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A Test of the Use of Timber Wolf (Canis lupus) Urine to Reduce Coyote (Canis latrans) Depredation Rates on Loggerhead Sea Turle (Caretta caretta) Nests

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To the Dean of the Graduate School:

We are submitting a thesis written by Michael Wauson entitled A Test of the Use of Timber Wolf (*Canis lupus*) Urine to Reduce Coyote (*Canis latrans*) Depredation Rates on Loggerhead Sea Turtle (*Caretta caretta*) Nests.

We recommend acceptance in partial fulfillment of the requirements for the degree of Masters of Science in Biology.



Jack E. DeRochi, Dean, Graduate School

A TEST OF THE USE OF TIMBER WOLF (*CANIS LUPUS*) URINE TO REDUCE COYOTE (*CANIS* LATRANS) DEPREDATION RATES ON LOGGERHEAD SEA TURTLE (*CARETTA* CARETTA) NESTS

A Thesis

Presented to the Faculty of the College of Arts and Sciences in partial Fulfillment

of the

Requirements for the Degree

of

Master of Science

In Biology

Winthrop University

May, 2019

By

Michael Wauson

Abstract

Loggerhead sea turtles are currently listed as vulnerable by the International Union of Conservation of Nature (IUCN) with a decreasing population trend. Over the past four years, coyotes (*Canis latrans*) have depredated 24.18% of loggerhead sea turtle (*Caretta caretta*) nests on the night they were laid on South Island beach at the Tom Yawkey Wildlife Center, near Georgetown, SC. This has resulted in an estimated 4,002 eggs lost each year there. Over that time, a South Carolina Department of Natural Resources (SCDNR) Turtle Technician Team patrolled the beach at dawn every morning to cage and catalog loggerhead eggs and nests but were unable to cost-effectively protect the nests the night the eggs are laid. To test a new method to dissuade coyote depredation, I used dispensers filled with wolf urine to simulate timber wolf (*Canis lupus*) activity on seven sections of the beach and left seven sections untreated as controls. There was an apparent depression in depredation rates where urine was present compared to that of the control areas. The results suggest this may be an example of exploitative competition in the absence of interference competition. Furthermore, there may be kairomones in the wolf urine that allow the exploitative competition to exist even when coyotes haven't been exposed to wolves in many generations. With daily teams patrolling the beaches already, the use of wolf urine as a deterrent could be an inexpensive, non-invasive way of reducing coyote depredation on loggerhead nests elsewhere.

With access to the DNR's large data set I was able to test if there were any naturally occurring potential influences that affected loggerhead nesting or coyote depredation behavior. I was able to determine that nocturnal atmospheric conditions, mean daily temperature, nocturnal precipitation, nocturnal wind conditions, moon phase

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grouping and nocturnal tide types had no effect on or correlation with loggerhead nesting or coyote depredation behavior.

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Introduction

Loggerhead sea turtles (*Caretta caretta*) are found throughout the subtropical and temperate regions of the Mediterranean Sea and Pacific, Indian and Atlantic Oceans (IUCN 2017A). This global distribution has made them the flagship species for sea turtle conservation. In South Carolina alone, there are more than 1,100 participants, most of them volunteers, working to secure a future for these majestic animals (SCDNR 2013). The infatuation with sea turtles goes beyond just conservation work and can be seen in merchandise such as t-shirts and jewelry as well as in the children's movies <u>A Turtle's Tale</u> and <u>Finding Nemo</u>. Even with the public awareness about the need to protect loggerheads and other sea turtles, populations are in decline, necessitating continued conservation work.

Conservation efforts focused on protecting loggerhead sea turtle nests have had an extensive history since they were listed as threatened throughout their range under the Endangered Species Act of 1973 (National Marine Fisheries Service (NMFS) 2008). However, most of those conservation methods do not protect the nests the night the eggs are laid, a point at which the eggs are particularly susceptible to predators such as coyotes, raccoons (*Procyon lotor*), boars (*Sus scrofa*), and ghost crabs (*Ocypode quadrata*) (Engeman *et al.* 2006). To prevent this depredation, a technique is required to be implemented to deter depredation 24/7 but have no effect on the turtles nesting.

The objective of this project was to tested a method to mitigate depredation by coyotes, which is presently the most significant natural threat to loggerhead turtle nests on South Island beach at the Tom Yawkey Wildlife Center near Georgetown, South Carolina. Coyotes account for 85 to 90 percent of lost eggs every year on South Island

beach (South Carolina Department of Natural Resources (SCDNR) 2017). All such losses occur the night the eggs are laid (Pers. Obs). A technique that reduces the depredation of nests by coyotes could potentially allow thousands more eggs to hatch. Therefore, I tested an affordable method to protect sea turtle nests the night they are laid, which has the potential to be used in the conservation of loggerhead and other sea turtle species.

Natural History of Loggerhead Sea Turtles

Lifecycle Stages

The loggerhead sea turtle's lifecycle can be broken down into six stages: 1) eggs, 2) hatchlings, 3) post-hatchlings, 4) oceanic juveniles, 5) neritic juveniles, and 6) adults (NMFS 2008) (Figure 1). The nesting season runs from late April through early September, with hatching occurring between late June and early November (NMFS 2008). Once the eggs are laid, they take on average 55-60 days to hatch (SCDNR Marine Turtle Conservation Program 2013), with the pivotal temperature in the nest, defined as the temperature that produces an equal number of males and females, being 29°C (NMFS 2008).



Figure 1. A simplified general locational life cycle of Atlantic Loggerheads (modified from Bolten 2003).

Loggerhead hatchlings require four to seven days to emerge from the sand after hatching (Koen *et al.* 1994). Hatchlings usually emerge en masse at night. It is hypothesized that they time emergence with the lowering of the sand temperature below a certain point, which usually occurs after sunset (Moran *et al.* 1999). Once hatchlings emerge from the sand, they head straight for the ocean, using the slope of the beach and the reflection of light from the stars and the moon off the ocean surface, compared to dark dunes, as a guide (Parker 1922). Many beachfront communities that have sea turtle nests on their shores require beachside lights-out curfews during the hatching season to ensure hatchlings do not become confused by artificial lighting (U.S. Fish and Wildlife Service 1978). It has been shown that nesting females will nest in front of tall dark objects on urban beaches to mitigate the effects of artificial light (Salmon *et al.* 1995). Once in the water, the 4 cm long hatchlings (measured as the length of the carapace) begin a 20 to 30-hour swimming frenzy (post-hatchling life stage) that takes them away from the coast into the open ocean, coming to rest in *Sargassum spp.*, dead terrestrial vegetation and/or debris in pelagic drift lines formed in current convergences (Carr 1986, NMFS and FWS 1991, NOAA Fisheries 2017). This starts the oceanic juvenile stage.

