A FEASIBILITY STUDY OF THE POPULATION STRUCTURE AND HABITAT USAGE OF WINTER FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS*) IN THE HUDSON RIVER ESTUARY INVESTIGATED THROUGH OTOLITH MICROCHEMISTRY

A Final Report of the Tibor T. Polgar Fellowship Program

George W. Jackman

Polgar Fellow

CUNY Graduate Center
365 Fifth Avenue
New York, NY 10016-4309

Project Co-Advisors:

John Waldman
Queens College
65-30 Kissena Blvd
Flushing, NY 11367

Karin E. Limburg
SUNY ESF
1 Forestry Drive
Syracuse, NY 13210

Jackman, G.W., J. Waldman, and K.E. Limburg. 2011. A Feasibility Study of the Population Structure and Habitat Usage of Winter Flounder (*Pseudopleuronectes americanus*), in the Hudson River Estuary Investigated through Otolith Microchemistry. Section I: 36 pp. In. D.J. Yozzo, S.H. Fernald, and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2009. Hudson River Foundation.

ABSTRACT

A preliminary investigation was conducted to determine the feasibility of using otolith microchemistry as a stock discrimination method, and as a means to unravel pertinent life history questions relating to winter flounder (*Pseudopleuronectes americanus*), in the Hudson River Estuary (HRE). Previous studies have demonstrated that trace elements absorbed by fish from the environment can be used as site-specific signatures, which can be utilized as natural habitat tags to track the movement of winter flounder through environmental exposure to reconstruct important life history patterns. In line with this approach, otoliths from juvenile winter flounder had been collected from five locations in the HRE over a three year period, and were analyzed with laser ablation inductively coupled mass spectroscopy (LA ICPMS) using 18 isotopes from 9 different elements. Testing revealed that chemically distinct signals, identifiable as patterns of trace elements specific to the local geochemical and anthropogenic history of the area were detectable. These chemical signals remained regionally identifiable and relatively stable on an annual basis. Chemical analysis of all fish sampled from each location revealed detectable of levels of Cu, Pb, and Zn, and that these elements were often absorbed in high concentrations. Additional investigation also demonstrated that there are apparent correlations with Sr, Ba, and Mn associated with certain life stages of developing winter flounder, which show promise to corroborate the origins of fish, and characterize the quality of their habitat. Consequently, the results of this research demonstrate that further development of site-specific signals is warranted, and that LA ICPMS has strong potential towards the reconstruction life history patterns of winter flounder in the HRE using of otolith microchemistry.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

The winter flounder, (*Pseudopleuronectes americanus*), is a dextral demersal flatfish that is seasonally distributed throughout the coastal waters and estuaries of the Northwest Atlantic, with the exception of the George's Bank population, which retains a uniquely marine identity throughout its life. These ecologically and economically important fish are managed in US waters as three distinct stocks indentified as the Gulf of Maine, George's Bank, and the Southern New England/Mid-Atlantic Bight (SNE/MAB) populations. The SNE/MAB stock is considered an estuarine dependent stock, recruiting from the shallow nearshore waters of New York, New Jersey, Connecticut, Rhode Island, and New Jersey. Though all three stocks have experienced steep population declines largely attributed to human origin, the SNE/MAB fishery has been the most negatively impacted. The status of the SNE/MAB stock is precarious, considered severely depleted in an over-fished condition, and currently protected under a commercial moratorium (ASMFC 2009).

The Hudson River Estuary (HRE) is prominently centered within the SNE/MAB stock's geographic range, existing as the largest estuary within the stock's sweep, and critically linked to its renewal as a major spawning and nursery habitat. However, the HRE has been transformed over time as a consequence of its human history, enduring extense urbanization and industrialization over time that has physically altered its contours and chemically compromised its waters. Consequently, the combined influence of inshore habitat degradation, commercial over-exploitation, and a newly resurgent suite of predators have synergistically impacted the spawning stock biomass and recruitment potential of winter flounder at a critical time. The ensuing loss of this once plentiful fish is likely to reverberate through the ecosystem as bio-energetic interruptions in the trophic transfer since winter flounder perform a pivotal role in the food-web, linking small invertebrates with larger piscivorous organisms. However, correlating a loss of ecosystem functioning to the decline of winter flounder is difficult to quantify, but the economic value of lost livelihoods and rich traditions that have faded along with the dwindling populations are not.

Life History Patterns

Management of the winter flounder fishery requires accurate assessment and careful management of the standing stock, as well as a thorough understanding of seasonal movement patterns and habitat linkages. This information has traditionally been obtained through periodic trawl surveys and large-scale mark-recapture methods (e.g., Lobell 1939; Perlmutter 1947; Saila 1961; Pearcy 1962; Howe and Coates 1975; Phelan 1992) that have generally described the SNE/MAB stock of winter flounder as an estuarine dependent, seasonally distributed population that engages in limited spawning migrations correlated with water temperature. Following this seasonal pattern, sexually mature individuals move inshore in the autumn, overwinter in the sediments, commence spawning in late winter and early spring, and subsequently exit the back bays and estuaries soon after spawning. Behavioral plasticity has been demonstrated through divergent patterns of estuarine usage with sexually mature fish captured on the coastal shelf during spawning season in variant states of reproductive and metabolic condition (Phelan 1992; Wuenschel et al. 2009), and also through inshore observations well into the summer (Olla 1969).

Estuarine usage is critically linked to the SNE/MAB stock's life cycle, and is presumably an adaptive trait that developed over time through enhanced fitness. Studies have shown that adult winter flounder home to the sheltered shoals of the upper estuary, which provide protection from predation and fast growth for the maturing juveniles, resulting in higher recruitment success than coastal habitat (Pearcy 1962). Landward circulation patterns in back bay areas are partially responsible for this success, and have been shown to retain demersal eggs and planktonic larvae, preventing them from being swept from the estuary to a more uncertain future (Pearcy 1962).

During larval development, flounder juveniles undergo metamorphosis and left eye migration, which transforms the bilaterally symmetrical larvae into a fully formed flatfish with both eyes situated on its right side (Jearld et al. 1992). Complete metamorphosis is achieved when juveniles settle onto the benthos. At this stage, the newly settled juveniles demonstrate limited movement for their first few months (Saucerman and Deegan 1991; Buckley et al. 2008), though they trend towards deeper water with age (Able and Fahay 1998). The limited ontogenetic movement and the natal nursery fidelity exhibited by young-of-year (YOY) winter flounder provides the basis for employing otolith microchemistry, because unique exposure patterns derived from the ambient environment during the philopatric period provide identifiable chemical tags to map the estuary. Once the site-specific signatures have been developed, they can be used to track the movements and natal origins of adult fish.

Habitat Contamination

Large estuaries are complex environments that have long been utilized by many species of fish for spawning and/or nursery habitat to exploit the rich productivity and the survival advantages conferred to their offspring; however, Edgar et al. (2000) describe temperate estuaries as some of the most anthropogenically degraded habitats on earth. The HRE is typical in this regard, being home to a diverse assemblage of fish, and as well as having been subjected to extensive levels of development, modification, and pollution. The highest levels of alteration and contamination generally occur in upper estuary regions, and according to Meng et al. (2005), are also the same locations where winter flounder maintain the greatest juvenile abundance and growth rates.

Disregarding the physical degradation, chemically compromised habitat is a byproduct of the human settlement history and industrial activity. As a result, concentrations of anthropogenic contaminants are often location specific, commensurate with levels of human disturbance. Some of these anthropogenic chemicals are absorbed by fish along with other naturally occurring elements, and deposited within their calcified otoliths to become valuable tracers of environmental exposure, allowing habitat inferences (Arslan and Secor 2005). These anthropogenic tracers consequently provide a scientific benefit by increasing the spatial resolution of site-specific signals in regions of similar geochemical and watershed processes when utilizing otolith microchemistry.

What Are Otoliths?

Otoliths are calcified ear bones located within the inner ear of telost fish. These bones are integral to the fish's sensory system, relating inertial innervations as spatial information. Each fish maintains three pairs of otoliths, known as the sagittae, asterisci, and lapilli (Figure 1).

