level effects of toxicants in the aquatic ecosystems. Journal of Aquatic Ecosystem Stress and Recovery 7:145-153.

Weis, J. S., G. Smith, T. Zhou, C. Bass, and P. Weis. 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. BioScience 51:209-217.

Weis, J.S., T. Zhou, J. Skurnick, and P. Weis. 2003. Evaluating prey capture by larval mummichogs, (*Fundulus heteroclitus*) as a potential biomarker for contaminants. Marine Environmental Research 55:27-38.

Weis, P., Barrett, K.R., Proctor, T., Bopp, R.F., 2005. Studies of a contaminated brackish marsh in the Hackensack Meadowlands of northeastern New Jersey: An assessment of natural recovery. Marine Pollution Bulletin 50:1405-1415.

Weis, P. and J. T. F. Ashley. 2007. Contaminants in fish of the Hackensack Meadowlands, New Jersey: size, sex, and seasonal relationships as related to health risks. Archives of Environmental Contaminant Toxicology 52:80-89.

Williams, S.D. 2006. Bioaccumulation patterns of organochlorines in young-of-the-year bluefish (*Pomatomus saltatrix*) in the Hudson River Estuary. Florida Agricultural and Mechanical University, Tallahasee, Florida.

Zhou, T., R. Scali, and J.S. Weis. 2001. Effects of methylmercury on ontongeny of prey capture ability and growth in three populations of larval *Fundulus heteroclitus*. Archives of Environmental Contamination and Toxicology 41:47-54.

Table 2.1 – Size differences of YOY Bluefish from HR and TK sites.

	Source of variance	df	F	p
Bluefish collected in July 2002				
ANOVA				
	Total length (mm) HR vs TK	1	0.30	0.593
	Weight (g) HR vs TK	1	0.06	0.807
MANOVA				
	Total length (mm) and weight (g) HR vs TK	2	0.459	0.640
Bluefish collected in Fall 2002 and 2003				
ANOVA				
	2002 versus 2003			
	Total length (mm) HR	1	0.48	0.492
	Weight (g) HR	1	2.10	0.151
	Total length (mm) TK	1	0.56	0.457
	Weight (g) TK	1	0.33	0.566
	2002/2003 HR versus 2002/2003 TK			
	Total length (mm) HR vs TK	1	71.46	< **0.0001**
	Weight (g) HR vs TK	1	38.54	**<0.0001**
MANOVA				
	Total length (mm) and weight (g) HR vs TK	2	36.56	**<0.0001**

p-values in bold were statistically significant (<0.05)

Table 2.2 – Contaminant concentration differences of HR versus TK bluefish and their stomach content

ANOVA	Source of variance	df	F	p
	HR versus TK bluefish[a]			
	tPCB	1	30.30	**<0.0001**
	tDDTs	1	26.47	**<0.0001**
	Hg	1	15.17	**0.001**
	HR versus TK menhaden and mummichog from stomachs[b]			
	tPCB	3	8.95	**<0.0001**
	tDDTs	3	14.51	**<0.0001**

[a] 2002/2003 HR and 2002 TK bluefish (tPCB and tDDT); 2003 HR and TK bluefish (Hg)

[b] menhaden and mummichog collected from stomachs of 2003 HR and TK bluefish

Table 2.3 – Contaminant concentration differences of prey (menhaden and mummichog) from field and from bluefish stomachs.

ANOVA	Source of variance	df	F	p
	tPCB (Field versus Stomach)			
	HR menhaden 2003	1	1.50	0.240
	HR mummichog 2003	1	3.73	0.089
	HR menhaden 2007	1	8.33	**0.010**
	tDDTs (Field versus Stomach)			
	HR menhaden 2003	1	1.18	0.295
	HR mummichog 2003	1	11.92	**0.009**
	HR menhaden 2007	1	7.80	**0.012**
MANOVA				
	tPCB & tDDTs (Field versus Stomach)			
	HR menhaden 2003	2	7.22	**0.007**
	HR mummichog 2003	2	18.60	**0.002**
	HR menhaden 2007	2	4.20	**0.033**
	TK menhaden 2003	2	2.14	0.181
	TK mummichog 2003	2	1.283	0.329

p-values in bold were statistically significant (<0.05)

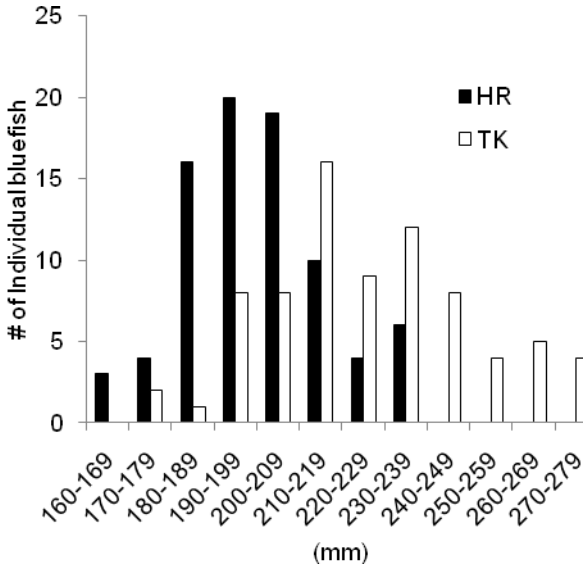

Figure 2.1 Total length (mm) frequency distribution of 2002/2003 HR & TK bluefish

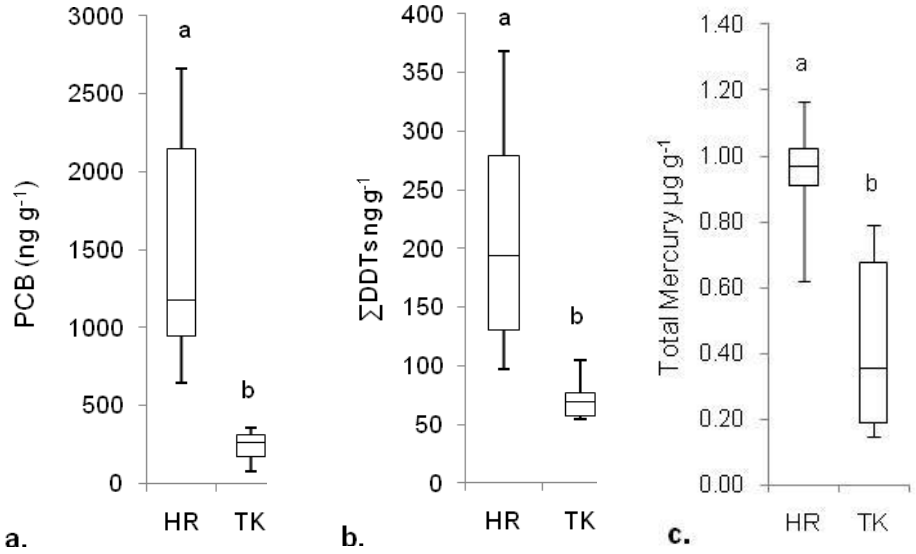

Figure 2.2 Contaminant concentrations of HR and TK field bluefish

a. tPCB (Arochlor sum) concentrations (ng g^{-1}) **b.** tDDTs (Sum of DDTs and metabolites) (ng g^{-1}) **c.** Hg (total mercury) (µg g^{-1}). Significant difference is indicated by an asterisk *. 2002/2003 HR and 2002 TK bluefish used for tPCB and tDDT; 2003 HR and TK bluefish used for Hg.

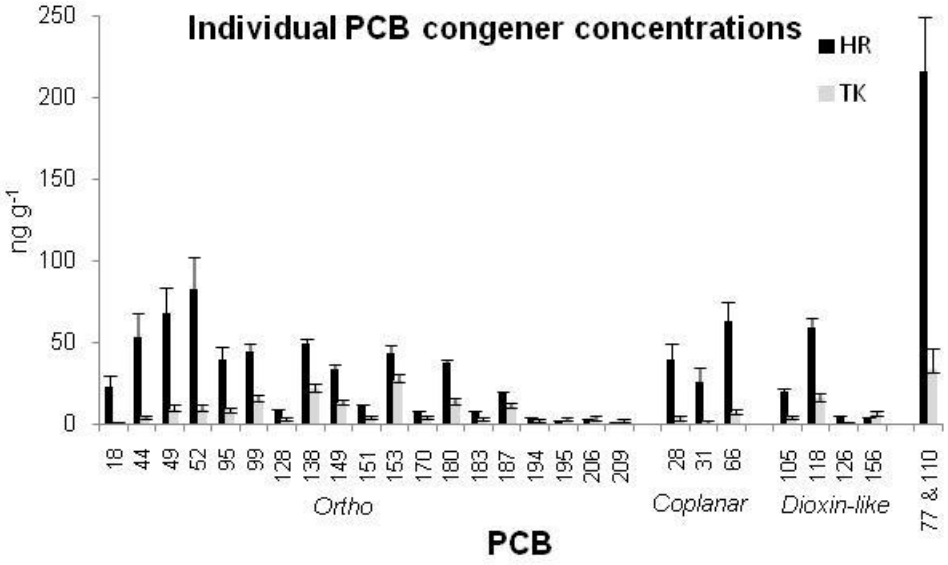

Figure 2.3 Mean concentration (ng g^{-1}) of individual PCB congeners in HR & TK field bluefish. The congeners are separated into ortho-substituted, coplanar and dioxin-like. The sum of PCB 77 and 110 is presented because they co-elute.

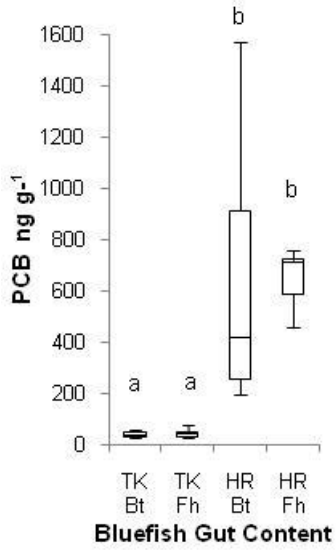

Figure 2.4 tPCB concentrations (ng g^{-1}) of 2003 HR & TK field-caught bluefish stomach content. Menhaden (Bt) and mummichog (Fh). Box plots with different letters are significantly different (ANOVA with Fisher's LSD pairwise comparison).

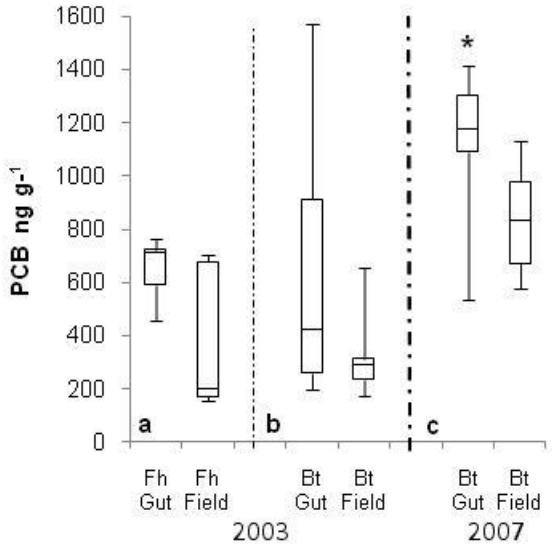

Figure 2.5 tPCB concentrations (ng g^{-1}) of bluefish stomach content.

a. mummichog collected from stomach content of 2003 HR field caught bluefish (Fh gut) and 2003 field-caught mummichog (Fh field);

b. menhaden collected from stomach content of 2003 HR field caught bluefish (Bt gut)

c. 2003 field-caught menhaden (Bt field); menhaden collected from stomach content of 2007 HR field caught bluefish (Bt gut) and 2007 field-caught menhaden (Bt field). Stomach and field prey from each species and year were compared separately via one-way ANOVA. Significant differences between concentrations in stomach content and field caught prey are indicated with an asterisk *.

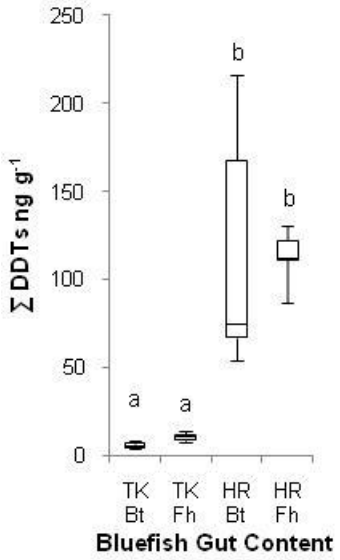

Figure 2.6 tDDT concentrations (ng g^{-1}) of 2003 HR & TK field-caught bluefish stomach content. Menhaden (Bt) and mummichog (Fh). Box plots with different letters are significantly different (ANOVA with Fisher's LSD pairwise comparison).

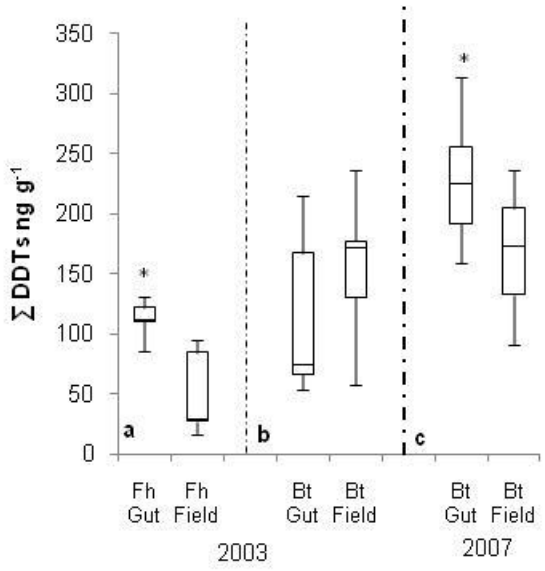

Figure 2.7 tDDTs (ng g^{-1}) of bluefish stomach content.

a. mummichog collected from stomach content of 2003 HR field caught bluefish (Fh gut) and 2003 field-caught mummichog (Fh field);

b. menhaden collected from stomach content of 2003 HR field caught bluefish (Bt gut)

c. 2003 field-caught menhaden (Bt field); menhaden collected from stomach content of 2007 HR field caught bluefish (Bt gut) and 2007 field-caught menhaden (Bt field). Stomach and field prey from each species and year were compared separately via one-way ANOVA. Significant differences between concentrations in stomach content and field caught prey are indicated with an asterisk *.

CHAPTER 3

Altered neurotransmitters and thyroid follicles in young-of-the-year bluefish, *Pomatomus saltatrix*, from a contaminated estuary, Hackensack River, NJ

Introduction

Exposure of aquatic organisms to xenobiotics including metals and persistent organic pollutants via water, sediment, and food has been shown to have numerous sublethal effects, including altered behavior and growth (Weis et al. 2001, Clotfelther et al. 2004, Scott and Sloman 2004, Zala and Penn 2004). Subtle changes in behavior because of contaminants can have severe implications for survival. PCBs, mercury, DDTs and various other persistent organic pollutants and metals have been shown to alter behaviors of fishes and other aquatic organisms, including locomotion, orientation, schooling, predator avoidance, feeding motivation, and prey capture rate and capture success (Sandheinrich and Atchison 1989, Little and Finger 1990, Weis et al. 2001, Webber and Haines 2003, Perez and Wallace 2004).

Physiology and behavior are closely coupled and altered behavior may be a result of a contaminant targeting a specific physiological pathway or a mixture of contaminants impacting multiple pathways and processes. Endocrine disruption, such as altered thyroid status, by metals and organic pollutants may cause altered behavior (Sloman et al. 2003, Scott and Sloman 2004, Sloman et al. 2006). At different developmental states, thyroid hormones control various physiological functions (Janz 2000, Schnitzler el al. 2008). Teleost thyroid follicles produce thyroxin (T4) that is essential for the regulation

of growth, development, nutrient utilization, behavior and metabolism. Fish grow faster and are healthier when thyroid hormone levels are high (Power et al. 2001). Comeau et al. (2001) concluded that thyroid hormones may be crucial to migration success of Atlantic cod by facilitating metabolism, sensory physiology and swimming capacity. Neurobehavioral development is contingent on proper thyroid hormone synthesis (Porterfield 1994, Porterfield 2000, Howdeshell 2002). Thus, altered thyroid hormones can lead to neurological impairment, especially during early life stages when critical stages in brain development are occurring.

Xenobiotics may alter hormone synthesis as agonists or antagonists, may bind to receptor sites, alter transport in the blood, or alter the deiodinases that metabolize thyroid hormones (Eales and Brown 1993, Zhou et al. 1999a, Adams et al. 2000, Weis et al. 2001, Li et al. 2008). Impairment of the ability to synthesize T4 will lead to a drop in T4 blood levels after thyroglobulin reserves are depleted (Zhou et al. 1999a). TSH from the pituitary gland stimulates the thyroid gland to secrete T4, which is the precursor to tri-iodothyronine, or T3, the biologically active hormone in teleost fishes (Leatherland 1994). In response to this decrease in T4, the pituitary will secrete more TSH, which causes enlargement, budding, and irregularity of the thyroid gland, commonly known as a goiter (Leatherland et al. 1978).

PCBs and organic pesticides such as DDT and its metabolites have been experimentally shown to alter thyroid hormones (Brouwer et al. 1998; Schnitzler et al. 2008). PCBs, particularly coplanar and dioxin-like congeners, have a halogenated, two phenol ring structure similar to the active thyroid hormones T4 and T3. Consequently,

they will bind readily to the aryl hydrocarbon (Ah) receptor (a ligand-activated transcription factor involved in the regulation of several genes that control a variety of developmental and physiological events), to thyroid hormone binding proteins, and to the thyroid hormone receptor (Landers and Bunce 1991, Porterfield 1994, Simon et al. 2007). PCBs are actually thought to have a higher affinity for binding to the serum carrier protein than the thyroid hormone itself and therefore can alter the thyroid hormone carrying capacity of the blood (Eales and Brown 1993, Porterfield 1994). Exposure to PCBs and dioxins often results in reduced serum T4 levels, normal T3 levels, increased TSH and thyroid enlargement (Porterfield 1994, Brown et al. 2004).

While goiters are common in vertebrates with an iodine deficiency (iodine is needed to synthesize T4), marine teleosts are unlikely to have iodine deficiency, and therefore, the presence of a goiter in a marine teleost can be used as a sensitive indicator of exposure to environmental toxicants (Eales and Brown 1993, Leatherland 1994). Abnormal thyroid histology has been found to be a more sensitive response than other sublethal effects. However, at sufficient exposure levels histological impairment has been correlated with various responses including the concentrations of circulating thyroid hormones and changes in developmental stages, behavior and reproduction (Patino et al. 2003, Tietge et al. 2005, Opitz et al. 2006, Carlsson and Norrgren, 2007). Studies have shown hypertrophied or goiterous thyroid follicles and altered thyroid hormone levels in fish exposed to contaminants in the laboratory and in the field (Leatherland 1994, Zhou et al. 1999a, Zhou et al. 1999b). Mummichogs, *Fundulus heteroclitus*, from Piles Creek, a highly contaminated site near Newark Bay, NJ, were found to have irregularly shaped

and greatly enlarged thyroid follicles, reduced hormone levels and altered behavior (locomotion, feeding and predator avoidance) compared to those from a reference site.

In addition to affecting the endocrine system, toxicants may also alter neurotransmitter levels. Atlantic croaker, *Micropogonias undulatus*, exposed to PCBs exhibited both impaired thyroid function and altered neurochemistry and, ultimately, disruption of the reproductive neuroendocrine function (Khan and Thomas 1996, Khan and Thomas 2006, Leroy et al. 2006). Neurotransmitters such as norepinephrine (NE), serotonin (5-HT) and dopamine (DA) and its metabolite dihydroxyphenylacetic acid (DOPAC) are associated with various behaviors including locomotion, conditioned responses and feeding (Weis et al. 2001, Grippo and Heath 2003a). The synthesis, storage, or release of neurotransmitters, synthesis of receptor sites, reuptake mechanisms and postsynaptic action have all been shown to be affected by exposure to toxicants. The mummichogs from Piles Creek, NJ, that had altered behavior and abnormal thyroid status also had decreased 5-HT levels (Smith et al. 1995, Weis et al. 2001).

Increases and decreases in levels of neurotransmitters including DA, 5-HT, and NE and their metabolites have been shown to have a variety of effects on behavior, although the studies correlating changes in brain monoamines and behavior in fish, are limited and often conflicting. Rainbow trout exposed to lindane were found to have altered 5-HT levels and metabolism in various regions of the brain (Rozados et al. 1991, Aldegunde et al. 1999). Fingerman and Russell (1980) found that a 24 hour exposure to Aroclor mixture 1242 significantly decreased levels of DA and NE and led to increased swimming activity in Gulf killifish, *Fundulus grandis*. They described locomotor

activity as a good indicator of neurotoxicity because it reflects the functional status of the nervous system.

Lead exposure generated increases in NE and 5-HT, feeding time, and number of miscues in juvenile fathead minnows (Weber et al. 1991). On the other hand, copper exposure decreased levels of DA and 5-HT and impaired food intake and locomotion in carp, and mercury decreased 5-HT in the hypothalamus and impaired motor control of tilapia (Tsai et al. 1995). Mummichogs from a polluted site (Piles Creek) had lower concentrations of 5-HT and its metabolite 5-HIAA in their medullas, along with reduced activity and feeding success (Smith et al. 1995). In a follow up study, methylmercury (MeHg) was shown to alter spontaneous activity and alter concentrations of DA, 5-HT and their metabolites in mummichog larvae from Piles Creek (Zhou et al. 1999b).

It has previously been shown in the laboratory experiment that YOY TK bluefish accumulate very high levels of PCBs, mercury, and chlorinated pesticides when fed prey from the contaminated (HR) estuary. After a few months of feeding on contaminated prey, fish showed a significant reduction in appetite, swimming activity, and growth (Chapter 1). In addition, the YOY bluefish collected from HR were found to be smaller in size that the TK field caught bluefish. Moreover, the percentage of HR YOY fish caught with food in the gut was very low (29%) compared to YOY bluefish reported from other regions, suggesting that reduced feeding also occurs in the wild population (Chapter 2).

The purpose of the present study was to examine possible mechanisms underlying the reduced activity, feeding, and growth. PCBs, DDTs, and methylmercury are known

endocrine disruptors and neurotoxins and it is possible that the exposure to these and other xenobiotics may produce alterations in the pituitary-thyroid axis and/or neurotransmitters (Weis et al. 2001). We hypothesized that YOY bluefish collected from HR and the YOY bluefish fed HR prey in the 2004 laboratory experiment would have enlarged, irregular thyroid follicles (an indication of reduced T4 concentrations) and could help explain the reduced activity and feeding observed in these fish.

We expected to find reduced neurotransmitter concentrations, particularly DA and 5HT, in both the HR field-caught bluefish and the HR-fed bluefish. PCBs have been shown to alter levels of biogenic monoamines, particularly DA, which is associated with altered behavior in fish (Fingerman and Russel 1980, Shain et al. 1991). Exposure to mercury has also been linked to alterations of behavior and levels of DA, 5-HT and their metabolites in fish (Tsai et al. 1995, Zhou et al. 1999b). Studies examining the physiological and behavioral impacts from exposure to contaminants are limited in fish and there is still a large gap in understanding how the sublethal effects are interconnected.

Materials and Methods

Study sites and sample collections

Young of the year bluefish were collected from study sites, Newark Bay, Hackensack River (HR) and Great Bay, Tuckerton, NJ (TK) between September 19 and October 11, 2006 for thyroid analysis and between September 6 and September 29, 2007 for neurotransmitter analysis. Bluefish were collected via a bottom trawl net and hook

and line. HR fish were collected at the same area in the lower part of the river that was sampled in a survey by the New Jersey Meadowlands Commission ("T3" (N 40˚ 45.1'/W 74˚ 05.0') (Konsevick and Bragin 2007). TK collections were performed off the beach of Graveling Point (N 39˚ 32.4' / W 74˚ 23.2'). Fish from each study site were collected during daylight hours at various tidal periods.

The laboratory bluefish were sacrificed after the behavioral experiments and immediately frozen (whole) and stored in a -80˚C freezer until analysis.

Thyroid histology

Field Bluefish

Twelve fish of similar fork length from each site were sacrificed and prepared for thyroid histology. The mean fork length of the HR field caught and TK field caught bluefish used for thyroid analysis was 188 ± 4 mm (range 169-210 mm) and 183 ± 5 mm (range 165-215mm), respectively. The ventral portion of the head was immediately removed and preserved in 10% formalin and transferred to 70% ethanol for preservation. They were decalcified, dehydrated, embedded in paraffin, and sliced sagittally near the midline, mounted on slides and stained with hematoxylin and eosin. The unencapulsated thyroid follicles are dispersed throughout the connective tissue of the lower jaw along the ventral aorta. The follicles are lined by a single layer of epithelial cells of varying height and form and are filled with colloid. Thyroid follicle morphology (cross-sectional area of follicles and epithelial cell heights) was measured utilizing a Carl Zeiss-Axiocam MRc5 camera and Carl Zeiss-Axioskop 2 plus light microscope and UTHSCSA Image Tool

version 3.0 software. The size of the follicles varied considerably and small follicles were common to both populations therefore the ten largest follicles were measured (Zhou et al. 1999a; Zhou et al. 2000). The cross-sectional area of each follicle was recorded and the cell height was measured in four places equally spaced around the circumference of the follicle.

Laboratory HR and TK fed Bluefish

Ten HR-fed and ten TK-fed bluefish of similar fork length from the laboratory experiments were analyzed for thyroid follicle area. The mean fork length of the HR-fed and TK-fed bluefish used for thyroid analysis was 221 ± 5 mm (range 199-240 mm) and 228 ±6 mm (range 199-255), respectively. To preserve the thyroid tissue, the ventral jaw of the fish was dissected while frozen using a small hacksaw and placed in a solution of 10% formalin to defrost and then transferred to a 70% ethanol solution for preservation. They were then processed and the follicle area was measured using the same procedure described above for the field bluefish samples. Since the tissue samples were frozen prior to preservation the clarity of the histological slides was not sufficient enough to get accurate measurements of the epithelial cell height; therefore only follicle area measurements were taken.

Neurotransmitters

Field Bluefish

Fifteen fish of similar fork length from TK and HR site were sacrificed and processed for neurotransmitter analysis. The mean fork length of the HR field caught and

TK field caught bluefish was 187 ± 6 mm (range 165 – 210 mm) and 184 ± 7 (range 171 – 220) mm, respectively. Their brains were dissected in the field, put on dry ice and stored at -80˚ C. Each brain was placed in 0.5 ml 0.1 N perchloric acid, sonicated and centrifuged for 8 min at 1000×*g*. Supernatants were stored at −80 °C until analyzed by HPLC-EC to determine concentrations of dopamine (DA) and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), serotonin (5-hydroxytryptamine (5-HT)) and its main metabolite, 5-hydroxy-3-indoleacetic acid (HIAA), and norepinephrine (NE). DOPAC:DA ratios and HVA:DA ratios, an index for dopaminergic activity, as well as HIAA:5HT ratios, an index for serotonergic activity, were also calculated for each measurement (Zhou et al. 1999b). For HPLC-EC analysis, samples were loaded onto a Waters 717 plus autosampler (Waters Associates, Milford, MA), and the mobile phase was delivered at a constant rate of 1 ml/min by a Waters model 510 pump through a C18, 5 mm, 250 3 4.6 mm analytical column (Alltech, Deerfield, IL) placed in a column heater (35°C). The LC amperometric potential was set to 0.75 V with reference to an Ag–AgCl reference electrode, and the sensitivity of the detector was maintained at 2 nA. The mobile phase consisted of 0.1 M disodium phosphate, 0.1 M citrate, 0.15 mM EDTA, 1.4 mM octyl sodium sulfate, and 12% methanol. The signal from the detector was recorded, and the data were analyzed by the use of a Waters Millennium 2010 Chromatography Manager.Methods. The pellets were later digested in 1ml of 0.5 N NaOH for measurements of protein concentration using Bio-Rad (Hercules, CA) assay reagents.

Laboratory HR and TK fed Bluefish

Ten HR-fed and ten TK-fed lab fish of similar size previously sacrificed and stored frozen -80˚ C were used for neurotransmitter analysis. The mean fork length of the HR-fed and TK-fed bluefish was 221.20±4.91 mm and 227.8±6.36 mm, respectively. The brain was removed while still frozen and processed immediately utilizing the same procedure as that of the field bluefish.

Statistical analysis

All statistical analyses were performed on Minitab13.1 software program with significance level of $p \leq 0.05$. Means and standard errors are presented in the text and on the graphs.

Field HR and TK bluefish

The mean thyroid follicle area of the ten largest follicles per fish and the greatest thyroid follicle area of HR and TK bluefish were compared using a one-way ANOVA. The mean epithelial cell heights of the ten largest follicles per fish were compared using an ANCOVA with the follicle area as the covariate to account for correlation between follicle and cell size. The average epithelial cell height of the largest thyroid follicle was compared between sites using an ANCOVA with the follicle area as the covariate to correct for correlation between follicle and cell size. A pairwise comparison test was also performed comparing the mean epithelial cell heights of the ten largest follicles per fish

and the average epithelial cell height of the largest thyroid follicle for HR and TK fish using Fisher's Least Significant Difference (LSD).

Neurotransmitter concentrations and metabolite activity ratios between the HR and TK bluefish were tested for significance using a one-way ANOVA for each variable: protein, DA, DOPAC, HVA, and 5-HT, HIAA, NE, DOPAC:DA, and HVA:DA, and HIAA:5HT.

Laboratory HR and TK fed bluefish

The average follicle area and greatest follicle area was compared between HR-fed and TK-fed bluefish using a one-way ANOVA.

Neurotransmitter concentrations and metabolite activity ratios between the HR-fed and TK-fed bluefish were tested for significance using a one-way ANOVA for each variable: protein, DA, DOPAC, HVA, and 5-HT, HIAA, NE, DOPAC, and HVA:DA, and HIAA:5HT.

A Pearson correlation coefficient was calculated comparing the mean of the ten largest thyroid follicle areas of each individual HR-fed bluefish to the concentration of their individual neurotransmitter concentration and the metabolic ratios, DOPAC, HVA, and 5-HT, HIAA, NE, DOPAC, and HVA:DA, and HIAA:5HT.

HR and TK fed versus HR and TK field-caught bluefish

One-way ANOVA tests were performed comparing the mean area of the largest thyroid follicle, the mean area of the ten largest follicles, and each neurotransmitter

concentration among the HR and TK field fish and HR-fed and TK-fed bluefish with pairwise comparisons using Fisher's Least Significant Difference (LSD).

Results

Thyroid histology

HR and TK field-caught bluefish

Thyroid follicles of HR field caught bluefish were noticeably enlarged and irregular, and often the colloid was partially depleted (Figure 3.1).

Epithelial cell height was positively correlated with the area of the follicle when analyzing both the largest follicle per fish and the ten largest follicles per fish. Utilizing follicle area as a covariate, ANCOVA revealed that the epithelial cell height of the largest follicle of each fish was significantly greater in the HR fish than TK fish ($F = 180.90$; $p < 0.0001$) and that the average cell height of the 10 largest follicles per fish was significantly greater in the HR fish than TK fish ($F = 15.20$; $p = 0.001$). There was no difference found between the epithelial cell height of the largest follicles of HR bluefish and the mean of the cell height of the 10 largest follicles of HR bluefish. This was also true for the TK bluefish (Figure 3.2).

The mean area of the largest HR follicle was significantly larger than mean area of the largest TK follicle per bluefish ($F=10.70$; $p=0.004$) (Figure 3.3). The mean area of the ten largest follicles was significantly larger in HR than in TK fish ($F=38.32$; $p<0.0001$) (Figure 3.3).

Laboratory HR- and TK-fed bluefish

The mean area of the largest HR-fed bluefish follicle was significantly larger than mean area of the largest TK-fed bluefish follicle per bluefish (F=5.47; p=0.031) (Figure 3.4). The mean area of the ten largest follicles was significantly larger in HR-fed than in TK-fed fish (F=29.82; p<0.0001) (Figure 3.4).

HR- and TK-fed versus HR and TK field-caught bluefish

The mean of the ten largest thyroid follicle areas of HR-fed bluefish (0.022±0.0016 mm^2) was significantly greater than the HR field caught bluefish (0.0099±0.00090 mm^2) (F=45.50, p<0.0001). The mean of the ten largest thyroid follicle areas of the TK-fed bluefish (0.012±0.0012 mm^2) was significantly greater than the TK field caught bluefish (0.0060±0.00047 mm^2) (F=15.93, p< 0.0001) (Figure 3.5). The mean of the largest thyroid follicle area of HR-fed bluefish (0.0512±0.0074 mm^2) was not significantly greater than the HR field caught bluefish (0.0375±0.0063 mm^2) (F=2.06, p=0.168). The mean of the largest thyroid follicle area of the TK-fed bluefish (0.0297±0.0056 mm^2) was significantly greater than the TK field caught bluefish (0.015±0.0026 mm^2) (F=5.88, p=0.026) (Figure 3.5).

Neurotransmitters

HR and TK field-caught bluefish

The mean concentration of protein (mg/L) was not significantly different between HR and TK brains (p = 0.161). However, the mean concentration of the DA metabolites

DOPAC and HVA was significantly lower in the HR fish than in the TK fish ($p < 0.0001$). The concentrations of DA, 5-HT and its metabolite HIAA were not significantly different ($p = 0.163$, 0.507 and 0.188, respectively) although they were lower in the HR fish (Figure 3.6a and 3.6b). NE concentrations were not significantly different, but there was a trend that HR was greater than TK ($p = 0.091$) (Figure 3.6a). The dopaminergic activity levels (ratios of DOPAC:DA and HVA: DA) of HR fish were significantly lower than that of TK fish for both metabolites DOPAC and HVA ($p = 0.002$ and 0.008, respectively). However, there was no difference in serotoninergic activity, HIAA:5HT ($p = 0.121$) (Figure 3.7).

Laboratory HR- and TK-fed bluefish

The mean concentration of protein (mg L^{-1}) was not significantly different between HR and TK brains ($p = 0.073$). Comparison of the HR-fed and TK-fed neurotransmitters revealed that all except the dopamine metabolite HVA were significantly higher in the HR-fed bluefish (Figure 3.8). The mean HVA concentration in the HR-fed bluefish was higher than that of TK-fed, but not quite significant ($p =0.067$). There were no differences found in metabolic activity (DOPAC:DA, HVA:DA and HIAA:5HT) for either dopamine or serotonin.

HR- and TK-fed versus HR and TK field-caught bluefish

A pairwise comparison using Fisher's (LSD) revealed significant differences among the four groups (Figure 3.9). TK-field fish had significantly higher concentrations of the dopamine metabolites DOPAC and HVA than the other three groups. HR-fed lab fish

contained significantly higher concentrations of serotonin and its metabolite HIAA, DA and NE than the other three groups. TK field had higher concentrations of dopamine, but lower concentrations of NE than TK-fed, HR-fed and HR-field fish (Figure 3.9). In addition, the TK-field fish also displayed significantly higher dopaminergic and serotonergic activity than all the other groups (Figure 3.10). This increased dopaminergic activity explains the significantly greater concentrations of the dopamine metabolites DOPAC and HVA.

