INFLUENCE OF DIET AND ENVIRONMENTAL WATER ON THE CARBON AND OXYGEN ISOTOPIC SIGNATURES OF SEABIRD EGGSHELL CARBONATE

Fred Charles Schaffner and Peter Koenraad Swart

ABSTRACT

The carbon and oxygen isotopic ratios of carbonate samples from eggshells of seven species of seabirds were examined in relation to information on the birds’ foraging habits and geographic distribution, in order to determine how the isotopic signatures of eggshell carbonate are influenced by the feeding ecology of the females which laid the eggs. Results indicated a gradient of increasing relative abundance of the heavier isotopes with degree of participation in the marine ecosystem, from freshwater to estuarine to offshore to pelagic. For carbon, the isotopic gradient originates from the different isotopic signatures of the base source material (particulate organic carbon, or POC) suspended in meteoric versus oceanic water, and the mixing of these waters in the coastal zone, compounded by trophic biomagnification of $^{13}$C relative abundance through the marine food web. For oxygen, the isotopic gradient reflects the isotopic signatures of the source water ingested by the birds, itself reflecting the isotopic signatures and mixing of meteoric and oceanic water in the coastal zone. For consumers at similar levels of the food web, in this case high end consumers such as piscivores, isotopic information can provide reliable inferences about relative participation in marine versus freshwater type ecosystems at the time of eggshell formation. Carbon isotopic information can also provide information on the trophic status of consumers occupying similar foraging habitats. For freshwater and terrestrial feeders, $^{18}$O values can provide information on the latitude of occurrence of the birds at the time of eggshell formation, due to the characteristic signature of local meteoric water. The stable isotope methodology presented here can complement conventional dietary analyses, as well as provide useful information on its own, and adds an additional dimension to the study of avian foraging ecology. This approach will be particularly useful in places where frequent visits are impractical. An advantage of this methodology is that it is non-destructive. No live specimens, nor remains thereof, need be taken.

Recent developments in the study of stable isotopes have shown that the dietary patterns of animals can be revealed by the isotopic signatures of their tissues (Keith et al., 1964; DeNiro and Epstein, 1978a; 1978b; Hackney and Haines, 1980; Rau and Anderson, 1981; Schoeninger and DeNiro, 1984; Fry and Sherr, 1984; Peterson and Fry, 1987). Such information has been used in ecological studies of extant vertebrates, as well as in paleoecological studies (Land et al., 1980; Sullivan and Krueger, 1981; Tauber, 1981; von Schirnding et al., 1982; Longinelli, 1984; Luz et al., 1984; Hobson and Schwarcz, 1986; DeNiro, 1987; Hobson, 1987; Koch et al., 1989). Natural abundances of stable isotopes are useful not only as ecological dietary tracers in single species studies, but also for the elucidation of patterns of water uptake (Sternberg and Swart, 1987) and organic and inorganic material flow through communities and food webs, beginning with abiotic sources through to high end consumers (Haines and Montague, 1979; Hackney and Haines, 1980; Rau et al., 1983; Schildowski et al., 1983; Peterson et al., 1985; DeNiro, 1987; Mizutani and Wada, 1988).

Concurrently, yet largely independent of the developments in the study of the natural abundance of stable isotopes, there has been increasing interest in the feeding ecology of marine birds (Croxall et al., 1984; Croxall, 1987). Unfortunately, because of the costly and often logistically difficult nature of both pelagic and coastal shipboard ornithology, and the difficulty in making substantive ob-
servations of foraging from land, the at-sea feeding ecology of marine birds has remained somewhat under-represented. Rather than direct field observations of feeding, research has focused largely on dietary studies dependent on the analysis of stomach contents, regurgitations, or pellets collected at nesting colonies. A variety of arbitrary comparative dietary indices have been devised, but all are biased by differential rates of digestion, in favor of hard items and against soft-bodied food items. Conclusions about the prey base of particular seabird species could change, depending on whether comparisons were made based on prey mass, volume, or number of food items (Hyslop, 1980; Duffy and Jackson, 1986). For example, Furness et al. (1984) found that cephalopod beaks remained intact and accumulated in seabird crops long after the soft tissues had been digested. Lethal sampling for stomach contents is disruptive and wasteful, and is inadequate for comparisons among individuals, while sampling of regurgitations is obviously biased by the often substantial individual variability in tolerance to handling. Moreover, dietary sampling at nesting colonies usually cannot provide information on the food habits of females during the critical period of egg formation, a very important time in the life of a female, and ultimately of great importance to the entire population.

With the above considerations in mind, we sought an isotopic method which could provide useful information about the feeding ecology of freshwater, estuarine, and marine birds, and which, at the same time would be simple and non-traumatic to the birds, involve neither sacrificing nor invasive sampling, and make use of material which is readily available. In particular, we were interested in obtaining information which could (1) provide a measure of relative degree of participation in the marine ecosystem, from freshwater to estuarine to offshore to pelagic, for sympatrically nesting species assemblages, (2) provide a means of discerning the trophic status of species foraging in the same habitat, and (3) provide information relevant to the foraging habits of females during the critical period of egg formation. We sought also a methodology which could complement conventional dietary analyses, as well as providing useful information itself. Herein we evaluate the feasibility of such a method: the salvage of eggshell fragments from nesting colonies, for analysis of the carbon and oxygen isotopic composition of the eggshell carbonate. We demonstrate the influence of diet and environmental water on the carbon and oxygen isotopic signatures of seabird eggshell carbonate by comparing this isotopic data to information already available on the feeding habits of birds at these colonies during the same nesting seasons when our eggshell samples were collected.