The sagittae are the largest of the three, and the ones most often used for age and growth studies as well as micro-chemical investigations. Formation of the sagittae occurs by precipitation of calcium carbonate, typically in the form of aragonite onto the protein otolin to create a protein/crystalline matrix (Degens et al. 1969). Crystallization is accomplished through an acellular process that renders the otolith metabolically inert once formed (Campana and Nielson 1985). Growth proceeds along axes in daily and seasonal increments, controlled by exogenous and endogenous rhythms that provide otoliths with chronological properties considered unparalleled in the animal world (Campana and Thorrold 2001).

Periodic growth provides another benefit by forming a sequential record of chemical exposure that facilitates tracking fish movement (Secor et al. 1995). During otolith synthesis, divalent elements absorbed from the external environment are substituted for Ca^{2+} or deposited into the interstitial spaces of the structural matrix (Dove et al. 1996; Dove and Kingsford 1998). Therefore, fish occurring in specific geographic locations will be exposed to chemically unique combinations of elements during otolith formation in relative proportion to that found in the environment (Campana 1999). When combined with age information, the reconstruction of life history patterns through chemical chronology can be used as a natural tag to ascertain habitat usage (Elsdon et al. 2008); overcoming many of the logistical problems associated with conventional mark-recapture, acoustic methods, or even sophisticated genetic techniques.

Otoliths unfortunately have one major drawback; fish must be sacrificed to retrieve the information.

Otoliths as Natural Tags

Winter flounder are undoubtedly a well studied marine organism, and yet despite extensive research, uncertainties still remain in fully comprehending the patterns of genetic exchange between populations, and their seasonal habitats. This uncertainty is not uncommon in the marine realm since the connectivity between estuarine and coastal populations of fish is often poorly understood, but fundamental to the study of population dynamics as well as the design of effective management and conservation strategies (Gillanders 2002). Difficulty is encountered in determining migration pathways and movement patterns simply because of the opacity of the environment, which inhibits direct observation.

Research attempts to overcome environmental restrictions often require the implementation of large-scale tagging and tracking programs that are logistically prohibitive, and unable to detail natal origins due to size and mortality constraints imposed by immature fish. Telemetry provides a good alternative to tracking adult movement, but requires a sufficient quantity of expensive electronic tags and receivers that are limited by range. Sophisticated genetic techniques measure gene flow between populations, but can be confounded by low levels of genetic introgression that mask stock partitioning and also require an extensive sampling regimen to ensure all possible demes are characterized for accurate assignment. Hence, most tracking methods have an inherent weakness that limits their effectiveness in resolving movement patterns, or uncoupling the genetic connectivity between populations, and as a result, the origins of most marine organisms remain effectively unknown (Thorrold et al. 2001).

The application of otolith microchemistry with laser ablation inductively coupled mass spectrometry (LA-ICPMS) overcomes inherent weaknesses and logistic difficulties associated with the aforementioned methods by treating elemental ratios as natural habitat tags to effectively track fish movements through environmental exposure. Chemical signatures can act as natural tags for several different life-history stages, and these chemical profiles can then be related to different environments to link groups of fish through space and time (Elsdon et al.2008). Movement patterns and stock origins can be determined by variations in otolith microchemistry, and have proven effective in discriminating among populations of fish (e.g. Dove et al. 1996; Gillanders and Kingsford 2000; Gillanders 2002; Walther et al. 2008).

METHODS

This study tests the feasibility of using otolith microchemistry to develop site-specific signatures from elemental ratios in the otoliths of juvenile winter flounder with the express hope of using otolith microchemistry to unravel pertinent life history questions. Juveniles were selected for analysis due to their tendency for site fidelity upon settlement, and were collected from the Arthur Kill, South Brooklyn, Port Jersey, Manhasset and Little Neck Bay from 2005-2008 (Figure 2). Using the otoliths removed from juvenile winter flounder, the immediate goals of this study were to determine: (**1)** if site-specific elemental signatures can be developed, (**2)** if these chemical signatures exhibit temporal (i.e., across years) and spatial stability, (**3)** the resolution of the chemical signal, and **(4)** discovery of the microchemical characteristics of winter flounder otoliths using the LA-ICPMS.

Sample fish were collected by beach seining utilizing a 10 meter and 66 meter net, and 3 meter beam trawl. The region from which samples were obtained determines collection methods. Within the New York Harbor region, fish were collected by the United States Army Corps of Engineers, New York District (USACE-NYD) and

Henningson, Durham and Richardson (HDR) Engineering, Inc., using a 3-meter beam trawl. Western Long Island Sound juveniles were collected using a 66-m haul seine in conjunction with the New York State Department of Environmental Conservation (NYSDEC), Diadromous Fish Unit's Beach Seine Survey. All samples were packaged, marked and stored on ice in the field until they could be frozen.

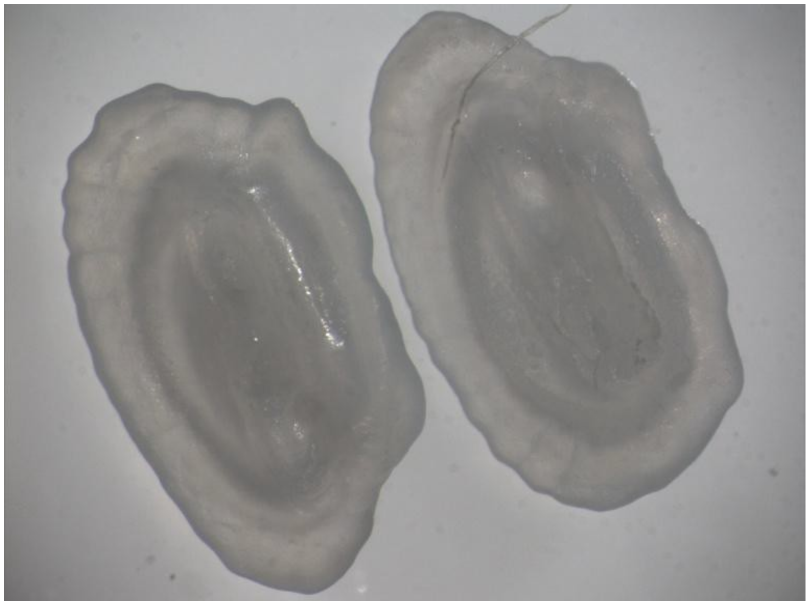

Figure 1. Sagittal otoliths removed from a winter flounder prior to polishing.

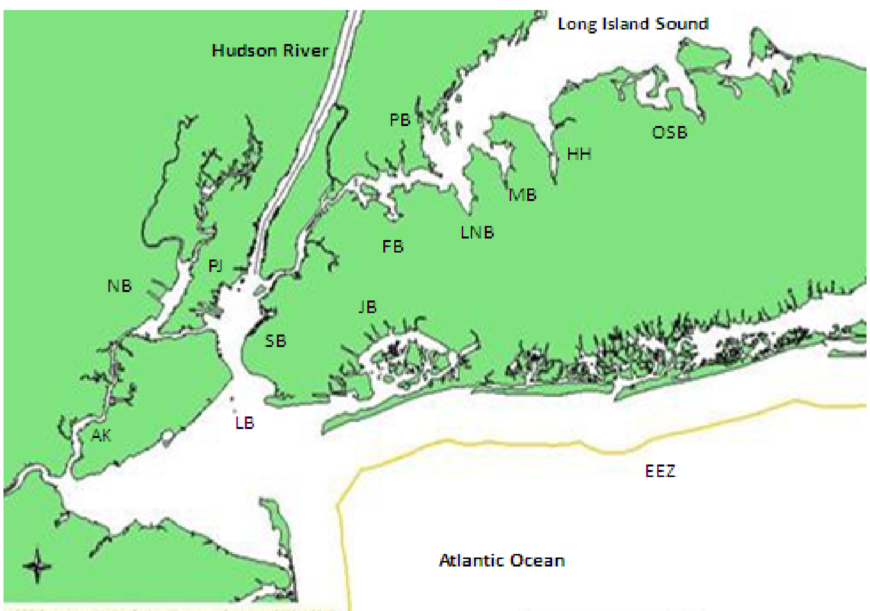

Figure 2. HRE sampling sites from left to right: AK: Arthur Kill, PJ: Port Jersey, SB: South Brooklyn, LNB: Little Neck Bay, MB: Manhasset Bay.