The juveniles continue to ride the current convergences for the next 7 to 11.5 years, growing to between 46 and 64 cm in carapace length (NMFS and FWS 1991). During this life stage, juveniles increase in length approximately 2.9 to 5.4 cm a year on average (Snover 2002). Juveniles consume a variety of organisms including cnidarians, salps, pelagic snails, jellyfish, barnacles and crabs (Bjorndal 1997). Hatchling survival probability to their second year is still unknown but is thought to be relatively low (NMFS and FWS 1991). This part of the juvenile stage is often referred to as the lost years due to the inability to track the hatchlings and young juveniles in the open ocean until 2014. In 2014 Mansfield et al. showed that the juveniles rarely travel in continental shelf waters, frequently leave currents associated with the North Atlantic Subtropical Gyre, select surface water habitats that most likely provide a thermal benefit or refuge which supports growth, foraging and survival. Their annual survival probability between the ages of 2 and 6 is 91.1 percent (NMFS and FWS 1991).

The neritic zone is defined as nearshore and estuarine waters, with depths less than 200 meters, along continental margins and shelves. During the neritic juvenile life stage, the turtles main prey items include jellyfish, mollusks, crabs, sea pens and fish bycatch from shrimp trawlers (Burke *et al.* 1993, Plotkin *et al.* 1993, Seney 2003). The carapace length of loggerheads in this phase ranges from 46 to 87 cm, with a growth rate of 1.8 to

2.1 cm/year (Bjorndal *et al.* 2001). Individuals stay in this stage between 14 and 24 years, depending on their size when they entered it. During the neritic juvenile stage, the annual survivorship estimates drop to 64.3 Percent (Bjorndal *et al.* 2003).

Loggerheads are sexually mature when their carapace length reaches 90 cm, which usually occurs between the ages of 17 and 33 years (MarineBio 2013, Drakes 2012). As adults, loggerheads are considered the largest hard-shelled turtles, weighing around 114 kg (NMFS 2008). Adults can move between the oceanic and neritic zones and feed mostly on *Janthina spp*. (small to medium-sized pelagic or planktonic sea snails), *Velella velella* (pelagic hydrozoans), *Lepas spp*. (gooseneck barnacles), *Planes spp*. (*crabs*), and *Pyrosoma* spp. (free-floating colonial tunicates) (Seney and Musick 2007, Parker *et al.* 2005).

As a result of nesting on land, there is a knowledge gap between male and female life history's. Females are easily tagged when nesting on the beach, and that data shows that adult females spend as much as 25 years in the neritic zone (Dahlen *et al.* 2000). Females are estimated to be reproductively viable for 25 years, but that may be an underestimate due to tag loss and incomplete surveys (NMFS 2008). There is little data on the movement and lifespan of adult male loggerheads because of the difficulty in locating and tagging them (Loggerhead Marinelife Center 2017). The annual survival probability of an adult loggerhead is estimated at 85 percent (Heppell *et al.* 2003).

Nesting Behavior

When females nest, they come up on the beach at night and lay between 100 and 126 eggs above the high tide line. As they move, the females leave clear carapace and

elbow joint marks in the sand (Figure 2). Once a nesting site has been chosen, the females turn around to face the ocean and begin to dig their nests. To excavate the egg chamber, the females use their hind flippers to dig a flask-like chamber around 60 cm in depth and 23-26 cm in width (Carthy et al. in Miller et al. 2003) (Figure 3). The actual nest size correlates with several measurements of the nesting female such as carapace length, width, and reproductive output (Carthy et al. in Miller et al. 2003). When selecting a nesting site, females must balance between nesting too close to the ocean, which could lead to inundation of the nest, and too far inland, which brings increased threats of nest depredation, hatchling misorientation and predation of the female (Wood and Bjorndal 2000). What determines the exact nest location is still poorly known. It is believed that a nest site is chosen when several threshold cues possibly slope, salinity and temperature among other environmental factors are reached (Wood and Bjorndal 2000). Reproductive females will usually return to the same beach to nest every two to three years, but the interval ranges from one to seven years (U.S. Fish and Wildlife Service 2018).



Figure 2. Nesting Loggerhead Tracks (Michael Wauson)



Figure 3. Loggerhead nesting image (Preserve Hawai'i).

Over their lifespan, loggerheads contend with a wide variety of predators. In the nest, loggerheads are vulnerable to crabs, ants, canids (including domestic dogs, foxes and coyotes), boars, raccoons, and both domestic cats and bobcats (Engeman *et al.* 2006). As the eggs hatch and the hatchlings are on their way to the water, they contend with the predators listed above as well as snakes, crows, gulls, and several species of raptors. Once in the water, hatchlings are vulnerable to birds and large predatory fish. As the turtles grow, their predator list shrinks to killer whales (*Orcinus orca*) and a few species of sharks such as tiger sharks (*Galeocerdo cuvier*) (Heithaus *et al.* 2008).

Conservation Issues

Loggerhead sea turtles are listed as vulnerable by the International Union for Conservation of Nature (IUCN) with a population that is declining. The main reason for this decline is human activity (IUCN 2017A). Humans harvest loggerheads for consumption (A), but commercial fishing (longline and trawling) also results in loggerhead bycatch (B). Other human-generated threats include marine deposition of garbage (C), pollution from agricultural and automotive runoff and oil spills (D), beach changes, which includes artificial lighting, coastal armoring (sea walls, rock revetments, and sandbags), beach erosion (E), human beach activities and human introduced predatory invasive species (F), and climate change (G) (U.S. Fish and Wildlife Service 1978).

A) Consumption

Human consumption has had a significant effect on loggerheads and other sea turtle species in North America. multiple studies in Baja California Sur, Mexico, focused on human consumption of loggerheads. One found that 45.5% of 1041 loggerhead carcasses located had been harvested for meat (Mancini and Koch 2009). The consumption of turtle meat seems to be related to local cultural factors, as it was consumed most often during the Christian fasting period of Lent (Mancini and Koch 2009, Peckham *et al.* 2008). Mancini and Koch (2009) and Peckham *et al.* (2008) also found multiple local, regional, and international black-market areas developed for the sale and consumption of sea turtle meat. Human consumption of turtles is not limited just to North America but is also

practiced in many countries including Madagascar, Mozambique, and South Africa to list a few (IOSEA Marine Turtle MoU 2013).

