Size determination of trophic level in Atlantic Cod *(Gadus morhua)* from the Gulf of Maine and Georges Bank stocks using stable isotope analysis

Honors Thesis
Presented to the College of Agriculture and Life Sciences,
Cornell University
in Partial Fulfillment of the Requirements for the
Biological Sciences Honors Program

by

Serina Moheed
May 2020

William E. Bemis, Jed Sparks

Abstract

Atlantic Cod (*Gadus morhua*) has historically been an important commercial and recreational fish species in New England. There are two main stocks: the Gulf of Maine (GOM) and Georges Bank (GB) which are considered inshore and offshore, respectively. Stable isotope analysis of ^{15}N was conducted on tissues to gain insights into the trophic level and diet of the fish. This study was conducted to test the hypothesis that stable isotope analysis of different tissues would significantly differ by stock, and that the size of the fish would predict the trophic level the fish was feeding at. All fish examined were from the GOM or GB stocks; we used two location parameters (stock and statistical area) and tested their interaction with $\delta^{15}N$ separately. Three different tissues were used for isotope analysis: caudal fin clips, muscle, and otoliths. By analyzing three statistical models, we found that the more specific location parameter, statistical area, was part of a significant interaction with total length and tissue type that influences $\delta^{15}N$; stock did not have a significant influence on any relationship. Total length by itself was not a significant variable affecting $\delta^{15}N$; other studies have confirmed it is more indicative of food web interactions than species trophic level. Testing between tissue type showed that otolith $\delta^{15}N$ measurements were significantly different from fin clips and muscle tissue; this could have impacts on archaeological studies. The baseline of variation for the $\delta^{15}N$ of each tissue type was determined, but further validation with more samples is needed.

Introduction

The New England region of the United States has a rich and complicated history with fisheries spanning the last 400 years. Fisheries have provided a crucial food source for the earliest colonists since the 1600's; settlers of the New England region established Atlantic Cod (*Gadus morhua*) as the first fishery due to its large and easily fished populations, especially around the region of Georges Bank (Jensen, 1967; Serchuk and Wigley, 1992). As a productive ecosystem separating the Gulf of Maine from the Atlantic Ocean, Georges Bank represents a notable and prolific fishery resource. It was soon after that the fishery for Atlantic Cod gained momentum, marking the commercialization of arguably one of the most important fishery species of all time.

Historically, Atlantic Cod have been distinguished into stocks, which are populations isolated geographically and ecologically from other populations of the same species. Within the context of this paper, the two notable cod stocks of the Northeastern United States are the inshore Gulf of Maine stock (GOM) and offshore Georges Bank stock (GB; **Fig. 1**). As defined by NOAA, the GOM stock extends from Cape Cod east to the border between the United States and Canada, and to the coast of Maine in the north. The GB stock is a "transboundary stock" crossing the United States Exclusive Economic Zone and is fished by both the United States and Canada (NOAA, 2013). Beginning in the 1970s, Atlantic Cod was overfished over the next decade, leading to a collapse and moratorium on fishing in two large areas of Georges Bank. Overfishing resulted in sparse recruitment for cod, and despite intensive management for more than two decades, cod stocks have still not recovered to their historic levels of abundance (Serchuck et al., 1992; NOAA, 2020).

There are inherent differences in environmental factors associated with inshore and offshore cod stocks, driven by temperature, depth, and different rates of nutrient mixing in the ocean. Georges Bank is a shallower, more ecologically productive area than the deeper waters of the Gulf of Maine, and this can contribute to differences in growth and age at maturity between the two stocks (O'Brien, 1999). As a result, GB cod generally have higher growth rates than do GOM cod (ICES 2005). There are genetic differences between the two stocks (Ruzzante et al., 1998; Wirgin et al., 2007), along with differences in larval growth rates, which is a highly selected trait (Purchase and Brown, 2000). This suggests that there may be other important ecological differences between the GOM and GB stocks that could inform management practices.

When mature, large Atlantic Cod are among the top predators in benthic ecosystems. The diet of cod changes ontogenically. As larvae, phytoplankton dominate the diet; juveniles mostly eat invertebrates and crustaceans and adults are piscivores, consuming invertebrates and smaller fish (Klein-MacPhee, 2002; Smith and Link, 2010). When prey populations change, cod adapt their diet and are thought to be opportunistic feeders (Link and Garrison, 2002). The shift from pelagic to more benthic prey is attributed to the increase in mouth size of mature cod (Bergstad et al., 1987; Klein-MacPhee, 2002). Therefore because of their increasing size and the energetic advantage of larger prey, cod eat bigger organisms within the taxon and higher up the food chain as they grow older (Link et al., 2009).

Historically, gut content analyses were the only methods to examine the diet and construct food webs of organisms (Perkins et al., 2014). It is known that consuming prey will enrich the tissues of the consumer with certain elements that are then used for metabolism and physiological functions, such as growth. Enrichment in this case refers to the ratio between the

heavier isotope to the lighter, more common one; an example of this is $^{15}N/^{14}N$ ($\delta^{15}N$) (Madigan et al., 2012). Measuring this enrichment with stable isotope analysis has been accepted as a simple and informative method for analyzing the diet and trophic interactions of animals. Examining $\delta\ ^{15}N$ in particular is widely known to be related to diet, with predators accumulating the ^{15}N of their prey as they move up in trophic level and then distributing that within their own tissues (Deniro and Epstein, 1981; Cabana et al., 1994; Perkins et al., 2014). Given that there are differences in metabolic turnover rates, there is intrinsic variation in the ^{15}N isotope value of an individual depending on the tissue being examined (Ankjærø et al., 2012). Establishing the range of variation that can occur in one fish can help future studies account for this intrinsic variation among tissues.

