CONTAMINANT EXPOSURE AND SUBLETHAL EFFECTS IN A PISCIVOROUS SEABIRD

by

GABRIELLE LEIGH ROBINSON

(Under the Direction of Sonia Hernandez)

ABSTRACT

We measured mercury and polychlorinated biphenyls (PCBs) in least terns (*Sternula antillarum*) across coastal Georgia to determine concentrations and sublethal effects. We are the first to document the transport of Aroclor 1268, a PCB mixture that was released exclusively at the Linden Chemical Plant (LCP) Superfund site in Brunswick, GA, at least 70 km south and 110 km north of its point source. Mean mercury in chick feathers and sediment, as well as egg Aroclor 1268 concentrations, were highest at sites closest to LCP. Mercury exposure was associated with biomarkers of both developmental instability and immunosuppression. Primary feather and wing chord symmetry in chicks both decreased with increasing primary feather mercury concentration. Heterophil/lymphocyte ratios in chick blood decreased with increasing fecal mercury. Increased prevalence (2.9%, *n* =7) of teratogenic deformities in the Savannah River least tern colony was also associated with elevated mercury. We also found that predator management substantially increased least tern nest success.

INDEX WORDS: Least tern, Aroclor 1268, Mercury, PCBs, Sublethal effects, Fluctuating asymmetry, Teratogenic deformities, Heterophil-lymphocyte ratios, Predator management, Predation

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

This M.S. thesis is in manuscript form. The present chapter (Chapter 1) consists of a comprehensive literature review on the topics included in this thesis research. Chapters 2-4 take manuscript form, each to be separately submitted to peer-reviewed academic journals, and are structured with the typical manuscript sections: ‘introduction,’ ‘methods,’ ‘results,’ ‘discussion,’ and ‘conclusion.’ Because chapters 2-4 are in manuscript form, the figures and tables used for each manuscript are bundled at the end of each chapter/appendix, but references for the entire document are combined in the bibliography at the end of the document. Chapter 2 covers the general distribution of mercury and polychlorinated biphenyls (PCBs) in least tern samples across the Georgia coast. Chapter 3 describes the sublethal health and reproductive effects associated with mercury and PCBs. The ‘results’ and ‘discussion’ sections in chapter 3 are combined to increase the ease of readability, and to minimize redundancy that would result from separating results from their discussion in this chapter. Chapter 4 reports how predator management significantly increased nest success at least tern colonies in Georgia. Chapter 5 is the conclusion, which briefly restates the major findings of this study.

Least Terns

Introduction

The least tern (*Sternula antillarum*) is a ground-nesting, colonial waterbird species that breeds along coastal and riverine shores of the United States. Least terns are a relatively long-lived species, with banded individuals of up to 24 years of age having been recovered[1]. Nests are shallow scrapes in the sand or gravel, and clutch size ranges from one to three eggs. Least terns may have multiple nesting attempts throughout the breeding season (April through August) as nests fail or chicks are lost, but double-brooding does not occur[2].

Diet

Least terns feed by plunging into the surface of shallow (< 1-4 m depth) water to capture prey swimming near the surface[3]. Over fifty species of fish have been documented as prey items of least terns[4], but based on several studies done on fish dropped by adults in breeding colonies it is thought that least terns will take any small (between two and nine cm in length), shallow-bodied (no more than 1.5 cm. in body depth), non-spiny fish, that swims near the surface (upper 15 cm of water column[2]) in shallow waters[3,5-7]. The diet of the Atlantic coast population varies by geographic location, and includes sand lance (*Ammodytes* spp.), herring (*Clupea* spp.), and hake (*Urophycis* spp.), in New England; anchovy (*Engraulis eurystole*), menhaden (*Brevoortia tyrannus*), mummichog (*Fundulus heteroclitus*), crustaceans, and silversides (*Menidia* spp.), in the mid-Atlantic and southeastern U.S.; and Gulf menhaden (*Brevoortia patronus*) and bay anchovy (*Anchoa mitchilli*) on the Gulf coast[8]. Although least terns feed primarily on small fish, they may also take shrimp and occasionally other aquatic invertebrates, such as swimming insects and marine worms[2].

Foraging Distance

Foraging habitat of least terns in marine habitats consists primarily of creek and river mouths, tidal marshes, estuaries, bays, lagoons, or lakes, but rarely they may feed offshore (especially *S. a. browni*)[2]. Forage habitat varies more widely with the interior population and includes fields, marshes, reservoirs, gravel or sand pits, rivers, ponds, sloughs, or streams[2]. Documented foraging distance by breeding adults from the nesting colony vary by subspecies, but in general least terns have been shown to travel no further than 3-12 km from their breeding colonies to forage[5,9]. The interior population (*S. a. athalassos*) generally forage within 100-300 m of the nesting colony in riverine habitats, but rarely have been shown to forage up to 4.5 km from the nesting colony in the Platte and Missouri River systems[7,10]. However, *S. a. anthalassos* in Oklahoma were shown to travel up to 12 km from the breeding colony to reach suitable foraging habitat[11]. In Georgia specifically, least terns were shown to forage no more than 4.9 km from the breeding colony[12].

Habitat

Historically, least terns have preferred to breed primarily on wide stretches of beach remote to human disturbance, with sandy or gravelly substrate and sparse to no vegetation[13]. Typically colonies occurred on mainland or barrier island beaches. Least tern habitat sites in Georgia were characterized by little vegetation with lower plant height, more wrack cover, higher elevation, and a wide berm[14]. However, as ideal habitat became increasingly developed throughout the 20th century, least terns began using poorer-quality natural habitat, as well as man-made sites. Although least terns prefer areas remote to human activity, they will often nest in areas that are suitable, except for the presence of off-road vehicle traffic, when no other suitable habitat exists[13]

Conservation

Three subspecies of least terns exist in the U.S., two of which are federally endangered. The eastern subspecies (*S. a. antillarum*) breeds on shores from southern Maine to southern Florida, and along the Gulf of Mexico coast. The eastern subspecies is not federally-listed, but is state-listed as: rare (proposed for state protection) in Georgia and North Carolina; special-concern in Massachusetts and Virginia; threatened in Florida, South Carolina, Connecticut, Maryland, New Hampshire, and Rhode Island; endangered in Delaware, Maine, New York, and New Jersey[15].

The federally endangered interior subspecies (*S. a. athalassos*) breeds primarily along the Platte, Ohio, Missouri, and Mississippi Rivers and their tributaries, but also along several other rivers, lakes, and reservoirs in the interior U.S. The endangered California subspecies (*S. a. browni*) breeds from the San Francisco Bay south to the Baja peninsula. Breeding also occurs locally along the Baja peninsula and Pacific coast in Mexico, and locally on islands in Central and South America, and the Caribbean. All subspecies migrate and over-winter in Central and South America, but little is known of specific winter locations and habits[2].

Least terns were nearly extirpated in the late 1800s mostly due to feather collection for women's hats, but populations slowly began increasing again after the 1918 Migratory Bird Treaty Act was passed, disallowing the sale, possession, or take of any migratory bird species, including the least tern[2]. However, since the mid-twentieth century many populations appear to be in overall decline owing to a variety of other conservation threats including human disturbance, habitat loss, breeding colony flooding due to dam release in the interior, increased predation pressure, and potentially climate change and associated sea level rise[2,16]. Because least terns are relatively long-lived, there is often a lag in time between the events resulting in poor productivity and the observed effects in the population, and therefore assessing productivity is an essential component of population monitoring for this species[2].

Human disturbance

Human disturbance at least tern colonies is a significant factor affecting both productivity and colony site selection[17-19]. When coastal development increases, as does off-road vehicular traffic, pedestrian traffic, and human recreational activity in close proximity to nesting colonies. Many authors have shown that human disturbance has negative impacts on colonial nesting waterbirds of various species including least terns[18-23]. The tendency of many eastern least tern colonies, especially in the northeast, to breed on mainland beaches as opposed to island/offshore beaches, certainly increases their vulnerability to human disturbance[13]. Disturbance can not only decrease productivity when nests are abandoned, chicks killed, or eggs addled by pedestrian and vehicle traffic, but disturbance can also factor into habitat selection, parental behavior, and nest predation[18,19,21,23,24]. Minimizing human disturbance is a common management tool used for beach-nesting shorebirds of concern, including least terns, and typically includes symbolic fencing and posting of breeding areas, steel cables to prevent vehicle access, and beach closures where necessary during the breeding season[2].

Habitat loss and manmade nesting sites

The loss of breeding habitat has been an increasing threat to least tern populations across their range since the mid-1900s. Much coastal habitat has been lost to development, or otherwise rendered useless as breeding habitat due to frequent human disturbance, as well as vehicular and pedestrian traffic. As a result, manmade dredge spoil islands[25,26] and flat gravel rooftops of large buildings[26-28] have been used by least terns (especially *S. a. antillarum*) for breeding in coastal areas more and more frequently in the past several decades. Although manmade environments can provide least terns with refugia from the human disturbance and tidal flooding often experienced by beach-nesting colonies, manmade habitats can also pose unique threats to survival and productivity. On rooftops, extreme temperatures (both high and low) threaten both egg and chick survival, while eggs and chicks are also at risk of rolling or falling off precipices at the edge of rooftops that lack parapets[26]. On dredge spoil islands, predation may dramatically increase due to inflated predator populations that tend to thrive in areas close to human settlement, including those of feral hogs, feral dogs and cats, grackle spp. and corvid spp., coyotes, skunks, foxes, and raccoons[26]. However, as coastal development persists, once-ideal nesting habitat will continue to be lost or degraded. Consequently, evaluating recruitment and productivity on manmade substrates will become increasingly important when considering incorporating manmade colony sites into conservation and management plans.

Another conservation threat unique to the interior population (*S. a. athalassos*) is associated with surface water management practices. The interior population historically bred on the frequently scoured sandbars of rivers and their tributaries. However, as water diversion and reservoir-construction for irrigation increased, water flow and natural annual flooding decreased, causing riparian sandbars to become overgrown with vegetation rendering them unsuitable as least tern breeding habitat[29]. More recently, sand pit lakes accompanied by bare, sandy, spoil mounds created by sand and gravel mining along river floodplains are now being used as breeding habitat by interior least terns[30]. These, as well as other manmade islands in interior rivers, are being intensively managed for least tern and other endangered shorebird conservation[31]. However, untimely flooding due to the controlled release of water from reservoirs along dammed rivers has been responsible for the flooding of riverine colonies and great losses in productivity[32].

Food-source contamination

Contamination in food sources of terns and other avian predators has been and will continue to be an area of concern as long as harmful elements and chemicals are introduced into, or are persistent in, the environment. Contaminant concentrations are often amplified top level consumers due to trophic biomagnification, and thus least terns may be at greater risk of exposure to contaminants and their harmful effects[33-36].

The American Ornithologists' Union identified the effects of contaminants on productivity as a research priority for least terns[2]. Not only is *S. a. antillarum* in decline and state-listed in many states across its range, but both the California (*S. a. browni*) and the interior (*S. a. anthalassos*) least tern subspecies are federally endangered[2]. Therefore, understanding and quantifying the effects of persistent contaminants on the health and productivity of least terns is paramount in order to bolster conservation efforts nationwide. This information is critical for making effective management decisions, especially where least terns are nesting on manmade structures, such as dredge-spoil islands and rooftops[26], in contaminated areas. For example, manmade islands on the Platte River in Nebraska are constructed and managed specifically to encourage least tern colonization, while most of the Platte River floodplain is heavily-developed for agriculture[30], making this habitat susceptible to pesticide contamination from agricultural run-off[37]. If managers for terns and other wetland birds had data showing decreased productivity due to contamination of breeding sites, alternative suitable habitat could be deliberately created *away* from contaminated areas, or at least not deliberately created or managed *in* contaminated areas[38]. For this reason, along with the least tern's conservation status in the U.S. and its potential utility as a bioindicator of ecosystem health in aquatic systems where it occurs, the need to investigate the role of contaminants in least tern health and productivity remains.

Predation

A wide array of both avian and mammalian species have been identified as least tern nest or chick predators. These predators include, but are not limited to, crows and ravens (*Corvus* spp.), gulls (*Larus* spp.), black skimmer (*Rynchops niger)*, gull-billed tern (*Gelochelidon nilotica*), boat-tailed grackle (*Quiscalus major)*, great blue heron (*Ardea herodias*), black-crowned night heron (*Nycticorax nycticorax*), sanderling (*Calidris alba*), ruddy turnstone (*Arenaria interpres*), loggerhead shrike (*Lanius ludovicianus*), great horned owl (*Bubo virginianus*), peregrine falcon (*Falco peregrinus*), northern harrier (*Circus cyaneus*), American kestrel (*Falco sparverius*), coyote (*Canis latrans*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), opossum (*Didelphis virginiana*), nine-banded armadillo (*Dasypus novemcinctus*), European rabbit (*Oryctolagus cuniculus*), brown rats (*Rattus norvegicus*), mink (*Neovison vison*), red fox (*Vulpes vulpes*), ghost crab (*Ocypode quadrata*), catfish (*Ictalurus* spp.), feral dogs (*Canis lupus familiaris)*, cats (*Felis catus*), and hogs (*Sus scrofa*)[2,39-45]. Fish crows (*Corvus ossifragus)* and raccoons were identified by several authors as significant nest predators of least terns in Georgia[12,14,26,46]. American crows were identified as nest predators at a least tern colony in Boston Harbor using remote-sensing nest cameras and artificial nests. These researchers noted that eggshell fragments were usually found in association with crow nest predation, but not other documented predators (brown rats and European rabbits), and this observation could help identify nest predators in the absence of tracks or other mammalian sign[45]. Ring-billed gulls have been shown to be significant predators of both least tern eggs and chicks[41]. Bobcats (*Lynx rufus)* are a known nest predator of American Oystercatchers[47], and although not previously documented as a least tern nest predator, were responsible for maiming and killing at least three least tern chicks in the present study.

Urbanization and human population growth contributes to least tern habitat loss, but can also contribute to increased predation pressure, especially by predator species that have adapted to exploit resources available in areas of human development[48]. Predators such as coyotes, raccoons, opossums, foxes, feral dogs and cats, gulls, grackles, and crows, are often commensal with humans, being "subsidized" by anthropogenic food sources. Subsidized species tend to be generalists or opportunists, and will exploit a variety of resources provided by the donor (in this case, humans), which often leads to an increase in reproduction of the recipient species[49,50]. These subsidized populations are often greatly inflated, especially in developed areas, where there is not only an abundance of food, but also an absence of natural predators. Nest predation is expected to increase after an introduction (especially on an island) of a non-native nest predator species, or following habitat modification that is favorable to a generalist nest predator species[51].

Corvids tend to concentrate around predictable food sources[51], and this could increase predation pressure to least tern colonies that occur in close proximity. Potential predictable food sources that may attract crows to tern colonies include human landfills, garbage dumpsters, crowded beaches, or the shorebird colonies themselves. Corvids have proven a significant shorebird nest and chick predator[47,52-54].

Predator management

Introduction:

An efficient nest predator can nearly obliterate an entire least tern breeding colony in a single night, especially in the case of terrestrial predators[2]. Repeated disturbance by predators may also cause colony site abandonment[2]. Because subsidized predator populations are unnaturally inflated in many shorebird breeding habitats, and because predation can be a significant (and indeed often the dominant) determinant of reproductive success in colonial nesting shorebirds, managing predators is becoming an increasingly valuable conservation tool for ground-nesting shorebird species[54]. Predator management is accomplished by two fundamental means: one involves preventing predator access, while the other involves physically removing predators from the vicinity of the shorebird nesting colony. Both means of predation management have pros and cons. Attempts to exclude predators from a nesting colony are not always successful, as predators may learn to infiltrate exclusion barriers. The benefits of predator exclusion are that 1) it is often less labor-intensive and less costly, and 2) it is non-lethal, making it easier to accomplish with minimal permitting or concern for public opinion. Predator removal tends to be more labor-intensive and costly[55], and is often done by lethal means, thereby requiring the proper permits as well as close regard to public safety as well as the potential for public protest[54]. However, predator removal can be much more effective than predator exclusion, especially when the majority of the predation is being carried out by one or just a few individuals from the predator populations.

Nest exclosures:

Means of predator exclusion for ground-nesting shorebirds generally include either 1) small nest exclosures erected right at an individual nest site which provide protection from most terrestrial and sometimes even avian predators[54,56], or 2) long fences spanning the perimeter of an entire nesting area that exclude only terrestrial predators[57]. Nest exclosures are most often used with non-colonial nesting species (such as plovers), who use ambulation rather than flight as a primary means of locomotion to access their nest site, allowing them to run along the ground through the exclosure when in transit to and from the nest. Chicks of these species tend to be superprecocial, leaving the nest site within hours of hatching, so that nest exclosures provide protection only to eggs but not to the flightless chicks once they're hatched. The other type of predator exclosure is a long barricade (typically a fence) that is erected around the entire breeding area, usually of a colonially-nesting species (e.g., tern spp.). The use of electric fences, in particular, have been shown more and more recently as an effective means to exclude most terrestrial predators[25,44,58-61]. Electric fences have also been successfully used to protect individual nests of non-colonial species, in particular piping plovers (*Charadrius melodus*) in Massachusetts[53,62]. While perimeter fences do not exclude avian predators like nest exclosures do, they will usually provide protection from terrestrial predators for a significant proportion of the colony's flightless chicks. This is because the flightless chicks of colonial-nesting species (e.g., terns, skimmers) are much less mobile before fledging than the offspring of superprecocial non-colonial shorebird species (e.g., plovers, oystercatchers), and tend to remain within the colony (and therefore within the fence exclosure) until fledging.

Corvid Control:

Attempted management for crow predation on threatened or endangered shorebird species includes both lethal and non-lethal methods. Some non-lethal methods include the use of scarecrows and other scare tactics or hazing techniques, such as "bird bombs" or other types of repellents, which have limited efficacy and can also negatively impact the prey species of interest and therefore are not always ideal[63]. Crow effigies have been successfully employed to disperse crows from roosting areas[64], but only limited documentation exists that crow effigies can also be effective in minimizing predation at shorebird colonies, and this has only been observed but not quantified[52,63]. Perhaps the most common non-lethal method of corvid predation control (which also has had limited success) is the use of taste-aversion, usually by means of lacing bait eggs with methiocarb or carbamylcholine chloride, which are ill-inducing taste deterrents[63,65,66].

Lethal methods of corvid control are generally found to be more effective and have longer-lasting effects. By law, corvids and other "black birds" are considered pest species and there is a standing depredation order in place that allows take of these species which would normally be prohibited (or require special federal permit granted for exceptional circumstances only) by the Migratory Bird Act. Code of Federal Regulations, Title 50: Wildlife and Fisheries, Part 21.43 (50 CFR 21.43) states that federal permit is not required for take of yellow-headed blackbirds, red-winged blackbirds, rusty blackbirds, cowbirds, all grackles, crows, and magpies, when these species are found depredating plants or animals (including wildlife) of human interest. Therefore, lethal means of corvid control near shorebird nesting areas have been employed, often with reasonable success. The two most common and effective methods of lethal corvid control are sharp-shooting and poisoning with toxicant-laden bait[63]. Sharp-shooting is the removal of target individuals by use of firearms, and has proven one of the most effective means of removing problem corvids preying on shorebird nests or chicks[53,62,63]. The most commonly-used toxicant used in poisoning is 3-chloro-4-methylbenzenamine HCL (DRC-1339). As with taste aversion chemicals, DRC-1339 is applied to bait eggs (usually quail or chicken eggs) in areas where corvids are feeding on shorebird eggs. DRC-1339 is acutely toxic in starlings and corvids, generally causing renal failure in 1-2 days, but was found essentially harmless to house sparrows and all raptors that were tested[63]. This method is designed to single out not only individual species, but individuals within a species that are habitually targeting shorebird nests.

Persistent environmental pollutants

Persistent environmental pollutants (POPs) are organic compounds that persist in the environment due their resistance to environmental processes (e.g., photodegradation) and to biodegradation, and have a strong tendency to bioaccumulate and biomagnify in ecosystems. The vast majority of POPs are anthropogenic in source. POPs may be released into the environment as industrial waste during production, or by use of a product that contains them (e.g., pesticides), POPs are an environmental and public health risk due their toxicity, longevity, potential for long-distance transport, and the range of sublethal health effects they have on living organisms, including humans[67].

Polychlorinated biphenyls

Polychlorniated biphenyls (PCBs) are one type of organochlorine POP that consist of two benzene rings attached by a single carbon (C) bond, that have at least one or more chlorine (Cl) atom attached to various locations around each benzene ring ($C_{12}H_{10-n}Cl_n$). The arrangement of the Cl atoms around each benzene ring gives the specific PCB congener its name (e.g., “2, 2’, 4, 4’ tetrachlorobiphenyl” has Cl atoms attached to the second and fourth carbon atoms on each benzene ring)[68]. PCBs were mass-produced and used throughout the world, especially in industrially-developed nations, with an estimated 1.5 billion tons produced in the U.S. alone from 1930-1975[69]. In America, the trade name for PCBs was “Aroclor,” along with a number identifying the number of carbon atoms and percent weight of chlorine in each particular mixture (e.g., Aroclor 1254 has 12 carbon atoms and is 54% chlorine by weight)[69]. Owing to their chemical properties that include extremely low water solubility and vapor pressure, a low dielectric constant, and high thermal stability, PCBs were very useful for a wide variety of industrial processes including (but not limited to) use as industrial lubricants, insulators, and coolants, hydraulic fluids, flame retardants, and dielectric fluids in transformers and capacitors. Their use in plasticizers attributed to a different variety of applications that include use in adhesives, sealants, caulking, wood finish, stabilizing additives to the coatings of electrical components, pesticide extenders, surgical implants, water-proofing compounds, and carbonless copy paper, to name just a few[69]. The chemical properties that make PCBs ideal for industrial use, such as their general inertness and very low water solubility, are the same properties that make them extremely persistent in the environment with a high bioaccumulation factor, or tendency to bioaccumulate[69]. Evidence of the persistence of PCBs in the environment began coming to light in the early 1970s, as did the concern for the affect of PCBs on public health, with several historic incidents of mass PCB poisoning to people occurring in Asia, such the Yusho incident and Yu-cheng disease[69]. Chronic effects to humans also became apparent in the early 1970s, leading the sole manufacturer of PCBs in the U.S., Monsanto, to voluntarily ban all open-ended and nominally-closed uses of PCBs[69]. The final ban on PCBs implemented in 1979, under the EPA's Toxic Substances Control Act, disallowed all manufacture, processing, commerce, and distribution of PCBs. Use of PCBs was also prohibited with this ban, except in cases of completely-closed systems such as electrical transformers, which have since been phased out completely[69].

Today PCBs are truly ubiquitous, continuously being found in plants and animals worldwide, largely due to their persistence and mobility in the environment. Their resistance to degradation and lipophilic nature allow PCBs to readily move up the food chain, and ultimately be transported worldwide by environmental processes and movement of contaminated biota[69]. Not only do PCBs degrade slowly in the environment, but depuration in biota is very slow, which contributes to a bioaccumulation factor ranging from 10x to 100x increases in the food web[69]. In the animal body, PCBs partition most strongly to fat, and are generally found in the highest concentrations in adipose tissue. Also noteworthy is that with increasing chlorination, PCBs (e.g. Aroclor 1268) become the more lipophilic (with lower water solubility and higher lipid-partitioning coefficient), resulting in lower excretion rates[69]. The position of the chlorines on the PCB congener also affects its retention in the body depending on the species of animal. PCB toxicity depends not only on the specific congener structure, but also the presence of impurities, which are often difficult to quantify after their manufacture and release into the environment[69].

Mercury

Anthropogenic release of mercury into the environment has been occurring for nearly 3,000 years. Cinnabar ore (consisting of sulfur and mercury) was used to make red paint and mined by the Phoenicians as early as 700 BC, and Roman slaves in Spain mined over 500 million pounds of mercury from the Almaden mine for over 2,500 years[69]. Then, during the California gold rush of the mid 1800s, mercury was used to separate gold from the miners' ore, and subsequently released into the California waterways. In the Sierra Nevadas alone, an estimated 50+ million pounds of mercury were expelled into waterways by prospectors[69]. More recently mercury contamination came from use of mercury by farmers in fungicide on seeds, use in production of felt for the felt hat industry, release from chlor-alkali plants and pulp paper mills, and combustion of fossil fuels, especially at coal-burning power plants[69]. Most historical uses of mercury have been banned or restricted at least in the westernized world, but residual mercury concentrations from these antiquated processes still exist in various environmental compartments, and influxes of mercury contamination in areas can occur due to acid rain and flooding of reservoirs that contain contaminated sediments. Furthermore, the combustion of fossil fuels, especially from coal-fired power plants is still a major source of mercury pollution[69].

Inorganic mercury is not generally harmful to biota, but methylation of inorganic mercury into methylmercury occurs naturally in the environment by bacterial metabolism, whereby inorganic mercury becomes bound to a methyl group and becomes a cation[69]. The methylmercury cation then has an affinity to bind to an anion, and tends to bind to hydroxide (OH^-), chloride (Cl^-), nitrate (NO_3^-), and sulfhydryl groups (SH^-). Because SH^- are a main functional group of cysteine amino acids, which make up animal proteins, methylmercury becomes bioavailable by binding to proteins, and will bioaccumulate in animals partitioning especially to muscle and other proteinaceous tissues[70]. This process can be facilitated by acidic conditions or by influxes of organic matter, occasionally resulting in background concentrations of mercury in sediments, alongside elevated concentrations in biota[69]. The environmental fate of mercury owes largely to its fast sorption to sediments, where it is strongly fixed. In aquatic environments, mercury sorbed to soil particles may be eventually volatilized, precipitated, leeched, or metabolized by bacteria into methylmercury and taken up by plants or benthic fauna. Methylmercury can be easily absorbed by many aquatic organisms, and once introduced into aquatic food chains bioaccumulates readily, partitioning mostly to muscle and liver tissue[69]. In terrestrial environments however, mercury fixes strongly to soil, has minimal absorption by plants, and the roots-to-shoots barrier for mercury in most plants limits mercury transfer from plant to terrestrial herbivore[69]. However, terrestrial species can still have high exposure rates to mercury from aquatic systems, as is the case with fish-eating birds[71].

A long history of mercury release into the environment, persistence in the environment, and atmospheric transport has made mercury a worldwide, ubiquitous, contaminant[69]. Highest concentrations of mercury in biota have been found in marine mammals and piscivorous birds. Pelagic bird species (e.g. albatross, shearwaters, storm petrels) however, often demonstrate elevated concentrations of mercury thought to be a product of natural (non-anthropogenic) mercury contamination rather than anthropogenic mercury pollution, when compared to terrestrial or near-shore piscivorous species that spend most of their time in closer proximity to anthropogenic sources[69].

