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The Ecology Of Winter Flounder From An Otolith Perspective

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THE ECOLOGY OF WINTER FLOUNDER FROM AN OTOLITH PERSPECTIVE

By

George W. Jackman

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the City University of New York

2015
This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT

THE ECOLOGY OF WINTER FLOUNDER FROM AN OTOLITH PERSPECTIVE

By

George W. Jackman

Advisors: John Waldman (CUNY Queens College) and Karin E. Limburg (SUNY ESF)

In this dissertation, sagittal otoliths were used as a lens to examine latent life history patterns in winter flounder (*Pseudopleuronectes americanus*) and also as a means of interpreting the species' relationship to their abiotic and biotic environment. Otoliths provide a unique and powerful perspective into the lives of the telost fishes, because they permanently record the spatial and temporal histories through sequential growth patterns from conception to capture. The patterns of growth and dormancy in the otolith are regulated by endogenous and exogenous rhythms, and as the otolith grows, trace elements are absorbed from the ambient environment and incorporated into the calcium carbonate (CaCO$_3$) matrix. Hence, concentric bands in the otolith reliably indicate age and growth conditions similar to the annuli in trees, whereas the chemical chronology contained within the CaCO$_3$ crystal can function as a geochemical tag, to permit retrospective tracking of a fish’s movements and reconstruction of its environmental history.
The first chapter in this study demonstrates that winter flounder sagittae are not morphologically, nor are they chemically identical, which is an essential distinction that sets them apart from those found in bilaterally symmetrical fishes. This finding has important implications when using otolith chemistry to investigate population structure of winter flounder and other flatfishes, because indiscriminate use of either otolith can bias statistical results. Consequently, method standardization is recommended when performing otolith chemistry in flatfishes. Furthermore, the results of this investigation provides evidence that the blind-side otolith in flatfishes may be absorbing chemicals differently from their eyed-side counterparts, though these results warrant further testing.

In the second chapter, otolith microchemistry is utilized to examine the fine-scale stock structure of winter flounder that were sampled from the coastal margins of Long Island and surrounding areas. Using otolith microchemistry in this manner, group membership was recognizable on a scale of tens of kilometers with a statistical accuracy that ranged from 83-87% depending upon spatial dimensions when re-classifying the specimens back to their location of capture. The second part of this chapter examines the feasibility of using otolith microchemistry with specific elemental markers to make qualitative assessments of inshore habitat of winter flounder. Through this investigation, it was revealed that some of the most chemically contaminated bodies of water still make important contributions to winter flounder recruitment, and juvenile growth in these systems can potentially exceed growth patterns in more pristine locations.

The last chapter of this dissertation looks at the age and growth structure of winter flounder in the Hudson River Estuary (HRE) and western Long Island Sound (WLIS) and compares those results to several large-scale surveys that were performed last century and
collectively form the historical record of winter flounder in New York waters. The ensuing results of this analysis show that winter flounder in the HRE and WLIS have incurred a faster growth rate, and larger, if not older fish comprise a greater percentage of the population than they did during the early and middle decades of the twentieth century. The increasing size-at-age shown by winter flounder in the HRE and WLIS correlates with a release from intra-specific competition and increasing pressure generated by size-selective mortality imposed by a resurgent and newly emerging suite of predators. Finally, the conclusions of this thesis summarize the results of these investigations and discuss potential directions for future research.
DEDICATION

To my family, especially my wife Aneta and my daughter Shannon
Acknowledgements

I would like to thank the CUNY Graduate Center and Queens College for providing me with this opportunity. I would also like to thank the following persons and institutions for providing invaluable assistance in obtaining specimens and/or answering persistent requests for information including: Henningson, Durham & Richardson, Inc. (HDR), United States Army Corps of Engineers (USACOE), Staff of the New York State Department of Environmental Conservation (NYSDEC), i.e., Julia Socrates, Jesse Hornstein, John Maniscalco, Alice Weber, and Peter Malaty. I also need to thank Deb Driscoll (SUNY ESF) for technical assistance while conducting LA-ICPMS analyses. Funding for this project was provided in part by Queens College Graduate Advisory Council, Hudson River Foundation Tibor T. Polgar Fellowship Program, and the Sounds Conservancy Quebec-Labrador Foundation Grant Program. This work is based in part upon research conducted at the Cornell High Energy Synchrotron Source (CHESS), which is supported by the National Science Foundation and the National Institutes of Health/National Institute of General Medical Sciences under NSF award DMR-00936384. I also want to thank the members of my Ph.D. committee, Gillian Stewart, Mike Hickerson and Isaac Wirgin for their sound advice, insight, interest, and support throughout the course of this study. I could not proceed without an extra special thanks to my co-advisors John Waldman and Karin Limburg for their countless hours of reflecting, reading, encouraging, and most of all, their patience throughout preparation of this thesis.
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INTRODUCTION AND BACKGROUND

The study species and their habitat

Winter flounder (*Pseudopleuronectes americanus*) are taxonomically classified under the order Pleuronectiformes. Membership in this order encompasses a diverse group of similar-shaped organisms that includes all flatfishes. Flatfishes as a group have evolved as unique set of organisms that are characteristically asymmetrical, having broad laterally compressed bodies lacking swim bladders. The morphology and negative buoyancy of these fishes makes them well suited to a demersal lifestyle, which allows them to readily forage on a variety of benthic invertebrates and small fish. Winter flounder are further assigned to the Pleuronectidae family, which contains 23 genera and 60 species of primarily dextral flatfishes maintaining a wide-ranging distribution, and inhabiting all oceans and seas of the Northern Hemisphere (Monroe 2005). The Pleuronectidae generally deposit pelagic eggs, but winter flounder are atypical in this regard, being the only member of the Pleuronectidae family to lay demersal eggs (Monroe 2005).

Upon hatching, winter flounder emerge from their eggs as bilaterally symmetrical, pelagic larvae, like those of all other flatfishes. After several weeks in the pelagic stage, the developing larvae undergo ontogenetic metamorphosis, during which time profound structural changes occur, and their left eye migrates from one side of the head to the other (Jearld et al. 1993). Once metamorphosis is complete, the newly transformed juveniles settle onto the substrate as dextral flatfish, having both eyes positioned on their right side (eyed-side) while contacting the substrate on their sinistral (blind-side). Settled winter flounder juveniles enter the benthic community primarily as stationary, ambush predators, positioned in the ecosystem as trophic intermediaries. As an intermediate link in the food chain, winter flounder perform a valuable ecological service.
by consuming a large mass of small fish and invertebrates, and as they become prey for larger piscivorous predators, transfer energy through the food chain. This is pivotal position in the trophic food-web and causes them to be subjected to high levels of predation, especially at the juvenile stage, which is thought to be one of the primary biotic causes of juvenile flatfish mortality (Gibson 1994; van der Veer et al. 1997).

Management of the winter flounder fishery in the United States occurs on a broad scale, wherein three main stocks are recognized. The stocks are identified as the Gulf of Maine (GOM), Georges Bank (GB) and the Southern New England/Mid Atlantic (SNE/MA). Winter flounder from the GB stock are fundamentally different than the other two stocks, tending to be larger and remaining offshore throughout their life, whereas fish associated with the GOM and the SNE/MA stocks generally make short, seasonal migrations to inshore waters for recruitment purposes (Pereira et al 1998). Recent research has shown evidence of coastal-spawning contingents of winter flounder within the SNE/MA and GOM stocks (Wuenschel et al 2009, DeCelles and Cadrin 2010). Nevertheless, it is generally accepted that the SNE/MA stock recruits from the shallow, nearshore waters within the state boundaries of New Jersey, New York Connecticut, Rhode Island, and Massachusetts (Howe and Coates 1977). The Hudson River Estuary (HRE) and the coastal margins of Long Island are geographically centered within the SNE/MA stock’s range, and comprise a network of interconnected rivers and embayments that spreads across a large landmass and extends eastward in a pattern of declining human density.

The estuarine habitat where the inshore populations of winter flounder spawn and mature are chemically complex environments where marine-derived nutrients are hydrodynamically mixed with exports of carbon and nitrogen introduced from terrestrial watersheds. Mobilization of autochthonous and allochthonous material into the estuaries occurs in seasonal pulses, and
forms the foundation of the local ecology by recharging primary and secondary production. As a result of the high rate of productivity and ready transport of nutrients through the food web, estuaries can support a large abundance and diversity of fish, invertebrates, and seagrasses (Beck et al. 2001). Though temperate estuaries rank among the most productive ecosystems on earth, they are also some of the most degraded (Edgar et al. 2000; Kennish 2004).

Winter flounder and other estuarine-dependent fish have developed behavioral reliance upon estuaries in order to exploit recruitment and growth advantages these habitats provide to their offspring (Manderson et al. 2004). Accordingly, the seasonal shift of winter flounder from coastal to inshore waters is specifically timed and spatially coordinated to regions of the estuary where hydrodynamic features maximize survivorship through larval retention and optimal growth during juvenile stages to survive a persistent predatory regime (Pearcy 1962; Crawford and Carey 1985; Crawford 1990). The spawning and nursery habitat of winter flounder are often situated near each other in the upper regions of the estuary, which is not surprising since these regions routinely show the highest levels of juvenile abundance while providing growth advantages over alternative areas (Meng et al. 2005). Positive recruitment in these areas is facilitated through the retention of eggs and larvae despite the net seaward movement of surface waters, because movement of bottom water is directed towards land (Pearcy 1962). The retention of eggs and larvae in the upper estuaries through hydrodynamic movement of bottom water may explain why winter flounder are the only member of the Pleuronectidae that lay demersal eggs. Consequently, recruitment success in these locales appears to imprint on winter flounder, and causes them to home to their natal nurseries. Despite evidence of straying behavior, estuarine-dependent winter flounder often return to spawn in the same waters where they were born and matured, which subsequently causes the population to be comprised of series of genetically
independent elements (Perlmutter 1947; Saila 1961; Phelan 1992). Spawning site fidelity has considerable implications in the population structure of marine fish species, because it is determined by constraints at early life-history stages (Sinclair 1988). Aside from the population structuring, homing to natal nurseries is believed to be an adaptive behavior that arose to maximize reproductive success by synchronizing the return of mature animals to the spawning grounds when conditions are optimal for egg and larval development and regulates the number of adults utilizing a given area (Legget 1977). Availability of quality inshore habitat is essential in flatfishes, because a positive relationship exists between the size of recruitment habitat and relative recruitment to the spawning stock biomass (Rijndorp et al. 1992). Unfortunately, much of the upper estuarine spawning habitat is also associated with the highest levels of human development and habitat alteration. Thus, the identification and protection of critical recruitment habitat is vitally important for winter flounder. In addition, when managing winter flounder at the stock level, it is important to recognize the genetic contribution provided by the relatively independent subpopulations and the respective recruitment habitat to ensure the long-term sustainability of the species in a changing environment.

**Using otoliths as a lens to infer and interpret life history patterns**

Otoliths are calcified ear stones located within the inner ear of teleost fishes that provide spatial orientation and acoustical information to these organisms. Sensory information is transmitted via inertial changes of the otolith within the endolymph through the action of sound or motion, which then causes the otolith to contact cilia and results in signal detection (Popper et al. 2005). Stimulation of the cilia bundles by the otolith movement transduces mechanical energy into electrochemical impulses. Teleosts have three pairs of otoliths, which are respectively
termed the la pilli, sagittae, and asterisci, though size and shape of each pair is governed by adaptive needs and systematic relationships. These ear stones are composed of approximately 96% calcium carbonate (CaCO$_3$), 3% protein, and trace element concentrations that comprise of less than 1% of the total mass (Campana et al. 1997). In non-ostariophysans, the sagittae are the largest of the set, and are the ones most often used for age and growth studies and chemical investigations. Formation of the sagittae occurs in the saccular endolymph by the precipitation of CaCO$_3$, typically in the polymorph of aragonite onto the protein otolin to form a protein-crystalline matrix (Degens et al. 1969). The principal substances involved in otolith growth are proteins, ionized calcium (Ca$^{2+}$), and bicarbonate ions (HCO$_3^-$) (Payan et al. 1999). The calcification process is dependent upon the composition of the endolymphatic fluid, which is regulated by the pH of the endolymph, and is determined by the concentration of bicarbonate ions (HCO$_3^-$) in the endolymph (Campana 1999). Otolith growth occurs through the diurnal deposition of Ca through an acellular process that renders otoliths metabolically inert once formed, and as a result cannot be resorbed during periods of stress, as are bone and scales (Mugiya et al. 1981; Campana and Nielson 1985). The capacity to age fish from otoliths corresponds with metabolic activity and protein synthesis, which is governed by endogenous and exogenous rhythms that reveal patterns of growth and dormancy at both daily and seasonal cycles. These periodic patterns are permanently recorded in the CaCO$_3$ crystals, and because of the regularity associated with increment formation, the chronometric properties of otoliths are considered unparalleled in the animal world (Campana and Thorrold 2001). As a result, the accuracy and precision of age estimates obtained from otoliths is widely recognized by fisheries scientists and managing agencies, and are routinely used in stock assessments. Scales can also be used for age and growth analyses purposes, but they are considered less reliable when aging
older fish due to growth cessation, loss leading to regeneration, or compaction of annuli (Beamish and McFarlane 1983). Though otoliths can be used in a variety of applications, otoliths have one major drawback; the fish must be sacrificed to retrieve the information.

The capacity for using otoliths chemistry in a variety of applications is derived from properties that govern formation of the otolith, which provides a chemical chronology to facilitate the tracking of fish movement through environmental exposure (Kalish 1989; Secor et al. 1995; Campana 1995). During increment synthesis, divalent elements in trace and minor concentrations are randomly substituted for Ca\(^{2+}\) or are deposited into the interstitial spaces of the structural matrix (Dove et al. 1996, Dove and Kingsford 1998), in relative relationship to that found in their ambient environment (Kalish 1989; Milton and Chenery 2001; Campana 2004; Walther et al. 2008). Hence, because otoliths are metabolically inert once formed, concentrations of elements absorbed from the ambient environment are permanently retained within the calcium/protein matrix. When combined with age information, the chemical record contained within otoliths can be used as a natural tag to ascertain fish movement and habitat usage (Elsdon et al. 2008). Thus, otolith chemistry can overcome many of the logistical dilemmas encountered through tracking fish with conventional methods. Additionally, the intrinsic properties of otoliths formation allow researchers to compare juvenile sections of otoliths with corresponding regions of adult otoliths. When used in this fashion, researchers have been able to effectively determine natal origins of mixed stocks of fish (Thorrold et al. 1998a, 1998b; Walther et al. 2008) and also to compare life history patterns to those of other fish (Secor et al. 1995; Limburg 2001).

The introduction of divalent elements into the otolith is not completely understood and often involves complex physiological processes. The basic pathway of the inorganic elements into the CaCO\(_3\) is from water to the blood plasma via the gills or intestines, then into the
endolymph, and finally into the crystalizing otolith (Campana 1999). However, small amounts of elements are assimilated into the otolith through dietary means (Limburg 1995). Regardless of the mode of entry, ambient concentration, pH, salinity, temperature, chemical structure, dissolved oxygen concentration, and physiological regulation all influence the rate of elemental uptake into the otolith.

**Otolith asymmetry in flatfishes**

All flatfishes undergo profound morphological changes during metamorphosis. During metamorphic transformation, changes occur in the cranium of flatfishes, and consequently all adult flatfishes have highly asymmetrical skulls with both eyes situated on one side of the head (Friedman 2008). However, despite the structural rearrangement of the fish, the otoliths remain in the conventional morphological positions, but are functionally rotated 90 degrees (Platt 1973). It is believed that body reorientation resulting from metamorphosis causes changes in the oculomotor and vestibular frames of reference, and that positional control and swimming ability are determined by both the utricular and sagittal otoliths (Helling et al. 2005). The physiological arrangement of the otoliths in flatfishes is also strongly associated with sound and vibration detection (Lowenstein 1971). More importantly, after the postural shift from the vertical to the horizontal plane, physical differences begin to appear between the sagittae in flatfishes. It is then conceivable that the morphological asymmetry of the sagittae, wherein the larger otolith is positioned on the blind side may benefit flatfish in the detection of prey through acoustical and mechanical vibrations that are transmitted through the substrate. Though the exact cause remains elusive, it appears that dimensional differences in the sagittae of flatfishes are an adaptation for life in the benthos, because with the exception of flatfishes, no other teleosts have asymmetrical
otoliths (Lychakov et al. 2008).

Flatfishes are also distinctive from pelagic fishes because they are in close association with both the water column and the sediments. The upper layers of marine sediments are complex boundaries where chemical kinetics activates an oxidation-reduction (redox) gradient capable of producing a change in oxidation states between chemical species. Thus, redox potential originating within the marine sediments - upon which flatfishes rest - may be partly responsible for the availability of certain elements in the blind-side otolith, whereas the eyed-side otolith may absorb elements from the water column similar to pelagic fishes.

**Project summary**

There are gaps in the body of knowledge pertaining to winter flounder biology, and because precipitous population declines are leading to a tenuous future, there is a pressing need to fill informational voids to ensure sustainability of the species. Accordingly, an important feature of this study involves an attempt to better understand winter flounder in relation to its environment while establishing a method to identify and protect critical recruitment habitat. To facilitate this objective, I used sagittal otoliths as a means of examining three pertinent aspects of winter flounder ecology: (1) the chemical and morphological asymmetry of winter flounder sagittae, (2) the fine-scale stock structure of inshore populations of winter flounder and recruitment habitat assessment through novel interpretations of otolith microchemistry, and (3) the dynamics driving the age and growth structure of winter flounder in the western Long Island Sound (WLIS) and Hudson River Estuary (HRE).

In chapter 1, I examined both the chemical and morphological asymmetry of winter flounder sagittae. In the physical investigation, I compared the total length of otolith pairs to
determine morphological relationship between the sagittae. In the chemical investigation, I examined concentrations of specific elements using laser ablation inductively coupled mass spectroscopy (LA-ICPMS) and scanning x-ray fluorescence (SXRF) to determine if morphological asymmetry in the sagittae corresponds to chemical asymmetry. I hypothesized that there would be differences in the patterns of chemical uptake given the morphological asymmetry and difference in the exposure to water sources (open water vs sediment pore water) by the eyed-sided and blind-side of the fish. Chapter 1 is an important precursor to future studies because if the otolith pairs are not chemically equivalent, then misclassification in the statistical analyses could occur, which could then potentially impact the classification rates when making population assignments. Bias in the classification investigations would then potentially skew interpretations of the results.

