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Fatty acids, stable isotopes, and regurgitate reveal diet differences between Lake Ontario and Lake Erie double-crested cormorants (*Phalacrocorax auritus*)

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ABSTRACT

A detailed understanding of the possible confounding effects of natural diet is important for ecotoxicological research where exposures cannot be controlled. The opportunistic feeding of double-crested cormorants (*Phalacrocorax auritus*) may limit the ability to draw toxicological conclusions where food sources are potentially contaminated, such as in some sites across the Great Lakes. Using a variety of methods to study diet may allow for the detection of finer differences that would be missed without these complementary approaches. We used regurgitates, essential fatty acids (EFA), and ^{13}C and ^{15}N stable isotopes to understand cormorant diets at sites in Lake Ontario (Hamilton Harbour) and Lake Erie. Lake Ontario cormorant diets as estimated with regurgitates consisted almost entirely of alewife (*Alosa pseudoharengus*; 51–56%) and round goby (*Neogobius melanostomus*; 25–42%). A higher number of fish species were identified in Lake Erie cormorant regurgitate, but overall diets were dominated by round goby (70%). Fatty acid profiles and stable isotope mixing models indicated large differences between the diets of cormorants from Lake Ontario and Lake Erie but not between sexes. Polymethylene-interrupted fatty acids (PMI-FA) were useful as indicators of secondary contribution of mussels; mass fractions were much higher in cormorants from Lake Erie, indicating those cormorants consumed higher amounts of prey that feed on invasive zebra and quagga mussels (*Dreissena* spp.), especially round goby. All methods used to study diet give incomplete and biased results, but combining traditional and biochemical methods can help generate a more complete picture than has been attempted previously.

Introduction

Waterbirds have long been used as sentinel species for aquatic environments across North America (Crump et al., 2016; Hebert et al., 2011; Yamashita et al., 1993). For example, double-crested cormorants (*Phalacrocorax auritus*; hereafter, cormorants) have been studied to understand the relationship between food web changes and avian botulism outbreaks (Hebert et al., 2014), the distributions and impacts of contaminants such as PCBs (Bishop et al., 2016) and DDT (Yamashita et al., 1993) and organochlorine contaminants (Somers et al., 1993). At many contaminated sites, such as the 'Areas of Concern' found on many of the Laurentian Great Lakes, exposure may result from airborne pollution (Somers et al., 2004), contaminated aquatic food resources (Marentette et al., 2010), or direct contact with contaminated sediment (Slater et al., 2008). Drawing conclusions between ecosystem or toxicological conditions and impacts on wildlife often necessitates a detailed understanding of a species' diet, along with an appreciation of whether and how it may vary across study sites. Cormorants are ideal sentinels for ecosystem toxicology studies as they breed across North America and are naturally exposed to a variety of environmental chemicals (Crump et al., 2016). Importantly, they avoid human foods, unlike herring gulls (Hebert et al., 2008), but they do forage flexibly on a wide variety of aquatic species. This opportunistic feeding therefore has the potential to complicate ongoing toxicological studies of this Great Lakes predator. Specifically, diet that differs among study sites, including control sites, may mask the true impacts of contaminants, yet similar diets among sites can reduce the number of variables that must be taken

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into account when assessing total exposure. Fine-scale studies of diet are therefore necessary for researchers to understand feeding ecology in ways that allow for broader ecotoxicological conclusions to be drawn when working with complex contaminated areas.

The variety of methods used to estimate cormorant diet can produce uncertainty, but a combination of approaches may offer the best way forward to interpret diet using different temporal and taxonomic perspectives (McInnes et al., 2016; Quillfeldt et al., 2011a). Regurgitated stomach contents are advantageous as they are easy to collect: both adults and chicks often vomit during disturbance, and regurgitated bodies are easier to identify to species than portions in pellets. However, serious biases can result from using only regurgitate (e.g. Alonso et al., 2013), and they integrate only one recent feeding. Biochemical approaches such as fatty acid and stable isotope analyses allow for inferences about diet that span and integrate over a much longer period of foraging than analyses of regurgitates (McInnes et al., 2016), and can add trophic perspectives to an understanding of diet. 'Essential' fatty acids cannot be synthesized, or synthesized efficiently, by vertebrates. They are formed by primary producers, then assimilated and retained by consumers from their prey (Hebert et al., 2006). Fatty acid compositions of different prey species are then partially reflected in their predators. Such trophic transfers of essential fatty acids have been recorded in various freshwater and marine food webs, including shags (Käkelä et al., 2007; Quillfeldt et al., 2011b).

Polymethylene-interrupted fatty acids (PMI-FA) are unusual C20 and C22 fatty acids produced in certain aquatic invertebrates and are especially high in invasive dreissenid mussels (Mezek et al., 2009, 2011). Because cormorants feed on fish that consume these mussels, including round goby (Marentette et al., 2010; Somers et al., 2003), PMI-FA should correlate with cormorants' relative secondary mussel intake and could therefore be an indicator of the presence of mussels in their extended diet.

Stable carbon (^{13}C) and nitrogen (^{15}N) isotopes have been widely used in trophic ecology and dietary studies (e.g. Hebert et al., 2014). In freshwater lakes, $\delta^{13}C$ is a good indicator of carbon sources to differentiate between benthic and pelagic primary production (France, 1995) whereas $\delta^{15}N$ is indicative of trophic position in the food web (Minagawa and Wada, 1984). Because there is a predictable trophic enrichment (discrimination factor) of $\delta^{13}C$ and $\delta^{15}N$ between a prey and its predator (Caut et al., 2009), predator diets can be estimated using mixing models (Parnell et al., 2010).

In Lake Ontario, introduced fish species such as alewife (*Alosa pseudoharengus*) and round goby (*Neogobius melanostomus*; syn. *Apollonia melanostoma*) form a large proportion of cormorant diet (Somers et al., 2003; Weseloh et al., 1995). Compared to Lake Ontario, shallower, warmer, and more nutrient-rich Lake Erie supports larger populations of percids, notably walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*) (Leach and Nepszy, 1976). However, introduced species have also likely caused declines in native benthic fish species in Lake Erie. For example, round goby was the most numerous and widespread small benthic fish in northern Lake Erie (Reid and Mandrak, 2008). These recent changes in fish populations have likely further affected cormorant diet.

In addition to site differences, individual or sex-based foraging patterns might also promote variation in cormorant diet; for example, males may forage deeper or farther from the colony (Anderson et al., 2004; Robinson et al., 2009). Several years after the round goby invasion, some cormorants fed on round goby (i.e. a new prey item) while others did not, reinforcing the idea that there are individual foraging differences within a population (Somers et al., 2003).

Our objectives for this study were to use four differing approaches (item counts from stomach regurgitates, fatty acid analyses (both methylene-interrupted FA and PMI-FA), and stable isotope analysis to generate a more complete understanding of cormorant diet at sites currently used for ecotoxicological studies. Additionally, as few studies have combined these four techniques, we aimed to understand whether exploration of a variety of methods would produce similar diet estimates.

Methods

Three similarly sized colonies of ground-nesting cormorants (each consisting of ~1000 breeding pairs) were sampled during the breeding and nesting seasons between mid-May and mid-July in 2009 and 2010. Colonies at Pier 27 (Eastport Drive, 43°17′01.0″N 79°47′38.0″W) and Farr Island (formerly located at 43°18′39.5″N 79°48′34″W but dismantled in late 2010) were located in Hamilton Harbour, western Lake Ontario (King et al., 2014). Mohawk or Gull Island (42°50′2″ N, 79°31′22″ W) is a National Wildlife Area in northeastern Lake Erie, near Rock Point at the mouth of the Grand River, ~2 km from shore (King and de Solla, 2010).

