Stable Isotope Analysis of Intrapopulation, Spatial, and Temporal Variation of Laughing Gull (*Larus atricilla*) Diets in the Virginia Coast Reserve and Jamaica Bay, New York

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ABSTRACT

In this study, the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values for blood, muscle, feather, and egg membranes were compared to examine temporal, interpopulation, and intrapopulation variation in the diets of Laughing Gulls (*Larus atricilla*) nesting in coastal Virginia and Jamaica Bay, New York. In addition, the stable isotopic compositions of Laughing Gull prey species were also measured. Stable isotopes are a powerful tool for the study of trophic linkages in dynamic coastal estuarine systems. The results reveal that there was a significant amount of intrapopulation variation in the diets of Laughing Gulls from both colonies. In addition, young Virginia Laughing Gulls consumed more foods of marine origin than did young from the New York colony. Results also indicate that young from the Virginia colony consumed proportionately more marine food items than did adult females during the period prior to egg laying. The stable isotopic compositions of blood and muscle from young Virginia Laughing Gulls indicate that there were two distinct feeding strategies employed by the parental gulls. Some young were fed a diet consisting of more estuarine species whereas other young were fed more marine food items. Based on sampling different portions of feathers from prefledged young, a temporal shift to a diet consisting of higher trophic level foods (higher $\delta^{15}N$ and $\delta^{13}C$ values) over time was observed for the young in the Virginia colony. No such temporal diet shift was apparent from feather analyses of the New York colony young. This study provides further insights into how stable isotope analysis can be applied to the study of avian feeding ecology.

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INTRODUCTION

The area that constitutes the Virginia Coast Reserve of the Long Term Ecological Research Project (VCR-LTER) is a highly dynamic coastal estuarine system. One of the goals of the VCR-LTER project is understanding the state of the system at present so that future changes can be recognized and evaluated. An understanding of the dynamics of ecosystem functioning is crucial to successful conservation (Odum 1959). Despite concern that a major state change occurred in this area during the 1930's (Hayden *et al*. 1991) and the likelihood that similar changes will occur again in the future, food web linkages in this area are poorly understood. Traditionally, theoretical perspectives of trophic relationships do not account for spatial and temporal variation in the diets of different populations of the same species. However, spatial and temporal variation in prey composition can have a large influence on the flows of nutrients, contaminants, and energy within the ecosystem.

In this study, Laughing Gulls (*Larus atricilla*) were used as a model organism because of their numerical abundance as a top trophic level consumer as well as their broad and opportunistic diet that includes foods of terrestrial, marine, and freshwater origin. Stable isotopes of carbon, nitrogen, and sulfur were analyzed in several types of Laughing Gull tissues as well as in their primary prey species to: (1) determine the feasibility of using different gull tissues in the evaluation of spatial, intrapopulation, and temporal variability in diet and trophic relationships, and (2) initiate the development of a stable isotope signature database for the entire VCR-LTER. Stable isotope analysis is a powerful tool for assessing certain changes in prey composition in space and time by top-

level consumers in coastal estuaries subject to ecosystem stressors. This technique is particularly valuable because only a small amount of tissue is necessary for analysis. Therefore, it is often possible to use stable isotope analysis to obtain valuable information about the diet of organisms without sacrificing the study organism. This advantage is particularly salient to studies examining the diets of endangered or threatened species.

This study examines dietary variation in Laughing Gulls on intrapopulation, spatial, and temporal scales. Spatially, the diets of Laughing Gulls nesting in coastal Virginia were compared to those of a Jamaica Bay, New York colony. Temporally, significant dietary shifts in nestlings were examined in both the coastal Virginia and Jamaica Bay colonies. In addition, the degree of intrapopulation dietary variation was also studied for adult and nestling Laughing Gulls in both colonies.

STABLE ISOTOPES:

In this study, a multiple stable isotope approach was used to insure that the trophic linkages were thoroughly analyzed. The use of stable isotope ratios in studies of food web dynamics is based on the fact that the stable isotopic ratio of the diet is reflected in the isotopic ratio of the consumer. As animals metabolize their food, stable isotopes undergo a process known as fractionation. Fractionation refers to an enrichment or depletion of the heavy isotope relative to the light isotope as a result of certain physical and chemical processes. Enrichment occurs when a greater amount of the heavier of the two forms of stable isotopes for a particular element (^{13}C, ^{15}N, ^{34}S) is retained in the tissues of the consumer while a greater amount of the lighter isotopes (^{12}C, ^{14}N, and ^{32}S) is lost. Although there are several theories regarding the physiological processes that lead to this

enrichment, there is no general agreement as to which is most likely (Michener and Schell 1994).

Carbon:

For carbon, the enrichment may occur owing to a preferential loss of $^{12}CO_2$ during the respiration process. Other possibilities are that there is a fractionation of the carbon during metabolic processes or that ^{13}C is preferentially absorbed during the digestion process. In light of the relatively small enrichment that occurs on average (0.5-1‰), carbon isotopic compositions are particularly useful in elucidating food webs in which there are several food sources that differ significantly in their $\delta^{13}C$ values (Michener and Schell 1994). For example, there are distinct differences in the $\delta^{13}C$ values of plants that use the Calvin cycle (C_3), Hatch-Slack cycle (C_4), and Crassulacean acid metabolism (CAM) photosynthetic pathways. These differences are due to differences in the degree of fractionation at the diffusion, dissolution, and the carboxylation steps of photosynthesis. As these plants are sources of primary productivity at the base of the food web, differences in the $\delta^{13}C$ values of plants utilizing these different pathways are then reflected throughout the food web (Lajtha and Marshall 1994).

When isotopic compositions are employed in the examination of diets and food web dynamics, it is important to realize that the isotopic compositions of different tissues of the study animal will reflect the diet of the animal during different temporal periods. Tissues that are rapidly replaced in the body will reflect the diet of the animal in the relatively recent past, and those tissues that do not have high turnover rates will reflect the diet over a longer span of time. This complication is also relevant to nitrogen and sulfur

isotopes (Michener and Schell 1994). Complexities arising from this effect can be lessened to some extent by either using a sample that consists of a homogenization of the entire organism, averaging the isotopic values obtained for several different tissue types, or by sampling different areas of a tissue (i.e. feathers and tusks) (Dieudonne 1998) that are deposited at different times. The present study examined the isotopic compositions of blood, muscle, egg membranes, and feathers. In addition, different portions of feathers were analyzed to examine the possibility that a temporal diet shift occurred in the young Laughing Gulls during the period of feather development. It is also important to conduct the isotopic analysis on several organisms within the examined species, as individuals of the same species eating approximately the same diet may vary in their isotopic compositions by up to 2‰ (Michener and Schell 1994). Numerous studies have examined and documented the utility of stable carbon isotopic analysis in studies of diet and trophic dynamics (Smith 1972, DeNiro and Epstein 1978, Tieszen *et al*. 1983, Jackson and Harkness 1987).

Nitrogen:

Like carbon isotopes, nitrogen isotopes are useful primarily in systems where there are two (or more) types of primary food sources that differ significantly in their $\delta^{15}N$ (such as aquatic versus terrestrial food sources). The approximately 3‰ enrichment in nitrogen with each successive trophic level makes the nitrogen isotope values particularly useful in the study of trophic dynamics (Michener and Schell 1994). The enrichment of nitrogen isotopes occurs either through the preferential excretion of ^{15}N-depleted nitrogen (i.e. higher in ^{14}N) in urea or ammonia or the preferential utilization of ^{15}N-enriched protein (or

through a combination of both processes) (Michener and Schell 1994). Specifically, it has been shown that nutritional stress may lead to significantly larger ^{15}N enrichment in birds (Hobson *et al*. 1993). This increased enrichment may be due to the physiological transfer of proteins from one part of the body to the other in response to nutritional stress (Hobson *et al*. 1993). The reactions involved in these movements may result in increased enrichment of nitrogen. Another possible explanation for this enrichment is that the types and amounts of amino acids may vary depending on whether a bird is experiencing nutritional stress (Hobson and Clark 1992). It has been shown that different amino acids have different stable isotopic signatures (Macko *et al*. 1983), so changes in the types and relative amounts of amino acids could very likely have a significant effect on the isotopic composition of the birds (Hobson and Clark 1992). Also, the utility of nitrogen isotopes in dietary studies may be limited by the extensive use of nitrogen fertilizers throughout the world. These fertilizers may affect the distribution of nitrogen isotopes in the primary food source (plants), and this variation will then be reflected throughout the food web (DeNiro and Epstein 1981). Several studies have utilized both carbon and nitrogen isotopic analyses in examination of trophic dynamics (Rau 1991-1992, Schoeninger and DeNiro 1984), and others have used only nitrogen isotopes (DeNiro and Epstein 1981, Minagawa and Wada 1984, Cabana and Rasmussen 1994).

Sulfur:

Although it appears that there is little or no enrichment in ^{34}S in animals relative to their diets, there is a very large difference between the isotopic ratios of seawater sulfate and sulfides (around 21‰ and –10‰, respectively) (Michener and Schell 1994). This

difference is very useful in studies of estuarine food web dynamics because one can readily distinguish between benthic and pelagic primary producers (benthic algae versus phytoplankton, for example). Like benthic producers, marsh plants tend to be richer in sulfur derived from sulfides, and they therefore characteristically have a less enriched $\delta^{34}S$ signal (Michener and Schell 1994). Several studies have utilized stable sulfur isotopes in conjunction with carbon and nitrogen analyses to examine the flow of organic matter through estuarine systems (Peterson and Howarth 1987, Neill and Cornwell 1992).

Background Literature for Feeding Biology-

Many studies have examined and documented aspects of the breeding biology of Laughing Gulls (Dinsmore and Schreiber 1974, Schreiber and Schreiber 1980, Schreiber *et al*. 1979). Dosch (1997) examined the diets of nestling Laughing Gulls in southern New Jersey using regurgitation samples and found that over eighty percent of the diet of these chicks by volume was composed of terrestrial food items. Like the Laughing Gulls examined in coastal Virginia in the present study, the New Jersey gulls nest on salt marsh islands, and it is therefore likely that food items of estuarine origin are closer in proximity to the nesting areas than foods available on the mainland. Dosch (1997) proposed that a possible explanation for the willingness of the parent gulls to fly longer distances to obtain inland food for their young is related to the lower salt content of terrestrial food items as compared to those of marine or estuarine origin. As Dosch (1997) also found a significant negative linear relationship between percent volume of marine food items in the diets of the young gulls and chick weight, he hypothesized that the salt gland of the young gulls is

not fully developed. Similarly, Johnston and Bildstein (1990) found evidence for the same physiological constraint in White Ibis (*Eudocimus albus*). Harriman (1967) and Dosch (1997) also found that increased salt consumption resulted in a negative effect on the growth and survival of young Laughing Gulls. These studies therefore suggest that adult Laughing Gulls may feed their young diets higher in foods of terrestrial, anthropogenic, and freshwater origin to reduce physiological stress on the immature osmoregulatory systems of the young.

In contrast, Annett (1987) found that adults that consumed large amounts of garbage (inland food) early in the breeding season began taking more small fish (especially anchovies) when the chicks hatched. Similarly, Pierotti and Annett (1990) found that seabirds that focused their feeding on mussels produced significantly larger and heavier young than generalists or those that specialized on petrels or garbage. They also had more eggs per clutch than birds using any of the other feeding strategies. Another finding of this study is that garbage specialists produced significantly more addled eggs (those that are either infertile or fail to develop completely), whereas mussel specialists produced significantly less addled eggs than birds specializing on other items. This study also documented the relatively higher success of seabirds feeding fish to their young as opposed to refuse. Fish and mussels are high in sulfonated amino acids, which are essential for the early stages of bone development in birds (Pierotti and Annett 1990). In contrast, refuse contains high amounts of connective tissue and fat as well as a great deal of relatively undigestable parts, and it is consequently low in the necessary sulfonated amino

acids. Hobson (1993) similarly documents seabird parents feeding their young diets consisting of higher proportions of fish.

Therefore, in contrast to the findings of studies indicating that the optimal feeding pattern for parent Laughing Gulls may be to feed their young fewer foods of marine origin (Harriman 1967, Dosch 1997, Dosch 1997), other studies have found evidence for increased breeding success in parents utilizing the opposite feeding pattern (Annett 1987, Pierotti and Annett 1990, Hobson 1993).

Many studies have utilized isotopic analyses of several different avian tissue types to examine various aspects of avian feeding biology. Schaffner and Swart (1991) established that the carbonate from eggshells could be used to assess certain aspects of the diet of the female at the time of egg formation. The relative reliance on marine and terrestrial food items as well as the relative trophic status of the females was established. In another study utilizing isotopic analyses of eggshell components, Hobson (1995) examined several different portions of whole eggs and determined their relative enrichments relative to the diet of the birds. The membrane inside an eggshell was found to be enriched by 3.5‰ for ^{15}N and 2.7‰ for ^{13}C relative to the diet of a carnivorous bird.

Stable isotope analyses have been utilized in studies examining various aspects of the feeding ecology of many avian species (Table 1). Many different tissue types were used in these studies, and several used isotopic analyses of tissues that were analyzed in the present study. Some studies focused their isotopic analyses on only one type of tissue, and others used several types of tissue to examine the diet of the birds over different temporal periods (Table 1). Tissues such as blood and muscle with a relatively high

metabolic activity, and thus turnover rate, reflect the diet of the animals in the relatively recent past, whereas some tissues, such as feathers, may reflect the diet only during the period in which the tissue was developing.