In chapter 2, examination of the stock structure of winter flounder in and around Long Island waters was performed with otolith microchemistry using the LA-ICPMS. Restricting the laser transects to the juvenile portion of the otolith enabled a direct comparison of the geochemical signatures among natal nurseries from the various age categories of our specimens. To facilitate discrimination between regions, the chemical concentration of eight elements in the respective otoliths were examined. The results of this chapter use the chemical concentrations as proxies to reconstruct seasonal movement patterns and habitat relationships, which then provides a level of insight into winter flounder biology and to our understanding of the stock structuring.

There is an ever present need to determine and understand stock structure in fish because it is central to any biological question and forms the basis of fisheries management, but defining stock structure in the marine realm is not an easy task either with conventional or modern approaches. In fact, current controversy exists regarding the population structure of winter
flounder as determined through genetic methods. Otolith microchemistry in contrast provides potential advantages over other techniques, because all telost fishes possess otoliths, which act as natural geochemical tags and from which group membership and habitat use can be inferred (Campana and Thorrold 2001). The main objective of this chapter is to investigate the feasibility of using otolith microchemistry with elemental markers to make quantitative assessments regarding the inshore population structure of winter flounder. New interpretations of otolith microchemistry using results from the LA-ICPMS and SXRF are introduced, which may allow fisheries scientists to infer differences in juvenile habitat quality.

In Chapter 3, the demographics of winter flounder in the HRE and WLIS was examined using otoliths to determine length-at-age and statistically compare those results to large-scale surveys performed decades ago, and which collectively form the historical record of winter flounder in New York waters. The hypothesis in this chapter proposes that as a result of declining density patterns, and an increasing presence of piscivorous predators, winter flounder will grow larger. The second part of this chapter used the data obtained from these analyses and examined abundance trends were to show that increased predation could be partly responsible for reduced winter flounder abundances.
CHAPTER 1

Life on the Bottom: the Chemical and Morphological Asymmetry of Winter Flounder 
(*Pseudopleuronectes americanus*) Sagittae

ABSTRACT

We observed that the paired winter flounder (*Pseudopleuronectes americanus*) sagittae are not morphologically or chemically identical. Statistically significant (p < 0.05) chemical asymmetry was detected between the sagittae in 5 of 8 elements (with three other elements, Sr, I, and Sn not statistically different). The blind-side otolith exhibited higher mean concentrations of Ba, Mn, Mg, Cu, and Zn trace elements than the eyed-side otolith. These asymmetries may be due to blind-side otoliths absorbing higher concentrations of transitional elements, partly as a result of environmental heterogeneity caused by redox reactions emanating from the sediments. This pattern may also account for element concentration levels that were significantly different between juveniles and both sub-adults as well as adults. The blind-side otolith may be absorbing higher concentrations of transitional elements partly as a result of environmental heterogeneity caused by redox reactions emanating from the sediments. Our results suggest that chemical uptake in members of an otolith pair is an asynchronous event, and that each potentially records the chemical history of their environment independently of the other. The physio-chemical pathway for entry into winter flounder otoliths is uncertain and warrants further testing. Our results have implications for other flatfish genera as well, and demonstrate the need for standardization of methodology when conducting otolith micro-chemical analyses on flatfishes.

Keywords: elemental uptake; metamorphosis; microchemistry; otolith; Pleuronectidae; sagittae
INTRODUCTION

Winter flounder (*Pseudopleuronectes americanus*) is a dextral flatfish found in coastal and estuarine environments of the northwest Atlantic ranging from Labrador to Delaware Bay, with centers of abundance located in the central portion of its range (Perlmutter 1947; Collete and Klein-McPhee 2002). Winter flounder, like all Pleuronectiformes, begin life as pelagic, bilaterally symmetrical larvae, but upon metamorphosis are transformed into asymmetrical, benthic flatfishes with laterally compressed bodies.

Located within the inner ear of all telosts are three sets of otoliths. Otoliths are paired calcium carbonate (CaCO$_3$) concretions that are necessary for spatial orientation and hearing. In non-ostariophyseans, the sagittae are the largest and are most often used for analysis. Growth of the sagittae is mediated by endogenous and exogenous rhythms operating over a range of temporal cycles (Campana and Nielson 1985). These rhythms cause patterns of incremental growth that enable estimation of age and chemical chronologies. During otolith growth, trace and minor elements from the external environment are introduced into the CaCO$_3$ matrix by substitution of Ca$^{+2}$, insertion into the interstitial spaces of the crystalline structure, or by binding to proteins (Miller et al. 2006). However, physiological controls may regulate pathways for some elements into the otolith (Sturrock et al. 2014). Despite the mode of entry, combinations of elemental ratios contained in the otolith have been used as geochemical tags to permit retrospective tracking of fish movements, reconstruction of the chemical environment, and discrimination among groups of fish (Elsdon et al. 2008).

Most applications of otolith microchemistry conducted on bilaterally symmetrical species have been predicated on the assumption that members of an otolith pair are absorbing elements equivalently from their environment, with each otolith indistinguishable from the other. This
assumption is reasonable because the sagittae in bilaterally symmetrical fishes are morphologically similar and the chemical environment for both (i.e., open water) is identical. Several studies have addressed the question of chemical equivalency in the sagittae of bilaterally symmetrical fishes but failed to detect significant differences between left and right otoliths (e.g., Gauldie 1996; Rooker et al. 2001). However, concern arises with the bilaterally asymmetrical flatfishes such as winter flounder, because unlike vertically oriented fishes, the sagittae are not morphologically equivalent and the chemical environment may not be identical for both.

The marine sediments upon which winter flounder rest and immerse themselves are chemically complex and reactive interfaces that may cause environmental heterogeneity on the respective sides of a flatfish. Given the difference in exposure to overlying water column vs. sediment porewater by its top and bottom sides, we hypothesize that winter flounder exhibit chemical asymmetry in their sagittae. Additionally, we hypothesize that adaptation to the benthic lifestyle produces morphological asymmetry as well. Thus, it needs to be determined if the sagittae of winter flounder exhibit chemical as well as morphological asymmetry.

**METHODS**

Winter flounder were collected (N = 115) from 13 locations within waters surrounding Long Island, NY, by trawling, seining, and angling (Fig. 1). Specimens ranged in size from 53.0 mm to 415 mm total length (TL). Upon extraction, otoliths were placed in 10% NaClO and rinsed in Milli-Q de-ionized water to remove impurities or adhering tissue. Otoliths were then air dried in a fume hood and stored in polypropylene vials. A deformed otolith was discovered during extractions and was stored with its complement for later use in the chemical analyses.
**Otolith morphology**

Seventy-seven adult and sub-adult otolith pairs were removed from specimens, which ranged in size from 92 – 415 mm. The otolith pairs were viewed under a Leica MZ7 dissecting microscope connected to a Schott 15000 LCD light source. Examinations were conducted between magnification settings of 6.5X and 16X and measurements were taken using the Leica Suite software program. Length measurements were taken at the point of greatest maximum length (mm) in the sagittal plane of each otolith (Fig. 2).

**Otolith chemistry**

A total of 38 winter flounder were subsetted from the original 115 and used in the chemical investigation. Size classes for this study were designated as follows: Young-of-the-year (YOY) specimens were identified using a New York State Department of Environmental Conservation (NYSDEC) protocol as follows: (YOY are identified as follows: Apr-May < 65 mm; Jun < 90mm; Jul < 115 mm; Aug < 140 mm; Sep-Nov < 165 mm), sub-adults (not considered YOY and < 250 mm TL), and adult specimens (>250 mm TL). This methodology resulted in the following categorizations of specimens used for chemical analysis: 12 YOY, 13 sub-adults, and 13 adults (Table 1).

The otolith pairs were embedded in EpoFix™ epoxy and allowed to dry until sufficiently hard to permit sectioning without risk of fracturing the otolith crystals. Sectioning was performed using a Buehler® Isomet™ low-speed saw with a diamond-wafering blade while immersed in Milli-Q water. Hand grinding and polishing was performed using a series of increasingly finer lapping films (3M®) ranging from 30 um - 0.05 um to remove excess overburden, and to polish the otolith to a desired degree of resolution.
The otoliths pairs were analyzed by laser ablation inductively coupled plasma mass spectroscopy (LA-ICPMS). In preparation for the LA-ICPMS, polished otoliths were mounted on petrographic slides with cyanoacrylate glue or double-sided adhesive tape and cleansed ultrasonically in Milli-Q water. Elemental concentrations of Ba$^{138}$, Cu$^{63}$, I$^{127}$, Mg$^{24}$, Mn$^{55}$, Pb$^{208}$, Sr$^{88}$, Sn$^{120}$, and Zn$^{64}$ were selected for analysis because of our interest in their relationship the external environment and concentrations in the otolith. Ablation was performed using a New Wave Research UP-193 (Electro Scientific Industries) 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). Laser spot size was 35 µm with a scan speed of 3 µm/sec, and a repetition rate of 10 Hz. A MACS-3 carbonate reference standard (USGS – Geochemical Reference Materials and Certificates) was used for correction calculations. Limits of detection (LOD) were calculated as mean concentration of the blank + 3 x standard deviation. When more than 25% of data points fell below LOD, that element was excluded from further analysis (Brazner et al. 2004). Calcium concentrations were checked by comparison to an in-house otolith standard (see Limburg et al. 2011), and with the MACS-3 standard. All elements were expressed as ratios of calcium to normalize concentrations with beam strength. For each otolith, a single ablation transect was run in the sagittal plane from the central primordium to the outer margins or from edge-to-edge, passing through the central primordium core. Transects for each pair were equivalent in direction and were mapped onto the growth axis alongside the succal groove to maintain consistency between scans. All hypotheses in this study were tested with a p-value of 0.05.

The deformed otolith and its complement were examined with scanning X-ray fluorescence microscopy (SXFM) at the Cornell High Energy Synchrotron Source, Ithaca, NY,
using the F3 bending magnet beamline (see Limburg et al. 2011 for further description of methods). Otolith preparation is similar to the LA-ICPMS methods, but samples were mounted on fused quartz disks. Specimens were rastered with a focused X-ray beam of ca. $10^{11}$ photons/second, across an energy spectrum of 2-16 KeV. Fluorescence counts were collected for 1-2 seconds with an energy dispersive Vortex silicon drift detector and fitted with an aluminum foil attenuator to damp Ca counts and increase sensitivity to trace elements. Transects were created in ArcMap to extract and transform the synchrotron data to develop high resolution, 2-D elemental maps that could be analyzed with ArcGIS.

**Statistical methods**

During chemical analyses, repeated measurements of reference standards and sample blanks were used to assess measurement precision of our selected elements. Relative standard deviation (RSD) was obtained from our reference standards, and was found to be consistent within elements, with values <10% for all elements, with the exception of Pb and Sn, which ranged between 10 and 15%, and I, which ranged between 15 and 20%. Adjusted sample counts for Pb were near or below LOD and were eliminated from further analyses.

Testing for chemical differences between the sagittae was performed with a paired t-test and a multivariate analysis of variance (MANOVA). The MANOVA was used to detect differences in the age-classes. Prior to any testing, normality assessments were conducted with the Shapiro-Wilkes (SW) test. Mean concentrations were examined for each element to determine which of the sagittal pair contained the higher element/Ca ratio. Extremely high values of Cu, Zn, Sn, and Sr in the leading and trailing edges as well as in the core regions were eliminated from the analyses because it was unknown if these results were due to contamination
or peculiarity of the LA-ICPMS. More importantly, extreme variations within a single otolith would cause a significant difference between the pairs.

Least squares regression analysis was performed comparing the eyed-side otolith to the blind-side otolith using a linear relationship \( \hat{Y}_{(\text{blind-side})} = mX_{(\text{eyed})} + b \) based upon an expected correlation between the otolith pairs. This approach models the scalar relationship between the eyed-side otolith as the dependent variable and the blind-side otolith as the explanatory variable and calculates the best-fitting line from the observed data to determine strength of the association between the variables. Diagnostic plots were created to view the fit of the regression line to the data and to discover potential outliers that might exert undue influence on the model.

**RESULTS**

*Otolith morphology*

Morphological investigation of 77 sagittal otolith pairs revealed that the blind-side otolith was larger than the eyed-side in 74% of the evaluations, whereas the eyed-side otolith was larger in 25% of the cases, with one pair equivalent in length. Results of the least squares linear regression comparing total length of the eyed-side otolith against total length of the blind-side otolith revealed a significant difference in the length dimensions between the otolith pairs (\( F = 2944.9, p < 2\times10^{-16} \)). No outliers were observed. The slope of the regression line differed significantly from 1, but showed a strong positive linear correlation. From these analyses, we found the lengths of otoliths pairs to be strongly correlated (\( R^2 = 0.975 \)), showing a high degree of proportionality and conforming to a linear equation \( \hat{Y}_{(\text{blind-side})} = 1.066 (X_{(\text{eyed})}) - 0.191 \) (Fig. 3).
**Otolith chemistry**

Statistical testing of raw elemental data revealed non-parametric distribution patterns. Data were subsequently log-transformed to improve normality and homogenize variances. Log-transformation showed improvement towards normality, but still failed both SW tests with a p-value < 0.01. Nevertheless, the paired t-test and MANOVA are considered robust to violations of normality and can be used under most practical conditions, especially with large data sets (Schmider et al. 2010; McGarigal et al. 2000). The paired t-tests performed on the pooled sample sets revealed significant differences for 5 of the 8 of the elements (Table 2). The MANOVA showed that significant differences among our sample set (Pillai’s Trace = 0.0766, F = 211.072, p-value <0.0001). Significant differences occurred among age-categories \( p_a \) for all elements and within most age-categories \( p_w \), with the exception of Sr and Ba.

Otolith pairs were then examined to determine which of the two expressed a higher mean concentration for each element (Table 3; Figs 4 & 5). In the pooled sample, the blind-side otolith exhibited higher mean concentrations for Ba, Cu, Mg, Mn, Sr, and Zn, whereas I and Sn expressed higher concentrations in the eyed-side otolith. Testing of mean concentration between otolith pairs was performed for the three age-categories. In these tests, the both sub-adults and adults showed similar results with higher mean concentrations on the blind side for all elements with the exception of Zn, which was found to be higher on the eyed-side otoliths. The YOY in contrast to the other two age-classes expressed higher mean values for I, Mn, and Sn in the eyed-side otolith. Thus, it appears that concentrations of I and Sn in the YOY population affected the outcome of the pooled sample, and weighed heavily on the overall results. For all the age-class comparisons, the elements Sr, Ba, Mg, and Cu were found to be higher in the blind-side otolith. Moreover, Sr, Ba, Mn, and Mg were found in higher concentrations in comparison to the Cu, I,
Sn, and Zn, which were often an order of magnitude or more lower in concentration.

The deformed otolith was unusual in appearance and structure, dotted with blue inclusions in contrast to its normal-appearing complement (Fig 6A). When this pair of otoliths was examined with SXFM, we discovered the blue formations contained elevated concentrations of Fe, which were not detected in the other (Fig. 6B). Corresponding graphs and 2-D images produced from synchrotron data reveal chemical asymmetry occurs in the otolith pairs among elements tested. For a more thorough discussion of the results of this chemical analysis see Limburg et al. (2015).

DISCUSSION

Our results show that the paired sagittae in winter flounder are neither morphologically nor chemically identical and thus cannot be used interchangeably. Morphological analyses show the blind-side otolith to be larger than the eyed-side otolith in 74% of the investigations, though length measurements between the otoliths pairs show a positive, linear relationship (Fig. 3). Strong correlation in the lengths measurements of the otolith pairs demonstrates a high degree of proportionality despite the dimensional differences between them. Reasons for the morphological asymmetry of flatfish sagittae are speculative, but positional control during lateralization processes and adaptation for life in the benthos has been suggested (Helling et al.2005; Schreiber 2006). Regardless of the exact cause, a change in the postural orientation in winter flounder introduces a corresponding asymmetry in the growth patterns of the sagittal otoliths that continues to diverge over time (Sogard 1991; Jearld et al. 1993; Helling et al. 2005).

In the chemical analysis, concentrations for most of the elements tested (Ba, Mn, Mg, Cu, and Zn) showed significant differences between the blind side and eyed-side sagittae, though
some did not (I, Sn, and Sr) (Table 2). Significant differences in elemental concentrations also occur among the different age-categories for all elements, though within age-categories show significant differences for most elements, with the exception of Sr and Ba (Table 2). Chemical changes between the sagittae of different age-categories are not unique to winter flounder. Ontogenetic changes in chemical chronologies have been reported in other flatfishes (Toole et al. 1993; de Pontual et al. 2003), and were also shown to occur in the respective age-categories of a bilaterally symmetrical species as well (Walther et al. 2010).

In general, the blind-side otolith of our specimens exhibited the highest chemical concentrations for all age classes with some variation to this trend for individual elements (Figs 4 & 5). The sub-adult and adult age-categories showed higher concentrations on the blind side for all elements with the exception of Zn. The YOY in contrast to other age-categories had higher concentrations for 5 of 8 elements on the blind side with Mn, Sn, and I higher on the eyed-side otolith. However, the high concentrations of I and Sn in the eyed-side of the YOY influence results in the pooled sample, because neither the sub-adults or the adults show higher concentrations of these metals in their blind-side otoliths (Table 3). Specific elements (e.g., Sr, Ba, Cu, and Mg), showed higher concentrations on the blind-side otolith for all age groups, whereas the others (Mn, I, Sn and Zn) did not. Why some metals react differently is unknown, but variability within age-classes may result from chemical properties of the metals or biological influence manifested by the fish. With transition metals (e.g., Cu, Mn, and Zn), protein binding may occur during otolith growth or bio-regulation of these potentially toxic elements may occur before introduction into the endolymph (Sturrock et al. 2012). Furthermore, it has been reported that drastic changes occurring during metamorphosis are liable to affect the otolith chemical patterns, irrespective of environmental conditions (de Pontual et al. 2003).
Other studies have suggested that morphological and chemical asymmetry between the sagittae in flatfishes results from a skew in relative rates of CaCO₃ accretion, similar to how the three otolith pairs (sagittae, asteriscii, and lapilli) integrate material differently from each other (Loher et al. 2008; Kajajian et al. 2013). Disparity in accretion rate between the sagittae likely contributes to the chemical asymmetry, but it is conceivable that environmental differences experienced by the respective sides of the fish add to the chemical asymmetry of the sagittae.

