Ecological tracers track changes in bird diets and possible routes of exposure to Type E Botulism

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ABSTRACT

Dreissenid mussels have become important components of the Great Lakes biological community since their introduction in the 1980s, but much remains to be understood regarding their effect on energy and nutrient flows in pelagic systems. Here, we report a new method that tracks incorporation of resources of molluskan origin into food webs used by aquatic birds. Biochemical tracers (fatty acids and stable carbon isotopes) are used to characterize species associated with pelagic and benthic food webs in Lake Ontario. Our focus is on the polymethylene-interrupted fatty acids (PMI-FAs) because previous research identified mollusks as their primary source. We found that PMI-FA mass fractions were greater in organisms associated with benthic (e.g. round goby) versus pelagic (e.g. alewife) food webs. Double-crested cormorants that had recently consumed benthic prey fish, i.e. goby, had greater proportions of PMI-FAs in their blood plasma than birds which showed no signs of recent goby ingestion. We did not detect an increase in mass fractions of PMI-FAs in cryogenically archived cormorant eggs following expansion of dreissenid mussels in Lake Ontario. However, following the introduction and expansion of round goby in the lake, PMI-FAs were detected at greater levels in cormorant eggs. These results illustrate how only after dreissenid mussel-facilitated establishment of round goby was the full extent of exotic species disruption of food webs manifested in fish-eating birds. These food web changes may be contributing to negative impacts on aquatic birds exemplified by the emergence of Botulism Type E as a significant mortality factor in this ecosystem.
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Introduction

Significant changes in the structure of Great Lakes aquatic communities have occurred through time, particularly in recent decades. A major factor contributing to these changes has been the introduction of exotic species. A notable example is the widespread colonization of the Great Lakes by dreissenid mussels (i.e. zebra mussel, *Dreissena polymorpha* and quagga mussel, *Dreissena bugensis*). Their introductions have resulted in a wide variety of physical (Johannsson et al., 2000), chemical (Hecky et al., 2004), and biological (Dermott, 2001; Johannsson et al., 2000; Nalepa et al., 2007) changes. Dreissenid mussel-mediated changes have led to a re-directing of pelagic production to the benthos resulting in ecosystem "benthification" (Mills et al., 2003). Pioneering organisms, such as dreissenids, that modify habitat and alter resource availability for other species in such significant ways have been described as ecosystem engineers (Jones et al., 1994).

The establishment of dreissenid mussels in the Great Lakes likely played an important role in facilitating the establishment of another invading species, the round goby (*Neogobius melanstomus*) (Barton et al., 2005; DeVanna et al., 2011; Ray and Corkum, 1997; Vanderploeg et al., 2002). These species co-evolved in the Ponto-Caspian region (Vanderploeg et al., 2002). While dreissenids are utilized as food by some native Great Lakes species (Madenjian et al., 2010), predators are limited to those that are morphologically adapted for crushing mussels. Therefore, many native species cannot make efficient use of this food resource. Conversely, round goby consume dreissenids routinely (Ghedotti et al., 1995; Ray and Corkum, 1997). Since its introduction in the 1990s, the round goby rapidly established abundant populations in all of the Great Lakes except Lake Superior (Gorman and Bunnell, 2011).

Mussel/round goby benthification of the Great Lakes, in conjunction with other factors, may be contributing to reductions in energy and nutrient flows in pelagic systems. For example, pelagic prey fish (e.g. alewife (*Alosa pseudoharengus*); rainbow smelt (*Osmerus mordax*)) declined greatly in all of the Great Lakes in recent decades (Gorman and Bunnell, 2011; Hebert et al., 2008; Roseman and Riley, 2009). In response to declines in availability of pelagic prey, higher trophic level predators may increase their reliance on benthic prey (Hebert et al., 2008; Rennie et al., 2009). To evaluate this possibility more thoroughly, effective tools are required for assessing temporal changes in diet.