Table 1. Previous studies of avian feeding ecology using stable isotope analysis.

SPECIES	STABLE ISOTOPES	TISSUES	STUDY
Western and Glaucous-winged Gulls	C	bone	Hobson 1987
Marbled Murrelets	C, N	muscle	Hobson 1990
Cormorants	C, N	muscle, bone, and feathers	Mizutani *et al* . 1991
Great Auks	N	bone	Hobson and Montevecchi 1991
Northern Saw-whet Owls	C	muscle and bone	Hobson and Sealy 1991
Red-billed Tropicbirds, White-tailed Tropicbirds, Sooty Terns, Laughing Gulls, Elegant Terns, Caspian Terns, and Western Grebes	C, O	eggshells	Schaffner and Swart 1991

Table 1. Previous studies of avian feeding ecology using stable isotope analysis, (continued).

SPECIES	STABLE ISOTOPES	TISSUES	STUDY
Weddell seabirds	C, N	muscle	Rau *et al*. 1992
High Arctic seabirds	C, N	muscle, bone	Hobson 1993
Crows	C	blood	Hobson and Clark 1993
Japanese Quail, Ross' Geese	C, N	muscle, blood, liver, feathers, bone	Hobson *et al*. 1993
Snow Geese	C, N	muscle	Aliskauskas and Hobson 1993
Canvasback Ducks	C, N	blood, muscle	Haramis *et al*. 1994
Auklets, Murrelets, Murres, Storm Petrels, Glaucous-winged Gulls, Mew Gulls, Northern Fulmars, Cormorants	C, N	muscle, bone	Hobson *et al.* 1994
Mallards, Japanese Quail, Prairie Falcons, Gyrfalcons	C, N	eggshells	Hobson 1995
Northern Fulmars	C, N	feathers	Thompson *et al*. 1995

Table 1. Previous studies of avian feeding ecology using stable isotope analysis, (continued).

SPECIES	STABLE ISOTOPES	TISSUES	STUDY
Cormorants, Western Gulls, Common Murres, Pigeon Guillemots, Auklets	C, N	eggs, muscle	Sydeman *et al* . 1997
Redhead Ducks	C (fatty acids)	fat (GC-IRMS)	Hammer *et al* . 1998
Glaucous Gulls	C, N	muscle, liver	Schmutz and Hobson 1998
Herring Gulls	C, N	eggs	Hebert *et al* . 1999

Although numerous studies have utilized isotopic analysis of tissues such as muscle, blood, and bone to examine the diets of many types of animals, the use of feathers in the examination of avian feeding ecology is a more recent and less thoroughly explored application of isotopic analysis and dietary studies. This is a particularly attractive tissue for this analysis, however, as it is simple and non-lethal. When using the stable isotope compositions of feathers to examine avian diets, it is important to know the extent of fractionation occurring during the production of feather tissue. Several studies have addressed this need by measuring diet-feather fractionation factors (Table 2).

Table 2. δ13C and δ15N diet-feather fractionation factors for various avian species.

SPECIES	FEATHERS	DIET-FEATHER FRACTIONATION FACTOR ($d^{13}C$)	DIET-FEATHER FRACTIONATION FACTOR ($d^{15}N$)	STUDY
Cormorant	primary	4.0	not reported	Mizutani *et al*. 1990
Black-tailed Gull	?	4.0	not reported	Mizutani *et al*. 1990
Cormorant	primary	3.6	3.6	Mizutani *et al*. 1991
Black-tailed Gull	primary	3.6	5.3	Mizutani *et al*. 1992
Nankeen Night Heron	primary	3.2	4.2	Mizutani *et al*. 1992
Great White Egret	nuptial plumage	3.1	3.9	Mizutani *et al*. 1992
Grey Heron	primary	3.4	4.3	Mizutani *et al*. 1992
Humboldt's Penguin	body	2.9	4.8	Mizutani *et al*. 1992
Cormorant	primary	3.8	3.7	Mizutani *et al*. 1992
Scarlet Ibis	primary	3.8	4.5	Mizutani *et al*. 1992
White Ibis	primary	2.5	4.3	Mizutani *et al*. 1992
Flamingo	primary	3.6	5.6	Mizutani *et al*. 1992
American Crow	primary	4.0	not done	Hobson and Clark 1992
domestic chicken	?	-0.4	1.1	Hobson and Clark 1992
Japanese Quail	?	1.4	1.6	Hobson and Clark 1992
Ring-billed Gull	primary	0.2	3.0	Hobson and Clark 1992
Peregrine Falcon	?	2.1	2.7	Hobson and Clark 1992

Table 2. δ13C and δ15N diet-feather fractionation factors for various avian species, (continued).

SPECIES	FEATHERS	DIET-FEATHER FRACTIONATION FACTOR ($d^{13}C$)	DIET-FEATHER FRACTIONATION FACTOR ($d^{15}N$)	STUDY
Common Murre	body	1.0	3.3	Thompson and Furness 1995
Arctic Tern	body	2.1	3.4	Thompson and Furness 1995
Broad-billed Prion	body	2.5	4.3	Thompson and Furness 1995
Subantarctic Skua	body	0.5	3.0	Thompson and Furness 1995
Cormorant	primary	2.6	4.9	Bearhop *et al*. 1999
Shag	primary	2.0	3.6	Bearhop *et al*. 1999
Goosander	primary	2.4	4.2	Bearhop *et al*. 1999

Like other tissues, feathers can be used to estimate the relative dietary contributions of marine and terrestrial food sources as well as in the determination of trophic level. Hobson (1992) found that the isotopic composition of growing feathers reflects the diet of the bird during feather production. In this study, captive crows were fed a uniform diet of known isotopic composition and the feather produced during this period was removed and isotopically analyzed. After the feather grown during the controlled diet was removed, the diet was changed to one with an isotopic composition that was distinctly different from the initial diet. After the crows consumed this diet for

some time, feather grown during this period was removed and isotopically analyzed. The isotopic composition of the diet at the time of feather growth was reflected in a consistent manner in the isotopic composition of the feathers. As birds do not produce all their feathers simultaneously, several feathers produced at different times can be removed from a single bird to examine temporal diet changes (Mizutani *et al*. 1990, Collier and Lyon 1991, Thompson and Furness 1995, Bearhop *et al*. 1999). Thompson and Furness (1995) examined intrafeather isotopic variations by analyzing base and tip portions of primary feathers. Although there was not a consistent and significant temporal dietary shift in the population as a whole, the base and tip portions of several feathers did differ significantly from one another, thus indicating that some individuals did undergo a pronounced dietary shift during feather formation. Several studies have utilized the fact that the isotopic composition of feathers reflects the diet during feather formation to identify and link breeding and wintering grounds for migratory songbirds (Chamberlain *et al.* 1997, Hobson and Wassenaar 1997).

Many studies have utilized stable isotopic analyses to examine the flow of organic matter through a wide variety of estuarine systems (Smith and Epstein 1970, Haines 1976, Haines 1976, Haines 1977, Haines and Montague 1979, Hackney and Haines 1980, Stephenson and Lyon 1982, Hughes and Sherr 1983, Peterson *et al*. 1985, Simenstad and Wissmar 1985, Peterson *et al*. 1986, Peterson and Howarth 1987, Neill and Cornwell 1992, Schacher and Wooldridge 1996, Kwak and Zedler 1997, Creach *et al*. 1997). In particular, many of these studies have sought to determine which source of primary productivity provides most of the nutrition for animals at the base of the estuarine food

web. The analysis of the stable isotopic compositions of carbon, nitrogen, and sulfur has been shown to be a robust method for the examination of trophic relationships in dynamic coastal estuarine systems. However, although the base of the estuarine food web has been well documented isotopically, far fewer studies have examined the influence of top trophic level consumers in detail. Because these higher level consumers are often larger, more mobile, and far ranging among habitats, they can have major impacts on the import and export of organic materials and contaminants.

Materials and Methods

FIELD COMPONENT:

During the summers of 1997 and 1998, samples of species known to serve as prey items for Laughing Gulls (Burger 1996, K. Brown, personal communication) were collected in the barrier islands and lagoonal marsh islands of the Virginia Coast Reserve in Northampton County, VA (figure 1).

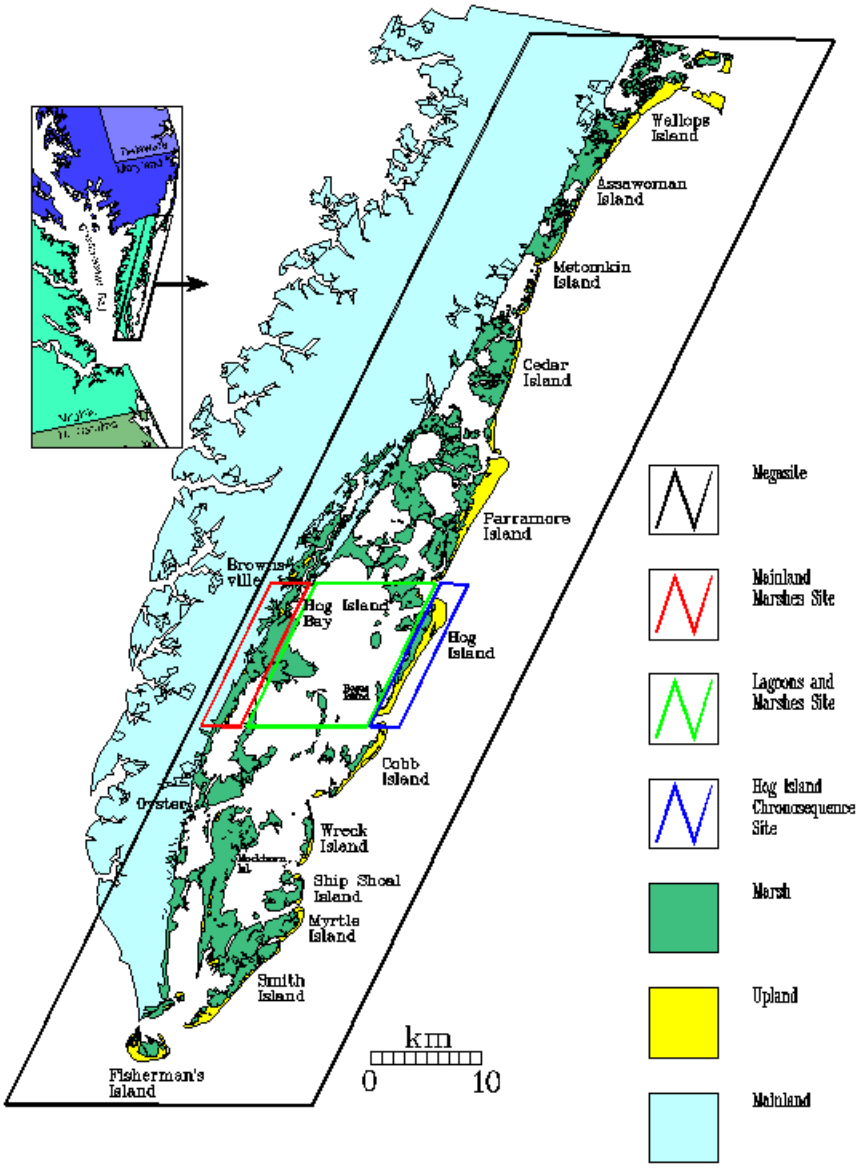


Figure 1: Map of the Virginia Coast Reserve.

In June-August of 1998, an intensive study of a breeding colony of Laughing Gulls on Egging Marsh was conducted. Egging Marsh is a small marsh island located near Hog Island (75.68 W, 37.46 N) (figure 1). Several hundred pairs of Laughing Gulls nested on Egging Marsh during the 1998 breeding season. After the eggs were laid in June, enclosures were constructed around fifteen of the nests using 30-60 cm high wire and wooden stakes. The enclosures were placed at a distance of at least 60 cm from the perimeter of the nests. Enclosed nests were selected because of their close proximity to a site from which visual observations of parental feeding were made.

At this time, eggs were also "floated" to determine the approximate time since laying (and thus also approximate time until hatching) (Hays and LeCroy 1971). At several other points prior to hatching the eggs were floated again to monitor their progress. A marking scheme was developed for all of the chicks within the enclosures so that each chick had a unique marking. As all chicks within the enclosures did not hatch at the same time, chicks were marked as they hatched to facilitate their assignment to nests. Markings consisted of two spots of nail polish on the left and right sides of each chick's head. Marks were periodically reapplied because of color fading. Eggshells of hatched chicks were also collected at all fifteen enclosed nests as well as from numerous other nests within the colony.

After hatching, marking, and collection of discarded eggshells, visual observations of parental feeding were made to identify the foods being delivered to young gulls. These observations were of limited success, as observational distances sufficient to avoid disturbance of the birds also made identification of food items extremely difficult.

