Reconstructing the life history of fishes using the stable isotope composition of eye lens laminae

by

Katherine Buell-Fleming

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Degree in Environmental Science with Distinction

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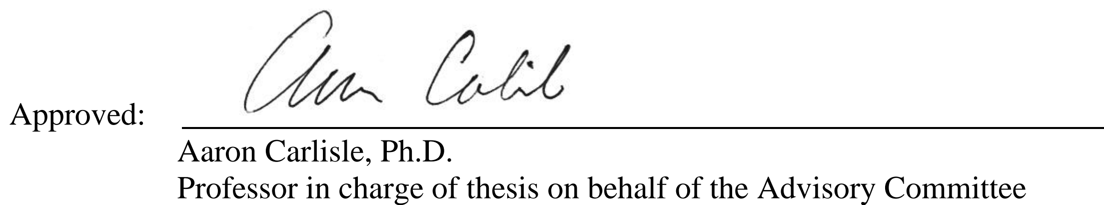

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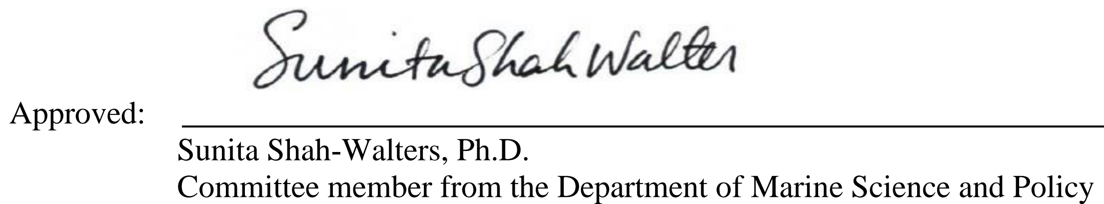

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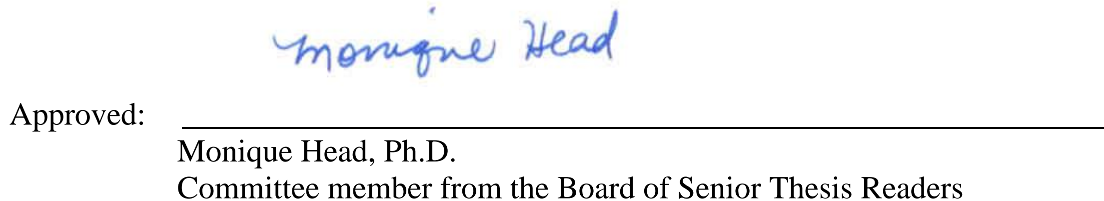

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ABSTRACT

In order to improve our understanding of fish ecology, behavior, and habitat utilization across ontogeny, researchers need approaches for understanding how these dynamics change throughout the lives of individual fishes. Stable isotope analysis (SIA), the comparison of isotope ratios in animal tissues to spatial variation in isotope values of prey and primary producer species throughout an environment, is one such tool that can be used to study animal migration and trophic ecology. However, researchers often use SIA of metabolically active tissues (e.g., muscle, liver) with faster turnover rates, which provides insight into the recent history of the animal. Analysis of eye lenses (laminae), a protein rich archival tissue that forms layers that do not change after deposition, is a newer method that is being developed. In this thesis, we used *Cynoscion regalis,* commonly known as Weakfish, in Delaware Bay as a model species to further explore the feasibility of stable isotope analysis on the laminae of individual fish in order to reconstruct their life histories. We analyzed the lamina from both eyes of 17 young of the year weakfish, measuring the carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$). We then compared the isotopic composition of Weakfish eye lenses to published SIA data from weakfish muscle samples collected in Delaware Bay during prior research. I found that $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in lens lamina where similar to those of muscle samples, suggesting this approach produces reasonable data for this species and location. There were distinct changes in $\delta^{13}C$, $\delta^{15}N$

and $\delta^{34}S$ values between lamina layers that were indicative of changes in habitat and dietary shifts through ontogeny.

Chapter 1

INTRODUCTION

1.1 History Weakfish in the Delaware Bay

Cynoscion regalis, commonly known as the weakfish, is the state fish of Delaware that has historically held great economic and ecological importance. In the early 2000s the populations of weakfish present in the Delaware Bay crashed due to overfishing and has since failed to recover. Weakfish have historically been one of the most abundant species in the estuaries and near shore waters of the Atlantic coast. It is valuable as a recreational species and highly important to various fisheries in the coastal region, including gillnet, pound net, haul-seine, and trawl fisheries (Mercer, 1989).

Weakfish range from Eastern Florida up to Massachusetts, though in times of high abundance can extend as far as the Gulf of Maine. They have an inshore and northerly migration in the spring, while migrating offshore and to the south in the fall (Shepherd & Grimes, 1983). This means that fisheries are practical on a seasonal basis. For example, in the Chesapeake Bay, weakfish generally are available from April through November (Shepherd & Grimes, 1983). North Carolina is thought to be where most of the over wintering grounds are located, though younger fish may stay closer to shore (Shepherd & Grimes, 1983). Weakfish feed on planktonic animals as larvae while they travel from their spawning area to nursery areas, which are in coastal rivers, bays sounds and estuaries where they remain until October or December of their first year. From there they migrate to the coast.

From 1950 to 1970 there was fluctuation in the number of commercial landings that ranged from three to nine million pounds (*ASMFC Stock Assessment Overview: Weakfish.* 2019). In the early 1970s there was a huge growth in the fishery which peaked in the 1980s (*ASMFC Stock Assessment Overview: Weakfish.* 2019). Since then, the commercial landings have drastically declined and have dropped to 102,492 pounds in 2018 (*ASMFC Stock Assessment Overview: Weakfish.* 2019). The 2019 stock assessment show that the weakfish populations have remained depleted since 2003 because the stock has remained below the spawning stock biomass threshold of 30% (*ASMFC Stock Assessment Overview: Weakfish.* 2019). There has also been a documented increase in mortality from the mid to late 2000s which could be impacting stock recovery despite fishing restriction (*ASMFC Stock Assessment Overview: Weakfish.* 2019).

Survivorship and population dynamics in most fish species are largely determined during the first few days to months of life of a given cohort of fish. As a result, understanding what happens during the early life history of a species in terms of habitat use and feeding of larval and juvenile stages is needed. This information is particularly important for helping foster effective conservation practices and predicting of future population dynamics.

1.2 Stable Isotope Analysis

The principle of stable isotope analysis (SIA) relies on the idea that naturally occurring endogenous markers, like stable isotopes, can be used to track movements of an individual and changes in their diet. In particular, this approach can be used to understand habitat use if there are spatial gradients in stable isotopes (e.g., isoscape, or a map of the isotopic values of primary producers in a region) is known (Hobson,

Barnett-Johnson, & Cerling, 2010). In order to effectively use the stable isotope composition of animal tissues to track animal migration, the stable isotope dynamics of a given tissue (i.e., turnover rates, fractionation) need to be understood (Hobson et al., 2010). Additionally, the physiological process though which metabolic routing of macronutrients to specific tissues needs to be understood, as well as the processes that change the isotopic ratios of the tissues when the animal is in equilibrium with the food web (Hobson et al., 2010). Finally, the turnover rate of the tissues must be known, a faster turnover rate will result in shorter time scale of dietary information, while metabolically inactive tissues, such as hair and feathers can be used to create timeseries of shifts in habitat and/or diet through time (Hobson et al., 2010). The eye lens is a metabolically inactive tissue that is now being explored as a recorder for life histories of individuals.