**Flatfish sagittae and environmental heterogeneity**

Flatfishes undergo profound morphological changes as they shift from a vertical to a horizontal orientation. During metamorphosis, structural rearrangement of the fish occurs, and yet the otoliths remain in conventional morphological position but are rotated 90 degrees (Platt 1973). Otolith shape also changes during metamorphosis. Prior to metamorphosis, the sagittae of larval winter flounder are virtually identical, round disks, but upon eye migration undergo divergent growth patterns, which causes changes in mass and shape (Sogard 1991 Helling et al. 2005). Completion of metamorphosis finds the fully formed winter flounder settling into the benthos with its blind-side proximal to the substrate while its eyed-side is directed towards the overlying water column. The sagittae of the transformed winter flounder are now positioned with the eyed-side otolith effectively stacked above the blind-side otolith. In this configuration, we believe that the chemical environment for the respective otoliths may not be identical and disparate patterns of chemical exposure may occur.

Marine sediments are complex interfaces where biogeochemical recycling processes cause redox reactions to occur. Hypoxic conditions cause the redox potential of divalent elements (e.g., Cu, Fe, Mn, Zn) to become activated, which liberates them from the sediments
(Calvert and Pedersen 1993; Kristiansen et al. 2002), and drives their availability for sediment-dwelling organisms (Baden and Eriksson 2006). Since organically rich marine sediments may become hypoxic within millimeters of substrate surface, the blind-side of a winter flounder, which is often in contact or immersed in these sediments, would presumably be more exposed to elemental flux than the eyed-side, causing uneven patterns of chemical uptake between the otoliths. However, the biological pathways for divalent cations into the endolymph are unknown at this point.

During the course of this investigation a deformed otolith was removed from the blind side of a mature winter flounder. The discovery of the deformed otolith was surprising as they have never been reported in winter flounder, and the fish was not exceptional, other than its otoliths. However, results from the SXFM had shown extreme chemical incongruence between the otolith pairs. While the cause of deformed otoliths in flatfishes is still undetermined, this unusual otolith highlights the potential for morphological and chemical disjunction to occur between paired sagittae during bio-mineralization processes and lends support to our findings regarding otolith asymmetry.

Thus, it is conceivable that higher chemical concentrations associated with the blind-side otoliths of winter flounder are influenced by both the disparity in patterns of carbonate accretion and environmental heterogeneity between the otolith pairs. Spatial discontinuity regarding ions and proteins within in the endolymph has been shown to correspond to variations in the chemical composition of the otolith (Payan et al. 1999). Accordingly, if a chemical gradient exists within the endolymph and within the otolith itself, then it may be feasible for a chemical incongruity to occur between otoliths pairs. However, we note that accumulation of metals in fish otoliths is a complex process and depends on a number of factors including environmental concentration
(Campana 1999), physiological state and growth rate of the individual fish, (Geffen et al. 1998), temperature (Elsdon and Gillanders 2004), pH (Payan et al. 1999; Takagi 2000), as well as sex, season, and reproductive condition (Kalish 1991; Sturrock et al. 2014).

Transitional elements and environmental concentration

Uncertainty arises when attempting to determine how accurately the external environment is reflected in the otolith and how much control biological factors exert over elemental uptake. Part of this uncertainty is caused by the tendency of some transitional elements (e.g., Mn, Cu, and Zn) to bind to otolith proteins or to become physiologically regulated because of their toxicity to the organism in high concentrations (Campana 1999; Miller et al. 2006; Sturrock et al. 2014). Complexity may also be introduced because growth rate may be associated with elemental concentrations within the otolith (e.g., Mn and Mg) (Sturrock et al. 2014; Limburg et al. 2015). Consequently, physiological influence on these metals could outweigh environmental signals in otoliths of marine fish. However, Arslan and Secor (2005) had shown a direct relationship between concentrations of transition and heavy metals in American eel (Anguilla rostrata) otoliths and their proximity to sites of known industrial contamination. In another study, Barbee et al. (2013) had reported elevated Cu in the otolith, wherein a threshold effect occurs and once Cu surpasses a certain level, variations in the patterns of uptake occur. It is therefore unlikely that otolith levels of transitional metals truly reflect environmental conditions in all but extreme conditions (Milton and Chenery 2001; Miller et al. 2006). Nevertheless, the waters surrounding New York City and other parts of Long Island from which these fish were sampled might indeed represent extreme conditions. These waterways are known to be some of the most contaminated on the east coast of the United States, possessing some of
the highest concentrations of transitional and post-transition metals (Turgeon and O’Connor 1991; Adams et al. 1998; NYCDEP 2012). However, despite the potential for bio-regulation, the association of Mn otolith concentrations and hypoxic conditions has been shown to occur in other fishes (Limburg et al. 2011; Mohan et al. 2014; Limburg et al. 2015).

Flatfishes and otolith chemistry

Overall, our results are similar to those obtained by Kajajian et al. (2013) with the left-eyed summer flounder (Paralichthys dentatus), and Loher et al. (2008) with the right-eyed Pacific halibut (Hippoglossus stenolepis) whose otolith pairs showed significant differences with regard to trace metals and stable isotope concentrations. In the Pacific halibut, Loher et al. (2008) only found the Sr to be significantly different between sagittae, whereas with the summer flounder, Kajajian et al. (2013) found Li, Mg and Sr to be significantly different. Though differences are apparent among the flatfish species, chemical incorporation into the otolith is known to be element and species-specific (de Pontual et al. 2003; Hamer and Jenkins 2007). The most important outcome in these studies is that morphological and chemical asymmetry was shown to occur in the sagittae of flatfish species exhibiting both dextral and sinistral orientation, and thus likely applies to other members of the Pleuronectiformes. However, previous studies have performed otolith chemistry on flatfishes and failed to mention which otolith was used during the analyses (de Pontual et al. 2000; de Pontual et al. 2003, Swearer et al. 2003; Vasconcelos et al. 2008; Leakey et al. 2009), or was restricted to the eyed-side otolith (Brown 2006). Procedural differences have pragmatic relevance because exclusive use of either a left or right otolith with a flatfish species will alter assignments in classification models. The highest accuracy in one mixed-stock analysis for summer flounder was obtained using the blind-side
otolith (Kajajian et al. 2013). Accordingly, our results when considered with those of Loher et al. (2008) and Kajajian et al. (2013) demonstrate that otolith chemistry with flatfishes should be performed using the blind-side otolith, in contrast to bilaterally symmetrical species whose otoliths can be used interchangeably.

CONCLUSIONS

In this study, we have shown that winter flounder otoliths exhibit both morphological and chemical asymmetry. At this time, it is unknown if disparity in chemical concentrations between the otolith pairs results from differences in the rates of CaCO₃ accretion or if environmental heterogeneity between the respective sides of a flatfish contributes to the chemical asymmetry. The evidence we have presented results from empirical testing, but we believe that in situ testing of redox chemistry with flatfishes is warranted.

ACKNOWLEDGEMENTS

We thank following people and organizations for their assistance during this study: Henningson, Durham, and Richardson (HDR), the United States Army Corps of Engineers (USACE), and the New York State Dept. of Environmental Conservation (NYS DEC) for providing winter flounder samples, Deb Driscoll (SUNY ESF) for assistance with sample analysis. This work is based in part upon research conducted at the Cornell High Energy Synchrotron Source (CHESS), which is supported by the National Science Foundation and the National Institutes of Health/National Institute of General Medical Sciences under NSF award DMR-00936384. We would also like to thank the CUNY Graduate Center for making this research possible.
Table 1.1. Length and age-class characteristics of 38 winter flounder samples used in the chemical analyses from 13 estuarine locations. TL = total length.

<table>
<thead>
<tr>
<th>Area</th>
<th>n</th>
<th>TL$_{mm}$ (mean ± sd)</th>
<th>Age-Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthur Kill</td>
<td>2</td>
<td>234.0 ± 91.2</td>
<td>1 adult &amp; 1 sub-adult</td>
</tr>
<tr>
<td>Bronx River</td>
<td>2</td>
<td>52.5 ± 6.4</td>
<td>2 YOY</td>
</tr>
<tr>
<td>Jamaica Bay</td>
<td>4</td>
<td>195.3 ± 163.9</td>
<td>2 adult &amp; 2 YOY</td>
</tr>
<tr>
<td>Huntington Harbor</td>
<td>1</td>
<td>330.2</td>
<td>1 adult</td>
</tr>
<tr>
<td>Hog Neck Bay</td>
<td>5</td>
<td>139.4 ± 6.4</td>
<td>5 sub-adult</td>
</tr>
<tr>
<td>Little Neck Bay</td>
<td>4</td>
<td>233.9 ± 97.9</td>
<td>3 adult &amp; 1 YOY</td>
</tr>
<tr>
<td>Lower Bay</td>
<td>1</td>
<td>226.0</td>
<td>1 sub-adult</td>
</tr>
<tr>
<td>Manhasset Bay</td>
<td>1</td>
<td>74.0</td>
<td>1 YOY</td>
</tr>
<tr>
<td>Moriches Bay</td>
<td>7</td>
<td>53.0 ± 1.8</td>
<td>7 YOY</td>
</tr>
<tr>
<td>Newark Bay</td>
<td>1</td>
<td>136.0</td>
<td>1 sub-adult</td>
</tr>
<tr>
<td>Oyster Bay</td>
<td>4</td>
<td>320.7 ± 52.1</td>
<td>4 adult</td>
</tr>
<tr>
<td>Port Jersey</td>
<td>2</td>
<td>137.0 ± 18.4</td>
<td>2 sub-adult</td>
</tr>
<tr>
<td>South Brooklyn</td>
<td>4</td>
<td>262.5 ± 74.9</td>
<td>4 adult</td>
</tr>
</tbody>
</table>
Table 1.2. Results of the paired t-test between blind side and eyed-side otolith are shown. Results of the MANOVAs show differences among and within age-classes.

<table>
<thead>
<tr>
<th>Left vs Right Otolith</th>
<th>t-ratio</th>
<th>t</th>
<th>S/NS</th>
<th>p_w</th>
<th>p_a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr</td>
<td>2.782</td>
<td>&lt;0.6340</td>
<td>NS</td>
<td>&lt;0.8527</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ba</td>
<td>4.632</td>
<td>&lt;0.0001</td>
<td>S</td>
<td>&lt;0.2180</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mn</td>
<td>1.388</td>
<td>&lt;0.0036</td>
<td>S</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mg</td>
<td>14.89</td>
<td>&lt;0.0001</td>
<td>S</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cu</td>
<td>4.411</td>
<td>&lt;0.0003</td>
<td>S</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Zn</td>
<td>6.329</td>
<td>&lt;0.0005</td>
<td>S</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>I</td>
<td>6.258</td>
<td>&lt;0.2921</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sn</td>
<td>143.46</td>
<td>&lt;0.6507</td>
<td>NS</td>
<td>&lt;0.0022</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

p_w = p-value within age groups, p_a = p-value among age groups, S/NS = Significant or non-significant
Table 1.3A. Tests of mean concentration in otolith pairs for the pooled sample results of adults, sub-adults and YOY reveals that the highest elemental concentrations occur the left otolith, with the exception of I and Sn, which show higher concentrations in the right otolith.

<table>
<thead>
<tr>
<th>Pooled</th>
<th>Left Otolith (mean ± sd)</th>
<th>Right Otolith (mean ± sd)</th>
<th>Difference</th>
<th>Side with higher concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr</td>
<td>3.51750 ± 1.0018</td>
<td>3.47239 ± 0.9526</td>
<td>0.03691</td>
<td>Left</td>
</tr>
<tr>
<td>Ba</td>
<td>0.01868 ± 0.0083</td>
<td>0.08553 ± 0.082</td>
<td>0.00038</td>
<td>Left</td>
</tr>
<tr>
<td>Mn</td>
<td>0.03180 ± 0.0326</td>
<td>0.03145 ± 0.0390</td>
<td>0.00044</td>
<td>Left</td>
</tr>
<tr>
<td>Mg</td>
<td>0.04605 ± 0.0421</td>
<td>0.04282 ± 0.0345</td>
<td>0.00916</td>
<td>Left</td>
</tr>
<tr>
<td>Cu</td>
<td>0.00114 ± 0.0021</td>
<td>0.00104 ± 0.0017</td>
<td>0.00010</td>
<td>Left</td>
</tr>
<tr>
<td>Zn</td>
<td>0.00528 ± 0.0096</td>
<td>0.00477 ± 0.0077</td>
<td>0.00051</td>
<td>Left</td>
</tr>
<tr>
<td>I</td>
<td>0.00280 ± 0.0020</td>
<td>0.00327 ± 0.0043</td>
<td>0.00047</td>
<td>Right</td>
</tr>
<tr>
<td>Sn</td>
<td>0.00029 ± 0.0013</td>
<td>0.00076 ± 0.0023</td>
<td>0.00047</td>
<td>Right</td>
</tr>
</tbody>
</table>
Table 1.3B. Comparison of left and right otoliths when sorted by age-class reveals that adult left otolith maintained the highest mean concentrations for most elements tested.

<table>
<thead>
<tr>
<th>Adults</th>
<th>Left Otolith (mean ± sd)</th>
<th>Right Otolith (mean ± sd)</th>
<th>Difference</th>
<th>Side with higher concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr</td>
<td>3.80234 ± 1.1040</td>
<td>3.78434 ± 1.0234</td>
<td>0.05894</td>
<td>Left</td>
</tr>
<tr>
<td>Ba</td>
<td>0.00610 ± 0.0038</td>
<td>0.00532 ± 0.0026</td>
<td>0.00078</td>
<td>Left</td>
</tr>
<tr>
<td>Mn</td>
<td>0.01808 ± 0.0266</td>
<td>0.01587 ± 0.0266</td>
<td>0.00221</td>
<td>Left</td>
</tr>
<tr>
<td>Mg</td>
<td>0.05251 ± 0.0273</td>
<td>0.03709 ± 0.0259</td>
<td>0.01542</td>
<td>Left</td>
</tr>
<tr>
<td>Cu</td>
<td>0.00166 ± 0.0019</td>
<td>0.00153 ± 0.0020</td>
<td>0.00013</td>
<td>Left</td>
</tr>
<tr>
<td>Zn</td>
<td>0.00361 ± 0.0079</td>
<td>0.00374 ± 0.0064</td>
<td>0.00013</td>
<td>Right</td>
</tr>
<tr>
<td>I</td>
<td>0.00206 ± 0.0017</td>
<td>0.00186 ± 0.0019</td>
<td>0.00020</td>
<td>Left</td>
</tr>
<tr>
<td>Sn</td>
<td>0.00015 ± 0.0001</td>
<td>0.00012 ± 0.0001</td>
<td>0.00003</td>
<td>Left</td>
</tr>
</tbody>
</table>
Table 1.3C. Comparisons between left and right otoliths of the sub-adult age-class reveals that the left otolith maintained the highest mean concentrations of most elements with the exception of Zn, which shows higher values in the right otolith.

<table>
<thead>
<tr>
<th>Sub-Adults</th>
<th>Left Otolith (mean ± sd)</th>
<th>Right Otolith (mean ± sd)</th>
<th>Difference</th>
<th>Side with higher concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr</td>
<td>3.56569 ± 1.0614</td>
<td>3.50415 ± 1.0038</td>
<td>0.06154</td>
<td>Left</td>
</tr>
<tr>
<td>Ba</td>
<td>0.01117 ± 0.0108</td>
<td>0.01090 ± 0.0104</td>
<td>0.00027</td>
<td>Left</td>
</tr>
<tr>
<td>Mn</td>
<td>0.01576 ± 0.0261</td>
<td>0.01560 ± 0.0243</td>
<td>0.00016</td>
<td>Left</td>
</tr>
<tr>
<td>Mg</td>
<td>0.05409 ± 0.0190</td>
<td>0.05135 ± 0.0207</td>
<td>0.00274</td>
<td>Left</td>
</tr>
<tr>
<td>Cu</td>
<td>0.00064 ± 0.0011</td>
<td>0.00061 ± 0.0009</td>
<td>0.00003</td>
<td>Left</td>
</tr>
<tr>
<td>Zn</td>
<td>0.00363 ± 0.0033</td>
<td>0.00367 ± 0.0030</td>
<td>0.00004</td>
<td>Right</td>
</tr>
<tr>
<td>I</td>
<td>0.00311 ± 0.0024</td>
<td>0.00262 ± 0.0016</td>
<td>0.00049</td>
<td>Left</td>
</tr>
<tr>
<td>Sn</td>
<td>0.00118 ± 0.0016</td>
<td>0.00095 ± 0.00023</td>
<td>0.00023</td>
<td>Left</td>
</tr>
</tbody>
</table>
Table 1.3D. Comparisons between left and right otoliths of the YOY age-class reveals that the 
YOY were the most equivocal of the age groups. Highest mean elemental concentrations occurs 
in the left otolith for Sr, Mg, & Zn, whereas Mn, Ba, Cu, I, and Sn are shown to be higher in the 
right otolith.