Thyroid/neurotransmitter correlations

The analysis using Pearson's correlation coefficient indicates a statistically significant linear relationship between the mean thyroid follicle areas of the individual ten HR-fed bluefish and 5HT (r=0.740, p=0.014). There was also a significantly positive correlation between the follicle area and the serotonin metabolite HIAA (r=0.654, p=0.040) and the dopamine metabolites, HVA and DOPAC (r=0.814, p=0.004 and r=0.842, p=0.002, respectively) (Figure 3.11). A statistically significant relationship was not found between the mean thyroid follicle area and DA, but there was a slight positive correlation (r=0.533, p=0.113) (Figure 3.11). There was no relationship found between the thyroid area and NE or the metabolic ratios, DOPAC:DA, HVA:DA and HIAA:5HT. In addition, there was no correlation found between the thyroid area and the fork length of the individual fish, indicating that size was not a factor in the differences of thyroid area.

Discussion

YOY bluefish residing in a contaminated estuary (HR) possessed enlarged, irregular thyroid follicles that were lined with epithelial cells of increased height compared to those from the relatively clean estuary (TK). The thyroid histology of the laboratory bluefish also revealed enlarged irregular follicles in the HR-fed bluefish compared to those fed TK prey. HR field caught fish had altered DA metabolites and dopaminergic activity, and HR-fed bluefish had significantly altered concentrations of neurotransmitters and their metabolites compared to their TK counterparts. These altered hormonal condition and neurotransmitter levels could underlie the abnormal behavior and ultimate fitness of these animals.

Thyroid status

Thyroid hormones are involved in many important processes in fish including smoltification, metamorphosis, osmoregulation and migration, in addition to influencing overall behavior and growth. Increased levels of the thyroid hormones T4 and T3 have been shown to increase fish growth and food consumption (Matty 1986, Moyle and Cech Jr. 1988), and regulate autumn migration in Atlantic cod, *Gadus morhua* (Comeau et al. 2001). The HR-fed bluefish had significantly reduced feeding, spontaneous swimming activity and growth in the laboratory (Chapter 1), and Hr field-caught fish showed evidence of reduced feeding and size (Chapter 2). YOY bluefish from HR field and bluefish fed HR prey also had elevated PCBs, mercury and DDTs compared to TK field

fish and TK-fed fish (Chapter 1 & 2). These are known endocrine-disrupting chemicals (Clotfelther et al. 2004, Baldigo et al. 2006). It is, therefore, plausible that a xenobiotic-induced thyroid alteration is causing maladaptive behavior in these fish when they are undergoing critical periods of rapid growth and development in the estuary (Juanes and Conover 1994b). Delayed growth and altered behavior and migration as a result of a disrupted thyroid system may have serious implications for their overwinter migration preparation and success.

The field fish and the lab fish analyzed in this study were not the same individuals used for contaminant analysis, and therefore we cannot directly compare the individual results. However, the field-caught bluefish were collected from the same site and during the same month as those used for contaminant analysis. These were also a subset from the collection of HR bluefish that were found to have a low percentage of food in their stomachs. The laboratory bluefish were a subset from the 2004 laboratory experiment. Therefore it is reasonable to compare the contaminant concentrations and behavior with the physiological findings. Histological thyroid alterations have been shown to be sensitive biomarkers of sublethal toxicant disruptions, and in some cases even more sensitive than alterations in behavior, development, reproduction, or thyroid hormone levels (Tietge et al. 2005, Opitz et al. 2006, Carlsson and Norrgren 2007). The presence of hypertrophied thyroid follicles in the HR bluefish and their high body burden of contaminants support the hypothesis that contaminant exposure may be causing thyroid disruption. However, there are other environmental factors that could be the cause of the goiter, such as salinity and diet and these must be taken into consideration.

The HR-field bluefish were residing in an estuarine system with an average salinity level (12ppt) much lower than the TK-field site (28 ppt). While goiters have been reported in hatchery fish fed a diet insufficient in iodine such as *Artemia sp.*, there are few reports of goiters in fish residing in their natural environment (Eales and Brown 1993; Solbakken et al. 2002). Fish residing in systems with low iodine levels, particulary predatory fish, have been found to obtain sufficient iodine from their prey (Eales and Brown 1993, Leatherland, 1981). Parker and Specker (1990) found that salinity and temperature did not affect whole animal thyroid hormone content in juvenile striped bass. The striped bass were held in salinity concentrations of 0-2, 10-12, and 28-30, which is consistent with the different salinity concentrations of HR and TK for the YOY bluefish. Juvenile striped bass reside in both sites (HR and TK) of this study and while they are not a perfect comparison since they spend more of their early life history in freshwater systems the study provides a good example of the effects of salinity on thyroid histology in estuarine fish species. Unfortunately, there are no studies examining the effects of salinity on the YOY bluefish directly; however, results of past studies indicate that the salinity is unlikely the cause of the goiters in this population.

Poor diet or food deprivation has also been correlated with altered thyroid hormones and histology. The HR-field bluefish diet at time of collection consisted primarily of menhaden and mummichog, which would be an adequate source of nutrition. However, the HR-field fish were often collected with empty stomachs when they should have been eating aggressively to prepare for southern migration. This reduced feeding could contribute to altered thyroid histology as seen in other studies in which fish were deprived of food (Leatherland 1982). However, these fish were unlikely to be starving,

and were probably just feeding less, similar to the lab fish. While the length and weight of the HR field fish was less than the TK field fish, the condition indices of the two groups were not different. This suggests that the HR fish were not starved and losing weight (which would have lowered their condition index) but that they had grown less in both length and weight.

In addition, HR-fed and TK-fed laboratory fish were held in the same water conditions with a mean salinity of 23.5 ppt. Therefore, differences in water conditions are not a factor in the case of the enlarged thyroids in the HR-fed bluefish. The HR-fed and TK-fed fish were also fed the same prey species (however, from different sites - HR and TK) for the entire experiment. The HR-fed fish did exhibit significantly reduced appetite during the last month of the exposure period and as a result their average food intake was less than the TK-fed bluefish. However, they were still consuming nearly two mummichogs per bluefish per feeding while the TK-fed bluefish were eating nearly three mummichogs per bluefish per feeding. The HR-fed bluefish were not food deprived; they were consuming less, which is conceivably a response to alterations in physiology. Therefore, when comparing the HR-fed to the TK-fed bluefish, the major factor that differentiates the two groups would be the exposure to the contaminants via the prey from the two sites. While we cannot state that this is the primary difference in the field fish, contaminant exposure is also a probable cause of the goiterous follicles in the HR-field fish.

The presence of a goiter (enlarged follicles) does not necessarily demonstrate alterations in hormone concentrations (Eales and Brown 1993). However, exposure to

PCBs and dioxins has been associated with reduced serum T4 levels and thyroid enlargement (Porterfield 1994, Brown et al. 2004). Schnitzler et al. (2008) found a correlation of increased follicle diameter and epithelial cell height and muscular T4 concentrations with increasing PCB and DDT tissue concentration. The concentrations of the endocrine disrupting PCBs in the HR-field and HR-fed bluefish are well above concentrations found in fish from previous studies that have been reported with both altered thyroid histology and circulating hormone concentrations ((Porterfield 1994, Brown et al. 2004). Therefore, we conclude that the presence of goiters in conjunction with the high body burdens of known endocrine disrupting xenobiotics in the HR fish makes thyroid hormone disruption highly likely.

Thyroid histology – HR- and TK-fed versus HR and TK field-caught bluefish

Laboratory fish had greater follicle size than the field bluefish, probably because they were sacrificed a month later than the field bluefish were collected, and were significantly larger. It is interesting that the mean of the largest follicle area is not significantly different between the HR-fed and HR-field fish. There was a much greater difference between the mean of the largest follicle area and the mean of the ten largest follicle areas of the HR-field fish compared to that in the HR-fed fish (6-fold and 2.3 fold, respectively). The HR-field bluefish already possessed some thyroid follicles that were as enlarged as those of the HR-fed bluefish, which were larger, older fish that had been exposed to contaminants for a month longer and had much higher body burdens of PCBs and Hg. This suggests that the alteration to the thyroid histology is sensitive, and

disruption may occur early on in exposure. Environmental factors such that the HR-field bluefish are experiencing may also contribute.

Analysis of the thyroid histology of the YOY bluefish throughout their estuarine stay would be an important next step to understanding how early into the exposure period these fish begin to experience disruptions and at what point hormonal, neurochemical and behavioral alterations may occur.

Neurotransmitters

Neurotransmitters - HR and TK field-caught bluefish

Dopaminergic activity (DOPAC:DA and HVA:DA) of the HR field bluefish was reduced significantly. The dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were 60% and 73% less, respectively, in the HR-field compared to the TK-field bluefish. These reduced concentrations and dopaminergic activity suggest that there is a reduction in the metabolism of dopamine. The mean concentration of dopamine was 20% lower in the HR-field fish compared to the TK, although the difference was not significant, suggesting that there may be reduction in either the synthesis or release of these chemicals as well. Decreased dopaminergic activity may be a response to a decreased release of dopamine, as a means to retain what dopamine levels are available. NE was 31% elevated in the HR-field bluefish, although this was not statistically significant. Analysis of the whole brain instead of discrete regions may have obscured changes in certain brain regions. More

region-specific analysis may have resulted in significant differences in DA, 5-HT and NE between the HR field and TK field fish. Mummichogs from a polluted site (Piles Creek) had lower concentrations of 5-HT and its metabolite 5-HIAA in their medullas but not in their cerebellums (Smith et al. 1995). Future studies should analyze neurotransmitters in specific regions of the brain, as there is evidence that different regions are more sensitive to certain contaminants (Khan and Thomas 2006, Lyng and Seegal 2008).

Neurotransmitters HR- and TK-fed bluefish

The neurotransmitter concentrations in the HR-fed bluefish were also significantly altered compared to the TK-fed bluefish. However, instead of a decrease in neurotransmitter concentrations as seen in the field fish, HR-fed fish has significantly elevated concentrations of all neurotransmitters and their metabolites except HVA (which was elevated but not quite significantly). There were no differences in metabolic activity. DA and 5-HT in the HR-fed fish appear to be metabolized at the same rate as in the TK-fed bluefish and therefore the increased concentrations of DA and 5-HT are generating increased concentrations of their metabolites, particularly DOPAC and HIAA. The serotonin system appears to be most affected, with a 70% increase in 5-HT and a 78% increase in HIAA in the HR-fed bluefish compared to the TK-fed bluefish. DA, DOPAC and HVA concentrations were 56%, 50% and 35% greater respectively in the HR-fed bluefish. NE was also significantly elevated in the exposed fish (33%), which is similar is to the results of the HR field-caught bluefish.

Exposure to contaminants, including mercury and PCBs, has been shown to damage the nervous system and alter neurotransmitters (Bemis and Seegall 1999, Zhou et al. 1999b, Weis et al. 2001, Grippo and Heath 2003a). However, in general, neurotoxicity of a contaminant and its impact on biogenic monoamines varies greatly depending on species, age, dosage and duration of exposure. Martiniuk et al. (accepted) showed that contaminants, including PCBs, have different modes of action and the potential to modulate brain transcripts required for the synthesis of DA and other catecholamines. The unpredictability of neurotransmitter response to contaminant exposure is further confirmed in the present study. HR field-caught and HR-fed bluefish were exposed to multiple xenobiotics at varying degrees of concentrations and are likely experiencing multiple neurotoxic effects, resulting in different neurochemical alterations.

Neurotransmitters - HR- and TK-fed versus HR and TK field-caught bluefish

When comparing the results of the lab fish to the results of the field fish, discrepancies in the alterations of neurotransmitters were striking. As stated in the thyroid comparison, the laboratory bluefish were sacrificed a month later than the field bluefish and therefore experienced longer contaminant exposure and were larger. The longer exposure period and higher body burdens of the HR-lab fish may be affecting the magnitude and direction of the effects on the neurotransmitters. Neurotransmitters are also notoriously variable in response to contaminants and the differences in results of the field fish versus the lab fish may be due to a multitude of unknown factors.

The TK field bluefish had greater concentrations of the DA metabolites, HVA and DOPAC, and increased dopaminergic and serotonic activity levels than the TK-fed, HR-fed and HR-field. The significant differences found between the TK-field and TK-fed lab fish indicate a possible response to the differences between residing in the laboratory and field. Water quality, diet, stress levels, activity levels and energy requirements may all be different in the field compared to the tank environment. Since the neurotransmitter levels in the reference fish from the field and the lab are conflicting, a more detailed discussion comparing the four groups (HR-fed, HR-field, TK-fed, TK-field) together would not be informative. We do not have a baseline for what is a "normal" concentration of each neurotransmitter for YOY bluefish and therefore comparison of the exposed fish to the reference fish is the only option. Further discussion only compares differences between the laboratory bluefish (HR-fed vs TK fed) and between the field bluefish (HR-field vs TK-field).

The comparisons of HR-fed to TK-fed fish neurotransmitters are in conflict with the comparisons of the field-caught bluefish with the exception of NE, which was elevated in both the HR-fed and the HR field-caught fish. HR field fish had reduced dopamine metabolic turnover, while the HR-fed lab fish did not have altered DA or 5-HT metabolic turnover; instead the metabolites were elevated because the DA and 5-HT were elevated. As stated above, these differences in neurochemical alterations between exposed and reference fish in the field and lab may be due to unknown stresses in the lab.

It is also possible that differences in chemical exposure and accumulation may be the cause of elevated neurotransmitters in the HR-fed fish and decreases in concentrations

and dopaminergic activity in the HR-field fish. The HR-field and HR-fed bluefish had elevated levels of both dioxin-like coplanar and non-coplanar ortho-substituted, PCBs, Hg, DDTs, and other pesticides (Chapter 4). These contaminants may target the fish's endocrine and nervous systems via distinct mechanisms. PCBs produce multiple effects in the brain depending on the degree of chlorination, including binding to the AhR receptor, directly affecting DA release, interfering with Ca^{2+}-dependent intercellular signaling pathways, and altering aromatase activity (Hany et al. 1999, Lyng and Seegal 2008). Coplanar, dioxin-like PCBs are linked to endocrine disruption and may impact the neurological system indirectly via thyroid disruption (Shain et al. 1991, Seegal et al. 1997, Fischer et al. 1998, Schantz and Widholm 2001). Non-coplanar congeners, once believed to be "non-toxic" are becoming more prevalent in the environment due to the biotransformation of the more highly chlorinated congeners, and their mode of action makes them more likely to be direct neurotoxins (Shain et al., 1991, Porterfield, 2000; Simon et al. 2007). Differences in neurotransmitter disruption as seen in the HR-field caught and the HR-fed bluefish may be a consequence of exposure to higher concentrations of certain PCB congeners or other contaminants with different toxicity mechanisms.

Overall, the lab fish had significantly higher concentrations of total PCBs and Hg than the HR-field bluefish (Chapter 1 and 2). The laboratory fish were fed prey from the same location that the Hackensack field bluefish were collected from; however, the diet of the field-caught bluefish consisted of other prey species as well, including grass shrimp, Atlantic silversides, juvenile white perch and juvenile striped bass (Chapter1). The PCB congener patterns varied considerably in the individual fish from the field and

varied considerably from the patterns in the HR-fed fish. The specific PCB congener distributions will be discussed in more detail in Chapter 4. In general, the lab fish have markedly higher concentrations of total PCBs and ortho-substituted congeners compared to the field bluefish, which may result in specific changes such as increases and decreases of certain neurotransmitters. The relationship between types of PCB congeners and their effects are explored in greater detail in Chapter 4 and the final discussion.

While it had been hypothesized that the neurotransmitter concentrations, particularly DA, would be reduced in both the HR field and HR-fed bluefish to explain the reduced activity, increases in neurotransmitters, as displayed in the HR-fed bluefish, have also observed in numerous studies of varying contaminants and developmental stages. Seegal et al. 1997 found that exposure to the ortho-substituted PCB 47 resulted in decreased dopamine while exposure to the coplanar PCB 77 led to increased DA. Morse et al. (1996) found altered serotonin systems including increased levels of HIAA and its metabolic rate in rats exposed gestationally and lactationally to the PCB Aroclor mixture 1254. Exposure to PCB Aroclor mixtures 1242 and 1254 inhibited GABA and DA uptake (Mariussen and Fonnum 2001). Mariunssen and Fonnum (2001) discuss that this inhibition may lead to apparent increased release of neurotransmitters due to the temporary high concentrations of neurotransmitters in the synaptic gap (Mariussen and Fonnum 2001). This could explain the observed increase in DA and 5-HT in the HR-fed bluefish, but it would not explain the increase in their metabolites.

Elevated Hg concentrations may also have impacted the neurotoxic response in the HR-fed bluefish. Crump and Trudeau (2009) summarize neurological effects of

MeHg on fish. MeHg has been linked in numerous studies to a decrease in 5-HT in the brains of fish and other organisms, but 5-HT was increased in larval mummichogs, which may be a sign that the developmental stage plays a factor in the impact (Zhou et al 1999b). On the other hand, DA and NE have been shown to increase in numerous studies from MeHg exposure, which correlates with the findings of the HR-fed bluefish (Tsai et al. 1995, Kirubagaran and Joy 1990, Tsuzuki 1981 and 1982, Zhou et al. 1999, Basu et al. 2005).

In the end, we cannot presume to understand exactly why these differences in neurotransmitter alterations occurred between the lab fish and the field fish - we can only hypothesize. Numerous additional studies controlling exposure time, contaminant mixtures and concentration levels would be required to get a better understanding. However, it is unlikely that even then we would have a definitive picture of the impact on the bluefish physiology in these estuarine systems. The results do illustrate the importance of assessing responses both in the lab and in the field when analyzing toxicant effects on an organism. It is apparent that both the lab-exposed fish and the field-exposed fish displayed altered concentrations of neurotransmitters compared to the reference fish. These alterations, whether they be increased or decreased, may be leading to disruption in behavior, including coordination, activity level and feeding ability.

Mixtures of toxicants such as those found in the Hackensack River may have additive, antagonistic, or even synergistic effect on the neurology and behavior of aquatic organisms. MeHg and PCBs, when combined, reduced dopamine levels in rat brains more than either chemical did alone and caused significant impairments in balance and

coordination, while neither chemical did alone (Bemis and Seegall 1999, Roegge et al. 2004). Fischer et al. (2008) also found that co-exposure to low doses of PCBs and MeHg increase developmental neurotoxic effects in mice. Thus, low concentrations of these xenobiotics that may not cause major behavioral or physiological changes on their own may do so together.

Thyroid and neurochemistry correlation

Altered thyroid status, neurochemistry, and behavior including memory, spontaneous activity, and feeding may be related (Donahue et al. 2004). Neurological impairments from thyroid disorders are similar to those from PCB or dioxin exposure; the neurotoxic mechanism(s) of these compounds may be the result of initial disruption of the thyroid system (Porterfield 2000). Organisms exposed to non-coplanar PCBs and those with hypothyroidism have been shown to have similar lowered levels of dopamine, and treatments with T4 raised the neurochemistry parameters back to normal (Porterfield 2000). Recent studies have begun to address the association between hormones and biogenic monoamines and behavior in fish. Growth hormones, dopaminergic activity and swimming activity were correlated in juvenile trout (Johansson et al. 2005) and Serbert et al. (2008) discussed the importance of thyroid hormones and dopamine in eel locomotion and migration. Piles Creek mummichogs also had altered thyroid hormones and histology in juxtaposition with altered neurotransmitters and behavior (Zhou et al. 1999a, Zhou et al. 2000).

A comparison of the concentrations of neurotransmitters in the HR-fed bluefish with the mean area of the thyroid follicles revealed significant positive linear correlations. Fish with more enlarged thyroid follicles had greater concentrations of 5-HT, HIAA, HVA and DOPAC. Interestingly, DA which was significantly elevated in the HR-fed fish, was not correlated with the follicle area, although there was an upward trend. HVA, on the other hand, was not significantly greater in the HR-fed fish, but did have a strong correlation with thyroid follicle size.

These correlations support the idea that the disruption of the thyroid system may be related to neurochemical alterations in the HR-fed bluefish. The disruptions are most likely the result of the exposure to endocrine disrupting toxicants including PCBs, MeHg, and DDTs. Studies of rats have shown similar correlations of disrupted thyroid systems generating increases in neurotransmitter concentrations, as seen in the HR-fed bluefish. Neonatal hypothyroidism elicited the increase of 5-HT and HIAA in forebrain, midbrain and hindbrain in rats (Savard et al 1983 and 1984). Rats prenatally exposed to the coplanar PCB 77 had reduced T4 and increased DA and DOPAC in the cortex (Harer et al. 2001). In the present study, NE was elevated in both the HR-field and HR-fed fish. However, the elevated NE concentration in the HR-fed bluefish was not found to be correlated with the altered thyroid histology. This suggests that the NE elevations are more likely a direct effect of neurotoxins such as Hg or ortho-substituted PCB congeners.

Unfortunately, the same individual field fish were not used for thyroid and neurochemical analysis, so the results could not be compared for similar correlations. Since most of the neurotransmitters and metabolites in the HR-field fish were reduced

compared to the TK-field fish, it would be interesting to see if there were any correlations with the thyroid sizes and learn if more enlarged thyroid follicles are associated with the production of less DOPAC, HVA, etc.

Conclusion

The aim of this study was to investigate possible mechanisms from contaminant exposure underlying changes in behavior and growth previously observed in YOY bluefish (Candelmo et al. in review). Fish from the contaminated estuary had abnormal thyroid follicles and reduced levels of dopamine metabolites, DOPAC and HVA, as well as reduced dopaminergic activity. Bluefish in the lab fed HR prey for four months also had abnormal, enlarged thyroid follicles but had elevated concentrations of neurotransmitters, including dopamine and its metabolite DOPAC, serotonin and its metabolite HIAA, and norepinephrine. Certain neurotransmitter concentrations were highly correlated with thyroid follicle enlargement, formulating associations between thyroid and neurochemical disruption. The present study, however, does not determine which toxicants are responsible for the neurological and thyroid impairments. Future work should examine the effects, mechanisms and pathways of disruption caused by specific PCB congeners and other contaminants. In addition, further research investigating whether the alterations are permanent would be beneficial. After emigration from the contaminated estuary, the fish will be exposed to lower levels of contaminants and their body burden may be reduced via depuration and growth dilution. If the thyroid and neurological impairments are reversible, then the fish may be able to recover.

However, there is evidence that neurochemical changes may persist even after the contaminant has depurated (Seegal et al. 1997). It is also believed that in winter the fish undergo a period of reduced feeding and utilize stored lipids, in which case the lipophilic PCBs, pesticides and meHg that were deposited in the lipids during the summer may be subsequently redistributed to the brain and other sensitive organs (Boon and Duinker 1985b, Jorgensen et al. 1997, Jorgensen et al. 1999), and result in further disruptions and affect recruitment to the adult population. Overall, the alterations of activity, feeding behavior, and growth from exposure to contaminants and disruptions of physiology may have detrimental effects on migratory competence, overwinter survival, and ultimately recruitment success of these exposed YOY bluefish.

REFERENCES

Adams, B. A., D. G. Cyr, and J. G. Eales. 2000. Thyroid hormone deiodination in tissues of American plaice, Hippoglossoides platessoides: characterization and short-term responses to polychlorinated biphenyls (PCBs) 77 and 126. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 127:367-378.

Aldegunde, M., J. L. Soengas, C. Ruibal, and M. D. Andres. 1999. Effects of chronic exposure to gamma-HCH (Lindane) on brain serotonergic and gabaergic systems, and serum cortisol and thyroxine levels of rainbow trout, Oncorhynchus mykiss. Fish Physiology and Biochemistry 20:325-330.

Baldigo, B. P., R. J. Sloan, S. B. Smith, N. D. Denslow, V. S. Blazer, and T. S. Gross. 2006. Polychlorinated biphenyls, mercury, and potential endocrine disruption in fish from the Hudson River, New York, USA. Aquatic Sciences 68:206-228.

Basu N, Stamler CJ, Loua KM, Chan HM. 2005. An interaspecies comparison of mercury inhibition on muscarinic acetylcholine receptor binding in the cerebral cortex and cerebellum. Toxicology and Applied Pharmacology 205:71–6.

Bemis, J. C. and R. F. Seegall. 1999. Polychlorinated biphenyls and methylmercury act synergistically to reduce rat brain dopamine content in vitro Environmental Health Perspectives 107:879-885.

Boon, J. P. and J. C. Duinker. 1985. Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (Solea solea) in relation to concentrations in water and to lipid metabolism under conditions of starvation. Aquatic Toxicology 7:119-134.

Brouwer, A., Morse, D. C., Lans, M. C., Schuur, A. G., Murk, A. J., Klasson-Wehler, E., Bergman, A., and Visser, T. J. 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. Toxicology Industrial Health 14: 59–84.

Brown, S. B., R. E. Evans, L. Vandenbyllardt, K. W. Finnson, V. P. Palace, A. S. Kane, A. Y. Yarechewski, and D. C. G. Muir. 2004. Altered thyroid status in lake trout (Salvelinus namaycush) exposed to co-planar 3,3', 4,4', 5-pentachorobiphenyl. Aquatic Toxicology 67:75-85.

Carlsson, G. and L. Norrgren. 2007. The impact of goitrogen 6-propylthiouracil (PTU) on West African clawed frog (*Wenopus tropicalis*) exposed during metamorphis. Aquatic Toxicology 82:55-62.

Clotfelther, E. D., A. M. Bell, and K. R. Levering. 2004. The role of animal behaviour in the study of endocrine-disrupting chemicals. Animal Behavior 68:665-676.

Comeau, L. A., S. E. Campana, G. A. Chouinard, and J. M. Hanson. 2001. Timing of Atlantic cod Gadus morhua seasonal migrations in relation to serum levels of gonadal and thyroidal hormones. Marine Ecology-Progress Series 221:245-253.

Crump K. L; Trudeau V. L. 2009. Mercury-induced reproductive impairment in fish. Environmental Toxicology and Chemistry 28:895-907.

Donahue, D. A., E. J. Dougherty, and L. A. Meserve. 2004. Influence of a combination of two tetrachlorobiphenyl congeners (PCB 47; PCB 77) on thyroid status, choline

acetyltransferase (ChAT) activity, and short- and long-term memory in 30-day-old Sprague-Dawley rats. Toxicology 203:99-107.

Eales, J. G. and S. B. Brown. 1993. Measurement and regulations of thyroidal status in teleost fish. Reviews in Fish Biology and Fisheries 3:299-347.

Fingerman, S. W. and L. C. Russel. 1980. Effects of the polychlorinated biphenyl Aroclor 1242 on locomotor activity and on the neurotransmitters dopamine and norepinephrine in the brain of the Gulf killifish, Fundulus grandis. Bulletin of Environmental Contaminants and Toxicology 25:267.

Fischer, L. J., R. F. Seegal, P. E. Ganey, I. N. Pessah, and P. R. S. Kodavanti. 1998. Symposium Overview: Toxicity of Non-Coplanar PCBs. Toxicological Sciences 41:49-61.

Fischer, C., Fredriksson, A. Eriksson, P. 2008. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244:157-165.

Grippo, M. A. and A. G. Heath. 2003. The effect of mercury on the feeding behavior of fathead minnows (Pimphales promelas) Ecotoxicology and Environmental Safety 55:187-198.

Hany, J., H. Lilienthal, A. Sarasin, A. Roth-Härer, A. Fastabend, L. Dunemann, W. Lichtensteiger, and G. Winneke. 1999. Developmental Exposure of Rats to a Reconstituted PCB Mixture or Aroclor 1254: Effects on Organ Weights, Aromatase Activity, Sex Hormone Levels, and Sweet Preference Behavior. Toxicology and Applied Pharmacology 158:231-243.

Howdeshell, K. L. 2002. A model of the development of the brain as a construct of the thyroid system. Environmental Health Perspectives 100:337-348.

Janz, D.M. (2000). Endocrine system. In: The Laboratory Fish, G.K.Ostrander, ed., Chap. 25. Academic Press, San Diego.

Johansson, V., Winberg, S., Björnsson, B.T., 2005. Growth hormone-induced stimulation of swimming and feeding behaviour of rainbow trout is abolished by the D1 dopamine antagonist SCH23390. General and Comparative Endocrinology 141: 58-65.

Jorgensen, E. H., I. C. Burkow, H. Foshaug, B. Killie, and K. Ingebrigtsen. 1997. Influence of lipid status on tissue distribution of the persistent organic pollutant octachlorostyrene in arctic char (*Salvelinus alpinus*). Comparitive Biochemistry and Physiology 118C:311-318.

Jorgensen, E. H., B. E. Bye, and M. Jobling. 1999. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (Salvelinus alpinus). Aquatic Toxicology 44:233-244.

Juanes, F. and D. O. Conover. 1994. Rapid Growth, High Feeding Rates, and Early Piscivory in Young-of-the-Year Bluefish (Pomatomus Saltatrix). Canadian Journal of Fisheries and Aquatic Sciences 51:1752-1761.

Juárez de Ku, L.M., Sharma-Stokkermans, M., Meserve, L.A., 1994. Thyroxine normalizes polychlorinated biphenyl (PCB) dose-related depression of choline acetyltransferase (ChAT) activity in hippocampus and basal forebrain of 15-day-old rats. Toxicology. 94:19-30.

Khan, I. A. and P. Thomas. 1996. Disruption of neuroendocrine function in atlantic croaker exposed to Aroclor 1254. Marine Environmental Research 42:145-149.

Khan, I. A. and P. Thomas. 2006. PCB congener-specific disruption of reproductive neuroendocrine function in Atlantic croaker. Marine Environmental Research 62:S25-S28.

Kirubagaran, R., Joy, K.P., 1989. Toxic effects of mercurials on thyroid function of the catfish, Clarias batrachus (L.). Ecotoxicology and Environmental Safety. 17:265-71.

Konsevick, E. and A. B. Bragin. 2007. Chemical characteristics of sediment collected as part of the fishery resource inventory of the lower Hackensack River within the Hackensack Meadowlands District: A comparative study 2001-2003 & 1987-1988.

Landers, J.P., Bunce, N.J., 1991. The Ah receptor and the mechansims of dioxin toxicity. Biochemical Journal 276: 273-287.

Leatherland, J.F., Sonstegard, R.A., 1981. Thyroid dysfunction in Great Lakes coho salmon, *Oncorhynchus kisutch* (Walbaum): seasonal and interlake differences in serum T3 uptake and serum total and free T4 and T3 levels. Journal of Fish Disease 4:413-423.

Leatherland, J. F. 1994. Reflections on the thyroidology of fishes from molecules to humankind. Guelph Ichthyology Review 2:1-67.

Leatherland, J. F., R. Moccia, and R. Sonstegard. 1978. Ultrastructure of the Thyroid Gland in Goitered Coho Salmon (Oncorhynchus kisutch). Cancer Research 38:149-158.

Leroy, K. D., P. Thomas, and I. A. Khan. 2006. Thyroid hormone status of Atlantic croaker exposed to Aroclor 1254 and selected PCB congeners. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 144:263-271.

Li, D., P. Xie, and X. Zhang. 2008. Changes in plasma thyroid hormones and cortisol levels in crucian carp (Carassius auratus) exposed to the extracted microcystins. Chemosphere 74:13-18.

Little, E. E. and S. E. Finger. 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environmental Toxicology and Chemistry 9:13-19.

Lyng, G. D. and R. F. Seegal. 2008. Polychlorinated biphenyl-induced oxidative stress in organotypic co-cultures: Experimental dopamine depletion prevents reductions in GABA. NeuroToxicology 29:301-308.

Mariussen, E., Fonnum, F., 2006. Neurochemical targets and behavioral effects of organohalogen compounds: an update. Critical Reviews in Toxicology 36:253-289.

Martyniuk, C.J., Sanchez, B., Szabo, N.J., Denslow, N.D., Sepúlveda, M.S., Aquatic Contaminants Alter Genes Involved in Neurotransmitter Synthesis and Gonadotropin Release in Largemouth bass. Aquat. Toxicol. In Press, Accepted Manuscript. 0166-445.

Matty, A. 1986. Nutrition, hormones and growth. Fish Physiology and Biochemistry 2:141-150.

Morse DC, Seegal RF, Borsch KO, Brouwer 1996. A Long-term alterations in regional brain serotonin metabolism following maternal polychlorinated biphenyl exposure in the rat. Neurotoxicology 17:631-638.

Moyle, P. B. and J. J. Cech Jr., editors. 1988. Fishes, an introduction to ichthyology. 2nd edition. Prentice Hall, Engelwood Cliffs.

Opitz, R., Hartmann, S., Blank, T., Braunbeck, T., Lutz, I., Kloas, W., 2006. Evaluation of histological and molecular endpoints for enhanced detection of thyroid system disruption in Xenopus laevis tadpoles. Toxicological Sciences 90:337-348.

Parker, S.J., Specker, J.L. 1990. Salinity and temperature effects on whole-animal thyroid hormone levels in larval and juvenile striped bass, *Morone saxatilis*. Fish Physiology and Biochemistry 8: 507-514.

Patino, R., Wainscott, M.R., Cruz-Li, E.I., Balakrishnan, S., McMurry, C., Blazer, V.S., Anderson, T.A., 2003. Effects of ammonium perchlorate on the reproductive performance and thyroid follicle histology of zebrafish. Environmental Toxicology and Chemistry 22:1115-1121.

Perez, M. H. and W. G. Wallace. 2004. Differences in prey capture in grass shrimp, Palaemonetes pugio, collected along an environmental impact gradient. Archives of Environmental Contamination and Toxicology 46:81-89.

Porterfield, S. P. 1994. Vulnerability of the Developing Brain to Thyroid Abnormalities: Environmental Insults to the Thyroid System. Environmental Health Perspectives 102:125-130.

Porterfield, S. P. 2000. Thyroidal dysfunction and environmental chemicals - Potential impact on brain development. Environmental Health Perspectives 108:433-438.

Power, D. M., L. Llewellyn, M. Faustino, M. A. Nowell, B. T. Bjornsson, I. E. Einarsdottir, A. V. M. Canario, and G. E. Sweeney. 2001. Thyroid hormones in growth and development of fish. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 130:447-459

Roegge, C. S., V. C. Wang, B. E. Powers, A. Y. Klintsova, S. Vilareal, W. T. Greenough, and S. L. Schantz. 2004. Motor impairment in rats exposed to PCBs and methylmercury during early development. Toxicological Sciences 77:315-324.

Roth-Härer, A., H. Lilienthal, M. Bubser, U. Kronthaler, W. R. Mundy, T. R. Ward, W. Schmidt, H. Winterhoff, and G. Winneke. 2001. Neurotransmitter concentrations and binding at dopamine receptors in rats after maternal exposure to 3,4,3',4'-tetrachlorobiphenyl: the role of reduced thyroid hormone concentrations. Environmental Toxicology and Pharmacology 9:103-115.