Regurgitates

Regurgitated stomach contents were collected from the ground (n = 55), or directly from adults (n = 27) or chicks (n = 130) of unknown sex (n = 212 regurgitated masses in total). Box trapping in these cormorant colonies, often for 3–4 days per week throughout the breeding season, was conducted for our concurrent study (King et al., 2014). This caused almost all adults to regurgitate onto or near their nest before flying away at our approach, and thus these fresh regurgitates were collected from all colonies throughout the season. We applied gentle abdominal stimulation to sample regurgitates from chicks (Somers et al., 2003). When two chicks from the same nest were sampled, the regurgitates were pooled into a single sample (3 cases) as these would not represent independent samples. Regurgitates were also taken opportunistically from adults when they regurgitated during blood sampling or trapping. Regurgitates were placed in plastic bags and kept on ice during the day, then frozen until analysis. Thawed regurgitates were weighed, and individuals identified to the lowest taxonomic level possible, usually species; heavily digested fish were recorded as unknown.

Trapping and blood sampling

We trapped reproductive adult cormorants directly on ground nests. Each site was sampled for up to a few days in a row, and the sampling was cycled consecutively among the colonies, so that all colonies were sampled evenly throughout the entire breeding season. Hence, if there were any consistent temporal shifts in diet of cormorants, the effect was even among all colonies. We used 1 × 1.5 × 0.5 m box traps made with chicken wire over a steel rod frame and a two-piece hinged wooden trigger connected to the back of the trap with clear monofilament line. Sampling and collection was approved by Environment Canada's Animal Care Committee and McMaster University's Animal Research Ethics Board (AUP # 08-06-31), cormorants were collected under a Ontario Ministry of Natural Resources Wildlife Scientific Collector's Authorization (# 1051384), and cormorants were banded under a Scientific Permit to Capture and Band Migratory Birds from the Canadian Wildlife Service (# 10529 AB).

Whole blood samples (up to 18 mL) were taken by brachial venipuncture from adult cormorants, stored on ice, then centrifuged for 6 min at 3200 rpm to separate plasma from red blood cell fractions (hematocrit). These components were then separately frozen in cryovials at −80 °C until analysis. We selected 39 hematocrit samples (15 from Pier 27, 14 from Farr Island, and 10 from Lake Erie) for fatty acid and stable isotope analyses, choosing samples with sufficient volumes that were evenly distributed throughout the breeding season.

Fatty acids and stable isotopes

Fatty acids were extracted by grinding freeze-dried red blood cell fractions in (2:1 vol:vol) chloroform:methanol and centrifuged to remove non-lipids; for details, see McMeans et al., 2012). Extracts were dried and re-weighed (using a Sartorious M5 microbalance with 1 μg

precision) so as to provide a gravimetric assessment of total lipid content. The remaining extract was then evaporated to dryness under N_2. The FA extracts were re-suspended in 1.5 mL toluene, and H_2SO_4/methanol (1%) was added to the tube before overnight methylation (16 h) in a water bath at 50 °C. The extract was then evaporated to dryness under N_2, and re-dissolved in 2 mL hexane for FA analysis. FA mass fractions (μg/mg dry weight of tissue extracted) were quantified using a capillary gas chromatograph (Agilent 6890 N) coupled with a flame ionization detector, using a Supelco SP-2560 column (100 m × 0.25 mm internal diameter × 0.20-μm-thick film). A 37-component FA standard (Supelco no. 47885-U) was used to identify and quantify FA in the samples.

Stable isotope analysis was performed at the Queen's Facility for Isotope Research, Queen's University. Between 0.3 and 0.5 mg (±0.001 mg) of freeze-dried hematocrit (not lipid extracted) were weighed into tin capsules on a semi-microbalance. The isotopic composition of organic carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in hematocrit were determined by flash combustion on an Elemental Analyser (Costech Instruments) coupled with a ConFlo III interface (ThermoFinnigan) to a Delta^{plus} XP isotope ratio mass spectrometer (IRMS; ThermoFinnigan). Stable isotope data were normalized using internal standards previously calibrated with international standards. Standards (ammonium sulfate USGS2: n = 4, graphite NBS21: n = 5, and graphite UC-1: n = 10) and in-house standards (Double-crested Cormorant feathers: n = 10, bovine blood: n = 5 and defatted Atlantic Salmon [*Salmo salar*] muscle tissue: n = 10) were used and were repeatable to within standard deviations (SD) better than 0.1‰, for $\delta^{15}N$ and $\delta^{13}C$. Standard deviation of duplicates of red blood cells averaged 0.07‰ for $\delta^{15}N$, 0.05‰ for $\delta^{13}C$. Stable isotope values are reported in per mil notation (‰) as divergence from international standards: Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N_2 (AIR) for nitrogen. Divergence is calculated according to the formula:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] * 1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample and standard.

Stable isotope values for main prey species were estimated from northeastern Lake Erie (Campbell et al., 2009), <50 km from our study site. Stable isotope values of fish can vary greatly if sampled within (Ryman, 2009) as opposed to outside (Yuille et al., 2012) Hamilton Harbour due to wastewater inputs which can dramatically increase $\delta^{15}N$ values (Cabana and Rasmussen, 1996). Values from outside the harbour were therefore taken from Yuille et al. (2012) and Ontario Ministry of Natural Resources (OMNR, unpublished data 2010 and 2011).

Molecular sexing

Adult cormorants used for fatty acid analysis were sexed following Fridolfsson and Ellegren (1999), in 10 μL volumes with added bovine serum albumin (BSA). PCR products were separated and visualized on 1% agarose gels stained with ethidium bromide.

Statistics and data analyses

The three types of regurgitates (from adults, chicks, and from the ground) were pooled within each site as we wanted to define overall diet at the colonies as opposed to partitioning it into chick and adult fractions (Quillfeldt et al., 2011a). Differences among sites in counts of round goby, alewife, and cyprinids were tested with chi square contingency tables. Other prey species that were found at small percentages (0.24–4.7%) in regurgitate were excluded from the analyses. We compared our alewife and round goby counts from our two Hamilton Harbour sites to those reported in Somers et al. (2003) using a chi square test.

Fatty acids that were either below detection limits (n = 10 fatty acids) or were not detected in a high proportion of samples (36–97% of samples, n = 4 fatty acids) in the cormorant hematocrit were excluded, and the remaining 33 fatty acids were included in further statistical analyses. Of these 33, six had a small proportion of values below the method detection limit (ranging from 2 to 14%, representing 1–5 blood samples in which they were not detected). For values below detection limits, the use of one-half detection limit or zeroes can create patterns where none exist (de Solla et al., 2012). To avoid this, missing concentrations were imputed with a Maximum Likelihood Estimation (MLE) spreadsheet (Villanueva, 2005) in Excel with the iterative Solver function. This method uses the individual detection limit for each fatty acid to calculate replacement values which fit along a log-transformed quantile normal plot of the unbiased population mean and variance with maximum log-likelihood. Values were then replaced sequentially according to their correlation with fatty acids that were detected in all samples, and each fatty acid then expressed as a percentage of the total fatty acids by weight for each individual (see de Solla et al., 2012 for details). These percentages were then used for principal components analysis (PCA) on correlations, where we included as variables only those fatty acids found at over 0.5% (n = 18; see Table 2 for the list of fatty acids used in the PCA). To highlight differences between benthic and pelagic species, we did not transform the data; the main prey items, round goby and alewife, are benthic and pelagic, respectively. Individual fatty acids, classes of fatty acids (e.g. n − 6 fatty acids), mass fractions, and percentages were compared among sites and sexes with ANOVA and Tukey-Kramer HSD tests when normally distributed, and Wilcoxon/Kruskal-Wallis tests when non-normally distributed. Values of $\delta^{15}N$ and $\delta^{13}C$ were compared among sites with ANOVA and Tukey-Kramer HSD tests when normally distributed ($\delta^{15}N$), and Wilcoxon/Kruskal-Wallis tests when non-normally distributed ($\delta^{13}C$). Two-factor ANOVA including site and sex was used as full model and simplified to the most parsimonious model.