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Traditional methods of assessing diet composition in birds often rely on examination of gut contents which usually requires lethal sampling (Johnson et al., 2010). However, ecological tracers, e.g. fatty acids and stable isotopes of carbon, offer alternative approaches for assessing diet (e.g. Hebert et al., 2008) and refinement of these methods to address specific dietary questions is ongoing. One promising group of tracers for assessing use of mollusks by higher trophic levels is the polymethylene-interrupted fatty acids (PMI-FAs) (Mezek et al., 2011). PMI-FAs are characterized by the presence of multiple methylene groups between adjacent double bonds in the aliphatic chain. This structure contrasts with other fatty acids that contain only one methylene group between adjacent double bonds. PMI-FAs have been found primarily in marine bivalves and gastropods (Budge et al., 2006, 2007; Saito, 2007), and they are also transferred to higher trophic level organisms that prey on these invertebrates (Budge et al., 2007). Recent work in the Great Lakes has identified PMI-FAs in freshwater mollusks and their predators but not in pelagic organisms (Mezek et al., 2011). Hence, this group of ecological tracers has potential as a marker of the degree to which higher trophic level organisms are utilizing benthic food webs in which mollusks are a major component.

Here, we quantify PMI-FAs in benthic and pelagic organisms from Lake Ontario and, for the first time, report on their occurrence in fish-eating birds. We further examine how diet composition can affect ecological tracer profiles in double-crested cormorant (*Phalacrocorax auritus*) tissue by quantifying $\delta^{13}C$ values and PMI-FA levels in blood plasma relative to recent diet evaluated through gut content analysis. Finally, we assess temporal trends in avian PMI-FA concentrations using archived cormorant eggs. Changes through time are interpreted relative to estimated date of arrival and population expansion of dreissenid mussels and round goby in Lake Ontario. We discuss implications of these food web alterations to aquatic birds emphasizing their role in contributing to the emergence of Type E Botulism as a significant avian mortality factor in this ecosystem.

Type E Botulism is one of seven strains of botulism. It is closely associated with aquatic environments and has been a cause of death primarily in fish-eating birds (CCWHC, 2012). *Clostridium botulinum* spores are widespread in sediments of the Great Lakes. Under anoxic conditions, in the presence of a nutrient substrate, and at optimum temperature, the spores germinate and produce botulinum toxin. The toxin can then be transferred through food webs causing mortality in consumers. Exposure to botulinum toxin through recent food intake kills birds quickly. Since 2002, Type E Botulism outbreaks have resulted in the deaths of thousands of aquatic birds on Lake Ontario during the breeding season and stopover periods on migration (Shutt et al., 2014–in this issue). For example, botulism-related mortality is likely responsible for the extirpation of Lake Ontario's breeding population of the great black-backed gull (*Larus marinus*) (Shutt et al., 2014–in this issue). Here, we use PMI-FAs to determine if there is evidence of temporal changes in fish-eating bird diets that may be making them more susceptible to botulism mortality.

Methods

Food web collections

Quagga mussels (n = 15) were collected from deep waters of eastern Lake Ontario in June 2007. PMI-FAs were extracted from combined foot and mantle tissues. Quagga mussels are the focus here as PMI-FA patterns are similar to those in zebra mussels (Mezek et al., 2011) and quagga mussels constitute the bulk of dreissenid biomass in Lake Ontario (Wilson et al., 2006). In July 2008, freshly caught alewife (n = 11) and round goby (n = 10) specimens were collected from the Kingston Basin (44.05N, 76.85W) in eastern Lake Ontario. These two species were selected as being representative of pelagic (alewife) and benthic (round goby) food webs. Whole frozen fish were homogenized cryogenically using a ball mill. These whole body homogenates were used for all analyses. Samples were stored at −85 °C until analyzed.

In 2009, breast muscle and blood plasma were collected from adult Caspian terns (*Hydroprogne caspia*, n = 2, August 12) and adult double-crested cormorants (*P. auritus*, n = 2, September 1) at False Duck Island (43.95N, 76.82W) in the Kingston Basin of eastern Lake Ontario. These birds showed signs of botulism intoxication, e.g. paralysis, inability to fly or walk, and inability to hold head upright. The birds were captured live by hand, killed in the field by decapitation, and immediately dissected, and tissue samples collected. Whole blood was collected from each adult and placed in a heparinized microcentrifuge tube. Tubes containing whole blood were centrifuged for 5 min at 14,000 rcf (relative centrifugal force) to separate red blood cells and plasma. Plasma samples were immediately placed in liquid nitrogen until they could be transferred to a −85 °C freezer in the laboratory. Sample sizes were small because of the limited availability of moribund birds, and this reflected the short window of opportunity for sampling affected birds prior to their death. All procedures involving the use and handling of animals were carried out according to protocols approved by the Animal Care Committee at the National Wildlife Research Centre (NWRC).

