Evidence for freshwater residency among Lake Ontario Atlantic salmon (*Salmo salar*) spawning in New York

Eric J. Guiry [a,b,c,*], Thomas C.A. Royle [d], Trevor J. Orchard [e], Suzanne Needs-Howarth [f,g,h], Dongya Y. Yang [d], Paul Szpak [a]

[a] *Department of Anthropology, Trent University, 1600 West Bank Drive, Peterborough, ON K9L 0G2, Canada*
[b] *School of Archaeology and Ancient History, University of Leicester, Mayor's Walk, Leicester LE1 7RH, United Kingdom*
[c] *Department of Anthropology, University of British Columbia, 6303 NW Marine Drive, Vancouver, BC V6T 1Z1, Canada*
[d] *Ancient DNA Laboratory, Department of Archaeology, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada*
[e] *Department of Anthropology, University of Toronto Mississauga, 3359 Mississauga Road, Mississauga, ON L5L 1C6, Canada*
[f] *Perca Zooarchaeological Research, Toronto, Canada*
[g] *The Archaeology Centre, University of Toronto, 19 Russell Street, Toronto, ON M5S 2S2, Canada*
[h] *Trent University Archaeological Research Centre, 1600 West Bank Drive, Peterborough, ON K9L 0G2, Canada*

ARTICLE INFO

ABSTRACT

Prior to their extirpation around 1900 CE, Lake Ontario hosted the world's largest freshwater Atlantic salmon (*Salmo salar*) fishery. Due to their early disappearance, questions remained about fundamental aspects of the species' biology, such as whether they belonged to sea-run (anadromous) or freshwater resident (potamodromous) ecotypes. Recent isotopic analyses have demonstrated that the complex of Atlantic salmon populations spawning in tributaries emptying along Lake Ontario's northern shores were potamodromous. However, no evidence has yet been gathered for Atlantic salmon migratory behaviour from Lake Ontario's southeastern region, where historical observations suggest both anadromous and potamodromous populations may have spawned. Here, we provide the first results for isotopic analyses of bone collagen from seven fish bones from archaeological sites (c. 1427 to 1600 CE) identified as Atlantic salmon through ancient DNA and zooarchaeological analyses. The results of the isotopic analyses confirm that at least some of the salmon spawning in tributaries emptying into Lake Ontario's southeastern shores were also potamodromous. Although further analyses are needed, this suggests anadromy may have been completely absent in Lake Ontario's complex of Atlantic salmon populations in recent centuries.

Introduction

Around the turn of the nineteenth century, when European colonization of the Lower Great Lakes region was intensifying, Lake Ontario hosted what was likely the world's largest complex of freshwater Atlantic salmon (*Salmo salar*) populations (Bogue, 2001; Dymond, et al., 2019; Parsons, 1973; Webster, 1982). These fish had long been both spiritually and nutritionally important for Indigenous peoples in the region and were quickly incorporated into the rapidly expanding European freshwater fisheries (Hawkins, et al., 2019; Recht, 1997; Tiro, 2016). However, extensive exploitation of this species, coupled with growing environmental deterioration (e.g., effluent, warming, invasive species, and blockage of spawning rivers; for review, see Dymond, et al., 2019) led to significant declines in spawning numbers by the 1850s (Adamson, 1857; Wilmot, 1869). From the mid-nineteenth century onward, the causes behind these sharply declining spawning numbers were hotly debated by period observers (for examples, see Adamson, 1857; Goode, 1884; King, 1866; Whitcher and Venning, 1870; DeKay, 1842; Edmunds, 1874). Concerns about this population collapse directly led to the development of one of North America's early major fish hatchery programs (1866–1883), which was aimed at rehabilitating Lake Ontario's Atlantic salmon stocks (Wilmot, 1869; Wilmot, 1870; Wilmot, 1880). Unfortunately, this early restoration attempt was unsuccessful (Wilmot, 1882; Wilmot, 1885), and the species was extirpated from the lake system by the end of the century (Dymond, et al., 2019; Parsons, 1973).

---

* Corresponding author.
  E-mail address: eguiry@lakeheadu.ca (E.J. Guiry).

With the last confirmed sighting of Lake Ontario Atlantic salmon occurring in 1899 (Cobb, 1900), the species disappeared before substantial or systematic studies could be performed (COSEWIC, 2011). A controversial question that remained unanswered was whether Lake Ontario's Atlantic salmon populations were (1) anadromous, migrating to the ocean and back, (2) potamodromous, living only in the freshwater environs of Lake Ontario, or (3) a mix of both (Carcao, 1986; Follett, 1932; Fox, 1930; Huntsman, 1944; Legendre et al., 1980; Parsons, 1973; Smith, 1892, 1995; Webster, 1982).

A number of studies have shed light on the migratory behaviour of Lake Ontario's Atlantic salmon using new analytical techniques that have become available over the intervening 120 years. In an early paper, Blair (1938) examined scale circuli patterns from three museum-curated Lake Ontario Atlantic salmon specimens from the nineteenth century to reconstruct mobility history and showed that these individuals were in fact potamodromous. Repetition of these analyses by Guiry et al., 2016, using scales from the composite representation of sea-run Miramichi Atlantic salmon as a comparator, were able to confirm Blair's (1938) findings and demonstrated that all five known museum skin mount specimens from Lake Ontario's native Atlantic salmon population were potamodromous. Guiry et al., 2016 also developed a second analytical approach, relying on stable carbon isotope ($\delta^{13}C$) composition of collagen, for assessing the migratory status of Lake Ontario's native Atlantic salmon population. This isotopic approach can be applied to the thousands of Late Holocene Atlantic salmon bones preserved in the Lake Ontario watershed archaeological record and can therefore offer a more detailed spatiotemporal perspective on the species' migratory behaviour. This approach is based on the observation that differences in carbon sources and cycling (for review, see Guiry, 2019) between the respective adult habitats of potamodromous and anadromous Atlantic salmon in the Lake Ontario/St. Lawrence River region enable the two behavioural types to be differentiated using their $\delta^{13}C$ values. To date, the stable carbon isotope composition of collagen from 60 Atlantic salmon bones from 8 sites dating to between 1300 and 1900 CE (common era) have been published (Guiry et al., 2016; van der Merwe et al., 2003). These data have provided clear evidence for potamodromy amongst salmon spawning across a large area of the lake.

While these studies indicate that most Atlantic salmon in Lake Ontario were behaviourally 'landlocked', the possibility exists that some spawning tributaries for which archaeological remains have not yet been analyzed were utilized by anadromous populations. All isotopic analyses to date have been on historical scales and archaeological bones (both isotopically directly comparable; Guiry and Hunt, 2020) collected from locations around the northern shore of Lake Ontario, where the majority of known Atlantic salmon spawning tributaries were situated (King, 1866; Parsons, 1973). This geographical concentration also reflects favourable taphonomic conditions at archaeological sites along Lake Ontario's northern shore (i.e., due to the more alkaline nature of soils in the region, favourable to bone preservation), as well as strong archaeological legislation, which has led to the recovery of large numbers of well-preserved fish bones.

