A TEST OF CONDITIONED FOOD AVERSION TO CONTROL RACCOON PREDATION ON THE EGGS OF GROUND-NESTING SHOREBIRD SPECIES ON THE BARRIER ISLANDS OF VIRGINIA

by

Joel D. Martin

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

In

Ecology

Approved:

_____	_____
Raymond D. Dueser Major Professor	Carl D. Cheney Committee Member
_____	_____
Nancy D. Moncrief Committee Member	Fred Provenza Committee Member

_____
Byron R. Burnham
Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2007

ABSTRACT

A Test of Conditioned Food Aversion to Control Raccoon Predation
on the Eggs of Ground-nesting Shorebird Species
on the Barrier Islands of Virginia

by

Joel D. Martin, Master of Science

Utah State University, 2007

Major Professor: Dr. Raymond D. Dueser
Department: Wildland Resources

To test the ability of estrogen-injected eggs to induce an aversion to untreated eggs in raccoons, pen, and island trials showed that 1) the average raccoon (*Procyon lotor*) reduced egg consumption, rather than food consumption, after consuming estrogen-treated eggs; 2) even though there were no conspicuous signs of aversion-inducing illness, an aversion formed generally within only a day or two of estrogen exposure; 3) averse animals not only reduced egg consumption, but apparently altered their foraging patterns; 4) prior exposure to untreated eggs impeded the formation of an egg aversion; 5) the aversion appeared likely to last longer than 21 days under ideal circumstances; 6) raccoons could not distinguish between estrogen-injected eggs and similar uninjected eggs; 7) an aversion to 1 type of egg did not appear to generalize to avoidance of other types of eggs as well; and 8) estrogen appeared

to be generally safe and effective for use with raccoons, with the possible exception of late-term pregnant females. Raccoons have a propensity to sample; and an egg aversion apparently depends on the taste or smell of the egg, the appearance of the egg, and the context in which the egg is found. So an aversion does not automatically generalize to eggs that are substantially different from the treated eggs. Taken together, these findings support the application of estrogen-induced aversive conditioning as a management tool, but also suggest that conditioned aversion is probably not a “magic bullet” for managing predation, and such field applications may need to be relatively complex in their design and execution.

(132 pages)

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Raymond Dueser, for his dedication and guidance, my wife, Laurie Baefsky, for supporting me emotionally and financially through this endeavor, Susan Durham for assistance in experimental design and statistical analysis, Dr. Mike Conover for inspiration in design, Ramona Skirpstunas, DVM, for help in designing tissue collection protocols and interpreting histological results, and committee members Dr. Carl Cheney, Dr. Fred Provenza, and Dr. Nancy Moncrief. Thanks to the Virginia Museum of Natural History for providing logistical support. Dr. Arthur Schwarzchild, Sue Rice, and the staff of the Eastern Shore of Virginia National Wildlife Refuge were instrumental in providing logistical support and a venue for my research. Thanks to my technicians, Robert Alonso, Scott Kupiec, Erika Miersma, Mekbeb Tessema, and Matthew Wirth for putting up with the heat, the bugs, and me.

This project was funded, in part, by the Virginia Coastal Zone Management Program at the Department of Environmental Quality through Grants NA04NOS4190060 and NA05NOS4191180 of the U.S. Department of Commerce, National Oceanic and Atmospheric Administration, under the Coastal Zone Management Act of 1972, as amended. The views expressed herein are those of the authors and do not necessarily reflect the views of the U.S. Department of Commerce, NOAA, or any of its subagencies. Support for this project was also supplied by the Berryman Institute West, the National Fish and Wildlife Foundation in cooperation with the Disney Company,

The Nature Conservancy, the University of Virginia's Long Term Ecological Research program, and the US Fish and Wildlife Service Eastern Shore of Virginia National Wildlife Refuge. Veterinary care was provided at no cost by Eastern Shore Animal Hospital.

Joel D. Martin

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Ground-nesting birds are declining worldwide because of habitat loss and fragmentation, overexploitation, and high rates of predation on the breeding grounds (Johnson and Stattersfield 1990, Martin et al. 1996, Blackburn et al. 2004). Introduced predators, expanding predator populations, and changes in land use have reduced the amount of nesting habitat available for waterbirds to a small fraction of what it was at the turn of the 20th century (Parnell et al. 1988, Helmers and Gratto-Trevor 1996). Seabirds and waterbirds in island ecosystems are especially sensitive to the effects of predation (Helmers and Gratto-Trevor 1996, Dobson 1998, Blackburn et al. 2004). Mammalian predators disturb nesting adults, destroy eggs, and kill both chicks and adults (Kadlec 1971, Birkhead and Nettleship 1995). The affected birds may fail to recruit young and even abandon prime nesting areas. Predation on eggs is a major cause of low breeding success (Conover 1990, Martin et al. 1996, Erwin et al. 2001).

Mammalian predators have extirpated or caused the decline of countless populations of island-nesting avian species (Burger and Gochfeld 1994, Blackburn et al. 2004). Kadlec (1971) reported that red foxes (*Vulpes vulpes*) and raccoons (*Procyon lotor*) introduced on islands off the Massachusetts coast eliminated the production of young herring gulls (*Larus argentatus*) and caused the total abandonment of several nesting sites. The introduction of mammalian predators has dramatically altered the avifaunas of entire archipelagos (e.g.,

Bailey 1992). Blackburn et al. (2004) determined that the probability of bird extinctions on each of 220 oceanic islands increased with the number of exotic predatory mammal species present.

Declining avian habitat quality and quantity, steadily declining interest in the hunting and trapping of furbearers, and the simultaneous spread of introduced predator species have accentuated the need for predator management (Birkhead and Nettleship 1995, Greenwood et al. 1995). Changes in land use and removal of top predators have favored an explosion of mesopredator populations, both in numbers and in range (Ratnaswamy et al. 1997). Historically, a common prescription for unwanted predation on birds has been predator removal (Neuman et al. 2004). Removals have mixed success depending on whether the removals are complete, the presence of alternate predators, and the rate of predator recolonization (Cote and Sutherland 1996). Complete predator removal programs are expensive, logistically difficult, and often controversial (Temple 1990, Bailey 1992, Cote and Sutherland 1996, Rosatte et al. 2007). Lethal predator control increasingly results in public opposition and litigation, which further complicates the management of predators and their prey (Goodrich and Buskirk 1995). Researchers have proposed a diverse array of nonlethal methods to reduce predation, including repellents (Hoover and Conover 2000, Shivik et al. 2003), fladry, electric fencing, scare devices, cages, exclosures (Estelle et al. 1996, Johnson and Oring 2002, Niehaus et al. 2004), supplemental feeding, alternate prey (Jimenez and Conover 2001), relocation, habitat modification (Carter and Bright

2002), barrier fencing (Murphy et al. 2003*a&b*, Lokemoen and Woodward 1993), nesting structures, construction of artificial islands and peninsulas, predator fertility control (Kirkpatrick and Frank 2005), modifying the predator community (Jimenez et al. 2001), protective umbrella or associational defense, and parasite introductions (Dobson 1998). Jimenez and Conover (2001) and others (e.g., Temple 1990, Witmer et al. 1996, Greenwood and Sovada 1996) have concluded that many of these techniques are expensive, controversial, inadequately tested or of limited applicability in most natural habitats, and none offer a panacea for enhancing avian recruitment.

The Virginia barrier islands support a diverse assemblage of nesting, migrating and wintering waterbirds, waterfowl, shorebirds, raptors, and songbirds (Williams et al. 1990). Sandy beaches, overwash fans, and sparsely-vegetated dunes provide extensive habitat for 27 colonial and beach-nesting species, including American oyster catchers (*Haematopus pallliatus*), black skimmers (*Rynchops niger*), brown pelicans (*Pelecanus occidentalis*), egrets (*Egretta* spp.), gulls (*Larus* spp.), herons (*Ardea* spp.), ibises (*Eudocimus albus, and Plegadis falcinellus*), plovers (*Charadrius* spp.), and terns (*Sterna* spp.) (Williams et al. 1990, Barrier Island Avian Partnership 1996). The piping plover (*C. melodus*) is “state and federal threatened”, and Wilson’s plover (*C*. *wilsonia*) is “state endangered” in Virginia (Virginia Department of Game and Inland Fisheries 1996). Most of these species nest on the ground, and are thus highly vulnerable to mammalian predation (Parnell et al. 1988). Many of these species have declined steadily, and sometimes dramatically, during the past 30 years

(Williams et al. 1990, Williams et al. 1996). The Conservation Action Plan for the Avian Communities in the Virginia Barrier Island System (Barrier Island Avian Partnership 1996) identified mammalian predators, particularly the raccoon and red fox, as a primary continuing threat to the success of avian conservation on these islands.

Increases in population and range of the raccoon since the 1970's have significantly reduced avian habitat suitability on the islands (Dueser et al. 1979, R. D. Dueser and N. D. Moncrief, Utah State University, unpublished data). A project has been underway since 1998 to develop ways to restore avian nesting habitat on the Virginia barrier islands through predator management (R. D. Dueser, Utah State University, personal communication). Annual trapping and removal of raccoons from 5 treatment islands have generally produced 1) reduced numbers of resident raccoons; 2) increased breeding populations for several avian species; 3) reduced rates of nest depredation (i.e. egg loss); and 4) higher nest productivity for those species for which productivity is monitored each year, such as American oyster-catchers and piping plovers (Dueser et al. 2000). At the same time, the total removal of raccoons challenges even professional trappers, and each year the one-to-a-few remaining (or recently arrived) individuals depredate a significant number of nests. The cost and effort of trapping per raccoon increases sharply as the number of raccoons is reduced (R. D. Dueser, personal communication). Thus there exists a need for a relatively low-cost, socially acceptable technology to reduce nest depredation by any remaining raccoons.

Most nonlethal techniques for predation management were developed for use in agricultural landscapes subject to intensive, mechanized management, and none are likely to be widely applicable on the Virginia barrier islands (R. D. Dueser, personal communication). Their utility on the barrier islands is limited by environmental conditions (i.e., windy, wet, and saline), logistical difficulties (e.g., fencing), inapplicability for colonial breeders (e.g., nesting structures), ethical concerns (e.g., modifying the predator community), and developmental status (e.g., predator fertility control and conditioned aversion) (Greenwood and Sovada 1996, Porton 2005). These limitations are accentuated by the remoteness and extent of the barrier islands (1,000 km^2), by the sparsely-vegetated, highly dynamic nature of the avian nesting habitats on the islands, and by the status of the Virginia Coast Reserve, which encompasses the barrier islands, as an International Union for the Conservation of Nature World Biosphere Reserve (Hayden et al. 1991, Jimenez et al. 2001).

There is widespread demand for a nonlethal remedy to reduce mammalian depredation on the eggs of ground-nesting birds, terrapins (*Malaclemys terrapin*), and sea turtles (various Chelonidae) (Nicolaus et al. 1989*b*, Ratnaswamy et al. 1997, Conover and Lyons 2003, Shivik et al. 2003). The most promising new technology is the use of conditioned food aversion (CFA) to “teach” nest predators to avoid the eggs of ground-nesting wildlife (Nicolaus et al. 1989*a*). CFA is an acquired dislike for the flavor of a food as a result of nausea following its consumption (Garcia et al. 1985). The use of oral estrogen to induce a food aversion appears to be the most promising

technology for accomplishing this management objective (Semel and Nicolaus 1992). Nicolaus et al. (1989*b*) reported that oral estrogen provided a nontoxic, but effective means of inducing a CFA in raccoons. Estrogen causes aversions specific to the flavor of the food it is placed in because estrogen has no flavor or smell of its own (Nicolaus et al. 1989*a*). Animals have been able to detect most other chemicals used in baits, which allowed them to distinguish between treated baits and untreated baits, and avoid the treated ones (Conover 1997). Rats and raccoons, as well as a host of other small and medium-sized predators, significantly reduced their consumption of eggs after consuming eggs containing estrogen (Nicolaus et al.1989*a*, *b,* Semel and Nicolaus 1992). Given the small number of field tests of this technology, and the mixed results of those trials, additional tests under relatively controlled, near-ideal circumstances were necessary to refine the design of this technology for use as a predator management tool.

A gut-defense system has evolved in animals that allows them to avoid consuming toxic plants and animals by detecting both flavors and emetic toxins and developing a CTA in response (Garcia et al. 1985). They require stimulation of the brain's emetic center, which resides in the lateral reticular formation of the medulla oblongata. In the case of illness, this emetic center is stimulated by either gastric irritation via the vagus nerve, or by blood-borne toxins which, in mammals, are sensed by cells in the area postrema in the bottom of the fourth ventricle of the medulla oblongata in the brain (Kiefer 1985). The gustatory afferent nerves converge directly on the lateral reticular

formation, and the signals from the olfactory afferent nerves are directed there as well by a more circuitous route (Kiefer 1985). This architecture allows the brain to make direct associations between illness and recently ingested foods, and causes responses to be reflexive (Kiefer 1985). Only poisons or events that cause nausea in this emetic system of the midbrain and brainstem will cause a CFA; other types of intestinal discomfort will not produce an aversion (Garcia et al. 1985). Food (taste) aversion is such a robust phenomenon that it occurs even when the toxin is introduced during deep anesthesia or tranquilization (Garcia et al. 1985).

Taste is the primary stimulus involved in conditioning a CFA, although weak place aversions can result as well from visual and olfactory clues associated with the feeding place (Garcia et al. 1985). The anatomical arrangement of nerves in the brain indicates taste plays a reflexive role in feeding while odor plays a plastic role (Garcia et al. 1985). For instance, both gustatory receptors and viscera send nerve fibers to the nucleus solitarius in the brainstem, while olfactory receptors send fibers to the limbic and paleocortical regions of the brain (Garcia et al. 1985).

Food aversions differ substantially from other conditioned aversions. Defense behaviors and place aversions result from sound and somatosensory stimuli (Garcia et al. 1985). This is known as the skin-defense system and is mediated by the convergence of the auditory and somatosensory pathways with the primary motor cortex in adjacent locations in the brain (Seeley et al. 2000).

Garcia (1989) reported that food aversions also differ from other aversions in that the interstimulus intervals between the unconditioned stimulus of taste and the feedback from nausea can be up to 2 hours and the aversion is usually acquired in 1 trial. Skin-defense systems require interstimulus intervals of at most a few seconds between the conditioned stimulus (sound or shock) and the unconditioned stimulus (defensive movement), as well as multiple repetitions (Garcia 1989). The gut-defense and skin-defense systems are mutually inhibitory, and it is difficult for animals to make connections across systems in a few trials (Garcia 1989).

The objective of this research was to design and test an aversion-based management tool to reduce predation on the eggs of ground-nesting wildlife. The central questions to establish the viability of estrogen-induced CFA as a predator management tool include: 1) Can raccoons distinguish between estrogen-injected eggs and uninjected eggs? 2) Do raccoons reduce egg consumption and change their foraging behavior in response to treated eggs? 3) Will oral estrogen, injected into surrogate eggs, cause an aversion to certain bird eggs in particular or bird eggs in general? 4) Will the aversion last long enough to cause treated raccoons to reject the eggs of the species of concern until the young hatch? 5) Will a large enough proportion of raccoons respond to the treatment? 6) What could cause the failure of the aversion? 7) What are the components of a successful food aversion application?

The experiments I used to address these questions ran from 19 May 2005 to 30 July 2005 and from 21 May 2006 through 9 August 2006.

LITERATURE CITED

Bailey, E. P. 1992. Red foxes, *Vulpes vulpes*, as biological control agents for introduced arctic foxes, *Alopex lagopus*, on Alaskan islands. The Canadian Field Naturalist 106:200-205.

Barrier Island Avian Partnership. 1996. Conservation action plan for the avian communities in the Virginia barrier islands system. Virginia Department of Environmental Quality. Richmond, Virginia, USA.

Birkhead, T. R., and D. N. Nettleship. 1995. Arctic fox influence on a seabird community in Labrador: a natural experiment. The Wilson Bulletin 107:397-412.

Blackburn, T. M., P. Cassey, R. P. Duncan, K. L. Evans, and K. J. Gaston. 2004. Avian extinction and mammalian introductions on oceanic islands. Science 305:1955-1957.

Burger, J., and M. Gochfeld. 1994. Predation and effects of humans on island-nesting seabirds. Pages 39-67 *in* D. N. Nettleship, J. Burger, and M. Gochfeld, editors. BirdLife conservation series no. 1. BirdLife International, Cambridge, UK.

Carter, S. P., and P. W. Bright. 2002. Habitat refuges as alternatives to predator control for the conservation of endangered Mauritian birds. Pages 71-78 *in* C. R. Veitch and M. N. Clout, editors. Proceedings of the International Conference on Eradication of Island Invasives. Occasional paper of the IUCN Species Survival Commission no. 27.

Conover, M. R. 1990. Reducing mammalian predation on eggs by using a conditioned taste aversion to deceive predators. Journal of Wildlife Management 54:360-365.

Conover, M. R. 1997. Behavioral principles governing conditioned food aversions based on deception. Pages 29-41 *in* J. R. Mason, editor. Repellents in wildlife management. Colorado State University, Fort Collins, Colorado, USA.

Conover, M. R., and K. S. Lyons. 2003. Reducing or delaying egg depredation by punishing free-ranging predators for opening eggs. Applied Animal Behaviour Science 83:177-185.

Cote, I. M., and W. J. Sutherland. 1996. The effectiveness of removing predators to protect bird populations. Conservation Biology 11:395-405.

Dobson, A. P. 1998. Restoring island ecosystems: The potential of parasites to control introduced mammals. Conservation Biology 2:31-39.

Dueser, R. D., W. C. Brown, G. S. Hogue, C. McCaffrey, S. A. McCuskey, and G. Hennessey. 1979. Mammals of the Virginia barrier islands. Journal of Mammalogy 60:425-429.

Dueser, R. D., O. Keišs, B. R. Truitt, and N. D. Moncrief. 2000. Avian habitat restoration on the Virginia barrier islands. Final Report to the Virginia Coastal Resources Management Program, Richmond, Virginia, USA.

Erwin, R. M., B. R. Truitt, and J. E. Jimenez. 2001. Ground-nesting waterbirds and mammalian carnivores in the Virginia barrier island region: running out of options. Journal of Coastal Research 17:292-296.

Estelle, V. B., T. J. Mabee, and A. H. Farmer. 1996. Effectiveness of predator exclosures for Pectoral Sandpiper nests in Alaska. Journal of Field Ornithology 67:447-452.

Garcia, J. 1989. Food for Tolman: Cognition and cathexis in concert. Pages 45-85 *in* T. Archer and L. Nilsson, editors. Aversion, avoidance and anxiety. Lawrence Erlbaum Associates, Hillsdale, New Jersey, USA.

Garcia, J., P. A. Lasiter, F. Bermudez-Rattoni, and D. A. Deems. 1985 A general theory of aversion learning. Pages 8-21 *in* N. S. Braveman and P. Bronstein, editors. Experimental assessments and clinical applications of conditioned food aversions. New York Academy of Science, New York, New York, USA.

Goodrich, J. M., and S. W. Buskirk. 1995. Control of abundant native vertebrates for conservation of endangered species. Conservation Biology 9:1357-1364.

Greenwood, R. J., A. B. Sargeant, D. H. Johnson, L. M. Cowardin, and T. L. Shaffer. 1995. Factors associated with duck nest success in the prairie pothole region of Canada. Wildlife Monographs 128.

Greenwood, R. J., and M. A. Sovada. 1996. Prairie duck populations and predation management. Transactions of the North American Wildlife and Natural Resources Conference 61:31-42.

Hayden, B. P., R. D. Dueser, J. T. Callahan, and H. H. Shugart. 1991. Long-term research at the Virginia Coast Reserve: Modeling a highly dynamic environment. BioScience 41:310-318.

Helmers, D. L., and C. L. Gratto-Trevor. 1996. Effects of predation on migratory shorebird recruitment. Transactions North American Wildlife and Natural Resource Conference 61:51-60.

Hoover, S. E., and M. R. Conover. 2000. Using eggs containing an irritating odor to teach mammalian predators to stop depredating eggs. Wildlife Society Bulletin 28:84-89.

Jimenez, J. E., M. R. Conover and T. A. Messmer. 2001. Exclusionary methods to reduce predation on ground-nesting birds and their nests. Berryman Institute Publication No. 20, Utah State University, Logan, Utah, USA.

Jimenez, J. E., and M. R. Conover. 2001. Ecological approaches to reduce predation on ground-nesting game birds and their nests. Wildlife Society Bulletin 29:62-69.

Johnson, T. H., and A. J. Stattersfield. 1990. A global review of island endemic birds. Ibis 132:167-180.

Johnson, M., and L. W. Oring. 2002. Are nest exclosures an effective tool in plover conservation? Waterbirds 25:184-190.

Kadlec, J. A. 1971. Effects of introducing foxes and raccoons on Herring gull colonies. Journal of Wildlife Management 35:625-635.

Kiefer, S. W. 1985. Neural mediation of conditioned food aversions. Pages 100-109 *in* N. S. Braveman and P. Bronstein, editors. Experimental assessments and clinical applications of conditioned food aversions. New York Academy of Sciences, New York, New York, USA.

Kirkpatrick, J. F., and K. M. Frank. 2005. Contraception in free-ranging wildlife. Pages 195-221 *in* C. S. Asa and I. J. Porton, editors. Wildlife contraception: Issues, methods, and applications. The Johns Hopkins University Press, Baltimore, Maryland, USA.

Lokemoen, J. T., and R. O. Woodward. 1993. An assessment of predator barriers and predator control to enhance duck nest success on peninsulas. Wildlife Society Bulletin 21:275-282.

Martin, T. E., I. J. Ball, and J. Tewksbury. 1996. Environmental perturbations and rates of nest predation in birds. Transactions North American Wildlife and Natural Resource Conference 61:43-49.

Murphy, R. K., R. J. Greenwood, J. S. Ivan, and K. A. Smith. 2003a. Predator exclusion methods for managing endangered shorebirds: are two barriers better than one? Waterbirds 26:156-159.

Murphy, R. K., I. M. G. Michaud, D. R. Prescott, J. S. Ivan, B. J. Anderson, and M. L. French-Pombier. 2003b. Predation on adult Piping Plovers at predator exclosure cages. Waterbirds 26:150-155

Neuman, K. K., G. W. Page, L. E. Stenzel, J. C. Warriner, and J. S. Warriner. 2004. Effect of mammalian predator management on Snowy plover breeding success. Waterbirds 27:257-263.

Nicolaus, L. K., P. V. Farmer, C. R. Gustavson, and J. C. Gustavson. 1989*a*. The potential of estrogen-based conditioned aversion in controlling depredation: A step closer toward the “magic bullet”. Applied Animal Behaviour Science 23:1-14.

Nicolaus, L. K., J. Herrera, J. C. Nicolaus, and C. R. Gustavson. 1989*b*. Ethinyl estradiol and generalized aversions to eggs among free-ranging predators. Applied Animal Behaviour Science 24:313-324.