Terns and cormorants have varied diets and take advantage of any readily available prey. However, there are differences in their foraging behavior. Caspian terns are plunge divers that feed within 0.5 m of the water's surface (Cuthbert and Wires, 1999). Double-crested cormorants dive to depths of at least 12 m (Hatch and Weseloh, 1999) to capture primarily fish prey (Johnson et al., 2010). Because of these differences in foraging ability, terns will consume benthic prey to a much more limited degree than cormorants. To characterize the fatty acid signatures typical of these species, we chose to analyze muscle tissue as it provides an integrated assessment of diet over a relatively long period of time; i.e. weeks (Hobson and Clark, 1992). Hence, even if there was commonality in recent diet resulting in similar botulism exposure in both species, muscle tissue would provide a longer-term assessment of diet typical of dietary differences between the species. To assess recent diet, PMI-FAs in blood plasma were measured in the same birds. PMI-FA levels in plasma likely reflected recent food intake (Käkelä et al., 2005, 2009).

Also in 2009, pre-fledged double-crested cormorant chicks (~21 days old) were killed by decapitation on Pigeon Island (44.07N, 76.55W) in the Kingston Basin of eastern Lake Ontario (n = 10, June 22). These collections were done as part of another study examining endoparasites in fish-eating birds. Whole blood was collected from each chick in an identical manner as described for adult birds. Chick stomach contents were also collected for later identification of cormorant prey (chicks obtain regurgitated food from their parents). As above, blood plasma was chosen for fatty acid and stable carbon isotope analysis because the signatures of these tracers in plasma are thought to most likely reflect that of recently ingested food, i.e. dietary intake over hours to days (Hobson and Clark, 1993; Käkelä et al., 2005, 2009). The degree to which plasma ecological tracer patterns reflected recent diet was tested by determining whether there were differences in the carbon isotope and fatty acid composition of plasma in chicks whose diets differed based upon gut content analysis.

Double-crested cormorant egg samples, collected from Pigeon Island in 1990, 1995, 2005, 2006, and 2008, were retrieved from the National Wildlife Specimen Bank at NWRC. Five eggs, each from a different nest, were collected for the 5 time points (total = 25 eggs). Archived samples consisted of homogenized whole egg contents (yolk and albumen) that had been stored at −40 °C since collection. Eggs sampled in 1990 represent a period soon after dreissenid mussels were first discovered in Lake Ontario (1989) but prior to population expansion (Mills et al., 2003). Eggs collected in 1995 represent a time when dreissenids were well established in the lake but round goby had not yet arrived (Mills et al., 2003). Egg samples from 2005 and later represent a period of high round goby abundance (Gorman and Bunnell, 2011; Johnson et al., 2010).

Sample analysis

Details regarding sample processing, lipid extraction, fatty acid methyl ester derivatization, and analysis by gas chromatography are provided in Mezek et al. (2011). Mass fractions ($\mu g \cdot mg^{-1}$ DW) of six PMI-FAs were measured: three 20 carbon chain PMI-FAs ($20{:}2n-9$, $20{:}2n-7$, $20{:}3n-6$) and three 22 carbon chain PMI-FAs ($22{:}2n-9$, $22{:}2n-7$, $22{:}3n-6$). These PMI-FAs were identical to those reported in Mezek et al. (2011). Limits of detection for individual PMI-FAs were 0.025 $\mu g \cdot mg^{-1}$ DW except for $22{:}3n-6$ which was 0.03 $\mu g \cdot mg^{-1}$ DW.

To allow detailed examination of fatty acid composition in fish, 36 other fatty acids were quantified in addition to the PMI-FAs. Individual fatty acid mass fraction data were expressed as percentages of total FA mass fraction. This approach facilitated comparisons across species. Details regarding the measurement of these fatty acids are provided elsewhere (Hebert et al., 2006, 2008). Principal component analysis (PCA) was used to summarize differences in fatty acid composition in pelagic (alewife) versus benthic (round goby) fish. PCA was done using correlation matrices and included 18 individual FAs (those with % values constituting at least 0.5% of total measured FAs) and total PMI-FAs. All statistical analyses (t-tests, correlation analysis, χ^2 analysis) were conducted using Statistica (Statsoft, Inc., 2005).