We applied a Bayesian stable isotope mixing model using the Monte Carlo Markov Chain method in the *siar* package (Parnell et al., 2010) in R 3.0.2 to estimate the percent of each species in cormorant diets. Values of $\delta^{15}N$ and $\delta^{13}C$ of individual birds were used in the model, whereas average values with error estimates (SD) were used for fish. The number of iterations in the model was 1×10^6 (4×10^5 initial iterations were discarded) and the thinning of the iterations was 60. Mean feasible proportions (%) and 95% credible intervals are reported. Discrimination factors between red blood cells of cormorants and their prey of 2.4 ± 0.7‰ for $\delta^{15}N$ and 0.7 ± 1.6‰ for $\delta^{13}C$ were used (Lavoie et al., 2012). The model was run with all fish species that were found in regurgitates except common mudpuppies (*Necturus maculosus*) for Lake Erie and emerald shiners (*Notropis atherinoides*) in Lake Ontario for which we were unable to find isotope values. In addition, we ran the model with only round goby and alewife due to their high abundance in the Great Lakes and their predominance in cormorant diets.

Results

Regurgitates

Eleven fish species and one amphibian species were identified in cormorant diet (Table 1). Differences in percentages by count of alewife, round goby, and cyprinids among sites were not significant (p = 0.22, 0.19, and 0.19, respectively). A significantly higher proportion of round goby and lower proportion of alewife were found in our study as compared with those reported by Somers et al. (2003); $\chi^2 = 139$, p < 0.0001, df = 1, n = 1461).

Methylene-interrupted fatty acids

The most abundant methylene-interrupted fatty acids found were palmitic acid (saturated, 16:0, 22.8%), followed by arachidonic acid (polyunsaturated, 20:4n − 6, 14.0%), then stearic acid (saturated, 18:0, 12.0%; Table 2). The most abundant monounsaturated fatty acid, and

Table 1
Total regurgitates and prey items collected as well as percent contribution (%) by number for each species identified in regurgitates of double-crested cormorants.

	Pier 27, Hamilton Harbour Lake Ontario	Farr Island, Hamilton Harbour Lake Ontario	Mohawk Island Lake Erie	Total
Regurgitates (n)	123	64	25	**212**
Total prey items	373	409	151	**933**
Round goby	25.4%	41.8%	70.1%	
Alewife	56.3%	51.3%	2%	
Cyprinid shiners[a]	13.4%	3.9%	13.9%	
Yellow perch	–	–	4.7%	
Smallmouth bass	–	–	2.7%	
Common mudpuppy	–	–	2.6%	
Other species	–	1.18[b]	1.98%[c]	
Too degraded	4.8%	1.7%	2%	

[a] Likely Emerald Shiner, *Notropis atherinoides*.
[b] 0.7% were bluegill (*Lepomis macrochirus*); goldfish (*Carassius auratus*) and white perch (*Morone americana*) were 0.24% each as they were only recorded once.
[c] Freshwater drum (*Aplodinotus grunniens*), white perch, and walleye were each found once and each made up 0.66%.

fourth overall highest, was oleic acid (18:1n − 9, 9.3%). Percentages of n − 6 fatty acids were higher in Lake Ontario cormorants than those from Lake Erie ($F = 9.81$, $df = 38$, $p < 0.001$). Polyunsaturated fatty acids (PUFA), as a percentage of all fatty acids, were higher at Lake Ontario, and significantly lower in Lake Erie samples ($F = 10.92$, $df = 38$, $p < 0.001$). This appears to be driven by several fatty acids, but especially 20:3n − 3, 22:2n − 6, and 20:2n − 6 (Table 2), which were found at approximately one-third the mass fractions at the Lake Erie colony.

The PCA two-component plot placed all Lake Erie cormorants in one cluster; the remaining cluster contained a mix from the two Lake

Table 2
Fatty acid profiles from double-crested cormorant (*Phalacrocorax auritus*) hematocrit from colonies in Ontario, Canada. Values (w/w percentages) are means ± SD from 39 cormorants. Asterisks (*) indicate fatty acids which are significantly different among sites, while [P] indicates fatty acids that were found above 0.5% and were included in the PCA. Abbreviations i and ai after the fatty acid name stand for iso and anteiso (carbon branches) respectively; c and t indicate *cis* and *trans* respectively.

	Pier 27, Hamilton Harbour Lake Ontario	Farr Island, Hamilton Harbour Lake Ontario	Mohawk Island Lake Erie	Overall
Saturated fatty acids (SAFA)				
14:0* [P]	1.49 ± 0.08	1.60 ± 0.08	1.22 ± 0.10	1.46 ± 0.34
15:0i*	0.22 ± 0.05	0.27 ± 0.06	0.17 ± 0.05	0.23 ± 0.06
15:ai	0.13 ± 0.04	0.10 ± 0.03	0.12 ± 0.05	0.11 ± 0.04
15:0*	0.44 ± 0.08	0.42 ± 0.06	0.30 ± 0.03	0.39 ± 0.08
16:0i*	0.20 ± 0.01	0.13 ± 0.10	0.33 ± 0.07	0.21 ± 0.10
16:0[P]	23.06 ± 1.36	22.58 ± 1.63	22.85 ± 2.01	22.83 ± 1.61
17:0* [P]	0.62 ± 0.07	0.61 ± 0.05	0.36 ± 0.04	0.55 ± 0.12
18:0[P]	11.67 ± 1.19	11.82 ± 1.82	12.85 ± 1.64	12.03 ± 1.59
20:0*	0.51 ± 0.08	0.39 ± 0.07	0.48 ± 0.13	0.46 ± 0.11
22:0*	0.47 ± 0.13	0.32 ± 0.08	0.38 ± 0.25	0.39 ± 0.17
24:0	0.30 ± 0.13	0.24 ± 0.09	0.19 ± 0.08	0.25 ± 0.11
Monounsaturated fatty acids (MUFA)				
15:1*	0.16 ± 0.04	0.13 ± 0.06	0.23 ± 0.13	0.17 ± 0.09
16:1n − 7* [P]	1.39 ± 0.29	1.67 ± 0.37	5.46 ± 2.46	2.53 ± 2.13
18:1n − 9t*	0.19 ± 0.05	0.18 ± 0.02	0.43 ± 0.11	0.25 ± 0.12
18:1n − 9c* [P]	9.61 ± 2.20	10.51 ± 2.28	7.23 ± 1.16	9.32 ± 2.36
18:1n − 7* [P]	6.65 ± 0.93	6.27 ± 0.59	8.33 ± 0.92	6.94 ± 1.16
20:1n − 9*	1.20 ± 0.29	1.06 ± 0.12	0.33 ± 0.08	0.93 ± 0.41
20:1n − 7*	0.13 ± 0.03	0.14 ± 0.02	0.21 ± 0.08	0.15 ± 0.05
22:1n − 9*	0.27 ± 0.07	0.22 ± 0.02	0.09 ± 0.04	0.21 ± 0.09
24:1n − 9* [P]	0.93 ± 0.26	0.74 ± 0.10	0.33 ± 0.09	0.71 ± 0.29
Polyunsaturated fatty acids (PUFA)				
18:2n − 6c* [P]	3.74 ± 0.57	3.45 ± 0.18	1.71 ± 0.23	3.12 ± 0.92
20:3n − 6*	0.47 ± 0.08	0.41 ± 0.02	0.24 ± 0.03	0.39 ± 0.10
18:3n − 6*	0.13 ± 0.02	0.15 ± 0.02	0.10 ± 0.02	0.13 ± 0.03
18:3n − 3* [P]	1.13 ± 0.28	1.28 ± 0.34	0.78 ± 0.30	1.09 ± 0.36
18:4n − 3[P]	0.50 ± 0.13	0.59 ± 0.20	0.66 ± 0.47	0.57 ± 0.28
20:3n − 3* [P]	0.67 ± 0.07	0.66 ± 0.06	0.22 ± 0.07	0.55 ± 0.20
20:4n − 6[P]	13.8 ± 1.60	14.65 ± 1.24	13.46 ± 2.09	14.05 ± 1.66
22:2n − 6*	0.18 ± 0.04	0.17 ± 0.02	0.05 ± 0.04	0.14 ± 0.06
20:2n − 6* [P]	0.86 ± 0.13	0.78 ± 0.04	0.28 ± 0.07	0.69 ± 0.26
20:5n − 3[P]	5.42 ± 1.25	5.59 ± 1.29	5.68 ± 1.59	5.55 ± 1.32
22:4n − 6[P]	1.97 ± 0.32	1.90 ± 0.23	2.10 ± 0.53	1.98 ± 0.36
22:5n − 3c* [P]	3.02 ± 0.31	2.93 ± 0.45	4.46 ± 0.52	3.36 ± 0.77
22:6n − 3[P]	8.18 ± 0.81	7.67 ± 0.85	8.01 ± 1.38	7.95 ± 1.00
Total n − 3	18.95	18.76	19.83	19.10
Total n − 6*	20.20	20.60	17.64	19.68
n − 3/n − 6 ratio	0.930	0.91	1.12	0.97
Total SAFA	39.22	38.59	39.41	39.04
Total MUFA	20.57	20.98	22.76	21.28
Total PUFA*	40.20	40.42	37.82	39.67

Ontario sites (Fig. 1). The first two principal components explained 62.1% of the variation (39.1% and 23.0%, respectively). Lake Erie cormorants were distinct compared to those from Lake Ontario, primarily due to the higher relative abundance of 22:5-3c, 18:1n−7, and 16:1n−7, which had loadings >−| 0.5| (Fig. 2). Conversely, the two Lake Ontario sites were strongly associated with increased 18:2n−6c, 20:3n−3, 20:2n−6, and others, which had loadings of >|0.5|. No separation between the Lake Ontario colonies was observed, nor were there sex-specific differences in fatty acid composition (21 females, 18 males).

