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1　　　**Deforestation caused abrupt shift in Great Lakes nitrogen cycle**

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21　　<u>Abstract</u>

22　　Despite the longstanding significance of North America's Great Lakes, little is known about their
23　　pre-industrial ecology. Here, we report on when and how humans first became a main driver of
24　　Lake Ontario's nutrient dynamics. Nitrogen isotope analyses of archaeological fish show that,
25　　prior to the 1830s, Lake Ontario's nitrogen cycle and the trophic ecology of its top predators had
26　　remained stable for at least 800 years, despite Indigenous and historical European agricultural
27　　land management across the region. An abrupt shift in the nitrogen isotope composition of Lake
28　　Ontario's fish community is evident in the early to mid-nineteenth century and reflects the
29　　initiation of industrial-scale forest clearance. These data show how the nitrogenous nutrient
30　　regimes of even the world's largest freshwater ecosystems can be highly sensitive to short-term
31　　watershed forest cover disturbances and indicate a profound shift in the relationship between
32　　humans and their environment.

33

34　　<u>Keywords</u>

35　　Historical Ecology, Archaeology, Great Lakes, Nitrogen Cycle, Fish, Deforestation

36

Introduction

Throughout the twentieth century, increased anthropogenic contributions of reactive nitrogen to freshwater environments have profoundly altered aquatic biogeochemical cycles and broader nutrient dynamics, resulting in widespread ecosystem dysfunction, loss of biodiversity, and degraded water quality (Smith and Schindler 2009). While numerous studies have documented how increasing anthropogenic nitrogen inputs are incorporated into aquatic food webs, promoting cultural eutrophication and altering trophic dynamics in the recent past (Schindler, et al. 2006), less consideration has been given to how historical and preindustrial populations have impacted aquatic ecosystems. In the context of recent debate about the timing of the origins of the Anthropocene (Lewis and Maslin 2015), in which past societies are considered as potential architects of the first broad-scale environmental changes, the role of humans as drivers of biogeochemical processes such as the nitrogen cycle is becoming increasingly important (Guiry, et al. 2018; Hadley, et al. 2010; Kintigh, et al. 2014).

For conservation and restoration efforts in freshwater ecosystems, where cultural eutrophication caused by increased nutrient loading poses one of the most significant threats globally (Smith and Schindler 2009), long-term retrospectives that document when and how human activities first began to alter natural ecosystem processes, and the nitrogen cycle in particular, could provide a valuable framework for evaluating which modern human activities pose the greatest risks (Canfield, et al. 2010; Humphries and Winemiller 2009). Paleolimnological proxy indicators based on the physical, biological, chemical, and isotopic compositions of sediments have long been used to establish trends in past aquatic productivity and water quality (Beeton 1965; Hodell and Schelske 1998; Jeffers, et al. 2015; Schelske, et al. 1983), but, because of potential taphonomic issues (Anderson 2014; Lu, et al. 2014) and because these approaches

60   usually do not incorporate consumers, they are unable to measure the direct impacts of

61   anthropogenic nitrogen loading on nutrient dynamics in the wider biotic community (e.g.,

62   invertebrates, fish, birds; although for a growing literature on invertebrate analyses see Anas, et

63   al. 2019; Frossard, et al. 2014; Perga 2010; Perga, et al. 2010; Schilder, et al. 2017; van

64   Hardenbroek, et al. 2010). Moreover, while many studies using isotopic analyses of museum-

65   archived vertebrate tissues have been able to make important contributions to understanding how

66   biotic communities have responded to human-caused environmental change during the twentieth

67   century (Fera, et al. 2017), a lack of suitable specimens from early historical and pre-industrial

68   time periods has, in most cases, prevented analyses of longer-term environmental variation in

69   vertebrate taxa (Szpak, et al. 2018). In this context, stable nitrogen isotope and other analyses of

70   ancient fish remains from archaeological repositories can provide an invaluable opportunity to

71   gain direct insight into how food web and nutrient dynamics within pre-industrial ecosystems

72   functioned and responded to impacts from human activities at varying temporal and spatial

73   scales (Guiry, et al. 2016a).

74   Using isotopic compositions of fish bone or scales has some interpretive advantages relative to

75   more commonly analyzed materials, such as bulk sedimentary organic matter, because fish stable

76   nitrogen isotope composition is linked firmly with a known ecological point (i.e. a particular

77   species with well-understood ecology and trophic affinity) and spatiotemporal framework

78   (Trueman and Moore 2007) and can be easily parsed to remove diagenetically altered samples

79   using robust quality-control criteria (DeNiro 1985; Szpak 2011). This is important because, in

80   contrast to analyses of bulk sedimentary organic matter, which is composed of a spatially,

81   temporally, and biologically heterogeneous mixture of sources of detritus (Lu, et al. 2014), with

82   fish bone or scales, the ability to compare "taxonomically anchored" isotopic patterns across

83 different parts of a food web will enable much higher-resolution interpretations of environmental

84 change at both the level of nutrient dynamics and the level of broader food web structures.

85 Moreover, with respect to measuring the isotopic compositions of ancient organic materials, the

86 ability to purify collagen extracted from bone (through pretreatment steps outlined below) and

87 assess the extent to which its constituent carbon and nitrogen are biogenic (e.g., using well-

88 established and precise $C:N_{Atomic}$ criteria (DeNiro 1985; Szpak 2011) provides an additional

89 advantage relative to other important paleontological materials (e.g., chitinous remains from sub

90 fossil invertebrates; Anas, et al. 2019) and makes bone and scale collagen particularly well suited

91 for inter-site comparisons of isotopic variation in biota across time and space. Moreover, because

92 collagen from bones and scales is constructed and remodeled slowly throughout the life of an

93 organism, the isotopic composition of collagen can provide a lifetime average record of dietary

94 intake and environmental conditions that is less susceptible to seasonal or short-term

95 idiosyncratic shifts in behavior (Hobson and Clark 1992). Bone collagen is therefore well suited

96 for reconstructing long-term trends in biology and ecology (Bump, et al. 2007) and can provide

97 an additional perspective with potential to complement and build on the success of previous,

98 well-established paleolimnological proxy indicators (e.g., physical, chemical, and isotopic

99 compositions of sediments and invertebrates; Beeton 1965; Hodell and Schelske 1998; Jeffers, et

100 al. 2015; Perga 2010; Schelske, et al. 1983).

101 We use stable nitrogen isotope compositions and peptide mass fingerprinting (Zooarchaeology

102 by Mass Spectrometry, hereafter, ZooMS) of late Holocene (900-2015 CE) fish bone collagen

103 from a large number of sites associated with Lake Ontario (Figure 1), the most easterly of the

104 Laurentian Great Lakes (hereafter, Great Lakes), to document long-term trends in regional

105 biogeochemical cycles. Results show that, in comparison with the industrial era, the nitrogen

106 cycle and trophic structure of this Great Lakes ecosystem remained remarkably stable until the

107 1830s, despite millennia of Indigenous agricultural and other land management, decades of

108 European settlement, and climatic fluctuations. After this time, increased N-loading from

109 forestry- and agriculture-induced soil erosion caused an unprecedented and abrupt bottom-up

110 shift throughout the entire aquatic ecosystem of Lake Ontario.

111 <u>Context</u>

112 With more than 20% of the world's surface freshwater, North America's Great Lakes are of

113 tremendous ecological, economic, and social interest (Sterner, et al. 2017). Since monitoring

114 programs began, the Great Lakes have experienced significant ecological and chemical changes

115 in response to pollution, hydrological controls, and species invasion (Allan, et al. 2013; Stewart,

116 et al. 2016). Paleolimnological studies show that recent industrial activities have had profound

117 impacts on Great Lakes productivity (Beeton 1965; Hodell and Schelske 1998; Jeffers, et al.

