APPENDIX A.

1 Methodological summary

1.1 Methodological Summary

2 Archaeological excavation and dating techniques for historical otoliths

- 2.1 Excavation of Smuttynose Island middens
- 2.2 Controlling for fish origin and harvest techniques of commercially-harvested cod
- 2.3 Low sample size considerations
- 2.4 References

3 Supplementary table and figures

Table A1: Average δ^{15} N and N content for five species Fig. A1: Schematic of protocol for δ^{15} N_{oto} Fig. A2. Visual comparison of uncleaned and cleaned fossil otoliths Fig. A3: Prince William Sound statistical areas (wild pink salmon locations) Fig. A4: L vs. R for wild pink salmon only Fig. A5: Otolith mass vs. δ^{15} N_{oto} for brown trout and rainbow trout Fig. A6: N content, otolith mass, and δ^{15} N for individual fish used in Fig. 9 Fig. A7: Otolith mass vs. fish length to reconstruct fish size Fig. A8. Example of a pitted fossil otolith.

Appendix 1: Methodological summary

We developed a novel method to analyze the δ^{15} N of otoliths by 1) cleaning and dissolution of otolith aragonite, 2) conversion of otolith organic matter to nitrate by persulfate oxidation, 3) conversion of nitrate to nitrous oxide, and 4) measurement of the isotopic composition of the nitrous oxide by GC-IRMS. Notes on the final protocol are included below (Section 1.1). The archaeological excavation and dating of historical cod otoliths and low sample size considerations are also discussed below (Sections 2.1-2.3). Our final protocol, as relates to the each of the analytical tests described in the main text, is included below:

<u>Cleaning reagent</u>: Sodium hypochlorite (NaOCl, 13% available chlorine)

Exposure time to NaOCI: 12 h, room temperature, on a continuous shaker

<u>Grain size</u>: crushing of modern otoliths is not necessary and they may be dissolved, oxidized, and analyzed intact. However, if crushing is required for experimental purposes, grain size does not affect $\delta^{15}N_{oto}$ measurements (other than that required for obtaining a representative sample of the otolith itself, i.e. goal of > 100 grains within a 4-5 mg subsample).

<u>Left versus right sagittal otolith</u>: either L or R otolith can be used for bilaterally symmetrical fishes (not yet tested for flatfishes).

Appendix 2: Archaeological excavation and dating techniques for fossil otoliths

2.1 Excavation of Smuttynose Island middens

Smuttynose Island (SI) is one of nine Isle of Shoals located seven miles off the coast of New Hampshire and Maine in the northeast U.S. From 1620-90, SI was the site of an English commercial enterprise in which fish were split, salted and dried on the shores of the island then shipped to European markets. The samples used in the present study were from Area A Block at a site located at 42.9823 °N x -70.6075 °W down to 90 cm in stratified terrestrial sediments. An upper horizon, dated to the late 17th century using redware smoking pipes and Lewis Binford regression analysis (Binford, 1962), was dated to 1640-1690. The redware method is based on rapidly changing pipe technology during this time. Pipe stem "bore" width indicates year of production with a precision of 10 years. The excavation was conducted by Nate Hamilton, an archaeologist and professor at the University of Southern Maine, Gorham, Maine and Robin Hadlock Seeley, Assistant Director of the Shoals Marine Laboratory, Appledore Island, Maine, from 2007 to 2013.

A thorough historical account of early 17th century fishing technology from nearby Richmond Island, Maine, suggests that fish were caught by handline fishing in doubleended shallops (Churchill, 1984). Shallops were small boats, ~ 20 ft long, generally with a fireplace, two masts, high walls. The boats were small enough to row yet large enough to hold a small crew (Churchill 1984).

2.2 Controlling for fish origin and harvest techniques of commercially-harvested cod

Because otoliths were excavated from middens of a former commercial fishing operation on Smuttynose Island (Isle of Shoals, York, Maine) during the 17th century, the precise location of each fish's capture cannot be known with certainty. Similarly, as large modern cod used in this study were obtained from a local fish market (Nassau Seafood, Princeton, NJ), only the broad general region of origin is documented, and the precise location of modern Gulf of Maine cod can also not be known with complete certainty. However, for reasons described below, the comparison of the two groups is a reasonable one.