Polymethylene-interrupted (PMI) fatty acids

The only PMI-FA detected in every sample was 20:3n−6 (Fig. 2). The other five PMI-FAs were detected at very low mass fractions and were not subject to further statistical analysis. Relative to total fatty acids, percentages of 20:3n−6 from Lake Erie were ~5–6 and up to 43 times higher than those from Lake Ontario ($F = 74.29$, $p < 0.0001$, Fig. 2). Mass fractions of 20:3n−6 showed a similar relationship ($F = 132.71$, $p < 0.0001$). There were no differences in 20:3n−6 between males and females.

Stable isotopes

Both $\delta^{13}C$ and $\delta^{15}N$ differed according to site (Fig. 3, Table 3). Results from Lake Erie cormorants were significantly different from Lake Ontario cormorants, with no difference between the two Lake Ontario sites (ANOVA, $\delta^{13}C$: $\chi^2 = 11.89$, $p = 0.0026$, $\delta^{15}N$: $F = 47.93$, $p < 0.0001$, Table 4). Sex was not a significant predictor term in the full model to explain variations of either $\delta^{13}C$ (two-factor ANOVA: $F = 0.12$, $p = 0.731$) or $\delta^{15}N$ ($F = 3.56$, $p = 0.068$) and was therefore removed for the final model (Table 4).

Mixing models revealed a high proportion of cyprinids in cormorant diet compared to round goby and alewife for the Lake Ontario sites, although there were no significant differences among proportions for Farr Island ($p > 0.05$, Table 5). The proportion of alewife in the diet was higher than round goby for both Hamilton sites but not significantly ($p > 0.05$). When including only 2 sources in the model, the proportion of alewife was slightly higher than round goby but not significant ($p > 0.05$). In Lake Erie, alewife and round goby showed higher proportional contributions to the cormorant diet than yellow perch and smallmouth bass (*Micropterus dolomieu*). When considering only 2 sources, the proportion of round goby was much higher than alewife ($p = 0.0021$, Table 5).

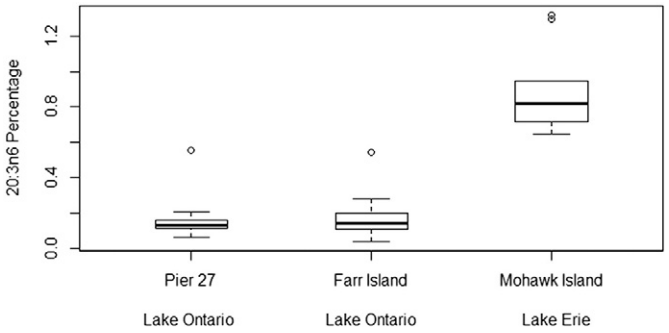

Fig. 2. Percentages of 20:3n−6, a polymethylene-interrupted (PMI) fatty acid in hematocrit of 39 cormorants from three sites in Ontario: Pier 27 and Farr Island, Hamilton Harbour, Lake Ontario, and Mohawk Island, Lake Erie. Percentage is calculated as concentrations of 20:3n6 divided by total concentration (total = 33 methylene-interrupted fatty acids used for analysis plus this one PMI-FA).

Discussion

Athough double-crested cormorants are largely piscivorous, they are otherwise opportunistic foragers, and will forage from both benthic and pelagic zones. Clarifying diet is therefore necessary to draw conclusions about exposure in a contaminated 'Area of Concern' such as Hamilton Harbour, where differentially contaminated prey fish species could potentially influence interpretations of contaminant effects. Our results show a low likelihood that diets at the two Lake Ontario sites could have contributed to differential contaminant exposure, and reinforce our conclusions that an observed increase in mutation rates (King et al., 2014), was associated with airborne pollution as opposed to differences in prey. Though it is not always discussed as a complicating factor in ecotoxicological studies, diet can explain variation in individual contaminant loads. Food ingestion is the main route of mercury exposure in seabirds, for example (Carravieri et al., 2013), and thus to link observed effects to airborne exposure, alternate explanations centred around differing diets must be ruled out. The use of four different methods to study diet is a more complete approach to fully assess the likelihood that diets differ between study sites, and allows for biases inherent in

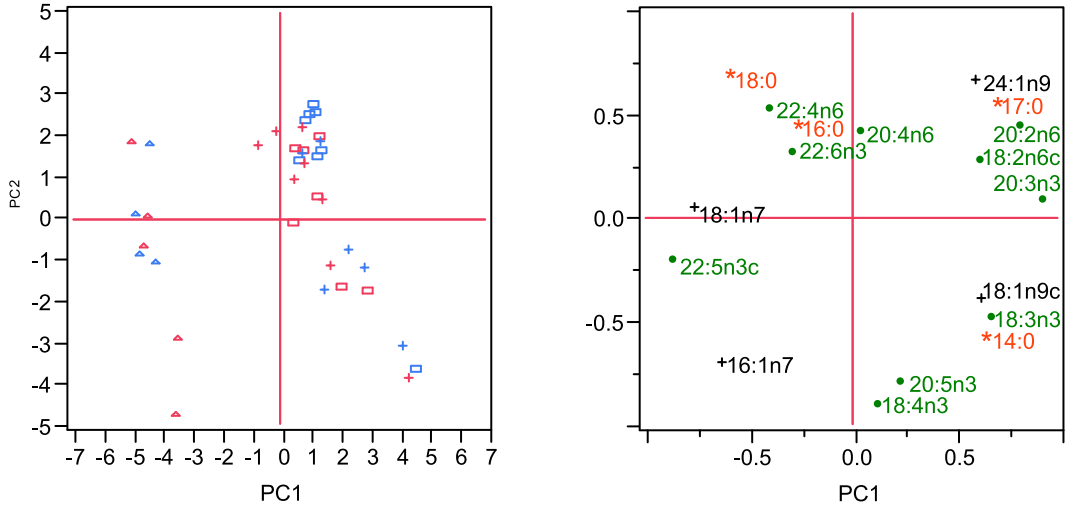

Fig. 1. PCA score plot (left) and fatty acid loadings on fatty acid percentages (right) in adult cormorant hematocrit. Cormorants from Pier 27 (Hamilton Harbour) are indicated with a rectangle, cormorants from Farr Island (Hamilton Harbour) are indicated with a cross, and cormorants from Lake Erie are indicated with a triangle. In the score plot, points representing females are pink (n = 21) and those representing males are blue (n = 18). In the loadings plot, fatty acids are identified by class: saturated in orange (with asterisks), monounsaturated in green (with circles), and polyunsaturated in black (with crosses).