1.3 Research Purpose

As estuary dependent fish, weakfish experience a wide range of estuarine habitats and environments during their estuarine residency as juveniles (Boutin & Targett, 2018). Fishery management priorities are emphasizing ecosystem-based fisheries management approaches which requires higher level of understanding of the variables that impact fish populations, including those in estuarine habitats used for nurseries, such as the Delaware Bay (Galvez, 2019). The utilization of different estuarine habitats during their residency can be studied using stable isotope analysis on archival tissues.

Eye lenses are metabolically inert tissues that are being evaluated as recorders of isotopic history for estuarine species such as the weakfish. Eye lenses are known to preserve lifetime isotopic records, with a new lens layer, called laminae, forming

consecutively around the outside of the lens like an onion as the animal grows (Peebles & Hollander, 2020). The proteins of the laminae reflect dietary isotope ratios while attenuated apoptosis ensures that the next laminae will reflect the new time period's isotopic signature (Peebles & Hollander, 2020). These ratios of $^{13}C/^{12}C$, $^{13}N/^{14}N$ or $^{34}S/^{32}S$ isotopes within a tissue sample ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) can potentially be used to track movement through an ecosystem, in this case the Delaware Bay. While the carbon and nitrogen values in eye laminae have been evaluated with higher frequency (Vecchio, 2020; Wallace, Hollander, & Peebles, 2014; Wei, Xin, Meng, & Na, 2020) analysis of sulfur isotope values are much less common (Bell-Tilcock et al., 2020)

I will be analyzing *Cynoscion regalis* samples collected from the Delaware Bay in 2020 to examine the stable isotope composition of consecutive laminae of weakfish eye lenses to create records of the fish's geographic history within the Delaware Bay. By comparing the isotope composition of the eye lenses to the known baseline isotopic levels of the Delaware Bay ecosystem, we will be able to track the movement of *Cynoscion regalis* over time. This will lead to better understanding of how juvenile weakfish utilize their estuarine habitat during their residency, which can help to guide population management practices and increase our understanding of the species.

1.4 Experimental Approach

In order to explore the feasibility of using eye lens laminae as recorders of geographic history in weakfish, and other estuarine species in general, I obtained the eye lenses of young of the year weakfish caught in the open waters of the Delaware Bay during the 2020 Delaware Department of Fish and Wildlife Trawl Survey. By

separating the lens laminae of the weakfish and performing stable isotope analysis on the separated lens laminae, I attempted to evaluate if the technique was a reasonable tool for reconstructing life history records of individual fishes. Based on prior work done by Dr. Steven Litvin on the isotope values weakfish muscle tissues caught in areas of the bay at different stages of life, I compared the results from the lens laminae to the baseline data provided by Dr. Litvin. Using Non-metric Multidimensional Scaling to group this data I was able to explore if there was distinct change in isotopic values between lens laminae, if it could be grouped by location within the bay, and if the lens laminae were able to be separated effectively for analysis.

1.5 Research Hypothesis and Objectives

The overall objective of this research was to explore the feasibility of use of stable isotope analysis on fish eye lenses to determine habitat usage and estuarine utilization by Weakfish in the Delaware Bay. In addition, I used these data to generate a preliminary assessment of estuarine utilization by Weakfish over time within the Delaware Bay.

Chapter 2

LITERATURE REVIEW

2.1 Stable Isotope Analysis in Ecology

The stable isotopic signatures of tissue can be utilized to understand the diet and habitat use of an organism within an ecosystem. The ratio of $^{13}C/^{12}C$ (i.e., $\delta^{13}C$), $^{13}N/^{14}N$ ($\delta^{15}N$), or $^{34}S/^{32}S$ ($\delta^{34}S$) isotopes within a tissue sample, is often used to determine diet organisms in situations where stable isotope dynamics within a particular tissue are understood. Stable isotope analysis can also be applied to describe the movement, habitat usage patterns and utilization of different sources of estuarine organic matter by Weakfish within the Delaware Bay (Galvez, 2019). The variability of organic matter in a habitat due to variation in the salinity gradients and geochemical processes are what create the isoscape of isotopic signatures that represent the local food webs (Litvin & Weinstein, 2004). The distribution of sulfur isotopes within an estuarine system is dependent on the salinity gradient of the area (Chumchal & Fry, 2011). There are zones of rapid $\delta^{34}S$ increase in areas of lower salinity (Chumchal & Fry, 2011). Plants will synthesize high $\delta^{34}S$ values close to those of sulfate with little fractionation (or change through the food web) (Chumchal & Fry, 2011). While benthic sediments have larger fractionation and results in lower faunal $\delta^{34}S$ values. (Chumchal & Fry, 2011). In research with salmon lens laminae Bell-Tilcock et al. (2020) characterized $\delta^{13}C$ as more enriched in marine environments than fresh water ones, $\delta^{15}N$ indicates trophic level, and is influenced by the diet of the species in question, and $\delta^{34}S$ values reflect macrophyte isotope characteristics, and are typically low in areas with long water residence time.

2.2 Lens Laminae as Recorders of Life History

Eye lenses are protein rich tissues that consist of metabolically inert optical proteins, which form layers sequentially over the fish's lifetime, akin to the rings on a tree. The concentric layers of the lens are called laminae. During the initial process of new lamina formation, the structural protein crystallin in the lens forms layers that reflect dietary isotope ratios as the animal grows. During the final development of the lamina attenuated apoptosis occurs, removing the organelles and nucleus from the fiber cells but leaving the crystallin and proteins behind (Wallace et. al, 2014). This traps existing isotopes that were initially associated with lamina formation, the next layer of laminae will then incorporate the dietary isotopes of the next time period, thereby creating a time series of dietary isotopes within the lens laminae. There has been correlation found between lens diameter and size of the fish, as laminae forming during periods of somatic grows (Bell-Tilcock et al., 2020). However, the rate of laminae formation and fractionation of laminae tissue is currently not well understood (Bell-Tilcock et al., 2020).

Prior research has indicated that stable isotope analysis of eye lens laminae is a viable method of showing life history records in vertebrate species. Wallace et. al (2014) explored efficacy of this method with the eye lens of four red grouper, three gag, eight red snapper and one white grunt. Low resolution isotopic screening showed strong potential as a record of broad- scale changes in the isotopic history of an individual fish. (Wallace et. al, 2014). Comparison of isotopic signatures in the left and right eye showed that there was little variation in values and indicated that variability between the left and right eyes were low (Bell-Tilcock et al., 2020), but that intra laminar variation could be a limit in the method (Wallace et al 2014). Research on SIA of lens laminae has also been performed on squids and demersal marine fishes

(Quaeck-Davies et al., 2018; Vecchio & Peebles, 2020; Wallace, Hollander, & Peebles, 2014; Wei, Xin, Meng, & Na, 2020) but little to no research has been performed on freshwater and estuarine fishes using this technique (Bell-Tilcock et al. 2020).