BACKGROUND AND PREDICTIONS

Most elements occur in at least two stable isotopes, with one species being far more common than the others (Hofb, 1980). Differences in isotope ratios of elements in the tissues of organisms (their isotopic signatures) can be used as natural ecological tracers which provide information as to the source of the elements. For animals, elements are mainly incorporated through ingestion of water and of organic and inorganic substances. Carbon and nitrogen are taken in primarily as food (see Food Webs, below). Water can be taken in either indirectly as the moisture in food, or by the direct ingestion of free water. Unless the localities of these two source waters are very different, the hydrogen and oxygen isotopic signatures of these waters will be similar (see HYDROLOGICAL CYCLE, below).

Notation. — Differences in the natural abundance levels of stable isotopes are measured using an isotope ratioing mass spectrometer. The unit of isotopic ratio measurement is the delta value (δ), expressed in parts per thousand (‰) relative to an arbitrary standard, and is described by the following equation:

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1,000 \text{ (‰)}$$

(1)

where R equals the ratio of the heavy (rare) isotope to the light (common) isotope in the sample and standard.
The international standards for light isotopes of elements of ecological interest are: carbon (\(^{13}\text{C}/^{12}\text{C}\), or \(\delta^{13}\text{C}\) – PDB (the calcium carbonate of \textit{Blechnum americana} from the Cretaceous PeeDee limestone formation)), nitrogen (\(^{15}\text{N}/^{14}\text{N}\), or \(\delta^{15}\text{N}\) – AIR (atmospheric nitrogen)), oxygen (\(^{18}\text{O}/^{16}\text{O}\), or \(\delta^{18}\text{O}\) – SMOW (Standard Mean Ocean Water) or PDB, and hydrogen (\(^{2}H/^{1}\text{H}\) (=D/H), or \(\delta\)D) – SMOW (Friedman and O’Neil, 1977; Hayes, 1982). Oxygen \(\delta^{18}\text{O}\) values can be related to the SMOW standard by the equation

\[
\delta^{18}\text{O}_{\text{SMOW}} = 1.0309 \times \delta^{18}\text{O}_{\text{PDB}} + 30.09.
\]

Applications of Stable Isotope Studies.—Food Webs. Studies of stable isotope natural abundances have proven extremely useful in tracking material flow through food chains. The \(\delta^{15}\text{N}\) values of bone collagen and \(\delta^{13}\text{C}\) values of whole body carbon and bone collagen have been used to determine the relative amounts of marine versus terrestrial and freshwater foods in the diets of a variety of animals including man (Parker, 1964; Fritz and Poplawski, 1974; Sullivan and Krueger, 1981; Tauber, 1981; Schoeninger et al., 1983; Fry et al., 1984; Fry and Sherr, 1984; Schoeninger and DeNiro, 1984; DeNiro, 1987). The \(\delta^{15}\text{N}\) and \(\delta^{13}\text{C}\) values of marine feeders are higher than those of their terrestrial counterparts. For nitrogen, this is thought to be due to a greater abundance of the heavier isotope in the plants at the base of marine food webs as compared to those at the base of terrestrial food webs, compounded by trophic effects (the preferential retention of the heavier isotope with each step in the food chain). The gradient for carbon is based largely on the different isotopic signature of the plants and their suspended decomposition products (particulate organic carbon, or POC) at the base of the respective food webs. Oceanic POC, based largely on marine phytoplankton with \(\delta^{13}\text{C}\) values averaging about \(-21\%_{\text{PDB}}\), can be distinguished from the POC suspended in freshwaters. Freshwater POC has \(\delta^{13}\text{C}\) values averaging about \(-26\%_{\text{PDB}}\), being largely influenced by terrestrial C3 plants due to runoff. Small but significant trophic effects, the increase in \(\delta^{13}\text{C}\) values of muscle and whole body carbon of animals relative to their diets, of about “1% per trophic level” (DeNiro and Epstein, 1976), generally have been observed (Williams and Gordon, 1970; Rau et al., 1983; Chisholm et al., 1982; Schoeninger et al., 1983; Fry et al., 1984; Fry and Sherr, 1984; Schoeninger and DeNiro, 1984; Peterson et al., 1985; DeNiro, 1987).

Although previous authors found little evidence of a trophic effect for bone collagen carbon (Schoeninger et al., 1983; Schoeninger and DeNiro, 1984; DeNiro, 1987), significant trophic effects for carbon isotopes of other tissues have been found (Chisholm et al., 1982; Fry et al., 1984; Peterson et al., 1985; DeNiro, 1987). For example, DeNiro and Epstein (1976; 1978a; 1978b), and Rau et al. (1983) found that the isotopic composition of whole body carbon or muscle carbon of an animal is on average enriched by about 1% relative to its diet, but that (1) individuals of a given species could differ by as much as 2%, (2) different components of the diet could be absorbed preferentially (which can account for some consumers being lighter than their diets), and (3) fractionations can differ by as much as 3% between different tissues in an individual. Von Schirnding et al. (1982) demonstrated that the isotopic compositions of dietary material is tracked (including discrimination between C3 and C4 plant diets) by the isotopic signature of the consumer’s eggshell carbonate and that the carbonate isotope ratios are stable for at least 10,500 years.