Otolith preparation

Otoliths were individually extracted using forceps after cutting through the cranial case. Any adhering tissue was removed. Otoliths were then rinsed in purified water, and air dried (Figure 2). Right sagittal otoliths were generally utilized unless one was lost or destroyed during processing. In those situations, the left otolith was substituted. After removal and cleaning, otoliths were embedded in EpoFix ™ epoxy and allowed to dry until sufficiently hard to allow sectioning without risk of fracture. Sectioning was accomplished using a low speed Isomet saw with a diamond blade immersed in de-ionized and purified water during cutting. Transverse sectioning is often used in otolith analysis, but due to the small size of juvenile otoliths, best results were obtained when otoliths were cut in the sagittal plane. Once sectioned, hand polishing was performed to gradually expose the primordium through the removal of the over-burden. Grinding and polishing were achieved using a series of increasingly finer silicon carbide lapping films until all etchings and scratch marks were removed. Fully prepared otoliths were then mounted on petrographic slides with cyanoacrylate glue, and labeled accordingly.

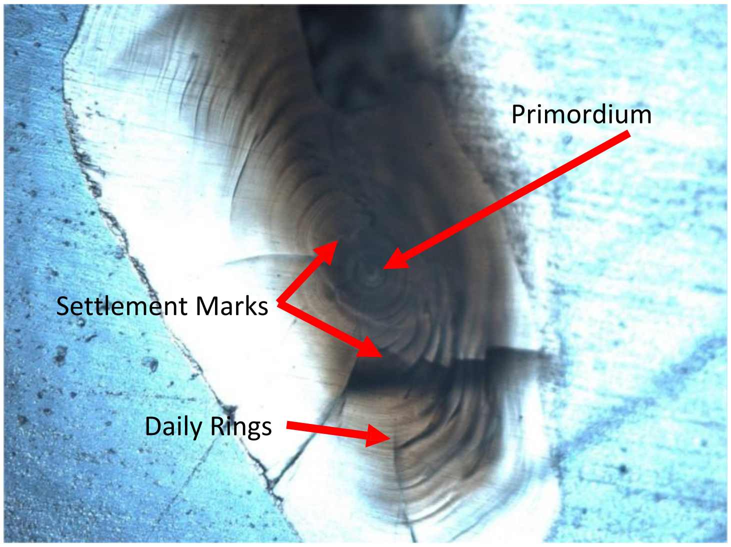

Figure 3. A winter flounder otolith cut through the sagittal plane and polished to reveal fine detail. The primordium, settlement marks, and daily increments are labeled.

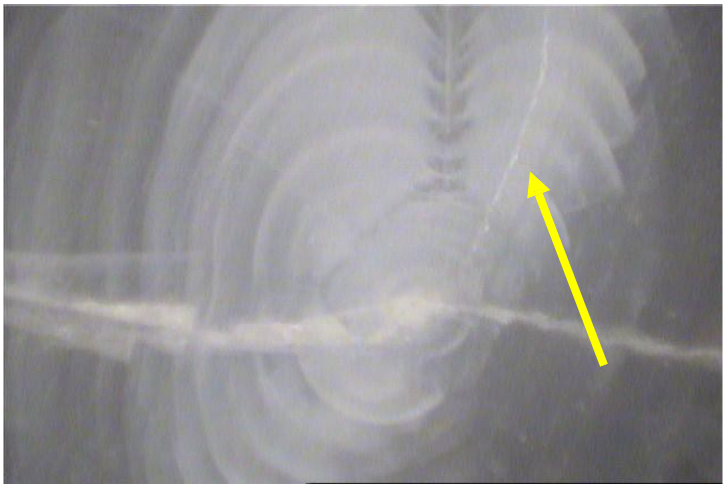
Figure 4. Laser transects followed the natural contour of the saggital ridge. Arrow is pointing to the ablative trench in the right portion of the image.

Otolith analysis

Ablation was performed with a New Wave Research UP-193 laser that utilizes a solid state 193 nm internally homogenized, flat beam, short pulse (UV) laser coupled to a Perkin- Elmer Elan (6000) DRC-e, plasma mass spectrometer (MS). Computer imaging allowed three-dimensional positioning of the sample and focal adjustments of the laser. Laser spot size was set at 35 μm with a scan speed of 3 μm per second, and the repetition rate was set at 10 Hz. Laser irradiance and fluence were monitored and maintained at 40 - 45 percent output, directing approximately 0.50 GW/cm^2 of ablative power to retain consistency. Laser scan paths were drawn onto a digital image of the sample prior to analysis with particular attention focused at the core and settlement region (Figure 3). Transects were generally initiated at the primordium, extending outwards along the rostral and post-rostral growth axes and following the natural contour of the sagittal ridge to the outer margin (Figure 4). Some exploratory scans were made in other directions to test for symmetry or contamination. Transects varied in length depending upon the size of the otolith. Replicates were integrated every 16.7 μm (3 um/second travel speed x 5.565 seconds/replicate). Multiple masses of Sr, Ca, Ba, Zn, Mn, Cu, Li, Rb and Pb were analyzed with the MS, but only the most common isotope of each element was used in this study: ^{42}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{86}Sr, ^{38}Ba, and ^{207}Pb.

The MS was interfaced with an Elan NT software package that integrates the MS with the laser. Inside the MS, a helium vacuum sweeps ablated material into a high-temperature argon plasma (8,000° C) ionizes the ablated materials, and causes ions to be introduced to the quadrupole mass spectrometer for quantification according to their mass to charge ratio. Blanks were run by firing the laser without opening the shutter, and were subtracted as background from the samples. A series of glass reference standards from the National Institute of Standards and Technology (NIST 610, 612, and 614) of known concentration were utilized to calibrate and correct for instrument drift each sample session. A calcium pellet composed of pulverized freshwater drum otoliths of known calcium concentration was used as a calcium standard and run with the NIST standards.

Drift correction is conducted, because the LA-ICPMS incurs variation of output inversely relating time and element concentrations. Correction calculations were derived using average values obtained from NIST and calcium standards run at the start, during sampling, and end of the sampling regimen. All elements were expressed as ratios of calcium to normalize concentrations with beam strength**,** and account for the elemental substitution of calcium during formation (Campana 1999; Elsdon and Gillanders 2004).

RESULTS

The LA-ICPMS was used over a four-day period. During this time, 31 otoliths were abalated in 54 scans. 18 isotopes of 9 elements were analyzed. Symmetry scans were performed on the same otolith to compare patterns of elemental uptake in relation to different axes.

Scan 6H2L-1 was performed on the postrostal otolith axis from a fish recovered in the Little Neck Bay. Scan 6H2L-2 was performed on the rostral axis, 180^{o} to 6H2L-1(Figure 5). Though some variability was displayed, an overall symmetry exists between the axes with respect to the chemistries occurring in the same growth bands.

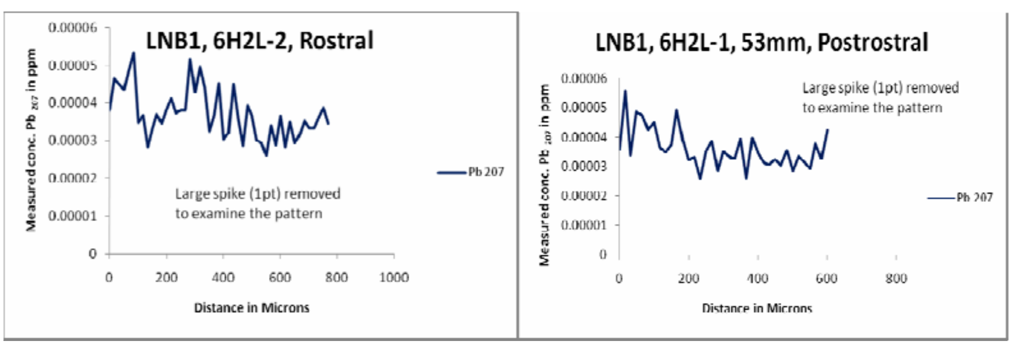

Figure 5. These plots demonstrate similarity in uptake of Pb on both rostral and postrostral axes.