Rozados, M. V., M. D. Andrés, and M. A. Aldegunde. 1991. Preliminary studies on the acute effect of lindane ([gamma]-HCH) on brain serotoninergic system in the rainbow trout Oncorhynchus mykiss. Aquatic Toxicology 19:33-40.

Sandheinrich, M. B. and G. J. Atchison. 1989. Sublethal copper effects on bluegill, *Lepomos macrochirus*, foraging behavior. Canadian Journal of Fisheries and Aquatic Science 46:1977-1984.

Savard, P., Mérand, Y., Di Paolo, T., Dupont, A. 1983. Effects of thyroid state on serotonin, 5-hydroxyindoleacetic acid and substance P contents in discrete brain nuclei of adult rats. Neuroscience 10: 1399-404.

Savard P, Merand Y, Di Paolo T, Dupont A. 1984. Effect of neonatal hypothyroidism on the serotonin system of the rat brain. Brain Research 30, 99–108.

Schantz, S. L. and J. J. Widholm. 2001. Cognitive effects of endocrine-disrupting chemicals in animals. Environmental Health Perspectives 109:1197-1206.

Schnitzler, J. G., E. Koutrakis, U. Siebert, J. P. Thomé, and K. Das. 2008. Effects of persistent organic pollutants on the thyroid function of the European sea bass (Dicentrarchus labrax) from the Aegean sea, is it an endocrine disruption? Marine Pollution Bulletin 56:1755-1764.

Scott, G. R. and K. A. Sloman. 2004. The effects of environmental pollutants on complex fish behavior: integrating behavioral and physiological indicators of toxicity Aquatic Toxicology 68:369-392.

Seegal, R. F., K. O. Brosch, and R. J. Okoniewski. 1997. Effects ofin Uteroand Lactational Exposure of the Laboratory Rat to 2,4,2',4'- and 3,4,3',4'-Tetrachlorobiphenyl on Dopamine Function. Toxicology and Applied Pharmacology 146:95-103.

Sebert, M. E., F. A. Weltzien, C. Moisan, C. Pasqualini, and S. Dufour. 2008. Dopaminergic systems in the European eel: characterization, brain distribution, and potential role in migration and reproduction. Hydrobiologia 602:27-46.

Shain, W., B. Bush, and R. Seegal. 1991. Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. Toxicology and Applied Pharmacology 111:33-42.

Simon, T., J. K. Britt, and R. C. James. 2007. Development of a neurotoxic equivalence scheme of relative potency for assessing the risk of PCB mixtures. Regulatory Toxicology and Pharmacology 48:148-170.

Sloman, K. A., G. R. Scott, Z. Diao, C. Rouleau, C. M. Wood, and D. G. McDonald. 2003. Cadmium affects the social behaviour of rainbow trout, Oncorhynchus mykiss. Aquatic Toxicology 65:171-185.

Sloman, K. A., D. C. Wilson, and S. Balshine, editors. 2006. Behaviour and physiology of fish. Elsevier, Amsterdam.

Smith, G., A. Khan, J. S. Weis, and P. Weis. 1995. Behavior and brain chemistry correlates in mummichogs (Fundulus heteroclitus) from polluted and unpolluted environments. Marine Environmental Research 39:329-333.

Thiruchelvam, M., E. K. Richfield, R. B. Baggs, A. W. Tank, and D. A. Cory-Slechta. 2000. The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: Implications for Parkinson's disease. Journal of Neuroscience 20:9207-9214.

Tietge, J. E., G. W. Holcombe, K. M. Flynn, P. A. Kosian, J. J. Korte, L. E. Anderson, D. C. Wolf, and S. J. Degitz. 2005. Metamorphic inhibition of Xenopus laevis by sodium perchlorate: Effects on development and thyroid histology. Environmental Toxicology and Chemistry 24:926-933.

Tsai, C., T. Jang, and L. Wang. 1995. Effects of mercury on serotonin concentration in the brain of tilapia, *Oreochromis mossambicus*. Neuroscience Letters 184:208-211.

Webber, H. M. and T. A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (Notemigonus crysoleucas). Environmental Toxicology and Chemistry 22:1556-1561.

Weber, D. N., A. Russo, D. B. Seale, and R. E. Spieler. 1991. Waterborne lead affects feeding neurotransmitter levels of juvenile fathead minnows (Pimephales promelas). Aquatic Toxicology 21:71-80.

Weis, J. S., G. Smith, T. Zhou, C. Bass, and P. Weis. 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. BioScience 51:209-217.

Zala, S. M. and D. J. Penn. 2004. Abnormal behaviours induced by chemical pollution: a review of the evidence and new challenges. Animal Behavior 68:649-664.

Zhou, T., H. John-Alder, and J. S. Weis. 1999a. Thyroidal status of mummichogs (*Fundulus heteroclitus*) from a polluted vs a reference habitat. Environmental Toxicology and Chemistry 18:2817-2823.

Zhou, T., H. John-Alder, J. S. Weis, and P. Weis. 2000. Endocrine disruption: thyroid dysfunction in mummichogs (*Fundulus heteroclitus*) from a polluted habitat. Marine Environmental Research 50:393-397.

Zhou, T., D. Rademacher, R. Steinpreis, and J. S. Weis. 1999b. Neurotransmitter levels in two populations of larval Fundulus heteroclitus after methylmercury exposure. Comparitive Biochemistry and Physiology Part C 124:287-294.

Yamano, Keisuke. 2005. The role of thyroid hormone in fish development with reference to aquaculture. Japan Agricultural Research Quarterly 39:161 – 168.

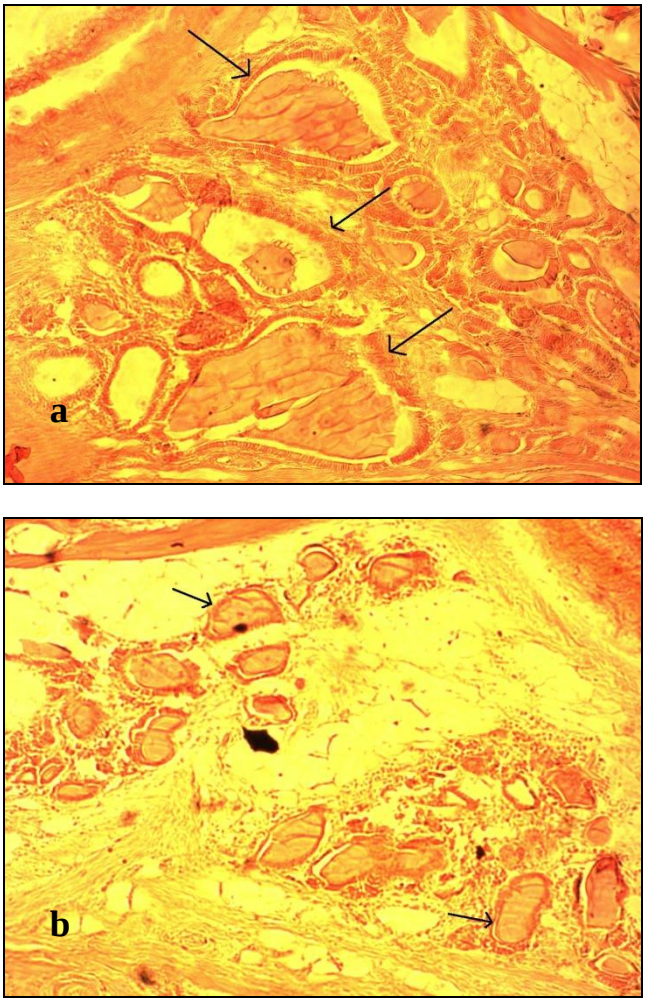

Figure 3.1 Images of thyroid tissue and follicles of bluefish. **a.** HR-field and **b.**TK-field. Picture taken at same magnification of 10x. Each photograph is of the largest follicles of those individual fish. Arrows point to largest follicles on HR and TK bluefish.

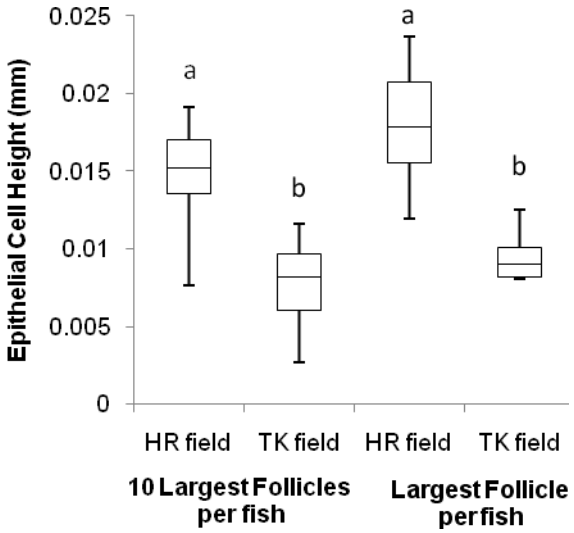

Figure 3.2 Mean epithelial cell height of thyroid follicles of field-caught bluefish. Mean epithelial cell height from the 10 largest thyroid follicles from 10 HR field and TK field bluefish and mean epithelial cell height from the largest follicle for each fish. Four cells were measured and averaged per follicle. The bars with different letters are significantly different (ANOVA, $p < 0.05$; pairwise comparison with Fisher's protected least significant difference).

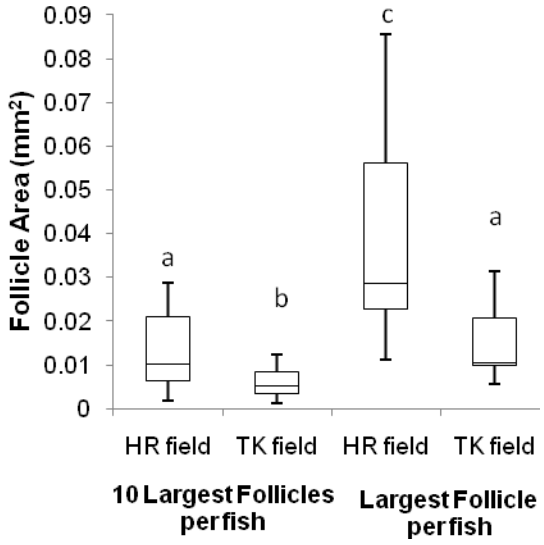

Figure 3.3 Mean cross-sectional thyroid follicle area of field-caught bluefish. Mean cross-sectional area of the ten largest thyroid follicles from 10 HR field and TK field fish and the mean area of the single largest thyroid follicle from 10 HR and TK field bluefish. Bars with different letters are significantly different (ANOVA, $p < 0.05$; pairwise comparison with Fisher's protected least significant difference).

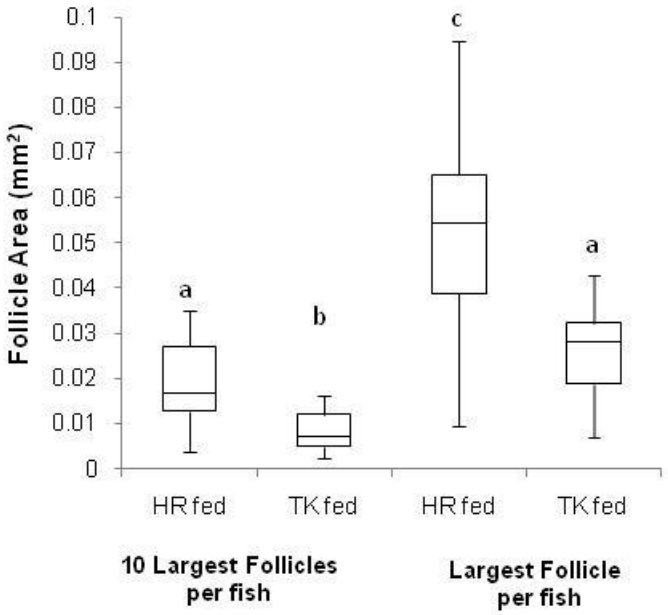

Figure 3.4 Mean cross-sectional thyroid follicles area of HR & TK-fed bluefish. Mean cross-sectional area of the ten largest thyroid follicles from 10 HR-fed and TK-fed fish and the mean area of the single largest thyroid follicle from 10 HR and TK fed bluefish. Bars with different letters are significantly different (ANOVA, $p < 0.05$; pairwise comparison with Fisher's protected least significant difference).

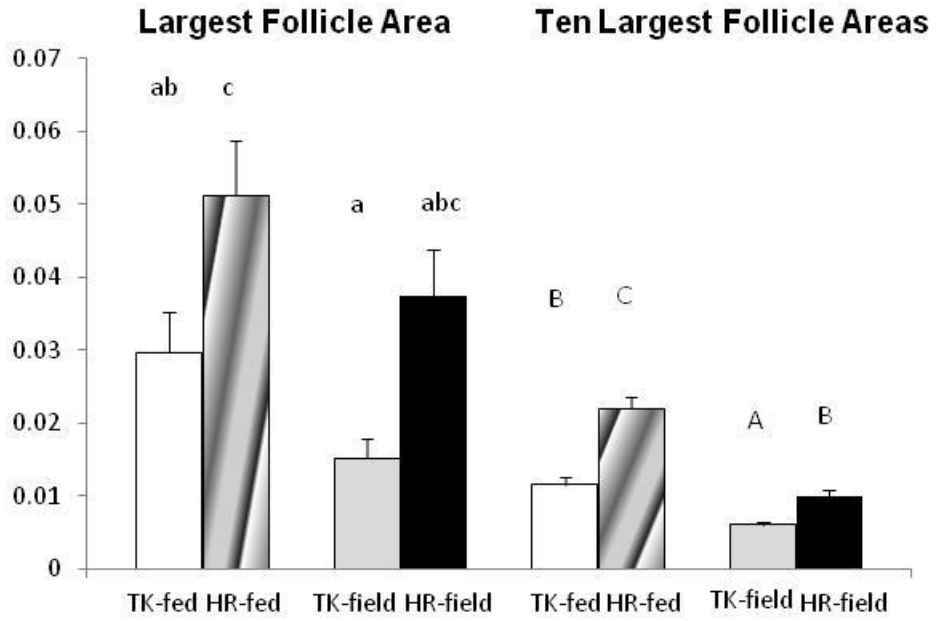

Figure 3.5 Mean cross-sectional thyroid follicle area of field & laboratory bluefish. Mean cross-sectional area of the ten largest thyroid follicles from HR-field and TK-field and HR-fed and TK-fed fish and the mean area of the single largest thyroid follicle from 10 HR and TK bluefish. Bars with different letters are significantly different (ANOVA, $p < 0.05$; pairwise comparison with Fisher's protected least significant difference).

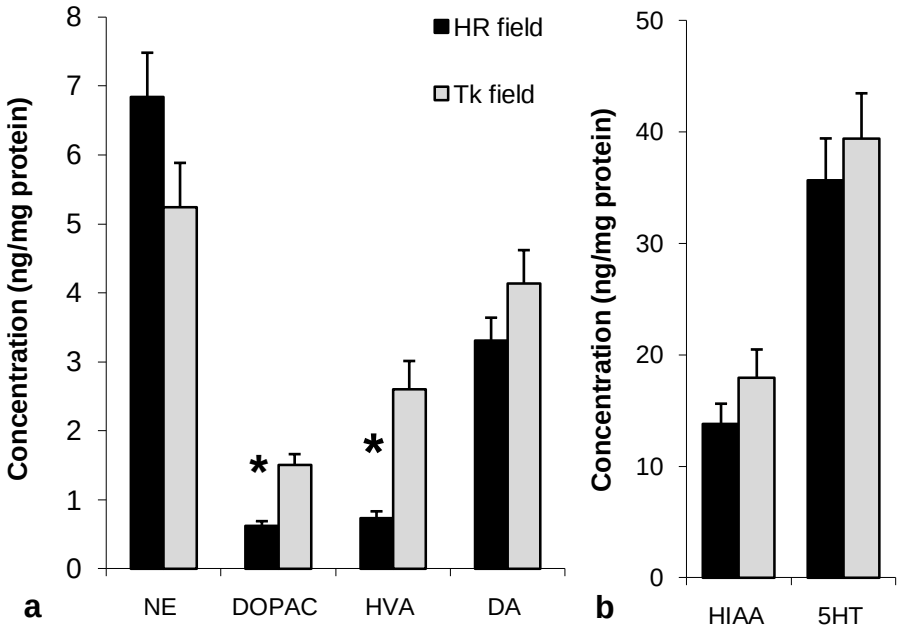

Figure 3.6 Neurotransmitter concentration of field-caught bluefish.

a. HR and TK bluefish concentrations of dopamine (DA), its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and serotonin (5-HT), its main metabolite, 5-hydroxy-3-indoleacetic acid (5-HIAA) and norepinephrine (NE).

b. HR and TK bluefish concentrations of serotonin (5-HT) and its main metabolite, 5-hydroxy-3-indoleacetic acid (5-HIAA). Significance level $p<0.05$. Significant differences between TK & HR are labeled with an asterisk *.

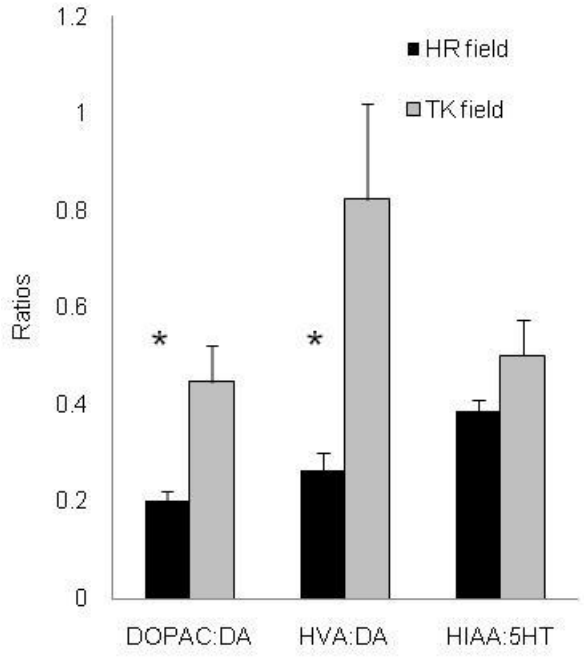

Figure 3.7 Neurotransmitter metobolic activity of field-caught bluefish. Dopaminergic activity levels (DOPAC:DA and HVA:DA) and serotonergic activity levels (HIAA:5HT) of HR and TK bluefish. Significance level $p<0.05$. Significant differences between TK & HR are labeled with an asterisk *.

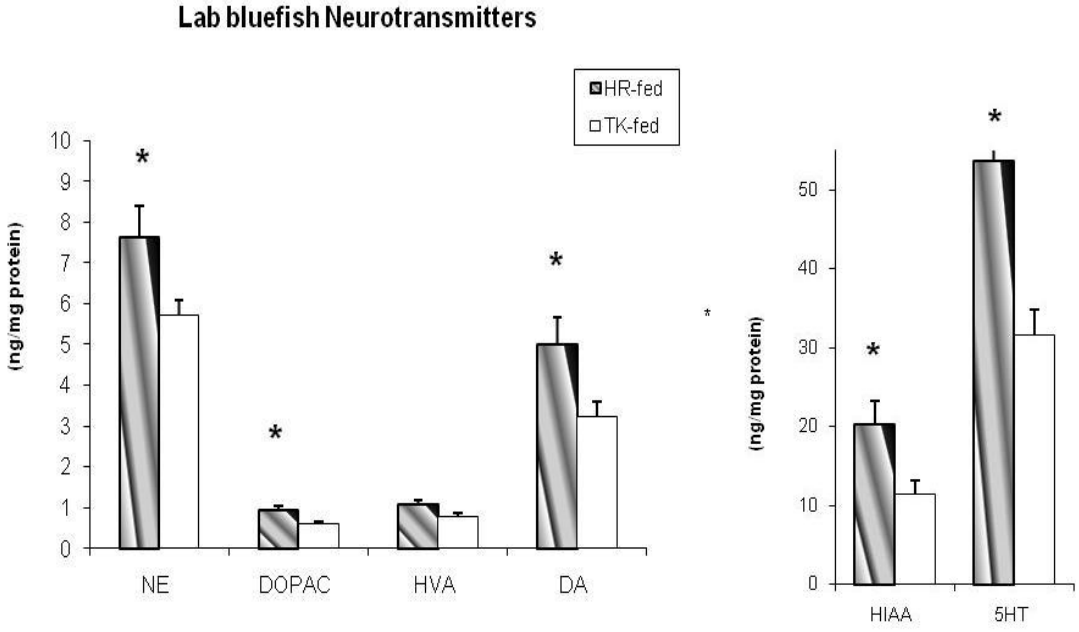

Figure 3.8 Neurotransmitter concentration levels of HR-fed & TK-fed lab bluefish. Abbreviations same as Figure 3.3. Significance level $p<0.05$. Significance difference found between TK & HR is labeled with an asterisk *.

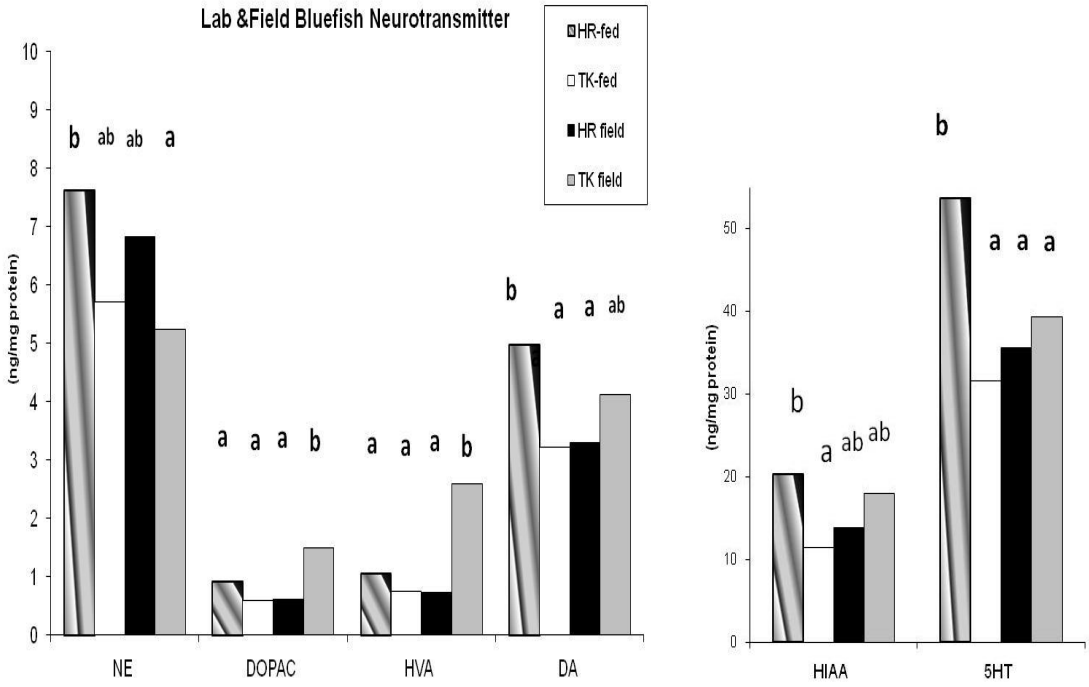

Figure 3.9 Field & lab bluefish neurotransmitter concentration levels. Abbreviations same as Figure 3.3. A pairwise comparison with Fisher's protected least significant difference was performed on each neurotransmitter separately. The bars for each neurotransmitter with different letters are significantly different from one another (ANOVA, $P < 0.0001$)

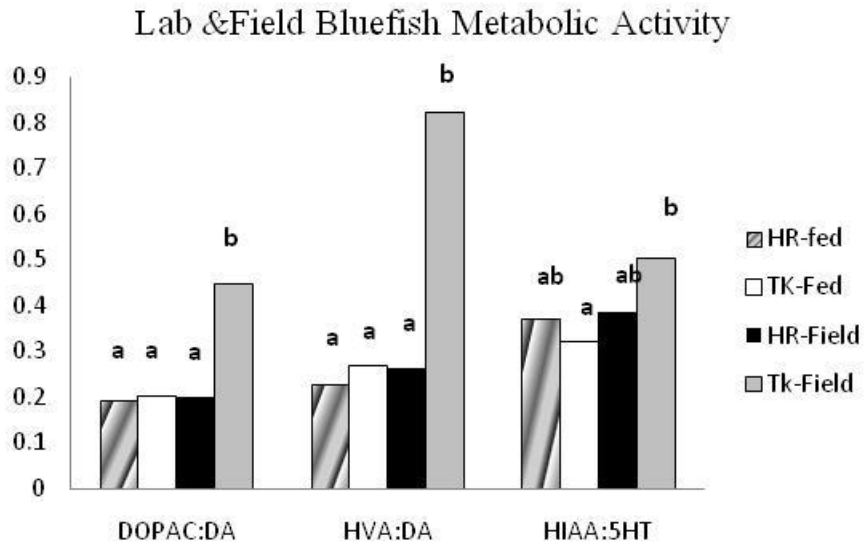

Figure 3.10 Neurotransmitter metobolic activity of field-caught & laboratory bluefish. Dopaminergic activity levels (DOPAC:DA and HVA:DA) and the serotonergic activity levels (HIAA:5HT) of HR and TK field, HR-fed and TK-fed lab bluefish. Significance level $p<0.05$. Significance difference found between TK & HR is labeled with an asterisk *.

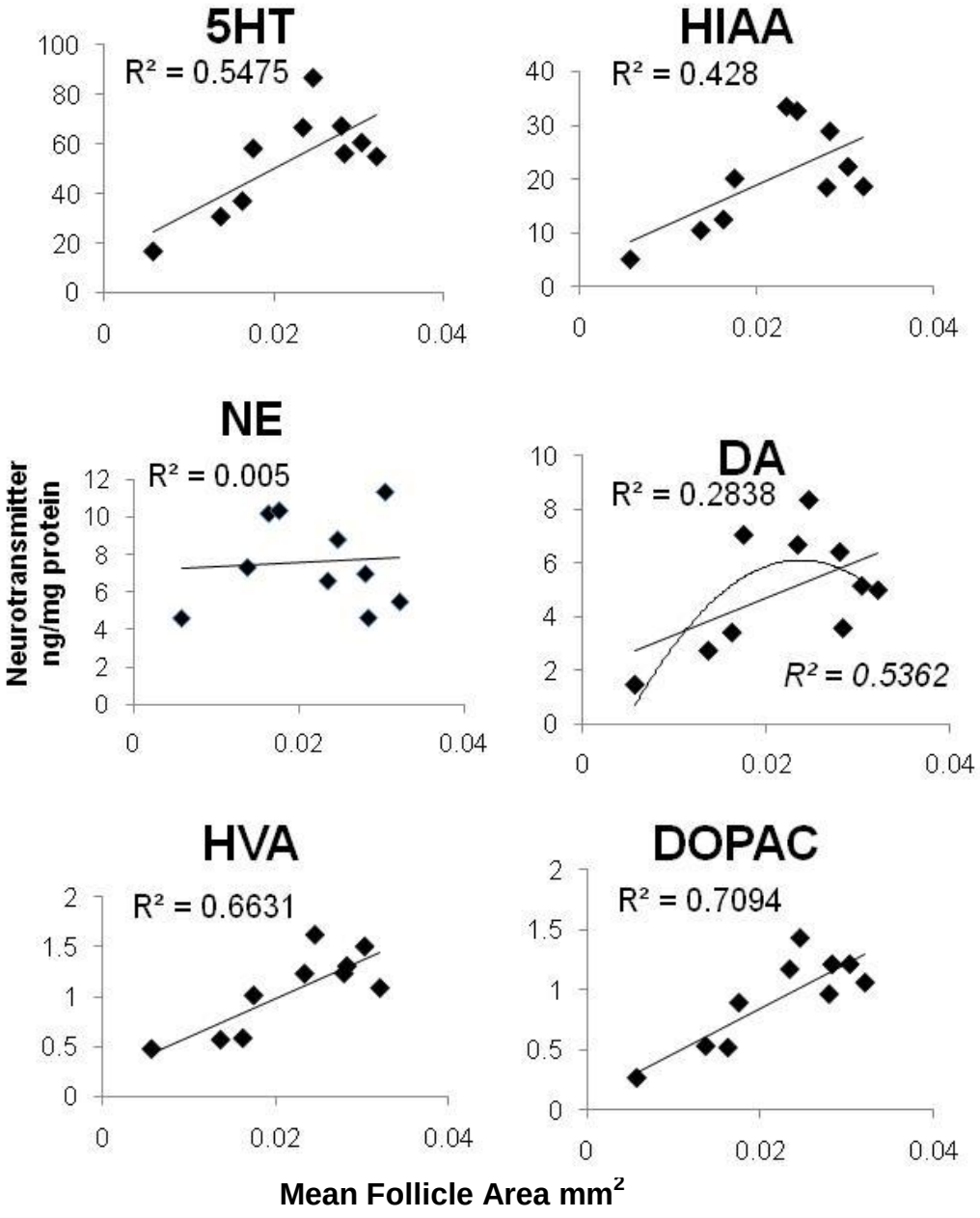

Figure 3.11 Correlation between mean thyroid follicle area & neurotransmitter concentrations of HR-fed & TK-fed bluefish.

CHAPTER 4

Congener specific PCB Accumulation:

A comparison of PCB fingerprints of YOY bluefish, *Pomatomus saltatrix* and their prey

Introduction

Understanding food web dynamics is important in determining the health of an ecosystem. Stomach content analysis is generally utilized to determine the diet of aquatic organisms, but stomachs may be empty or the contents partially or mostly digested requiring the use of scales or other hard parts for identification, which can be difficult or inaccurate. Also, soft tissue does not digest at the same rate as hard tissues, which could lead to inaccurate conclusions of the predator's diet (Bowen at al. 1993). In addition, stomach contents reflect food eaten at only one point in time and may not represent the entire picture. Newer techniques such as stable isotope analysis and fatty acid signatures can provide a more accurate understanding of feeding ecology.

Stable isotopes have been extensively used to provide information of trophic level and feeding location of aquatic organisms (Baeta et al. 2009). While $\delta^{13}C$ values tend to distinguish benthic/inshore food webs (more enriched $\delta^{13}C$ values) from pelagic/offshore food webs (less enriched $\delta^{13}C$ values) (Hobson et al. 1995, Baeta et al. 2009, Dennard et al. 2009), the $\delta^{15}N$ can be used to derive trophic positions in the food web, with enrichment of the isotope increasing linearly with the trophic levels (Post 2002, Baeta et al. 2009). This is an effective technique for studying the feeding habitat and trophic

levels of organisms, but it will generally not provide detailed information about the particular species consumed.

Fatty acids have also been used for more specific diet analysis. Fatty acids are deposited largely unmodified from prey to predator and create a fatty acid signature or fingerprint unique to each species (Iverson et al. 1997b). The fatty acids can be tracked as they move their way through the food web (Iverson et al. 1997b), and diet can be estimated from the fatty acids present and their proportions with respect to other fatty acids (Iverson et al. 2004). Proportions of specific prey species in the predator's diets can be estimated over time periods as long as a few weeks (Iverson et al. 1997a)compared with stomach content analysis which only provides information for the most recent feeding.

These biochemical analytical techniques are effective in diet analysis on their own or in conjunction with stomach content analysis and can also provide information about and organism's movements in certain habitats (Haas et al. 2009). It is possible that techniques utilizing the concentrations of persistent bioaccumulating xenobiotics, such as PCBs, in organisms residing in contaminated habitats could also shed light on their diets.

PCB congeners are accumulated at different concentrations depending on the predator, prey item, and location. PCB concentrations vary much more in estuaries than they do along shelf environments. Analysis of PCB fingerprints may reveal which congeners are preferentially accumulated in the different species and up the food chain (Kennish and Ruppel 1998, Deshpande et al. 2000). YOY bluefish from some contaminated areas contain distinct PCB fingerprints that may be indicative of

differential exposure (Kennish and Ruppel 1998, Deshpande et al. 2000). The variation of PCB concentrations in lipids of bluefish caught offshore is most likely due to the varying condition of the estuary where they spent their first summer. PCB congener patterns or "fingerprints" can provide information about the estuary where they resided. Bluefish switch diets to a piscivorous diet shortly after their entry into the estuaries. As voracious predators rapidly growing up to 1 mm per day, with a high lipid body content, it is likely that the bulk of their PCB body burden is due to trophic transfer (Juanes et al 1994). Therefore it may also be possible to utilize the PCB "fingerprints" that they acquire via feeding exposure to gain a better understanding of their diets.

The HR-fed bluefish and HR-field bluefish have elevated levels of PCBs compared to the TK-fed and TK-field bluefish (Chapter 1 and Chapter 2). Menhaden and mummichog from HR field and from HR bluefish gut also had elevated PCBs that are trophically transferred to the bluefish (Chapter 1 and Chapter 2). It is expected that the HR-fed bluefish will have obtained a PCB fingerprint pattern that reflects a combination of the diet (mummichog and menhaden) that they were fed over the four month feeding exposure experiment. The HR-field bluefish are likely to have fingerprints that reflect diet of a high percentage of menhaden since these were the prey species most frequently found in the stomach content of the field caught bluefish (Chapter 1). Greater variation in the PCB patterns seen in the field-caught fish is expected as their diets may vary each feeding period.

Methods

Study sites

Fish were collected from the contaminated Hackensack River-Newark Bay estuary (HR site) and the reference site in the Mullica River-Great Bay estuary complex near the Rutgers Tuckerton Marine Field Station (TK site). The HR fish samples were collected from study sites of the New Jersey Meadowlands Commission survey (T3 (N 40˚ 45.1'/W 74˚ 05.0'), T1, S2, and S3) (Konsevick and Bragin 2007). All TK fish collections for this study were at the beach or in the lagoons near Graveling Point (N 39˚ 32.4' / W 74˚ 23.2') or from the dock and shore of the Rutgers Tuckerton Marine Field station. Fish from each study site were collected during daylight hours at various tidal periods.

Bluefish samples

PCBs congeners were analyzed in twelve fillets (average weight 40.73 ± 2.69 g) of 2003 HR-field bluefish and ten TK-field fillets (average weight 32.39 ± 2.7 g). Ten whole TK-fed bluefish (average weight 120.65 ± 5.54 g) and ten whole HR-fed bluefish (average wet weight 97.46 ± 5.34 g), five fish from each of the four holding tanks, were analyzed for PCB congeners. Four whole bluefish caught in July from Tuckerton (T-zero), a small sub-sample of the fish used in the laboratory experiment, (average wet weight 16.57 ± 6.92 g) were also analyzed for PCB congeners.

Prey fish samples

Five whole TK and HR mummichogs and menhaden and ten HR silversides (the common prey items determined by stomach content analysis) collected by seine nets at the same sampling location and time as the bluefish from TK and HR, were also analyzed for PCB congeners. Average sample weights of HR mummichog, menhaden, and silversides were 4.63 ± 0.47 g, 4.89 ± 0.35 g, and 3.90 ± 0.34g respectively. Average sample weights of TK mummichog and menhaden were 4.02 ± 0.46 g and 5.05 ± 0.074 g, respectively.