It is thought that 17th century European fishers on Smuttynose Island were fishing the productive region known as "Whaleback", located 10 miles south of the Isle of Shoals (Nate Hamilton, University of Southern Maine, personal communication). To-date, the region (NOAA Statistical Area 513, in the western Gulf of Maine) of which Whaleback is one feature, is still one of the most productive fishing grounds in the Gulf of Maine, resulting in ~90% of commercial landings in 2014 (Palmer, 2014). Thus, although coastal cod stocks were decimated in the 18th century compared to historical abundances (Bolster, 2012; Alexander et al., 2009), the cod come from similar, and likely similarly coastal, geographic regions within the broader Gulf of Maine. Lastly, although cod otoliths were not sampled by scientists from each time period, it is not unreasonable to assume that both 17th century and 21st century fishers were similarly targeting the most marketable fish. In this way, the study here controls for the origins and fishing effort of both sets of commercially harvested cod.

2.3 Low sample size considerations

As mentioned in the main text, we do not have sufficient sampling to ensure that the historical fish are representative of the 17th century population at large. Thus, we interpret our data here as specific to these fish individuals, and aim to assess whether our findings make sense with respect to what is known about these fish.

2.4 References

- Alexander K. E., Leavenworth W. B., Cournane J., Cooper A. B., Claesson S., Brennan S., Smith G., Rains L., Magness K., Dunn R., Law T. K., Gee R., Jeffrey Bolster W. and Rosenberg A. A. (2009) Gulf of Maine cod in 1861: Historical analysis of fishery logbooks, with ecosystem implications. *Fish Fish.* 10, 428–449.
- Binford L. R. (1962) New Method of Calculating Dates from Kaolin Pipe Stem Samples. *Southeast. Archaeol. Conf. Newsl.* 9, 19–21.
- Bolster W. J. (2012) The Mortal Sea., Harvard University Press.
- Churchill E. A. (1984) A Most Ordinary Lot of Men: The Fishermen at Richmond Island, Maine, in the Early Seventeenth Century. *New Engl. Q.* **57**, 184–204.
- Palmer M. C. (2014) 2014 Assessment Update Report of the Gulf of Maine Atlantic Cod Stock.

Appendix 3: Supplemental figures and table

Table A1: Average δ^{15} N and N content for five species

- Fig. A1: Schematic of protocol for $\delta^{15}N_{oto}$
- Fig. A2. Visual comparison of uncleaned and cleaned fossil otoliths
- Fig. A3: Prince William Sound statistical areas (wild pink salmon locations)
- Fig. A4: L vs. R for wild pink salmon only
- Fig. A5: Otolith mass vs. $\delta^{15}N_{oto}$ for brown trout and rainbow trout Fig. A6: N content, otolith mass, and $\delta^{15}N$ for individual fish used in Fig. 9
- Fig. A7: Otolith mass vs. fish length to reconstruct fish size
- Fig. A8. Example of a pitted fossil otolith.

Table A1: Average δ^{15} N and N content for five species: Atlantic cod (*Gadus morhua*), brown trout (Salmo trutta), pink salmon (Oncorhynchus gorbuscha), rainbow trout (Oncorhynchus mykiss), and queen snapper (Etelis oculatus).

Species	Wild vs. Farmed	Crushed vs. Intact	Crushed otolith Cleaned or not cleaned	δ ¹⁵ N (‰)	N content (nmols N mg ⁻¹ otolith)	п
Atlantic cod	Farmed	Crushed	Clean	6.15 ± 0.29	21.3 ± 1.7	2
	Wild (historical) ¹	Crushed	Clean	10.05 ± 0.56	14.6 ± 1.1	4
	Wild (historical) ²	Crushed	Unclean	10.33 ± 0.17	19.8 ± 1.6	1
		Crushed	Clean	9.52 ± 0.27	15.8 ± 0.7	
	Wild (historical) ²	Crushed	Unclean	7.89 ± 0.16	14.3 ± 0.3	1
		Crushed	Clean	7.45 ± 0.38	14.6 ± 0.5	
	Wild $(modern)^3$	Crushed	Unclean	7.10 ± 0.58	25.2 ± 6.5	3
	Wild $(modern)^{3,4}$	Crushed	Clean	6.90 ± 0.33	15.8 ± 1.8	33
Brown trout	Farmed	Intact		11.49 ± 0.29	21.8 ± 2.4	13
Pink salmon	Wild	Intact		14.49 ± 0.38	32.0 ± 3.2	16
	Wild ⁵	Crushed	Clean	14.42 ± 0.27	17.1 ± 2.0	34
Rainbow trout	Farmed	Intact		11.28 ± 0.35	18.1 ± 3.9	10
Queen snapper	Wild	Crushed	Clean	14.40 ± 0.30	19.2 ± 2.0	20