Hydrological Cycle. Differences in the vapor pressure of the various isotopic species in water cause the heavy isotopes of hydrogen and oxygen, D and \(^{18}\text{O}\), to be preferentially retained in the liquid phase. This difference is described by the fractionation factor \((\alpha)\) and varies as a function of temperature (Kakuchi and Matsuo, 1979). Dansgaard (1964) has shown that the \(\delta^{18}\text{O}\) composition of rainwater averaged over an entire year varies linearly with the mean annual temperature difference between the place of evaporation and the place of deposition, giving rise to the observation that at higher altitudes and latitudes precipitation becomes isotopically depleted (Yurtsever and Gat, 1981). Global rain systems begin as cloud vapor evaporates from tropical oceanic reservoirs and becomes isotopically depleted relative to ocean water. As precipitation continues and the vapor moves polewards, the vapor itself becomes increasingly depleted, and as a result, subsequent precipitation condensing from the vapor becomes progressively lighter and lighter. This process is known as Rayleigh distillation, and accounts for progressive seasonal differences in the isotopic signatures of precipitation with increasing latitudes (see Kohler et al., 1989). The oxygen isotopic composition of pelagic oceanic water varies by only \(\pm 0.5\%\) worldwide, while meteoric water, the principal source of most bodies of freshwater, can be highly isotopically depleted (Craig, 1957; 1961; Dansgaard, 1964). Thus, the mixing of the coastal runoff meteoric water
with ocean water (mixed masses of water) forms the basis of our expectation of a positive gradient in the $\delta^{18}$O values of eggshell carbonate from freshwater consumers in isotopically depleted waters ($\delta^{18}$O values $< 0\%_{o}$SMOW) to full oceanic consumers in waters of about $3\%_{o}$SMOW. This gradient should be more pronounced at higher latitudes than in equatorial regions, due to the latitudinal trend in meteoric water (op. cit.).

Even for air breathing vertebrates, the oxygen isotopic signature of total body water (the "background signal") shows only minor short-term variability due to respiration and metabolism (Liison et al., 1949; Lifson and McClintock, 1966; Luz et al., 1984). Luz and Kolodny (1985) demonstrated that drastic environmental changes involving the rates of water and carbon dioxide turnover, atmospheric oxygen incorporation, and metabolism (without a change in the oxygen isotopic composition of the source water itself) have a negligible effect on the oxygen isotopic composition of vertebrate body water. Wolf et al. (1979) provided experimental evidence that ingestion of $^{18}$O-enriched water leads to similar enrichment in body water and tissues. Moreover, several studies of both free-living and captive vertebrates have demonstrated correlations between the $\delta^{18}$O composition of environmental water and inorganic tissues ($CO_{2}$ and $PO_{4}$ of mammalian apatite), as well as between the $\delta^{13}C$ composition of body water and inorganic tissues (e.g., Land et al., 1980; Longinelli, 1984; Luz et al., 1984; Luz and Kolodny, 1985). Thus, the evidence presently available indicates that the principal source of variation in the oxygen isotopic composition of both body water and inorganic tissues is the variation in isotopic composition of the environmental water ingested. In the absence of diagenesis, the oxygen isotopic signatures of inorganic tissues also are likely to have long-term stability. For example, Koch et al. (1989) were able to track the seasonal differences in oxygen isotopic signatures of environmental water using the hydroxyapatite of the dental laminae of Pleistocene proboscidean tusks and molars.

**Eggshell Formation.**—Eggshell formation and eggshell structure have been described by Simkiss (1961), Tyler (1969), Taylor (1970), and Mongin and Carter (1977). The eggshell consists of protein membranes upon which is laid calcium carbonate in the form of calcite. Most of the carbonate is provided for crystal formation by the mucosa of the shell gland via the action of carbonic anhydrase:

$$H_{2}O + CO_{2} \rightarrow H_{2}CO_{3} \rightarrow HCO_{3}^{-} + H^{+}.$$  \hspace{1cm} (4)

The bicarbonate ion is transported to the lumen of the shell gland for reaction with calcium, and the hydrogen ion is transported into the blood, resulting in short-term acidosis. The carbon and oxygen of the carbonate are thought to originate from recently ingested material, rather than stored tissues. Females feed continuously to, and often during, the process of eggshell formation, and digestion continues as the eggshell is formed. The process takes about 16 h for all birds.

**Predictions.**—Based on the above considerations, we make the following predictions for sampling carbon from a given tissue, in this case eggshell carbonate: (1) for consumers (in this case piscivores) at similar positions in their respective food webs, relative participation in the marine ecosystem will be indicated by a gradient in $\delta^{13}C$ values, becoming less negative from freshwater to pelagic, (2) species with the most variation in diets and feeding zones between individuals will exhibit the widest spread in $\delta^{13}C$ values, and (3) for species feeding in the same environment but at different levels in the food web (e.g., pelagic planktonivores versus pelagic piscivores), lower levels in the food web will be represented by more negative $\delta^{13}C$ values. Without trophic effects, the minimum between-species spread in $\delta^{13}C$ values between freshwater versus pelagic feeders will be approximately 5% (reflects POC bases of $26\%$ to $21\%$, see Table 1), and the within-species spread in $\delta^{13}C$ values for consumers which forage widely from freshwater areas to marine areas (e.g., Species 2, Caspian Terns, below) can be expected to be about 5% as well.

Similarly, for sampling oxygen from a given tissue, in this case eggshell carbonate, we predict that: (1) relative participation in the marine ecosystem will be manifested in a positive trend in $\delta^{18}$O values from freshwater to pelagic, and that (2) species with the most variation in feeding zones between individuals will also have the widest spread in $\delta^{18}$O values. Because the oxygen fixed in eggshell carbonate originates from local environmental water, we predict a 4% spread in $\delta^{18}$O values for our array of consumers (see Methods, below), from our freshwater feeder (Species 1) in environmental water of $-4\%_{o}$SMOW to our pelagic oceanic feeders (Species 5, 6, and 7) in environmental water of $0\%_{o}$SMOW (Table 1). Thus, the oxygen isotopic composition of eggshell carbonate will provide useful ecological information, in addition to the somewhat higher resolution information provided by eggshell carbonate carbon.