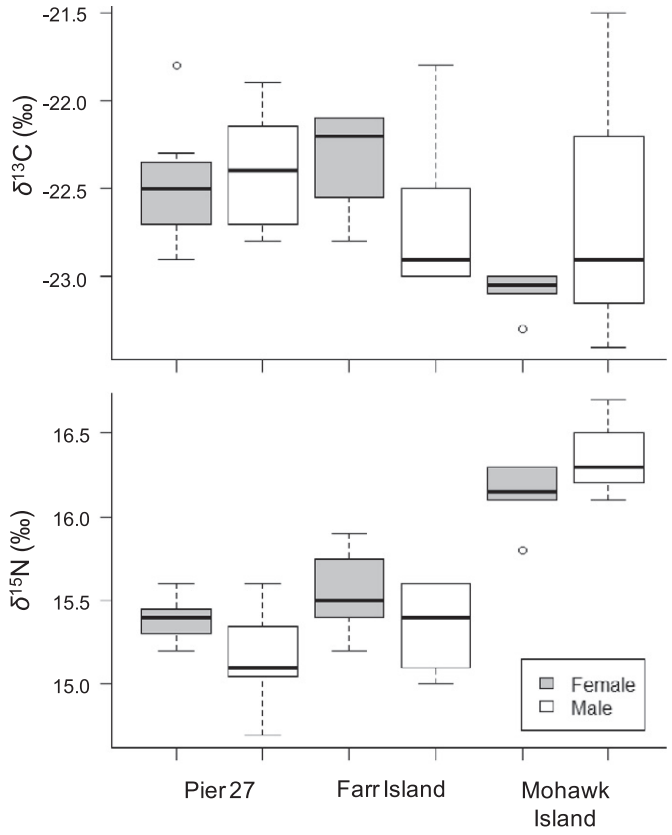

Fig. 3. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in cormorant cellular blood fractions (hematocrit) from three sites in Ontario: Pier 27 and Farr Island, Hamilton Harbour, Lake Ontario, and Mohawk Island, Lake Erie (n = 39).

each type of analysis to be overcome through the combination of methods.

Alewife has consistently been an important prey item for cormorants (Weseloh et al., 1995) since cormorants began recolonizing the Laurentian Great Lakes in the late 1970's (Wires and Cuthbert, 2006). Following the invasion of round goby into the Great Lakes around 2000, cormorants rapidly adapted their diets, with adults preying heavily on round goby (Johnson et al., 2010a). In eastern Lake Ontario, adult cormorant diets are now largely round goby (75–79%) with alewife being the second-most common species (Johnson et al., 2010a). In Hamilton Harbour, Somers et al. (2003) found 68–98% alewife and 0–21% round goby in chick regurgitates. We found round goby at significantly higher overall proportions in Hamilton Harbour (25–42% by colony), demonstrating their increasing importance since 2002. Alewife, which we found at 51–56% by colony in Hamilton Harbour, showed a decrease from 2002 concurrent with the increase in round goby. Lake Erie cormorant diets were composed mostly of round goby (70%). Round goby invaded Lake Erie before they were detected in Hamilton Harbour (Johnson et al., 2005; Walsh et al., 2007); their higher proportions in the Lake Erie cormorant diet may reflect higher population densities (>100/m^2 in Lake Erie compared to <35/m^2 in Hamilton Harbour, Steinhart et al., 2004; Vélez-Espino et al., 2010). Round goby populations may have been exhibiting a period of rapid initial growth during the first years of their colonization, so their percentage in cormorant diet may have been dramatically higher during that time (Johnson et al., 2010a).

Table 4
Analysis of variance results of the full (parametric) and reduced (non-parametric) models with site and sex as factors and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes from double-crested cormorants (*Phalacrocorax auritus*) hematocrit as dependent variables. Values (‰) are means ± SD from 39 cormorants.

	Full model				Final model		
	Terms	*df*	*F*	p	*df*	*F* or χ^2	p
$\delta^{13}C$	Site	2	6.69	0.0037	2	11.89[a]	0.0026
	Sex	1	0.12	0.731			
	Site * Sex	2	2.71	0.081			
	Residuals	33			36		
$\delta^{15}N$	Site	2	18.81	<0.0001	2	47.93[b]	<0.0001
	Sex	1	3.56	0.068			
	Site * Sex	2	3.17	0.055			
	Residuals	33			36		

[a] Chi-square (χ^2).
[b] F-ratio.

Increased proportions of round goby since diets were last analyzed by Somers et al. (2003) provides further evidence that invasive species have continued to modify food webs in Lake Ontario. Round goby have altered energy and contaminant pathways in the Great Lakes (Johnson et al., 2005; Marentette et al., 2010), and may have contributed to lethal Type E botulism outbreaks which have killed thousands of aquatic birds in recent years (Hebert et al., 2014). The increased consumption of round goby may have made cormorants more vulnerable to these outbreaks; during the course of field work we collected many ailing birds.

Because cormorants are highly opportunistic feeders, diet composition usually reflects species availability in a given area (Hatch and Weseloh, 1999) and can therefore serve to reflect relative numbers of most fish species, an important measure of ecosystem health. Years of environmental and ecological damage (reviewed in Dermott et al., 2007) have resulted in a highly degraded fish community in Hamilton Harbour with a much lower percentage of native fish than a healthy ecosystem would support (Brousseau and Randall, 2008). This altered fish community now influences cormorant diets in western Lake Ontario, partially because the total number of species available in the area is relatively low, and most native fish populations are too depleted to make up a large portion of cormorant diets. Our regurgitate results identified the same primary species found in Hamilton Harbour fish community surveys, confirming that variation in diet largely reflects different fish communities. Alewife is the most abundant prey fish species in Lake Ontario (Hebert et al., 2008) and the most common offshore species in Hamilton Harbour, comprising up to 62% of total catch (Brousseau and Randall, 2008). Yellow perch may be "virtually absent" in Hamilton Harbour; recent declines may be related to cormorant predation (reviewed in Brousseau and Randall, 2008), but Lake Erie supports larger populations of yellow perch (Bur et al., 1999; Burnett et al., 2002).

Regurgitate represents only a short-term time scale of a bird's diet (Johnson et al., 2010b). Major sources of bias can include the fact that adults usually only regurgitate food held in the proventriculus, and there can be differential digestion of the hard and soft portions of prey (Barrett et al., 2007). Perhaps reflecting these digestion rate differences, Johnson et al. (2010b) found alewife to be the most common prey item

Table 3
Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes from double-crested Cormorant (*Phalacrocorax auritus*) cellular blood fractions (hematocrit) from colonies in Ontario, Canada. Values (‰) are means ± SD from 39 cormorants. Asterisks indicate significant differences among sites and sites that share common letters (A and B) are not significantly different.

	Pier 27, Hamilton Harbour Lake Ontario	Farr Island, Hamilton Harbour Lake Ontario	Mohawk Island Lake Erie	Overall
$\delta^{13}C$*	-22.4 ± 0.3[A]	−22.5 ± 0.4[A]	−22.9 ± 0.5[B]	−22.6 ± 0.5
$\delta^{15}N$*	15.3 ± 0.2[A]	15.5 ± 0.3[A]	16.2 ± 0.2[B]	15.6 ± 0.5

Table 5
Mean diet composition (%; [low-high 95% credible intervals]) from double-crested cormorant (*Phalacrocorax auritus*) cellular blood fractions (hematocrit) using Bayesian mass balance dual-isotope mixing model with stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes. Differences among sources are based on probabilities of posterior draws. Sites that share common letters (A and B) show no significant differences among prey items.

	Pier 27, Hamilton Harbour[a] Lake Ontario						Farr Island, Hamilton Harbour[a] Lake Ontario						Mohawk Island[b] Lake Erie					
	All sources			2 sources			All sources			2 sources			All sources			2 sources		
Round goby	16.5	[3.9–29.8]	A	44.5	[28.8–59.2]	A	26.2	[9.9–42.6]	A	48.2	[32.5–63.9]	A	35.4	[1.6–63.7]	AB	92.6	[73.7–100.0]	A
Alewife	29.5	[16.7–42.8]	AB	55.5	[40.8–71.7]	A	32.6	[17.8–46.9]	A	51.8	[36.1–67.5]	A	38.1	[21.5–56.5]	A	7.4	[0–26.3]	B
Emerald shiner	54.0	[34.1–73.4]	B				41.2	[18.4–65.3]	A									
Yellow perch													8.3	[0–31.7]	B			
Smallmouth bass													18.2	[0–5.5]	B			

[a] Isotope values are from Yuille et al. (2012) and OMNR (unpublished data).
[b] Isotope values are from Campbell et al. (2009).

in stomach contents, but round goby were the most common in pellets. This observation suggests that our regurgitate results could also be biased towards higher proportions of alewife in the diet; the stable isotope model also provided complementary evidence that alewife were not significantly higher than round goby.