Effects of contaminants on birds

The effects of organochlorine and heavy metal exposure in birds have been documented, and include physical and physiological abnormalities in offspring, high nestling mortality, lowered hatch success due to embryo death and decreased fertility of eggs, decreased egg production, decreased growth rate, reduced egg volume and weight, thinning of eggshells, abnormal development of sex organs, immune system disorders, and abnormal incubating behavior[71-76]. While birds tend to be more resistant than to acute toxicity of PCBs than are mammals, they are often more likely to experience increased exposure, especially in the case of piscivorous birds (like seabirds) or communal scavengers (like crows)[69]. Most quantitative studies on the effects of different rates of PCB or mercury exposure on acute or chronic toxicity have taken place in laboratories, and the vast majority with gallinaceous species (which are not representative of most birds). Furthermore, much laboratory work has been done with chickens (*Gallus gallus*), though chickens are more sensitive to PCBs than other avian species[77]. Laboratory mercury or PCB feeding trials with other species have shown reduction in egg production and decreased egg fertility, and early stage embryo death, with chronic PCB exposure in quail, pheasant, and dove species[69]. Embryo death from exposure to 10 ppm Aroclor 1254 in ring-necked doves was found to increase with parental incubation, likely because PCB exposure negatively impacted parental attentiveness at nests[78]. Mercury feeding trials in mallard ducks resulted in reduced clutch size, an increase in embryo and duckling mortality, and hypersensitive avoidance behavior in ducklings[79-81]. Feeding trials have been conducted to show that both PCB and mercury exposure caused reduced growth rate, as well as increased liver-to-body-weight ratio (from PCBs)[82] and kidney lesions (from mercury)[83]. Contrasting studies with mallards, Atlantic puffins, and screech owls showed no reproductive impacts from certain PCB and mercury feeding trials, and the degree of sensitivity appears to be species- and congener-specific, as well as dependent on dosage and the form of the contaminant at time of exposure[69].

A long-term study published in 2011 of wild-caught captive white ibis dosed with methylmercury showed a decrease in productivity and an increase in homosexual behavior in dosed males[84]. Dosed males had a decreased rate of courtship and aggressive behavior, were approached less often by females, and spent more of the breeding season paired with other males than did control males. There were fewer productive nests from both male and female dosed birds (due to decreased egg production and unsuccessful male-male pair bonds), and fewer nestlings were produced by dosed males and females. While there was no overall significant difference in reproductive output, the authors speculated that decreased reproductive output would be more likely in wild populations where fewer nesting attempts are feasible than in a captive setting[84].

Impacts of contaminant exposure on avian reproduction have been clearly demonstrated in the laboratory. However, the extent to which contaminants are correlated with decreased productivity in wild populations, or responsible for population decline, is not well known because few studies attempt to relate reproductive success with contaminant levels. The most extensive series of investigations on this in the U.S. have been in the Great Lakes region where reproductive success of various waterbird species was significantly lower in areas known to be polluted than in relatively unpolluted areas[85,86]. However, these studies did not directly correlate overall reproductive success with contaminant concentrations or any other health or reproductive parameters.

Seabirds as indicators of environmental contamination

Introduction

The introduction of certain persistent contaminants into the environment has residual effects on ecosystems that last much longer than does the original source of pollution[73]. Additionally, many persisting contaminants have the tendency to bioaccumulate in higher trophic levels, thus resulting in the highest contaminant concentrations in top-tier predators, like piscivorous birds in a coastal ecosystem[87]. To make well-informed management decisions, we must quantify both the bioavailability of contaminants over time, and how the contaminants affect the health of the organisms in a system. However, the information gained about the relationship between contaminants and species in a specific system can also be used as a tool to detect and monitor pollution and its effects on ecosystem on a broader scale, as well as in other similar systems, thereby addressing ecological and public health in a more broadly applicable way[88]. This forms the basis for selecting certain organisms as indicator species to be used as an environmental management tool for assessing the health of ecosystems in a more timely and cost-effective way.

Colonial-nesting waterbirds could be ideal bioindicators (or beacons of environmental degradation) for a number of reasons, especially with regard to contamination[88,89]. The relative longevity and position at the top of the local food web of many colonial seabird species both facilitates the accumulation of detectable levels of contaminants in their bodies, as well as makes them heralds of contamination occurring in organisms at lower trophic levels[90]. Colonial seabird mobility and use of areas of high human impact afford them exposure to a variety of pollutants, and they often have extensive geographic ranges that allow for comparison between sites[88]. The colonial nesting strategy makes it easier for investigators to make observations and collect samples from a large number of individuals and their offspring with minimal effort[88]. Lastly, most species are relatively well-known, conspicuous, recognizable, and spark public interest, making colonial waterbirds ideal candidates for bioindicators[88]. Some useful organism-level and suborganismal traits (hereinafter "biomarkers") that have been used to signal individual or population stress in waterbirds are described here.

Eggs

Egg-shell thinning due to organochlorine pesticides like dichloro-diphenyl-trichloroethane (DDT) has caused widespread and detrimental impacts to bird populations in the past[73]. Additionally, eggshell thinning in correlation with PCB exposure has been documented in many species of waterbirds[76,91,92] However, organochlorines (including PCBs) can also affect egg volume and composition, which may in turn affect the fitness and survival of the offspring[93]. Length and maximum breadth can be measured on viable waterbird eggs in the field (without collection) to estimate volume and fresh weight[94]. Eggshell thickness can easily be measured from collected eggs using a high-precision micrometer[95].

PCB and mercury burden can be analyzed from collected eggs[33,35,90,91,96-100]. Egg production is a major outlet for contaminant deposition in birds. A female bird deposits approximately 40% of her total body burden of mercury into an egg one week before it is laid, making eggs a good proxy for contamination present in adult birds when sampling of adult birds is logistically or legally problematic or impossible[101,102]. However, mercury concentration found in chicks (with the exception of newly-hatched chicks) is attributed primarily to contaminated food ingestion, and contribution from the parent (egg) is negligible[103,104]. This is primarily due to the growth dilution effect, whereby the contaminant load inherited by the chick from the parent contributes less and less to the total body burden of the chick, and consequently food ingestion by the chick becomes the primary source of contaminants. Contaminant concentrations in the developing chick reflect the net balance in the rate of accumulation from diet via ingestion of contaminated food, and dilution by the rapid increase in body mass during the nestling period[105,106]. Contaminant burdens in chicks are highest at hatching and fledging, due to the growth dilution effect of the middle nestling period of rapid growth[107].

Feathers and feces

Feces and feathers can reveal Hg burden in birds[87,108]. Hg is ingested by adult birds through the diet and accumulates in tissues between molts until it is mobilized into the bloodstream and sequestered in feathers, via binding to keratin, during feather production[87]. Feather deposition is a primary elimination pathway for accumulated mercury body burden in birds[109]. Once bound to the feather matrix, Hg is very resistant to leaching[71,87] and external contamination[110]. Hg found in feathers is almost entirely in the form of methylmercury (the form present in body tissue), and methylmercury load in feathers is comparable to that found in body tissue[109,110]. For this reason, feathers have been well-established as a means for measuring Hg burden in birds[100,106,111-113]. PCBs can also be detected in feathers and other keratinous structures such as mammalian hair[114,115].

Studies have shown concentrations of PCBs found in feathers of various bird species to be highly correlative with those found in internal tissues[116,117].

Mercury is also eliminated in the feces, and mercury concentrations in the feces is a better reflection of mercury currently being ingested, rather than that of accumulated mercury[87,108]. Fecal and feather samples from colonial waterbird chicks are particularly useful in revealing local contamination because the uptake of contaminants (via ingested fish) is restricted to localized feeding grounds used during that given breeding season, and body burden in older chicks almost entirely excludes contamination acquired from the mother, who may have been exposed to contaminants in other locations[106,112].

Immune function

Immunosuppression and other immune system disorders have been associated with environmental contaminants in birds and there are various immunotoxicological techniques used to assess the impacts of contaminants on the health of birds[75]. T-lyphocyte function can be a useful indicator of contaminant exposure. For example, strong exposure-response associations were found between PCBs and T-cell mediated immunity in Caspian Terns (*Hydroprogne caspia*)[118], and chicken embryos injected with PCBs showed reduced lymphocyte counts[119]. Another immunotoxicological parameter is hemoparasite load, which is negatively correlated with immune function, and can cause sublethal effects in birds[120]. Collecting blood samples for white blood cell estimates can therefore be a non-lethal way to assess immunosuppression in relation to contaminant exposure in wild populations of waterbirds.

Reproductive success and nestling development

Monitoring the prevalence of teratogenic (congenital) deformities in offspring and quantifying colony reproductive performance are two simple ways to assess the impact of contaminants in seabirds. This type of biomarker data may be especially appealing due to the relative ease with which this information can be determined in a colonial species, particularly in one that is already being monitored[88]. These biomarkers may be especially useful because they can portend population-level impacts in advance[88]. Morphological anomalies such as bill and leg deformities, splayed legs, reduced eyes, and underdeveloped or loss of nestling feathers, have been observed on roseate (*Sterna dougallii)* and common tern (*Sterna hirundo*) chicks in correlation with PCB and heavy metal exposure[72]. Organochlorines have been correlated with reproductive impairment in a number of bird species[76,90,91,121], demonstrating that reproductive success (i.e. clutch size, hatch rate, fledging success) has the potential to be a useful biomarker. Organochlorine and heavy metals can also cause wasting (loss of condition) and reduced survival rate in offspring[73,90]. Incremental weight measurements of young birds can detect affected growth patterns and could therefore be used to signal negative impacts to reproduction from contaminants[88,122].

Fluctuating asymmetry

Finally, fluctuating asymmetry (FA) is the degree to which two complementary morphological features in a bilaterally-symmetric organism deviate from perfect symmetry, and is regarded as an indicator of developmental stability related to environmental stress[123]. Tarsus length (length of tarsometatarsus bone), nares length (distance between the distal end of the nares and bill tip), wing chord (distance between radial carpal joint and longest primary feather—or phalanges on a nestling bird—on a closed, unflattened wing), and lengths of complementary flight or tail feathers, are the most common measurements used to evaluate FA in birds. Contaminant exposure during development can act as the environmental stress that increases asymmetry in birds, and therefore FA can be a useful biomarker of pollution exposure[124-126].

A recent literature review on the topic suggests that colonial waterbird species make good bioindicators of environmental health, but recommends including a broad suite of biomarkers that incoporate both sub-organismal and organism-level biomarkers (including those described above) for the most sensitive and successful monitoring program[88].

History of a highly-contaminated estuary

Brunswick, GA is situated on the southern coast of Georgia and has several "Superfund" sites, designated by the Environmental Protection Agency's (EPA) National Priorities List (NPL) due to a history of industrial contamination of waterways with various persisting pollutants. These Superfund sites were designated for urgent remediation to comply with the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)[127]. The Linden Chemical Plant (LCP) Superfund site consists of about 220 ha adjacent to the Turtle River in Brunswick, which empties into the Atlantic Ocean. A petroleum refinery called ARCO Petroleum, fueled at first by coal and later by oil, operated at the LCP site from 1919-1935[128,129]. ARCO disposed of large volumes of petroleum products and wastes into the ground, contributing to the polycyclic aromatic hydrocarbon (PAH) pollution at the site[129]. Part of the site was then purchased by Georgia Power and operated as an oil-burning power plant from 1937-1950, when polychlorinated biphenyls (PCBs) were reportedly released by Georgia Power[129]. Another part of the site was purchased by Dixie O'brien Corporation to manufacture paint and varnish from 1941-1955. Dixie is alleged to have generated lead- and mercury-based wastes that may have been released at the LCP site during this time[129]. The site was also used as a chlor-alkali facility from 1955-1994, first by Allied Chemical (later Allied Signal), and then LCP Chemical-Georgia. Chlor-alkali plants produce chlorine gas using the mercury cell process, which involves caustic solutions, bleach, and hydrogen gas as a byproduct[129]. The PCB Aroclor 1268 was used to lubricate the high-voltage processing equipment at the facility[130]. Allied Chemical/LCP Chemical released mercury and Aroclor 1268, along with other chemicals, during this period[129]. Operations at the LCP site ceased in 1994, and the site was declared a NPL Superfund site by the Environmental Protection Agency. In 1998 Allied Signal (now Honeywell) bought the property back from the then bankrupt LCP estate, and Honeywell is still the current owner of the property, except for a small 1.2 ha parcel of the site that is still owned by Georgia Power[128].

Between 1919-1994, industrial activities of various companies at the LCP site resulted in the release of various persistent pollutants into the waterways of the Turtle River estuary, most prominently including the heavy metals mercury and lead, PCBs, and PAHs. Perhaps one of the most remarkable convictions was that LCP released at least 150-200 tons of mercury into the Purvis Creek.[129] Additionally, one highly-chlorinated PCB mixture known as Aroclor 1268 was discharged into waterways by LCP during this time[128,131]. Aroclor 1268 was purchased for industrial use by LCP from its sole-producer, Monsanto Chemical Company, and LCP is the only company in the eastern U.S. that used this specific PCB signature (Fig.1) (with Aroclor 1242, 1254, 1260, 1016, and others being much more common). Hence, when Aroclor 1268 is recovered in biota on the east coast, it can be traced back to this one specific site and timeframe of origin: LCP in Brunswick, GA[131].

Remediation efforts at LCP that occurred between 1994 and 1999, after the Superfund designation, removed contaminated soil from upland and marsh areas and replaced it with fresh soil[129]. Three operable units were designated, including marshland, upland soils, and groundwater. The EPA estimates that sediments in all of the Purvis Creek, and at least a one-mile portion of the Turtle River marshes, were contaminated. Contaminated sediment was excavated and removed from approximately 5 hectares within the marsh and approximately 800 linear meters of tidal marshland. Additionally, approximately 128,000 cubic meters of contaminated soil and industrial waste was removed from upland soils in 26 distinct geographic areas on the site during the late 1990s remediation. Groundwater monitoring has been going on since the early 2000s, during which mercury leakage and caustic brine pools were discovered on site. A phytoremediation project, aimed at suppressing the local groundwater table to prevent contaminated seepage, failed when the trees used for phytoremediation died[129]. In 2007 extraction wells were installed to begin a caustic brine pool remediation. Since then no other sediment remediation has occurred, and groundwater remediation feasibility assessments are still ongoing.

Early studies of biota at LCP

Despite remediation efforts at LCP, Aroclor 1268 congeners and mercury are still being recovered from sediments and organisms near LCP. Concentrations of Aroclor

1268 congeners were found to be several orders of magnitude higher in fish from these waters, than at reference sites[132]. Fish sampling done in the late 1990s in the Turtle River/Purvis Creek revealed that concentrations of Aroclor 1268 were highest in the benthic detritivorous forage fish species sampled, such as striped finger mullet (*Mugil cephalus*), which is a species seabirds and other predators typically feed on[130,132]. PCBs and mercury have also been found in LCP marsh sediments[133,134], while PCBs have been found in reptiles[130], invertebrates[130], and even in the needles of pine trees[135] here. PCBs have also been quantified in a few lower trophic-level bird species at LCP, including red-winged blackbirds (*Agelaius phoeniceus*), mottled ducks (*Anas fulvigula*), and boat-tailed grackles (*Quiscalus major*)[130].

Recent studies of biota at LCP

The most recent baseline ecological risk assessment for the LCP site (from 2011) found methylmercury hazard quotients which suggest that "potential adverse risk to the viability of piscivorous avian species in the LCP estuary is moderate[128]." The Georgia Department of Natural Resources (GDNR) is concerned with how contamination might be affecting protected waterbirds, especially those of higher trophic levels, that nest near LCP (Tim Keyes, GDNR, pers. comm.).

Clapper rails:

A series of recent studies were conducted on clapper rails (*Rallus longirostris*) in the marshes adjacent to LCP to determine the effects of mercury and PCB exposure[131,136-140]. Clapper rails are secretive, solitary marsh birds that feed primarily on benthic macroinvertebrates, especially fiddler crabs (*Uca* spp.). In one study, PCB and mercury concentrations were analyzed from sediments, fiddler crabs, adult clapper rail liver and muscle tissue, and clapper rail chicks, from the LCP marsh and at reference sites[136]. Mercury and PCB concentrations across all sample types were significantly higher at the LCP marsh than at reference sites. This study also demonstrated that mercury and PCBs in the LCP marsh increase via trophic transfer in a simple food web ending with clapper rails. Mercury and PCB concentrations in the LCP marsh increased significantly between both sediment and fiddler crab samples, and clapper rail adult and chick samples. However, while mercury was significantly higher in LCP sediments than in fiddler crabs, PCB concentrations did not differ significantly between LCP sediment and crabs[136].

Another study revealed that clapper rails in the LCP marsh had a high degree of degradation (breakage) to DNA strands, while clapper rails from nearby reference sites showed none[137]. Eggshell integrity from hatched eggs was also compromised in LCP rails[137]. Another study showed that clapper rails from the LCP marsh had altered bone mineral composition. Bones from LCP birds had significantly higher calcium (Ca) to phosphorus (P) ratios, and lower carbonate and acid phosphate content[139]. As bones mature bone mineral becomes more crystalline, Ca:P increases, and carbonate and acid phosphate content decreases. Therefore, the results of this study indicate that contaminant exposure increases the rate of bone maturation in LCP clapper rails[139].

Eggshell calcification is one of the endocrine-mediated mechanisms behind bone mineralization. Another clapper rail study analyzed eggshell thickness, microstructure, mineral composition, and chemical composition, and found that LCP clapper rails produced eggshells that were generally thinner and more brittle, and contained microstructural anomalies, when compared to clapper rails from reference sites[138]. These eggshell attributes are significant because they could contribute to decreased reproductive success. While eggshell PCB concentrations in this study were at background levels among all study sites, mercury concentrations were elevated in eggshells from the LCP, even though mercury exposure was not concluded to be the only factor contributing to compromised eggshell integrity[138].

These clapper rail studies were the first investigations of effects of contaminants on birds at LCP. They did not, however, attempt to link these results with population productivity or health. In light of these reports, clapper rails have been recently suggested as an indicator of marsh health at contaminated sites like LCP[136]. Piscivorous birds that feed exclusively on fish from waters near LCP may be even more threatened by contaminants (due to bioaccumulation in higher trophic levels) than invertebrate-eating clapper rails or any other species sampled there thus far. For example, PCB concentrations were analyzed from unviable eggs collected from three (piscivorous) tern species, including the federally-endangered California least tern subspecies, as well as from clapper rails in the San Francisco Bay in California over the course of several years[99]. Egg PCB concentrations did not differ significantly amongst tern species (but were curiously the highest in the smallest species, the least tern). However, egg PCB concentrations for all tern species were significantly higher than those for clapper rails[99]. Furthermore, colonial-nesting piscivorous birds may make a more practical bioindicator species than secretive marsh bird species like clapper rails due to the accessibility of large numbers of eggs and flightless chicks for study in a single breeding colony. However, no published reports exist on contaminant levels in piscivorous birds or the effects of contaminants on the productivity of any bird species, at LCP.

Marine mammals:

The traits that make marine mammals good indicator species for persistent contaminants in the ecosystems they inhabit resemble those that could potentially make least terns ideal bioindicators (e.g., a piscivorous diet, relatively long lifespan, and limited range during the breeding season). For this reason, the recent findings[141,142] in dolphins local to the LCP Superfund site could further enlighten health study results for least terns breeding near the LCP site.

Bottlenose dolphins (*Trusiops truncatus,* hereinafter "dolphins") are long-lived, apex predators in marine and estuarine habitats along the east coast of the U. S., and have an abundance of lipids in the form of blubber in which to accumulate lipophilic persistent organochlorines (including PCBs) and mercury. Microsatellite markers and mitochondrial DNA indicate that dolphins in certain regions are long-term residents, meaning that health effects to dolphins by long-term exposure to organochlorines in polluted areas is likely[141].

A pilot study that compared biopsies from Turtle River system (LCP) dolphins with those from Savannah River system (~90 km north) dolphins, found elevated concentrations of PCBs in LCP dolphins, and that the PCB mixture in Turtle River dolphins matched the congener pattern specific to Aroclor 1268[141]. Another investigation was launched to explore the relationship between individual dolphin ranging patterns (with respect to the LCP site), with PCB exposure and congener patterns in the dolphins' tissues[141]. The study was aimed at clarifying whether or not Sapelo dolphins (40 km to the northeast of LCP) had been exposed to LCP contaminants, and if Sapelo dolphin exposure resulted from PCB environmental transport, or by dolphin movements between the two areas[141].

Dolphins were categorized by photo-identification survey sightings and telemetry data as individuals with a “Brunswick,” “Sapelo,” or “Mixed” ranging pattern (if an individual had a ranging pattern that included both areas). Tissue samples were analyzed for persistent organic pollutants[141]. In males, total PCB concentration, total Aroclor 1268 concentration, and total Aroclor 1268 : total PCB congeners, were significantly higher in Brunswick dolphins than in Sapelo dolphins. However, concentrations of other persistent organic pollutant classes did not differ significantly across male ranging patterns. In females, only total Aroclor 1268 : total PCB congeners were significantly higher in Brunswick vs. Sapelo ranges. This data seems to support previous hypotheses that female dolphins offload the least lipophilic contaminants during lactation; because Aroclor 1268 is highly-chlorinated (and therefore more lipophilic) it may be offloaded at a lesser rate by lactating females when compared to the other PCBs. For both sexes, total Aroclor 1268 : total PCB congeners was negatively correlated with the mean sighting distance to the LCP site, demonstrating that Aroclor 1268 was negatively correlated with ranging distance from the point source (LCP)[141].

Ranging patterns of Brunswick-area dolphins showed that Sapelo dolphins had elevated concentrations of Aroclor 1268 but did not frequent the Brunswick area, indicating that these dolphins were likely exposed *in* the Sapelo area[141]. Furthermore, the PCB concentrations in Sapelo dolphins exceeded the previous record (held by dolphins in Florida) for highest known dolphin PCB concentrations in the southeastern U.S., even though the Sapelo area is thought of as a relatively pristine natural reserve[141]. This study revealed that LCP area dolphins are exposed to PCB concentrations 1.5 x higher than the highest documented concentrations recorded thus far in any cetacean worldwide. The cetacean record PCB concentration was previously held by Pacific killer whales (*Orcincus orca*), whose diet consists of higher trophic order organisms (e.g., seals, other cetaceans) when compared to the dolphin diet. This implicates the *proximity* of the Brunswick dolphins to the point source (as opposed to the trophic order of organisms in their diet) as the main reason for their higher PCB concentrations[141].

Annual survival rate for 6 neonates was 17% in one year of this study, compared to higher annual neonate survival (i.e. 95%, 80%) documented in other areas (S. Carolina and Florida, respectively), indicating that neonate survival may be limited due to PCB exposure in areas covered in this study, although further research is needed to establish annual survival rates of dolphins in this study area[141]. However, a concurrent related study conducted health evaluations on the same dolphins and found a positive correlation between immunosuppression and PCB concentrations in dolphins sampled[142]. Dolphins suffered anemia, as well as reduced thyroid hormone levels (including total thyroxine, free thyroxine, and triiodothyronine) that were negatively correlated with PCB concentrations from blubber samples. Additionally, T-lymphocyte proliferation and indices of innate immunity in sampled dolphins were negatively correlated with PCB concentration, demonstrating compromised immune function in PCB-exposed dolphins in waters in and around the LCP estuary[142].

Least terns in the LCP estuary

Least terns currently breed in several coastal locations in Georgia, with habitats consisting of barrier islands, manmade dredge spoil islands, and mainland rooftop colonies[26,27,46]. Least terns are considered rare in the state of Georgia, and productivity and numbers in Georgia have been declining[143]. Currently, one of the largest least tern breeding colonies in Georgia is located on a manmade dredge spoil island called Andrews Island, located in the Turtle River estuary less than 4 km from the LCP Superfund site. Because of concern over Georgia's rare birds breeding in such close proximity to LCP on Andrews Island, another dredge spoil island was created approximately 8 km farther out in the estuary. It was created to provide alternative breeding habitat for birds and divert breeding efforts away from the contaminated estuary (Tim Keyes, pers. comm.). Although several waterbird species do nest on this secondary dredge spoil island, least terns do not nest there but rather continue to colonize Andrews Island, likely outcompeted by the larger species (e.g., royal terns/*Thalasseus maximus*, laughing gulls/*Leucophaeus atricilla*) that nest in much larger numbers on the secondary island. Andrews Island requires regular management, such as disking to remove vegetation, in order to maintain suitable habitat for least tern (and other shorebird) breeding. It is for this reason that the Georgia DNR Nongame Conservation Division is keenly interested in the impacts that proximity to LCP has on least tern health and productivity in order to inform future management decisions at Andrews Island (Tim Keyes, pers. comm).

Summary and identification of data gaps

1) PCBs and mercury are known to cause developmental, reproductive, and immune system deficiencies in many species of birds, mostly demonstrated in the laboratory setting, but it is not clear how environmentally-relevant concentrations affect health and reproduction in a wild population.

2) Extremely high (and in some cases unprecedented) concentrations of PCBs and mercury have been found in the flora and fauna near the LCP Superfund site in Brunswick, GA. Health and reproductive impairment has been documented in some of these species (i.e., clapper rails and bottlenose dolphins). However, no piscivorous bird near LCP has been investigated for effects and concentrations of contaminants.

3) State conservation authorities need information regarding whether or not contamination should influence management decisions to encourage least tern nesting at Andrews Island despite its proximity to LCP, even as suitable and relatively undisturbed least tern breeding habitat is becoming more and more scarce.

Consequential gaps relating to the effect of contaminants on least terns exist in the literature and are the impetus for this research, which aims to determine the degree to which least terns in Georgia are exposed to mercury and PCBs, establish the health and reproductive effects of pollutants on least terns, and inform management decisions made for least terns in the state of Georgia (and potentially other regions with similar circumstances), especially in regard to Andrews Island.

CHAPTER 2

EXPOSURE TO MERCURY AND AROCLOR 1268 IN A FISH-EATING SEABIRD IN GEORGIA [1]

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[1] Robinson, G.L., Mills, G.L., Schweitzer, S.H., Hernandez, S.M. To be submitted to *Journal of Environmental Monitoring*)

Abstract

We investigated concentrations of mercury, and the unique PCB mixture Aroclor 1268, in least terns (*Sternula antillarum*) at a highly-contaminated estuary in coastal Georgia. This estuary is the location of the Linden Chemical Plant (LCP) Superfund site, where until 1994 industrial enterprises released effluent containing these contaminants. High concentrations of Aroclor 1268 and mercury are found in local biota, but no studies report concentrations in piscivorous birds. We collected egg samples, as well as feathers and feces from chicks, from breeding colonies across the Georgia coast to analyze contaminant loads. Mean Aroclor 1268 concentrations in eggs were highest at colonies in and just outside LCP, and decreased with increasing distance to LCP, which was expected as Aroclor 1268 was released only at LCP. Mean Hg concentrations of eggs varied little among sites, but eggs with the highest concentrations were found at sites closest to LCP, and in the Savannah River. Mercury in chick feathers and sediment samples were highest at the site closest to LCP, indicating that chicks are better gauges of local contamination than eggs. This was expected, as chicks are fed locally until fledging, while adults may accumulate contaminants over time. However, chick fecal Hg was highly variable within each colony, and did not differ significantly among colonies, indicating fecal samples are likely a better reflection of the variation of contaminant concentrations in the food items. Lastly, we report the transport of Aroclor 1268 ~110 km north, and ~70 km south from its point source (LCP); transport of this unique PCB mixture > 40 km has not previously been documented.

