EVALUATION OF WATER, SEDIMENT, AND PREY AS ROUTES OF EXPOSURE OF ATLANTIC TOMCOD TO AROMATIC HYDROCARBON POLLUTANTS IN THE HUDSON RIVER

A Final Report of the Tibor T. Polgar Fellowship Program

Allyce E. Nowak

Polgar Fellow

Biology Program
Iona College
New Rochelle, New York 10801

Project Advisors:

Joseph Stabile
Assistant Professor of Biology
Iona College
New Rochelle, New York 10801

Isaac Wirgin
Associate Professor of Environmental Medicine
New York University School of Medicine
Tuxedo, New York 10987

Nowak, A., Stabile, J., and Wirgin, I. 2001. Evaluation of water, sediment, and prey as routes of exposure of Atlantic tomcod to aromatic hydrocarbon pollutants in the Hudson River. Section I:25pp. In J.R. Waldman & W.C. Nieder (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2000. Hudson River Foundation, NY.

ABSTRACT

The Hudson River ecosystem is contaminated with organic pollutants such as dioxins (PCDDs), furans (PCDFs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). These xenobiotics are lipophilic and concentrate in organic particles in the water column that eventually settle to the benthos where they may accumulate to high levels. As a result, benthic fauna are often chronically exposed to high levels of aromatic hydrocarbon pollutants by a combination of dermal contact with contaminated sediments, water-borne absorption through the gills and skin, and gastrointestinal tract ingestion of contaminated prey. Yet, the relative importance of each of these routes of exposure to contaminants in environmentally-exposed benthic fish has not been investigated and will have important implications in designing monitoring and remediation programs and in evaluating the tissue-specific toxicity of these contaminants.

Studies have demonstrated that Atlantic tomcod, *Microgadus tomcod*, is a sensitive sentinel species to Hudson River contaminants. The Atlantic tomcod population within the Hudson River bioaccumulates high levels of organic pollutants as demonstrated by their elevated levels of hepatic PCDDs, PCDFs, PCBs, and PAH metabolites in bile, hepatic DNA adducts, and activation of the K-*ras* oncogene. Furthermore, the unusually high prevalence of hepatic tumors in tomcod livers is probably due to their exposure to mixtures of these and other pollutants that are found in the Hudson River sediments.

Our hypothesis is that prey serves as the primary route of exposure in Atlantic tomcod to aromatic hydrocarbon pollutants in the Hudson River. Our results show that CYP1A1 levels were very low for all routes of exposure. Prey may very well be the primary route of exposure, but due to high water temperatures and the possibility that the Atlantic tomcod did not eat while in the treatment groups, our results were inconclusive.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

The Hudson River is one of the best areas to study the effects of pollutants on aquatic biota. Aquatic ecosystems can be very sensitive to environmental contaminants because both water soluble and insoluble xenobiotics, or pollutants, can easily accumulate in high concentrations. This also holds true with lipophilic, or lipid loving contaminants, which concentrate in both organic and inorganic matter in the water and then settle to the bottom and aggregate with the organic, carbon-rich sediment. There these lipophilic pollutants are often resistant to physical, chemical, and biological degradation and can stay in the sediment for extended periods of time. Because these pollutants can persist in the environment, there can be numerous effects on the biota in these contaminated areas (Wirgin and Waldman 1998). This study was designed to determine the routes of exposure and to evaluate the effects of lipophilic contaminants on the Atlantic tomcod, *Microgadus tomcod*, from the Hudson River.

The Atlantic tomcod was chosen for this experiment because it is an excellent sentinel species. Atlantic tomcod are benthic fish and are exposed to the lipophilic contaminants that have settled into the Hudson River sediment. They also feed upon many benthic and potentially contaminated prey species. Atlantic tomcod are confined to their natal rivers. The Hudson River Atlantic tomcod migrate and complete their entire lifecycle in the Hudson River estuary (Klauda et al. 1988). Since they are confined to one river, there is little chance that the Atlantic tomcod would have been exposed to pollutants in other rivers. Atlantic tomcod also have high lipid content in their livers which tend to show the effects of lipophilic contaminants (Cormier et al. 1989). Hudson River Atlantic tomcod have high incidences of neoplastic liver tumors (Wirgin and

Waldman 1998). Other general reasons to use the Atlantic tomcod as a sentinel species are because they are very common in large estuaries, they are easy to maintain in the laboratory, and there have been previous studies on Atlantic tomcod and the effects of pollutants.

There are four types of organic pollutants found in the Hudson River. They are polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzo furans (PCDFs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) (O'Connor et al. 1982). The major source of PCDDs and PCDFs found in the Hudson River was a chemical plant on the Passaic River in New Jersey. This plant produced a herbicide from 1948 to 1969 that was the major component of Agent Orange (Bopp et al. 1991). As for the PAHs, there is no major point source. PAHs come from smoke, petroleum combustion, wood preserving plants, paint manufacturing, sewage plants, and many other industries. The PCBs found in the Hudson River have two major point sources, two General Electric plants located just north of Troy, New York. PCBs are found in a declining gradient in the Hudson River from Troy to the Battery at the southern tip of New York City (Feng et al. 1998).