PCB congener analysis was also performed on stomach content samples from HR and TK bluefish of whole prey or those with a significant identifiable portion of the body and organs remaining. Stomach content analyzed from HR bluefish included 10 menhaden and 6 mummichogs, average sample weights 4.45 ± 0.51 g and 3.97 ± 0.79 g, respectively. Stomach content of TK bluefish included seven mummichogs, five menhaden, and five silversides with average sample wet weight of 5.31 ± 0.54 g, 6.30 ± 0.86 g, and 4.53± 0.74 g, respectively.

PCB analysis

PCB and pesticide analysis was performed as per the guidelines of methods of Krahn et al.(1988),USEPA (1993), Sloan et al. (1993) and Deshpande et al. (2000). The samples were analyzed using an Agilent 5890 Gas Chromatograph (GC) with electron

capture detection (ECD) for 31 PCB congeners and 24 pesticide compounds listed in Table A.1 (Deshpande et al. 2000, Deshpande et al. 2002b).

Statistical analysis

Fingerprints

The concentrations of the following PCB congeners were used to create the PCB fingerprints, to compare concentrations of types of congeners and as variables in the principal component analysis; PCB 18, 28, 31, 44, 49, 52, 66, 95, 99, 105, 118, 126, 128, 138, 149, 151, 153, 156, 170, 180, 183, 187, 194, 195, 206, 209.

In order to compare the pattern of PCB congeners, but normalize for the actual concentration values, the percent of total concentration of the 26 congeners listed above was calculated for each congener in each sample. These values were used for statistical comparison and creation of PCB fingerprint charts.

Principal Component Analysis

A principal component analysis was performed utilizing the PCB congener percent concentrations for each individual sample. Various combinations of groups were compared and the PCB factor 1 and factor2 were plotted to determine any group clustering. The first principal component analysis (PCA1) was performed including HR-fed, HR menhaden and mummichog and T-zero to compare the HR-fed bluefish fingerprints to that of the two prey species they were fed during the experiment and the

fingerprint of the bluefish prior to exposure (T-zero). A second principal component analysis (PCA 2) was performed including, HR-field, HR menhaden and mummichog from stomachs, and HR silverside, to compare the fingerprints of the HR-field bluefish to the prey that was found in their stomachs (silverside was also included as it is another common prey item). A third principal component analysis (PCA 3) was performed, including T-zero, TK-fed and TK-field, TK mummichog and menhaden and TK mummichog, menhaden and silverside from stomachs to compare the fingerprints of the bluefish and prey from the reference site. The fourth principal component analysis (PCA 4) was performed including, HR-field, HR-fed, HR menhaden and mummichog, HR menhaden and mummichog from the stomach content, HR silverside, T-zero, TK-fed and TK-field. This final analysis was a comparison of all of the major bluefish groupings and the HR field-caught prey and prey from stomach content to examine similarities and differences among bluefish from different sites, lab versus field, and to compare the bluefish fingerprints to the prey fish and prey fish to one another. For the purpose of brevity for each PCA only the PCB congeners with loadings greater than 0.70 will be presented when discussing variable strength for each Factor score.

MANOVA and ANOVA

A MANOVA was applied using the Factor 1, Factor 2 and Factor 3 scores as variables. The Factor 1 PCA scores explained the majority of the variance and therefore these scores were used primarily to compare the specific groups of fish to one another. An ANOVA with Fisher's pairwise comparison was performed on the Factor 1 and

Factor 2 scores comparing the different groups of fish for each principal component analysis.

Z-scores

The factor 1 scores from the principal component analysis of HR-field, HR-fed, HR menhaden and mummichog, HR menhaden and mummichog stomach content, HR silverside, T-zero, TK-fed and TK-field were used to calculate the z-scores. The average Factor 1 score and standard deviation was calculated for HR field-caught menhaden, mummichog, silverside and the stomach content menhaden and mummichogs. These values were used to calculate z-scores comparing the individual HR-fed bluefish to the prey items that they were fed (menhaden and mummichog) and the individual HR field-caught bluefish to their stomach content (menhaden and mummichog) and the field-caught silverside. The z-score was calculated by subtracting the individual bluefish Factor 1 score from the average prey score and then dividing by the standard deviation of the prey average score. A z-score of ≤ 0.25 was accepted as a very strong relation; a score between 0.25 and 0.75 was viewed as a strong relation, a score between 0.75 and1.25 was accepted as a moderate relation and a score between 1.25 and 1.50 was accepted as a weak relation. A z-score ≥ 1.5 was viewed as no relation.

Results

Fingerprints

HR-fed

In the HR-fed bluefish the hexachlorinated congener PCB 153 was the most prominent peak followed by hexa-chlorinated PCB 138 and pentachlorinated PCB 118. In general, the moderately chlorinated congeners (hepta, hexa-, penta- and tetra-) were dominant (Figure 4.1, 4.2 and 4.3, A2.1, Table 4.1).

HR field-caught

The HR field-caught bluefish were dominated by tetra-chlorinated congeners (Figure 4.2, 4.3, 4.4, and A2.2). The moderately chlorinated penta-, hexa- and to a lesser degree the tri-homologues also appeared in the average top ten congeners concentrations (Figure 4.2 and 4.4, Table 4.1). Overall, PCB 52 was the most dominant except in HR-field fish #3. PCB 118 was also prominent. HR field #3 had a fingerprint similar to the HR-fed fingerprints with its dominant hexa-chlorinated congeners PCB 153 and PCB 138 (Figure 4.1 and 4.4).

HR-fed vs. HR field

The fingerprints of the HR-fed and HR-field bluefish appear substantially different. The top three congeners of these groups are different and only two of the top

five congeners in the HR-fed (PCB 118 and PCB 52) are the same as in the HR field. The overall pattern of the fingerprint is driven by these top five congeners (Figure 4.2, Table 4.1). The top five congeners and a comparison of all the PCB congeners of the HR-fed bluefish were hexa- dominant while that of the HR-field was tetra- dominant (Figure 4.2 and 4.3). Many of the congeners that were dominant in HR-fed (such as PCB 153 and PCB 138) were also present at substantial concentrations (in the top ten) in HR-field, but they were not the most prominent (Table 4.1). Also, the top congeners (PCB 52 and PCB 49) of the HR-field were also prominent in HR-fed. PCBs 118, 66 and 99 were also in the top ten of both HR-fed and HR-field. However, there were a few congeners in HR-fed which were not in the top ten in HR field and vice versa. PCBs 180, 187 and 149 were in the top ten for HR-fed, but not in HR-field. PCBs 44, 28 and 95 were in the top ten for HR-field, but not for HR-fed (Table 4.1). The tetra-homologue group was the highest (35%) in the HR field bluefish and the hexa-homologue group was the highest (31%) in the HR-fed bluefish (Figure 4.3).

HR mummichog

The hexa-chlorinated congener PCB 153 was the most prominent peak, followed by hexa-PCB 138 and penta-PCB 118; in general, the moderately chlorinated congeners (hexa-, penta- and tetra-) were dominant (Figure 4.5b, 4.6, A2.3, Table 4.1)

HR mummichog and HR-fed bluefish

The average mummichog fingerprint is very similar to the HR-fed bluefish fingerprint (Figure 4.1, 4.5 and 4.6). In fact, the top eight congeners of mummichog are identical to that of the HR-fed bluefish, except that PCB 180 has a higher concentration percentage than PCB 52 in the mummichog fingerprint. PCBs 28 and 49 are also prominent and in the top ten of the mummichog fingerprint, but not in the HR-fed. However, these congeners are the 11^{th} and 13^{th} most prominent congeners, in the HR-fed. Likewise, while PCBs 149 and 187 were in the top ten HR-fed congener list they are the 11^{th} and 12^{th} congeners in mummichog. The average percent concentration of homologue groups of the HR-fed bluefish was nearly identical to that of the mummichogs (Figure 4.3 and 4.6).

HR menhaden

In the menhaden samples, tetrachlorinated congener PCB 52 was the most prominent peak followed by tetra-PCB 49; in general, the moderately chlorinated congeners (tri-, tetra-, hexa-, and penta-) were dominant (Figure 4.5 and A2.4, Table 4.1).

HR menhaden vs HR-fed bluefish

The fingerprint of HR menhaden is not similar to the HR-fed bluefish fingerprint. The two top congeners in the HR-fed bluefish, the hexachlorinated PCB 153 and PCB 138, are not in the top ten most prevalent congeners of the HR menhaden. In addition, the more highly chlorinated congeners, heptachlorinated PCBs 180 and 187 are not prevalent in the menhaden, while the lighter tri-chlorinated congeners PCBs 28 and 31

are dominant in the menhaden but not in the bluefish. The same was found for the tetra-PCB 44 and penta-PCB 95 (Figure 4.5, Table 4.1).

HR menhaden in bluefish stomachs

In the menhaden in bluefish stomachs tetrachlorinated congener PCB 52 was the most prominent peak followed by tetra PCB 49. The lighter tetrachlorinated congeners were the most prominent, followed by the penta- and then the hexachlorinated congeners (Figure 4.7 and 4.8, Table 4.1).

HR menhaden in stomach vs HR field menhaden

Eight of the top ten PCB congeners in the HR-field and HR menhaden in bluefish stomachs were similar. Only PCB 31 and PCB 99 were different. PCB 52, 49 and 44 were in the top five of both the HR-field and the HR menhaden (Figure 4.7). The average percent concentration of the HR field bluefish was nearly identical to that of the menhaden from bluefish stomachs (Figure 4.8).

HR mummichog in bluefish stomachs

Congener PCB 153 was the most prominent peak followed by tetra-PCB 52 and penta-PCB 118. In general, the moderately chlorinated congeners (hexa-, penta- and tetra-) were dominant, as in the HR field-caught mummichog (Figure 4.7, Table 4.1).

HR mummichog in stomach vs HR field

There are differences between the HR mummichog in bluefish stomachs and the HR field mummichog fingerprints (Table 4.1). Only two congeners (PCB 118 and PCB 52) of the top five congeners in the stomach content mummichog fingerprint are the same as that in the HR field mummichog. The overall pattern of the fingerprint is driven by these top five congeners. Many of the congeners that were dominant in HR mummichog in stomachs were also present at substantial concentrations (in the top ten) in HR-field. In fact, the groups had all but four congeners (PCB 180, PCB 149, PCB 28, and PCB 44) in common in their top ten.

HR silverside

The average PCB congener fingerprint of the silverside revealed hexachlorinated PCB 153, PCB 149, and PCB 138 and the pentachlorinated PCB 99 as the dominant congeners. This fingerprint was distinct from the menhaden and mummichog fingerprint but more similar to mummichog (Figure 4.7).

TK bluefish and prey

Overall, the fingerprints of the bluefish (T-zero, TK-field) and those fed prey from TK (TK-fed) and the prey (mummichog and mehanden) collected from the TK reference site and during the experiment were very similar to one another (Figure 4.9). The hexa-chlorinated PCB 153 was the dominant congener in each, followed by PCB 138.

Principal Component Analysis and MANOVA

PCA 1 - HR-fed, HR field prey (menhaden and mummichog) and T-zero bluefish

A plot of individual fish sample Factor 1 and Factor 2 scores from the PCA 3 is presented in Figure 4.10. Factor 1 from the PCA of the group HR-fed, HR menhanden, HR mummichog and T-zero explains 78% of the variance, while factor 2 only explains 7%; therefore, only Factor 1 scores will be used to compare the groups to one another. An ANOVA with a Fisher's pairwise comparison of the group revealed that HR-fed and HR mummichog Factor 1 scores were not statistically different (Figure 4.10). The HR-fed cluster together tightly within the cluster of mummichog on the positive to zero side of the Factor 1 axis (Figure 4.10). HR-fed was significantly different from the HR menhaden and the T-zero bluefish. More negative Factor 1 scores as seen in the menhaden (Figure 4.10) were associated with relatively high percent concentration of PCBs 18-52 and 95 and lower levels of the hexa-, hepta-, and octachlorinated PCBs 128, 138, 153, 170-195 (Figure 4.5 and 4.9). The analysis reveals that the laboratory HR-fed bluefish fingerprint changed from the initial pattern (t-zero) and experienced an uptake of congener patterns similar to the HR mummichog that they were fed during the exposure period.

PCA 2 - HR-field, HR prey from bluefish stomach (menhaden and mummichog) and silverside (field)

A plot of individual fish sample Factor 1 and Factor 2 scores from the PCA 3 is presented in Figure 4.11. Factor 1 from the PCA of the group HR-field, HR prey from bluefish stomach (menhaden and mummichog) and silverside explains 50% of the variance, and Factor 2 explains 6%. A MANOVA with Factors 1, 2 and 3 scores as variables revealed significant differences among the groups ($F=9.39$; $p < 0.0001$). An ANOVA with a pairwise comparison the Factor 1 scores of the groups revealed that HR-field bluefish were significantly different from HR mummichog from stomach content and from silverside and but not statistically different from the HR menhaden from stomach content (Figure 4.11). More positive Factor 1 scores are associated with higher percent concentrations of tri- and tetra- PCBs 18-52 and lower levels of hexa- and hepta-PCBs 138, 153, 180 and 187. This analysis reveals that overall the HR-field bluefish PCB fingerprints are more similar to that of HR menhaden and less like mummichog and silverside, suggesting a menhaden-dominated diet. An ANOVA with pairwise comparison of Factor score 2 revealed HR-field bluefish were significantly different than menhaden and mummichog from stomachs, but not silverside (Figure 4.11). More positive Factor 2 scores as seen in menhaden from stomachs (Figure 4.11) are associated with lower percent concentrations of PCBs, 66, 99, 105, and 118 (Figure 4.7).

PCA 3 - TK-field, TK-fed, TK menhaden and TK mummichog, T-zero

A plot of individual fish sample Factor 1 and Factor 2 scores from the PCA 3 is presented in Figure 4.12. Factor 1 explained 21% of the variance, Factor 2 explained

15% of the variance and Factor 3 explained 7.5% of the variance. The MANOVA of Factor 1, Factor 2 and Factor 3 scores revealed significant differences among the groups ($F=5.99$, $p=0.001$). ANOVA of Factor 1 revealed significant differences between the bluefish and prey ($F=7.31$, $p<0.0001$). For Factor 1, TK-field, TK-fed and T-zero bluefish were not significantly different from one another. The TK-field, TK-fed, T-zero were significantly different from TK mummichog from field and stomachs, and menhaden from stomachs, but not from field menhaden or silversides. More Positive Factor 1 scores as seen in the mummichogs and menhaden from stomachs (Figure 4.12) were associated with elevated percent concentrations of the less chlorinated PCBs 28, 31, 44, and 66 and lower levels of PCB 138 (Figure 4.9).

ANOVA of Factor 2 revealed significant differences between the bluefish and prey ($F=12.69$; $p<0.0001$). For Factor 2, TK-field, TK-fed and T-zero bluefish were not significantly different from one another and were significantly different from all of the prey groups. The prey groups were not different from one another with the exception of TK menhaden field which was significantly different than mummichog from field and stomachs. More Positive Factor 2 scores as seen in the prey (Figure 4.12) were associated with lower percent concentrations of the pentachlorinated PCBs 95 and 99 and elevated levels of PCB 187 (Figure 4.9 and 4.12).

PCA 4 - HR-fed, HR-field, TK-fed, TK-field, T-zero bluefish, HR field prey and stomach content (menhaden, mummichog)

The plot of individual fish sample Factor 1 and Factor 2 scores from the PCA 4, Figure 4.13, although complex, shows some general groupings. Factor 1 explains 63% of the variance, Factor 2 explains 6%. A MANOVA of Factor scores 1, 2 and 3 revealed that there were significant differences among the groups ($F=18.56$, $p<0.0001$). ANOVA of Factor 1 showed significant differences among the groups of bluefish and their prey ($F=34.02$, $p<0.0001$). HR-fed was significantly different from all groups except, HR field-caught mummichog and the HR field-caught mummichog and mummichog from stomachs were not statistically different (Figure 4.13b). The HR-fed bluefish form a tight cluster near the origin on the positive side of the Factor 1 axis grouped with the mummichog and mummichog from stomachs (Figure 4.13a and b).

HR-field was significantly different from all groups except HR menhaden from stomachs (Figure 4.13a and c). The HR-field bluefish form a less compact cluster on the negative side of the Factor 1 axis along with the menhaden and menhaden from stomachs nearby (Figure 4.13a and c). HR field-caught menhaden were significantly different from all groups. TK-field, TK-fed and T-zero bluefish and HR silverside were not statistically different (Figure 4.13d). The TK-fed, TK field, T-zero bluefish, and silverside cluster tightly together on the positive Factor 1 axis (Figure 4.13a and d).

For Factor 1, ortho-substituted PCBs 44, 49, 52, 138, 153, and 187 and coplanar PCBs 28 and 31 had loadings greater than 0.90. Ortho-substituted PCBs 95 and 128 had loadings greater than 0.80. Ortho-substituted PCBs 18, and 170 had loadings greater than

0.70. More positive Factor 1 scores as seen in the HR-fed, TK and t-zero bluefish, and silversides (Figure 4.13a) was associated with higher percent concentrations of PCBs 128, 138, 153, 170 and 187 and lower levels of PCBs 18 to 52 and 95 (Figure 4.5, 4.7, and 4.9). More negative Factor 1 scores as seen in HR-Field bluefish and menhaden (Figure 4.13a) were associated with high levels of the PCBs 18 to 52 and 95 and lower levels of PCBs 128, 138, 153, 170 and 187 (Figure 4.5, 4.7, and 4.9). Overall, the analysis revealed that the HR-fed bluefish and HR-field bluefish fingerprints correlated with their most common prey items, field mummichog and menhaden from stomachs, respectively.

ANOVA of Factor 2 also showed significant differences ($F=9.91$, $p<0.0001$). HR-fed and HR-field were significantly different from each prey group and TK-field bluefish, but not from each other or from the TK-fed and T-zero bluefish. None of the prey items were significantly different from one another. For Factor 2, dioxin-like PCB 118 had a loading greater than 0.7. More negative Factor 2 scores as seen in most bluefish, except TK-field (Figure 4.13a) were associated with higher percent concentrations of the dioxin-like pentachlorinated PCB 118 (Figure 4.5, 4.7, and 4.9), while the prey such as menhaden from stomachs (Figure 4.7) had lower percent concentrations of PCB 118 and more positive Factor 2 scores (Figure 4.13a).

Z-scores

HR field compared to HR-fed

There was a much greater variation in the factor 1 scores of the PCB fingerprints of the individual field bluefish compared to that of the individual HR-fed bluefish. In the HR field bluefish the average factor 1 score was -0.82 ± 0.61 while the average factor 1 score of the HR-fed was 0.36 ± 0.15 (mean ± standard deviation).

HR fed compared to prey

Comparison of the individual factor scores of the HR-fed bluefish to the average factor scores of menhaden and mummichog using a z-score showed that the individual bluefish were all similar to the mummichogs (Table 4.2).

Eight out of ten of the individual z-scores comparing the individual HR-fed bluefish to the mummichog average score were very strong to strong, while two out of ten were moderately related. There was no relationship found to the menhaden; the z-score comparing the individual HR-fed bluefish to the menhaden average score ranged from 3.23 to 3.91. These results were also consistent with the results of the ANOVA and the qualitative comparison of the fingerprints.

HR field compared to prey

The z-scores comparing the HR field bluefish to the mummichog and menhaden average factor 1 scores varied considerably among individual fish (Table 4.2). HR field-caught bluefish #6 and 10 had a very strong relation to mehaden. HR field fish #2, 4, 7

and 11 had a strong relation to menhaden in stomachs. HR Field #1, 5 and 9 had a strong relation to menhaden and a moderate relation to mummichog. HR #12 had a moderate relationship to menhaden and a strong relationship to mummichogs. HR-field fish # 8 had only a weak relationship to menhaden. HR field fish #3 had a weak relation to mummichog and a strong relation to silversides, and was the only individual with no relationship to menhaden. This is seen in the pattern of the individual fingerprint, which resembles that of the mummichog, silverside and HR-fed.

Coplanar, dioxin-like and ortho-substituted PCB congeners

The concentration of ortho-substituted congeners in the HR-fed bluefish was more than two-fold that of the HR field bluefish (Figure 4.14). The HR-fed had very elevated concentrations (>150 ng g^{-1}) of the moderately weighted ortho congeners such as PCBs 118 and 153, but also had substantial concentrations (>100 ng g^{-1}) of the lighter and heavier congeners like PCB 52 and PCB 180 (Figure 4.15a). In contrast, the HR-field bluefish had substantial concentrations of the lighter ortho congeners PCBs 52 and 49 (Figure 4.15b). The concentration of dioxin-like congeners in the HR-fed bluefish was more than two-fold that of the HR field bluefish (Figure 4.14). The concentration of coplanar congeners in the HR-fed bluefish was greater than the HR field bluefish (Figure 4.14). The concentrations of the dioxin-like PCB 118 and the coplanar PCB 66 were greater than100 ng g^{-1} in the HR-fed bluefish while they were only approximately 50 ng g^{-1} in the HR field (Figure 4.15).

Discussion

The PCB fingerprint patterns in the HR-field bluefish, HR-fed bluefish and HR prey fish reflect their diets, trophic level and habitat selection. In general, the concentration of PCBs increases with trophic level and this increase is often associated with increasing percentages of more highly chlorinated congeners (Matsuo et al. 2009). This is reflected in the patterns seen in the bluefish and their prey. Organisms at higher trophic levels are dominated by tetra – to hexachlorinated congeners while the lower trophic levels have higher ratios of di- and trichlorinated congeners (Matsuo et al. 2009). The less chlorinated, less hydrophobic congeners are more available in the open water and via uptake through gills and the heavier, higher chlorinated, less soluble, more liphophilic congeners are more likely to adsorb to particles and be suspended in the sediment (Feldman and Titus 2001, Dimou et al. 2006).

The HR menhaden fingerprint pattern is dominated by the lighter tri- congeners such as PCB 52 and PCB 49. This reflects the juvenile menhaden diet that is primarily phytoplankton; however, they are also capable of feeding on zooplankton, benthic diatoms, and organic detritus (Lippson 1995, Lewis 1994, Helm et al 2008). The HR menhaden caught in the field had a pattern significantly different from those collected from the HR bluefish stomachs, which may be a result of different feeding selection by the individual fish. The menhaden in the stomachs were closer to the mummichog in the principal component analysis which suggests some benthic feeding. The menhaden in the stomachs also had greater total PCB concentrations than those in the field (Chapter 1). The elevated concentrations may due to a preference for grazing on benthic diatoms

and detritus which resulted in the accumulation of different congeners and behavioral alterations that made them more susceptible to predation (Chapter 1).

The mummichog fingerprint varied significantly from the menhaden. The heavier, more highly chlorinated congeners were more dominant, which reflects their diet and habitat. Mummichogs are benthic feeding omnivores and their diet includes detritus, small crustaceans, diatoms, algae, and insects (larvae and adults). They feed along the mudflats and on the marsh surface at high tide and may also be exposed via contact and ingestion of contaminated sediments. Their PCB fingerprint pattern, including the tetra-, penta- and hexachlorinated congeners, correlates with their benthic lifestyle (Helm et al. 2008).

Silversides are generally pelagic plankton feeders whose diet can include copepods, mysids, small decapods, amphipods, cladocerans, fish eggs, young squid, annelids, and molluscan larvae, as well as insects, algae and diatoms mixed with sand and mud (Fry et al. 2008). The silverside Factor 1 scores were different than both the mummichog and the menhaden; however, the fingerprint of the HR silversides is dominated by penta- and hexachlorinated congeners, similar to the HR mummichog. This suggests that the silversides and mummichogs in HR are feeding on similar prey items, which is inconsistent with other studies. Stable isotope and stomach content comparison of mummichog and silverside in an estuary north of Boston, MA, revealed consistent differences in their diets (Fry et al. 2008). Mummichogs as benthic feeders consumed an abundance of detritus, amphipods and isopods; silversides consumed more planktonic copepods, shrimp and insects, but also consumed items from the benthic food web (Fry et al. 2008). While their overall diets generally differ, they do overlap and it is

possible that the HR silversides consume a higher percentage of items from the benthic food web. Mummichogs and silversides were caught together in high abundance with a seine net along the tidal marsh flats of the Hackensack River, so it is likely that they are both utilizing this area for food and shelter. Juvenile menhaden, on the other hand, were more commonly collected with the bottom trawl in the open water, which is related to the less chlorinated congeners. Overall the menhaden fingerprint seems to vary the most compared to the mummichogs, silverside and the bluefish. PCB fingerprint patterns may not be established as strongly in the less chlorinated, less bioaccumulative congeners and therefore fluctuations in diets and feeding locations could cause regular fluctuations in their PCB fingerprints.

The HR-fed bluefish were fed both mummichog and menhaden during the laboratory feeding experiment. However, instead of having a fingerprint that is a compilation of the two prey fingerprints, the fingerprint is similar to the HR mummichog and unlike the HR menhaden. While the HR-fed bluefish were fed equal amounts of menhaden and mummichogs for the first three months, only mummichogs were used for the qualitative feeding experiments conducted during the last month of exposure. The congener pattern of these mummichogs obscured any menhaden fingerprint that may have been present before the last month. The lower chlorinated congeners from the menhaden may have been eliminated from the tissue. Since more highly chlorinated congeners accumulate at faster rates, the penta- and hexa- pattern from the mummichog may have always been more dominant in the bluefish (Gobas et al. 1999, Matsuo et al. 2009). The fingerprints of all of the HR-fed bluefish were changed from what they had been at the start of the experiment (T-zero) and were also nearly identical to one another.

The lab results provide evidence that if individual bluefish are feeding on similar prey items in the field then their PCB fingerprints will be similar to one another and to the prey that they have been consuming at least for the last month. Further laboratory experiments involving different combinations of prey and weekly PCB analyses would be important to better understanding the strength and elasticity of the fingerprints. A menhaden fingerprint may be eliminated much faster from a bluefish or another predator than a mummichog fingerprint.

Stomach content analysis of HR field-caught bluefish in 2003 and 2007 revealed that menhaden is their dominant prey item (approximately 70% weight) at the end of the summer (Chapter 1 and Chapter 2). This is also reflected in their average PCB fingerprints which is likewise dominated by the tetra chlorinate congeners. Some individual fish such as HR field #2, 4, 6, 10, and 11 appear to be consistently feeding on menhaden while others such as HR field #12 may have a more varied diet.

The comparison of the HR-fed bluefish to the HR-field bluefish fingerprints illustrates the differences in their diets. This comparison may be compromised because the HR field bluefish were analyzed as fillets while the HR-fed were whole fish. However, bluefish muscle is high in lipids compared to other species (Deshpande et al. 2002) and analysis of total lipid percentage of HR bluefish fillets and livers of HR bluefish revealed a higher percentage of lipids in the fillets (unpublished). Therefore, the difference between fillet and whole-fish concentrations may be negligible, as seen in other studies. Mummichog from Newark Bay and Hudson River had different PCB congener distribution patterns between sites, but had similar within site patterns in their

muscle, liver and gonads (Monosson et al. 2003). In addition, the fillets represent a significant amount of the wet weight of the bluefish and the pattern of congener concentrations accumulated in the fillet would obscure any slight differences in congener concentrations from the other organs.

It is generally accepted that the intake efficiency or assimilation of hydrophobic and organic chemicals increases with increasing log Kow or chlorination and then this assimilation rate will decrease for the most highly chlorinated (hepta- and octa-) congeners (Gobas et al. 1999, USEPA 1999c, Leblanc et al. 2006). Less chlorinated PCBs do not bioconcentrate as efficiently and are more readily metabolized and excreted (Giesy and Kannan 2002, Magnusson et al. 2006). This could be a partial explanation for the greater concentrations of total PCBs in the HR-fed bluefish compared to that in the HR field-caught bluefish (Chapter 1 and Chapter 2). The higher concentrations of moderately chlorinated penta-, hexa-, and hepta- congeners in the mummichogs are likely to bioaccumulate more than the tri- and tetra- congeners from the menhaden. Feeding on mummichogs may have led to increased bioaccumulation of total PCBs in the HR-fed bluefish compared to the HR field bluefish, which fed more frequently on menhaden. Prey selection of menhaden over mummichog or even silversides could result in lower body burdens in bluefish. This contradicts the expectation that a menhaden diet would result in greater exposure and accumulation because they are high in lipids and therefore more likely to have higher body burden. Instead, menhaden may be the less detrimental prey if the congeners in their tissue are less chlorinated, toxic and bioaccumulative and more likely to be eliminated.

The higher percentage of lower chlorinated congeners in the HR-field bluefish may also be attributed to uptake via gills from the contaminated water. The HR-fed bluefish were held in clean water and were only exposed via trophic transfer. Nevertheless, HR-fed fish still accumulated high concentrations (greater or equal to those in the HR-field) of lower congeners as well through their diet of menhaden and mummichog, therefore, accumulation via trophic transfer is likely dominating the fingerprint.

PCB congener fingerprints for diet analysis of the TK bluefish and their prey are distinct. Overall the patterns in each group of fish, bluefish and prey, are much more similar to one another than in the HR fish. The differences that are seen in the principal component analysis provide some correlations with their specific feeding history or habitat selection, but are inconsistent. The Factor 2 scores do appear to be a representation of trophic levels; greater more positive scores (menhaden and silverside) representing lower tropic levels and lower more negative scores (TK-fed bluefish) representing higher trophic levels.

For the Factor 1 scores the TK-field bluefish congener patterns are similar to the silverside and menhaden which is consistent with the results of the gut content analysis of the TK bluefish in which both species were approximately 36% of their diet by weight (Chapter 1). The TK-fed, on the other hand, are also similar to silverside and menhaden and not to mummichog, despite the fact that they were fed only mummichog for the last month and never any silverside. Mummichog are showing an affinity for higher percent concentrations of the less chlorinated congeners than the other prey or bluefish which is

inconsistent with the results of the HR fish in which menhaden and bluefish possessed the higher percent concentrations of these congeners. The mean total PCB concentrations of the TK mummichog from the field and stomachs is greater than that of the menhaden (Chapter 1) which is likely do to the mummichogs accumulation of contaminants that are present in the sediment. The mummichogs are likely being exposed to higher concentrations of all of the congeners than the menhaden and are therefore able to accumulated low levels of even the light congeners. The concentrations of PCBs in the water and plankton in TK may be so low that the menhaden are not exposed to the less chlorinated, less bioaccumulative congeners at high enough concentrations to contribute to their body burden. The results do support the principle that the moderately chlorinated congeners will have a greater assimilation rate, as these are the congeners at greatest concentrations in the bluefish and the prey. Overall, the concentrations of many of the congeners in the TK fish were very low, particularly in the prey, making it difficult to distinguish differences among species. The concentrations in the prey may be too low to create representative fingerprints, particularly the lighter less chlorinated congeners. It appears that the use of PCB fingerprints for specific diet identification is not an accurate application at sites of low contamination levels such as TK; however, it could be suitable to establish trophic levels.

Conclusion

PCB fingerprint patterns were analyzed to gain a better understanding of the foraging ecology of bluefish residing in the Hackensack River. PCB fingerprints can

provide a means to identify which estuary fish migrated out of after they have joined the adult population and, possibly, how much these fish from contaminated estuaries are contributing to the stock. This study shows that they can also provide insight into which prey species are contributing to the overall body burden of the predator. Congener specific PCB studies are important to understand the consequences of exposure. Toxicity of PCB congeners depends on the position and the number of chlorines. Prey availability and selection can influence the trophic exposure to certain congeners and the magnitude of sublethal effects in the bluefish population. The HR-fed bluefish had significantly greater body burdens of both the endocrine disrupting dioxin-like congeners and the more highly chlorinated neurotoxic ortho-substituted congeners than the HR field fish. This suggests that a diet dominated by mummichog may be more detrimental to the fitness of the bluefish compared to a diet of menhaden. If a shift in diet were to occur in the field due to a decline in the menhaden population, then the sublethal effects on YOY bluefish in HR might be more deleterious than what has already been observed.

REFERENCES

Able, K. W. and M. P. Fahay. 1998. The first year in the life of estuarine fishes in the Middle Atlantic Bight. Rutgers University Press, New Brunswick, NJ

Adams, B. A., D. G. Cyr, and J. G. Eales. 2000. Thyroid hormone deiodination in tissues of American plaice, Hippoglossoides platessoides: characterization and short-term responses to polychlorinated biphenyls (PCBs) 77 and 126. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 127:367-378.

Aldegunde, M., J. L. Soengas, C. Ruibal, and M. D. Andres. 1999. Effects of chronic exposure to gamma-HCH (Lindane) on brain serotonergic and gabaergic systems, and serum cortisol and thyroxine levels of rainbow trout, Oncorhynchus mykiss. Fish Physiology and Biochemistry 20:325-330.

Armstrong, T. N., T. J. Iannuzzi, J. B. Thelen, D. F. Ludwig, and C. E. Firstenberg. 2005. Characterization of Chemical Contamination in Shallow-Water Estuarine Habitats of an Industrialized River. Part II. Metals. Pages 35-52 Soil & Sediment Contamination. Taylor & Francis Ltd.

ASMFC. 2003. Population Assessment and the Short-term Stock Projections of the Bluefish Population. A Report to the Atlantic States Marine Fisheries Commission and the Mid-Atlantic Fishery Management Council Monitoring Committee. Atlantic States Marine Fisheries Commission.

Baeta, A., I. Valiela, F. Rossi, R. Pinto, P. Richard, N. Niquil, and J. C. Marques. 2009. Eutrophication and trophic structure in response to the presence of the eelgrass Zostera noltii. Marine Biology 156:2107-2120.

Baldigo, B. P., R. J. Sloan, S. B. Smith, N. D. Denslow, V. S. Blazer, and T. S. Gross. 2006. Polychlorinated biphenyls, mercury, and potential endocrine disruption in fish from the Hudson River, New York, USA. Aquatic Sciences 68:206-228.

Bemis, J. C. and R. F. Seegall. 1999. Polychlorinated biphenyls and methylmercury act synergistically to reduce rat brain dopamine content in vitro Environmental Health Perspectives 107:879-885.

Boon, J. P. and J. C. Duinker. 1985a. Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (*Solea solea*) in relation to concentrations in water and to lipid metabolism under conditions of starvation. Aquatic Toxicology 7:119-134.

Boon, J. P. and J. C. Duinker. 1985b. Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (Solea solea) in relation to concentrations in water and to lipid metabolism under conditions of starvation. . Aquatic Toxicology 7:119-134.

Bragin, B., J. Misuik, C. A. Woolcott, K. R. Barrett, and R. Jusino-Atresino. 2005. A fishery resource inventory of the lower Hackensack River within the Hackensack meadowlands district: a comparative study 2001-2003 vs 1987-1988. New Jersey Meadowlands Commission Meadowlands Environmental Research Institute.

Brown, S. B., R. E. Evans, L. Vandenbyllardt, K. W. Finnson, V. P. Palace, A. S. Kane, A. Y. Yarechewski, and D. C. G. Muir. 2004. Altered thyroid status in lake trout

(Salvelinus namaycush) exposed to co-planar 3,3', 4,4', 5-pentachorobiphenyl. Aquatic Toxicology 67:75-85.