<table>
<thead>
<tr>
<th>YOY</th>
<th>Left Otolith (mean ± sd)</th>
<th>Right Otolith (mean ± sd)</th>
<th>Difference</th>
<th>Side with higher concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr</td>
<td>2.97849 ± 0.8339</td>
<td>2.91731 ± 0.7121</td>
<td>0.06118</td>
<td>Left</td>
</tr>
<tr>
<td>Ba</td>
<td>0.01989 ± 0.0083</td>
<td>0.01976 ± 0.0080</td>
<td>0.0004</td>
<td>Left</td>
</tr>
<tr>
<td>Mn</td>
<td>0.03123 ± 0.0357</td>
<td>0.03263 ± 0.0358</td>
<td>0.00140</td>
<td>Right</td>
</tr>
<tr>
<td>Mg</td>
<td>0.10001 ± 0.0391</td>
<td>0.09430 ± 0.0377</td>
<td>0.00571</td>
<td>Left</td>
</tr>
<tr>
<td>Cu</td>
<td>0.00097 ± 0.0009</td>
<td>0.00096 ± 0.0009</td>
<td>0.00001</td>
<td>Left</td>
</tr>
<tr>
<td>Zn</td>
<td>0.01001 ± 0.0129</td>
<td>0.00727 ± 0.0049</td>
<td>0.00274</td>
<td>Left</td>
</tr>
<tr>
<td>I</td>
<td>0.00334 ± 0.0016</td>
<td>0.00538 ± 0.0065</td>
<td>0.00204</td>
<td>Right</td>
</tr>
<tr>
<td>Sn</td>
<td>0.00043 ± 0.0011</td>
<td>0.00100 ± 0.0023</td>
<td>0.00057</td>
<td>Right</td>
</tr>
</tbody>
</table>
Figure 1.1. Samples were obtained from 13 locations around Long Island. The locations are as follows: Lower Bay (1), South Brooklyn (2), Arthur Kill (3), Newark Bay (4), Port Jersey (5), Jamaica Bay (6), Little Neck Bay (7), Manhasset Bay (8), Hempstead Harbor (9), Oyster Bay (10), Huntington Harbor (11), Hog Neck Bay (12), Moriches Bay (13)
**Figure 1.2.** Comparison of a pair of sagittal otoliths from an adult winter flounder. The top otolith shows the blind-side otolith and bottom is the eyed-side otolith. The blind-side otolith is 7.462 mm, whereas the eyed-side otolith is 7.018 mm. Notice the difference in the location of growth centers in a mature winter flounder’s sagittae.
Figure 1.3. Linear regression of the morphological relationship between left vs. right otolith (mm TL) for 77 otolith pairs. Solid red line represents the slope of the regression, and the blue dotted line is a 1:1 line representing perfect symmetry between the blind-side otolith and the eyed-side otolith.
Figure 1.4. The figure shows mean concentration of otolith pairs, which were sorted by age, class, and element. Each figure represents a separate elemental concentration. The dark bar on the left represents the blind-side otolith, whereas the lighter bar on the right represents the eyed-side otolith. The figures correspond to (A) Strontium (Sr), (B) Barium (Ba), (C) Manganese (Mn), and (D) Magnesium (Mg).
Figure 1.5. The figure shows mean concentration values of the otolith pairs, which were sorted by age, class, and element. Each figure represents a separate elemental concentration. The dark bar on the left represents the blind-side otolith, whereas the lighter bar on the right represents the eyed-side otolith. The figures correspond to (A) Copper (Cu), (B) Zinc (Zn), (C) Iodine (I), and (D) Tin (Sn).
Figure 1.6. A) A pair of winter flounder otoliths removed from an adult winter flounder captured in Little Neck Bay. Note the difference in structure between the pair as well as the blue inclusions in the deformed otolith. The discovery of this aberrant otolith prompted testing for heavy and transitional metals as well as chemical asymmetry in sagittal otoliths. B) Chemical differences between the pair are shown with respect to Fe.
CHAPTER 2

Fine-Scale Structure of Inshore Populations of Winter Flounder Examined Through Otolith Microchemistry

ABSTRACT

We used otolith microchemistry to investigate the stock structure of winter flounder \((Pseudopleuronectes americanus)\) from New York Harbor, western Long Island Sound, and eastern Long Island. Using laser ablation inductively coupled mass spectroscopy and scanning X-ray fluorescence microscopy we detected significant differences among regions, sites, and years. We obtained 83% re-classification accuracy to our sampling locations. When we reduced the scale from the regional level to the specific embayments we sampled, we obtained an even greater 87% re-classification accuracy. These results suggest that within-region heterogeneity and similarity among regions may have confounded the results. However, an even higher accuracy might have been achieved if we had a more temporally cohesive sample set. The high rate of statistical accuracy in our methods allows us to infer that winter flounder exhibit high rates of natal homing, which is fundamental to population structuring. We also assessed the habitat quality by using the elements Mn and Mg as proxies for hypoxia and metabolic growth rates among sample sites. This study not only supports the validity of otolith microchemistry for winter flounder stock discrimination, but we advance it another step with novel interpretations of trace elemental chemistry. Thus, otolith microchemistry can be a predictor of quality recruitment habitat for winter flounder.
INTRODUCTION

Winter flounder (*Pseudopleuronectes americanus*) is a widely distributed flatfish found in the coastal waters and inshore of the northwest Atlantic, and is most common in the central portions of its range (Perlmutter 1947). In United States waters, winter flounder are managed as three stocks, which are identified as the Southern New England/Middle Atlantic (SNE/MA), Gulf of Maine (GOM), and Georges Bank (GB) (Pereira et al. 1999). The SNE/MA and GOM stock are usually described as estuarine dependent, whereas the GB stock is recognized as an offshore population lacking estuarine requirements for recruitment purposes (Howe and Coates 1975). Aside from the behavioral differences, minor morphological discrepancies have been identified among the three-stocks (Lux et al. 1970; Howe and Coates 1975; Pierce and Howe 1977).

More recently, genetic analyses found little fine-scale heterogeneity across the range of winter flounder populations, which supports the broad-scale, three-stock designation currently utilized by management agencies (Wirgin et al. 2014). The results of this study are at odds with earlier genetic analyses that suggested winter flounder populations are more localized in structure (Crivello et al. 2004; Buckley et al. 2008; O’Leary et al. 2013). While these studies have contributed to our understanding of genetic connectivity amongst winter flounder populations, in the marine environment low levels of gene flow can confound results, while also being unable to show natal origins and direct habitat association. In addition to the discord over stock structuring, recent research has shown departures from commonly accepted patterns of reproductive movements and estuarine residency (Wuenschel et al. 2009; DeCelles and Cadrin 2010; Sagarese and Frisk 2011; and Fairchild 2013).

Much of the knowledge pertaining to winter flounder biology has been amassed through
large-scale tagging surveys conducted in the past century. While traditional tagging methods are relatively effective at detailing population structure and movement patterns, they have limitations and also cannot reveal natal origins, and potentially disclose more about fishing pressure than the fish themselves.

Otolith microchemistry in contrast the aforementioned methods has great potential to resolve mixed-stock analyses and determine movement patterns, because results are derived directly from geochemical cues contained within each fish’s otoliths, (Walther and Thorrold 2010; Turner et al. 2015). Three pairs of otoliths are possessed by all telosts, which collectively provide inertial positioning and acoustical information to these fishes. The sagittal otoliths used in this study are paired calcium carbonate (CaCO$_3$) concretions primarily composed of aragonite, which is a polymorph of CaCO$_3$ (Mugiya et al. 1981). During otolith growth, trace elements absorbed from the external environment are introduced into the CaCO$_3$ matrix by substituting for Ca$^{2+}$, or are inserted into the interstitial spaces of the crystalline structure (Campana 1999). Recent research shows some transition metals may be introduced into the CaCO$_3$ structure by binding to otolith proteins (Miller et al. 2006; Sturrock et al. 2012). Regardless of the mechanism of entry into the otolith, trace elements when normalized to Ca can provide a permanent chronological record of the fish’s exposure to different environments to offer insights into movement patterns (Campana and Thorrold 2001). There is however an a priori assumption when conducting otolith microchemistry that fish obtained from chemically distinct waters will record recognizable signatures in the growth increments. Accordingly, group membership and habitat use can be inferred from chemical ratios recorded in the otolith.

The Hudson River Estuary (HRE) and the coastal margins of Long Island, New York have been historically important recruitment habitat for the SNE/MA stock of winter flounder
(Lobell 1939; Perlmutter 1947; Poole 1966; Phelan 1992). In this study we examined the population structure of winter flounder from these regions using otolith microchemistry with a multi-elemental set of markers. No previous studies exist using otolith microchemistry on winter flounder from the SNE/MA stock to examine population structure and also to assess their habitat, although otolith microchemistry has been used on other flatfishes (Brown et al. 2006, Vasconcelos et al. 2007). More importantly, with winter founder populations currently at an all-time low (ASMFC 2014), there is an unyielding need to understand genetic connections among populations, and to identify and assess critical recruitment habitat. Therefore, the main objective of this study is to use otolith microchemistry with beam-based methods to: (1) investigate the feasibility of discriminating between groups of fish from different regions, (2) infer life-history patterns that lead to stock structuring in winter flounder, (3) apply new interpretations of otolith microchemistry to assess differences in juvenile habitat quality.

**METHODS**

*Study System and sample collection*

Fifty-three winter flounder were collected from 13 locations around Long Island, and an additional 5 were analyzed from NOAA collections acquired from Georges Bank for use as an out-group (Figure 1). All specimens were obtained by trawling, seining, and angling from 2006 – 2010, and ranged in size from 48 mm to 520 mm total length (TL). Young-of-the-year (YOY) specimens were identified using a New York State Department of Environmental Conservation (NYSDEC) protocol based upon size at time of capture: Apr-May < 65mm; Jun < 90mm; Jul < 115mm; Aug < 140mm; Sep-Nov < 165mm). Accordingly, 16 specimens were classified as juveniles, whereas the remaining fish were classified as either sub-adult (non-YOY and <250
mm) or adult (>250) winter flounder (Table 1.) Specimens were assigned to regions of capture as follows: New York Harbor (NYH), western Long Island Sound (WLIS), eastern Long Island (ELI), and Georges Bank (GB). The three New York regions were geographically delineated as follows: (1) New York Harbor included all sites west of the Bronx River and south to the Lower Bay, (2) Western Long Island Sound included all sampling site from the Bronx River east to Huntington Harbor, and (3) Eastern long Island sites consisted of Hog Neck Bay within the greater Peconic Bay and Moriches Bay.

**Otolith chemistry**

Upon extraction, otoliths were rinsed in Milli-Q de-ionized water, transferred to 10% NaClO, re-rinsed in Milli-Q water, and air-dried in a laminar fume hood. The otoliths were next embedded in EpoFix™ epoxy to permit sectioning without risk of fracturing the otolith crystals. Sectioning was performed using a low speed Buehler® Isomet™ low-speed saw with a diamond-wafering blade while immersed in Milli-Q water as a lubricant. Once sectioned, samples were ultrasonicated in Milli-Q water. Hand grinding and polishing was performed using a series of increasingly finer lapping films (3M®) ranging from 30 um to 0.05 um to remove excess overburden, and polish the otolith until the desired degree of resolution as achieved. Polished otoliths were mounted on petrographic slides and ultrasonicated again.

In order to interpret differences in the chemical signatures unique to the specific regions and to enhance spatial separation between collections, we used a multi-elemental set of markers, which included the following: Mg²⁺, Mn⁵⁺, Cu⁶⁺, Zn⁶⁺, Sr⁸⁸, Ba¹³⁸, Pb²⁰⁸, and I¹²⁷, all normalized to Ca⁴³. Elemental concentrations of these elements were quantified with a laser ablation inductively coupled plasma mass spectroscopy (LA-ICPMS) system. Ablation was performed
using a New Wave Research UP-193 (Electro Scientific Industries) 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). Laser spot size was set at 35 µm with a scan speed of 3 µm/sec, and a repetition rate set at 10 Hz. Reference standards were run at the start and completion of each session, and routinely in between. A glass reference standard (MACS-3) was used for calibration and correction calculations. Precision of the elemental ratios was analyzed for limits of detection (LOD), and relative standard deviation (RSD). Data were converted into concentration values using known concentrations from an in-house standard made from pulverized otoliths of freshwater drum (*Aplodinotus grunniens*) and pressed into a pellet (Limburg et al. 2011) and from a MACS-3 standard (Wolf and Wilson 2007). All elements were expressed as ratios of Ca to normalize elemental concentrations with beam strength, and account for the substitution of Ca during formation.

The blind-side (left) otolith was used in all LA-ICPMS analyses, because recent studies have determined that flatfish sagittae are chemically dissimilar (Loher et al. 2008) and the blind-side otolith results in the highest classification accuracies when attempting to re-assign samples back to their sampling location (Kajajian et al. 2013). Jackman et al. (in publication) discovered that the highest mean concentrations of most elements tested occur in the blind side otolith of winter flounder.

In a second method, 15 otoliths from 5 regions (Georges Bank, the Arthur Kill, Newark Bay, Peconic Bay, and Little Neck Bay) were analyzed with synchrotron-based scanning X-ray fluorescence microscopy (SXFM) at the Cornell High Energy Synchrotron Source, Ithaca, NY, using the F3 bending magnet beamline (see Limburg et al. 2011 for further description of methods). When preparing samples for the synchrotron, otoliths were processed similar to the
LA ICPMS, but were mounted on fused quartz disks (clean material) instead of ordinary petrographic slides; this reduced contamination effects, as the X-ray beam penetrates not only the otolith, but also anything behind it (glass slide). Specimens were rastered with a focused X-ray beam of ca. $10^{11}$ photons/second, across an energy spectrum of 2-16 KeV. Fluorescence counts were collected for 1-2 seconds per pixel with an energy dispersive vortex silicon drift detector fitted with an aluminum foil attenuator to damp Ca counts and increase sensitivity to trace elements. This procedure permitted us to develop high resolution, 2-D elemental maps that could be analyzed with ArcGIS. Elements that could be mapped in this study included Ca, Mn, Fe, Cu, Br, and Sr. Lighter elements such as Mg are not possible to quantify with this technique.

**Statistical methods**

Data were assessed for normality prior to performing other statistical analyses. Non-parametric distributions were log-transformed to improve normality and to homogenize the variance. Multivariate analysis of variance (MANOVA) was used to assess elemental differences among sites and years using Pillai’s trace as a test for statistical significance. Mean differences in elemental concentrations among sites were examined using analysis of variance (ANOVA) with Tukey’s Honest Significant Difference (HSD) as a post-hoc, multiple comparison procedure. Where appropriate, Bonferroni corrections were applied to control family-wise error potentially arising from multiple comparisons. Principal component analysis (PCA) was used as an ordination technique to reduce the complexity of the synchrotron data and to examine the underlying structure in order to assess relationships between sites and elements. Prior to PCA ordination, data were standardized to reconcile varying scales of measurement within the elemental values (Baxter 1995). Loadings matrices were used to assess the principal components.
and to provide an interpretation of each principal component. Discriminant function analyses (DFA) were then employed to discriminate among groups of fish and to assign membership based upon elemental concentrations. Equality of variance-covariance matrices was assessed with Bartlett’s test to determine which DFA (linear or quadratic) would be used in the analyses (Shaffler et al. 2009). Classification success rate of the DFA was determined through cross-validation methods using a jackknife (leave-one out) resampling technique with assignments of equal probability, because prior information is unknown (White and Ruttenberg 2007).

To account for the juvenile period, chemical concentrations were examined along a 1200 um transect running 600 um on either side of the core in both rostral and post-rostral directions for all samples. The 600 um transect distance ensures that YOY regions of the otolith were included in the analyses for all age classes. Within this transect, we interpreted variations in Mn:Ca as evidence of exposure to hypoxia, defined as dissolved oxygen < 2 mg/L; this is a recent interpretation that has a basis in biogeochemistry and has strong statistical support (Limburg et al. 2015). In addition, we interpreted the ratio of magnesium to calcium (Mg:Ca) as an index of growth. Otolith Mg:Ca is known to be under physiological regulation (Kalish 1989), and when daily growth increments of flounder otoliths were compared to Mg:Ca ratios, a positive correlation was found (KL, unpublished data). Thus, we interpret elevated Mn:Ca ratios (above a threshold 0.10 Mn:Ca x1000 ) as evidence of a fish experiencing hypoxia, and Mg:Ca ratios as being proportional to growth rate. We further hypothesize that higher growth rates indicate better habitat, all else being equal. To assess this assumption we examined daily growth from four sampling locations and examined the correlations with Mg concentrations.
RESULTS

Statistical results

Shapiro-Wilk (SW) tests conducted for each of the elements tested revealed that the data were not normally distributed (p < 0.0001). Log-transformation improved results, but most elemental distributions still failed normality tests (p < 0.01). Homogeneity of variance was determined with Bartlett’s test (K-squared = 20.91, p-value < 0.0001), which indicates that quadratic discriminant function analysis (QDFA) was appropriate, as it is robust to deviations from normality (McGarigal et al. 2000).

Results of the MANOVA testing showed that significant differences exist between regions (Pillai’s trace = 0.799, F = 167.53, p-value < 0.0001) and among years (Pillai’s Trace = 1.223, F = 95.16, p-value < 0.0001) (Table 2). However, the F-value for the Pillai’s trace was nearly two times lower among years than among regions, which demonstrates that the regional difference is greater than the inter-annual difference. Test of mean concentrations for each element performed with an ANOVA and Tukey HSD showed that significant variation exists among sites and elements (Table 2, Figures 2, 3, & 4). The results obtained from these analyses indicate that some elemental concentrations were similar at the regional scale (Table 2). In these tests, NYH showed the highest mean concentrations for Mn and Pb, WLIS showed highest mean concentrations for Mg, Cu, Z, and I, and ELI showed the highest concentrations for Sr and Ba. Site similarity was shown between NYH and WLIS for Sr and Mn. Similarity was observed between NYH and ELI for Zn and I. No regional sites were comparable for Ba, Cu, and Pb. The QDFA performed at the regional level achieved an overall classification accuracy of 83.2% with regional classifications as follows: NYH = 77%, WLIS = 86%, ELI = 91% (Figure 5). In the
regional QDFA, NYH achieved the lowest score, whereas ELI was the highest, and WLIS was intermediate between the two. The length of the biplot rays (Figure 5) indicates relative strength of the respective elements when making assignments. At the regional level, Mg appears to contribute the most discriminatory power to the analysis.

**Discrimination methods**

A second QDFA was performed using the specific embayments where the specimens were captured; in this case, an overall classification accuracy of 87.3% was achieved (assignment accuracies: AK 88.2%, BR = 92.2%, HNB = 89.1%, JB = 83.1%, LB = 100%, LNB = 81.7%, MB = 88.2%; Figure 6). Higher levels of accuracy were most likely because the NYH complex is a heterogeneous environment, which then introduces a higher degree of variability to the analyses and because of overlap in the regional assignments, as shown by the 50% confidence intervals (Figure 5). At the local level Mg, Mn and Ba appear to contribute equally to the re-assignment classifications, based upon the length of the biplot rays (Figure 6).

**Synchrotron analysis**

Elemental distributions of the synchrotron SXFM data also revealed non-normal distributions (p-value < 0.001) for all elements. Standardization of the data failed to meet multivariate normality when evaluated with subsequent SW tests. Principal component analysis of mean synchrotron values for each element revealed that the first two components explain 87.1% in the system as determined from the loadings matrix (Figure 7). Principal component 1 loaded heaviest on Cu and Mn. Principal component 2 was largely a function of Br, Fe, Sr and Zn, and Sr, with Sr inversely related to the Br, Fe, and Zn (Figure 7). Newark Bay exhibited the
highest mean values for Br, Cu, Fe, and Mn. Georges Bank, the fully marine out-group, had the highest Sr values. Little Neck Bay, Jamaica Bay, and Peconic Bay grouped together. The Arthur Kill was central in the principal component biplot. Newark Bay had the highest mean concentrations in all elements tested, with the exception of Sr.

When examining the high resolution, 2-D elemental maps that were created from the synchrotron data, we noticed similar patterns with regard to Mn in the seasonal growth regions of the otolith for Newark Bay (Figure 8). We show Mn maps from Georges Bank otoliths as a comparison (Figure 9). Graphs of the elemental concentrations of Mn show similar patterns in many of the same areas (Figure 10). Also, Zn was elevated in the core region from four fish captured in Moriches Bay, though concentrations were not similar.