There are a number of reasons to anticipate that, if anadromous Atlantic salmon spawned in the Lake Ontario watershed in recent centuries, they may have used tributaries along the lake's southeastern shores. First, the tributaries emptying along the southeastern shores on Lake Ontario would have been the closest and most accessible for salmon returning from the St. Lawrence River. Second, the large size of Lake Ontario's watershed, with its many waterways flowing into and out of subsidiary lakes, coupled with Atlantic salmon's behavioural plasticity, means it is possible that different sub-populations within the lake could exhibit a range of migratory behaviours. This may be supported by historical accounts, which suggest that phenotypically divergent Atlantic salmon populations spawned in Lake Ontario's southeastern tributaries. While there were nineteenth-century observers who firmly believed that Lake Ontario's Atlantic salmon were either entirely anadromous (e.g., DeKay, 1842; Talbot, 1824; Wright, 1892) or entirely potamodromous (e.g., Clinton, 1822; Goode, 1884; Simcoe, 1911), there were many whose first-hand experience with these fish led them to believe that both types were present in New York (e.g., Adamson, 1857; Atkins, 1872; Edmunds, 1874; Nettle, 1857). A compelling piece of evidence suggesting that both potamodromous and anadromous ecotypes were present in Lake Ontario comes from the observations of J. A. Mathewson, who fished Atlantic salmon on the New York side of Lake Ontario for >50 years. Mathewson's accounts describe how fish returning to three tributaries in New York to spawn were notably different in character and quality, with some being "long and slim" (Deer Creek), some "short and chubby" (Grindstone Creek), and some "heavy" (Salmon River) (Goode, 1884, 473-474). The variation in characteristics observed by Mathewson could be consistent with the physical appearance of Atlantic salmon that had undergone both long and short migrations and could be suggestive of a mix between anadromous and potamodromous salmon stocks spawning in New York (Parsons, 1973).

Historical observations also appear to suggest that some rivers may have hosted two temporally segregated spawning seasons (Parsons, 1973). Comprehensive reviews of historical observations have established that the primary period for Lake Ontario Atlantic salmon spawning was during the fall, and mostly between October and November (Dymond, et al., 2019). However, substantial variation in eyewitness accounts for the timing of upstream migration, both between and within tributaries, confounded early attempts to generate a unified overview of spawning behaviour in Lake Ontario Atlantic salmon (e.g., Edmunds, 1874; Goode, 1884; Smith, 1892). There is sufficient evidence from these accounts to confirm that in some rivers, such as the Salmon and Oswego (e.g., Goode, 1884; Jackson, 2016; Simpson, 1949; Smith, 1892) in New York and the Credit (Wilmot, 1872) in Ontario, Atlantic salmon moved upstream during spring (between April and July), in addition to fall. The nature of these spring upstream movements remains unclear. While some have interpreted these movements as a second, spring spawning run (Huntsman, 1944; Parsons, 1973), others draw on additional historical observations to compellingly argue that a more parsimonious explanation is that they simply reflect a natural instinct for Lake Ontario's Atlantic salmon to occasionally swim into spring freshets (Dymond, et al., 2019). However, observations from Samuel Wilmot, describing differences in the physical characteristics of fish swimming upstream in the spring ("bright and silvery in color, rich, fat, and in prime condition") and fall ("dark in color, lean, lank, out of condition") (Wilmot, 1872, 79), provide additional clues that, again, seem consistent with expectations for the physical conditions of separate potamodromous and anadromous populations returning to spawn. Although Wilmot was a keen and knowledgeable observer of Lake Ontario's salmon, it is also possible that his observation of leaner salmon in the fall simply reflects a change in the appearance of salmon that had already spawned (i.e., kelts) but had not yet returned to the lake.

In this study, we investigate the possibility that anadromous populations of Atlantic salmon may have utilized tributaries emptying along Lake Ontario's southeastern shores in recent centuries. To test this hypothesis, we performed stable carbon and nitrogen isotopic analyses on seven vertebrae from two archaeological sites in New York's Oswego River and Sandy Creek drainages that have been identified as Atlantic salmon through zooarchaeological and ancient DNA (aDNA) analyses. The results of our analysis suggest that at least some of the Atlantic salmon spawning in tributaries draining into southeastern Lake Ontario were potamodromous.

Methods

Sample collection

Seven fish vertebrae were collected from archaeological assemblages from two sites curated at the New York State Museum (Albany, NY, USA; Fig. 1). Five of these vertebrae are from the Diable site (NYSM 665), an ancestral Oneida village dating to c. 1570–1600 CE (Jones, 2008; Weiskotten, 1986). The Diable site is located near Oneida Creek, a formerly productive spawning ground for Atlantic salmon (Webster, 1982) up river (~50 km SSE) of Oneida Lake, which is accessed from Lake Ontario via the Oswego River. While Oneida Lake itself hosted a highly productive Atlantic salmon fishery, the disappearance of the species shortly after the construction of locks blocking access to Lake Ontario at the turn of the nineteenth century (Jackson, 2016) suggests that salmon spawning in this area were downstream migrants. The remaining two vertebrae are from the Toles site (NYSM 9349; also known as the Durfee site), a Jefferson County Iroquoian village dating to c. 1427–1560 CE (Abel, et al., 2019). The Toles site is located on Taylor Brook, a tributary of South Sandy Creek, which is accessed from Lake Ontario via Sandy Creek. All seven specimens were identified as Atlantic salmon with a high degree of certainty by two ichthyozooarchaeological experts (co-authors TJO, SNH) through morphological comparisons with reference specimens held in the Deborah J. Berg Faunal Collection at the Department of Anthropology, University of Toronto Mississauga (Mississauga, ON, Canada).

Ideally, our analyses would be performed on cranial bones, which allow for an accurate estimation of how many individual fish are included in a given sample. Unfortunately, due to their lower bone density, cranial bones from Atlantic salmon are extremely rare in comparison with those from other non-salmonid fish taxa at archaeological sites (Hawkins, et al., 2019). It was therefore necessary for our analyses to utilize vertebrae, which are typically much more abundant archaeologically due to their higher bone density (Butler and Chatters, 1994; Hawkins, et al., 2019; Lubinski, 1996). However, aside from the atlas, penultimate, and ultimate vertebrae, salmonid vertebrae do not have morphological features allowing for the differentiation of individuals. In order to mitigate this issue, where possible, samples were selected from different archaeological contexts in order to reduce the likelihood of sampling more than one bone per individual. Therefore, while the minimum number of individual fish represented by samples from each site is technically one, we believe that it is likely that our sample represents multiple individuals at each site.
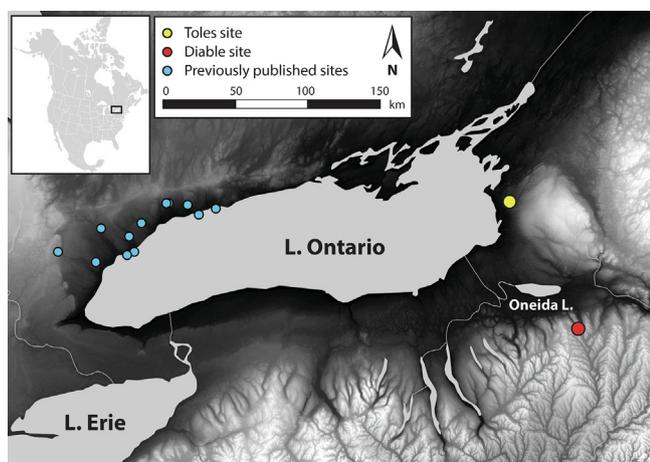

Fig. 1. Map of study region showing the locations of the Toles and Diable sites relative to other sites with previously published isotopic data.

If the Atlantic salmon bones recovered from the Diable and Toles are from fish caught elsewhere and then transported to these sites, it is possible that they may not originate from the Lake Ontario population. However, because the sites are located within a short travelling distance (less than 1–2 h walking) of known Atlantic salmon spawning sites in the Lake Ontario watershed, the long-distance transportation of fish from other drainages offers a less parsimonious explanation for these specimens' origins. In this context, we believe that the Atlantic salmon bones at Diable and Toles are derived from fish caught locally within the immediate watershed, at least partly during their spawning period, when they would have been easiest to catch (Hawkins, et al., 2019).