The three goals of this project are to: 1) elucidate whether there are significant differences in the trophic dynamics between the GOM and GB stocks of Atlantic Cod through $\delta^{15}N$ analysis ; 2) confirm whether $\delta^{15}N$ stable isotope analysis can be used to relate fish size to the trophic level of the organism; and 3) make intraspecies comparisons to understand the range of $\delta^{15}N$ variation between different tissues of the same individuals.

Hypotheses:

I predict that due to the different locations paired with unique environmental characteristics, the GOM and GB stocks of Atlantic Cod will show significant differences in $\delta^{15}N$ values. I also expect that total length will be a significant and reliable indicator of trophic level of the fish, because size is so closely linked to the physical needs of feeding.

Methods:

Collection

Fifty-six whole specimens of Atlantic Cod were retrieved during NOAA cruises during Spring and Fall 2019. All fish samples were caught by NEFSC using either random stratified bottom trawl surveys and a 4-seam bottom trawl with rockhopper gear (Politis et al., 2014) or bottom longline surveys within the Gulf of Maine (McElroy et al., 2019). Except for one fish (SM-2019-44) which was not included in any analyses, all were from either the Gulf of Maine (GOM) or the Georges Bank (GB) stocks.

Dissection

Specimens were frozen until dissection. During dissection, length (standard and total) and weight measurements were recorded, and each fish was photographed. Eight samples were taken from each specimen: 1) a fin clip from the caudal fin; 2) a muscle plug near the tail; 3) skin patch; 4) otolith (right side); 5) eye lens (right side); 6) a piece of the cleithrum; 7) liver; 8) stomach tissue and its contents (placed in individual vials if identifiable). The locations from which each tissue was taken was standardized as much as possible.

Stable Isotope Analysis

All tissue samples were placed in labeled vials and frozen at -20 degrees Celsius. Samples were then freeze-dried at -45 degrees Celsius for one week. Each tissue sample was ground into a homogenous mixture using liquid nitrogen, and a weighed subsample of 1 mg ± 0.1 mg was packaged into 4x6 tin capsules (EA Consumables). Samples of otoliths were weighed to 10 mg ± 0.1 mg and packaged into 5x8 tin capsules (EA Consumables). The samples were combusted using a Carlo Erba Elemental Analyzer (Italy) outfitted with a PN150

autosampler (Costech, California, USA). The elemental analyzer was coupled to a Thermo Scientific Isotope Ratio Mass Spectrometer (Bremen, Germany) via a ConfLo IV (Thermo Scientific, Bremen, Germany). Both ^{13}C and ^{15}N fractionations were measured for each tissue type, however only the $\delta^{15}N$ data was used for analysis.

Statistical Analysis

One objective was to evaluate whether the location at which a specimen was collected influences the $\delta^{15}N$ values of the tissues. Within the dataset, there were two location parameters for each fish: a category for stocks (GOM or GB), which were separated based on oceanographic characteristics, and statistical area groupings that could be further divided within stocks. The classification of statistical areas, as seen in **Fig. 2**, follows the NOAA Northeast Fisheries Science Center guidelines (Palmer and Wigley, 2007). There was no overlap between statistical area and stock (**Table 1**).

Data from the fin clip, muscle, and otolith samples were analyzed (otolith data was not recovered for three individuals). Three models were created to explore the influence of size (total length), location (stock and statistical area), and tissue type (fin clip, muscle, and otolith) on the $\delta^{15}N$ isotope data. Models started with all possible interactions between terms, and then non-significant terms were taken out unless they were a part of a significant, higher order interaction. Linear regressions were run on the $\delta^{15}N$ data vs. total length to characterize slope of the data points as related to statistical area and stock (**Fig. 3a and 3b**). A Bonferroni Correction Factor was utilized to show significance despite the sheer number of comparisons made between tissues. A Least Squares Mean Student's t-test was run with the terms of the third model. Assumptions were met for all tests. Data were organized in Microsoft Excel, and all statistical

analyses and graphs were run using JMP Pro 14. The significance level used for all statistical tests was $\alpha = 0.05$.

Results:

To understand the influence of size of the fish and location on the value of δ15N in different tissues, I created three statistical models.

Model 1

The first model included tissue type, total length, and statistical area in response to the $\delta^{15}N$ data (**Table 2a**). I found that the triple interaction term between the three factors was significant ($p = 0.024$). This means that the effect of total length on $\delta^{15}N$ depends on the statistical area from which the fish came, and the tissue type examined. This can be seen clearly in **Fig. 3a** which shows linear regressions fitted to the data points and divided by statistical area and tissue type. The slopes are positive and negative depending on the statistical area; the trend for otoliths is notably different than for muscle and fin clips, and has a lower isotopic range.