Information about the diets of individual Laughing Gulls was therefore obtained primarily through collection and analysis of regurgitations of the stomach contents of chicks. In this procedure, chicks in the colony (both from within and outside the enclosures) were handled for approximately five minutes and any regurgitations were collected and analyzed for content. Regurgitations were collected and maintained on ice until they could be placed in freezers for storage. This procedure provides a means whereby specific food items can be identified and their relative contributions to total diet may be quantified. However, analysis of regurgitations in dietary studies has several drawbacks. First, identifications of gut contents can be very difficult, as prey items are often partially digested when the regurgitation is obtained. Second, certain prey items that are rapidly digested may not be accurately represented in regurgitation contents, whereas the dietary importance of those that are relatively resistant to digestion may be overestimated (Michener and Schell 1994)

Like the visual observations, regurgitations were of limited success. Throughout the breeding season, attempts to obtain regurgitations from nestlings throughout the colony were made approximately five times per week. Fifteen regurgitations were obtained during this period. In addition, certain nestlings were more prone to regurgitate than others. As a result, specific data about the diets of individual young were obtained for fewer than fifteen young. Observations and collection of regurgitation were continued until the chicks were between three and four weeks of age. At this point, five of the nests had failed or been abandoned. A United States Geological Survey Patuxent Wildlife Research Center (USGS PWRC) veterinarian then collected blood and pectoral muscle

samples from twelve chicks from the enclosed nests. At least one nestling was sampled from each nest. Blood (1 cc) was taken from the medial tarsal vein using a syringe, and small amounts of pectoral muscle tissue (0.5-1.0 mg) were removed using a biopsy needle. These samples were also stored on ice until they could be moved to freezers. At the time of the muscle and blood collection, the fifth primary feather was also removed from each of the twelve sampled chicks. The fifth primary feather was also removed from the other chicks within the enclosures as well as chicks within the rest of the colony (49 total feathers). The colony was visited periodically several times in the week after the sampling procedure to insure that all chicks recovered from the sampling.

Based on organisms observed in the regurgitations as well as remnants of food items observed on and collected from nests, live specimens of prey that contributed to the diet of the Laughing Gull chicks were collected. Many species of crabs, fish, insects (from mainland, marshes, and barrier islands), mollusks, and other marine invertebrates were collected near Egging Marsh and stored in freezers (Table A-1). To allow comparisons of Laughing Gulls between locations, feather and prey samples were also obtained by Dr. Kevin Brown from a colony of Laughing Gulls nesting in the Jamaica Bay Wildlife Refuge in Jamaica Bay, New York during the summer of 1998 (figure 2).

Figure 2: Map of Jamaica Bay Wildlife Refuge and its surroundings.

Additional samples were obtained of species that are probably not part of the diet of the gulls; these samples have been analyzed to provide data for an overall database of information about trophic relationships for the organisms within the VCR-LTER (Table A-1).

LABORATORY ANALYSES:

Blood, muscle, feather, and eggshell membranes from Laughing Gulls as well as prey samples were analyzed for their stable isotopic compositions using an elemental analyzer (EA) connected to a Micromass Optima Isotope Ratio Mass Spectrometer (IRMS). Before these analyses were conducted, however, the samples were prepared. Feathers and eggshells were cleaned with water to remove any external debris. The

connective tissue inside the base of the feathers was also removed and this area was thoroughly cleaned. The waxy sheath covering the base of the feathers was also removed. The membrane portion of the eggshells was separated from the hard outer shell portion. Muscle tissue was removed from all prey samples. Eggshell membranes, prey muscle tissue, as well as juvenile Laughing Gull muscle and blood samples were then dried for several days at 60° C. After drying, each sample was ground into a fine powder and homogenized.

After washing, a test was conducted to determine which portion of the feathers was most appropriate for isotopic analysis. For several feathers, sections from the proximal and distal ends were removed. Then, the rachis and barb portions of these sections were isotopically analyzed separately to determine whether the rachis and barbs from the same area on the same feather had significantly different isotopic compositions. After it was determined that rachis and barb samples taken from the same area on the same feather did not have significantly different isotopic compositions, the barb portions of all feathers were removed from the rachis. The rachis portion was then used in the rest of the feather analyses, as it was far easier to sample than the barb portions. As all feathers were taken from chicks of approximately three to four weeks of age, most feathers were around 15 cm in length. Therefore, feathers between 13 and 17 cm in length were preferentially sampled. Smaller feathers, although rare, were sometimes sampled when those larger than 13 cm were not available. For these samples, feather length was noted. With feathers averaging 15 cm in length, 2 cm was removed from both the proximal and distal ends of the feather. The ends were removed so that the subdermal portion of the

feather was not sampled. This was done to attempt to reduce any variation between the base and tip portions so that isotopic differences observed between these two portions could be attributed to a change in diet rather than a difference in microenvironment (inside versus external on the bird). The same amount (2 cm) was also removed from the tip of the feathers so that the proximal and distal samples were taken from equivalent areas of the proximal and distal ends. After the ends were removed, samples were taken for carbon and nitrogen isotopic analysis from the base and tips of all feathers sampled. For some feathers, the proximal and distal ends were also sampled for sulfur isotopic composition. Some feathers were not sampled for sulfur, however, as the amount of feather required for sulfur analysis made sequential sampling along the entire length of the feathers impossible. In this sequential sampling procedure, a carbon and nitrogen sample was taken every 5mm along the length of feathers with significantly different isotopic compositions at the proximal and distal ends. The proximal and distal sampling, as well as the sequential sampling, was conducted on feathers from both the coastal Virginia and Jamaica Bay, New York colonies.

A test was also conducted to address the possibility that the presence of waxes or lipids may influence the carbon isotopic signature of the feathers. Birds often deposit lipid-rich substances on feathers as waterproofing. As lipids have significantly more depleted isotopic compositions than other compounds (Michener and Schell 1994), the presence of these substances on feathers could significantly influence the carbon isotope values obtained. Therefore, different areas of several different feathers were reflux extracted with distilled dichloromethane for approximately 30 minutes to remove all lipids. After

refluxing, the feathers were rinsed several times in clean dichloromethane to remove lipids released by the reflux process. Feathers were then allowed to dry. The treated samples were then isotopically analyzed, as were equivalent samples (from the same feathers and same areas on the examined feathers) that did not undergo the lipid treatment. Isotopic values for the treated and untreated samples were then compared to determine whether the presence of lipid significantly influenced the isotopic values obtained. There was no significant difference between the isotopic compositions of the treated and untreated feathers.

After sample preparation was complete, each sample was weighed out (0.4-0.6 mg for a carbon and nitrogen sample and 5.0 mg for a sulfur sample) and placed in a tin boat, sealed, and loaded into the sample carousel on the EA (Micromass Optima IRMS). The goal of preparation is the conversion of the organic samples into suitable gases that can then be analyzed by the mass spectrometer. Carbon is converted to CO_2, nitrogen to N_2, and sulfur to SO_2. The reproducibility of isotopic measurements varies depending on the specific technique and instruments used, but it is usually around plus or minus 0.2‰ for carbon, nitrogen, and sulfur measurements. Isotopic ratios of samples (R_{sa}) were compared to the isotopic ratio of a standard for that element (R_{std}). R is the ratio of the heavy isotope to that of the light isotope of the element, and differences in the ratios are expressed in 'delta' (δ) notation and are reported in per mil (‰):

$$\delta^{N}E\ (‰) = (R_{sa}/R_{std} - 1) * 1000$$

The standard for carbon is Pee Dee Belemnite (PDB), which is a Cretaceous age cephalopod from the Pee Dee formation of South Carolina. The standard for nitrogen is atmospheric molecular nitrogen, and sulfur samples are compared to troilite of the Canyon Diablo Meteorite (CDT) (Lajtha and Michener 1994). By definition, the δ values of all the standards are equal to zero.

STATISTICAL ANALYSES:

Stable isotopic values of muscle and blood from juvenile Laughing Gulls within the enclosures at the Virginia colony were plotted and an R^2 value was calculated to determine the degree of linear correlation between muscle and blood values from individual birds. The plots revealed two distinct groups within the isotopic values of the juvenile gulls. Therefore, a t-test was used to determine whether the two groups were statistically distinct. The muscle and blood samples were also analyzed using a one-way analysis of variance (ANOVA) to determine whether there was significant variation among the carbon, nitrogen, and sulfur isotopic values obtained for muscle and blood. This analysis was performed separately with values obtained for the muscle $\delta^{13}C$, muscle $\delta^{15}N$, blood $\delta^{13}C$, blood $\delta^{15}N$, and blood $\delta^{34}S$. Separate t-tests were used to determine whether there were statistically significant differences between carbon and nitrogen isotopic compositions of the muscle and blood.

A one-way analysis of variance (ANOVA) was performed to determine whether there was a significant amount of variation among the isotopic values of the egg membranes. This analysis was done separately for carbon, nitrogen, and sulfur. The carbon and nitrogen values for the egg membranes were also plotted so that a R^2 value could be

calculated using a linear regression model. All R^2 values were compared to the critical levels of r obtained from Zuwaylif (1980).

Correlation between the blood and feather isotopic values of the marked juveniles from the Virginia colony was determined by plotting blood and feather values from the individual gulls and calculating an R^2 value from the linear regression model. The strength of this correlation may indicate differences in the diet-tissue fractionation factors between blood and feather tissues.

The isotopic values obtained for egg membranes and feathers taken from adults and young at the Virginia colony, respectively, were utilized to compare the isotopic signatures of adult and young Laughing Gulls in this colony. Before this could be done, however, diet-tissue fractionation factors obtained by previous studies for avian egg membranes and feathers were used to account for differences in the degree of fractionation that occurs during the production of these two tissue types. Hobson (1995) found diet-egg membrane fractionation factors of 2.7‰ for carbon and 3.5‰ for nitrogen for a carnivorous bird. The $\delta^{13}C$ and $\delta^{15}N$ values for egg membranes were then corrected based on these fractionation factors to produce an isotopic values indicative of the diet of the adult female Laughing Gulls at the time of egg formation. As many more studies have examined diet-feather fractionation factors, mean fractionation values for carbon and nitrogen were obtained from the eleven waterbird primary feather values (Table 2). This mean value was then used to obtain the approximate carbon and nitrogen isotopic values of the diet that the young gulls were being fed during primary feather growth. The corrected egg membrane and feather values were then compared using a t-test to

determine whether there were significant differences between the diets of the adult and young Laughing Gulls from the Virginia colony.

Paired t-tests were performed to determine whether there was a significant difference in the isotopic compositions of carbon, nitrogen, and sulfur for the base and tip portions of the feathers. This was done independently for both Virginia and New York colony feathers. T-tests were also performed to compare the isotopic compositions of the New York and Virginia colony feathers. This analysis was done separately for carbon, nitrogen, and sulfur. Isotopic compositions of feathers obtained from birds within the enclosures were compared to those from birds outside the enclosures using a t-test. This analysis was done to ensure that the isotopic values of tissues obtained from enclosed birds were a representative sample of the colony.

An F-test was performed to determine whether there was a significant difference between the variances in the isotopic compositions of feathers obtained from the Virginia and New York colonies. This analysis was done separately for the carbon, nitrogen, and sulfur compositions of both the base and tip portions. F-tests were also used to compare variances in the stable isotopic compositions of the base and tip portions of the feathers analyzed. This analysis was done for carbon, nitrogen, and sulfur on feathers obtained from both the New York and Virginia colonies.

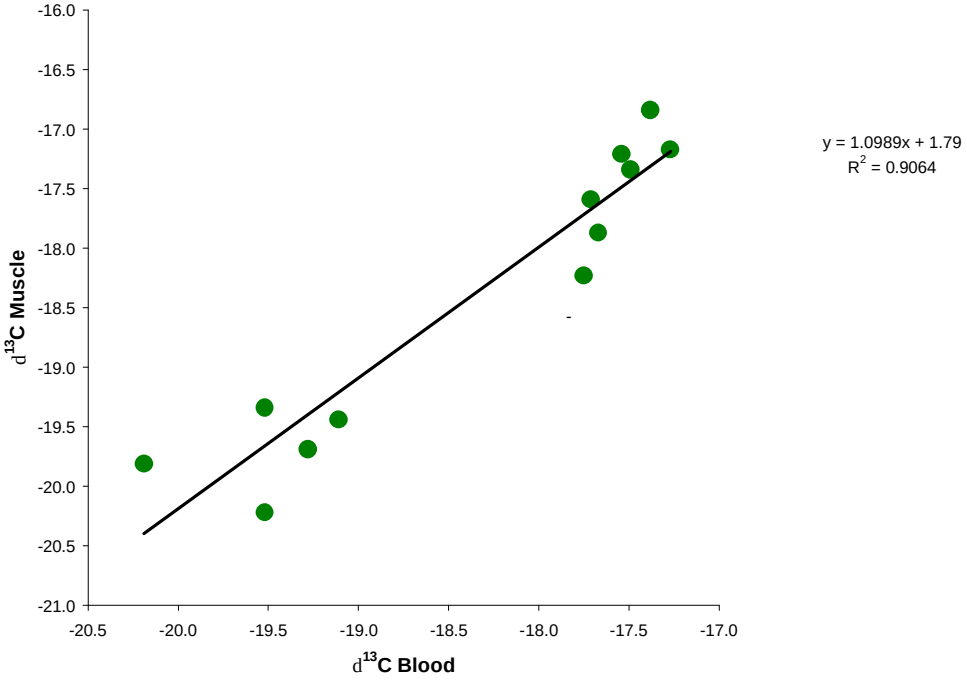
For the results of all the statistical analyses, a P value less than 0.05 was required for a result to be considered statistically significant. Results were often tested for significance at lower P values to indicate relative strength of significance. In addition, as

many statistical tests were performed in the analysis, testing for significance at lower P values also reduced the probability of obtaining a false significance result.