B) Commercial Fishing

Two activities associated with commercial fisheries that result in significant negative effects on loggerhead populations include long-lining and trawling. In 2000, an estimated 200,000 loggerhead sea turtles were caught on the hooks of longlines, resulting in tens of thousands of moralities (Lewison 2004). Longlines used for swordfishing have the largest effect on loggerheads due to their placement near the surface of the water column (Taylor and Haplin 2008). The consequences of being caught on a longline can vary. If the turtle is unable to reach the surface, it will drown. If it does not drown, the long-term effects of being hooked in the mouth or flipper are relatively unknown but could be disfigurement and reduced mobility.

Trawling has a major impact on loggerhead populations. In the late 1980's, Turtle Excluder Devices (TEDs) were developed and implemented by the U.S. and numerous other fishing fleets. TEDs dramatically decreased loggerhead losses, but fishermen and nations that do not implement TEDs are still a threat to loggerheads (Crowder *et al.* 1995). It is estimated that 30,000 loggerheads are caught by trawlers in the Mediterranean Sea per year, with a 25 percent mortality rate (Sala *et al.* 2011).

C) Marine Deposition of Garbage

Marine deposition of garbage has serious consequences for marine life. For example, at least 267 species of organisms are known to be negatively affected by plastic debris, and its accumulation over the decades has resulted in the deaths of millions of animals each year (Moore 2008). In the case of sea turtles, they apparently mistake some plastic items for jellyfish, which are among their primary food sources. Oceanic juveniles are particularly vulnerable to death from ingested plastics. Pham *et al.* (2017) found 20 of the 24 sampled loggerheads had consumed garbage containing plastic debris; the animals averaged 15.83 (S.E. \pm 6.09) items of plastic in their stomachs. In total the items had a mean dry weight of 1.07g (S.E. \pm 0.41), an amount that can prove fatal (Pham *et al.* 2017).

D) Marine Pollutants

Marine pollutants have a substantial effect not only on sea turtles directly, but also on their food sources. Many of the pollutants affecting aquatic life come from agricultural and automotive runoff as well as from oil spills (Ley-Quiñónez *et al.* 2011). The chemicals from runoff result in bioaccumulation and biomagnification of pollutants in the prey items of loggerhead turtles, which results in possible illnesses and diseases to the turtles during the course of their lives (Maffucci *et al.* 2009). A potentially lethal or debilitating illness is fibropapilloma tumors, which develop predominately in fibrous tissue, but can affect all kinds of soft tissues; the tumors have recently been linked to the chelonid herpesvirus 5 (Rossi *et al.* 2015) and are suspected to cause debilitating cutaneous infection (Lackovich *et al.* 1999). Oil spills, on the other hand, have more immediate effects on turtles such as increased susceptibility to infection, and can result in severely altered blood chemistry when oil is ingested. Oil spills

also result in the loss of potential nesting sites when oil washes onto beaches (Lutcavage *et al.* 1995, Lauritsen *et al.* 2017).

E) Beach Changes

Beach changes such as coastal armoring such as sea walls, and erosion have a dramatic effect on loggerhead nesting. When coastal armoring is built to protect human coastal infrastructure, the altered beach structure results in fewer successful nesting emergences compared to natural dune areas (Mosier and Witherington 2001). Because loggerheads are philopatric – females return to nest on the beach where they hatched – beach erosion (natural or human influenced) is another threat (Stiebens *et al.* 2013). It can take a loggerhead 33 years to reach sexual maturity; thus, the beach where a female hatched could be gone or modified in such ways that she is unable to nest where she was hatched. This forces the female to choose between an unknown beach that may not be conducive to the survival of her offspring and risking nesting on the modified armored beach (Mosier and Witherington 2001).

F) Other Human Beach Activities and Invasive Species

Beach activities and invasive species are interrelated in that domestic and feral dogs and cats are known to dig up nests and eat hatchlings (Turkozan *et al.* 2003, Hilmer *et al.* 2010). Human and animal activity at night can keep nesting females from leaving the ocean to nest or disrupt a female while she is laying her eggs or digging her nest to the point where she leaves the beach. Another threat is recreational equipment such as chairs on the beach, boats on the beach

or in the water, and other debris that can deter nesting females (U.S. Fish and Wildlife Service 1978).

G) Climate Change

Climate change exacerbates some of the issues listed above, especially beach erosion and human attempts at coastal armoring in the face of rising sea levels and larger storms. One of the primary effects of climate change is increased mean annual temperatures. As the temperature of the beaches increases, so does the ratio of female to male turtles that are produced (Abella *et al.* 2007). This is because loggerheads like most turtles, sex is based on temperature-dependent sex determination (temperature of each egg in the nest will determine the sex of that individual), eggs incubated below 27.7 Celsius will be male and anything over 31 Celsius will produce females. Anything between that range with produce either or. As the temperatures increases, it forms a potential genetic bottleneck as the sex ratio becomes more and more skewed towards females; however, there is evidence that as temperatures rise, the nesting season will begin earlier in the year, perhaps mitigating this effect (Thaler and Fuentes 2016, Weishampel *et al.* 2008).

Coyotes

Natural History of Coyotes

Coyotes (*Canis latrans*) are native to the plains and southwest deserts of North America, but over the last 150 years they have spread across the United States (Bozarth *et al.* 2011). Moving east, they took two primary paths: the northern front went through the Great Lakes region and the southern front moved through the Gulf States (Bozarth *et al.* 2011). As they moved, the northern and southern groups contended with different competitors and environmental pressures. In their original home ranges, coyotes had to cope with timber wolves (*Canis lupus*), mountain lions (*Puma concolor*), black bears (*Ursus americanus*), and brown bears (*Ursus arctos*). As they moved east, the northern group encountered and interbred with the Great Lakes population of timber wolves, according to DNA evidence (Bozarth *et al.* 2011). In contrast, as they traveled east, the southern front no longer had to contend with timber wolves but encountered the red wolf (*Canis lupus rufus*), a subspecies native to the southeastern United States (IUCN 2017B). Red wolves resemble coyotes in body size, prey preference and social group behavior (U.S. Fish & Wildlife Service 2016). Thus, there is a large niche overlap between the two species, and they are known to interbreed (Wayne and Jenks 1991).

