USING STABLE-ISOTOPE ANALYSIS TO IDENTIFY ENDOGENOUS AND EXOGENOUS SOURCES OF NUTRIENTS IN EGGS OF MIGRATORY BIRDS: APPLICATIONS TO GREAT LAKES CONTAMINANTS RESEARCH

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ABSTRACT.—Stable-isotope analyses (carbon [13C/12C] and sulfur [34S/32S]) were performed on eggs of two migratory fish-eating birds, Caspian Terns (Sterna caspia) and Double-crested Cormorants (Phalacrocorax auritus) collected from Lake Ontario, Canada, to examine the extent to which nutrient reserves acquired on marine wintering grounds are transferred to eggs laid on freshwater breeding grounds. In order to establish isotopic patterns typical of eggs of birds using marine and freshwater C-3 biomes, eggs of Herring Gulls (Larus argentatus), a year-round resident on the Great Lakes, and those of Caspian Terns and Herring Gulls, breeding respectively in the Gulf Coast of Texas and Atlantic Coast of Canada, were analyzed isotopically. Individual egg components showed distinct isotope values that were similar for both migratory and nonmigratory birds breeding in a freshwater biome and significantly lighter than those breeding in a marine biome. Hence, there appeared to be little evidence for significant nutrient transfer between the two biomes. The intermediate isotope values shown for egg components of Herring Gulls breeding on the Atlantic Coast suggest nutrient input from terrestrial as well as marine sources. Our results indicate the utility of stable-isotope analysis for tracing endogenous and exogenous contributions to reproduction in birds and further validate the use of migratory birds as indicators of breeding area contaminant levels and their effects on the Great Lakes. Received 16 October 1996, accepted 14 February 1997.

HIGH LEVELS OF CONTAMINANTS present in eggs of Double-crested Cormorants (Phalacrocorax auritus) and Herring Gulls (Larus argentatus) have been linked to the low reproductive success shown by these species in the Great Lakes in the early 1970s (Gilbertson 1974, Weseloh et al. 1983). These species, together with Caspian Terns (Sterna caspia) have been studied extensively in the Great Lakes Basin as part of the Great Lakes colonial waterbird contaminant monitoring program initiated in 1971 by the Canadian Wildlife Service (Mineau et al. 1984; Government of Canada 1991; Bishop et al. 1992a,b; Pettit et al. 1994a,b). Over the past 20 years, however, contaminant levels have dropped substantially and breeding populations have increased dramatically (Mineau et al 1984; Ewins et al 1994b; Weseloh et al 1994, 1995). During this time, the use of migratory birds as indicators of local contaminant exposure on the Great Lakes breeding grounds seldom has been questioned for two reasons. First, spatial patterns of contaminant levels in the eggs of these birds were similar to those in fish (Suns et al. 1991), sediments (Frank et al. 1977, 1979), and eggs of other piscivorous birds (Bishop et al. 1992a,b; Yamashita et al. 1993; Pettit et al. 1994a,b). Second, in the 1970s and 1980s, the Great Lakes ecosystem was one of the most contaminated freshwater systems in the world, and levels generally were much higher than those at wintering grounds elsewhere (Nriagu and Simmons 1984). Therefore, the contribution of contaminants from wintering grounds to eggs laid in the Great Lakes was thought to be minimal.

Levels of most persistent contaminants in fish and bird species in the Great Lakes have declined to a plateau since the mid-1980s (Mineau et al. 1984, Borgmann and Whittle 1991). Small but detectable temporal fluctuations in residue levels remain in bird eggs (Bishop et al. 1992a,b; Sun et al. 1991). The use of migratory birds as indicators of local contaminant exposure on the Great Lakes breeding grounds is not in question anymore. However, the contribution of contaminants from wintering grounds to eggs laid in the Great Lakes was thought to be minimal.

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These fluctuations probably are due to many different factors, including variation in local feeding conditions, availability of forage fish (Ewins et al. 1994b), and the influence of weather-induced changes that might affect rates of contaminant uptake in biota (Smith 1995). The contribution of endogenous nutrients, and hence lipophilic persistent contaminants from wintering grounds, is another potential source of variation influencing temporal trends in contaminant levels in eggs of these migratory species. However, techniques for tracing the origin of nutrient input into eggs from endogenous reserves acquired on the wintering grounds have been unavailable until recently. In this study, we evaluate the use of stable-isotope analysis of eggs for tracing nutrient transfer between biomes and hence of the relative importance of endogenous and exogenous nutrient resources to reproduction.