Niehaus, A. C., D. R. Ruthrauff, and B. J. McCaffery. 2004. Response of predators to Western Sandpiper nest exclosures. Waterbirds 27:79-82.

Parnell, J. F., D. G. Ainley, H. Blokpoel, B. Cain, T. W. Custer, J. L. Dusi, S. Kress, J. A. Kushlan, W. E. Southern, L. E. Stenzel, and B. C. Thompson. 1988. Colonial waterbird management in North America. Colonial Waterbirds 11:129-169.

Porton, I. J. 2005. The ethics of wildlife contraception. Pages 3-16 *in* C. S. Asa and I. J. Porton, editors. Wildlife contraception: Issues, methods, and applications. The Johns Hopkins University Press, Baltimore, Maryland, USA.

Ratnaswamy, M. J., R. J. Warren, M. T. Kramer, and M. D. Adam. 1997. Comparisons of lethal and nonlethal techniques to reduce raccoon depredation of sea turtle nests. Journal of Wildlife Management 61:368-376.

Rosatte, R., S. Kirk, D. Donovan, M. Allan, L. Bruce, T. Buchanan, and C. Davies. 2007. Raccoon density and movement after population reduction to control rabies. Journal of Wildlife Management 71:2373-2378.

Seeley, R. R., T. D. Stephens, and P. Tate. 2000. Pages 390-395 *in* Anatomy and physiology. Fifth edition. McGraw-Hill, Boston, Massachusetts, USA.

Semel, B., and L. K. Nicolaus. 1992. Estrogen-based aversion to eggs among free-ranging raccoons. Ecological Applications 2:439-449.

Shivik, J. A., A. Treves, and P. Callahan. 2003. Nonlethal techniques for managing predation: Primary and secondary repellents. Conservation Biology 17:1531-1537.

Temple, S. A. 1990. The nasty necessity: Eradicating exotics. Conservation Biology 4:113-115.

Virginia Department of Game and Inland Fisheries. 1996. Special Status Faunal Species in Virginia. Richmond, Virginia, USA.

Williams, B., B. Akers, M. Beck, R. A. Beck, and J. Via. 1996. The 1996 beach-nesting and colonial waterbird survey of the Virginia barrier islands. Raven 68:85-88.

Williams, B., R. A. Beck, B. Akers, and J. Via. 1990. Longitudinal surveys of the beach-nesting and colonial waterbirds of the Virginia barrier islands. Virginia Journal of Science 41:380-388.

Witmer, G. W., J. L. Bucknall, T. H. Fritts, and D. G. Moreno. 1996. Predator management to protect endangered avian species. Transactions North American Wildlife and Natural Resource Conference 61:102-108.

CHAPTER 2

PEN TRIALS OF ESTROGEN-INDUCED EGG AVERSION IN RACCOONS[1]

ABSTRACT

Aversive conditioning is a promising but unproven nonlethal approach to reducing mammalian depredation on the eggs of ground-nesting birds, terrapins, and sea turtles. Most aversive agents can be detected by taste or smell and cause predators to avoid treated baits. This research tested the efficacy and short-term health effects of oral estrogen in eggs as an aversive agent for raccoons (*Procyon lotor*). In 2005, I gave untreated eggs to 16 raccoons for 6 days, followed by 12 feedings of estrogen-injected eggs roughly every other day for 22 days. Most of the raccoons reduced their consumption of eggs at some point during the trial. Five animals died during the trial, none of which could be directly attributed to the effects of estrogen. In 2006, I gave estrogen-injected eggs to 9 treatment raccoons for 7 feedings over 14 days, then gave a combination of 2 estrogen-injected eggs, 2 untreated eggs, and 2 carrier-only injected eggs for 7 feedings over the next 14 days. Nine control animals received carrier-only injected eggs for the first 7 feedings, and 2 untreated eggs plus 4 carrier-only injected eggs for the next 7 feedings. All 9 treatment animals reduced their egg consumption, but not their food consumption, in response to the treatment. Other than 1 control animal that rejected 2 eggs early in the trial, the control animals ate every egg. No raccoons distinguished between estrogen-injected eggs and uninjected eggs. Two

[1] Coauthored by Joel D. Martin, Raymond D. Dueser, and Nancy D. Moncrief.

animals died during the trial, one due to complications from a failed pregnancy, possibly induced by estrogen consumption. Estrogen is a relatively safe and effective aversive agent and a full-scale field trial of estrogen is likely to be productive. Raccoons cannot detect estrogen in eggs. Previous exposure of raccoons to eggs may make it more difficult to establish an effective aversion to eggs. Although most raccoons appear likely to exhibit an aversion to eggs following ingestion of treated eggs, no specific dosage is expected to be universally effective.

INTRODUCTION

Environmental conditions (e.g., windy, wet, and saline), logistical difficulties (e.g., fencing), inapplicability for colonial breeders (e.g., nest cages and nesting structures), detrimental side effects (e.g., hazing), developmental status (e.g., predator fertility control), or ethical concerns (e.g., modifying the predator community) limit the utility of most current nonlethal techniques to reduce egg predation in many locations (Greenwood and Sovada 1996, Porton 2005). Aversive conditioning is a promising but unproven nonlethal approach to reducing mammalian depredation on the eggs of ground-nesting birds, terrapins, and sea turtles (Nicolaus et al. 1989*b*, Ratnaswamy et al. 1997, Conover and Lyons 2003, Shivik et al. 2003). The most promising new technology is the use of conditioned food aversion to “teach” mammalian nest predators such as, raccoons (*Procyon lotor*) to avoid the eggs of ground-nesting wildlife (Nicolaus et al. 1989*a*).

The literature suggests an ideal aversive compound would 1) produce a severe short-term illness in the predator (Nicolaus et al. 1989*b*); 2) have an effective (illness-producing) dose far below the lethal dose (Gill et al. 2000); 3) cause this illness with a brief time delay (~2 hours) to allow the predator to consume an effective dose of the compound (Conover 1997); 4) be undetectable to the predator when present at appropriate concentrations in a bait (Conover 1984, Gill et al. 2000); 5) be chemically stable in baits when distributed under field conditions (Nicolaus et al. 1992); 6) produce no chronic or long-lasting health effects (Gill et al. 2000); 7) work equally well for both solitary and colonial nesters; and 8) be deployed outside the actual nest or colony (Conover 1990, Conover and Lyons 2003). The expectation is that predators will develop an aversion to treated eggs, will generalize this aversion to untreated eggs, and will cease depredating all eggs.

Researchers have proposed and tested a host of potential aversive compounds for this application with raccoons, including emetine dihydrochloride (Conover 1989, 1990), cinnamamide and thiabendazole (Gill et al. 2000), carbachol (Cox et al. 2004), pulegone (Conover and Lyons 2003), and oral estrogen (Nicolaus et al. 1989*a*). Most have proven ineffective, effective for only a short duration, difficult to deploy safely, laden with side effects, or toxic in the environment (Conover 1990). A major stumbling block is that most aversive agents can be detected by taste or smell and cause predators to easily avoid treated baits (Conover 1997). Oral estrogen appears to be the most promising of these compounds (Semel and Nicolaus 1992). Nicolaus et al. (1989*b*)

reported that it provides a nontoxic, but effective means of inducing a conditioned aversion in raccoons. Oral estrogen was also effective with a host of small and medium-sized predators that reduced their consumption of eggs after consuming surrogate eggs containing estrogen (Nicolaus et al. 1989*a*, *b*, Semel and Nicolaus 1992). Estrogen is the best known aversive agent because it has no taste or smell, so animals avert to the salient features of the food rather than the treatment (Semel and Nicolaus 1992).

A number of uncertainties limited the use of estrogen-induced aversive conditioning as a management tool. Examples are: 1) the severity and duration of any illness resulting from estrogen ingestion; 2) the appropriate estrogen concentration for deployment in surrogate eggs; 3) the detectability of estrogen in surrogate eggs at that concentration; 4) lethal or chronic health effects resulting from the ingestion of an effective dose; and 5) the effect of experience with untreated eggs prior to exposure to treated eggs.

I conducted this research to further test the efficacy and short-term health effects of oral estrogen as an aversive agent for raccoons, and to work out the logistics of using various types of eggs to deliver an effective dose. I conducted 2 pen trials with captive raccoons. I designed the 2005 pilot pen trial to 1) test for variability among raccoons in their response to estrogen-treated eggs under controlled conditions; 2) observe the severity and duration of any illness resulting from estrogen ingestion; and 3) adapt the baiting procedure for use with Japanese quail (*Coturnix japonica*) eggs. Based on the 2005 pilot pen trial results, I designed the 2006 pen trial to test the practical

viability of estrogen-induced conditioned aversion as a predation management tool. Specifically, I wanted to learn: 1) Do individual raccoons reduce egg consumption, rather than food consumption in general, after consuming estrogen-treated eggs? 2) Can raccoons distinguish between estrogen-injected eggs and similar uninjected eggs? 3) Is the average raccoon likely to respond to the treatment? 4) Does prior exposure to untreated eggs impede the formation of an egg aversion? 5) Is estrogen safe and effective for use with raccoons?

PILOT PEN TRIAL 2005

Methods

Animal care.—I constructed an 18-cage pen facility in a forested, rural setting in Northampton County, Virginia, USA (Figure 1). There were 3 pens, each consisting of 6 cages made of pressure-treated lumber and wire. Each cage was a cube 1.2 meters per side with its floor 0.9 meters off the ground. The floors were made of ½-inch hardware cloth, and the walls and ceiling were made of 2-inch mesh kennel wire (Figure 2). I outfitted each cage with a 10-gallon plastic den box, 1-liter water bottle, set of food bowls, and a “pacifier” designed to provide a diversion from chewing on the wooden framework (Figure 3). The pacifier consisted of a 20-cm length of 1.8-cm inside diameter, schedule 40, polyvinyl chloride (PVC) pipe smeared with 10 ml Food Lion peanut butter on the inside. The pens had a roof (flat in 2005, sloped in 2006) of 6-mil black plastic sheeting to provide protection from sun and rain. During feeding events, I removed the food bowls and water bottles from each cage,

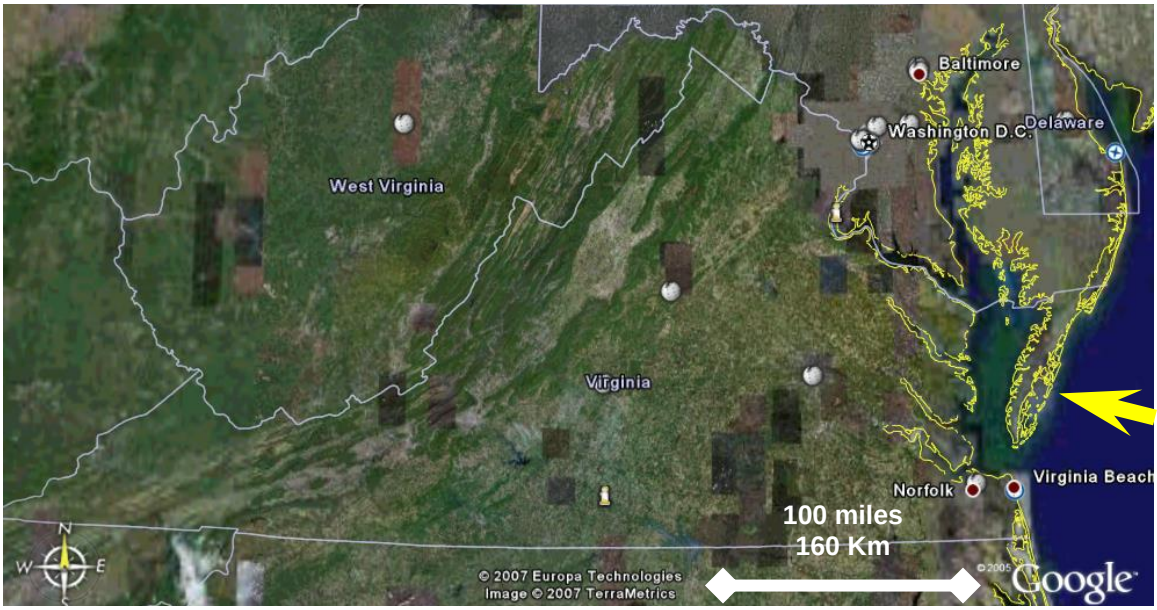

Figure 1. Pen trials of aversive conditioning in raccoons were conducted in Northampton County on the Eastern Shore of Virginia in 2005 and 2006. Copyright 2007 TerraMetrics, Inc. http://www.truearth.com

Figure 2. The campus of Raccoon State University (RSU), "… where learning is just a matter of experience" (photo by author 2006).

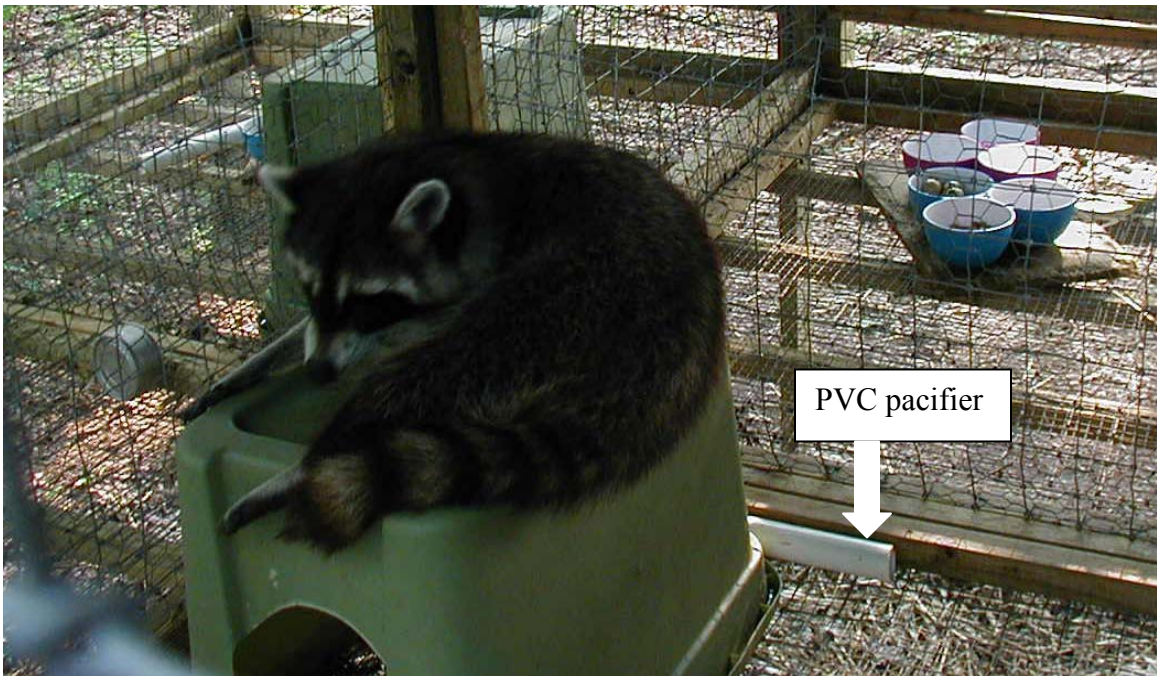

Figure 3. One of 18 RSU behavioral ecology students resting between classes (photo by author 2005).

cleaned and refilled them, and scooped feces from each cage with a small shovel. Each animal retreated to the den box or corner of the cage during feeding and cleaning operations. I returned food bowls containing dog food and new treated or fresh eggs to the cages in as short a time as possible. I pressure washed the cages every second or third day. I covered feces, spilled food, and egg drippings under the pens with hydrated lime after every washing.

I stocked this pen facility with 18 raccoons live-trapped on 5 nearby mainland sites between 10 June 2005 and 21 June 2005. Each was sedated with an intramuscular injection of Ace-Ketamine (0.2 ml/kg body mass) (ketamine concentration 100 mg/ml; acepromazine concentration 10 mg/ml) (Kreeger et al. 2002). Each was sexed, weighed, ear-tagged, individually caged, and monitored daily for general appearance and well-being. I used only

yearlings and adults in the trials, with an equal mix of males and females. The animals were maintained on a daily ration of 120 g of dry dog food (Purina Dog Chow and Purina Hunter's Choice Dog Food) and provided water ad libitum. With a crude protein content $\geq$ 18.0% and a crude fat content $\geq$ 6.5%, these foods provided a diet on which 7 of the raccoons were able to maintain or gain weight. I recorded daily food and water consumption data for each animal to ensure that they were adequately provisioned and made frequent observations of how the study animals interacted with the dog food and eggs. I conducted all research in compliance with Utah State University International Animal Care and Use Committee protocols (#952).

Egg preparation.—I used ethinyl estradiol, a powdered form of estrogen obtained from Spectrum Chemical Manufacturing Corporation (Gardena, CA). I used a flour-water mixture as the estrogen carrier as per Semel and Nicolaus (1992). The carrier is used to facilitate injection of the estrogen into the egg, keep the estrogen suspended in the yolk, and prevent the estrogen from losing potency by becoming bound with albumen (Nicolaus et al. 1989*a*, Nicolaus et al. 1992). To prepare this mixture for injection into Japanese quail eggs, I made a semi-liquid paste by mixing just enough plain bleached wheat flour with 200 ml cold water while constantly stirring. I blended 100 ml of this paste with 1 gram of estrogen powder weighed out on a milligram laboratory scale. I prepared the eggs by using a 30-ml plastic syringe with a 16-gauge needle to pierce the shell at the tapered end and remove 2 ml of the contents, both yolk and albumen. I then injected a 1-ml plug (0.5 ml on 2 occasions) of the estrogen

flour paste mixture (10 mg/ml) using a 3-ml syringe with a 16-gauge needle thrust into the yolk. I then sealed the needle hole using a glass stirring rod dipped in melted paraffin. I refrigerated the eggs until used, usually 1 or 2 days.

Treatments.—The pen trial in 2005 consisted of 2 phases (Figure 4).

1) Preconditioning phase (23 Jun 2005 to 28 Jun 2005; days 1-5): I gave each raccoon 4 untreated quail eggs for 4 days, and 2 untreated quail eggs for 2 days, along with 120 g dry dog food. I included this phase to ensure that all of the raccoons were experienced with eating eggs.

2) Conditioning phase (29 Jun 2005 to 20 Jul 2005: days 6-27): I designated 16 raccoons as treatment animals and 2 randomly selected individuals as controls (Figure 5). Every other day, in addition to dog food, the treatment animals also received variable numbers (4–24) of treated quail eggs. All eggs were presented at the normal feeding time between 1700 and 1800 hours. Most individuals fed readily and without delay, even while being observed; a few waited until the caretakers had departed for the evening before beginning to feed. I made observations of feeding behavior in fading sunlight and postfeeding behavior under red filtered light after sunset. At 0800 hours the next day, I recorded egg condition as “intact” or “consumed” and recorded food and water consumption for each animal for the previous 24 hours.

Necropsy. —At the conclusion of the trial, I sedated the 13 remaining raccoons (five died), and euthanized them with cardiac injections of Beuthanasia D®. On 24 July 2005, I necropsied all 18 animals and collected tissues to have tested for general condition and evidence of parasitic disease.

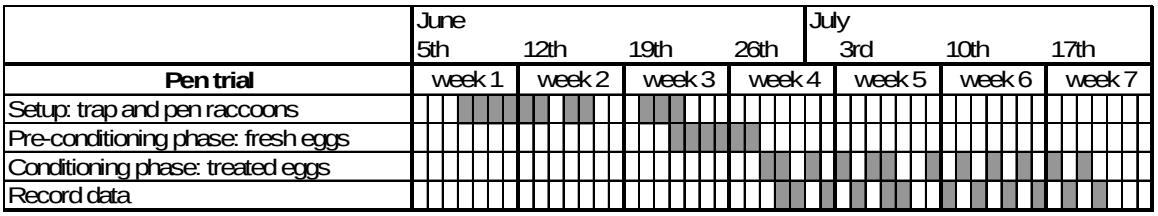

Figure 4. Sequence and timing of 2005 estrogen-induced aversive conditioning pilot pen trial.

Treatment 18** Female	Treatment 17 Female	Treatment 16 Male
Male 1** Treatment	Male 2 Treatment	Female 3 Treatment

Treatment 15** Female	Control 14** Male	Treatment 13 Female
Female 4** Treatment	Male 5 Control	Male 6 Treatment

Treatment 12 Female	Treatment 11 Female	Treatment 10 Male
Male 7 Treatment	Female 8 Treatment	Male 9 Treatment

** early death

Figure 5. Distribution of raccoons among pens and cages during the 2005 pilot pen trial.

Dr. Ramona Skirpstunas, DVM, at the Utah Veterinary Diagnostic Laboratory at Utah State University, performed histopathology analysis of the frozen tissues. Five animals died during the trials: #1 on 20 July 2005, #4 on 11 July 2005, #14 on 15 July 2005, #15 on 3 July 2005, and #18 on 5 July 2005.

Data analysis.—I graphed the egg consumption of each raccoon in 3 dimensions to demonstrate effect as well as variability. No statistical tests were appropriate due to insufficient numbers of controls and low sample size. I averaged daily doses for the raccoons that rejected eggs and those that did not. I graphed estrogen exposure against net change in body mass.

Results

The behavior of the caged animals was highly variable. Some individuals showed immediate interest in their food at each feeding, while others did not.

Some chewed the wooden framework of their cages while others did not, and some habitually stole their neighbors' pacifiers through the wire. Some growled and acted aggressively toward pen mates and caretakers; some seemed passive and lethargic; and others were social, nonaggressive, and curious.

The raccoons quickly learned to manipulate and consume eggs. Eleven of the 18 raccoons (61%) ate every untreated egg from the first day of the preconditioning phase. All 18 individuals ate all available untreated eggs by day 4. The raccoons used a variety of methods for eating eggs, but all attempted to consume the entire contents of the egg. They usually bit off one end and licked out the contents, and sometimes ate the shell. Some individuals simply crunched up and swallowed the entire egg, while others spit out the chewed shell. There was no apparent discrimination between yolk and albumen, and no obvious attempt on the part of the treatment animals to avoid ingesting the estrogen plug. The 2 raccoons that ate every egg usually ate all of the dog food as well. The 14 animals that rejected eggs often did not eat all of their dog food.