Model 2

The second model examined whether stock, a broader and more ecologically important measure of location, influenced the stable nitrogen isotopic data. I explored a model with the triple interaction of tissue type, stock, and total length in response to $\delta^{15}N$; however, this interaction was not significant and was removed from subsequent models. As seen in **Table 2b**, the second model examined the influence of tissue type, stock, and total length on the isotopic data. Among other terms, the interaction between stock and tissue type was most notable. **Fig. 4** shows the corresponding Least Square Means Plot for this interaction; there is a difference

between GOM and GB stocks for each tissue, however further analysis revealed this difference to not be significant.

Model 3

Based on the visual difference between otolith isotopic data compared to the muscle and fin clip data, I examined a third model including tissue type, statistical area, and total length in response to the $\delta^{15}N$ data. This model was similar to Model 1 but contained fewer interactions (**Table 2c**). I found that the interaction term between statistical area and tissue type was significant. To test between tissues, a Least Square Means Difference Student's t-test was run; the resulting matrix produced every possible combination of statistical area and tissue type compared pairwise. From the ordered difference report, it was shown that there was overlap in similarity between fin clips and muscle pairings, however there was no overlap with either to the otolith groups; all of these pairwise comparisons with the otolith measurements were statistically significant ($p < 0.0001$). Keeping in mind the number of comparisons run, a Bonferroni Correction Factor ($\alpha = 0.05$) was calculated using the formula:

Alpha level / Number of comparisons made = new alpha level needed for significance

(0.05) / (59 otolith comparisons) = 0.0008

The *p*-value of 0.0001 for the otolith comparisons is still less than the new significance level of 0.0008; therefore, even with the Bonferroni Correction, the differences between the otolith data compared to the other two tissues is still significant.

Range of Variation

The ranges of variation of $\delta^{15}N$ are: fin clips (12.06-16.37), muscle (11.81-14.12), and otoliths (4.40-11.50). **Fig. 5** shows a boxplot visualizing the variation in $\delta^{15}N$ for the three tissue types; it is notable that the otolith range is significantly lower than the range for fin clip or muscle tissue.

Discussion

The location of capture, in conjunction with total length and tissue examined, is a significant factor influencing the stable nitrogen isotopic value. As seen in **Fig. 3a** the size distribution of sampled fish per statistical area was not equal. Notably, sampled fish from statistical area 522 were relatively few (n=4) and of larger size compared to statistical area 551 (n=17); this discrepancy in sample sizes and size of fish should be considered when comparing statistical areas to each other. As noted earlier, stable isotope values will vary by location naturally based on the intrinsic environmental differences and trophic dynamics in the area. In fact, some other studies have used the difference in isotope values to track migratory animals or fish crossing over into different regions (Hansson et al., 1997; Bergstad et al., 2008), or even to distinguish the difference between wild and farmed fish (Dempson and Power, 2004).

While I saw no significant influence of stock on the isotope data, the *p*-values for interactions between stock and tissue type (Model 2) were borderline significant. This indicates that potentially with more data and more equal size distribution, the stocks would significantly differ by tissue type. The similarities between the $\delta^{15}N$ values of the two stocks divided by tissue type can be seen in **Fig. 3b**; the linear regressions visually and statistically are not significantly different. It was worth noting that the broader measure of location in our study (stock) did not

yield a significant relationship with the $\delta^{15}N$ data while the more specific parameter (statistical area) did. Area 551, the most offshore area, was significantly different from the other statistical areas. It would be interesting to compare environmental conditions of the statistical areas to explore why this would be the case.

Although total length by itself did not significantly influence the $\delta^{15}N$ value, and in turn the trophic level of the organism, it was still a notable factor to consider within interactions. Other studies have shown that total length was not the significant indicator of trophic dynamics as expected (Jennings et al., 2002; Ramsvatn and Pedersen, 2012; Perkins et al., 2014). In fact, one study uses trout to show that variation in $\delta^{15}N$ in different lakes accounts for food web length and interactions more than the species trophic level (Cabana and Rasmussen, 1994). Although I expected a direct relationship with total length and enriched $\delta^{15}N$ levels indicative of higher trophic levels, this is a much more complicated relationship that still needs further study.

One of the most interesting results was testing between tissues to determine if $\delta^{15}N$ values of the fin clips, muscle tissue, and otoliths were significantly different from each other. The third statistical model showed that the otolith stable isotope data *was* significantly different from both muscle and fin clip data, even those from the same statistical area. This could be explained by how $\delta^{15}N$ is incorporated at varying rates by different tissues, often measured using turnover rates. Other studies have examined the turnover rates and have concluded that the variation could be due to differences in metabolic activity between tissues (Ankjærø, 2012; Madigan et al., 2012). Muscle and caudal fin tissues of a fish grow rapidly, and most likely reflect the present diet of the fish. In contrast, otoliths grow incrementally over the fish's lifetime; therefore, it makes sense that the $\delta^{15}N$ data collected from it would reflect a different point of the fish's life. Noting this difference for otoliths is important because in archaeological

studies, otoliths and bones are typically the only parts of the fish left after the tissues degrades. Being aware that these body parts could have different stable isotope measurements offers researchers another variable to consider when using them in long-term studies.