118 2015; Meyers 2003; Schelske and Hodell 1991b; Schelske and Hodell 1995; Schelske, et al.

119 1988; Schelske, et al. 1983), especially through increased phosphorus and nitrogen loading that

120 led to eutrophication of Lake Ontario and Lake Erie by the twentieth century (Hodell and

121 Schelske 1998; Lu, et al. 2010). With respect to nutrient dynamics, particularly the nitrogen

122 cycle, which plays a vital role in primary production in naturally oligotrophic ecosystems such as

123 Lake Ontario (Lean and Knowles 1987; Leggett, et al. 2000), the extent to which these changes

124 represent a departure from longer-term trends in broader food web structure and nutrient

125 dynamics remains unclear (Sterner, et al. 2017; Supplementary Information [SI] 1). But this

126 extent could be determined through isotopic reconstructions of past nutrient and food web

127 dynamics using historical or archaeological fish specimens, as these can provide a

128 complementary isotopic record for ancient environmental variation.

129   Human activities have modified the cycling of nitrogen in aquatic environments across the globe,

130   contributing to the ongoing threat of cultural eutrophication (Gruber and Galloway 2008; Smith

131   and Schindler 2009). The majority of these impacts occur when anthropogenic nitrogen inputs

132   and/or chemical or physical changes to an environment alter the conditions regulating the

133   nitrogen cycle, thereby disrupting the flow of nitrogenous nutrients through an ecosystem

134   (Kendall, et al. 2007). Modeling how nitrogen moves into and through aquatic ecosystems is,

135   therefore, of considerable importance for conservation efforts seeking to restore heavily

136   impacted watersheds (Denk, et al. 2017). With respect to broad-scale human impacts, such

137   factors as climate change, acid rain, nutrient loading, and invasive species introductions can

138   significantly alter the biogeochemical processes that balance an aquatic ecosystem's nitrogen

139   cycle and often, in turn, cause detectable changes in the isotopic composition of nitrogen pools

140   that are available to support aquatic life (Anderson and Cabana 2005; Botrel, et al. 2014; Lake, et

141   al. 2001). Stable nitrogen isotopic compositions of consumer tissues show a stepwise increase,

142   by roughly 3–4‰, in δ^{15}N values with each trophic level step up in a food web and have

143   therefore traditionally provided a powerful indicator for food web interactions in ecology and

144   archaeology (Post 2002). However, because any impact on the δ^{15}N of aquatic nitrogen pools at

145   the base of a food web (i.e., in various pools of dissolved inorganic nitrogen [DIN]) is passed up

146   the trophic ladder to producers (phytoplankton) and their consumers (invertebrates and fish),

147   isotopic analyses of tissues from aquatic producers and consumers can also serve as a highly

148   integrative indicator for anthropogenic impacts on nutrient dynamics, in addition to food web

149   structure, in aquatic environments (Hoffman, et al. 2012; Morrissey, et al. 2013). While a

150   majority of isotopic research has approached the question of changing freshwater nutrient

151   dynamics through analyses of sedimentary organic matter (e.g., Dubois, et al. 2018; Talbot

152   2001), a growing number of studies are demonstrating that δ^{15}N compositions of invertebrates

153   and fish can provide a highly sensitive record for environmental change (e.g., Anas, et al. 2019;

154   Fera, et al. 2017; Lumb and Johnson 2012; Perga, et al. 2010).

155   The natural abundance of ^{15}N in DIN is largely controlled by three factors (for reviews see,

156   Finlay and Kendall 2007; Guiry 2019; Kendall 1998; Kendall, et al. 2007; Talbot 2001):

157   productivity (Hodell and Schelske 1998; Schelske and Hodell 1991a), environmental conditions

158   (temperature, oxygenation, and pH; Finlay and Kendall 2007; Knowles 1982), and nitrogen

159   inputs (quantity and isotopic composition; Heaton 1986; Lake, et al. 2001). These factors are

160   necessarily interrelated because, for instance, increasing the input of reactive nitrogen can

161   increase productivity, and this can affect environmental conditions that regulate other parts of the

162   nitrogen cycle (Finlay and Kendall 2007). Moreover, nitrogen-limitation rates can influence

163   plankton community composition, which is an integral component of determining the presence

164   of nitrogen-fixing bacteria (Gu 2009) that can further modify the isotopic composition of an

165   aquatic ecosystem by introducing isotopically distinctive atmospheric nitrogen. Human activity

166   should affect those factors controlling the nitrogen cycle and its isotopic composition (for review

167   see Guiry 2019) by: 1) contributing reactive nitrogen (i.e., through inputs directly from sewage,

168   agriculture, and soil erosion – typically, but not always, leading to elevated biota δ^{15}N (Anderson

169   and Cabana 2005; Morrissey, et al. 2013)), which itself leads to increasing productivity (creating

170   greater nitrogen demand and thereby possibly promoting the importance of N-fixing algae –

171   leading to higher and lower biota δ^{15}N, respectively(Gu 2009; Pennock, et al. 1996)), and 2)

172   changing chemical conditions, such as pH and oxygen levels, that regulate transformations

173   between important forms of reactive nitrogen (altering the dynamics for nitrification and

174   denitrification – with potential to push biota δ^{15}N higher or lower (Collister and Hayes 1991;

175   Sebilo, et al. 2006)).

176   With the longest history of European and Indigenous agricultural land management and

177   settlement among the Great Lakes, Lake Ontario and its watershed are an ideal context in which

178   to investigate how large-scale human activities can impact major freshwater environments over

179   time (Hodell and Schelske 1998). Because Lake Ontario receives a large amount of its water

180   from land runoff, it is particularly sensitive to broader ecological processes and human

181   disturbances occurring across the watershed, particularly in terrestrial and riparian zones.

182   Moreover, the significant and highly visible environmental degradation of Lake Ontario's

183   watershed throughout the twentieth century, as well as its close proximity to major population

184   centers, has spurred considerable research into its ecological structure (e.g., Bogue 2001; Estepp

185   and Reavie 2015; Meyers 2003; Stewart, et al. 2016). For this reason, the lake has a relatively

186   well-documented ecological and biogeochemical history, with numerous isotopic studies of

187   modern fish identifying recent trends in community structure, trophic dynamics, and energy

188   flows (Colborne, et al. 2016; Fera, et al. 2017; Kiriluk, et al. 1995; Lumb and Johnson 2012;

189   Rush, et al. 2012; Yuille, et al. 2015). The longest-term of these studies, however, only offers a

190   70-year retrospective, one that postdates intensification of European settlement of Lake Ontario's

191   watershed by more than 150 years.

192   While a number of important isotopic studies have helped to document longer-term variability in

193   Lake Ontario's nitrogenous nutrient dynamics (SI 1), these have focused on isotopic

194   compositions of organic matter in lake sediments rather than specific taxa. With respect to the

195   last millennium, the timeframe over which human land management would have intensified,

196   most (95%) of these analyses focus on the period after the mid-nineteenth century and show

197   substantial change in the nitrogen isotopic composition of organic matter settling out of Lake

198   Ontario's water column (SI 1; Figure S1, Figure 2; Hodell and Schelske 1998; McFadden, et al.

199   2004). Only a small fraction (5%; *n*=8 of 178) of analyses cover the early historical period

200   (1600s-1850 CE), when European activities would have started impacting the region, and pre-

201   contact period, when Indigenous farming and other land management activities were accelerating

202   across the watershed (i.e., starting with the Late Woodland, c. 900 CE). Moreover, only one lake

203   sediment isotopic dataset spans both time frames and is not consistent with the others (SI 1,

204   Figure 2). While these data provide an invaluable macro-scale perspective on general patterns in

205   nutrient dynamics in Lake Ontario, they cannot be used to assess the consequences of shifting

206   nitrogenous nutrient regimes for the broader food web (invertebrates and fish). Moreover, the

207   coarse temporal resolution of the lake sediment isotopic record prior to 1850 prevents detailed

208   analyses of when the human activities began to impact the nitrogen cycle and to what extent

209   early historical and Indigenous activities may have been important drivers for change. In this

210   context, isotopic analyses of archaeological fish can provide a complementary line of evidence

211   for nutrient dynamics and food web structure.