**Materials and Methods.**

We salvaged eggshells of hatched and abandoned eggs of seven species of seabirds. The species chosen for study are known to represent a qualitative gradient of feeding zones, from freshwater to pelagic. Each eggshell sampled represents a different female, e.g., we sampled one eggshell per nest.

We collected eggshell fragments of Species 1 at Sweetwater Reservoir, San Diego County, California (32°41'N, 116°60'W) (see Grizzle, 1984). Eggshell fragments of Species 2 and 3 were collected at the
Table 1. Isotopic composition of particulate organic carbon (POC) and environmental water end point source materials in the freshwater/terrestrial to marine gradients at San Diego and Puerto Rico

<table>
<thead>
<tr>
<th>Endpoint source material composition*</th>
<th>Terrestrial/freshwater</th>
<th>Pelagic oceanic</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>D^13C_POC of POC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Diego</td>
<td>−26.0‰</td>
<td>−21.0‰</td>
<td>+5.0‰</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>−26.0‰</td>
<td>−21.0‰</td>
<td>+5.0‰</td>
</tr>
<tr>
<td>D^18O env of environmental water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Diego</td>
<td>−4.0‰</td>
<td>0.0‰</td>
<td>+4.0‰</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>−2.0‰</td>
<td>0.0‰</td>
<td>+2.0‰</td>
</tr>
</tbody>
</table>

* Established in the literature. For carbon (D^13C_POC of POC) see Williams and Gordon, 1970; Hackney and Haines, 1980; Chisholm et al., 1982; Rau et al., 1983; Schoening et al., 1983; Fry et al., 1984; Fry and Sherr, 1984; Schoening and DeNiro, 1984; Peterson et al., 1985; DeNiro, 1987. For oxygen (D^18O env of environmental water) see Craig, 1957; 1961; Dansgaard, 1964; Yurtsever, 1975; Fontes, 1981; Gat and Coudrain, 1981; Yurtsever and Gat, 1981.

tern nesting colonies located at the south end of San Diego Bay, San Diego County, California (32°36'N, 117°07'W) (see Schaffner, 1982; 1985; 1986; 1990a). Eggshell fragments of Species 4–7 were collected at nesting colonies located on several cayos in the Culebra Archipelago, Culebra National Wildlife Refuge, Puerto Rico (18°20'N, 65°18'W) (see Kepler and Kepler, 1978; Furniss, 1983; Schaffner, 1988; 1990; Pennycuick et al., 1990). The San Diego samples (Species 1–3) were collected during May–July of 1980–1983, and the Culebra samples (Species 4–7) were collected during May–July of 1984–1988. Both collections were concurrent with studies, by Schaffner and others, of the breeding biology and ecology of these populations of these species at these sites. The diets and feeding zones of these birds can be characterized as follows:

1. Western Grebe (Aechmophorus occidentalis). Adult body mass = 1,500 g. A migratory freshwater forager which occasionally feeds in estuarine areas. Highly piscivorous, feeding primarily on small fishes such as threadfin shad (Dorosoma pretense) and bluegill (Lepomis macrochirus) and perhaps also crayfish (Astacus sp.) at our study site during the period of eggshell collection (Bent, 1919; Nuechterlein, 1981; Grizzle, 1984; Schaffner, unpubl.).

2. Caspian Tern (Sterna caspia). Adult body mass = 650 g. A generalist forager which takes a wide variety of prey types. It is primarily estuarine, but ranges very broadly from freshwater to littoral areas. Based on food remains found at nest sites early in incubation, prey species of breeders at our sample site during the period of eggshell collection included the estuarine topsmelt (Atherinops affinis), as well as a variety of other prey, including freshwater species such as crayfish (Astacus sp.), bullfrog (Rana catesbeiana), bluegill (L. macrochirus), smallmouth bass (Micropterus dolomieu), the freshwater/estuarine threadfin shad (D. pretense), an inshore marine sculpin (Arctidens sp.), California halibut (Paralichthys californicus), black surfperch (Embiotoca jacksoni), and occasionally, the offshore northern anchovy (Engraulis mordax). Observations of dawn flight lines of birds departing the nesting colony suggested that individuals used particular foraging areas routinely (Schaffner, 1982; unpubl.; Ohlendorf et al., 1985; Hayes and Schaffner, 1986).

3. Elegant Tern (Sterna elegans). Adult body mass = 235 g. Offshore marine, ranging from inshore to near pelagic. Never forages in freshwater areas. These birds are highly gregarious, and forage in highly structured social groups. Foraging birds from our sample site during the period of eggshell collection specialized on a single species, the offshore northern anchovy (E. mordax) (about 10 cm body length) (Schaffner, 1982; 1985; 1986; 1990a).

4. Laughing Gulls (Larus atricilla). Adult body mass = 325 g. A generalist marine forager, feeding on baits from inshore areas to the pelagic environment. Birds from this tropical island locality were observed fishing very far offshore, as well as scavenging along the shoreline during the period of eggshell collection (Schaffner, unpubl.). Schaffner also observed food remains at nest sites of incubating parents, including dwarf herring (Jenkinsia lamprotaenia), redear herring (Harengula humeralis), false pilchard (H. clupeola), silversides (Jenkinsia spp.), and garbage. A few individuals were observed drinking freshwater from rain puddles. Additional general information on this species can be found in Bent (1921), Cobleitzz (1985), Kepler and Kepler (1978), and Furniss (1983).