Stable isotope analysis and food web interactions of Atlantic Cod should be examined in future studies with a broader sample set. Taking into account the low numbers of cod available for research, making interspecies comparisons with ecologically similar fish such as Haddock might also illuminate the complexities associated with stable isotope studies and food web interactions. By comparing trophic dynamics of two species of fish , we could get insights into feeding ecology and differing environmental variables that impact their life histories. With global warming and climate change drastically altering the oceans, understanding the mechanisms and factors behind our food sources is vital to ensure that we can continue to rely on them in the future.

Conclusion:

In terms of location, statistical area influences the $\delta^{15}N$ data of Atlantic cod more than the stock the fish came from. Additional samples and a more uniform size distribution of individuals, as well as repetition of this study using ecologically similar fish like Haddock, might shed light on the difference between the two location parameters. Total length is not a significant factor influencing $\delta^{15}N$ *by itself*, however in conjunction with other variables such as statistical area and tissue type, size can predict the trophic level of a fish. Tissue to tissue comparisons of $\delta^{15}N$ data showed that otoliths are significantly different from both fin clips and muscle tissue; this has implications for long term archaeological studies and future experiments using otoliths as a means of comparison.

Acknowledgements:

I would like to thank my advisors, William E. Bemis and Jed Sparks for being willing to take on this project and for guiding me every step of the way. Special thanks to Kim Sparks for her ever-present help in preparing and running the many, many isotope samples. I would also like to thank our collaborators at NOAA Fisheries, Mark Wuenschel and John Galbraith who helped procure the fish that we used in this project. Finally, I would like to thank the Jane E. Brody Undergraduate Research Award and the Dextra Undergraduate Research Endowment Fund for partially funding this project.

References

Ankjærø, T., Christensen, J., & Grønkjær, P. 2012. Tissue-specific turnover rates and trophic enrichment of stable N and C isotopes in juvenile Atlantic Cod Gadus morhua fed three different diets. Marine Ecology Progress Series, 461, 197–209.

Bergstad, O. A., Jørgensen, T., Knutsen, J. A., & Berge, J. A. 2008. Site fidelity of Atlantic Cod Gadus morhua L. as deduced from telemetry and stable isotope studies. Journal of Fish Biology, 72(1), 131–142.

Bergstad, O., Jørgensen, T., & Dragesund, O. 1987. Life history and ecology of the gadoid resources of the Barents Sea. Fisheries Research, 5(2-3), 119–161.

Cabana, G., & Rasmussen, J. B. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature, 372(6503), 255–257.

Dempson, J. B., & Power, M. 2004. Use of stable isotopes to distinguish farmed from wild Atlantic salmon, Salmo salar. Ecology of Freshwater Fish, 13(3), 176–184.

Deniro, M. J., & Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica Et Cosmochimica Acta, 45(3), 341–351.

Hansson, S., Hobbie, J. E., Elmgren, R., Larsson, U., Fry, B., & Johansson, S. 1997. The Stable Nitrogen Isotope Ratio As A Marker Of Food-Web Interactions And Fish Migration. Ecology, 78(7), 2249–2257.

ICES. 2005. Spawning and life history information for North Atlantic Cod stocks. ICES Cooperative Research Report, No. 274. 152 pp.

Jennings, S., Pinnegar, J. K., Polunin, N. V. C., & Boon, T. W. 2002. Weak cross-species relationships between body size and trophic level belie powerful size-based trophic structuring in fish communities. Journal of Animal Ecology, 70(6), 934–944.

Jensen, A. C. 1967. A Brief History of the New England Offshore Fisheries. A Brief History of the New England Offshore Fisheries. Washington D.C.

Klein-MacPhee, G. 2002. Cods. Family Gadidae. Pages 223-261 IN Bigelow and Schroeder's Fishes of the Gulf of Maine, 3rd ed. Edited by B. B. Collette and G. Klein-MacPhee. Smithsonian Institution Press, Washington.

Link, J. S., Bogstad, B., Sparholt, H., & Lilly, G. R. 2009. Trophic role of Atlantic Cod in the ecosystem. Fish and Fisheries, 10(1), 58–87.

Link, J. S., & Garrison, L. P. 2002. Trophic ecology of Atlantic Cod Gadus morhua on the northeast US continental shelf. Marine Ecology Progress Series, 227, 109–123.

Madigan, D. J., Litvin, S. Y., Popp, B. N., Carlisle, A. B., Farwell, C. J., & Block, B. A. 2012. Tissue Turnover Rates and Isotopic Trophic Discrimination Factors in the Endothermic Teleost, Pacific Bluefin Tuna (Thunnus orientalis). PLoS ONE, 7(11).

McElroy, D., O'Brien, L., Blaylock, J., Martin, M. H., Rago, P. J., Hoey, J. H., & Sheremet, V. A. 2019. Design, Implementation, and Results of a Cooperative Research Gulf of Maine Longline Survey, 2014-2017. Woods Hole, MA: US Department of Commerce.

NOAA. 2013. 55Th Northeast Regional Stock Assessment Workshop (55th Saw) Assessment Summary Report. Woods Hole, MA.