All of these pollutants fall under the category of aromatic hydrocarbons, or AHs. Aromatic hydrocarbon pollutants induce the production of cytochrome P450 (CYP1A1) in Atlantic Tomcod. CYP1A1 is critical in the activation and detoxification of some aromatic hydrocarbon pollutants. The process of induction initiates or increases the production of an enzyme or other protein, in this case CYP1A1, at the level of gene transcription or translation. For CYP1A1 to be induced, the aromatic hydrocarbons must first bind to a receptor. These receptors are called aromatic

hydrocarbon receptors, or AhRs and are found in Atlantic tomcod cells. The receptor is a soluble, intracellular protein that regulates the induction of cytochrome P4501A. As shown in Figure 1, the AhR recognizes and intracellularly binds the aromatic hydrocarbon compounds. These aromatic hydrocarbons, or ligands, bind to the AhR in the cytoplasm of the cell and force two molecules of heat shock protein 90 to be released along with a chaperone molecule. Then the ligand bound receptor is carried into the nucleus of the cell where it binds to a transcription factor aromatic receptor nuclear translocator (ARNT). This binding activates the AhR complex and the complex binds with enhancer elements in the promoter region on the CYP1A1 gene. The CYP1A1 gene is a biomarker, or a gene that measures an actual biological effect. It is this biomarker that is used to quantify the effect of aromatic hydrocarbons on the Atlantic tomcod.

Once CYP1A1 is induced in the Atlantic tomcod cells, it metabolizes the PAHs into reactive electrophiles which can either be eliminated from the body when they conjugate with other cellular molecules, or they can react with cellular DNA to form DNA adducts which can eventually lead to DNA mutations (Wirgin and Waldman 1998). It is because of these DNA mutations that it is critical to know the routes of exposure and how the contaminants can affect a species.

Figure 2 (Yuan et al. 2001) shows the level of CYP1A1 expression in Atlantic tomcod in previous experiments from River Mile 0 to River Mile 90. Some of the highest levels of CYP1A1 induction are found at the tip of New York. Previous studies on the Atlantic tomcod have shown that it is indeed a sensitive indicator of environmental

