

An Analysis of Possible Genotoxic Exposure in Adult and Juvenile Royal Terns in North Carolina, USA

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Abstract.—We studied possible genotoxic exposure in Royal Terns (*Sterna maxima*) by collecting blood from adults and juveniles at five breeding colonies in coastal North Carolina in 1999. These colonies are located in three estuarine systems (Core Sound, Pamlico Sound, and Cape Fear River), each subjected to different contaminant loads. DNA in red blood cells was analyzed using the comet assay to determine levels of DNA strand breaks, a technique previously not applied to birds. In addition, we weighed each bird and estimated its fat reserves as an indication of nutritional health. Gross health assessments showed no significant differences between study sites, both for adult and juvenile terns. The comet assay indicated that blood cells from the adult and juvenile terns from two Core Sound colonies, Wainwright and Sand Bag Islands, had significantly higher levels of apparent DNA damage than the remaining study sites. Based on previously published studies of sediment contaminants, the Core Sound colonies have relatively low overall pollutant loads, a finding contrary to the expected result based on the DNA damage. Plausible explanations for these findings are that birds from the Core Sound are exposed to an undetected genotoxic contaminant(s) or that birds from the more polluted sites have had an adaptive response to the contaminant exposure. *Received 30 October 2000, accepted 25 March 2001.*

Key Words.—Royal Terns, genotoxins, North Carolina, comet assay, environmental pollutants.

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We investigated the potential effect of genotoxic exposure in Royal Terns (*Sterna maxima*) while breeding in estuaries along the coast of North Carolina. Genotoxins are pollutants that damage DNA and can accumulate through the food chain rendering high trophic-level feeders particularly at risk (Gardner *et al.* 1991; French *et al.* 1996; Boon *et al.* 1998; White *et al.* 1998). Seabirds are known bioindicators of the health of marine ecosystems (Bourne 1976; Furness and Camphuysen 1997) and top predators such as terns and gulls are potentially excellent indicator species for the assessment of genotoxic exposure.

Royal Terns were selected for this study because they breed in three areas of North Carolina that are known to receive different levels of pollutants that cause genotoxic damage, a large number of known-age birds exist in these colonies, thereby providing a unique opportunity to assess contaminant exposure over time and, in the early 1990s, juvenile birds from one colony were observed with foot and bill deformities (T. Augspurger, pers. comm.). Moreover, the Royal Terns in North Carolina have been declining over the past 23 years. According to Parnell *et al.* (1997), there were 9,755 Royal Tern nests in North Carolina in 1977. This num-

ber increased in the 1980's, but by 1999 the number dropped to 12,519 (NC Colonial Waterbird Database, unpublished data). The number of breeding terns can fluctuate from year to year depending on weather conditions and nest site availability. However, ten-year averages beginning in 1977 indicate that there has been a 13% drop in Royal Tern numbers in the last decade.

To assess possible genotoxic exposure, blood was collected from adult and juvenile Royal Terns at five colonies in three estuarine systems in North Carolina. The blood was analyzed by the comet assay in order to determine levels of DNA strand breaks. Comet assays have been used to assess DNA damage, both in laboratory and field studies, and on a variety of cell types (for a review see Fairbairn *et al.* 1995).

Previous studies using the comet assay on other organisms have indicated that the amount of strand breaks correlates well with exposure to genotoxic agents (Theodorakis *et al.* 1994; Shugart 1988; Meyers-Schone *et al.* 1993; Mitchelmore and Chipman 1998a, b; Leroy *et al.* 1996; Collins *et al.* 1997; Fairbairn *et al.* 1995; Nacci *et al.* 1996; Pandrangi *et al.* 1995). The biological effects of genotoxic agents are mutations, altered gene expres-

sion, aneuploidy, oncogene activation, sister chromatid exchanges, creation of micronuclei, and translocations. These changes can lead to cell death, fertility decline, protein dysfunction, developmental abnormalities, physiological impairment, cancer, and death. At the population level, prolonged exposure to genotoxins can lead to altered genotypic diversity, altered age class structures, decreased reproductive success, decreased populations and possibly extirpation of species (Anderson *et al.* 1994; Shugart 1995; Shugart and Theodorakis 1996).