212   Materials and methods

213   *Sample description*: Sampling focused on three taxa in the family Salmonidae: lake trout

214   (*Salvelinus namaycush*; *n*=222), Atlantic salmon (*Salmo salar*; *n*=158), and whitefishes

215   (*Coregonus* spp.; primarily *C. clupeaformis* and *C. artedi*, see below; *n*=277; SI 2, Table S1).

216   These taxa were selected to provide a highly integrated record of changes to Lake Ontario's

217   nitrogen cycle and trophic structure. The upper trophic position and longer lifespan (5−15 years)

218   as well as pelagic foraging and highly mobile behavior of these species means that their diets

219   will incorporate a broad range of resources from across the lake (Fera, et al. 2017; Guiry, et al.

220 2016a; Rush, et al. 2012), providing a time-averaged perspective on processes affecting the

221 nitrogen cycle at a lake-wide, multi-seasonal scale. A comparison of isotopic variation among

222 three taxa, each with a different behavioral strategy and trophic position (Fera, et al. 2017; Holm,

223 et al. 2009; Mumby, et al. 2018), should make isotopic trends associated with dietary shifts at the

224 species level (as opposed to changes at the base of the food web) clearly discernible and,

225 therefore, these data also give a long-term perspective on the stability of Lake Ontario's food

226 web structure.

227 With the exception of nine Atlantic salmon samples, all archaeological specimens are from sites

228 within the Lake Ontario watershed. As Lake Ontario is the only known source of Atlantic

229 salmon, specimens from this species at sites outside the watershed were also included because

230 they represent individuals originating from Lake Ontario. Where possible, archaeological fish

231 bone specimens were selected based on minimum number of individual counts per

232 archaeological context in order to avoid sampling the same individual multiple times. For all

233 species, archaeological sampling efforts targeted bone specimens from adult-sized fish in order

234 to exclude juveniles, who may feed at a lower trophic level. All specimens were examined by

235 one or more of three zooarchaeologists (S.N.-H., A.H., T.O.) with specific expertise in the

236 identification through comparative osteology of archaeological fish bones from relevant taxa in

237 the Great Lakes region (Hawkins, et al. 2019). The archaeological samples are largely derived

238 from fish vertebra, because they are more ubiquitous than fish cranial and pectoral girdle bones

239 in the archaeological collections. Zooarchaeologists determined species for each specimen where

240 possible, based on clear morphological differences between taxa. Due to overlaps in osteology

241 and the possibility of hybrids, morphological examination of *Coregonus* vertebrae and many

242 *Coregonus* cranial and pectoral girdle bones cannot be confidently used to assign species-level

243   identification in the Lake Ontario watershed. However, of the five Coregonus species that are

244   native to Lake Ontario, only two, lake whitefish (*C. clupeaformis*) and cisco (*C. artedi*), inhabit

245   waters shallow enough to have been broadly accessible using fishing technologies (birch bark

246   canoes and gill nets) available in the pre-contact past, and it is therefore highly likely that

247   archaeological *Coregonus* of that time are composed of these taxa. Some bones in the genera

248   *Salvelinus* and *Salmo* are also morphologically similar to each other, resulting in additional

249   higher-level taxonomic identifications (including to *Salmo salar/Salvelinus namaycush* and

250   Salmonidae). In cases where a species-level taxonomic identification was not achieved with a

251   high degree of certainty, we undertook ZooMS analyses (*n*=292) to confirm identifications,

252   comparing the archaeological samples with reference sequences we generated for *S. salar*, S.

253   namaycush, brook trout (*Salvelinus fontinalis*), round whitefish (*Prosopium cylindraceum*), C.

254   clupeaformis, and *C. artedi* (Supporting Information 4 Table S1).

255   Dating for pre-contact specimens is based on published (SI 2, Table S2) radiocarbon dates,

256   ceramic seriations, and reconstructed village occupation sequences (e.g., Williamson 2014), to

257   occupations within 100-year bins. Excellent chronological control for data from Indigenous

258   archaeological sites reflects the highly standardized lifecycle in which settlements were built,

259   occupied, decommissioned, and left behind in favor of relocation every 10–30 years (Warrick

260   2008). The relative dearth of data from the seventeenth and eighteenth centuries reflects changes

261   in Indigenous settlement patterns during this period. Reduced sample sizes in the nineteenth

262   century reflect lower frequencies for fish bone at Euro-Canadian archaeological sites. Historical

263   fish scale samples of adult specimens of known date were taken from museum-archived

264   collections with assistance from an Ontario fish identification expert (E.H.). Analyses of scales

265   from museum-archived *Coregonus* specimens include both *C. clupeaformis* and *C. artedi*

266   because: 1) these two species likely account for the majority of archaeological specimens and

267   will therefore be directly comparable; and 2), based on δ^{15}N values, they appear to have fed at

268   the same trophic level in early twentieth-century Lake Ontario. For data sourced from the

269   literature (*n*=834; (Colborne, et al. 2016; Fera, et al. 2017; Guiry, et al. 2016a; Kiriluk, et al.

270   1995; Rush, et al. 2012; van der Merwe, et al. 2003; Yuille, et al. 2015)), fork length, when

271   available, was used to exclude juveniles, which may feed at a lower trophic level.

272   *Sample preparation:* Samples weighing between 30 and 300 mg were cut from bone specimens,

273   residual bone lipids were removed with a series of 2:1 chloroform:methanol ultrasonic baths

274   (solution refreshed every 10 minutes until visible signs of reaction ceased), and the samples were

275   then left to air dry (Guiry, et al. 2016b). Bone samples were then demineralized in 0.5 M

276   hydrochloric acid (HCl) for several days at 4°C and then rinsed to neutrality in Milli-Q water.

277   Humic acids and other base soluble contaminants from the burial environment were removed

278   from demineralized bone samples with successive treatments in 0.1 M sodium hydroxide in an

279   ultrasonic bath (solution refreshed every 15 minutes until visible signs of reactions ceased) and

280   then rinsed to neutrality in Milli-Q water (Szpak, et al. 2017a). Bone samples were then

281   solubilized in 10^{-3} M HCl in an oven at 65°C for 48 hours. The resulting solutions were

282   centrifuged, after which the solubilized collagen fraction was transferred to a new tube and then

283   frozen and lyophilized.