303 **NOAA.** 2020. Atlantic Cod. https://www.fisheries.noaa.gov/species/atlantic-cod (accessed 11
304 March 2020)

305 **O'Brien, L.** 1999. Factors Influencing Rate of Sexual Maturity and the Effect on Spawning
306 Stock for Georges Bank and Gulf of Maine Atlantic Cod Gadus morhua Stocks. Journal
307 of Northwest Atlantic Fishery Science, 25, 179–203.

308 **Palmer, M. C., & Wigley, S. E.** 2007. Northeast Fisheries Science Center Reference Document
309 07-22Validating the Stock Apportionment of Commercial Fisheries Landings Using
310 Positional Data from Vessel Monitoring Systems (Vms). Woods Hole, MA: NOAA
311 Fisheries Science Center.

312 **Perkins, M. J., Mcdonald, R. A., Veen, F. J. V., Kelly, S. D., Rees, G., & Bearhop, S.** 2014.
313 Application of Nitrogen and Carbon Stable Isotopes (δ15N and δ13C) to Quantify Food
314 Chain Length and Trophic Structure. PLoS ONE, 9(3).

315 **Politis, P. J., Galbraith, J. K., Kostovick, P., & Brown, R. W.** 2014. Northeast Fisheries
316 Science Center. Bottom trawl survey protocols for the NOAA Ship Henry B. Bigelow,
317 Northeast Fisheries Science Center bottom trawl survey protocols for the NOAA Ship
318 Henry B. Bigelow. Woods Hole, MA: US Department of Commerce.

319 **Purchase, C. F., & Brown, J. A.** 2000. Interpopulation differences in growth rates and food
320 conversion efficiencies of young Grand Banks and Gulf of Maine Atlantic Cod (Gadus
321 morhua). Canadian Journal of Fisheries and Aquatic Sciences, 57(11), 2223–2229.

322 **Ramsvatn, S., & Pedersen, T.** 2012. Ontogenetic niche changes in haddock Melanogrammus
323 aeglefinus reflected by stable isotope signatures, δ13C and δ15N. Marine Ecology
324 Progress Series, 451, 175–185.

Ruzzante, D. E., Taggart, C. T., & Cook, D. 1998. A nuclear DNA basis for shelf- and bank-scale population structure in northwest Atlantic Cod (Gadus morhua): Labrador to Georges Bank. Molecular Ecology, 7(12), 1663–1680.

Serchuk, F. M., & Wigley, S. E. 1992. Assessment and Management of the Georges Bank Cod Fishery: An Historical Review and Evaluation. Journal of Northwest Atlantic Fishery Science, 13, 25–52.

Smith, B. E., & Link, J. S. 2010. The Trophic Dynamics of 50 Finfish and 2 Squid Species on the Northeast Us Continental Shelf. Woods Hole, MA: NOAA.

Wirgin, I., Kovach, A. I., Maceda, L., Roy, N. K., Waldman, J., & Berlinsky, D. L. 2007. Stock Identification of Atlantic Cod in U.S. Waters Using Microsatellite and Single Nucleotide Polymorphism DNA Analyses. Transactions of the American Fisheries Society, 136(2), 375–391.

Table 1. Division of stocks and statistical areas within the dataset, referencing with Figure 2.

Stock	Gulf of Maine	Georges Bank
Statistical Areas	513, 514, 515	522, 551

Table 2a, 2b, and 2c. The three models and the factors that were included in each, along with their significance level. The Source box is an output from JMP.

a) Model 1

Source	LogWorth	PValue
Tissue 2	8.247	0.00000
Stat Area*Tissue 2	5.640	0.00000
Stat Area	2.600	0.00251
Total Length (cm)*Stat Area*Tissue 2	1.622	0.02386
Total Length (cm)	1.183	0.06555
Total Length (cm)*Stat Area	0.445	0.35887
Tissue 2*Total Length (cm)	0.001	0.99657

b) Model 2

Source	LogWorth	PValue
Tissue 2	62.828	0.00000
STOCK*Tissue 2	5.532	0.00000
Total Length (cm)	1.803	0.01575
STOCK	0.399	0.39885

c) Model 3

Source	LogWorth	PValue
Tissue 2	59.936	0.00000
Stat Area*Tissue 2	5.481	0.00000
Stat Area	2.277	0.00529
Total Length (cm)	1.343	0.04543