The comet assay can detect strand breaks created both by direct-acting and indirect-acting xenobiotics (Mitchelmore and Chipman 1998a). DNA modifications by pollutants can result in strand breaks either by directly damaging the DNA or by inducing repair mechanisms. Production of strand breaks correlates well with the mutagenic and carcinogenic properties of environmental pollutants (Mitchelmore and Chipman 1998b). The comet assay is a nonspecific technique; it cannot determine causative agents of strand breaks. The technique can, however, be utilized as an initial indicator of genetic damage for which further studies would be necessary to elucidate the circumstances that led to the strand breaks.

The comet assay has been used to assess genotoxic exposure in a large number of vertebrate and invertebrate species, and we believe this technique can be useful for birds, especially for high trophic-level species such as terns. Here, we test the hypothesis that Royal Terns from more polluted areas should exhibit more DNA damage than terns from less polluted areas. Assessing the effects of pollutants on Royal Terns using the comet assay demonstrates another method for monitoring high trophic-level species as bioindicators for environmental health.

STUDY AREA AND METHODS

Study Sites

The five breeding colonies selected for this study are on estuarine non-barrier islands that have been covered with dredge material deposited at various times in their history (Fig. 1). The islands are managed either by the National Audubon Society (Ferry Slip and Wainwright Islands) or the North Carolina Wildlife Resources Com-

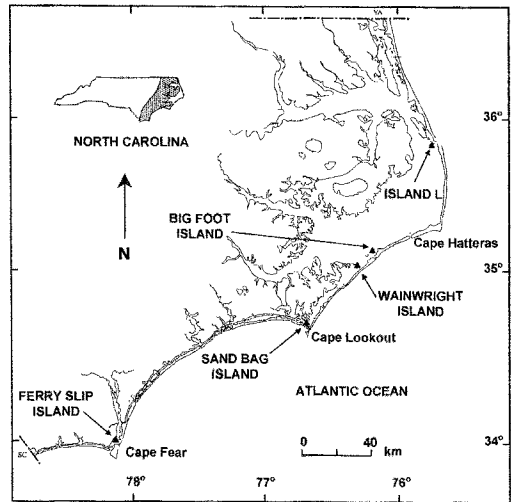


Figure 1. Map of Coastal North Carolina showing the locations of Royal Tern breeding colonies discussed in the text.

mission (NCWRC; Sand Bag, Big Foot, and Island L) and terns have bred on these islands or nearby islands since the 1970s (Parnell and Soots 1979; Parnell *et al.* 1995). The NCWRC maintains colonial waterbird census data (beginning in the 1970's) on all sites used in this study (NC Colonial Waterbird Database, unpublished data). Royal Terns require bare or nearly bare sandy nesting sites (Parnell and Soots 1979); birds will only nest on islands that are in an early stage of succession. The terns have strong fidelity to a local area. If a colony is forced to move, the terns will use a nearby suitable site (Parnell and Soots 1979; W. Golder, pers. comm.).

Factors Used To Determine Site Ranking

The five sites used in this study were ranked from cleanest to most polluted as determined from previously published studies on sediment and water quality analyses completed by the Environmental Protection Agency (EPA) and the Center for Marine Science Research, University of North Carolina at Wilmington (Hackney *et al.* 1998; EPA 1998a, b). Concentrations of contaminants in sediments were averaged over several sampling sites found within a 35-km radius of the study sites during a period of time from 1994 to 1997 (Table 1; Hackney *et al.* 1998). A 35 km radius was selected to encompass the most likely foraging range of the terns (Buckley and Buckley 1972; Swartz 1987).

Concentrations of several individual contaminants in surrounding sediments were considered as well as total contaminant loads. A calculation of total toxicity units (Σ TU) determined the total contaminant load at a particular site. The Σ TU provide a means for evaluating the cumulative effects of sub-lethal contaminants. A Σ TU below one is considered "uncontaminated", a value between one and four is considered "moderate contamination", and a value above four is "highly contaminated" (Hackney *et al.* 1998).