5. Sooty Tern (Sterna fuscata). Adult body mass = 180 g. Pelagic. Seldom feeds within sight of land. Schaffner observed juvenile fishes and especially small squids, in the regurgitations of incubating adults during 1984–1985. Invertebrates, primarily small squids (body lengths up to 8 cm), compose over half the diet (J. Saliva, pers. comm., and see also Harrison et al., 1983). Kepler and Kepler (1978), and Furniss (1983) have previously described the study site.

6. White-rumped Tropicbird (Phaethon lepturus). Adult body mass = 370 g. Pelagic. Birds from our study area during the period of eggshell collection took atherinomorph fishes, particularly the flyingfish Parexocoetus brachypnema (Schaffner, 1988; 1990; unpubl.; Fuller et al., 1989), with admixtures of squid. During 1984–1986 fish represented 75–90% of the individual food items of pre-breeding and
incubating adults, the balance being large squid (body lengths to 17 cm). During this same period chick regurgitations contained 64–73% fish by item, and regurgitations from chick-rearing adults contained 80–87% fish by item (Schaffner, 1988). Additional information on this species can be found in Stonehouse (1962), Kepler and Kepler (1978), and Furniss (1983).

7. Red-billed Tropicbird (*Phaethon aethereus*). Adult body mass = 630 g. Pelagic. Birds from our study area during the period of our eggshell collection fed largely on flyingfishes, particularly *Hirundichthys* spp. and *Cypselurus* spp., with admixtures of large squid (body lengths to 25 cm) (Schaffner, 1988; unpbl.). Additional information on this species can be found in Stonehouse (1962), Kepler and Kepler (1978), and Furniss (1983).

The eggshell fragments, seven from each species, were crushed to a fine powder and bleached (1.5% NaOCl for 1 h) to oxidise the organic fraction. Replicate analyses showed that this procedure had a negligible effect on the isotopic composition of the eggshells. The carbonate was reacted with phosphoric acid at 50°C to generate CO₂ using the modified method of McCrea (1950). Isotope ratios were measured using a Finnigan Mat 251 mass spectrometer and expressed in delta (δ) values relative to the PDB (C) and SMOW (O) standards, with conventional corrections for contributions of masses 45 and 46 (Craig, 1957), modified for a triple collector mass spectrometer. External reproducibility for our instrument is approximately 0.10% for δ¹⁸O and 0.05% for δ¹³C.

During the period of eggshell collection and feeding studies, we salvaged samples of breast muscle from dead Elegant Tern and White-tailed Tropicbird chicks, whole body muscle from their food species, and food species of Caspian Terns. We combusted them to generate CO₂ for ¹³C analysis. We extracted the organic fractions (membranes) of some larger eggshell fragments with 10% HCl, and combusted them to generate CO₂ for ¹⁸O analysis. Samples of blood water from chick and adult White-tailed Tropicbirds were collected and reacted using the guanidine-HCl method (Wong et al., 1987) to generate CO₂ for ¹⁸O analysis.

**RESULTS**

The isotopic signatures of eggshell inorganic carbon (Fig. 1) ranged from a mean of −18.19‰ (SD = 0.61) for the freshwater Western Grebe, up to −3.86‰ (SD = 0.60) and −4.10‰ (SD = 0.48) for the tropicbirds. The total range observed (about 14%), as well as the 9% range between Western Grebes and Caspian Terns, and the 11% range between Western Grebes and Elegant Terns, were greater than the 5% range in carbon values which would result from the POC freshwater-pelagic gradient alone (Table 1). The within-species range in δ¹³C values for Elegant Terns was narrow, while that for Caspian Terns was wide (Fig. 1).
Table 2. Isotopic composition (‰, PDB) of organic carbon from two seabirds and the food species of four seabirds. Common names of food fish species are given in the Methods bird species synopses. Analyses of adult regurgitations (White-tailed Tropicbirds), and observations of food items dropped at nest sites early in the incubation period (terns) indicated that adults were utilizing these same food species (Schaffner, 1982; 1986, 1988; Ohlendorf et al., 1985; Hayes and Schaffner, 1986).

<table>
<thead>
<tr>
<th>Species</th>
<th>$\delta^{13}C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>White-tailed Tropicbird chick (breast muscle)</td>
<td>-15.56</td>
</tr>
<tr>
<td>Food species*</td>
<td></td>
</tr>
<tr>
<td>Squid (large adult)</td>
<td>-16.96</td>
</tr>
<tr>
<td>Cypselurus spp.</td>
<td>-17.25</td>
</tr>
<tr>
<td>Parexocoetus brachypterus</td>
<td>-17.75</td>
</tr>
<tr>
<td>Elegant Tern chick (breast muscle)</td>
<td>-18.81</td>
</tr>
<tr>
<td>Food species*</td>
<td></td>
</tr>
<tr>
<td>Engraulis mordax</td>
<td>-19.31</td>
</tr>
<tr>
<td>Caspian Tern food species†</td>
<td></td>
</tr>
<tr>
<td>Paralichthys californicus</td>
<td>-14.59</td>
</tr>
<tr>
<td>Atherinops affinis</td>
<td>-15.30</td>
</tr>
<tr>
<td>Arctidius sp.</td>
<td>-19.78</td>
</tr>
<tr>
<td>Dorosoma pretense†</td>
<td>-23.89</td>
</tr>
<tr>
<td>Lepomis macrochirus‡</td>
<td>-26.80</td>
</tr>
</tbody>
</table>

* Food samples from regurgitations.
† These fishes were also food species of Western Grebes nesting at Sweeterwater Reservoir, San Diego County, California (Schaffner, unpubl. 1982–1983).