Coyotes are relatively small canids weighing between 9 to 22.7 kg. Their lifespan in the wild is 6-8 years, on average (Bekoff 1977). Carlson (2008) found that coyotes are usually monogamous, maintaining pair bonds for many years. They are reproductively capable as early as ten months of age, but adults (34 months or older) have the highest fecundity. They have from three to seven pups per litter on average. Pups are usually born between March and May after a 60 to 63 day gestation period (Carlson 2008) and leave the parental territory as early as six to nine months of age or remain as subordinates in the pack. Both parents protect the territory and provide food for the pups (Gier 1968).

Coyote pack size varies based on food availability. The more abundant the food source, the larger the pack tends to be. A pack is made up of the alpha breeding pair, young of the alpha, and other individuals (not offspring of the alpha pair) that have been accepted into the pack (Bekoff and Wells 1980). Usually only the alpha male and female

breed (Carlson 2008). There seems to be no correlation between pack size and territory size (Bekoff and Wells 1986).

Coyotes are omnivores with a broad diet that includes small mammals, birds, livestock, pets (cats and dogs), fruits, vegetables, carrion, and eggs (Pennsylvania Game Commission 2014). In pairs or a pack, coyotes can take down large ungulate prey such as mule deer (Bowen 1981). Mountain lions and wolves are the two largest predatory threats to coyotes in terms of numbers of animals killed in their native range (Merkle *et al.* 2009; Washington Department of Fish & Wildlife).

Coyotes are considered a nuisance and invasive species in South Carolina; SCDNR encourages the hunting of coyotes (SCDNR 2015). The coyotes' ability to adapt to a wide range of diets and habitats makes them very versatile predators, and is why it is believed that coyotes can fill the niche left vacant with the elimination of the region's top predator, the red wolf. The coyote's ability to exploitation food options in its environment is not limited to wild game, but can also extend to domestic animals, resulting in a substantial financial problem for farmers. In 2000, coyotes were responsible for a loss of over ten million dollars due to calf predation alone in the Eastern United States (Houden 2004).

Coyotes can be significant predators on sea turtle nests. For example, on the Baja California Peninsula in Mexico, 81.4 percent of monitored loggerhead nests were depredated by coyotes (Mendez-Rodriguez and Alvarez-Castaneda 2016). In the conclusion of that paper, Mendez-Rodrigues and Alvarez-Castaneda (2016) state that coyotes should be considered an important predator of sea turtles.

The Study Site

History of Tom Yawkey Wildlife Center

South, North, Cat and Sand Islands make up the Tom Yawkey Wildlife Center off the coast of Georgetown, South Carolina (Figure 4). The preserve was established in 1976 by Tom Yawkey, the former owner of the Boston Red Sox. The preserve is 97.12 Km^2 (24,000 acres) (SCDNR Public Lands 2016) in area. It includes marshes, wetlands, maritime forests and beaches.

The preserve has a rich history dating back before colonial times with the Pee Dee, Samtee, Sampit, See Wee, Waccamaw, and Winyah Native Americans inhabiting the area. The Archaeology and Anthropology Institute at University of South Carolina has dated one of the sites on Cat Island going back as early as 1500 B.C. (SCDNR 2004 Tom Yawkey Wildlife Center).

During the Civil War, South, Cat and North Islands were fortified with forts and batteries (SCDNR 2004). A few of the military landmarks can be seen today on Cat and North Islands. After the war, Confederate General Edward Porter Alexander, Bill Yawkey and Joseph Wheeler bought land on North and South Island and turned it into a waterfowl hunter's paradise. Powerful men of the time, including President Grover Cleveland, frequented the islands for the rich hunting grounds (SCDNR 2004). Bill Yawkey eventually became the sole owner of the islands and upon his death, he bequeathed the land to his nephew and namesake of the preserve, Tom Yawkey (SCDNR 2004). In the late 1930's, Tom began to change the focus of his land from hunting to conservation aimed at creating a waterfowl refuge. Tom Yawkey died on July 9th, 1976. His will bequeathed his South Island Plantation to the State Wildlife and Marine Resources Department, which cares for it to this day (SCDNR 2004).



Figure 4. The Tom Yawkey Preserve divided into its four islands (Eskew 2012).

History of Loggerheads on South Island

The first survey for loggerhead sea turtles on South Island beach was in 1977. Since that time, there have been continuous conservation and management plans in place. South Island beach is 5.88 km long and averaged 241 loggerhead nests per year from 2015-2018. Over the last three years (2015-2017), an average of 4,140 eggs on South Island beach (15.86 percent of the eggs laid per season) have been depredated each year by coyotes. A mean of 69 eggs are destroyed each time a coyote raids a nest (SCDNR unpublished).

Three apex terrestrial predators, coyotes, bobcats (*Lynx rufus*), and American alligators (*Alligator mississippiensis*) call the Yawkey preserve home. Since 2015, there have been no documented bobcat or alligator depredations of loggerhead nests on South Island beach (SCDNR unpublished). SCDNR does however report that in 2009 and 2013, there were egg and nest losses on South Island beach due to "other" causes that did not fall under the usual ghost crab, coyote, research, tidal, or racoon losses (SCDNR 2017).

History of Loggerhead and Coyote interactions and conservation techniques in the Yawkey Preserve

Coyotes have become major predators on loggerhead eggs on the Yawkey Islands since their first appearance in 2006 (Eskew 2012). As of 2009, they were responsible for 52% of loggerhead egg losses in the preserve (SCDNR 2010). The methods that have been tried to protect loggerhead nests on the Yawkey Islands from coyote depredation have met with varying degrees of success. Techniques employed include using highpitched sounds near turtle nests, but that showed no detectable effect on depredation levels (Pers. Comm., Jamie Dozier, Certified Wildlife Biologist and Project Leader at the Tom Yawkey Wildlife Center). Nightly patrols by humans worked well to deter coyote depredation but resulted in a massive number of man-hours and, as a consequence, were expensive and could not be sustained (Eskew 2012). Caging the nests the morning after the eggs are laid is extremely effective at dissuading coyotes and raccoons, but this method still leaves the nest vulnerable the night the eggs are laid (Pers. Obs.). It is

believed that several coyote packs occupy areas in the Tom Yawkey Preserve but that a single pack patrols the South Island beach and that the beach constitutes most of their territory (Pers. Comm., Jamie Dozier). Removal of this coyote pack is the most recent control attempt and has had very limited success; it required a large number of man-hours due to the terrain and failed because of the canids' rapid learning to avoid traps (Pers. Comm., Jamie Dozier).