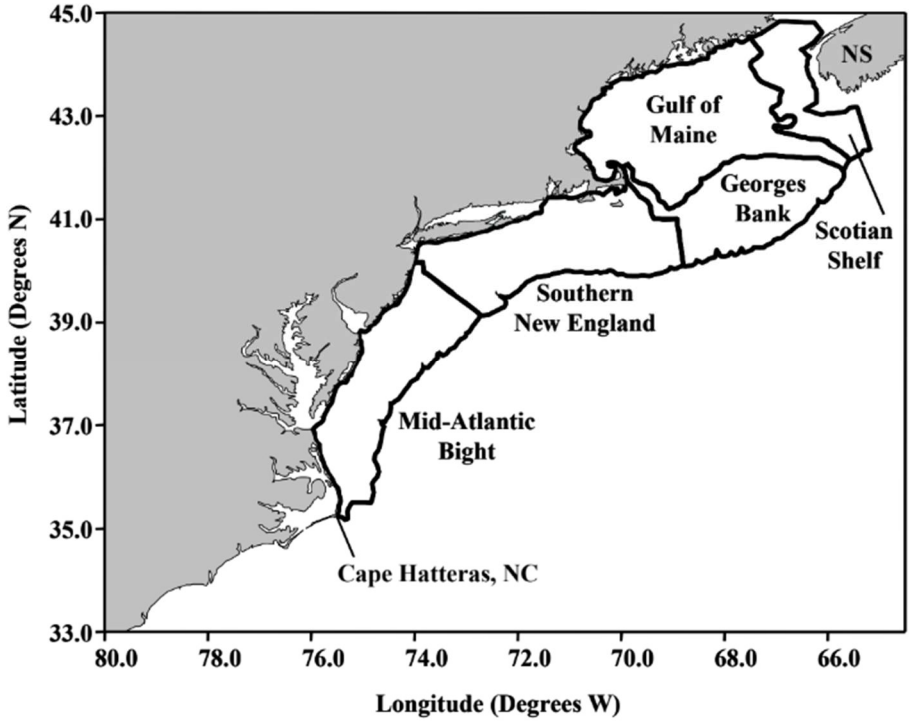
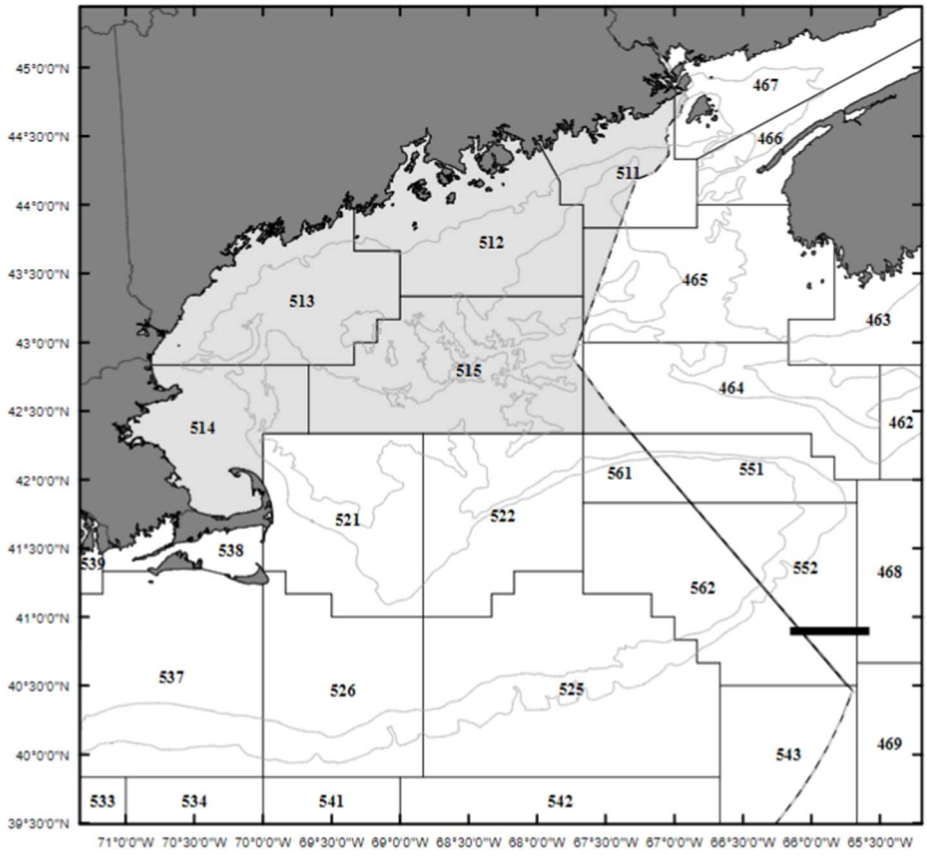


Figure 1. Map showing the boundaries of the Gulf of Maine and Georges Banks in relation to each other; GOM is inshore whereas GB is offshore. Reprinted from *The Trophic Dynamics of 50 Finfish and 2 Squid Species on the Northeast US Continental Shelf* (pg. 44), by B. E. Smith and J. S. Link, 2010, Woods Hole, MA: NOAA. Copyright by NOAA.



372

373 **Figure 2.** The statistical areas used for commercial fishing; the shaded areas represent those

374 designated to the GOM stock. The fish studied here were from areas 513, 514, 515, 522, and

375 551. Reprinted from the *55th Northeast Regional Stock Assessment Workshop (55th Saw)*

376 *Assessment Summary Report* (pg. 20), by NOAA, 2013, Woods Hole, MA: NOAA. Copyright by