2.3 Weakfish Isotopes and Isoscape of Delaware Bay

Variability in the distribution and abundance of two macrophytes (primary producers) in Delaware Bay lead to spatial variation in baseline sulfur isotope content throughout the Delaware Bay. The Delaware Bay can be roughly divided into open water and marshes with tidal creeks. The lower marshes of the Delaware bay are dominated by Smooth Cordgrass, *Spartina alterniflora* and the upper marshes are dominated by the invasive species, Common Reed, *Phragmites australis*, specifically in the Alloway creeks region of the Bay there is a there is a monoculture of *P. australis*, as well as in the southern region of the Bay on the southern Delaware shore of the Bay (Litvin & Weinstein, 2003). Benthic microalgae and phytoplankton are other primary producers in the system (Litvin & Weinstein, 2003). Litvin and Weinstein (2003) showed that isotopic values of weakfish muscle tissue were bounded by the $\delta^{13}C$ and $\delta^{34}S$ values of the primary producers of the Delaware Bay (2004). In muscle tissue from juvenile weakfish caught in the lower Delaware bay area there were more enriched carbon isotope values (i.e. higher $\delta^{13}C$) compared to other collection locations (Litvin & Weinstein, 2004). Fish caught in the open bay displayed relatively depleted (lower $\delta^{34}S$) sulfur isotopes (Litvin & Weinstein, 2004). Galvez (2019) noted enriched $\delta^{15}N$ values in juvenile weakfish from the upper bay.

Chapter 3

METHODS

3.1 Sample Collection and Description

All of the fish used in the research were young of the year weakfish captured in the 2020 Delaware Fish and Wildlife Trawl Survey in the months of November and October. Their total length ranged between 11.2 and 16 centimeters. Their weight ranged from 13 to 46 grams. Based on their size they are likely to be young of the year at the cusp of moving out the open ocean. All fish were caught in the lower outer bay area of the Delaware Bay. 17 fish were prepared for analysis, the number of laminae ranged from 4 to 12 layers per lens. While the diameter of the lens (equating a larger number of lamina) and the age of the fish has been observed to be correlated (Wallace, Hollander, & Peebles, 2014) I experienced difficulty separating the lamina at a fine enough resolution to see clear correlation between number of lamina and size of fish.

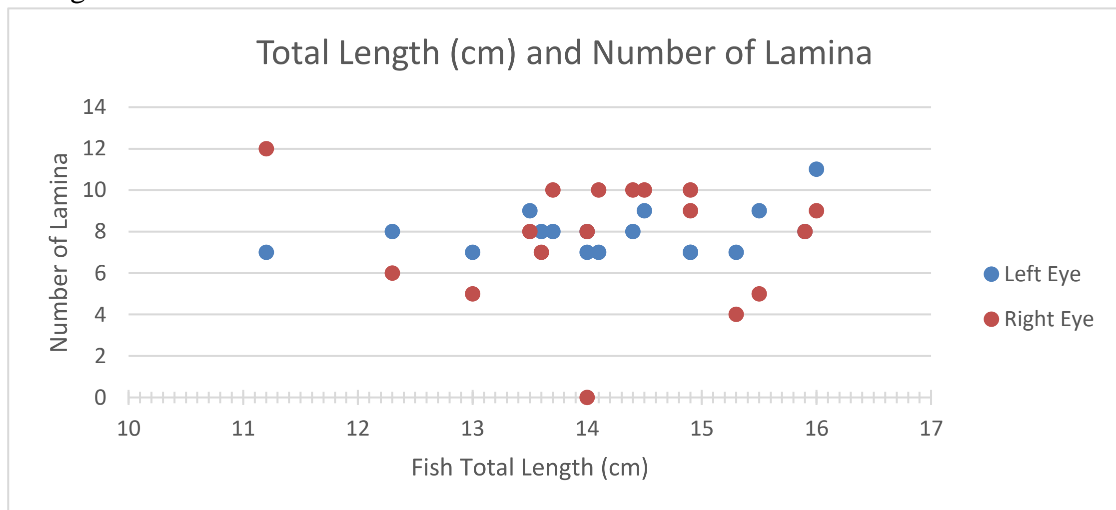

Figure 1. The number of laminae separated for each eye lens based on the total length of the fish in cm, there is little to no correlation between fish size and number of laminae separated

3.2 Lens Dissection

The eye was removed from the weakfish body after the specimen was partially thawed. The eye was removed from the body using fine point tweezers. These tweezers were then rinsed with deionized water and used to remove the lens from the eyeball. The sclera of the eyeball was pierced using the pair of fine nosed tweezers and pulled apart using an accompanying pair. The lens was located within the viscera as a semitransparent white orb, which was then placed into a petri dish to be rinsed initially with deionized water. The lens was then moved with the petri dish under a stereo microscope, where the delamination was performed. Delamination methods were taken from the work of (Bell-Tilcock et al., 2021; Peebles & Hollander, 2020; Quaeck-Davies et al., 2018; Wallace et al., 2014; Wei et al., 2020). While deionized water was initially used sparingly in the delamination process, it proved too difficult to separate the lens laminae into finer layers without crushing the lens. Due to this, the lens was kept moist using deionized water throughout the delamination process to improve delamination, as done in (Wallace et al., 2014).

Delamination was performed using fine nosed tweezers and a pair of forceps. Each lens has what appears to be a seam running along the orb, which was utilized to separate the layers. Holding the lens steady with the forceps, the fine nosed tweezers were used to gently separate the laminae away from the core of the lens starting at the seam using a brushing motion. A lamina was considered separated when an entire layer came away from the lens, leaving behind the lens as a spherical orb once again. Drops of deionized water were added periodically to keep the lens moist.

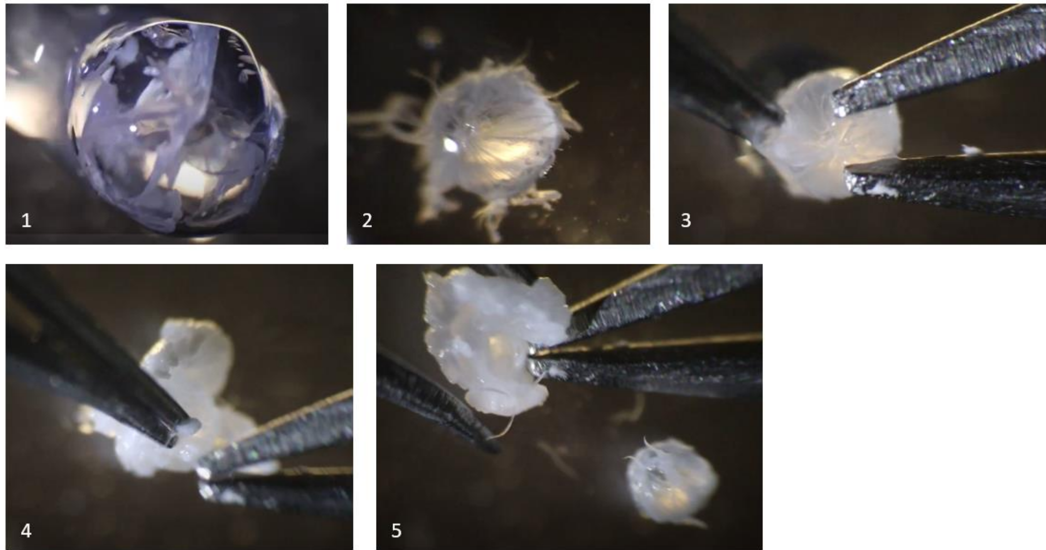

Figure 2. The process of Delamination. 1) Depicts a whole lens before delamination. 2) Shows post initial laminae removal. 3) Two tweezers are utilized to gently separate laminae from base core. 4) Laminae is pried from core in its entirety. 5) Lens laminae is placed in a tin capsule.