Choice of tissue type for fatty acid and stable isotope analyses

We chose to analyze the cellular fraction of blood (hematocrit), a medium-term storage component (Hobson and Clark, 1993) because plasma might not integrate enough long-term information about diet, and would skew results towards recent consumption. Longer term measures such as analysis of adipose tissue are logistically difficult, require sacrificing birds and would likely integrate diet information from wintering locations or migration, thus complicating interpretation. In isotope studies in birds, blood cells are thought to integrate up to two months of diet (Hobson and Clark, 1993), so this timeline may be similar for fatty acid analysis. Red blood cells have an approximate life span of 28–35 d in chickens, 35–45 d in pigeons, 33–35 d in quail (Thrall et al., 2012) and 39–42 d in ducks (Brace and Altland, 1956; Campbell et al., 2010). Cormorant blood cell half-life was 27 days as estimated using an allometric equation based on body weight and carbon half-life in whole blood and red blood cells (Carleton and Martínez del Rio, 2005). This figure results in a 94% renewal (4 half-lives) of carbon in 107 days. Based on this evidence, we estimate cormorant hematocrit integrated diet information for ~1 (50% renewal) to 3 (94% renewal) months. Because most blood samples were taken in late May and end of June, they would therefore represent at least 50% of diet beginning from late-April to end of May, when the adults were at the colony nesting and feeding chicks. The time we examined covered mostly the chick-feeding period (though various nests in the colony are at different stages at any one time) such that the timing of fatty acid profiles partially corresponds with the periods during which we collected regurgitates.

Fatty acid profiles

FA results provided support for the dominance of main prey items at each site. In captive herring gulls (*Larus argentatus*), Käkelä et al. (2009) demonstrated that certain fatty acids responded to changes in diet reliably, while others were modified enough by metabolism to represent little value in determining diet. Individual fatty acids that responded to diet changes in Käkelä et al. (2009) may thus be among the best indicators of diet in cormorants as well. The FA 18:1n − 7 originates in microbes and was higher in cormorants from Lake Erie. Concentrations in round goby were among the highest in multiple fish species analyzed by Czesny et al. (2011). Cormorant diets at Lake Erie are dominated by benthic round goby, so higher concentrations of 18:1n − 7 in Lake Erie hematocrit support results obtained from regurgitates. FA 20:1n − 9 is a long chain monounsaturated fatty acid derived from zooplankton (Käkelä et al., 2007) and a good indicator of diet (Käkelä et al., 2009). It was higher in alewife than round goby (Czesny et al., 2011), and ~4× higher by percentage in the Lake Ontario cormorants, providing further support for the importance of alewife as a prey item at Lake Ontario but not Lake Erie. Of the PUFA, the biggest two drivers of differences between Lake Ontario and Lake Erie were 20:3n − 3 and 20:2n − 6.

Several studies have used fatty acid analyses in various ways to assess diet in seabirds and waterbirds. Seasonal diet changes from squid to crustaceans were reflected in fatty acids of thin-billed prion (*Pachyptila belcheri*) regurgitates (Quillfeldt et al., 2011a). European shag (*P. aristotelis*) plasma demonstrated diets with low inter-individual variability dominated by pelagic fish species (Käkelä et al., 2007). Our fatty acid profiles also appear to show little inter-individual variability in diet, judging by the small amounts of variability within colonies overshadowed by larger differences among lakes. Rather than specializing on preferred species, cormorants in our study appear to have foraged opportunistically on the species that were the most abundant, easiest to capture, or both, as is probably the case for alewife and round goby.

PMI fatty acids and mussel-based food web

Lake Erie cormorants had relatively high concentrations and percentages of the PMI-FA 20:3n − 6. In zebra and quagga mussels (*Dreissena* spp.) from Lake Ontario, PMI-FAs make up 4–6% of total fatty acids (Mezek et al., 2011). PMI-FAs are transferred and stored in organisms that consume mussels, and in species that then secondarily consume them. Mussel residues can thus be traced through aquatic food webs, and PMI-FAs have been detected in seals, walruses (Budge et al., 2007) and polar bears (Thiemann et al., 2007). Käkelä et al. (2007) reported one PMI-FA in *P. aristotelis* plasma at a mean molar percentage of 0.08 (20:2NMID), representing 20:2n − 7 and 20:2n − 9. These PMI-FAs were detected in some cormorants in our study, but we also observed a high percentage of non-detected values. This could be a reflection of the different relative amounts of individual PMI-FAs available in these two very different food webs, or may result from differences in tissue concentrations (plasma versus hematocrit). Hebert et al. (2014) reported up to five PMI-FAs in cormorant muscle and plasma, notably 22:2n − 9 and 22:3n-6 in muscle and 20:3n − 6 in plasma. Higher percentages of 20:3n − 6 in birds from Lake Erie in our study suggest cormorants at this site secondarily consume much higher proportions of mussels. This finding is likely because round goby in Lake Erie consume almost entirely mussels (92% of prey items in Andraso et al., 2011).

Stable isotopes

Cormorant isotopic values of Lake Ontario were distinct from those of Lake Erie. This could be due to either different proportions of specific prey fish in cormorants' diets between lakes or to different isotopic compositions of the two food webs. Pelagic fish such as alewife (obligate planktivores) are usually more negative in $\delta^{13}C$ than benthic round

goby in Lake Erie (Campbell et al., 2009) and Lake Ontario (Rush et al., 2012; Yuille et al., 2012). Moreover, alewife seem to have a lower trophic level than round goby as measured by $\delta^{15}N$; values for round goby in Lake Erie near our study site were found to be in between −20.9‰ ± 1.3 SD and −18.8‰ ± 1.6 SD for $\delta^{13}C$ and between 13.6‰ ± 0.6 SD and 14.1‰ ± 0.5 SD for $\delta^{15}N$, whereas alewife were −22.7‰ ± 2.0 SD for $\delta^{13}C$ and 12.3‰ ± 0.6 SD for $\delta^{15}N$ (Campbell et al., 2009). Mixing models resulted in a similar contribution of alewife and round goby to the diet of cormorants in Lake Erie when yellow perch and smallmouth bass were included. However, a much higher proportion of round goby was estimated when only alewife and round goby were included in the model. For cormorants in Lake Ontario, isotopic values are very different when looking at fish within as opposed to outside the harbour. Fish values in $\delta^{15}N$ within the harbour (Ryman, 2009) are much higher than outside the harbour near Bronte, 10–20 km northeast of Hamilton Harbour (Yuille et al., 2012) or near Niagara, 60 km east of Hamilton Harbour (Rush et al., 2012). Higher $\delta^{15}N$ values are a consequence of the large amount of nitrogen-containing human waste released from wastewater treatment plants (Cabana and Rasmussen, 1996). Values of $\delta^{15}N$ in fish are therefore higher than those measured in cormorants which suggests that cormorant feed heavily outside the harbour.

The cyprinid emerald shiner ($\delta^{13}C$: −22.2‰ ± 0.6 SD, $\delta^{15}N$: 15.3‰ ± 0.8 SD) interestingly fell directly within the isotopic signature of cormorants from Hamilton (after correcting for the discrimination factor between prey and predator) which resulted in a higher proportion of this species in their estimated diet (Table 5). Even if emerald shiner are a very small proportion of regurgitates (3.9–13.4%, Table 1), the isotopic signature tends to overestimate the proportion of this species in the mixing model. Alewife (−22.9‰ ± 2.0 SD for $\delta^{13}C$ and 14.7‰ ± 1.6 SD for $\delta^{15}N$) and round goby (−21.3‰ ± 1.5 standard error (SE) for $\delta^{13}C$ and 13.5‰ ± 0.7 SE for $\delta^{15}N$, Yuille et al., 2012; OMNR, unpublished data) appear less important in the model. After removing emerald shiner from the model, alewife were slightly more important than round goby although not significantly. Thus, the two-source mixing model tends to agree with regurgitates and fatty acids results, especially for Lake Erie.