Introduction

Persistent organic pollutants (POPs) and heavy metals are environmental contaminants that, once released, remain in sediments and ecosystems long after their introduction[69]. POPs and heavy metals are not readily degraded by natural processes, and tend to bioaccumulate (increase in concentration when transferred from environmental substrate to living organism), and in some cases biomagnify (increase in concentration when transferring from one trophic level to the next), in the ecosystems they contaminate[69]. Because of their longevity, bioavailability, and documented harmful effects on living organisms, the presence and concentrations of POPs and heavy metals in the environment are of great interest not only to public health authorities, but also to wildlife and habitat management authorities[128]. Sampling of sediment and wildlife prior to, and immediately following, remediation of contaminated areas is common. However, the extent of transport and long-term bioaccumulation in food webs of contaminants in years following remediation is not always well understood.

Both POP and mercury contamination are threats to human and wildlife health and reproduction. For example, effects of POPs and heavy metal exposure in birds include decreased egg production, abnormal incubating behavior, reduced egg volume and weight, thinning of eggshells, lowered hatch success due to embryonic death and decreased fertility of eggs, physical and physiological abnormalities in offspring, decreased growth rate, abnormal development of sex organs, immune system disorders, and high nestling mortality[71-76]. The chronic effects of PCB and mercury exposure to humans include immunosuppression, increased risk of cancer, and a variety of reproductive problems in men and women, as well as developmental and neurological problems in developing fetuses and children[129]. The risks posed to public health and the environment by mercury and PCB contamination make monitoring their transport and biomagnification in the ecosystem over time imperative.

The Linden Chemical Plant (LCP) site in Brunswick, GA is designated a "Superfund" area by the Environmental Protection Agency (EPA)'s National Priorities List. Superfund sites are considered by EPA to be the highest priority for remediation to comply with the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)[69]. The LCP Superfund site consists of ~ 220 hectares that includes the Purvis Creek, which empties into the adjacent mouth of the Turtle River, which in turn empties into the Atlantic Ocean (Fig. 2.1). From 1919-1994, various industrial enterprises operated at the LCP site including a coal- and later oil-fired petroleum refinery, an oil-fired power plant, a paint and varnish manufacturer, a chlor-alkali facility, and for most of the latter half of the century a chemical plant (LCP), before the site was shut down and declared a Superfund site in 1994[129]. Industrial activity at the LCP site resulted in the release of various persistent pollutants into the waterways of the Turtle River estuary, including lead and polycyclic aromatic hydrocarbons (PAHs), but primarily polychlorinated biphenyls (PCBs) and mercury[129]. LCP alone was estimated to have released 150-200 tons of mercury into the Purvis Creek[131]. Additionally, LCP used a highly-chlorinated PCB mixture known as Aroclor 1268 that was discharged into waterways[128]. Aroclor 1268 was purchased for industrial use by LCP from its sole producer, Monsanto Chemical Company, and LCP is the only site in the eastern U.S. where this specific PCB signature was used. Thus, the origin of Aroclor 1268 recovered in biota on the east coast is unambiguous, making Aroclor 1268 a hallmark of LCP pollution in the eastern U.S[131].

The remediation efforts at LCP that occurred between 1994 and 1999, after the Superfund designation, removed contaminated soil from upland and marsh areas and replaced it with uncontaminated soil[129]. The EPA estimates that sediments throughout the Purvis Creek, and in at least a 1.6 km portion of the Turtle River marshes, were contaminated. Contaminated sediment was excavated and removed from approximately 5 hectares within the marsh and approximately 800 linear meters of tidal marshland[129]. Additionally, approximately 128,000 cubic meters of contaminated soil and industrial waste was removed from upland soils. Groundwater remediation feasibility assessments are still ongoing[129]. Despite remediation efforts at LCP, Aroclor 1268 and mercury are still being found in marsh sediments and organisms near LCP[133,134]. Aroclor 1268 has also been documented in sea turtles[144,145] and other reptiles[130], cetaceans[146], invertebrates[130,147], and even in the needles of pine trees[135] at LCP. Fish tissue samples collected in the late 1990s in the Turtle River/Purvis Creek revealed that concentration of Aroclor 1268 was several orders of magnitude higher when compared with fish from reference sites[132]. Concentrations of Aroclor 1268 were highest in the benthic detritivorous forage fish species sampled, such as striped finger mullet (*Mugil cephalus*), which is a species many seabird species feed upon[130,132]. PCBs were also quantified in several lower trophic-level avian species at LCP, including red-winged blackbirds (*Agelaius phoeniceus*), mottled ducks (*Anas fulvigula*), and boat-tailed grackles (*Quiscalus major*)[130].

More recently, bioaccumulation and biomagnification of mercury and PCBs in the LCP marsh was demonstrated in clapper rails (*Rallus longirostris*) and their primary prey item, fiddler crabs (*Uca* spp.)[136]. Concentrations of Aroclor 1268 and mercury were significantly higher in fiddler crabs than in marsh sediments, and an even further increase existed between the crabs and the clapper rails. Aroclor 1268 and mercury concentrations in clapper rail feathers, livers, muscle tissue, and chicks, were highest in birds from the LCP marsh when compared to birds from other nearby reference sites[136]. Another recent study of bottlenose dolphins (*Trusiops truncatus*) was the first report of the transport of Aroclor 1268 from LCP as far away as Sapelo Sound, located another 40 km up the coastline from the Brunswick Estuary (where LCP is located)[141]. The ranging patterns of the dolphins sampled were characterized by radio telemetry and photo identification over the course of several years to ensure that the concentrations measured in blubber samples from these animals were acquired locally; thus it was presumed that concentrations of Aroclor 1268 in Sapelo Sound dolphins resulted primarily from foraging activities within the Sapelo Sound. Dolphins from both the Brunswick Estuary and the Sapelo Sound had concentrations of Aroclor 1268 in their blubber that exceeded any previous PCB concentrations documented in any cetacean worldwide. This was the first documentation of Aroclor 1268 outside of the Brunswick estuary. Notably, Sapelo Sound was previously thought to be a relatively pristine habitat, with the nearby Sapelo Island National Estuarine Research Reserve often cited as a reference site in ecological research[141]. Dolphins in these studies were sampled as far as 40 km to the north of LCP[141], but there have been no reports of the transport of Aroclor 1268 in biota across the rest of the approximately 160 km of Georgia coastline.

Seabirds have long been used as a sentinel species for contamination of the aquatic environments in which they live[87]. Because PCBs and mercury are not easily metabolized by living organisms, they are often stored for long periods of time in adipose, or proteinaceous organ and muscle tissue, respectively[69,70]. Seabirds tend to harbor some of the highest concentrations of contaminants, in part due to their high trophic status and their relatively long lifespans (which can be decades-long)[87]. However, because their geographical ranges are often extensive, contaminants found in seabirds may have origins that include a wide variety of global regions. For example, the eastern subspecies of least tern (*Sternula antillarum antillarum*) winters in Central and South America, and nests along the eastern and Gulf shores of the U.S. Thus, longevity, a high trophic status, and a wide geographic range, are all characteristics that increase exposure to contaminants by seabirds, but which also confound the determination of contaminant origin. However, the flightless chicks of colonial nesting seabirds provide a reflection of local contamination. Because chicks are hatched, fed, and raised on site, contaminants recovered in chicks are of relatively local origin[87]. Contaminant origin would be especially localized in chicks of a species that forages in very close proximity to the breeding colony site.

Least terns breed along the U.S. east coast, including locations in coastal Georgia. Least terns are migratory, colonially-nesting, relatively long-lived (the oldest recovered banded least tern was 24 years old), and typically forage in close proximity (within 5 km in Georgia[12]) to their breeding colony[2]. Least terns feed almost exclusively on shallow-bodied (< 1.5 cm. in body depth) and non-spiny fish between 2-9 cm in length that swim in the upper 15 cm of shallow waters[2,3,5-7]. Because of their high trophic position as piscivores, their localized foraging strategy during the breeding season, and their relative longevity, least terns are a species likely to demonstrate increased exposure to, and biomagnification of, environmental contaminants local to their breeding grounds. Least terns are also state-listed as ‘rare’ in GA, making the negative impacts of contaminants to least tern survival and recruitment a conservation concern. We aimed to determine the degree to which least terns breeding on the coast of Georgia were exposed to contaminants associated with the LCP Superfund site, and if proximity of the breeding colony to the LCP site affected exposure. We hypothesized that least terns would have lower concentrations of mercury and Aroclor 1268 in their tissues as distance of the breeding colony from LCP increased. We also hypothesized that because Aroclor 1268 was released at LCP and no other point source in the eastern U.S., that only least terns breeding in colonies close to LCP would have Aroclor 1268 in their tissues. We also aimed to explore how concentrations of contaminants in various sample types (eggs, feathers, and feces) related to one another, and to concentrations of contaminants in local sediments.

Methods

Sites and field sampling

We sampled a total of six different least tern nesting colonies that were spread across the entire Georgia coast from May-August in 2011 and 2012. We sampled Andrews Island (ANDR), the Savannah River dredge spoil site (SARI), a Publix grocery store rooftop (PUBL), the Cumberland Island National Seashore (CINS), and Little St. Simon’s Island (LSSI) in both years, but the breeding colony on Pelican Spit (PESP) was only active and sampled in 2011 (Fig. 2.2). CINS and LSSI were natural barrier island beach colonies, remote to human disturbance but prone to tidal flooding. ANDR and SARI were located on manmade dredge spoil sites, unaffected by tidal flux but located in close proximity to urbanized areas. The PUBL colony was located on top of a flat, gravel, rooftop, in the city of Kingsland, GA. PESP was located on a sand bar located ~ 1 km offshore from St. Simon's Island, GA. We collected sediment samples from several locations in the Turtle River estuary near our ANDR colony (~5 km from LCP), in Christmas Creek and the Atlantic Ocean directly flanking either side of our CINS colony (~70 km south of LCP), and in the Savannah River along the south border of our SARI colony (~110 km north of LCP).

We monitored each colony every three days throughout the season. We located nests and marked them with labeled tongue depressors ~ 2 m from the nest. We collected eggs throughout the breeding season in both years, usually following nest abandonment due to random events such as partial depredation, flooding and nest over-wash, or colony abandonment after dark by adults trying to avoid predation by nocturnal predators (mostly great-horned owls, *Bubo virginianus*). We wrapped collected eggs in aluminum foil and stored them frozen in plastic tubes until processed. We individually identified chicks upon hatching with a uniquely numbered aluminum leg band and monitored each chick throughout nestling. We regularly monitored the health and growth rate of chicks (see chapter 3), and during routine handling of chicks we opportunistically collected fecal samples, which we stored in plastic centrifuge tubes. We collected the second primary feather from both wings of each chick when primaries were > 50% grown, typically within a few days of the chick fledging, ~15-18 days of age. We chose the second primary because it is one of the largest feathers and one of the first flight feathers to grow in, making it a major deposition site for mercury during chick development[148].

Total mercury analysis

We lyophilized whole eggs, fecal, and sediment samples to a constant weight, and then ground and homogenized them using solvent-cleaned glass rods. We divided each egg sample into separate plastic tubes for subsequent PCB and total Hg (THg) analysis. We cleaned feathers once with a 1% liquinox solution and again with GC-grade methanol, with each cleaning being preceded and followed by a rinse with Milli-Q H_2O (18 MΩ deionized water). Finally, we washed feathers in hexane to remove external contamination, freeze-dried them, and cut them into fragments to fit into the mercury analyzer boats. We cleaned chick carcasses with a 1% liquinox solution, preceded and followed by a Milli-Q H_2O rinse.

We separated a subsample from each homogenized sample for analysis. Mean (and range in parentheses) subsample dry weights used for each sample type were: 0.0618 g (0.0128 – 0.1680 g) for eggs, 0.0180 g (0.0003 – 0.1689 g) for feces, 0.0085 g (0.0003 – 0.0196 g) for feathers, 0.0945 g (0.0139 – 0.1826 g) for carcasses, and 0.0924 g (0.0341 – 0.1975 g) for sediment. We analyzed subsamples for THg content by thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry (DMA 80; Milestone, Monroe, CT, USA), according to U.S. Environmental Protection Agency (EPA) method 7473. For quality assurance, each group of 10 samples included a replicate, blank, and two standard reference materials (SRM; TORT-2 lobster hepatopancreas and DOLT-4 dogfish liver, National Research Council of Canada, Ottawa, ON.) The instrument was calibrated using the solid SRMs (TORT-2 and

DOLT-4). Method detection limits (MDLs; threefold the standard deviation of procedural blanks) averaged 0.0004 ppm (0.3679 ppb) dry mass. Mean percent recoveries (± standard deviation) of THg for the SRMs TORT-2 and DOLT-4 were 103.2 ± 5.7, and 98.9 ± 4.1, respectively.

PCB analysis

We adopted methods to extract PCB congeners from feathers from previous work[114,115]. Specifically, we dried feathers and then cut them into fragments to increase surface area and optimize PCB extraction. We placed the feathers in a cleaned glass vial with 8ml 4M HCl and 5ml dichloromethane (DCM), and incubated them overnight in a 40°C H_2O water bath. We transferred the DCM layer to a 20ml vial with a Teflon-lined cap. We repeated the extraction with an additional 3ml DCM in order to optimize PCB extraction from the feather medium, and then combined the two extracts. To remove traces of HCl in the DCM extracts, we filtered samples through 500 mg of anhydrous Na_2SO_4. The DCM was then concentrated to 1ml under nitrogen evaporation. We used a hydrocarbon and moisture trap to prevent contamination of the analyte from the nitrogen stream.

We used columns of acidified silica to remove residual organic material from the extract. We prepared the acidified silica by slowly adding 27 ml of concentrated sulfuric acid to 50 g of Silica Gel 60 (70-230 mesh; EM Science), while stirring to maintain homogeneity, and we stirred the mixture for another 60 minutes following the acid addition. We weighed approximately 780 mg of acidified silica for each sample, and added it to a 6 ml glass column lined with a Teflon frit. We conditioned columns with 2 ml hexane:DCM, and 2 ml hexane. We added 1 ml hexane to the extract to create a nonpolar eluent of 1:1 DCM/hexane. We loaded the 2 ml samples onto the silica column, eluted them with 4 ml 1:1 DCM/hexane, and collected eluate in a clean glass vial. We used nitrogen to concentrate the eluate to 50 µl, and then we added hexane to bring the final volume to 1 ml. Due to the continued presence of residual hydrocarbons and other non-PCB organic material, a second cleanup step was necessary. We prepared a second set of silica columns, as described above, and the samples were eluted with 4ml hexane. We evaporated the final eluate to dryness with a gentle nitrogen stream, and then added hexane to a final volume of 1 ml with methanol (100% gas chromatography (GC) grade).

For GC analyses, we used an Agilent (Atlanta, GA) 6890 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm I.D., 0.25 um film thickness; J&W Scientific, Folsom, CA), electronic pressure control (EPC), and an electron capture detector (ECD). We introduced samples via auto-injection at 250°C, splitless mode. Chromatographic conditions were: 54°C, held isothermal for one minute; 54-160°C at 20°C min^{-1}, held isothermal for 5 minutes; then 160°-270°C at 3°C min^{-1}, held isothermal for 3 minutes. We held pressure constant at 11 psi. We quantified samples using a six-point calibration curve derived from dilutions of certified congener (Ultra Scientific) and Aroclor 1268 (Supelco; CAS# 11100-14-4) standards. We identified congeners based on retention time, and elution order, relative to the standards. We used previous analyses performed on GC-MS to confirm identification of Aroclor 1268 congeners[136]. We determined all 12 congeners in the Aroclor 1268 mixture with a minimum detection of 17.53 ng g^{-1}. All selected congener peaks were at least 25% of the highest Aroclor component. We replicated ten percent of the samples.

Statistical analysis

None of our sample types had contaminant concentrations that were normally-distributed, but all contaminant distributions were successfully transformed to fit a normal distribution, which is standard procedure for these types of analyses[149]. We used a square root transformation to normalize egg mercury, egg Aroclor 1268, and chick whole body Aroclor 1268 concentrations. We used a log10 transformation to normalize mercury concentrations in feathers, chick whole bodies, and feces. We used a post-hoc Tukey's Honestly Significant Difference (HSD) test to determine pairwise differences ($\alpha = 0.05$) in mean contaminant concentrations among sites and years. We used R statistical computing program for all statistical analyses[150].

Results

PCBs in egg samples

We measured PCB concentrations in a total of 104 least tern eggs across six sites and two consecutive years. We analyzed total PCBs, individual Aroclor 1268 congeners (Table 2.1), and total Aroclor 1268. Because least terns did not nest successfully at every site in both years and sample sizes were therefore uneven between sites, and due to the possible differences between years in contaminant availability to least terns, we report results from each year individually (Table 2.2). However, analysis of variance (ANOVA) indicated that there was no significant effect of year on egg Aroclor 1268 concentrations ($F_{1,102} = 2.0053$, $p = 0.1598$). With combined years, in ascending order of distance from LCP, mean and median total concentrations (respectively) of Aroclor 1268 in eggs were 4,266 ppb and 3,843 ppb at ANDR, 4,758 ppb and 2,869 ppb at PESP, 2,257 ppb and 1,364 ppb at LSSI, 2,769 ppb and 2,037 ppb at CINS, 1,193 ppb and 1,303 ppb at PUBL, and 544 pbb and 284 ppb at SARI. Because ANOVA indicated that there was a significant effect of collection site on egg Aroclor 1268 concentrations ($F_{5,98} = 12.798$, $p < 0.0001$), a follow-up Tukey's HSD was needed to define pairwise differences among sites. PESP had significantly higher egg Aroclor 1268 concentrations than LSSI ($p = 0.0507$), PUBL ($p = 0.0050$), and SARI ($p < 0.001$), and ANDR had significantly higher egg Aroclor 1268 concentrations than both PUBL ($p = 0.0071$) and SARI ($p < 0.001$). In addition, egg Aroclor 1268 concentrations were higher at CINS ($p < 0.001$) and LSSI ($p = 0.0051$) than at SARI, but neither differed significantly from any other sites (Fig. 2.3).

Mercury in egg samples

We analyzed a total of 170 eggs for mercury across all six sites between 2011 and 2012, and analyzed years separately (Table 2.3) as well as pooled. For pooled years, in ascending order of distance from LCP, mean (and median in parentheses) THg concentrations (respectively) in eggs were 1,057 ppb and 991 pbb at ANDR, 1,080 ppb and 964 ppb at PESP, 1,098 ppb and 936 ppb at LSSI, 965 ppb and 943 ppb at CINS, 830 ppb and 697 pbb at PUBL, and 994 ppb and 829 pbb at SARI. An ANOVA showed that neither collection site ($F_{5,164} = 1.0312$, $p = 0.4011$) nor year ($F_{1,168} = 2.4753$, $p = 0.1175$) had a significant effect on egg mercury.

Chick fecal mercury

We analyzed 102 chick fecal samples, from five different sites, for total mercury (Table 2.4). With pooled years, mean and median fecal mercury concentrations (respectively) were 103 ppb and 81 pbb at ANDR, 81 ppb and 49 ppb at PESP, 7 ppb and 7 ppb at CINS, 78 ppb and 62 ppb at PUBL, and 74 ppb and 57 pbb at SARI. There was no effect of collection year on fecal mercury ($F_{1,100} = 3.4674$, $p = 0.0655$). Due to predation and flooding, some colonies either did not successfully hatch chicks, or hatched very few chicks (e.g., LSSI, CINS). Because CINS had a vastly inferior fecal sample size (*n* = 2) that was unlikely to be representative of mean fecal mercury from a colony at this site, we omitted CINS from the analysis. There was no difference in fecal mercury among colony sites ($F_{3,96} = 1.0023$, $p = 0.3953$) (Fig. 2.4).

Chick feather mercury

We analyzed the second primary feather (P2) from 97 chicks, from four different sites, for total mercury (Table 2.5). With years pooled, mean and median mercury concentrations (respectively) in P2 feathers were 3,190 ppb and 3,080 ppb at ANDR, 1,581 ppb and 1,540 ppb at PESP, 2,205 ppb and 1,830 ppb at PUBL, and 1,560 ppb and 1,660 ppb at SARI. With years pooled, P2 mercury was significantly higher at ANDR than at PESP ($p < 0.001$), SARI ($p < 0.001$), and PUBL ($p = 0.0195$), but there was no significant difference in P2 mercury among PESP, SARI and PUBL. ANOVA indicated that there was a significant difference in P2 mercury concentrations among sites ($F_{3,93} = 9.6903$, $p < 0.0001$), but also between years ($p < 0.0001$), so years were separated for statistical analysis. Collection site significantly affected feather mercury in both 2011 ($F_{1,35} = 21.322$, $p < 0.0001$) and 2012 ($F_{2,57} = 5.5546$, $p = 0.0063$). Only PESP and SARI produced chicks that survived long enough to grow primary feathers in 2011, and PESP chicks had significantly higher P2 mercury than SARI chicks ($p < 0.0001$) (Fig. 2.5). In 2012, ANDR chicks had higher P2 mercury than both SARI chicks ($p = 0.0060$) and PUBL chicks ($p = 0.0160$), but SARI and PUBL chicks did not differ significantly from one another ($p = 0.9732$) (Fig. 2.6).

Chick whole body mercury and Aroclor 1268

In 2012, fifteen chick carcasses were collected from three different colonies (Table 2.6). Chicks were between the ages of 7-12 days, with the exception of one that was 15-16 days old. Chicks were found dead in or on the perimeter of the colony, either from unknown causes, from predation, or from falling from the rooftop at PUBL. Mean chick Aroclor 1268 concentration differed among sites ($F_{2,12} = 19.524$, $p = 0.0002$) with concentrations at ANDR (3,299 ppb) significantly exceeding those at PUBL (418 ppb, $p = 0.0010$) and SARI (484 ppb, $p < 0.0001$) (Fig. 2.7). There were no significant differences among sites in total mercury in chick carcasses ($F_{2,12} = 2.8423$, $p = 0.0976$).

Discussion

Transport of a unique PCB mixture

Aroclor 1268 is a unique PCB mixture that is exclusively linked to LCP pollution in the eastern U.S. The sole producer of this highly-chlorinated PCB mixture was Monsanto, and the only place Aroclor 1268 was used and released into the environment was at LCP Chemical Company in Brusnwick, GA. As far as we are aware, we are the first to document the transport of Aroclor 1268 as far north as the South Carolina border (SARI breeding colony) and as far south Kinglsand, GA (PUBL colony) and the Cumberland Island National Seashore (CINS colony), which border the state of Florida. While a number of studies since the 1990s have investigated the bioaccumulation of Aroclor 1268 in biota local to LCP, only within the last two years has Aroclor 1268 been identified in organisms local to areas beyond the Turtle River estuary. Concentrations of Aroclor1268 (exceeding any previous reports of PCB concentrations in any cetacean worldwide) were found in the blubber of dolphins with feeding ranges both in Brunswick, GA and in Sapelo Island National Estuarine Research Reserve (SINERR)[141,151]. SINERR has long been thought of as a relatively pristine habitat, used as a reference site in many ecological studies. Sapelo Island is ~40 km north of Brunswick, GA, and we report here the spread of Aroclor 1268 at least as far as 110 km north of Brunswick, in Savannah, and 70 km south of Brunswick, in Kingsland, GA. Aroclor 1268 concentrations in eggs reached over 2,000 ppb at PUBL, over 3,000 ppb at SARI, and over 9,000 ppb at CINS, which were the three sites farthest from Brunswick. We also report Aroclor 1268 contamination around Cumberland Island National Seashore, one of the premier national seashores in the eastern U.S., which is also considered a relatively pristine reference site for biological research.

Implications for least terns and other birds

The Aroclor 1268 and mercury concentrations we found in least tern samples along the Georgia coast reach and exceed those associated with adverse effects in some studies. A great deal of variability in sensitivity to PCBs and mercury exists among bird species, and even sometimes within individuals of the same species. Mean concentrations of mercury and Aroclor 1268 may not warrant concern at all sites in our study, but the range of concentrations of these contaminants at our study sites are cause for concern. For example, several studies found that the total PCB concentration in eggs associated with reduction in hatch success in common terns (*Sterna hirundo*) was 7,000 – 10,000 ppb[90,151,152]. Aroclor 1268 egg concentration ranges exceed 7,000 ppb at all sites except PUBL and SARI, the two sampling sites farthest from LCP. The upper range of Aroclor 1268 concentrations at our two closest sampling sites reached over 14,000 ppb at PESP, and over 16,000 ppb at ANDR. A review by Eisler[153] reports that in birds, egg mercury concentrations as low as 1,500 ppb, and feather mercury concentrations as low as 5,000 ppb, have been associated with adverse effects. Ranges of egg mercury concentrations in least tern eggs exceeded 1,500 ppb at each site either in 2011, in 2012, or in both years (Table 2.3), and the range of feather mercury concentration at ANDR exceeded 5,000 ppb. More research is needed to identify sublethal health and reproductive effects to birds posed by the elevated exposure to these contaminants along the Georgia coast.

Comparison of sample types for gauging local contamination

We sampled least tern eggs, chick feathers, chick feces, and chick whole bodies, and each sample type reflects contamination local to the collection site in a different way. Female birds sequester mercury and PCBs into eggs[101,154], feather growth is a major depuration pathway for heavy metals in birds[155], and birds excrete heavy metals in feces[108]. Contaminant concentration found in seabird chicks (with the exception of newly-hatched chicks) is attributed primarily to contaminated food ingestion, while contribution from the parent (egg) is negligible[104,148]. This is largely due to the growth dilution effect, whereby as the chick grows the contaminant load inherited by the chick from the parent contributes less and less to the total body burden of contaminants in the chick, while contaminant ingestion by the chick becomes the primary contributing source to the total body burden. Meanwhile, overall contaminant concentration in the chick is being diluted by rapid increase in body mass during the nestling period[105,106]. For mercury exposure, it has been suggested that the best sample type to take from a chick is the feather, because feather growth is one of the primary mercury depuration pathways in birds, and thus chick feathers represent an archive of mercury contamination throughout the chick's entire nestling period[112]. This is because chicks develop contour feathers (which include body, wing, and tail feathers) throughout the nestling period, and there is a constant blood supply to each feather until it is fully-grown. Therefore, because feathers are growing throughout the nestling period and mercury is sequestered in feathers, a feather pulled from a chick just before fledging represents an archive of the mercury exposure that the chick has experienced throughout its life up until that point. In contrast, eggs are the only sample type we collected that come directly from the adult bird. Least terns are migratory and relatively long-lived. Therefore adult least terns could be introducing contaminants into their eggs that have been acquired elsewhere, in any number of locations where the adult fed throughout the years of its life. However, egg samples could still provide an adequate proxy for local pollution in some cases, especially in those where the pollutants have a very limited geographic range and history of release, as is the case with Aroclor 1268. For example, in our study Aroclor 1268 concentrations in least tern eggs significantly decreased with increasing distance from LCP, which can be explained by the low probability of least terns in our study being exposed to Aroclor 1268 anywhere other than at our study sites.