Once a lens lamina was separated out it would be placed in a pre-weighed 5x6 mm tin capsule to desiccate. Lamina were continually separated from the lens until the core was reached, and it was physically impossible to separate a finer layer from the core without destroying the entire structure. What remained was termed the core and placed in its own capsule to desiccate. Many cores were lost in the transfer process and were ultimately discarded due to consideration for maternal signature contamination, as recommended through personal communication with Drs. Miranda Bell-Tilcock and Steven Litvin.

Before delamination the lens diameter was roughly measured using the stage micrometer.

There is little difference in the carbon, nitrogen and sulfur isotopic ratio between the left and right eye (Peebles & Hollander, 2020). As such, in order to reach the required mass needed for analysis as dictated by the Center for Stable Isotope Biogeochemistry at UC Berkeley the adjacent lens layers were combined within a 4x6mm tin capsule in order to reach the mass required. The left eye lenses were combined together and used for sulfur analysis while the right eye lens was combined together and used for combined carbon and nitrogen analysis. The minimum mass needed for carbon and nitrogen was around 300 micrograms and the goal mass for the sulfur samples was around 500 micrograms. The expected CNS percentages used for the stable isotope analysis was taken from Miranda Bell-Tilcock's work with salmon lens laminae: carbon ~26%, nitrogen ~7%, and sulfur was estimated at ~2%.

3.3 Data Analysis

Time series of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were generated for the laminae sampled for each fish. In RStudio using the vegan package a non-metric multidimensional analysis was run on the innermost laminae for each fish and the outermost laminae of each fish assuming that the oldest (innermost) and youngest (outermost) layers should be different. Data provided by Dr. Steven Litvin was used as "base line" values of average $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values from muscle tissue of juvenile weakfish to act as markers of isotope values based on location. The juvenile fish were caught in the Upper Bay Marsh, Mid Bay Marsh, Lower Bay Marsh, Upper Outer Bay, Mid Outer Bay and Lower Outer Bay of the Delaware Bay. The NMDS produced an ordination based on the dissimilarity of the isotopic values for the laminae and the muscle tissue of the fish from various locations in the bay. The dissimilarity was measured using the Bray-Curtis Dissimilarity equation in RStudio to calculate distance.

Chapter 4

RESULTS

I found that there was clear variation in the stable isotope composition of different lamina layers in the weakfish. There was high variation in carbon, nitrogen, and sulfur values within individual fish and between fish.

Table 1. Sample Size, Mean, Standard Deviation, Maximum and Minimum of Laminae Across Fish.

		d34S				
	Row	n	mean	SD	max	min
Oldest	1	19	14.74	1.335889	16.94	11.27
	2	19	14.25	1.644498	16.84	10.90
	3	14	13.65	2.448357	16.86	7.35
	4	6	12.60	3.846629	15.71	5.04
	5	1	15.24	n/a	15.24	15.24
	6	n/a				
Newest	7	n/a				
		d13C				
	Row	n	mean	SD	min	max
Oldest	1	16	-19.16	3.014788	-23.91	-16.38
	2	16	-19.31	2.723116	-24.77	-16.32
	3	14	-19.3893	2.415955	-23.37	-16.4
	4	10	-21.097	2.301135	-24.07	-18.27
	5	7	-18.8657	2.767061	-24.34	-16.65
	6	2	-19.88	5.529575	-23.79	-15.97
Newest	7	1	-16.19	n/a	-16.19	-16.19
		d15N				
		n	mean	SD	min	max
	Row	16	14.31688	1.823709	11.04	17.64
Oldest	1	16	13.64375	1.858461	9.7	16.63
	2	13	13.61923	1.66246	9.7	16.06
	3	10	14.532	1.690488	11.36	16.84
	4	7	14.34833	2.212342	12.25	18.41
	5	2	15.595	2.100107	14.11	17.08
	6	1	12.91	n/a	12.91	12.91
Newest	7					

		C: N				
	Row	mean	SD			
Oldest	1	2.957288	0.053159			
	2	2.872428	0.244095			
	3	2.574833	0.687231			
	4	2.945194	0.062744			
	5	2.913875	0.064994			
	6	2.900342	0.054638			
Newest	7	2.904262	0			

Table 1. The Population size, Average, Standard Deviation, Maximum and Minimum of the as $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ for each laminae layer across the fish that were analyzed. As well as the mean and standard deviation for the C:N ratio. The values on average were under 3, which indicates that there is not contamination of the carbon values from some substance outside of the tissues analyzed.

The C:N ratios on average fell below three, which indicates that there was low lipid content and values represented protein, which is the tissue constituent that we are interested in.

Looking at time series of each isotope individually we do see some trends and clustering of the values between laminae. Because there were unequal numbers of samples from the left and right eyes due to the difference in mass requirements for the isotope analysis, we had higher resolution (i.e. more layers) for carbon and nitrogen values compared to sulfur. We can see that there is variation among laminae and among the individual fish across the time series (youngest to oldest layers).