Stable-isotope analysis of tissues can provide valuable information on the diets of individuals and populations (von Schirnding et al. 1982; Hobson, 1987, 1990; Mizutani et al. 1990; Schaffner and Swart 1991; Hobson and Sealy 1991; Hobson et al. 1994). This approach is based on the premise that stable-isotope ratios of various elements in a consumer's tissues ultimately are related to those in its diet (DeNiro and Epstein 1978, 1981). Conventional methods of diet analysis provide a relatively short-term and detailed assessment of dietary intake. Stable-isotope analysis complements these methods by allowing time-integrated dietary estimates and the evaluation of assimilated versus ingested dietary contributions (Tieszen et al. 1983, Hobson 1993). Stable-isotope analysis also may be particularly useful in determining the origin of nutrients in consumer tissues in cases where access to isotopically distinct sources of food occurs. Using this approach, the relative contributions of terrestrial and marine protein to consumer diets have been established for both modern and ancient food webs because $^{13}$C abundance in marine food webs typically is enriched over that of terrestrial C-3 food webs (Chisholm et al. 1982, Schoeninger and DeNiro 1984; Hobson and Collier 1984; Hobson 1987, 1990; Mizutani et al. 1990). Other elements with stable-isotope values that are typically enriched in marine versus terrestrial food webs include sulfur, hydrogen, and nitrogen (Fry and Sherr 1988, Michener and Schell 1994). The occurrence of isotopically distinct inputs to various systems also forms the basis for using stable-isotope measurements to trace sources of pollutants in food webs (see Macko and Ostrom 1994).

Dietary shifts in birds from terrestrial or freshwater C-3 to marine ecosystems (and vice versa) influence isotopic signatures in a variety of tissues (Hobson 1987, 1990; Mizutani et al. 1990; Thompson and Furness 1995). Bird eggs are particularly amenable to this type of analysis because nutrients required for egg production are derived from the diet of the laying female (von Schirnding et al. 1982, Schaffner and Swart 1991, Hobson 1995, Jarman et al. 1996). In their recent examination of seabird eggs and marine prey, Jarman et al. (1996) suggested that the combined use of contaminant and stable-isotope analyses of egg proteins and lipids could provide information on the allocation of endogenous versus exogenous lipid reserves to eggs. This, together with the isotopic investigations of Hobson (1995) using captive-raised birds, clearly suggests that stable isotopic analyses of various egg components can provide information on the source of nutrients in egg production. Our objective was to test the hypothesis that migratory birds wintering in marine areas acquire body-nutrient reserves that ultimately are transferred to their eggs laid on freshwater breeding grounds.

**Materials and Methods**

**Study sites and collections.**—Eggs of two migratory, fish-eating colonial waterbirds were selected: Double-crested Cormorant and Caspian Tern. Both species migrate from their wintering grounds in the Atlantic and Gulf Coast states (and the Caribbean Basin, in the case of terns) and arrive in April to breed in the Great Lakes in Canada (L'Arrive and Blokpoel 1988, Dolbeer 1991, Weseloh and Ewins 1994). For comparative purposes, we also analyzed eggs of Caspian Terns breeding in a marine environment of coastal Texas. Stable-isotope values for the eggs of Herring Gulls, one of the few nonmigratory, colonial fish-eating birds that breed on the Great Lakes (Moore 1976, Gilman et al. 1977, Weseloh 1984), were compared with those of nonmigratory Herring Gulls collected from a marine environment on the Atlantic Coast of Canada. In general, a transfer of marine-derived nutrients to eggs laid in a freshwater or terrestrial C-3 biome would be indicated by a substantial enrichment of $^{13}$C or $^{34}$S values above those expected.
from nonmigratory birds that feed exclusively in these latter areas.