Magnesium shows some correlation with metabolic activity and somatic growth. Highest concentrations of Mg appear in the pre and post-settlement regions of the otoliths, which is also where the fastest growth occurs (Figures 11&12). Specimens obtained from the Bronx River estuary, Little Neck Bay, and Jamaica Bay respectively exhibited highest mean concentrations. Daily growth was measured to assess relationships among four sampling locations (figure 13). In this part of the study, Moriches Bay has the lowest concentration of Mg and also the lowest growth rate (Figures 14). Although the Bronx River estuary showed the highest mean concentrations of Mg, the highest daily growth rates in the four tested locations was found in Little Neck Bay and Jamaica Bay respectively (Table 3). The relationship between Mg and growth rate among bays shows a general trend, but the association appears more dynamic than can be expressed by linear regression and implies other environmental conditions mediate otolith concentrations of Mg.
DISCUSSION

The existing controversy over the scale of genetic relatedness among populations of winter flounder along with evidence of coastal spawning prompted the desire to resolve these uncertainties through otolith microchemistry. With this objective in mind, two different methods were employed to distinguish between groups of winter flounder on a regional and local level. In the first method, we used LA-ICPMS with eight trace elements (Mg$^{24}$, Mn$^{55}$, Cu$^{63}$, Zn$^{64}$, Sr$^{88}$, Ba$^{138}$, Pb$^{208}$, and I$^{127}$, normalized to Ca$^{43}$) on juvenile sections of winter flounder otoliths. From our results, we were able to differentiate amongst three regions (NYH, WLIS, ELI) with an 83.2% reclassification rate to region of capture (Figure 5). A higher classification accuracy of 87.3% was achieved when we reduced the spatial dimensions to specific embayments from which our specimens were obtained (Figure 6). We believe that the increased accuracy was due to the environmental heterogeneity and varying rates of water quality in NYH, which likely confounded results at the regional level. The highest classification accuracy was assigned to the ELI region, whereas the WLIS (86%) was intermediate between NYH (77%) and ELI (91%). However, our specimens were obtained over a five-year period (2006 – 2010), and significant inter-annual difference was shown. Thus, the among-region difference was nearly twice as large as the among-year variation as shown by comparison of the values obtained from the Pillai’s trace for both tests, which indicates that regional variation was greater than the annual variation within the chemical signatures. Accordingly, classification accuracy would likely have increased if we had a more temporally cohesive sample collection and the effects of inter-annual variation were removed (Gillanders 2002; Walther and Thorrold 2009; Sturrock 2012). In the second method, we used SXFM to assess whole otolith transects to discriminate between winter flounder from five regions using five elements: Br, Cu, Fe, Mn, and Zn (Figure 7). In this analysis, we
were able to show distinct groupings based upon regional differences in the otolith chemistry through ordination techniques. Consequently, as a result of both analyses we were able to show that discrimination among groups of winter flounder is readily achievable with a relatively high degree of accuracy at a spatial scale of tens of kilometers. Similar results have been obtained with otolith chemistry methods on other flatfish species (Brown 2006; Reis-Santos et al. 2008; Vasconcelos et al. 2008). More recently, Bailey et al. (2015) showed successful separation of winter flounder groups collected in New England using solution based otolith microchemistry techniques.

**Regional differences in sampling locations**

Estuaries are complex environments where intrinsic physical and chemical reactions strongly influence the express characteristics found in these bodies of water. Our sampling regions (NYH, WLIS, and ELI) were selected to exploit variance in geomorphology as well as differing levels of anthropogenic influence corresponding to patterns of human population density. The patterns of human density and development show a declining trend in a west-to-east gradient. In the westernmost region, NYH is a heavily industrialized and urbanized estuary with an extensive network of navigation channels leading to ports that are among the busiest in the world (Wilber et al. 2013). New York Harbor is unlike the other regions in this study, because geologically distinct bands of bedrock mix with glacial deposits and fluvial sediments deposited from four major rivers, which then merge with tidal waters from the Atlantic Ocean and the Long Island Sound (USGS 2003). In contrast, Long Island’s landmass is comprised entirely of glacial till with substrates ranging in size from clay to boulders (Coates 1973). Thus, the sample sites within the ELI and WLIS regions are fairly uniform in geologic origin in contrast to NYH,
but all three regions differ in the levels of human density and anthropogenic influence. Lower classification rates in NYH are presumably reflective of the high degree of spatial and chemical variation within the region, whereas geologic homogeneity combined with less human disturbance likely accounts for higher classification accuracy obtained for ELI.

**Geochemical relationships among regions**

In our study, certain elements often associated with anthropogenic activity, e.g., Cu, Zn, and Pb (Campana et al. 2001) helped to increase the spatial separation between selected regions, and may provide significant value in assessing habitat quality. However, despite the historical levels of contamination in NYH in regards to these elements (Chilrud 1996), our results indicate that specimens from the WLIS region have the highest concentrations of Cu, I, Mg, and Zn. Invariably, WLIS sites consist of relatively small, shallow bays, receiving high input from sewage overflows which contributes to the high concentrations of transition and post-transition metals found within these bays (Turgeon and O'Connor 1991; NYCDEP 2012). The bathymetry and hydrodynamics of these waterways tends to aggravate the impaired conditions, as recirculation of East River water into the WLIS increases pollution levels and residence times (Viera 2000). New York Harbor in contrast is exposed to higher levels of flushing imparted by strong currents and tides, with the exception of Jamaica Bay and Newark Bay, which represents some of the most contaminated areas in the region (Adams et al. 1998; HEP 2011). In general, the lower an estuary's flushing rate to the open ocean and the smaller its volume for diluting pollutants, the greater its susceptibility to retain dissolved chemicals (NOAA 1988). However, deeper depths and varying levels of salinity in NYH and WLIS contribute to high degrees of stratification in the water column, which is a precursor for hypoxia.
Research has shown that transition and post-transition metals, e.g., Cu, Mn, Pb, and Zn in fish otoliths may not be reflective of the external environment because these metals are bound to otolith and blood proteins (Miller et al. 2006; Sturrock et al. 2012). However, Cu, Pb and Zn differed significantly in our samples at the regional scale, which suggests that physiological control is not the only cause of variability. Our samples routinely show high peaks of Cu, Pb and Zn in core regions (Figure 10). Barbee et al. (2013) reported elevated Cu in otoliths of embryos in response to exposure to varying concentrations of Cu during the egg stage with treated sediments. They determined that Cu uptake is variable, suggesting that Cu in otoliths can be used to track a history of exposure to elevated Cu levels in the environment. If winter flounder eggs were deposited in upper embayments in the same locality as point sources of contamination, concentrations would likely increase beyond threshold limits, and would account for spiking observed in core regions and elsewhere in the transect patterns. Previous research has shown that American eel (*Anguilla rostrata*) otoliths from the Hudson River Estuary retain significantly higher concentrations of both transitional and heavy metals in close proximity to areas of known contamination (Arslan and Secor 2005). Another study has shown that dietary Zn was the major source of Zn incorporated into the otoliths (Rinaldi and Gagnon 2008; 2010). However, if the otolith concentrations of Cu, Pb, and Zn are the product of physiological regulation, they may reflect a food-chain effect from contaminated benthos and still retain value in discerning quality habitat. In the synchrotron data we saw that Newark Bay recorded the highest concentration for Cu, Zn, Br, Fe and Mn (Figure 7). Considering the impaired water quality in the bay (Adams 1998), these results are not surprising, and whether they indicate a dietary pathway or food chain effect, or not, these metals are clearly represented in our specimen’s otoliths. Thus, we cannot invalidate use of these elements in our methods.
In general, NYH and WLIS otoliths contained the highest concentrations of most elements, but ELI region had the highest concentrations of both Ba and Sr. While it is known that levels of Ba are correlated with fluvial sediments (Li and Chan 1979), and even though NYH has four rivers of significance as a potential source of Ba, fish from Long Island estuaries have been shown to contain low Ca levels in otoliths, which translates into a higher element:Ca ratio (Turner 2014). Ample study has shown that variations of both Sr and Ba in the physical environment are reflected by changes in the otolith and are relatively proportional to ambient levels (Secor and Rooker 2000; Kraus and Secor 2004; Miller 2009; Miller 2011). Inverse relationships of Sr and Ba have been used to demonstrate diadromous fish movements under the assumption that Sr is greater in marine environments and Ba is greater in fresh water, a condition that holds in the Hudson River and Long Island (Turner 2014).

Manganese also shows significant difference among regions, though NYH and WLIS show similar concentrations. In juvenile sections of the otolith, elevated concentrations of Mn appear in the core and settlement regions of many specimens. Manganese in fish otoliths has been linked to hypoxic conditions and anoxic sediments (Mohan et al. 2014; Limburg et al. 2011; 2015). In Chesapeake Bay, concentrations of Mn dominate the bay water in summer when anoxia ensues in bottom water, and surficial sediments are positively correlated to levels of Mn in the water column (Dorval et al. 2007). Furthermore, manganese is abundant in non-sandy marine sediments, and can be readily mobilized in response to redox kinetics under hypoxic conditions (Baden and Eriksson 2006). In Connecticut harbors, winter flounder eggs were located on fine mud substrates (Schultz et al. 2007), and Manderson et al. (2003) collected the highest numbers of newly settled winter flounder in organically rich habitats. Composition of sediments would likely correspond to different patterns of Mn in the core and settlement region.
found in many of our otoliths. Since highest abundances of Mn are found in non-sandy substrates, Mn could potentially describe sediment types and would be useful for tracking winter flounder movements through hypoxic events or when resting on sediments where hypoxic pore water can liberate Mn from the sediments into the winter flounder otolith as a result of redox potential (Jackman et al. in publication; Limburg et al. 2015). When we expanded the laser transects beyond the juvenile portions of the otolith, high concentrations of Mn appear in summer growth increments and are unmistakably pronounced in specimens from Newark Bay and WLIS (Figures 8 &10). While we do not contend that these specimens remained in hypoxic waters throughout their growing season, it is likely that they encountered hypoxic conditions out-migrating. In comparison, fish from Georges Bank do not show the same patterns of Mn in their otoliths as those from Newark Bay and WLIS (Figures 9). Overall, we believe Mn to be valuable tracer of hypoxic water, and it shows potential of indicating organic substrates, or higher maternal investment into the egg, all of which provide useful information when making qualitative comparisons between habitats.

Our results show highest concentrations of Mg in the fast-accreting core and post-settlement regions of the otolith then decline afterwards, which correlates to high metabolic activity in juvenile fish (Figures 11 & 12). Moreover, it has been shown that concentrations of Mg in otoliths do not change in response to external concentrations of Mg in the water or diet, which indicates that Mg is likely physiologically regulated, and is not a reliable environmental indicator (Bath et al. 2000; Woodcock et al. 2012; Sturrock et al. 2014). However because Mg is associated with physiological regulation, and thus metabolic activity, there is strong potential for using Mg as a predictor of habitat quality by using Mg as a proxy for metabolic activity and comparing Mg concentrations among sampling locations. By determining daily growth rates
among sampling locations, we discovered that Mg concentrations correlate with locations of highest concentrations and negatively associated with the locations of lowest concentration, but the connection is not linear, and may indicate other environmental factors are associated with otolith concentrations of Mg and somatic growth. Closer examination showed that the Bronx River, Little Neck Bay, and Jamaica Bay exhibited the highest concentrations of Mg, and yet these three locations are considered to have impaired water quality attributed to human effluent emanating from combined sewer overflows (NYCDEP 2012; HEP 2011). In contrast, Moriches Bay has the lowest concentrations of Mg and the lowest growth rate. High concentrations of Mg in the core and pre-settlement regions may be attributed to ontogenetic shifts and maternal investment (e.g. egg size) (Ruttenberg et al. 2005). In lab-reared larval winter flounder, tanks with high algal growth were significantly larger than those raised in clear water despite being fed the same diet (King and Howell 1997). Thus, it is conceivable that either maternal investment or eutrophic conditions contributes to this phenomenon, and may provides a growth advantage for winter flounder during a critical time period when they are most vulnerable. In winter flounder, a critical period occurs in the first few weeks of life when larval mortality is twofold higher than for older larvae (Pearcy 1962). In another study, Chambers and Legget (1987) showed that size of winter flounder larvae is the most important predictor of metamorphosis. Therefore, in spite of the impaired water quality emanating from degraded, eutrophic systems, these highly perturbed sites may offer viable contributions to winter flounder recruitment, which during population contractions is vitally important. However, a tradeoff would later occur, as growth rates of winter flounder are negatively impacted by hypoxic waters (Stierhoff et al. 2006). Nevertheless, this initial attempt to use Mg concentrations in the otolith as a proxy to assess winter flounder habitat requires further investigation.
**Population regulation**

Populations of fishes appear to be physically defined at the early life history phases and in relation to well-defined geographic or hydrodynamic features that create adaptive patterns of population persistence (Sinclair and Isles 1987). Adaptive patterns relating hydrodynamics to life history has been shown with estuarine-dependent populations of winter flounder, since the highest egg densities in NYH occur in relatively shallow non-channel regions (Wilber et al. 2013). Protected shoal waters are important for winter flounder recruitment, because tidal circulation patterns cause retention of eggs and larvae within the upper estuarine locations (Pearcy 1962; Crawford and Carey 1985, Carey 1990; Pereira 1999). The same dynamics also appear to be responsible for settlement patterns (Chant et al. 2000; Curran and Able 2002; Manderson et al. 2003; Schultz et al. 2007. However, settlement locations would need to support the trophic requirements of the fast growing winter flounder recruits. Examination of our winter flounder otoliths confirms high metabolic activity with distinctly large growth rings that correspond to high concentrations of Mn and Mg. The highest concentrations of Mg are often in the settlement regions of the otolith, which has been previously linked to metabolic activity (Ruttenberg et al. 2005; Hamer and Jenkins 2007; Woodcock et al. 2012) (Figure 12).

Overall, when viewing winter flounder otoliths with respect to specific elemental markers, we are able to make assessments of life history patterns and habitat quality. Our results also show a fairly high degree of success in classifying juvenile sections of otoliths to their region of capture. These results infer that natal homing and natal nursery fidelity contribute to the relatively high classification rates and bolsters previous studies that contend that inshore winter flounder recruitment is the product of localized spawning, from which discrete population units are formed (e.g., Lobell 1939; Perlmutter 1947; SAILA 1961; Howe and Coates 1975, Pierce
and Howe 1977; Phelan 1992). The methods used in this study are similar to the techniques used by Thorrold et al. (2001) to demonstrate natal homing in weakfish by comparing the geochemical signatures in otolith cores of spawning fish to those of juveniles captured in the same waters. Logically, it can be assumed that the extent to which winter flounder make seasonal migrations to home to natal estuaries is likely an adaptive behavior that incurs benefit to the progeny and potentially isolates gene flow between embayments. The restriction of gene flow induced by the capacity for natal homing in mobile marine species tends to regulate spatial population structure and connectivity between habitats (Bradbury et al. 2008). Accordingly, our results imply that the inshore population structure of winter flounder is ordered by the degree of natal homing though wandering is probably important to the maintenance of allelic diversity, at the meta-population level, as well as the local level. Vagrancy, transients, and mixing between groups of fish would account for some portion of misclassification in our results. In addition, site heterogeneity, as well as inherent variation within the fish contribute to within-region variation, and provide explanation for the lower classification accuracy assigned to NYH. Also, at the regional level some similarity of geologic and other watershed influences as well as the ubiquity of chemical contaminants sourced from human populations likely accounts for some spatial overlap among regions.
Table 2.1. Length and age-class characteristics of winter flounder sampled from the various locations around Long Island and the Hudson River Estuary.

<table>
<thead>
<tr>
<th>AREA</th>
<th>NUMBER</th>
<th>TL (mm) (mean ±)</th>
<th>REGION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthur Kill (AK)</td>
<td>6</td>
<td>186 ± 71.4</td>
<td>NYH</td>
</tr>
<tr>
<td>Bronx River (BX)</td>
<td>3</td>
<td>53 ± 4.7</td>
<td>WLIS</td>
</tr>
<tr>
<td>Jamaica Bay (JB)</td>
<td>6</td>
<td>185 ± 138.6</td>
<td>NYH</td>
</tr>
<tr>
<td>Huntington Harbor</td>
<td>1</td>
<td>330</td>
<td>WEST</td>
</tr>
<tr>
<td>Peconic Bay (PB)</td>
<td>7</td>
<td>139.4 ± 6.4</td>
<td>EAST</td>
</tr>
<tr>
<td>Little Neck Bay LNB)</td>
<td>5</td>
<td>243 ± 131.38</td>
<td>WEST</td>
</tr>
<tr>
<td>Lower Bay (LB)</td>
<td>1</td>
<td>89</td>
<td>NYH</td>
</tr>
<tr>
<td>Manhasset Bay (MB)</td>
<td>1</td>
<td>74.0</td>
<td>WEST</td>
</tr>
<tr>
<td>Moriches Bay (MOR)</td>
<td>7</td>
<td>53 ± 1.8</td>
<td>EAST</td>
</tr>
<tr>
<td>Newark Bay (NB)</td>
<td>5</td>
<td>212 ± 70.1</td>
<td>NYH</td>
</tr>
<tr>
<td>Oyster Bay (OB)</td>
<td>4</td>
<td>322 ± 52.1</td>
<td>WEST</td>
</tr>
<tr>
<td>Port Jersey (PJ)</td>
<td>3</td>
<td>184 ± 83.4</td>
<td>NYH</td>
</tr>
<tr>
<td>South Brooklyn (SB)</td>
<td>4</td>
<td>253 ± 68.51</td>
<td>NYH</td>
</tr>
<tr>
<td>George Bank (GB)</td>
<td>5</td>
<td>410 ± 95.39</td>
<td>GB</td>
</tr>
</tbody>
</table>
Table 2.2. Comparison of elemental concentrations among sample sites within the three regions as tested with ANOVA with Tukey HSD as a post hoc test. Similarity among sites is determined by the Tukey HSD test.