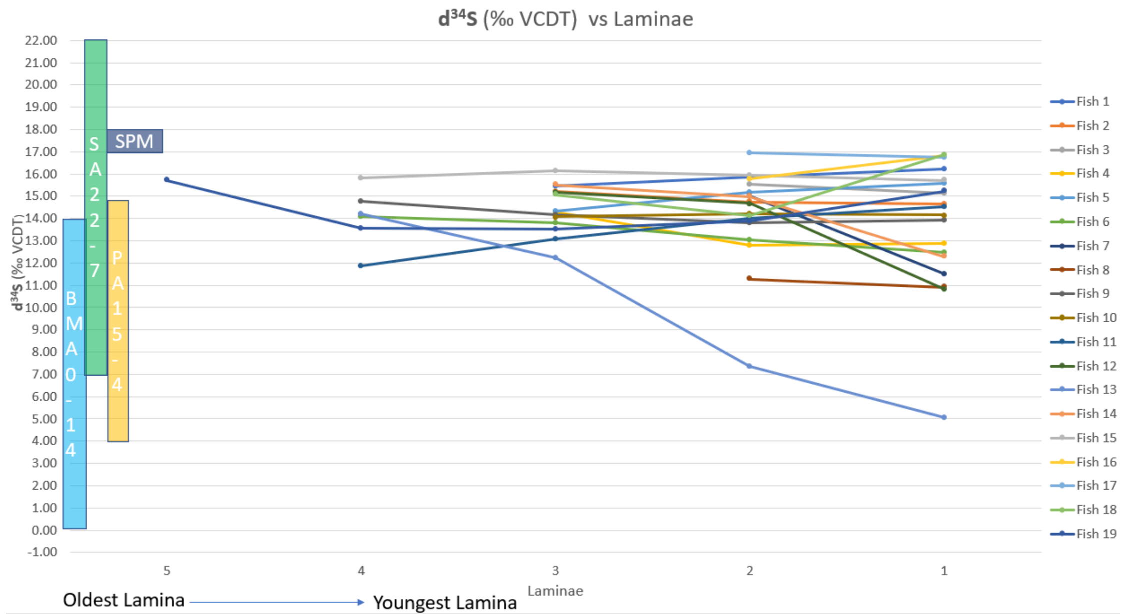

Figure 3. Shows the $\delta^{34}S$ values of each lamina arranged in a time series from oldest (innermost) lamina to newest (outermost) lamina. The bars to the left represent the baseline sulfur isotope values of the predominate primary producers in the Delaware bay area, Benthic Micro algae (BMA), Suspended Particulate Matter (SPM) *P. australis* (Pa), and *S. alterniflora* (Sa) taken from (Litvin & Weinstein, 2004). There is a cluster of newest lamina sulfur isotope values between 14 ‰ VCDT and 14 ‰ VCDT, and another cluster between 10 ‰ VCDT and 13 ‰ VCDT. Fish 13 shows a drastic depletion in sulfur value from its oldest lamina to newest lamina, falling from 14.2 ‰ VCDT to 5.05 ‰ VCDT.

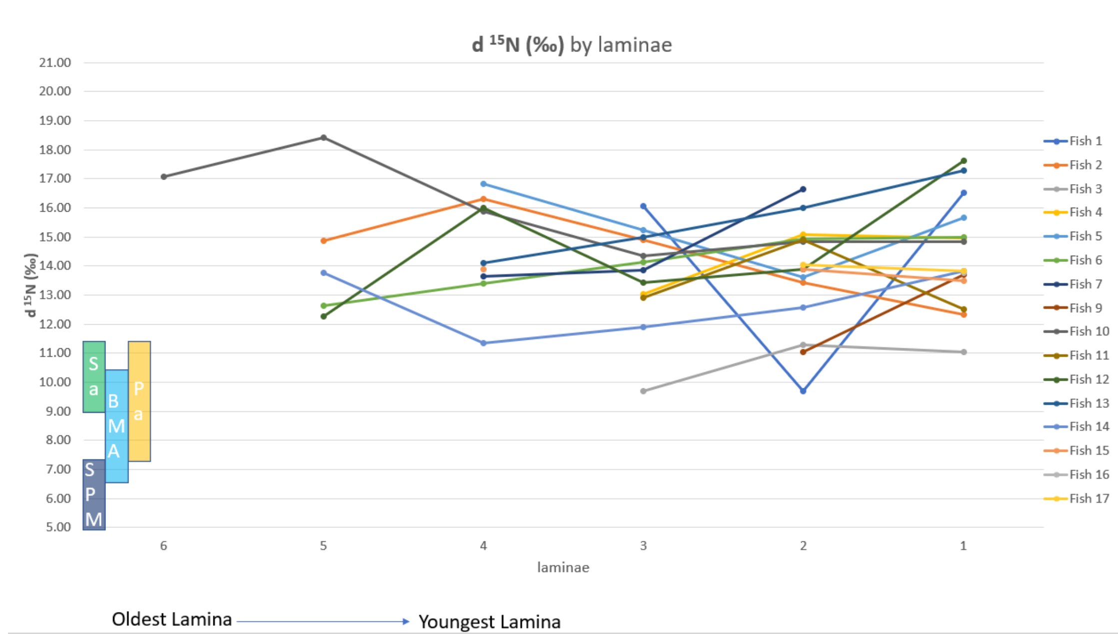

Figure 4. The $\delta^{15}N$ values of each lamina arranged in a time series from oldest (innermost) lamina to newest (outermost) lamina. The bars to the left represent the baseline nitrogen isotope values of the predominate primary producers in the Delaware bay area, Benthic Micro algae (BMA), Suspended Particulate Matter (SPM) *P. australis* (Pa), and *S. alterniflora* (Sa) taken from (Litvin & Weinstein, 2004). There is little overlap between the nitrogen values of the primary producers and the lamina samples.

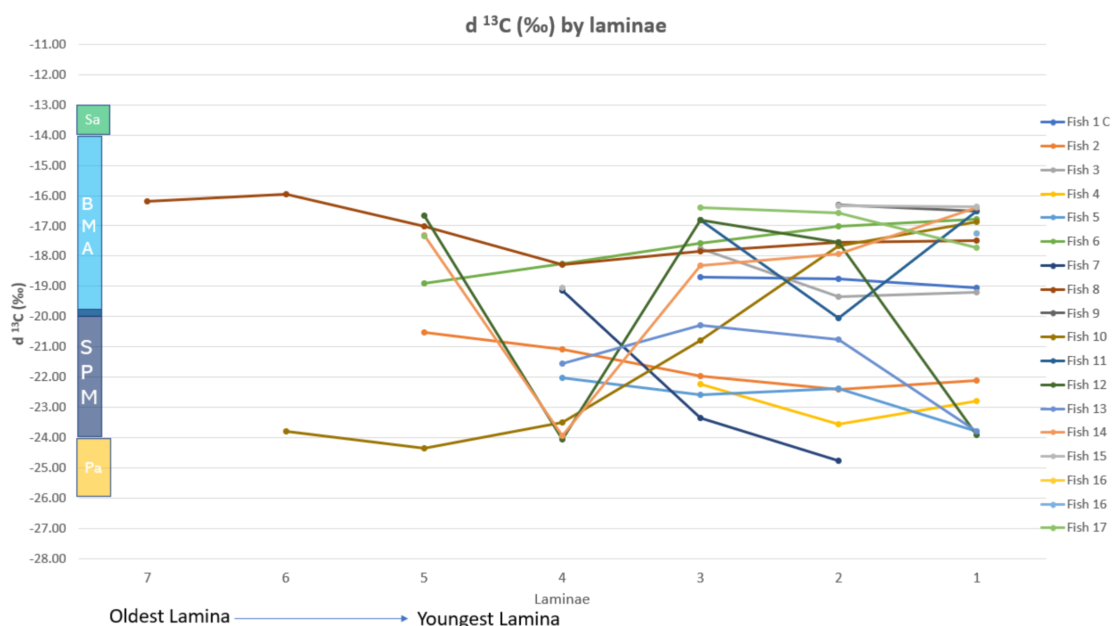

Figure 5. The $\delta^{13}C$ values of each lamina arranged in a time series from oldest (innermost) lamina to newest (outermost) lamina. The bars to the left represent the baseline carbon isotope values of the predominate primary producers in the Delaware bay area, Benthic Micro algae (BMA), Suspended Particulate Matter (SPM) *P. australis* (Pa), and *S. alterniflora* (Sa) taken from (Litvin & Weinstein, 2004). Carbon isotope values overlap with carbon isotope values from the SPM and BMA

The greatest variation within individuals was in the $\delta^{13}C$. Laminae carbon isotope values ranged between -25 ‰ and -16 ‰ while $\delta^{34}S$ for all fish (except number 13) ranged from 18‰ and 11‰. In all fish $\delta^{15}N$ ranged between 9.7‰ and 18.41‰. Fish 13 displayed unusual values only in $\delta^{34}S$, showing a large decease in sulfur ‰, dropping from 14.02‰ in its innermost lamina, and therefore oldest sample, to 5.04 ‰ in its outermost sample.