RESULTS

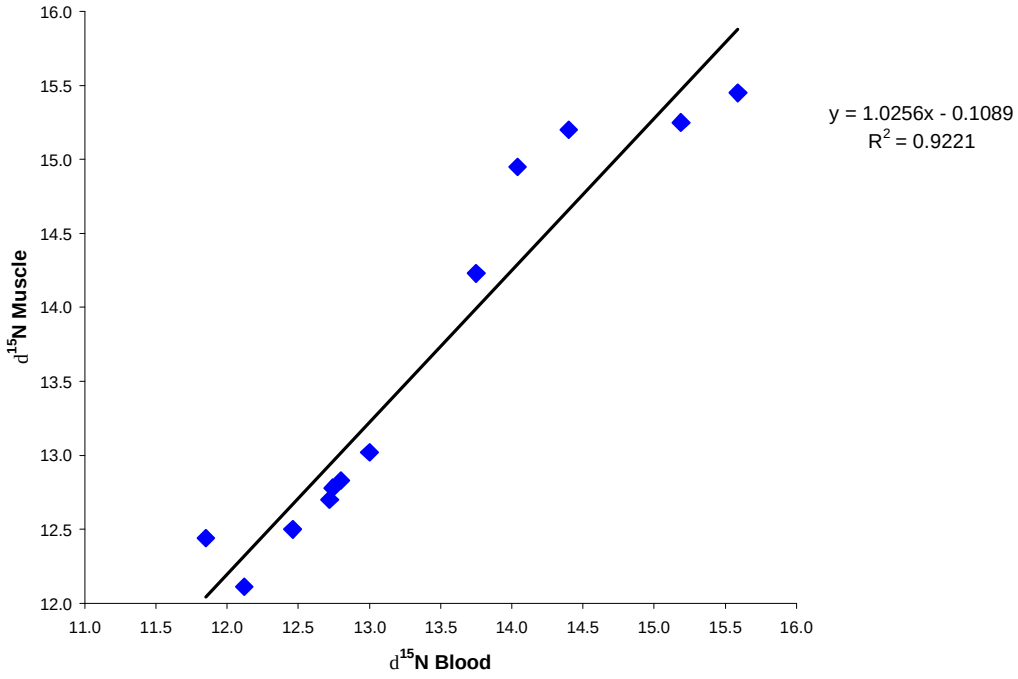
Results of the stable isotopic analysis of juvenile Laughing Gull blood and muscle from the Virginia colony indicate that the carbon isotopic compositions of blood and muscle are highly significantly correlated (model: linear; R^2= 0.91; P<0.01; Fig. 3).

FIGURE 3. Relationship between $\delta^{13}C$ values in Laughing Gull muscle and blood tissues



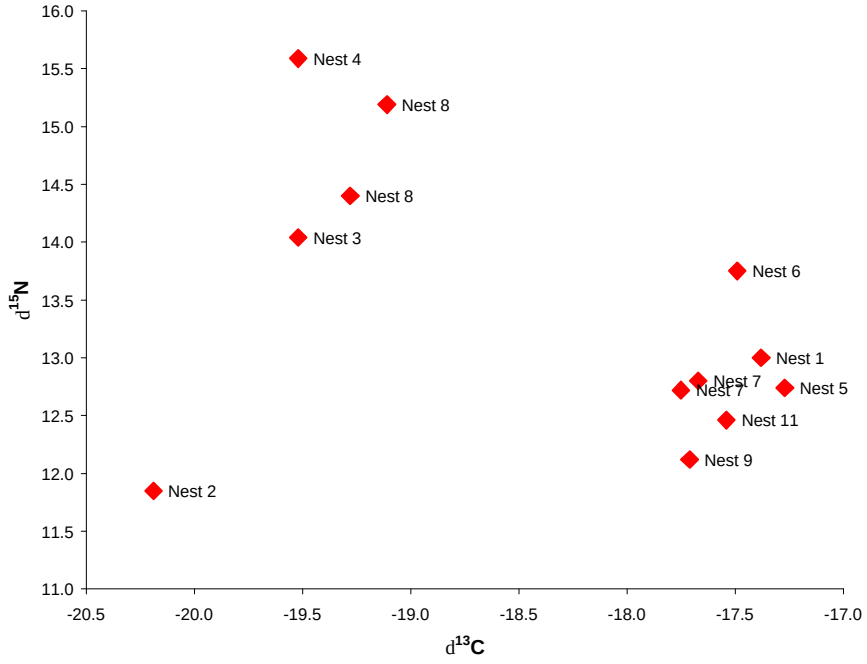
Similarly, the nitrogen isotopic values of the Laughing Gull muscle and blood are highly significantly correlated (model: linear; R^2= 0.92; P<0.01; Fig. 4).

FIGURE 4. Relationship between $\delta^{15}N$ values of Laughing Gull muscle and blood tissues



Graphs of the isotopic values of the muscle and blood reveal two distinct clusters (nest two was considered an outlier), one consisting of birds whose tissues are more depleted in carbon and more enriched in nitrogen and one with relatively more enriched carbon values and less enriched nitrogen values (Fig. 5).

FIGURE 5. Relationship between $\delta^{13}C$ and $\delta^{15}N$ values in Laughing Gull blood



Results of a t-test indicate that these two groups are indeed statistically different. The differences observed between the carbon isotopic values are very highly significant ($t = 11.5$; $df = 10$; $P<0.001$), and the difference observed in the nitrogen and sulfur values of the two groups are also significantly different ($t_{nitrogen} = 2.41$; $t_{sulfur} = 1.85$; $df = 10$; $P<0.05$).

The results of another series of paired t-tests indicate that there is not a statistically significant difference between the muscle and blood carbon isotopic values, but that there is a significant difference between the muscle and blood nitrogen isotopic values ($t = 2.25$; $df = 11$; $P<0.05$). Results of the one-way analysis of variance indicate that there is a significant amount of intrapopulation variation among both the carbon and nitrogen isotopic compositions of the muscle and blood samples. The one-way analysis of variance conducted on the egg membrane isotopic values similarly revealed that there was a significant amount of intrapopulation variation in the isotopic values of the membranes. This significance was indicated for carbon, nitrogen, and sulfur isotopes. The carbon, nitrogen, and sulfur isotopic compositions of feathers obtained from the Virginia and New York Laughing Gulls also had a significant amount of intrapopulation variation (Table 3).

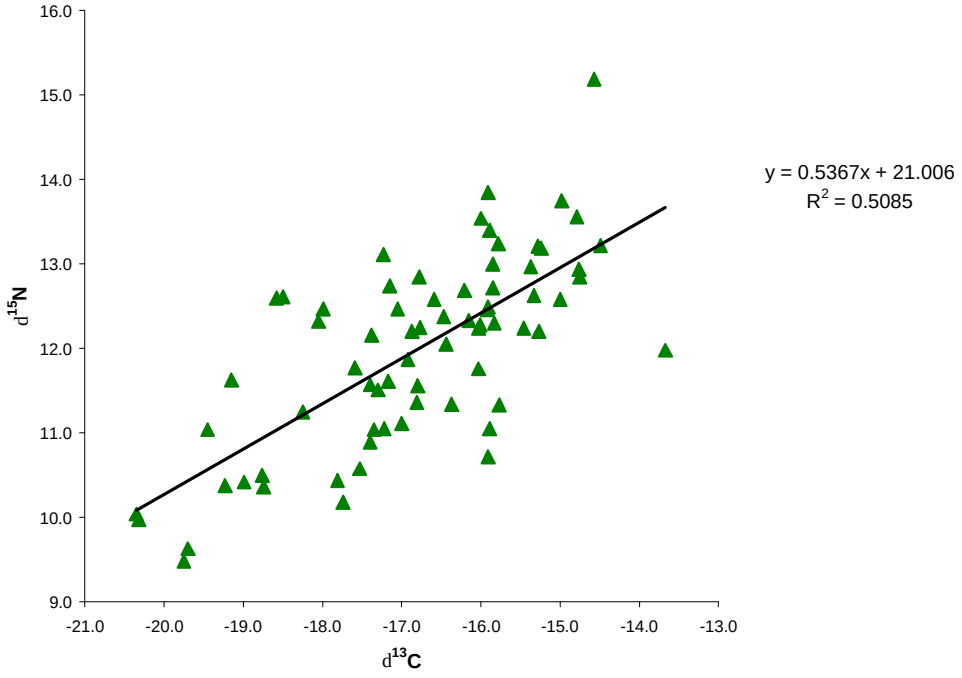
Table 3. Analysis of variance results for Laughing Gull blood, muscle, feather, and egg membrane isotopic values

TISSUE	F ($\delta^{13}C$)	F ($\delta^{15}N$)	F ($\delta^{34}S$)	DF	P<
blood[a]	1.12	1.44	1.59	11	0.01
muscle[a]	1.49	1.65	NA	11	0.01
VA feathers[a]	0.711	1.49	1.91	48	0.01
NY feathers[a]	0.397	1.12	3.51	19	0.01
egg membrane[b]	2.23	1.72	2.70	81	0.01

[a] from young
[b] from adult females

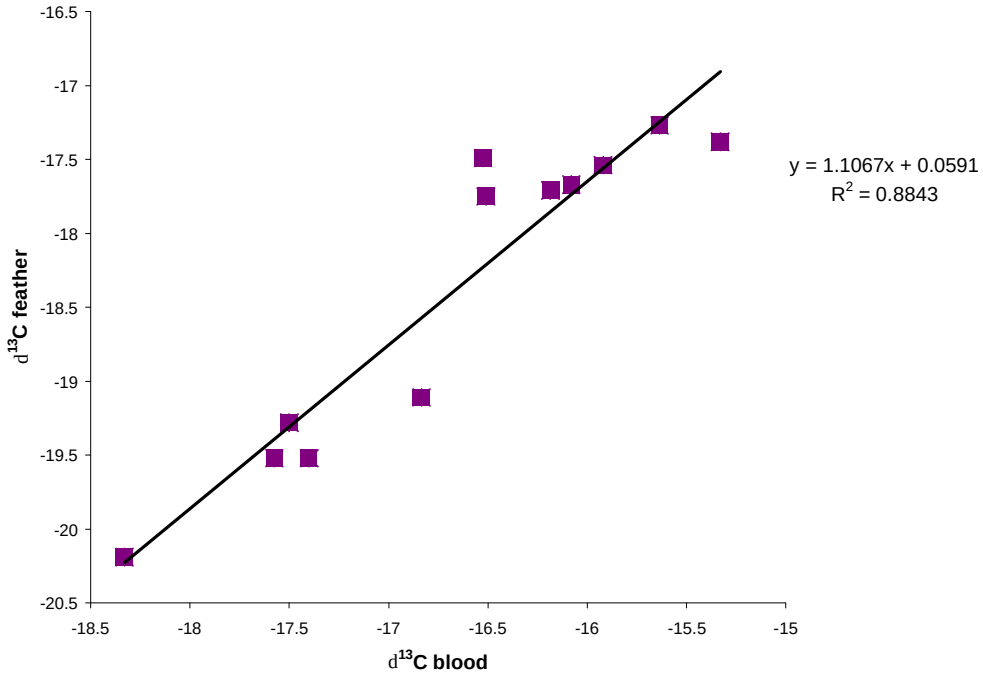
Also, the carbon and nitrogen isotopic compositions of the egg membranes are highly significantly correlated (model: linear; R^2=0.51; P<0.01; Fig. 6).

FIGURE 6. Relationship between $\delta^{13}C$ and $\delta^{15}N$ in Laughing Gull Egg Membranes



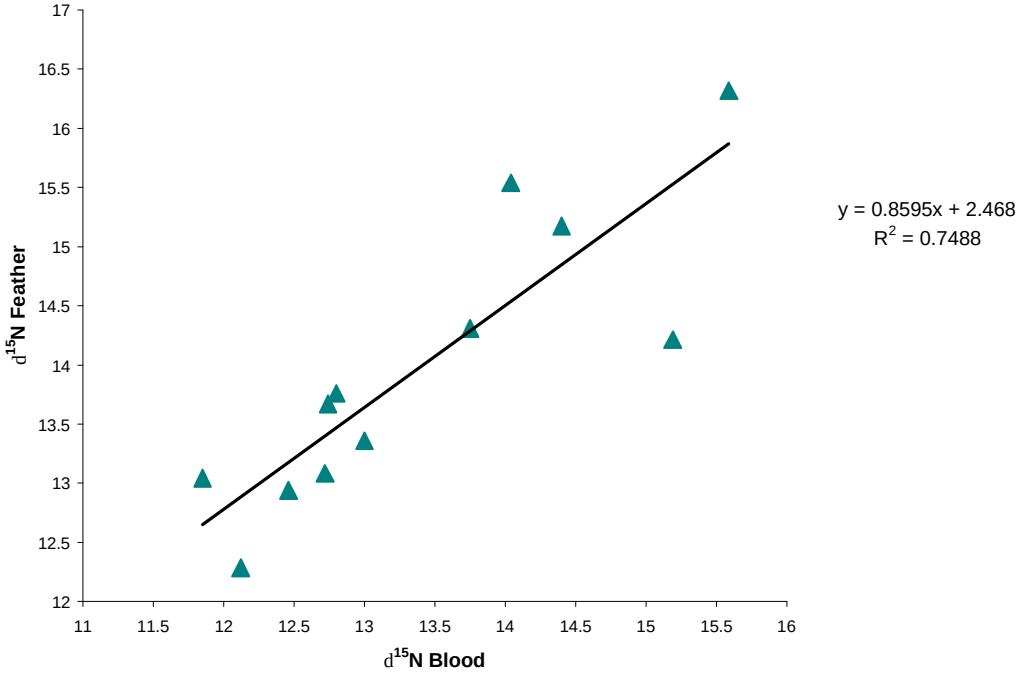
The carbon isotopic values of feathers and blood taken from the same bird reveal a high degree of linear correlation between the isotopic values of these two tissues. (Fig. 7).