Stable carbon isotopes ($^{13}C/^{12}C$) were analyzed to provide an independent measure of the degree to which organisms may have been utilizing pelagic versus benthic/littoral habitats (France, 1995). Carbon stable isotopes were measured in alewife, round goby, cormorant chick blood plasma samples, and archived double-crested cormorant eggs. This involved encapsulation of 1.0 ± 0.2 mg (DW) in tin cups (6×4 mm). Stable carbon isotope measurements were conducted at the University of Ottawa's G.G. Hatch Stable Isotope Laboratory using standard isotope ratio mass spectrometry, as described in Hebert et al. (1999) and Braune (2007). Briefly, samples were freeze-dried and lipids removed using three 2:1 chloroform:methanol rinses. These lipid-free subsamples were used for stable carbon isotope analysis. Encapsulated samples were combusted at 1800 °C in a VarioEL III elemental analyzer (Elementar, Hanau Germany) followed by on-line analysis by continuous flow with a Delta Plus Advantage isotope ratio mass spectrometer coupled to a ConFlo II (Thermo Scientific, Bremen Germany). Stable carbon isotope results (‰) were expressed in delta (δ) notation, $\delta = ((Rx - Rstd) / Rstd) * 1000$, where 'R' is the ratio of the abundance of the heavy (^{13}C) to the light (^{12}C) isotope, (x) denotes the ratio in a sample and 'std' denotes the ratio in a standard (i.e., Pee Dee Belemnite limestone). Analytical precision, based upon repeated measurements of a standard was $\pm 0.2‰$.

Results

Mean mass fractions (± 1 SD) of individual PMI-FAs and total PMI-FAs in quagga mussel, alewife, round goby, Caspian tern muscle, cormorant muscle, tern plasma, and cormorant plasma are shown in Fig. 1. PMI-FA levels in quagga mussel were dominated by $22{:}2n-9$ and $22{:}3n-6$. These PMI-FAs were also present in organisms associated with benthic food webs, i.e. round goby and double-crested cormorant. In organisms primarily associated with pelagic food webs, i.e. alewife and Caspian tern, very little of these PMI-FAs were found in samples (i.e. whole body/muscle) indicative of long-term "typical" diet. Total PMI mass fractions in round goby were approximately 13 times greater than those in alewife. In bird muscle, total PMI levels were approximately four times greater in cormorants than terns (t-test, $t(2) = -6.3$, $p = 0.03$). Although total PMI mass fractions in plasma were lower in terns than cormorants, this difference was not statistically significant (t-test, $t(2) = -2.5$, $p = 0.14$).

Examination of overall fatty acid composition (% of total) in pelagic (alewife) and benthic (round goby) fish revealed further differences. PCA scores separated the species along principal component 1 (PC1 explained 42% of the variance in the fatty acid percent composition data) (Fig. 2). Mean PC1 scores were significantly different between the two species (Welch's t-test, $t(19) = -9.5$, $p < 0.001$). Round goby were characterized by greater percent contributions of total PMIs, palmitoleic acid ($16{:}1n-7$), and omega-3 docosapentaenoic acid (DPA) whereas alewife had greater proportions of the shorter chain saturated fatty acids (14:0, 15:0, 17:0), eicosenoic acid ($20{:}1n-9$), and oleic acid ($18{:}1n-9$). PC1 scores for individual fish were correlated with their $\delta^{13}C$ values ($r(19) = 0.76$, $p < 0.001$; Fig. 2).