Sex differences in diet

Some fatty acids showed minor sex-based differences in diet; those that best separated females from males included those originating in benthic and intestinal bacteria (Käkelä et al., 2009). This result may suggest higher consumption of round goby by females, or different gut microbial compositions between sexes. Previous stable isotope work suggested males from Lake Ontario foraged at a slightly lower trophic level than females (Robinson et al., 2009). However, we found no sex-based differences within sites for either $\delta^{15}N$ or $\delta^{13}C$, although statistical power may have been low.

Conclusion

Use of regurgitates allows for identification of prey species recently consumed, while fatty acids, PMI-FAs, and stable isotopes provide for detailed comparisons of trophic levels across individuals and colonies. These four non-lethal methods use only blood samples and regurgitate. Combining these methods can more accurately describe diet than has been done previously, as verifying or questioning the results from one method helps to overcome biases inherent in any one method. Multi-method resolution ultimately improves studies using wild species such as cormorants because differences in diet and therefore trophic positions are known to affect contaminant patterns in waterbirds in the Great Lakes (Hebert and Weseloh, 2006). As cormorant prey will likely continue to shift along with future food web alterations, diet examinations using traditional and biochemical methods can help track these feeding changes and improve interpretations of toxicological effects.

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References

Alonso, H., Granadeiro, J.P., Ramos, J.A., Catry, P., 2013. Use the backbone of your samples: fish vertebrae reduces biases associated with otoliths in seabird diet studies. J. Ornithol. 154, 883–886.
Anderson, C.D., Roby, D.D., Collis, K., 2004. Foraging patterns of male and female double-crested cormorants nesting in the Columbia River estuary. Can. J. Zool. 82, 541–554.
Andraso, G.M., Ganger, M.T., Adamczyk, J., 2011. Size-selective predation by round gobies (*Neogobius melanostomus*) on dreissenid mussels in the field. J. Great Lakes Res. 37, 298–304.
Barrett, R.T., Camphuysen, K.C.J., Anker-Nilssen, T., Chardine, J.W., Furness, R.W., Garthe, S., Hüppop, O., Leopold, M.F., Montevecchi, W.A., Veit, R.R., 2007. Diet studies of seabirds: a review and recommendations. ICES J. Mar. Sci. 64, 1675–1691.
Bishop, C.A., Trudeau, S., Janz, D., Hughes, K.D., 2016. Contaminant concentrations and biomarkers in 21-day old herring gulls (*Larus argentatus*) and double-crested cormorants (*Phalacrocorax auritus*) from eastern Lake Ontario, and from Hamilton Harbour in western Lake Ontario in 1989 and 1990. Aquat. Ecosyst. Health Manag. 19, 181–191.
Brace, K.C., Altland, P.D., 1956. Life span of the duck and chicken erythrocyte as determined with C^{14}. Proc. Soc. Exp. Biol. Med. 92, 615–617.
Brousseau, C.M., Randall, R.G., 2008. Assessment of long-term trends in the littoral fish community of Hamilton Harbour using an index of biotic integrity. Can. Tech. Rep. Fish. Aquat. Sci. 2811, 102.
Budge, S.M., Springer, A.M., Iverson, S.J., Sheffield, G., 2007. Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. Mar. Ecol. Prog. Ser. 336, 305–309.
Bur, M.T., Tinnirello, S.L., Lovell, C.D., Tyson, J.T., 1999. Diet of the double-crested cormorant in western Lake Erie. Symposium on Double-Crested Cormorants: Population Status and Management Issues in the Midwest. Milwaukee, WI, pp. 72–85.
Burnett, J.A.D., Ringler, N.H., Lantry, B.F., Johnson, J.H., 2002. Double-crested cormorant predation on yellow perch in the Eastern Basin of Lake Ontario. J. Great Lakes Res. 28, 202–211.
Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Natl. Acad. Sci. U. S. A. 93, 10844–10847.
Campbell, L.M., Thacker, R., Barton, D., Muir, D.C.G., Greenwood, D., Hecky, R.E., 2009. Re-engineering the eastern Lake Erie littoral food web: the trophic function of non-indigenous Ponto-Caspian species. J. Great Lakes Res. 35, 224–231.
Campbell, Smith, Zimmerman, 2010. Chapter 124: hematology of waterfowl and raptors. In: Weiss, D.J., Wardrop, K.J. (Eds.), Schalm's Veterinary Hematology.
Carleton, S.A., Martínez del Rio, C., 2005. The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). Oecologia 144, 226–232.
Carravieri, A., Bustamante, P., Churlaud, C., Cherel, Y., 2013. Penguins as bioindicators of mercury contamination in the Southern Ocean: birds from the Kerguelen Islands as a case study. Sci. Total Environ. 454–455, 141–148.
Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. 46, 443–453.
Crump, D., Farhat, A., Chiu, S., Williams, K.L., Jones, S.P., Langlois, V.S., 2016. Use of a novel double-crested cormorant ToxChip PCR Array and the EROD assay to determine effects of environmental contaminants in primary hepatocytes. Environ. Sci. Technol. 50, 3265–3274.
Czesny, S.J., Rinchard, J., Hanson, S.D., Dettmers, J.M., Dabrowski, K., Smith, R., 2011. Fatty acid signatures of Lake Michigan prey fish and invertebrates: among-species differences and spatiotemporal variability. Can. J. Fish. Aquat. Sci. 68, 1211–1230.
Dermott, R., Johannsson, O., Munawar, M., Bonnell, R., Bowen, K., Burley, M., Fitzpatrick, M., Gerlofsma, J., Niblock, H., 2007. Assessment of lower food web in Hamilton Harbour, Lake Ontario, 2002–2004. Can. Tech. Rep. Fish. Aquat. Sci. 2729 (120 pp.).
de Solla, S.R., Struger, J., McDaniel, T.V., 2012. Detection limits can influence the interpretation of pesticide monitoring data in Canadian surface waters. Chemosphere 86, 565–571.
France, R.L., 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol. Oceanogr. 40, 1310–1313.
Fridolfsson, A., Ellegren, H., 1999. A simple and universal method for molecular sexing of non-ratite birds. J. Avian Biol. 30, 116–121.
Hatch, J.J., Weseloh, D.V.(.C.)., 1999. Double-Crested Cormorant. Birds North Am. Online.
Hebert, C., Weseloh, D., 2006. Adjusting for temporal change in trophic position results in reduced rates of contaminant decline. Environ. Sci. Technol. 40, 5624–5628.
Hebert, C.E., Arts, M.T., Weseloh, D.V.C., 2006. Ecological tracers can quantify food web structure and change. Environ. Sci. Technol. 40, 5618–5623.