<table>
<thead>
<tr>
<th>ELEMENTS</th>
<th>F</th>
<th>p-value</th>
<th>SITE SIMILARITY</th>
<th>HIGH MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr:Ca</td>
<td>121.10</td>
<td>p = &lt;0.0001</td>
<td>NYH &amp; WLIS</td>
<td>ELI</td>
</tr>
<tr>
<td>Ba:Ca</td>
<td>282.70</td>
<td>p = &lt;0.0001</td>
<td>NONE</td>
<td>ELI</td>
</tr>
<tr>
<td>Mn:Ca</td>
<td>109.95</td>
<td>p = &lt;0.0001</td>
<td>NYH &amp; WLIS</td>
<td>NYH</td>
</tr>
<tr>
<td>Mg:Ca</td>
<td>369.10</td>
<td>p = &lt;0.0001</td>
<td>WLIS</td>
<td>WLIS</td>
</tr>
<tr>
<td>Cu:Ca</td>
<td>83.83</td>
<td>p = &lt;0.0001</td>
<td>NONE</td>
<td>WLIS</td>
</tr>
<tr>
<td>Zn:Ca</td>
<td>64.73</td>
<td>p = &lt;0.0001</td>
<td>NYH &amp; ELI</td>
<td>WLIS</td>
</tr>
<tr>
<td>Pb:Ca</td>
<td>16.85</td>
<td>p = &lt;0.0001</td>
<td>NONE</td>
<td>NYH</td>
</tr>
<tr>
<td>I:Ca</td>
<td>88.10</td>
<td>p = &lt;0.0001</td>
<td>NYH &amp; ELI</td>
<td>WLIS</td>
</tr>
</tbody>
</table>
Table 2.3. Comparison of daily growth (mm) in YOY winter flounder captured in four different bodies of water vs mean otolith concentration of Mg.

<table>
<thead>
<tr>
<th>BAY</th>
<th>DAILY GROWTH</th>
<th>mean Mg:Ca x 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNB</td>
<td>0.53</td>
<td>0.088</td>
</tr>
<tr>
<td>JB</td>
<td>0.51</td>
<td>0.091</td>
</tr>
<tr>
<td>BR</td>
<td>0.46</td>
<td>0.090</td>
</tr>
<tr>
<td>MOR</td>
<td>0.38</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Little Neck Bay (LNB), Jamaica Bay (JB), Bronx River (BR), Moriches Bay (MOR)
Figure 2.1. Samples were obtained from 13 locations from 3 regions around Long Island and Georges Bank. Sample locations are as follows: Arthur Kill (AK), George Bank (GB), Hempstead Harbor (Hem), Hog Neck Bay (HNB), Huntington Harbor (HH) Jamaica Bay (JB), Little Neck Bay (LNB), Lower Bay (LB), Manhasset Bay (MB), Moriches Bay (Mor), Oyster Bay (OB), Newark Bay (NB), Port Jersey (PJ), South Brooklyn (SB).
Figure 2.2. Regional levels of (A) strontium, (B) barium, (C) manganese, and (D) magnesium in relation to the three regions in the study: eastern Long Island (East), New York Harbor (NYH), and western Long Island Sound (West).
Figure 2.3. Regional levels of (A) copper, (B) zinc, (C) lead, and (D) iodine in relation to the three regions: eastern Long Island (East), New York Harbor (NYH), and western Long Island Sound (West).
Figure 2.4. Concentrations of (A) strontium, (B) barium, (C) manganese, and (D) magnesium as shown in 8 embayments: Arthur Kill (AK), Bronx River (BR), Jamaica Bay (JB), Little Neck Bay (LB), Manhassett Bay (MB), Newark Bay (NB), Peconic Bay (PB), and Port Jersey (PJ).
Figure 2.5. QDFA plot of juvenile sections of otoliths using 8 elemental markers (Sr, Ba, Mn, Mg, Cu, Zn, Pb and I), with equal probabilities assigned to each region. Classification accuracy to region of capture resulted in 83.2% accuracy. The ellipses signify the 50% density function. Note the NYH, WLIS, and ELI centroid are within each group's orbit.
Figure 2.6. QDFA plot of juvenile sections of otoliths using 8 elemental markers (Sr, Ba, Mn, Mg, Cu, Zn, Pb, & I) with equal probabilities assigned to each location. Overall, 87.3% classification accuracy to location of capture was achieved. The 50% centroids were removed to display detail.
Figure 2.7. A PCA of the synchrotron data. Arthur Kill (AK), Georges Bank (GB), Jamaica Bay (JB), Little Neck Bay (LNB), Newark Bay (NB), Peconic Bay (PB) are shown in relation to the elements used in the analysis.
Figure 2.8. An SXRF map of manganese showing elevated concentrations in growth regions of
winter flounder otoliths. Axis increments are in mm, and “samz” and “samx” are the names of
the motors that move the sample in the vertical and horizontal directions relative to the X-ray
beam, respectively. These three specimens represented by images (A), (B), and (C) were all
captured in Newark Bay in the spring. Second growth period from the previous summer seems to
show two distinct periods of hypoxia.
Figure 2.9. An SXRF map of manganese (in comparison to figure 2.8) showing low concentrations in growth regions of winter flounder otoliths. The axes are labeled in mm and “samz” and “samx” are the names of the motors that move the sample in the vertical and horizontal directions relative to the X-ray beam, respectively. (A) These two fish were captured on Georges Bank and there isn’t any discernible pattern with respect to Mn in contrast to figure 2.8.
Figure 2.10. These graphs show similar patterns of Mn in the previous growing season. The peaks are likely associated with hypoxia. Specimens were captured in the bodies of water as follows: (A) Newark Bay, (B) Newark Bay, (C) Newark Bay, and (D) Huntington Harbor showing a similar pattern.
Figure 2.11. These graphs show patterns of Mg from four juvenile fish captured in (A) Bronx River Estuary (B) Jamaica Bay, (C) Little Neck Bay, and (D) another specimen from Little Neck Bay. Notice that the patterns of highest concentration are centered around the core and settlement regions of the otolith.
Figure 2.12. This image shows a graph of Mg overlaying its corresponding otolith. The highest Mg concentrations occur in the core and post-settlement region of growth.
Figure 2.13. Daily rings clearly visible in this image were used to assess growth rates among capture locations
Figure 2.14. The image shows the relationship between daily growth rate of fish (mm) and Mg concentrations in the same locations. Trend lines show the highest Mg concentrations corresponding to the highest growth rates, whereas Moriches Bay shows the lowest growth rate and lowest levels of Mg.
CHAPTER 3

Is Winter Flounder Growth in the New York City region Driven by Density Patterns and Predation?

ABSTRACT

Within the Western Long Island Sound (WLIS) and Hudson River Estuary (HRE), age-1+ and older winter flounder (*Pseudopleuronectes americanus*) have declined over the past thirty years. Herein, we compared the size at age of winter flounder in this region to the historical record and discovered that these fish are now larger at age, and older fish comprise a larger segment of the population in contrast to large-scale surveys that were conducted in New York waters decades ago. The changing growth effect was observed in our field sample, WLIS recreational fishery data, and state agency monitoring data. Simultaneous to the winter flounder decline, we show the rise in a suite of predators that were not as abundant or absent decades ago. We hypothesize that the changing growth dynamics we observed in winter flounder in the WLIS and HRE is a compensatory response to decreasing intra-specific competition manifested from the combined affects of fishing and predation, and from size-selective mortality exerted by this newly emerging and resurgent predatory regime. This study additionally provides evidence of changing ecological and evolutionary conditions confronting winter flounder when stocks are at a depressed level.
INTRODUCTION

Winter flounder (*Pseudopleuronectes americanus*) is a demersal flatfish found on the continental shelf and estuaries of the Northwest Atlantic, primarily from New Jersey to New Brunswick. The species lives approximately 15 years and attains a maximum length of 670 mm (Collette and Klein-MacPhee 2002). Though highly important to both the commercial and recreational fisheries, winter flounder has experienced major declines within much of its range, with the SNE/MA stock most affected (ASMFC 2009). Consequently, winter flounder from the Southern New England/ Mid-Atlantic (SNE/MA) stock are currently considered overfished with the spawning stock biomass well below sustainable thresholds (ASMFC 2009, 2014).

In New York, winter flounder once supported a thriving inshore recreational fishery, with 7.8 million fish landed in 1984 (ASMFC 2000). It is notable that prior to 1988 no regulations existed for the recreational winter flounder fishery in New York, and yet the species still sustained large spring and fall harvests. However, since the 1980s recreational catches have dwindled, and increasingly draconian regulations have failed to spur recovery. In fact, a recent analysis of effective population size of winter flounder in Long Island bays has shown a continuing downward trend (O’Leary et al. 2013; ASMFC 2014). A number of causes for reductions in the SNE/MA stock or some of its regional components have been proposed, including overfishing, estuarine degradation, effects of climate change (including consequent ecological interactions), and a burgeoning predator assemblage (ASMFC 2009, 2011; Gibson 2013).

One area within New York waters that received occasional study of winter flounder in New York beginning in the 1930s is the western Long Island Sound (WLIS) (Lobell 1939; Perlmutter 1947). Since 1984, winter flounder abundance in Long Island Sound (LIS) has been
continuously monitored through the Long Island Sound Trawl Survey (LISTS) conducted by the Connecticut Department of Energy and Environmental Protection (CTDEEP), and beach seine surveys (BSS) monitoring young-of-the-year (YOY) conducted by the New York State Department of Environmental Conservation (NYSDEC).

In this study, we compared winter flounder abundance from the CTDEEP survey with the NYSDEC survey and found a surprising relationship. In the Long Island Sound (LIS), WLIS, and Hudson River Estuary (HRE) regions, non-juvenile winter flounder experienced significant declines ($p < 0.001$) between 1984 and 2014. In contrast, during the same time period young-of-year (YOY) winter flounder in the WLIS and HRE did not show a statistically significant decline ($p = 0.716$). This relationship suggests a recurring loss of individuals between the YOY and adult stages. One possible reason for the missing size class is an increase in predators. Increased abundances of a suite of predators e.g. striped bass, striped sea robins, summer flounder, Double-crested Cormorants that are known to prey upon maturing flatfish, occurred concomitant to the winter flounder declines (ASMFC 2011). Subsequently, reduced numbers of winter flounder and an increased predator assemblage suggest that decreased intraspecific competition combined with size-selective mortality might be reflected in faster growth rates for contemporary winter flounder populations.

To test the hypothesis that increased predation could be partly responsible for reduced winter flounder abundances, we (1) compared demographics to test for density dependent responses to fishing and predation, and (2) analyzed abundance trends.
METHODS

Study site

In this study, 640 winter flounder were sampled from 11 locations in WLIS and HRE (Figure 1). Hereafter, our data set is referred to as the WLIS/HRE data. With the exception of YOY, all specimens were sampled in winter and spring from 2006 to 2010. Young-of-year were obtained by seining in summer. Older specimens were obtained via otter trawling and angling. Individuals were not separated by sex since some of the adult fish were obtained as “racks” (filleted carcasses) from head boats. All specimens were measured for total length (TL, mm), and placed on ice until they could be frozen at -20°C.

Sagittal otoliths were extracted from thawed specimens and rinsed in Milli-Q de-ionized water to remove impurities or adhering tissue. After initial rinses, otoliths were transferred to 10% NaClO for 3 min, rinsed in Milli-Q water, allowed to air dry in a laminar fume hood, and stored in polystyrene snap cap vials until further use. Aging of otoliths was performed under a Leica MZ75 dissecting microscope illuminated with a Schott 1500 LCD light source. Whole otoliths were examined between 6.5X and 16X for best resolution of seasonal growth increments. Next, otoliths were embedded in EpoFix™ epoxy to permit sectioning without risk of fracturing the otolith crystals. Sections were marked prior to cutting to ensure core regions were captured. Sectioning was performed using a Buehler® Isomet™ low-speed saw with a diamond-wafering blade immersed in Milli-Q water making transverse cuts that resulted in (~1mm) section of the otolith. Hand grinding and polishing was performed using a series of increasingly finer lapping films (3M®) ranging from 30 um to 5 um to remove excess overburden, and to improve resolution of the annuli or other distinguishing marks. Otolith
sections were also examined under the dissecting microscope with power settings between 16X and 32X.

Age estimates were performed three times for each otolith or until age discrepancies were resolved. Initial readings were performed using whole blind-side (left) and eyed-side (right) otoliths without prior knowledge of TL, or date of capture to avoid biasing the estimate. All YOY specimens were classified as age-0. By convention, January 1st birthdates were assigned to age-1+ fish. Since all of our age-1+ samples were captured in winter and spring we did not have to account for seasonal differences in increment formation. Eyed-side otoliths of specimens estimated to be age-3+, or any equivocal estimates were later sectioned in concordance with Berry et al. (1965). When uncertainty in the estimate remained, the left otolith was sectioned as well to ensure accuracy in the age estimate. Age estimates of sectioned otoliths were taken on the axis alongside the sulcal groove since splits and checks tend to coalesce into more defined annuli near the sulcus (ASMFC 2012).

**Historical Winter Flounder data**

We compared our WLIS/HRE collection to three surveys that collectively comprise the historical record of winter flounder in New York waters. The Perlmutter (1947) survey extended from Maine to New Jersey, wherein 10,809 winter flounder were tagged and measured. Within New York waters, 4,091 specimens were tagged and measured in the following locations: (1) LIS off Mattinecock Point, (2) LIS off Port Jefferson, (3) LIS off Huntington Harbor, (4) Gardiner’s Bay, (5) Peconic Bay, (6) off the Hamptons and Montauk, (7) Shinnecock Bay, and (8) the Great South Bay. In the Lobell (1939) survey, 957 specimens were collected from the recreational fishery in Peconic Bay, Moriches Bay, Shinnecock Bay, and the WLIS. The Poole
(1966) survey sampled 2,400 winter flounder from Peconic Bay, Shinnecock Bay, Moriches Bay, and Great South Bay.

The von Bertalanffy growth function (VBGF) models somatic growth in fish populations and is the most commonly used length-age model (Quinn and Deriso 1999). Data for the VBGF was derived utilizing size-at-age determined from otolith annuli, which was then used to compare growth rates between studies. The VBGF replicates curvilinear growth of both the immature and mature stages of fish growth, and is expressed by the formula: \( L_t = L_\infty \left(1 - e^{(-K(t-t_0))}\right) \) where \( L_t \) = Length at age t, \( L_\infty \) = asymptotic average length, \( K \) = Brody growth rate coefficient with units as \((\text{year}^{-1})\), and \( t_0 \) = an integration constant, interpreted as the average age when \( L = 0 \). The K parameter does not represent a growth rate but instead measures how quickly the asymptote is approached. Confidence intervals (CI) were obtained by bootstrapping, which re-samples the data with replacement, and is used to control for normality and stability. Resultant VBGF parameter estimates from WLIS/HRE2+ were compared with a VBGF constructed from the Poole (1966) data. Additional VBGFs could not be generated for the Lobell (1939) and Pelrmutter (1947) data since these surveys did not include length-at-age data. One-way analysis of variance (ANOVA) was used to test the null hypothesis that size-at-age and growth of fish remained the same for all studies and time periods. Tukey’s HSD was used as a post-hoc test to determine which groups differed. The VBGF and graphical data pertaining to the VBGF were created using the FSA, FSAdata, and NLS tools packages imported into R® version 3.1.1(R Core Team 2014).

Statistical analyses were performed on the time series of abundances for winter flounder and other species from data maintained by the CTDEEP, NYSDEC, and New York City Audubon. Linear models were developed using time as the explanatory variable and respective
species as the response variable to estimate coefficients and provide a test of the null hypothesis based upon \( B_0 = 0 \). The catch composition of the WLIS and HRE bays conducted by the BSS from 1984 – 2014 required a principal component analysis (PCA) to reduce data dimensionality and to help explain variability and complexity. Prior to performing the PCA, data were standardized to increase multivariate normality (Shapiro-Wilks test of untransformed data (\( p = \) <0.0001). Statistical and graphical analysis was performed on the CTDEEP LISTS that have been conducted in the LIS from 1984 – 2014. Statistical and graphical analyses were also performed on Double-crested Cormorant nesting surveys data (1980 - 2014), which was provided by NYC Audubon. All statistical and graphical analyses were performed in R® version 3.1.1 (R Core Team 2014).

**RESULTS**

*Size and ages of contemporary WLIS/HRE Winter Flounder*

Forty percent of the WLIS/HRE collection consisted of immature individuals of less than 100 mm (Table 1; Figure 2A). We removed yearlings and YOY from the WLIS/HRE data sets to improve correspondence with the historical data, and subsequently refer to the transformed dataset as the WLIS/HRE 2+ (Figure 2B). The age distribution of the WLIS/HRE sample set is shown (Figure 2C). In the historical surveys, specimens were obtained by trawling (unless specified), which generally allows younger, smaller fish to escape due to mesh size. Thus, in our study, with YOY and yearlings removed, the TL/frequency shifts to the 300-350 mm range and comprise the highest percentage in sample population, whereas age-4 fish form the most dominant cohort (Figure 2D).
Size and ages of Winter Flounder in historical studies

Analysis of Lobell’s (1939) recreational fishery data indicates that in the WLIS, the most commonly caught flounder size via angling was in the 200 – 225 mm range (Figure 3A). Winter flounder in the 200 - 225 mm size class were also the most commonly caught in other regions around Long Island at the time (Figure 3B). In the Lobell (1939), age-3 fish were the most dominant age-class of winter flounder caught in the recreational fishery in the WLIS (Figure 3C). In comparison, contemporary party boat data from the WLIS/HRE data shows the most commonly caught winter flounder was in the 300 - 325 mm range (Figure 3D). Thus, an approximately 100-mm size increase has occurred in the winter flounder caught via angling.

Graphical representation of the WLIS recreation fishery data - past and present - shows both the shift in abundance and the size of fish captured (Figure 4.) Comparison between Lobell (1939) and the WLIS/HRE demonstrates that for the first two years the median size-at-age for the WLIS region is similar for both periods (Table 2). However by the third year, growth rates increase for the WLIS/HRE sample, and are comparable to the Peconic Bay region.

In the Perlmutter (1947) survey, the most commonly caught flounder in Long Island Sound were in the 250 - 275 mm range (Figure 5A). In the WLIS the mode was the 250 - 275 mm range (Figure 5B). When all Long Island waters (Atlantic side as well as the Sound) were pooled, the result was similar to both the LIS and WLIS with modal size in the 250 - 275 mm range (Figure 5C). However, Perlmutter (1947) did not report the age of fish in his study other than stating that fish in New York waters in the size range 203 - 254 mm were 2-3 years old, and were mostly mature.
Since we could not compare the WLIS/HRE region directly with the Poole (1966) survey, we combined the results from the four bays (Figure 6). The modal size range of flounder caught in this survey was 275 – 300 mm (Figure 6A) and age-3 and age-4 individuals were the most commonly caught (Figure 6B). This survey was the only one of the historical surveys that provided both age and TL for each specimen. Comparison of the summary statistics between the WLIS/HRE (Table 1) and the Poole (1966) data (Table 3) shows that age-2 and age-3 individuals in the 1960s were larger than the comparable age classes of the WLIS/HRE. However, beginning at age-4, the mean and median size-at-age of the WLIS/HRE exceed those of Poole’s (1966) survey.