Gut contents of double-crested cormorant chicks revealed differences in recent diet among birds. Six of the 10 chicks had round goby remains in their stomachs (categorized as "goby" chicks); the other 4 did not ("non-goby" chicks.). Plasma %PMI values were greater in chicks having round goby in their stomachs (Welch's t-test, $t(8) = 4.6$,
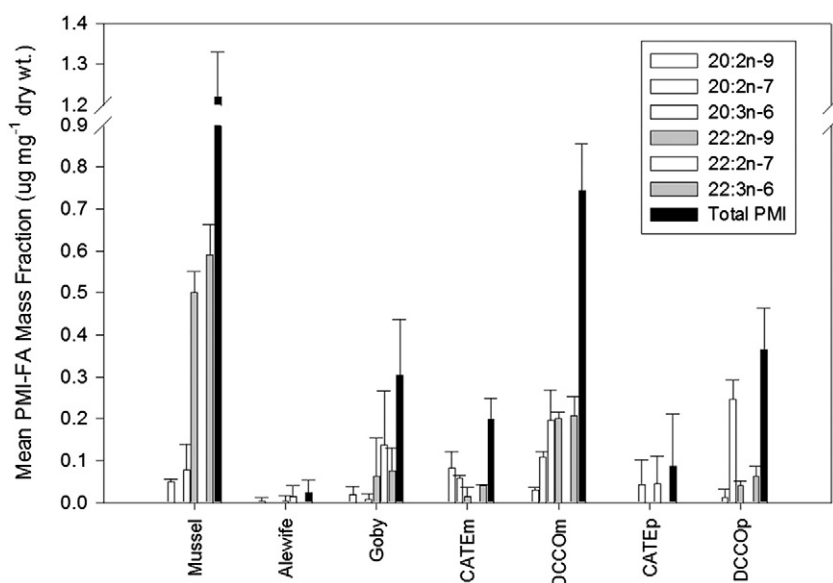
Fig. 1. Mean (± 1 SD) mass fraction of polymethylene-interrupted fatty acids (PMI-FAs) in species associated with benthic and pelagic food webs in Lake Ontario, Canada. Species shown are quagga mussel (whole body excluding shell), alewife (whole body), round goby (whole body), Caspian tern (CATEm, breast muscle), double-crested cormorant (DCCOm, breast muscle), tern (CATEp, plasma), and cormorant (DCCOp, plasma). PMI-FAs with higher mass fractions in dreissenids are shaded gray, and other PMI-FAs are shown in white. Total PMI-FA values are shaded black. The PMI-FAs are given in the legend and displayed from left to right in the bar graphs.
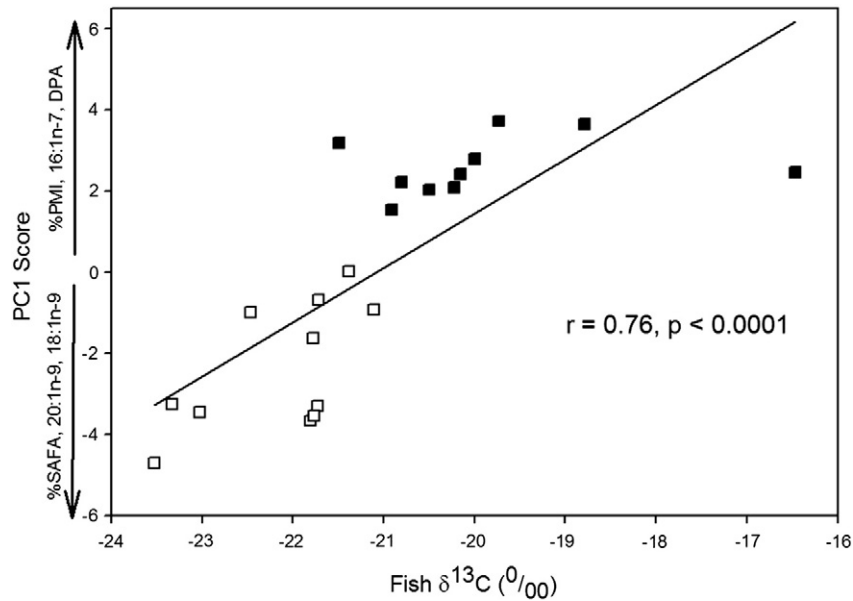
Fig. 2. Correlation between principal component scores and $\delta^{13}C$ values in benthic (round goby − solid squares) and pelagic (alewife − open squares) prey fish from Lake Ontario ($r(19) = 0.76$, $p < 0.001$). Principal component analysis was conducted using percent composition data for 18 individual fatty acids and total PMIs.