Eggs of Double-crested Cormorants, Caspian Terns, and Herring Gulls were collected from Pigeon Island, Lake Ontario (44°04′N, 76°33′W). In addition, Caspian Tern eggs were collected from nests on spoil islands in the Laguna Madre of Texas (26°21′N, 97°18′W), and Herring Gull eggs were collected from two islands located on the Atlantic coast of Canada: Kent Island (44°35′N, 66°45′W) in the Bay of Fundy, and Gull Island (47°16′N, 52°46′W) in Witless Bay, Newfoundland.

One egg per nest was collected from freshly completed clutches. Although no attempt was made to randomize collection, sampling was opportunistic and likely unbiased because observers walked throughout colonies during collections. Egg “freshness” was determined by the appearance of newly deposited calcium carbonate residue around the shell and by flotation. Thus, all eggs collected probably were within the first 10 days of incubation. Each egg was wrapped in aluminum foil and frozen at −20°C within six h of collection. Within Lake Ontario, Herring Gull eggs were collected on 27 April 1994 (n = 7) and 14 May 1993 (n = 5), and Caspian Tern eggs were collected on 5 May 1994 (n = 12) and 14 May 1994 (n = 4). Because no significant difference in stable-isotope ratios between collection dates was observed for any egg component, values were pooled within species. To examine the influence of the amount of time spent on freshwater breeding areas to changes in isotope ratios, one Double-crested Cormorant egg was collected from each of 10 randomly selected nests containing early clutches (14 May 1993) and from each of six nests containing late clutches (10 June 1993). Caspian Tern eggs also were collected from the Gulf of Texas on 10 May 1993 (n = 10), and Herring Gull eggs were collected from Kent Island on 6 June 1993 (n = 5) and from Gull Island on May 23, 1994 (n = 12).

**Stable-isotope analysis.**—Eggs, eggshell, albumen, and yolk samples were separated by hand; subsamples of yolk were obtained with a syringe. Eggshells were rinsed in distilled water, allowed to dry at room temperature (after shell membranes were removed), and powdered using a mortar and pestle. Yolk was similarly powdered after freeze drying. Yolk lipids were rinsed in distilled water, allowed to dry at room temperature (after shell membranes were removed), and likely unbiased because observers walked throughout colonies during collections. Egg “freshness” was determined by the appearance of newly deposited calcium carbonate residue around the shell and by flotation. Thus, all eggs collected probably were within the first 10 days of incubation. Each egg was wrapped in aluminum foil and frozen at −20°C within six h of collection. Within Lake Ontario, Herring Gull eggs were collected on 27 April 1994 (n = 7) and 14 May 1993 (n = 5), and Caspian Tern eggs were collected on 5 May 1994 (n = 12) and 14 May 1994 (n = 4). Because no significant difference in stable-isotope ratios between collection dates was observed for any egg component, values were pooled within species. To examine the influence of the amount of time spent on freshwater breeding areas to changes in isotope ratios, one Double-crested Cormorant egg was collected from each of 10 randomly selected nests containing early clutches (14 May 1993) and from each of six nests containing late clutches (10 June 1993). Caspian Tern eggs also were collected from the Gulf of Texas on 10 May 1993 (n = 10), and Herring Gull eggs were collected from Kent Island on 6 June 1993 (n = 5) and from Gull Island on May 23, 1994 (n = 12).

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Powdered lipid-free yolk (hitherto referred to as yolk), yolk lipid, and eggshell samples for stable-carbon isotope analysis were placed in tin cups and introduced into a Europa Robo Prep combustion system interfaced with a Europa Tracermat continuous-flow isotope-ratio mass spectrometer. Eggshell carbonates were reacted with phosphoric acid under vacuum to evolve CO₂ for direct isotopic analysis using a VG OPTIMA mass spectrometer. Yolk samples for stable-sulfur isotope analysis were decomposed to sulfate by nitric-acid digestion, nitrate fusion, and barium precipitation, followed by thermal decomposition to sulfur dioxide before being analyzed using the VG OPTIMA mass spectrometer.