Eggs could plausibly provide a decent proxy for local pollution of a contaminant that like mercury, which is geographically widespread and has a long history of both natural and anthropogenic deposition, if a bird’s molting pattern in taken into consideration. As stated previously, feather growth is one of the primary elimination pathways for mercury in birds. Thus, if the adults of the study species had recently undergone molt (loss and subsequent regrowth of feathers) before egg collection, the eggs may be a fairly accurate representation of local mercury contamination, given that the bird recently eliminated the majority of its overall body burden of mercury through the molt. For example, least terns molt twice a year: once in the late winter/early spring (~ Jan – Mar), and then again during the breeding season (~ May – Aug)[2]. A study of a gull species, which belong to the family most closely related to that of terns, found that 93% of an adult's total mercury body burden was deposited into feathers[155]. Least terns arrive at their breeding colonies in April after spring migration, at which point they would have recently finished their spring molt[2]. The onset of egg-laying is in early May[2]. An adult gull will deposit up to 40% of its total body burden of mercury into an egg within a few days of laying it[102]. Therefore, in the case of least terns in our study, one could infer that the eggs we collected, which were laid by adults who recently finished spring molt, should be a reasonable representation of local mercury concentrations. However, egg mercury concentrations in our study did *not* follow the same trends as feather or sediment mercury concentrations. With the exclusion of site/year combinations with inferior egg sample sizes, PUBL was the only site in our study that had significantly lower mean egg mercury concentration than any other sites. In 2011 there were no significant differences in egg mercury between ANDR, PESP, LSSI, CINS, or SARI, but PUBL was not sampled for eggs in 2011 due to logistical issues in the field. In 2012, only ANDR, SARI, and PUBL were sampled for eggs, and PUBL had significantly lower egg mercury concentrations than ANDR or SARI. In summary, egg mercury in our study was consistent across all sites except PUBL. To the contrary, feather mercury was significantly higher in both years at sites in closest proximity to LCP than at sites farthest from LCP (PESP > SARI in 2011, and ANDR > PUBL and SARI in 2012). This pattern of feather mercury concentrations aligned closely with that of sediment mercury concentrations, which were also much higher at sites closest to LCP (ANDR = 1000 ppb) than at other sites (CINS = 11 pbb, SARI = 48 ppb). This suggests that feather mercury is indeed a better indicator of local mercury pollution than are eggs. Furthermore, our results indicate that mercury pollution studies that sample eggs vs. those that sample chick feathers from the same sites may yield conflicting results. Although many studies have demonstrated that feathers developed by birds at the sampling site can provide a fairly accurate reflection of local mercury pollution at that site[103,105,111,155-157], in many cases egg samples are used to determine the degree of mercury exposure birds are experiencing at a particular site[33,35,87,98,158,159]. Eggs are likely a favored sample type because they are easy to collect and do not require the capturing or handling of the sampled birds. However, our data indicate that investigators should use caution when interpreting concentrations from egg samples to evaluate the extent of uptake of mercury from the local habitat by birds, especially in migratory, or far-ranging, bird species.

We also note that in our study, eggs from the same clutch/adult did not necessarily have similar contaminant concentrations, especially in the case of Aroclor 1268. For example, a 2-egg nest at LSSI had Aroclor 1268 concentrations of 5,307 ppb in one egg, and 704 ppb in the other, at a SARI nest 1,838 ppb and 13 ppb, and at a CINS nest 5,302 ppb and 9,125 ppb. Mercury, by contrast, was within a few hundred ppb between eggs in almost all cases where both eggs in a clutch were analyzed. We do not have a large enough sample size to expound upon these disparities statistically, but regardless, investigators should consider these results, particularly when interpreting data that use contaminant concentrations in a collected egg to explain effects (e.g., hatch success, observable adverse effects), or lack thereof, in other eggs or chicks from the same clutch.[90,160,161].

Our results also indicate that fecal samples do not accurately convey overall mercury exposure to birds from a particular site, but may better convey the range of mercury concentrations in food items delivered to chicks at a particular site. Mean fecal mercury concentrations in chicks did not differ among sites in either year, or with years combined, whether or not inferior sample sizes for a site/year combination excluded from statistical analysis. However, the range of mercury concentrations was extremely varied within each site in all cases. This is most likely a result of the fecal sample representing only capturing a "snap-shot" of the overall mercury exposure to a chick, because the mercury content in a fecal sample is largely influenced by the mercury content of the chick's last meal at the time of sampling. Over fifty species of fish have been documented as prey items of least terns[4], with the most common prey species in the mid-Atlantic and southeast being anchovy (*Engraulis eurystole*), menhaden (*Brevoortia tyrannus*), mummichog (*Fundulus heteroclitus*), and silverside (*Menidia* spp.)[8]. Because least terns consume a high diversity of prey species, the variability in mercury content in least tern prey items would likely also be high. Finally, if mercury concentrations are highly variable between prey items at sampling sites, overall differences in mercury exposure to birds between sites may be obscured.

Conclusions

We determined that the transport of Aroclor 1268 from the LCP area is far more extensive than expected or previously known. Mercury is also pervasive along the Georgia coast, especially in the Turtle River and Savannah River estuaries. This presence of elevated concentrations of these contaminants is important information for resource managers, as well as for researchers at preserves like SINERR, Little St. Simon's Island,

and Cumberland Island National Seashore, where myriad ecological research projects are staged each year. Concentrations of both mercury and PCBs in many of our least tern feather and egg samples have reached or exceeded those levels documented in the literature to cause adverse effects in birds. Because least terns are piscivorous birds at the top of their local food web, elevated exposure to, and adverse effects of, contaminants in this species indicates elevated exposure and possible effects in lower trophic order species, which may warrant further investigation into species of concern. Furthermore, the implications our results have for least terns, as a species of statewide conservation concern in Georgia and in many other eastern states, demand further attention to adverse health and reproductive effects in this species.

Figures:

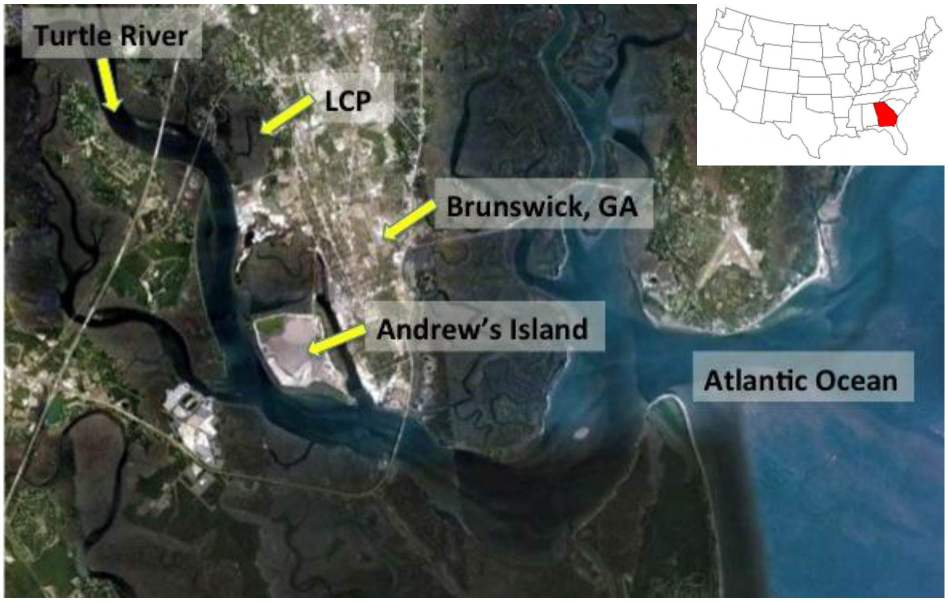

Figure 2.1. Location of Linden Chemical Plant (LCP) Superfund site and the Andrew's dredge spoil island in the Turtle River estuary in Brunswick, Georgia.

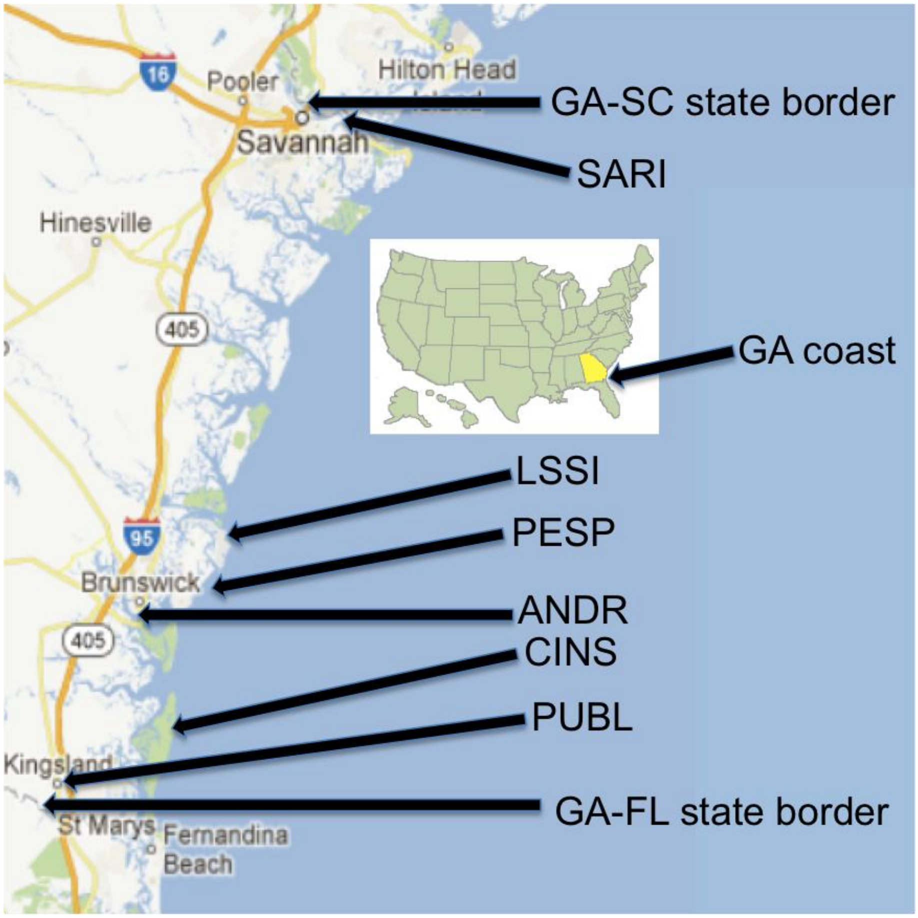

Figure 2.2. Locations of least tern nesting colonies along ~150 km of Georgia (GA) coastline: Savannah River dredge spoil area in the Savannah River estuary (SARI), Little St. Simon's barrier island (LSSI), Pelican Spit sandbar (PESP), Andrew's dredge spoil island in the Turtle River estuary (ANDR), Cumberland Island National Seashore barrier island (CINS), and the Publix grocery store rooftop in Kingsland, GA (PUBL). The locations of the state borders Georgia shares with South Carolina (SC) and Florida (FL) are also shown.

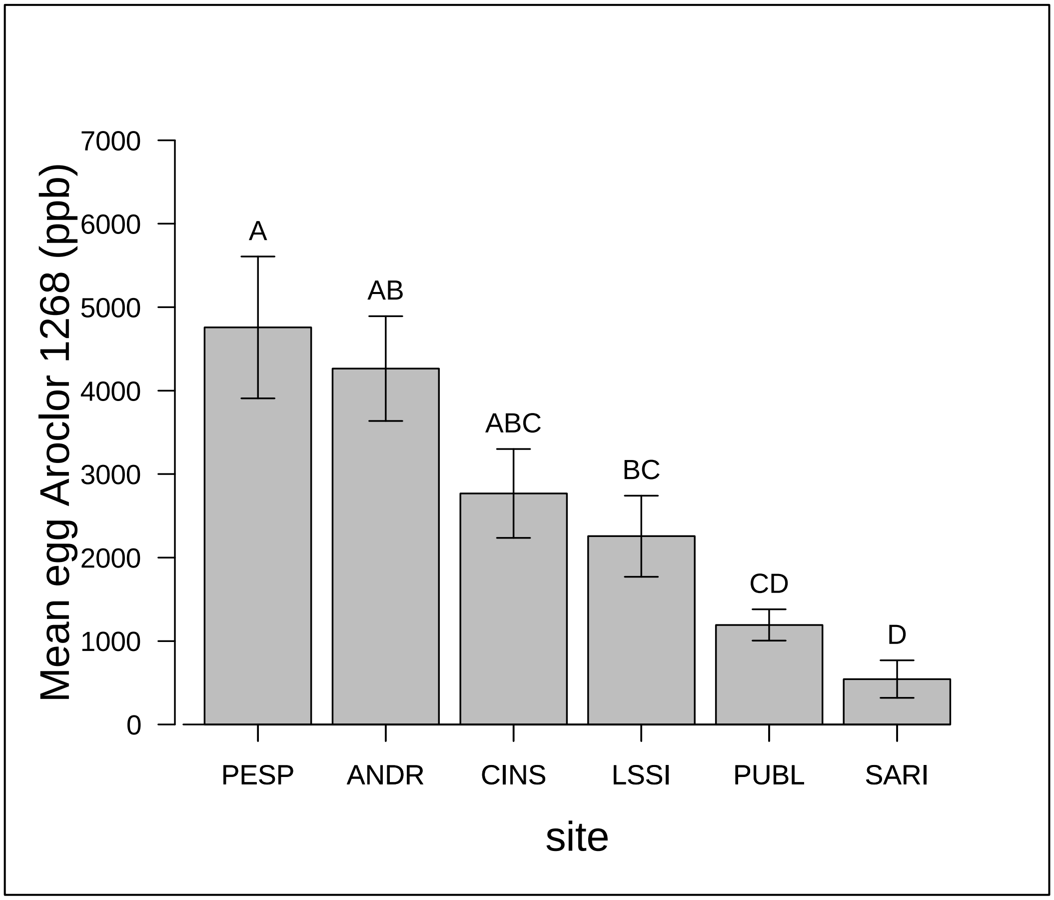

Figure 2.3. Mean total Aroclor 1268 concentrations in least tern eggs among colony sites in Georgia in 2011 and 2012. Colony sites, in order of increasing distance to the Linden Chemical Plant (LCP) Superfund site, were Andrew's dredge spoil island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI). Standard error bars are shown, and means that share a common letter are not statistically different, and those that do not are statistically different ($\alpha = 0.05$).

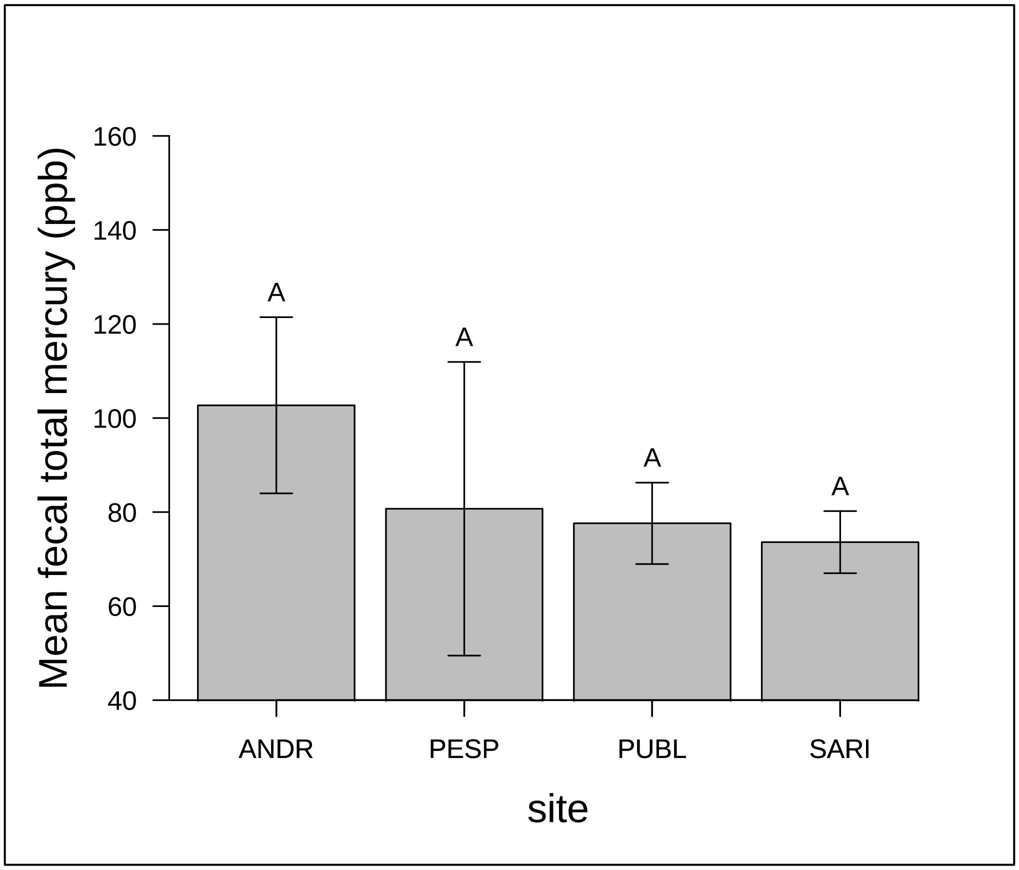

Figure 2.4. Mean total mercury concentrations in least tern chick feces across nesting colony sites in Georgia in 2011 and 2012. Colony sites, in order of increasing distance to the Linden Chemical Plant (LCP) Superfund site, were Andrew's dredge spoil island (ANDR), Pelican Spit sandbar (PESP), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI). Standard error bars are shown, and means that share a common letter are not statistically different, and those that do not are statistically different ($\alpha = 0.05$).

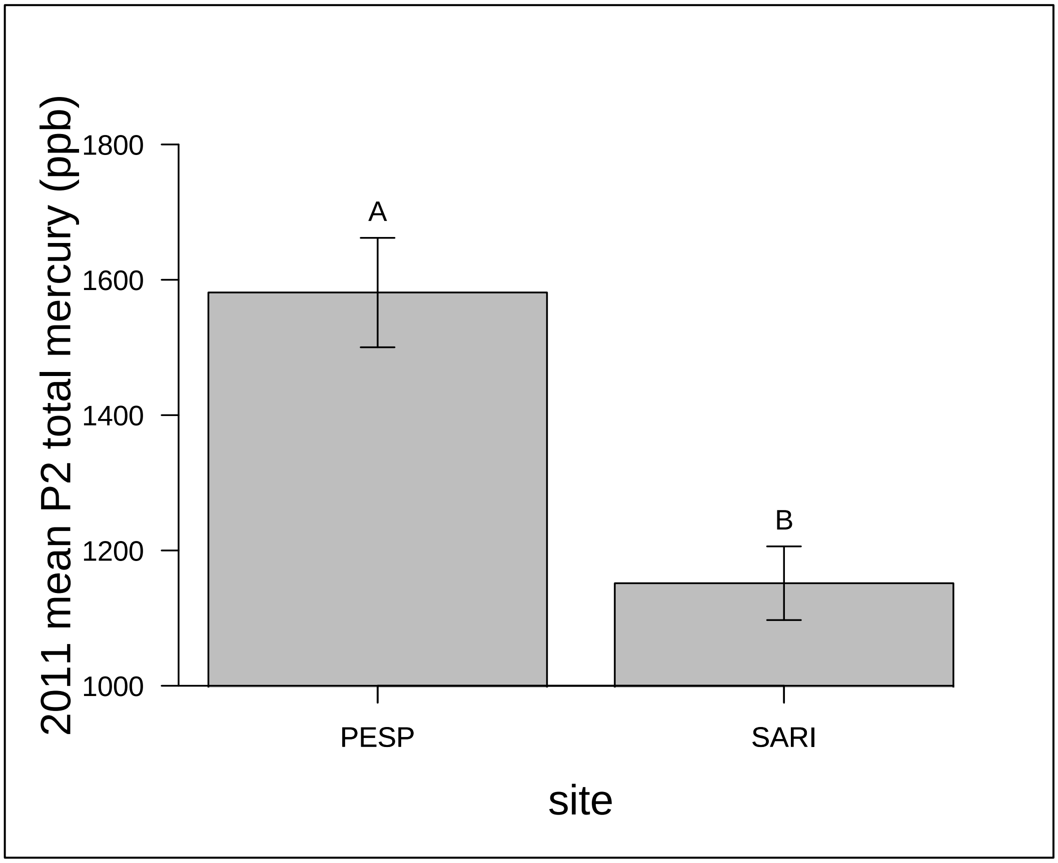

Figure 2.5. Mean total mercury concentrations in least tern chick second primary (P2) feathers among nesting colony sites in Georgia in 2011. Colony sites, in order of increasing distance to the Linden Chemical Plant (LCP) Superfund site, were Pelican Spit sandbar (PESP) and the Savannah River dredge spoil area (SARI). Standard error bars are shown, and means that share a common letter are not statistically different, and those that do not are statistically different ($\alpha = 0.05$).

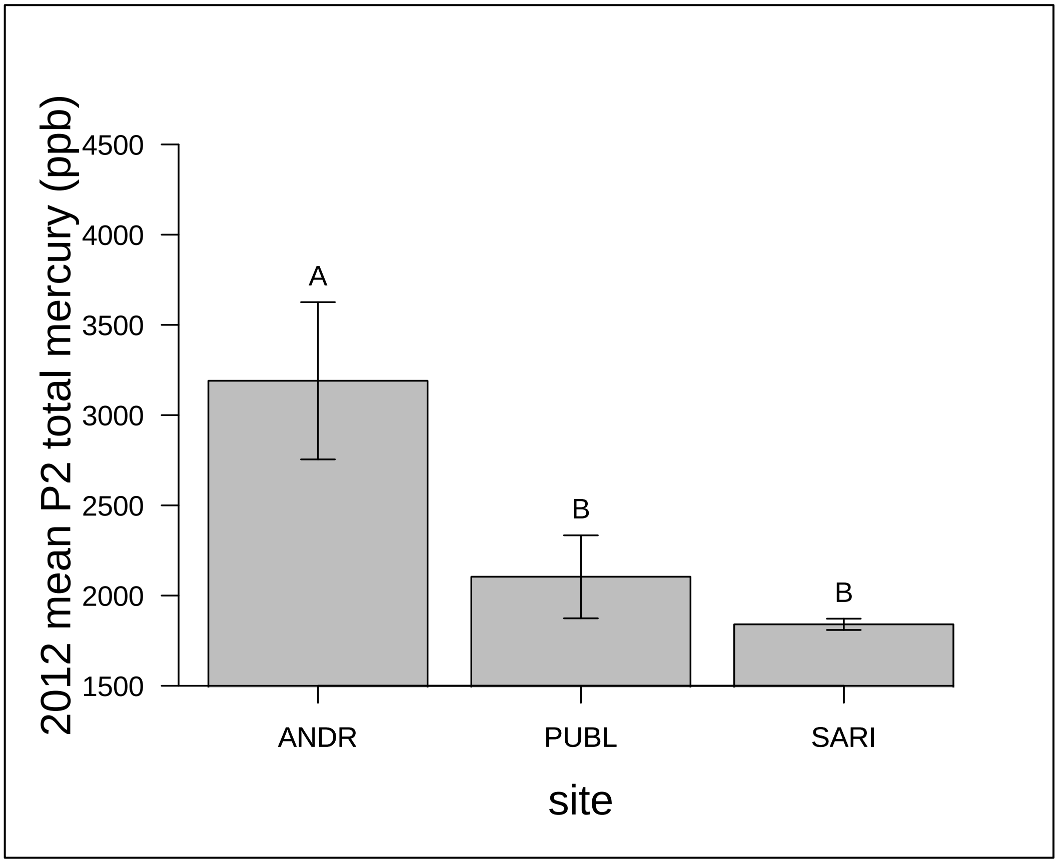

Figure 2.6. Mean total mercury concentrations in least tern chick second primary (P2) feathers among nesting colony sites in Georgia in 2012. Colony sites, in order of increasing distance to the Linden Chemical Plant (LCP) Superfund site, were Andrew's dredge spoil island (ANDR), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI). Standard error bars are shown, and means that share a common letter are not statistically different, and those that do not are statistically different ($\alpha = 0.05$).

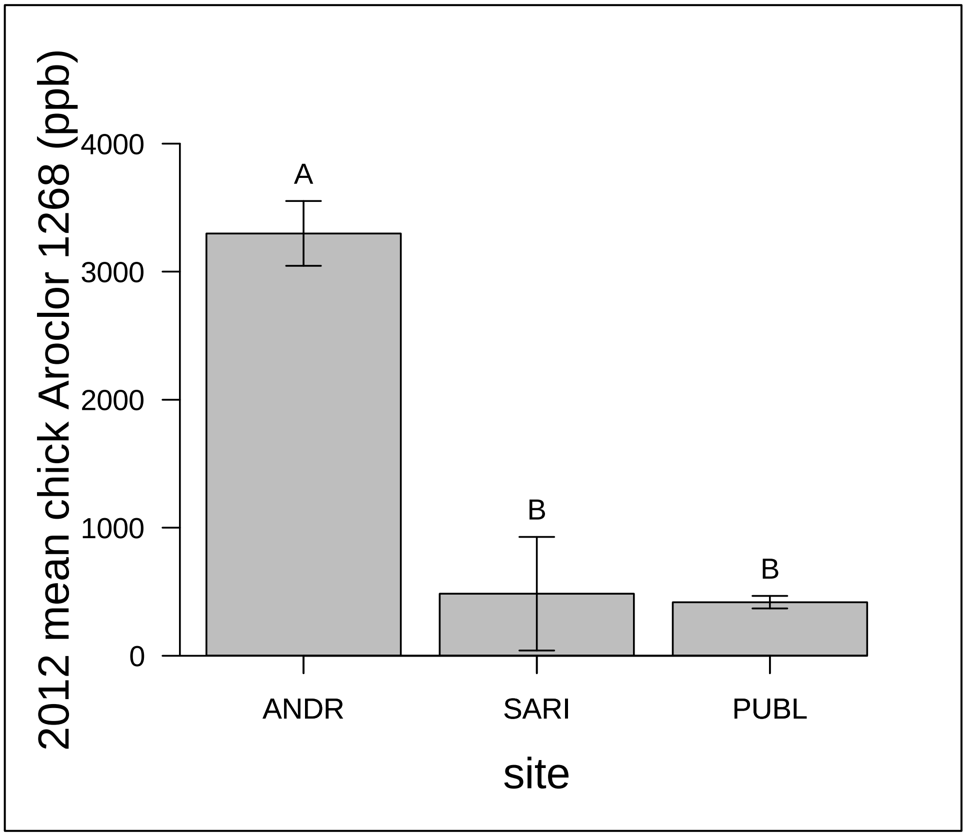

Figure 2.7. Mean total Aroclor 1268 concentrations in least tern chick carcasses in among nesting colony sites in Georgia in 2012. Colony sites, in order of increasing distance to the Linden Chemical Plant (LCP) Superfund site, were Andrew's dredge spoil island (ANDR), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI). Standard error bars are shown, and means that share a common letter are not statistically different, and those that do not are statistically different ($\alpha = 0.05$).