**Von Bertalanffy growth function**

Von Bertalanffy growth functions were constructed for both the WLIS/HRE2+ and Poole (1966) surveys (Figures 7 and 8). Ninety-five percent confidence intervals (CIs) were developed using 200 iterations of bootstrapping (Table 4). For the WLIS/HRE2+ data, bootstrapped parameters estimated from the VBGF are listed as follows: theoretical starting point ($t_0 = 0.241$ mm), maximum asymptotic average length ($L_\infty = 446.0$ mm), and Brody growth coefficient ($K = 0.334$). The WLIS/HRE VBGF shows a steeply increasing curve that exhibits asymptotic inflection in the 300 – 400 mm range, and suggests a high growth rate between year-classes (Figure 7). The VBGF fit to Poole’s (1966) data indicates a shallower curve that approaches asymptotic inflection in the 275 – 350 mm range (Figure 8). The parameters for the Poole (1966) VBGF are as follows: theoretical starting point ($t_0 = -1.915$), maximum asymptotic average length $L_\infty = 377.0$ mm, and Brody growth coefficient ($K = 0.273$). Estimates of $L_\infty$ derived from the
WLIS/HRE data were 69 mm larger and show a faster time to asymptotic inflection than those determined from the Poole (1966) survey (Table 4). Confidence intervals are tighter relative to the curve of the parameter estimates for the WLIS/HRE model in comparison to the Poole (1966) VBGF.

**Size comparison among studies**

Among all surveys, median TL of winter flounder was greatest in the WLIS/HRE2+ (305 mm) (Table 5; Figure 9). Comparing mean TL rather than medians, WLIS/HRE 2+ (290.0 mm) and the Poole (1966) data (290.07 mm) are nearly identical (Table 5). Additionally, the mean TL of WLIS/HRE samples is 46 mm greater than reported by Perlmutter (1947) and 66.6 mm greater than the Lobell (1939) survey for WLIS.

Box and whisker plots representing the one-way ANOVA and Tukey HSD reveals a significant difference among surveys (F=102.1, p < 0.001). Both the Lobell (1939) and Perlmutter (1947) data sets are similar, whereas the Poole (1966) and the WLIS/HRE are comparable to each other (Figure 10). Box and whisker plots of the one-way ANOVA in the WLIS demonstrated a significant difference between Lobell (1939), Perlmutter (1947), and WLIS/HRE2+ data sets (F=173.2, p < 0.001), with the WLIS/HRE 2+ having the highest mean average (Figure 10).

Overall, our results show that larger, older specimens comprise a larger proportion of the sample population compared to multiple surveys from the 20th century. Age estimates of the WLIS/HRE ranges from age-0 through age-7. This age range corresponds well with the other studies except in the WLIS where Lobell (1939) did not report any specimens from the WLIS over age-5 (Figure 5).
**Trends and potential predators**

In the LIS, non-YOY winter flounder collected in spring trawl surveys by CTDEEP from 1984 – 2013 showed a significant decrease in numbers in the LIS waters (p < 0.001; Figure 10A). In the 2013 Long Island Sound Trawl Survey, winter flounder were at record lows levels in the 30-year history of survey (Gottschall and Pacileo 2013). Concurrent with this 30-year winter flounder decline, striped bass (*Morone saxatilis*) abundance increased significantly (p < 0.001) (Figures 10B, spring survey; 10C, fall survey), as did summer flounder (*Paralichthys dentatus*) in spring (p < 0.001) (Figure 11A) and fall tows (p < 0.001) (Figure 11B), as well as striped sea robin (*Prionotus evolans*) (p = 0.0129) (Figure 11C).

In the WLIS, NYSDEC BSS results were more variable, showing no significant change in winter flounder YOY production (p = 0.716), whereas non-YOY winter flounder show a significant decrease (p < 0.001), and winter flounder CPUE remains statistically unchanged (p = 0.521) (Figure 12). Principal component analysis of the BSS data shows Atlantic menhaden (*Brevortia tyrannus*), bay anchovy (*Anchoa mitchilli*), and silversides (*Menidia menidia*) loading heavily on the first principal axis (Figure 13). Summer flounder and northern sea robin (*Prionotus carolinus*) appear to vary inversely with striped sea robin. Striped bass and bluefish (*Pomatomus saltatrix*) load mostly on the second principal component axis. Winter flounder load equidistant to principal components 1 and 2.

Double-crested Cormorant (*Phalacrocorax auritus*) nesting sites have increased in New York Harbor (NYH) from zero prior to 1986, to a high of 1806 nests surveyed in 1995. Overall, there is an increasing trend of cormorant nesting sites in the NYH regions (p < 0.01). Each nest represents a breeding pair of cormorants, but does not account for immature or non-nesting birds (Figure 14).
DISCUSSION

With the exception of YOY, winter flounder in the WLIS and HRE has noticeably declined since the early 1980s, though the remaining population appears to be compensating to the density reduction by growing larger and faster than the specimens captured in several large-scale surveys conducted in the early and middle periods of the 20th century. These surveys were the first attempts establish a baseline of winter flounder populations in New York waters and have come to represent the historical record of these fish. We were able to demonstrate a change in the growth dynamics by comparing size-at-age and growth rates of winter flounder recently captured in the WLIS and HRE to those listed in the historical record. Not only have winter flounder in the WLIS and HRE increased in size-at-age, and incurred a faster growth rate, but older individuals have also become proportionately more abundant in the population in comparison to the aforementioned surveys. More specifically, we were able to demonstrate that the length-frequency of winter flounder in the WLIS has increased nearly 100 mm in comparison to the Lobell (1939) survey (Figures 3 and 4), whereas, the length-frequency of the WLIS/HRE in comparison to the Perlmutter (1947) survey shows an increase in all regions by 50 mm (Figure 5). In contrast to Poole (1966) survey, winter flounder in the WLIS/HRE have increased in size by 25 mm (Figure 6). These frequencies are not precise differences, but represent categorical increments when binning results of the comparison. Although winter flounder are sexually dimorphic, with females growing faster and larger than males (Poole 1966; Collette and Klein-McPhee 2002), we did not sort our sample by sex. The lack of separate sexing of our specimens doesn’t impact our results, because Lobell’s (1939) specimens were not sorted by sex, and Perlmutter’s (1947) use of scale texture as a means of sex determination was invalidated by Bejda and Phelan (1998).
When using the VBGF to compare the WLIS/HRE age-2+ to the Poole (1966) survey, we observe that our specimens have incurred a faster growth rate and are predicted to be larger at theoretical maximum length (Figure 7 and 8). The growth curve in the VBGF models somatic growth, and shows the time to asymptotic inflection – as denoted by the Brody coefficient (K) is faster, and the curve is steeper. The steepness of the VBGF curve indicates the fastest growth in the immature and intermediate-aged specimens, which is true for most fish. However, the growth curve generated from the WLIS/HRE2+ specimens is steeper than that produced in the Poole (1966) VBGF.

Notable size differences have previously been reported regarding winter flounder captured from different regions within New York waters (Lobell 1939; Neville and Perlmutter 1940; Perlmutter 1947; Poole 1966). For instance, Lobell (1939) reported that winter flounder from Peconic Bay were larger at age and grew faster than either Shinnecock-Moriches or WLIS specimens. Poole (1966) supported Lobell’s (1939) conclusions, and attributed the size variation to location-specific genetic and environmental differences. However, divergent growth trajectories appearing in individuals captured decades later from the same waters imply changing ecological conditions within those locations. While comparing our specimens to those documented in the historical record, we noticed a change in the growth dynamics over time, which corresponds temporally to both population declines and with a simultaneous rise in a suite of winter flounder predators. Consequently, we believe that compensatory growth stemming from a reduction in the intensity of intraspecific competition and directional selection imposed by a resurgent regime of piscivorous predators that were not as abundant or simply not present decades earlier contribute to the changing growth conditions for winter flounder in the WLIS and HRE. Other studies involving flatfishes have shown that decreases in population
density would cause a lessening in the intensity of intraspecific competition, which would be manifested as an increased growth rate and increased size-at-age (Rijnsdorp and van Leeuwen 1996). Thus, with less competition for food, fish tend to grow larger. Alternatively, human exploitation, which targets larger fish, would present fishery-induced evolution (FIE) as an opposing selective pressure to patterns of low density. However, an expanded predator regime would potentially generate selection for larger sizes that countervails the effects of FIE, and compounds the density-dependent relationships.

How might the historical fishing pressure and ecological changes be related? Winter flounder harvests were consistent from 1937 to the mid 1960s (Poole 1969). In fact, winter flounder fishing during this period was considered the most important angling activity in New York (Lobell 1939; Poole 1969). From the 1930s onward, angling for winter flounder increased proportionately to a burgeoning human population, while commercial trawling also increased both inshore and offshore, and fyke and pound nets were commonly seen throughout the inshore waterways of Long Island as well. For many years both the recreational and commercial winter flounder fisheries collectively exploited the same waters and maintained robust harvests with little regulation. Then suddenly, changes began to occur in the winter flounder fishery during the 1980s. In 1982, both recreational and commercial harvests were at or near peak levels (ASMFC 2012). Soon after, winter flounder populations began a downward decline and stringent efforts to reduce fishing mortality have failed to revive this once vibrant fishery. The most intriguing aspect of the decline in winter flounder was that it coincidently occurred when a suite of piscivorous predators became more prevalent in the same waters.
**Long-term monitoring of winter flounder and other species**

Long-term monitoring by NYSDEC and CTDEEP provides a method of measuring trends within the inshore ecosystems of the WLIS, HRE, and surrounding regions. Results from CTDEEP LISTS conducted from 1986 – 2013 show statistically significant increases in striped bass, summer flounder, and striped sea robin numbers that coincide with declining quantities of winter flounder (p < 0.001) (Figures 10 and 11). However, the results from the NYSDEC BSS conducted in the WLIS and HRE from 1984 - 2014 show a contrasting trend for winter flounder (Figure 12). The majority of winter flounder captured in this survey are also YOY, and though recruitment over time has been highly variable, YOY abundances remain statistically unchanged (p = 0.72). Additionally, age-1+ flounder in the BSS are seen to be declining (p < 0.001), but the overall CPUE for all flounder captured remains statistically unchanged (p = 0.52), (Figure 12).

Principal component analysis of the BSS in the WLIS and HRE shows an ecosystem dominated by forage species (Figure 13). Atlantic menhaden, silversides, and bay anchovy load highest on the first principal component, and high abundances of these species are correlated with periods of high winter flounder recruitment. In the Great South Bay, Nuttal et al. (2011) demonstrated a switch from a demersal food web to a more pelagic state. High abundance of pelagic forage species may provide refuge for maturing winter flounder from piscivory, as the pelagic species provide optimal forage for bluefish (*Pomatomus saltatrix*), and become increasingly important for maturing striped bass as well (Buckel and McKown 2002). However, it has been shown that the abundance and distribution of YOY winter flounder are poorly correlated to the abundance of other species and abiotic factors, likely because estuarine systems are so dynamic (Fairchild et al. 2008). Nonetheless, in spite of declining numbers of non-YOY
age classes, recruitment in the WLIS and Jamaica Bay, though highly variable, does not show a statistically significant decrease.

It is somewhat surprising that winter flounder recruitment in the WLIS and Jamaica Bay have remained statistically unchanged given the declining numbers of yearlings and older individuals. However, the way in which recruitment depends on stock size is unresolved (Anderson 1988), and in general flatfish show a relatively weak relationship between spawner abundance and recruitment (Myers and Barrowman 1996). Recruitment variability has been observed in other flatfish species, and it appears that year-to-year fluctuations may be intrinsic to these fishes (Rijnsdorp et al. 2005; Kennedy et al. 2007b; Kennedy et al. 2009), though environmental variation may impact age-categories differently (Rjinsdopr 1991; Rideout and Morgan 2007). In winter flounder, reproductive potential has been shown to vary within and across stocks, and that recruitment dynamics and reproductive potential are insufficiently explained by spawning stock biomass, because female size is considered the most important predictor of egg production (McElroy et al. 2013). In addition, since fast-growing winter flounder mature earlier and exhibit larger size at age, they achieve higher fecundity over the course of their lives (Bertram et al. 1993). Thus, in spite of the variability as shown by the BSS, it appears that increased fecundity from larger females and earlier maturation schedules is counteracting the loss in spawner abundance. However, a loss of spawner abundance is not a sustainable across time, because even though recruitment may vary about a constant average level over a restricted range of stock size, it is not biologically possible for a population to maintain high juvenile production at low stock sizes (Iles 1994).
Fishery induced evolution

Prior to the commercial moratorium in 2009, winter flounder in the SNE/MA were fully exploited throughout their range both commercially and recreationally. They now remain at an all-time low, currently regarded as overfished, partially recruited beginning at age-2, and fully recruited by age-4 (ASMFC 2012, 2014). Fishing negatively impacts fish because it greatly increases the mortality rate of the target species, and is often directed towards the largest and fastest growing individuals in a population (Law 2000; Conover and Munch 2002; Law 2007; Kuparinen et al. 2009). As a consequence, fisheries become selective processes that have potential to cause changes in exploited populations (Law 2000; Heino and Godø 2002).

Theoretical predictions and empirical studies of FIE show that size-selective harvests targeting larger individuals will lead to slower growth rates, higher reproductive investment, and maturation at younger ages (Walsh and Reznick 2008). With regard to flatfish, three species in the North Sea were shown to have incurred reduced growth, and after more than a century it was determined that FIE is responsible for the effect (Rijnsdorp and van Leeuwen 1996; Millner and Whiting 1996).

In New York waters, recreational restrictions for winter flounder were first implemented in 1988 with a minimum size of 8 inches, and over time the limits were incrementally increased to the current limit of 12 inches (in 2006). Then in 2009, a two-fish per angler possession limit was applied. It could be argued that increases in the size-at-age in our data results from imposed size restrictions and harvest regulations. However, while sampling on board the party boats, all winter flounder caught by anglers were measured, including sublegal specimens, and are factored into the calculations. Furthermore, despite decades of commercial and recreational fishing, size increases in the WLIS/HRE contradict predictions of reduced size-at-age caused by FIE. Since
fish size has been shown to evolve inversely to the size bias imposed by fishing (Conover and Munch 2002), we can assume that other forces are generating changes in the growth rate. Fishing contributes to kinetic changes in the population by leading to a reduction in intra-specific competition (Law 2000), and because of the flexibility in the growth of fish, declining densities would be primarily manifested as an increased growth rate, which is a function of food availability (Beverton and Holt 1957). Density reduction means that less energy is required to meet nutritional demands and more energy may be devoted to both somatic growth and reproduction. For exploited stocks, changes in the biotic environment can result in new selection pressures through changed predation regimes or resource availability (Heino and Godø 2002). As a result of reduced density and changing predation regimes, the combination of compensatory growth and size-selective mortality can alter growth dynamics (Sogard 1997).

**Predation**

Predation is a major source of mortality for juvenile winter flounder (Buckley 1989). Upon hatching winter flounder larvae are vulnerable to a variety of invertebrate predators (Pereira et al. 1998), though it believed that fish in general are most susceptible to predation during post-larval stages (Anderson 1988). Following settlement, the juvenile winter flounder face a spectrum of invertebrate, fish, and bird predators that typically narrows in scope and scale, as they grow larger (Manderson et al. 1999). The concept of predatory fields was proposed by Folkvord and Hunter (1986), wherein they demonstrated that a higher percentage of survivors escape attack from a variety of predators simply by growing larger. Thus, the intensity of predation mortality on maturing juveniles would potentially impose a strong selective force that would cause them to grow larger to escape this pressure. Various studies have documented the
affect of predators on winter flounder. In the study of estuarine populations of winter flounder, Pearcy (1962) had suggested that density-dependent growth in winter flounder was triggered by summer flounder, and that cormorants also posed a threat to maturing juveniles. Another study stated that winter flounder mortality was highest when YOY bluefish were abundant (Howe et al. 1976). With the potential for high rates of mortality, Bertram et al. (1993) suggested that size selective predation is the cause of increased growth rates in winter flounder (Bertram et al. 1993).

Striped bass represent a significant source of mortality on winter flounder, and with coastal migrating striped bass increasing from an estimated 32 million in the early 1980’s to a high of 245 million in 1994 (ASMFC 2003; ASMFC 2013), their sudden abundance is likely to have significantly impacted already exploited populations of winter flounder throughout their range. The timing of the precipitous rise in striped bass is most curious, because it occurred around the same time when winter flounder began to decline.

Double-crested Cormorants are another predator of young winter flounder whose numbers have drastically increased in the Northeast when pollution controls reduced the level of contaminants in the food chain, and the birds were given federal and state protection (Vermeer and Rankin 1984; USFWS 2006). Subsequently, Double-crested Cormorant nesting sites in New York Harbor have increased over the past thirty years from none in 1980 to a high of 2,006 in 2013 (NYC Audubon 2013). Furthermore, each nest represents two adult birds, and these numbers do not account for non-nesting birds or juveniles (Figure 16). The effect of cormorants on flatfishes was shown by a study conducted in the Wadden Sea, which shows that in the most seaward roosts, YOY flatfish compose a major portion of the diet (Leopold et al. 1998). Another
study had shown that cormorant prey densities were significantly reduced in relationship to the proximity of their colonies (Birt et al. 1987).

Warming water temperatures may also be increasing predation of winter flounder juveniles by sand shrimp (*Crangon septemspinosa*). Sand shrimp are active predators of newly settled winter flounder juveniles and complete refuge from this form of predation isn’t obtained until size greater than 25 mm TL are attained (Taylor 2003). It has been demonstrated that sand shrimp are more active, and maintain higher attack rates at higher temperatures (Witting and Able 1995; Taylor 2003; Taylor and Collie 2003), but are least effective at low densities of winter flounder YOY (Taylor and Danilla 2005).

It is wholly conceivable that increased growth rates in winter flounder are advanced by density changes and a burgeoning presence of co-occurring piscivorous predators that prey upon all but the oldest and largest life stages. An expanding predator assemblage would actively select maturing flatfish. All indications show that winter flounder have been declining since the early 1980’s, while striped bass, summer flounder, sea robins, and cormorants have been increasing in New York waters during the same time period, not to mention several invasive crab species. The escalating threat posed by increasing predator populations was acknowledged by the ASMFC (2011), and thus from settlement to maturity, winter flounder confront a gauntlet of predators that would present a strong selective pressure on reduced populations and cause fast-growing genotypes to advance more rapidly through the most vulnerable stages to escape predation. Those that survive the larval stage undergo strong selective mortality that acts to remove slower growing fish (Meekan et al. 2006). Consequently, there is a consistent preference for small-sized prey by a variety of piscivorous fishes (Juanes 1994), though natural mortality typically decreases with increasing fish size as the prey outgrow their predators (Heino and Godø 2002).
In particular, predation on flatfishes has been shown to be size-selective, concentrated on smaller size classes of flatfish (Ellis and Gibson 1995), which may also contribute to recruitment variability (Baily 1994).