284   The protein fraction of fish scale is also composed primarily of collagen, which is

285   compositionally and isotopically comparable to fish bone collagen (Guiry, et al. 2016a; Trueman

286   and Moore 2007). Fish scales samples underwent a succession of three rinses in Milli-Q water in

287   an ultrasonic bath for five minutes each (Guiry, et al. 2016a). Scale samples were then soaked for

288   five minutes in 1.0 M HCl in an ultrasonic bath to remove the mineral phase of the scale's

289 external plate as well as any microscopic debris that may have become adhered to the external

290 surfaces of the fish specimens during preservation and storage. Scale samples were then rinsed to

291 neutrality in Milli-Q water in an ultrasonic bath and left to air dry. The historical fish specimens

292 had been preserved using formalin fixation and then stored in ethanol. While this form of

293 preservation is known to introduce small quantities of carbon, which can slightly alter the stable

294 carbon isotope composition of biological tissues, formalin and ethanol provide no new sources of

295 nitrogen and therefore have a minimal influence the nitrogen isotopic composition of preserved

296 fish scales. To confirm this, we also compared the percent carbon-to-nitrogen ratio (C:N) of

297 historical scales with that of modern fish scales (see below; (Szpak 2011)) to establish that

298 diagenetic nitrogen is not present.

299 *Isotopic analyses*: The stable nitrogen isotope composition as well as percent carbon and

300 nitrogen were measured on 0.5 mg samples of bone and scale collagen using an Elementar Vario

301 MICRO cube elemental analyzer coupled via continuous flow to an Isoprime isotope ratio mass

302 spectrometer in the Archaeology Chemistry Laboratory at the University of British Columbia,

303 Canada. Duplicate or triplicate analyses were performed on 14% of samples. For a full account

304 of procedures used for calibration of isotopic values as well as isotopic uncertainty calculations

305 see SI 3. Analytical uncertainty for $\delta^{15}N$ measurements was +0.20‰ (Szpak, et al. 2017b; SI 3).

306 The following data quality criteria were applied for establishing the integrity of $\delta^{15}N$

307 measurements from bone and scale collagen (DeNiro 1985): 1) % carbon and % nitrogen values

308 above 13% and 4.8%, respectively; and 2) atomic C:N values falling between 2.9 and 3.6.

309 *ZooMS analyses*: Collagen was rehydrated with approximately 100 µL 50 mM ammonium

310 bicarbonate per milligram and each digested with 0.4 µg sequencing grade trypsin (Promega,

311 UK) overnight at 37$^{\text{o}}$C. Initial attempts were made at directly spotting these digests through

dilution in 0.1% trifluoroacetic acid (TFA; Buckley, et al. 2018), but many required ZipTip

purification with C18 solid phase extraction and rehydration in 0.1% TFA (Buckley, et al. 2009).

Samples were spotted onto a stainless-steel Matrix Assisted Laser Desorption Ionization

(MALDI) target plate with equal volume of 10 mg/mL alpha-cyano hydroxycinnamic acid in

50% acetonitrile/0.1% TFA and allowed to air dry. Dried spots were then analyzed using a

Bruker Ultraflex II MALDI mass spectrometer over the *m/z* range 700-3700 with up to 2,000

laser acquisitions for each sample. Peptide mass fingerprints were then compared with those of

standard reference material via the identification of genus- or species-specific biomarkers (SI 4).

Statistical analyses: For statistical comparisons of fish $\delta^{15}N$ values, each taxon (i.e., lake trout,

Atlantic salmon, whitefishes) was compared separately by time periods binned by 100 (pre-1925

CE) and 25 (post-1925 to present) year groups (SI 5, Table S1) using PAST version 3.22. Bin

timeframes were selected with a view to maximizing sample size per bin. To assess whether fish

$\delta^{15}N$ values changed over time we first compared pre-1800 bins to one another to establish

whether significant differences occur within each fish taxon between sequential time frames.

After finding that no significant differences occur (SI 5, Table S2), we then grouped all pre-1775

(preindustrial) bins and compared this individually to all succeeding time periods (SI 5, Table

S3). For each bin, normality of distribution was first tested using a Shapiro–Wilk's test (SI 5,

Table S1). For datasets where all time periods were distributed normally, a one-way ANOVA

was used, and homogeneity of variance was assessed using a Levene's test. A post hoc Dunnett's

T3 test or a post hoc Tukey's HSD test was used to compare groups with variances that were

equal or unequal, respectively. For data sets where not all time periods were distributed

normally, Mann-Whitney U tests with Bonferroni corrected p values were used to compare

groups.

335   Results

336   Of the samples with sufficient collagen (*n*=607 of 662) for isotopic analyses, 96% (total *n*=582)

337   produced acceptable C:N, %C, and %N values satisfying quality control criteria. Interpretations

338   also include previously published data from archaeological (*n*=68; (Guiry, et al. 2016a; van der

339   Merwe, et al. 2003)) and modern (post-1958 CE; *n*=819; (Colborne, et al. 2016; Fera, et al. 2017;

340   Kiriluk, et al. 1995; Lumb and Johnson 2012; Mumby, et al. 2018; Rush, et al. 2012; Yuille, et

341   al. 2015)) specimens. ZooMS analyses of 290 samples confirmed or helped to refine 244 non-

342   species taxonomic identifications provided through bone morphology analyses by

343   zooarchaeologists (it confirmed the identification for 225 of these 244 specimens and refined it

344   to the level of species for the remaining 19). In a further 20 cases, specimens were reassigned to

345   another taxon, namely, 12 to lake trout (*Salvelinus namaycush*) (previously assigned with

346   varying degrees of confidence to the species or genus level in *Salmo* [*n*=8], *Salvelinus* [*n*=2], or

347   *Coregonus* [*n*=2]); four to Atlantic salmon (*Salmo salar*) (previously identified with varying

348   degrees of confidence as *Salvelinus namaycush*); four to whitefish (*C. clupeaformis* or *C. artedi*)

349   (previously assigned with varying degrees of confidence to the species or genus level in *Salmo*

350   *salar* [n = 1], *Salvelinus fontinalis* [n = 2], or *Salvelinus namaycush* [n = 1]). In 15 other cases,

351   ZooMS could not reassign specimens (previously assigned with varying degrees of confidence to

352   the Salmonidae family level [n = 3] or to the species or genus level in *Salmo* [*n*=4], *Salvelinus*

353   [*n*=1], or *Coregonus* [*n*=4]) to one of the four species and one genus used in this study. Based on

354   variation in peptide mass fingerprints, these samples likely derive from at least eleven other

355   species.

356   Stable nitrogen isotope compositions of bone and scale collagen from archaeological and

357   historical lake trout (*n*=196), Atlantic salmon (*n*=147), and whitefishes (*n*=239) are summarized

in the SI 5, Table S1 and presented in full in Figure 2 and SI 2. Given the large and well-dated sample (n=1469) from archaeological sites (n=42) and from museum-archived specimens and contemporary fisheries research (n=<50 locations) across a broad geographical range, we believe that the temporal trends observed in fish bone and scale collagen δ^{15}N are representative of processes occurring in Lake Ontario's open water ecosystem at a regional scale.

Discussion and conclusion

1200–1830 CE: Fish δ^{15}N shows remarkably little variation between 1200 CE and 1830 CE (Figure 2), despite centuries of Indigenous and European land management in the Lake Ontario watershed, and provides a unique perspective on the long-term stability of a Great Lakes ecosystem. Prior to the nineteenth century, mean δ^{15}N values binned by taxon and at 100-year intervals vary by less than 0.4‰ and show no statistically significant differences (SI 5, Tables S1 and S2). This long-term stasis, over at least 600 years, suggests that the nitrogen cycle and nitrogenous nutrient inputs for Lake Ontario were highly stable during the past millennium. Analyses of two additional Atlantic salmon specimens (not shown in Figure 2) from earlier archaeological deposits suggests that this stability extends further back in time to at least 900 CE. However, due to a paucity of samples from the earlier time period, discussion will focus on the post–1200 CE time frame. A comparison of mean δ^{15}N for pre-1800 bins (n=4 see SI 5, Table S1) shows steady offsets between species: lake trout +1.6±0.2‰ > Atlantic salmon +1.3±0.2‰ > whitefishes. Replication of this pattern across all three taxa, encompassing multiple trophic levels, suggests that stability was also characteristic of interspecific trophic relationships throughout the broader pelagic food web during this period.