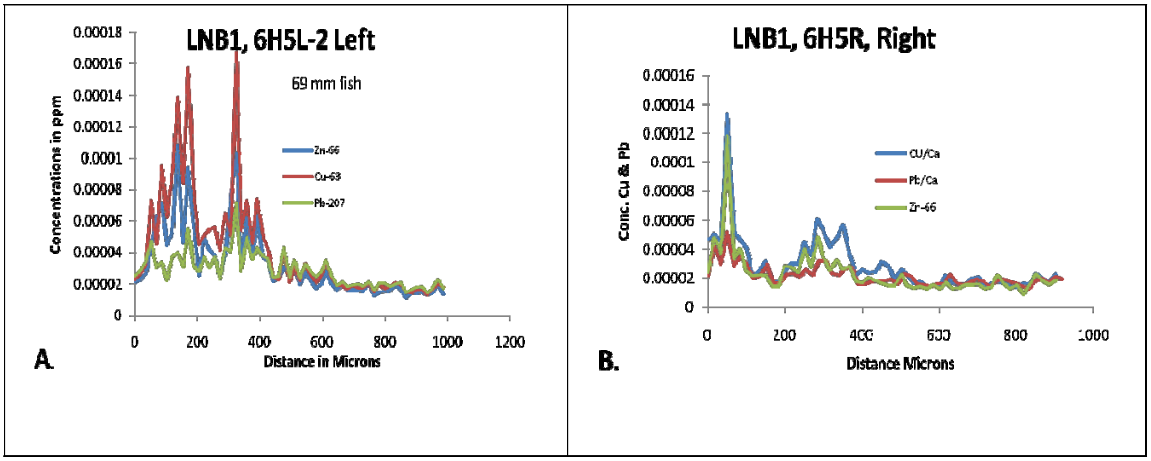

Figure 6. These plots compare chemistries of the left and right otoliths removed from the same fish. Box A. The left otolith (blind side) shows a more extreme pattern. Box B. the right otolith shows asymmetry exists in patterns of uptake, but the concentrations are similar.

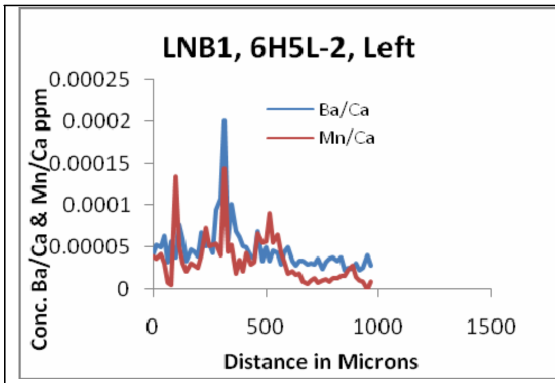

Figure 7a. The above graph shows the patterns of Ba & Mn in the left otolith.

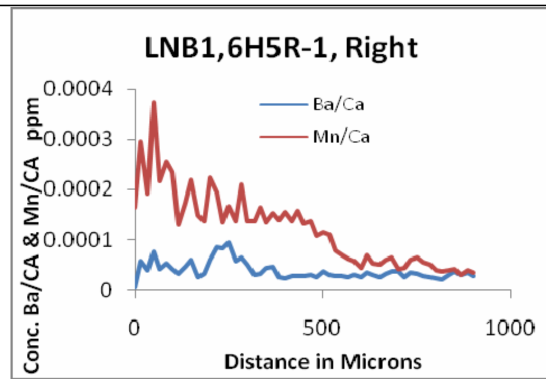

Figure 7b. This graph shows the patterns of Ba & Mn in the right otolith.

Tests were conducted between otolith pairs to determine the similarity of uptake patterns (Figure 6 & 7). These tests show that the left and right otoliths are not perfectly symmetrical, but there is an overall similarity in patterns and concentrations with Cu, Pb, and Zn; however, Mn and Ba differs considerably between left and right otoliths. Tests were also conducted on the cyanoacrylate glue to determine if the glue that was used for mounting otoliths onto the slides introduced any contamination. Glue tests produced negative values for Cu, Pb, Mn, and Zn. The glue is effectively neutral in elemental composition showing no contamination of samples (Figure 8).

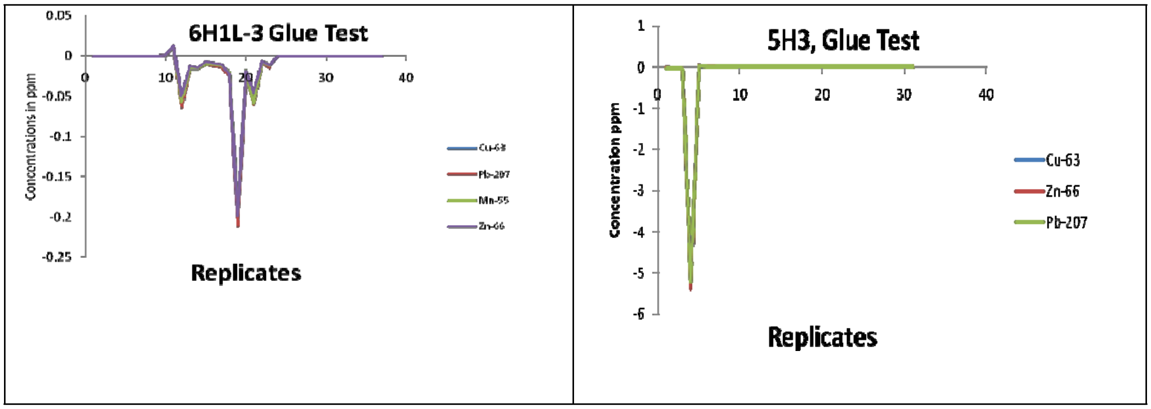

Figure 8. The results of two samples tested for contamination from the glue. The results obtained demonstrate that the cyanoacrylate glue is effectively neutral and not a source of contamination.

Copper, Lead, and Zinc

An apparently deformed otolith was removed from a fish captured in Little Neck Bay, and appeared as a crystalline structure impregnated with blue dots (Figure 9). This finding prompted testing for transition and heavy metals, and demonstrated that varying concentrations of Cu, Pb, and Zn are prevalent in all sample otoliths, oftentimes in high concentrations. High concentrations were manifested as sharp spikes in comparison to the baseline concentrations (Figure 10). This effect was particularly noticeable on samples recovered from Little Neck Bay where a low baseline was contrasted with high spiking concentrations. The western locales such as the Arthur Kill, South Brooklyn, and Port Jersey samples showed higher overall concentrations, but with similar patterns of Cu, Zn, and Pb accumulation localized to narrow zones on the otolith. The Cu concentrations were highest, followed by Zn, and Pb, but all three elements exhibited similar absorption patterns. High concentrations were not limited to any particular region or structure within the otoliths, but it appeared that the core and settlement regions often registered higher concentrations than other portions of the otolith.

However, the otolith margins in some cases did exhibit high concentrations. Test scans were conducted to determine if the “edge effect” was real, but reverse and parallel scans produced similar patterns. The cause of this effect remains unknown.

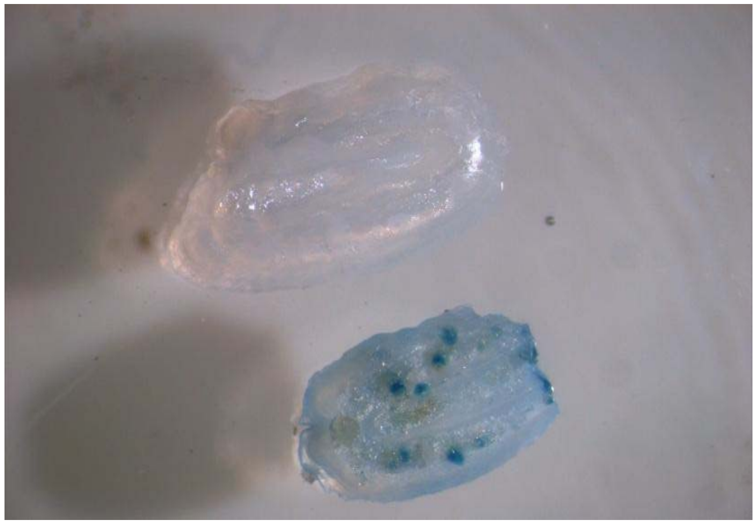

Figure 9. A pair of otoliths removed from a fish caught in Little Neck Bay. This deformed otolith prompted testing for copper, lead, and zinc.