$p = 0.003$). Plasma %PMI values were positively correlated with plasma $\delta^{13}C$ values ($r(8) = 0.83$, $p < 0.001$; Fig. 3).
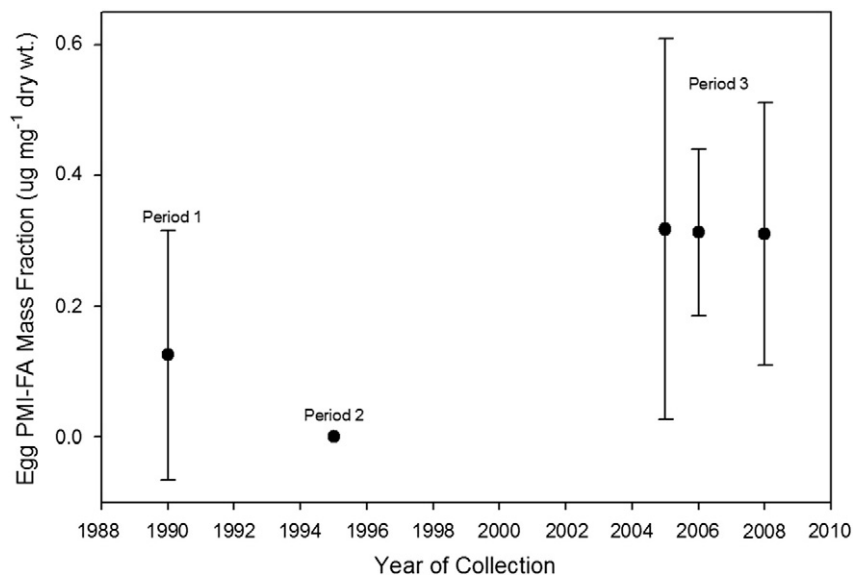
Cormorant eggs from Pigeon Island exhibited differences in PMI-FA levels through time (Fig. 4). PMI-FAs that dominated in mollusk tissue, i.e. $22:2n-9$ and $22:3n-6$, were found in 2/5 egg samples collected in 1990 and 0/5 egg samples collected in 1995. These PMI-FAs were found in 3/5, 5/5, and 4/5 eggs collected in 2005, 2006, and 2008, respectively. The proportion of eggs with detectable levels of "dreissenid mussel" PMI-FAs ($22:2n-9$ and $22:3n-6$) was greater in the late period (2005, 2006, 2008) compared to the earlier period (1990, 1995) (χ^2 $(1, n = 25) = 8.77$, $p = 0.003$). Mean sum egg mass fractions of the "mussel" PMI-FAs showed a significant increase between the early (1990, 1995) and late (2005, 2006, 2008) periods (Welch's t-test, $t(23) = -3.6$, $p = 0.002$). Mean egg $\delta^{13}C$ values were not different between these two periods (Welch's t-test, $t(23) = 1.2$, $p = 0.25$).

Discussion

Here we used whole body and muscle polymethylene-interrupted fatty acid measurements to provide an integrative measure of diet over an extended period. As such, they provided insights into character-istic differences in PMI-FA patterns in organisms utilizing benthic, i.e. littoral molluskan-based food webs versus pelagic food webs. Levels of PMI-FAs were greater in organisms associated with benthic food webs, i.e. dreissenids, goby, and cormorant, than those more closely linked to pelagic food webs, i.e. alewife and tern. However, the small number of samples taken from terns and cormorants points to the fact that results for these groups should be interpreted cautiously. Clearly, there is a need for further sampling of birds to confirm the patterns ob-served here. Nonetheless, our overall findings for invertebrates, fish, and birds were consistent with previous studies (Budge et al., 2007; Mezek
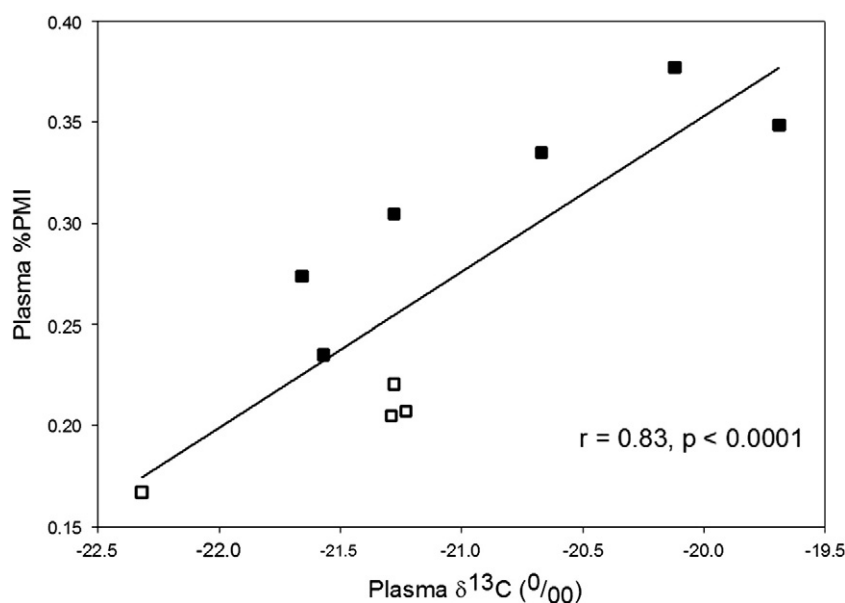
Fig. 3. Correlation between %PMI values in double-crested cormorant chick plasma and plasma $\delta^{13}C$ values ($r(8) = 0.83$, $p < 0.001$). Solid squares indicate chicks that had round goby remains in their stomachs at the time of collection. Open squares represent chicks that did not contain round goby.