A wealth of historical and paleoenvironmental research on the Lake Ontario watershed (Beeton 1965; Bogue 2001; Estepp and Reavie 2015; Hodell and Schelske 1998; Jeffers, et al. 2015;

381 Meyers 2003; Schelske and Hodell 1991b; Schelske and Hodell 1995; Schelske, et al. 1988;

382 Schelske, et al. 1983; Wood 2000) provides an excellent opportunity to contextualize and

383 consider the implications of this long-term stability. Proxies for past environmental conditions in

384 other, smaller lakes in the same region document anthropogenic disturbances, at least as early as

385 the thirteenth century CE, resulting from Indigenous land clearance and farming activities (e.g.,

386 Ekdahl, et al. 2004). These studies suggest that Indigenous land management practices, including

387 the use of fire for forest clearance followed by crop cultivation (Munoz and Gajewski 2010), had

388 a significant impact on soil erosion, sedimentation, and nutrient loading, causing early cultural

389 eutrophication of aquatic environments in the region. European settlement of the Lake Ontario

390 watershed began in the seventeenth century (Gentilcore 1984). Settlement expansion in the

391 nineteenth century was accompanied by intensive commercial fishing (1800 onward; Bogue

392 2001), diversion of the Niagara River and work on the Erie and Welland canals (1820s;

393 McIlwraith 1976; Wood 2000), broad-scale land clearance for lumber and agriculture (1840s–

394 1850s; Head 1975; Lower, et al. 1938), and wetland loss due to mining and other activities

395 (1880s; Bogue 2001). Paleolimnological studies suggest that productivity of Lake Ontario's

396 phytoplankton community began to grow slowly with the first European settlement, with more

397 substantial impacts occurring in the mid-nineteenth century, probably in response to nutrient

398 loading from soil erosion following deforestation (e.g., Estepp and Reavie 2015; Hodell, et al.

399 1998; Schelske 1991; SI 1). Despite clear historical and paleolimnological evidence for earlier

400 anthropogenic impacts on nutrient dynamics (e.g., nitrogen and phosphorus loading, pollen

401 species change) in other areas of the watershed (Bunting, et al. 1998; Duthie and Sreenivasa

402 1971; Ekdahl, et al. 2004; Ekdahl, et al. 2007; Munoz and Gajewski 2010; Schelske, et al. 1983;

403 Yang, et al. 1993), archaeological fish stable nitrogen isotopic compositions demonstrate that

404 Lake Ontario's nitrogen cycle and food web structure remained unchanged until the 1830s. In

405 that context, and given the sensitivity of aquatic nitrogen cycles to human disturbances

406 (Anderson and Cabana 2005; Botrel, et al. 2014; Lake, et al. 2001), these data underscore Lake

407 Ontario's stability throughout much of the last millennium and demonstrate a degree of

408 resilience at the level of the nitrogen cycle in large lake ecosystems to a variety of human

409 impacts, even when sustained over long time spans.

410 The resilience of Lake Ontario's nitrogen cycle has important implications for understanding

411 human impacts on freshwater ecosystems in the recent and more ancient past. It is now well

412 known that industrial activities over the past 150 years have fundamentally altered global

413 nutrient distributions and biogeochemical cycles (Galloway and Cowling 2002). Within the

414 context of debate about the onset of the Anthropocene (Lewis and Maslin 2015), there is a

415 growing consensus that "pristine environments" probably did not exist where humans were

416 present, because there was always some form of impact from human activities (Heckenberger, et

417 al. 2003). This perspective has been supported by archaeological research showing that ancient

418 societies, particularly those that used agriculture, had significant impacts at both local and

419 regional scales on terrestrial and aquatic nutrient cycles for millennia (Curtis, et al. 1998; Guiry,

420 et al. 2018; Hadley, et al. 2010). Evaluating the role of past human activities as drivers of

421 ecological change has been a priority for archaeologists (Kintigh, et al. 2014), but is complicated

422 by the fact that ancient anthropogenic impacts on the environment are spatiotemporally

423 heterogeneous and occur along a continuum of intensity (from very significant to ephemeral).

424 For ecologists, this issue represents a source of uncertainty for retrospective studies where

425 archaeological or paleobiological information is not available to establish when and to what

426 extent past human activities have impacted a particular environment or biotic community

427   (Holmes 2006; Lyman and Cannon 2004). In contrast to the cautionary tone of the debate about

428   the existence of "pristine" environments, our data demonstrate that, in fact, nutrient dynamics

429   and trophic relationships in larger aquatic environments can remain unchanged despite centuries

430   of moderate human land use.

431   *Post-1830:* Our analyses of archaeological and historical fish also show that this long-term

432   stability was profoundly altered when Lake Ontario's nutrient pools experienced an abrupt shift

433   in stable nitrogen isotope composition, becoming enriched in ^{15}N throughout the pelagic

434   ecosystem. During the early to mid-nineteenth-century, fish δ^{15}N values underwent a

435   simultaneous, statistically significant elevation across multiple trophic levels, a difference that

436   has been sustained throughout the twentieth century and to this day (SI 4, Table S3). Atlantic

437   salmon, for instance, which had previously had a highly conserved distribution of δ^{15}N values,

438   show an elevation of +3‰ in well-dated mid-nineteenth-century museum specimens. Although a

439   paucity of fish samples from the later nineteenth and early to mid-twentieth century precludes

440   detailed comparison, these data show similar overall trends (positive shifts between intraspecific

441   δ^{15}N means for pre-1800 and post-1990, ranging from +5.3‰ to +6.2‰, for all fish species) to

442   those observed in Lake Ontario's isotopic records from sediment cores (+5.5‰ to +5.6‰ for c.

443   1850 to 1990s; Hodell and Schelske 1998; Figure 2). This further indicates that the observed

444   upward pattern in fish δ^{15}N predominately reflects a bottom-up shift in baseline δ^{15}N rather than

445   changes to fish trophic behavior.

446   It is nonetheless apparent from these data that human activities have resulted in meaningful

447   behavioral changes, including increased niche overlap between Lake Ontario's salmonid

448   communities. Prior to 1800, there is minimal overlap between the δ^{15}N ranges for each species,

449   with overlaps of only 0.1‰ between Atlantic salmon and whitefishes and only 0.9‰ between

450 Atlantic salmon and lake trout over a 600-year period. In contrast, in the twentieth century, there

451 is substantial overlap between $\delta^{15}N$ ranges for all species in all 25-year temporal bins (overlap

452 ranges from 1.7 to 4.7‰). However, caution is required when interpreting trends in variation

453 across time in this dataset. Differences in tissue turnover rates for sample materials between pre-

454 nineteenth- (mainly collagen from bones – slow turnover) and twentieth-century (mainly

455 previously published muscle from modern fish – fast turnover) time frames make detailed

456 analysis of these trends difficult because tissues with faster turnover rates are inherently more

457 likely to produce greater isotopic variation reflecting seasonal or other short-term dietary

458 aberrations. With that caveat in mind, we argue that it is likely that the greater isotopic variation

459 in twentieth-century specimens reflects changes in energy pathways and foraging behavior in

460 response to major anthropogenic environmental disruptions (e.g., impacts from invasive species,

461 extirpations, or over fishing; Bogue 2001; Colborne, et al. 2016; Dymond, et al. 2019; Fera, et al.

462 2017; Mills, et al. 2003).