The Present Project

I proposed to test a novel method to reduce coyote depredation on sea turtle nests on the Yawkey Islands based on research by Merkle et al. in 2009 on the timber wolves reintroduced to Yellowstone National Park in 1995, and their interactions with coyotes. That study provided an important opportunity to understand the competitive interactions between coyotes and timber wolves. Competition can be broken down into two main components: interference and exploitative (Smallegange *et al.* 2006). Interference competition occurs when organisms directly vie for resources. One type of interference competition is intraguild competition, where one competitor kills and sometimes eats the other, a form of predation. For example, Merkle *et al.* (2009) documented 337 interactions where wolves dominated (i.e., killed or forced coyotes to leave the immediate area) in 91 percent of the interactions and proved lethal to coyotes in 7 percent of those meetings (Merkle *et al.* 2009).

Exploitative competition results from organisms indirectly competing with other species for resources and consuming those resources to the point where they are denied to other species. For instance, territory marking by wolves where coyotes in these areas of high wolf use exhibited decreased rest and increase the time spent in vigilance activities (Switalski 2003, Lang *et al.* 2013). Exploitative competition may be the result of interference competition with a particular species. In the studies above (Switalski 2003) the coyotes initially had more confrontations with wolves, but as time passed, the coyotes were seen less and less near wolves. This could have been due to a greater food availability with the change in seasons, or because interference competition needs to be present to create exploitative competition. To keep the one-sided (exploitative) competition in balance an animal may require the competitor to physically be present.

The level of exploitative competition between animals varies in locations due to dietary overlap and prey distribution and abundance (Fonju 2011). This was seen in a study in Alaska where coyotes and wolves were studied but wolves primarily fed on moose and coyotes fed on small mammals; with less dietary overlap there was less competition between the two organisms (Thurber *et al.* 1992). In the North Fork area of northwestern Montana, however, where dietary overlap was higher, there was an observed decrease in the coyote population as the wolf population increased, suggesting a higher level of competition (Arjo *et al.* 2002). Another observation involving canids in exploitative competition is between African wild dogs (*Lycaon pictus*) and spotted hyenas (*Crocuta crocuta*), where high dietary overlap resulted in a negative correlation in wild dog and hyena densities (Creel and Creel 1996).

These studies were central to my understanding of exploitative competition between coyotes and wolves, and I hypothesized that lessons learned from them could be applied to the conservation of loggerhead sea turtles. Thus, I simulated wolf "presence" and activity on South Island beach using wolf urine dispensers to test whether wolf urine would deter coyote depredation of loggerhead sea turtle nests.

Methods and Materials

Layout and Execution

From May 11th to May 17th, 2018, I established control, partial control and treatment areas on South Island beach at the Tom Yawkey Wildlife Center, S.C. There were seven 200-meter long control areas, thirteen 100-meter long "partial-control" or "buffer" areas, and seven 200-meter long treatment areas. Areas designated as partial controls were required between the treatment and control sites because it was unclear how far the wolf urine scent would disperse.

Each treatment and control area required 50 wolf urine dispensers which were constructed following the manufacturer's instructions (Maine Outdoor Solutions). Each dispenser consisted of one plastic bottle containing 44.36 mL of wolf urine attached to a 25.4 cm wooden post using hardware provided by the supplier. Posts were placed 4 meters apart, with the container section on the seaward side and the wooden support post facing the landward side. The dispensers formed a 4100 m line running on top of the first dune line, and parallel to the shoreline. A transect at each end of the treatment group ran perpendicular from the dune line down to the "king tide" line (which marks the extent of higher than normal tides brought on by specific alignments of the moon and sun) (see Appendix 1). The perpendicular line also was furnished with dispensers 4 m apart, per the manufacturers' instructions. In some cases, there was only room for one dispenser to be placed before the king tide line, but in other areas, up to three were set. Stakes were placed every 50 meters to indicate the type of treatment area (treatment, control, or partial control) a nest was discovered in.

The South Carolina Department of Natural Resources Turtle Technician Team collect and provided all the data dealing with loggerhead nest dates, location, depredation events (including predator identification), counts of eggs, nests depredated, and nest losses. Data collection for my work started when the first loggerhead nest appeared on the beach after the dispensers were in place. The team would arrive on the beach just before sunrise or, due to tide variability, an hour or two later when the high tide passed. The team used four-wheelers to patrol the length of South Island beach in search of "turtle crawls" as well as predator prints. When a crawl was located, the team drew a perpendicular line through the turtle's trail above the high tide line to ensure that the crawl was not logged twice. The team then followed the crawl to determine whether a nest was constructed.

If a nest was located intact, it was caged using a metal mesh box that enclosed the nests on the tops and sides with spacing to allow hatchlings to pass through. For depredated nests, the team removed all broken eggs from the nest and searched the surrounding area for broken eggs. Once all the depredated eggs were counted, the remaining intact eggs were removed from the nest, cleaned and relocated to a nearby man-made nest at the dune line and buried about one meter down into the sand. The team then determined the predator by the paw prints left in the sand. The teams were trained and used diagrams to compare and identify prints. The GPS location of the original and relocated nests, along with the egg counts of depredated nests, were recorded. I traveled to the preserve bi-weekly to collect the data from the technician team and to inspect and fill the urine dispensers.

I visited to the beach to collect the data and refill/ replace dispensers between May 17th to August 11th. The dispensers were filled at 30-day intervals with 44.6 mL of urine following the manufacturer's guidelines until July 14th when they began to be filled biweekly. I changed the filling interval because the urine in the dispensers lasted approximately 9 days, not 33 days as expected based on the manufacturer's instructions. At the bottom of each filled vial, moist colored sand was found that seemed to hold the scent of the urine after the rest of the liquid evaporated. Between visits to the island, dispensers were sometimes lost either due to burial from beach sand movement or to tides and wind. This loss happened to about five dispensers per section. I searched for and when possible restored these but if the dispensers could not be located, new ones were constructed to replace them.