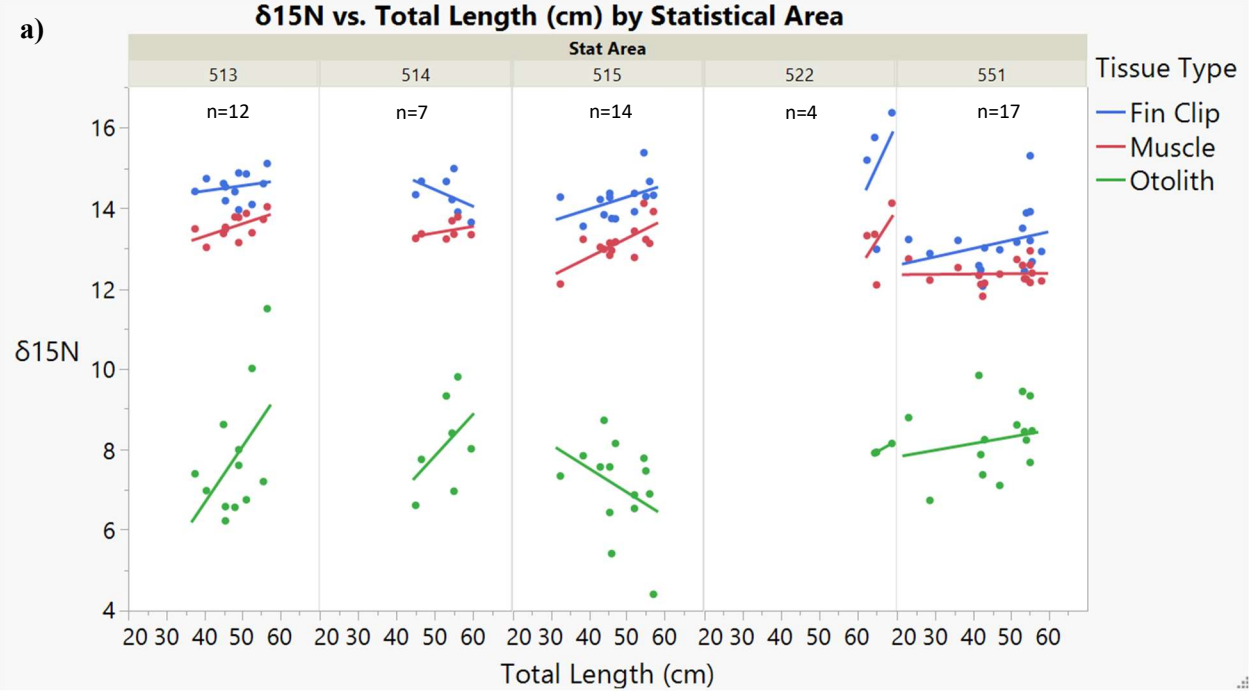
377 NOAA.

378

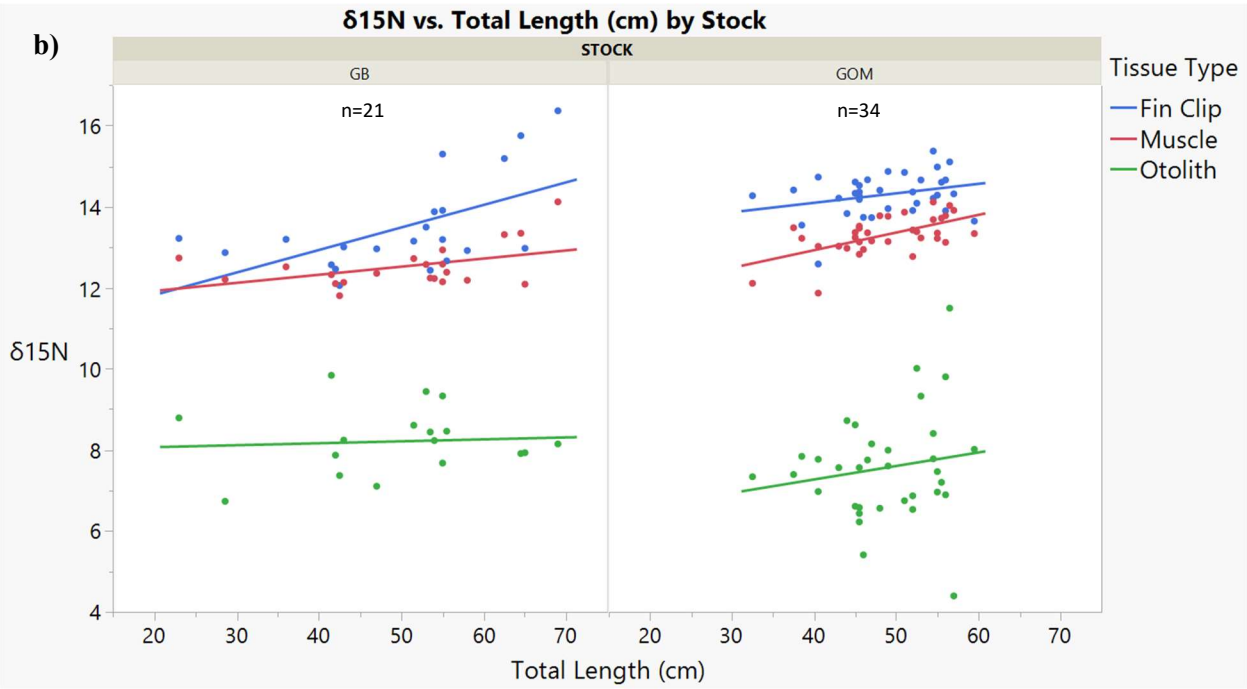
379

380

381



382



383

384 **Figure 3a and b.** Graphs showing total length vs. $\delta^{15}N$ for each tissue type by (5a) statistical
385 area and (5b) stock. The colored lines are linear regressions for each tissue type that best fit the
386 data. Sample sizes for each statistical area and stock are noted (one fish sample, SM-2019-39,
387 had an associated stock, but not a listed statistical area).