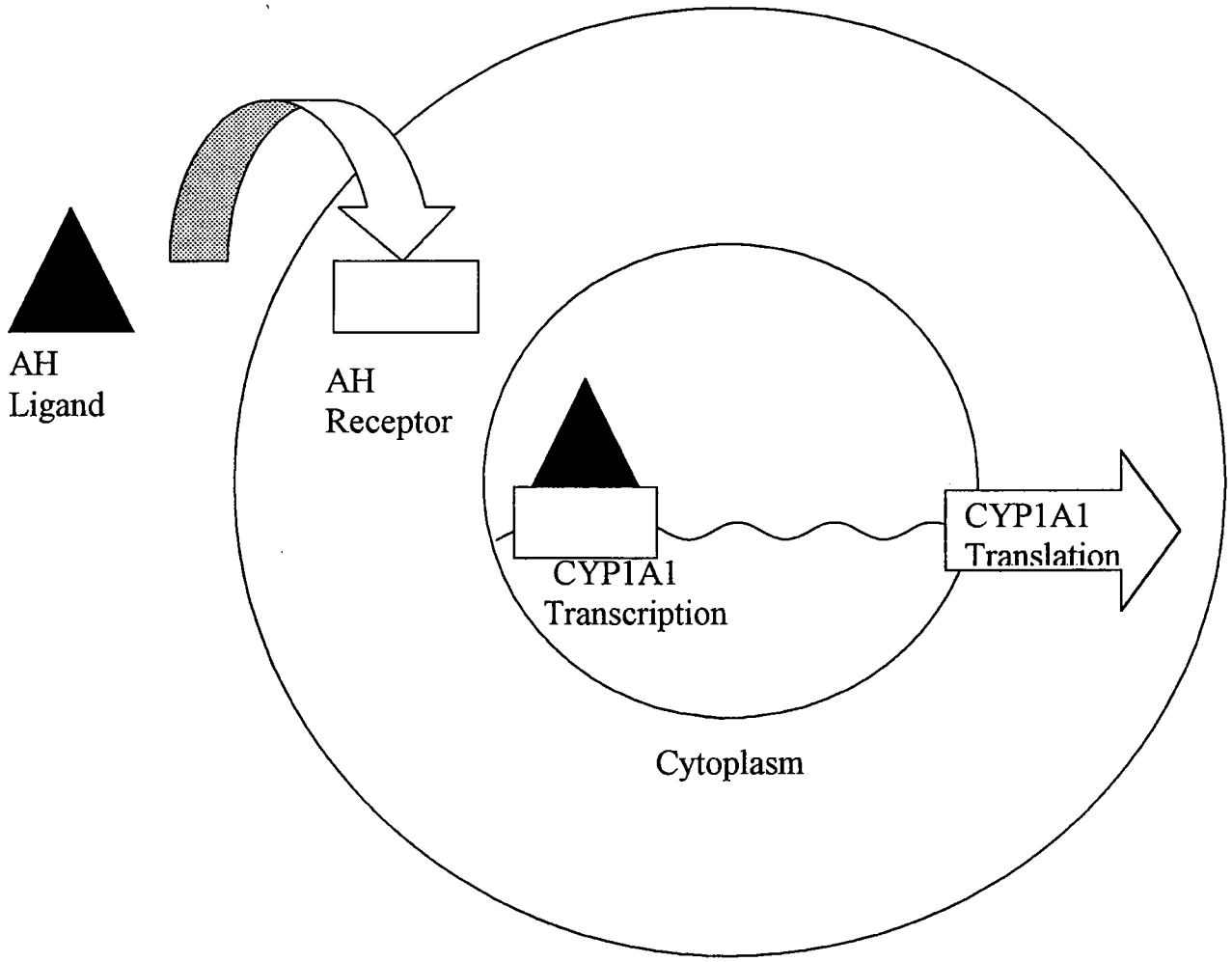

Figure 1. Process of CYP1A1 expression

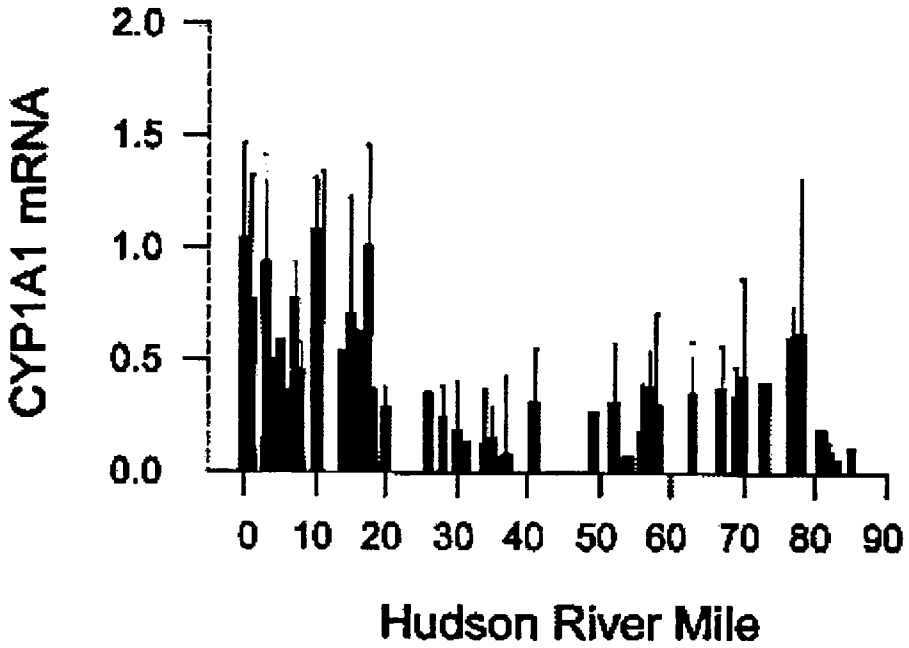

Figure 2. Levels of CYP1A1 induction in the Hudson River from River Mile 0-90 (Yuan et al. 2001)

quality (McLaren et al. 1988). Figure 3 shows the number of hepatic DNA adducts in five different rivers. The high prevalence of DNA adducts in Atlantic tomcod show that the levels of CYP1A1 are high enough to react with cellular DNA to form adducts. The Margaree is the most pristine river (Figure 3) and the Hudson River is the least pristine (Wirgin et al. 1994). There is a relatively high number of hepatic DNA adducts found in the Miramichi River Atlantic tomcod because of dioxins that are released from a paper mill along the Miramichi River. There are also very high numbers of hepatic DNA adducts in the Hudson River Atlantic tomcod, an occurrence that is consistent with the high levels of PAHs and other aromatic hydrocarbon pollutants found in the Hudson River.

Due to the fact that these xenobiotics tend to settle in the Hudson River sediment, the aquatic biota is exposed to these high levels of lipophilic contaminants. The Atlantic tomcod can be exposed to these lipophilic contaminants through dermal contact with the sediment, water-borne absorption through the skin and gills, and through the gastrointestinal tract via contaminated prey. Though it is possible for contaminants to be absorbed through any of these routes, the primary route of exposure has not been determined. The objective of this study is to determine the primary route of exposure of aromatic hydrocarbon pollutants in Atlantic tomcod. Because the xenobiotics accumulate in the sediment, and the Atlantic tomcod feed on benthic prey, our hypothesis was that the prey, as opposed to the water or the sediment, would be the primary route of pollutant exposure in the Atlantic tomcod. This hypothesis was based on the process of biomagnification. As the xenobiotics move up the food chain, they will accumulate in