Fig. 4. Annual mean mass fractions (± 1 SD) of dreissenid mussel-associated PMI-FAs in double-crested cormorant eggs (n = 5 per year) collected from Pigeon Island (eastern Lake Ontario). Period 1 is pre-dreissenid mussel population expansion; Period 2 is post-dreissenid mussel population expansion, pre-round goby introduction; Period 3 is post-round goby population expansion. PMI-FA levels in eggs were greater in period 3 than in periods 1 and 2 (Welch's t-test, t(23) = −3.6, p = 0.002).

et al., 2011) highlighting the utility of PMI-FAs as tracers of biomolecules originating from molluskan sources (Budge et al., 2006, 2007). Furthermore, PMI-FAs were among the most useful FAs in terms of differentiating benthic and pelagic prey fish species. Percent contribution of PMI-FAs to total FA levels was greater in benthic fish than pelagic fish. Other FAs were also useful in separating alewife and round goby. For example, high levels of palmitoleic acid ($16:1n-7$) have been found in sediments (Goedkoop et al., 2000) possibly accounting for the greater proportions observed in sediment-associated round goby. Conversely, percent contribution of eicosenoic acid ($20:1n-9$) was greater in alewife than round goby. In marine systems, this particular FA has been linked to pelagic food sources as it is found in high proportions in the wax ester lipid stores of calanoid copepods (Ackman et al., 1980; Wold et al., 2011). However, it must be noted that most freshwater copepods store their lipids as triacylglycerols, not wax esters, so testing this interpretation will require more research to characterize the fatty acid composition of freshwater food webs. Regardless, based upon available information, results for these other FAs corroborate the interpretation of the PMI-FA data. Although these other FAs are useful markers of the degree to which the fish species utilized benthic versus pelagic food webs, only the PMI-FAs are able to provide a specific indication of whether resources of molluskan origin were utilized.

Carbon isotopes provided an independent measure of the degree to which organisms used pelagic versus littoral/benthic resources (France, 1995). $\delta^{13}C$ values are expected to become more negative with increased reliance on pelagic food webs (France, 1995). The concurrent increase (i.e. less negative values) in fish $\delta^{13}C$ and %PMI-FA values supported the use of PMI-FAs as indicators of the consumption of benthic resources. Thus, the PMI-FA, FA, and stable isotope data indicated that round goby were dependent on benthic resources originating, at least in part, from molluskan sources. This confirms previous goby diet studies (Ghedotti et al., 1995; Ray and Corkum, 1997).

Investigation of PMI-FAs in avian samples revealed that they contributed more significantly to the overall FA composition of blood plasma taken from double-crested cormorant chicks with round goby in their stomachs. $\delta^{13}C$ values were correlated with %PMI-FA values in plasma indicating that greater utilization of benthic resources, i.e. round goby, resulted in an increase in plasma $\delta^{13}C$ (i.e. less negative values) and %PMI-FA values. Thus, from this perspective also, PMI-FAs appear to be useful in assessing the degree to which fish-eating birds may be linked to benthic/mollusk food webs through the consumption of round goby.

The biochemical composition of Great Lakes fish-eating bird eggs reflects the local environment (Hobson et al., 1997) and their fatty acid composition mirrors the diet of the laying female (Hebert et al., 2006, 2008). Hence, increased utilization of mollusk-round goby food sources by fish-eating birds will be reflected in the fatty acid composition of their eggs. Here, we examined temporal changes in levels of PMI-FAs in double-crested cormorant eggs collected from the same site from three periods: 1) 1990, prior to expansion of dreissenid mussel populations, 2) 1995, post dreissenid mussel population expansion but prior to round goby introduction, and 3) 2005 and later, post round goby population expansion. Surprisingly, PMI-FAs were found in 2/5 eggs collected in 1990. Native mollusks are known to contain PMI-FAs (Mezek et al., 2011) so it is possible that this was the source of PMI-FAs in those egg samples. Undoubtedly, native mollusks have always been consumed to some extent by higher trophic level predators. However, lack of PMI-FAs in the 1995 egg samples indicated that, even after the establishment of dreissenids, uptake of PMI-FAs of molluskan origin by fish-eating birds was rare. The fact that no increase in the prevalence of PMI-FAs was found between periods 1 and 2 indicated that the presence of dreissenid mussels by themselves was not sufficient to increase PMI exposure in fish-eating birds. However, after the introduction and expansion of round goby populations, the frequency with which PMI-FAs were found in eggs increased and egg PMI-FA mass fractions were greater.