Ancient DNA analysis

Although relatively abundant at many archaeological sites in the northern half of the Lake Ontario watershed, salmon bones appear to be rare in archaeological fish bone assemblages from New York. For this reason, while being confident in the morphology-based taxonomic identifications, we opted to conduct aDNA analysis to verify the zooarchaeological identifications assigned to the remains. Sample decontamination, DNA extraction, and PCR setup were all performed in a dedicated ancient DNA laboratory in the Department of Archaeology at Simon Fraser University (Burnaby, BC, Canada). To reduce the likelihood of contamination, strict contamination control protocols, including the separation of the pre-PCR and post-PCR laboratories, the use of protective clothing, and the regular cleaning of work surfaces with bleach, were adhered to throughout the analysis (Cooper and Poinar, 2000; Yang and Watt, 2005). Ancient DNA analysis was conducted on 11–57 mg subsamples of bone removed from each vertebra. Following Speller et al. (2012), all the samples were decontaminated prior to DNA extraction through a combination of chemical decontamination and exposure to UV irradiation. For chemical decontamination, each sample was submerged in a commercial bleach solution ($\approx$5% w/v NaOCl) for 8 min, rinsed in distilled water for 1 min, and then submerged in distilled water for 10 min. Following chemical decontamination, the samples were UV irradiated for 30 min on two sides in a UV crosslinker. The decontaminated samples were then added to 3.5 mL of lysis buffer (0.5 M EDTA pH 8.0, 0.25% SDS, and 0.5 mg/mL proteinase K) and incubated overnight at 50 °C in a rotating hybridization oven. DNA was then extracted from the samples using a modified silica-spin column method (Yang, et al., 1998; Yang, et al., 2008). DNA extractions were performed in a single batch that included all seven vertebrae and a single blank extraction control.

Following established protocols (Yang, et al., 2004; Yang and Speller, 2006), a 168 bp fragment of the mitochondrial *cytochrome b* (*cytb*) gene and 255 bp fragment of the mitochondrial control region (D-loop) useful for salmonid species identification were amplified with published primers (Table 1). These fragments were either co-amplified in a single 35 µL reaction volume (Yang and Speller, 2006) or amplified individually through a singleplex PCR conducted with a 30 µL reaction volume (Yang et al., 2004). The reaction volumes for both the singleplex and co-amplification PCRs included 1.5× PCR Gold Buffer (Applied Biosystems, Carlsbad, CA, USA), 2 mM MgCl₂, 0.2 mM of each dNTP, 1 mg/mL BSA, 3 µL DNA solution, and 0.75 U AmpliTaq Gold (Applied Biosystems, Carlsbad, CA, USA). The co-amplification PCRs also contained 0.6 µM of each D-loop primer and 0.3 µM of each *cytb*, while the singleplex PCRs included 0.3 µM of each *cytb* or D-loop primer. For both the co-amplification and singleplex PCRs, the thermocycling program consisted of an initial denaturation step at 95 °C for 12 min, followed by 60 cycles at 95 °C for 30 s (denaturation), 54 (singleplex PCRs) or 56 (co-amplification PCRs) °C for 30 s (annealing), and 70 °C for 40 s (extension), and a final extension step

Table 1
Primers used for the PCR amplification of fragments of *cytochrome b* and D-loop.

Primer[1]	Locus	Sequence (5′-3′)	Amplicon Size	Reference
cytB5 (F)	*cytb*	AAAATCGCTAATGACGCACTAGTCGA	168 bp	Yang et al. (2004)
cytB6 (R)	*cytb*	GCAGACAGAGGAAAAAGCTGTTGA		Yang et al. (2004)
Smc7 (F)	D-loop	AACCCCTAAACCAGGAAGTCTCAA	255 bp	Yang et al. (2004)
Smc8 (R)	D-loop	CGTCTTAACAGCTTCAGTGTTATGCT		Yang et al. (2004)

[1] F and R indicate the forward and reverse primers in a primer pair, respectively.

at 72 °C for 7 min. A negative PCR control was included in each PCR run in order to detect instances of contamination. Each combination of primers was applied to the blank extraction control.

Following amplification, 3–5 μL of PCR product from each sample was pre-stained with SYBR Green I (Life Technologies, Carlsbad, CA, USA), electrophoresed on a 2% agarose gel, and visualized with a Dark Reader transilluminator (Clare Chemical Research, Dolores, CO, USA). All successfully amplified *cytb* or D-loop fragments were directly sequenced with the forward or reverse amplification primers at Eurofins Genomics (Toronto, ON, Canada). The obtained sequences were visually edited, trimmed to remove the primer sequences, and compiled in ChromasPro v2.1.8 (Technelysium, Brisbane, QLD, Australia). To determine their closest taxonomic match, the edited sequences were compared against reference sequences accessioned in GenBank (Sayers, et al., 2019) through BLASTn searches (Altschul, et al., 1990). Following Royle et al. (2020), multiple alignments of the obtained ancient *cytb* and D-loop sequences as well as reference sequences from all salmonine species (Atlantic salmon, lake trout [*Salvelinus namaycush*], and brook trout [*Salvelinus fontinalis*]) native to the Lake Ontario watershed (Crossman and Van Meter, 1979) were conducted with ClustalW (Thompson, et al., 1994) through BioEdit v 7.2.5 (Hall, 1999). Each alignment also included a huchen (*Hucho hucho*) reference sequence, which was used as an outgroup in the phylogenetic analyses. In BioEdit, the obtained alignments were edited by eye and the sequences were trimmed to the same length. For each marker, a maximum-likelihood tree was subsequently constructed in PHYML v3.1 (Guindon, et al., 2010) through the PHYML Online web server (Guindon, et al., 2005) using 1000 bootstrap replications and a nucleotide substitution model chosen with Smart Model Selection (Lefort, et al., 2017) using the Akaike information criterion (HKY85 for *cytochrome b*; TN93 + G for D-loop). The obtained trees were visualized and annotated with iTOL v5.1.1 (Letunic and Bork, 2019). A sample was assigned to a species if the obtained sequences closely resembled reference sequences from a single species and differed significantly from other closely related taxa (Yang, et al., 2004).

Isotopic analyses

Bone collagen was extracted from the specimens following a Longin (1971) method modified as follows. Bone samples (50–100 mg) were treated with a 2:1 chloroform–methanol solution in a ultrasonic bath (solution refreshed every 15 min until it remained clear) to remove residual bone lipids (Folch, et al., 1957; Guiry et al., 2016b). Samples were then demineralized in 0.5 M HCl at room temperature. Demineralized samples were then neutralized in Type 1 water before being treated with 0.1 M NaOH in an ultrasonic bath (solution refreshed every 15 min until it remained clear) to remove base-soluble contaminants (e.g., humic acids). Samples were again neutralized in Type 1 water and then solubilized in a 10^{-3} HCl solution (~pH 3) in an oven at 70 °C for 48 h. Sample solutions were then centrifuged, after which the solubilized fraction was transferred to a new vial, which was frozen and lyophilized.