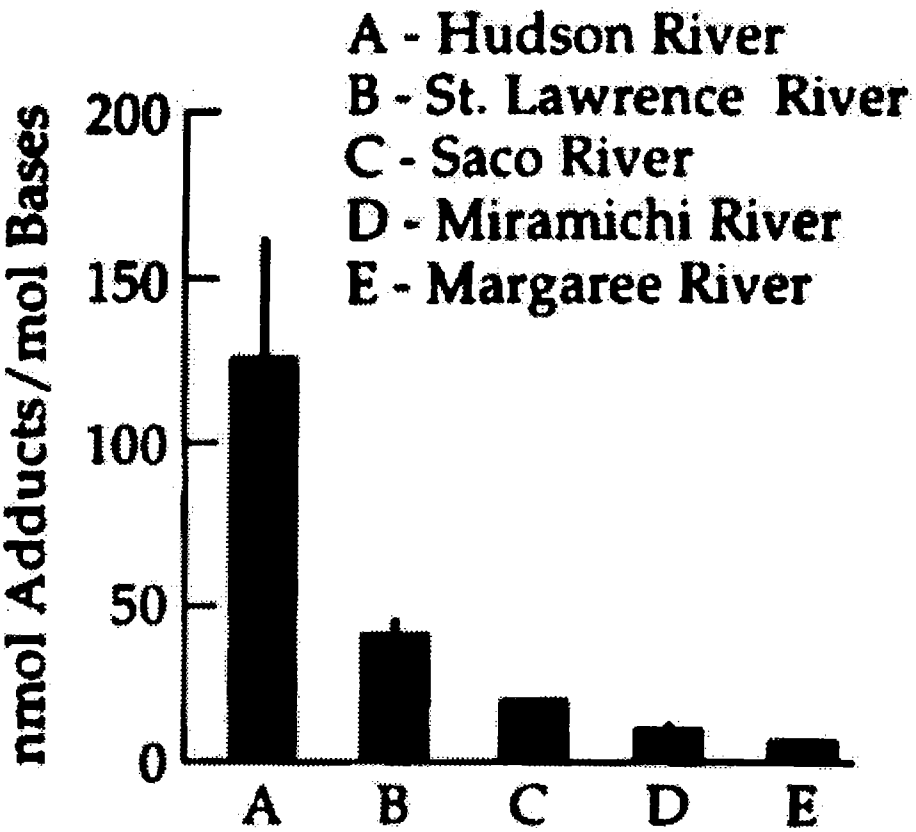

Figure 3. Number of hepatic DNA adducts in five rivers (Wirgin and Waldman 1998)

higher concentrations in the predatory animals. To determine the route of exposure, samples of gills, liver, intestine, and skin of juvenile Atlantic tomcod were collected from fish that were exposed under controlled conditions, to contaminated Hudson River water, sediment, and prey individually and in combination.

METHODS

Experimental Design

Atlantic tomcod Samples

All of the sample treatments and one sample collection of Atlantic tomcod were made at the River Project. The River Project is a non-profit organization that is dedicated to public education and scientific research on the ecology of the Hudson River. The River Project is located at Pier 26 on the lower west side of Manhattan at River Mile 2. The River Project allowed us to set up and maintain all of our treatment tanks on site.

Approximately two hundred non-exposed, or naive, juvenile Atlantic tomcod were obtained from Dr. Chris Chambers from the National Marine Fisheries Service (NMFS) in Sandy Hook, New Jersey. These juvenile Atlantic tomcod were the F_1 progeny of Hudson River parents that had been collected off the coast of Garrison, New York and mated in January of 2000. All of the Atlantic tomcod used in experimental treatments were these juvenile naive F_1 progeny. In addition, 23 of these F_1 progeny Atlantic tomcod were put into a killitrap and lowered off the edge of the River Project Pier and were used as a positive control. Also, 25 Hudson River Atlantic tomcod were caught off of the River Project Pier with unbaited killitraps. These Atlantic tomcod served as another positive control, or environmentally-exposed tomcod.

Treatments

Between 23 and 25 F_1 progeny, juvenile Atlantic tomcod were allotted into eight treatment tanks, each containing three matrices. There were individual treatments, combination treatments, and one negative control. Every combination of clean and Hudson River water, clean and Hudson River sediment, and market and Hudson River shrimp were used. Combinations of matrices are shown in Table 1. For the individual

Tank Number	Water	Sediment	Prey
Individual Treatments			
1	Clean	Clean	Clean
2	Hudson River	Clean	Clean
3	Clean	Hudson River	Clean
4	Clean	Clean	Hudson River
Combination Treatments			
5	Hudson River	Hudson River	Clean
6	Clean	Hudson River	Hudson River
7	Hudson River	Clean	Hudson River
8	Hudson River	Hudson River	Hudson River

Table 1. Experimental Treatment Matrix Setups

treatments, the tanks only contained one source of Hudson River pollutant, while the combination tanks contained two or three. Hudson River water was pumped in from under the River Project pier into the River Project laboratory into the tanks that required