Unlike egg PMI-FA patterns, egg $\delta^{13}C$ values did not change through time. The reason for this difference is not clear but $\delta^{13}C$ values may be influenced by factors that are not related to changes in the utilization of mollusk-based food webs (e.g. rates of primary production, benthic versus littoral habitat use, Suess Effect). The fact that changes in PMI-FAs were observed indicates the potential usefulness of PMI-FAs as a more specific marker of change in the degree of utilization of mollusk-based food webs by birds.

Following introduction into the Great Lakes, dreissenid mussels were not utilized efficiently by native fish species. In fact, consumption of dreissenids by native species may have resulted in adverse effects on fish growth and condition (Pothoven and Madenjian, 2008). Round goby, however, exploited the abundant dreissenid food resource and their populations expanded rapidly (Jude, 2001; Walsh et al., 2007). Consumption of dreissenid mussels by round goby has likely provided an effective conduit for the transfer of dreissenid mussel-related biomolecules to higher trophic levels (Johnson et al., 2005; Jude et al.,

1995; Weimer and Sowinski, 1999). In eastern Lake Ontario, round goby have become a significant component of the diets of higher trophic level fish (Dietrich et al., 2006) and birds (Johnson et al., 2010), constituting 75–80% of the double-crested cormorant diet in Lake Ontario within two years of their first being consumed by that species (Johnson et al., 2010).

While round goby provide a source of energy, protein, lipids, and other essential nutrients to high trophic level species, they may also be responsible for enhancing exposure to less desirable biomolecules. For example, since 2002, annual outbreaks of Type E Botulism have occurred on Lake Ontario; but prior to then, this disease was not recorded. Type E Botulism outbreaks have also been noted on Lakes Erie, Huron, and Michigan (CCWHC, 2012). In the past, when lake food webs were dominated by pelagic organisms, transfer of botulinum toxin to higher trophic levels may have been less likely to occur. These conditions could account for the absence of Type E Botulism-related mortality on Lake Ontario prior to 2002. However, with the "benthification" of Lake Ontario, benthic/molluskan-based food webs have become more important. In the cormorants studied here, significant shifts from pelagic to benthic food webs were manifested in egg PMI-FA signatures only after dreissenids facilitated the introduction of the round goby. Arrival of the round goby may have provided a new efficient link for the transfer of benthic biomolecules, including botulinum toxin, to fish-eating birds. On Lake Ontario, post-mortem examination of birds that had succumbed to botulism revealed that round goby were often found in their stomachs (CCWHC, 2012). The fact that plasma total PMI-FA levels in botulism-intoxicated adult cormorants and Caspian terns were not significantly different was interesting in this regard. Plasma PMI-FA values indicated that food recently consumed by these birds was more similar than what was expected based upon longer-term "typical" diets inferred from the analysis of muscle tissue. This observation highlights the possible vulnerability of surface-feeding birds to botulism even if they only occasionally use food resources connected with benthic food webs.

As demonstrated here through the use of PMI-FAs, increased transfer of biomolecules originating from benthic environments, may in part be responsible for the increase in botulism-related mortality in aquatic birds. However, other factors are also likely playing a role in the changing incidence of Type E Botulism outbreaks on the lower Great Lakes (Byappanahalli and Whitman, 2009; Lafrancois et al., 2011; Pérez-Fuentetaja et al., 2011). Regardless, the emergence of Type E Botulism as a significant mortality factor in this system represents an increased threat to the health of aquatic birds. As emphasized by Ricciardi (2001), complex combinations of interactions among species may render ecological impacts of exotic species unpredictable. In the Great Lakes, possible connections between exotic species introductions and disease emergence were unforeseen. However, through the application of ecological tracers, we have developed a better understanding of how successive exotic species introductions can exacerbate changes in pathways of energy and nutrient transfer possibly contributing to alterations in disease dynamics.

Acknowledgments

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