The 16 treatment raccoons exhibited substantial variability in the consumption of treated eggs during the conditioning phase (Figure 6). Eleven individuals exhibited reduced egg consumption after 1 or more feedings of treated eggs. Three individuals (#'s 1, 3, and 8) exhibited reduced egg consumption on days subsequent to 1 feeding of 4 treated eggs (12 mg/kg cumulative estrogen exposure). One animal (#17) reduced egg consumption after 2 feedings totaling 8 eggs (24 mg/kg cumulative exposure), 4 animals (#'s 2, 10, 11, and 16) after 5 or 6 feedings totaling 42 to 66 eggs (63-111 mg/kg

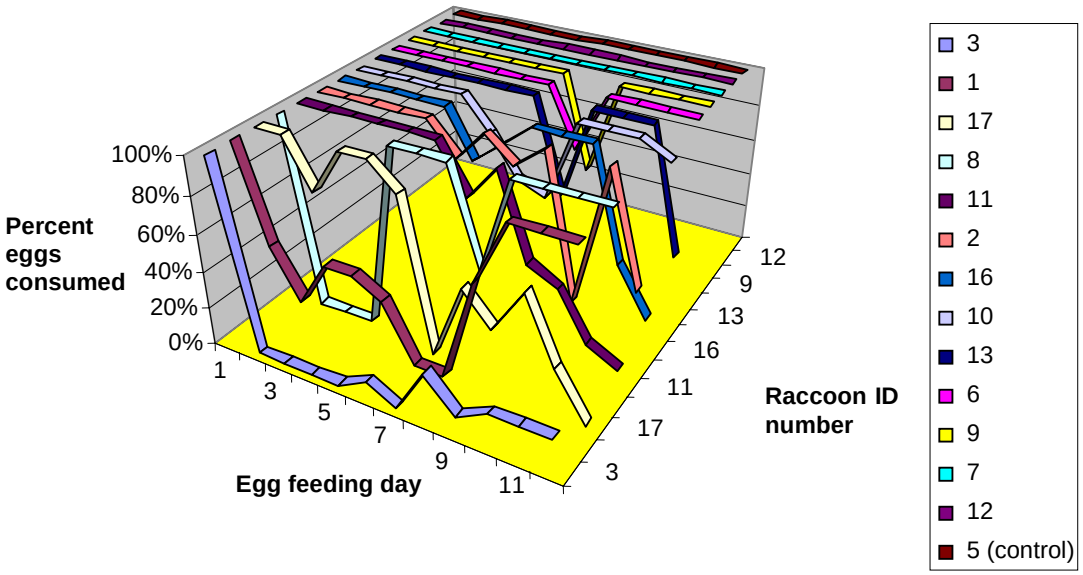

Figure 6. Egg consumption per feeding day (n = 12) for the 13 treatment animals and 1 control that remained alive at the end of the 2005 pilot pen trial. Raccoon ID numbers refer to animal numbers listed in text.

exposure), and 3 animals (#'s 6, 9, and 13) after 7 feedings totaling 74 eggs (122 mg/kg exposure). Every individual that exhibited reduced egg consumption subsequently "sampled" eggs on 1 or more occasions, and raccoon 8 resumed eating eggs after only a 2-day layoff.

Two animals (#'s 7 and 12) were still consuming every egg – a total of 122 treated eggs (198 mg/kg cumulative exposure) – by the end of the trial. The average daily dose received by the 6 raccoons that consumed less than 75% of treated eggs was 13.0 mg/kg (SE 2.84). The average daily dose received by raccoons 7 and 12, which consumed 100% of treated eggs, was 28.3 mg/kg and 20.5 mg/kg, respectively.

Although darkness limited direct observations, the treatment raccoons exhibited few if any outward signs of illness or discomfort in the 2 hours after

eating estrogen-injected eggs. The animals exibited no conspicuous signs of distress such as vomiting, retreat to the den box, or males with soprano vocalizations. They suffered occasional bouts of diarrhea with no apparent connection to the ingestion of estrogen. Some animals may have reduced their activity, but inherent behavioral variability among individuals and only 1 surviving control made it impossible to pinpoint any clear symptoms resulting from estrogen ingestion.

All of the animals bore at least a few ticks at the outset, but each appeared healthy and vigorous. Nevertheless, 2 males (animals 1 and 14) and 3 females (#'s 4, 15, and 18), 2 of them pregnant, died during the trial after surviving in the cages for intervals of 9 to 18 days. Interestingly, 2 of these animals were housed in adjacent cages in pen 1, and 3 were housed in adjacent cages in pen 2 (Figure 5). One of the control animals, a male (#14), also died. One female (#4) carried 3 embryos 79 mm in length, and another female (#18) carried 4 embryos 30 mm in length, at the time of death.

The raccoons varied in body-mass dynamics between the beginning and end of the trial. Males weighed an average of 4.5 kg (SE 0.24), and females 3.8 kg (SE 0.15), at the beginning of the trial. Eleven animals lost an average of 1.08 kg (SE 0.17) during the trial, including 1 of the control males which lost 2.2 kg before it died. Six raccoons gained an average of 1.07 kg (SE 0.21), and 1 did not change. Percentage weight loss between genders did not differ. Assuming little or no spillage of egg contents, I estimated maximum values of daily estrogen ingestion per individual, with unknown but probably small error.

I detected no relationship between estrogen exposure and net change in body mass (Figure 7). The 5 animals that received the highest average doses (above 18 mg/kg/day) tended to lose weight or stay the same, but 11 animals that received either intermediate or low average doses either lost or gained. I also did not detect a relationship between estrogen exposure and raccoon survival (Figure 7). One of the control animals gained weight and thrived, while the other lost weight and died. The 4 treatment raccoons that died during the trial consumed smaller daily and cumulative doses of estrogen than 7 of the treatment raccoons that lived through the trial. Animal #1 died after 12 feedings totaling 69 eggs (164 mg/kg exposure), animal #4 died after 7 feedings totaling 26 eggs (50 mg/kg exposure), animal #15 died after 3 feedings totaling 12 eggs

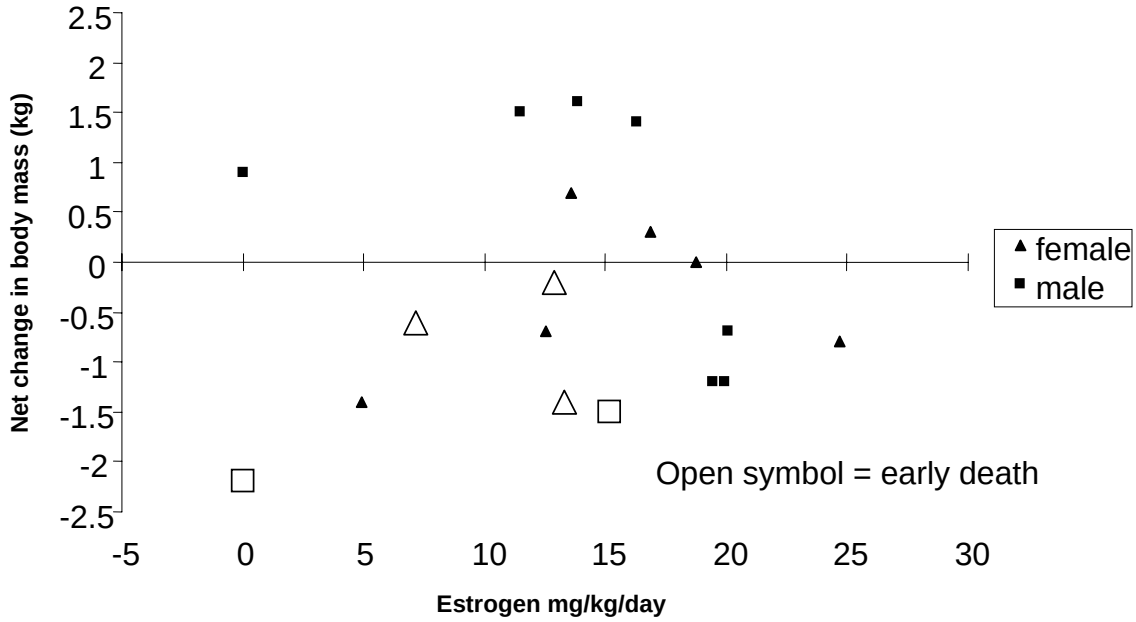

Figure 7. Overall net change in body mass (kg) between the beginning and end of the 2005 pilot pen trial as a function of average daily estrogen consumption (mg/kg/day).

(39 mg/kg exposure), and animal #18 died after 4 feedings totaling 16 eggs (53 mg/kg exposure).

Necropsy results.—The 2005 necropsies yielded incomplete and poorly preserved sets of tissues, making it unlikely that any subtle health consequences of the estrogen exposure could be identified. I was, therefore, unable to quantify any changes in the internal organs of the raccoons due to estrogen ingestion. I had only 1 surviving control animal (out of 2 males) for comparison, and most of the animals had varying degrees of organ damage due to parasitism and other diseases. All of the tested individuals exhibited lesions indicating myocardial compromise, chronic liver disease, and heavy intestinal parasitism (R. Skirpstunas, unpublished report). Thus, despite appearances, these raccoons suffered serious chronic health conditions. The 5 animals that died early showed similar symptoms just prior to death, including extreme lethargy and refusal to eat. No definitive cause of death could be identified for these individuals, but each had lesions consistent with chronic cardiac inflammation, which is evidence of serious cardiac insufficiency similar to that reported in raccoons with *Sarcocystis neurona* infection (Skirpstunas, unpublished report). I was unable to test for the presence of canine distemper virus, rabies virus, or other causes of neurological disease. I found no gross abnormalities visible in the reproductive organs.

Discussion

Difficulties.—I used only 2 control animals because I assumed that once raccoons had been exposed to eggs and had developed a preference for their

taste, they would continue to consume them before dog food. The literature supported this assumption (Semel and Nicolaus 1992), and all the raccoons quickly learned to eat eggs in the preconditioning phase. However, using only 2 control animals proved insufficient because 1 of these animals died before the end of the experiment, and differences in condition and behavior among animals made comparisons difficult.

The results of the pen trial reflected a high degree of variability among individual raccoons in their behaviors and responses to the estrogen treatment. The observed variability may reflect several possible sources of influence. These include the following examples:

1. Normal variation among individuals – Gustavson and Gustavson (1985) state that behavioral and other types of variation among individuals is often cited as a challenge in running pen trials with wild-caught animals.
2. Learned safety – Preconditioning with untreated eggs may initially have “taught” the raccoons that eggs were safe to eat, thereby confusing some of them as to what was making them ill during the conditioning phase and increasing the variability in behavior rather than standardizing it (Kulat and Rozin 1973). I considered this a mistake in technique until I was able to repeat this test in 2006.
3. Confusion over source of illness – Presentation of the treated eggs together with dog food on the first treatment day did not differ from the regimen of untreated eggs and dog food given during the pretreatment,

and may have prevented the raccoons from determining the cause of their discomfort.

4. Restricted food availability – Feeding just enough dog food to meet a raccoon's nutritional needs may have turned a free-choice trial into a no-choice trial once the dog food was gone, making it less likely that an animal would refuse eggs even though they caused illness (Conover 1997).

5. High estrogen concentration – My penned animals were likely consuming doses far in excess of what would be considered most effective. Most of the pilot trial eggs contained 1.0 mg of estrogen per ml (the eggs given on days 6 and 7 of the treatment phase contained 0.5 mg/ml). I used the same dose per egg that Semel and Nicolaus (1992) used; however, the quail eggs were 20% as large as a chicken egg so the concentration of estrogen was 5 times as high. On first consideration, the higher doses consumed by 2 raccoons that never rejected eggs mirrors the results of Semel and Nicolaus (1992). They reported large doses (30 mg/egg or 0.6 mg/ml) as being less effective than smaller ones (10 mg/egg or 0.2 mg/ml) at inducing an aversion in free-ranging raccoons, because they thought raccoons could detect the higher dose and thus avoid them. The quail eggs contained 1.0 mg/ml doses, yet the raccoons still ate them, which argues against detection being the reason for the lowered effectiveness.

I attribute these results to lack of choice due to confinement, confusion as to the source of illness due to pretreatment, restricted food availability, or variable resistance to the effects of estrogen, rather than ability to detect and avoid higher doses.

In spite of the high variability, estrogen safely induced an aversion in raccoons to eggs. Eleven out of 13 surviving animals rejected eggs at some point, in spite of all of the reasons listed above why they shouldn't have. Furthermore, since all the raccoons learned to eat eggs very quickly and consumed them completely, it is likely that free-ranging raccoons would approach surrogate eggs readily and consume the estrogen plug.

The lack of obvious symptoms makes it difficult to surmise what effects the raccoons experienced from large doses of estrogen. Raccoons ate dog food, drank water, and engaged in normal behaviors in spite of whatever discomfort they experienced. This lack of obvious suffering recommends estrogen-induced conditioned aversion as a humane treatment as long as it causes strong CFAs. Semel and Nicolaus (1992) witnessed head shaking behaviors that were not evident in my experiment.

Health effects.—All 18 raccoons began the experiment in apparent good health. However, the effects of estrogen and pen stress may have combined with pre-eisting disease to cause some of these raccoons to experience weight loss or death. Some individuals thrived on pen life and even gained weight despite large doses of estrogen, so it was not inherently toxic to raccoons.

Estrogen did not appear to play a direct role in body mass dynamics. Food consumption roughly correlated with body mass; raccoons that gained weight ate more than raccoons that lost weight. The raccoons that ate the largest quantities of food also ingested the most estrogen. The raccoons that ate the largest quantities of estrogen gained the most weight and did not die.

I identified 3 major problems with the pilot pen trial. Problem #1: Five animals in adjacent cages died during the trial, possibly from the stress of penning combined with organ damage due to high parasite loads. All 5 early deaths occurred in cages adjacent to another early death, suggesting at least the possibility of a communicable disease, although none was identified. The only 2 pregnant females died early, which opens the possibility that complications from estrogen-induced abortions may have contributed to their deaths. Raccoons that died consumed fewer estrogen-injected eggs per day than many of the ones that survived, suggesting that the early deaths were due to causes other than large doses of estrogen.

Problem #2: I found several drawbacks to using flour as the estrogen carrier. I could smell the flour-estrogen mixture, so I assume raccoons could as well. Also, this mixture began to coagulate and clog the hypodermic needle after about an hour, when the gluten became stringy. The mixture had to be used immediately and could not be stored. Outside of refrigeration, the dough began to ferment in less than 24 hours and either blew off the wax plug or cracked the egg from the pressure. Semel and Nicolaus (1992) found the flour-estrogen mixture they used often settled and adhered to the shell of the egg.

Problem #3: Commercially available Japanese quail eggs resemble the size and coloration of the eggs of many species of shorebirds (Baicich and Harrison 1997) and would make good surrogates for any shorebird eggs in a field application (Conover and Lyons 2003). Unfortunately, quail eggs are expensive ($3.75 per 24 from an Asian grocer), only 20% the volume of a medium chicken egg (~10ml as opposed to ~50ml), and difficult to acquire in the quantities needed to run such an experiment (I used over 3,000 quail eggs in 2005). In spite of their small size, quail eggs presented no real technical challenge in handling and injecting, although most of the yolk was often removed to make room for the 1-ml estrogen plug. To avoid detectibility I found it best to use smaller doses in small eggs, remove albumen in preference to yolk, and then inject the plug directly into the yolk. Many of the raccoons rejected quail eggs as food during the experiment, so they were effective when used as a part of the treatment. The limited availability of quail eggs presented the greatest challenge. The results of the 2005 pilot pen trial led to refinements in the methods for 2006.

PEN TRIAL 2006

Methods

Animal care.—I stocked the 18-cage pen facility in Northampton County, Virginia, with 10 raccoons live-trapped on the Skidmore Island section of the Eastern Shore of Virginia National Wildlife Refuge, and 8 from the nearby mainland section of the Refuge. I carried out multiple randomizations using a coin toss to balance the treatment between genders and source populations.

I assigned 5 males and 4 females to the treatment group, and 4 males and 5 females to the control group. Prior to caging, a veterinarian examined each animal visually and treated it with 3 doses over 3 days (50 mg/kg) of the drug fenbendazole (Panacur®) in an effort to reduce the health effects of potentially heavy loads of internal parasites. I used only adults (between the ages of 1 and 7 years according to tooth aging) in the trials, with an equal mix of males and females. I sexed, weighed, and caged each animal individually, and monitored it daily for general appearance and well-being. Unlike in 2005, I sedated no raccoons until the end of the trial except for 2 treatment animals that were taken for veterinary care and died early. I assigned each animal randomly to a cage and to the treatment or control group, and caged it within sight of 5 other raccoons, both control and treatment (Figure 8). Each animal received a sufficient daily ration (140 g) of dry dog food (Home Valu Field Chunks; 18% protein, 6% fat) for them to gain weight, and water ad libitum. I kept records of food and water consumption for each animal. I also kept records of stool characteristics, and attempted to record behaviors related to stress level to prevent the inclusion of animals likely to die during the experiment (Broom

Control	Treatment	Treatment
18 Female	17 Female	16 Male
Male 1	Female 2	Male 3
Control	Control	Treatment

Control	Treatment	Treatment
15 Female	14 Female	13** Female
Female 4	Male 5	Male 6
Control	Control	Treatment

Control	Control	Treatment
12 Male	11 Female	10 Female
Male 7**	Male 8	Male 9
Treatment	Control	Treatment

** early death

Figure 8. Distribution of raccoons among pens and cages during the 2006 pen trial.

1991); however, behavior was so variable among individual raccoons that quantifying stress level in a systematic manner proved impossible in the time frame of the study.

Egg preparation.—Because I was dissatisfied with the flour-water estrogen carrier in 2005, I sought an alternative carrier in 2006. I tested a group of likely carriers, including wheat flour, potato starch, guar gum, rice starch, cornstarch, gum Arabic, gelatin, pectin, tapioca starch, and arrowroot starch. Each of these food thickeners was cooked up with water and then tasted and smelled by a panel of judges consisting of myself and 3 technicians. The gels made from tapioca starch and arrowroot starch were the only ones that none of us could detect by taste or smell. Cooking arrowroot starch resulted in a gel with a smoother, more even consistency, and it remained injectable after being stored in the refrigerator overnight, so I chose it as the new carrier. Furthermore, I left a sample outside in humid 35º C heat for several days with no signs of spoilage. The raccoons did not distinguish between injected and uninjected eggs, so arrowroot starch gel was a good carrier.

Carrier preparation.—To prepare the powdered estrogen for injection, I made a gel by mixing 20 g of arrowroot powder with 500 ml cold water and heating on a stove at low heat while constantly stirring. Once the solution cleared and gelled, I allowed it to cool and blended 500 ml of the gel with 5.00 g of estrogen powder. I then prepared the eggs as in 2005; however, in 2006, I used chicken eggs because the 2005 pen trial had already shown the efficacy of quail eggs. Cost and time drove this decision.

Treatments.—The 2006 pen trial consisted of 3 phases (Figure 9):

1) Acclimation phase (11 Jun 2006 to 6 Jul 2006): Depending on the date of capture, the acclimation lasted between 7 and 26 days. The raccoons were fed only dog food and water (and small amounts – 5 g – of peanut butter in the pacifiers) during this time. Except for the veterinary care described above, animal husbandry in 2006 was similar to 2005. This phase was longer than planned because of difficulty in acquiring an adequate supply of estrogen.

2) Treatment phase (7 Jul 2006 to 20 Jul 2006; days 1-14): I injected each medium white chicken egg with 1 ml of a mixture of estrogen (10 mg/ml) and arrowroot gel. I gave 6 estrogen-injected eggs without dog food to the treatment animals on the eighth day after the last pen was filled. I gave only dog food on the ninth day. I gave 6 treated eggs along with dog food every other day for the next 12 days (6 egg feedings). I gave eggs injected with gel, but without estrogen to the 9 control animals on the same schedule. I presented all eggs at the normal feeding time between 1700 and 1800 hours. At 0900 hours the next day, I recorded egg condition as “intact” or “consumed”, and recorded food and water consumption for each animal. I did not pre-epose the raccoons

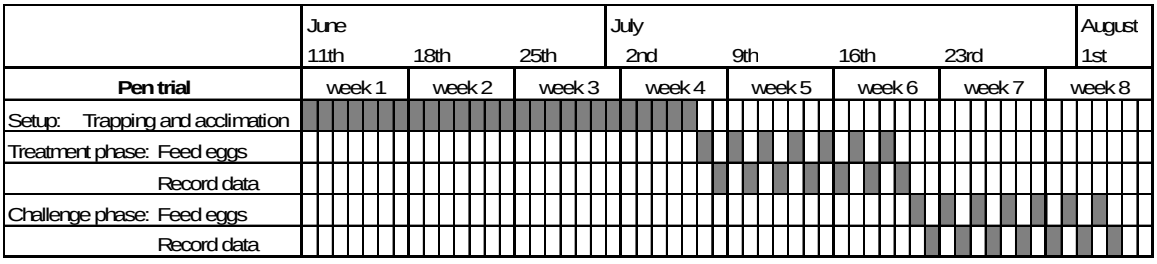

Figure 9. Sequence and timing of 2006 estrogen-induced aversive conditioning pen trial.

to eggs in 2006 because pre-eposure appeared to delay or prevent the onset of an aversion to eggs in 2005. I converted data on consumption to approximate caloric values using the caloric densities of 1.5 Calories per gram for chicken eggs (Carey et al. 1980), and 3.17 Calories per gram for dog food (Dzanis 1998). Because there was some spillage of both egg contents and dog food, I reported the consumption values as maximal values; actual intake might have been somewhat less in many cases. I gave a large number of medium chicken eggs (12 to 18) to each of 2 control animals on 1 occasion during the treatment phase to see how many they could consume.

3) Challenge phase (21 Jul 2006 to 3 Aug 2006; days 15-28): I designed the challenge phase to test the ability of raccoons to discriminate among fresh eggs, estrogen-injected eggs, and carrier-only injected eggs. During the last 14 days (7 egg feedings), each treatment raccoon received 2 eggs of each type marked with pencil. I tallied the number of each type left undamaged, minus all instances where all 6 eggs were left undamaged, for each treatment day.

Necropsy.—I collaborated with Dr. Ramona Skirpstunas DVM to design a systematic tissue collection protocol tailored to the 2006 pen trial (Table 1). At the conclusion of the study, I sedated each remaining raccoon and euthanized it with a jugular injection of Beuthanasia D. I harvested tissue sets to test for general condition, the presence of lesions, and endoparasitic infections. I extracted a premolar to section for age. I visually compared the appearance of tissues and organs between treatment and control animals, and sent tissues to the Utah Veterinary Diagnostic Laboratory for histopathology diagnosis by Dr.

Table 1. Tissue preservation protocol in 2006.

	10% formalin	freeze fresh
Skeletal muscle	yes	yes
Lung	yes	yes
Heart	yes	yes
Liver	yes	yes
Spleen	yes	yes
Kidney	yes	yes
Brain	yes	yes
Bladder	yes	yes
Large intestine	yes	yes
Small intestine	yes	yes
Stomach	yes	yes
Thyroid	yes	no
Adrenals	yes	no
Pituitary	yes	no
Bone marrow	yes	no
Eyeball	no	yes

Skirpstunas. I cut tissues into 1-cm^3 blocks, except for bone marrow, which was taken by splitting a 2-cm section of femur, and preserved them by fixing in 10% buffered formalin or freezing.