Data from the Index of Watershed Indicators (EPA 1998a) and Toxic Release Inventory (TRI, EPA 1998b) were used as an additional assessment of potential pol-

Table 1. Sediment contaminants at sites and site ranking based on potential pollutant exposure; 1 is the most polluted while 5 is the cleanest. Data are means derived from samples taken from 1994 to 1997 as reported by Hackney *et al.* (1998). Acronyms are: Σ TU—Total Toxicity Units, PAHs—Polycyclic aromatic hydrocarbon, PCBs—Polychlorinated biphenyls, Σ Metals—sum of metals (cadmium, chromium, mercury, nickel, zinc, antimony, arsenic, copper, lead, and silver), IWI—EPA Index of Watershed Indicators.

| Colony | Site Ranking | Σ TU | PAHs (ppb) | PCBs (ppb) | Σ metals (ppm) | IWI Rank |
|------------|--------------|-------------|------------|------------|-----------------------|----------|
| Ferry Slip | 1 | 3.81 | 313 | 5.98 | 149 | 3 |
| Island L | 2 | 3.52 | 201 | 4.44 | 138 | 2 |
| Wainwright | 3 | 2.85 | 170 | 3.88 | 106 | 1 |
| Sand Bag | 4 | 1.15 | 19 | 3.14 | 45 | 1 |
| Big Foot | 5 | 0.89 | 7 | 2.66 | 30 | na |

lutant exposure. The Index of Watershed Indicators ranks watersheds on a scale of 1 to 6 with one indicating a "better quality" watershed and six indicating a watershed with "serious problems" (EPA 1998a). The TRI lists the amounts and types of regulated toxic compounds released directly into surface waters from permitted facilities (EPA 1998b).

Environmental Ranking of Sites

The colony with the highest contaminant exposure was Ferry Slip Island (Fig. 1; 33°58'N, 77°56'W), in the Cape Fear River (Table 1). Previous investigations on the island in the 1990's revealed foot and bill deformities in chicks (T. Augspurger, pers. comm.). The average Σ TU at Ferry Slip Island approaches 4, which is "highly contaminated" (Table 1, Hackney *et al.* 1998). Polycyclic aromatic hydrocarbon concentrations were highest and chromium and nickel concentrations were high at this site (Hackney *et al.* 1998). Polycyclic aromatic hydrocarbons, chromium, and nickel are all genotoxins (Anderson *et al.* 1998). Chromium and nickel are also known to cause cancer (Anderson *et al.* 1998; International Agency for Research on Cancer 1990). In 1996, permitted industrial users in New Hanover, Brunswick, and Bladen Counties released more than 1.5 million pounds of EPA regulated toxic chemicals directly into the river (EPA 1998b). EPA ranked the Lower Cape Fear and the Northeast Cape Fear rivers as 3, indicating "less serious problems" in the watershed (Table 1, EPA 1998a).

Island L (Fig. 1; 35°46'N, 75°34'W), near Oregon Inlet, was the next most polluted study site. The Σ TU in surrounding sediments is in the high "moderately contaminated" range (Table 1). Chromium and nickel concentrations were highest at this site (Hackney *et al.* 1998). Island L is located between the Albemarle and Pamlico Sounds. Total toxic releases into surface waters from surrounding counties (Dare, Tyrell, Perquimans, Chowan, and Washington Counties) were approximately 100,000 pounds (680,000 kg) from 1988 to 1997 (EPA 1998b). EPA ranked the Albemarle Sound as 2, suggesting a "better quality" watershed. There were not enough data available to rank the Pamlico Sound (Table 1, EPA 1998a).

Wainwright Island (Fig. 1, 34°44'N, 76°41'W), in Core Sound, was the third most polluted of the five study sites (Table 1). The Σ TU in surrounding sediments indicates "moderate" contamination (Table 1, Hackney *et al.* 1998). There were no toxic releases into surface waters from surrounding counties (Carteret and Pamlico) from 1988 to 1997 (EPA 1998b). The sound was ranked as 1, indicating a "better quality" watershed (Table 1, EPA 1998a).

Sand Bag Island (34°40'N, 76°32'W) is in Core Sound (Fig. 1). Sediment Σ TU around the study site were slightly higher than "uncontaminated" (Table 1, Hackney *et al.* 1998). Rankings for Sand Bag are the same as for Wainwright (EPA 1998a, b).