463 The interspecific synchronicity and magnitude of this shift could have been caused by multiple

464 related processes (Figure 3), including changes in the nitrogen cycle (at the phytoplankton-DIN

465 pool level) favoring nitrogen transformations that retain ^{15}N and changes in nitrogen inputs to the

466 system that include ^{15}N-enriched nutrients (Finlay and Kendall 2007; Kendall 1998; Kendall, et

467 al. 2007; Talbot 2001). Detailed historical and archaeological dating provides a robust temporal

468 framework to evaluate which of these variables, and therefore what type of human activity, drove

469 this initial ecosystem-wide ^{15}N enrichment in Lake Ontario's food web. At archaeological sites

470 with occupation dates as late as 1832, many fish show no sign of $\delta^{15}N$ elevation for their

471 respective taxon, whereas a museum specimen dating from no later than 1857 shows

472 significantly elevated $\delta^{15}N$ relative to the preceding centuries. In addition, many specimens from

473   archaeological contexts with date ranges spanning this *terminus ante quem* (1832) and *terminus*

474   *post quem* (1857) show elevated $\delta^{15}N$ values. These data therefore provide a temporal bracket for

475   the initiation of change in Lake Ontario's nitrogen cycle, demonstrating that the shift occurred

476   abruptly (within the context of the stability of the preceding 900 years), within a relatively short,

477   15–25-year window (1830s–1850s). This timeframe coincides precisely with the emergence of

478   broad-scale land clearance for agriculture and timber harvesting in the Lake Ontario watershed.

479   From the 1830s onward, especially around the middle of the century, historical analyses

480   document how forestry activities, namely severe cutting as well as widespread burning of the

481   remaining slash, radiated outward from the Lake Ontario waterfront to encompass much of the

482   watershed. These forestry activities accelerated over time due to increased demand for timber

483   (Head 1975) and cleared land, improved export capacity (canals and railroad; Lower, et al. 1938;

484   McIlwraith 1976), and growing sawmill infrastructure (Head 1975; Wood 2000).

485   During this period, between the 1820s and 1850s, the then-province of Upper Canada (which

486   included the Ontario portion of the Lake Ontario watershed) had the fastest growing population

487   in all of North America (Lewis 2001). Prior to 1830, newly settled farmers of European heritage

488   focused on subsistence (Kelly 1973; Kelly 1975) and put considerable effort into clearing land

489   for cultivation through cutting and burning (Ball 1979). However, relative to the mid-nineteenth

490   century, these early farming activities were patchy, small in scale, and diversified in purpose,

491   with most of the land granted to settlers remaining forested (Kelly 1975). Moreover, much of the

492   land that was cleared retained stabilizing features of the previous forest, including stumps, root

493   mats, and stones, for up to a decade, and sometimes much longer, while under cultivation and

494   these would have mitigated issues with soil erosion and nutrient loss (Ball 1979; Kelly 1975).

495   During this early period, in which the farmers were themselves clearing their land, care was

496   often taken throughout the processes, from cutting trees to sowing crops (delaying ploughing), to

497   further minimize soil nutrient loss (Ball 1979; Kelly 1971). As farms became established,

498   however, stumps were removed, ploughs were more widely employed to break up the soil, and

499   more land came under cultivation. Throughout the 1830s and 1840s, the agricultural system of

500   Upper Canada was dominated by plough-intensive (three or more times per biennial fallow) and

501   highly profitable monocropping of wheat (Kelly 1971; Kelly 1973). The focus on a wheat-

502   fallow-wheat agriculture system, as well as labor-saving furrow drainage and general avoidance

503   of manure-based crop fertilization (until the 1850s) were widely blamed for rapid depletion of

504   soil nutrients as well as erosion across the region (Kelly 1971; Kelly 1975). In this context, prior

505   to the 1880s, when a wider appreciation developed among farmers in the Lake Ontario watershed

506   of the risks of higher runoff-to-percolation ratios that come with overly cleared lands, farmland

507   was subjected to a damaging seasonal cycle of spring floods, washing away nutrient-rich organic

508   materials, and summer droughts, parching what soil remained (Kelly 1975).

509   The isotopic composition of reactive forms of nitrogen in terrestrial ecosystems (i.e., soil

510   nutrients including ammonia and nitrate), particularly those used for agriculture, is typically

511   elevated relative to aquatic nitrogen sources (i.e., DIN) due to intense soil denitrification (Botrel,

512   et al. 2014; Heaton 1986; Kendall 1998). In this context, the most parsimonious explanation for a

513   broad-scale isotopic shift in Lake Ontario's nitrogen cycle is that there was an increase in ^{15}N-

514   enriched nutrient inputs coming directly from soil erosion following deforestation during the

515   1840s in Lake Ontario's watershed area (Figure 3). This interpretation is supported by

516   paleolimnological data (diatoms, sediment accumulation rates, and elemental and isotopic

517   compositions of organic matter) from across the Lake Ontario watershed that records an uptick in

518   nutrient loading from soil erosion runoff over the course of the mid-nineteenth century (Hodell

519 and Schelske 1998). The relatively early timeframe for the observed shift in fish nitrogen

520 isotopic compositions also suggests that its cause lay in new nutrient inputs originating primarily

521 within the local watershed area, rather than from other Great Lakes, because upstream

522 watersheds such as Lake Erie had not been as extensively settled by this time.

523 While inputs of ^{15}N-enriched terrestrial nutrients, released from eroding soils across the

524 watershed, could easily result in the observed isotopic pattern, additional related processes,

525 occurring both on land and in the water, could further contribute to the elevation of the stable

526 nitrogen isotopic composition of Lake Ontario's biota (Figure 3). Higher soil erosion rates would

527 increase mobilization of terrestrial nitrate across oxygen-poor ecotones (i.e., riparian/wetland

528 zones), promoting bacterial denitrification, leaving the residual nutrients that are eventually

529 delivered to Lake Ontario relatively enriched in ^{15}N (Bowden 1987; Knowles 1982; McClain, et

530 al. 2003). Increases in anthropogenic soil disturbance from farming and use of fertilizers

531 (pushing the nitrogen cycle to be more open; Szpak 2014), as well as changes to pH in aquatic

532 environments, may promote volatilization of ammonia before or during nutrient transport to

533 Lake Ontario's food web (Kendall 1998). Increased phosphorous loading from soil runoff would

534 also increase primary productivity, and the resulting bottom-up shift would have increased

535 demand on Lake Ontario's pool of available reactive N, thereby reducing discrimination against

536 ^{15}N during assimilation of DIN by phytoplankton (Hodell and Schelske 1998). Regardless of

537 which of these processes were most facilitative of the nitrogen isotopic patterning in Lake

538 Ontario's fish community, the timing (between 1832 and 1857) and abruptness of the ecosystem-

539 wide shift indicates that the first human activity to have broad-scale impacts on nitrogenous

540 nutrient pathways in the Great Lakes was likely deforestation.

541   It is also worth noting that the time period covered by this study includes significant global

542   climatic fluctuations, including the Medieval Climate Anomaly (MCA; 950–1250 CE) and the

543   Little Ice Age (LIA; 1300–1850 CE). Paleolimnological studies of Lake Ontario (McFadden, et

544   al. 2005), Lake Erie (Finkelstein and Davis 2006), and other lakes in the local (Keizer, et al.

545   2015; Mullins, et al. 2011; Paquette and Gajewski 2013; Stager, et al. 2017) and broader region

546   (Laird, et al. 2012) show that climatic variability associated with both the MCA and LIA did

547   have some impact on aquatic and terrestrial environments across the Great Lakes and St.