FIGURE 7. **Relationship between $\delta^{13}C$ in Laughing Gull feather and blood tissues**



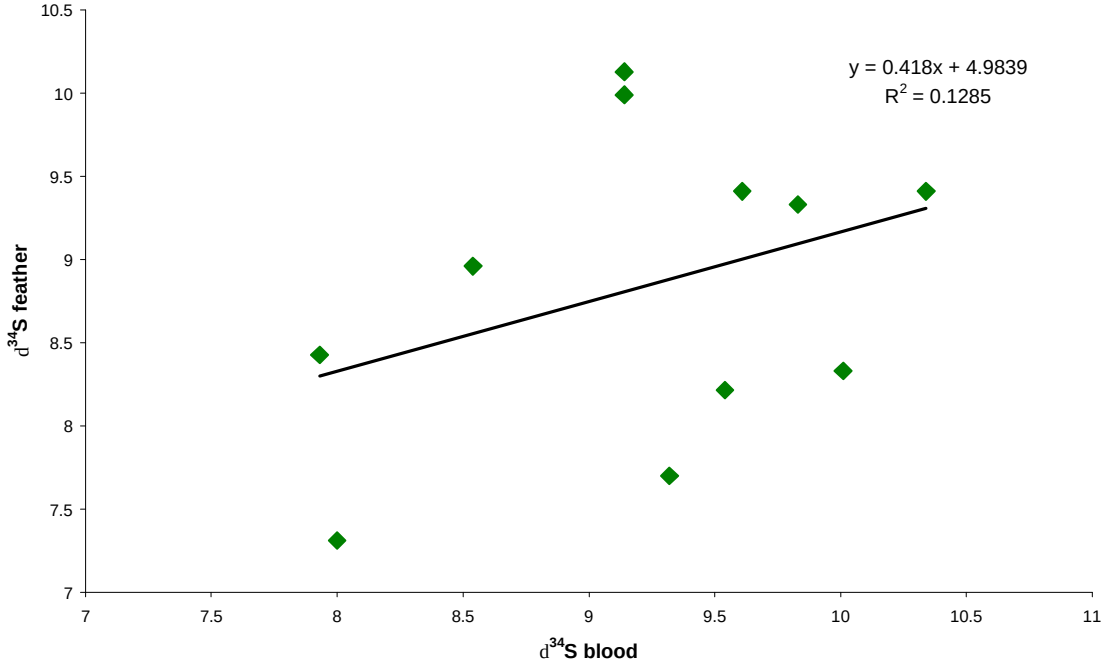
The nitrogen isotopic compositions of feathers and blood are also significantly correlated (Fig. 8).

FIGURE 8. **Relationship between $\delta^{15}N$ in Laughing Gull blood and feather tissues**



Results of these plots indicate that for both carbon and nitrogen, the isotopic compositions of feathers and blood taken from individual birds are highly correlated (model: linear; R^2 for carbon = 0.88, R^2 for nitrogen = 0.75; $P<0.01$). However, the sulfur isotopic compositions of the feathers and blood were not significantly correlated (model: linear; $R^2= 0.13$; $P>0.05$) (Fig. 9):

FIGURE 9. Relationship between $\delta^{34}S$ of Laughing Gull blood and feather tissues



Results of the sequential feather sampling indicate that this procedure can be used to track significant changes in the carbon and nitrogen isotopic compositions of feathers (Figures 10 and 11).

Figure 10. Sequential $d^{13}C$ sampling of young Virginia Laughing Gull feathers

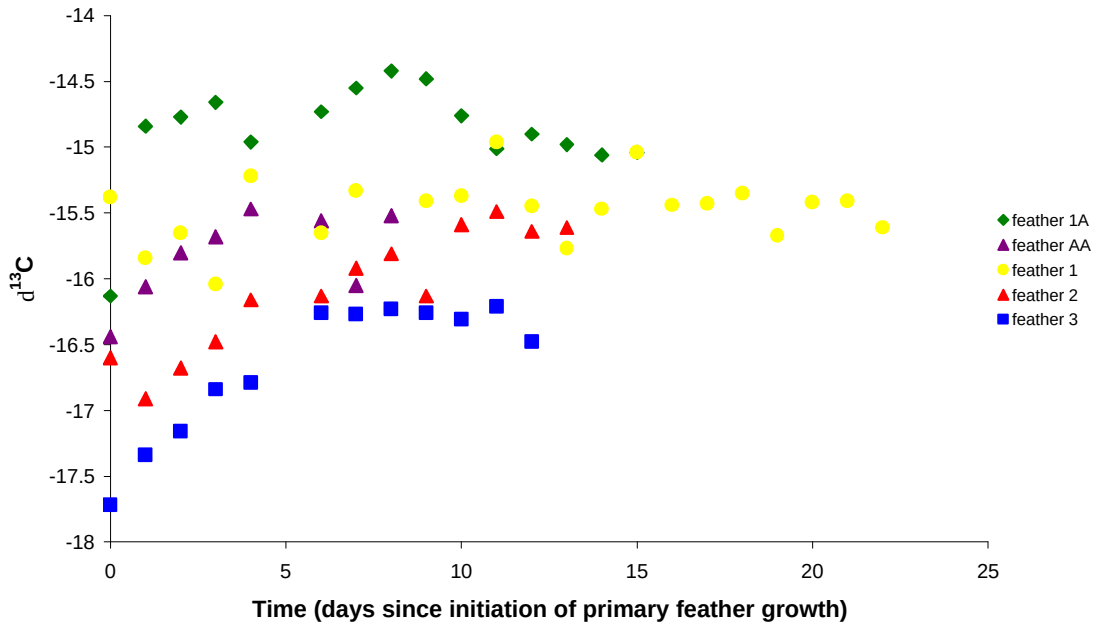
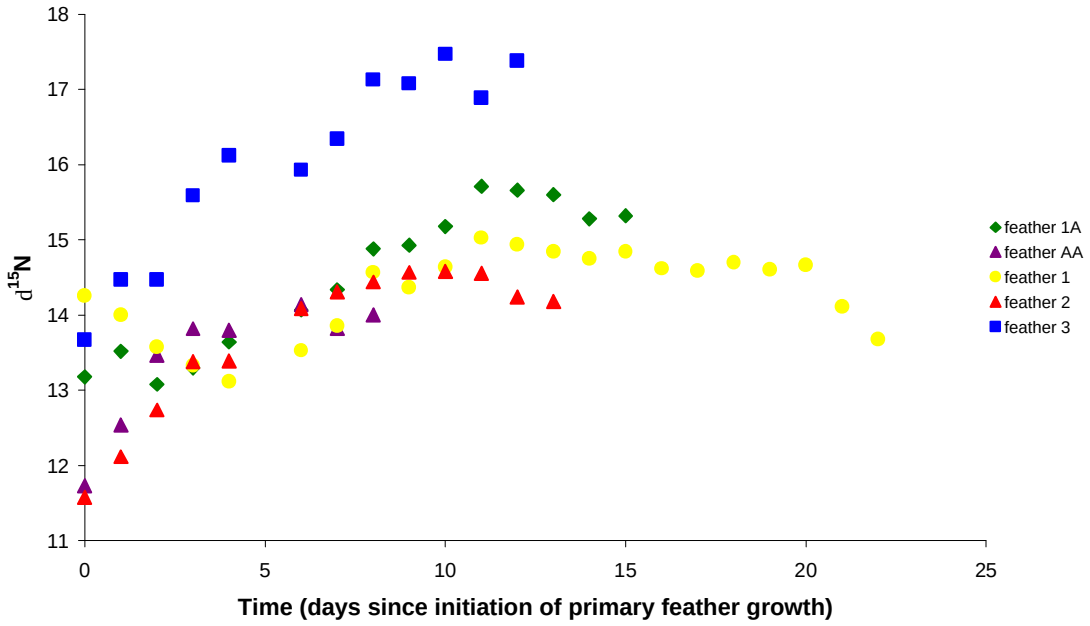


Figure 11. Sequential $d^{15}N$ sampling of young Virginia Laughing Gull feathers



Results of the t-tests comparing the isotopic values of feather base and tip portions from the Virginia colony indicate that the portions taken from the base of feathers were very highly significantly (t carbon = 6.11; t nitrogen = 4.44; df = 48; $P<0.001$) enriched in both carbon and nitrogen relative to the corresponding tip portions of the same feathers. Unlike the carbon and nitrogen isotopes, however, the t-test revealed no statistically significant difference in the sulfur isotopic compositions of base and tip portions sampled from the same feathers (table 4).

Table 4. Relationship between stable isotopic compositions of base and tip portions of Virginia colony feathers (mean +/- standard error).

FEATHER PORTION	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
TIP	-16.6 +/-0.14 (N=49)	13.6 +/- 0.20 (N=49)	9.5 +/- 0.26 (N=37)
BASE	-15.9 +/- 0.12 (N=49)	14.3 +/- 0.19 (N=49)	9.9 +/- 0.28 (N=36)

Results of the same analysis performed on the isotopic values of the New York colony feathers reveal that there is no significant difference between the carbon, nitrogen, or sulfur isotopic compositions of base and tip portions (table 5).

Table 5. Relationship between stable isotopic compositions of base and tip portions of New York feathers (mean +/- standard error).

FEATHER PORTION	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
TIP	-16.9 +/- 0.16 (N=20)	10.7 +/- 0.26 (N=20)	8.5 +/- 0.47 (N=20)
BASE	-16.8 +/- 0.15 (N=20)	10.6 +/- 0.24 (N=20)	7.9 +/- 0.42 (N=20)

A mean isotopic value was obtained for each feather by taking the mean of the base and tip portions. Using these mean values, the New York and Virginia colony feathers were compared to determine whether there were significant differences between the isotopic values of feathers taken from birds in the two colonies. Results of the t-tests indicate that the Virginia Laughing Gull feathers were very significantly ($t = 2.88$; $df = 68$; $P<0.01$) enriched in carbon relative to the New York feathers. Virginia feathers were also very highly significantly (t nitrogen = 10.6; df nitrogen = 68; $P<0.01$; t sulfur = 2.98; df sulfur = 40; $P<0.01$) more enriched in nitrogen and sulfur than feathers taken from Laughing Gulls in the New York colony (table 6).

Table 6. Relationship between the stable isotopic compositions of Virginia and New York feathers (mean +/- standard error).

LOCATION	$d^{13}C$	$d^{15}N$	$d^{34}S$
Virginia	-16.2 +/-0.12 (N=49)	13.9 +/- 0.17 (N=49)	9.7 +/- 0.23 (N=36)
New York	-16.8 +/- 0.14 (N=20)	10.7 +/- 0.24 (N=20)	8.2 +/- 0.42 (N=20)

Another series of t-tests was performed to determine whether there was a significant difference between the isotopic compositions of feathers from birds within the

enclosures and those of birds that were not enclosed. Results indicate that the carbon isotopic composition of feathers from birds in the enclosures were significantly ($t = 1.97$; $df = 47$; $P<0.05$) more enriched than the carbon isotopic values of feathers from birds outside the enclosures. There were no other significant differences between the isotopic values of the feathers from enclosed and non-enclosed birds.

Results from a series of F-tests indicate that there is no statistically significant difference in the variances of the carbon, nitrogen, or sulfur isotopic values of base and tip portions of feathers obtained from either the Virginia or the New York colonies.

Differences in degrees of dietary variation were also examined on a spatial scale, as the variances for the New York feathers were compared to those of the Virginia feathers. F-tests were used to determine whether there was a significant difference in the amount of variance in isotopic values obtained from the New York and Virginia feathers. Results indicate that the variance in the carbon isotopic compositions of the tip portions of Virginia feathers was significantly ($F = 2.00$; $df = 48, 19$; $P<0.05$) greater than the variance in the New York feathers. Although none of the other analyses in this series revealed statistically significant differences (P values all > 0.05), it is interesting to note that the variances were higher in the carbon and nitrogen values of the Virginia feathers (both base and tips), and the sulfur variance was higher for both base and tip portions in the New York feathers.

Temporal intrapopulation variation in diets was examined for both the New York and the Virginia colonies by comparing the variances of the base and tip portions of the feathers. Results from a series of F-tests indicate that there is no statistically significant

difference in the variances of the carbon, nitrogen, or sulfur isotopic values of base and tip portions of feathers obtained from either the Virginia or the New York colonies.

DISCUSSION

The results of this study reveal a great deal about intrapopulation and interpopulation variation in the diets of Laughing Gulls nesting in coastal Virginia and New York. Muscle and blood samples taken from the young Virginia gulls were isotopically very similar to one another. As muscle tissue generally has a slower turnover rate than blood, it reflects the diet of organisms over a longer time span than that of blood. The fact that the muscle and blood samples are so similar indicates that there was no significant change in diet in the time periods reflected in the muscle and blood tissues. Without controlled tests to measure the turnover rates of muscle and blood in Laughing Gulls, however, it is difficult to ascertain the length of the temporal periods reflected in muscle and blood tissue. Therefore, although these data indicate that there was no significant diet change between the times the muscle and blood samples were produced by the birds, without knowledge of how rapidly these tissues are replaced in young Laughing Gulls in the wild, the time period over which the diet did not change significantly cannot be precisely assessed.