The cleanest site in this study was Big Foot Island (Fig. 1, 35°09'N, 76°00'W), near Ocracoke (Table 1). Mean Σ TU from 1994 to 1997 was <1, indicating that the site is considered "uncontaminated" (Table 1, Hackney *et al.* 1998). There were no toxic releases into surface waters in surrounding counties (Hyde, Carteret, and Pamlico) from 1988 to 1997 (EPA 1998b). The Pamlico Sound is not ranked due to insufficient data (Table 1, EPA 1998a).

Sampling Procedures

Adult terns were trapped from 24 May to 7 June 1999 with a hoop trap at the nest or with a mist net placed near the colony. The ages of 50 recaptured banded adults were determined from banding records. Prefledgling juveniles were herded into 1 m high fence corals for banding and were sampled from 24 June to 1 July 1999. Juveniles from Big Foot Island were captured on 28 July 1999. As a measure of gross nutritional health, the adult birds were weighed and fat reserves were visually estimated by classifying the amount of fat in the interclavicular region (0 = no fat, 1 = small amount of fat, 2 = moderate amount of fat, and 3 = large amount of fat). The terns were placed in a cloth bag and mass determined to the nearest g with a Pesola scale.

Blood was collected from a tern by swabbing a toe with 70% isopropyl alcohol, then pricking the toe next to the nail with a 25-gauge sterile needle and 5 to 40 μ l of blood was collected in heparinized capillary tubes. Blood was transferred to two tubes each containing 1 ml sterile saline buffer (0.9% NaCl) and sodium citrate (3.8%). Blood samples were kept on ice in the field and maintained at 4°C in the laboratory.

Comet Assay

A 20 μ l aliquot from each blood sample was mixed with 30 ml of Hank's Balanced Salt Solution (Gibco BRL) and 50 μ l of 0.5% trypan blue for viability analysis (Lillie 1969). Cell concentration was determined with a hemocytometer. Our procedure for the comet assay was modified from the protocol described by Singh *et al.* (1988). Approximately 200 blood cells were mixed with 75 μ l of 0.5% low-melting-point (37°C) agarose (Fisher Scientific) spread on a microscope slide pre-coated with 1% normal-melting-point agarose (Fisher Scientific),

and covered with a cover slip. Once the agarose had solidified, the cover slip was removed and another layer of 0.5% low-melting-point agarose was added to the top of the slide and covered with a cover slip. After the gel had solidified a second time, the cover slip was removed and the slide was immersed in cold lysing buffer (2.5M NaCl, 100mM EDTA, 10mM Tris, 10% DMSO, 1% Triton X-100, pH 10.0) for one hour at 4°C. The slide was then placed in fresh alkaline DNA unwinding solution (0.3N NaOH, 1mM EDTA, pH > 13.0) for 10 minutes. Gel electrophoresis was performed in the unwinding solution for 10 minutes at 255mA (0.6 V/cm). The slide was neutralized in 0.4M Tris, pH 7.4, dipped in cold 100% ethanol, and allowed to air dry. Triplicate slides were made for each tern. All steps were performed in an unlit room to minimize possible UV damage. A dehumidifier was used to aid in gel polymerization. Each electrophoresis run contained an internal standard of farm-raised quail's blood. The quail blood was collected in the same manner as the tern blood but was flash frozen with ethanol soaked dry ice and maintained at -70°C in 100 µl aliquots until assayed. All blood samples were assayed within four days of collection.

All slides were stained with 300 µl Sybr-green solution (Molecular Probes; excitation 254 nm, emission 520 nm) and analyzed at Integrated Laboratory Systems (Research Triangle Park, North Carolina) with an epifluorescent microscope (Olympus BX60) equipped with an imaging system that utilized Komet 3.0 software (Kinetic Imaging). Fifty cells were analyzed per slide giving a total of 150 cells scored per individual tern. For each cell, the software determined the percentage of DNA present in the head (nucleus) and tail (DNA that had migrated away from the nucleus), the length of the migration of the tail, and tail moment (% of DNA in tail × tail length/100).