Accordingly, when confronting an assemblage of predators and nutritional demands are not limiting, it is likely that the mortality rate experienced by faster growing fish decreases, and selection will cause a shift towards larger size-at-age. In a study focusing on the effects of predation, Walsh and Reznick (2008) demonstrated strong genetic differences in the life-history traits for populations under predation. In that study, fish under predation pressure matured earlier and produced more offspring than the control group. Therefore, we believe that both human exploitation and increased predation have contributed to waning winter flounder numbers, and the combination of relaxed intraspecific competition and size-selective mortality would synergistically foster faster growth in juvenile fish as response mechanism to escape predation vulnerability.
Table 3.1: Winter flounder sampled from the western Long Island Sound and Hudson River Estuary between 2006 and 2010 are represented the WLIS/HRE data set. Statistical information pertaining to each age class is shown in the table.

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>mean TL</th>
<th>sd</th>
<th>min</th>
<th>median</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>255</td>
<td>59.3</td>
<td>13.2</td>
<td>33.0</td>
<td>59.0</td>
<td>97.0</td>
</tr>
<tr>
<td>1</td>
<td>97</td>
<td>122.7</td>
<td>27.7</td>
<td>73.0</td>
<td>123.0</td>
<td>196.0</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>198.9</td>
<td>33.5</td>
<td>119.0</td>
<td>206.0</td>
<td>299.0</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>264.7</td>
<td>30.8</td>
<td>185.0</td>
<td>265.0</td>
<td>332.0</td>
</tr>
<tr>
<td>4</td>
<td>105</td>
<td>321.4</td>
<td>21.7</td>
<td>260.4</td>
<td>320.0</td>
<td>380.0</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>351.8</td>
<td>26.0</td>
<td>260.4</td>
<td>355.0</td>
<td>390.0</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>377.2</td>
<td>26.1</td>
<td>298.5</td>
<td>381.4</td>
<td>415.0</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>408.5</td>
<td>3.7</td>
<td>406.4</td>
<td>406.4</td>
<td>412.8</td>
</tr>
</tbody>
</table>
Table 3.2. Median length (mm) of winter flounder in the sport fishery as reported by Lobell (1939) and for the WLIS/HRE.

<table>
<thead>
<tr>
<th>Age</th>
<th>Peconic Bay</th>
<th>Shinn-Mor</th>
<th>WLIS</th>
<th>WLIS/HRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>N/A</td>
<td>201</td>
<td>200</td>
<td>206</td>
</tr>
<tr>
<td>3</td>
<td>274</td>
<td>243</td>
<td>285</td>
<td>265</td>
</tr>
<tr>
<td>4</td>
<td>320</td>
<td>272</td>
<td>276</td>
<td>320</td>
</tr>
<tr>
<td>5</td>
<td>357</td>
<td>310</td>
<td>340</td>
<td>355</td>
</tr>
<tr>
<td>6</td>
<td>391</td>
<td>340</td>
<td>N/A</td>
<td>381</td>
</tr>
<tr>
<td>7</td>
<td>414</td>
<td>N/A</td>
<td>N/A</td>
<td>404</td>
</tr>
<tr>
<td>8</td>
<td>440</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Table 3.3. Statistical data pertaining to the winter flounder samples from the Poole (1966) data set are shown in the table.

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>mean TL</th>
<th>sd</th>
<th>min</th>
<th>median</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>127</td>
<td>247.0</td>
<td>30.5</td>
<td>165</td>
<td>250.0</td>
<td>315</td>
</tr>
<tr>
<td>3</td>
<td>347</td>
<td>279.1</td>
<td>32.8</td>
<td>170</td>
<td>280.0</td>
<td>365</td>
</tr>
<tr>
<td>4</td>
<td>344</td>
<td>302.4</td>
<td>35.2</td>
<td>195</td>
<td>305.0</td>
<td>375</td>
</tr>
<tr>
<td>5</td>
<td>152</td>
<td>317.6</td>
<td>35.2</td>
<td>240</td>
<td>315.0</td>
<td>395</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>339.4</td>
<td>43.8</td>
<td>260</td>
<td>337.5</td>
<td>420</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>355.0</td>
<td>72.1</td>
<td>275</td>
<td>375.0</td>
<td>415</td>
</tr>
</tbody>
</table>
Table 3.4. Comparison of the Poole (1966) study to the WLIS/HRE2+ using von Bertalanffy growth function (VBGF) parameters to model somatic growth. The CI was developed from 200 iterations of bootstrapping.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Poole</th>
<th>sd</th>
<th>WLIS/HRE2+</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(\infty)</td>
<td>377.045</td>
<td>25.688</td>
<td>445.963</td>
<td>21.690</td>
</tr>
<tr>
<td>K</td>
<td>0.237</td>
<td>0.082</td>
<td>0.334</td>
<td>0.048</td>
</tr>
<tr>
<td>t(0)</td>
<td>-1.915</td>
<td>0.761</td>
<td>0.241</td>
<td>0.165</td>
</tr>
</tbody>
</table>

95% LCI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Poole</th>
<th>sd</th>
<th>WLIS/HRE2+</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(\infty)</td>
<td>345.921</td>
<td>N/A</td>
<td>407.810</td>
<td>N/A</td>
</tr>
<tr>
<td>K</td>
<td>0.148</td>
<td>N/A</td>
<td>0.251</td>
<td>N/A</td>
</tr>
<tr>
<td>t(0)</td>
<td>-3.603</td>
<td>N/A</td>
<td>-0.861</td>
<td>N/A</td>
</tr>
</tbody>
</table>

95% UCI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Poole</th>
<th>sd</th>
<th>WLIS/HRE2+</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(\infty)</td>
<td>449.282</td>
<td>N/A</td>
<td>497.349</td>
<td>N/A</td>
</tr>
<tr>
<td>K</td>
<td>0.424</td>
<td>N/A</td>
<td>0.434</td>
<td>N/A</td>
</tr>
<tr>
<td>t(0)</td>
<td>-0.856</td>
<td>N/A</td>
<td>-0.509</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 3.4 legend L\(\infty\) = maximum asymptotic average length, K = Brody growth coefficient, t\(0\) = theoretical starting point, sd = standard deviation.
Table 3.5. Statistical comparisons made between regions within the surveys to the WLIS/HRE dataset. Regions are represented as follows: LI = Long Island, LIS = Long Island Sound, LISS = Long Island South Shore, WLIS = Western Long Island Sound, HRE = Hudson River Estuary. N = number, all other statistical values represent TL of fish in mm.

<table>
<thead>
<tr>
<th>Survey</th>
<th>N</th>
<th>mean</th>
<th>min</th>
<th>median</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobell LI</td>
<td>957</td>
<td>264.8</td>
<td>160</td>
<td>250</td>
<td>440</td>
</tr>
<tr>
<td>Lobell WLIS</td>
<td>337</td>
<td>223.4</td>
<td>160</td>
<td>220</td>
<td>370</td>
</tr>
<tr>
<td>Perlmutter LI</td>
<td>4095</td>
<td>265.4</td>
<td>125</td>
<td>240</td>
<td>500</td>
</tr>
<tr>
<td>Perlmutter LIS</td>
<td>1100</td>
<td>244.0</td>
<td>155</td>
<td>240</td>
<td>500</td>
</tr>
<tr>
<td>Perlmutter WLIS</td>
<td>604</td>
<td>267.0</td>
<td>185</td>
<td>260</td>
<td>500</td>
</tr>
<tr>
<td>Poole LISS</td>
<td>999</td>
<td>290.7</td>
<td>165</td>
<td>290</td>
<td>420</td>
</tr>
<tr>
<td>WLIS/HRE</td>
<td>288</td>
<td>290.0</td>
<td>119</td>
<td>305</td>
<td>415</td>
</tr>
</tbody>
</table>

* One 500 mm specimen was caught in the WLIS by Perlmutter (1947) and it subsequently affects the other Perlmutter (1947) sites in the LIS.
Figure 3.1. Sampling sites for the WLIS/HRE are numbered as follows: 1 = Arthur Kill, 2 = Port Jersey, 3 = Newark Bay, 4 = South Brooklyn, 5 = Lower Bay, 6 = Jamaica Bay, 7 = Little Neck Bay, 8 = Eastchester Bay, 9 = Manhasset Bay, 10 = Hempstead Harbor, 11 = Oyster Bay, 12 = Huntington Harbor. Embayments sampled in the historical data are as follows: MP = Matinecock Point, PJ = Port Jefferson, PB = Peconic Bay, SB = Shinnecock Bay, MB = Moriches Bay, GSB = Great South Bay.
Figure 3.2. Size frequency histograms of (A) the WLIS/HRE data set and (B) WLIS/HRE age-2+ fish, and age frequencies of (C) WLIS/HRE and (D) WLIS/HRE age-2 fish and older.
Figure 3.3. Comparison of winter flounder from the WLIS sport fishery showing: (A) Length-frequency histogram of the Lobell (1939) sport fishery in the WLIS, with a mode at 225 mm. (B) Length-frequency histogram of the sport fishery from all regions of Long Island (Lobell 1939), with most dominant class of fish in the 200-250 mm range. (C) Three year-old fish formed the most dominant age group of the WLIS sport fishery reported by Lobell (1939). (D) Total-length frequency histogram of fish from party boats from 2006 – 2010 in the WLIS with a mode of 300-350 mm.
Figure 3.4. Comparison the total length distribution from angling results in the WLIS as reported by Lobel (1939) and those from contemporary party boat activity. The party boat data was normalized to the Lobell (1939) data to more effectively compare data sets.
Figure 3.5. Length/frequency distributions of the Perlmutter’s (1947) survey conducted in New York waters shown by region. (A) Length/frequency in the Long Island Sound, (B) Length/frequency in the WLIS, and (C) Length/frequency results from all Long Island waters.
Figure 3.6. Total length-frequency distribution (A) and age/frequency histograms (B) of the Poole (1966) survey conducted in four south shore Long Island bays, e.g., Great South Bay, Moriches Bay, Shinnecock Bay, and Peconic Bay.
Figure 3.7. The VBGF constructed from the WLIS/HRE2+ data. Confidence intervals (red line) were developed by bootstrap estimation through random resampling of the data with replacement. (A) Bootstrap distribution of the $L_\infty$ parameter and the CI, (B) Bootstrap distribution of the Brody growth coefficient and CI, (C) Bootstrap distribution of the $t_0$ and CI, (D) Depiction of the VBGF (solid line) showing the 95% CI (dashed lines).
Figure 3.8. VBGF created from the Poole (1966) survey. Confidence intervals (red line) were developed by bootstrap estimation through random re-sampling of the data with replacement. (A) Bootstrap distribution of the $L_\infty$ and CI (B) Bootstrap distribution of the Brody growth coefficient and CI (C) Bootstrap distribution of the $t_0$ and CI (D) Depiction of the VBGF (solid line) showing the 95% CI (dashed lines).
Figure 3.9. VBGF created from the Poole (1966) survey. (A) An age jittered VBGF of the Poole (1966) data. The $L_\infty$ for this data are 377.0 mm with the Brody growth coefficient of $K= 0.273$
(B) An age jittered depiction of the WLIS/HRE age-2+ data. The $L_\infty$ for this data are 445.1 mm with the Brody growth coefficient of $K= 0.334$. 
Figure 3.10. (A) Results of a one-way ANOVA comparing the total length (mm) with the historical data and the WLIS/HRE2+ data. (B) Comparison limited to the WLIS. Boxes represent the interquartile range of the study results; the line in the box represents the median; whiskers represent the range of the data; and open circles represent outliers.
**Figure 3.10A.** Long Island Sound Spring Trawl Survey conducted by CTDEEP from 1984 - 2013. The geometric mean average per tow of Winter Flounder shows a declining trend.
Figure 3.10B. CT DEEP LISTS spring trawl comparison of Striped Bass and Winter Flounder over the 30-year period. Values represent geometric mean values per tow.
Figure 3.10C. CT DEEP LISTS fall trawls of Striped Bass and Winter Flounder over the 30-year period. Values represent geometric mean averages per tow.
Figure 3.11A. CT DEEP LISTS spring trawls of Summer Flounder and Winter Flounder over the 30-year period. Values represent geometric mean values per tow.
Figure 3.11B. CT DEEP LISTS fall trawls of Summer flounder and Winter flounder over the 30-year period. Values represent geometric mean per tow.
Figure 3.11C. CT DEEP LISTS fall trawls of Striped Sea Robin and Winter Flounder over the 30-year period. Values represent geometric mean per tow.
Figure 3.12. Results of the NYSDEC Beach Seine Surveys conducted from 1984 through 2014. (A) The YOY show no significant difference over time (p = 0.716), whereas (B) the number of non-YOY are declining (p < 0.001), and (C) CPUE is trending slightly downward but remains statistically unchanged (p = 0.521).
Figure 3.13. PCA of NYSDEC BSS in the WLIS. Standardized values (std) of the first two principal components shown as a vector plot of the following species: striped bass = SB, winter flounder = WF, northern sea robin = NSR, summer flounder = SF, American menhaden = AM, bay anchovy = BA, bluefish = BF, silverside = SS, striped sea robin = SSR. 40.6% of the variation is caused by the first two components.
Figure 3.14. Number of Double-crested Cormorants nest sites in New York Harbor from 1982 – 2014 according to data obtained from NYC Audubon. Each nest site represents two birds and doesn’t account for juveniles or non-nesters.
CONCLUSIONS

This research endeavor has shown that otoliths can provide retrospective insight into the life history of winter flounder in order to resolve mixed stock analyses, assess the quality of the habitat usage, and determine differences within winter flounder ecology that would not be readily manifested through other methods. The data in chapter 1 demonstrates that winter flounder sagittae are both morphologically and chemically distinct from each other and are therefore not identical. The chemical asymmetry in winter flounder sagittae is suggestive of differences in the availability of ions in the sediment pore-water vs. in the overlying water column. The discovery of deformed otoliths also illustrates that the bio-mineralization processes and chemical uptake within the otoliths of a flatfish are potentially decoupled, and function independent of one another. These discoveries have important implications for winter flounder and other flatfishes when conducting otolith chemistry, and suggest a protocol be adopted, because indiscriminant use of either otolith may skew statistical results. Furthermore, because beam-based analyses are destructive, the blind-side otolith should be retained for otolith chemistry while the remaining otolith could be preserved for age and growth studies. More importantly, method standardization would ensure uniformity in the results when performing otolith chemistry on flatfishes.

In chapter 2, I was able to discriminate amongst groups of winter flounder at both the regional and the local level. This result shows that otolith microchemistry can be used to resolve stock structure of winter flounder at a variety of scales. In addition, the relatively high re-classification rates to location of capture are the result of similarity in juvenile sections of the otoliths, which implies a high degree of natal nursery fidelity is expressed by the spawning adults. The ramifications of this finding is relevant to winter flounder stock structure, because the
capacity for homing or site fidelity in mobile marine species tends to regulate population structuring and connectivity between habitats (Bradbury et al. 2008). The relatively high results in the re-classifications tend to support previous studies, which established that the inshore winter flounder recruitment is the product of localized spawning from which discrete population units are formed. Natal homing is believed to have evolved to maximize reproductive success by synchronizing the return of mature adults to spawning grounds when conditions are optimal to ensure recruitment success and by regulating habitat usage – to avoid under or overutilization of habitat (Legget 1977). Therefore, the results obtained in chapter 2 imply that the inshore population structure of winter flounder is ordered by the degree of natal homing and juvenile philopatry. Localized spawning, wandering, and coastal spawning probably remain essential to the maintenance of allelic diversity, and likely account for the stock structure at the coast-wide level, as well as the local level. Consequently, marine fish are expected to show low genetic differentiation and high gene flow due to large population sizes, broad geographic distributions, high fecundity and high dispersal potential (DeWoody & Avise 2000).

In the second part of chapter 2, the results demonstrate that habitat assessments can be successfully conducted with otolith chemistry. In this chapter it was demonstrated that Mn is valuable in identifying oxygen deprived water bodies and may potentially be useful in identifying spawning location through sediment type. Magnesium is another element that shows strong value in assessing habitat condition and was found most often in fast-accreting sections of the otolith. Correlations can be made with metabolic activity and growth rates using Mg. In this part of the study the highest mean levels of Mg were discovered in locations that also showed the fastest daily growth rates, whereas lowest mean Mg concentrations were found in an embayment with low daily growth rates. There are probably other mitigating factors such as maternal
investment, which contributes to the result, but this approach, though promising requires further research. Nevertheless, the findings in this part of the chapter may help identify and define critical winter flounder recruitment habitat at a crucial time.

In chapter 3, the results of my analyses demonstrate that winter flounder in the WLIS/HRE have incurred a faster growth rate and older fish are found in higher frequencies when viewed in comparison to flounder data listed in the historical record. Otoliths were used to create a size-at-age index to enable comparisons between recent captures of winter flounder with those recorded in the historical record. Consequently, changing growth dynamics within the same locality implies a shifting environmental condition. Despite the negative affects that may be attributed to anthropogenically-impaired habitat, winter flounder have increased in size, which has important ramifications in the recruitment of future generations. Studies of this nature are essential because accurate age determination and growth dynamics of exploited fish is vital to successful fisheries management. Since the shift in growth is apparently correlated to a release from intra-specific competition and in response to predation, then accordingly predation pressure needs to be factored into management decisions and policies directly relating to winter flounder populations and future stock assessments. This finding strongly suggests that we cannot manage a species in isolation but need to view fish from an ecological perspective and manage them from an ecosystem level.

In closing, otolith geochemistry has been used in a wide variety of studies, and yet many uncertainties still persist regarding elemental incorporation and the pathways of transitional and post-transitional metals into otolith crystals. More research needs to examine the affects of redox potential from marine sediments and their correspondence with otolith elemental uptake in flatfishes and other species. Empirical studies using in situ caging might better address these
questions rather than laboratory settings, which cannot replicate the complexities found in natural environments. More importantly research should be directed towards qualitative assessments of habitat in other species to determine if these findings hold true in other genera. In addition, the effect of increasing winter flounder growth should be investigated in other regions to determine if flounder from other populations are growing larger from decreased intra-specific competition and directional selection from size-selective mortality. Lastly, in this research I have demonstrated the value of using otolith analyses on winter flounder to answer questions pertaining to population structure in winter flounder, but better resolution would most likely be achieved from a complementary approach that incorporates otolith chemistry with other techniques such as telemetry, tagging, and genetics to produce a more robust and cohesive study.
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