¹ well preserved historical otoliths

² suboptimal preservation of these historical otoliths

³ cod otolith standard (CDS); note, for wild commercially harvested cod (a different collection of otoliths than the otoliths used for making standards), the mean was $7.88 \pm 0.86\%$ and 16.1 ± 1.6 nmol N mg⁻¹ (n = 7)

⁴ cod otolith standard (CDS) across eight sample batches

⁵ pink salmon otolith standard (PSS) across eleven sample batches

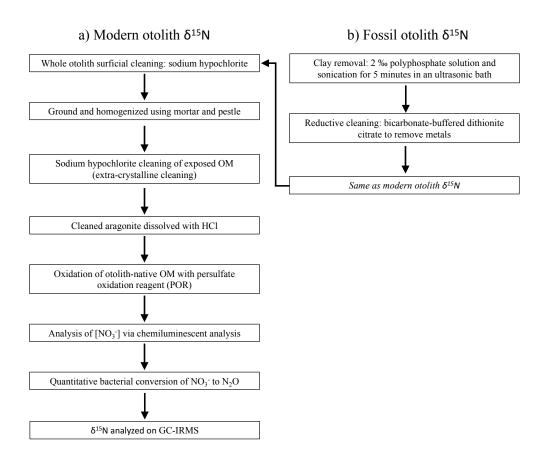


Fig. A1. Schematic for $\delta^{15}N_{oto}$ analysis of modern (a) and fossil (b) otoliths.

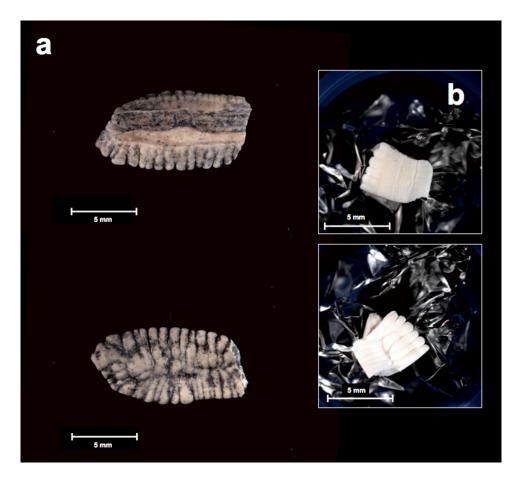


Fig. A2. **Visual comparison of uncleaned and cleaned fossil otoliths.** (a) An otolith recovered from a 17th century midden showing sediment and discoloration on the surface of the otolith. (b) Piece of a different 17th century otolith after sodium polyphosphate cleaning to remove clays, bicarbonate-buffered dithionite citrate cleaning to remove metals, and sodium hypochlorite oxidative cleaning to remove organic material. Otoliths are shown sulcus side up in the top half of the figure and sulcus side down in the bottom half of the figure.

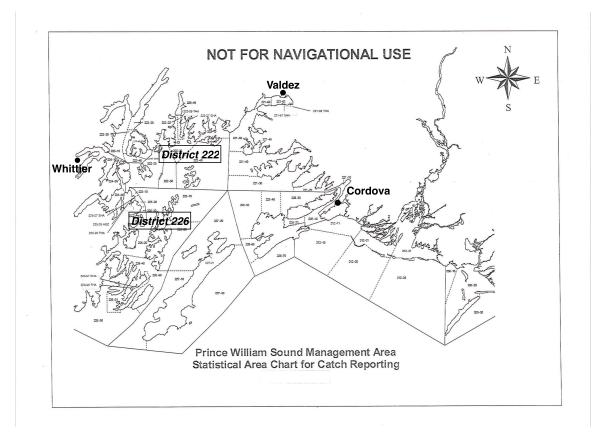


Fig. A3. **Prince William Sound statistical areas (wild pink salmon locations).** Statistical districts for Prince William Sound, Central Alaska (courtesy of Alaska Department of Fish and Game, Cordova branch). Pink salmon from Figure 6 were harvested from Districts 222 and 226 on the same day.