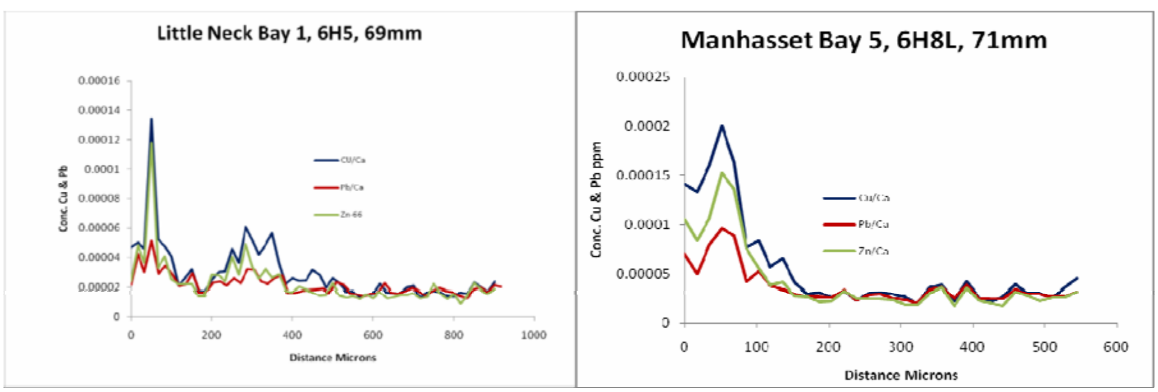

Figure 10a. Patterns of Cu, Pb, and Zn uptake from Little Neck and Manhasset Bay.

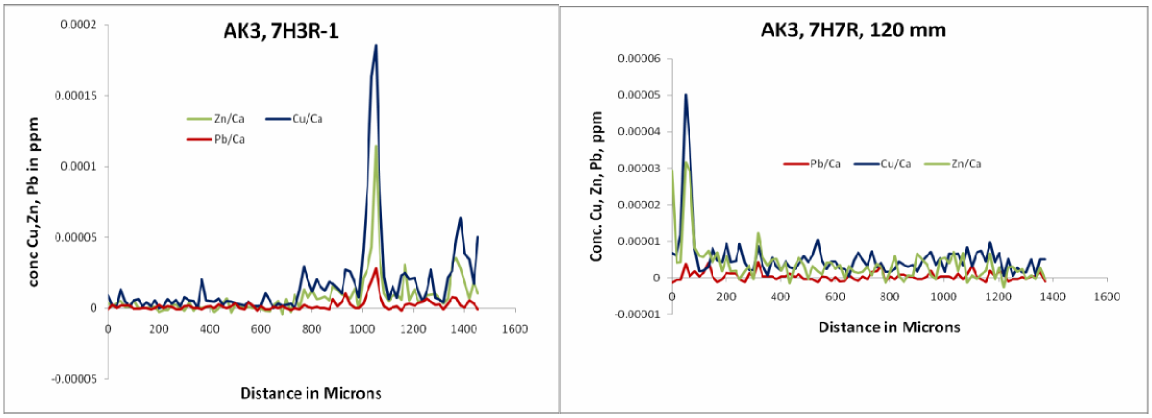

Figure 10b. Patterns of Cu, Pb, and Zn from the Arthur Kill. Peaks are not restricted to just core regions

Strontium

Sr was present in higher concentrations than other elements in all samples. The basic pattern expressed by Sr is a general trend from lower to higher concentrations when initiating the scan at the focus and extending outwards towards the margin. This approach correlates distance to time, or equivalently, the age of the fish (Figure 11). Thus, the shallow western Long Island Sound embayments show a steeper trend over time compared to the Arthur Kill. This pattern is repeated in all otoliths tested.

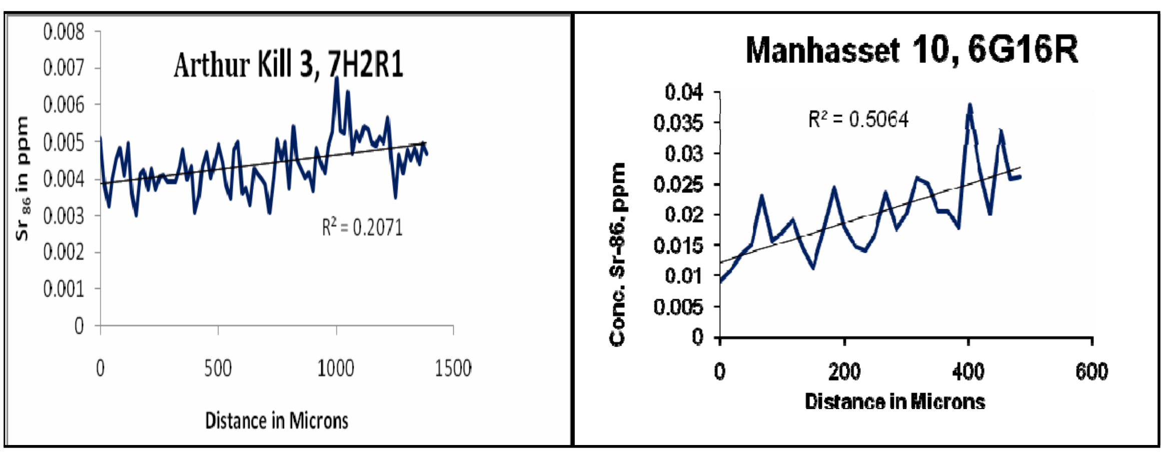

Figure 11. Sr measured in ppm is used as a proxy for salinity. Notice the higher trend with distance (age).

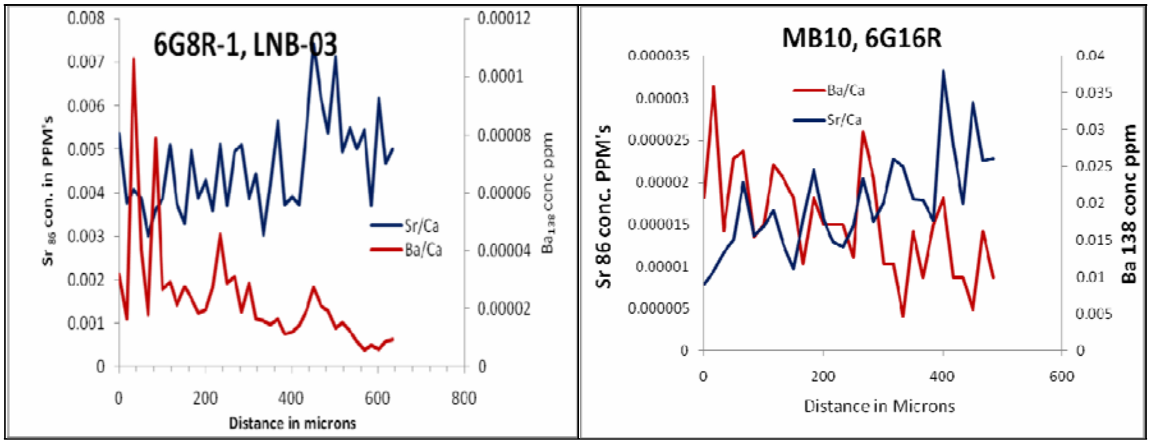

Figure 12. Sr is contrasted with Ba. In Little Neck and Manhasset Bays, Ba and Sr co-vary inversely with Sr increasing and Ba decreasing over time. In the Arthur Kill this trend is less evident.