Data analysis.—I graphed the egg consumption of each raccoon in 3 dimensions to demonstrate effect as well as variability. I compared mean egg consumption between control and treatment animals using a paired 2-sample for means t-test. I also used the paired 2-sample for means t-tests to compare dog food consumption between the 2 groups and to compare the differences in consumption rates of 3 different egg treatments by the treatment animals.
I graphed average daily egg consumption per raccoon for treatment animals in 2005 and 2006 and control animals in 2006, and graphed the same data averaged over both raccoons and days as well. Both of these graphs included standard errors. I graphed estrogen exposure against net change in body mass

and average food consumption versus net change in body mass. I graphed testes sizes of treatment and control animals with standard errors although sample sizes were too small and age range too large to detect a statistically significant difference.

Results

As the 2 control animals that were given large numbers of eggs became satiated, they ate yolk in preference to albumen, and spilled large quantities of egg contents. The rest of the raccoons spilled less because their supply of eggs was limited to 6 per feeding. As in 2005, measures of egg consumption or estrogen ingestion represent the maximum possible exposure. During the challenge phase, 4 out of 9 control raccoons ate dog food before eggs, 3 ate eggs first, and 2 alternated which they ate first. Not surprisingly, all of the treatment animals ate dog food before eggs. As in 2005, these animals exhibited no conspicuous signs of illness or distress after consuming estrogen, such as vomiting or odd behavior. I perceived the only change as a decrease in activity level of some individuals.

Eight of the 9 control raccoons ate every egg they received; raccoon (#1), a shy male, skipped 2 eggs out of 6 on day 2 of the treatment phase (Figure 10). Six out of 7 treatment animals rejected some eggs a minimum of 3 times during the treatment phase (egg feeding days 1-7), and 7 out of 7 animals rejected eggs a minimum of 4 times during the challenge phase (Figure 11). The control animals consumed an average of 6.0 eggs per feeding day during the 14-day challenge phase (egg feeding days 8-14), while the treatment

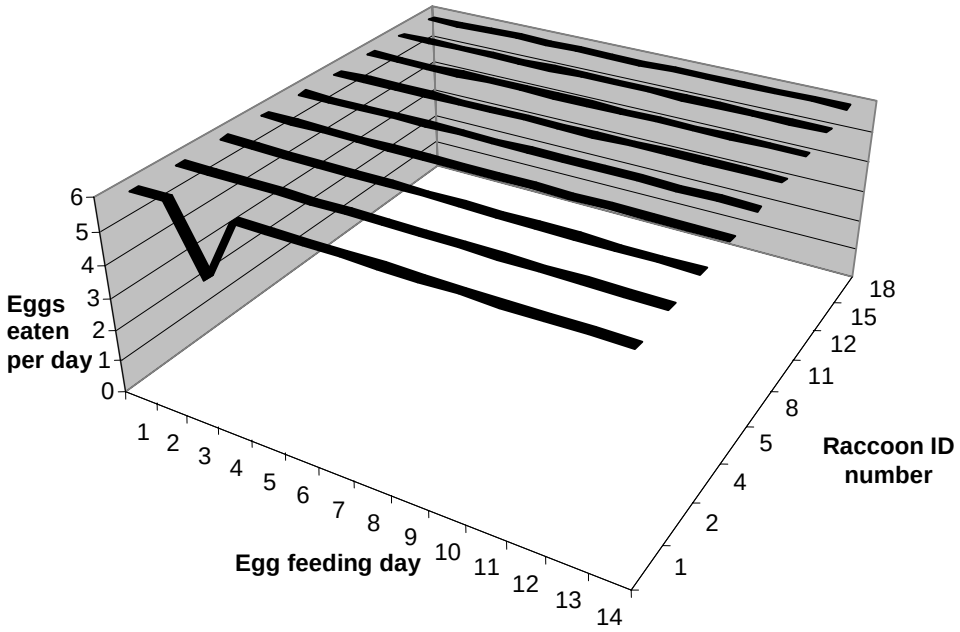

Figure 10. Egg consumption per feeding day (n=14) for the 9 control animals in the 2006 pen trial. Feeding days 1-7 were the treatment phase, and days 8-14 were the challenge period. Raccoon ID numbers refer to animal numbers listed in the text.

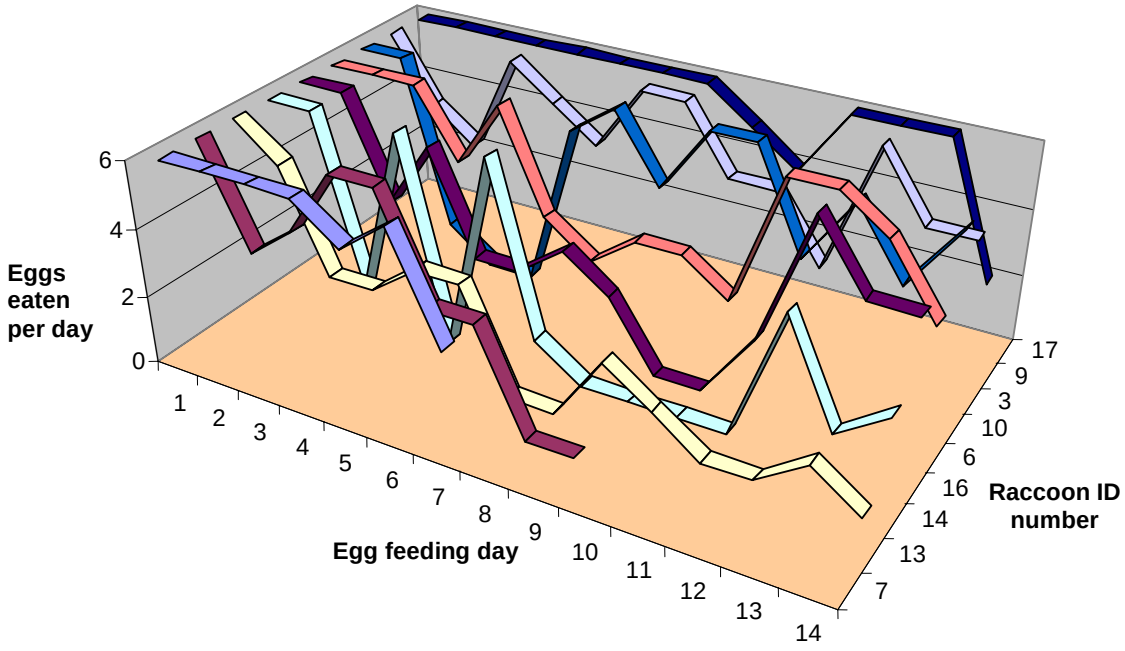

Figure 11. Egg consumption per feeding day (n=14) for the 9 treatment animals in the 2006 pen trial. Feeding days 1-7 were the treatment phase, and days 8-14 were the challenge period. Raccoon ID numbers refer to animal numbers listed in the text.

animals consumed an average of only 3.1 eggs (t_6 = 10.00, P = 0.00003). The control animals ate an average of 130 g of dog food per day during the 14-day challenge phase, while the treatment animals ate an average of 135 g per day. If anything, the treatment animals exhibited a somewhat higher feeding rate than the controls (t_{12} = -2.06, P = 0.061). The cumulative estrogen dose received by the 7 surviving treatment animals ranged from 80 mg/kg to 128 mg/kg over a 14-day exposure period. The cumulative dose received before eggs were rejected ranged from 15 mg/kg to 116 mg/kg.

Raccoons did not distinguish between estrogen-injected eggs and uninjected eggs. The means for the instances of rejection of the 3 types of eggs in the challenge period did not differ (Figure 12). Comparisons between fresh and estrogen, estrogen and carrier, and carrier and fresh gave values of

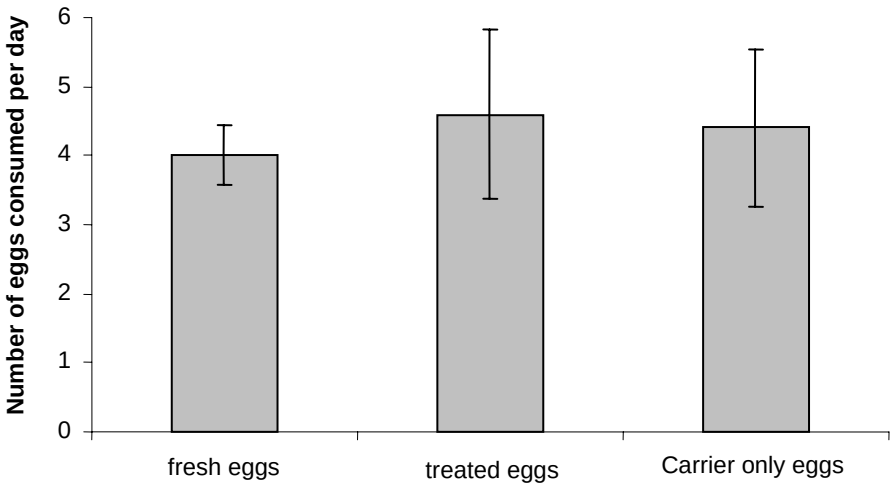

Figure 12. Daily consumption for each type of egg (fresh, treated, and carrier-only) averaged over the 7-day challenge period in the 2006 pen trial (± 1 SE).

t_6 = -0.496 (P = 0.637), t_6 = 0.167 (P = 0.873), and t_6 = -0.452 (P = 0.667), respectively.

In 2005, I calculated egg consumption for 12 treatment raccoons and 1 control raccoon (4 treatment and 1 control died early) with 12 data points collected over a period of 18 days. I fed them untreated eggs for 1 week prior to receiving estrogen-injected eggs. In 2006, I collected 14 days of data from 7 treatment animals (2 died) and 9 control animals over a period of 27 days (eggs given every other day) (Figure 13).

The average cumulative estrogen dose exposure in 2006, 410 mg/kg (SE 22.8, n =7), was larger than the average cumulative dose of 328 mg/kg (SE 82.0, n =13) received in 2005, and the response in 2006 was greater and more consistent (Figure 14).

In 2006, 2 treatment animals died during the challenge phase: a female (#13) on day 5 due to sepsis from a failed late-term pregnancy (4 130-mm

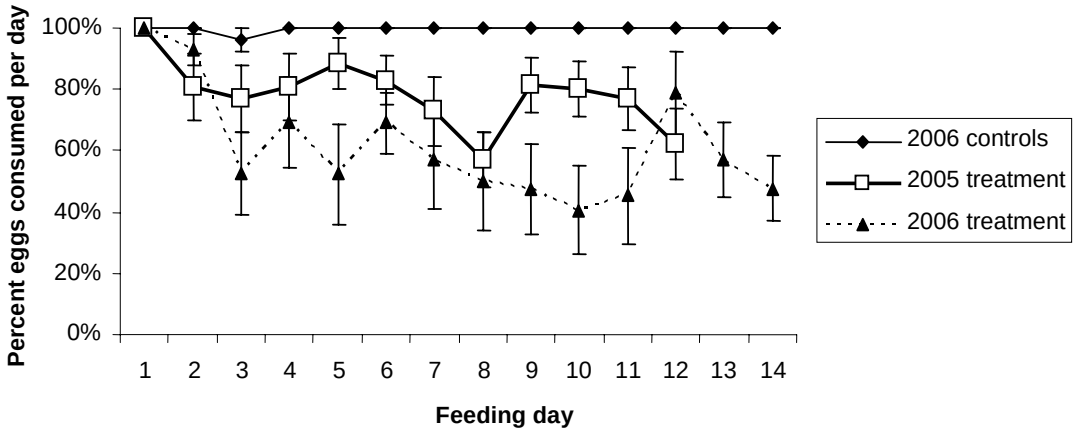

Figure 13. Average daily egg consumption per raccoon (± 1 SE) for 2005 and 2006.

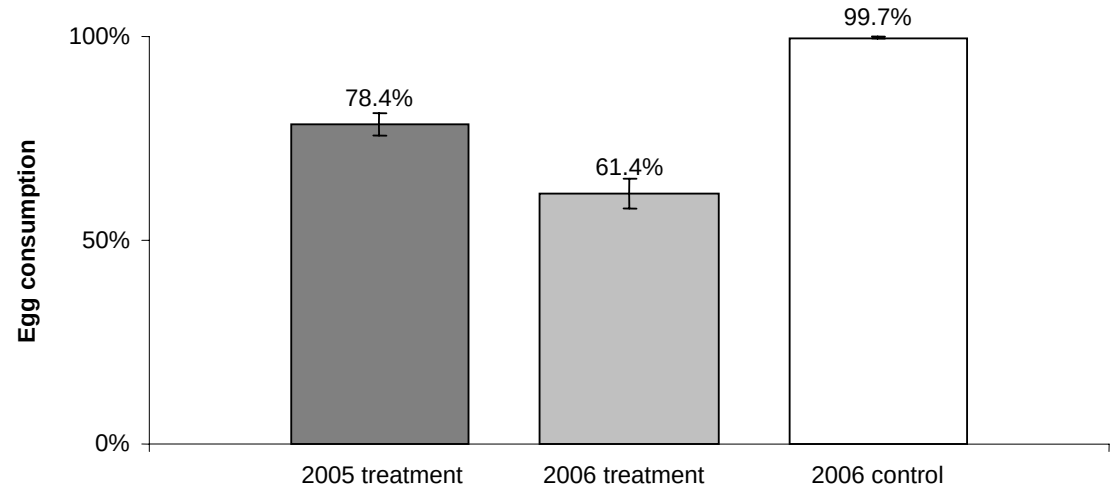

Figure 14. Total average egg consumption per raccoon per day (± 1 SE) for 2005 and 2006.

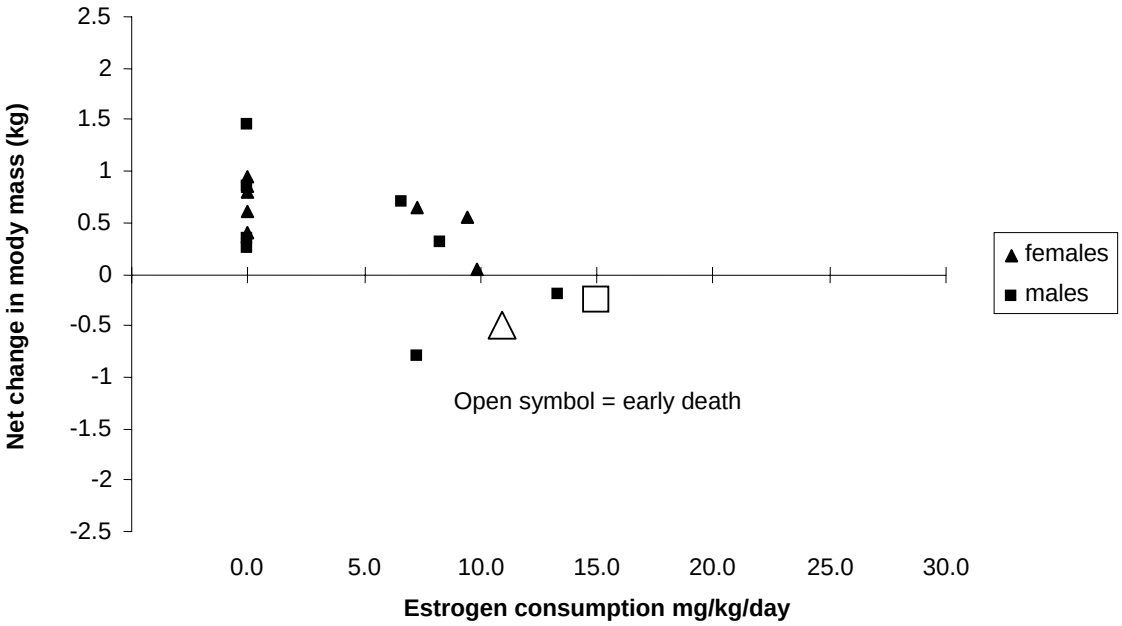

Figure 15. Net change in body mass (kg) between the beginning and end of the 2006 pen trial as a function of average daily estrogen consumption (mg/kg/day).

embryos) and a male (#7) on day 2 due to a prolapsed rectum. The 2 animals that died early got higher average daily doses of estrogen than most of the other treatment animals (female = 11 mg/day, male = 15 mg/day) (Figure 15). No control animals died during the 2006 trial.

Body mass dynamics differed somewhat between treatment and control animals. Treatment animals exhibited somewhat less increase in body mass on average than control animals. The control animals weighed an average of 3.8 kg (SE 0.23), and the treatment animals 3.9 kg (SE 0.16), at the beginning of the trial. The controls gained an average of 0.72 kg (SE 0.124) in body mass (18.4%). Five of the treatment animals gained an average of 0.45 kg (SE 0.121), and 4 lost an average of 0.44 kg (SE 0.138). The 4 animals that lost weight (except for 1 male that stopped eating for 2 days before he died) ate

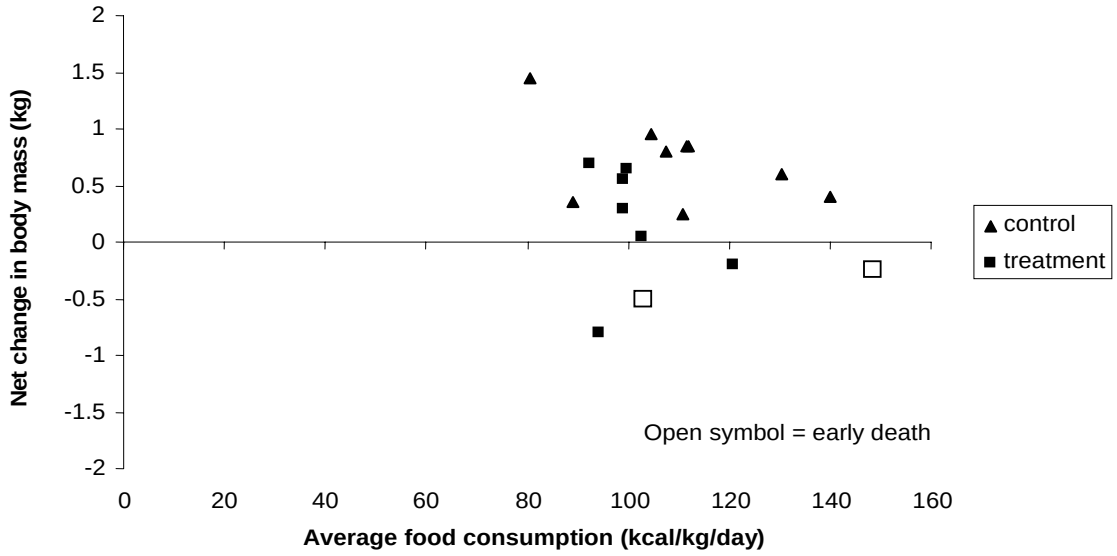

Figure 16. Overall change in body mass (kg) between the beginning and end of the 2006 pen trial as a function of average daily food consumption (kcal/kg/day).

fewer than 130 kcal /kg/day, and the ones that gained mass ate more than 80 kcal/kg/day (Figure 16). All 4 weight losers were treatment animals.

I only tested for estrogen influence on male reproductive organs. To determine whether estrogen consumption changed testes size in the males, testes measurements for 2005 and 2006 were combined for comparison. The testes of the treatment animals were actually larger on average than those of the control males, although the standard errors overlap (Figure 17). At least over the time period of the pen trials, estrogen exposure appeared not to influence testes size. No gross abnormalities were apparent in the reproductive organs of the females.

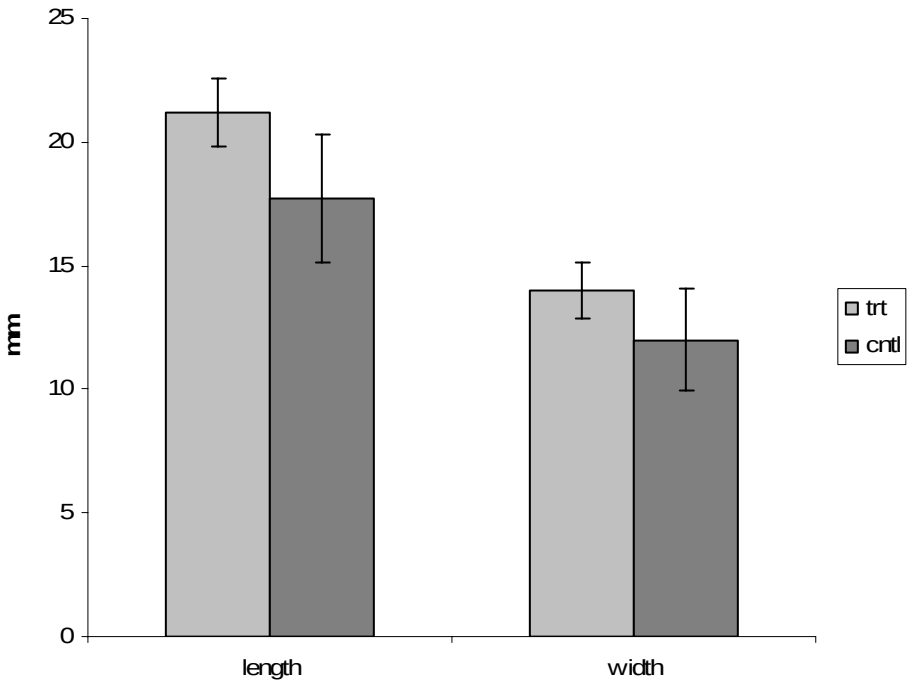

Figure 17. Testes size (± 1 SE) for 11 treatment and 4 control males (2005 and 2006 combined).

As in 2005, necropsy revealed that the raccoons were laden with endoparasites and long-standing, chronic mild to moderate organ damage (R. Skirpstunas, unpublished report). The pathologist found Sarcocytosis (*Sarcocystis* sp.) in the heart and skeletal muscle of several animals, but did not consider it a pathological condition. The pathologist found lesions possibly attributable to at least 3 protozoan organisms widespread. The pathologist considered intestinal parasite loads low and of no clinical significance. Seven of the caged raccoons exhibited dermatophytosis (ringworm infection) by the end of the trial, likely resulting from the stress of capture and containment. As in 2005, we were unable to detect any tissue or organ damage directly attributable to the effects of estrogen ingestion.

Discussion

Effectiveness.—As in Semel and Nicolaus (1992), with no limitation on availability, raccoons will break and eat very large numbers of eggs. This illustrates how raccoons can be so very damaging to colonial-breeding ground nesters (Hartman et al. 1997).

Raccoons in the treatment group became averse to eating eggs rather than dog food. This result agreed with the findings of Semel and Nicolaus (1992). The treatment animals ate slightly more dog food than the controls while their egg consumption was significantly less. Their survival ability was not impaired by an estrogen-induced aversion to eggs. The control animals ate less dog food during the challenge period presumably because they were satiating on eggs.

A minimum cumulative exposure between 15 and 116 mg/kg given in daily doses of 15 mg/kg caused individual raccoons to reject eggs. This corresponds to 6 eggs per day, each containing 10 mg of estrogen, over 1 to 8 days. Thus, 2 weeks of treatment should be sufficient to bring about a reduction in egg predation using this method under cage conditions. Semel and Nicolaus (1992) noted a large drop in both eggs consumed and the number of raccoons present after a 10-night conditioning period.