Tables:

Table 2.1. Mean concentrations (ppb) of Aroclor 1268 congeners in least tern eggs among nesting colony sites in Georgia in 2011 and 2012. Congeners are presented in the Ballschmiter-Zell (BZ) numbering system. Colony sites, in order of increasing distance to the Linden Chemical Plant (LCP) Superfund site, were Andrew's dredge spoil island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI).

Site	**[BZ-187]**	**[BZ-202]**	**[BZ-201]**	**[BZ-180]**	**[BZ-200]**	**[BZ-196]**	**[BZ-208]**	**[BZ-207]**	**[BZ-194]**	**[BZ-206]**	**[BZ-209]**
ANDR	895	364	75	149	796	640	302	82	196	681	85
PESP	1,009	446	80	176	839	707	316	82	228	788	89
LSSI	533	175	49	93	445	322	123	38	97	347	35
CINS	496	232	76	99	540	541	401	58	131	621	64
PUBL	210	109	24	231	172	148	62	19	61	142	15
SARI	135	80	20	64	149	135	50	20	52	232	19

Table 2.2. Summary of total Aroclor 1268 concentrations in least tern eggs among nesting colony sites in Georgia in 2011 and 2012; *n* is the sample size in number of eggs analyzed, and distance from each nesting colony to the Linden Chemical Plant (LCP) Superfund site is listed. Colony sites were Andrew's dredge spoil island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI).

Site	**Distance from LCP (km)**	***n***	**egg [Aroclor 1268] (ppb) RANGE**	**egg [Aroclor 1268] (ppb) MEDIAN**	**egg [Aroclor 1268] (ppb) MEAN**
2011					
ANDR	5	10	2,183 - 5,054	3,468	3,611
PESP	30	20	1,310 - 14,580	2,869	4,758
LSSI	40	16	619 - 7,062	1,364	2,257
CINS	42	19	13 - 9,125	1,991	2,624
SARI	110	6	13 - 1,838	303	481
2012					
ANDR	5	13	1,294 - 16,329	3,962	4,769
CINS	42	1	-	-	5,525
PUBL	70	9	445 - 2,158	1,303	1,193
SARI	110	10	192 - 3,395	284	538

Table 2.3. Summary of total mercury concentrations ([THg]) in least tern eggs among nest colony sites in Georgia in 2011 and 2012; *n* is the sample size in number of eggs analyzed, and distance from each nesting colony to the Linden Chemical Plant (LCP) Superfund site is listed. Colony sites were Andrew's dredge spoil island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI).

Site	**Distance from LCP (km)**	***n***	**egg [THg] (ppb) RANGE**	**egg [THg] (ppb) MEDIAN**	**egg [THg] (ppb) MEAN**
2011					
ANDR	5	10	488 - 773	719	687
PESP	30	20	351 - 2,290	964	1080
LSSI	40	16	404 - 1,340	879	874
CINS	42	19	350 - 1,379	893	846
SARI	110	22	362 - 2,360	599	736
2012					
ANDR	5	33	773 - 2,190	1,130	1,169
LSSI	40	3	1,590 - 2,810	2,470	2,290
CINS	42	4	1,450 - 1,860	1,775	1,715
PUBL	70	18	338 - 1,780	697	830
SARI	110	25	764 - 3,770	1,050	1,221

Table 2.4. Summary of total mercury concentrations ([THg]) in least tern chick feces among nest colony sites in Georgia in 2011 and 2012; *n* is the sample size in number of eggs analyzed, and distance from each nesting colony to the Linden Chemical Plant (LCP) Superfund site is listed. Colony sites were Andrew's dredge spoil island (ANDR), Pelican Spit sandbar (PESP), Cumberland Island National Seashore barrier island (CINS), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI).

Site	**Distance from LCP (km)**	***n***	**fecal [THg] (ppb) RANGE**	**fecal [THg] (ppb) MEDIAN**	**fecal [THg] (ppb) MEAN**
2011					
PESP	30	10	22 - 358	49	81
CINS	42	2	6 - 9	7	7
PUBL	70	4	23 - 52	33	35
SARI	110	20	34 - 203	56	80
2012					
ANDR	5	21	23 - 417	81	103
PUBL	70	25	14 - 175	66	84
SARI	100	20	14 - 158	58	67

Table 2.5. Summary of total mercury concentrations ([THg]) in least tern chick second primary feathers (P2) among nest colony sites in Georgia in 2011 and 2012; *n* is the sample size in number of eggs analyzed, and distance from each nesting colony to the Linden Chemical Plant (LCP) Superfund site is listed. Colony sites were Andrew's dredge spoil island (ANDR), Pelican Spit sandbar (PESP), Cumberland Island National Seashore barrier island (CINS), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI).

Site	**Distance from LCP (km)**	***n***	**P2 [THg] (ppb) RANGE**	**P2 [THg] (ppb) MEDIAN**	**P2 [THg] (ppb) MEAN**
2011					
PESP	25	17	1,030 - 2,260	1,540	1,581
SARI	110	20	832 - 1,660	1,115	1,152
2012					
ANDR	5	11	715 - 5,950	3,080	3,190
PUBL	70	20	873 - 4,360	1,830	2,105
SARI	110	29	1,570 - 2,280	1,820	1,841

Table 2.6. Summary of total mercury concentrations ([THg]) and total Aroclor 1268 concentrations in least tern chick whole body (carcasses) among nest colony sites in Georgia in 2012; *n* is the sample size in number of carcasses analyzed, and distance from each nesting colony to the Linden Chemical Plant (LCP) Superfund site is listed. Colony sites were Andrew's dredge spoil island (ANDR), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI).

Site	**Distance from LCP (km)**	***n***	**body [THg] (ppb) RANGE**	**body [THg] (ppb) MEDIAN**	**body [THg] (ppb) MEAN**	**body [Aroclor 1268] (ppb) RANGE**	**body [Aroclor 1268] (ppb) MEDIAN**	**body [Aroclor 1268] (ppb) MEAN**
ANDR	5	5	820 - 1,090	939	937	2,643 - 3,906	3,465	3,299
PUBL	70	5	294 - 1,260	569	631	308 - 574	443	418
SARI	110	5	598 - 1,000	823	789	0 - 2,255	0	484

CHAPTER 3

SUBLETHAL EFFECTS ASSOCIATED WITH MERCURY AND PCB EXPOSURE IN LEAST TERNS [1]

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[1] Robinson, G.L., Mills, G.L., Schweitzer, S.H., Hernandez, S.M. To be submitted to *Environmental Pollution*

Abstract

We studied six least tern (*Sternula antillarum*) nesting colonies on the Georgia coast to assess the impacts of contamination on least tern health and reproduction. Least terns are piscivorous, colonially nesting, migratory seabirds, that forage close (typically within 5 km) to breeding colonies. One least tern nesting colony in Georgia is located in the same estuary as the Linden Chemical Plant (LCP) Superfund site. An estimated 200 tons of mercury and the highly-chlorinated polychlorinated biphenyl (PCB) Aroclor 1268, which are persistent pollutants that biomagnify in the food web, were released at LCP from 1919-1994. We investigated the relationship between biomarkers including egg volume and weight, eggshell thickness, prevalence of congenital deformities, fluctuating asymmetry, chick growth rate, and lymphocyte estimates and differential counts, with concentrations of mercury and PCBs in feces, feathers, and eggs, collected during the 2011 and 2012 nesting seasons. Symmetry in chick wing chord and second primary feather decreased significantly with increasing feather mercury concentration, associating mercury exposure with developmental instability in our study. Heterophil/lymphocyte ratios also decreased significantly with increasing mercury concentrations in chicks, associating mercury exposure with immunosuppression in least terns. We also found that increased prevalence (2.9%, *n* = 7) of congenital deformities (especially limb deformities) at one colony appeared to be linked to mercury. Least tern in Georgia suffer sublethal health effects associated with contamination.

Introduction

Persistent organic pollutants (POPs) and heavy metals are environmental contaminants that both bioaccumulate and biomagnify, resulting in their fixture in the ecosystems they pollute long after their introduction[69]. Polychlorinated biphenyls (PCBs) are one type of POP strictly anthropogenic in source, often released into the environment via industrial practices[68]. In contrast, some heavy metals, such as mercury, have natural sources (e.g., volcanic eruptions), but are present in elevated concentrations in most areas due to anthropogenic contamination (e.g., coal-fired power plants)[69]. PCBs and mercury have been shown to have adverse effects on the health, reproduction, and survival of many organisms, including humans and birds[69]. While the release of these toxic substances has been dramatically curbed in the U.S. since the early 1970s, concurrent with the establishment of the Environmental Protection Agency (EPA) and the passing of landmark environmental legislation, their persistence and propensity to accumulate in biota has made them a continuing threat to ecosystem health to date.

Piscivorous seabirds, in particular, tend to harbor some of the highest concentrations of persistent pollutants, largely due to their high trophic position in marine food webs, longevity, and wide geographical range that exposes them to high rates of contaminant exposure[87]. For example, gull and tern species have been shown to have PCB body burdens comparable to those of bald eagles (*Haliaeetus leucocephalus*), a bird of an even higher trophic position that feeds on both fish as well as other fish-eating birds[162]. The young of colonial seabirds most accurately reflect local pollution because they are fed locally before fledging, and have not yet had time to concentrate contaminants from abroad as adults with broad ranging patterns can. In addition, colonial seabirds are easy to sample because eggs and flightless chicks in nesting colonies are easy to access and concentrated in number.

While lethal doses of toxic substances are usually easily demonstrated in laboratory studies, and acute toxicity has a clearly observable outcome in organisms (mortality), sublethal effects from subacute doses of the same chemicals can be more difficult to discern. Subacute doses are most often encountered by wild animals in the environment; thus, understanding sublethal effects, in addition to acute toxicity, is critical when investigating wild populations exposed to environmentally relevant concentrations of contaminants. Sublethal effects of organochlorine and heavy metal exposure in birds include decreased egg production, reduced egg volume and weight, thinning of eggshells, abnormal incubating behavior, lowered hatch success due to embryo death and decreased fertility of eggs, physical and physiological abnormalities in offspring, high nestling mortality, decreased nestling growth rate, abnormal development of sex organs, and immune system disorders[71-76]. Most quantitative studies on the effects of different rates of PCB or mercury exposure on acute or chronic toxicity have taken place in laboratories, and mostly on gallinaceous species (not necessarily representative of most species). In particular, much laboratory work has been performed on chickens (*Gallus gallus*), even though chickens appear to be more highly sensitive when compared with other avian species[77]. The degree of sensitivity to PCBs and heavy metals in birds appears to be species-specific, dependent both on dose and the form the contaminant takes at the time of exposure[69]. While experimental laboratory studies of the effects of contaminants in birds have provided invaluable and foundational rationale upon which to base field research, more information is needed on the effects of sublethal, environmentally relevant doses on wild bird populations, and how exposure relates to impaired reproductive success and survival in wild birds.

Here we investigate the relationship between various health and reproductive parameters of least tern (*Sternula antillarum*) chicks, and the contaminant burden carried by chicks, on coastal Georgia. Least terns are long-lived, colonial-nesting, fish-eating seabirds, that forage within close proximity (typically < 5 km in Georgia) to their nesting colony[2]. These attributes make least terns likely to have elevated exposure to contaminants (due to diet), a high accumulation of contaminants stored in tissues (due to longevity), and chicks whose contaminant loads reflect local pollution (due to localized foraging habits during breeding).

The Georgia coast has a history of environmental pollution, particularly in Brunswick, Georgia’s industrial areas. The LCP Superfund site in Bruswick, GA, consists of about 220 ha adjacent to the Turtle River estuary, which empties into the Atlantic Ocean. The site was an active industrial site from 1919 until 1994, when it was closed down and declared a “Superfund” site. Superfund sites are those on the Environmental Protection Agency’s (EPA) National Priorities List as areas prioritized for remediation. A coal-, and later oil-fired petroleum refinery, an oil-fired power plant, a paint and varnish manufacturer, and for the last 40 years a chlor-alkali facility, were all in operation at LCP and responsible for the release of lead, mercury, polycyclic aromatic hydrocarbons (PAHs), and PCBs into the Turtle River[129]. One of the biggest offenders was the chlor-alkali manufacturer, LCP Chemical, that released mercury via the mercury cell process involved in chlor-alkali manufacturing, as well as the unique and highly-chlorinated PCB Aroclor 1268 used to lubricate equipment. LCP is the only company in the eastern U.S. to purchase and use Aroclor 1268 from its sole manufacturer, Monsanto Chemical Company. Hence, when Aroclor 1268 is found in biota on the east coast of the

U.S., its origin can be traced back to Brunswick, Georgia[131]. Most of the pollution at the LCP site consisted of large quantities of Aroclor 1268, as well as an estimated 200 tons of mercury released into the Turtle River estuary[129]. Remediation efforts at LCP, involving the removal of some of the most highly-contaminated soil off-site, occurred between 1994-1999, and groundwater remediation feasibility assessments are still ongoing[129].

Despite remediation, Aroclor 1268 congeners and mercury are still being recovered from sediments and organisms near LCP. Fish tissue collected in the late 1990s from the Turtle River had concentrations of Aroclor 1268 congeners several orders of magnitude higher than concentrations in fish from reference sites[130,132]. Aroclor 1268 has also been quantified in a few lower trophic-level bird species at LCP, including red-winged blackbirds (*Agelaius phoeniceus*), mottled ducks (*Anas fulvigula*), and boat-tailed grackles (*Quiscalus major*)[130], and extensively in clapper rails (*Rallus longirostris*)[131,136-140]. Most recently, Aroclor 1268 concentrations in bottlenose dolphins (*Trusiops truncatus*) within ~40 km of LCP exceeded PCB concentrations on record in any cetacean worldwide[141,142]. However, the extent to which fish-eating birds in this area are exposed to Aroclor 1268 has not been published.

In addition to pollution associated with LCP, the Savannah River in north Georgia has a long history of industrial pollution. According to the EPA's Toxic Release Inventory (TRI), the Savannah River is the fourth most polluted river in the United States[163], and elevated concentrations of POPs have been found recently in fish offshore from Savannah, Georgia[164,165].

PCBs are strongly lipophilic, partition almost entirely to fat tissues in organisms, and can be stored and accumulate over long periods of time[69]. Mercury partitions most strongly to proteinaceous tissues, including body organs and muscle, and is also sequestered in keratinous structures such as hair or feathers[103]. Because eggs are high in both protein and lipid, egg production is a major elimination pathway for both mercury and PCBs in birds[101,154]. For example, adult gulls (closely-related to terns) will deposit up to 40% of their total body burden of mercury into an egg within days of laying it[102] and Arctic terns (*Sterna paradisaea*) will deposit 45% of their total body burden of PCBs into their eggs[166]. Therefore, contaminant loads in eggs can be a proxy for contaminant loads in adult female birds. However, most of the contaminant load found in seabird chicks (excluding newly-hatched chicks) is attributed to contaminated food ingestion, while the proportion attributed to contribution from the parent (egg) is negligible due to the growth dilution effect[103,104]. As a chick grows, the contaminant load inherited by the chick from the parent contributes less and less to the total body burden of contaminants in the chick, and contaminant ingestion by the chick becomes the primary contributing source to the total body burden. Meanwhile, overall contaminant concentration in the chick is diluted by rapid growth during the nestling period[105]. Thus, tissue samples taken from older chicks better reflect local contamination than do samples (such as eggs) taken from adults.

In addition to egg production, feather growth is one of the primary mercury depuration pathways in birds. For example, seabird adults and chicks may sequester 93% and 91%, respectively, of their total mercury body burden into feathers[167]. Chick feathers in particular are ideal indicators of local mercury pollution, because chicks grow contour feathers (which include body, wing, and tail feathers) throughout the nestling period. During feather growth, there is a constant blood supply to each feather until it is fully-grown, and mercury is deposited continuously into feathers via the bloodstream[112]. Therefore, a feather taken from a chick just before fledging represents an archive of that individual's mercury exposure throughout its life up until that point, and that exposure results exclusively from mercury ingestion from the natal habitat. Birds will also excrete mercury in feces[108], so fecal samples can reflect current mercury load in seabird chicks.

Developing a contaminant exposure monitoring scheme appropriate for least terns, and determining what degrees of contaminant exposure adversely affect health and productivity in least terns, is critical for least tern management. This information should be an integral part of habitat and resource management for least terns for several reasons. First, the least tern is a species of conservation concern that is either federally endangered, state-listed, or otherwise in decline, across its range and in all three subspecies[2]. Therefore, even subtle impacts to the population, such as sublethal effects that impair productivity, may be consequential. Second, it is likely that least terns have an elevated degree of exposure to contaminants due to their high trophic position. Finally, most least tern natural nest habitat has been lost or degraded due to coastal development, human disturbance, invasive or subsidized predators, or damming and controlled release of water resulting in flooding of nesting colonies[2]. Because of loss of suitable natural habitat, least terns are often encouraged by management practices to breed on artificial habitat such as on manmade islands[30] created in contaminated rivers (e.g., Platte River in Nebraska[37,168]), or on dredge spoil islands[26] and rooftops[27] located in urban areas, which may further increase their exposure to contaminants.

We aimed to determine if health and reproductive biomarkers (eggshell thickness, egg volume and fresh weight, fluctuating asymmetry, prevalence of teratogenicity, and immune function parameters) were affected by contaminant exposure to inform monitoring schemes and management decisions for least terns in relation to contaminated habitat. Sublethal effects of contaminants on least terns are a conservation concern.

Although only high dietary concentrations of PCBs can cause eggshell thinning[169], much lower concentrations of other organochlorines, such as DDE, have been responsible for devastating reductions in eggshell thickness in wild birds[170]. Furthermore, the effects of egg PCB and DDE concentrations on eggshell thinning have often been indistinguishable in past field studies[171], warranting further investigation into the effects of PCBs on eggshell thickness. In addition, dietary exposure to methylmercury in the laboratory has caused birds to lay shell-less eggs[172]. Therefore, we hypothesized that there may be a reduction in eggshell thickness in relation to mercury and PCB exposure. We hypothesized that egg fresh weight and volume may be decreased in eggs contaminated with PCBs and mercury[172]. Growth rate in birds may decrease with mercury exposure[173,174], so we hypothesized we may see a decreased growth rate in chicks with increased mercury exposure. Leukocyte estimates and differential counts are immune function parameters that can be used to indicate stress in birds. In general, leukocytosis (or increased leukocytes in the blood) occurs when an immune response is mounted, and in birds usually indicates inflammation, toxicosis, hemorrhaging, or leukemia. However, the differential presence of the various types of leukocytes can also aid diagnostics. For example, monocytes are primarily involved in phagocytosis, heterophils fight bacterial infection, eosinophils fight parasitic infection, and lymphocytes are integral in T-cell- and antibody-mediated responses. Immune function can be compromised with exposure to environmental contaminants[75]. For example, several parameters of immunosuppression, including heterophil/lymphocyte ratios, were significantly correlated with PCB-exposure in Great Lakes Caspian terns[118]. Therefore, we expected that leukocyte estimates and differential counts would be influenced by contaminant exposure in our study.

Fluctuating asymmetry (FA) is the non-directional deviation from perfect symmetry between two complementary morphological features on a bilaterally symmetrical organism[175]. It is caused by developmental instability due to environmental stress. Tarsus, wing chord, and primary feather lengths are common measurements used to measure the symmetry of living birds in the field. FA increases significantly in birds with environmental stress associated with nutritional quality[126], antigen exposure[176], habitat disturbance[177], and forest fragmentation[178]. In addition, FA is shown to increase with increasing exposure to environmental pollution. For example, there was a positive correlation between FA in the third primary feather and concentrations of several organochlorines, including one PCB, in the blood of glaucous gulls (*Larus hyperboreus*) breeding in the Arctic.[124]. Similarly, the degree of FA in retrix feather mass in great tits (*Parus major*) in a site polluted with heavy metals was significantly higher than those in reference sites[126]. Therefore, we expected FA in least tern tarsus, wing chord, and primary feather measurements, to increase with increasing contaminant load.

We chose this suite of parameters as biomarkers of contaminant exposure in least terns because all have been shown in previous avian studies to be affected by contaminant exposure, and they all can be sampled with minimally invasive procedures.

Contaminant concentrations and associated health effects can also be determined by euthanizing and collecting birds from the wild, and analyzing body organ contaminant concentrations, and identifying gross lesions associated with contaminant exposure. However, techniques that require the sacrifice of the study subjects are often impossible, or less desirable, when the subject is of conservation concern. Least terns are rare in Georgia, necessitating non-lethal and minimally invasive techniques for investigating contaminant loads and sublethal effects in this species.

Methods

Colony Sites

We monitored six least tern nesting colonies along the Georgia coast from May-August, 2011 and 2012 (Fig. 3.1). Cumberland Island National Seashore (CINS) and Little St. Simon's Island (LSSI) are both natural barrier island sites, and Pelican Spit (PESP) was a sandbar located ~1 km offshore from St. Simon's Island. These natural sites were remote from human disturbance, but susceptible to tidal flooding and storm surge. Andrew's Island (ANDR) and the Savannah River confined dredge disposal area (SARI) are both manmade dredge spoil sites and were not susceptible to tidal flooding or storm surge, but were in close proximity to urban centers. The Publix (PUBL) colony was located on top of a Publix grocery store rooftop in Kingsland, Georgia. The ANDR colony was in closest proximity (~5 km) from the LCP Superfund site, located in the Turtle River estuary (Fig. 3.2).

Nest Monitoring

We monitored nests and chicks at each colony every three days throughout the breeding season. We located nests and marked them with labeled tongue depressors placed ~ 2 m from the nest. We collected eggs throughout the breeding season in both years, usually following nest abandonment due to natural random events such as partial depredation, flooding and nest over-wash, or evening colony abandonment by adults fleeing from nocturnal predators (mostly great horned owls, *Bubo virginianus*). Therefore, no bias existed toward unhatched, unviable eggs. We wrapped collected eggs in aluminum foil and stored them frozen in plastic bags until processed. When a nest was found, we floated eggs to determine stage of incubation[179] . Nests that remained >10 days after the expected hatch date were also assumed abandoned and collected. Hatch date was estimated on a 21-day incubation period[2]. To estimate fresh weight and volume, we measured length and maximum breadth (to the 0.001 mm) of eggs when nests were found using digital calipers (Tresna 8 inch, 0-200 mm, high precision digital calipers, Series: EC10, ID: 110-202)[94]. We averaged together volume and fresh weight of multiple eggs from a single clutch together to represent a single egg volume and fresh weight value for each nest, just in case fresh weight and volume of eggs in a single brood might vary by order in which each egg was laid.

Eggshell thickness

In the lab, we carefully rinsed the shell of each collected egg with ultra-pure water, cut the eggs open along the vertical midline, and scraped out the yolk into a clean plastic tube using stainless steel dissecting tools. We cleaned dissection tools using 70% isopropyl alcohol and Kim-wipes between each egg dissection. If an embryo was discernible, we inspected it and recorded any visible abnormalities. We used dissecting tools, and a spray bottle filled with ultra-pure water, to rinse out any egg contents stuck to the insides of shells. We measured each half of the eggshell (to 0.015 mm) on three equidistant locations along the midline, using a Starrett® dial indicator pocket gauge (micrometer) No. 1010M (9 mm). We used the average of these six measurements to represent the thickness measurement for each egg.

Blood smears

We bled each chick once, typically at ~10-15 days old, via the brachial artery, using 30-gauge bevel-tipped needles. We drew several drops of blood up into heparinized microhematocrit capillary tubes, and created blood smears using a drop of blood on a microscope slide, in standard manner[180]. We fixed blood smears with methanol several hours later. We sent blood smears to Dr. Carolyn Cray, University of Miami's Comparative Pathology Laboratory, for analysis. Blood smears were analyzed by a single observer, for hemoparasites, leukocyte estimates, and leukocyte differential counts.

Chick samples, growth, and morphometric measurements

Upon hatching, we banded each chick with a uniquely-numbered USGS Bird Banding Lab aluminum leg band to identify each chick throughout the nestling period. We collected fecal samples from chicks opportunistically throughout the nestling period during routine chick handling, and stored them frozen in plastic centrifuge tubes. Fecal samples were not collected if they were contaminated with substrate from the site. We collected the left and right second primary feather (P2) of each chick when primaries were > 50% grown in, typically within a few days of fledging age, ~15-18 days of age. We also collected chicks found dead for contaminant analysis.

We took morphometric measurements of chicks at least once, typically on the same day that feathers were collected. We took complementary measurements of tarsus length, nares, wing chord, and web length of collected P2 feathers, on each chick. We used high precision digital calipers to measure tarsus, nares, and P2 lengths (to the nearest 0.001 mm). A wing ruler was used to measure wing chord to the nearest 0.5 mm. To minimize observer error, a single observer took all morphometric measurements used in the symmetry analysis. We weighed (to 0.1 g) chicks on each visit (at 3-day intervals) using a digital scale. We plotted chick growth rates and found them to be linear until about 15-16 days of age, at which point growth plateaued. Therefore, we used the first 15 days of growth for each chick to calculate a slope that was used to represent the growth rate for each chick.

Contaminant analysis

We quantified total mercury concentrations in eggs, feathers, feces, and chick carcasses, and total PCBs and individual Aroclor 1268 congeners in eggs and chick carcasses, according to previously described methods (see chapter 2).

Egg fresh weight and volume estimates

We estimated fresh weight (g) and volume (cc) of eggs using a previously-established method[94] which multiplies a predetermined constant (K) by egg length (L) and maximum breadth (B) squared: $K \, x \, L \, x \, B^2$, to estimate egg fresh weight and volume within 2%. We used K_v (constant used for volume) and K_w (constant used for fresh weight) values established for white terns (*Gygis alba rothschildi*), which was the closest species to least terns for which we could find K_v and K_w values already established from previous work (K_v = 0.520, and K_w = 0.546[94]).

Statistical analysis

Many of our parameters were ratios or contaminant concentrations, and were therefore not normally distributed. All non-normal parameters were successfully transformed to fit a normal distribution and meet the assumptions required for parametric statistical analysis. We used a square root transformation for concentrations of mercury and Aroclor 1268 in eggs, as well as for eggshell thickness measurements. A log10 transformation normalized mercury concentrations in feathers, chick whole bodies, and feces. We calculated % perfect symmetry between complementary measurements (tarsus length, wing chord, and primary 2 feather web length) for each chick. All symmetry ratios, as well as the blood heterophil/lymphocyte ratio, were normalized using an arcsine-square root (*180/pi) transformation.

We used a linear regression model and forward selection to test the effects of colony site, year, and contaminant concentrations in various sample types, on each response variable. First we confirmed that there was no interaction between sampling year and any of the other predictor variables, before moving on to compare models. For eggs, we tested the effects of site, year, distance from LCP, total egg mercury concentration, and egg Aroclor 1268 concentration, on egg fresh weight and volume, and eggshell thickness. For chicks, we tested the effects of site, year, distance from LCP, and feather and fecal total mercury concentrations, on growth rate, symmetry ratio of tarsus length, wing chord, nares, and P2 web length, as well as on leucocyte estimates, heterophil, lymphocyte, monocyte, eosinophil, and basophil differentials, and on heterophil/lymphocyte ratio. We used R statistical computing program[150] for all statistical analyses, and a significance level of $\alpha = 0.05$.