Barium

Ba was discovered in all regions and tended to have higher concentrations near the core, decreasing in relation to time. Ba generally varies inversely with Sr, though there is much variability within and among all regions (Figure 12); however, the New York Harbor regions seemed to have less predictable patterns. The eastern bays, draining into Long Island Sound, showed a decreasing trend that is not as apparent in the western locations.

Manganese

Mn appears correlated with settlement and post-settlement growth. Mn patterns vary among estuaries, and seem to show a relationship with post-settlement growth (Figure 13). Levels of Mn were highest at settlement, trending downwards with age. A physio-chemical relationship seems to exist between the newly transformed juveniles and the sediments since this pattern was generated by all samples from all locations.

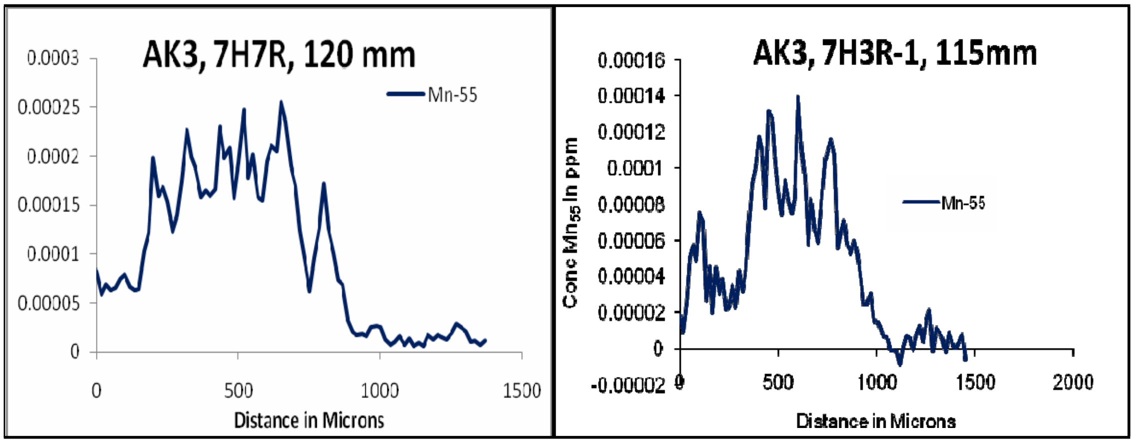

Figure 13. Patterns of Mn peaks that correspond with settlement and post-settlement growth periods. Similar patterning was repeated in most samples.

DISCUSSION

Stocks of winter flounder are at an all time low, with the SNE/MAB stock in a precarious state, facing an uncertain future. The collapse of this once robust fishery highlights the pressing need to conserve and protect remnant populations and essential habitat to ensure the prompt rebuilding of these depleted stocks to healthy levels, as well as the effective management of the fishery once sustainable levels are achieved. Accomplishing these objectives requires a thorough understanding of the genetic linkages between populations and the migratory connection between regions of seasonal residence. The coupling of LA- ICPMS with otolith microchemistry provides a novel approach to overcome the logistical difficulties associated with traditional tracking methods by allowing patterns of elemental uptake to act as chemical tags to track fish movement. Since these natural tags are carried by all teleost fish, differences in the exposure history derived from a combination of ontological and environmental conditions allows otoliths to be used as stock discriminators when these differences are detected (Campana 1999).

This investigation tested the feasibility of using otolith microchemistry to track winter flounder movement in the HRE through environmental exposure. The qualitative results appear to demonstrate that spatial heterogeneity and temporal stability of site-specific signals exist. Signal variability and resolution scales hundreds of meters to kilometers, with variation detected within and among sampled locations. These patterns appeared stable on an annual basis, with some variation discovered within locations. Signal discrimination is a function of geochemical as well as watershed processes, but we have observed that it is also enhanced by metals of anthropogenic origin i.e. Cu, Pb, and Zn. Variability within locations was shown by these elements, and was even detected within otolith pairs removed from the same fish. Differences between otoliths were particularly evident with Sr, Ba, and Mn. However, accumulation of metals in fish otoliths depends on a number of factors, including the concentration in the environment, bioavailability, the physiological state of the individual fish, and the growth rate of the individual fish (Geffen et al. 1998). A more refined and mathematically robust quantitative analysis would be required to resolve variability within and among locations, as well as within the fish.

Unlike bilaterally symmetrical fish, winter flounder are laterally compressed, with one side in constant contact with the sediment, whereas the eye side is exposed to the water column. This environmental relationship is likely responsible for the morphological variation in winter flounder otoliths, which become increasingly asymmetrical between the left and right sides with age (Sogard 1991). Tests were conducted to determine if differences in the asymmetry of otolith growth and orientation are reflected in the patterns of elemental uptake. Our limited testing shows non-uniformity and nonsymmetrical absorption patterns within in the otoliths pairs tested. Though the patterns of uptake are somewhat similar over the life of the fish, the patterns produced on the blind side (left) otolith reveal significant differences when compared with the eye side (right). It appears that the blind side of a winter flounder may absorb higher concentrations of certain elements through direct contact with the sediment, whereas the eye side appears to absorb more from the water column. Patterns produced by Ba in the left otoliths appear to exhibit higher concentrations, and more striking patterns than those of the right side.

Sr patterns contrast those of Ba, with right otoliths revealing more striking patterns than the left. This finding suggests that uptake within the otoliths is influenced by its position within the fish and the availability of element with relation to the ambient environment. This finding requires further testing, but it appears that winter flounder absorb elements through disparate uptake mechanisms, unlike pelagic fish.

Tests conducted to determine the symmetry and uniformity of metal uptake in the growth increments between rostral and post-rostral axes seem to show a similarity in patterns of uptake. This seems logical since incremental growth accretes outward along growth axes simultaneously. As the otolith grows, divalent elements are deposited into the calcium matrix roughly proportional to that in the ambient environment. The patterns of uptake between different axes within the same otolith are relatively uniform and symmetrical.

Strontium and Barium

Experimental evidence indicates that Sr and Ba are correlated with salinity. A positive trend is detectable by changes in the Sr/Ca ratios in the otolith microchemistry (e.g. Limburg 1995; Secor et al. 1995; Secor and Rooker 2005; Limburg 2001; Elsdon and Gillanders 2004; but see Kraus and Secor 2004, who demonstrated that Sr:Ca in otoliths is governed by Sr:Ca in water). These studies demonstrate that discounting temperature as a variable; strontium concentrations are reflective of both the salinity (i.e., Sr is often higher relative to Ca in saline waters) and ambient levels in the environment. Ba concentrations contrast with Sr, being generally greater in brackish water than those in seawater which is due to local geology (Bath et al. 2000; DeVries et al. 2005). This inverse relationship allows Sr to be employed as a proxy for salinity (Limburg 1995; Kraus and Secor 2004), whereas Ba concentrations are useful in estimating freshwater. Ba concentrations are known to be released from the sediments and clays of rivers, and the concentrations in the HRE are typical in this regard, due to the combined influence of both the Hudson and Raritan Rivers (Li and Chan 1979). Mobilization of large volumes of riverine sediments by both rivers would be delivered in pulses due to seasonal hydrology, and would explain the higher and more variable Ba concentrations in the Arthur Kill region compared with those of Little Neck and Manhasset Bay. Ba concentrations should decrease with distance from these large rivers and freshwater input, as was demonstrated by the patterns shown from Manhasset and Little Neck Bay.

Higher salinity corresponds with the lower regions of the estuary. Using the relationship of Sr and Ba as a proxy for salinity, it appears that juvenile winter flounder demonstrate movement towards higher salinity coincident with age as reflected by the increasing levels of Sr and the reverse trend exhibited by Ba in all samples. Sr and Ba exhibit higher concentrations and more variability in New York Harbor regions compared to Manhasset and Little Neck Bay, likely due to large pulses of riverine sediment derived from the Hudson and Raritan Rivers. Fish from Manhasset and Little Neck Bay in contrast to the Harbor regions show a greater increase in salinity over a shorter time period. This patterns correlates to the shorter distance from back-bay to the Long Island Sound.