For the breast muscle of chicks, our results (Table 2) suggest a trophic effect of 0.50 to 2.19‰ between the consumer and its diet, and a total trophic effect of 3.00 to 6.00‰ between the birds and POC. In addition, a difference of ca. 9–11‰ between the birds' organic (muscle) and inorganic (eggshell carbonate) carbon is indicated (Tables 2, 3, Fig. 1). The $\delta^{13}C$ values of Caspian Tern food species varied widely, over a range of 12.12‰, and two freshwater food species common to both Caspian Terns and Western Grebes were highly negative ($-23.89‰$ and $-26.80‰$). For all species, the $\delta^{13}C$ values for their eggshell organic fractions were substantially lighter than their inorganic fractions (Table 3), except for two anomalous values for Western Grebes. The total difference between the $\delta^{13}C$ values for the birds' eggshell carbonate and the POC assumed to be at the bases of their food webs varied from 7.81‰ for Western Grebes (freshwater) to 17.20‰ for White-tailed Tropicbirds (pelagic) (assumes POC values of $-26.0‰$ and $-21‰$ respectively).

We urge caution in the interpretation of the variability of carbon isotope ratios of the organic fractions (membranes) of our samples of eggshells (see Table 3). These samples were collected opportunistically, and the most variable values (within-egg organic-inorganic differences, $\Delta_{O-I}$, Table 3) were from abandoned Western Grebe and Caspian Tern eggs. These eggs failed at unknown stages of development, as indicated by the highly variable degrees of bacterial decomposition of the egg contents. Eggshell membranes are the gas exchange organs of the developing chick, and experience substantial modifications during the incubation period. They change from thin and delicate structures at the time of laying, to thick and leathery after hatching, when they presumably contain substantially increased amounts of collagen. Captive studies under controlled conditions are needed to pinpoint the effects of embryo development on membrane carbon isotope ratios, but it is important to note that most of our results (Table 3) for hatched eggs (=matured membranes) are similar to those of Sullivan and Krueger (1981), who found a ca. 8‰ difference between the organic and inorganic fractions of mammalian bone.
Table 3. The $\delta^{18}$O values of individual eggshells from which both organics and inorganics were analysed. Samples WG-1 through WG-7, and CT-1 through CT-3 were from abandoned eggs which failed at an unknown stage of incubation and exhibited highly variable levels of bacterial decomposition of the egg contents, whereas CT-6, ET-1 through ET-4, and WT-6 were from hatched eggs with mature membranes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample</th>
<th>$\delta^{18}$O Organics</th>
<th>$\delta^{18}$O Inorganics</th>
<th>$\delta^{18}$O Eos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Grebes</td>
<td>WG-1</td>
<td>-20.22</td>
<td>-18.37</td>
<td>1.85</td>
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<tr>
<td></td>
<td>WG-2</td>
<td>-17.95</td>
<td>-18.03</td>
<td>-0.08</td>
</tr>
<tr>
<td></td>
<td>WG-3</td>
<td>-24.34</td>
<td>-17.87</td>
<td>6.47</td>
</tr>
<tr>
<td></td>
<td>WG-7</td>
<td>-25.22</td>
<td>-17.36</td>
<td>7.86</td>
</tr>
<tr>
<td>Caspian Terns</td>
<td>CT-1</td>
<td>-19.31</td>
<td>-11.90</td>
<td>7.41</td>
</tr>
<tr>
<td></td>
<td>CT-2</td>
<td>-13.81</td>
<td>-8.20</td>
<td>5.61</td>
</tr>
<tr>
<td></td>
<td>CT-3</td>
<td>-13.55</td>
<td>-8.51</td>
<td>5.04</td>
</tr>
<tr>
<td></td>
<td>CT-6</td>
<td>-20.44</td>
<td>-11.07</td>
<td>9.37</td>
</tr>
<tr>
<td>Elegant Terns</td>
<td>ET-1</td>
<td>-15.89</td>
<td>-7.47</td>
<td>8.42</td>
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<tr>
<td></td>
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<td></td>
<td>ET-4</td>
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</tr>
<tr>
<td>White-tailed Tropicbirds</td>
<td>WT-6</td>
<td>-13.64</td>
<td>-4.88</td>
<td>8.76</td>
</tr>
</tbody>
</table>

The $\delta^{18}$O values of the eggshell carbonate of our seabird samples (Fig. 2) varied from 24.49‰$_{SMOW}$ (SD = 0.58) for the freshwater Western Grebes, to 28.54‰, 28.81‰, and 28.57‰ (SDs = 0.26, 0.36, and 0.49) for the pelagic Sooty Terns, White-tailed Tropicbirds, and Red-billed Tropicbirds, respectively. This 4‰ range between species means is identical to the 4‰ difference in their source waters (Table 1). Note particularly, the 2‰ within-species range in $\delta^{18}$O values of Laughing Gulls, which is identical to the total range between the freshwater and pelagic marine source water signatures in Puerto Rico (Table 1).

Figure 2. Oxygen isotopic composition of seabird eggshell carbonate; N = 7 for each species.
DISCUSSION

Carbon.—The carbon isotopic ratios of our samples of eggshells increased over a range of 14%, from freshwater (Species 1) to estuarine (Species 2) to offshore (Species 3) to pelagic foragers (Species 5–7) (Fig. 1), with one apparent outlier, Sooty Terns (Species 5), in the pelagic group. This trend, and the outlier, can be explained on the basis of (1) the carbon signatures of the source material and (2) trophic effects.