Buckel, J. A. and D. O. Conover. 1996. Gastric evacuation rates of piscivorous young-of-the-year bluefish. Transactions of the American Fisheries Society 125:591-599.

Buckel, J. A. and D. O. Conover. 1997. Movements, feeding periods, and daily ration of piscivorous young-of-the-year bluefish, Pomatomus saltatrix, in the Hudson River estuary. Fishery Bulletin 95:665-679.

Buckel, J. A., D. O. Conover, N. D. Steinberg, and K. A. McKown. 1999a. Impact of age-0 bluefish (Pomatomus saltatrix) predation on age-0 fishes in the Hudson River estuary: evidence for density-dependent loss of juvenile striped bass (Morone saxatilis). Canadian Journal of Fisheries and Aquatic Sciences 56:275-287.

Buckel, J. A., M. J. Fogarty, and D. O. Conover. 1999b. Foraging habits of bluefish, Pomatomus saltatrix, on the US east coast continental shelf. Fishery Bulletin 97:758-775.

Buckel, J. A., B. H. Letcher, and D. O. Conover. 1998. Effects of a delayed onset of piscivory on the size of age-0 bluefish. Transactions of the American Fisheries Society 127:576-587.

Buckel, J. A. and K. A. McKown. 2002. Competition between juvenile striped bass and bluefish: resource partitioning and growth rate. Marine Ecology-Progress Series 234:191-204.

Candelmo, A., C. , A. Deshpande, B. Dockum, J. Samson, P. Weis, and J. S. Weis. in review. Trophic transfer and effects on young-of-the-year bluefish, *Pomatomus saltatrix*, consuming contaminated prey.

Carlsson, G. and L. Norrgren. 2007. The impact of goitrogen 6-propylthiouracil (PTU) on West African clawed frog (*Wenopus tropicalis*) exposed during metamorphis. Aquatic Toxicology 82:55-62.

Clotfelther, E. D., A. M. Bell, and K. R. Levering. 2004. The role of animal behaviour in the study of endocrine-disrupting chemicals. Animal Behavior 68:665-676.

Comeau, L. A., S. E. Campana, G. A. Chouinard, and J. M. Hanson. 2001. Timing of Atlantic cod Gadus morhua seasonal migrations in relation to serum levels of gonadal and thyroidal hormones. Marine Ecology-Progress Series 221:245-253.

Crisp, T. M., E. D. Clegg, R. L. Cooper, W. P. Wood, D. G. Anderson, K. P. Baetcke, J. L. Hoffmann, M. S. Morrow, D. J. Rodier, J. E. Schaeffer, L. W. Touart, M. G. Zeeman, and Y. M. Patel. 1998. Environmental Endocrine Disruption: An Effects Assessment and Analysis. Environmental Health Perspectives 106:11-56.

Dennard, S. T., B. C. McMeans, and A. T. Fisk. 2009. Preliminary assessment of Greenland halibut diet in Cumberland Sound using stable isotopes. Polar Biology 32:941-945.

Deshpande, A. D., A. F. J. Draxler, V. S. Zdanowicz, M. E. Schrock, and A. J. Paulson. 2002a. Contaminant levels in the muscle of four species of fish important to the recreational fishery of the New York Bight Apex. Marine Pollution Bulletin 44:164-171.

Deshpande, A. D., A. F. J. Draxler, V. S. Zdanowicz, M. E. Schrock, A. J. Paulson, T. W. Finneran, B. L. Sharack, K. Corbo, L. Arlen, E. A. Leimburg, B. W. Dockum, R.

A. Pikanowski, B. May, and L. B. Rosman. 2000. Contaminant levels in muscle of four species of recreational fish from the New York Bight Apex. NOAA Technical Memorandum NMFS-NE-157, NOAA, Woods Hole, MA.

Deshpande, A. D., R. J. Huggett, and R. A. Halbrook. 2002b. Polycyclic aromatic hydrocarbon metabolites in the bile of a territorial benthic fish, oyster toadfish (*Opsanus tau*) from the Elizabeth River, Virginia. Archives of Environmental Contamination and Toxicology 42:43-52.

Dimou, K. N., T. L. Su, R. I. Hires, and R. Miskewitz. 2006. Distribution of polychlorinated biphenyls in the Newark Bay Estuary. Journal of Hazardous Materials 136:103-110.

Donahue, D. A., E. J. Dougherty, and L. A. Meserve. 2004. Influence of a combination of two tetrachlorobiphenyl congeners (PCB 47; PCB 77) on thyroid status, choline acetyltransferase (ChAT) activity, and short- and long-term memory in 30-day-old Sprague-Dawley rats. Toxicology 203:99-107.

Dorea, J. G. 2008. Persistent, bioaccumulative, and toxic substances in fish: Human health considerations. Science of the Total Environment 200:93-114.

Eales, J. G. and S. B. Brown. 1993. Measurement and regulations of thyroidal status in teleost fish. Reviews in Fish Biology and Fisheries 3:299-347.

Eriksson, P. and A. Fredriksson. 1996. Developmental neurotoxicity of four ortho-substituded PCBs in neonatal mouse. Environmental Toxicology and Pharmacology 1:155-165.

Feldman, R. S. and J. E. Titus. 2001. Polychlorinated biphenyl accumulation differs among pumpkinseed sunfish during experimental field exposure:the role of invertebrate prey. Aquatic Toxicology 51:389-404.

Fingerman, S. W. and L. C. Russel. 1980. Effects of the polychlorinated biphenyl Aroclor 1242 on locomotor activity and on the neurotransmitters dopamine and norepinephrine in the brain of the Gulf killifish, Fundulus grandis. Bulletin of Environmental Contaminants and Toxicology 25:267.

Fischer, C., A. Fredriksson, and P. Eriksson. 2008. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244:157-165.

Fischer, L. J., R. F. Seegal, P. E. Ganey, I. N. Pessah, and P. R. S. Kodavanti. 1998. Symposium Overview: Toxicity of Non-Coplanar PCBs. Toxicological Sciences 41:49-61.

Gartland, J., R. J. Latour, A. D. Halvorson, and H. M. Austin. 2006. Diet composition of young-of-the-year bluefish in the lower Chesapeake Bay and the coastal ocean of Virginia. Transactions of the American Fisheries Society 135:371-378.

Giesy, J. P. and K. Kannan. 2002. Dioxin-like and non-dioxinlike effects of polychlorinated biphenyls: Implications for risk assessment. Lake Reservoir Research Management 7:139-181.

Gobas, F. A., J. B. Wilcockson, R. W. Russell, and G. D. Haffner. 1999. Mechanisms of biomagnification in fish under laboratory and field conditions. Environmental Science and Technology 33:133-141.

Grippo, M. A. and A. G. Heath. 2003a. The effect of mercury on the feeding behavior of fathead minnows (Pimphales promelas) Ecotoxocology and Environmental Safety 55:187-198.

Grippo, M. A. and A. G. Heath. 2003b. The effect of mercury on the feeding behavior of fathead minnows (Pimphales promelas) Ecotoxicology and Environmental Safety 55:187-198.

Haas, H. L., C. J. Freeman, J. M. Logan, L. Deegan, and E. F. Gaines. 2009. Examining mummichog growth and movement: Are some individuals making intra-season migrations to optimize growth? Journal of Experimental Marine Biology and Ecology 369:8-16.

Hany, J., H. Lilienthal, A. Sarasin, A. Roth-Härer, A. Fastabend, L. Dunemann, W. Lichtensteiger, and G. Winneke. 1999. Developmental Exposure of Rats to a Reconstituted PCB Mixture or Aroclor 1254: Effects on Organ Weights, Aromatase Activity, Sex Hormone Levels, and Sweet Preference Behavior. Toxicology and Applied Pharmacology 158:231-243.

Harvey, J., L. Harwell, and J. K. Summers. 2008. Contaminant concentrations in whole-body fish and shellfish from US estuaries. Environmental Monitoring and Assessment 137:403-412.

Hauge, P., J. Bukowski, P. Morton, M. Boriek, J. McClain, and G. Casey. 1990. Polychlorinated biphenyls (PCBs), chlordane, and DDTs in selected fish and shellfish from New Jersey waters, 1986–1987:Results from New Jerseys Toxics in Biota Monitoring Program.
.*in* T. NJ Dept of Environ Protection, editor. Available from http://www.state.nj.us/dep/dsr/pcb86-87.pdf.

Hinck, J. E., C. J. Schmitt, K. A. Chojnacki, and D. E. Tillitt. 2009. Environmental contaminants in freshwater fish and their risk to piscivorous wildlife based on a national monitoring program. Environmental Monitoring and Assessment 152:469-494.

Hobson, K. A., W. G. Ambrose, and P. E. Renaud. 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: Insights from delta C-13 and delta N-15 analysis. Marine Ecology-Progress Series 128:1-10.

Howdeshell, K. L. 2002. A model of the development of the brain as a construct of the thyroid system. Environmental Health Perspectives 100:337-348.

Iannuzzi, T. J., T. N. Armstrong, J. B. Thelen, D. F. Ludwig, and C. E. Firstenberg. 2005. Characterization of chemical contamination in shallow-water estuarine habitats of an industrialized river. Part 1: Organic compounds. Soil & Sediment Contamination 14:13-33.

Iverson, S. J., J. P. Y. Arnould, and I. L. Boyd. 1997a. Milk fatty acid signatures indicate both major and minor shifts in the diet of lactating Antarctic fur seals. Canadian Journal of Zoology-Revue Canadienne De Zoologie 75:188-197.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecological Monographs 74:211-235.

Iverson, S. J., K. J. Frost, and L. F. Lowry. 1997b. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology-Progress Series 151:255-271.

Johansson, V., S. Winberg, and B. T. Björnsson. 2005. Growth hormone-induced stimulation of swimming and feeding behaviour of rainbow trout is abolished by the D1 dopamine antagonist SCH23390. General and Comparative Endocrinology 141:58-65.

Jorgensen, E. H., I. C. Burkow, H. Foshaug, B. Killie, and K. Ingebrigtsen. 1997. Influence of lipid status on tissue distribution of the persistent organic pollutant octachlorostyrene in arctic char (*Salvelinus alpinus*). Comparitive Biochemistry and Physiology 118C:311-318.

Jorgensen, E. H., B. E. Bye, and M. Jobling. 1999. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (Salvelinus alpinus). Aquatic Toxicology 44:233-244.

Jørgensen, E. H., B. E. Bye, and M. Jobling. 1999. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (*Salvelinus alpinus*). Aquatic Toxicology 44:233-244.

Juanes, F. and D. O. Conover. 1994a. Piscivory and Prey Size Selection in Young-of-the-Year Bluefish - Predator Preference or Size-Dependent Capture Success. Marine Ecology-Progress Series 114:59-69.

Juanes, F. and D. O. Conover. 1994b. Rapid Growth, High Feeding Rates, and Early Piscivory in Young-of-the-Year Bluefish (Pomatomus Saltatrix). Canadian Journal of Fisheries and Aquatic Sciences 51:1752-1761.

Kennish, M. J. and B. E. Ruppel. 1998. Organochlorine contamination in selected estuarine and coastal marine finfish and shellfish of New Jersey. Water Air Soil Pollution 101:123-136.

Khan, I. A. and P. Thomas. 1996. Disruption of neuroendocrine function in atlantic croaker exposed to Aroclor 1254. Marine Environmental Research 42:145-149.

Khan, I. A. and P. Thomas. 2006. PCB congener-specific disruption of reproductive neuroendocrine function in Atlantic croaker. Marine Environmental Research 62:S25-S28.

Kodavanti, P. R. S., D. S. Shin, H. A. Tilson, and G. J. Harry. 1993. Comparative Effects of Two Polychlorinated Biphenyl Congeners on Calcium Homeostasis in Rat Cerebellar Granule Cells. Toxicology and Applied Pharmacology 123:97-106.

Kodavanti, P. R. S. and T. R. Ward. 2005. Differential effects of commercial polybrominated diphenyl ether and polychlorinated biphenyl mixtures on intracellular signaling in rat brain in vitro. Toxicological Sciences 85:952-962.

Konsevick, E. and A. B. Bragin. 2007. Chemical characteristics of sediment collected as part of the fishery resource inventory of the lower Hackensack River within the Hackensack Meadowlands District: A comparative study 2001-2003 & 1987-1988.

Kraus, M. L. and D. B. Kraus. 1986. Differences in effects of mercury on predator avoidance in two populations of grass shrimp, Palaemonetes pugio. Marine Environmental Research 18:277-289.

Leatherland, J. F. 1994. Reflections on the thyroidology of fishes from molecules to humankind. Guelph Ichthyology Review 2:1-67.

Leatherland, J. F., R. Moccia, and R. Sonstegard. 1978. Ultrastructure of the Thyroid Gland in Goitered Coho Salmon (Oncorhynchus kisutch). Cancer Res 38:149-158.

Leblanc, L. A., J. A. Buckel, D. O. Conover, and B. J. Brownawell. 2006. Tests of bioaccumulation models for polychlorinated biphenyl compounds: A study of young-of-the-year bluefish in the Hudson River estuary, USA. Environmental Toxicology and Chemistry 25:2067-2076.

Leroy, K. D., P. Thomas, and I. A. Khan. 2006. Thyroid hormone status of Atlantic croaker exposed to Aroclor 1254 and selected PCB congeners. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 144:263-271.

Lewis, M. 2002. 2002 review of the Atlantic States Marine Fisheries Commission fishery management plan for bluefish.*in* W. Atlantic States Marine Fisheries Commission, DC, editor.

Li, D., P. Xie, and X. Zhang. 2008. Changes in plasma thyroid hormones and cortisol levels in crucian carp (Carassius auratus) exposed to the extracted microcystins. Chemosphere 74:13-18.

Li, S. X., L. F. Zhou, H. J. Wang, Y. G. Liang, J. B. Chang, M. H. Xiong, Y. C. Zhang, and J. X. Hu. 2009. Feeding habits and habitats preferences affecting mercury bioaccumulation in 37 subtropical fish species from Wujiang River, China (vol 18, pg 204, 2008). Ecotoxicology 18:384-384.

Little, E. E. and S. E. Finger. 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environmental Toxicology and Chemistry 9:13-19.

Long, E. R., D. D. MacDonald, S. L. Smith, and F. D. Calder. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management 19:81-97.

Lyng, G. D. and R. F. Seegal. 2008. Polychlorinated biphenyl-induced oxidative stress in organotypic co-cultures: Experimental dopamine depletion prevents reductions in GABA. NeuroToxicology 29:301-308.

Magnusson, K., R. Ekelund, R. Grabie, and P. A. Bergqvist. 2006. Bioaccumulation of PCB congeners in marine benthic infauna Marine Environmental Research 61:379-395.

Mariussen, E. and F. Fonnum. 2006. Neurochemical Targets and Behavioral Effects of Organohalogen Compounds: An Update. Critical Reviews in Toxicology 36:253-289.

Matsuo, H., M. Kawano, K. Omori, K. Nakajima, and I. Takeuchi. 2009. Stable isotope-guided analysis of congener-specific PCB concentrations in a Japanese coastal food web. Marine Pollution Bulletin 58:1615-1623.

Matty, A. 1986. Nutrition, hormones and growth. Fish Physiology and Biochemistry 2:141-150.

McBride, R. S. and D. O. Conover. 1991. Recruitment of young-of-the-year bluefish, *Pomatomas saltatrix* to the New York Bight: variation in abundance and growth of spring-and summer-spawned cohorts. Marine Ecology Progress Series 78:205-216.

Mesa, M. G., T. P. Gasomski, and J. H. Peterson. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. Journal of Fish Biology 45 Supplement A:81-96.

Monosson, E., J. T. F. Ashley, A. E. McElroy, D. Woltering, and A. A. Elskus. 2003. PCB congener distributions in muscle, liver and gonad of *Fundulus heteroclitus* from the lower Hudson River Estuary and Newark Bay Chemosphere 52:777-787.

Moyle, P. B. and J. J. Cech Jr., editors. 1988. Fishes, an introduction to ichthyology. 2nd edition. Prentice Hall, Engelwood Cliffs.

O'Connor, J. M., J. B. Klotz, and T. J. Kneip. 1982. Sources, sinks and distribution of organic contaminations in the New York Bight ecosystem. Estuarine Research Federation, Columbia, SC.

O'Neill, H. J. and K. M. Kieley. 1992. Polynuclear aromatic hydrocarbons: Atlantic region data summary and review. Technical report 92-01, Conservation and Protection Atlantic Region, Moneton, New Brunswick.

Opitz, R., T. Braunbeck, C. Bogi, D. B. Pickford, G. Nentwig, J. Oehlmann, O. Tooi, I. Lutz, and W. Kloas. 2005. Description and initial evaluation of a Xenopus metamorphosis assay for detection of thyroid system-disrupting activities of environmental compounds. 24:653.

Opitz, R., Hartmann, S., Blank, T., Braunbeck, T., Lutz, I., Kloas, W., 2006. Evaluation of histological and molecular endpoints for enhanced detection of thyroid system disruption in Xenopus laevis tadpoles. Toxicological Sciences 90:337-348.

Osius, N., W. Karmaus, H. Kruse, and J. Witten. 1999. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. Environmental Health Perspectives 107:843-849.

Patino, R., M. R. Wainscott, E. I. Cruz-Li, S. Balakrishnan, C. McMurry, V. S. Blazer, and T. A. Anderson. 2003. Effects of ammonium perchlorate on the reproductive performance and thyroid follicle histology of zebrafish. Page 1115.

Perez, M. H. and W. G. Wallace. 2004. Differences in prey capture in grass shrimp, Palaemonetes pugio, collected along an environmental impact gradient. Archives of Environmental Contamination and Toxicology 46:81-89.

Porterfield, S. P. 1994. Vulnerability of the Developing Brain to Thyroid Abnormalities: Environmental Insults to the Thyroid System. Environmental Health Perspectives 102:125-130.

Porterfield, S. P. 2000. Thyroidal dysfunction and environmental chemicals - Potential impact on brain development. Environmental Health Perspectives 108:433-438.

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

Power, D. M., L. Llewellyn, M. Faustino, M. A. Nowell, B. T. Bjornsson, I. E. Einarsdottir, A. V. Canario, and G. E. Sweeney. 2001. Thyroid hormones in growth and development of fish. Page 447.

Roegge, C. S., V. C. Wang, B. E. Powers, A. Y. Klintsova, S. Vilareal, W. T. Greenough, and S. L. Schantz. 2004. Motor impairment in rats exposed to PCBs and methylmercury during early development. Toxicological Sciences 77:315-324.

Roth-Härer, A., H. Lilienthal, M. Bubser, U. Kronthaler, W. R. Mundy, T. R. Ward, W. Schmidt, H. Winterhoff, and G. Winneke. 2001. Neurotransmitter concentrations and binding at dopamine receptors in rats after maternal exposure to 3,4,3',4'-tetrachlorobiphenyl: the role of reduced thyroid hormone concentrations. Environmental Toxicology and Pharmacology 9:103-115.

Rozados, M. V., M. D. Andrés, and M. A. Aldegunde. 1991. Preliminary studies on the acute effect of lindane ([gamma]-HCH) on brain serotoninergic system in the rainbow trout Oncorhynchus mykiss. Aquatic Toxicology 19:33-40.

Sandheinrich, M. B. and G. J. Atchison. 1989. Sublethal copper effects on bluegill, *Lepomos macrochirus*, foraging behavior. Canadian Journal of Fisheries and Aquatic Science 46:1977-1984.

Schantz, S. L. and J. J. Widholm. 2001. Cognitive effects of endocrine-disrupting chemicals in animals. Environmental Health Perspectives 109:1197-1206.

Scharf, F. S., J. A. Buckel, F. Juanes, and D. O. Conover. 1998. Predation by juvenile piscivorous bluefish (Pomatomus saltatrix): the influence of prey to predator size ratio and prey type on predator capture success and prey profitability. Canadian Journal of Fisheries and Aquatic Sciences 55:1695-1703.

Schmidt, K., C. E. W. Steinburg, S. Pflugmacher, and G. Staaks. 2004. Xenobiotic substances such as PCB mixtures (Arochlor 1254) and TBT can influence swimming behavior and biotransformation activity (GST) of carp (Cyprinus carpio). Environmental Toxicology 19:460-470.

Scott, G. R. and K. A. Sloman. 2004. The effects of environmental pollutants on complex fish behavior: integrating behavioral and physiological indicators of toxicity Aquatic Toxicology 68:369-392.

Seegal, R. F., K. O. Brosch, and R. J. Okoniewski. 1997. Effects ofin Uteroand Lactational Exposure of the Laboratory Rat to 2,4,2',4'- and 3,4,3',4'-Tetrachlorobiphenyl on Dopamine Function. Toxicology and Applied Pharmacology 146:95-103.

Shain, W., B. Bush, and R. Seegal. 1991. Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. Toxicology and Applied Pharmacology 111:33-42.

Shepherd, G. R. and D. B. Packer. 2006. Essential fish habitat source document: Bluefish, Pomatomas saltatrix, life history and habitat characteristics. 2nd edition. NOAA Tech. Mem. NMFS-NE-144.

Sloman, K. A., G. R. Scott, Z. Diao, C. Rouleau, C. M. Wood, and D. G. McDonald. 2003. Cadmium affects the social behaviour of rainbow trout, Oncorhynchus mykiss. Aquatic Toxicology 65:171-185.

Sloman, K. A., D. C. Wilson, and S. Balshine, editors. 2006. Behaviour and physiology of fish. Elsevier, Amsterdam.

Smith, G., A. Khan, J. S. Weis, and P. Weis. 1995. Behavior and brain chemistry correlates in mummichogs (Fundulus heteroclitus) from polluted and unpolluted environments. Marine Environmental Research 39:329-333.

Smith, G. M. and J. S. Weis. 1997. Predator/prey interactions in Fundulus heteroclitus: effects of living in a polluted environment. Journal of Experimental Marine Biology and Ecology 209:75-87.

Smith, S. J., S. J. Iverson, and W. D. Bowen. 1997. Fatty acid signatures and classification trees: new tools for investigating the foraging ecology of seals. Canadian Journal of Fisheries and Aquatic Sciences 54:1377-1386.

Stewart, P., J. Reihman, E. Londy, T. Darvill, and J. Pagano. 2000. Prenatal PCB exposure and postnatal behavioral assessment scale (NBAS) performance. Neurotoxicology and Teratology 22:21-29.

Tietge, J. E., G. W. Holcombe, K. M. Flynn, P. A. Kosian, J. J. Korte, L. E. Anderson, D. C. Wolf, and S. J. Degitz. 2005. Metamorphic inhibition of Xenopus laevis by sodium perchlorate: Effects on development and thyroid histology. Environmental Toxicology and Chemistry 24:926-933.

Tsai, C., T. Jang, and L. Wang. 1995. Effects of mercury on serotonin concentration in the brain of tilapia, *Oreochromis mossambicus*. Neuroscience Letters 184:208-211.

USEPA. 1999a. Guidance for assessing chemical contaminant data for use in fish advisories. Vol. 2. Risk assessment and fish consumption limits, 3rd ed. EPA 823-B-99-008. US EPA, Office of Water, Washington, DC.

USEPA. 1999b. Mercury update: impact on fish advisories. EPA-823-F-99-019. US EPA, Office of Water, Washington, DC.

USEPA. 1999c. Polychlorinated biphenyls (PCBs) update: Impact on fish advisories. EPA-823-F-99-019. US EPA, Office of Water, Washington, DC.

USEPA. 2000. Guidance for assessing chemical contamination data for use in fish advisories. Vol. 2. Risk assessment and fish consumption limits, 3rd ed. EPA 823-B-00-008. Office of Water, Washington, DC.

USEPA. 2001. Mercury update: Impact on fish advisories. EPA-823-F-01-011. US EPA, Office of Water, Washington, DC.

USFDA. 1993. National Shellfish Sanitation Program. Guide for the control of molluscan shellfish. US Food and Drug Administration,Washington, DC

Varanasi, U. and J. E. Stein. 1991. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environmental Health Perspectives 90:93-100.

Webber, H. M. and T. A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (Notemigonus crysoleucas). Environmental Toxicology and Chemistry 22:1556-1561.

Weber, D. N., A. Russo, D. B. Seale, and R. E. Spieler. 1991. Waterborne lead affects feeding neurotransmitter levels of juvenile fathead minnows (Pimephales promelas). Aquatic Toxicology 21:71-80.

Weinand-Härer, A., H. Lilienthal, K.-A. Bucholski, and G. Winneke. 1997. Behavioral effects of maternal exposure to an ortho-chlorinated or a coplanar PCB congener in rats. Environmental Toxicology and Pharmacology 3:97-103.

Weis, J. S. 2002. Tolerance to environmental contaminants in the mummichog, *Fundulus heteroclitus*. Human and Ecological Risk Assessment 8:933-953.

Weis, J. S., G. Smith, T. Zhou, and C. Bass. 2000. Predator/prey interactions: a link between the individual level and both higher and lower level effects of toxicants in the aquatic ecosystems. Journal of Aquatic Ecosystem Stress and Recovery 7:145-153.

Weis, J. S., G. Smith, T. Zhou, C. Bass, and P. Weis. 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. BioScience 51:209-217.

Weis, J. S., T. Zhou, J. Skurnick, and P. Weis. 2003. Evaluating prey capture by larval mummichogs, (Fundulus heterclitus) as a potential biomarker for contaminants. Marine Environmental Research 55:27-38.

Weis, P. and J. T. F. Ashley. 2007. Contaminants in fish of the Hackensack Meadowlands, New Jersey: size, sex, and seasonal relationships as related to health risks. Archives of Environmental Contaminant Toxicology 52:80-89.

Williams, S. D. 2006. Bioaccumulation patterns of organochlorines in young-of-the-year bluefish (Pomatomus saltatrix) in the Hudson River Estuary. . Florida Agricultural and Mechanical University, Tallahasee, Florida.

Zala, S. M. and D. J. Penn. 2004. Abnormal behaviours induced by chemical pollution: a review of the evidence and new challenges. Animal Behavior 68:649-664.

Zhou, T., H. John-Alder, and J. S. Weis. 1999a. Thyroidal status of mummichogs (*Fundulus heteroclitus*) from a polluted vs a reference habitat. Environmental Toxicology and Chemistry 18:2817-2823.

Zhou, T., H. John-Alder, J. S. Weis, and P. Weis. 2000. Endocrine disruption: thyroid dysfunction in mummichogs (*Fundulus heteroclitus*) from a polluted habitat. Marine Environmental Research 50:393-397.

Zhou, T., D. Rademacher, R. Steinpreis, and J. S. Weis. 1999b. Neurotransmitter levels in two populations of larval Fundulus heteroclitus after methylmercury exposure. Comparitive Biochemistry and Physiology Part C 124:287-294.

Table 4.1 – Top ten PCB congeners by concentration in HR bluefish, field-caught prey and prey from stomach content

Top ten Congeners	HR-fed	homologue	HR field	homologue	HR mummichog	homologue	HR menhaden	homologue	HR Gut mummichog	homologue	HR Gut menhaden	homologue
1	**BZ 153**	hexa	**BZ 52**	tetra	**BZ 153**	hexa	**BZ 52**	tetra	**BZ 153**	hexa	**BZ 52**	tetra
2	**BZ 138**	hexa	**BZ 49**	tetra	**BZ 138**	hexa	**BZ 49**	tetra	**BZ 52**	tetra	**BZ 49**	tetra
3	**BZ 118****	penta	**BZ 66***	tetra	**BZ 118****	penta	**BZ 44**	tetra	**BZ 118****	penta	**BZ 44**	tetra
4	**BZ 52**	tetra	**BZ 118****	penta	**BZ 180**	hepta	**BZ 95**	penta	**BZ 138**	hexa	**BZ 153**	hexa
5	**BZ 180**	hepta	**BZ 44**	tetra	**BZ 52**	tetra	**BZ 66***	tetra	**BZ 99**	penta	**BZ 95**	penta
6	**BZ 66***	tetra	**BZ 138**	hexa	**BZ 66***	tetra	**BZ 28***	tri	**BZ 49**	tetra	**BZ 66***	tetra
7	**BZ 99**	penta	**BZ 99**	penta	**BZ 99**	penta	**BZ 118****	penta	**BZ 95**	penta	**BZ 118****	penta
8	**BZ 49**	tetra	**BZ 153**	hexa	**BZ 49**	tetra	**BZ 31***	tri	**BZ 180**	hepta	**BZ 138**	hexa
9	**BZ 187**	hepta	**BZ 28***	tri	**BZ 44**	tetra	**BZ 99**	penta	**BZ 149**	hexa	**BZ 28***	tri
10	**BZ 149**	hexa	**BZ 95**	penta	**BZ 28***	tri	**BZ 149**	hexa	**BZ 66***	tetra	**BZ 31***	tri

* Coplaner PCB congeners

**Dioxin-like PCB congeners

Table 4.2 – Absolute Z-scores of individual bluefish compared to their prey

HR-fed Fish #

Prey	1	2	3	4	5	6	7	8	9	10	Average
Mummichog	0.48	0.17	0.92	0.71	1.11	0.0	0.31	0.33	0.32	0.22	0.35
Menhaden	3.62	3.46	3.82	3.72	3.91	3.38	3.53	3.54	3.23	3.28	3.55

HR-field Fish #

Prey	1	2	3	4	5	6	7	8	9	10	11	12	Average
Mummichog gut	1.10	3.86	1.44	1.73	0.80	1.89	1.51	5.17	1.08	2.34	3.15	0.60	1.82
Menhaden gut	0.60	0.71	1.80	0.30	0.74	0.22	0.40	1.33	0.61	0.01	0.37	0.83	0.26
Silverside	2.04	3.57	0.63	2.39	1.87	2.48	2.27	4.30	2.03	2.73	3.17	1.76	2.44

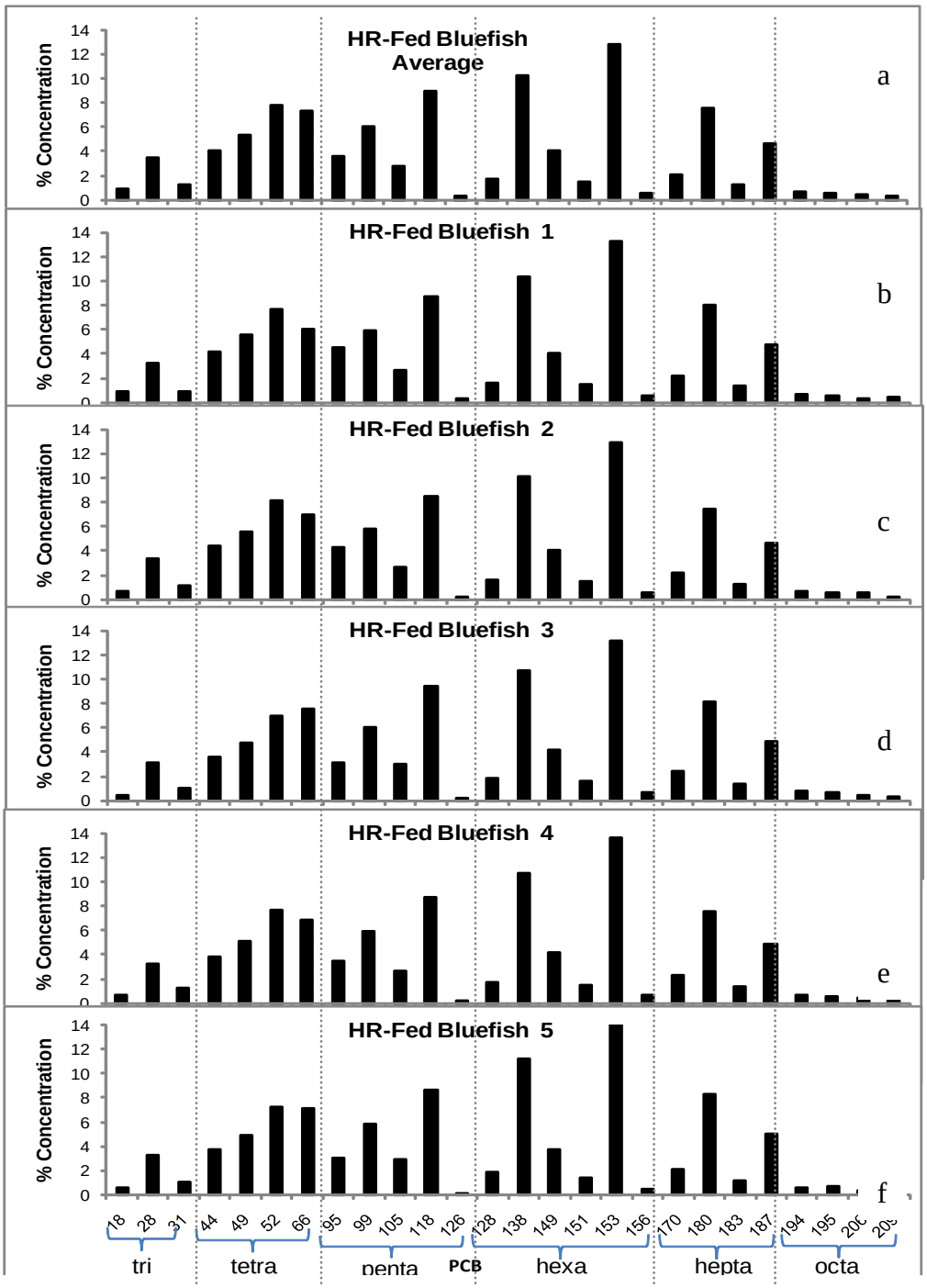

Figure 4.1 a. Mean PCB congener percent concentration fingerprint of the HR-fed bluefish b – f. PCB congener percent concentration fingerprint of the individual HR-fed bluefish # 1-5 PCB fingerprints for HR-fed #6-10 are in the Appendix 2.

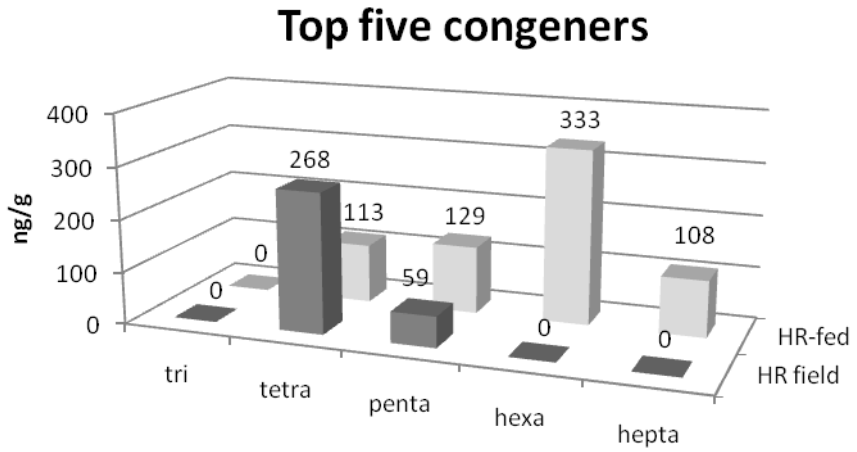

Figure 4.2 Mean concentration (ng g^{-1}) of each homologue group (tri – hepta) for the average top five congeners of HR-fed and HR field bluefish.