Copper, Lead, and Zinc

During otolith removal, a deformed left otolith (blind side) was removed from an adult flounder collected in Little Neck Bay. Its appearance was crystalline in appearance without the typical banding in normal otoliths, impregnated with blue dots, whereas the matching otolith appeared normal. It was presumed to be composed of vaterite, which is identifiable by its glassy (Tómas et al. 2004), and has been shown to be the principal polymorph of aberrant otoliths (Campana 1999; Tómas et al. 2004). Deformed otoliths are uncommon. Mugiya (1971) documented the first aberrant otolith, describing it as a sagittal otolith composed of vaterite, removed from the blind side of a flatfish, which is similar to our findings, but without the blue dots. These dots were presumed to be caused by Cu contamination since vaterite forms micro-pellets maintaining less free energy and chemical stability than either calcite or aragonite in the presence of Cu^{2+} (Nassrallah-Aboukais et al. 1999). Disregarding the actual cause, our deformed otolith was a serendipitous find, which prompted testing for heavy metals. Our analyses show that Cu, Zn, and Pb are present in all samples tested, with the Arthur Kill, Little Neck Bay and Manhasset Bay exhibiting high concentrations in the core regions and peripheral rings of the otolith. The large surface to volume ratio of eggs and larvae may be correlated with the uptake of heavy metals in these particular regions of the otolith, or perhaps the eggs and/or larvae were simply exposed to very high levels of contaminants at specific sites. In contrast, very low levels of metal contamination were present in the ovaries of winter flounder captured in the western Long Island Sound (Nelson et al. 1991), which demonstrates that the gravid female is not the source of contamination in flounder eggs. Higher baseline concentrations of Cu, Zn, and Pb were apparent from the Arthur Kill than either Little Neck or Manhasset Bays, and were not always restricted to the juvenile stage. This is not surprising since Cu is associated with human activity, and some of the highest concentrations in the northeast are found in New York Harbor, decreasing with distance from this vicinity (Turgeon and O'Connor 1991).

Metals of anthropogenic origin such as Cu, Zn, and Pb are known to gain entry into the ecosystem system from marinas, ships, and sewer outflows. Boating and shipping activity is largely responsible for the significant concentrations of metal contamination in the vicinity of marinas (Turner et al. 2008). The principal components of marine bottom paint are Cu, Zn, and Pb, and are readily leached from particles of spent anti-fouling paint (Singh and Turner 2009). The patterns of Cu, Zn, and Pb returned from our analyses reflect constituent metal concentrations found in marine paint, which suggests that marinas are indeed a source of entry for these contaminants. Cu is the most abundant element in marine bottom, acting as the principal inorganic biocide followed by levels of Zn and Pb, which are used to control the erosion rate, and boost the toxicity of Cu by 200 times (Waterman et al. 2005; Turner et al. 2008). Since metal concentrations in otoliths have been shown to be correlated to levels found in the ambient environment, we can assume that winter flounder are coming into contact with these elements at specific locations in the estuary.

Manganese

Settlement occurs in winter flounder as they exit the pelagic habitat to begin life as bottom dwelling flatfish. This transitional period is perceptible in the otolith as asymmetrical formations emanating from the periphery of the core section, known as settlement marks. Using the settlement marks as temporal signposts, we noticed that Mn concentrations elevate upon settlement or soon after, then decline over time. We speculate that when juvenile winter flounder settle onto the benthos, the hypoxic pore water associated with benthic sediments activates the redox potential, which drives Mn^{2+} from these sediments, and into the immature winter flounder that retain a large surfaced to volume ratio, since the upper layers of marine sediments are often enriched in oxidized manganese as a result of biogeochemical processes (Kristiansen et al. 2002), which becomes available as Mn^{2+} in water when reduced by hypoxic/anoxic conditions in the sediment (Baden and Eriksson 2006). During hypoxia, the bioavailability of Mn^{2+} in the bottom or pore water can increase by several orders of magnitude and become liberated from the sediments, causing Mn to become available to a sediment dwelling organism (Balzer 1982; Valkirs et al. 1994). In addition, rapid growth upon settlement corresponds to the patterns of Mn uptake detected (opaque zones), and metabolic inactivity (translucent zones), which have been shown by Hamer and Jenkins (2007) to be influenced by otolith accretion rates and metabolism. High concentrations later in life are presumably attributed to proportionate levels of Mn exposure or possible relationship to hypoxic conditions, again causing positive redox flux from the sediments. In future studies, differences in Mn patterns may prove useful indicators for substrate composition, and hypoxic zones. Controlled experiments should be used to test for correlations among

Mn, substrate type, and hypoxia.

This investigation has concluded that spatial heterogeneity and temporal stability of site specific signals exist within the HRE. This finding provides positive incentive to proceed in the elemental characterization of winter flounder otoliths, and to continue using otolith microchemistry to help illuminate patterns of connectivity in winter flounder.

ACKNOWLEDGEMENTS

I would like to thank following people and organizations for their all their help and guidance during this study. My project advisor Dr. John Waldman initially introduced me to the topic, and has guided me to the many resources and contacts necessary to undertake this endeavor. My co- advisor Dr. Karin Limburg has provided immense support in so many ways, has answered a multitude of emails, and has helped clarify the many intricacies associated with the science of otolitihology. I would like to thank the Hudson River Foundation for the funding, and the support for this project. I would also like to thank Henningson, Durham, and Richardson (HDR), the United States Army Corps of Engineers (USACE), and the New York State Dept. of Environmental Conservation (NYS DEC) (specifically Julia Socrates and her team) for providing winter flounder samples without which this study would have never occurred. Support for the LA-ICPMS analysis came from NSF grant DEB-021383 and NSF grant DBI-0722942 to K. Limburg. I would also like to thank Deb Driscoll (SUNY ESF) for her tremendous help with the operation of the LA-ICPMS.

REFERENCES

Able, K.W. and M.P. Fahay. 1998. Winter flounder *Pseudopleuronectes americanus* (Walbaum), the first year in the life of estuarine fishes in the Middle Atlantic Bight. Rutgers University Press, New Brunswick, New Jersey.

Arslan, Z. and D.H. Secor. 2005. Analysis of trace transition elements and heavy metals in fish otoliths as tracers of habitat use by American eels in the Hudson River Estuary. Estuaries and Coasts 28: 382-393.

Atlantic States Marine Fisheries Commission (ASMFC), 2009. Addendum I to Amendment 1 to the Interstate Fishery Management Plan for the Inshore Stocks of Winter Flounder.

Baden, S.P. and S.P. Eriksson. 2006. Roles, routes, and effects of manganese in crustaceans. Oceanography and Marine Biology: An Annual Review 44:61-83.

Balzer, W. 1982. On the distribution of iron and manganese at the sediment/water interface: thermodynamic versus kinetic control. Geochemica et Cosmochimica Acta 46:1153-1161.

Bath G.E., S.R. Thorrold, C.M. Jones, S.E. Campana, J.W. McLaren, and J.W.H. Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. Geochimica et Cosmochimica Acta, 64: 1705–1714.

Buckley, L., J. Collie, A.E. Kaplan, and J. Crivello. 2008. Winter flounder larval genetic population structure in Narragansett Bay, RI: Recruitment to juvenile young-of-the-year. Estuaries and Coasts 31:745–754.

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188:263-297.

Campana, S.E. and J.D. Nielson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014-1032

Campana, S.E., and S.R. Thorrold. 2001. Otoliths, increments and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences 58: 30-38.

Degens, E.T., W.G. Deuser, and R.L. Haedrich. 1969. Molecular structure and composition of otoltihs. Marine Biology 2:105-113.

DeVries, M.C., B.M. Gillanders, T.S. Elsdon. 2005. Facilitation of barium uptake into fish otoliths: Influence of strontium concentration and salinity. Geochimica et Cosmochimica Acta 69:4061-4072.

Dove, S.G., B.M. Gillanders, and M.J. Kingsford. 1996. An investigation of chronological differences in the deposition of trace elements in the otoliths of two temperate reef fishes. Journal of Experimental Marine Biology and Ecology 205: 15-33.