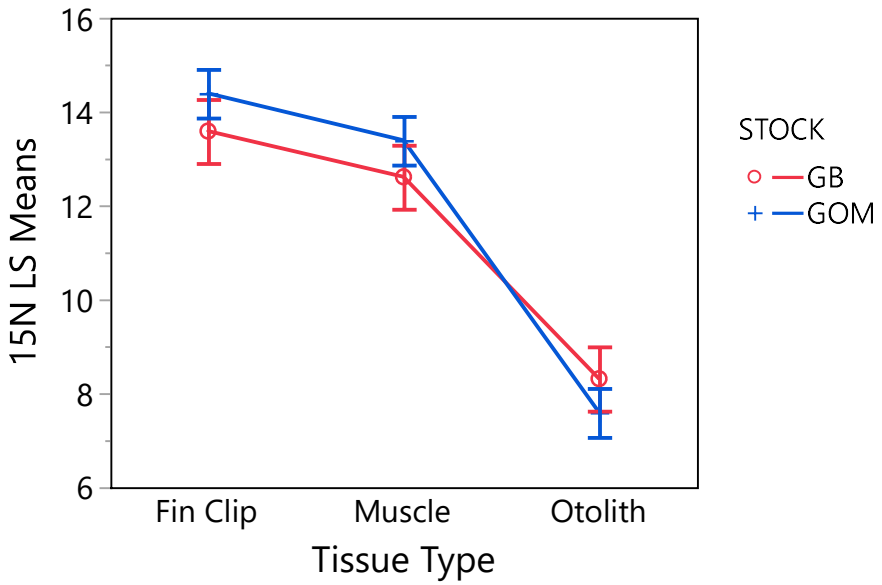
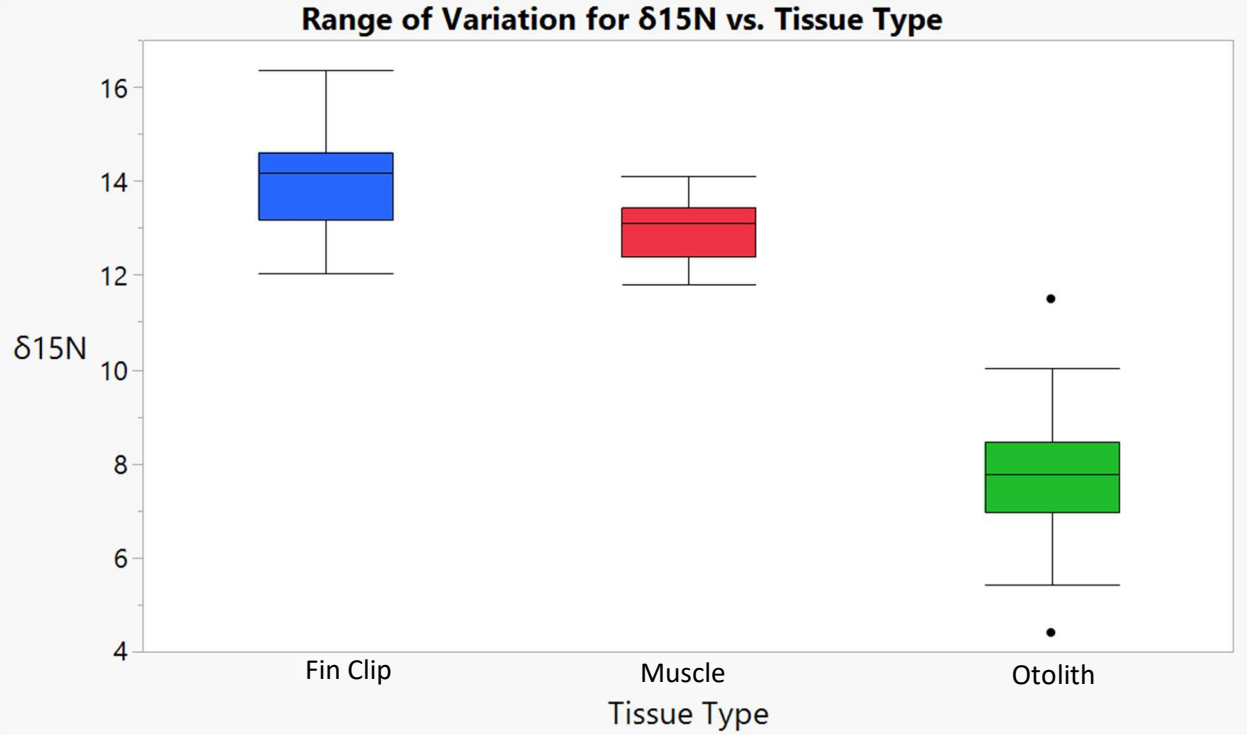


Figure 4. The Least Square Means Plot from the output of Model 2, showing how the stocks differ between stable isotope value of each tissue type. This difference was shown to be statistically nonsignificant.



410

411 **Figure 5.** These boxplots show the range of variation of $\delta^{15}N$ for each tissue type. The

412 disconnected dots on the otolith boxplot indicate outlier points. The difference between the

413 otolith range of variation compared to the fin clip and muscle groups is significant.