To ensure that our storage time did not cause DNA damage, quail blood was assayed on days one through five after collection. To ensure that assay conditions could detect differences in levels of DNA damage, quail blood was heated to 55°C and held at that temperature for 5, 10 and 20 minutes. The damaged cells were compared with undamaged control quail blood cells. The damaged and control blood cells were analyzed with the same assay conditions as the Royal Tern blood.

One-way analysis of variance (ANOVA) with a Tukey-Kramer test ($\alpha = 0.05$) was used to determine statistical significance between sites based on tail moment. The Tukey-Kramer test is similar to the Student's t-test except it allows multiple comparisons and is more conservative (Sall and Lehman 1996). The nonparametric Kruskal-Wallis ANOVA was used when tests for normality were not met by the data. A simple regression ANOVA was calculated to determine degree of correlation between data sets. All statistical analyses were completed with the JMP IN software program (Version 3.2.1, SAS Institute, Inc., 1996).

RESULTS

Health Assessments

Health assessments of adult Royal Terns were completed at Ferry Slip, Sand Bag, Wainwright Islands and Island L. Adult body mass, which ranged from a mean of 476 g

($N = 18$) at Island L to 504 g ($N = 18$) at Sand Bag Island, and did not vary significantly between the study sites (ANOVA, $F_{3,79} = 2.57$, n.s.). The scores for estimating mean fat reserves also did not differ between the sites (Kruskal-Wallis ANOVA, $H_{3,79} = 12.42$, n.s.) and ranged only from 0 (no fat) to 1 (little fat). During annual banding efforts in 1998 and 1999, juveniles were visually inspected for overt developmental deformities; none were reported from any study site.

Comet Assay

There were no significant differences between mean tail moments of the comet assays internal standards (ANOVA, $F_{26,1225} = 1.27$, n.s.). All individual internal standard tail moment means fell within the 95% confidence range of the overall internal standard mean. No difference was detected in levels of DNA damage in quail red blood cells during storage for at least five days after sample collection (ANOVA, $F_{4,374} = 0.42$, n.s.). A clear increase in damage was detected in the heat-exposed quail blood (ANOVA, $F_{3,399} = 886$, $P < 0.0001$).

Mean tail moment values per individual were used to generate the mean tail moment score for study sites. Apparent DNA damage, as measured by tail moment, was significantly higher in adults from the two Core Sound sites, Wainwright and Sand Bag Islands than at Ferry Slip Island and Island L (Fig. 2; ANOVA, $F_{3,81} = 18.6$, $P < 0.0001$). Tail moment did not vary significantly between the Core Sound Sites or between Ferry Slip and Island L.

To ascertain if accumulation of contaminants over time was affecting levels of DNA damage, adult age was plotted against mean tail moment values. The terns ranged in age from 4 to 20 years. No correlation existed between mean tail moment age of all adult terns ($r^2_{48} = 0.005$). Also, no correlation was found between adult age and tail moment within individual study sites. No correlation existed between adult mass and mean tail moment values among individuals from all sites and within sites ($r^2_{78} = 0.032$).

For the juveniles, apparent DNA damage, as determined by mean tail moment,

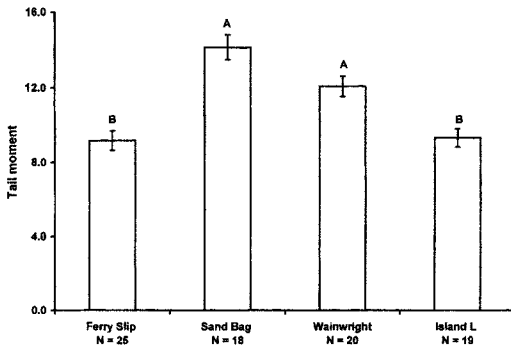


Figure 2. Measurements of tail moment (% DNA in tail \times tail length/100) in adult Royal Terns from four breeding colonies in North Carolina, 1999. Shared letters between bars indicate no significant differences while different letters indicate significant differences (ANOVA, $P < 0.05$).