By using a mix of treated, carrier-injected, and fresh eggs, I was able to determine that the raccoons were averting to the taste of egg rather than the smell or taste of the carrier, or the smell or taste of estrogen. There was no apparent discrimination between types of egg treatments by the raccoons. Therefore, estrogen and arrowroot gel should be effective when used in any type of egg.

All of the raccoons in 2005 ate untreated eggs before they consumed treated eggs. In 2006, the raccoons first ate eggs that were injected with estrogen. The drop in egg consumption rates from 2005 to 2006 supports the idea that learned safety reduces the potential effectiveness of any conditioned aversion; although Semel and Nicolaus (1992) found that many free-ranging raccoons developed a strong CFA in spite of previous exposure to eggs. Another difference in feeding protocols between 2005 and 2006 may also be partially responsible for the difference in average egg consumption in these years. The 2005 animals always received dog food at the same time as eggs, during both the preconditioning and conditioning phases. During each egg

feeding in 2006 except the first one, I gave dog food at the same time as eggs because free-ranging raccoons have other foods available, and lack of choice in a pen situation could have prompted them to sample whatever they had available, even if it made them sick (Conover 1989). I made the first feeding eggs-only to avoid confusing the treatment animals about the source of their illness. The lack of variation in feeding protocol in 2005 may have confused the animals as to the source of their discomfort (Garcia 1989). This strongly suggests that the deployment of treated eggs must precede the actual breeding season in order to achieve the greatest result.

Safety.—I attributed none of the deaths in either year directly to the treatment. The large variety of parasites and health problems, combined with pen stress, likely caused the deaths rather than toxic effects of estrogen (R. Skirpstunas, personal communication); however, estrogen may have exacerbated some of these conditions.

No pregnancies were successful in any animals in either pen trial. High doses of estrogen prevent and terminate pregnancies in wild animals (Asa 2005). Out of 2 pregnant animals in 2005 and 1 in 2006, all 3 died. I did not establish cause of death for the 2 in 2005, but the death of the 2006 female could be attributed to sepsis due to inability to resorb late-term embryos. Estrogen treatment of females with late term pregnancy may increase their chances of dying from complications.

All of the control animals gained weight. Five of the 9 treatment animals gained weight. Four out of 9 treatment animals in 2006 lost weight during the

trial. Two died during the trial; they stopped eating for at least 2 days before dying. Estrogen consumption does not necessarily cause weight loss, but it may exacerbate pre-eisting conditions that can lead to loss of weight. The weight gain shows that the raccoons were sufficiently cared for to thrive.

Even though estrogen can disrupt reproductive processes, it is one of the safest known effective aversive agents; the LD_{50} for estrogen in rats is 1200 mg/kg (Gill et al. 2000). I was surprised that raccoons showed no outward signs of illness after eating treated eggs. In humans, the most frequent and unpleasant symptom of using estrogen is nausea (Murad and Haynes 1980). Large doses can cause anorexia, vomiting, mild diarrhea, and edema in humans (Murad and Haynes 1980). In humans, the nausea caused by estrogen rarely interferes with eating and does not cause a loss of weight (Murad and Haynes 1980). I chose ethinyl estradiol, a synthetic form of estrogen, for this study because it is the most active oral preparation of estrogen known (Murad and Haynes 1980).

At the conclusion of the study, the large number of ringworm cases demonstrated the speed with which a communicable disease could spread among penned animals. Ringworm does not typically affect healthy animals, which suggests that pen stress, parasites, and possibly high doses of estrogen suppressed immune function (Blecha 2000, R. Skirpstunas, unpublished report).

Response variability.—Some of the possible causes of the failure of an aversion to form or to persist include the following: 1) Previous exposure or

learned safety—Raccoons that have previously eaten eggs without ill effects are less likely to develop an aversion, and the aversion would likely be less persistent. Learned safety appears to be a complicating factor in using conditioned aversion as a management tool (Kulat and Rozin 1973); 2) Social learning—Raccoons in the presence of unaverted animals may be more likely to sample eggs (Semel and Nicolaus 1992); 3) Naturally resistant animals—Some raccoons may not respond to estrogen; 4) Restricted alternate food availability—If eggs are the only available food, it may not matter if they are treated; 5) Poor technique—Concurrent presentation of treated and untreated eggs can prevent or slow the onset of aversion (Conover 1997); too high concentration of estrogen in eggs may allow animals to detect the treatment (Semel and Nicolaus 1992); and the wrong carrier may allow animals to detect the treatment.

I found no data available on the time required for raccoons to break down or excrete estrogen (clearance time). If the clearance time for raccoons is comparable to dogs, then it should be approximately 0.1 mg/day/kg (Batista et al. 2005). It is unknown whether clearance time for 17-alpha ethinyl estradiol in raccoons is concentration-dependent, or whether an oral dose is completely absorbed and what factors affect absorption. This means that over the time period of this trial the doses may or may not have been additive. Uncertainty about the actual estrogen intake of the raccoons due to spillage and lack of information on absorption efficiency and clearance times make it impossible to determine what a standard minimum effective dose would be. Nicolaus et al.

(1989*b*) reported that the aversion response to estrogen-laced baits was dose dependent with 40 mg/kg being most effective in captive rats. This study showed that 15 to 120 mg/kg was an effective oral aversive dose for raccoons in the 4-kg range.

Several factors may have influenced the results in my pen trials: 1) Confinement may have altered behavior and caused stress, which suppressed immune function (Blecha 2000), which may have in turn facilitated the transmission of parasites and disease; 2) Boredom and lack of choice due to the inability to forage or leave the vicinity of treated eggs may have forced unusual behaviors, such as eating treated eggs the animals knew would make them sick (Conover 1989); 3) The wide range of personalities and behaviors exhibited by wild-caught animals increased the expected variability and increased the number of replications required for meaningful statistical analysis; and 4) Using wild-caught animals made it impossible to distinguish between normal health problems, cage-stress induced problems, and the potential health consequences of estrogen ingestion. In spite of these limitations, the 2 pen trials were worthwhile in that they reasserted the efficacy of estrogen as an aversive agent when used in eggs; demonstrated that learned safety could delay or prevent the acquisition of a food aversion; and demonstrated the inability of raccoons to discern the difference between treated and untreated eggs.

CONCLUSIONS

In the 2005 pilot pen trial, most of the raccoons reduced their consumption of eggs at some point during the trial. Five animals died during the trial; none of these deaths could be directly attributed to the effects of estrogen. In the 2006 pen trial, all 9 treatment animals reduced their egg consumption, in response to the treatment, but not their food consumption. Other than 1 animal that rejected 2 eggs early in the trial, the control animals ate every egg. No raccoons distinguished between estrogen-injected eggs and similar uninjected eggs. Two treatment animals died during the trial. One of the deaths could be attributed to complications from a failed pregnancy, possibly induced by estrogen consumption.

Very large doses of estrogen were less effective in conditioning an egg aversion than many eggs containing 10 mg of estrogen. I found that these very large doses did not impair the health of most animals. Even though there were no conspicuous signs of aversion-inducing illness, an aversion formed generally within only a day or two of estrogen exposure. Prior exposure to untreated eggs impeded the formation of an egg aversion. Estrogen appeared to be generally safe and effective for use with raccoons, with the possible exception of late-term pregnant females.

I conclude that estrogen is a safe and effective aversive agent and a full-scale field trial of estrogen is likely to be productive. The difference in response between the animals in 2005 and 2006 indicated previous exposure to eggs may have made it more difficult to establish an effective aversion to eggs.

Although most raccoons appear likely to exhibit an aversion to eggs following ingestion of estrogen-treated eggs, no specific dosage is expected to be universally effective.

LITERATURE CITED

Asa, C. S. 2005. Types of contraception: the choices. Pages 29-52 *in* C. Asa and I. J. Porton, editors. Wildlife contraception: Issues, methods, and applications. The Johns Hopkins University Press, Baltimore, Maryland, USA.

Baicich, P. J., and J. O. Harrison. 1997. A guide to the nests, eggs, and nestlings of North American birds. Academic Press / Natural World, San Diego, California, USA.

Batista, M. R., M. S. Smith, W. L. Snead, C. C. Connally, D. B. Lacy, and M. C. Moore. 2005. Chronic estradiol and progesterone treatment in the conscious dog: Effects on insulin sensitivity and the response to hypoglycemia. American Journal of Physiology - Regulatory Integrative and Comparative Physiology 289:1064-1073.

Blecha, F. 2000. Immune system response to stress. Pages 111-121 *in* G. P. Moberg and J. A. Mench, editors. The biology of animal stress. CAB International, Wallingford, UK.

Broom, D. M. 1991. Animal welfare: Concepts and measurement. Journal of Animal Science 69:4167-4175.

Carey, C., H. Rahn, and P. Parisi. 1980. Calories, water, lipid, and yolk in avian eggs. Condor 82:335-343.

Conover, M. R. 1984. Response of birds to different types of food repellents. Journal of Applied Ecology 21:437-443.

Conover, M. R. 1989. Potential compounds for establishing conditioned food aversions in raccoons. Wildlife Society Bulletin 17:430-435.

Conover, M. R. 1990. Reducing mammalian predation on eggs by using a conditioned taste aversion to deceive predators. Journal of Wildlife Management 54:360-365.

Conover, M. R. 1997. Behavioral principles governing conditioned food aversions based on deception: Pages 29-41 *in* J. R. Mason, editor. Repellents in wildlife management. Colorado State University, Fort Collins, Colorado, USA.

Conover, M. R., and K. S. Lyons. 2003. Reducing or delaying egg depredation by punishing free-ranging predators for opening eggs. Applied Animal Behaviour Science 83:177-185.

Cox, S. Baker, D. Macdonald, and M. Berdoy. 2004. Protecting egg prey from carrion crows: The potential of aversive conditioning. Applied Animal Behaviour Science 87:325-342.

Dzanis, D. A. 1998. Interpreting pet food labels—part 1: General rules. FDA Veterinarian Newsletter 13(6). <http://www.fda.gov/cvm/petlabel.html>. Accessed 14 Jun 2007.

Garcia, J. 1989. Food for Tolman: cognition and cathexis in concert. Pages 45-85 *in* T. Archer and L. Nilsson, editors. Aversion, avoidance and anxiety. Lawrence Erlbaum Associates, Hillsdale, New Jersey, USA.

Gill, E. L., A. Whiterow, and D. P. Cowan. 2000. A comparative assessment of potential conditioned taste aversion agents for vertebrate management. Applied Animal Behaviour Science 67:229-240.

Greenwood, R. J., and M. A. Sovada. 1996. Prairie duck populations and predation management. Transactions of the North American Wildlife and Natural Resources Conference 61:31-42.

Gustavson, C. R., and J. C. Gustavson. 1985. Predation control using conditioned food aversion methodology: theory, practice, and implications. Annals of the New York Academy of Sciences 443:348-356.

Hartman, L. H., A. J. Gaston, and D. S. Eastman. 1997. Raccoon predation on Ancient murrelets on East Limestone Island, British Columbia. Journal of Wildlife Management 61:377-388.

Kreeger, T. J., J. M. Arnemo, and J. P. Raath. 2002. Page 241 *in* Handbook of wildlife chemical immobilization. International edition. Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA.

Kulat, J. W., and P. Rozin. 1973. “Learned safety” as a mechanism in long-delay taste-aversion learning in rats. Journal of Comparative and Physiological Psychology 83:198-207.

Murad, F., and R. C. Haynes, Jr. 1980. Estrogens and progestins. Pages 1420-1447 *in* A. G. Gilman, L. S. Goodman, and A. Goodman, editors. Goodman and Gilman’s The pharmacological basis of therapeutics. Sixth edition. Macmillan Publishing Company, New York, New York, USA.

Nicolaus, L. K., M. Crowe, and R. Lundquist. 1992. Oral estrogen retains potency as an aversion agent in eggs: Implications to studies of community ecology and wildlife management. Physiology and Behavior 51:1281-1284.

Nicolaus, L. K., P. V. Farmer, C.R. Gustavson, and J. C. Gustavson. 1989*a*. The potential of estrogen-based conditioned aversion in controlling depredation: A step closer toward the “magic bullet”. Applied Animal Behaviour Science 23:1-14

Nicolaus, L. K., J. Herrera, J. C. Nicolaus, and C. R. Gustavson. 1989*b*. Ethinyl estradiol and generalized aversions to eggs among free-ranging predators. Applied Animal Behaviour Science 24:313-324.

Porton, I. J. 2005. The ethics of wildlife contraception. Pages 3-16 *in* C. S. Asa and I. J. Porton, editors. Wildlife Contraception: Issues, methods, and applications. The Johns Hopkins University Press, Baltimore, Maryland, USA.

Ratnaswamy, M. J., R. J. Warren, M. T. Kramer, and M. D. Adam. 1997. Comparisons of lethal and nonlethal techniques to reduce raccoon depredation of sea turtle nests. Journal of Wildlife Management 61:368-376.

Semel, B., and L. K. Nicolaus. 1992. Estrogen-based aversion to eggs among free-ranging raccoons. Ecological Applications 2:439-449.

Shivik, J. A., A. Treves, and P. Callahan. 2003. Nonlethal techniques for managing predation: primary and secondary repellents. Conservation Biology 17:1531-1537.

CHAPTER 3

ISLAND TRIALS OF ESTROGEN-INDUCED AVERSIVE CONDITIONING IN RACCOONS[2]

ABSTRACT

Aversive conditioning is a promising but unproven nonlethal approach to reducing mammalian depredation on the eggs of ground-nesting birds, terrapins, and sea turtles. Oral estrogen has proven undetectable by taste or smell yet causes predators to avoid treated baits. This research tested the efficacy of eggs containing oral estrogen in a field setting as an aversive agent for raccoons (*Procyon lotor*). I ran 2 field studies on Skidmore Island, a short-term field trial in 2005, and a more refined, redesigned field trial in 2006. I used artificial nest "colonies" and automatic cameras to determine 1) the rate of encounter between tagged raccoons and artificial colonies, 2) variability among individuals in their propensity to consume eggs, and 3) consumption rates of both treated and untreated eggs. I used radio telemetry to see if individual raccoons were more likely to visit colonies close to their sleeping areas.

In 2005, I trapped, marked, and released 22 raccoons on Skidmore Island, 10 of which I radio-collared. I deployed estrogen-injected eggs in 4 artificial colonies for 11 days, followed by untreated eggs for 10 days, and checked them every day. Consumption of eggs dropped from 75 to 20 in the first 3 days. In 2006, I trapped and marked 22 raccoons on Skidmore Island, 10 of which I radio-collared and re-released on Skidmore (plus 2 kits released with their

[2] Coauthored by Joel D. Martin, Raymond D. Dueser, and Nancy D. Moncrief.

mother) as the study population. I deployed estrogen-injected eggs in 6 artificial colonies for 13 days followed by a mix of treated and untreated eggs for 19 days. The raccoons reduced their egg consumption from 123 eggs to 42 eggs over the first 6 days of treatment, and photos revealed a concurrent reduction in colony visitation as well. Some raccoons had much higher visitation rates than others, and a few raccoons visited colonies on one side of the island but not on the other. The raccoons did not discriminate among estrogen-injected eggs, carrier-only injected eggs, and fresh eggs. No radio-collared raccoons died during or within 4 months after the trials. Estrogen is an effective aversive agent and has promise as an egg predation management tool in locations with limited predator populations.

INTRODUCTION

Several field trials of estrogen-induced conditioned food aversion (CFA) have been conducted under less-than-ideal circumstances, some with positive and some with negative results (Nicolaus et al. 1989, Semel and Nicolaus 1992, Ratnaswamy et al. 1997). In the first known field trial of estrogen as an aversive agent, conducted at 21 sites along the Mississippi River in Illinois and Iowa, Nicolaus et al. (1989) tested the ability of estrogen-injected domestic chicken eggs to reduce consumption of eggs by a suite of mammalian egg predators. Nicolaus et al. (1989) observed that 1) the predators did not discriminate between eggs containing 5.6 mg estrogen and untreated eggs; 2) at least some of the predators that visited the treatment sites developed an aversion to

chicken eggs; and 3) there was a reduction in predation on both treated and untreated eggs.

Semel and Nicolaus (1992) used video and nighttime observations of marked, free-ranging raccoons (*Procyon lotor*) eating estrogen-injected chicken eggs in an effort to determine optimum dosage per egg, effective dosage per predator, the optimum frequency of treatment required to induce an aversion, the conditions that influence the generalization of an aversion to other foods, longevity of the aversion, and the conditions likely to influence longevity. They found that 1) a few eggs containing 30 mg of estrogen were less effective in conditioning an egg aversion than many eggs containing 10 mg of estrogen; 2) even raccoons with prior experience eating untreated eggs developed an aversion to them; 3) the aversion did not depend on location or surrounding scent cues; 4) aversions persisted in treated raccoons that were present while untreated individuals consumed untreated eggs; and 5) estrogen dosages between 22.4 and 32.9 mg kg^{-1} per animal caused no obvious detrimental health effects. Semel and Nicolaus (1992) concluded after a second year of study that some of the animals retained some aversion from the previous summer and that raccoons also quickly reacquired aversions that had faded from the previous year.

In an experiment where the investigators assumed all eggs must be equal in the eyes of raccoons, Ratnaswamy et al. (1997) used estrogen-injected chicken eggs placed on the dunes of a barrier beach in Florida to induce an aversion in raccoons to sea turtle eggs (various Chelonidae) before the turtles'

breeding season. When the consumption of treated eggs by an unknown number of raccoons from a large population failed to prevent depredation of turtle nests, Ratnaswamy et al. (1997) concluded that conditioned aversion did not work to protect the eggs of sea turtles from raccoons. However, Ratnaswamy et al. (1997) failed to reduce the raccoon population on their study site to any meaningful extent, so the first problem was a large population of highly mobile individuals with the potential to continually replace any animals that may have been averted. Second, they used chicken eggs as surrogates for sea turtle eggs. Chicken eggs are a different size, shape, and hard-shelled, whereas turtle eggs are leathery. They may be too different in every aspect except color to prompt a raccoon to associate the two. Finally, they assumed that eggs placed on top of a dune could not be differentiated from a buried nest. Ratnaswamy et al.'s (1997) results were negative mainly due to methodological constraints, and they suggested that the adoption of CFA in the management of turtle egg predation awaits further research. Meanwhile, the reaction of the wildlife management community was to lose interest in what was once considered a major breakthrough in wildlife damage management technology. Given the mixed results of the field trials published to date, I concluded an additional test under relatively controlled, near-ideal circumstances in order to determine effective methods for inducing CFA in raccoons using oral estrogen.

The Virginia barrier islands represent an ideal system for further testing estrogen-based conditioned aversion technology (Conover 1997): 1) Raccoons (*Procyon lotor*) are the most abundant mammalian predators on these islands;

2) There is a diverse community of beach-nesting avian species; 3) The simple nest structures of these species are easily mimicked with artificial “scrapes” on the beach; and 4) The eggs of these species are reasonably mimicked using eggs of domestic fowl such as Japanese quail (*Coturnix japonica*).

The objective of this research was thus to design and test an aversion-based management tool to reduce predation by raccoons on the eggs of ground-nesting wildlife. The central questions to establish the viability of estrogen-induced CFA as a predation management tool are as follows: 1) Do individual raccoons reduce egg consumption or change their foraging behavior in response to the consumption of treated eggs? 2) How quickly does an aversion form? 3) Will the aversion last long enough to cause treated raccoons to reject the eggs of the species of concern until the young hatch? 4) Can raccoons distinguish between estrogen-injected eggs and similar uninjected eggs? 5) Does an aversion to one type of egg generalize to avoidance of other types of eggs? 6) Is the average raccoon likely to respond to the treatment? 7) What are possible causes of a failure of the aversion? 8) What are the necessary components of a successful CFA field application?

STUDY SITE

Skidmore Island is located near the southern tip of the Delmarva Peninsula (Figure 18) and is part of the Eastern Shore of Virginia National Wildlife Refuge. Measuring ~44 ha in area, Skidmore is one of the smaller of the Virginia coastal islands. The island is actually located in the estuary behind the ocean-facing barrier islands (Figure 19), but a narrow sand beach circles

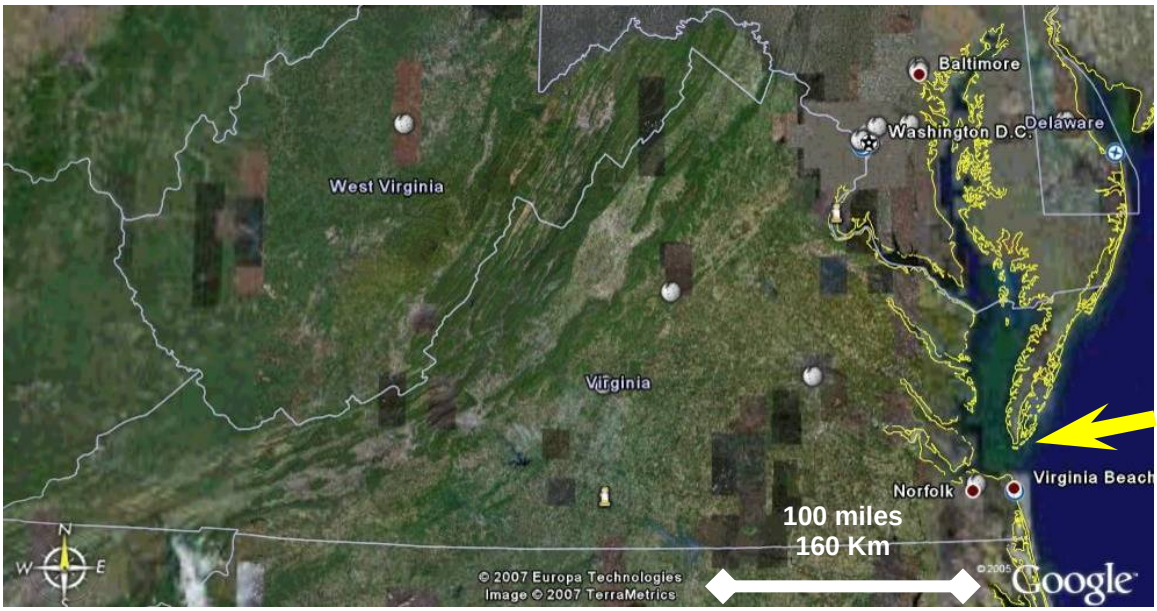

Figure 18. The island trials in 2005 and 2006 were conducted on Skidmore Island, Virginia, a section of the Eastern Shore of Virginia National Wildlife Refuge. Copyright 2007 TerraMetrics, Inc. http://www.truearth.com

~75% of the way around the island, on the west, north, and east sides. The upland portion of the island (above mean high tide) measures ~16 ha. Skidmore supports several hectares of mixed pine, cedar, and deciduous forest, an extensive tall shrub thicket, extensive grassland, and a broad expanse of tidal marsh (Figure 20). Refuge Manager Susan Rice granted written permission to conduct field trials of estrogen-induced aversive conditioning on Skidmore Island.