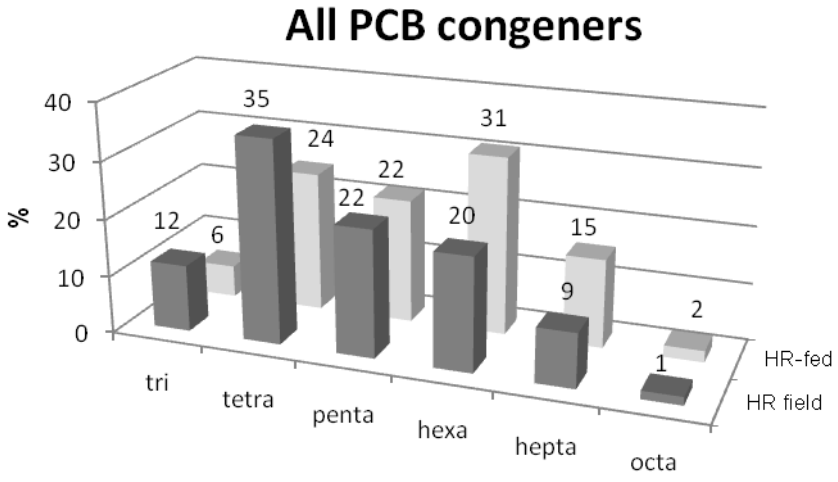

Figure 4.3 Mean percent concentration of each homologue group for the average of all congeners of HR-fed & HR field bluefish.

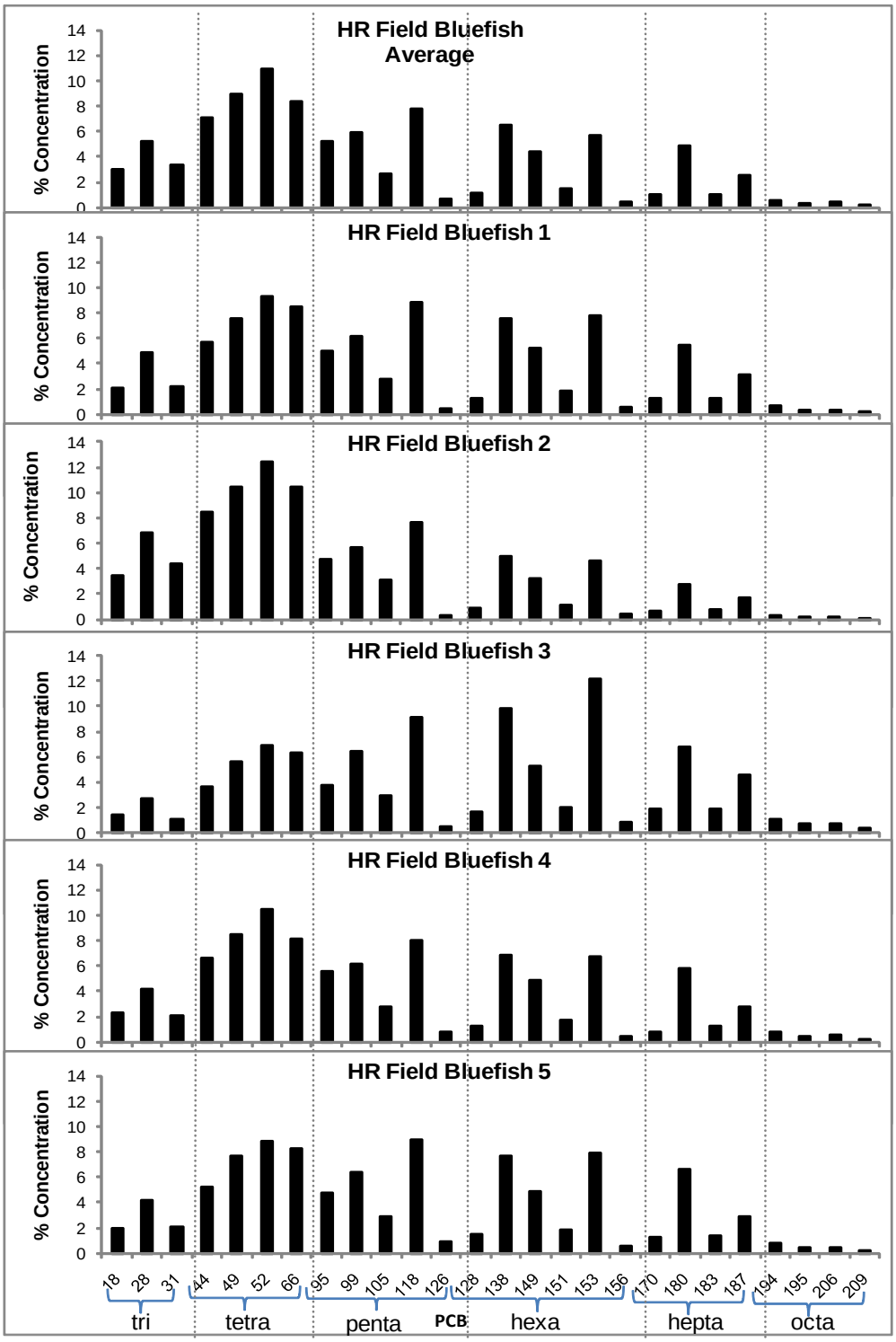

Figure 4.4 a. Mean PCB congener percent concentration fingerprint of the HR field-caught bluefish. **b – f**. PCB congener percent concentration fingerprint of the individual HR field bluefish # 1-5 PCB fingerprints for HR field #6-12 are in the Appendix 2.

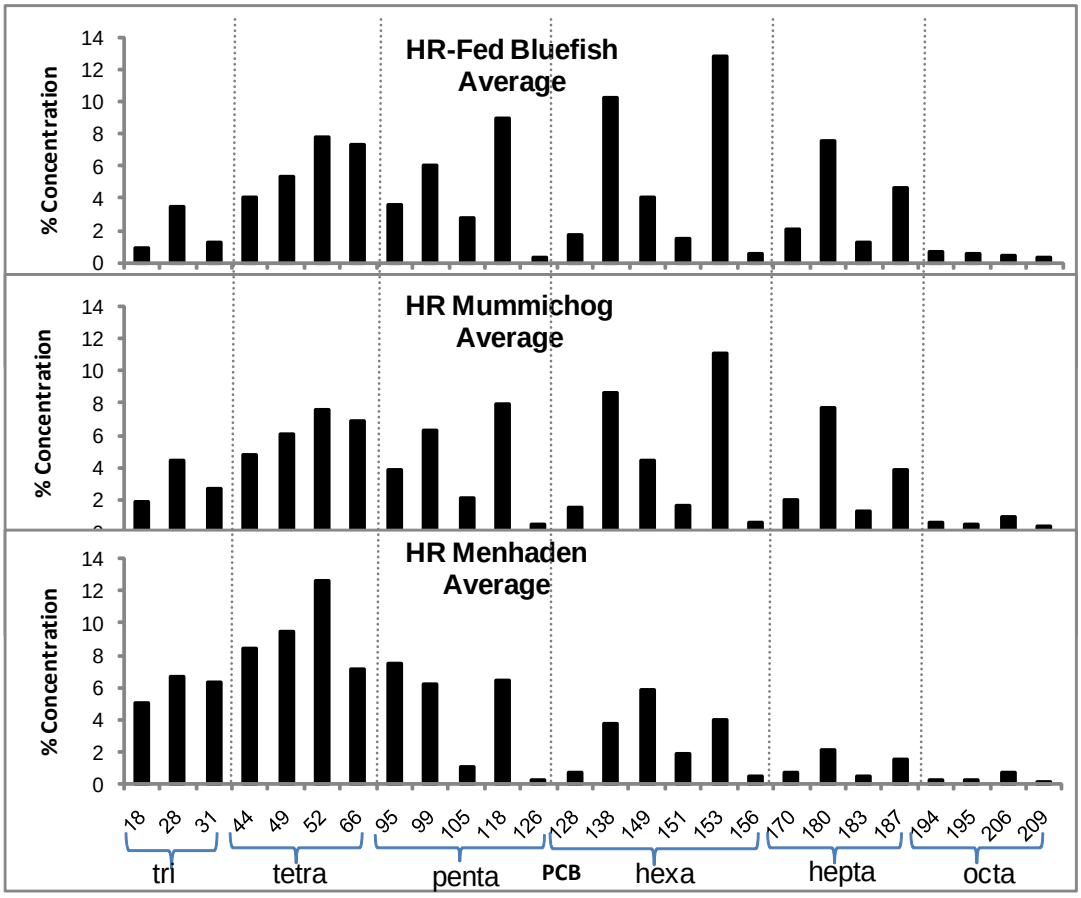

Figure 4.5 Mean PCB congener percent concentration fingerprint of HR-fed bluefish and prey

a. HR-fed bluefish

b. HR field-caught mummichog

c. HR field-caught menhaden

Individual fingerprints of five HR mummichog and menhaden is in Appendix 2.

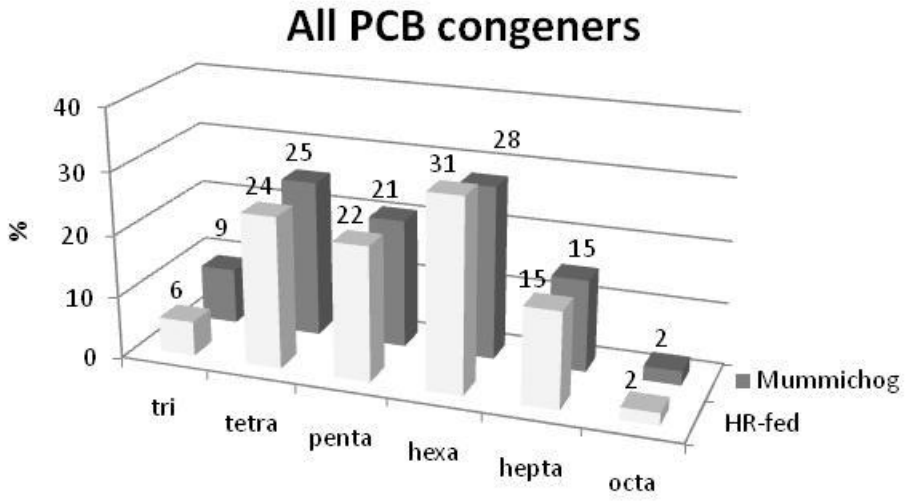

Figure 4.6 Mean percent concentration of each homologue group for the average of all congeners of HR-fed and HR mummichog.

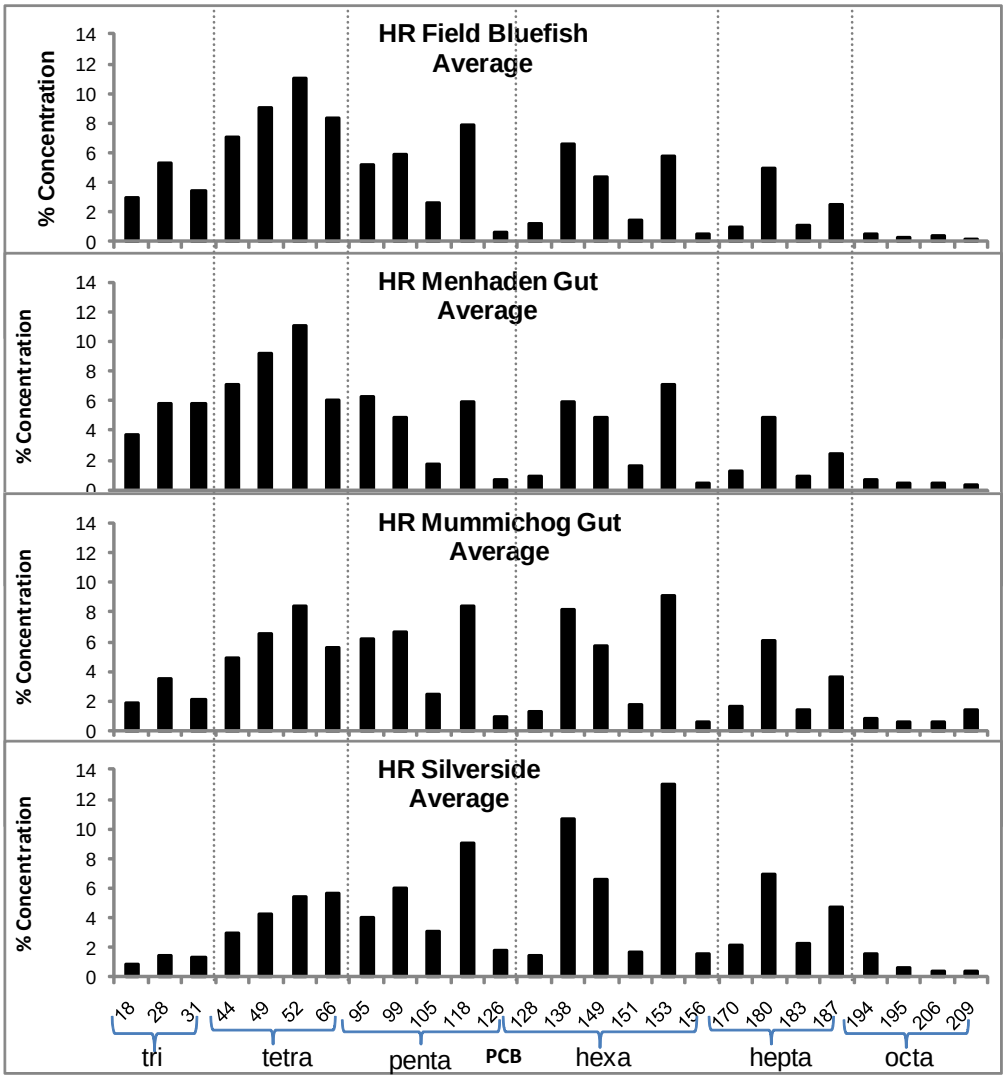

Figure 4.7 Mean PCB congener percent concentration fingerprint of HR field bluefish and stomach content

a. HR field bluefish

b. HR mummichog from the bluefish stomach content

c. HR menhaden from the bluefish stomach content

d. HR field collected silverside

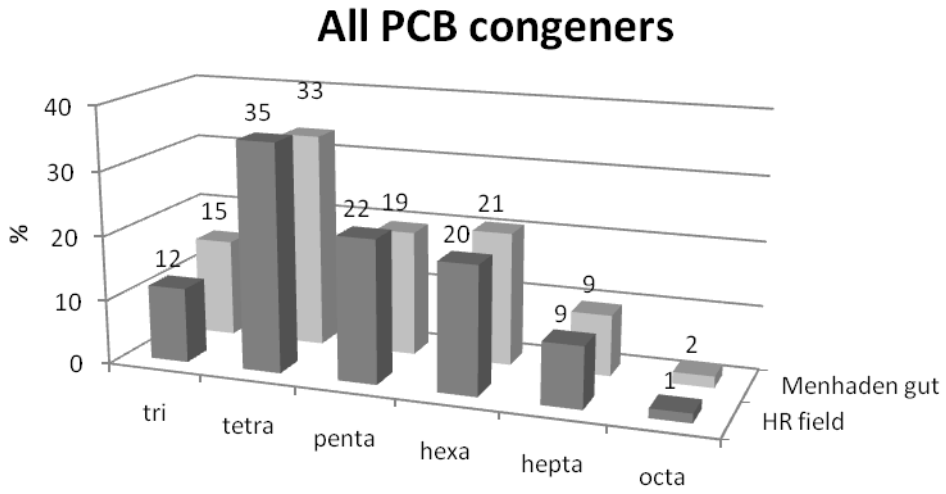

Figure 4.8 Mean percent concentration of each homologue group for the average of all congeners of HR field-caught bluefish and HR menhaden gut content.

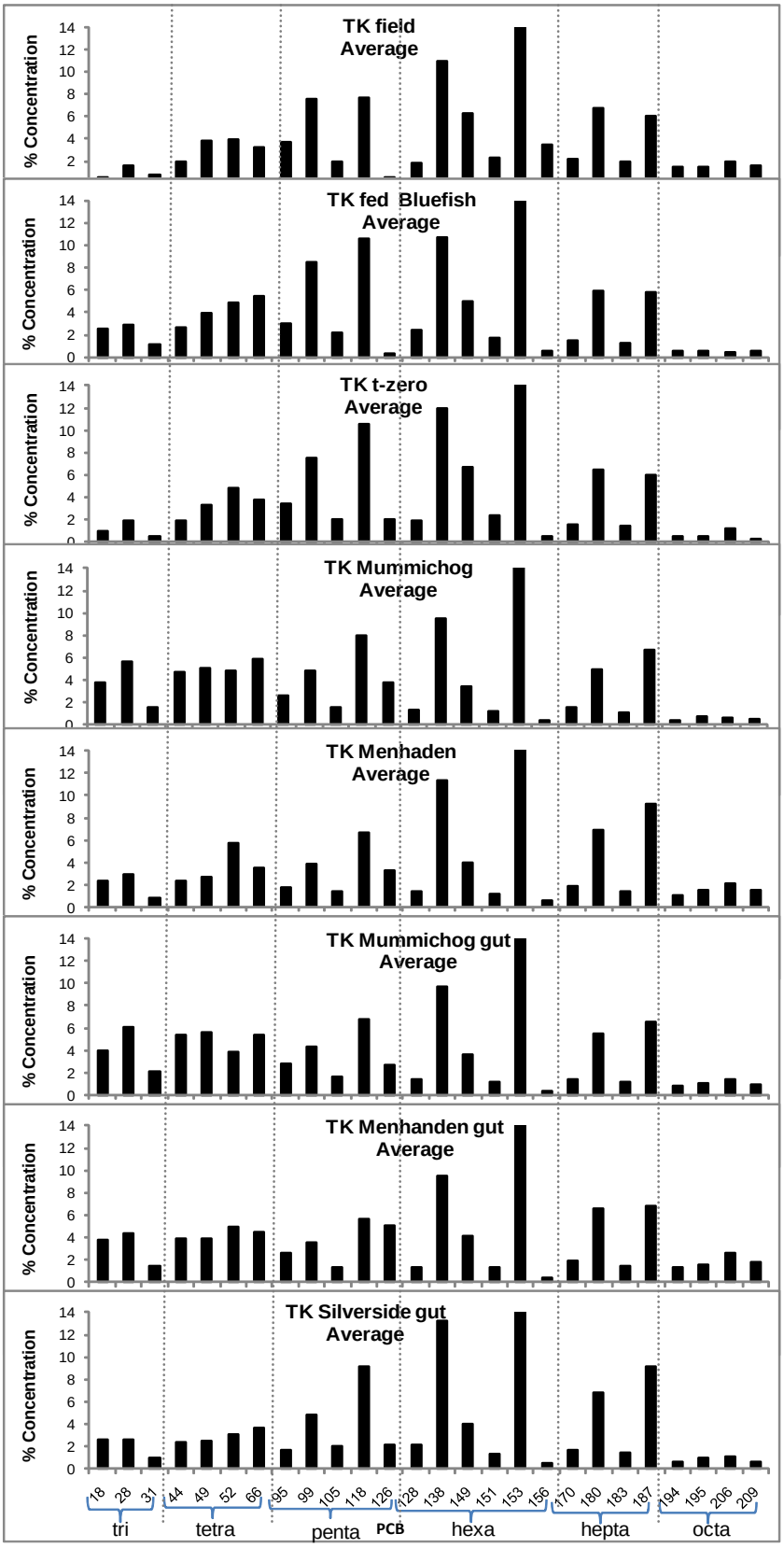
TK field Average
TK fed Bluefish Average
TK t-zero Average
TK Mummichog Average
TK Menhaden Average
TK Mummichog gut Average
TK Menhanden gut Average
TK Silverside gut Average
% Concentration
0
2
4
6
8
10
12
14
18 28 31 44 49 52 66 95 99 105 118 126 128 138 149 151 153 156 170 180 183 187 194 195 206 209
tri
tetra
penta
PCB
hexa
hepta
octa

Figure 4.9 Mean PCB congener percent concentration fingerprint of TK bluefish and prey

a. TK field bluefish.

b. TK-fed bluefish.

c. T-zero bluefish.

d. TK field-caught mummichog

e. TK field-caught menhaden

f. TK mummichog from stomach of TK bluefish

g. TK menhaden from stomach of TK bluefish

h. TK silverside from stomach of TK bluefish

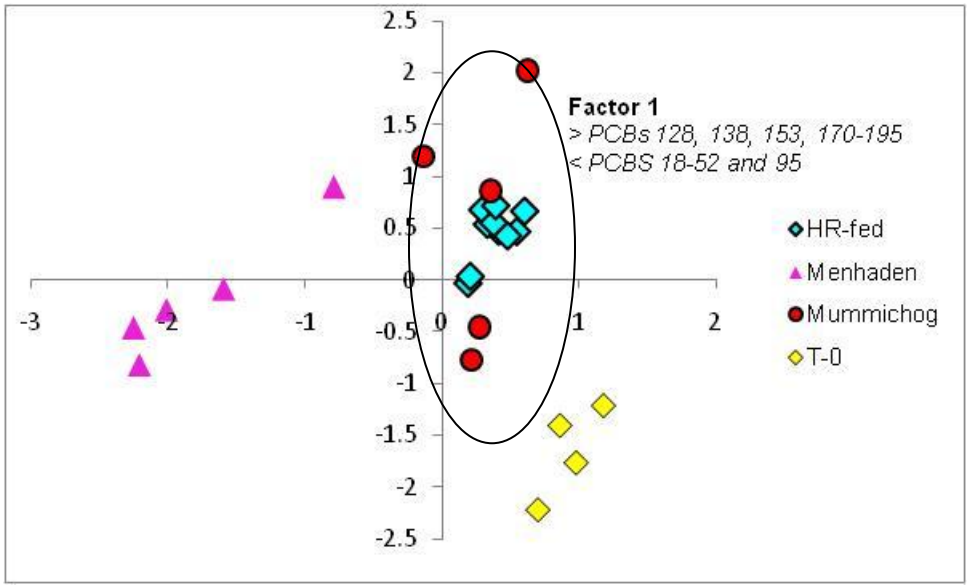

Figure 4.10 Principal component analysis (PCA 1) of HR-fed bluefish and prey. HR-fed, HR field prey (menhaden and mummichog) and T-zero. Plot of Factor 1 scores (x-axis) and Factor 2 scores (y-axis). The oval represents Factor 1 scores that are not statistically different. PCB congeners with contributing loads to the respective Factor scores are presented in italics.

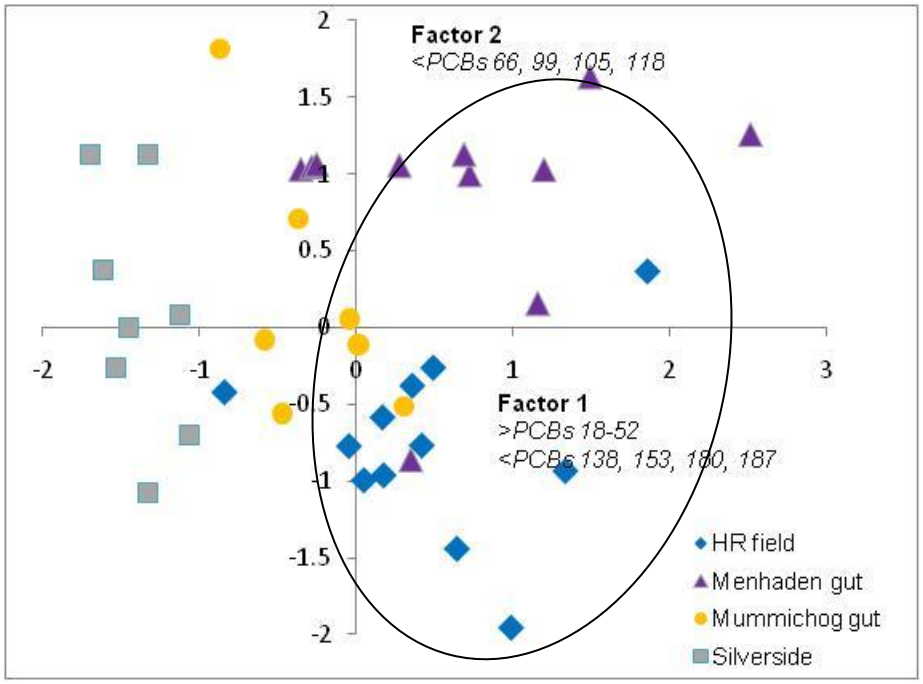

Figure 4.11 Principal component analysis (PCA 2) of HR-field bluefish and prey. HR-field, HR prey from bluefish stomach (menhaden and mummichog) and silverside (field). Plot of Factor 1 scores (x-axis) and Factor 2 scores (y-axis). The oval represents Factor 1 scores that are not statistically different. PCB congeners with contributing loads to the respective Factor scores are presented in italics.

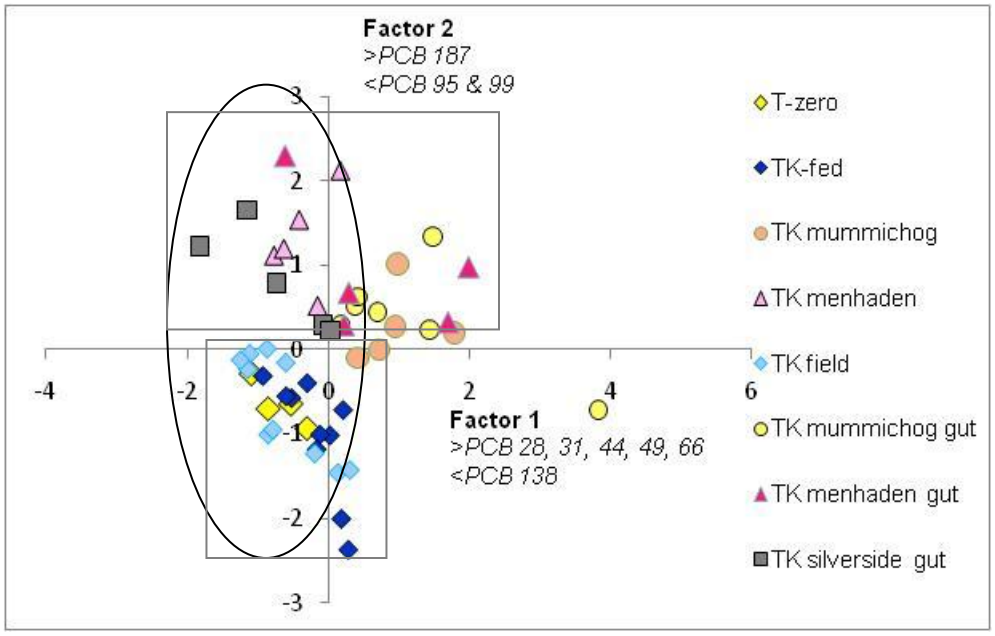

Figure 4.12 Principal component analysis (PCA 3) of TK bluefish and prey. TK-field, TK-fed, TK menhaden and TK mummichog, T-zero. Plot of Factor 1 scores (x-axis) and Factor 2 scores (y-axis). The oval represents Factor 1 scores that are not statistically different. Rectangle represents Factor 2 scores that are not statistically different. PCB congeners with contributing loads to the respective Factor scores are presented in italics.

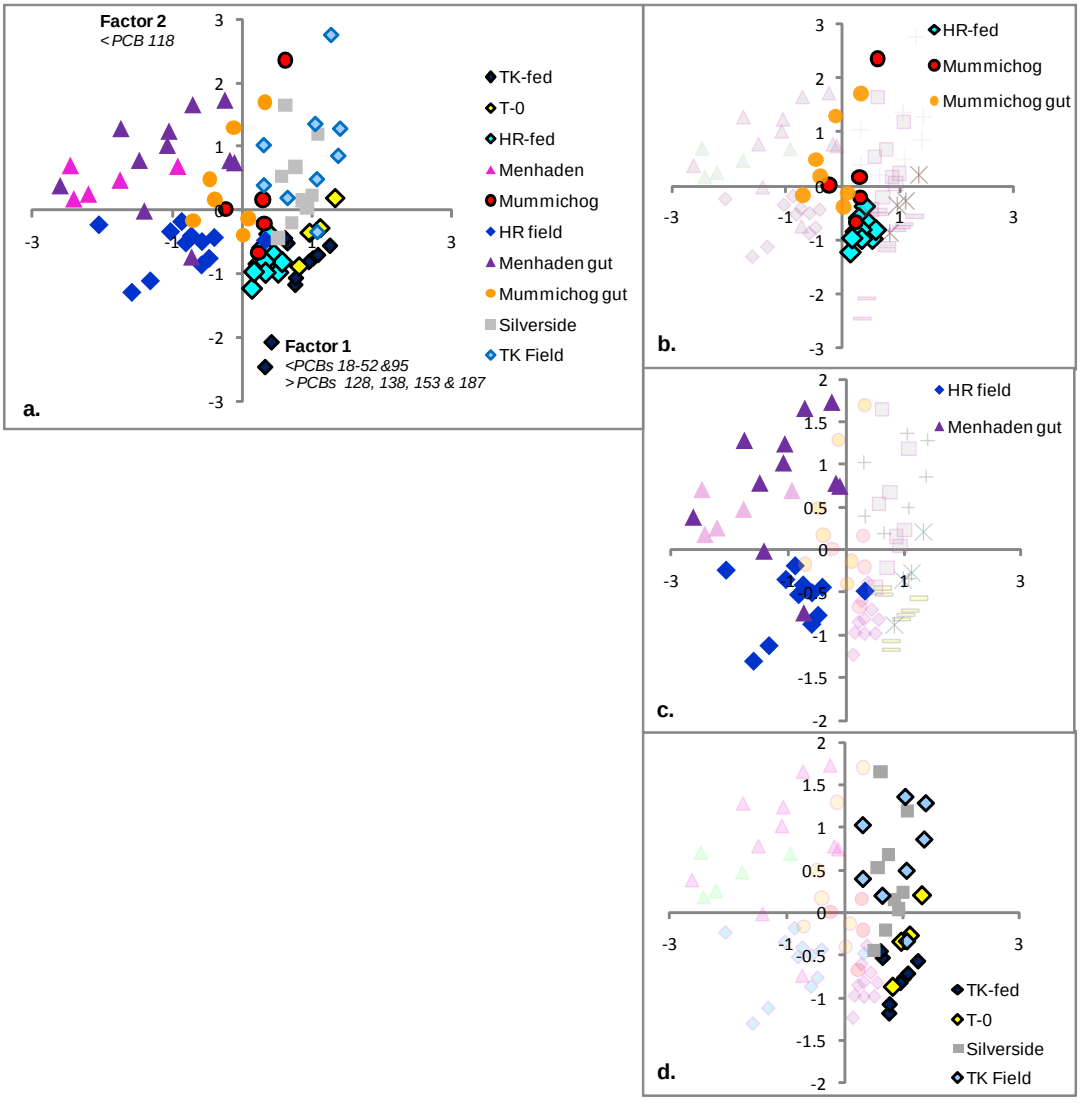

Figure 4.13 Principal component analysis (PCA 4) of HR and TK groups. **a.** HR-fed, HR-field, TK-fed, TK-field, T-zero bluefish, HR field prey and stomach content (menhaden, mummichog). Plot of Factor 1 scores (x-axis) and Factor 2 scores (y-axis). **b.** Graph 13a with only HR-fed bluefish, HR mummichog from field and stomachs highlighted. **c.** Graph 13b with only HR-field bluefish, HR field menhaden highlighted.**d.** Graph 13a with only TK-fed, T-zero, TK-fed, and HR silverside, highlighted. Groups highlight in **b-d** are not statistically different from one another.

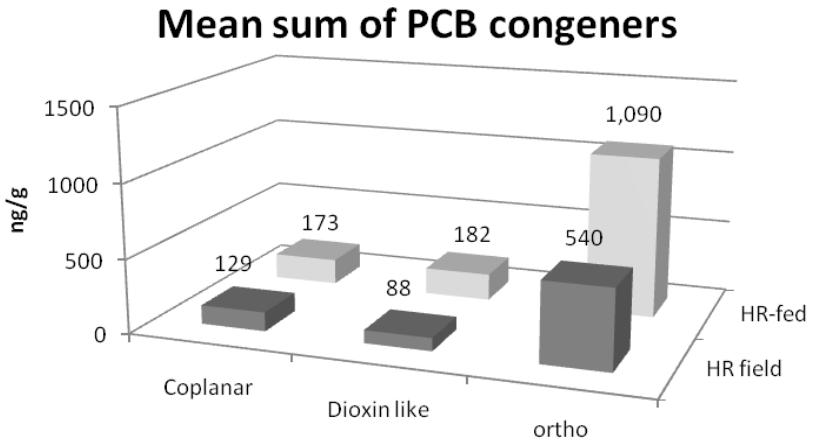

Figure 4.14 Mean concentration (ng g^{-1}) of each type of congener (coplanar, dioxin-like and ortho) of HR-fed and HR field bluefish.

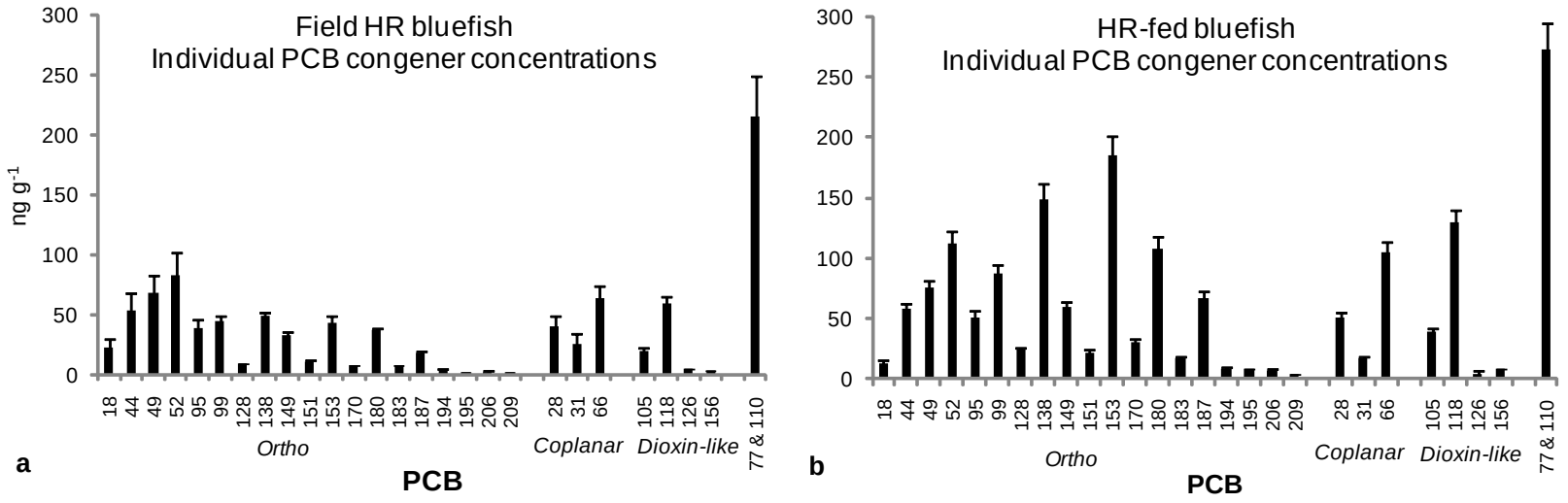

Figure 4.15 Mean concentration (ng g-1) of each specific congener grouped by each type of congener (coplanar, dioxin-like and ortho)

a. HR-fed

b. HR field bluefish

FINAL DISCUSSION & CONCLUSION

Estuaries serve as nurseries for many species of juvenile fish, including young-of-the-year bluefish, providing food and shelter during vital months of growth and development. However, if the YOY utilize a contaminated estuary such as the Hackensack River the exposure may result in sublethal effects that could be ultimately detrimental to their recruitment success. This study examined the accumulation of three particularly deleterious toxicants, PCBs, DDTs, and Hg in YOY bluefish and their prey and the impacts on bluefish behavior, growth, and physiology, including thyroid morphology and neurotransmitter concentrations. The examination of multiple sublethal effects is imperative to comprehending ecological impacts of exposure. In addition, this project's amalgamation of laboratory studies with field observations provides evidence of alterations at the cellular, organismal and community level.