Data I analyzed but not collected by the turtle techs included high and low tide levels (m), mean daily temperatures (C), percent lunar visibility, nocturnal atmospheric conditions (clear sky, overcast, mixed), whether there was nocturnal rain, and nocturnal wind direction. This data was analyzed to determine if there is an ideal time to perform night patrols in regards to when loggerheads are most likely to nest or coyotes to depredate. All data listed above were from May 17th to August 9th between 2015 to 2018. In addition, I analyzed loggerhead nest locations, depredation events by an identified predator, all these forms of data were collected by the turtle techs. The wind direction, mean daily temperature, atmospheric conditions, and precipitations data came from Weather Underground. The tidal data came from Tides4Fishing and lunar data from Timeanddate.com.

Statistical Analysis

To test the effect of wolf urine on the depredation of loggerhead nests, I initially broke down the data into two sets, 9 days and 30 days. I broke it down this way because urine on the Yawkey Islands during the summer evaporated in 9 days. The 30day blocks were the intervals when I filled the dispensers. Partial control areas were initially analyzed as separate entities but were found to be statistically indistinguishable from the control areas using a Fisher Exact Test. Based on that, I combined the partial control data with the control data forming the Total Control group. From there, I compared the two-groups (Total Control and Treatment) at the 30-day interval and then at the 9-Day interval using a Chi-Squared test and Fishers Exact Test respectively.

For the below tests, I compared 2018 data with the combined data for 2015 through 2017 to determine if 2018 was unique. If the tests before showed no difference I combined the data of the four years to determine the effects of some climate elements and tides affect loggerhead nesting behavior. I used a Kruskal-Wallis Test to determine if atmospheric conditions (overcast sky, clear, or mixed (50% overcast)) affected the number of loggerhead nests laid per night. I used Mann-Whitney U tests to determine whether nightly precipitation and wind direction (landward or seaward) affected the number of nests laid. I used Spearman's rho tests to determine whether there was a correlation between: mean daily temperature and the number of nests laid; mean daily temperature and the number of depredated nests per night; and percent moon visibility and the number of nests laid.

To determine the effects of the same climate elements had on coyote depredation events on loggerhead nests. I again ensured I could combine the 4 years but excluded the

treatment area data for 2018. I then used Chi-squared tests to determine if nocturnal wind direction affected the number of nests depredation by coyotes per night, if nocturnal tide types affected the number of nests depredated by coyotes per night, if nocturnal rain affected the number of nests depredated by coyotes per night, and if nocturnal atmospheric conditions affected the number of nests depredated by coyotes per night.

Results

Wolf Urine Effectiveness

The proportion of nests made and depredated during my data collection did not differ between control and partial control conditions (Fisher Exact Test, p=0.2718, ns) (Table 1). That result allowed the two groups to be combined into a single entity, "total control".

	Non-depredated	Depredated	Total	
Control	39	12	51	
Partial Control	15	9	24	
Marginal Column	54	21	75	
Totals				

Table 1. Comparison of all coyote depredation events in control and partial control areas over the entire experiment, based on 30-day intervals between dispenser refilling's (Fisher exact test, p=0.2718, ns)

From there I determined there was no difference in number of nests depredated by coyotes between the total control and the treatment areas (Fisher Exact Test, p=1, ns)

(Table 2).

	Non-Depredated	Depredated	Total
Total Control	54	21	75
Treatment	30	12	42
Totals	78	33	117

Table 2. Comparison of number of loggerhead nests made and coyote depredation events between the total control and treatment areas for the entirety of the experiment (30-day time interval) (Fisher exact test, p=1, ns)

I once more made a comparison, this time between depredation rates during the 9-

day interval following a refill. I confirmed that again control and partial control areas

could be combined, this time for 9-day intervals (Chi-Squared, $\chi^2=.0078$, df=1, p=0.929,

ns) (Table 3).

	Non-Depredated	Depredated	Total
Partial Control	8	5	13
Control	12	8	20
Totals	20	13	33

Table 3. Table shows that for 9-Day grouping, control and Partial control can be combined to for a Total Control again using a Chi-Squared Test. $\chi 2=.0078$, df=1, p=.929

Finally, I compared the total control areas versus the treatment areas for the 9-day interval. I found a no significant depressing effect of the urine on coyote nest depredations in the treatment areas compared to the Total control areas (Fisher Exact Test, p=0.0759, ns) (Table 4).

	Non-Depredated	Depredated	Total
Total Control	20	13	33
Treatment	10	1	11
Totals	30	14	44

 Table 4. Shows the 9-Day grouping comparing Total control nest and depredations to the

 Treatment using a Fisher Exact Test (p=.0759).

Other Variables Tested

I found no differences between 2018 and the prior three years for either number of nests laid or number of nests depredated. (Chi-squared test, $\chi^2 = 0.012$, df= 1, p= 0.913, ns) (Table 5). Since they were not statistically distinguishable from one another, the nesting data and depredation data were combined in later tests.

	Nests Laid	Depredation Events	Total
2015-2017	659	160	819
2018	160	38	198
Totals	819	198	1017

Table 5. Comparison of loggerhead nest data and coyote depredation event data for the 2015 through 2017 season versus 2018 season (Chi-square, $\chi^2 = 0.012$, df=1, p=0.913, ns)

Variables That May Affect Loggerhead Sea Turtle Nesting Behavior

Across all four years (2015 through 2018), none of the tested variables had an effect on, or correlation with, the number of loggerhead nests laid per night on South Island beach.

- Nocturnal atmospheric conditions (Kruskal-Wallis Test, χ² = 4.639, df= 2, p= 0.098)
- Mean daily temperature (Spearman's rho, P = .023, p = 0.569, ns)

- Nocturnal precipitation (Mann-Whitney U Test, $\chi^2 = -1.298$, p=0.194, ns)
- Nocturnal wind direction (Kruskal-Wallis Test, $\chi^2 = .809$, df=2, p=0.667, ns)
- Moon phase group (Kruskal-Wallis Test, $\chi^2 = 2.233$, df=3, p=0.525, ns)
- Nocturnal tide types (Chi-squared, $\chi^2 = 0.0$, df=1, p=1, ns)

Variables That May Affect Coyote Depredation of Loggerhead Sea Turtle Nest Behavior

Across all four years (2015 through 2018) but not including data from the treatment area in 2018, none of the tested variables had an effect on, or correlation with, the number of loggerhead nests laid per night on South Island beach.