In comparing figure 3 and figure 4 you can see that the distribution of isotopic values for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in both the innermost and the outer most layers are in keeping with the range in isotopic values that were found in Litvin & Weinstein, 2004 research on juvenile weakfish muscle tissue that were captured in various regions of the Delaware Bay and connecting estuaries. It can be seen that the weakfish lens values have the widest ranges in the innermost layers especially in regard to sulfur and carbon, while the outermost layer had a shorter range in sulfur values and an equivalent range in nitrogen values.

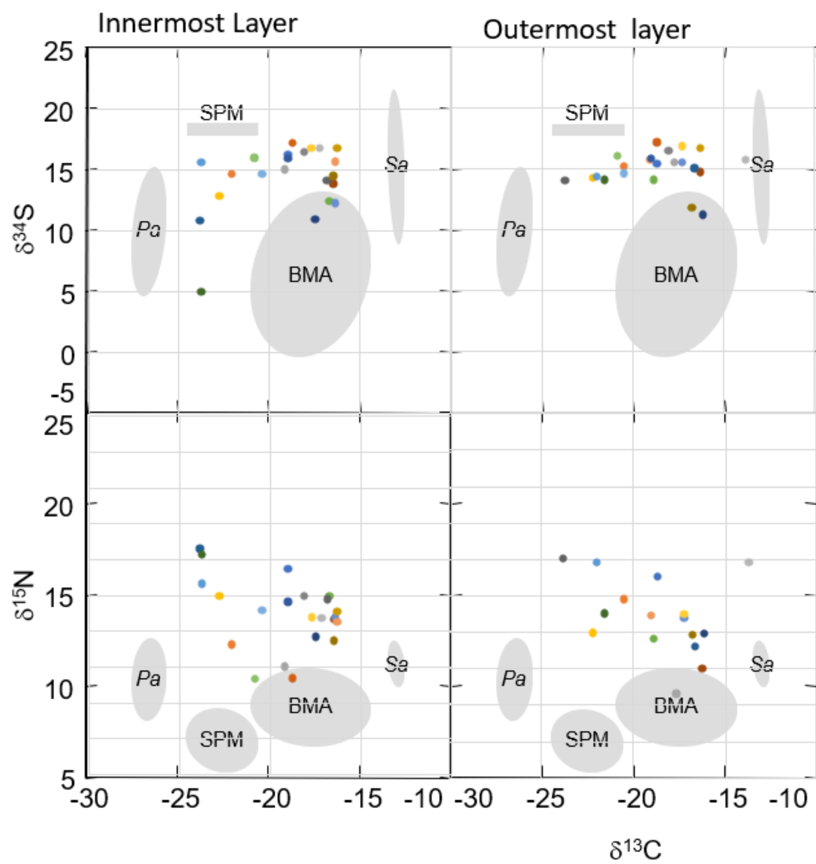

Figure 6. The δ^{13}C, δ^{15}N and δ^{34}S values for the innermost sampled layer for all fish and the outermost sampled layer for all fish on a biplot contrasted with the as δ^{13}C, δ^{15}N and δ^{34}S values for primary producers in the Delaware Bay area taken from (Litvin & Weinstein, 2004).

Fig. 2. Dual-isotope plots of $\delta^{15}N$ on $\delta^{13}C$ and $\delta^{34}S$ on $\delta^{13}C$ comparing weakfish, *Cynoscion regalis*, constituting the post-settlement baseline (<60 mm standard length (SL) except those in Alloway Creek, 60–100 mm SL; see the text) collected in lower Bay creeks (□) (*a*), lower Bay open waters (▷) (*b*), Mad Horse Creek (◇) (*b*), mid-Bay open waters (△) (*d*), Alloway Creek (○) (*e*), and upper Bay open waters (◁) (*f*), with those of primary producers (phytoplankton measured as suspended particulate matter (SPM), benthic microalgae (BMA), *Phragmites australis* (*Pa*), and *Spartina alterniflora* (*Sa*)). Ellipses represent mean values ±1 standard deviation.

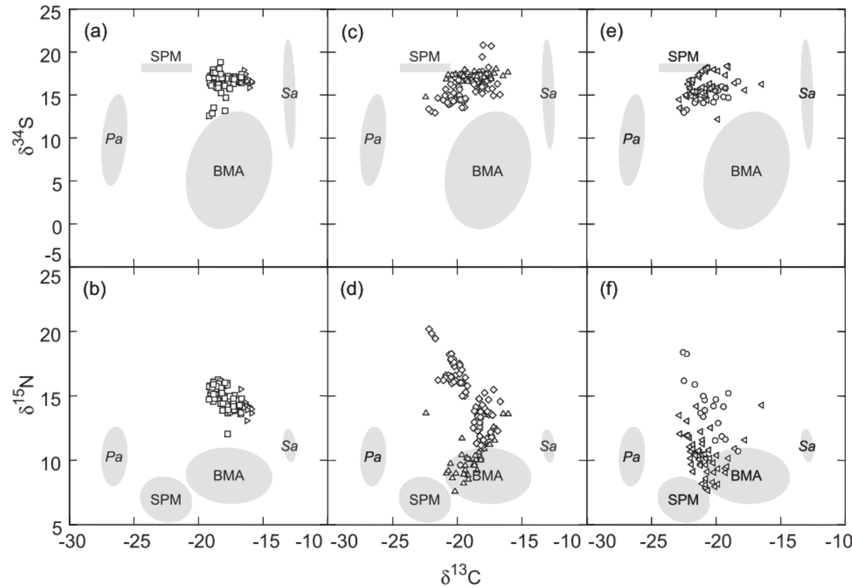

Figure 7. Bi-plot taken from (Litvin & Weinstein, 2004) for comparison. Graphs show the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values for muscle tissue sampled from juvenile weakfish at different locations within the Delaware Bay in addition to the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ for primary producers in the bay region. The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ found in the laminae are in keeping with the muscle tissue isotopic values.

The NDMS on the innermost layers of every fish resulted in grouping based on the values of the baseline muscle tissue isotopic ratios, which are located in areas in the Delaware Bay. The NMDS of the innermost layers resulted in a stress value of 0.0258, classed as stress type 1 indicating weak ties, two convergent solutions were found after 20 tries. The NMDS of the outermost layers gave a stress value of 0.0159, a stress type of 1 meaning weak ties in the data, and two convergent solutions found after 20 tries.