Hudson River water. All the tanks that required Hudson River sediment were lined with sediment that was collected right on site at the River Project with bottom grabs. Past studies have shown that environmentally-exposed Atlantic tomcod collected from the River Project site have had high levels of CYP1A1 expression. Reports show that sites along the Hudson River exhibit some of the highest PAH concentration of any U.S. estuary (NOAA Technical Memorandum 1987). For this reason it was assumed that sediment collected from the River Project would have high levels of PAHs. The clean sediment came from Quogue Canal in eastern Long Island, New York. The Hudson River shrimp was collected in killitraps on site at the River Project. The clean shrimp was purchased at a local market. Atlantic tomcod were fed approximately one square inch cubes of frozen shrimp, and about the same amount of fresh shrimp. The clean water was New York City tap water that was dechlorinated overnight, and then treated with Instant Ocean to obtain a salinity between 20 and 22 parts per thousand. The Atlantic tomcod juveniles were kept in these treatment tanks for approximately twenty days and then sacrificed.

One tank served as a negative control. It contained clean water, clean sediment, and market shrimp. There were also two positive controls. One consisted of wild, environmentally exposed Atlantic tomcod collected at the River Project. The other positive control was a group of F_1 progeny that were reintroduced into the Hudson River at the River Project in closed off cages for about twenty days. These cages had been used to collect tomcod in the past as well as shrimp and were suspended in the water.

Treatment tanks were kept in two large basins on the floor of the River Project. There were four treatment tanks in each basin. The two basins had a flow through system

in which Hudson River water was constantly circulated thereby cooling the treatment tanks. By pumping water straight from the Hudson River the temperature of the water in the treatment tanks was as close as possible to the temperature of the Hudson River on a particular day. The water ideally should have remained between 17 and 19°C. But due to a heat wave, a chiller was placed in one of the basins and the water surrounding the treatment tanks was circulated. But the chiller could only lower the temperature of such a volume of water to approximately 22°C, and the flow through system was replaced.

As for the treatment tanks that required clean water, one fourth of the clean water was changed approximately every three days in order to keep the ammonia levels low. The pH of the clean water and the Hudson River water treatment tanks was taken every day to assure that the pH levels were stable. The temperature of each treatment tank was also recorded daily. Also, all of the treatment takes were aerated to provide the Atlantic tomcod with a constant supply of oxygen.

Tissue Sample Collection

The liver, gills, intestine, and skin near the mouth were collected for the negative and two positive controls. For all the other treatment tank fish, only the livers were collected. The RNA isolated from the livers served as a positive control because previous studies have shown that hepatic CYP1A1 is highly induced in Hudson River juvenile Atlantic tomcod. Once the tissue samples were collected, they were snap frozen in dry ice and stored at -80 degrees Celsius until processed.

Tissue Sample Processing

The RNA of all the tissue samples was isolated using the Ultraspec RNA Isolation system (Biotecx). All of the livers were isolated first, followed by the gills, intestine, and skin. The RNA was then purified and its concentration determined by UV spectrophotometry. Then the integrity of the RNA was evaluated through Northern Gel analyses. Only the RNA samples that exhibited intact 18S and 28S rRNA bands were used for gene expression analysis. Then the CYP1A1 mRNA expression was quantified by using dot blot analyses. The RNA was then directly applied to a membrane and hybridized to a P^{32} radiolabeled CYP1A1 probe. The membrane was then stripped of the CYP1A1 probe and then re-hybridized with P^{32} to quantify 18SrRNA levels. This process of normalization to a housekeeping gene allows for better sample analysis because the optical density, O.D., of the18SrRNA is used to normalize the amount of RNA in each dot of the dot blot. Therefore the O.D. of CYP1A1 accurately reflects the actual expression of CYP1A1 and not the difference in the RNA loaded per sample. This membrane was then exposed to a phosphoimager screen and then scanned (Molecular Dynamics STORM 860 Scanner Control). The level of CYP1A1 induction was quantified by densitometry (Molecular Dynamics Image Quant). Statistical analyses were performed using a one way ANOVA.

RESULTS

Figure 4 shows the level of hepatic CYP1A1 induction for all of the treatment groups normalized for 18SrRNA. Table 2 is the legend for Figure 4 and explains the

abbreviations used in the graph. These results show that there was little to no induction via any combination of Hudson River water, sediment, and prey. Also, there was no statistically significant difference between and among the treatment and control groups. The negative control showed an optical density of approximately 0.19, while the treatment group containing all Hudson River matrices showed approximately 0.16. The treatment group containing only the Hudson River prey had an optical density of 0.08. The environmentally-exposed Atlantic tomcod showed a value of 0.30, while the Atlantic tomcod sampled from the Hudson River showed an optical density of 0.28.