Results and discussion

Teratogenic deformities in chicks

We found chicks at ANDR and SARI in 2012 that had physical deformities, mostly of the hind limbs, but also one with curvature of the spine (Figure 3). All but two of the deformed chicks were found on hatch-day or within one day of hatch-day, and not seen again thereafter. The two that survived beyond hatch-day had clubbed feet but were mobile, but neither survived to fledge. We presumed that all deformed chicks were either depredated, or died due to internal problems, because we searched the colony and the perimeter for chicks thoroughly on each visit. The chicks with splayed legs or the spine curvature were unable to ambulate when we found them. If chicks died at the nest site, the parent birds may have carried them off site (nest sanitation), such that we would not have recovered their bodies. Because deformed chicks disappeared soon after hatching, we were unable to collect fecal or primary feather samples from them. However, we analyzed breast feathers from two recently hatched sibling chicks at the SARI colony; one had severely splayed legs and the other appeared normal, but neither survived. Total mercury concentrations in the down feathers of these chicks were 7,030 ppb in the deformed individual, and 9,490 ppb in the sibling of the deformed individual. We analyzed whole bodies of two other chicks found on hatch-day: one had clubbed feet and difficulty breathing and was euthanized, and the other died naturally just after hatching. These chicks had whole body mercury concentrations of 2,770 ppb and 3,590 ppb, respectively, yet total PCB (including Aroclor 1268) concentrations were relatively low in both chicks (277 ppb and 501 ppb, respectively).

We found a 2.9% rate (7 of 238 chicks) and a 0.01% rate (1 of 145 chicks) of teratogenic deformities in chicks in 2012 at SARI and ANDR, respectively. The 2.9% rate of deformities at SARI is high relative to other studies that reported high rates of physical deformities associated with highly-polluted areas (e.g., 0.5% - 2.5% of terns and other waterbird species in the Great Lakes region and in the New York Bight were deformed[72,181]), and the 0.01% rate of deformity at ANDR is high when compared with a reported background prevalence of 0.005% (or 1 in 20,000)[86]. According to EPA's recent Toxic Release Inventory (TRI), the Savannah River (location of SARI colony) is the fourth most polluted river in the United States[163], and PCBs, metabolites of dichlorodiphenyltrichloroethane (DDT), and polycyclic aromatic hydrocarbons (PAHs) have been found in sediment and fish from the Savannah River estuary[165]. Because the Savannah River is highly polluted and birds there are likely exposed to other contaminants, and because most deformed chicks did not survive long enough to provide samples, we cannot confirm causality in this case. However, because the eggs analyzed with the highest concentrations of mercury across sites were from the SARI colony (up to 3,770 ppb), and due to the high concentrations of mercury in the feathers and whole bodies of the few deformed chicks we were able to sample, mercury appears to be associated with the high rate of physical deformities we observed. We recommend a further investigation into the cause of physical deformities in least terns at the SARI site, currently the largest nesting colony of least terns in the state of Georgia, that is managed specifically to attract breeding least terns for mitigation purposes.

Fluctuating Asymmetry in Chicks

We investigated effects of collection site, collection year, distance to LCP, primary 2 feather (P2) mercury, and fecal mercury, on the developmental symmetry of the tarsus length, wing chord, nares, and P2 web length (Table 3.1). None of the predictor variables had an effect on tarsus or nares symmetry. WC symmetry increased significantly with increasing P2 feather mercury ($F_{1,89} = 5.373$, $p = 0.0227$) (Fig. 3.4), but was not affected by any of the other predictor variables. Because we found an effect collection year on P2 symmetry, it was added to all other models, but site, distance, and fecal mercury still had no effect on P2 symmetry. P2 feather mercury was the only other predictor variable that had an effect on P2 feather symmetry, and when P2 feather mercury and year were modeled together, year no longer had an effect. P2 feather symmetry (with year as a covariate) decreased significantly with increasing P2 feather mercury in chicks ($F_{1, 1, 92} = 6.8314$, $p = 0.0105$) (Fig. 3.5).

Fluctuating asymmetry (FA) is caused by instability during early development, and is often found in association with exposure to contaminants in birds. For example, fluctuating asymmetry in two insectivorous European songbirds (*Parus major* and *Ficedula hypoleuca*) increased significantly in the tarsus and third primary feather, but not in the outermost retrix (tail) feather, with downstream breeding proximity to a copper smelt[182]. Jenssen et. al found that European shag (*Phalacrocorax aristotelis*) had higher degrees of FA in wing bone length, in association with concentrations of several different PCB congeners in the liver, but that tarsus length did not appear to be affected by PCB concentrations[125]. Similarly, we found that increasing mercury concentrations in primary feathers were associated with an increase in FA in least tern wing chord and primary feather web length, but not tarsus or nares length. Although it is possible that tarsus and nares length symmetry are affected by mercury exposure in least terns, our study failed to demonstrate that. However, our results suggest that wing chord and primary feather web length asymmetry are more suitable biomarkers of mercury exposure than tarsus and nares symmetry in least terns.

Chick hematological parameters

Here we report leukocyte estimates and differential counts across colony sites in our Georgia least tern population, as no reference values for these parameters in least terns exist to our knowledge (Table 3.2). We found that least tern hematological parameters were similar to those documented for common terns (*Sterna hirundo*) in Massachusetts[183], fairy terns (*Sterna nereis*) in captivity (isis.org), and sooty terns (*Onychoprion fuscatus*) in Hawaii[184] (Table 3.3). While heterophils are the most abundant leukocyte in the blood of many domestic bird species, others such as most passerine species have naturally lower heterophil/lymphocyte (H/L) ratios[185], as would appear to be the case with terns (family Sternidae). Least terns in our study had fewer heterophils than lymphocytes in the blood (ratio of ~ 0.5:1), and this ratio is comparable to those found in common terns, fairy terns, and sooty terns. Although H/L ratios can vary depending on age, the tern studies we cite here include both adults and chicks, indicating that the low H/L is not an effect age in this case. No hemoparasites were found in any of our chicks, which is likely due to the short time chicks were exposed to insect vectors before sampled. However, adult common terns from Massachusetts were also free of hemoparasites[183], so it is possible the hemoparasites are less common in terns. Indeed, Ricklefs (1992) found that hemoparasite load appeared to be related to taxonomic group, as hemoparasite load was inversely related to incubation period, suggesting that altricial species have less capacity to resist hemoparasite infection than precocial species (e.g. terns)[186].

We assessed the effect of collection site, collection year, fecal mercury concentration, feather mercury concentration, and distance from LCP, on total white blood cell count estimates, as well as monocyte, lymphocyte, heterophil, basophil, and eosinophil differential counts (Table 3.4). We prepared and analyzed blood smears from 109 chicks at PESP, SARI, and PUBL, in 2011. Of the 109 chicks we measured blood parameters for in 2011, we measured fecal mercury for 27 and feather mercury concentrations for 34. We collected and analyzed blood smears for 131 chicks from ANDR, SARI, and PUBL, in 2012. Of the 131 chicks we measured blood parameters for in 2012, we measured fecal mercury for 47 and feather mercury for 59.

Leukocyte estimates:

Leukocyte estimates ranged from 1,000-15,000/µl, with a mean of 7,088/µl, and median of 7,000/µl. Both year ($F_{1, 238} = 16.509$, $p < 0.0001$) and site ($F_{3, 236} = 3.611$, $p = 0.0071$) affected chick leukocyte estimates; therefore, these variables were added to all other models as covariates. Feather mercury and fecal mercury had no significant effect on leukocyte estimates. Because not every colony fledged young in both years, we sampled different sites each year. Therefore, we analyzed leukocyte estimates by year to disentangle the effects of year and site. Site did not have a significant effect on leukocyte estimates in either 2011 ($F_{2, 106} = 0.4162$, $p = 0.6606$) or 2012 ($F_{2, 128} = 1.4242$, $p = 0.2388$). Similarly, when year and site were both included in the model, only year ($p < 0.0001$) remained a significant predictor variable while site was no longer significant ($p =$

0.3776). This ndicated that sampling year had a true effect on leukocyte estimates, while the difference in leukocyte estimates among sampling sites was just an artifact of differing sampling regimes between years. Mean leukocyte estimate in chicks was 7,569/µl in 2011, and 6,687/µl in 2012.

<u>Leukocyte differentials:</u>

None of the predictor variables (collection site, collection year, fecal mercury, feather mercury, or distance from LCP) affected percentages of lymphocytes or heterophils in the blood. Only three chicks had > 0% basophils, (two chicks with 1%, and one chick with 2% basophils). Only 0.8% ($n = 2$) of all chicks had > 2 % monocytes, and 91% of all chicks had 0% monocytes. Because basophils and monocytes were absent from most chicks, or were in a negligible proportion of total leukocytes, the existence of this variable in the population was insufficient for modeling. Monocytes and basophils are generally uncommon in avian peripheral blood, and our results are consistent with this norm[185]. Eosinophil % in blood varied significantly between years ($F_{1, 238} = 31.598$, $p < 0.0001$) and among sites ($F_{3, 236} = 4.9854$, $p = 0.0007$). Therefore, year and site were added to all other models as covariates, but no other variables affected eosinophils significantly. Year and site were correlated because only certain sites were sampled each year, so we tested effects of variables separately between years. Site had no effect on eosinophils in 2011 ($F_{2, 106} = 0.2808$, $p = 0.7557$) or in 2012 ($F_{2, 128} = 0.9256$, $p = 0.4305$), indicating that sampling year had the true effect on eosinophils and that the effect of site was just an artifact of sampling sites varying between years, as was the case with total leukocyte counts.

Heterophil/Lymphocyte Ratios:

Collection site, collection year, feather mercury, and distance from LCP, had no significant effect on heterophil/lymphocyte (H/L) ratio. However, H/L ratios decreased significantly ($F_{1, 61} = 5.6235$, $p = 0.0209$) with fecal mercury concentration in chicks (Fig. 3.6). In general, *elevated* H/L ratios are typically associated with a stress response in birds, and are a much more reliable indicator than absolute heterophilias (increase in heterophils only) or lymphopenias (decrease in lymphocytes only) are[187]. Heterophils and lymphocytes are the two most common leukocytes in avian blood, and the differential between these two cell types illustrates the difference between the immediate, fast-acting, generalized immune response (e.g., infection, injury, stress) of the heterophil, and a longer-term, antibody-mediated response of the lymphocyte[118]. An increase in heterophils (heterophilia) and a decrease in lymphocytes (lymphopenia), and the resulting increase in H/L ratio, are hematological hallmarks of an avian stress response[185,187]. This increase is particularly marked in species with normally low H/L ratios, as appears to be the case with the several tern species mentioned here, as well as least terns in the present study. Therefore, the decrease in H/L ratio we observed that was significantly related to increasing fecal mercury concentrations in chicks, could be an indication that chicks with elevated mercury exposure were unable to mount a proper response to the stress typically associated with being captured, handled, and bled[188].

Chick Growth

Plotting growth rates of individual chicks revealed that chick growth rates were linear up until the age of ~16 days, when growth began to plateau (Fig. 3.7). Because growth in the first 16 days was linear, we calculated the slope of the 16-day growth curve for each chick, and we used that slope to represent the growth response variable in our models. We calculated a 16-day growth curve for 135 chicks, of which 61 had associated feather mercury and 54 had associated fecal mercury. None of the predictor variables significantly affected chick growth through day 16 (Table 3.1). We also used chick weight at 17-19 days of age as a response variable in our models, upon which none of the predictor variables had a significant effect. Reduced food intake accompanied by decreased growth rate are both hallmarks of acute mercury toxicity in birds, and they are also generally associated with progressive weakness in the limbs, inability to fly or ambulate, and lack of coordination in muscle movements, ultimately leading to death [71,189]. We did not expect acute toxicity, which is most often demonstrated in laboratory dosing experiments, because least terns in our study were exposed to sublethal concentrations through the food web. However, mercury has been shown to reduce nestling growth rate in birds exposed to environmentally relevant, subacute concentrations, in the wild[173]. We did not see this in our study, which may mean that least terns are less sensitive to mercury exposure in regard to nestling growth, or that mercury concentrations our chicks were environmentally exposed to were no high enough to effect nestling growth. However, we could only monitor growth rate until fledging occurred (~ 20 days), and we suspect that least tern chicks may suffer abnormal weight loss after fledging occurs, in response to elevated mercury exposure. There were at least seven chicks in our study that were drastically underweight after 20 days of age; all but one (from PUBL) were from the SARI colony (where we also had the highest rate of teratogenic deformities). Least tern chicks will generally gain weight steadily throughout nestling, then peak at weights between ~ 36-42 g between 17-20 days, before losing a very small amount of weight just before fledging[2]. Most of our chicks followed this trend and generally weighed 37-40g just before fledging, but the mean weight and age of the seven chicks that were markedly underweight in our study was 32.8 g at 20.3 days of age. This is suggestive that weight loss associated with mercury exposure could occur in least terns near- or post-fledge, but our evidence is limited. The reason for a delayed growth rate response associated with mercury exposure could be that the majority of mercury being consumed by chicks during the nestling period is sequestered via the bloodstream into growing feathers, thereby decreasing the mercury circulating in the blood that could contribute to physiological changes that inhibit growth. For example, Spalding et. al found that great egret (*Ardea albus*) chicks dosed in captivity with methylmercury suffered appetite and weight loss only after feather growth was completed, even though their maximum mercury exposure occurred weeks before[174].

Because PCB concentrations (dominated by Aroclor 1268) in eggs decreased significantly with increasing distance from the LCP Superfund site (see chapter 2), we can consider distance to LCP (or colony site) a good proxy for relative PCB exposure to chicks. Our results indicate that PCB exposure did not significantly affect 16-day chick growth rate, because site and distance to LCP had no effect on nestling growth in our models. Most studies have shown that PCB exposure does not affect nestling growth rate[190-192]. Other studies show that, as with mercury toxicity, acutely toxic doses of PCBs cause the cessation of feeding and weight loss in birds shortly before death[77,193]. There have been mixed results in the literature however, regarding the effects of sublethal PCB doses on chick growth rate, most of which involve chickens in the laboratory. While some studies show no effect on body weight from PCB exposure[190], others found that chickens dosed with PCBs would experience decreased weight gain, but not always immediately. For example, Rehfeld (1971)[194] found that a 2.5-week PCB dosing regiment caused depressed weight gain, but Platonow and Funnell (1971)[195] found an effect on weight gain only after 6-9 weeks of dosing. Furthermore, a study on American kestrels exposed in ovo to PCBs showed an *increase* in growth during the offspring's first year, but only in males. Thus, while most studies indicate there should be no change in growth rate with PCB exposure, the 16-day period before fledging during which our least tern chick growth rates were measured may not have been ample time to demonstrate depression in growth rate after prolonged exposure to PCBs.

Eggs

<u>Fresh weight and volume:</u>

We measured egg fresh weight and volume from 840 nests in 2011 and 834 nests in 2012. Egg fresh weight and volumes were averaged for eggs within the same nest, in order to account for any effect of laying order on fresh weight or volume within a brood. There was no effect of year ($F_{1,\,1672} = 2.4115$, $p = 0.1206$) or site ($F_{5,\,1668} = 1.4349$, $p = 0.2086$) on egg fresh weight or volume. Mean egg fresh weight in our population (across sites in both years) was 9.027 g, and mean egg volume was 8.597 cc (Table 3.5). Using the same constants from our study, we calculated egg fresh weight and volume from mean egg measurements documented in previous studies. A report from 1921 provided mean measurements of least tern egg specimens from the U. S. National Museum, which had the highest estimated egg fresh weight and volume[196], followed by eggs in our study, followed by eggs from Massachusetts in a 1997 report[2], followed by eggs collected in California from a 1974 report[197], and followed by eggs with the lowest fresh weight and volume collected in Oklahoma from a 1985 report[198] (Table 3.6). Without access to the actual data from these studies, we cannot expound upon whether or not these differences are statistically significant. However this trend in egg volume and fresh weight relative to collection year is reminiscent of trends previously documented whereby bird egg volume and weight is highest before excessive dichlorodiphenyltrichloroethane (DDT) use in the sampling area, and lowest during and right after periods of DDT use, and gradually increases after DDT was banned in the area[199].

Only a subsample of eggs that were measured in the field for fresh weight and volume were subsequently collected for contaminant analysis. We measured eggs immediately upon discovering a new nest, but most nests were subsequently lost to predation, flooding, or other causes, and therefore eggs were never collected for contaminant analysis or eggshell thickness measurements. From the subsample of eggs that were measured and subsequently collected, we regressed egg fresh weight and volume of 88 eggs against predictor variables in 2011 and 2012; 44 had both mercury and PCBs measured, and an additional 40 had mercury alone measured. Egg fresh weight and volume were not significantly affected by any of the predictor variables (collection site, distance from LCP, year, total Aroclor 1268 concentration, and THg). A reduction in egg fresh weight and volume has been associated with mercury exposure in ring-necked pheasants (*Phasianus colchicus*)[172], but we did not observe this relationship with least terns in our study. It is possible that pheasants are more sensitive to mercury exposure than other species. For example, Burger and Gochfeld (1997) reviewed the concentrations of mercury associated with adverse effects in birds, and showed that while mercury concentrations of 150 – 4,500 ppb in pheasant eggs were associated with reduced hatch rate and chick survival, similar effects in mallards, black ducks, and common terns, were induced only at higher concentrations that ranged from 1,500 – 16,500 ppb[112].

Eggshell thickness:

We measured the eggshell thickness of 537 eggs between 2011 and 2012, and of those, 65 were analyzed for both PCBs and mercury, plus an additional 35 for mercury only. Because both collection year ($F_{1, 535} = 251.76$, $p < 0.0001$) and site ($F_{5, 531} = 38.512$, $p < 0.0001$) affected eggshell thickness, they were included in all the models. Eggshell thickness across sites was significantly higher in 2011 ($\mu = 0.223$ mm, $n = 198$) than in 2012 ($\mu = 0.188$ mm, $n = 339$). Neither mercury ($F_{1, 98} = 0.6011$, $p = 0.44$) nor PCBs ($F_{1, 55} = 3.216$, $p = 0.0784$) affected eggshell thickness, but rather eggshell thickness appeared to depend more upon site, being highest at PESP, CINS, and LSSI (the natural barrier island and sandbar sites), and lowest at ANDR, SARI, and PUBL (the manmade sites) (Fig. 3.8). This variation in eggshell thickness among sites resembles the variation in proximity of breeding colonies to coastal waters. For example, the PESP colony was located on a sandbar offshore from a barrier island, and the CINS and LSSI colonies were both located on barrier islands. These colonies had the thickest eggshells, and were also located immediately adjacent to coastal waters. This contrasts with ANDR, SARI, and PUBL, which had the thinnest eggshells, and were also located relatively inland. ANDR and SARI were both located in estuaries in the Turtle and Savannah Rivers, respectively, and each was ~ 11 km from coastal waters. PUBL was a rooftop colony located even farther inland, with only tidal creeks for foraging in the immediate vicinity, and ~17 km from the nearest coastal waters. It appears that eggshell thickness in our study may have been related to colony habitat type (natural vs. manmade), which is perhaps linked proximity to quality foraging habitat, although distance traveled by least terns to forage, and forage habitat quality, were not quantified in the present study.

Our results indicated that mercury and PCB exposure did not significantly affect eggshell thickness. Various studies have shown that dietary exposure to methylmercury has little to no impact on eggshell thickness[79,200-202]. Although other organochlorines like DDT are notoriously known for eggshell thinning, most previous work suggests that PCBs do not have this effect[193,203,204], except at extremely high dietary doses (e.g., 150,000 ppb)[169].

Conclusions

We found that the biomarkers best correlated to contaminant exposure in least terns in our study were fluctuating asymmetry (FA) of both the wing chord and the second primary feather web length, which each increased with elevated mercury concentration in the primary 2 feathers. This evidence supports our hypothesis, and agrees with previous work that shows that contaminant exposure increases FA, indicating that contaminants cause instability during early development.

We found an unprecedented rate of debilitating physical deformities in least tern chicks from the Savannah River (SARI), and a high prevalence in chicks from the Turtle River estuary (ANDR). Teratogenic deformities result from a disruption of normal developmental processes, and were associated with mercury exposure in this study. Special attention should be given to the possible causes, as well as the regularity in occurrence, of high rates of teratogenic morphological deformities in chicks, especially in the Turtle River and Savannah River estuaries where deformed chicks were observed in this study.

We found that H/L ratios in least tern chicks decreased with increasing fecal mercury, suggesting that contaminant exposure may have been inhibiting immune function, suppressing least tern chicks' ability to mount a proper stress response while being handled and bled. However, interpretations of H/L can vary, and many factors have been known to influence it, so while our results suggest an impaired immune response in association with mercury exposure, this conclusion is not unequivocal, and invites further investigation.

It appeared that eosinophil counts were unaffected by mercury, and monocyte, basophils, and hemoparasites were essentially non-existent. To the best of our knowledge, we are also the first to report leukocyte estimates and differential counts for least terns.

We found that eggshell thickness, egg fresh weight and volume, chick tarsus length, and chick pre-fledging growth rate did not appear to be affected by mercury or PCB exposure in least terns. (However, post-fledge weight loss in relation to mercury and PCB exposure deserves additional attention.) These parameters in least tern chicks may have been unaffected in our study because local concentrations of contaminants were not elevated enough to have an effect. However, it is also possible that some of these parameters are not good biomarkers for mercury and PCB exposure (as is probably the case with eggshell thickness), or that some are affected only in certain species, or at higher dietary doses that are generally used in controlled laboratory experiments but that are not typically encountered through environmental exposure (as is likely the case for egg fresh weight and volume).

We suggest that the egg parameters we measured may not be ideal biomarkers for field studies aiming to quantify effects of local contaminant exposure for two reasons: (1) often the concentrations known from previous studies to cause these effects are not environmentally relevant, and (2) eggs are produced by adult birds, who may be harboring burdens of contaminants that have been acquired throughout life and across their geographical range. Contaminant concentrations from our study showed (see chapter 2) that eggs had comparable mercury concentrations across sites, while the distribution of chick feather mercury and sediment mercury distribution across sites was in close agreement, indicating that samples taken from locally-fed chicks are better indicators of local pollution.

Figures:

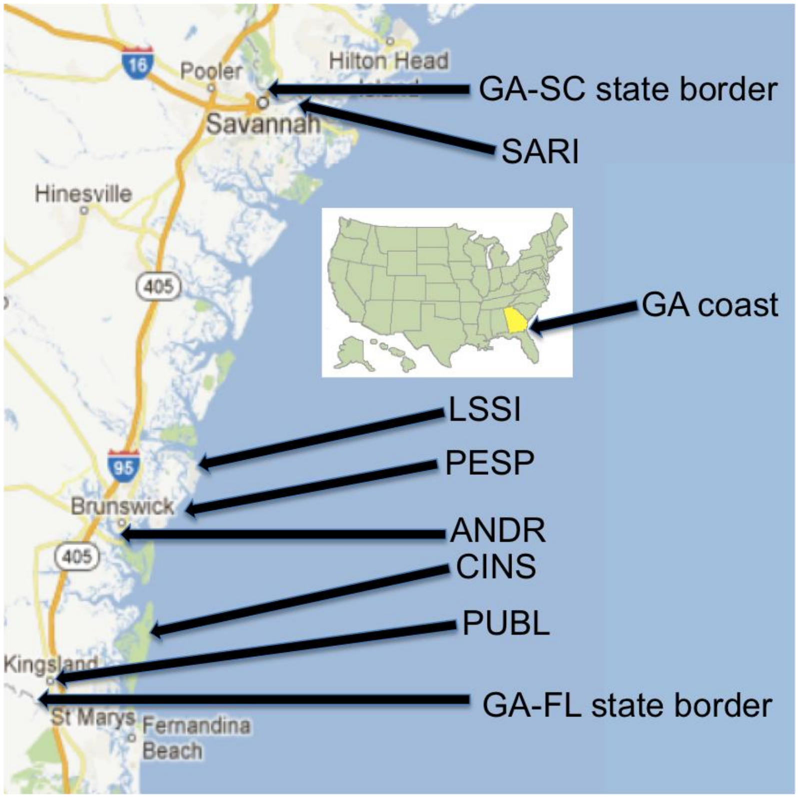

Figure 3.1. Locations of least tern nesting colonies along ~150 km of Georgia (GA) coastline. Colony sites were: Savannah River dredge spoil area in the Savannah River estuary (SARI), Little St. Simon's barrier island (LSSI), Pelican Spit sandbar (PESP), Andrew's dredge spoil island in the Turtle River estuary (ANDR), Cumberland Island National Seashore barrier island (CINS), and the Publix grocery store rooftop in Kingsland, GA (PUBL). The locations of the state borders Georgia shares with South Carolina (SC) and Florida (FL) are also shown.

Figure 3.2. Location of Linden Chemical Plant (LCP) Superfund site in the Turtle River estuary in Brunswick, Georgia, and the location of Andrew's dredge spoil island. Andrew's Island is the location of the largest least tern nesting colony in Georgia, and is one of the sites we sampled in 2011 and 2012.

Figure 3.3. Examples of teratogenic deformities we observed in least tern chicks in Georgia in 2012. From left: severely splayed legs, hind limb deformity, severely clubbed feet, and spine curvature.

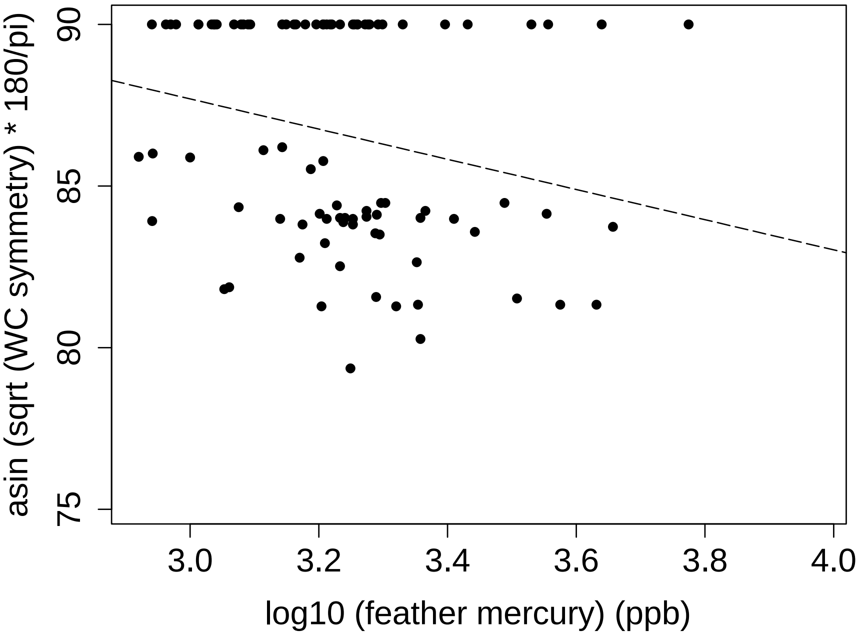

Figure 3.4. Wing chord (WC) symmetry in least tern chicks sampled in 2011 and 2012 decreased significantly with increasing total mercury concentration in the second primary feather (P2) ($F_{1, 89} = 5.373$, $p = 0.0227$). To meet statistical assumptions of normality, wing chord symmetry ratios were arcsine square root (*180/pi) transformed and P2 feather mercury concentrations were log10 transformed prior to analysis.