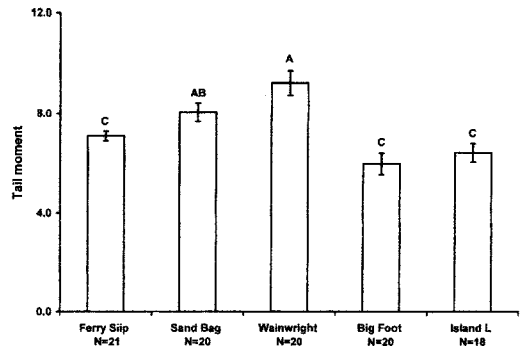


Figure 3. Measurements of tail moment (% DNA in tail \times tail length/100) in juvenile Royal Terns from five breeding colonies in North Carolina, 1999. Letters as in Fig. 2.

was lowest at Big Foot Island (Fig. 3; ANOVA, $F_{4,97} = 11.8$, $P < 0.0001$). Tail moment was highest in juveniles from the two Core Sound sites, Wainwright and Sand Bag Islands (Fig. 3). Tail moment values for juveniles from Big Foot and Island L were significantly smaller than the scores from both Core Sound islands. While the scores from Ferry Slip Island were significantly smaller than those from Wainwright, they did not differ from any other island (Fig. 3). When tail moment values were compared between adult and juvenile Royal Terns from the same site, the adult scores were higher in all cases (ANOVA, $F_{8,180} = 29.7$, $P < 0.0001$).

DISCUSSION

The apparent DNA damage detected in Royal Tern blood most likely was caused by a genotoxic agent and is not an artifact. Internal standard controls indicate that the electrophoresis was consistent between all samples. The assay conditions used in this study were able to detect differences in levels of DNA damage as indicated by heat damaged quail blood cells. In addition, all Royal Tern blood was analyzed within four days of collection; therefore storage conditions did not contribute to any DNA damage detected.

Tail moment did not correlate with increasing age in the adults. It is unlikely that the difference observed between adults and juveniles is due to an accumulating genotox-

in. The differences could be due to diet, as the adults are capable of consuming larger prey. It also is possible that the juvenile terns are protected from the effects of genotoxin exposure. Metabolic rates of juvenile birds are generally higher than adults (Vleck and Bucher 1998). The enhanced metabolic rate in young birds could protect them as they may be able to process toxins faster.

Levels of DNA damage followed the same pattern between sites with greater damage in the adult and juvenile terns from the two Core Sound colonies, Wainwright and Sand Bag Islands, than in all other sites (Figs. 2 and 3). This result was unexpected because these two colonies are ranked as relatively clean of pollutants (Table 1). The tail moment scores did not correlate with overall pollutant exposure or with the concentration of any single contaminant present in sediments surrounding the sites. However, these findings agree with Wickliffe and Bickham (1998), who used flow cytometry to assess DNA damage in red blood cells collected from pelicans residing on Ferry Slip and Wainwright Islands. Contrary to their expectations, they found that DNA damage was significantly higher in the blood cells from the pelicans on Wainwright Island.

Possible Mechanisms for Genotoxic Exposure

There are several likely explanations for our findings. One is that the terns and/or

their prey are not foraging close to the breeding colonies, which could render surrounding sediment contaminant level information ineffectual in predicting possible pollutant responses. However, Buckley and Buckley (1972) found that Royal Terns in North Carolina and Virginia usually forage near their breeding colonies and occasionally 20 to 30 km up tidal rivers. Schwartz (1987) determined that Royal Terns mainly forage up to 22 km from their breeding colonies. Thus, terns in this study are likely to forage near their breeding sites and the genotoxicity results require alternate explanations.

Royal Tern diet consists primarily of fish (Bent 1921; Buckley and Buckley 1972, 1974; Erwin 1977). Both benthic and surface dwelling fish are consumed by Royal Terns (Buckley and Buckley 1972; E. Wambach, pers. comm.). Prey fish are small, ranging in size up to 160 mm in length (Bent 1921; E. Wambach, pers. comm.). Studies have shown that there is a seasonal movement into, an associated residence time within, and then movement out of estuaries by ocean-spawned species in North Carolina (Weinstein *et al.* 1980; Swartz *et al.* 1981; Rozas and Hackney 1983, 1984; Carolina Power and Light 1985). The size and type fish consumed during the sampling period are generally found in shallow marsh areas of the upper to mid portions of the estuaries (Swartz *et al.* 1981; Rozas and Hackney 1983, 1984; Carolina Power and Light 1994). Therefore, it is likely that prey species are feeding in local estuaries. However, fish are mobile and it is possible that the observed genotoxic effects are due to pollutants found farther from the study sites.