Again, based on Conover's (1997) review of the environmental conditions favorable for a field test of aversive conditioning, Skidmore was an ideal venue for this field trial: 1) The island is surrounded by open water for at least 0.4 km in every direction, and thus represented a relatively isolated experimental system; 2) There was sufficient sparsely-vegetated sand surface

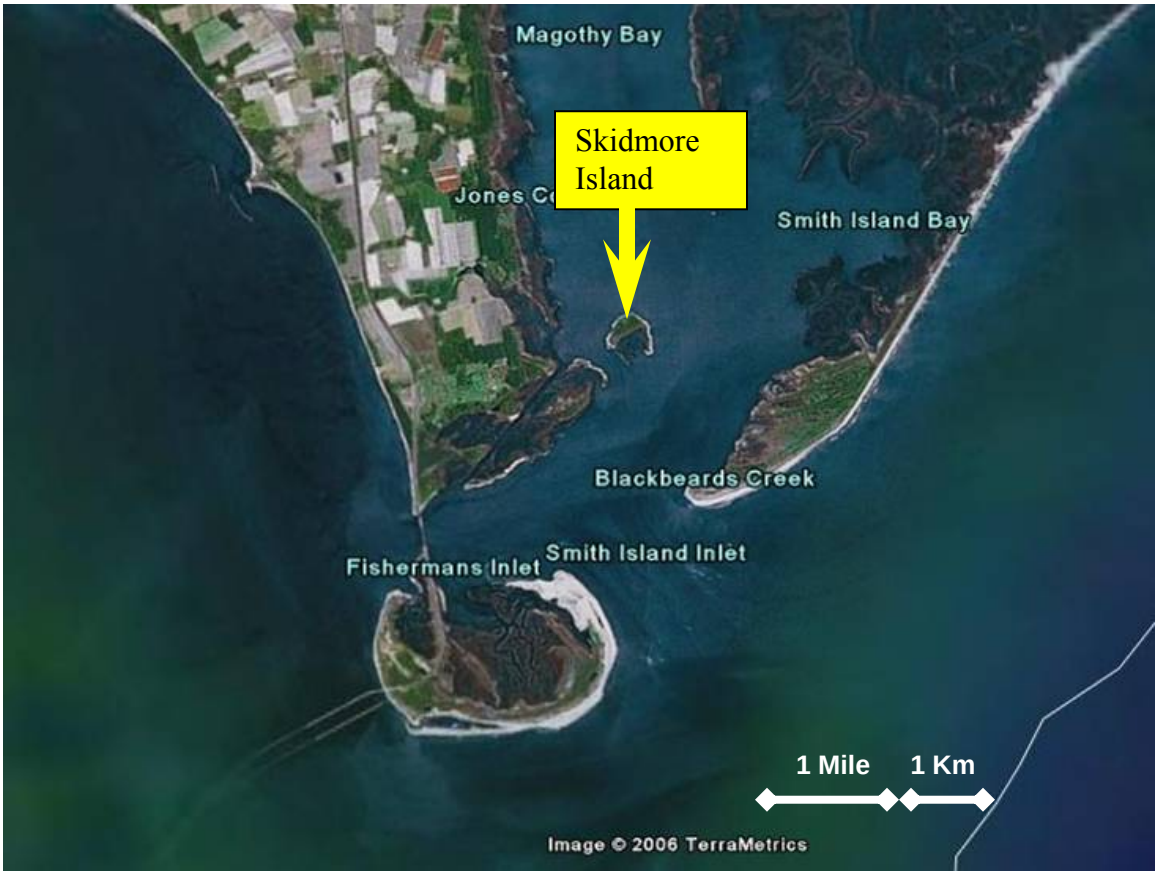

Figure 19. Location of Skidmore Island, Virginia. Copyright 2007 TerraMetrics, Inc. http://www.truearth.com

Figure 20. A closer view of Skidmore Island looking north at low tide in early spring 2007 (photo by Ray Gefken, USFWS).

suitable both for establishing artificial nest scrapes and for definitive track identification; 3) There were no known ground-nesting birds, and no nesting colonies, that might have been disrupted by research activity; 4) The island harbored a large resident raccoon population; 5) The river otter (*Lutra canadensis*) was the only other mammalian predator species detected (one sighting in 2005); 6) Alternative predator food supplies were abundant in the marsh; and 7) The island was uninhabited and public access was restricted, particularly during the blood-sucking-arthropod-rich summer bird nesting season.

I conducted 2 field trials of CFA in raccoons on Skidmore Island to determine the feasibility and potential expense of implementing such management: a pilot trial in summer 2005 and a complete field trial in summer 2006. These projects represent necessary steps in preparation for a full-scale field application of this technology on the Virginia barrier Islands.

PILOT FIELD TRIAL 2005

Methods

Treatments.— The 2005 pilot field trial consisted of 3 phases (Figure 21):

1) Pretreatment phase (19 May 2005 to 8 Jun 2005): I knew from observing tracks that raccoons were present on Skidmore Island, but I had no idea at the outset how many individuals might be resident or how often they might come and go on the island. I trapped initially to deploy radio collars (model HPLM–2180M, Wildlife Materials, Inc., Murphysboro, IL) and apply ear tags. Later during the treatment and challenge phases, I trapped to enumerate

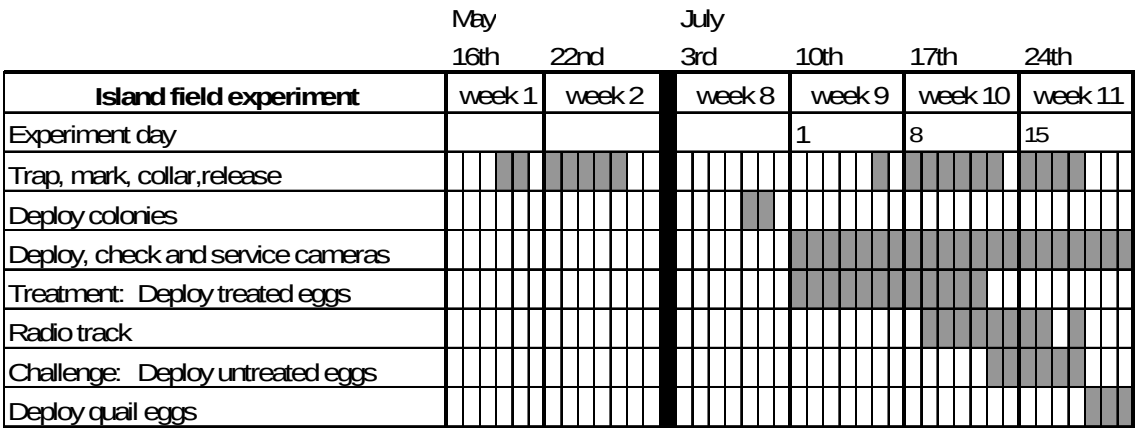

Figure 21. Sequence and timing of 2005 estrogen-induced aversive conditioning pilot field trial on Skidmore Island.

the population. I used 80 x 30 x 25 cm wire cage traps (Tomahawk Live Trap Company, Inc., Tomahawk, WI) baited with apple, marshmallows, pancake syrup, and canned fish. I captured 10 adult raccoons in 87 trap nights over 8 days (19 May 2005 to 26 May 2005), and an additional 4 adults in 414 trap nights during the period of 15 July 2005 to 27 July 2005. This was concurrent with the treatment and challenge phases of the study. The last adult was captured on 26 July 2005 (Table 2).

I sedated each adult with an injection of Ketamine 100 mg/ml and Acepromazine 10 mg/ml (0.2ml/kg body mass) (Kreeger et al. 2002), determined sex, weighed, and examined each for general condition. I fitted each animal with a flexible, numbered, color-coded polyurethane ear tag in each ear (Y-Tex, Livestock Concepts, Inc., Hawarden, IA) to facilitate photographic identification, and radio-collared 10 individuals to allow day-time monitoring of locations. I physically restrained 7 juveniles (all captured between 15 Jul 2005 and 28 Jul 2005), ear-tagged them with numbered metal tags

Table 2. Skidmore Island raccoon capture data (2005).

Tag #	Gender	Age	Capture date	Body mass (kg)
1	M	Adult	5/20/2005	3.0
2	M	Adult	5/21/2005	4.5
6	M	Adult	5/23/2005	3.8
4	F	Adult	5/24/2005	3.4
3	F	Adult	5/24/2005	3.4
7	M	Adult	5/24/2005	4.5
12	F	Adult	5/25/2005	3.0
11	F	Adult	5/25/2005	3.5
13	F	Adult	5/26/2005	3.2
10	F	Adult	5/27/2005	3.2
14	F	Adult	7/16/2005	3.8
15	F	Adult	7/16/2005	4.2
17	M	Adult	7/16/2005	4.0
19	F	Adult	7/26/2005	3.4
1136-7	F	Kit	7/19/2005	1.1
Escaped	?	Kit	7/20/2005	?
1139	?	Kit	7/25/2005	?
1140-1	?	Kit	7/28/2005	?
1142	M	Kit	7/22/2005	1.5
1143-4	M	Kit	7/23/2005	1.1
1145-6	M	Kit	7/23/2005	1.1
1135-6	F	Kit	7/28/2005	?

(style 893, size 4, National Band and Tag Co., Newport, KY), and released them. An eighth juvenile escaped without tags. I released each raccoon at the point of capture. I radio-monitored animal locations once per day on 18 July 2005 through 27 July 2005 (not including 26 July) to obtain information on daytime bed sites. I conducted all research in compliance with Utah State University International Animal Care and Use Committee protocols (#952).

On 6 June 2005 to 8 June 2005, I set out a 400-m transect of artificial nests (i.e., scrapes) on the upper beach to obtain baseline information on egg predation. I established nests at 40 random locations along the transect. I supplied each nest with 2 Japanese quail eggs for 2 days, and checked for

egg predation and predator tracks for 2 successive mornings. I replaced missing eggs on the first morning. Japanese quail eggs are similar in size and coloration to the eggs of many species of shorebirds (Baicich and Harrison 1997). Using them in the pretreatment phase of the island trial demonstrated what some of the technical challenges of using them might be. Unfortunately, they are expensive ($3.75 per 24 from an Asian grocer), only 20% the volume of a medium chicken egg (~10 ml as opposed to ~50 ml), and difficult to acquire in the quantities needed to run such an experiment (I used over 3,000 quail eggs in a concurrent pen trial).

2) Treatment phase (10 Jul 2005 to 21 Jul 2005; days 1-12):

I established 4 artificial nest colonies on the southeastern peninsula of Skidmore Island, each consisting of 18 shallow scrapes in the sand at least 1m apart (Figure 22). Each colony encompassed an area of approximately 60 m^2 and was shaped to fit within its trackable surface or clearing. I stocked each scrape with 2 estrogen-injected medium (50 ml volume) chicken eggs. I injected each egg with 1 ml of a mixture of powdered estrogen (Spectrum Chemicals & Laboratory Products, Gardena, CA) and a semi-liquid flour paste carrier as per Semel and Nicolaus (1992) (10 mg of estrogen per ml of carrier). I prepared medium chicken eggs by using a 30-ml plastic syringe with a 16-gauge needle to pierce the shell at the tapered end and suck out 2 ml of the contents, mostly yolk. I then injected the eggs with the estrogen-gel mixture using a 3-ml syringe with a 16-gauge needle thrust into the yolk. I sealed the resulting needle hole with molten paraffin. I refrigerated the eggs for 1–2 days until deployed.

Figure 22. Part of an artificial colony showing scrapes, nest cages, and a TrailMaster infrared beam generator (photo by author 2005).

I checked and replenished treated eggs daily for 12 days, recording the numbers and locations of eggs eaten or damaged and the apparent cause of egg loss. After the first 5 days, I surrounded each nest with a round cage of 5-cm mesh wire 25 cm tall and 25 cm in diameter to discourage depredation by American crows (*Corvus brachyrhynchos*) and gulls (*Larus* spp.) (Figure 23). I removed the cages and placed them beside each nest on day 12 and reinstalled them around the eggs on day 15.

3) Challenge phase (22 Jul 2005 to 30 Jul 2005; days 13-21): I ran a 9-day challenge phase to test for persistence of any aversion. I replaced treated eggs with 2 fresh chicken eggs per nest for 6 days and 2 fresh quail eggs for 3 days. I deployed 2 automatic trail monitors with 35-mm still cameras (TrailMaster® model TM1550, Goodson and Associates, Inc., Lenexa, KS)

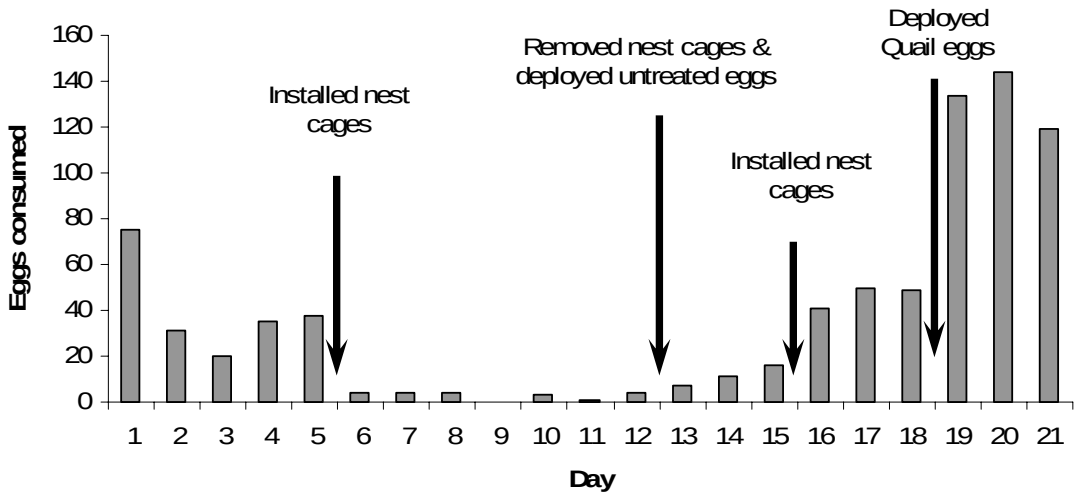

Figure 23. Total daily predation on chicken eggs by raccoons in 4 artificial colonies (2005).

aimed across the center of each of 3 artificial colonies, and 1 camera on the fourth colony (I only had 7 workable camera sets) to photograph and identify individual raccoons, as well as any other nest predators that visited the colonies. I loaded the cameras with ISO 200 color film and set them to operate 24 hours a day with 30 seconds minimum between shots. The cameras operated daily 10 July 2005 through 30 July 2005. I restocked the cameras with film as necessary.

Results

Intensive trapping (501 trap nights) established a minimum population of 14 adult and 8 juvenile raccoons, for a density of 0.5 raccoons per hectare overall, or 1.4 raccoons per hectare of upland (Table 1). Of the 14 adults, 5 were male and 9 were female. Out of 8 juveniles there were 3 males, 2 females, and 3 of unknown gender. The adult males weighed 4.0 kg on average

(SE 0.28), the adult females weighed 3.5 kg on average (SE 0.12), and the 4 juveniles I was able to weigh averaged 1.2 kg (SE 0.10). One of the animals had originally been radio-collared on Skidmore in 2001 and was fitted with a new collar. The first animal that I radio-collared, an adult male, left the island before the treatment phase of the trial began. From radio-tracking, I determined that this animal swam across 0.4 km of open water from Skidmore to Holly Bluff Island by the first day of the trial (10 Jul 2005). It then crossed 0.15 km across the Intracoastal Waterway and traveled ~3 km over land to the southern tip of the mainland a few days later.

A trio of crows picked up and cached almost every quail egg deployed on the beach transect during the pretreatment phase. This prompted me to use chicken eggs instead of quail eggs in the colonies during the treatment phase because they are too large for a crow to carry. Using chicken eggs lowered the cost and increased the resolution of the data because 1 raccoon by itself cannot eat an entire colony of chicken eggs. In the last 3 days of the experiment, quail eggs were consumed in larger numbers due in part to their small size (a medium chicken egg is ~5 times larger than a quail egg).

The cameras produced approximately 900 photographs. Only 38 photos showed raccoons that could be reliably identified as individuals from their ear tags and other attributes. The cameras shot the first photo with an identifiable raccoon on day 4, the second on day 17. Ear tags proved inadequate to always identify individual raccoons in the still photos. Many of the photographs show animals besides raccoons, including crows, gulls, a purple grackle (*Quiscalus*

quiscula), a river otter, and even a diamondback terrapin (*Malaclemys terrapin*) passing through on her way to nest.

Raccoons could still reach through the wire and roll out the eggs, but crows were afraid to approach the cages and only a few gulls could access the eggs. Raccoon consumption of eggs during the first 3 treatment days declined from 75 to 31 to 20 (Figure 23). It increased to 35 on day 4, and 38 on day 5, just before the cages were deployed. Consumption then dropped to 4 eggs or fewer for the next 7 days. The day before the cages were removed and untreated eggs deployed, raccoons ate 4 of the eggs. After the cages were removed consumption increased to 7, 11, and 16 eggs on days 13, 14, and 15. After the nest cages were re-installed on day 15, total consumption jumped to 41, 50, and 49 eggs on days 16, 17, and 18 (Figure 23). When the quail eggs were deployed, consumption jumped to 134, 144, and 119 on days 19, 20, and 21. Placing wire cages around the nests on day 5 stopped crow predation and drastically reduced gull predation (Figure 24).

Radio-telemetry revealed that most of the raccoons showed no particular affinity for any single location, but were most likely to be found near the middle of the upland portion of the island (Figure 25). No radio-collared animals died during the trial, and no other marked animals were discovered dead.

Discussion

Circumstances.—The upland population density of 1.4 raccoons per hectare on Skidmore Island was 3 times that of nearby Parramore Island (3,440

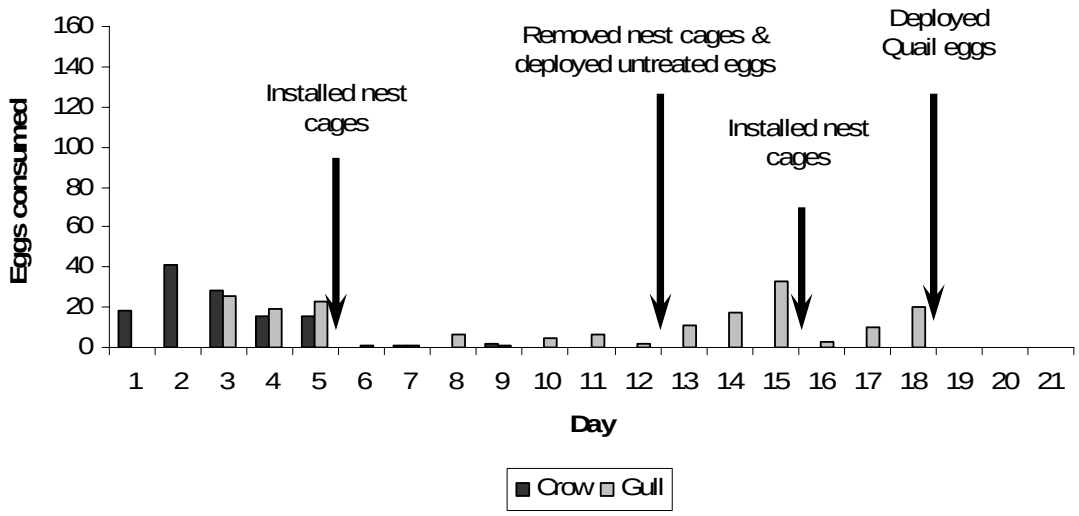

Figure 24. Total daily predation on chicken eggs by crows and gulls in 4 artificial nest colonies (2005).

ha of upland), which is considered high at 0.4 individuals per hectare (Hanlon et al. 1989). The pilot field trial was thus conducted against a background of exceptionally high raccoon abundance.

Effectiveness.—The rapid decline in egg consumption over the first 3 days of the treatment phase indicated that interest was diminished and was consistent with the formation of a conditioned aversion, which apparently took only a few days. The spike in consumption that occurred on days 4 and 5 was perhaps due to discovery of the colonies by 1 or more untreated animals. Because of the lack of raccoon photos early in the trial, I had no certain way to determine whether this spike was due to rapid extinction of the aversion or the discovery of the colonies by naïve animals. Nevertheless, because this jump was driven by the 2 most distant colonies on the peninsula, I suspect 1 or more naïve animals walked through the marsh and bypassed the first 2 colonies to

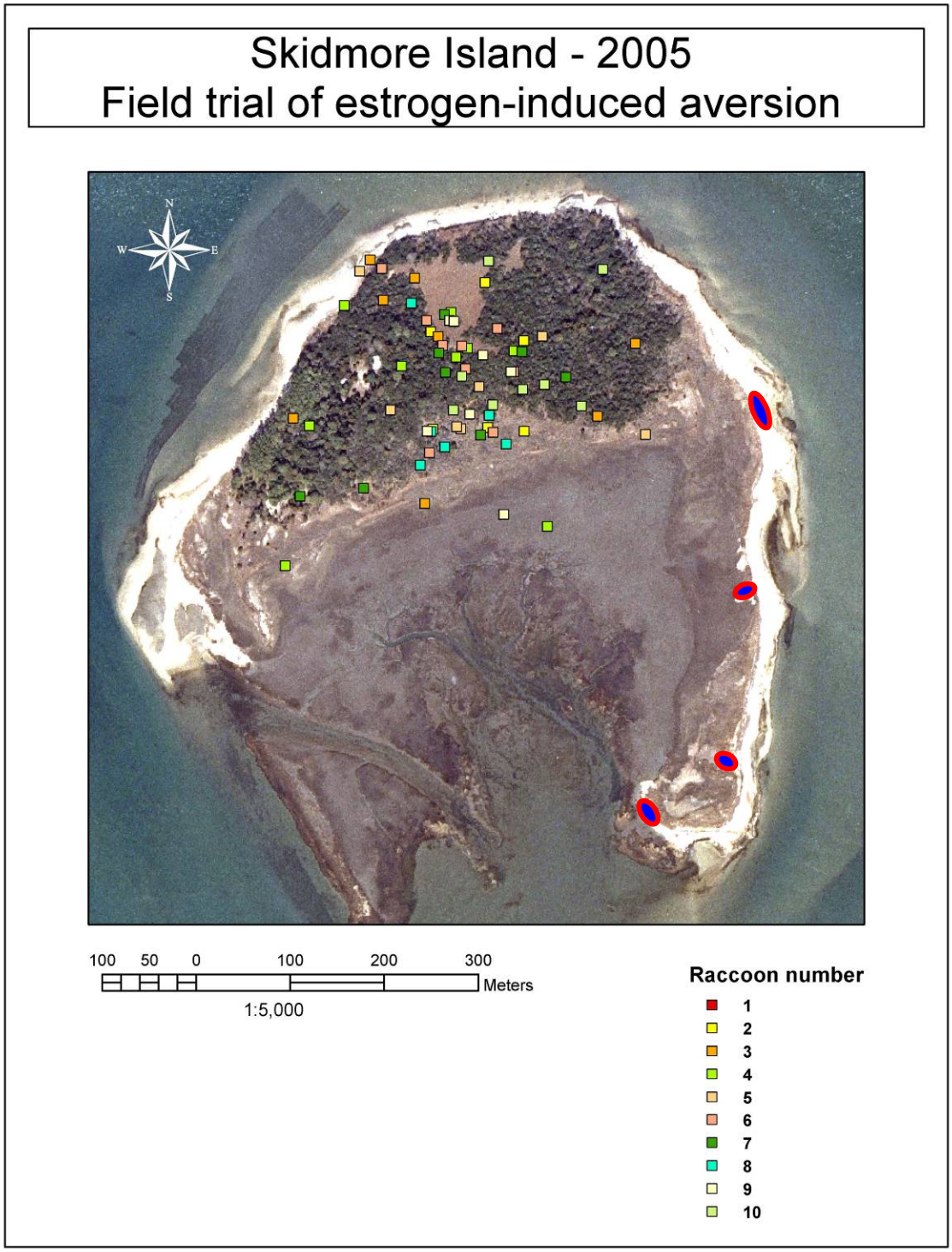

Figure 25. Raccoon telemetry locations in 2005 with colony locations marked in red and blue.

discover eggs they had not yet experienced. Consumption remained low on days 6–12, while the nest cages were in place, likely due to either an aversive effect or because the nest cages altered behavior. Although the photographic evidence was too sparse to provide guidance, the even larger increase in consumption after the nest cages were re-installed on day 15 implies the cages were not the main cause of the earlier decline in consumption.