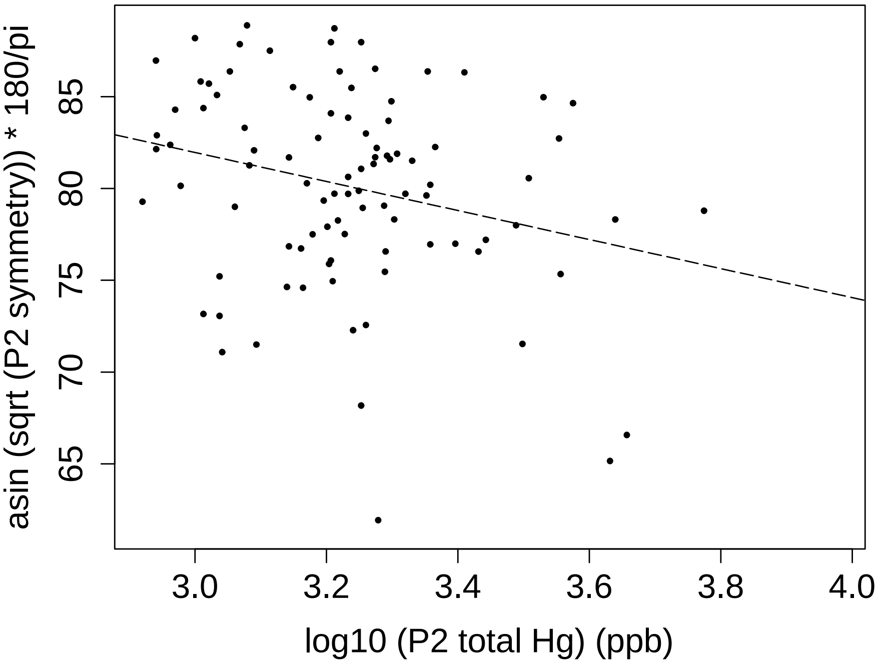

Figure 3.5. Second primary feather (P2) symmetry in least tern chicks sampled in Georgia in 2011 and 2012 decreased significantly with increasing total mercury concentration in P2 ($F_{1,\ 92}$ = 6.8314, p = 0.0105). To meet statistical assumptions of normality, P2 feather symmetry ratios were arcsine square root (*180/pi) transformed and P2 feather mercury concentrations were log10 transformed prior to analysis.

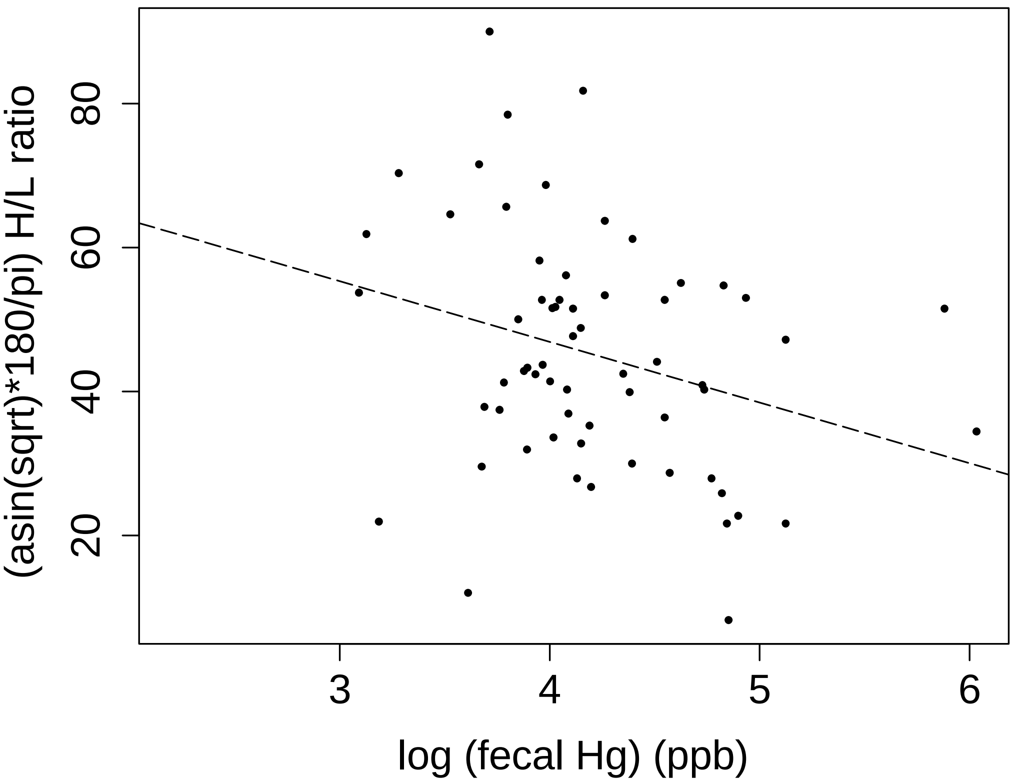

Figure 3.6. Heterophil/lymphocyte ratios (H/L) in blood of least terns chicks sampled in Georgia in 2011 and 2012 decreased significantly with increasing total mercury concentration in the second primary feather (P2) ($F_{1,\ 61}$ = 5.6235, *p* = 0.0209). To meet statistical assumptions of normality, H/L ratios were arcsine square root (*180/pi) transformed and P2 feather mercury concentrations were log10 transformed prior to analysis.

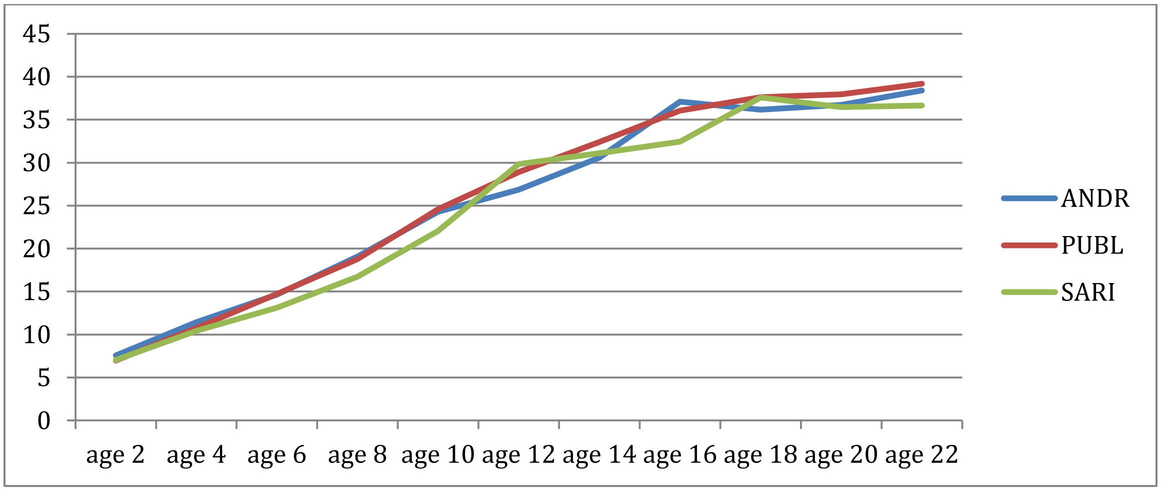

Figure 3.7. Mean growth rates of nestling least terns among nesting colony sites in Georgia in 2012. Colony sites were: Andrew's Island (ANDR), Publix grocery store rooftop (PUBL), and the Savannah River dredge spoil area (SARI). Nestling mass (g) on the *y* axis is plotted against nestling age (days post-hatch) on the *x* axis. Note that growth plateaus at the age of approximately 16 days, and therefore only slope of growth rate to day 16 were used in regression models to assess the response of nestling growth rate to predictor variables, including total mercury concentrations in feathers and feces.

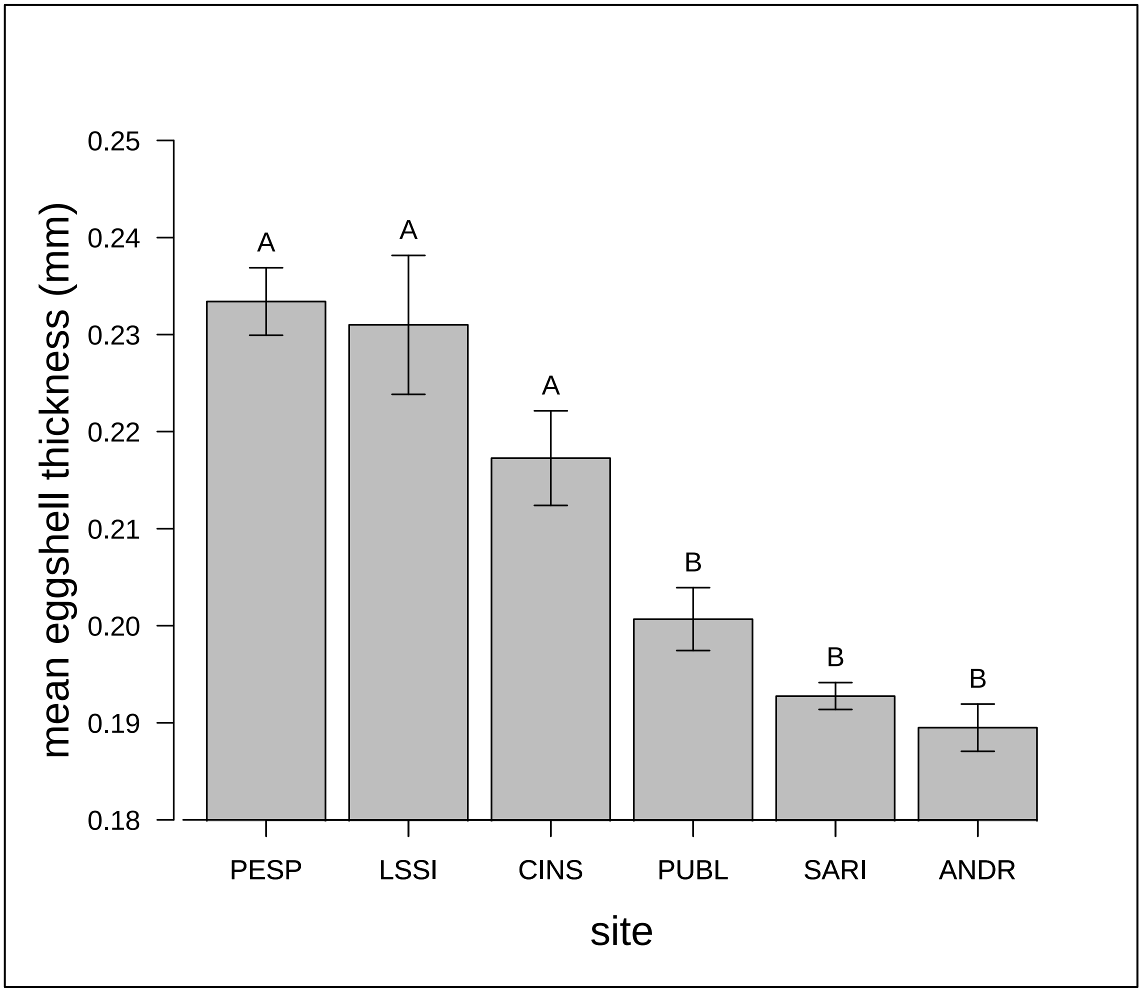

Figure 3.8. Mean eggshell thickness of least tern eggs among nesting colony sites in Georgia in 2011 and 2012. Colony sites were Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), Publix grocery store rooftop (PUBL), Savannah River dredge spoil area (SARI), and Andrew's dredge spoil island (ANDR). Standard error bars are shown, and means that share a common letter are not statistically different, and those that do not are statistically different (α = 0.05). There was an effect of colony site on eggshell thickness ($F_{5, 531} = 38.512$, $p < 0.0001$), and eggshell thickness was lower at manmade sites (PUBL, SARI, and ANDR) than at natural sites (PESP, LSSI, and CINS).

Tables:

Table 3.1. Relationships between predictor variables and response variables in least tern chicks sampled in Georgia in 2011 and 2012. Predictor variables modeled were: collection year, nesting colony site, distance of nesting colony to the contaminated Linden Chemical Plant (LCP) Superfund site, and total mercury concentrations (Hg) in least tern chick feces and second primary feathers. Response variables were the symmetry ratios of least tern chick tarsus, wing chord, nares, and second primary feather (P2) measurements, and the slope of least tern chick growth rate during the first 16 days post-hatch. Relationships are represented using *p* values, with those that are statistically significant at $\alpha = 0.05$ shown in bold typeface.

	tarsus symmetry	wing chord symmetry	nares symmetry	P2 symmetry	growth
year	0.7328	0.0754	0.7306	**0.0357**	0.1119
site	0.9021	0.2549	0.3921	0.2494	0.9380
distance	0.6590	0.1008	0.2029	0.3725	0.9927
fecal Hg	0.0553	0.4016	0.3842	0.7591	0.3963
feather Hg	0.0658	**0.0227**	0.5068	**0.0105**	0.0853

Table 3.2. Mean, standard deviation (SD), median, and range, in white blood cell (leukocyte) estimates (WBC), and heterophil, lymphocyte, eosinophil, basophil, and monocyte differentials, in least tern chicks in Georgia in 2011 and 2012 (*n* =240).

	Mean	**SD**	**Median**	**Range**
WBC estimate (x 10^3/µL)	7.091	1.716	7	1 – 15
heterophil (%)	34.924	15.172	35	2 – 78
lymphocyte (%)	62.183	15.796	63	22 – 98
eosinophil (%)	2.490	2.907	2	0 – 19
basophil (%)	0.016	0.154	0	0 – 2
monocyte (%)	0.668	6.402	0	0 – 2

Table 3.3. Mean white blood cell (leukocyte) estimates (WBC), and heterophil, lymphocyte, eosinophil, basophil, and monocyte differentials, in adult common terns (*Sterna hirundo*, "COTE") from Cape Cod (*n* =33)[183], captive adult fairy terns at zoos (*Sterna nereis,* "FATE") (*n* = 2) (isis.org), and sooty tern (*Onychoprion fuscatus*, "SOTE") chicks in Hawaii (*n* = 35)[184].

	Mean (COTE)	**Mean (FATE)**	**Mean (SOTE)**
WBC estimate (x 10^3/μL)	10	10	33
heterophil (%)	38	33	22.9
lymphocyte (%)	51	61	70.1
eosinophil (%)	9.1	0.05	5.1
basophil (%)	0.13	0.01	0.8
monocyte (%)	5.6	-	1.2

Table 3.4. Relationships between predictor variables and response variables in least tern chicks sampled in Georgia in 2011 and 2012. Predictor variables modeled were: collection year, nesting colony site, distance of nesting colony to the contaminated Linden Chemical Plant (LCP) Superfund site, and total mercury concentrations (Hg) in least tern chick feces and second primary feathers. Response variables were hematological parameters: total leukocyte estimates, and heterophil, lymphocyte, and eosinophil differentials, and the heterophil/lymphocyte ratio. Relationships are represented using *p* values, with those that are statistically significant at $\alpha = 0.05$ shown in bold typeface.

	total leukocyte	% heterophil	% lymphocyte	% eosinophil	heterophil/ lymphocyte ratio
year	**0.0150**	0.1752	0.5547	**0.0003**	0.2078
site	**0.0062**	0.4564	0.7536	**0.0005**	0.5747
distance	0.2282	0.5171	0.5986	0.0763	0.4252
fecal Hg	0.1579	0.1663	0.1197	0.1542	**0.0209**
feather Hg	0.6153	0.3741	0.3031	0.2922	0.9297

Table 3.5. Mean least tern egg fresh weight (g) and volume (cc) across nesting colonies in GA in 2011 and 2012. Colony sites were Andrew's dredge spoil island (ANDR), Pelican Spit sandbar (PESP), Little St. Simon's barrier island (LSSI), Cumberland Island National Seashore barrier island (CINS), Publix grocery store rooftop (PUBL), and Savannah River dredge area (SARI). Note: PESP was only sampled in 2011.

Site	Mean 2011 fresh wt.	Mean 2011 volume	Mean 2012 fresh wt.	Mean 2012 volume
ANDR	9.175	8.738	9.057	8.626
PESP	9.022	8.593	--	--
LSSI	9.182	8.745	9.038	8.607
CINS	9.080	8.648	8.885	8.462
PUBL	8.899	8.475	8.819	8.399
SARI	9.126	8.691	9.009	8.580

Table 3.6. Mean egg fresh weight (g) and volume (cc) in least tern eggs, reported over time. Egg fresh weight and volume was calculated from measurements of least tern eggs from a museum collection in 1921[196], from California in 1974[197], Oklahoma in 1985[198], and Massachusetts in 1997[2], and compared with fresh weight and volume of eggs from the current study, collected from least terns in Georgia from 2011-2012. Dichlorodiphenyltrichloroethane (DDT) use in the US took place between the 1940s and 1972, when it was banned. Eggshell thickness trends in many bird species have followed the pattern observable here in least tern eggs, with eggshell thickness decreasing during and following periods of DDT use, and gradually increasing after DDT use was banned.

Collection site and year of report	***n***	**Mean egg fresh wt.**	**Mean egg volume**
museum collection (1921)	63	9.347	8.902
CA (1974)	122	8.886	8.462
OK (1985)	59	8.809	8.390
MA (1997)	202	8.925	8.500
GA (2011-2012)	840	9.027	8.597

CHAPTER 4

PREDATOR MANAGEMENT GREATLY INCREASES NEST SUCCESS AT LEAST TERN (*STERNULA ANTILLARUM*) COLONIES IN GEORGIA[1]

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[1] Robinson, G.L., Schweitzer, S.H., Hernandez, S.M. To be submitted to *Condor*

Abstract

Urbanization and human encroachment upon wildlife habitat is inexorable, and poses a variety of challenges to native wildlife species. One such challenge for many species is the food provisioning, and the subsequent overpopulation, of predator species. For example, least terns (*Sternula antillarum*) are piscivorous seabirds that are in decline in the eastern U.S., while the interior and California subspecies are federally endangered. Decline of this species since the second half of the twentieth century has been largely due to habitat loss or degradation, human disturbance at coastal breeding colonies, and predation by many species whose populations are artificially inflated due to anthropogenic food provisioning. We document the efficacy of several predator management techniques, including use of electric fencing, crow effigies, ghost crab traps, and lethal crow removal. In 2011, 4 of the 5 least tern breeding colonies we monitored in this study suffered total reproductive failure due to predation. None of these colonies fledged young, and nest success estimates ranged from 0%-7%. We implemented predator management at one colony mid-way through the season in 2011, and the nest success estimate here was 51%. In 2012, we implemented predator management at two of these colonies for the entire season, and nest success estimates were 82% and 66%. Use of positive-negative conductive electric mesh fencing and crow removal were very effective at increasing nest success, and based on our results reproductive success at these sites seems unlikely in the future without predator management. We also document an instance of bobcat (*Lynx rufus*) predation on least tern chicks, which has not been reported previously.

Introduction

Urbanization and human development of wildlife habitat often creates challenges to the survival and recruitment of native species that include habitat loss and degradation, introduction of non-native invasive species, and increased disease transmission[48,205]. However, one other often overlooked impact of urbanization to native species is the proliferation of species commensal with human communities[48]. These species, whether native or introduced, can become problematic when their population growth is unrestricted due to resource provisioning by anthropogenic sources. Naturally, species compete with others for resources, or may exploit other species as resources themselves, but this relationship can quickly become out of balance when populations are allowed to increase unchecked[206]. These provisioned, or “subsidized” species tend to be generalists or opportunists, and will exploit a variety of resources provided by the donor (in this case, humans), often leading to an increase in reproduction of the recipient species. These subsidized populations are often greatly inflated, especially in developed areas, where there is not only an abundance of food, but also an absence of natural predators[49,50].

Colonial, ground-nesting seabirds, such as least terns (*Sternula antillarum*), have many conservation threats that include habitat loss and habitat degradation, food source contamination, human disturbance that hinders or inhibits productivity, and nest predation by a large number of species. Species such as gulls (*Larus* spp.), crows (*Corvus spp.*), grackles (*Quiscalus spp.*), coyotes (*Canis latrans*), raccoons (*Procyon lotor*), opossums (*Didelphis virginiana*), nine-banded armadillo (*Dasypus novemcinctus*), striped skunk (*Mephitis mephitis*), European rabbit (*Oryctolagus cuniculus*), mink (*Neovison vison*), red fox (*Vulpes vulpes*), ghost crab (*Ocypode quadrata*), and feral dogs (*Canis* *lupus familiaris)*, cats (*Felis catus*), and hogs (*Sus scrofa*) are all documented nest and chick predators of least terns that may also be commensal with humans and take advantage of anthropogenic food sources[2,39-45]. Nest predation will increase after an introduction (especially to an island) of a non-native nest predator species, or following habitat modification that is favorable to a generalist nest predator species[51]. Therefore, the conservation threat to least terns presented by overabundance of nest predator populations due to anthropogenic food source provisioning, is particularly marked.

Least tern populations are declining on the east coast of the U.S. (subspecies *S. a. antillarum*) and are state-listed in most states. Furthermore, least terns are federally endangered in the interior U.S. (subspecies *S. a. athalassos*) and in California (subspecies *S. a. browni*)[2]. An efficient nest predator can nearly obliterate an entire least tern breeding colony in a single night, especially in the case of terrestrial predators[2]. Repeated disturbance by predators may also cause colony site abandonment[2]. Because subsidized predator populations are inflated in many least tern breeding habitats, and because predation can be a significant (and indeed often the dominant) determinant of reproductive success these colonial ground-nesting birds, managing predators is becoming an increasingly valuable conservation tool for species like least terns[54].

Predator removal is one management option, but tends to be labor-intensive and costly[55], and is often done by lethal means, thereby requiring the proper permits as well as close regard to public safety as well as the potential for public protest[54]. Another option is use of barricades preventing access of terrestrial predators to the nesting area[57]. The use of electric fences, in particular, is increasing and has been demonstrated as an effective means to exclude most terrestrial predators[25,44,58-61]. Corvids cannot be excluded using nesting colony barriers and are a significant predator to chicks and eggs of ground-nesting birds like least terns[47,52-54]. Legally, corvids and other "black birds" are considered pest species and there is a standing depredation order in place that allows the take of these species that would otherwise be prohibited (or require special federal permit granted for exceptional circumstances only) by the Migratory Bird Act. Code of Federal Regulations, Title 50: Wildlife and Fisheries, Part 21.43 (50 CFR 21.43) states that federal permit is not required for take of yellow-headed blackbirds, red-winged blackbirds, rusty blackbirds, cowbirds, all grackles, crows, and magpies, when these species are found depredating plants or animals (including wildlife) of human interest. While taste aversion or hazing techniques may have limited success minimizing predation of least terns and other ground-nesting birds, lethal methods of corvid control are generally the most effective and long-lasting[52,66].

As coastal development and urbanization persists, species like least terns will continue to face threats posed by habitat loss, human disturbance, compromised reproductive success due to the overabundance of subsidized predators. It seems that habitat loss is irrepressible, while resolving issues of human disturbance is straightforward albeit contentious, but preventing substantial impacts to productivity by predation has proven to be more complicated[54]. The present study was not designed to test the effects of predator management strategies on least tern hatch success, but rather this report results from an extempore attempt to prevent the total reproductive failure of least tern colonies being sampled for a different study. Therefore the experimental design is ad hoc, but the predators we documented, strategies we implemented, and the resulting increase in least tern hatch success, are pertinent and timely data that could benefit and inform least tern conservation management decisions.

Methods

Sites and field sampling

We monitored least tern breeding colonies on the Georgia coast from May-August, 2011 and 2012, as part of an ecotoxicology study on the effects of environmental pollutants on the health and productivity of least terns. Two colonies were located at natural sites and two were located on manmade dredged-material (spoil) sites. Cumberland Island National Seashore (CINS) and Little St. Simon's Island (LSSI) are both relatively undeveloped barrier islands, at which least terns attempt to breed each annually. The breeding colonies on each of these islands are remote to human access points and are therefore exposed to minimal to no human disturbance. The two manmade colony sites were Andrew's Island (ANDR), a dredge spoil island located in the mouth of the Turtle River in the city of Brunswick, Georgia, and the Savannah River dredge spoil area (SARI) located in the mouth of the Savannah River near Savannah, Georgia. Both of these colony sites are managed specifically to encourage least tern colonization, are in close proximity to urban centers, but are closed to the public and protected from disturbance by site workers. LSSI and CINS are both islands where raccoons, coyotes, and feral hogs are present. Both ANDR and SARI are contiguous with the mainland, and are therefore accessible to all mainland predators.

We monitored nesting colonies every three days throughout the breeding season. We marked nests with uniquely-labeled tongue depressors ~ 1 m from the nest bowl. We floated eggs[207] to determine incubation stage when new nests were found, and we estimated hatch date based on a 21-day incubation period[2]. We checked eggs in a nest for pipping, which usually begins one to three days before hatching[2], on each visit following the fifteenth day of incubation. We recorded pipping nests as hatched nests even if chicks were not found in the nest bowl on the visit following pipping, except in situations where chicks died while pipping. We also considered a nest successful if we found new (one- to two-day old) chicks < 1 meter of the nest bowl, and if the nest was within several days of its estimated hatch date on the previous visit. We ascribed unknown fate to nests that were active and approaching the estimated hatch date when they were last observed, but for which no chicks were found associated with the nest bowl. Nests that disappeared more than a few days prior to the estimated hatch date, or that showed clear signs of predation, over-wash, or abandonment, we considered to have failed.

We considered nest failure “unknown” unless clear signs of failure met the following criteria: We assigned a nest fate of over-wash when a nest was missing in a recently over-washed or inundated area, or if eggs were found displaced from a nest that was recently over-washed (evidenced by wet sand). We assigned nest failure by coyote or raccoon/armadillo only if tracks were found leading up to the nest bowl, or within ~ 15 cm (or 6 in) of the nest bowl. Thus, we fated many nests “unknown,” even when we strongly suspected mammalian predation. Raccoons and armadillos often had indistinguishable tracks in the soft sand, and other times both species’ tracks were intermingled in the colony, making it difficult to distinguish which nests were depredated by which species. Therefore, we lumped raccoon and armadillo depredation into one nest fate. We ascribed very few nests as crow-depredated, because crows did not leave any sign at a nest bowl. However, we did observe crows depredating nests, and the nests were fated as such. We only ascribed ghost crab predation when ghost crab tracks, or a fresh ghost crab burrow, were found on top of the nest site. We determined that chicks died while pipping, when eggs were found with dead, partially-hatched, chicks. We determined a nest was abandoned when eggs were found cold to the touch or wet, cracked, partially buried in the substrate, or displaced from an active nest bowl, for more than two consecutive visits.

Predator management techniques

Electric fences:

In response to catastrophic rates of predation at least tern breeding colonies in 2011, we implemented predator management in 2012. We installed temporary electric fences (Premier Pos/Neg ElectroStop® 10/42/12, 42" x 164'), that were powered throughout the least tern nesting season by solar-powered energizers (Premier PRS 200 Solar Energizer), around the perimeters of all four breeding colonies in 2012 (Fig. 4.1). The fences were 107 cm (42 in) tall, and consisted of 10 horizontal strands with plastic vertical struts every 30 cm and PVC posts every 3.8 m (12.5 ft).