Stable-isotope concentrations were expressed in δ notation as the deviation from standards in parts per thousand (%) according to the following relationship:

\[ \delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000, \]  

where X is 13C or 34S and R is the corresponding ratio 13C/12C or 34S/32S. R̄ standard values for 13C and 34S correspond to the Pee Dee Belemnite (PDB) standard and Canyon Diabolo standard, respectively. Using laboratory internal standards for organic material, analytical error was estimated to be ±0.3‰ for 13C samples run on the Robo Prep, ±0.1% for carbonates run on the VG OPTIMA, and ±0.3‰ for sulfur samples.

**Statistical analyses.**—Simultaneous comparisons of mean δ13C and δ34S values in egg yolk among species and sites were performed using a multivariate analysis of variance (MANOVA) and Wilks’ Lambda as the test statistic. Mean δ13C values for yolk lipid and eggshell carbonate were compared among species and sites using a one-way analysis of variance (ANOVA). For all egg components, Tukey multiple range test was used to identify the source of variation when significant differences were detected among species and sites. Unless stated otherwise, all tests of significance refer to this test. Statistical analyses were conducted using SAS version 6.04 (SAS 1985). All tests were two-tailed, and statistical significance was defined as \( P < 0.05 \).

**RESULTS**

**Marine-freshwater comparison.**—Stable-carbon isotope values differed among egg components, with eggshell carbonates showing the most enriched and yolk lipids the most depleted values (Table 1). Overall, significant differences among all species and sites were detected in values of δ13C and δ34S in yolk (MANOVA, Wilks’ Lambda \( F = 55.42, \text{df} = 10 \) and 104, \( P < 0.0001 \)), and in values of δ13C in yolk lipids (ANOVA, \( F = 38.31, \text{df} = 6 \) and 61, \( P < 0.0001 \)) and eggshell carbonates (ANOVA, \( F = 49.56, \text{df} = 5 \) and 53, \( P < 0.0001 \)). Furthermore, these differences fit a consistent pattern; i.e. egg components of Caspian Terns from coastal Texas were significantly more enriched in 13C and 34S than those species, including Caspian Terns, that bred at Lake Ontario (Tukey tests throughout; Table 1, Fig. 1). Herring Gulls breeding off Newfoundland tended to show intermediate values of δ13C and δ34S between those breeding
### Table 1. Stable-isotope analysis (± SD, range and number of eggs analyzed in parentheses) of egg components. Within columns, means with the same letter are not significantly different (P > 0.05; Tukey test). For Double-crested Cormorants, early and late clutches are indicated by E and L, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Yolk δ¹³C (%o)</th>
<th>Yolk lipid δ¹³C (%o)</th>
<th>Carbonate δ¹³C (%o)</th>
<th>Yolk δ³⁴S (%o)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lake Ontario</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Double-crested Cormorant E</td>
<td>-22.8 ± 1.2ab</td>
<td>-25.3 ± 0.9b</td>
<td>-11.2 ± 0.5b</td>
<td>4.4 ± 0.5b</td>
</tr>
<tr>
<td>Double-crested Cormorant L</td>
<td>-23.9 ± 0.7bc</td>
<td>-25.7 ± 0.6bc</td>
<td>-11.4 ± 0.4b</td>
<td>4.9 ± 0.2b</td>
</tr>
<tr>
<td>Caspian Tern</td>
<td>-25.0 ± 1.2c</td>
<td>-27.0 ± 1.5c</td>
<td>-11.6 ± 0.8b</td>
<td>5.0 ± 0.6b</td>
</tr>
<tr>
<td>Herring Gull</td>
<td>-24.0 ± 1.0bc</td>
<td>-26.2 ± 0.7bc</td>
<td>-11.0 ± 0.8b</td>
<td>4.6 ± 0.5b</td>
</tr>
<tr>
<td>Herring Gull (Lake Ontario)</td>
<td>-21.8 ± 0.6a</td>
<td>-23.2 ± 0.6a</td>
<td>-9.4 ± 2.3a</td>
<td>—</td>
</tr>
<tr>
<td>Herring Gull (Newfoundland)</td>
<td>-21.7 ± 1.3a</td>
<td>-25.6 ± 0.7bc</td>
<td>-6.4 to -11.4, 5</td>
<td>10.7 ± 2.0a</td>
</tr>
<tr>
<td>Herring Gull (Coastal Texas)</td>
<td>-18.6 ± 1.0b</td>
<td>-21.2 ± 1.3d</td>
<td>-5.4 ± 1.5b</td>
<td>16.0 ± 1.3b</td>
</tr>
</tbody>
</table>