Carbon and nitrogen elemental and isotopic compositions were measured on 0.5 mg subsamples of the extracted collagen using an Elemental Analyzer 300 (Eurovector, Pavia, Italy) coupled via continuous flow to a Horizon Isotope Ratio Mass Spectrometer (Nu Instruments, Wrexham, UK) at the Water Quality Research Centre at Trent University (Peterborough, ON, Canada). All samples were analyzed in duplicate. Stable carbon and nitrogen isotope compositions were calibrated relative to VPDB (carbon) and AIR (nitrogen), respectively, using a two-point calibration anchored to USGS40 and USGS41a (Electronic Supplementary Materials (ESM) Table S1; Qi, et al., 2003; Qi, et al., 2016). Standard deviations and numbers for calibration standards are reported in ESM Table S2. Analytical accuracy was monitored using three internal check standards (ESM Table S1). Mean and standard deviations for check standards are reported in ESM Table S3. Standard deviations for all duplicates are reported in ESM Table S4. Calculated for carbon and nitrogen ($\delta^{15}N$) isotopic compositions, respectively: random errors ($uR_{(w)}$) were ±0.06‰ and ±0.15‰; systematic errors ($u_{(bias)}$) were ±0.09‰ and ±0.14‰; standard uncertainty was ±0.11‰ and ±0.21‰ (Szpak, et al., 2017).

Collagen quality was assessed using established criteria for ancient collagen (Ambrose, 1990; DeNiro, 1985; van Klinken, 1999). Isotopic compositions from archaeological bone collagen are deemed acceptable if they are accompanied by elemental concentrations of >13% and 4.8% carbon and nitrogen, respectively, collagen yields >1%, and a C:N atomic ratio between 2.9 and 3.6.

Results

Ancient DNA analysis

The targeted 168 bp fragment of *cytb* and 255 bp fragment of D-loop were both successfully amplified from all seven samples (100% amplification success rate). This amplification success rate is comparable to that obtained for these fragments from Atlantic salmon and lake trout remains (98.36%, *n* = 61) from the thirteenth-century CE Antrex (AjGv-38) site, near Lake Ontario's northern shore (Royle et al., 2020). These high amplification success rates suggest that, similar to other regions (for review, see Oosting et al., 2019), DNA is generally well preserved in archaeological fish remains from the Lake Ontario watershed. No DNA was amplified from either the blank extraction control or the negative PCR controls, suggesting a lack of systematic contamination (Cooper and Poinar, 2000).

BLASTn searches indicated that the *cytb* and D-loop sequences obtained from each sample most closely resembled Atlantic salmon reference sequences. The maximum-likelihood trees constructed for the *cytb* (Fig. 2) and D-loop (Fig. 3) sequences yielded similar results. The sequences obtained from the samples formed a strongly supported (>99% bootstrap support values) monophyletic group with all the Atlantic salmon reference sequences in both maximum-likelihood trees (Figs. 2 and 3). The similarity between the *cytb* and D-loop sequences obtained from the archaeological samples and Atlantic salmon reference sequences strongly supports the morphology-based identification
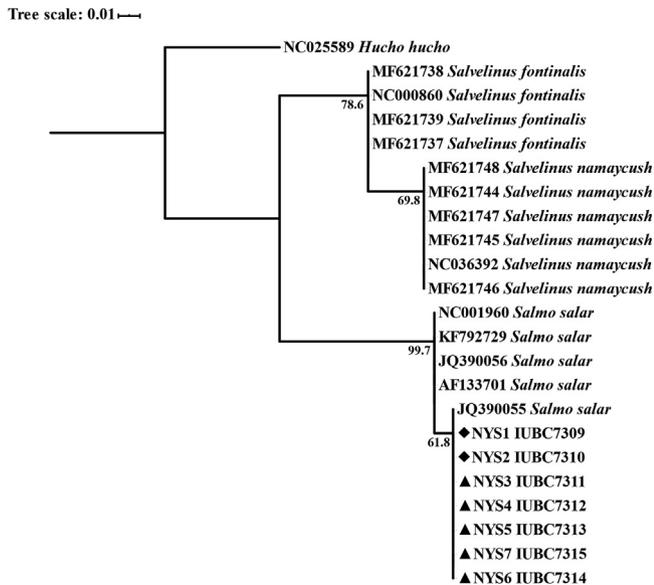
Tree scale: 0.01

Fig. 2. Maximum-likelihood phylogenetic trees documenting the relationship between the *cytochrome b* sequences obtained from the analyzed archaeological specimens from the Toles (denoted with diamonds) and Diable (denoted with triangles) sites and reference sequences from salmonine species native to Lake Ontario (GenBank accession numbers provided). The aDNA (NYS#) and isotope (IUBC#) lab numbers for each of the archaeological specimens are provided. The tree was rooted using a huchen (*Hucho hucho*) reference sequence as an outgroup. The numbers at nodes indicate the bootstrap support values for nodes with values ≥50% after 1000 bootstrap replications. The scale bar represents the number of nucleotide substitutions per site.
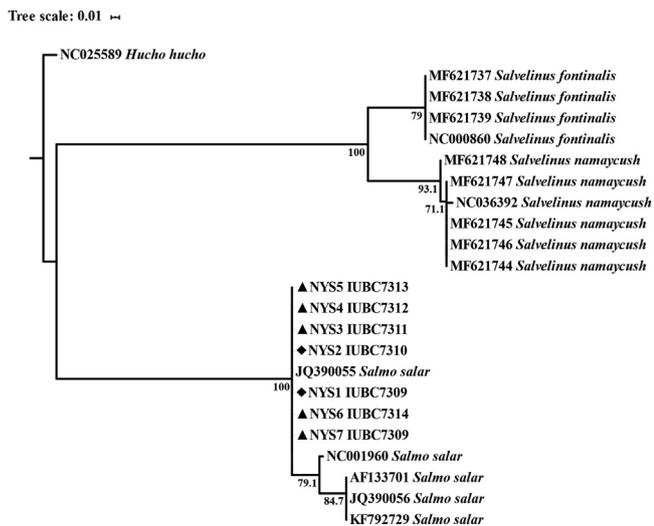

Tree scale: 0.01

Fig. 3. Maximum-likelihood phylogenetic trees documenting the relationship between the D-loop sequences obtained from the analyzed archaeological specimens from the Toles (denoted with diamonds) and Diable (denoted with triangles) sites and reference sequences from salmonine species native to Lake Ontario (GenBank accession numbers provided). The aDNA (NYS#) and isotope (IUBC#) lab numbers for each of the archaeological specimens are provided. The tree was rooted using a huchen (*Hucho hucho*) reference sequence as an outgroup. The numbers at nodes indicate the bootstrap support values for nodes with values ≥50% after 1000 bootstrap replications. The scale bar represents the number of nucleotide substitutions per site.

of these samples as Atlantic salmon. The concordant species identities assigned to the samples through the analysis of two different markers provide additional support for the authenticity of the aDNA results (Yang, et al., 2004; Yang and Speller, 2006). The D-loop and *cytb* sequences obtained from each of the samples have

been deposited in GenBank, under accession numbers MN707925–MN707938.

Isotopic analyses

Results from stable isotope and elemental analyses are presented in Fig. 4 and reported in full in ESM Table S5. All samples (n = 7) passed all collagen quality controls, with an average C:N$_{atomic}$ of 3.16 ± 0.04, indicating that collagen has not been contaminated with residual lipids or materials from the burial environment. Samples from the Diable (n = 5, δ^{13}C = −19.9 ± 0.3‰; δ^{15}N = +10.5 ± 0.2‰) and Toles (n = 2, δ^{13}C = −19.8 ± 0.1‰; δ^{15}N = +10.3 ± 0.2‰) sites had similar mean δ^{13}C and δ^{15}N values, which differed by 0.10‰ and 0.21‰, respectively (less than analytical uncertainty).