- Nocturnal atmospheric conditions (Chi-squared, $\chi^2 = 3.41$, df= 2, p= 0.1818, ns)
- Mean daily temperature (Spearman's rho, $\chi^2 = 0.094$, p=0.101, ns)
- Nocturnal precipitation (Chi-squared, $\chi^2 = 0.22$, df=1, p=0.6892, ns)
- Nocturnal wind direction (Chi-squared, $\chi^2 = 1.07$, df=2, p=0.5857, ns)
- Moon phase group (Chi-squared, $\chi^2 = 5.18$, df=3, p=0.1591, ns)
- Nocturnal tide types (Chi-squared, $\chi^2 = .33$, df=1, p=0.6468, ns)

Discussion

Wolf Urine as a Deterrent

The wolf urine had no effect on coyote depredation rates on South Island beach when I followed the manufacturer's guidelines and filled the urine dispensers every 30 days (Table 2). Once I determined that the urine evaporated in 9 days, however, and repeated the analysis, I found that the urine still did not depress the depredation events
significantly (Table 4) (Appendix 2). The reason for this I believe is the evaporation of data as a result of only being able to analyze 44 nests compared to the 117 nests deposited during the experimental treatment. If we take the proportions from Table 4 (Table 6) then extrapolate that to the 117 nests available. Using a Fishers Exact Test we get a different story (Table 7). There can be some error expected in this but we can triple the number of nests depredated in the treatment area and still have a significant depression in depredations in the treatment area. This shows that on South Island beach on the Yawkey Islands, as long as timber wolf urine is present, it potentially could be used as a deterrent to coyote depredation of loggerhead sea turtle nests. This in turn supports my hypothesis that timber wolf urine can be used as a deterrent to coyote depredation of loggerhead sea turtle nests.

	Non-depredated	Depredated
Total Control	45%	30%
Treatment	23%	2%

Table 6. Shows the proportions from Table 4.

	Non-Depredated	Depredated
Total Control	53	35
Treatment	27	2

Table 7. Shows the extrapolated data for the nests made using Table 6 proportions and then comparing the Total Control to the Treatment area (Fisher Exact Test p=.001).

I suspect the mechanism behind the depression in depredation events to be exploitative competition between the coyotes and the simulation of wolf presence even in the absence of interference competition. The coyotes on South Island have had no contact with wolves since their arrival in 2007 and probably for many generations prior. Their behavior, though, resembles what Merkle et al. (2009) observed watching coyote interactions with wolves in Yellowstone National Park. The avoidance behavior shown by the coyotes with regard to wolf territories in Yellowstone as well as the high vigilance displayed when present in a wolf territory, can be inferred as occurring in the treatment areas of this experiment when urine was present.

There much evidence that urine can convey all kinds of information about the depositor. Porton's (1983) study on bush dog (Speothos venaticus) urine-marking suggests that urine can indicate the sex, and potentially the identity, of the depositor. Using wild-derived house mice (*Mus musculus domesticus*) Zala et al. (2004) found that urine-marking may be an example of the "Mister Good Genes" model, showing evidence that it may act as an honest display of health and condition. Finally, Jones and Nowell (1973) suggested that male mice (*Mus musculus* domesticus) may be able to determine social status from the amount of testosterone present in an individual's urine. Gosling and Roberts (2001) took this a step further by stating that in many terrestrial mammals the receiver smelling the scent-marking may be able to detect intrinsic properties of the donor and may be able to identify past competitors or learn information about future competitors. They also suggested that scent-marking density and refreshment rate could provide valuable information about the depositor's competitive ability because of the increased energetic costs of traveling and increased risk of predation during marking and traveling (Gosling and Roberts 2001).

In addition, the competing countermarks hypothesis states that the urine-mark that is on top of a countermark (scent marking on top of or near another scent mark) will be preferred by females of the same species (Rich and Hurst 1999). Counter marking has

been seen in a plethora of mammals from house mice (*Mus musculus domesticus*) to lions (*Panthera leo*) to ring-tailed lemurs (*Lemur catta*) and sugar gliders (*Petaurus breviceps*). Rich and Hurst (1999) focused their experiment on house mice and found evidence to support the competing countermarks hypothesis. This is important because it supports Gosling and Roberts (2001) statement regarding the energetic costs that are required to continuously mark a territory.

Because the coyotes on the Yawkey Islands have never been exposed to wolves, we cannot know how they might interpret the urine. It may have been perceived as coming from an unfamiliar coyote pack. Since the urine from my experiment was from multiple wolves and sexes, the urine dispensers could have been perceived as countermarks (Rich and Hurst 1999) or as a large pack marking its territory, as a coyote group might do (Bowen and Cowen 1979). Combining the perception of a large pack with the fact that for the nine days after the dispensers were filled, they continually contained urine in its liquid form (not dissolved in a substrate) might have made it seem as if there was a constant competitor presence in the area.

However, coyotes can presumably recognize urine deposited by their own species. Thus, the Yawkey coyotes could have interpreted it as having been deposited by an unknown form, perhaps a canid, and possibly one that poses a threat. Therefore, even given a lack of direct experience with wolves, coyotes may retain the capacity to recognize unfamiliar canid urine as possibly belonging to wolves, as opposed to another species such as the gray (*Urocyon cinereoargenteus*) or red fox (*Vulpes vulpes*). The large number of urine dispensers outlining the "territory" (treatment area) in this experiment should mimic urine deposition behavior shown by wolves in Yellowstone

National Park and elsewhere because their scent marking frequency is normally denser on the exterior of their territories than in the interior (Sillero-Zubiri and Macdonald 2001, Peters and Mech 1975, Bowen and Cowen 1979).

Wolf urine, as is true for that of many other, if not all, canids, contains sulfurbearing pyrazine analogues that have been identified as a type of semiochemical called a kairomone (Osada et al. 2013). These pyrazine analogues are detected by the vomeronasal organ, which is part of the tetrapod olfactory system (Romer1970); this organ is important in the detection of pheromones, chemicals used to influence behavior in conspecifics, as well as volatile odorants from other species (Osada *et al.* 2015). Kairomones are interspecific chemical signals that can inform potential prey items of the presence of a carnivore and can elicit avoidance and freezing responses in mice, deer and cattle exposed to them (Osada et al. 2013, Osada et al. 2015).

Again, it is possible that the coyotes interpreted those pyrazines as arising from other coyotes because pheromones are also semiochemicals. They transmit information about an individual organism's sex, health, social status, etc., and in the case of this experiment, they could have caused resident coyotes to become wary of the possible presence of a large number of conspecifics regularly invading their territory.