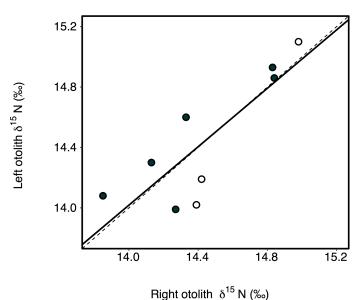


Fig. A4. L vs. R δ^{15} N_{oto} for wild pink salmon only (Fig. 5 shows L vs. R for brown trout, rainbow trout, wild pink salmon). Empty symbols are crushed otoliths; crushed otoliths are intact symbols. For crushed otolith comparisons, the L otolith was crushed whereas the R otolith was intact. Solid black line is the regression (slope = 0.97, $r^2 = 0.67$); dashed line is 1:1 line through the origin.

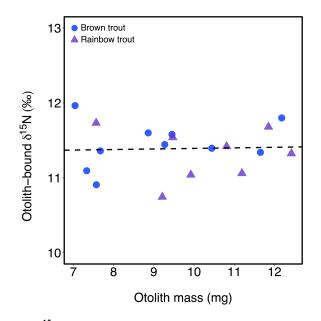


Fig. A5. Otolith mass versus $\delta^{15}N_{oto}$ of farm-raised brown trout and rainbow trout. Both species were reared in the same controlled, aquaculture setting on the same commercial aquaculture feed. Dashed line represents the least squares regression of all points.

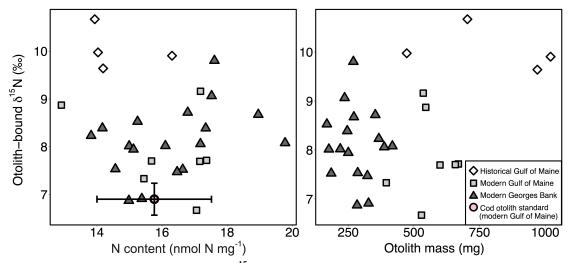


Fig. A6. N content, otolith mass, and δ^{15} N for individual fish used in Fig. 9. N content (a) and otolith mass (b) vs. δ^{15} N for individual Atlantic cod otoliths analyzed in Figure 9 from the modern Gulf of Maine (gray squares), modern Georges Bank (gray triangles), and historical Gulf of Maine (white diamonds). The long term mean of the cod otolith standard is also plotted (round symbol).

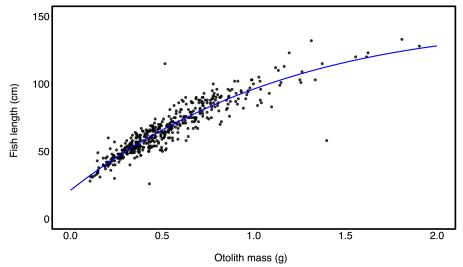


Fig. A7. **Otolith mass vs. fish length to reconstruct fish size**. Length (cm) vs. otolith weight (g) for Western Gulf of Maine cod (data from the Massachusetts Division of Marine Fisheries). An exponential model explains the relationship between fish length (cm) as a function of otolith size (g): $f(x) = 249.5 * e^{(-0.168x)} - 228.3 * e^{(-0.5957x)} (r^2 = 0.86)$.

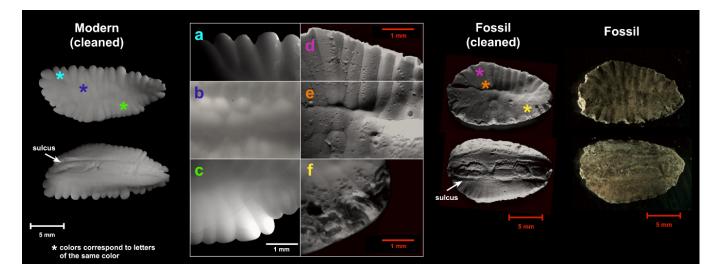


Fig. A8. **Example of a pitted fossil otolith.** Left-most otolith is a modern otolith; right-most otolith shows a fossil otolith after cleaning and before cleaning. Rounded features (a) on the dorsal part of the modern otolith are missing in the fossil otolith (d); the surface of the modern otolith is smooth (b) whereas dimples, pores, and grooves are visible on the surface of the cleaned fossil otolith (e); and rounded lobes on the ventral side of the modern otolith (c) are chipped away to expose the otolith interior of the fossil sample (f). White arrows show the sulcus acusticus in both modern and fossil otoliths.