Together, samples from both sites had an average δ^{13}C of −19. 8 ± 0.2‰ (range = −19.7‰ to −20.4‰). These δ^{13}C values fall squarely within the range of −20.6‰ and −18.8‰ (n = 65; mean = −19.9 ± 0.5‰; from 9 sites) for archaeological and historical Atlantic salmon samples collected from drainages emptying along the northern shores of Lake Ontario (Guiry et al., 2016; van der Merwe et al., 2003) and well outside of the observed range of −16.6‰ to −14.0‰ (n = 1887) observed for collagen from archaeological and modern (Suess-corrected) anadromous Atlantic salmon (Bocherens, et al., 2014; Dixon, et al., 2015; Dixon, et al., 2012; Drucker and Bocherens, 2004; Guiry et al., 2016b; MacKenzie, et al., 2012; Müldner and Richards, 2007).

Stable nitrogen isotope compositions are typically measured in tandem with δ^{13}C, and, while these compositions are not the focus of this study, we provide them nonetheless as they may be useful for future comparative research. Stable nitrogen isotope compositions can provide information about the trophic level at which an organism fed (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). However, due to greater complexity of nitrogen sources and nitrogen cycling in freshwater ecosystems, ecological interpretations based on δ^{15}N from archaeological fish require a substantial isotopic baseline (Guiry, 2019; Guiry et al., 2020), which is not yet available for the Lake Ontario watershed. The δ^{15}N values for sam-
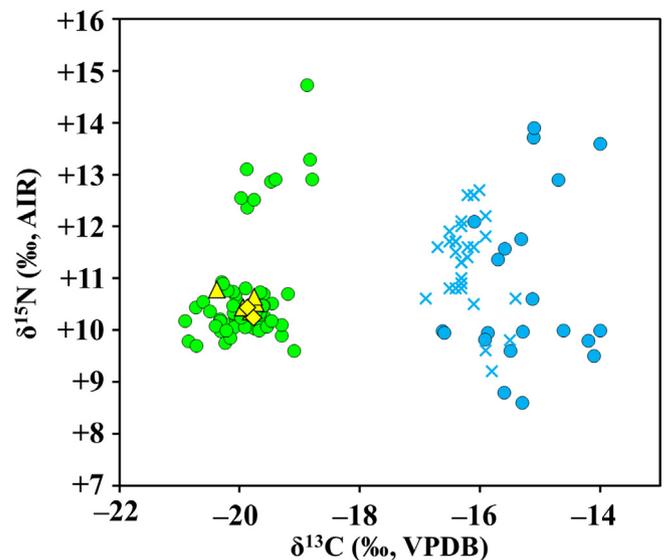

Fig. 4. Stable carbon and nitrogen isotope values for Atlantic salmon from the Toles (diamonds) and Diable (triangles) sites shown with baseline data from potamodromous (archaeological and historical sites from Lake Ontario, shown as green/open circles) and anadromous (modern shown as x's; archaeological shown by blue/solid circles) Atlantic salmon (Dixon et al., 2012; Guiry et al., 2016; MacKenzie et al., 2012; van der Merwe et al., 2003).

ples from the Diable and Toles sites ($n = 7$; mean $\delta^{15}N$ = +10.5 ± 0.2‰; range = +10.2‰ to +10.8‰) also fall within the range of +8.9‰ to +11.4‰ ($n = 56$; mean = +10.3 ± 0.3‰, 7 sites) previously observed in archaeological Atlantic salmon bone collagen from Lake Ontario (Guiry et al., 2016; van der Merwe et al., 2003), suggesting that individuals spawning on the northern and southern sides of Lake Ontario filled a similar trophic niche.

Discussion

The rapid decline and extirpation of Atlantic salmon from the Lake Ontario watershed continues to generate scholarly and public interest due both to its historical significance (Dymond, et al., 2019; Hutchings, et al., 2019; Stewart, et al., 2017; Tiro, 2016) and ongoing efforts to reintroduce the species to the region (Glass, 2010; He, et al., 2015; Houde, 2015; Houde, et al., 2017; Randall, 2010). The findings of this study can help to provide a more complete understanding of the behavioural ecology of Lake Ontario's native complex of Atlantic salmon populations. In demonstrating that at least some of the salmon spawning in the Oswego River and Oneida Lake region as well as the Sandy Creek region of New York were potamodromous, our results show that Atlantic salmon spawning in tributaries along the southeastern shore Lake Ontario were similar to their counterparts spawning in tributaries along the lake's northern shore. Our identification of Atlantic salmon at the fifteenth- to seventeenth-century Diable and Toles sites also indicates that Lake Ontario salmon fishing has a deep history amongst New York Iroquoians, as is the case among their Ontario Iroquoian neighbours (for a review of Ontario Iroquoian salmon use, see Hawkins, et al., 2019).

Our data provide additional context for interpreting eyewitness accounts, which remain the primary resource for our understanding of the Lake Ontario watershed's Atlantic salmon populations and their place in its pre-industrial ecology (Dymond, et al., 2019). First, we can re-examine historical observations of multiple (i.e., spring and fall) upstream migrations and their interpretation as potential evidence for the presence of both anadromous and potamodromous Atlantic salmon populations in Lake Ontario (Huntsman, 1944; Parsons, 1973). The Oswego River system, where five of our samples originate, was one of the most productive spawning habitats for Lake Ontario's Atlantic salmon and is one of the locations for which there are multiple accounts of spring upstream movements. For this reason, if anadromous Atlantic salmon were still returning to Lake Ontario in recent centuries, and practiced temporally segregated spawning with their potamodromous counterparts (Huntsman, 1944; Parsons, 1973), the Oswego watershed is an ideal location in which to search. In this context, our analyses, showing only potamodromous salmon, suggest that multi-season upstream movements were not related to the presence of anadromous and potamodromous populations sharing the same tributary.

Using data generated for another study (Guiry, et al., 2020), we are also able to more closely consider Samuel Wilmot's observations describing differences in the physical characteristics of the Credit and Humber rivers Atlantic salmon swimming upstream in the spring ("good condition") and fall ("poor condition") (Wilmot, 1872, 79), which have also been highlighted as support for the idea that there may have been separate potamodromous and anadromous populations returning to spawn (Huntsman, 1944; Parsons, 1973). Isotopic analyses of Atlantic salmon specimens from the Antrex (c. 1250–1300 CE, see Archaeological Services Inc, 2010; Braun, 2010; $n = 21$, $\delta^{13}C$ = –19.6‰) and Emmerson Springs (AkGx-5; c. 1550–1580 CE, see Hawkins, 2004; $n = 2$, $\delta^{13}C$ = –19.7‰) sites in the Credit River drainage and the Seed-Barker site (AkGv-1; c. 1530–1560 CE, see Burgar, 2005; $n = 12$, $\delta^{13}C$ = –19.8

‰) in the Humber River drainage (Guiry, et al., 2020) show that all samples came from potamodromous fish, suggesting that Wilmot's observations probably do not indicate the presence of anadromous populations returning to spawn on the northern shore of Lake Ontario. Samuel Wilmot was an astute observer, and his accounts about seasonal changes in Atlantic salmon condition can still provide valuable insights into other aspect of the species' ecology in the Lake Ontario watershed. For instance, if Atlantic salmon coloration, size, and overall condition suggested to Wilmot that fish were better fed and healthier in spring, it could be an indication that winter and early spring were superior feeding times for this species in Lake Ontario. However, it remains possible that his observations of inter-seasonal differences in the physical condition of salmon reflect the change in the appearance of pre- and post-spawning fish. Moreover, it is difficult to assess the extent to which Wilmot's observations (primarily from the, 1860s to the 1880s) are representative of longer-term patterns in Lake Ontario Atlantic salmon behaviour, given that the populations had already been severely impacted by commercial fishing and habitat destruction. Taken together, this isotopic evidence from archaeological fish from the Oswego, Credit, and Humber river systems supports Dymond and colleagues' (2019) hypothesis that spring upstream migrations simply reflect the Lake Ontario Atlantic salmon's instinct to swim into freshets, rather than a second spring spawning run.