Feather and blood isotopic values were also highly correlated among the marked Virginia young. On average, the $\delta^{13}C$ of the feathers appears to be about 1.5‰ to 2.0‰ more enriched than the blood samples taken from the same bird. Similarly, the $\delta^{15}N$ of

feathers appear to be about 0.4‰ more enriched than the blood values. On average, there appears to be little difference in enrichment of sulfur isotopes in blood and feather samples taken from the same individual. These blood-feather enrichment factors are valuable as they may allow the estimation of the isotopic composition of the blood from birds for which only feather samples were taken. This is especially valuable to studies seeking to emphasize non-destructive and non-invasive mechanisms for isotopic analysis of birds. Minimally invasive methods of obtaining tissues for isotopic analysis are especially valuable to studies examining the diets of endangered or threatened species.

The muscle and blood isotopic values also reveal that there appear to be two statistically distinct feeding patterns represented in the enclosed nests of the Virginia colony. One group had more enriched $\delta^{13}C$ values, and less enriched $\delta^{15}N$ and $\delta^{34}S$ values relative to the other group. Perhaps the group with more enriched $\delta^{13}C$ values (and less enriched $\delta^{15}N$ and $\delta^{34}S$) was consuming a diet higher in estuarine prey items, and the group with more $\delta^{15}N$ and $\delta^{34}S$ enrichment and less enriched $\delta^{13}C$ values represents a group feeding more heavily on marine prey items. It is also interesting to note that for two marked nests (7 and 8) more than one chick was sampled. Tissues from chicks in the same nests had very similar isotopic compositions and therefore fell into the same clusters. At the same time, the nests are not separated into the two distinct feeding patterns based on proximity to one another. Nests from different areas in the marked portion of the colony are randomly represented in the two feeding strategy groups (Fig. 12).

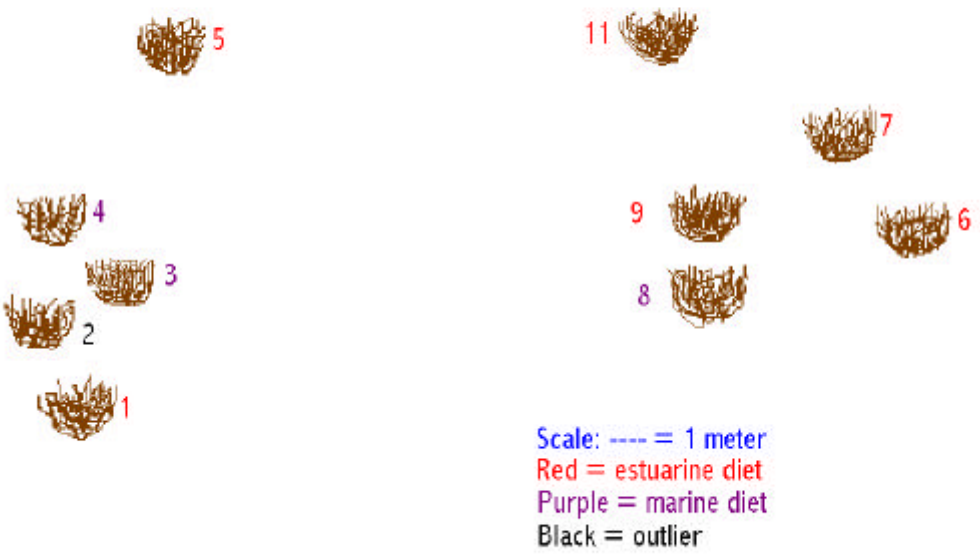


Figure 12: Spatial distribution of marked nests within the Virginia colony.

If adult Laughing Gulls nesting near one another learned foraging strategies from one another, one would perhaps expect that nests displaying the two distinct feeding strategies would be clustered spatially within the colony. This spatial clustering would also be expected if adults from nearby nests often foraged in social groups. The fact that these spatial clusters are not present therefore indicates that individual adult preferences for food types and foraging areas are probably not determined by the feeding behavior of

adults nesting nearby. This result also indicates that adults in this colony do not choose nest location based on some perceived foraging similarity with other nearby nesting adults.

The results indicate that the $\delta^{13}C$ of the feather base portions from enclosed Virginia colony young were significantly less enriched than these values from the non-enclosed young. It is not clear why there should be any difference in the isotopic compositions of the enclosed and non-enclosed young. As there were no other significant differences between the isotope values of the enclosed and non-enclosed young, it is likely that the difference seen in the $\delta^{13}C$ values of the feather bases is an artifact.

In terms of intrapopulation variation in diets, the results of the muscle and blood isotopic analyses indicate that parent Laughing Gulls in the Virginia colony appear to develop distinct preferences for certain food sources. If parent Laughing Gulls had no preference as to what was brought to the nest for the young, one would expect that the diets, and therefore isotopic compositions, of the young gulls would be not be statistically distinct. Different parents may find it easier or more efficient to feed in certain locations, at certain times, or on certain prey and these differences will therefore be reflected in the amounts and types of food brought back to the young. These preferences may be acquired either through learning from other birds or learning from personal experiences. As eggs are produced by females, they therefore reflect the isotopic composition of the diet of the female during the period of egg formation. The amount of variation in the isotopic compositions of the egg membranes examined in this study indicate that there was significant variation in the diets of the maternal Laughing Gulls in the period during which the eggs were formed. There is a highly significant linear correlation in the egg membrane

$\delta^{13}C$ and $\delta^{15}N$ values. Some of the adult females have relatively more depleted $\delta^{13}C$ and $\delta^{15}N$ values, indicating a higher proportion of foods of terrestrial origin in the diet. In contrast, the egg membranes of some females had more enriched $\delta^{13}C$ and $\delta^{15}N$ values, thus indicating a diet higher in foods of marine origin.

The results indicate that although there is widespread heterogeneity in feeding behavior within the population as a whole, there is also homogeneity of feeding behavior at the individual level. The large-scale heterogeneity is evident from the highly significant results of the analyses of variance. The smaller-scale homogeneity is indicated by the distinct feeding clusters apparent in the isotopic compositions of blood taken from Virginia young. The clusters indicate that at the individual or mating pair level, the parent Laughing Gulls were feeding in a more homogenous manner. If their feeding was more heterogeneous, the distinct differences in foraging strategies would not be apparent from the blood isotopic values. Perhaps paired males and females learn preferred foraging locations or food types from one another.

As some studies have found a tendency among seabirds to switch to a diet relatively high in fish and low in foods of terrestrial origin after chick hatching, it is interesting to compare isotope values from adult females during the period prior to egg laying to the isotope values of feathers taken from nestlings that are being fed by their parents. On average, the diet-primary feather fractionation factor for waterbirds is 3.05‰ for carbon and 4.22‰ for nitrogen. According to Hobson (1995), the diet-egg membrane fractionation factor for a carnivorous bird is 2.7‰ for carbon and 3.5‰ for nitrogen. Using these diet-tissue fractionation factors, it is possible to compare the isotope values of

the egg membranes (representing the diet of adult females) to the isotope values of the feathers (representing the diet of nestlings). As these fractionation factors were not measured for Laughing Gulls specifically, however, the results of this comparison must be interpreted cautiously. After correction for fractionation, the average diet of the adult females has a $\delta^{13}C$ value of –19.52‰ and a $\delta^{15}N$ value of 8.59‰. The average $\delta^{34}S$ for the adult female Laughing Gull diet is 6.22‰. The corrected values obtained for young Laughing Gulls based on the $\delta^{13}C$ of feathers (-19.22‰) is quite similar, although the average $\delta^{15}N$ value of the young (9.72‰) is significantly larger ($t = 4.04$; $df = 131$; $P<0.001$), indicating a diet consisting of either higher trophic level foods or more foods of marine origin. The corrected $\delta^{34}S$ of the young feathers (9.69‰) is also significantly ($t = 11.2$; $df = 116$; $P<0.001$) larger than the corrected value for the adult egg membranes. This indicates that the juveniles may be fed a diet higher in foods of marine origin than adults take for themselves prior to the hatching period. These data do not support the findings of previous studies indicating that parents may compensate for immature salt glands in young by providing a diet lower in foods of marine origin (Dosch 1997). The carbon values for the young and adults may not be significantly different as the adults may have been consuming a diet consisting of a mixture of foods of estuarine and terrestrial origin. The carbon isotopic signatures of these food sources, when combined, would be indistinguishable from a mostly marine-based diet. These results therefore indicate that adult Laughing Gulls may feed their young more foods from the marine environment than they would eat themselves. It is not clear whether the parents also switch to a diet higher in marine foods after hatching or if they simply bring their young more marine foods than

they eat themselves. This question could be resolved by isotopically analyzing tissues obtained from adult Laughing Gulls in the Virginia colony during the period after hatching.

Although the isotopic compositions of the muscle and blood samples give some indication that there was no significant dietary change, the results of the analyses on the base and tip feather portions indicate that there was an overall statistically significant shift in the diets of the young Virginia birds between the initiation of primary feather formation (at approximately one week of age) and near fledging (at approximately three to four weeks of age).

Overall, the stable carbon and nitrogen isotopic compositions of the feathers became more enriched over time. The base portions of the feathers (representing more recent diet) were more enriched in carbon and nitrogen isotopes relative to the tip portions (representing the diet after the initiation of primary feather growth). This pattern could be indicative of a shift to more marine foods and less terrestrial foods over time, however the fact that the sulfur isotopic composition of the feathers does not shift significantly from base to tip fails to support this scenario. As nitrogen and carbon isotopes both undergo enrichment as they move up a system trophically and sulfur isotopes appear to undergo no such change, the results suggest that the shift reflects an increase in the trophic level of the diet. Results of the analysis of the stable isotopic compositions of a variety of organisms in the VCR-LTER system indicate that trophic level (and therefore $\delta^{15}N$ and $\delta^{13}C$) increases intraspecifically with increasing body size. Perhaps as the young gulls grow, they are gradually capable of consuming larger prey items that therefore have higher $\delta^{15}N$ and $\delta^{13}C$

values. It is also possible that a food source that was not available to the colony during the initial stages of primary feather formation later became available and was utilized by the colony. As this food source was utilized by the parents for feeding their young, therefore, the isotopic compositions of the feather being produced at that later time would reflect the influence of this new food source. It is also possible that a food source that was available to parent gulls earlier in the nesting period became unavailable as time passed. As this study was only conducted during one breeding season, it is also possible that a food source that is not normally available to the gulls was available in 1998 and therefore altered their diet (and therefore isotopic compositions). The dietary shift observed through the isotopic composition of the feathers may therefore be unique to the breeding season during which the feather samples were taken. The results of the analysis of the Virginia colony feathers do not, however, support the results of earlier studies (Dosch 1997) that postulated that partially developed salt glands in young gulls necessitate increased feeding of terrestrial food items in the early stages of nestling growth. Isotopic analyses on base and tip portions of feathers from young in the New York colony indicate that there was no significant dietary shift during the period of primary feather formation. Although there was isotopic variation among feathers, the feather base and tip portions were not significantly different isotopically. Like the Virginia colony, however, it is possible that significant dietary shifts occur in some years and not in others due to temporal differences in food availability. However, the 1998 data indicate that there was a significant dietary shift in young from the Virginia colony whereas there was no such shift in the New York colony.

As the stable isotopic signature shift most likely represents a shift to higher trophic level foods over time, it is not clear why such a change would take place only at the Virginia colony. The young gulls in both colonies would become more capable of consuming larger food items as they matured. It is possible that the shift to larger and higher trophic level marine food sources was not observed in the New York birds because they do not consume a diet as high in foods of marine origin. This difference is substantiated by the results of comparing the isotopic values of the New York and Virginia colony feathers.

These data indicate that although the New York gulls do utilize marine food sources to some extent, that they are more reliant on a diet that is very highly significantly less enriched in $\delta^{15}N$ and $\delta^{34}S$ and that is highly significantly less enriched in $\delta^{13}C$. This indicates that young gulls from the New York colony consume less foods of marine origin and more of terrestrial origin than the Virginia colony birds. If these terrestrial foods were of anthropogenic origin, one would not necessarily expect their isotope signals to change during the course of the nesting season. The New York birds may also consume proportionately more mainland insects, and as most of these insects feed directly on the primary producers throughout the season, one would also not expect that their trophic levels would increase during the summer season.

Variance was also used as an indication of relative intrapopulation or interpopulation variation in diets. Temporal intrapopulation variation in diets was examined for both the New York and the Virginia colonies by comparing the variances of the base and tip portions of the feathers. Significantly more variance in either the base or

the tip portions may indicate a temporal shift within the colony to a diet either higher or lower in food source variety. This difference may then be attributed to the developmental stages to which the temporal periods correspond. There was no statistically significant base/tip difference for either the New York or Virginia colonies.

Differences in degrees of dietary variation were also examined on a spatial scale, as the variances for the New York feathers were compared to those of the Virginia feathers. The variance in the $\delta^{13}C$ values of the Virginia feather tips was significantly higher than the New York tips. This difference could be due either to more variance in the $\delta^{13}C$ food sources available to the Virginia gulls, or it could be an indication that during the period of primary feather growth initiation, young birds from the Virginia colony were fed a wider variety of food sources than the New York colony young. It is also possible that this result is an artifact.

The results of the stable isotopic analysis of Virginia Coast Reserve (VCR) organisms reveal that marine, estuarine, and terrestrial organisms can be reliably separated based on their stable isotopic compositions (Table A-1). Also, trophic levels within each of these systems are apparent, especially from the nitrogen isotope values. It is also clear that certain species have more intraspecific variation in their isotopic compositions. These differences in level of variation are indicative of differences in the amount of intraspecific dietary variation that exists between species living in the same environments.