At least 5% of this trend can be explained solely as a result of differences in the carbon isotopic ratios of the particulate organic carbon (POC) source material at the bases of the respective food webs (Fry et al., 1984; Fry and Sherr, 1984). On this basis alone, it is not surprising that the species with the most wide-ranging foraging areas, e.g., Laughing Gulls, and most especially, Caspian Terns, have the widest ranges of δ13C values in their eggshell carbonate. Note particularly the 5‰ spread in δ13C for Caspian Terns, which themselves forage in areas ranging from freshwater to full seawater.

A large portion of the positive trend amongst our array of seabirds, 11‰, was found between the first and third species, the freshwater Western Grebe and the marine (but not truly pelagic) Elegant Tern. Thus, the freshwater to oceanic water mixing model alone cannot explain the entire spread amongst our range of consumers. We believe trophic effects (= the preferential retention of the heavier isotope with each step in the food chain) to be responsible for much of the remainder of the freshwater to pelagic spread.

In a study of the carbon isotope ratios of eggshells of the African Ostrich (Struthio camelus), von Schirnding et al. (1982) found a 2.1‰ trophic effect between the birds’ diet (lucerne, oats, natural forage, and maize) and their organic carbon. This finding agrees with the 0.50‰ to 2.19‰ trophic effect observed in our seabirds (Table 2, Fig. 3). These authors also reported a 16.2‰ difference between the birds’ diets and their eggshell carbonate, and a 14.1‰ difference between the eggshell organic and inorganic fractions. These differences are greater than those of most of our samples (see Tables 2 and 3, Fig. 3). However, the physiochemical pathways responsible for these effects are not well-understood, and will only be identified through captive studies under controlled conditions. A potential contributor which warrants further examination is the gassification associated with the digestion of carbohydrates. Our birds are largely protein feeders, unlike ostriches, which are carbohydrate feeders. By exporting light carbon, digestive gassification of carbohydrates could isotopically enrich the carbon reservoir which is available within the bird for carbonate precipitation. Other potential modifiers such as respiration and exchange with atmospheric CO2 are likely to be similar for most species of birds.

Because most of the trophic steps in aquatic and marine food chains occur from POC through phytoplankton and zooplankton microfauna to schooling squids and fishes, a greater total trophic effect is expected amongst a group of consumers exploiting resources spread through these levels than for a group of predators whose prey all share the same POC-microfaunal base. Moreover, the total number of sub-macrofaunal trophic levels is greater in the marine environment than in the freshwater environment (Schoeninger et al., 1983; Fry et al., 1984; Fry and Sherr, 1984; Schoeninger and DeNiro, 1984; DeNiro, 1987). Macrofaunal predators including seabirds, sharks, tunas, pinnipeds and toothed cetaceans, which feed on prey that are already high in the food web, occupy a narrower range of higher trophic levels as compared to their counterparts feeding at microfaunal levels and lower (see Mearns, 1982; Rau et al., 1983). Means of δ13C values for
Figure 3. Flow chart diagram of paths traveled by carbon and oxygen (inset) from the environment to incorporation in the consumer bird’s eggshell carbonate. Carbon paths for protein feeders (piscivorous seabirds) and herbivores are given. Values (δ values) are given in ‰ PDB for carbon and ‰ SMOW for oxygen. Representative piscivores are the offshore marine Elegant Terns (ET) and pelagic White-tailed Tropicbird (WT) from this study. Prey compositions by item are 100% fish for Elegant Terns, and 85% fish, 15% squid for White-tailed Tropicbirds (see text, and Tables 1, 2, and 3). Herbivore values are taken from von Schirnding et al. (1982) for African Ostriches at Elands Bay, South Africa. Path of oxygen is the same for all vertebrates. The symbol Δ indicates the change in isotope ratio of carbon or oxygen with a given step in the path towards the specified end consumer’s eggshell carbonate.
our species (Fig. 1) support this assessment, in the greater than 5‰ spread from freshwater to full seawater (from Western Grebes, to Caspian Terns, to Elegant Terns). Our three exclusively pelagic feeders (Sooty Terns, White-tailed Tropicbirds, Red-billed Tropicbirds) take prey which are themselves highly predatory. The two tropicbirds are predominantly piscivorous, whereas at least half the diet of Sooty Terns is composed of invertebrates (small squid) (Stonehouse, 1962; Kepler and Kepler, 1978; Furniss, 1983; Harrison et al., 1983). Furthermore, Sooty Terns, being of considerably smaller body size than the tropicbirds, are obliged to take much smaller prey which are themselves necessarily lower in the marine pelagic food web (see Mearns, 1982). This results in more negative $\delta^{13}C$ values for Sooty Terns (Fig. 1). The wide range of $\delta^{13}C$ values, for the generalist Laughing Gulls is the result of feeding on prey which occupy a diversity of levels in the food chain and which also occur over a wide range of the meteoric to oceanic mixing gradient.

In summary, we have made several conclusions based on our sampling of carbon from seabird eggshell carbonate: (1) For consumers at equivalent positions in their own respective food chains (e.g., high end consumers such as piscivores), relative participation in the marine ecosystem is manifested in a gradient in $\delta^{13}C$ values of at least 5‰, becoming less negative from freshwater to pelagic. (2) More diverse feeding zones result in wider within-species spreads in $\delta^{13}C$ values, and the effect of feeding on prey which occupy several levels of the food web is to further enhance the within-species spread. (3) For species feeding in the same environment, but at different levels in the food web, those feeding at lower levels are distinguished by more negative $\delta^{13}C$ values.

The ecologically relevant information available from $\delta^{13}C$ values will be most useful in studies of assemblages of sympatric breeders, as the extent of the freshwater to pelagic water mixing gradient will vary with proximity to local freshwater sources and bottom depth, which affect the volumes of the two source waters mixed.