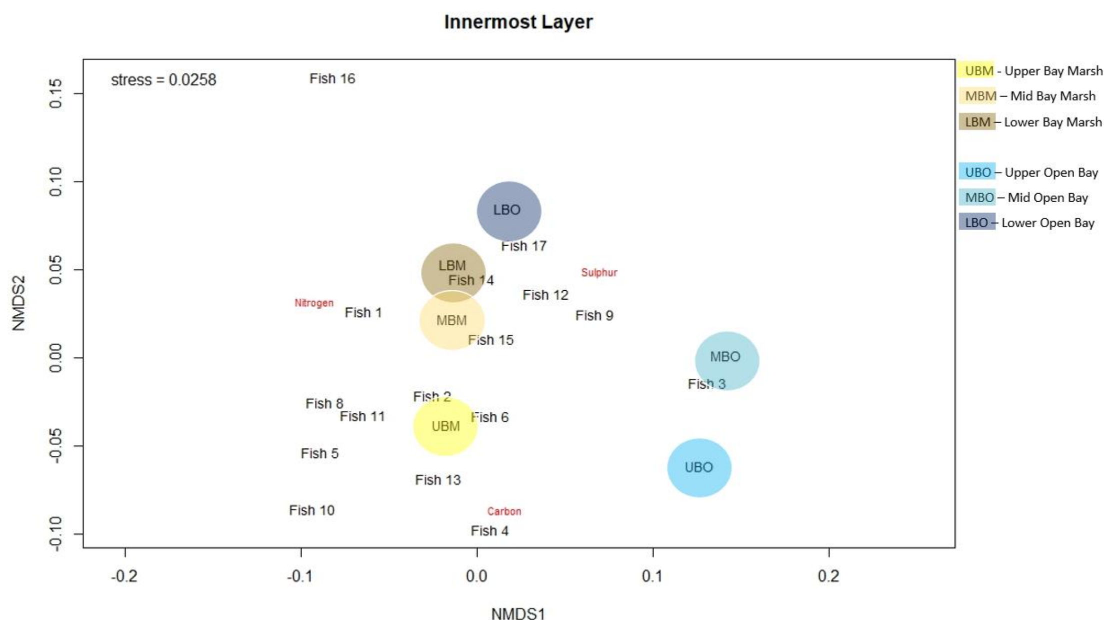

Figure 8. Results of non-metric multidimensional scaling of innermost lens laminae of each fish and the average isotopic values for muscle tissues of fish sampled from specific locations in the Delaware Bay (Upper Bay Marsh, Mid Bay Marsh, Lower Bay Marsh, Upper Outer Bay, Mid Outer Bay and Lower Out Bay). Samples are grouped based on similarity, where proximity indicates increased similarity in values. A majority of the fish are clustered around the fish caught in the marsh areas.

The NMDS grouped most of the laminae with fish caught in the marsh areas of the bay. Seven fish clustered together with the fish from the upper bay marsh. Six fish were grouped together with the fish from the mid bay marsh, lower bay marsh and the lower outer bay, which were themselves grouped together. Fish 3 was the only fish grouped in the outer bay area, being most similar to the mid outer bay fish. Fish 16 was a far outlier, and not grouped with any fish.

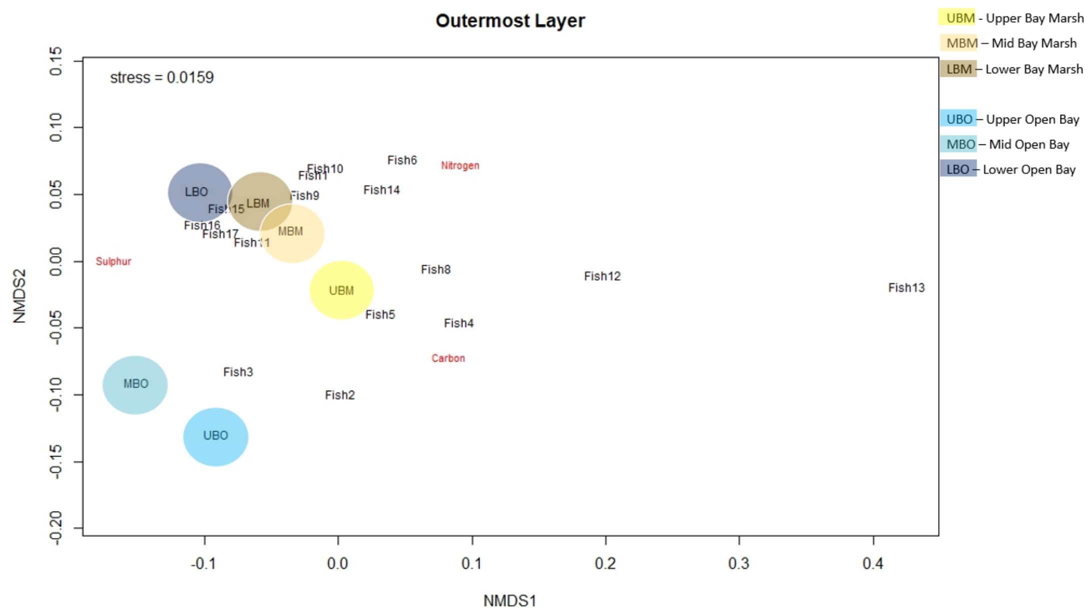

Figure 9. Results of the non-metric Multidimensional Scaling analysis of outermost lens laminae of each fish and the average isotopic values for muscle tissues of fish sampled from specific locations in the Delaware Bay (Upper Bay Marsh, Mid Bay Marsh, Lower Bay Marsh, Upper Outer Bay, Mid Outer Bay and Lower Out Bay). Samples are grouped based on similarity, where proximity indicates increased similarity in values. A majority of the fish are clustered around the fish caught in the marsh areas.

The NMDS on the outermost layer of the laminae grouped the majority of the fish laminae as similar in isotopic value to the fish caught in the marsh areas of the Bay. The lamina for fish 16 is no longer placed as an outlier, though the laminae for Fish 12 and Fish 13 are outliers. Fish 3 and Fish 2's outermost laminae are grouped closer to the outer bay areas than their innermost layers. Eleven of the fish are grouped with the lower outer bay, mid bay marsh and lower bay marsh, which are once again grouped with each other. Both NMDS analysis gave stress values below the threshold

of 0.05, which indicates that NMDS gives “an excellent representation with no prospect of misinterpretation.” (Dexter, Rollwagen-Bollens, & Bollens, 2018).