Conclusions

Overall, stable isotope analysis is a strong indicator of trophic level, as well as the degree of intrapopulation, interpopulation, and temporal variation in the diets of avian species. The isotopic compositions of feathers obtained from the Virginia and New York colonies are significantly different, thus indicating that the Laughing Gulls in the Virginia colony consumed a significantly different diet from Laughing Gulls nesting in the New York colony. The Virginia colony birds consumed significantly more marine foods than did the New York colony birds. Young gulls from the Virginia colony also underwent a significant dietary shift during the period between primary feather initiation and fledging to a diet consisting of organisms at higher trophic levels. Young gulls in the New York colony underwent no such diet shift. This may be due to the fact that the New York colony nestlings were fed a diet consisting of more foods of terrestrial origin, and one would not expect that these food sources would increase trophically over the course of the summer season. There was also a significant amount of intrapopulation dietary variation. This variation was observed for adults from the Virginia colony as well as nestlings from both the Virginia and New York colonies. Two distinct feeding strategies were observed based on the muscle and blood samples taken from Virginia colony nestlings. One group was fed a diet consisting of more foods of estuarine origin, while the other group was fed more marine prey items. The results also indicate that adult Laughing Gulls consume more terrestrial foods during the period prior to egg laying than they feed their nestlings. Further study would be necessary to determine whether the adults also switch to a diet higher in foods of marine and estuarine origin after hatching.

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APPENDIX A

Stable isotopic compositions of organisms from the Virginia and New York systems

Table A-1. Stable isotopic compositions of organisms from the Virginia and New York systems.

Scientific Name	Common Name	N	Location	$d^{13}C$ mean, SE, range	$d^{15}N$ mean, SE, range	$d^{34}S$ mean, SE, range
Panopeus herbstii	Black-fingered mud crab	3	VA	-14.2, 0.187, 0.640	11.4, 0.171, 0.590	9.7, 0.360, 1.21
Callinectes sapidus	Blue crab	6	VA	-14.6, 0.189, 1.31	11.5, 1.00, 5.69	11.4, 1.15, 7.91
Sesarma reticulatum	Marsh crab	3	VA	-13.3, 0.630, 1.97	10.1, 0.270, 0.830	8.6, 1.63, 5.00
Ocypode quadrata	Ghost crab	5	VA	-16.2, 0.465, 2.56	11.0, 0.692, 4.13	13.6, 0.938, 5.21
Limulus polyphemus	Horseshoe crab conglomerate	1	VA	-13.2	10.3	not enough sample
Acrididae (family)	marsh grasshopper	3 (2 $\delta^{34}S$)	VA	-13.5, 0.432, 1.49	8.2, 0.611, 2.10	-4.0, 0.758, 1.52
Spartina patens		1	VA	-12.9	9.8	9.92
Spatina alterniflora	Salt marsh cordgrass	1	VA	-12.7	12.1	-2.80
Tabanidae (family)	Greenhead	3 (1 $\delta^{34}S$)	VA	-14.6, 1.02, 3.47	12.8, 0.444, 1.44	4.12
Fundulus heteroclitus	Mummichog	4 (3 $\delta^{34}S$)	VA	-13.6, 0.747, 3.49	14.1, 0.199, 0.880	11.0, 0.652, 2.20
Gobiosoma bosci	Naked goby	3 (2 $\delta^{34}S$)	VA	-13.6, 0.375, 1.22	13.9, 0.106, 0.360	12.7, 0.845, 1.69
Pomatomous saltatrix	Bluefish	3	VA	-14.4, 0.139, 0.480	13.5, 0.118, 0.390	17.0, 1.13, 3.44
Leiostomus xanthurus	Spot	1	VA	-12.5	12.9	14.1
Uca pugilator	Sand fiddler	3	VA	-13.0, 0.541, 1.81	4.2, 0.951, 3.18	17.7, 0.576, 1.87
Uca pugnax	Mud fiddler	3 (2 $\delta^{34}S$)	VA	-16.0, 0.139, 0.480	5.2, 0.383, 1.24	8.9, 1.55, 3.11
Arenaeus cribrarius	Speckled crab	3	VA	-17.3, 0.0667, 0.200	11.3, 0.321, 1.06	16.7, 0.740, 2.27
Ovalipes ocellatus	Lady crab	1	VA	-17.7	11.0	17.9
Pagarus spp.	Hermit crab	2	VA	-18.8, 1.93, 3.85	10.6, 0.235, 0.470	17.9, 0.480, 0.959
Tenodera aridifoliasinensis	Praying mantis	2 (1 $\delta^{34}S$)	VA	-24.0, 0.565, 1.13	9.1, 0.415, 0.830	13.4
Littorina irrorata	Periwinkle snail	3	VA	-14.3, 0.157, 0.540	10.8, 0.0433, 0.130	14.7, 0.887, 2.96
Ilyanassa obsoleta	Mud snail	3	VA	-15.7, 0.183, 0.630	10.4, 0.0917, 0.300	14.0, 0.402, 1.23
Trachinotus carolinus	Florida Pompano	3	VA	-18.1, 0.191, 0.640	13.0, 0.00667, 0.0200	17.1, 0.540, 1.70
Menticirrhus americanus	Southern Kingfish	3	VA	-17.8, 0.205, 0.710	13.2, 0.163, 0.560	19.7, 0.664, 2.19
Fundulus majalis	Striped Killifish	3	VA	-17.5, 0.224, 0.700	13.0, 0.0670, 0.230	18.8, 0.301, 1.01
Menticirrhus saxatilis	Northern Kingfish	3	VA	-14.3, 0.164, 0.520	9.4, 0.350, 1.19	17.8, 0.801, 2.76
Lucania parva	Rainwater Killifish	3 (1 $\delta^{34}S$)	VA	-14.3, 0.419, 1.35	9.2, 1.40, 0.451	17.2
Cyprinodon variegatus	Sheepshead Minnow	3	VA	-14.3, 0.257, 0.860	6.8, 0.0698, 0.230	16.0, 0.913, 2.86
Menidia menidia	Atlantic Silverside	6	VA	-16.9, 0.182, 1.16	13.2, 0.216, 1.40	16.6, 0.737, 5.43
Diopatra cuprea	Polychaete	4	VA	-13.8, 0.104, 0.500	12.0, 0.149, 0.520	19.4, 0.551, 2.54
Geukensia demissa	Ribbed Mussel	6	VA	-17.3, 0.108, 0.660	8.2, 0.313, 1.85	13.3, 1.79, 10.6
Mercenaria mercenaria	Hard clam	4	VA	-17.4, 0.671, 2.82	8.8, 0.798, 3.40	14.9, 1.63, 6.86
Menidia menidia	Silverside	2	NY	-13.0, 0.240, 0.480	13.2, 0.460, 0.920	10.7, 0.294, 0.589
Fundulus spp.	Killifish	3 (2 $\delta^{15}N$)	NY	-13.0, 1.33, 4.60	13.9, 0.210, 0.420	12.0, 0.561, 1.81
Brevoortia tyrannus	Menhaden	1	NY	-16.3		13.3
Limulus polyphemus	horseshoe crab eggs	1	NY	-16.8	5.4	12.3
	Japanese Shore Crab	1	NY	-13.2	13.2	15.2
Libinia spp.	Spider Crab	1	NY	-14.2	11.7	14.8
Panopeus herbstii	Mud Crab	2 (1 $\delta^{34}S$)	NY	-12.5, 0.650, 1.30	12.0, 0.860, 1.72	15.0
Mercenaria mercenaria	Hard clam	1	NY	-15.5	4.6	10.8
Oligochaeta (class)	Earthworm	3	NY	-25.4, 1.27, 4.01	5.4, 0.251, 0.84	4.4, 0.316, 1.07
Blattidae (family)	Cockroach	3	NY	-19.4, 0.213, 0.640	3.4, 1.67, 5.17	3.9, 0.191, 0.610
Scarabaeidae (family)	Scarab beetle	3	NY	-29.9, 0.695, 2.12	4.5, 1.13, 3.86	6.7, 0.0777, 0.262

APPENDIX B

Raw isotope data

Table B-1. Isotopic values for muscle and blood from young Virginia colony Laughing Gulls

Sample Type	Sample ID	$d^{13}C$	$d^{15}N$	$d^{34}S$
muscle	nest 1	-16.8	13.0	9.8
muscle	nest 2	-19.8	12.4	
muscle	nest 3	-19.3	15.0	
muscle	nest 4	-20.2	15.5	
muscle	nest 5	-17.2	12.8	
muscle	nest 6	-17.3	14.2	
muscle	nest 7	-17.9	12.8	
muscle	nest 7 (RG)	-18.2	12.7	
muscle	nest 8	-19.7	15.2	
muscle	nest 8 (GR)	-19.4	15.3	
muscle	nest 9	-17.6	12.1	
muscle	nest 11	-17.2	12.5	
blood	nest 1	-17.4	13.0	11.3
blood	nest 2	-20.2	11.9	10.9
blood	nest 3	-19.5	14.0	10.7
blood	nest 4	-19.5	15.6	10.5
blood	nest 5	-17.3	12.7	6.7
blood	nest 6	-17.5	13.8	10.2
blood	nest 7	-17.7	12.8	9.3
blood	nest 7 (RG)	-17.8	12.7	8.6
blood	nest 8	-19.3	14.4	10.0
blood	nest 8 (GR)	-19.1	15.2	10.4
blood	nest 9	-17.7	12.1	8.7
blood	nest 11	-17.5	12.5	10.0

Table B-1. Isotopic values for muscle and blood from young Virginia colony Laughing Gulls, continued.

Sample Type	Sample ID	$d^{13}C$	$d^{15}N$	$d^{34}S$
blood	nest 1	-17.4	13.0	11.3
blood	nest 5	-17.3	12.7	6.7
blood	nest 6	-17.5	13.8	10.2
blood	nest 7	-17.7	12.8	9.3
blood	nest 7 (RG)	-17.8	12.7	8.6
blood	nest 9	-17.7	12.1	8.7
blood	nest 11	-17.5	12.5	10.0
blood	nest 2	-20.2	11.9	10.9
blood	nest 3	-19.5	14.0	10.7
blood	nest 4	-19.5	15.6	10.5
blood	nest 8	-19.3	14.4	10.0
blood	nest 8 (GR)	-19.1	15.2	10.4

Table B-2. Isotopic values for Virginia colony Laughing Gull egg membranes with estimations of $\delta^{13}C$ and $\delta^{15}N$ of diets based on diet-tissue fractionation factors.

Sample Type	Sample ID	$d^{13}C$	$d^{13}C$ diet	$d^{15}N$	$d^{15}N$ diet	$d^{34}S$
Egg Membrane	1	-13.7	-16.4	12.0	8.5	3.7
Egg Membrane	2	-15.8	-18.5	12.3	8.8	3.8
Egg Membrane	3	-17.1	-19.8	12.5	9.0	5.4
Egg Membrane	4	-14.8	-17.5	12.9	9.4	5.4
Egg Membrane	5	-15.0	-17.7	12.6	9.1	5.3
Egg Membrane	6	-14.5	-17.2	13.2	9.7	6.1
Egg Membrane	7	-15.8	-18.5	13.2	9.7	7.0
Egg Membrane	8	-16.0	-18.7	12.2	8.7	4.0
Egg Membrane	9	-15.9	-18.6	12.5	9.0	4.8
Egg Membrane	10	-15.9	-18.6	13.0	9.5	2.5
Egg Membrane	11	-18.7	-21.4	10.4	6.9	3.6
Egg Membrane	12	-19.8	-22.5	9.5	6.0	7.8
Egg Membrane	13	-16.4	-19.1	12.1	8.6	6.5
Egg Membrane	14	-17.0	-19.7	11.1	7.6	5.5
Egg Membrane	15	-19.0	-21.7	10.4	6.9	4.8
Egg Membrane	16	-20.4	-23.1	10.0	6.5	4.0
Egg Membrane	17	-16.4	-19.1	11.3	7.8	3.8
Egg Membrane	18	-17.6	-20.3	11.8	8.3	7.7
Egg Membrane	19	-16.9	-19.6	12.2	8.7	5.4
Egg Membrane	20	-15.0	-17.7	13.8	10.3	7.2
Egg Membrane	21	-17.2	-19.9	13.1	9.6	9.2
Egg Membrane	22	-17.7	-20.4	10.2	6.7	5.4
Egg Membrane	23	-15.9	-18.6	12.5	9.0	5.9
Egg Membrane	24	-15.3	-18.0	12.6	9.1	4.3
Egg Membrane	25	-17.4	-20.1	12.2	8.7	6.1
Egg Membrane	26	-16.2	-18.9	12.7	9.2	5.9
Egg Membrane	27	-15.2	-17.9	13.2	9.7	4.9
Egg Membrane	28	-16.5	-19.2	12.4	8.9	5.9
Egg Membrane	29	-18.0	-20.7	12.5	9.0	5.8
Egg Membrane	30	-16.6	-19.3	12.6	9.1	6.4

Table B-2. Isotopic values for Virginia colony Laughing Gull egg membranes with estimations of $\delta^{13}C$ and $\delta^{15}N$ of diets based on diet-tissue fractionation factors, continued.