The modest increase in egg consumption on days 13–15, after the nest cages were removed from around the eggs and the untreated (challenge) eggs were deployed, was due to 1) sampling by averse animals, 2) the discovery of the eggs by naïve, nonaverse raccoons, or 3) the undeterred access to the eggs. In fact, all three of these factors probably played a role. The further increase in egg consumption on days 16–18 after the nest cages were re-installed on day 15 was due either to the arrival of naïve animals or to the failure of the aversion. Again, the extraordinary abundance of raccoons and lack of photographs complicated interpretation of these results.

The large increase in egg consumption after the untreated quail eggs were deployed on day 18 was likely due to the much smaller size of these eggs; chicken eggs are 5 times larger. The higher egg consumption in days 19-21 actually represented a smaller total volume of egg contents consumed than on days 16-18. It is also likely that quail eggs represented novel prey, and either their appearance, or the moving of the cages may have attracted even averse raccoons to the colonies and prompted them to sample eggs.

Ultimately, the nest cages proved effective in selectively reducing egg predation by avian predators, but their addition and removal during the field trial may have influenced the behavior of the raccoons. However, because the installation of nest cages was followed by both a decline (day 6) and an increase (day 16) in egg consumption, I concluded that the nest cages had no restrictive influence on raccoon foraging in the artificial colonies.

Many of the eggs that were not quickly consumed spoiled in the summer heat. Initially, I only replaced depredated eggs, so an unknown number of eggs sat spoiled in the nests for up to 4 days, which also may have affected predation rates. The raccoons may have developed an aversion to spoiled eggs and resumed eating eggs when fresh ones were put out.

Confounding factors.—I continued to trap and mark raccoons during the treatment and challenge phases in an effort to get an accurate estimate of actual population size. This concurrent trapping may have altered the outcome of the experiment. For example, it may inadvertently have caused some of the raccoons to shy away from the wire nest cages due to their resemblance to the wire traps. It may also have removed certain raccoons from the population for a night and prevented them from accessing the colonies. Either of these results could have reduced colony visitation and egg consumption, making reductions in consumption artificially high.

The TrailMaster cameras were sufficiently effective in allowing identification of individual raccoons to suggest that photos can be useful in identifying egg predators. This experience led me to rethink how to deploy the

cameras to make them more effective in the 2006 field trial. The radio telemetry showed that the collared animals could be found mostly in the forest near the center of the island during the day. None of the raccoons was found on the southeast peninsula where all of the colonies were located. This seemed to decrease the likelihood that some raccoons would find the colonies right away and increase the likelihood of spikes in the data.

Conclusion.—The results of the pilot island trial were promising, but not definitive, due to the short duration of the experiment and a variety of technical problems. A successful field application would therefore necessitate locating eggs so that every raccoon on an island has access to them. In addition, islands with large populations of predators are poor candidates for conditioned aversion since every animal averted could be replaced by a naïve individual.

FIELD TRIAL 2006

Methods

Setup.—I established 6 artificial nest "colonies" on Skidmore Island on 21 June 2006, each with 18 shallow scrapes in the sand spaced >1 m apart. Storm erosion of the already narrow beach during winter 2006 forced me to move the colony sites to higher grassy areas behind the beach. Each colony encompassed a rectangular area of approximately 45 m^2 between a pair of parallel 5-cm mesh wire drift fences 7.5 m long by 0.6 m tall (Figure 26). Some colonies had to be prepared by cutting sparse vegetation and placing extra sand in the nest locations to facilitate tracking. I deployed nest cages and TrailMaster cameras 16 days prior to setting out treated eggs to allow the

Figure 26. Aerial view of artificial colony 5 and surrounding habitat (photo by Erika Miersma 2006).

raccoons to acclimate to their presence, prevent avian depredation, and compile baseline photographic data. I maintained the colonies daily by baiting, sweeping tracks, and checking cameras (Figure 27). I deployed 1 camera aimed across each of 2 access routes into each colony to photograph and identify the individual raccoons that visited the colony (Figure 28). I set the cameras to operate from 1900 to 0800 hours with a minimum of 5 minutes between shots using ISO 200 color film.

Using the same procedures as in 2005, I live-trapped 22 raccoons (20 adults and 2 juveniles) in trapping locations away from the colonies (Table 3). I stopped trapping just before I released the 10 study animals back onto the island to reduce the chance of affecting their behavior. I was confident I had captured the entire adult population after 311 trap nights. The 12 cameras gave

Figure 27. Pulling maintenance on artificial colony 1 (photo by author 2006).

Figure 28. Drift fence and TrailMaster camera setup in tamper resistant box (photo by author 2006).

Table 3. Skidmore Island raccoon capture data (2006).

Tag #	gender	age	capture date	mass	fate
21-21	M	Adult	6/13/06	4.1	Island trial
25-6	M	Adult	6/13/06	4.2	Island trial
16-16	F	Adult	6/12/06	2.8	Island trial
19-19	F	Adult	6/12/06	3.2	Island trial
8-8	M	Adult	6/12/06	3.8	Island trial
20-20	M	Adult	6/12/06	2.9	Island trial
10-10	F	Adult	6/13/06	3.1	Island trial
12-21	F	Adult	6/12/06	2.8	Island trial
12-12	F	Adult	6/12/06	3.2	Island trial
18-18	F	Adult	6/12/06	3.3	Island trial
953-4	M	Kit	6/18/06	1.0	Island trial
955-6	F	Kit	6/18/06	1.0	Island trial
Pen 1	M	Adult	6/17/06	4.5	pen trial
Pen 2	F	Adult	6/13/06	4.0	pen trial
Pen 3	M	Adult	6/21/06	4.1	pen trial
Pen 4	F	Adult	6/12/06	3.8	pen trial
Pen 5	M	Adult	6/12/06	3.3	pen trial
Pen 7	M	Adult	6/12/06	4.3	pen trial
Pen 11	F	Adult	6/12/06	3.4	pen trial
Pen 12	M	Adult	6/12/06	4.7	pen trial
Pen 17	F	Adult	6/13/06	3.7	pen trial
Pen 18	F	Adult	6/13/06	3.7	pen trial

additional evidence that this was the case. I also reasoned that continued trapping during the trial could cause the raccoons to shy away from the wire mesh of the drift fencing and nest cages, possibly altering the results of the trial.

I brought each animal to a pen facility on the mainland, and 10 adults were selected at random to be returned to Skidmore as the study population. I kept no animal on the mainland for more than 10 days. The 2 kits were released with their mother. Each adult was sedated, fitted with 2 color-coded, numbered ear tags and a radio collar as in 2005, and marked with fur dye to facilitate photographic identification. I clipped tail and body hair in patterns and

painted the fur with Nyanzol D fur dye (Figure 29). The dye was prepared by mixing 10 g Nyanzol D crystals (Greenville Colorants, Inc.) with 100 ml of boiling 70% methyl alcohol. I then added 20 ml of 20% hydrogen peroxide developer from a local beauty supply to the cooled solution. A local veterinarian (Dr. Lance Mayfield, DVM, from Eastern Shore Animal Hospital) gave each raccoon a wellness check and treated each with 3 doses over 3 days (50 mg/kg) of the drug fenbendazole (Panacur®) in an effort to reduce the health effects of potentially heavy loads of internal parasites.

I released the 10 marked animals simultaneously on the northeastern corner of the island on 22 June 2006. I limited the island population to 10

Figure 29. Raccoon ready for release with dye markings, ear tags and radio collar (photo by Robert Alonso 2006).

animals to prevent the treated raccoons from being continually replaced by naïve animals, which would destroy the resolution of the data. The released population provided a density of 1.6 raccoons per hectare of upland. I released a population of raccoons with a similar sex ratio (4♂♂:6♀♀) to that of the 2005 adult population (5♂♂:9♀♀). I radio-monitored the released individuals periodically on 19 occasions between days 10 and 49 to determine their locations. I used ten of the remaining adults in a concurrent pen trial.

Treatment.— The 2006 Skidmore Island field trial consisted of 4 phases (Figure 30): 1) Calibration phase (22 Jun 2006 to 6 July 2006; days 1-15): I set the cameras up and began operating them on 20 June 2006. I bracketed each colony by parallel drift fences with the open sides of the square located at natural access points. I positioned 1 camera to shoot across each open end to capture a date-stamped, color photo of essentially every visitor to the colony. I released the raccoons on 22 June 2006 (day 1) with the colonies and nest

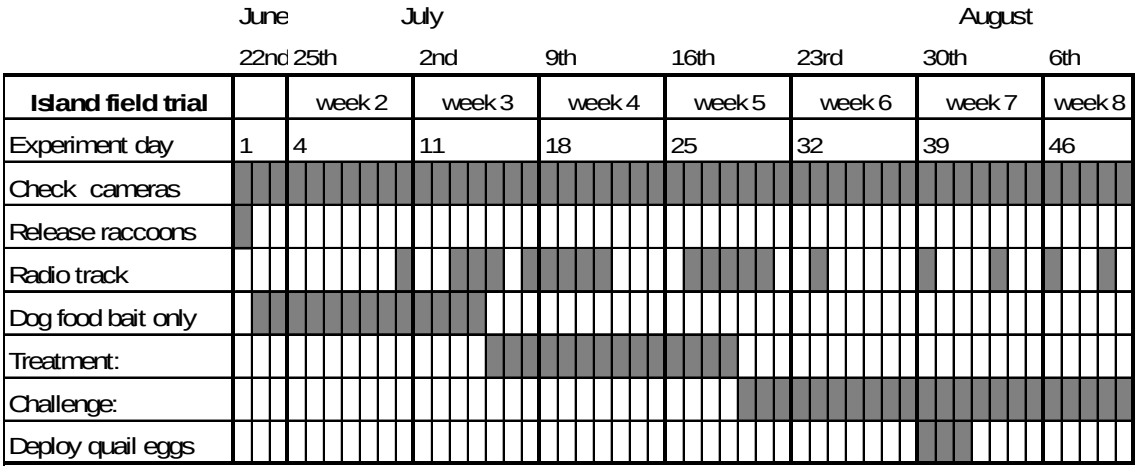

Figure 30. Sequence and timing of 2006 estrogen-induced aversive conditioning field trial on Skidmore Island.

cages already in place. To maintain their interest in checking the colonies, I sprinkled 2 or 3 nests in 3 to 6 colonies with ~25g of dry kibble per nest for the 15 days between releasing the raccoons and beginning the treatment. This amounted to enough to satisfy 1 or 2 raccoons if they managed to find it all before the others.

2) Treatment phase (7 Jul 2006 to 19 Jul 2006; days 16-28): I tested for the rate of onset and efficacy of any conditioned aversion. I stopped feeding dog food on day 15 (6 Jul 2006), and deployed estrogen-injected eggs in the colonies on day 16 (7 Jul 2006). I stocked each scrape initially with 2 treated medium chicken eggs (36 eggs per colony). Ten raccoons could not locate and consume anywhere close to this many eggs (216 per day), even on the first day of treated eggs with no aversion (they ate 123 eggs on day 17). I reduced the number to 1 egg per nest on day 20 (11 Jul 2006) and recorded data as "number" of eggs damaged or consumed rather than "percentage." I injected the eggs with a mixture of estrogen (10 mg/egg) and arrowroot gel (see complete description below). I checked and replenished the treated eggs every day, recording the numbers and locations of eggs eaten or damaged and the predator responsible (i.e., raccoon, rodent, crow, gull, ghost crab, unknown). I left no egg in a nest more than 2 nights for the first 10 days to avoid spoilage. After 10 days, I replaced every egg every day due to my perception of a difference in odor between treated and untreated eggs after 2 days of exposure to daytime temperatures around 35° C.

3) Challenge phase (20 Jul 2006–29 Jul 2006): I tested for the persistence of any conditioned aversion established during the treatment phase. After 13 days of placing estrogen-injected eggs in the nests, I deployed 9 treated eggs at each colony entrance to serve as “guard eggs” (M. R. Conover, Utah State University, personal communication), while 9 carrier-only injected eggs and 9 fresh eggs were placed at random in the 18 colony nests. Each egg was marked with a pencil to designate its contents as carrier-only (C), fresh (F) or treatment (X).

4) Postchallenge phase (30 Jul 2006–10 Aug 2006): I tested for generality of any conditioned aversion established by the estrogen treatment. I placed a pair of Japanese quail eggs in each nest along with a chicken egg for 3 days (days 39–41), whereupon I removed them and returned the colonies to the challenge-phase configuration for 5 days. I then exchanged the positions of the fresh and carrier-only eggs with the treated guard eggs on day 47 so that the “guard” eggs were now untreated and the nest eggs contained estrogen. This configuration lasted 3 days before the experiment ended on day 50.

During the summer of 2005, I used the flour-water mixture as per Semel and Nicolaus (1992), which involved mixing enough water with about 100 ml of white flour to reach an injectable consistency. This mixture had several drawbacks. I could smell it, so I was sure raccoons could as well. It also began to coagulate and clog the hypodermic needle after about an hour when the gluten became stringy, which meant that the mix had to be used immediately and could not be stored. Outside of refrigeration, the dough began to ferment in

less than 24 hours and either blew off the wax plug or cracked the egg from the pressure.

In 2006, I tested a group of likely carriers which included wheat flour, potato starch, tapioca starch, guar gum, rice starch, arrowroot starch, cornstarch, gum Arabic, gelatin, and pectin. I cooked each of these food thickeners with water, and a panel of judges consisting of myself and my 3 technicians tasted and smelled each mixture. None of us could detect the gels resulting from tapioca starch or arrowroot starch by taste or smell. I chose the arrowroot starch as the new carrier because it had a smoother, more even consistency, remained injectable after being stored in the refrigerator overnight and did not spoil easily. I left a sample outside in 35º C heat for several days with no signs of spoilage. However, it was difficult for me to distinguish between the clear carrier-estrogen plugs and the contents of the eggs. To solve this problem, I added 6 drops of blue food coloring to each 500-ml batch of carrier. This provided a color contrast with the egg contents, which allowed me to detect whether a plug had been consumed or missed.

I made the new gel carrier by mixing 20 g of arrowroot powder with 500 ml of cold water and heating while constantly stirring until the solution gelled. Once it cooled, I added the gel to a blender carafe along with 6 drops of blue food coloring and 5.00 grams of estrogen powder weighed on a milligram laboratory scale. I prepared medium chicken eggs as in 2005.

Data Analysis

The 2005 island trial results suggested that 2 to 4 days of exposure to estrogen-injected eggs was sufficient to establish an aversion. The 2005 egg consumption data have a large spike 2 days after the eggs were initially set out, possibly due to the later discovery of eggs by 1 or more animals. Therefore, I assumed in 2006 that each raccoon required 4 days maximum to establish an aversion sufficient to alter its behavior. For purposes of statistical analysis of 2006 data, the 6-day period from the day the eggs were set out to 4 days after the major spike was designated the "treatment period." The 12 days prior to setting out eggs was the "pretreatment period," and the 12 days following the "treatment period" was the "post-treatment period" (Figure 31). This data structure allowed me to test for a difference in raccoon visitation to the colonies

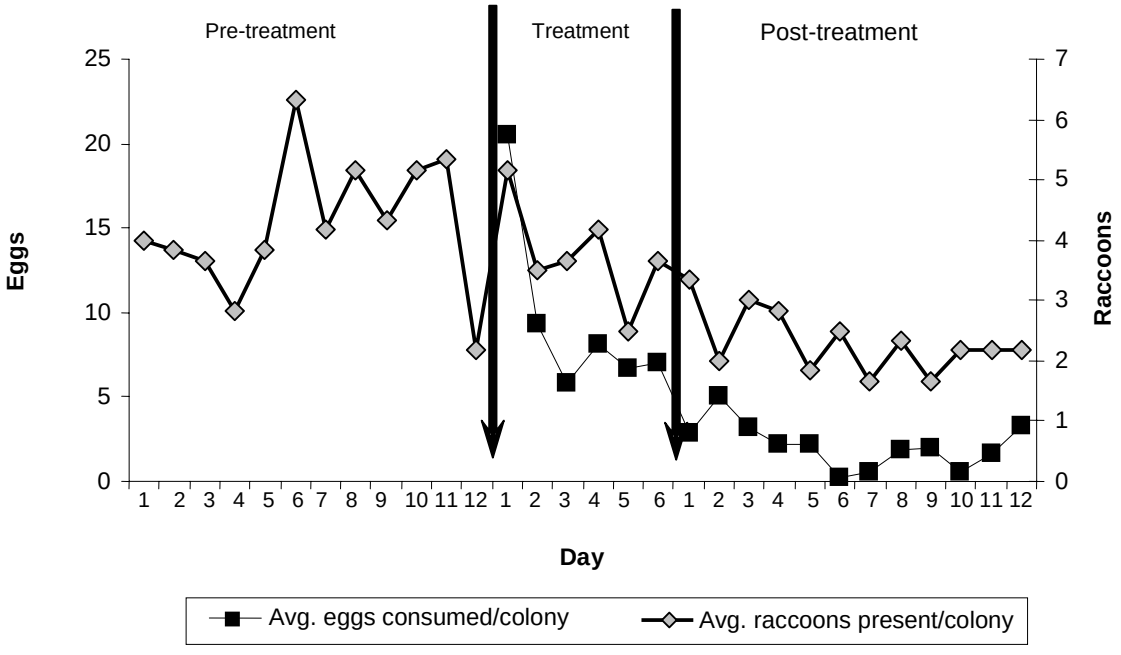

Figure 31. Average daily egg consumption and average daily number of raccoons present in colonies 1-6 (2006).

(a surrogate for behavior) before and after the establishment of an aversion to eggs. I assumed that once the aversion was established, the behavior of the raccoons would have stabilized somewhat at a new level.

I chose the locations of colony sites based on logistical workability and maximum island coverage rather than apparent suitability as bird habitat (Figure 32) because the available sites were limited due to vegetation, limited open sand, and the small size of the island. As a result, only 4 of the colonies (#'s 2, 4, 5, and 6) represent the type of beach or American beach-grass (*Ammophila breviligulata*) habitat which ground-nesting birds might actually use to nest. Therefore, the results speak more to the behavior of raccoons than the qualities of the colonies. I originally intended to move colony locations periodically to test whether raccoons associated estrogen-induced illness with the location where the eggs were encountered, but moving colonies proved to be impractical. I tallied the number of visits to each colony by each raccoon during each period based on visitation data from the photos. I assessed the difference in the mean number of visits during the pretreatment period and the post-treatment period using an analysis of variance of a one-way treatment design with period (pre and post) as a fixed-effects factor in a mixed model with raccoon, colony, raccoon by colony interaction, raccoon by period interaction, and colony by period interaction as random-effects factors. I evaluated assumptions of normality and homogeneity of variance using graphical assessment of residuals. I generated the data analysis using the MIXED procedure in SAS/STAT software (SAS Institute 2006).

I simply graphed the egg consumption data as there was no simultaneous control for comparison. I separated the egg consumption and raccoon visitation data for colonies 1 and 3 from colonies 2, 4, 5, and 6 because the latter were more like the habitat in which ground-nesting birds might occur – i.e., sparse beach grass in sandy areas away from trees and shrubs. Colonies 1 and 3 more closely represented typical raccoon denning habitat within or adjacent to forest. This blocking was useful in demonstrating that visitation was not always correlated with egg consumption.

Results

Intensive trapping (311 trap nights) over 12 days established a minimum raccoon population of 20 adults, all recaptured from 2005, and 2 juveniles. Photos revealed 2 more juveniles for a density of 0.5 raccoons per hectare overall, or 1.5 raccoons per hectare of upland (Table 2). Of the 20 adults, 9 were male and 11 were female. There were 1 juvenile male, 1 female, and 2 of unknown gender. The adult males weighed 4.0 kg on average (SE 0.19) at capture, the adult females weighed 3.4 kg (SE 0.12), and the 2 juveniles each weighed 1.0 kg.

Radio telemetry indicated that the 10 raccoons slept in various locations around the island (Figure 32), although several animals inhabited mostly one side or the other. For example, raccoon #5 slept in locations clustered around colony 3, raccoon #6 always slept near colonies 5 and 6, and raccoon #8 slept mostly away from colonies 5 and 6.