Our findings also provide a point of reference for contextualizing Mathewson's example (Goode, 1884) of systematic variation in Atlantic salmon spawning in three New York rivers. Mathewson's account of Atlantic salmon spawning in the Salmon River and in Deer and Grindstone creeks gives details of phenotypic differences at the inter-population level that could reflect different migratory strategies (Parsons, 1973). Mathewson's description of Atlantic salmon spawning in Deer Creek as "long and thin" in appearance is particularly interesting as this would be more consistent with an anadromous population that has lost significant weight during a long return migration from the Atlantic Ocean (Belding, 1934). Unfortunately, we have been unable to locate archaeological Atlantic salmon remains from the Deer Creek area and so could not directly test the hypothesis that salmon spawning in this drainage were anadromous. However, because the example provided by Mathewson deals with spawning rivers and creeks that are separated by less than 5 km at their point of entry into Lake Ontario, it is equally possible that other tributaries in New York could have hosted a similar amount of phenotypic diversity, potentially including use by anadromous Atlantic salmon. Our results come from the Oswego River and Sandy Creek systems, which empty into Lake Ontario about 65 km and 14 km west and east of Deer Creek, respectively, and suggest that salmon spawning in these locations were not anadromous. The Oswego River was an important access point for Atlantic salmon spawning in a large portion of New York's Lake Ontario watershed, including Oneida Lake along the Oneida River to the east and Onondaga, Cayuga, and Seneca lakes along the Seneca River to the west. While our results demonstrate potamodromy for salmon returning to the Oneida Lake (Diable) and Sandy Creek (Toles) watersheds, the immense size of the broader Oswego River and Sandy Creek systems leaves open the possibility that other spawning areas could have hosted anadromous Atlantic salmon.

Conclusion

This study provides the first evidence for the migratory behaviour of Atlantic salmon spawning in the Oswego River and Sandy Creek systems, which hosted one of Lake Ontario's most productive salmon fisheries prior to the species' extirpation in the nineteenth

century (Dymond, et al., 2019; Webster, 1982). A number of historical observations (e.g., Goode, 1884; Jackson, 2016; Webster, 1982) seem to suggest that, if anadromous Atlantic salmon were still migrating to spawn in Lake Ontario's tributaries in recent centuries, they may have favoured river systems emptying along the lake's southeastern shores, in what is now New York State. Our analyses show that salmon in at least two spawning grounds, one in the Oswego River system and the Oneida Lake drainage and one in the Sandy Creek drainage, were potamodromous. These findings are concordant with those generated from the growing body of data (Guiry et al., 2016; van der Merwe et al., 2003) from sites in drainages along the northern shores of Lake Ontario. Taken together, these data suggest that in recent centuries, anadromy may have been totally absent among the complex of Atlantic salmon populations inhabiting Lake Ontario. While archaeological fish bones suitable for isotopic analyses appear to be less common in New York, future work on additional Atlantic salmon specimens, if and when they are discovered, could help to further test this hypothesis.

CRediT authorship contribution statement

Eric J. Guiry: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing. **Thomas C.A. Royle:** Investigation, Formal analysis, Data curation, Visualization, Writing - original draft, Writing - review & editing. **Trevor J. Orchard:** Investigation, Writing - review & editing. **Suzanne Needs-Howarth:** Investigation, Writing - review & editing. **Dongya Y. Yang:** Resources, Writing - review & editing. **Paul Szpak:** Resources, Writing - review & editing.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2020.05.009.

References

Abel, T.J., Vavrasek, J.L., Hart, J.P., 2019. Radiocarbon dating the Iroquoian occupation of Northern New York. Am. Antiq. 84 (4), 748–761.

Adamson, W.A., 1857. The decrease, restoration, and preservation of salmon in Canada. Can. J. Industry Sci. Arts 2 (7), 1–7.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410.

Ambrose, S.H., 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. J. Archaeol. Sci. 17 (4), 431–451.

Archaeological Services Inc, 2010. Report on the Salvage Excavation of the Antrex Site (AjGv-38) City of Mississauga, Regional Municipality of Peel, Ontario. Report on file at the Ministry of Heritage, Sport, Tourism and Culture Industries, Toronto.

Atkins, C.G., 1872. On the salmon of eastern North America, and its artificial culture. Report of the Commissioner for 1872 and 1873, Part II. United States Commission of Fish and Fisheries, Washington, D.C, pp. 226–335.

Belding, D.L., 1934. The cause of the high mortality in the Atlantic salmon after spawning. Trans. Am. Fish. Soc. 64 (1), 219–224.

Blair, A.A., 1938. Scales of Lake Ontario salmon indicate a land-locked form. Copeia 1938 (4), 206.

Bocherens, H., Baryshnikov, G., Van Neer, W., 2014. Were bears or lions involved in salmon accumulation in the Middle Palaeolithic of the Caucasus? An isotopic investigation in Kudaro 3 cave. Quat. Int. 339–340, 112–118.

Bogue, M.B., 2001. Fishing the Great Lakes: An Environmental History, 1783–1933. The University of Wisconsin Press, Madison.

Braun, G., 2010. Technological choices: ceramic manufacture and use at the Antrex site (AjGv-38). Ontario Archaeol. 89–90, 69–96.

Burgar, R.W.C., 2005. Seed-Barker Site (AkGv-1). Report on file at the Toronto and Region Conservation Authority, Vaughan.

Butler, V.L., Chatters, J.C., 1994. The role of bone density in structuring prehistoric salmon bone assemblages. J. Archaeol. Sci. 21 (3), 413–424.

Carcao, G., 1986. Atlantic Salmon in the Great Lakes Basin: A History of its Extirpation and Attempted Restortation. Report on file at the Ramsay Wright Laboratories. University of Toronto, Toronto.

Clinton, D., 1822. Letters on the Natural History and Internal Resources of the State of New York. E. Bliss & E. White, New York.

Cobb, J.N., 1900. The commercial fisheries of Lake Erie, Lake Ontario, and Niagara and St. Lawrence River. In: Davis, B.H., Weed, W.R., Babcock, C.H., Thompson, E., Holden, H.S. (Eds.), Fifth Annual Report of the Commissioners of Fisheries, Game and Forests of the State of New York. Commissioners of Fisheries, Game and Forests of the State of New York, Albany, pp. 189–239.

Cooper, A., Poinar, H.N., 2000. Ancient DNA: do it right or not at all. Science 289 (5482), 1139.

COSEWIC, 2011. COSEWIC Assessment and Status Report on the Atlantic Salmon in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa.

Crossman, E.J., Van Meter, H.D., 1979. Annotated List of the Fishes of the Lake Ontario Watershed. Great Lakes Fishery Commission, Ann Arbor.

DeKay, J.E., 1842. Zoology of New York; or, The New York Fauna; Comprising Detailed Descriptions of All the Animals Hitherto Observed within the State of New York, with Brief Notices of those Occasionally Found Near its Borders, and Accompanied by Appropriate Illustrations, Part IV, Fishes. W. & A. White & J. Visscher, Albany.

DeNiro, M.J., 1985. Postmortem preservation and alteration of *in vivo* bone collagen isotope ratios in relation to palaeodietary reconstruction. Nature 317, 806–809.

DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta 45 (3), 341–351.

Dixon, H.J., Dempson, J.B., Power, M., 2015. Assessing the use of different marine growth zones of adult Atlantic salmon scales for studying marine trophic ecology with stable isotope analysis. Fish. Res. 164, 112–119.