Contaminants

Concentrations

As expected, the bluefish and prey fish from Hackensack (HR) had elevated PCB, DDT and Hg concentrations compared to Tuckerton (TK). In addition, the laboratory bluefish that were fed prey from HR from July to November also had elevated concentrations of PCBs, DDTs, and Hg compared to those fed prey from TK. While the elevated body burdens in the HR fish were anticipated, the Hg concentrations in the TK-fed and TK field bluefish were higher than one would have expected from fish residing in a "pristine" site. Many were well above the EPA tolerance level (0.47 $\mu g\ g^{-1}$) and one higher than the FDA 1.0 $\mu g\ g^{-1}$ no consumption level. The wide range in concentrations

in the TK field bluefish is likely explained by variation in diets (Li et al. 2009). Mercury accumulation has been correlated with feeding behavior and habitat preference. Carnivorous fish, residing in or feeding on organisms that reside in benthic habitats have been associated with greater mercury bioaccumulation rates (Li et al 2009). Analysis of sediment from the lower Mullica River (within a couple kilometers of our Graveling Point site) found average mercury sediment concentrations of 0.32 μg g^{-1} and maximum values of 0.46 μg g^{-1} (Armstrong et al. 2005). While these mercury concentrations are not as high as those reported from sediment of the Hackensack River and Newark Bay estuaries (ranging from 1.5-8.7 μg g^{-1}) (Armstrong et al. 2005, Konsevick and Bragin 2007), they could biomagnify in the bluefish and other top predators in TK. It is also possible that some of the collected bluefish from TK had previously migrated down from northern urban estuaries, particularly the individual with a concentration of 1.22 μg g^{-1}. Unlike mercury, the TK-fed and field bluefish did not contain unexpectedly high concentrations of PCBs and DDTs. The tPCB and tDDT sediment concentrations in the Mullica River analysis were only 36 ng g^{-1} and 5.4 ng g^{-1}, respectively, compared to concentrations of tPCBs ranging from 91-1078 ng g^{-1} in the Newark Bay estuaries and tDDT concentrations as high as 151 ng g^{-1} reported in the Passaic River (Iannuzzi et al. 2005).

Biomagnification

Based on previous studies, it was predicted that the body burden of the bluefish from HR would be 3-4 fold greater than their prey (Rasmussen et al. 1990; Leblanc et al. 2006). Overall, the HR-fed and HR-field bluefish bioaccumulated PCBs, DDTs, and Hg 2-4 times that of their prey, with the exception of tPCB in the HR-fed bluefish, which

was 7-fold that of the prey. The biomagnification factors in the TK fed and TK field bluefish were much higher than expected for each contaminant, but particularly for the Hg. These higher biomagnifications factors may be due to differences in metabolic elimination pathways in the less exposed TK bluefish compared to the HR bluefish. Both tPCB and tDDT were biomagnified in the TK-fed and TK field bluefish 6-10 times that of their prey and Hg was biomagnified 37-45 times that of their prey. The fact that the mercury biomagnifies in the TK bluefish to such a great degree suggests that it is methylmercury. Methylation of mercury is more likely to occur during the warmer summer months, producing the more bioavailable methylmercury (Newman and Unger 2003). The biomagnification in the TK bluefish led to tPCB and Hg concentrations that fall within the toxicity literature threshold range summarized by Hinck et al. (2009). These fish may also be experiencing subtle sublethal effects, and their consumption could be detrimental to top predators including birds and even humans (Hinck et al. 2009). The human health risk from consuming fish, especially self-caught fish is an important issue. Relationships have been reported between mercury and PCB levels in fish, fish consumption by pregnant women, and deficits in neurobehavioral development in children (Stewart et al. 2000, Dorea 2008). In order for fish advisories to be effective it is necessary to have information on contaminant loads in the fish that subsistence and recreational fishermen are catching. As observed in the Hg concentrations of the TK bluefish, the location of catch is not sufficient to determining the edibility of a fish. In addition, because larger bluefish are migratory, the location of catch does not denote prior residency and won't indicate contaminant exposure (Gartland et al. 2006).

Prey Concentrations

The tPCB and tDDT concentrations of menhaden and mummichogs from bluefish stomachs were significantly higher than that of these prey species caught in the field. Previous laboratory studies supported the hypothesis that contaminant concentrations would be greater in captured prey due to increased predation rate (Kraus and Kraus 1986, Smith and Weis 1997, Smith et al. 1997, Weis et al. 2000, Webber and Haines 2003, Scott and Sloman 2004). Grass shrimp were more vulnerable to predation from mummichogs after Hg exposure, and mummichogs from polluted Piles Creek, NJ were more susceptible to predation by blue crabs compared to reference organisms (Kraus and Kraus 1986, Smith and Weis 1997). The results of the present study provide a field validation that increased contamination impairs predator avoidance behavior, making prey more vulnerable. This preferential selection of more highly contaminated prey may be a result of poor predator avoidance in the prey and also impaired prey capture ability of the bluefish requiring them to focus on the slower more contaminated prey for feeding success. When determining biomagnification factors and exposure concentrations, studies should analyze the prey from the predator's stomachs. Future behavioral experiments assessing the predator avoidance capability of juvenile menhaden, the dominant prey of the HR bluefish, would provide a clearer picture of the predator/prey interactions occurring in this estuary.

Fillet versus whole fish

The mean PCB, DDT and Hg concentrations in the HR-fed bluefish were greater than those found in bluefish from HR. However, since the laboratory fish were analyzed as whole-fish and the field fish were analyzed as fillets, they cannot be compared directly

without some caution. It is often assumed that due to the inclusion of fatty internal organs such as the liver and intestines the whole fish PCB concentration will be higher than the fillet. One of the few studies in the literature reveal ratios of 1:1.5 and 1:1.7 fillet to whole-fish PCB concentrations for rainbow trout and coho salmon, respectively (Amrheim et al. 1999). Bluefish muscle is high in lipids compared to other species (Deshpande et al. 2002) and analysis of total lipid percentage of YOY bluefish fillets and livers of bluefish revealed a higher percentage of lipids in the fillets (unpublished). Therefore, the difference between fillet and whole-fish concentrations may be comparitively less in the YOY bluefish. In addition, the Hg analysis was performed on a 2g section of muscle tissue from both the lab fish and the field fish and, consistent with the PCB and DDT results, the mean concentration of total Hg in the HR-fed bluefish was significantly greater than in the HR field-caught bluefish. The tPCB, tDDT and Hg concentrations in HR-fed were all approximately 1.5 times that of the HR field-caught bluefish. While the mean concentrations of the field fish are less than the lab fish, the tPCB concentrations of nine of the twenty two individuals from the field fall within the range of lab fish. The bluefish in the lab were sacrificed a month later than the bluefish collected from the field. However, the laboratory feeding exposure began July 16th and previous studies have shown that bluefish immigrate into the estuaries as early as late May/early June so the difference in exposure time for the field versus lab fish was probably negligible. Also, assessment of YOY bluefish in the Hudson River in two separate studies found relatively low variation in concentration/wet weight throughout the summer months despite increased exposure time and size (Leblanc et al. 2006, Williams 2006). Therefore, it is unlikely that the sacrifice date is the cause of the differences in

concentrations between lab and field HR fish. Greater contaminant concentrations of the HR-fed laboratory fish may be a result of being fed to satiation daily with mummichogs and menhaden. In addition, fish in the field would be expending much more energy than those swimming in a small tank in the lab and being fed frozen prey. Increased activity, metabolic activity and excretion could possibly lead to increased elimination of the contaminants particulary the less lipophilic contaminants including the less chlorinated PCB congeners and inorganic mercury (Newman and Unger 2003). Also, individual bluefish in the field would be less likely to feed consistently and on the same prey, which could explain the increased variance in the individual contaminant concentrations (Chapter 2). During the beginning of their residence in the estuary, HR-field bluefish would be consuming smaller prey and invertebrates, which would presumably have lower body burdens (Juanes and Conover 1994, Able and Fahay 1998, Buckel et al. 1998).

Prey selection can have a substantial impact on the final body burden of the individual bluefish. One would think that the consumption of more highly contaminated impaired prey as described in Chapter 2 would lead to higher trophic transfer; however, the average HR-field bluefish body burdens is still lower than the HR-fed.

The lower mean contaminant concentrations of the HR field bluefish, compared to those in the laboratory feeding experiment (HR-fed) may also be a consequence of increased mortality in the more highly contaminated bluefish due to starvation and/or predation. These individuals may have impaired prey capture and predator avoidance behavior as seen in the prey fish and other studies (Kraus and Kraus 1986, Smith and Weis 1997, Webber and Haines 2003, Perez and Wallace 2004). The high frequency of empty stomachs implies that they are not eating much. Irregular swimming behavior,

poor schooling ability, and slower movement would make them more conspicuous, less protected and less able to escape predators. If the more highly contaminated HR-field bluefish are exhibiting these disruptions, then they will be likely targets for larger predators in the estuary such as striped bass and larger bluefish.

One final point regarding the discrepancy in the field versus lab bluefish body burdens is that different prey species accumulate a unique pattern of PCB congeners based on their feeding behavior and habitat (Chapter 4). Menhaden are more likely to transfer less chlorinated PCB congeners than silversides and mummichogs. These congeners have lower K_{ow} value and are therefore less bioaccumulative and more easily metabolized and eliminated (Gobas et al. 1999, Leblanc et al. 2006). As a result, individual bluefish with a higher preference for menhaden may have lower body burdens. Since menhaden were the dominant prey item of the of the HR-field bluefish and the field bluefish had a PCB fingerprint that was very similar to menhaden, the differences in the accumulation of certain PCBs can be a strong argument for the disparity in body burdens. The HR-fed bluefish, on the other hand, were fed primarily mummichog during the last month of the feeding exposure and accumulated a fingerprint similar to the mummichog with more highly chlorinated, bioaccumulative, PCB congeners and higher body burdens.

Overall, the concentrations of tPCB and Hg in both the HR-fed and HR-field fish and even the HR prey were particularly high compared to other studies of bluefish and other organisms in the Northeast. PCB concentrations of the HR bluefish were three-fold greater than that in YOY bluefish from the Hudson River (Williams 2006). Harvey et al. (2008) conducted a survey of nationwide estuaries and the highest tPCB and Hg concentrations reported was 1160 ng g^{-1} and 0.96 µg g^{-1}, respectively. These values are

all below the body burdens of the HR bluefish, confirming that, while the Hackensack River is improving, the organisms are still heavily impacted, as Weis and Ashley (2007) also reported. Overall, the tPCB, Hg, and tDDT concentrations of the HR bluefish and prey were all within or much greater than the literature-based toxicity threshold limits summarized by Hinck et al. (2009) in a USGS national monitoring program (tPCB 110-480 ng g^{-1}; Hg 0.1-0.3 µg g^{-1}; and tDDT 150-3000 ng g^{-1}). At these exposure levels, it is not surprising that these fish are experiencing sublethal effects.

Behavior and Growth

As predicted, the TK bluefish fed contaminated prey exhibited reduced activity and consumption. Exposure to contaminated food in this laboratory study led to decreased appetite or motivation to feed. Since the feeding experiments in this study employed frozen prey, the ability to capture prey, frequency of strikes and handling time were not measured, but may also be an issue.

At the end of the summer, YOY bluefish are primarily piscivores and known to be voracious, feeding whenever the opportunity arises. It was expected that most of the bluefish caught during daylight hours would have fish in their stomachs; however, this was not the case. Only 29% of the HR bluefish collected in the fall over the 3 collection years had food in their stomachs, which is inconsistent with results from previous studies conducted in less contaminated estuaries. These fish should still be feeding readily in preparing for fall migration and the high percentage of empty stomachs indicates sublethal behavioral disruptions (Buckel and Conover 1997).

It was expected that the HR bluefish would have reduced growth and condition. At the end of the 4 month feeding exposure period, both total length and weight were smaller in the HR-fed bluefish, demonstrating reduced growth. This was also true for the field-caught HR bluefish. Since both the length and the weight of the HR-fed bluefish were smaller, the condition index, which is a proportion of length and weight, was not different than that of TK bluefish. This suggests that these fish were not experiencing a sudden weight loss due to recent reduced feeding, but that they had been gradually growing less in both length and weight over a period of time, as a result of a long term reduction of food consumption or hormone disruption or a combination of both.

TK field caught bluefish were 14.9% longer and weighed 44.4% more than the HR field caught bluefish (Chapter2). In contrast, in the laboratory study the size differences were considerably less. The TK-fed bluefish were only 4.55% longer and weighed 12.81% more than the HR-fed bluefish (Chapter 2). The field bluefish appear to be experiencing a more substantial difference in growth rate than observed in the laboratory, which is representative of the greater variance of conditions in the field and the additional energy expenditure required to feed and survive in the wild. While prey capture rate and handling time were not measured, numerous studies have found that exposure to contaminants can lead to deleterious effects on predatory ability of fish (Sandheinrich and Atchison 1989, Buckel, et al. 1999, Weis et al. 2001, Weis et al. 2003). Reduced motivation to feed in conjunction with hindered prey capture and handling would lead to a significantly reduced diet and growth in the wild fish. Overall, both the lab and field fish appear to be poor predators,which is reflected in their smaller size.

Bluefish from HR and those fed contaminated prey displayed alterations in thyroid histology compared to the TK counterparts. Histological thyroid alterations have been shown to be sensitive biomarkers of sublethal toxicant disruptions, in some cases even more sensitive than alterations in behavior, development, reproduction and hormone circulation (Patino et al. 2003, Opitz et al. 2005, Tiege et al. 2005, Opitz et al 2006, Carlsson and Norrgren 2007). Thyroid status has been correlated with metabolism and growth, and linked through feedback of the central nervous systems with behavior including activity rate, orientation, and migration (Crisp et al. 1998, Porterfield 2000, Donahue et al. 2004). Therefore, early signs of histological alterations could be bioindicators of further disruptions (Porterfield 2000, Donahue et al. 2004). A time series examination of thyroid histology of YOY bluefish throughout their estuarine stay could provide insight into how early effects can be seen.

Growth hormones would be another important factor to analyze in the YOY bluefish from HR and fed HR prey. Exposure to endocrine-disrupting contaminants is likely impairing more than just the thyroid system. While reduced feeding as a result of a neurological or thyroid hormonal imbalance could indirectly decrease growth rate, there may also be direct effects on growth hormones due to inhibition or mimicking by contaminants. Growth hormones help regulate appetite and food conversion efficiency in fish and have also been correlated with dopaminergic activity and swimming activity in juvenile trout (Matty 1986, Johansson et al. 2005). Exposure to a mixture of contaminants each with multiple mechanisms of toxicity will result in organisms undergoing numerous physiological and behavioral malfunctions, many of which are

interrelated. Future research should investigate the correlations between various hormone disruptions, neurological impairments and behavior.

The final factor that was assessed in the bluefish was neurotransmitter concentrations. We hypothesized that the reduced activity and feeding in the bluefish indicated reduced levels of monoamines, particularly dopamine and serotonin, because of the elevated concentrations of the known neurotoxins, PCBs and Hg. Due to the higher concentrations in the HR-fed bluefish, we expected to see more substantial disruptions in these fish. However, the divergent results between the HR-fed and HR-field were not anticipated. Possible causes for the differences in neurochemical disruption between the lab and field bluefish have been discussed thoroughly in Chapter 3. It is difficult to arrive at one explanation because neurotransmitter concentrations are often inconsistent from species to species, from study to study and from specific brain regions and th differences in conditions and stressors in the field versus lab led were likely a factor.

One argument that was not discussed in great detail is that congener-specific accumulation and prey selection may be influencing the neurotoxicity of exposure. The differences may be a consequence of exposure to higher concentrations of certain PCB congeners with different toxicity mechanisms in the HR-fed bluefish compared to the HR-field fish (Chapter 3, Chapter 4).

The HR bluefish had elevated levels of both dioxin-like coplanar and non-coplanar ortho-substituted, PCBs, Hg, DDTs, and other pesticides (Chapter 4). These contaminants may target the fish's endocrine and nervous systems via distinct mechanisms. The coplanar and dioxin-like PCBs are linked to endocrine disruption and may be disrupting the neurological system indirectly via hormone disruption, while the

non-coplanar, ortho-substituted congeners are considered to be direct neurotoxins (Shain et al. 1991, Seegal et al. 1997, Fischer et al. 1998, Schantz and Widholm 2001). The HR-fed bluefish had greater concentrations of each of these groups of PCBs. The concentration of the dioxin-like PCB 118 and the coplanar PCB 66 were two- to three-fold greater in the HR-fed bluefish. In addition, the concentrations of penta-, hexa- and heptachlorinated, ortho-substituted were also two- to four-fold greater in the HR-fed bluefish. Ortho-substituted congeners that were of particularly high concentrations in the HR-fed bluefish but not the HR-field included PCBs 99, 138, 153, 180 and 187. Congener-specific studies are limited, particularly in fish; however, it is accepted that certain congeners have difference modes of action and toxicity equivalent factors. In general, the more highly chlorinated congeners have often been associated with greater neurological and behavioral impairments (Stewart et al. 2000). It is certainly plausible that the exposure to higher concentrations of one or a combination of all of the above-mentioned congeners could be the driving factor in the differences observed between the neurotransmitter alterations in the HR-fed compared to the HR-field bluefish. For example, the ortho-substituted PCB 47 has been shown to lead to a decrease in dopamine concentrations in rats, while the highly potent dioxin-like PCB 77 increases dopamine concentrations and has also been linked with reduced thyroid function and T4 levels and altered behavior (Seegal et al. 1997, Weinand-Härer et al. 1997, Roth-Härer et al. 2001). We cannot make a direct comparison to these results since PCB 47 was not tested in our samples and further analysis revealed that the concentration contribution of PCB 77, which coeluted with PCB 110, was minimal. Nevertheless, we can speculate that the high concentrations of dioxin-like and coplanar PCBs 118 and 66 may have stimulated

the increase in dopamine and other monoamines in the HR-fed bluefish, while the HR-field bluefish were impaired primarily by the dominant ortho-substituted PCBs 49 and 52 leading to reduced dopamine, similar to the rats exposed to PCB 47.

PCBs 138, 153, 180 and 187 have been associated with thyroid disruption in rodents and fish and could be indirectly impairing the neurodevelopment of HR-fed fish (Kodavanti et al. 1993, Osius et al. 1999, Kodavanti and Ward 2005, Leroy et al. 2006). The ortho-substituted PCB 153, which has the greatest body burden in the HR-fed bluefish, has been associated with altered behavior and neurotransmitter concentrations and critical developmental neurotoxic effects (Eriksson and Fredriksson 1996, Fischer et al. 1998, Mariussen and Fonnum 2006, Fischer et al. 2008). A neurotoxic equivalence scheme developed by Simon et al. (2007) assigned the PCBs 66, 99, 118, 138, 153, 180 and 187, which are prevalent in HR-fed, but not in the HR-field bluefish, relatively low neurotoxic equivalency potency values, ranging from 0.09 – 0.36 on a scale of 0 to 1; however the combination of high concentrations of each of these congeners could lead to drastic alterations. While PCB 52 and PCB 110 have been labeled as a very active neurotoxins (Seegal et. al 1998, Simon et al 2007), these congeners were found in high concentrations in both the HR-fed and HR-field bluefish and therefore would not explain the differences in neurochemical alterations; however, they are likely interacting with the other congeners and contaminants (Bowman et al. 1981, Eriksson and Fredriksson, 1996; Fischer et al. 1998, Holene et al. 1998, Mariussen et al. 1999, Simon et al. 2007, Fischer et al. 2008). More specific congener studies would be necessary to explain the differences in neurotoxicity and their effects on the overall health of the YOY bluefish.

Results indicate that diet can play a major role in the contaminant impact. It is possible that a switch in diet from menhaden to mummichog could lead to more drastic neurobehavioral effects. Overall, both the HR-fed and HR-field bluefish exhibited altered monoamine levels compared to the TK reference fish, which could be responsible for disruptions in their behavior and fitness.

This project provides data that correlates numerous sublethal effects from contaminant exposure to juvenile teleost fish. Clearly, more work is needed examining the interrelationships between thyroid hormone status, neurochemistry, behavior, and specific contaminant exposure in the YOY bluefish and other organisms in this estuary.

One can draw a parallel of the results of this project to the findings in mummichogs in the contaminated Piles Creek, NJ. Piles Creek, a tributary of the Arthur Kill south of Newark Bay, is impacted with similar toxicants as the Hackensack River. While bluefish and mummichog differ in life history, body structure, feeding, and reproductive behavior, their behavioral and physiological responses to contaminant exposure were analogous. The Piles Creek mummichog had elevated mercury, reduced growth, slower activity level, impaired prey capture ability, reduced motivation to feed, and impaired predator avoidance compared to the TK reference mummichogs (Smith and Weis 1997, Weis and Ashley 2007). They also had enlarged, irregular thyroid follicles with greater epithelial cell height and higher plasma thyroxine (T4) level, significantly lower concentrations of serotonin and its metabolites and an overall shorter life span (Smith et al. 1995, Zhou et al. 1999, Weis et al. 2001). In contrast to the Piles Creek mummichog population, the grass shrimp population is more abundant and contains larger individuals than the reference site, TK, and their predator avoidance behavior is

unaffected (Kraus and Kraus 1986). The proliferation of the grass shrimp population was attributed to the poor predation ability of the mummichog (Weis et al. 2000). YOY bluefish predation has been associated with impacts on prey population density and may also be influencing the prey populations in the HR estuary (Buckel et al. 1999, Scharf et al. 2002). The impaired predatory ability of the bluefish could facilitate the persistence of the mummichog population and other prey species. Understanding the community dynamics in these contaminated estuaries is important to assessing the impacts on each species.

Conclusion

Monitoring of bluefish migrating from contaminated estuaries along the mid-Atlantic Bight would provide essential information needed to better understand the true contribution of YOY bluefish to the adult population. As discussed earlier, the YOY HR bluefish are smaller, slower, with irregular swimming ability and reduced predatory ability in addition to altered thyroid status and neurochemistry. Each of these factors alone could reduce their chance of a successful migration, overwinter survival and recruitment into the adult population. Therefore, it is reasonable to expect that a combination of all of these impairments will be highly detrimental. Recent stock assessments of bluefish have reported increases since 1999 and the bluefish stock has been declared rebuilt and no longer considered overfished (Baldigo et al. 2006, Shepherd et al. 2006, Shepherd and Packer 2006). If impaired fish such as those emigrating out of the HR estuary or other contaminated estuaries such as the Hudson River and New Bedford Harbor are included in these stock assessments this may lead to inaccurately

inflated numbers, as the probability of mortality due to starvation or predation is high. In addition, as likely prey they will transfer their high concentrations of PCBs, Hg, and other contaminants to top predators, including humans. Assessing the habitat quality of a region and establishing a connection to the status of the fisheries is essential. Overall, a better understanding of the fitness of the young-of the year bluefish when they migrate from contaminated estuaries will help implement improved management plans and fishery advisories for this and other species.

REFERENCES

Amrhein, J.F., C.A. Stow, C. Wible. 1999. Whole-fish versus filet polychlorinated-biphenyl concentrations: an analysis using classification and regression tree models. Environmental Toxicology and Chemistry 18:1817-1823.

Able, K. W. and M. P. Fahay. 1998. The first year in the life of estuarine fishes in the Middle Atlantic Bight. Rutgers University Press, New Brunswick, NJ

Adams, B. A., D. G. Cyr, and J. G. Eales. 2000. Thyroid hormone deiodination in tissues of American plaice, Hippoglossoides platessoides: characterization and short-term responses to polychlorinated biphenyls (PCBs) 77 and 126. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 127:367-378.

Aldegunde, M., J. L. Soengas, C. Ruibal, and M. D. Andres. 1999. Effects of chronic exposure to gamma-HCH (Lindane) on brain serotonergic and gabaergic systems, and serum cortisol and thyroxine levels of rainbow trout, Oncorhynchus mykiss. Fish Physiology and Biochemistry 20:325-330.

Armstrong, T. N., T. J. Iannuzzi, J. B. Thelen, D. F. Ludwig, and C. E. Firstenberg. 2005. Characterization of Chemical Contamination in Shallow-Water Estuarine Habitats of an Industrialized River. Part II. Metals. Pages 35-52 Soil & Sediment Contamination. Taylor & Francis Ltd.

ASMFC. 2003. Population Assessment and the Short-term Stock Projections of the Bluefish Population. A Report to the Atlantic States Marine Fisheries Commission and the Mid-Atlantic Fishery Management Council Monitoring Committee. Atlantic States Marine Fisheries Commission.

Baeta, A., I. Valiela, F. Rossi, R. Pinto, P. Richard, N. Niquil, and J. C. Marques. 2009. Eutrophication and trophic structure in response to the presence of the eelgrass Zostera noltii. Marine Biology 156:2107-2120.

Baldigo, B. P., R. J. Sloan, S. B. Smith, N. D. Denslow, V. S. Blazer, and T. S. Gross. 2006. Polychlorinated biphenyls, mercury, and potential endocrine disruption in fish from the Hudson River, New York, USA. Aquatic Sciences 68:206-228.

Bemis, J. C. and R. F. Seegall. 1999. Polychlorinated biphenyls and methylmercury act synergistically to reduce rat brain dopamine content in vitro Environmental Health Perspectives 107:879-885.

Boon, J. P. and J. C. Duinker. 1985a. Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (*Solea solea*) in relation to concentrations in water and to lipid metabolism under conditions of starvation. Aquatic Toxicology 7:119-134.

Boon, J. P. and J. C. Duinker. 1985b. Kinetics of polychlorinated biphenyl (PCB) components in juvenile sole (Solea solea) in relation to concentrations in water and to lipid metabolism under conditions of starvation. . Aquatic Toxicology 7:119-134.

Bragin, B., J. Misuik, C. A. Woolcott, K. R. Barrett, and R. Jusino-Atresino. 2005. A fishery resource inventory of the lower Hackensack River within the Hackensack meadowlands district: a comparative study 2001-2003 vs 1987-1988. New Jersey Meadowlands Commission Meadowlands Environmental Research Institute.

Brown, S. B., R. E. Evans, L. Vandenbyllardt, K. W. Finnson, V. P. Palace, A. S. Kane, A. Y. Yarechewski, and D. C. G. Muir. 2004. Altered thyroid status in lake trout (Salvelinus namaycush) exposed to co-planar 3,3', 4,4', 5-pentachorobiphenyl. Aquatic Toxicology 67:75-85.

Buckel, J. A. and D. O. Conover. 1996. Gastric evacuation rates of piscivorous young-of-the-year bluefish. Transactions of the American Fisheries Society 125:591-599.

Buckel, J. A. and D. O. Conover. 1997. Movements, feeding periods, and daily ration of piscivorous young-of-the-year bluefish, Pomatomus saltatrix, in the Hudson River estuary. Fishery Bulletin 95:665-679.

Buckel, J. A., D. O. Conover, N. D. Steinberg, and K. A. McKown. 1999a. Impact of age-0 bluefish (Pomatomus saltatrix) predation on age-0 fishes in the Hudson River estuary: evidence for density-dependent loss of juvenile striped bass (Morone saxatilis). Canadian Journal of Fisheries and Aquatic Sciences 56:275-287.

Buckel, J. A., M. J. Fogarty, and D. O. Conover. 1999b. Foraging habits of bluefish, Pomatomus saltatrix, on the US east coast continental shelf. Fishery Bulletin 97:758-775.

Buckel, J. A., B. H. Letcher, and D. O. Conover. 1998. Effects of a delayed onset of piscivory on the size of age-0 bluefish. Transactions of the American Fisheries Society 127:576-587.

Buckel, J. A. and K. A. McKown. 2002. Competition between juvenile striped bass and bluefish: resource partitioning and growth rate. Marine Ecology-Progress Series 234:191-204.

Candelmo, A., C. , A. Deshpande, B. Dockum, J. Samson, P. Weis, and J. S. Weis. in review. Trophic transfer and effects on young-of-the-year bluefish, *Pomatomus saltatrix*, consuming contaminated prey.

Carlsson, G. and L. Norrgren. 2007. The impact of goitrogen 6-propylthiouracil (PTU) on West African clawed frog (*Wenopus tropicalis*) exposed during metamorphis. Aquatic Toxicology 82:55-62.

Clotfelther, E. D., A. M. Bell, and K. R. Levering. 2004. The role of animal behaviour in the study of endocrine-disrupting chemicals.
Animal Behavior 68:665-676.

Comeau, L. A., S. E. Campana, G. A. Chouinard, and J. M. Hanson. 2001. Timing of Atlantic cod Gadus morhua seasonal migrations in relation to serum levels of gonadal and thyroidal hormones. Marine Ecology-Progress Series 221:245-253.

Dennard, S. T., B. C. McMeans, and A. T. Fisk. 2009. Preliminary assessment of Greenland halibut diet in Cumberland Sound using stable isotopes. Polar Biology 32:941-945.

Deshpande, A. D., A. F. J. Draxler, V. S. Zdanowicz, M. E. Schrock, and A. J. Paulson. 2002a. Contaminant levels in the muscle of four species of fish important to the recreational fishery of the New York Bight Apex. Marine Pollution Bulletin 44:164-171.

Deshpande, A. D., A. F. J. Draxler, V. S. Zdanowicz, M. E. Schrock, A. J. Paulson, T. W. Finneran, B. L. Sharack, K. Corbo, L. Arlen, E. A. Leimburg, B. W. Dockum, R. A. Pikanowski, B. May, and L. B. Rosman. 2000. Contaminant levels in muscle

of four species of recreational fish from the New York Bight Apex. NOAA Technical Memorandum NMFS-NE-157, NOAA, Woods Hole, MA.

Deshpande, A. D., R. J. Huggett, and R. A. Halbrook. 2002b. Polycyclic aromatic hydrocarbon metabolites in the bile of a territorial benthic fish, oyster toadfish (*Opsanus tau*) from the Elizabeth River, Virginia. Archives of Environmental Contamination and Toxicology 42:43-52.

Dimou, K. N., T. L. Su, R. I. Hires, and R. Miskewitz. 2006. Distribution of polychlorinated biphenyls in the Newark Bay Estuary. Journal of Hazardous Materials 136:103-110.

Donahue, D. A., E. J. Dougherty, and L. A. Meserve. 2004. Influence of a combination of two tetrachlorobiphenyl congeners (PCB 47; PCB 77) on thyroid status, choline acetyltransferase (ChAT) activity, and short- and long-term memory in 30-day-old Sprague-Dawley rats. Toxicology 203:99-107.

Eales, J. G. and S. B. Brown. 1993. Measurement and regulations of thyroidal status in teleost fish. Reviews in Fish Biology and Fisheries 3:299-347.

Eriksson, P. and A. Fredriksson. 1996. Developmental neurotoxicity of four ortho-substituded PCBs in neonatal mouse. Environmental Toxicology and Pharmacology 1:155-165.

Feldman, R. S. and J. E. Titus. 2001. Polychlorinated biphenyl accumulation differs among pumpkinseed sunfish during experimental field exposure:the role of invertebrate prey. Aquatic Toxicology 51:389-404.

Fingerman, S. W. and L. C. Russel. 1980. Effects of the polychlorinated biphenyl Aroclor 1242 on locomotor activity and on the neurotransmitters dopamine and norepinephrine in the brain of the Gulf killifish, Fundulus grandis. Bulletin of Environmental Contaminants and Toxicology 25:267.

Fischer, C., A. Fredriksson, and P. Eriksson. 2008. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244:157-165.

Fischer, L. J., R. F. Seegal, P. E. Ganey, I. N. Pessah, and P. R. S. Kodavanti. 1998. Symposium Overview: Toxicity of Non-Coplanar PCBs. Toxicological Sciences 41:49-61.

Gartland, J., R. J. Latour, A. D. Halvorson, and H. M. Austin. 2006. Diet composition of young-of-the-year bluefish in the lower Chesapeake Bay and the coastal ocean of Virginia. Transactions of the American Fisheries Society 135:371-378.

Giesy, J. P. and K. Kannan. 2002. Dioxin-like and non-dioxinlike effects of polychlorinated biphenyls: Implications for risk assessment. Lake Reservoir Research Management 7:139-181.

Gobas, F. A., J. B. Wilcockson, R. W. Russell, and G. D. Haffner. 1999. Mechanisms of biomagnification in fish under laboratory and field conditions. Environmental Science and Technology 33:133-141.

Grippo, M. A. and A. G. Heath. 2003a. The effect of mercury on the feeding behavior of fathead minnows (Pimphales promelas) Ecotoxicology and Environmental Safety 55:187-198.

Grippo, M. A. and A. G. Heath. 2003b. The effect of mercury on the feeding behavior of fathead minnows (Pimphales promelas) Ecotoxocology and Environmental Safety 55:187-198.

Haas, H. L., C. J. Freeman, J. M. Logan, L. Deegan, and E. F. Gaines. 2009. Examining mummichog growth and movement: Are some individuals making intra-season migrations to optimize growth? Journal of Experimental Marine Biology and Ecology 369:8-16.

Hany, J., H. Lilienthal, A. Sarasin, A. Roth-Härer, A. Fastabend, L. Dunemann, W. Lichtensteiger, and G. Winneke. 1999. Developmental Exposure of Rats to a Reconstituted PCB Mixture or Aroclor 1254: Effects on Organ Weights, Aromatase Activity, Sex Hormone Levels, and Sweet Preference Behavior. Toxicology and Applied Pharmacology 158:231-243.

Hauge, P., J. Bukowski, P. Morton, M. Boriek, J. McClain, and G. Casey. 1990. Polychlorinated biphenyls (PCBs), chlordane, and DDTs in selected fish and shellfish from New Jersey waters, 1986–1987:Results from New Jerseys Toxics in Biota Monitoring Program.
.*in* T. NJ Dept of Environ Protection, editor. Available from http://www.state.nj.us/dep/dsr/pcb86-87.pdf.