Sample Type	Sample ID	$d^{13}C$	$d^{13}C$ diet	$d^{15}N$	$d^{15}N$ diet	$d^{34}S$
Egg Membrane	31	-16.8	-19.5	11.6	8.1	5.8
Egg Membrane	32	-16.8	-19.5	12.3	8.8	5.5
Egg Membrane	33	-19.5	-22.2	11.0	7.5	5.5
Egg Membrane	34	-18.5	-21.2	12.6	9.1	6.9
Egg Membrane	35	-19.2	-21.9	11.6	8.1	5.2
Egg Membrane	36	-18.6	-21.3	12.6	9.1	6.0
Egg Membrane	37	-16.0	-18.7	12.3	8.8	5.8
Egg Membrane	38	-16.8	-19.5	12.9	9.4	5.7
Egg Membrane	39	-19.2	-21.9	10.4	6.9	5.2
Egg Membrane	40	-18.1	-20.8	12.3	8.8	5.6
Egg Membrane	41	-16.8	-19.5	11.4	7.9	4.3
Egg Membrane	42	-14.6	-17.3	15.2	11.7	7.6
Egg Membrane	43	-16.0	-18.7	13.5	10.0	7.2
Egg Membrane	44	-15.3	-18.0	13.2	9.7	6.3
Egg Membrane	45	-18.3	-21.0	11.3	7.8	5.2
Egg Membrane	46	-17.2	-19.9	12.7	9.2	6.4
Egg Membrane	47	-14.8	-17.5	13.6	10.1	6.7
Egg Membrane	48	-16.9	-19.6	11.9	8.4	6.4
Egg Membrane	49	-19.7	-22.4	9.6	6.1	3.4
Egg Membrane	50	-15.5	-18.2	12.2	8.7	6.2
Egg Membrane	51	-16.2	-18.9	12.3	8.8	4.4
Egg Membrane	52	-17.8	-20.5	10.4	6.9	3.9
Egg Membrane	53	-17.2	-19.9	11.6	8.1	5.0
Egg Membrane	54	-16.0	-18.7	11.8	8.3	5.0
Egg Membrane	55	-18.8	-21.5	10.5	7.0	10.4
Egg Membrane	56	-15.9	-18.6	13.4	9.9	8.3
Egg Membrane	57	-17.2	-19.9	11.1	7.6	7.3
Egg Membrane	58	-17.3	-20.0	11.5	8.0	6.3
Egg Membrane	59	-17.4	-20.1	11.0	7.5	6.1
Egg Membrane	60	-15.8	-18.5	11.3	7.8	6.3

Table B-2. Isotopic values for Virginia colony Laughing Gull egg membranes with estimations of $\delta^{13}C$ and $\delta^{15}N$ of diets based on diet-tissue fractionation factors, continued.

Sample Type	Sample ID	$d^{13}C$	$d^{13}C$ diet	$d^{15}N$	$d^{15}N$ diet	$d^{34}S$
Egg Membrane	61	-15.4	-18.1	13.0	9.5	7.6
Egg Membrane	62	-15.9	-18.6	13.9	10.4	9.5
Egg Membrane	63	-20.3	-23.0	10.0	6.5	6.6
Egg Membrane	64	-14.8	-17.5	12.9	9.4	7.4
Egg Membrane	65	-15.3	-18.0	12.2	8.7	6.1
Egg Membrane	66	-15.9	-18.6	10.7	7.2	5.9
Egg Membrane	67	-17.4	-20.1	11.6	8.1	5.0
Egg Membrane	68	-17.4	-20.1	10.9	7.4	6.7
Egg Membrane	69	-15.9	-18.6	11.1	7.6	7.2
Egg Membrane	70	-17.5	-20.2	10.6	7.1	5.6
Egg Membrane	71	-15.9	-18.6	12.7	9.2	8.3
Egg Membrane	n. 2 c.1, 72	-17.0	-19.7	11.2	7.7	7.0
Egg Membrane	n. 9, 73	-15.8	-18.5	12.1	8.6	7.5
Egg Membrane	n. 7, 74	-16.4	-19.1	11.1	7.6	6.9
Egg Membrane	n. 9 c. 3, 75	-16.5	-19.2	12.9	9.4	8.1
Egg Membrane	n. 9 c. 2, 76	-17.2	-19.9	13.2	9.7	8.5
Egg Membrane	n. 8 c. 1, 77	-17.1	-19.8	11.7	8.2	6.6
Egg Membrane	n. 8 c. 2, 78	-18.5	-21.2	9.8	6.3	5.0
Egg Membrane	n. 15, 79	-14.5	-17.2	12.5	9.0	6.7
Egg Membrane	n. 1 XX, 80	-15.1	-17.8	13.2	9.7	8.2
Egg Membrane	old n. 6, 81	-20.1	-22.8	17.4	13.9	10.9
Egg Membrane	new n. 6, 82	-18.4	-21.1	15.6	12.1	11.2

Table B-3. Results of lipid removal treatments on feather isotopic values.

Treated	$d^{13}C$	$d^{15}N$	**Not-treated**	$d^{13}C$	$d^{15}N$
quill tip	-15.8	13.4	quill tip	-15.8	13.4
quill base	-15.9	14.5	quill base	-16.2	13.9
downy tip	-16.8	13.9	downy tip	-16.4	13.7
downy base	-16.1	14.3	downy base	-16.3	14.0
quill tip	-15.6	11.9	quill tip	-15.8	12.5
quill base	-15.6	14.1	quill base	-15.2	14.5
downy tip	-16.1	12.5	downy tip	-16.1	12.3
downy base	-15.2	14.6	downy base	-15.1	14.6
			quill tip	-15.0	12.4
downy tip	-16.2	12.9	downy tip	-15.1	12.5
downy base	-15.1	14.6	downy base	-14.8	15.0

Table B-4. Stable isotopic values of feathers from young Virginia colony Laughing Gulls

Feather ID	$\delta^{13}C$ tip	$\delta^{15}N$ tip	$\delta^{34}S$ tip	$\delta^{13}C$ base	$\delta^{15}N$ base	$\delta^{34}S$ base	$\delta^{13}C$ mean	$\delta^{15}N$ mean	$\delta^{34}S$ mean
AA	-17.8	11.2		-15.7	13.2		-16.7	12.2	
BB	-16.3	14.4		-15.5	13.9		-15.9	14.1	
CC	-16.9	11.9		-16.2	13.2		-16.6	12.5	
DD	-16.1	14.7		-15.5	14.4		-15.8	14.5	
EE	-16.2	14.7		-16.3	12.9		-16.2	13.8	
FF	-15.2	16.2		-15.3	15.9		-15.3	16.0	
1	-15.8	12.5		-14.9	13.6		-15.3	13.1	
2	-16.9	11.7	7.4	-15.4	13.9	9.2	-16.1	12.8	8.3
3	-17.7	14.3	9.4	-16.2	17.1	12.1	-17.0	15.7	10.8
A	-15.2	13.0	7.5	-15.1	14.7	9.8	-15.1	13.9	8.7
B	-16.8	14.2	7.9	-16.3	13.1	8.2	-16.5	13.7	8.1
C	-17.3	14.7	11.0	-17.3	16.0	11.5	-17.3	15.3	11.3
D	-16.4	13.8	9.4	-16.5	13.2	9.2	-16.4	13.5	9.3
E	-17.3	15.1	10.4	-14.4	14.8	8.6	-15.9	14.9	9.5
F	-16.9	13.4	10.1	-15.8	13.8	10.3	-16.4	13.6	10.2
G	-14.1	13.6	9.6	-14.5	15.5	10.9	-14.3	14.5	10.2
H	-17.2	13.0	6.6	-15.9	15.9	7.8	-16.6	14.4	7.2
I	-15.6	13.6	9.3	-15.7	15.3	10.6	-15.7	14.5	10.0
J	-14.6	12.1	8.2	-14.2	13.2	8.3	-14.4	12.7	8.2
1	-17.6	13.0	8.2	-16.4	13.5	8.4	-17.0	13.2	8.3
2	-15.6	16.1	11.9	-15.4	14.7	10.9	-15.5	15.4	11.4
3	-16.2	15.9	11.5	-16.4	16.4	11.8	-16.3	16.2	11.6
4	-16.3	12.0	8.6	-16.0	13.6	9.1	-16.1	12.8	8.9
5	-15.7	10.8	7.30	-15.6	11.8	7.3	-15.6	11.3	7.32
6	-17.2	14.0	10.6	-16.3	13.7	9.9	-16.8	13.9	10.3
7	-16.8	12.5	10.5	-16.3	13.9	10.7	-16.5	13.2	10.6
8	-16.7	12.9	10.7	-15.5	14.3	11.0	-16.1	13.6	10.8
9	-14.2	14.3	10.1	-14.1	14.8	11.1	-14.1	14.5	10.6
10	-17.6	13.6	11.1	-16.8	15.0	12.5	-17.2	14.3	11.8

Table B-4. Stable isotopic values of feathers from young Virginia colony Laughing Gulls, continued.

Feather ID	$\delta^{13}C$ tip	$\delta^{15}N$ tip	$\delta^{34}S$ tip	$\delta^{13}C$ base	$\delta^{15}N$ base	$\delta^{34}S$ base	$\delta^{13}C$ mean	$\delta^{15}N$ mean	$\delta^{34}S$ mean
1A	-16.2	12.5		-15.3	14.5		-15.7	13.5	
1B	-16.5	14.9		-16.2	15.1		-16.3	15.0	
1C	-17.0	16.1		-16.1	16.7		-16.6	16.4	
1D	-16.8	14.2		-15.8	15.1		-16.3	14.6	
1E	-17.8	15.0		-16.4	15.1		-17.1	15.0	
RG-7	-16.3	13.5	9.8	-16.8	12.7	8.6	-16.5	13.1	9.18
2 (12 cm)	-18.9	11.8	8.1	-17.7	14.3	10.1	-18.3	13.0	9.08
GO	-15.6	11.2	8.7	-16.0	12.8	8.1	-15.8	12.0	8.41
3	-17.7	14.2	9.4	-17.1	16.9	10.9	-17.4	15.5	10.2
7	-16.5	13.4	9.7	-15.7	14.1	9.9	-16.1	13.8	9.77
GR8 (12 cm)	-17.7	15.2	8.5	-16.0	13.2	9.4	-16.8	14.2	8.95
8	-17.3	14.3	9.1	-17.7	16.1	12.6	-17.5	15.2	10.9
XG	-18.4	12.2	6.8	-16.3	12.0	6.7	-17.4	12.1	6.73
9 (12 cm)	-16.3	12.5	9.7	-16.0	12.1	6.2	-16.2	12.3	7.97
1 (11 cm)	-15.7	12.6	8.6	-15.0	14.1	12.0	-15.3	13.4	10.3
GB (11 cm)	-15.6	11.8	13.6	-16.0	13.2	8.6	-15.8	12.5	11.1
11 (11 cm)	-16.3	12.8	10.4	-15.6	13.0	11.7	-15.9	12.9	11.0
4 (9 cm)	-17.5	16.3	9.1	-17.6	16.3	11.4	-17.6	16.3	10.3
5	-16.1	13.6	13.2	-15.2	13.8	10.8	-15.6	13.7	12.0
6	-16.8	13.8	8.4	-16.3	14.8	NA	-16.5	14.3	NA

Table B-5. Stable isotopic values of feathers from young New York colony Laughing Gulls.

Feather ID	$\delta^{13}C$ tip	$\delta^{15}N$ tip	$\delta^{34}S$ tip	$\delta^{13}C$ base	$\delta^{15}N$ base	$\delta^{34}S$ base	$\delta^{13}C$ mean	$\delta^{15}N$ mean	$\delta^{34}S$ mean
17-1 (35)	-17.0	11.6	13.6	-15.9	10.5	9.9	-16.5	11.1	11.7
20-1 (35)	-18.1	8.82	6.0	-18.0	9.14	5.3	-18.0	8.98	5.6
17-3 (33)	-16.5	12.0	9.8	-16.2	12.6	11.2	-16.4	12.3	10.5
18-1 (34)	-16.8	11.7	8.7	-15.7	11.6	8.7	-16.3	11.6	8.7
256-1 (29)	-15.6	11.0	6.9	-16.4	10.2	6.4	-16.0	10.6	6.6
152-3 (29)	-16.9	12.9	11.5	-17.6	11.9	10.7	-17.3	12.4	11.1
167-1 (31)	-16.7	8.76	5.5	-16.8	9.23	5.5	-16.8	9.00	5.5
19-3 (35)	-16.2	11.0	8.2	-16.4	10.3	6.8	-16.3	10.6	7.5
32-1 (35)	-17.0	10.9	8.9	-16.6	9.60	6.5	-16.8	10.2	7.7
19-2 (34)	-16.4	11.9	10.7	-16.2	11.7	8.4	-16.3	11.8	9.5
167-2 (30)	-17.3	8.94	5.8	-17.5	9.03	6.3	-17.4	8.99	6.0
36-1 (34)	-15.7	10.4	6.3	-15.9	11.8	7.3	-15.8	11.1	6.8
20-3 (33)	-18.2	9.03	7.0	-17.4	10.3	6.8	-17.8	9.65	6.9
32-3 (34)	-17.0	10.4	8.7	-17.1	9.66	7.1	-17.0	10.0	7.9
18-2(34)	-16.7	10.7	7.7	-16.4	11.4	8.4	-16.5	11.1	8.0
35-2 (33)	-17.5	10.6	8.8	-17.6	11.0	8.8	-17.5	10.8	8.8
35-1 (33)	-17.7	11.6	10.8	-17.5	10.9	9.3	-17.6	11.2	10.1
256-2 (29) 12 cm	-16.1	10.7	6.7	-16.5	10.0	6.4	-16.3	10.3	6.5
36-2 (33)	-16.8	9.63	8.0	-17.2	9.94	6.6	-17.0	9.79	7.3
152-2 (31)	-16.9	11.6	9.5	-17.4	12.2	11.6	-17.1	11.9	10.6