Dixon, H.J., Power, M., Dempson, J.B., Sheehan, T.F., Chaput, G., 2012. Characterizing the trophic position and shift in Atlantic salmon (*Salmo salar*) from freshwater to marine life-cycle phases using stable isotopes. ICES J. Marine 69 (9), 1646–1655.

Drucker, D., Bocherens, H., 2004. Carbon and nitrogen stable isotopes as tracers of change in diet breadth during Middle and Upper Palaeolithic in Europe. Int. J. Osteoarchaeol. 14 (3–4), 162–177.

Dymond, J.R., MacKay, H.H., Burridge, M.E., Holm, E., Bird, P.W., 2019. The history of the Atlantic Salmon in Lake Ontario. Aqu. Ecosyst. Health Manage. 22 (33), 305–315.

Edmunds, M., 1874. Obstructions in the tributaries of Lake Champlain. Report of the Commissioner for 1872 and 1873. Goverment Printing Office, Washington, D.C., pp. 622–629.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226 (1), 497–509.

Follett, R., 1932. Salmo salar of the St. Lawrence River. Trans. Am. Fish. Soc. 62 (1), 366–368.

Fox, W.S., 1930. The literature of *Salmo salar* in Lake Ontario and tributary streams. Trans. Royal Soc. Can. 24, 45–55.

Glass, C., 2010. An Evaluation of the Reintroduction of Atlantic Salmon to Lake Ontario and its Tributaries (Master's thesis). University of Waterloo, Waterloo, Canada.

Goode, G.B., 1884. The Fisheries and Fishery Industries of the United States, Section I, Natural History of Useful Aquatic Animals. Government Printing Office, Washington, D.C.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Sys. Biol. 59 (3), 307–321.

Guindon, S., Lethiec, F., Duroux, P., Gascuel, O., 2005. PHYML Online—a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res. 33 (S2), W557–W559.

Guiry, E., 2019. Complexities of stable carbon and nitrogen isotope biogeochemistry in ancient freshwater ecosystems: Implications for the study of past subsistence and environmental change. Front. Ecol. Evol. 7, 313.

Guiry, E.J., Buckley, M., Orchard, T.J., Hawkins, A.L., Needs-Howarth, S., Holm, E., Szpak, P., 2020. Deforestation caused abrupt shift in Great Lakes nitrogen cycle. Limnol. Oceanogr. https://doi.org/10.1002/lno.11428(n/a).

Guiry, E.J., Hunt, B.P.V., 2020. Integrating fish scale and bone isotopic compositions for 'deep time' retrospective studies. Marine Environ. Res. 160, 104982.

Guiry, E.J., Needs-Howarth, S., Friedland, K.D., Hawkins, A.L., Szpak, P., Macdonald, R., Courtemanche, M., Holm, E., Richards, M.P., 2016. Lake Ontario salmon (*Salmo salar*) were not migratory: a long-standing historical debate solved through stable isotope analysis. Sci. Rep. 6, 36249.

Guiry, E, Royle, T.C.A., Matson, R.G., Ward, H., Weir, T., Waber, N., Brown, T.J., Hunt, B.P.V., Price, M.H.H., Finney, B.P., Kaeriyama, M., Qin, Y., Yang, D.Y., Szpak, P., 2020. Differentiating salmonid migratory ecotypes through stable isotope analysis of collagen: Archaeological and ecological applications. PloS One 15, (4) e0232180.

Guiry, E.J., Szpak, P., Richards, M.P., 2016b. Effects of lipid extraction and ultrafiltration on stable carbon and nitrogen isotopic compositions of fish bone collagen. Rapid Commun. Mass Spectrom. 30 (13), 1591–1600.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hawkins, A.L., 2004. Report on the 2004 Investigations at the Emmerson Springs Village (AkGx-5), Town of Halton Hills, Ontario under Licence P081-002 and P081-004. Report on file at the Ministry of Heritage, Sport, Tourism and Culture Industries, Toronto.

Hawkins, A.L., Needs-Howarth, S., Orchard, T.J., Guiry, E.J., 2019. Beyond the local fishing hole: a preliminary study of pan-regional fishing in southern Ontario (ca. 1000 CE to 1750 CE). J. Archaeolog. Sci.: Rep. 24, 856–868.

He, X., Wilson, C.C., Wellband, K.W., Houde, A.L.S., Neff, B.D., Heath, D.D., 2015. Transcriptional profiling of two Atlantic salmon strains: implications for reintroduction into Lake Ontario. Conserv. Genet. 16 (2), 277–287.

Houde, A.L.S., 2015. Restoration of Native Biodiversity in Altered Environments: Reintroduction of Atlantic salmon into Lake Ontario. Restoration of Native Biodiversity in Altered Environments: Reintroduction of Atlantic Salmon into Lake Ontario (Ph.D. thesis). University Western Ontario, London, Canada.

Houde, A.L.S., Wilson, C.C., Neff, B.D., 2017. Performance of four salmonids species in competition with Atlantic salmon. J. Great Lakes Res. 43 (1), 211–215.

Huntsman, A.G., 1944. Why did the Lake Ontario salmon disappear? Trans. Royal Soc. Can. 38, 83–102.

Hutchings, J.A., Ardren, W.R., Barlaup, B.T., Bergman, E., Clarke, K.D., Greenberg, L.A., Lake, C., Piironen, J., Sirois, P., Sundt-Hansen, L.E., Fraser, D.J., 2019. Life-history variability and conservation status of landlocked Atlantic salmon: an overview. Can. J. Fish. Aquat. Sci. 76 (10), 1697–1708.

Jackson, J.R., 2016. Oneida Lake BC (before Cornell). In: Rudstam, L.G., Mills, E.L., Jackson, J.R., Stewart, D.J. (Eds.), Oneida Lake: Long-term Dynamics of a Managed Ecosystem and its Fishery. American Fisheries Society, Bethesda, pp. 31–52.

Jones, E.E., 2008. Iroquois Population History and Settlement Ecology, AD 1500-1700 (Ph.D. thesis). Pennsylvania State University, United States of America.

King, W.R., 1866. The Sportsman and Naturalist in Canada or Notes on the Natural History of the Game, Game Birds, and Fish of that Country. Hurst and Blackett, London.

Lefort, V., Longueville, J.-E., Gascuel, O., 2017. SMS: Smart Model Selection in PhyML. Mol. Biol. Evol. 34 (9), 2422–2424.

Legendre, V., Mongeau, J.-R., Leclerc, J., Briseboise, J., 1980. Les Salmonidés des Eaux de la Plaine de Montréal. Ministère du Loisir de la Chasse et de la Pêche, Service de l'Amenagement et de l'Exploitation de la Faune, Région Administrative de Montréal, Montreal.

Letunic, I., Bork, P., 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res. 47 (W1), W256–W259.

Longin, R., 1971. New method of collagen extraction for radiocarbon dating. Nature 230, 241–242.

Lubinski, P., 1996. Fish heads, fish heads: an experiment on differential bone preservation in a salmonid fish. J. Archaeol. Sci. 23 (2), 175–181.

MacKenzie, K.M., Trueman, C.N., Palmer, M.R., Moore, A., Ibbotson, A.T., Beaumont, W.R.C., Davidson, I.C., 2012. Stable isotopes reveal age-dependent trophic level and spatial segregation during adult marine feeding in populations of salmon. ICES J. Mar. Sci. 69 (9), 1637–1645.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochim. Cosmochim. Acta 48 (5), 1135–1140.

Müldner, G., Richards, M.P., 2007. Stable isotope evidence for 1500 years of human diet at the city of York, UK. Am. J. Phys. Anthropol. 133 (1), 682–697.