Figure 4 also contains standard error bars to determine the error produced in each of these analyses. Standard error bars do not only show the maximum error, but also the minimum error. To determine the minimum error, one has to look at the standard error bars as extending downwards. In this case the data suggests that there is minimum CYP1A1 induction in the positive control group 9 of environmentally exposed Atlantic tomcod, as well as minimum CYP1A1 induction in the positive control group 10 of re-exposed Atlantic tomcod. Taking this data into account, all of the treatment groups show very little to no CYP1A1 induction at all. Levels can range from an optical density of 0 to 0.1.

DISCUSSION

The primary result of this experiment was that there were no differences among and between treatment groups and controls. It was expected that the treatment tanks

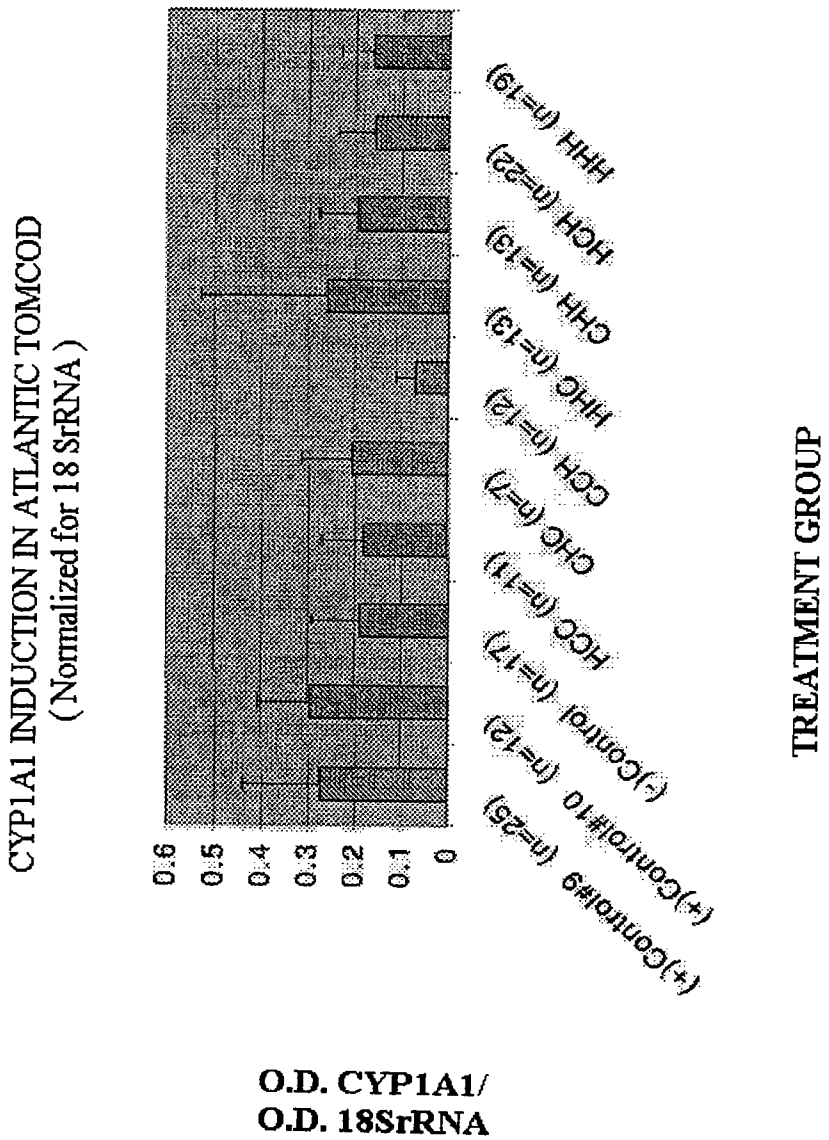

Figure 4. Levels of hepatic CYP1A1 induction with standard error bars

+ Control #9	Environmentally-Exposed Atlantic tomcod
+ Control #10	Re-exposed Atlantic tomcod
- Control	Clean Water, Clean Sediment, and Clean Prey
HCC	Contaminated Water, Clean Sediment, and Clean Prey
CHC	Clean Water, Contaminated Sediment, and Clean Prey
CCH	Clean Water, Clean Sediment, and Contaminated Prey
HHC	Contaminated Water, Contaminated Sediment, and Clean Prey
CHH	Clean Water, Contaminated Sediment, and Contaminated Prey
HCH	Contaminated Water, Clean Sediment, and Contaminated Prey
HHH	Contaminated Water, Contaminated Sediment, and Contaminated Prey

Table 2. Legend for hepatic CYP1A1 induction in Atlantic tomcod

containing Hudson River prey would show the highest levels of CYP1A1 expression. But the treatment tank containing Hudson River prey did not show higher levels of CYP1A1 expression compared to other treatment tanks containing non-contaminated prey. If anything, the contaminated prey treatment groups showed the lowest levels of CYP1A1 induction as compared to treatment groups containing polluted sediment and water. Levels of CYP1A1 induction were so low that it is likely that there was no induction at all and the resulting levels of CYP1A1 induction found in the Hudson River Atlantic tomcod stemmed from inter-individual variation. Results suggest that the Hudson River is in fact *not* polluted enough to induce CYP1A1, there were problems with experimental design, or that CYP1A1 should not be used as a biomarker of pollution levels in the Hudson River. It is unlikely that the Hudson River is not polluted due to reports on the level of pollutants published in the NOAA Technical Memorandum and due to other studies performed on Hudson River Atlantic tomcod and the levels of CYP1A1 induction, such as Yuan et al. (1992) and Warren (2001).