**Oxygen.** — The oxygen isotopic compositions of the seabird eggshells (Fig. 2) also follow a gradient consistent with a meteoric to oceanic water mixing model (Table 1). Two conclusions similar to those for carbon are drawn: (1) Relative participation in the marine ecosystem is manifested in a positive gradient in $\delta^{18}O$ values, corresponding to the difference between local meteoric water and pelagic oceanic water (here 4‰ for San Diego and 2‰ for Puerto Rico) from freshwater foragers to pelagic foragers (Fig. 3). (2) Species with the most diverse diets and the most diverse feeding zones also have the widest spread in $\delta^{18}O$ values.

The mixing of the coastal runoff meteoric water with oceanic water forms the basis of the positive gradient in the $\delta^{18}O$ values of eggshell carbonate from freshwater consumers to full oceanic (pelagic) consumers, and its extent can be expected to vary with proximity to freshwater sources and bottom depth (again, affecting the volumes of the two waters mixed) in the coastal zone. The average $\delta^{18}O$ value for meteoric water is approximately $-4%o_{SMOW}$ to $-5%o_{SMOW}$ at 32°N (San Diego), and $-2%o_{SMOW}$ at 18°N (Puerto Rico) (Yurtsever and Gat, 1981). Thus, the freshwater and coastal samples we chose were at approximately the minimum latitudes at which meteoric water has a distinct $\delta^{18}O$ signature. The coastal meteoric to oceanic water mixing gradients at higher latitudes will have progressively wider spreads and steeper slopes (Fig. 4). The $\delta^{18}O$ values of our samples are isotopically enriched by about 28.5‰ relative to their source water (Fig. 3), suggesting a metabolic effect of roughly +2‰ to +4‰ relative to the equilibrium effect of the precipitation of calcite at 39–40°C, the body temperatures of these birds (see Kak-
Figure 4. Expected variation of $\delta^{18}O$ values of source water in the meteoric to oceanic mixing gradient with mean annual temperature and latitude (after Dansgaard, 1964; and Yurtsever and Gat, 1981).

iuchi and Matsuo, 1979). Such effects are likely to be the same for all birds. The $\delta^{18}O$ values of White-tailed Tropicbird blood water was found to be $0.10\%_{\text{SMOW}}$ (SD = 0.572, N = 19), similar to its $0.0\%_{\text{SMOW}}$ source water.

The combined carbon-oxygen isotopic signature line (Fig. 5) illustrates the concurrent directionality of trend, although neither gradient directly affects the other. At progressively higher latitudes the slope of such a line will increase, as the spread in $\delta^{18}O$ values widens, while the spread in $\delta^{13}C$ values is little changed. Thus, at low latitudes, the resolution of the ecological information available from $\delta^{18}O$ values will be low, but will increase with increasing latitude, while the resolution available from $\delta^{13}C$ values will be constant at all latitudes.

The methodology we have presented can provide valuable information in comparative studies of the feeding ecology of a wide array of sympatrically breeding marine and aquatic birds. Because the oxygen isotope ratios of meteoric water become progressively lighter with increasing latitude (see Yurtsever and Gat, 1981), this methodology will be especially useful in studies of coastal species occurring at higher latitudes. Carbon isotopic values alone also can be useful for distinguishing consumer trophic status in feeding studies of terrestrial, aquatic, and marine animals (Schoeninger et al., 1983; Peterson et al., 1985). In addition, the examination of $\delta^{18}O$ values of the eggshell carbonate from freshwater and terrestrial birds can be a useful forensic tool for wildlife law enforcement authorities interested in determining the actual latitude of origin of eggs of protected or prohibited species found in private collections.

The isotopic signatures of whole-body carbon and most single-tissue carbon integrate the isotopic signatures of carbon ingested during the period in which the tissue was accumulated. They will reflect the animal's diet over a fairly large portion of its life span. However, isotopic signatures of eggshell carbonate reflect
the female’s diet over the narrow time window immediately prior to egglaying (see Eggshell formation, above). Western Grebes for example, are migratory and feed in marine and estuarine areas during the nonbreeding season, yet the isotopic signatures of their eggshell carbonate are indicative of the freshwater areas to which they return during nesting. These considerations argue strongly against a within-bird mixing model. Similarly, sampling eggshell carbonate is the only method, other than drawing fresh blood water, which can provide information on the isotopic signature of ingested water over relatively short time spans, as blood water quickly equilibrates with its source water.

The stable isotope methodology presented here can compliment conventional dietary analyses, as well as provide useful information on its own, and adds an additional dimension to the study of avian foraging ecology. This approach will be particularly useful in places where frequent visits are impractical. An advantage of this methodology is that it is non-destructive. No live specimens, nor remains thereof, need be taken.

ACKNOWLEDGMENTS

We thank the USFWS Culebra National Wildlife Refuge and the Western Salt Company, Chula Vista, California for access to study sites in Puerto Rico and San Diego, B. Grizzle and J. Salvia for assistance with field collections, and S. Harrison, L. Sternberg and D. Price for assistance with laboratory analyses. We also thank J. P. Croxall, B. Fry, P. Koch, C. J. Pennycook, L. Sternberg, and W. Tang for comments on a preliminary draft of the manuscript. This research was partially supported by the Sea and Sky Foundation through a cooperative agreement with the USFWS Patuxent Wildlife Research Center (F.C.S.), by the Rosenstiel School of Marine and Atmospheric Sciences Stable Isotope Laboratory, and National Science Foundation grant number EAR 84-17424 to P.K.S.
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DATE ACCEPTED: July 24, 1990.