Nettle, R., 1857. The Salmon Fisheries of the St. Lawrence and its Tributaries. John Lovell, Montreal.

Oosting, T., Star, B., Barrett, J.H., Wellenreuther, M., Ritchie, P.A., Rawlence, N.J., 2019. Unlocking the potential of ancient fish DNA in the genomic era. Evol. Appl. 12 (8), 1513–1522.

Parsons, J.W., 1973. History of Salmon in the Great Lakes, 1850–1970. United States Department of the Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Washington, D.C.

Qi, H., Coplen, T.B., Geilmann, H., Brand, W.A., Böhlke, J., 2003. Two new organic reference materials for δ^{13}C and δ^{15}N measurements and a new value for the δ^{13}C of NBS 22 oil. Rapid Commun. Mass Spectrom. 17 (22), 2483–2487.

Qi, H., Coplen, T.B., Mroczkowski, S.J., Brand, W.A., Brandes, L., Geilmann, H., Schimmelmann, A., 2016. A new organic reference material, l-glutamic acid, USGS41a, for δ^{13}C and δ^{15}N measurements – a replacement for USGS41. Rapid Commun. Mass Spectrom. 30 (7), 859–866.

Randall, R.G., 2010. Quantifying the Fluvial Habitat Needed for the Re-Introduction of Atlantic salmon (*Salmo salar*) in Lake Ontario Canadian Science Advisory Secretariat Research Document 2009/110. Department of Fisheries and Oceans, Ottawa.

Recht, M., 1997. The role of fishing in the Iroquois economy, 1600–1792. N. Y. History 76 (4), 429–454.

Royle, T.C.A., Zhang, H., Guiry, E.J., Orchard, T.J., Needs-Howarth, S., Yang, D.Y., 2020. Investigating the sex-selectivity of a Middle Ontario Iroquoian Atlantic salmon (*Salmo salar*) and lake trout (*Salvelinus namaycush*) fishery through ancient DNA analysis. J. Archaeolog. Sci.: Rep. 31, 102301.

Sayers, E.W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K.D., Karsch-Mizrachi, I., 2019. GenBank. Nucleic Acids Res. 47 (D1), D94–D99.

Simcoe, E., 1911. The Diary of Mrs. John Graves Simcoe, Wife of the First Lieutenant-Governor of the Province of Upper Canada, 1792-6. William Briggs, Toronto.

Simpson, E.M., 1949. Mexico, Mother of Towns: Fragments of Local History. J. W. Clement Company, Buffalo.

Smith, H.M., 1892. Report on an investigation of the fisheries of Lake Ontario. Bull. U. S. Fish Comm. 10, 177–215.

Smith, S.H., 1995. Early Changes in the Fish Community of Lake Ontario. Great Lakes Fishery Commission, Ann Arbor.

Speller, C.F., Hauser, L., Lepofsky, D., Moore, J., Rodrigues, A.T., Moss, M.L., McKechnie, I., Yang, D.Y., 2012. High potential for using DNA from ancient herring bones to inform modern fisheries management and conservation. PLoS One 7, (11) e51122.

Stewart, T.J., Todd, A., LaPan, S.R., 2017. Fish Community Objectives for Lake Ontario. Great Lakes Fishery Commission, Ann Arbor.

Szpak, P., Metcalfe, J.Z., Macdonald, R.A., 2017. Best practices for calibrating and reporting stable isotope measurements in archaeology. J. Archaeolog. Sci.: Rep. 13, 609–616.

Talbot, E.A., 1824. Five Years' Residence in the Canadas: Including a Tour through Part of the United States of America, in the Year 1823. Longman, Hurst, Rees, Orme, Brown and Green, London.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22 (22), 4673–4680.

Tiro, K.M., 2016. A sorry tale: natives, settlers, and the salmon of Lake Ontario, 1780–1900. Historical J. 59 (4), 1001–1025.

van der Merwe, N.J., Williamson, R.F., Pfeiffer, S., Thomas, S.C., Allegretto, K.O., 2003. The Moatfield ossuary: isotopic dietary analysis of an Iroquoian community, using dental tissue. J. Anthropol. Archaeol. 22 (3), 245–261.

van Klinken, G.J., 1999. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. J. Archaeol. Sci. 26 (6), 687–695.

Webster, D.A., 1982. Early history of the Atlantic salmon in New York. New York Fish Game J. 29 (1), 26–44.

Weiskotten, D.N., 1986. Summary of Excavations at the Diable Site - MSV-2 Stockbridge. Manuscript on file at the New York State Museum, Albany.

Wilmot, S., 1869. Appendix No. 6. Annual Report of the Department of Marine and Fisheries for the Year 1868. Hunter, Rose & Co., Ottawa, pp. 84–99.

Wilmot, S., 1870. Report of S. Wilmot, Esq., on the fish-breeding establishment at Newcastle, Ontario, during the season of 1869, Appendix No. 9. Annual Report of the Department of Marine and Fisheries for the Year Ending June 30th 1869. I.B. Taylor, Ottawa, pp. 39–65.

Wilmot, S., 1872. Appendix I, Report of S. Wilmot, Esq., on the fish-breeding establishment at Newcastle, Ontario, during the season of 1871. Annual Report of the Department of Marine and Fisheries for the Year Ending June 30th 1871. I.B. Taylor, Ottawa, pp. 76–89.

Wilmot, S., 1880. Report of Samuel Wilmot, Esq., on the several fish-breeding establishments and fish culture in Canada during the season of 1879. Twelfth Annual Report of the Department of Marine and Fisheries for the Year 1879. Maclean, Roger, & Co., Ottawa, pp. 5–21.

Wilmot, S., 1882. Report of Samuel Wilmot, Esq., on fish-breeding operations in the Dominion of Canada, during the year 1881, with remarks on the decline of the salmon fisheries. Fourteenth Annual Report of the Minister of Marine Fisheries for the Year 1881. Maclean, Roger, & Co., Ottawa, pp. 3–47.

Wilmot, S., 1885. Fish Breeding, 1884. Annual Report of the Department of Fisheries, Dominion of Canada For the Year 1884. Maclean, Roger & Co., Ottawa, pp. 5–43.

Whitcher, W.F., Venning, W.H., 1870. Appendix No. 9, Special report of Messrs. Whitcher and Venning, on fish breeding, at Newcastle, Ontario. Annual Report of the Department of Marine and Fisheries for the Year Ending June 30th 1869. I.B. Taylor, Ottawa, pp. 66–69.

Wright, R.R., 1892. Preliminary Report on the Fish and Fisheries of Ontario. Warwick & Sons, Toronto.

Yang, D.Y., Cannon, A., Saunders, S.R., 2004. DNA species identification of archaeological salmon bone from the Pacific Northwest Coast of North America. J. Archaeol. Sci. 31 (5), 619–631.

Yang, D.Y., Eng, B., Waye, J.S., Dudar, J.C., Saunders, S.R., 1998. Improved DNA extraction from ancient bones using silica-based spin columns. Am. J. Phys. Anthropol. 105 (4), 539–543.

Yang, D.Y., Liu, L., Chen, X., Speller, C.F., 2008. Wild or domesticated: DNA analysis of ancient water buffalo remains from north China. J. Archaeol. Sci. 35 (10), 2778–2785.

Yang, D.Y., Speller, C.F., 2006. Co-amplification of cytochrome b and D-loop mtDNA fragments for the identification of degraded DNA samples. Mol. Ecol. Notes 6 (3), 605–608.

Yang, D.Y., Watt, K., 2005. Contamination controls when preparing archaeological remains for ancient DNA analysis. J. Archaeol. Sci. 32 (3), 331–336.