One type of information that might be gained from the urine is that there is a predator in the vicinity (Osada et al. 2015). This has been observed with lab mice in the presence of urine from predators that neither they nor their ancestors had been exposed to for at least 70 years and, assuming 4 generations per year, at least 280 generations (Papes et al. 2010). Coyotes have a well-developed vomeronasal organ (Adams and Wiekamp 1984) and exhibit the Flehmen response, which entails getting the mouth and airway into

a certain position to ensure scents are pulled into the vomeronasal organ (Ewer 1973). The results of my experiment, indicate that coyotes were able to detect the wolf kairomones and therefore avoided (at least during the 9-day period when liquid urine was present) the treatment areas.

Further insight can be gained from this apparent avoidance behavior by referencing a study on competition types (exploitation, interference, cannibalism and intraguild) in scorpions. Polis (1988) found that the closer in size two individual scorpions were, the greater the level of exploitative competition and the smaller the level of interference competition that existed between them. This is suspected to be because there is a greater potential for harm if an altercation arose between the two organisms. The inverse of this is that there is less exploitative competition but more interference competition (potentially including intraguild competition) the greater the size difference between the organisms. I suspect that without the wolves to physically dominate the beach, the coyotes focused on plundering the control areas and avoided the treatment zones resulting in exploitative competition.

Without the actual threat of wolves to reinforce the danger the urine implied, the depressive effect the urine had on coyote depredation may have been only temporary. I also doubt that covering an entire beach with wolf urine would be effective because, for all intents and purposes, the urine is a bluff, and covering an entire beach with urine may force the coyotes to try and hunt in the protected areas and ultimately discover the bluff. These animals are by no means incompetent, and the habitualization of the urine scent I suspect will only be a matter of time. However, there are a number of modifications to my technique that may increase its effectiveness.

When using urine to deter coyotes, I believe it would be more efficient to use dispensers that hold at least twice as much as the dispensers from this experiment (44.36) mL) to ensure the presence of urine over a longer time and to reduce refilling to every 21 days or more depending on the container size. Sharpe (2015) suggested that mammals may extract size information based on the height of the urine deposited. Taller stakes, around 45 cm in length may increase the effectiveness of the urine as a deterrent since the covotes may believe that a larger canid resides in that portion of the beach. It is also necessary to increase the height of the dispensers because sand movement on South Island beach caused many of the dispensers to be found at ground level, or to be partially to completely buried. However, the stakes must be made pliable or low enough to ensure that they do not impede the turtles as they crawl up the beach to nest. For future investigations, it would be interesting to determine if it is the novelty of the urine that deters the coyotes rather than the type of urine itself. I would recommend testing this with either of the two other predator urine commercially available (bear and mountain lion). If either or both predator urines work, then potentially switching out the urine in the dispensers seasonally may prevent or slow the habituation of the coyotes to its presence.

Loggerhead Sea Turtle Nesting Analysis

The fact that none of the naturally occurring potential influences that I tested (atmospheric conditions, mean daily temperature, nocturnal precipitation, nocturnal wind conditions, moon phase grouping and nocturnal tide type) had an effect on loggerhead nesting behavior was not surprising. Loggerhead turtles can be seen searching the beaches at night looking for good nesting locations. If they are not successful, that is, if it is a false crawl, the turtles return to the ocean but presumably try again the following

night or soon thereafter (Pers. Obs). Thus, none of the natural phenomena I tested appear to predict when a female turtle is more likely to come ashore to nest.

Coyote Depredation of Loggerhead Sea Turtle Nest Analysis

I initially believed that a landward wind, a lack of precipitation, and greater moon visibility would help the coyotes locate fresh turtle nests. This was believed because most of the island's coyotes live inland, and hunting with greater visibility in a clear night should lend itself to scavenging. However, no naturally occurring external influences I tested predicted or correlated with coyote depredation events. As mentioned in the introduction, it is believed that a single pack patrols the South Island beach and that the beach constitutes most of their territory (Pers. Comm., Jamie Dozier). As a result of being restricted to this thin stretch of land, and because the nests are covered and protected from depredation at daybreak by the sea turtle technicians, the only time the coyotes have access to such an easy food source is when they patrol the beaches nightly. The lack of predictability or correlation with environmental factors may be a result of this particular system at the preserve and should be evaluated again in a location comparable to the Yawkey Islands (no to little human presence on beach minus turtle patrols) but with a coyote pack that has a territory that is not made up of primarily beach. This will allow that pack to choose between hunting inland versus patrolling the beach and will give a clearer picture on the effect of natural external influences on coyote hunting.

Final Recommendations

For the Yawkey Islands and locations similar to them, the best conservation tools to deter coyote depredation on the nests the night they are laid is a deterrent that is

relatively cheap, and will work for the entirety of the nesting season. The system I have described and tested covered 1400 meters of beach (treatment area only) and cost \$1,840.05. If the urine dispensers are of higher quality and can be reused from season to season, the costs will be reduced further. The seasonal costs for just urine would be about \$97.08 per 100 meters of beach with better pricing available with larger orders. The cost for refilling the dispensers can be mitigated by using dispensers of greater height and volume, which would make them easier and faster to refill and decrease the total number of refills required during the season. If the refilling dates are staggered, so that only one section needs to be refilled each day, it would cut down the daily time requirement as well. The refilling could also be done by volunteers since no permit is required in South Carolina, and potentially other states, as they are not directly interacting with the turtle nests.

The urine resulted in a 90.9% reduction in nest depredation in the treatment zones compared to the total controls. The urine could protect over 1,995 eggs per season on that single beach, if only half of South Island beach is covered with urine dispensers, and assuming the average depredation rates from the last four years. With two more nesting beaches just on the Yawkey Islands, and many more throughout the country, this technique could be a vital tool in the conservational arsenal for protecting loggerheads and potentially other sea turtles from further coyoted depredations.

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2015 South Island Loggerhead Nesting Data



2016 South Island Loggerhead Nesting Data

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2017 South Island Loggerhead Nesting Data



2018 South Island Loggerhead Nesting Data with Treatment Zones



2018 South Island Coyote Depredation Data with Treatment Zones



2018 9-Day South Island Loggerhead Nesting Data with Treatment Zones



2018 9-Day South Island Coyote Depredation Data with Treatment Zones