Throughout this experiment there were several factors that may have contributed to the very low levels of CYP1A1 expression in all treatment tanks and controls. Firstly, although the juvenile Atlantic tomcod seemed to have grown in size, there was no way to be certain that they were actually feeding upon the contaminated prey. The Atlantic tomcod were measured after sacrificing but not before. Also, there was some evidence of cannibalism on dead tomcod in the tanks.

Secondly, the environmentally exposed Atlantic tomcod caught in killitraps from the Hudson River were held by River Project volunteers until it was possible to sacrifice them. The Atlantic tomcod were held for approximately six days before sacrificing. PAHs metabolize quickly and for this reason the PAHs may have been depurated in the few days the Atlantic tomcod were kept in the cages.

Another reason CYP1A1 induction levels may have been very low is due to the high mortality experienced by the fish. Out of the approximately 23 fish put in each experimental tank, only a portion of the fish in each treatment tank survived and were sacrificed for use in tissue processing. High mortality may stem from the tomcod not consuming prey, cannibalism, and environmental stress.

Optimally, the treatment tanks should be at 17°C, but due to a heat wave, temperatures varied between 19° and 25°C. Efforts to keep the treatment tank water cool with a chiller were not successful and the elevated water temperatures may have caused the high mortality rates in the Atlantic tomcod. The Hudson River is the southernmost spawning ground for the Atlantic tomcod species. The Atlantic tomcod are adapted to cooler temperatures. An increase in temperature of the Hudson River water may have been enough to produce high mortality rates. High water temperatures may not have only

lead to high mortality but may also be the reason for very low CYP1A1 expression. If the Atlantic tomcod were environmentally stressed due to high temperatures, the CYP1A1 pathway may have shut down. A previous study by Courtenay et al. (1994) concluded that an increase in temperature did not affect CYP1A1 expression, but his study was conducted on Atlantic tomcod in water temperatures ranging from 0 to 10°C. Another study by Kloepper and Stegeman (1992) on *Fundulus heteroclitus* showed that temperature did not have an effect on CYP1A1 expression. But again, their experiments dealt with water temperatures between 6° and 16°C. In both of these studies the fish were kept in water much cooler than the water our juvenile Atlantic tomcod in this experiment. We do not discount that the extremely high water temperatures in the treatment groups led to lowered CYP1A1 induction. There have not been enough studies conducted on elevated temperatures and the effects on CYP1A1 expression to confirm or deny this. A single run of each treatment group and each control was performed. A second run would have been performed, if time allowed. Two treatment groups, the negative control and the group containing clean water, Hudson River sediment, and clean prey, had to be repeated because all the Atlantic tomcod died. Also, the environmentally exposed positive control had to be redone due to the fact that all of the tomcod escaped from the killitrap. When these fish were sacrificed, there may have been very low levels of CYP1A1 induction due to the fact that the Atlantic tomcod may have not been able to feed on prey while in the traps.

Another reason CYP1A1 induction varied among treatment groups may be due to inter-individual variation. The Atlantic tomcod from the Hudson River are a genetically varied outbred population. This genetic variation would allow some tomcod to have a

higher susceptibility to the pollutants than others. Some Atlantic tomcod may have had genetic differences that might have led to low levels of CYP1A1 induction. In Figure 1 the high error bars indicate high levels of genetic variability among Atlantic tomcod from the Hudson River. This inter-individual variation allows for some slight CYP1A1 induction. This induction is very negligible and should not be considered CYP1A1 induction at all since it stems from genetic variability. The positive control Atlantic tomcod caught in traps from the Hudson showed slightly higher levels of CYP1A1 induction compared to the tomcod in the experimental treatment groups, possibly due to the fact that these fish were from an outbred population in the Hudson River and not from the lab reared fish used in the treatment groups. This may have been a factor for these differences, but this is doubtful due to the fact that the tomcod were subject to very high water temperatures.

If this experiment were repeated it would be necessary to make changes in the experimental design. One of the most important changes in experimental design would be to regulate the water temperature of the treatment tanks. The ability to keep the temperature of the water low may result in a lower mortality rate, an increase in appetite, and an increase in CYP1A1 expression. To determine whether or not the Atlantic tomcod were consuming prey items, an analysis of stomach contents would also be beneficial. By analyzing the stomach contents it could be determined if the environmentally exposed Atlantic tomcod in killitraps were feeding or not. Also, having liquid nitrogen and dry ice available would be advantageous. This would allow the Atlantic tomcod from the Hudson River to be frozen immediately after they were caught. By freezing the tissue immediately, there would be less time for the PAHs to depurate.