Hebert, C., Weseloh, D., Idrissi, A., Arts, M., O'Gorman, R., Gorman, O.T., Locke, B., Madenjian, C.P., Roseman, E.F., 2008. Restoring piscivorous fish populations in the Laurentian Great Lakes causes seabird dietary change. Ecology 89, 891–897.
Hebert, C.E., Weseloh, D.V.C., Macmillan, S., Campbell, D., Nordstrom, W., 2011. Metals and polycyclic aromatic hydrocarbons in colonial waterbird eggs from Lake Athabasca and the Peace-Athabasca Delta, Canada. Environ. Toxicol. Chem. 30, 1178–1183.
Hebert, C.E., Chao, J., Crump, D., Johnson, T.B., Rudy, M.D., Sverko, E., Williams, K., Zaruk, D., Arts, M.T., 2014. Ecological tracers track changes in bird diets and possible routes of exposure to type E botulism. J. Great Lakes Res. 40, 64–70.
Hobson, K.A., Clark, R., 1993. Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. Auk 110, 638–641.
Johnson, T.B., Bunnell, D.B., Knight, C.T., 2005. A potential new energy pathway in Central Lake Erie: the round goby connection. J. Great Lakes Res. 31, 238–251.
Johnson, J.H., Ross, R.M., McCullough, R.D., Mathers, A., 2010a. Diet shift of double-crested cormorants in eastern Lake Ontario associated with the expansion of the invasive round goby. J. Great Lakes Res. 36, 242–247.
Johnson, J.H., Ross, R.M., McCullough, R.D., Mathers, A., 2010b. A comparative analysis of double-crested cormorant diets from stomachs and pellets from two Lake Ontario colonies. J. Freshw. Ecol. 25, 37–41.
Käkelä, A., Furness, R., Kelly, A., Strandberg, U., Waldron, S., Käkelä, R., 2007. Fatty acid signatures and stable isotopes as dietary indicators in North Sea seabirds. Mar. Ecol. Prog. Ser. 342, 291–301.
Käkelä, R., Furness, R.W., Kahle, S., Becker, P.H., Käkelä, A., 2009. Fatty acid signatures in seabird plasma are a complex function of diet composition: a captive feeding trial with herring gulls. Funct. Ecol. 23, 141–149.
King, L.E., de Solla, S.R., 2010. Successful renesting of Caspian terns on Mohawk Island, Lake Erie, after complete colony failure. Ontario Birds 28, 158–165.
King, L.E., de Solla, S.R., Small, J.M., Sverko, E., Quinn, J.S., 2014. Microsatellite DNA mutations in double-crested cormorants (*Phalacrocorax auritus*) associated with exposure to PAH-containing industrial air pollution. Environ. Sci. Technol. 48, 11637–11645.
Lavoie, R.A., Rail, J.F., Lean, D.R.S., 2012. Diet composition of seabirds from Corossol Island, Canada, using direct dietary and stable isotope analyses. Waterbirds 35, 402–419.
Leach, J.H., Nepszy, S.J., 1976. The fish community of Lake Erie. J. Fish. Res. Board Can. 33, 622–638.
Marentette, J.R., Gooderham, K.L., McMaster, M.E., Ng, T., Parrott, J.L., Wilson, J.Y., Wood, C.M., Balshine, S., 2010. Signatures of contamination in invasive round gobies (*Neogobius melanostomus*): a double strike for ecosystem health? Ecotoxicol. Environ. Saf. 73, 1755–1764.
McInnes, J.C., Raymond, B., Phillips, R.a., Jarman, S.N., Lea, M.-A., Alderman, R., 2016. A review of methods used to analyse albatross diets—assessing priorities across their range. ICES J. Mar. Sci. 73, 2125–2137.
McMeans, B.C., Arts, M.T., Rush, S.A., Fisk, A.T., 2012. Seasonal patterns in fatty acids of *Calanus hyperboreus* (Copepoda, Calanoida) from Cumberland Sound, Baffin Island, Nunavut. Mar. Biol. 159, 1095–1105.
Mezek, T., Arts, M., Sverko, E., Fisk, A.T., 2009. Sample preparation methods for the analysis of poly-methylene interrupted fatty acids. Verh. Internat. Verein Limnol. 30, 903–906.
Mezek, T., Sverko, E., Rudy, M.D., Zaruk, D., Capretta, A., Hebert, C.E., Fisk, A.T., McGoldrick, D.J., Newton, T.J., Sutton, T.M., Koops, M.A., Muir, A.M., Johnson, T.B., Ebener, M.P., Arts, M., 2011. Polymethylene-interrupted fatty acids: biomarkers for native and exotic mussels in the Laurentian Great Lakes. J. Great Lakes Res. 37, 289–297.
Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta 48, 1135–1140.
Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS One 5, e9672.
Quillfeldt, P., Masello, J.F., Brickle, P., Martin-Creuzburg, D., 2011a. Fatty acid signatures of stomach contents reflect inter- and intra-annual changes in diet of a small pelagic seabird, the thin-billed prion *Pachyptila belcheri*. Mar. Biol. 158, 1805–1813.
Quillfeldt, P., Schroff, S., van Noordwijk, H., Michalik, A., Ludynia, K., Masello, J., 2011b. Flexible foraging behaviour of a sexually dimorphic seabird: large males do not always dive deep. Mar. Ecol. Prog. Ser. 428, 271–287.
Reid, S.M., Mandrak, N.E., 2008. Historical changes in the distribution of threatened channel darter (*Percina copelandi*) in Lake Erie with general observations on the beach fish assemblage. J. Great Lakes Res. 34, 324–333.
Robinson, S.A., Forbes, M.R., Hebert, C.E., 2009. Parasitism, mercury contamination, and stable isotopes in fish-eating double-crested cormorants: no support for the co-ingestion hypothesis. Can. J. Zool. 87, 740–747.
Rush, S.A., Paterson, G., Johnson, T.B., Drouillard, K.G., Haffner, G.D., Hebert, C.E., Arts, M.T., McGoldrick, D.J., Backus, S.M., Lantry, B.F., Lantry, J.R., Schaner, T., Fisk, A.T., 2012. Long-term impacts of invasive species on a native top predator in a large lake system. Freshw. Biol. 57, 2342–2355.
Ryman, J., 2009. Spatial and Temporal Food web Dynamics of a Contaminated Lake Ontario Embayment, Hamilton Harbour. (M.Sc. Thesis). University of Waterloo.
Slater, G.F., Cowie, B.R., Harper, N., Droppo, I.G., 2008. Variation in PAH inputs and microbial community in surface sediments of Hamilton Harbour: implications to remediation and monitoring. Environ. Pollut. 153, 60–70.
Somers, J., Goski, B., Barbeau, J., Barrett, M., 1993. Accumulation of organochlorine contaminants in double-crested cormorants. Environ. Pollut. 80, 17–23.
Somers, C.M., Lozer, M., Kjoss, V.A., Quinn, J.S., 2003. The invasive round goby (*Neogobius melanostomus*) in the diet of nestling double-crested cormorants (*Phalacrocorax auritus*) in Hamilton Harbour, Lake Ontario. J. Great Lakes Res. 29, 392–399.
Somers, C.M., McCarry, B.E., Malek, F., Quinn, J.S., 2004. Reduction of particulate air pollution lowers the risk of heritable mutations in mice. Science 304, 1008–1010.
Steinhart, G.B., Stein, R.A., Marschall, E.A., 2004. High growth rate of young-of-the-year smallmouth bass in Lake Erie: a result of the round goby invasion? J. Great Lakes Res. 30, 381–389.
Thiemann, G.W., Budge, S.M., Iverson, S.J., Stirling, I., 2007. Unusual fatty acid biomarkers reveal age-and sex-specific foraging in polar bears (*Ursus maritimus*). Can. J. Zool. 85, 505–517.
Thrall, M.A., Baker, D.C., Lassen, E.D., 2012. Veterinary Hematology and Clinical Chemistry. second ed. Wiley-Blackwell.
Vélez-Espino, L.A., Koops, M.A., Balshine, S., 2010. Invasion dynamics of round goby (*Neogobius melanostomus*) in Hamilton Harbour, Lake Ontario. Biol. Invasions 12, 3861–3875.
Villanueva, P., 2005. MLE-Based Procedure for Left-Censored Data (Microsoft Excel spreadsheet). Unpublished report, U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, D.C.
Walsh, M.G., Dittman, D.E., Gorman, R.O., 2007. Occurrence and food habits of the round goby in the profundal zone of southwestern Lake Ontario. J. Great Lakes Res. 33, 83–92.
Weseloh, D.V., Ewins, P.J., Struger, J., Mineau, P., Bishop, C.A., Postupalsky, S., Ludwig, J.P., 1995. Double-crested cormorants of the Great Lakes: changes in population size, breeding distribution and reproductive output between 1913 and 1991. Colon. Waterbirds 18, 48–59.
Wires, L.R., Cuthbert, F.J., 2006. Historic populations of the double-crested cormorant (*Phalacrocorax auritus*): implications for conservation and management in the 21st century. Waterbirds 29, 9–37.
Yamashita, N., Tanabe, S., Ludwig, J.P., Kurita, H., Ludwig, M.E., Tatsukawa, R., 1993. Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and Caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. Environ. Pollut. 79, 163–173.
Yuille, M.J., Johnson, T.B., Arnott, S.E., Campbell, L.M., 2012. *Hemimysis anomala* in Lake Ontario food webs: stable isotope analysis of nearshore communities. J. Great Lakes Res. 38, 86–92.