548   Lawrence River region. For instance, evidence from flux in biogenic silica sedimentation rates

549   and changes in diatom (e.g., *Fragelaria* spp.) communities (Finkelstein, et al. 2005; McFadden,

550   et al. 2005) demonstrates that these periods of climatic change were felt in the Lake Ontario

551   watershed over the past 1000 years. Climatic variability could affect Lake Ontario's nitrogen

552   cycle and the nitrogen isotopic composition of fish through changes in adjacent wetland size

553   (important sites for denitrification; Bowden 1987; McClain, et al. 2003), terrestrial runoff

554   impacts (Anderson and Cabana 2005; Heaton 1986), fluctuating dissolved oxygen levels

555   (controlling nitrification and denitrification rates; Knowles 1982), and changes to productivity

556   and thermally regulated stratification (seasonal draw-down of DIN; Hodell and Schelske 1998).

557   Assuming that the influence of these potential sources of environmental variation did not cancel

558   one another out, our data suggest that late Holocene climatic variation did not influence Lake

559   Ontario's nitrogen cycle or trophic structure.

560   The temporal as well as taxonomical detail offered by isotopic data from archaeological fish

561   specimens has provided a framework for higher resolution interpretation of food web dynamics

562   as well as the timing and likely cause of the first human impacts on Lake Ontario's nitrogen

563   cycle. While previous isotopic analyses of organic matter from sediments (SI 1 and Figure 2;

564 Hodell and Schelske 1998; McFadden, et al. 2004) have demonstrated that Lake Ontario's

565 nitrogenous nutrients were becoming ^{15}N enriched during the nineteenth century, temporal

566 differences between datasets as well as a dearth of pre-1850 data prevented detailed analyses of

567 the processes which drove this change in lake-wide nitrogen cycling (SI 1). In addition, although

568 a variety of paleoenvironmental indicators (e.g., biogenic silica; Schelske 1991; Schelske and

569 Hodell 1991b; Schelske, et al. 1988) have shown human activities, such as deforestation, had

570 begun to have major nutrient (particularly P) loading impacts on the Lake Ontario watershed

571 from the mid-nineteenth century onward (although see, McAndrews and Boyko 1972; Schelske,

572 et al. 1983), our results demonstrate that Lake Ontario's nitrogen cycle had already been

573 substantially impacted decades earlier. Finally, by analyzing material from known taxa, we have

574 been able to document the impact that these changes in lake-wide nutrient dynamics had on long-

575 term food web structure.

576 *Anthropological and Ecological Implications:* This study is the first detailed analysis of long-

577 term isotopic variation in freshwater fish. Results suggest that, by altering aquatic nutrient inputs,

578 nineteenth-century European forestry practices in the lower Great Lakes region, whether focused

579 on timber extraction or producing cleared land for agriculture, left a durable isotopic signature on

580 fish in Lake Ontario. While the findings of this research focus on the Great Lakes watershed, the

581 implications are global; human impacts on aquatic nutrient dynamics, particularly the nitrogen

582 cycle, may be detectable in archaeological fish remains wherever land management has

583 extensively modified the balance of nutrient exchange between local terrestrial and aquatic

584 landscapes. Our results are particularly germane to recent discussion in conservation, calling for

585 increased attention to how human impacts on land may be a driver of environmental

586 deterioration in adjacent aquatic ecosystems (Abell 2002). For this reason, our findings have

587 significant potential to serve as a model for research that seeks to understand fundamental

588 changes in the way past societies, through forestry and other land management practices,

589 affected aquatic nutrient dynamics around the globe (Jenny, et al. 2019).

590 With respect to debate on the origins of the Anthropocene, our results provide an important

591 waypoint for the earliest lasting anthropogenic alteration of one of the world's great freshwater

592 ecosystems. By identifying the tipping point at which human activities began to reorganize

593 nutrient dynamics in the Great Lakes region, our results do not reveal a "golden spike" indictor

594 for the starting point of the Anthropocene (Lewis and Maslin 2015), but, rather, provide a new

595 signpost along the path of the global expansion of industrial impacts. These changes to Lake

596 Ontario nonetheless represent a profound shift in the way humans have impacted the Great Lakes

597 environment and illustrate some important anthropological and ecological considerations for how

598 we can approach aquatic human–environment relationships in archaeology and historical ecology

599 moving forward.

600 From an ecological perspective, an ecosystem-wide, bottom-up alteration of the nitrogen cycle of

601 one of the world's largest freshwater environments over the course of only one or two decades,

602 as shown here for Lake Ontario, demonstrates an unprecedented shift in the nature and scale of

603 how humans cause environmental change. Understanding how humans have shaped earth's

604 biological and physical systems remains an important archaeological and ecological challenge

605 because finding lasting evidence of human impacts within complex regional or global systems is

606 rare. This is particularly true for our understanding of ancient environmental nutrient dynamics,

607 such as the cycling of nitrogen throughout an ecosystem, which, although fundamental to the

608 success of human societies both today and in the past, preserves relatively few traces of change.

609 Not only do our findings demonstrate how isotopic analyses of archaeological fauna can provide

610 a clearer overview of ancient nutrient dynamics, they do so in one of the most enigmatic arenas

611 of human–environment interaction, namely, aquatic ecosystems. Even today, in comparison with

612 terrestrial environments, surprisingly little is known about how escalating human exploitation of

613 the world's aquatic environments will impact these vital ecosystems in the long term (Abell

614 2002). In that context, deeper retrospectives that are based on integrating results of contemporary

615 studies with data from both historical and archaeological resources, as demonstrated by this

616 study, have the potential to significantly enhance our understanding of the sensitivity of different

617 environments to human disturbances (Barak, et al. 2016; Dubois, et al. 2018; Jeffers, et al. 2015;

618 McLauchlan, et al. 2013).

619 From an anthropological perspective, approaching questions of human–environment interactions

620 from the vantage point of nutrient dynamics could provide a novel outlook on the role of humans

621 as drivers of ecological change. While the relationship between humans and their environment

622 has traditionally been framed against a backdrop of such factors as technological change and the

623 development of social complexity as sources of evidence for macro-scale exploitation of

624 environmental resources (Kintigh, et al. 2014), there is an increasing recognition that an

625 understanding of how humans have influenced their landscape at a molecular level can provide

626 powerful new lines of evidence for previously invisible but profound shifts in human–

627 environment relations (Guiry, et al. 2018). In particular, throughout most times and places in

628 human history, the carrying capacity of local terrestrial and aquatic ecosystems, which are the

629 environmental foundations upon which societies are sustained, has hinged on the maintenance of

630 well-balanced biogeochemical cycles and nutrient regimes. With respect to lake and other

631 freshwater ecosystems, which have been and continue to be of pivotal importance to many

632 cultures, it is possible to use isotopic analyses of local fauna to look back in time and pinpoint