Dove S.G., and M.J. Kingsford. 1998. Use of otoliths and eye lenses for measuring trace-metal incorporation in fishes: A biogeographic study. Marine Biology 130:377-387.

Edgar, G.J., N.S. Barrett, D.J. Graddon, and P.R Last. 2000. The conservation significance of estuaries: A classification of Tasmanian estuaries using ecological, physical and demographic attributes as a case study. Biological Conservation 92: 383–397.

Elsdon, T.S. and B.M. Gillanders. 2004. Fish otolith chemistry influenced by exposure to multiple environmental variables. Journal of Experimental Marine Biology and Ecology 313:269-284.

Elsdon, T.S., B.K. Wells, S.E. Campana, B.M. Gillanders, C.M. Jones, K.E. Limburg, D.H. Secor, S.R. Thorrold, and B.D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations, and inferences. Oceanography and Marine Biology: an Annual Review 46: 297-330.

Geffen, A.J., N.J.G. Pearce, and W.T. Perkins 1998. Metal concentrations in fish otoliths in relation to body composition after laboratory exposure to mercury and lead. Marine Ecology Progress Series 165:235-245.

Gillanders, B.M. and M.J. Kingsford. 2000. Elemental fingerprints of otoliths of fish may distinguish estuarine nursery habitats. Marine Ecology Progress Series 201:273-286.

Gillanders, B.M. 2002. Connectivity between juvenile and adult fish populations: do adults remain near their recruitment estuaries? Marine Ecology Progress Series 240:215-223.

Hamer, P.A. and G.P. Jenkins. 2007. Comparison of spatial variation in otolith chemistry of two fish species and relationships with water chemistry and otolith growth. Journal of Fish Biology 71:1035-1055.

Howe, A.B. and P.G Coates. 1975. Winter flounder movements, growth and mortality off Massachusetts. Transactions of the American Fisheries Society 104:13-29.

Jearld, A. Jr, S.L. Sass, and M.F. Davis. 1992. Early growth, behavior and otolith development of the winter flounder, *Pseudopleuronectes americanus*. Fishery Bulletin 91:65-75.

Kraus, R.T. and D. H. Secor. 2004. Incorporation of strontium in otoliths of an estuarine fish. Journal of Experimental Marine Biology and Ecology 302:85-106.

Kristiansen, K.D., E. Kristensen and E. M. H. Jensen. 2002. The influence of water column hypoxia on the behavior of manganese and iron in sandy coastal marine sediment. Estuarine, Coastal and Shelf Science 55:645-654.

Li, Y.U., and L.H. Chan. 1979. Desorption of Ba and ^{226}Ra from river-borne sediments in the Hudson Estuary. Earth and Planetary Science Letters 43:343-350.

Limburg, K.E., 1995. Otolith strontium traces migratory histories of juvenile American shad, Alosa sapidissima. Marine Ecology Progress Series, 119:25-35.

Limburg, K.E. 2001. Through the gauntlet again: demographic restructuring of American shad by migration. Ecology 82: 1584-1596.

Lobell, M.J. 1939. A biological survey of the saltwaters of Long I sland, 1938. Report on certain fishes. Winter flounder (*Pseudopleuronectes americanus*). 28th Annual. Report, New York Conservation Department, Part 1 No. 14:63-93.

Meng, L., G. Cicchetti, and S. Raciti. 2005. Relationships between juvenile winter flounder and multiple-scale variation in Narragansett Bay, Rhode Island. Transactions of the American Fisheries Society 135:1509-1519.

Mugiya, Y. 1971. On aberrant sagittass of telostean fishes. Japanese Journal of Ichthyology 19:11-14.

Nassrallah-Aboukais, N., A. Boughriet, J.C. Fischer M. Wartel, H.R. Langelin, and A. Aboukais. 1998. Transformation of Vaterite into Cubic Calcite in the Presence of Copper(II) Species. Journal of Chemical Society 92: 3211-3216.

Nassrallah-Aboukais, N., A. Boughriet, J. Laureyns, L. Gengembre, and A. Aboukaïs. 1999. Stabilization of the Vaterite Structure in the Presence Of Copper(II): Thermodynamic and Spectroscopic Studies. Chemistry of Materials 11:44-51.

Nelson, D.A., J.E. Miller, D. Rusanowsky, R.A. Greig, G.R. Sennefelder, R. Mercaldo-Allen, C. Kuropat, E. Gould, F.P. Thurburg, and A. Calabrese. 1991. Comparative reproductive success of winter flounder in Long Island Sound: A three year study (biology, biochemistry, and chemistry). Estuaries 14:318-331.

Olla, B.L., R. Wicklund, and S. Wilk. 1969. Behavior of winter flounder in a natural habitat. Transaction of the American Fisheries Society 98:717-720

Pearcy, W.G. 1962. Ecology of an estuarine population of winter New York Bight: science and management. Bulletin of the Bingham Oceanographic Collection, Yale University 18:39-64.

Perlmutter, A. 1947. The blackback flounder and its fishery in New England and New York. Bulletin Bingham Oceanographic Collection Yale University, 92 pgs.

Phelan, B.A. 1992. Winter flounder movements in the inner New York Bight. Transactions of the American Fisheries Society 121:777-784.

Saila, S.B., 1961. A study of winter flounder movements. Limnology and Oceanography 6:292-298.

Saucerman, S. and L.A. Deegan. 1991. Lateral and cross-channel movement of young-of-the- year winter flounder *(Pseudopleuronectes americanus) in* Waquoit Bay, Massachusetts. Estuaries 14:440-446.

Secor, D.H., A. Henderson-Arzopalo, and P.M Piccoli. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? Journal of Experimental Marine Biolology and Ecology 192:15-33.

Secor D.H., and J.R. Rooker. 2005. Connectivity in the life histories of fishes that use estuaries. Estuarine, Coastal and Shelf Science 64: 1-3

Singh, N. and A. Turner. 2009. Trace metals in antifouling paint particles and their heterogeneous contamination of coastal sediments. Marine Pollution Bulletin 58:555-564.

Sogard, S. 1991. Interpretation of otolith microstructure in juvenile winter flounder (*Pseudopleuronectes americanus*): Ontogenetic development, daily increment validation, and somatic growth relationships. Canadian Journal of Fisheries and Aquatic Sciences 48:1862-1871.

Thorrold, S.J., C. Latkocy, P.K. Swart, and C.M. Jones. 2001. Natal homing in a marine fish metapopulation. Science 291:297-299.

Tómas, J, A.J. Geffen, I.S. Allen, and J. Berges. 2004. Analysis of the soluble matrix of vaterite otoliths of juvenile herring (*Clupea harengus*): do crystalline otoliths have less protein? Comparative Biochemistry and Physiology 139:301-308.

Turgeon, D.D., and T.P. O'Connor. 1991. Long Island Sound: Distributions, trends and effects of chemical contamination. Estuaries 3:279-289.

Turner, A., S. Fitzer, and G.A. Glegg. 2008. Impacts of boat paint chips on the distribution and availability of copper in an English ria. Environmental Pollution 151, 176–181.

Valkirs, A.O., B.M. Davidson, L.L. Kear LL, R.L. Fransham, A.R. Zirino, and J.G Grovhoug. 1994. Environmental effects from in- water hull cleaning of ablative copper anti-fouling coatings. Technical Document 2662, Naval Command, Control, and Ocean Surveillance Center, San Diego, CA.

Walther, B.D., S.R. Thorrold, and J.E. Olney. 2008. Geochemical signatures in otoliths record natal origins of American shad. Transactions of the American Fisheries Society 137:57-69.

Waterman, B.T., B. Daehne, S. Sievers, R. Dannenberg, J.C. Overbeke, J.W. Klijnstra, and O. Heemken. 2005. Bioassays and selected chemical analysis of biocide-free antifouling coatings. Chemosphere 60:1530-1541.

Wuenschel, M.J., K.W. Able, and D. Byrne. 2009. Seasonal patterns of winter flounder, (*Pseudopleuronectes americanus*) abundance and reproductive condition on the New York Bight continental shelf. Journal of Fish Biology 74:1508–1524