*Lipids removed.

in Lake Ontario and Caspian Terns breeding in the Gulf of Texas (Table 1, Fig. 1). Significant differences were detected in δ¹³C and δ³⁴S values in yolk of Herring Gull eggs collected from Lake Ontario and Newfoundland (MANOVA, Wilks’ Lambda F = 60.33, df = 2 and 17, P < 0.0001). No differences were detected for δ¹³C values in yolk lipids of Herring Gull eggs from Lake Ontario versus Newfoundland. However, differences were significant between mean yolk

![Diagram](image-url)

**Fig. 1.** Stable carbon- and sulfur-isotope values (± SD) of lipid-removed egg yolk of birds from Lake Ontario, Newfoundland and the Texas coast. CATE, Caspian Tern; HEGU, Herring Gull; DCCO, Double-crested Cormorant.
δ¹³C values for these two sites and New Brunswick (ANOVA, F = 33.64, df = 2 and 26, P < 0.0001). Significant differences also were detected in δ¹³C values in eggshell carbonates of Herring Gull eggs collected from Lake Ontario and New Brunswick (ANOVA, F = 4.80, df = 1 and 15, P = 0.045).

Lake Ontario comparison.—For species breeding on Lake Ontario, the isotope signatures were typical of C-3 freshwater biomes (Table 1, Fig. 1). Generally, Double-crested Cormorant eggs showed more enriched δ¹³C values than did Caspian Tern eggs. Marginally significant differences were detected in δ¹³C and δ³⁴S in yolk between early versus late-laid Double-crested Cormorant eggs (MANOVA, Wilks’ Lambda F = 4.11, df = 2 and 13, P = 0.042), with late-laid eggs showing δ¹³C and δ³⁴S values similar to those for Herring Gulls and Caspian Terns on Lake Ontario. The results of the MANOVA and Tukey tests (denoted by superscripts in Table 1) may not agree because the former test, which is more rigorous, compares means for two different variables simultaneously (e.g. δ¹³C and δ³⁴S values in yolk), and the latter test compares means for one variable across all sites sampled. No significant differences were found between early and late-laid cormorant eggs in δ¹³C of yolk lipids and eggshell carbonates. Consequently, for the purpose of interspecific comparisons on Lake Ontario, these two groups were pooled for both egg components (see below).

Overall, significant differences occurred in δ¹³C and δ³⁴S values in yolk among species breeding on Lake Ontario (MANOVA, Wilks’ Lambda F = 3.78, df = 6 and 72, P = 0.0025; not shown in Table 1). When δ¹³C and δ³⁴S values were analyzed separately among species, early laid Double-crested Cormorant eggs were significantly more enriched in values of δ¹³C in yolk than were values of δ¹³C in Caspian Tern eggs. No significant differences occurred in values of δ³⁴S among Lake Ontario species. Significant differences also were found for δ¹³C values in lipid among eggs of Caspian Terns, Herring Gulls, and Double-crested Cormorants (pooled) in Lake Ontario (ANOVA, F = 8.41, df = 2 and 38, P = 0.0009); upon further investigation, only lipid values in eggs of Double-crested Cormorants (pooled) and Caspian Terns were significantly different, whereas values for both of these species were not significantly different from eggs of Herring Gulls (not shown in Table 1). There were no significant differences in values of δ¹³C in eggshell carbonates among species found in Lake Ontario.