Although there were flaws in the experimental design, an unanticipated heat wave, and data showing low levels of CYP1A1 induction, this experiment was among the first to lay a foundation for future work, quantify the effects of different routes of exposure on the level of AH contaminants, and the first using environmentally relevant routes of exposure. With improvement to the experimental design and under more controlled conditions, an experiment of this nature would be worthwhile to try again.

ACKNOWLEDGMENTS

I would like to thank the Chuck Nieder, John Waldman, and the Hudson River Foundation for granting me this opportunity to participate in the Tibor T. Polgar Fellowship Program. Thank you to the technical staff at the Nelson Institute of Environmental Medicine who assisted me this past summer. Thank you to Dr. Chris Chambers of the National Marine Fisheries Reserve for supplying us with all the juvenile Atlantic tomcod, and to the River Project staff; Director Cathy Drew, Tim Parsons, Sarah Lindrop, Anu Carroll, Teldert Welch, and Xiu Mei Dong. I would like to thank my two advisors, Drs. Isaac Wirgin and Joseph Stabile, for all their guidance and patience. And to Nyasha Warren, thank you for teaching me invaluable skills and procedures. I would also like to thank Dr. Charles Sackerson and Br. Novak for reviewing and commenting on my paper.

REFERENCES

Bopp R.F., M.L. Gross, H. Tong, H.J. Simpson, S.J. Monson, B.L. Deck, and F.C. Moser. 1991. A major incident of dioxin contamination: sediments of New Jersey estuaries. Environ. Sci. Technol. 25:951.

Cormier S.M., R.N. Racine, C.E. Smith, W.P. Dey, and T.H. Peck. 1989. Hepatocellular carcinoma and fatty infiltration in the Atlantic tomcod, *Microgadus tomcod* (Walbaum). J Fish Dis. 12:105.

Courtenay S, P.J. Williams, C. Grunwald, B. Konkle, T.L. Ong, and I. Wirgin. 1994. Assessment of within-group variation in CYP1A1 mRNA inducibility in environmentally exposed and chemically treated Atlantic tomcod. Environ. Health. Perspect. 102(Suppl 12):85.

Feng H, J.K. Cochran, H. Lwiza, B.J. Brownawell, and D.J. Hirschberg. 1998. Distribution of heavy metal and PCB contaminants in the sediments of an urban estuary: the Hudson River. Mar. Environ. Res. 45:69.

Klauda R.J., R.E. Moos, and R.E. Schmidt. 1988. Life history of Atlantic tomcod, *Microgadus tomcod*, in the Hudson River estuary, with emphasis on spatio-temporal distribution and movements. Pages 219-251 in C.L. Smith, editor. Fisheries Research in the Hudson River. State University of New York Press, Albany.

Klopper-Sams, P.J., and J.J. Stegmann. 1992. Effects of temperature acclimation on the expression of hepatic cytochrome P4501A mRNA and protein in the fish *Fundulus heteroclitus*. Archives of Biochemistry and Biophysics 38.

McLaren J.B., T.H. Peck, W.P. Dey, and M. Gardinier. 1988. Biology of Atlantic tomcod in the Hudson River estuary. Pages 102-112 in Barnthouse L.W., R.J. Klauda, D.S. Vaughan, R.L. Kendall, editors. Science, Law, and Hudson River Power Plants. Am. Fish Soc. Monogr. 4.

NOAA Technical Memorandum NOS OMA 44. 1988. National status and trends program for marine environmental quality: Progress report: a summary of selected data on chemical contaminants in sediments collected during 1984-1987. Rockville MD, NOAA.

O'Connor J.M., J.B. Klotz, and T.J. Kneip. 1982. Sources, sinks, and distribution of organic contaminants in the New York Bight in G.F. Mayer, editor. Ecological Stress and the New York Bight: science and management. Estuarine Research Foundation, Columbia.

Warren N. 2001. Evaluation of water, sediment, and prey as routes of exposure of Atlantic tomcod (*Microgadus tomcod*) to aromatic hydrocarbon pollutants in the Hudson River. Master's thesis. New York University, New York, New York.

Wirgin I.I., C. Grunwald, S. Courtenay, G.L. Kreamer, W.L. Reichert, and J.E. Stein. 1994. A biomarker approach to assessing xenobiotic exposure in Atlantic tomcod from the North American Atlantic coast. Environ. Health Perspect. 102:764.

Wirgin I.I. and Waldman J.R. 1998. Organismic responses to contaminated aquatic systems: Four case histories from the Hudson River. Pages 1563-1579 in W.N. Rom, editor. Environmental and Occupational Medicine. Lippincott-Raven Publishers, Philadelphia.

Yuan Z., M. Wirgin, S. Courtenay, M. Ikonomou, and I. Wirgin. 2001. Is hepatic cytochrome P4501A1 expression predictive of hepatic burdens of dioxins, furans, and PCBs in Atlantic tomcod from the Hudson River estuary? Aquatic Toxicology (in press).