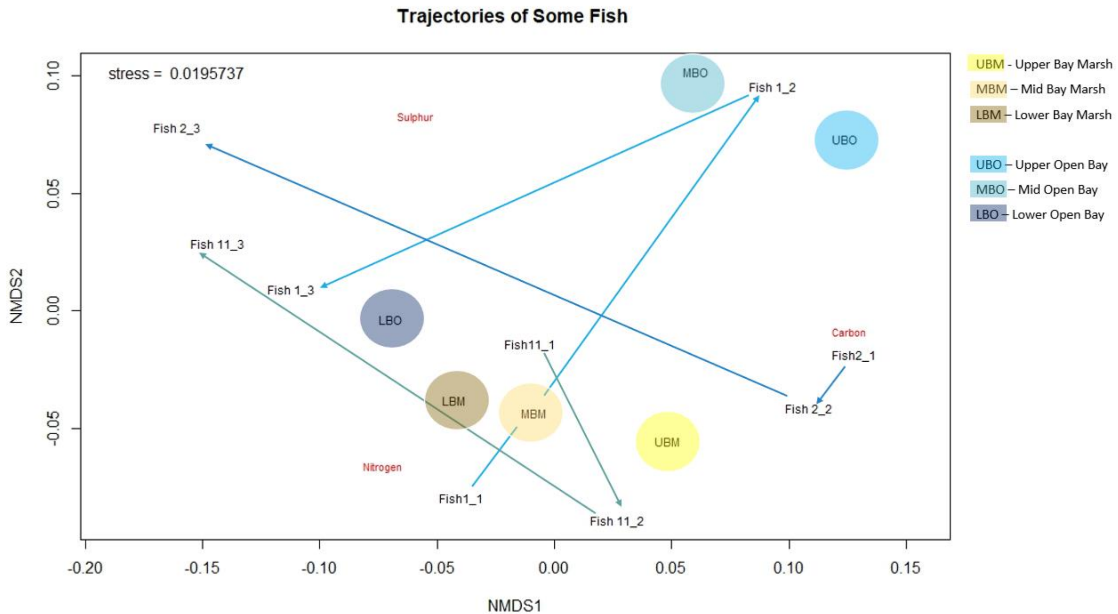

Figure 10. Proposed trajectory of selected fish between each lamina sample based on NMDS run on all laminae sampled for fish 1, 2, and 11 with muscle tissue isotopic values from fish caught in the (Upper Bay Marsh, Mid Bay Marsh, Lower Bay Marsh, Upper Outer Bay, Mid Outer Bay and Lower Out Bay of the Delaware Bay. Arrows indicate movement over time of individual fish based on grouping of laminae.

Looking at three select fish shows the potential for using NMDS grouping to infer movement throughout the Delaware Bay over time by specific fish. The three fish were selected based on the variation in grouping between lamina over time based on a NMDS performed on all fish and lamina layers. They show the more dramatic changes in lamina layer location. Fish 1 showed greatest change in laminae grouping.

The innermost layer was grouped with the fish from the lower outer bay, the second lamina sample grouped with the mid outer bay and the outermost sample was grouped with the mid bay marsh. Fish 2 was grouped as more dissimilar to most of the locator fish, its innermost layer appeared to be dissimilar to the other locator fish, however, it was grouped closest to the upper bay marsh sample for its second and outermost layer. Fish 11's innermost layer grouped closes to the lower outer bay sample. The second layer was closes to the upper bay marsh and the outermost layer was closest to the mid and lower bay marsh.

Chapter 5

Discussion and Conclusion

5.1 Discussion

From the time series of individual isotopes, we can see that there is clear variation in isotopic values between lens laminae in an individual and also across individuals. From the time series we can see some clustering, especially in the outermost (most recently formed) laminae for each isotope. These clusters in isotope ratios could be used to assume similar habitat utilization later in life history for those fish. The majority of the fish showed consistent isotopic values both with each other and with the isotope values found in muscle tissue from (Litvin & Weinstein, 2004, 2011). However, the differences in fractionation between muscle tissue and lens tissue are not known in this context and that will impact our understanding of the findings. While the timeseries do indicate that there is variation in the isotope values, a more holistic approach is needed to better understand the patterns and how they could be extrapolated for reconstructing life histories.

The biplots of the innermost and outermost laminae for each fish, when compared to the biplots in Figure 7 (Litvin & Weinstein 2011) further reaffirm a similar range in isotope values between the lens laminae and muscle tissue. The sulfur isotope values are bounded by the sulfur values of marsh macrophytes *Phragmites australis* and *Spartina alterniflora* which may indicate the influence of that primary producer on the isotopic values of the tissues. *P. australis* was more present in the marshes while *S. alterniflora* is more indicative of the lower bay areas. The nitrogen values of the tissues are more enriched than the primary producers of the bay, which is likely due to fractionation and the trophic level of the sample fish. Changes in nitrogen

values between lens lamina layer could indicate shifts in trophic level as the fish grow in size and shift their diet (Galvez, 2019). Based on Galvez's (2019) research on the diet of juvenile weakfish and the relationship to isotopic values in muscle and liver tissue, nitrogen values were best explained by season and location of the juvenile weakfish, he noted enriched nitrogen values in weakfish from the upper bay which he attributed to anthropogenic input from industry. The carbon values were consistent with some overlap to the macrophytes and suspended particulate matter and benthic micro algae (Litvin & Weinstein, 2004)

In order to fully take a holistic approach, the NMDS was run, which grouped the lens samples with muscle tissue samples that were geolocated. The majority of the fish were grouped with the marsh located samples for both their innermost layer and outermost layer which implies that the fish may have only very recently moved to the lower outer bay where they were captured. It indicates that they spent the majority of the juvenile life time in the marsh areas of the bay before moving towards the open ocean, which is in keeping with previous research on the species (Fry & Chumchal, 2011; Galvez, 2019; Litvin & Weinstein, 2004; Weinstein, Litvin, Bosley, Fuller, & Wainright, 2011) However it should be noted that the trophic level of the geolocated fish, which were juveniles, was possibly lower than the trophic level of the fish whose lenses were sampled, which could impact analysis of the nitrogen isotope values. The difference in fractionation between muscle and lens tissue may also have influenced the results.

The NMDS of all laminae of three individual fish compared to the baseline values of the muscle tissue showcases a very preliminary way to infer the trajectory of fish movement throughout the bay. However, there are still many unknowns that could

influence the data (such as differences in fractionation, trophic level). From the three fish selected I could see differences in habitat utilization. However, Fish 1 seemed to have started its earliest stages in the outer bay areas and then moved up into the marshes before it was captured out in the open bay, which is not in keeping with the expected behaviors of the weakfish (Fry & Chumchal, 2011; Galvez, 2019; Litvin & Weinstein, 2004; Weinstein, Litvin, Bosley, Fuller, & Wainright, 2011). So, this process should be performed on a greater sample size to better refine the method.

5.2 Conclusions and Future Work

It is apparent that utilization of stable isotope analysis on lens laminae is a viable option that needs to be further explored for use in life history reconstruction of estuarine species.

For future work, in order to better improve the effectiveness of this method a greater sample of baseline data for both the model species sample and the primary producers in the Delaware Bay should be collected. Further refinement is needed for the methods by which the laminae can be separated. A more delicate touch would lend itself to a greater number of laminae layers per lens. Generating a way of performing stable isotope analysis on tissue at smaller masses would result in finer analysis of the separate laminae and could result in a finer resolution in time scale. Additionally, there needs to be more exploration in the turnover rate of lens laminae, if the time that it takes for a new lens to form could be identified in weakfish species, which could be used to estimate the number of laminae present in a lens, and potentially the age of the fish. Conversely, knowing the age of the fish using the von Bertalanffy growth

function to estimate age would assist in predicting the number of laminae that should be present per eye lens (Lowerre, Chittenden, & Barbieri, 1995). A method of standardizing laminae thickness between layers and a way of understanding of the temporal scale that the laminae represent would also improve accuracy of interpretation. Furthermore, research should be done on the differences in fractionation between lens tissue and muscle tissue. If these aspects of the process are better understood it would greatly enhance the precision of this technique in recreating life histories of the weakfish, and from there, any estuarine species with a similar lens structure.

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