**DISCUSSION**

**Stable-isotope values in freshwater versus marine environments.**—The distinct isotopic segregation in egg components among Caspian Terns breeding in the marine environment of Texas and Double-crested Cormorants and Caspian Terns breeding on Lake Ontario provides little evidence for significant transfer of endogenous reserves from marine wintering sites by cormorants and terns to their eggs laid in a freshwater ecosystem. The isotopic evidence for this is based on inferences from our investigation involving stable isotopes of two elements and of three egg components. Our use of several egg components was based on an interest in evidence for mobilization of both proteins and lipids from endogenous reserves. Although birds might be expected to synthesize egg proteins directly from their diet, systematic declines in somatic protein in response to protein demands during egg production have been demonstrated in waterfowl (Alisauskas and Ankney 1992; see also Houston et al. 1995, Williams 1996). In such cases, the occurrence of carbon or sulfur from proteins originating in body reserves that were in turn derived from areas separate from the breeding grounds is possible. Lipid-nutrient storage prior to reproduction has been demonstrated in waterfowl (Alisauskas and Ankney 1992; see Perrins 1996), and it was this component of eggs that we assumed would have the greatest likelihood of providing isotopic evidence for transfer between marine and freshwater systems by migrating birds. Our δ¹³C analysis of shell carbonate was performed to test whether carbon isotope values of eggshell are directly related to isotope values in local foods. Unlike calcium that may be mobilized from medullary bone during shell formation, carbon for the calcium carbonate fraction of shell is derived directly from plasma through the metabolism of recently assimilated food (Simkiss and Tyler 1958).

The average δ¹³C values of egg yolk, yolk lipids, and eggshell carbonates for birds breeding on Lake Ontario were −24.1, −26.2, and
-11.3%, respectively, and the difference between these values and the respective values for eggs of Caspian Terns breeding on the Gulf coast of Texas (Table 1) were remarkably consistent, ranging from 5.0 to 5.9%. This isotopic difference between marine and freshwater endpoints is consistent with carbon-isotope gradients previously measured between primary production in each of these biomes (Fry and Sherr 1988, Schaffner and Swart 1991). However, considerable variation in δ¹³C values of biota in freshwater systems also have been documented (France 1995, Hecky and Hesslein 1995). Nevertheless, our inferences based on δ¹³C values are corroborated by δ³⁴S analyses and, for all egg components that we examined, there was no isotopic evidence for significant transfer of nutrients from marine to freshwater locations. Further support for this inference was provided by the similar carbon and sulfur isotope values for Lake Ontario Herring Gull, Caspian Tern, and Double-crested Cormorant eggs, because Herring Gulls were resident year-round and were expected to provide an entirely non-marine signal.

We also were interested in determining if there was evidence for changes in the isotope signatures of egg components between birds laying eggs shortly after arrival and those laying eggs later. A shift toward isotopically lighter isotope values of egg components later in the season might indicate that birds had mobilized nutrients from wintering grounds into their eggs. Our comparison of stable-isotope ratios in early Double-crested Cormorant eggs and those laid four weeks later indicated a slight but significant decrease in δ¹³C and a slight increase in δ³⁴S values in yolk only. It is difficult to know whether the δ¹³C changes provide evidence for a transfer of marine-derived nutrients from wintering grounds because even the early eggs showed δ¹³C (and δ³⁴S) values typical of the local food web (described below). Although isotopic values of components of cormorant eggs varied among individuals (Table 1), we detected no isotopic overlap between cormorant eggs and those of Caspian Terns breeding exclusively in the marine biome of coastal Texas. Enrichment in yolk δ³⁴S values for late clutches actually was opposite to the trend expected if marine-derived reserves had been used primarily in early clutches. Bulk protein sources for carbon and sulfur differ, with sulfur occurring specifically in protein pathways involving sulfur-bearing amino acids (e.g. cystein) compared with carbon that occurs in all amino acids. As such, seasonal changes in stable-carbon and sulfur isotope values may not necessarily be correlated.

It is also possible that diets of breeding cormorants changed seasonally (see below). Another variable complicating interpretation of the cormorant data is that the entire population does not winter consistently in marine environments. Most of the recoveries of juvenile Double-crested Cormorants (72%, age 1 to 2 years) banded on Lake Ontario in 1986 occurred during the winter months on the mid-Atlantic Coast and the Gulf Coast portions of Florida, Mississippi, and Louisiana (Weseloh and Ewins 1994). The remainder of the recoveries occurred farther inland in Mississippi, Louisiana, and Texas, where Double-crested Cormorants have been observed increasingly in the last 20 years feeding at freshwater lakes (Campo et al. 1993) and fish farms (Glahn and Stickley 1995, Jackson and Jackson 1995). If these patterns are typical of adults, then most Double-crested Cormorants would be expected to have endogenous reserves with isotopic signatures characteristic of a marine biome prior to arriving on the breeding grounds, provided that few reserves were acquired during migratory stopovers in freshwater habitats (Hobson and Clark 1992).