Hinck, J. E., C. J. Schmitt, K. A. Chojnacki, and D. E. Tillitt. 2009. Environmental contaminants in freshwater fish and their risk to piscivorous wildlife based on a national monitoring program. Environmental Monitoring and Assessment 152:469-494.

Hobson, K. A., W. G. Ambrose, and P. E. Renaud. 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: Insights from delta C-13 and delta N-15 analysis. Marine Ecology-Progress Series 128:1-10.

Howdeshell, K. L. 2002. A model of the development of the brain as a construct of the thyroid system. Environmental Health Perspectives 100:337-348.

Iannuzzi, T. J., T. N. Armstrong, J. B. Thelen, D. F. Ludwig, and C. E. Firstenberg. 2005. Characterization of chemical contamination in shallow-water estuarine habitats of an industrialized river. Part 1: Organic compounds. Soil & Sediment Contamination 14:13-33.

Iverson, S. J., J. P. Y. Arnould, and I. L. Boyd. 1997a. Milk fatty acid signatures indicate both major and minor shifts in the diet of lactating Antarctic fur seals. Canadian Journal of Zoology-Revue Canadienne De Zoologie 75:188-197.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecological Monographs 74:211-235.

Iverson, S. J., K. J. Frost, and L. F. Lowry. 1997b. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology-Progress Series 151:255-271.

Johansson, V., S. Winberg, and B. T. Björnsson. 2005. Growth hormone-induced stimulation of swimming and feeding behaviour of rainbow trout is abolished by the D1 dopamine antagonist SCH23390. General and Comparative Endocrinology 141:58-65.

Jorgensen, E. H., I. C. Burkow, H. Foshaug, B. Killie, and K. Ingebrigtsen. 1997. Influence of lipid status on tissue distribution of the persistent organic pollutant octachlorostyrene in arctic char (*Salvelinus alpinus*). Comparitive Biochemistry and Physiology 118C:311-318.

Jorgensen, E. H., B. E. Bye, and M. Jobling. 1999. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (Salvelinus alpinus). Aquatic Toxicology 44:233-244.

Jørgensen, E. H., B. E. Bye, and M. Jobling. 1999. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (*Salvelinus alpinus*). Aquatic Toxicology 44:233-244.

Juanes, F. and D. O. Conover. 1994a. Piscivory and Prey Size Selection in Young-of-the-Year Bluefish - Predator Preference or Size-Dependent Capture Success. Marine Ecology-Progress Series 114:59-69.

Juanes, F. and D. O. Conover. 1994b. Rapid Growth, High Feeding Rates, and Early Piscivory in Young-of-the-Year Bluefish (Pomatomus Saltatrix). Canadian Journal of Fisheries and Aquatic Sciences 51:1752-1761.

Kennish, M. J. and B. E. Ruppel. 1998. Organochlorine contamination in selected estuarine and coastal marine finfish and shellfish of New Jersey. Water Air Soil Pollution 101:123-136.

Khan, I. A. and P. Thomas. 1996. Disruption of neuroendocrine function in atlantic croaker exposed to Aroclor 1254. Marine Environmental Research 42:145-149.

Khan, I. A. and P. Thomas. 2006. PCB congener-specific disruption of reproductive neuroendocrine function in Atlantic croaker. Marine Environmental Research 62:S25-S28.

Kodavanti, P. R. S., D. S. Shin, H. A. Tilson, and G. J. Harry. 1993. Comparative Effects of Two Polychlorinated Biphenyl Congeners on Calcium Homeostasis in Rat Cerebellar Granule Cells. Toxicology and Applied Pharmacology 123:97-106.

Kodavanti, P. R. S. and T. R. Ward. 2005. Differential effects of commercial polybrominated diphenyl ether and polychlorinated biphenyl mixtures on intracellular signaling in rat brain in vitro. Toxicological Sciences 85:952-962.

Konsevick, E. and A. B. Bragin. 2007. Chemical characteristics of sediment collected as part of the fishery resource inventory of the lower Hackensack River within the Hackensack Meadowlands District: A comparative study 2001-2003 & 1987-1988.

Kraus, M. L. and D. B. Kraus. 1986. Differences in effects of mercury on predator avoidance in two populations of grass shrimp, Palaemonetes pugio. Marine Environmental Research 18:277-289.

Leatherland, J. F. 1994. Reflections on the thyroidology of fishes from molecules to humankind. Guelph Ichthyology Review 2:1-67.

Leatherland, J. F., R. Moccia, and R. Sonstegard. 1978. Ultrastructure of the Thyroid Gland in Goitered Coho Salmon (Oncorhynchus kisutch). Cancer Res 38:149-158.

Leblanc, L. A., J. A. Buckel, D. O. Conover, and B. J. Brownawell. 2006. Tests of bioaccumulation models for polychlorinated biphenyl compounds: A study of

young-of-the-year bluefish in the Hudson River estuary, USA. Environmental Toxicology and Chemistry 25:2067-2076.

Leroy, K. D., P. Thomas, and I. A. Khan. 2006. Thyroid hormone status of Atlantic croaker exposed to Aroclor 1254 and selected PCB congeners. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 144:263-271.

Lewis, M. 2002. 2002 review of the Atlantic States Marine Fisheries Commission fishery management plan for bluefish.*in* W. Atlantic States Marine Fisheries Commission, DC, editor.

Li, D., P. Xie, and X. Zhang. 2008. Changes in plasma thyroid hormones and cortisol levels in crucian carp (Carassius auratus) exposed to the extracted microcystins. Chemosphere 74:13-18.

Li, S. X., L. F. Zhou, H. J. Wang, Y. G. Liang, J. B. Chang, M. H. Xiong, Y. C. Zhang, and J. X. Hu. 2009. Feeding habits and habitats preferences affecting mercury bioaccumulation in 37 subtropical fish species from Wujiang River, China (vol 18, pg 204, 2008). Ecotoxicology 18:384-384.

Little, E. E. and S. E. Finger. 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environmental Toxicology and Chemistry 9:13-19.

Long, E. R., D. D. MacDonald, S. L. Smith, and F. D. Calder. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management 19:81-97.

Lyng, G. D. and R. F. Seegal. 2008. Polychlorinated biphenyl-induced oxidative stress in organotypic co-cultures: Experimental dopamine depletion prevents reductions in GABA. NeuroToxicology 29:301-308.

Magnusson, K., R. Ekelund, R. Grabie, and P. A. Bergqvist. 2006. Bioaccumulation of PCB congeners in marine benthic infauna Marine Environmental Research 61:379-395.

Mariussen, E. and F. Fonnum. 2006. Neurochemical Targets and Behavioral Effects of Organohalogen Compounds: An Update. Critical Reviews in Toxicology 36:253-289.

Matsuo, H., M. Kawano, K. Omori, K. Nakajima, and I. Takeuchi. 2009. Stable isotope-guided analysis of congener-specific PCB concentrations in a Japanese coastal food web. Marine Pollution Bulletin 58:1615-1623.

Matty, A. 1986. Nutrition, hormones and growth. Fish Physiology and Biochemistry 2:141-150.

McBride, R. S. and D. O. Conover. 1991. Recruitment of young-of-the-year bluefish, *Pomatomas saltatrix* to the New York Bight: variation in abundance and growth of spring-and summer-spawned cohorts. Marine Ecology Progress Series 78:205-216.

Mesa, M. G., T. P. Gasomski, and J. H. Peterson. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. Journal of Fish Biology 45 Supplement A:81-96.

Monosson, E., J. T. F. Ashley, A. E. McElroy, D. Woltering, and A. A. Elskus. 2003. PCB congener distributions in muscle, liver and gonad of *Fundulus heteroclitus* from the lower Hudson River Estuary and Newark Bay Chemosphere 52:777-787.

Moyle, P. B. and J. J. Cech Jr., editors. 1988. Fishes, an introduction to ichthyology. 2nd edition. Prentice Hall, Engelwood Cliffs.

O'Connor, J. M., J. B. Klotz, and T. J. Kneip. 1982. Sources, sinks and distribution of organic contaminations in the New York Bight ecosystem. Estuarine Research Federation, Columbia, SC.

O'Neill, H. J. and K. M. Kieley. 1992. Polynuclear aromatic hydrocarbons: Atlantic region data summary and review. Technical report 92-01, Conservation and Protection Atlantic Region, Moneton, New Brunswick.

Opitz, R., Hartmann, S., Blank, T., Braunbeck, T., Lutz, I., Kloas, W., 2006. Evaluation of histological and molecular endpoints for enhanced detection of thyroid system disruption in Xenopus laevis tadpoles. Toxicological Sciences 90:337-348.

Osius, N., W. Karmaus, H. Kruse, and J. Witten. 1999. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. Environmental Health Perspectives 107:843-849.

Perez, M. H. and W. G. Wallace. 2004. Differences in prey capture in grass shrimp, Palaemonetes pugio, collected along an environmental impact gradient. Archives of Environmental Contamination and Toxicology 46:81-89.

Porterfield, S. P. 1994. Vulnerability of the Developing Brain to Thyroid Abnormalities: Environmental Insults to the Thyroid System. Environmental Health Perspectives 102:125-130.

Porterfield, S. P. 2000. Thyroidal dysfunction and environmental chemicals - Potential impact on brain development. Environmental Health Perspectives 108:433-438.

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

Power, D. M., L. Llewellyn, M. Faustino, M. A. Nowell, B. T. Bjornsson, I. E. Einarsdottir, A. V. Canario, and G. E. Sweeney. 2001. Thyroid hormones in growth and development of fish. Page 447.

Roegge, C. S., V. C. Wang, B. E. Powers, A. Y. Klintsova, S. Vilareal, W. T. Greenough, and S. L. Schantz. 2004. Motor impairment in rats exposed to PCBs and methylmercury during early development. Toxicological Sciences 77:315-324.

Roth-Härer, A., H. Lilienthal, M. Bubser, U. Kronthaler, W. R. Mundy, T. R. Ward, W. Schmidt, H. Winterhoff, and G. Winneke. 2001. Neurotransmitter concentrations and binding at dopamine receptors in rats after maternal exposure to 3,4,3',4'-tetrachlorobiphenyl: the role of reduced thyroid hormone concentrations. Environmental Toxicology and Pharmacology 9:103-115.

Rozados, M. V., M. D. Andrés, and M. A. Aldegunde. 1991. Preliminary studies on the acute effect of lindane ([gamma]-HCH) on brain serotoninergic system in the rainbow trout Oncorhynchus mykiss. Aquatic Toxicology 19:33-40.

Sandheinrich, M. B. and G. J. Atchison. 1989. Sublethal copper effects on bluegill, *Lepomos macrochirus*, foraging behavior. Canadian Journal of Fisheries and Aquatic Science 46:1977-1984.

Schantz, S. L. and J. J. Widholm. 2001. Cognitive effects of endocrine-disrupting chemicals in animals. Environmental Health Perspectives 109:1197-1206.

Scharf, F. S., J. A. Buckel, F. Juanes, and D. O. Conover. 1998. Predation by juvenile piscivorous bluefish (Pomatomus saltatrix): the influence of prey to predator size

ratio and prey type on predator capture success and prey profitability. Canadian Journal of Fisheries and Aquatic Sciences 55:1695-1703.

Schmidt, K., C. E. W. Steinburg, S. Pflugmacher, and G. Staaks. 2004. Xenobiotic substances such as PCB mixtures (Arochlor 1254) and TBT can influence swimming behavior and biotransformation activity (GST) of carp (Cyprinus carpio) Environmental Toxicology 19:460-470.

Scott, G. R. and K. A. Sloman. 2004. The effects of environmental pollutants on complex fish behavior: integrating behavioral and physiological indicators of toxicity Aquatic Toxicology 68:369-392.

Seegal, R. F., K. O. Brosch, and R. J. Okoniewski. 1997. Effects ofin Uteroand Lactational Exposure of the Laboratory Rat to 2,4,2',4'- and 3,4,3',4'-Tetrachlorobiphenyl on Dopamine Function. Toxicology and Applied Pharmacology 146:95-103.

Shain, W., B. Bush, and R. Seegal. 1991. Neurotoxicity of polychlorinated biphenyls: Structure-activity relationship of individual congeners. Toxicology and Applied Pharmacology 111:33-42.

Shepherd, G. R. and D. B. Packer. 2006. Essential fish habitat source document: Bluefish, Pomatomas saltatrix, life history and habitat characteristics. 2nd edition. NOAA Tech. Mem. NMFS-NE-144.

Sloman, K. A., G. R. Scott, Z. Diao, C. Rouleau, C. M. Wood, and D. G. McDonald. 2003. Cadmium affects the social behaviour of rainbow trout, Oncorhynchus mykiss. Aquatic Toxicology 65:171-185.

Sloman, K. A., D. C. Wilson, and S. Balshine, editors. 2006. Behaviour and physiology of fish. Elsevier, Amsterdam.

Smith, G., A. Khan, J. S. Weis, and P. Weis. 1995. Behavior and brain chemistry correlates in mummichogs (Fundulus heteroclitus) from polluted and unpolluted environments. Marine Environmental Research 39:329-333.

Smith, G. M. and J. S. Weis. 1997. Predator/prey interactions in Fundulus heteroclitus: effects of living in a polluted environment. Journal of Experimental Marine Biology and Ecology 209:75-87.

Smith, S. J., S. J. Iverson, and W. D. Bowen. 1997. Fatty acid signatures and classification trees: new tools for investigating the foraging ecology of seals. Canadian Journal of Fisheries and Aquatic Sciences 54:1377-1386.

Stewart, P., J. Reihman, E. Londy, T. Darvill, and J. Pagano. 2000. Prenatal PCB exposure and postnatal behavioral assessment scale (NBAS) performance. Neurotoxicology and Teratology 22:21-29.

Tietge, J. E., G. W. Holcombe, K. M. Flynn, P. A. Kosian, J. J. Korte, L. E. Anderson, D. C. Wolf, and S. J. Degitz. 2005. Metamorphic inhibition of Xenopus laevis by sodium perchlorate: Effects on development and thyroid histology. Environmental Toxicology and Chemistry 24:926-933.

Tsai, C., T. Jang, and L. Wang. 1995. Effects of mercury on serotonin concentration in the brain of tilapia, *Oreochromis mossambicus*. Neuroscience Letters 184:208-211.

USEPA. 1999a. Guidance for assessing chemical contaminant data for use in fish advisories. Vol. 2. Risk assessment and fish consumption limits, 3rd ed. EPA 823-B-99-008. US EPA, Office of Water, Washington, DC.

USEPA. 1999b. Mercury update: impact on fish advisories. EPA-823-F-99-019. US EPA, Office of Water, Washington, DC.

USEPA. 1999c. Polychlorinated biphenyls (PCBs) update: Impact on fish advisories. EPA-823-F-99-019. US EPA, Office of Water, Washington, DC.

USEPA. 2000. Guidance for assessing chemical contamination data for use in fish advisories. Vol. 2. Risk assessment and fish consumption limits, 3rd ed. EPA 823-B-00-008. Office of Water, Washington, DC.

USEPA. 2001. Mercury update: Impact on fish advisories. EPA-823-F-01-011. US EPA, Office of Water, Washington, DC.

USFDA. 1993. National Shellfish Sanitation Program. Guide for the control of molluscan shellfish. US Food and Drug Administration,Washington, DC

Varanasi, U. and J. E. Stein. 1991. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environmental Health Perspectives 90:93-100.

Webber, H. M. and T. A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (Notemigonus crysoleucas). Environmental Toxicology and Chemistry 22:1556-1561.

Weber, D. N., A. Russo, D. B. Seale, and R. E. Spieler. 1991. Waterborne lead affects feeding neurotransmitter levels of juvenile fathead minnows (Pimephales promelas). Aquatic Toxicology 21:71-80.

Weinand-Härer, A., H. Lilienthal, K.-A. Bucholski, and G. Winneke. 1997. Behavioral effects of maternal exposure to an ortho-chlorinated or a coplanar PCB congener in rats. Environmental Toxicology and Pharmacology 3:97-103.

Weis, J. S. 2002. Tolerance to environmental contaminants in the mummichog, *Fundulus heteroclitus*. Human and Ecological Risk Assessment 8:933-953.

Weis, J. S., G. Smith, T. Zhou, and C. Bass. 2000. Predator/prey interactions: a link between the individual level and both higher and lower level effects of toxicants in the aquatic ecosystems. Journal of Aquatic Ecosystem Stress and Recovery 7:145-153.

Weis, J. S., G. Smith, T. Zhou, C. Bass, and P. Weis. 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. BioScience 51:209-217.

Weis, J. S., T. Zhou, J. Skurnick, and P. Weis. 2003. Evaluating prey capture by larval mummichogs, (Fundulus heterclitus) as a potential biomarker for contaminants. Marine Environmental Research 55:27-38.

Weis, P. and J. T. F. Ashley. 2007. Contaminants in fish of the Hackensack Meadowlands, New Jersey: size, sex, and seasonal relationships as related to health risks. Archives of Environmental Contaminant Toxicology 52:80-89.

Williams, S. D. 2006. Bioaccumulation patterns of organochlorines in young-of-the-year bluefish (Pomatomus saltatrix) in the Hudson River Estuary. . Florida Agricultural and Mechanical University, Tallahasee, Florida.

Zala, S. M. and D. J. Penn. 2004. Abnormal behaviours induced by chemical pollution: a review of the evidence and new challenges. Animal Behavior 68:649-664.

Zhou, T., H. John-Alder, and J. S. Weis. 1999a. Thyroidal status of mummichogs (*Fundulus heteroclitus*) from a polluted vs a reference habitat. Environmental Toxicology and Chemistry 18:2817-2823.

Zhou, T., H. John-Alder, J. S. Weis, and P. Weis. 2000. Endocrine disruption: thyroid dysfunction in mummichogs (*Fundulus heteroclitus*) from a polluted habitat. Marine Environmental Research 50:393-397.

Zhou, T., D. Rademacher, R. Steinpreis, and J. S. Weis. 1999b. Neurotransmitter levels in two populations of larval Fundulus heteroclitus after methylmercury exposure. Comparitive Biochemistry and Physiology Part C 124:287-294.

APPENDICES

APPENDIX 1

Table A.1 Specific PCB congers (BZ-benzene) and pesticides that were analyzed.

PCBs	Pesticides
BZ-8	HCB
BZ-18 [a]	b-BHC
BZ-28 [a]	Lindane
BZ-31	Heptachlor
BZ-44 [a]	Aldrin
BZ-49	Heptachlor Epoxide
BZ-52 [a]	Oxyclordane
BZ-66 [a]	g-Chlordane
BZ-77	o,p'-DDE
BZ-95	a-Chlordane
BZ-99	t-Nonachlor
BZ-101	p,p'-DDE
BZ-105 [a]	o,p'-DDD
BZ-110	Endrin
BZ-118 [a]	Endosulfan II
BZ-126	o,p'-DDT
BZ-128 [a]	Endosulfan Sulfate
BZ-138 [a]	p,p'-DDT
BZ-149	a-BHC
BZ-151	Endosulfan I
BZ-153 [a]	Dieldrin
BZ-156	cis-Nonachlor
BZ-169	p,p'-DDD
BZ-170 [a]	Mirex
BZ-180 [a]	
BZ-183	
BZ-187 [a]	
BZ-194	
BZ-195 [a]	
BZ-206 [a]	
BZ-209 [a]	

[a]PCBs that were used for the Aroclor estimate

APPENDIX 2

Individual bluefish and prey PCB fingerprints

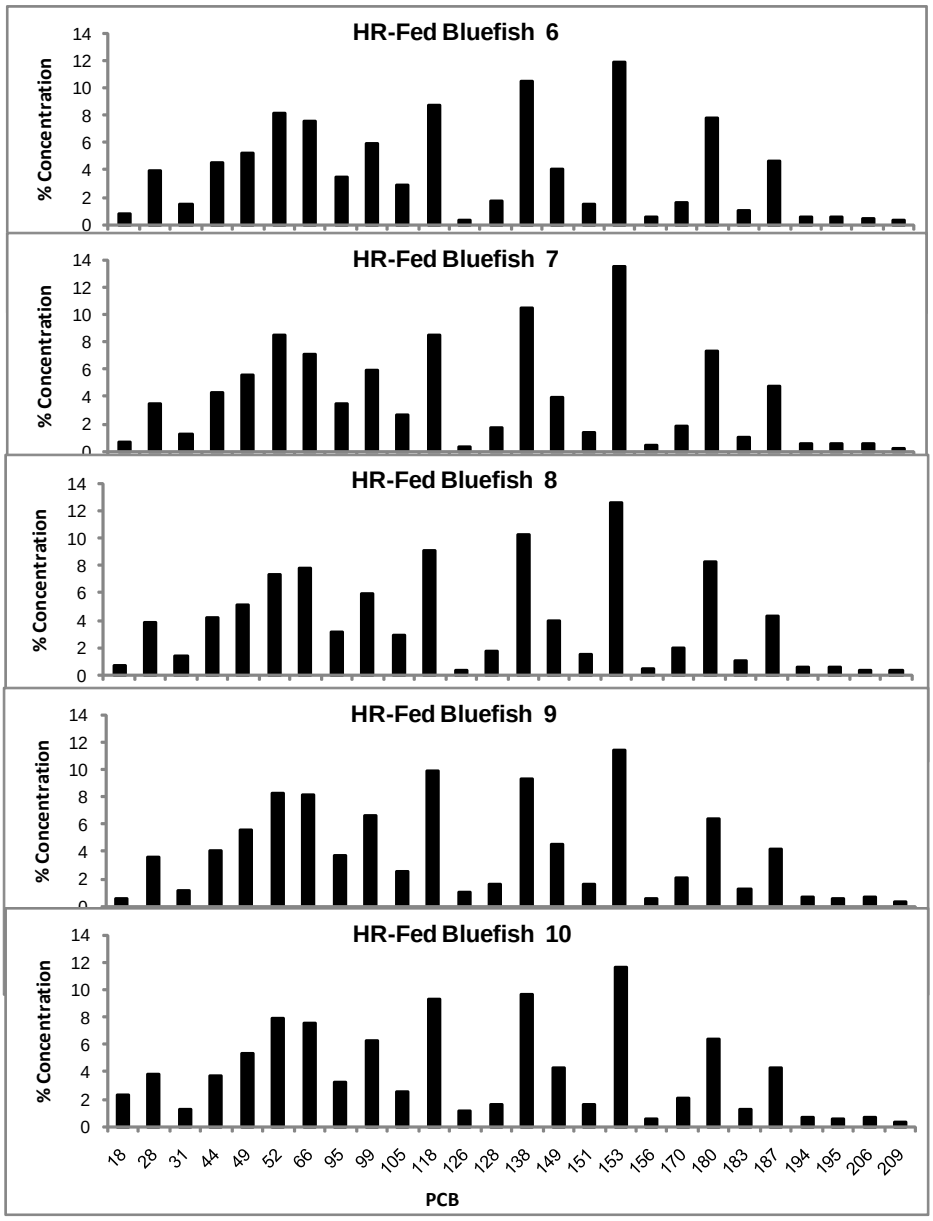

Figure A2.1 PCB fingerprint of individual HR-fed bluefish

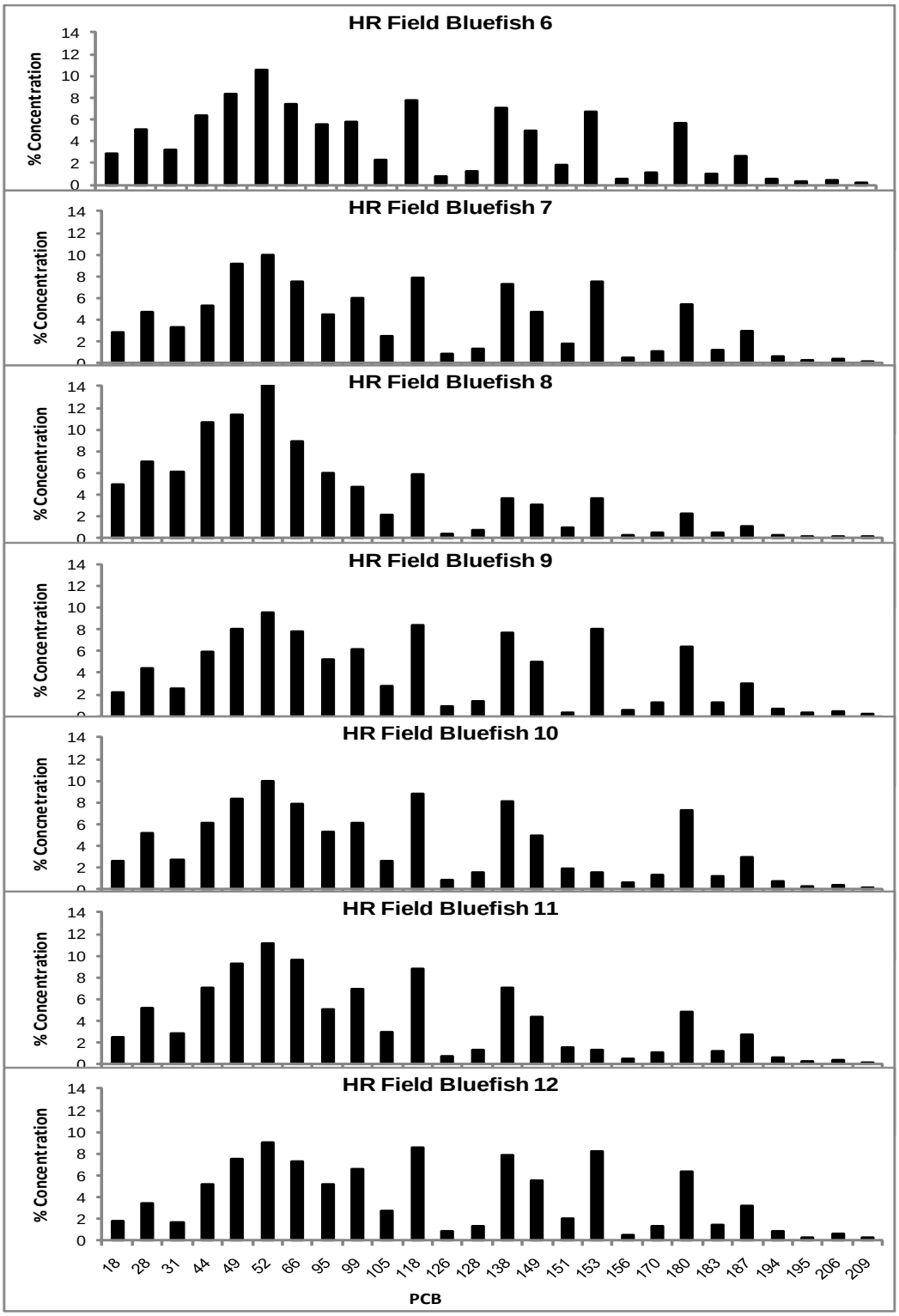

Figure A2.2 PCB fingerprint of individual HR-field bluefish

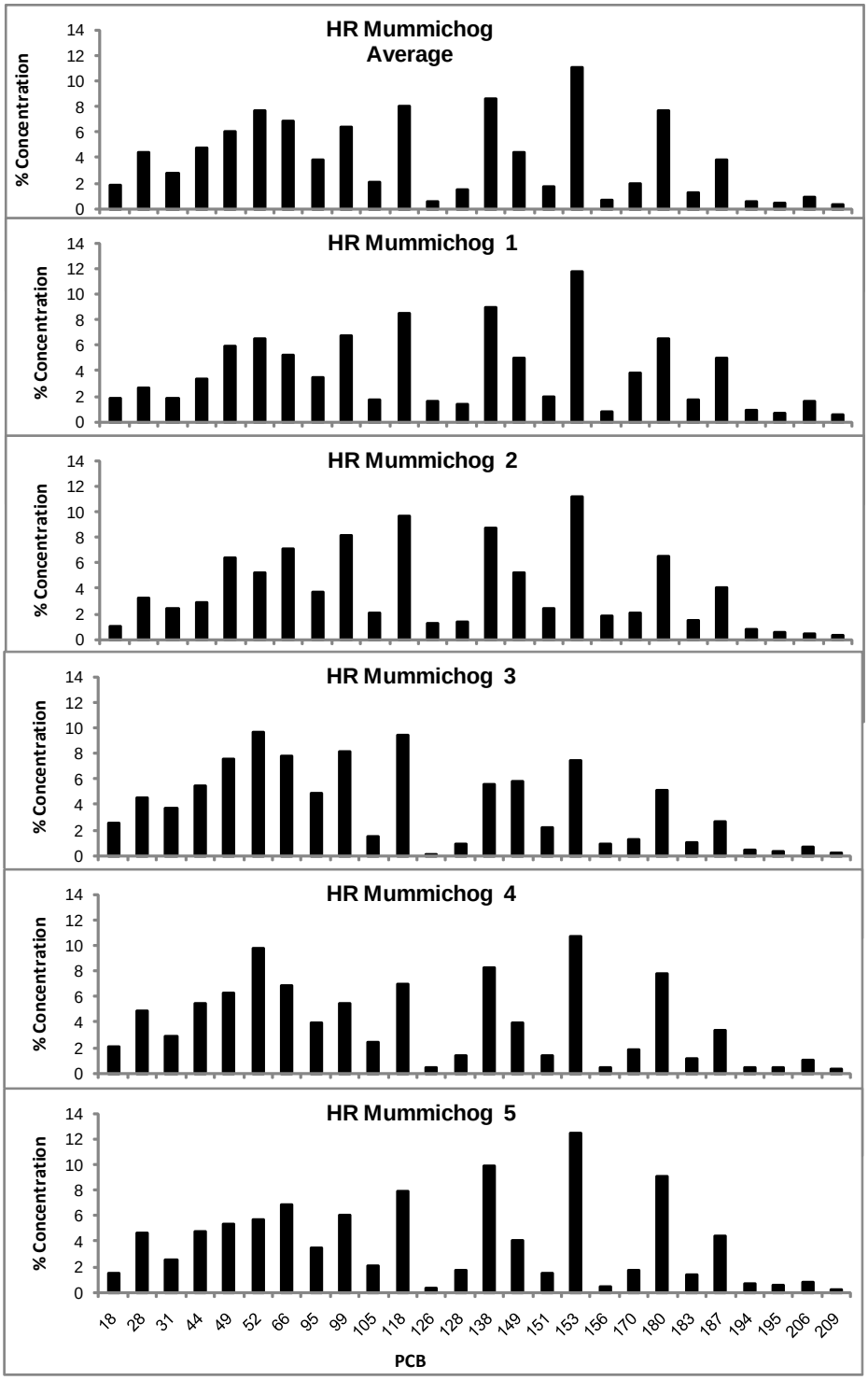

Figure A2.3 PCB fingerprint of individual HR mummichog

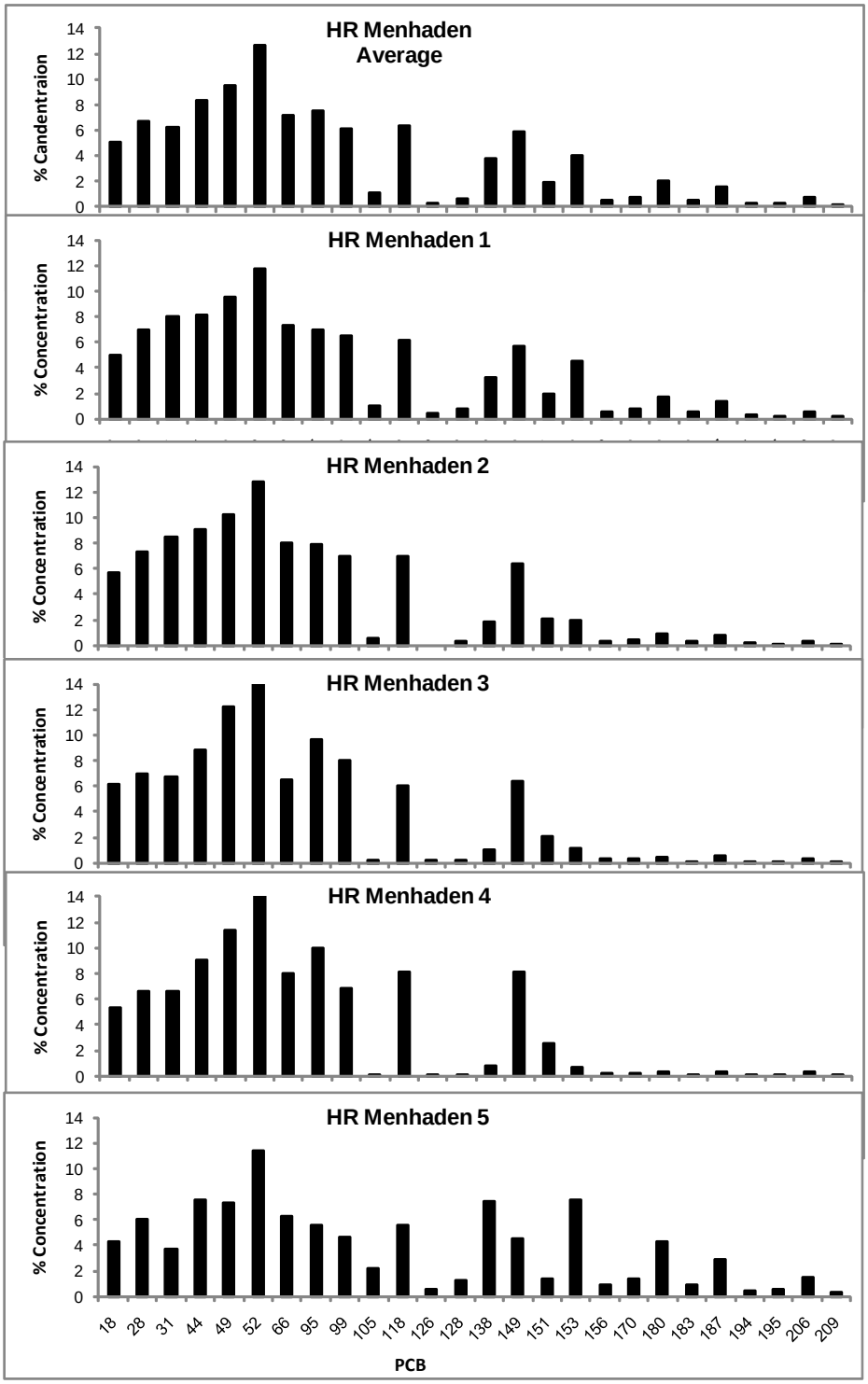

Figure A2.4 PCB fingerprint of individual HR menhaden

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Candelmo, A., V.G. Guida, S. Williams and D. Wieczorek. 2007. Palatability to the Fisheries Ecosystem of the Invasive Tunicate Didemnum sp. Draft Report of the Trophic biochemistry of invasive tunicate(s) in northeastern US Invasive Tunicate Research Project to the Ecosystem Assessment Division, Office of Habitat Conservation, NOAA Fisheries. J.J. Howard Laboratory, NEFSC.12 pp.