Royal Terns are migratory and it is feasible that the genotoxic effects observed are due to pollutants from areas other than North Carolina. However, the juvenile terns in this study hatched in North Carolina and had been exposed to pollutants only from local estuaries. Also, DNA damage did not increase with adult age therefore, it is unlikely that the observed results in the blood are due to genotoxins from other areas.

It is conceivable that abiotic contaminant loads at a site are not indicative of the con-

taminant concentrations found in the local food chain at each site. The bioavailability of the contaminants within the study area is unknown. However, food web studies have shown that abiotic pollutant loads can be transferred to the biota (including piscivorous birds) of that system (Den-Besten *et al.* 1996; Stab *et al.* 1996; Hope *et al.* 1997; Nisbet 1998; Sample and Suter 1999). Also, terns have been used as bioindicators of contaminants in aquatic food chains (Scharenberg 1991; Fox *et al.* 1991). Therefore, it is likely that environmental contaminants are passing through the food chain to the terns. A contaminant screening of the Royal Terns and their prey species could aid in validating this assumption, but was beyond the scope of this investigation.

Another possible explanation for our results is that individual birds from the more contaminated areas may have experienced an adaptive response to pollutant exposure. If true, terns from the polluted sites should have less DNA damage than birds from the cleaner sites. One response of organisms exposed to ionizing radiation is enhanced DNA repair (Protic *et al.* 1989; Wolff 1996; Saves and Masson 1998; Maeda *et al.* 1999). Organisms may enhance metabolic detoxification by turning on various enzymatic pathways such as metallothionein (Kagi and Kojima 1987; Roesijadi 1992) and the P450-dependent monooxygenase enzyme system (McCarthy and Shugart 1990; Huggett *et al.* 1992). Also, because many birds molt at least once a year, sequestering metals in feathers is a method of reducing heavy metal body burdens (Honda *et al.* 1986; Braune 1987; Walsh 1990; Burger *et al.* 1994).

Another form of adaptive response to pollutant exposure relies on the fixation of genetically based traits at the population level, which alters genotypic diversity (Anderson *et al.* 1994; Anderson and Wild 1994; Bickham and Smolen 1994; Guttman 1994; Shugart 1995; Shugart and Theodorakis 1996; Bickham *et al.* 2000). Laboratory toxicity studies have indicated that chemically induced mortality, fecundity, and amount of DNA damage were dependent on genotype, which suggests that selection for certain alle-

les during pollutant exposure can occur (Nevo *et al.* 1983; Gillespie and Guttman 1989; Changon and Guttman 1989; Bickham and Smolen 1994; Theodorakis *et al.* 1998, 1999; Bickham *et al.* 2000). For a similar process to occur in a long-lived species like terns would require high selective pressure, minimal gene flow between populations, and consistent pollutant levels at the sites over many years. The developmental deformities observed on Ferry Slip Island may indicate that selective pressures at that site could have been high. Analysis of bird banding information from recaptured adult Royal Terns in our study revealed that regional philopatry could be as high as 80% with the percentage returning to the Cape Fear River area to breed possibly even higher at 94%. Thus, it is reasonable to postulate that Royal Terns from the more polluted sites may have had an adaptive response to contaminant exposure at the population level. Genetic analyses would be required to assess the validity of this hypothesis.

The last, and most likely, explanation for the results described here is that the birds from the Core Sound colonies are exposed to an undetected contaminant(s). The type and amount of pesticides present at each study site is unknown. Also, the terns have not been analyzed for the presence of genotoxins. The apparent DNA damage found in the Core Sound terns has the potential to have profound adverse biological effects on individual birds as well as the population as a whole. Further study and monitoring of tern colonies are needed to understand the potential health risks of pollutants in North Carolina estuaries and coastal ecosystems.

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