Further evidence that cormorants breeding in the 1990s in eastern Lake Ontario did not draw markedly on endogenous reserves accumulated on their wintering grounds is provided by a contaminant marker. Mirex released until 1978 at Oswego, New York and the Niagara River (Kaiser 1978) still is detected consistently and at relatively high levels in wildlife tissues sampled from Lake Ontario compared with the other Great Lakes (International Joint Commission1985, Struger et al. 1993, Ewins et al. 1994b). Mirex concentrations in Double-crested Cormorant eggs collected from Pigeon Island in 1990 ranged from 0.475 to 0.607 µg/g (Canadian Wildlife Service unpubl. data). In the southeastern United States in the early 1970s, mirex was used to control the spread of fire ants (Committee for Agricultural Science and Technology 1976). Since its ban in the United States in 1978 (Environmental Protection Agency 1977), however, mirex levels in wildlife
tissues have dropped dramatically. Reports of organochlorines in avian tissues in many parts of the southern United States indicate that mirex is detected infrequently (White et al. 1980, 1983), is found at relatively low concentrations compared with piscivorous species on the Great Lakes (King et al. 1987), or generally falls below the limit of detection, usually 0.1 µg/g wet weight (King and Kynitsky 1986). Within the last 10 years, few contaminants studies in these regions have reported significant levels of mirex in bird tissues. Therefore, the comparatively high levels of mirex for both early and late cormorant eggs at Lake Ontario in the 1990s provide further support that these migratory birds readily accumulate organochlorines on the breeding grounds and largely use nutrients acquired there for egg production.

The possibility that some cormorants may not have wintered exclusively in a marine biome could equally be applied to Caspian Terns (R. Pierotti pers. comm.). However, without dealing exclusively with marked individuals or using extremely large sample sizes, it is impossible to know whether we have encompassed individuals wintering in marine biomes exclusively. Further, we were interested in determining if there was positive isotopic evidence for a marine contribution to eggs laid at freshwater breeding grounds and so obtained a minimum estimate for this effect. The absence of this effect in our data does not preclude the possibility that some individuals may have wintered inland.

Stable-isotope ratios and diet.—Although we did not make direct isotopic measurements of food-web components in each of the three areas where eggs were obtained, other isotopic studies provide data for comparison with our results. In order to link isotopic measurements of egg components with the expected diet of laying females, it is necessary to apply diet-tissue isotope fractionation factors for each tissue of interest (Hobson and Clark 1992). Applying the appropriate fractionation factors derived by Hobson (1995) between carnivore diets and egg yolk, yolk lipid, and eggshell carbonates to the average egg component values reported above, we estimated dietary δ¹³C values to be −24.1, −22.8, and −22.5‰, respectively. These values are typically enriched by about 2‰ compared with those obtained by Kiriluk et al. (1995) for components of the pelagic food web of Lake Ontario. Such an enrichment is consistent with a more benthic- or inshore-based diet among the bird species that we examined (e.g. Ewins et al. 1994b, France 1995, Hecky and Hesslein 1995). However, Kiriluk et al. (1995) did not extract lipids from their samples prior to isotope analysis. Because lipids are isotopically lighter in ¹³C than in other tissues, Kiriluk et al.’s δ¹³C values may be biased toward more negative values compared with ours (Monteiro et al. 1991, Sholto-Douglas et al. 1991).