633 where, when, and in that context perhaps even how, human activities reached a turning point to
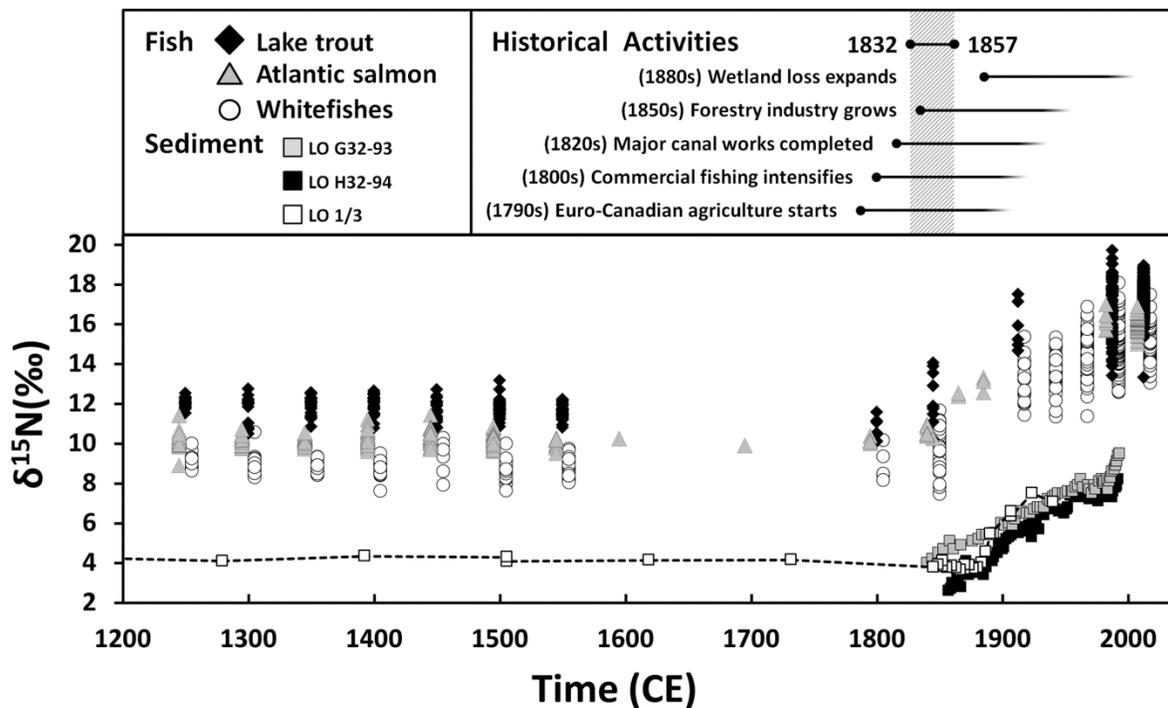
634 become dominant drivers in their ecosystem.

635

636 <u>Acknowledgments</u>

662

Figure 1. Map of study area showing locations of archaeological sites from which fish bone

samples were collected. Map data from NASA and ESRI.

665

Figure 2. Stable nitrogen isotope values for fish (*n*=1469) versus time. Data are binned by 50-

year (archaeological) and 25-year (historical/modern) intervals. For fish data sourced from the

literature (Colborne, et al. 2016; Fera, et al. 2017; Guiry, et al. 2016a; Kiriluk, et al. 1995; Lumb

and Johnson 2012; Mumby, et al. 2018; Rush, et al. 2012; van der Merwe, et al. 2003; Yuille, et

al. 2015). For sediment data sourced from the literature: LO G32-93, LO H32-94 (Hodell and

Schelske 1998), and LO 1/3 (McFadden, et al. 2004).

Pre 1830-50s

TERRESTRIAL N
↓ nutrient export
↓ $\delta^{15}N$
- • ↓ denitrification
- • ↓ NH_3 volatalization

AQUATIC N
↓ nutrient inputs
↓ $\delta^{15}N$
- • ↓ productivity
- • >N:P

Post 1830-50s

TERRESTRIAL N
↑ nutrient export
↑ $\delta^{15}N$
- • ↑ denitrification
- • ↑ NH_3 volatalization

AQUATIC N
↑ nutrient inputs
↑ $\delta^{15}N$
- • ↑ productivity
- • <N:P
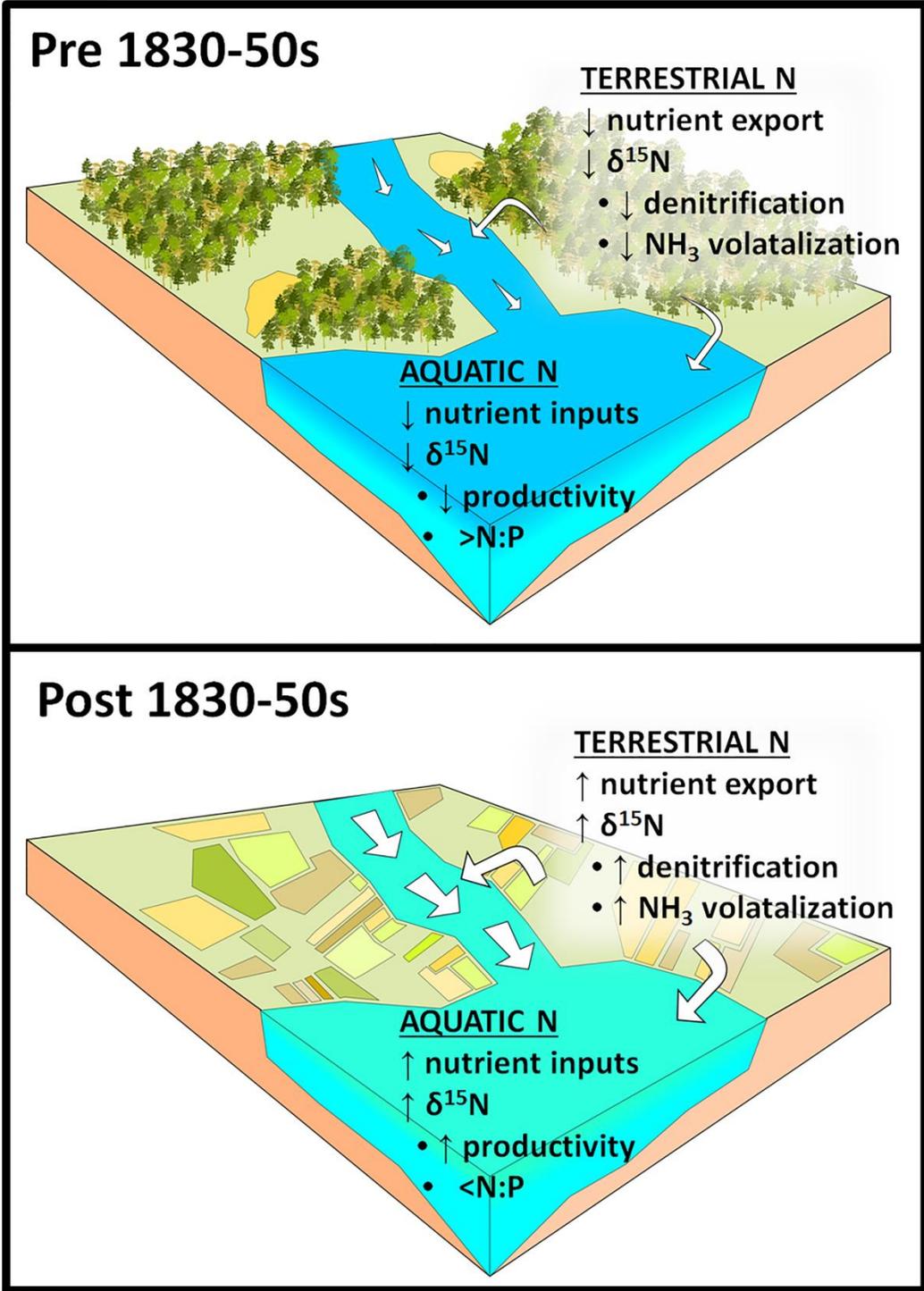
Figure 3. Schematic diagram of nutrient regime shifts (nitrogen sources and isotopic compositions) that could be associated with the intensification of deforestation and agriculture in the Lake Ontario watershed during the early to mid-nineteenth century.

678   Supplementary Information

679   **SI1** Paleoenvironmental context

680   **SI2** Isotopic results

681   **SI3** Calibration and analytical uncertainty

682   **SI4** Collagen peptide finger-printing results

683   **SI5** Statistical results

684

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