We checked the voltage of fences (consistently 7-10 kV) with a voltmeter during each visit to ensure that they were consistently maintaining a voltage adequate to deter predators. We also affixed “scent caps” to conductive strands along the fence at regular (~ 15 m) intervals. We made scent caps by attaching plastic or aluminum bottle caps to the fence with zip ties. We regularly baited the scent caps with some combination of fish oil, fish paste, and canned sardines, so they maintained a pungent odor throughout the least tern breeding season (Fig. 4.2). The purpose of the scent caps was to increase the likelihood that a predator’s first encounter with the fence would result in a shock, thereby deterring the predator from the area immediately, and lessening the chance that it would attempt to jump the fence, or otherwise infiltrate the breeding colony.

One colony in 2011 was protected by electric fencing, but of a different variety. SARI 13 was surrounded by a 3-strand electric cattle fence. This fence consisted of three horizontal conductive strands that ran between vertical wooden stakes. The fence was ~0.75 m in height.

Crow effigies:

The ANDR colony experienced total reproductive failure and subsequent colony site abandonment due almost entirely to crow predation in 2011 (see ‘Results’ section below). Therefore, we attempted to deter crows from preying on least tern eggs using crow effigies. We purchased life-sized crow decoys with outspread wings, covered in real feathers, from several Halloween product distributors. We hung crow effigies upside down from metal posts, and arranged a total of 10 effigies around the perimeter of the tern colony. We also crowned the top of each effigy post with a pointed, sharpened tongue depressor, to keep potential avian predators from roosting on the posts (Fig. 4.3).

Crow removal:

An animal damage control professional removed 31 crows from the ANDR least tern colony area between 9 – 25 May 2012, during four occasions. Crows were killed with a 12-guage shotgun. We had observed this group of crows preying on least tern eggs in 2012, and it seemed likely that they are the same crows that caused complete reproductive failure at ANDR in 2011. Initially, our intention was to condition the crows to avoid the least tern colony by killing several of them in front of the rest of their group.

However, the remaining crows continued to prey on least tern eggs until 31 were removed, after which no crows were observed in the area for the rest of the season.

Ghost crab traps:

We constructed inexpensive traps using chicken wire to capture ghost crabs in least tern colonies. We made two types of traps that were similar conceptually, whereby the crab enters the trap through a cylinder that is not flush with the bottom of the inside of the trap, and thus the crab does not crawl back through the cylinder to get out of the trap once it enters. One trap had a single cylinder leading into it that was meant to be placed in a single occupied ghost crab hole to capture its occupant, while the other trap had several cylinders leading into it from the ground level, and was baited to attract and capture multiple ghost crabs at once (Fig. 4.4).

Statistical Analysis

We used Program MARK to estimate daily survival rate for seven nest survival groups: ANDR (2011), SARI 12A (2011), SARI 13 (2011)*, LSSI (2011), CINS (2011), ANDR (2012)*, and SARI 14 (2012)*. The asterisk (*) indicates colonies that were provided with electric fencing. In 2012, least tern colonies did not establish at LSSI and CINS due to a series of abnormally high tide and storm surge events, and therefore these sites are not included in the analysis. There were 87 encounter histories, or number of days during the season that nests were monitored. We used a 21-day incubation period to convert daily survival rates to point estimates of nest success for each group.

Results

Point estimates of nest success, along with causes of nest failure, are listed for all sites in 2011 and 2012 (Table 1). In 2011, nest success estimates were between 0.00001

(0%) and 0.06869 (7%) at the unmanaged sites (ANDR, LSSI, CINS, SARI 12), while nest success was 0.51315 (51%) at the managed site (SARI 13). In 2012, nest success at managed sites was 0.66488 (67%) at ANDR and 0.81606 (81%) at SARI 14 (Fig. 4.5).

Four (CINS, LSSI, ANDR, and SARI 12A) of the five colonies we monitored in 2011 suffered complete reproductive failure due to predation. There were ~40 pairs of least terns at LSSI, and we monitored 65 nests there. Raccoon and armadillo predation was identified as the primary cause of nest failure at 63% of nests, and ghost crab predation at 11% of the nests. Least terns began nesting at LSSI during the first week of May, and had completely abandoned the colony site by the end of May, following heavy predation by raccoons and armadillos. Approximately 50 pairs attempted nesting at ANDR in 2011, and we monitored 43 nests. Although we only witnessed fish crows take eggs from least tern nests on two occasions, we observed crows flying through the colony looking for eggs throughout every visit to the colony. We expect that all of the unknown nest failures (84%, n = 34) were caused by fish crow depredation. It was common during our visits to observe one or more crows flying through the colony several times an hour, being mobbed by adult terns. Coyote tracks were also associated with five depredated nests toward the end of May. Fish crows preyed on this colony incessantly, until the least terns finally abandoned the site in late May. Finally, ~ 40-50 pairs nested at CINS, and nesting continued throughout the season, even though only 7 of 137 (5%) of nests hatched. None of the hatched chicks survived more than ~ 7 days. The main cause of nest failure at CINS was coyote depredation. Coyote tracks could only be identified within 15 cm of the nest bowl for 41 (30%) of the failed nests, but we are confident that most of the 45 unknown nest failures were also caused by coyote depredation, but tracks were not visible right at the nest bowl at the time of the observation. Ghost crab predation was attributed to 20 nest failures (15%), and over-wash was attributed to 23 (17%) of the nest failures. We are the first to document bobcat (*Lynx rufus*) predation of least tern chicks. Three least tern chicks were found maimed and killed in the colony one morning in early July, with fresh bobcat tracks at the site (Fig. 4.6). Finally, we estimated there to be at least 200 nesting pairs at the SARI 12A colony in the Savannah River, and a total of 194 nests were monitored. Only one nest (< 1%) hatched, and only six (3%) failures could be clearly attributed to coyote predation. However, the colony site was constantly covered in both coyote and feral hog tracks, and we are confident that most of the 167 nest failures (86%) with an unknown cause could be attributed to either feral hog or coyote predation.

The only colony in 2011 with predator management (a standard three-stranded electric cattle fence) was the SARI 13 colony. Of the 48 monitored nests, 30 (63%) hatched and 17 (35%) failed due to unknown causes, and one (2%) was abandoned. However, fairly regularly we observed broken fence strands associated with feral hog tracks into the colony, as well as coyote tracks crossing the colony. It appeared that coyotes jumped the fence (which was ~ 1 m tall), and hogs occasionally broke through the fence. It did not appear that either coyotes or hogs were preying on nests. Coyote and hog tracks typically led across the colony with little meandering, so it did not appear that either potential predator had recognized the food source or was intending to forage for least tern eggs.

In 2012 the ANDR and SARI 14 colonies were protected by Premier electric mesh fencing. We estimated that nearly 300 pairs of least terns nested at ANDR in 2012, and we monitored 344 nests. Of the monitored nests, 150 (51%) hatched, 91 (26%) failed due to unknown causes, 56 (16%) were abandoned, 23 chicks died while pipping, 22 (6%) could be attributed to crow predation before the offending group of crows was removed, and 2 (>1%) were flooded during heavy tropical depression rainfall. We also observed great-horned owl depredated chicks, and observed the owl roosting on the edge of the colony on several night visits. We estimated that between 200- 250 pairs nested at SARI 14 in 2012. We monitored 306 nests, 169 (55%) of which hatched. Nest failure was attributed to abandonment for 65 nests (21%), death during pipping for 5 nests (2%), and unknown causes for 67 nests (22%).

Discussion

Electric fencing significantly increased nest success in our study. All sites lacking predator management in our study had nest success point estimates of 0-7%, while managed sites had nest success of 51%-82%. Although least terns never established a colony on LSSI in 2012, the electric fence that we erected on LSSI protected a black skimmer (*Rynchops niger*) colony that successfully fledged ~50 offspring. Black skimmers that attempted to nest in the same area in 2011 with the least terns, also failed due to raccoon and armadillo predation. The 2012 season was the first year site managers have on record that black skimmers were successful on LSSI, even though they attempt to nest there annually (Scott Coleman, Little St. Simon’s, pers. comm.). Our results provide strong evidence that without predator management, least tern colonies at our study sites would be unsuccessful, making predator management a necessity for least tern recruitment in Georgia.

Crow removal was critical in achieving least tern reproductive success at ANDR. There was only one group of offending crows, and once most of them were removed we observed no additional crow disturbance to the least tern colony for the rest of the season. Crow predation caused the highest rate of nest failure we observed across sites (and thus the lowest daily survival rate), with many nests observed on only a single occasion before disappearing. Crow removal in this study was relatively simple and cost-effective, with all problem crows removed in only four visits. However, our site was closed to the public, so the use of firearms to remove crows was safe and exempt from the possibility of public protest. The type of crow removal we used would likely be more complicated, or impossible, at some least tern colonies, such as those on public beaches. However, our results indicate that crows can very rapidly cause the collapse and abandonment of least tern colonies, and therefore crows should be considered a serious threat to least tern conservation. If the use of firearms to remove crows is not an option, mangers should consider other lethal forms of crow control, that include taste aversion conditioning[63,65] or poisoning via toxicant-laden eggs[53,62].

We found crow effigies to be unsuccessful in deterring crows. We first deployed crow effigies at ANDR in mid-May 2011. We witnessed an immediate response from crows. Just minutes after we had erected one of the effigies, two crows began flying in close circles around the effigy, and then began calling. Other crows flew in and soon a flock that we estimated to be ~30-40 crows were circling above the effigy, calling. This continued for a couple minutes, before the crows all flew off together. It is possible that the effigies did deter crows from the tern colony in 2011, but by then most of the least tern nests had failed and the site was abandoned soon after. Therefore, we cannot say whether the crows were avoiding the area due to the effigies, or if they just frequented the area less because the food source was gone. Regardless, the crow effigies did not reduce crow predation in the 2012 season. Crows continued unrelenting predation of the least tern colonies until most of the crows had been removed. Crow effigies have been successfully employed to disperse crows from roosting areas[64], but only a few records of effigies minimizing predation at seabird colonies exist[52,63].

Between the time that crow removal at ANDR was completed, and the time that the great horned owl began preying on the colony, daily survival rate of nests was at or near 100% at ANDR (Fig. 4.7). Not only did we find tern chicks that appeared to have been killed by the owl, but the owl was also implicated in the surge of chick mortality, that occurred during pipping, observed during this period. All 23 observations of death during pipping occurred after the owl began preying on the colony. We suspected that death during pipping occurred when eggs would hatch overnight in nests of adults who had abandoned the colony for the evening to avoid predation by the owl. We confirmed nighttime abandonment during several evening visits during which we saw many (but not all) adults leaving the colony site just before dark. Owl predation should be considered a grave threat to least tern colonies. Of 145 chicks that we documented at ANDR, we documented only ~20 (14%) that reached fledgling age. We found many chicks dead and dismembered in the colony after the owl appeared, and we suspect that many chicks were depredated by the owl. Not only will owls kill chicks and increase nest abandonment, but owls can also kill adult least terns, which will have a much greater impact on the population. Suitable owl roosting habitat near a least tern colony should be minimized whenever possible (e.g., removal of trees, sign posts, or other tall structures, in close proximity to the colony). Federal permits can be acquired to trap and remove problematic owls from tern colony sites, and should be considered if owl roost removal or hazing tactics are ineffective or impossible.

Conclusions

Predation has become the primary driver for low productivity of least terns at many sites throughout the U.S. Populations of predator species with anthropogenic food provisioning often become inflated, creating an unnatural predation pressure on other non-commensal, native, and especially colonial, species such as least terns. For the least tern conservation issues of habitat loss and human disturbance, manmade habitat can be created, and human disturbance minimized, through management practices. However, approaches to management of what is arguably the most significant threat to least tern conservation, predation, are not always well understood by many wildlife managers, and too often overlooked. The use of electric fencing is a relatively cost-effective strategy (one-time cost of ~$2,500 - $5,000, depending on colony size) for increasing nest success for least terns, and should be accepted more broadly as a tool to bolster least tern recruitment, especially in areas where the species has state or federal conservation status. In addition, lethal corvid control may be necessary in some areas, as corvids in our study took least tern nests at a higher rate than any other predator we documented. Predator management for least terns and other beach-nesting species has not yet been a regular practice in Georgia previous to our study. Our results strongly indicate that predator management at these colonies will be a necessity for even meager reproductive success in the future. Furthermore, we suggest a closer investigation of least tern productivity in the absence of predator management elsewhere in the country, as it may also be critically threatened by subsidized predator populations.

Figures:

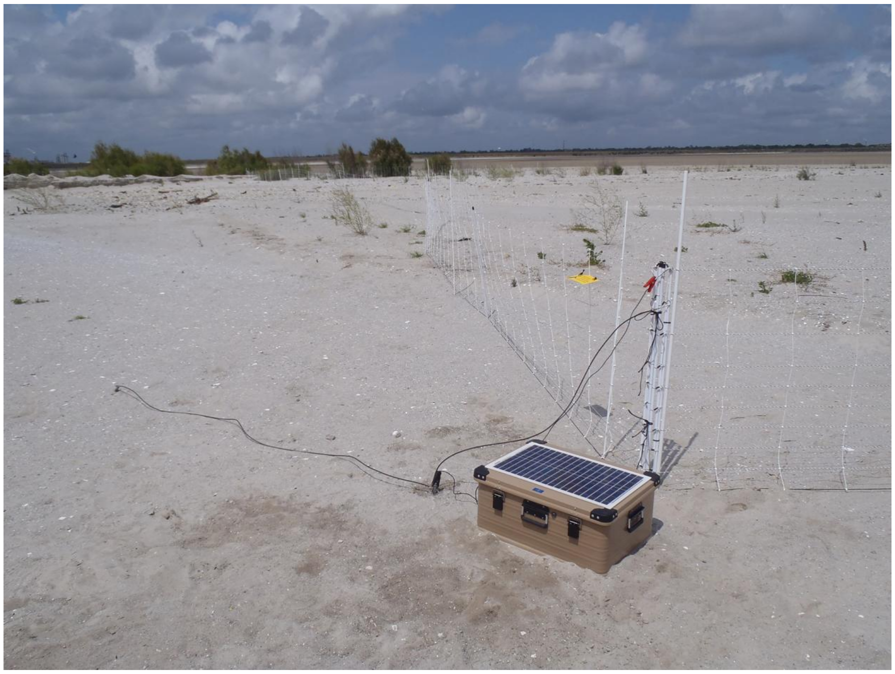

Figure 4.1. Premier Pos-Neg® electric mesh fencing with solar-powered energizer, installed along the perimeter of a least tern colony in Georgia in 2012. Electric fencing of this kind greatly increased nest success at least tern colonies in Georgia by minimizing nest predation by mammalian predators.

Figure 4.2. Scent cap placed on a conductive wire of an electric fence installed around the perimeter of a least tern colony in 2012. The scent cap has fish paste inside as bait, and is being sprayed with fish oil to refresh the scent. Scent caps were placed on fences at ~15 m intervals, and at ~ 0.3 m from the ground. The purpose of placing baited scent caps on the conductive wires of electric fencing was to increase the probability that a mammalian predator's first encounter with the least tern colony would result in a deterring electric shock, thereby decreasing the probability that the predator would attempt to infiltrate the fenced-in colony.

Figure 4.3. A crow effigy used in attempt to deter fish crow (*Corvus ossifragus*) predators from least tern colonies in Georgia in 2011 and 2012. A pointed stick crowns the post in order to prevent avian predators from using the post as a roost.

Figure 4.4. Homemade traps for trapping ghost crab nest predators at least tern colonies in 2011 and 2012: a trap made for baiting and catching multiple crabs (a), and a trap made for catching a single crab from an active burrow; this trap is shown sitting beside an active ghost crab hole (b).

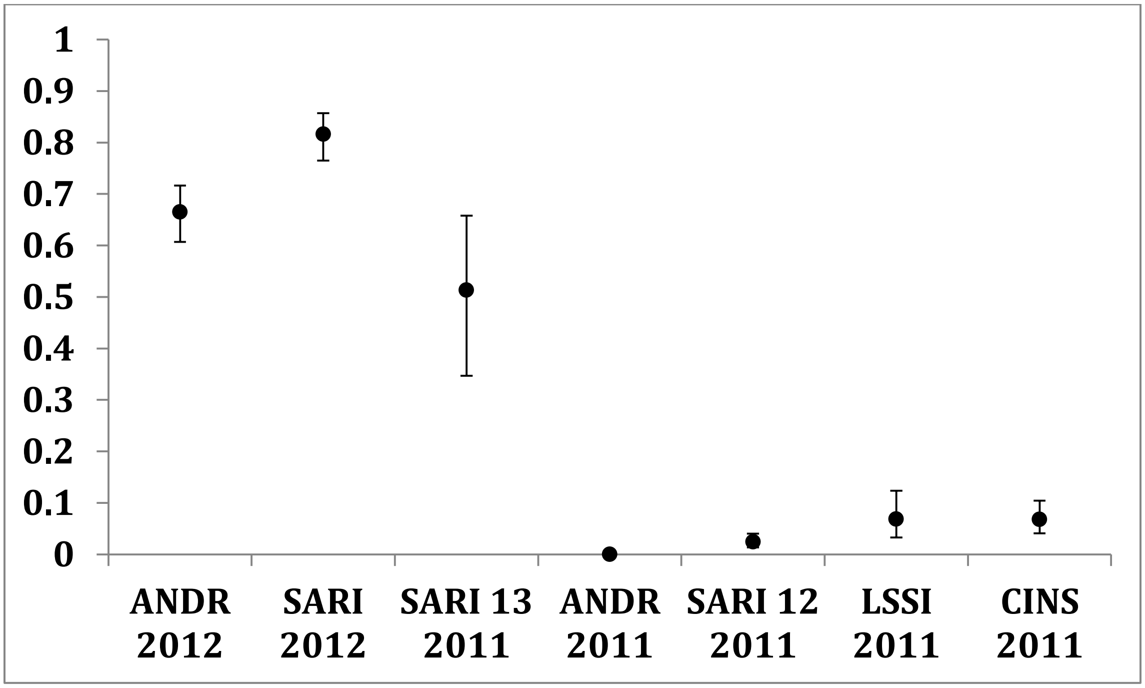

Figure 4.5. Point estimates, with 95% confidence intervals, of least tern nest success in Georgia in 2011 and 2012. Least tern nesting colony and sampling year are shown on the *x* axis, and point estimates of nest success are shown on the *y* axis. Nesting colonies were at Andrew's Island (ANDR), the Savannah River dredge spoil area (SARI), Little St. Simon's Island (LSSI), and Cumberland Island National Seashore (CINS). ANDR and SARI were managed for mammalian predators in 2012 using Premier Pos-Neg® electric mesh fencing with a solar-powered energizer. In 2011, SARI was managed using a standard 3-stranded electric cattle fence, but no other sites were managed for predators. Least tern nest success was significantly higher at managed colonies (ANDR 2012, SARI 2012, and SARI 2011)

Figure 4.6. We are the first to report least tern chick predation by a bobcat (*Lynx rufus*). Three least tern chicks were found maimed and killed within the nesting colony in July of 2011 at Cumberland Island National Seashore, and all chicks were closely associated with bobcat footprints.

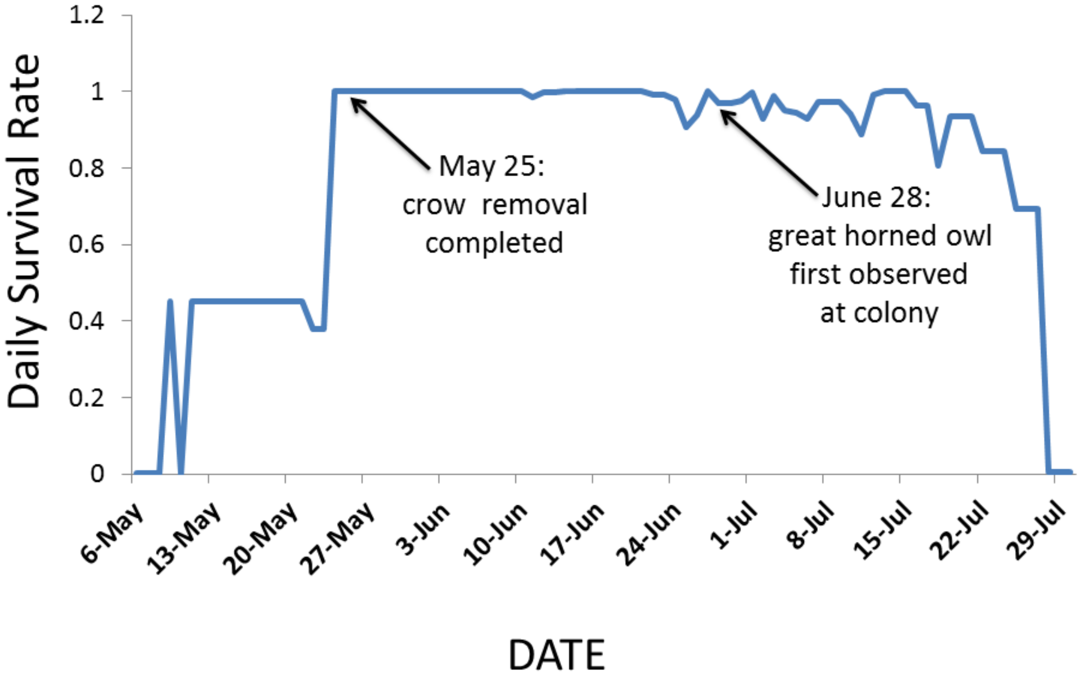

Figure 4.7. Daily survival rate for least tern nests over the course of the breeding season at Andrew's Island in 2012. Arrows indicate the date we completed crow removal at the nesting colony (May 25th), as well as the date that the great horned owl (*Bubo virginianus*) was first visually observed at the nesting colony (June 28th), which had followed several visits during which least tern chicks that appeared to be owl-depredated were found.

Tables:

Table 1. Summary of nest success and nest fate of least terns nesting in Georgia in 2011 and 2012. Nesting colony sites were Andrew's Island (ANDR), the Savannah River dredge spoil area (SARI 12, 13, and 14), Little St. Simon's Island (LSSI), and Cumberland Island National Seashore (CINS). Colonies that received predator management are shown in blue typeface. ANDR and SARI 14 were managed for mammalian predators in 2012 using Premier Pos-Neg® electric mesh fencing with a solar-powered energizer. In 2011, SARI 13 was managed using a standard 3-stranded electric cattle fence, while no other sites received management. Least tern colony nest success point estimates are listed, along with total nests monitored, number of monitored nests that hatched, and number of monitored nests that failed for each cause of failure: over-wash (includes flooding), predation by coyote, predation by raccoon/armadillo, predation by ghost crab, predation by fish crow, died while pipping, abandoned for unknown reasons, or unknown (nest missing with no evidence of the cause of failure). Colonies that received predator management had higher point estimates of nest success and total number of hatched nests

site and year	nest success	total nests	hatch	overwash	coyote	raccoon/ armadillo	crab	crow	pipping	unknown abandon	unknown
ANDR 2011	0.0001	43	0	0	5	0	0	2	0	0	36
LSSI 2011	0.0687	65	1	4	0	41	7	0	0	2	10
CINS 2011	0.0676	137	7	23	41	0	20	0	0	0	45
SARI 12 2011	0.0243	194	1	0	6	0	0	0	0	20	167
SARI 13 2011	0.5131	48	30	0	0	0	0	0	0	1	17
ANDR 2012	0.6649	344	150	2	0	0	0	22	23	56	91
SARI 14 2012	0.8161	306	169	0	0	0	0	0	5	65	67

CHAPTER 5

CONCLUSIONS

We investigated concentrations of mercury, and the unique PCB mixture Aroclor 1268, in least terns (*Sternula antillarum*) at a highly-contaminated estuary in coastal Georgia. This estuary is the location of the LCP Superfund site, where until 1994 industrial enterprises released effluent containing these contaminants. High concentrations of Aroclor 1268 and mercury are found in local biota, but no studies report concentrations in piscivorous birds. We collected egg samples, as well as feathers and feces from chicks, from breeding colonies across the Georgia coast to analyze contaminant loads. Mean Aroclor 1268 concentrations in eggs were highest at colonies in and just outside LCP, and decreased with increasing distance to LCP, which was expected as Aroclor 1268 was released only at LCP. Mean Hg concentrations of eggs varied little among sites, but eggs with the highest concentrations were found at sites closest to LCP, and in the Savannah River. Mercury in chick feathers and sediment samples were highest at the site closest to LCP, indicating that chicks are better gauges of local contamination than eggs. This was expected, as chicks are fed locally until fledging, while adults may accumulate contaminants over time. However, chick fecal Hg was highly variable within each colony, and did not differ significantly among colonies, indicating fecal samples are likely a better reflection of the variation of contaminant concentrations in the food items. Lastly, we report the transport of Aroclor 1268 at least

~110 km north, and ~70 km south from its point source (LCP); transport of this unique PCB mixture > 40 km has not previously been documented.

We investigated the relationship between biomarkers including egg volume and weight, eggshell thickness, prevalence of congenital deformities, fluctuating asymmetry, chick growth rate, and lymphocyte estimates and differential counts, with concentrations of mercury and PCBs in least tern feces, feathers, and eggs, collected during the 2011 and 2012 breeding seasons. Symmetry in the wing chord and the primary 2 feather of least tern chicks decreased significantly with increasing feather mercury concentration, associating mercury exposure with developmental instability in least terns. Heterophil/lymphocyte ratios also decreased significantly with increasing mercury concentrations in chicks, indicating that mercury exposure may be associated with immunosuppression in least terns. We also found that increased prevalence (2.9%, $n = 7$) of congenital deformities (especially limb deformities) at one colony appeared to be linked to mercury.

Urbanization and human encroachment upon wildlife habitat is inexorable, and poses a variety of challenges to native wildlife species. One such challenge for many species is the food provisioning, and the subsequent overpopulation, of predator species. Least terns are in decline in the eastern U.S., while the interior and California subspecies are federally endangered. Decline of this species since the second half of the 20th century has been largely due to habitat loss or degradation, human disturbance at coastal breeding colonies, and predation by many species whose populations are artificially inflated due to anthropogenic food provisioning. We document the efficacy of several predator management techniques, which include the use of electric fencing, crow effigies, ghost crab traps, and lethal crow removal. In 2011, 4 of the 5 least tern nesting colonies we monitored in this study suffered total reproductive failure due to predation. None of these colonies fledged young, and nest success estimates ranged from 0%-7%. We implemented predator management at one colony mid-way through the season in 2011, and the nest success estimate here was 51%. In 2012, we implemented predator management at two of these colonies for the entire season, and nest success estimates were 82% and 67%. We found the use of positive-negative conductive electric mesh fencing, as well as crow removal, to be very effective at increasing nest success, and based on our results reproductive success at these sites seems very unlikely or minimal in the future without predator management. We also document an instance of bobcat (*Lynx rufus*) predation on least tern chicks, which has not yet been reported to our knowledge.

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