In Lake Ontario, observed differences among species in δ¹³C values in eggs may be related to differences in diet and foraging location during egg formation. Based on analyses of regurgitated pellets, Double-crested Cormorants, Caspian Terns, and Herring Gulls foraging in Lake Ontario consume a wide variety of prey items. Double-crested Cormorants and Caspian Terns are piscivores with diets dominated by shallow and warmwater fish (Ewins et al. 1994b), but cormorants appear to have a more varied diet, taking more benthic prey species, such as brown bullhead (*Ictalurus nebulosus*), white sucker (*Catostomus commersoni*), snails, and crayfish. Herring Gulls on the Great Lakes are opportunistic predators, feeding primarily on fish but also on terrestrial items including small mammals, refuse, and plant material (Fox et al 1990, Ewins et al. 1994a). Using the diet-tissue isotope fractionation factors estimated by Hobson (1995), the δ¹³C dietary values for Caspian Terns breeding in coastal Texas were estimated to be −18.5, −17.8, and −17.0‰ based on egg yolk, yolk lipid, and eggshell carbonates, respectively. These values were similar to δ¹³C values found for fish (δ = −17.0‰) and benthic filter-feeders (δ = −18.2‰) at offshore areas in the Gulf of Mexico (see Fry and Sherr 1988). Fry et al. (1977) examined δ¹³C values in coastal surface sediments from the Upper Laguna Madre region close to our Caspian Tern colony and obtained evidence for carbon derived from both seagrass and plankton. The derived δ¹³C values for the diets of laying Caspian Terns in this area are entirely consistent with the enriched δ¹³C values (range −12.5 to −17.2‰) measured by Fry et al. (1977) for this inshore coastal area, providing additional evidence that these birds may preferentially be feeding inshore.

In our study, Herring Gull eggs from Newfoundland and New Brunswick originally were
chosen to establish isotope values for eggs that were typical of gulls feeding in a marine biome. However, for both carbon and sulfur, gulls from these two regions showed stable-isotope values for both isotopes that were intermediate between those expected for gulls feeding in marine and terrestrial or freshwater C-3 food webs (Dickson 1986, Hobson 1987, this study). As a result of the large decline of northern cod (Gadus morhua) stocks off the coast of Newfoundland (Hutchins and Myers 1994), and a subsequent moratorium imposed on the Canadian fishing industry in 1992, fish offal that had been readily available to breeding Herring Gulls is no longer abundant (but see Pierotti and Annett 1987). Consequently, Herring Gulls, now more than ever, may be depending on refuse as a dietary source in Newfoundland (J. W. Chardine pers. comm, G. Fox pers. comm; see also Pierotti and Annet 1990). Additional isotopic studies designed to monitor changes in the diets of gulls in coastal areas would be valuable (e.g. Pierotti and Annet 1987). Herring Gulls also show considerable individual variation in prey selection or specialization (Pierotti and Annette 1990, 1991), and this was reflected in the range of isotope signatures in eggs of Herring Gulls in Newfoundland and New Brunswick (Table 1). Clearly, stable-isotope analysis of eggs represents a new and effective means of determining dietary differences among laying females within and among populations.

Stable-sulfur isotope ratios.—The use of a second element, sulfur, complemented the use of carbon in identifying sources of nutrients in the components of eggs. To date, no studies have investigated the relationship between stable-sulfur isotope values in dietary and various egg components. However, δ²⁷S values determined for the eggs of birds breeding on Lake Ontario were typical of those found in freshwater biomes in temperate regions, and little fractionation between diet and eggs is expected (Nriagu 1968, Nriagu and Coker 1976, Fry 1988, Krouse 1988). Similarly, the highly enriched δ²⁷S values determined for Caspian Tern eggs from a coastal marine area are consistent with values found in other marine food webs (Jackson and Gough 1988, Michener and Schell 1994). The large difference in δ²⁷S values of sulphates derived from terrestrial and marine sources (see Nriagu et al. 1991) suggests that this isotope actually is more useful than carbon in delineating sources of nutrients to birds moving between terrestrial and marine biomes. Previously, this isotope was more difficult to measure compared with carbon, a situation that has changed with the advent of continuous-flow isotope ratio mass spectrometers.

Conclusions.—Our study has demonstrated how a stable-isotope approach can be used to gain insight into the origins of nutrients from which eggs of piscivorous birds are formed. In addition, we have illustrated how this technique can be used as an aid in the interpretation of contaminant data derived from the analysis of bird eggs in freshwater and marine systems and, more importantly, how one might use the isotope technique to evaluate evidence for the transfer of nutrients and stored contaminants between these systems. This study adds yet another application to the rapidly expanding field of using stable-isotope and contaminant analyses together in ecosystem-level studies (e.g. Spies et al 1989, Broman et al 1992, Rolff et al 1993, Kidd et al 1995, Muir et al 1995, Jarman et al 1996).

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