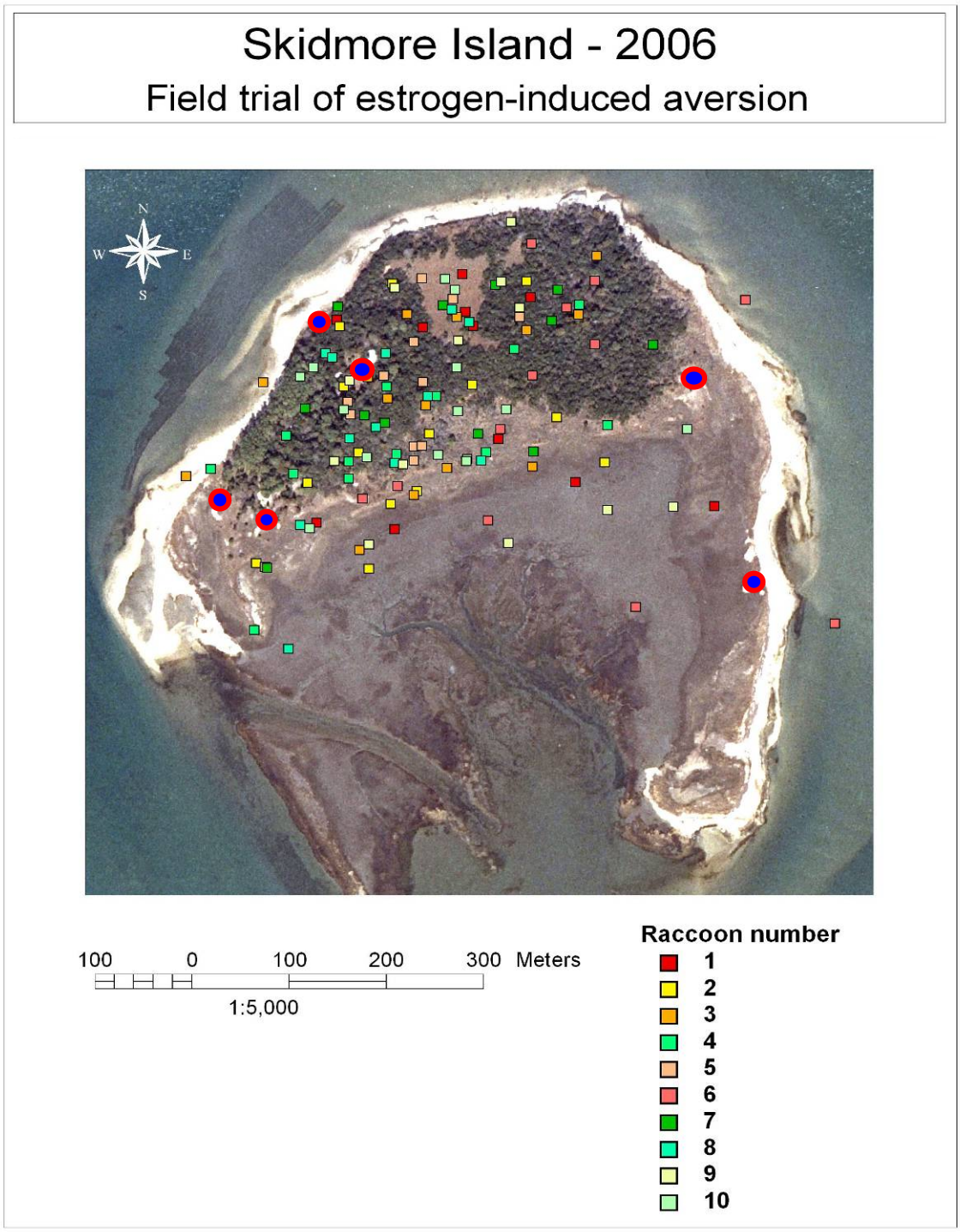

Figure 32. Raccoon telemetry locations in 2006 with colony locations marked in red and blue.

All 10 raccoons were photographed repeatedly; 2,341 photographs contained identifiable raccoons. The average number of recognizable photographs per individual was 209 (range 52-330). Cameras documented a pair of unmarked juveniles 12 times in colonies 5 and 6 between day 12 (3 Jul 2006) and day 42 (2 Aug 2006). Three new unmarked adult raccoons were photographed on Skidmore Island for the first time during the challenge phase, 2 on day 35, and 1 on day 36. These animals likely were new arrivals to the island rather than hold-outs that had avoided trapping. Even the marked raccoon with the lowest visitation was photographed on 27 out of 49 days, with 5 days being the longest absence by any marked individual. One of the unmarked animals was photographed only in colony 3 on days 36-50. On day 35,1 unmarked raccoon was photographed in colonies 1, 3, and 4, and 1 unmarked animal visited colonies 1, 2, 3, and 4. These raccoons could be distinguished from each other by tail markings and pelage color even though they did not have ear tags.

The raccoons reduced their consumption of chicken eggs in response to the treatment (days 17-29; Figure 33). The total number of eggs consumed in all 6 colonies dropped from a high of 123 on the first day of the treatment phase to 56 on the second day, a 54% decline overnight, and 35 on the third. Consumption continued to drop through day 11, and remained low until the 3 new, unmarked adults appeared on day 35 (Figure 33). Consumption spiked in all 6 colonies on day 36 before declining once again on days 37-39. The average number of eggs destroyed per raccoon present on each day

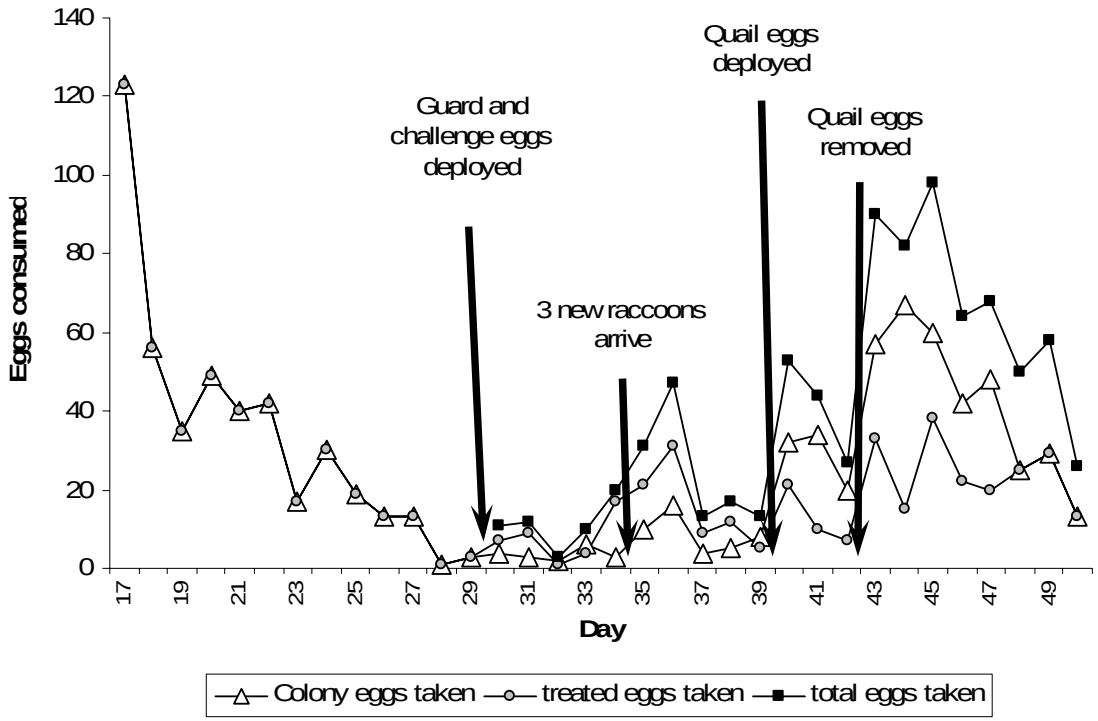

Figure 33. Total number of eggs consumed daily by raccoons in artificial colonies 1-6 (2006).

dropped from a high of 3.97 on the first day (day 17) to 0.3 on the last day of the treatment period (day 28, Figure 34). It then drifted back up to around 1.0 and spiked at 3.9 when the unmarked animals appeared.

The raccoons also changed their foraging behavior in response to treated eggs (Figure 35). Pretreatment visitation averaged 5.05 (SE = 0.432, n = 60) raccoons per colony per day and was greater ($F_{1,9.08} = 15.67$, P = 0.0032) than average post-treatment visitation of 2.22 (SE = 0.352, n = 60) raccoons per colony per day. Post-treatment visitation was lower for every animal, even though the differences were not significant in all cases (Figure 36).

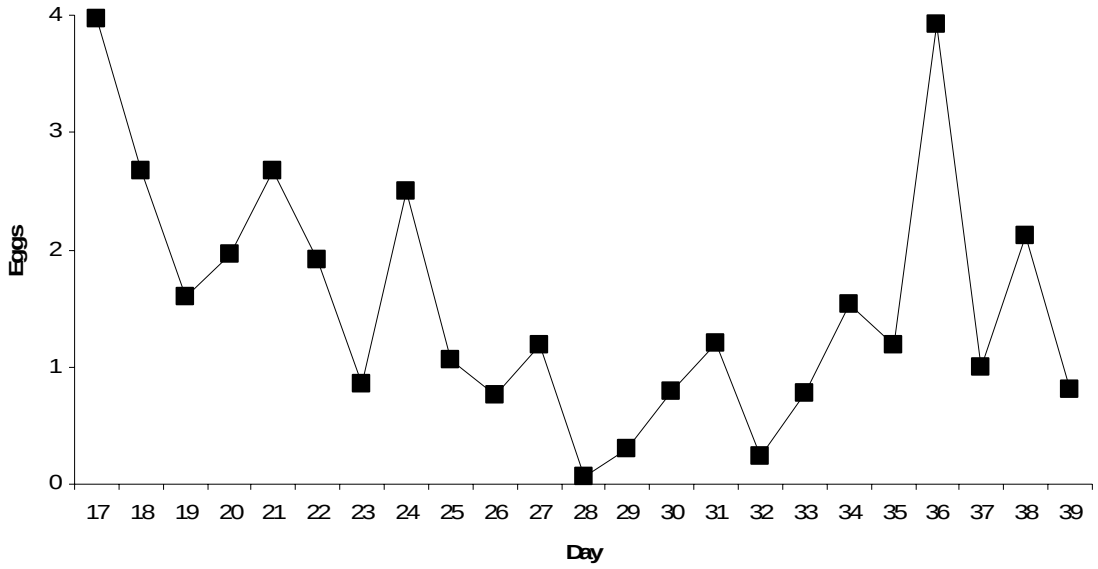

Figure 34. Average number of eggs consumed daily per raccoon in artificial colonies 1-6 (2006).

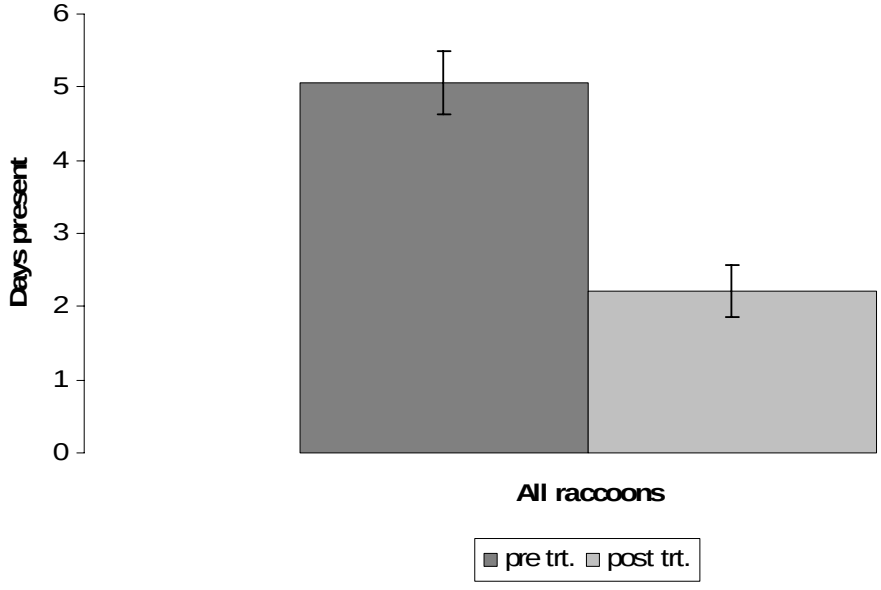

Figure 35. Average (± 1 SE) number of days on which all raccoons were present in artificial colonies 1-6 during the pretreatment and post-treatment periods, based on 2,341 photographs of identifiable raccoons (2006).

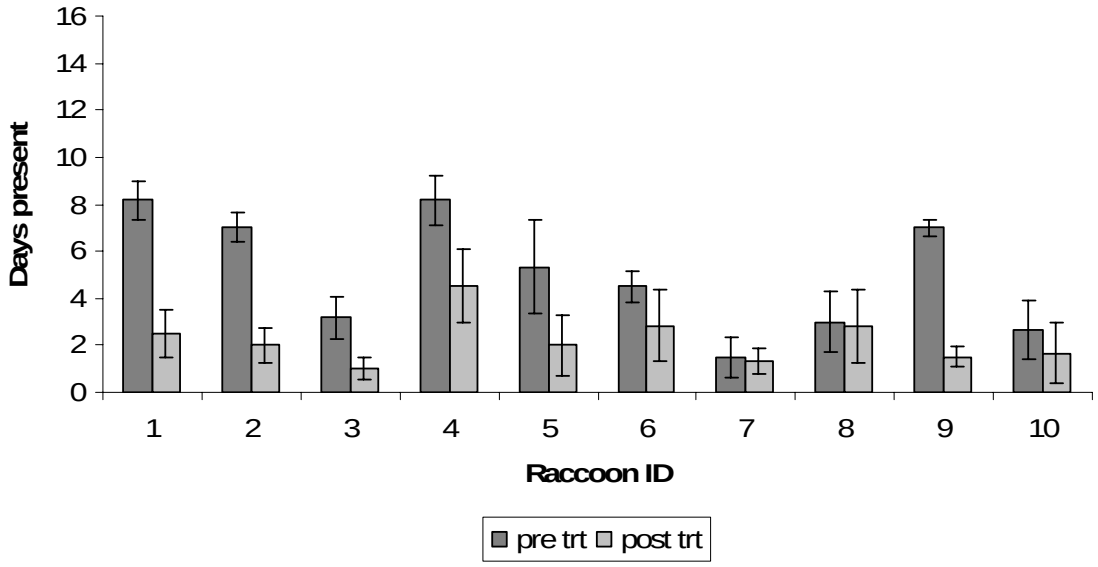

Figure 36. Average (± 1 SE) number of days on which individual raccoons (#'s 1-10) were present in artificial colonies 1-6 during the pretreatment and post-treatment periods, based on 2,341 photographs of identifiable raccoons (2006).

Egg consumption does not simply correlate with the number of raccoons present. On the colony sites (#'s 2, 4, 5, and 6) that most resembled ground-nesting bird habitat (Figure 37), egg consumption declined much more quickly than raccoon visitation (1 day as opposed to 6 days). It also declined on the sites which least resembled ground-nesting habitat, while visitation remained essentially unchanged (colonies 1 and 3, Figure 38). The spikes that occur on days 35 and 36 coincide with the first appearance of 3 unmarked adult raccoons in the photographs.

Average egg consumption was suppressed below the initial high of 4 eggs per raccoon from day 18 to day 35 (Figure 34). Due to the constraints of

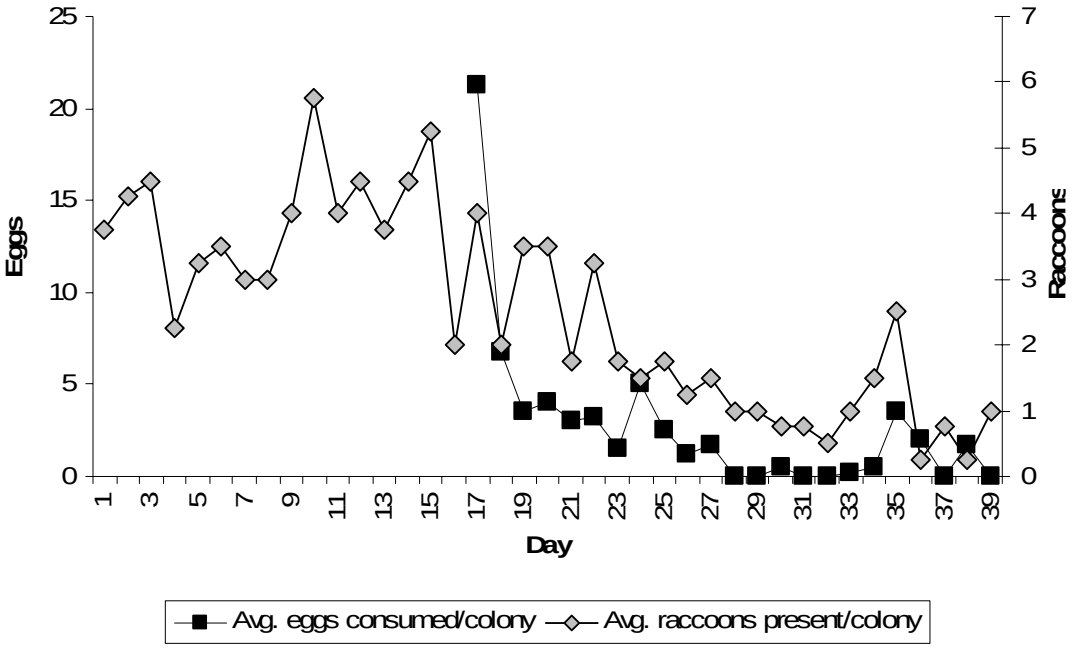

Figure 37. Average number of eggs consumed and average number of raccoons present per day in colonies 2, 4, 5, and 6 (2006).

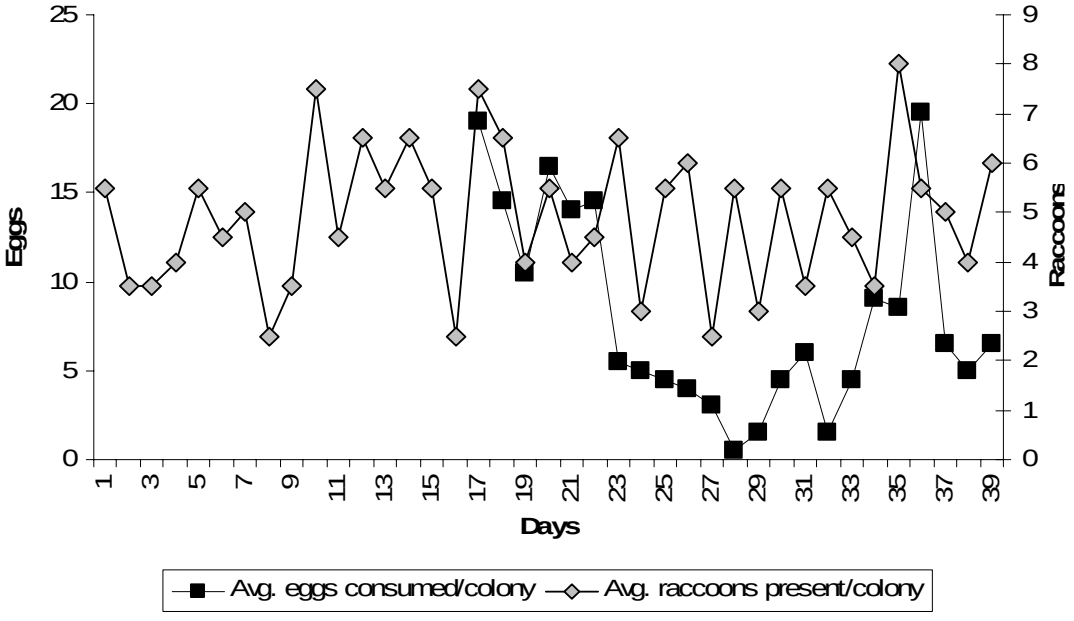

Figure 38. Average number of eggs consumed and average number of raccoons present per day in colonies 1 and 3 (2006).

time and experimental design, I could only demonstrate 21 days of suppressed egg consumption.

There was no indication that raccoons distinguished between estrogen-injected eggs and untreated eggs. The positions of the treated and untreated eggs were switched on day 31, so that untreated eggs were in the “guard” position and treated eggs were in the nests. If the raccoons could detect a difference between treated and untreated eggs, and favored one type over the other, then whichever location experienced higher consumption in the 3 days before the switch should have experienced lower consumption than the other location immediately after the switch. This did not happen (Figure 39).
The raccoons may have been in the process of detecting the difference based on position, but the change in consumption was much too slow to indicate an ability to differentiate between the treated and untreated eggs. The difference in mean consumption of control versus treated eggs in the 3 days before the switch was 3.89, and the difference in mean consumption of control versus treated eggs in the 3 days after the switch was -1.39 (t_{34}= 3.86, $p < 0.0002$). This appears to be evidence that some raccoons may have used a slow process of trial and error to figure out which eggs in which locations caused illness.

Likewise, the raccoons did not learn to avoid the estrogen-carrier plugs, suggesting that they could detect neither the carrier nor the estrogen. The number of plugs consumed varied over time but did not decrease (Figure 40). The raccoons consumed 54% of plugs on average (SE 3.3%) over 34 days.

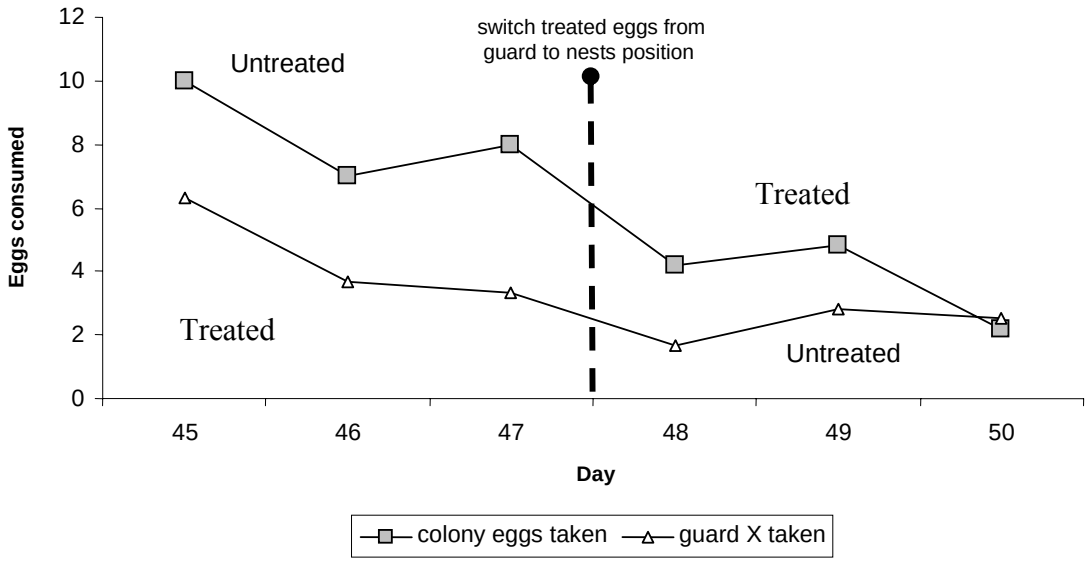

Figure 39. Number of eggs consumed before and after switching egg treatments between colony and guard positions (2006). If raccoons could detect a difference in the eggs based upon smell or taste, upon switching the eggs the consumption of guard eggs should have jumped up immediately, while the consumption of nest eggs should have dropped immediately. Thus the 2 lines on the graph should have crossed between days 47 and 48.

Plug consumption appeared to be a function of how completely eggs were consumed. Eggs that were licked dry almost never had a plug left intact. Eggs that were only partially consumed or spilled seemed to have about an even chance of containing a plug (Figure 40).

The CFA to chicken eggs did not appear to generalize to quail eggs. When I deployed 2 quail eggs in each nest on days 39-41 along with the untreated chicken eggs, raccoons ate 75% of the quail eggs (100% in colonies 1 and 3), and consumption of chicken eggs also increased somewhat. After the quail eggs were removed, the consumption of chicken eggs spiked (Figure 33).

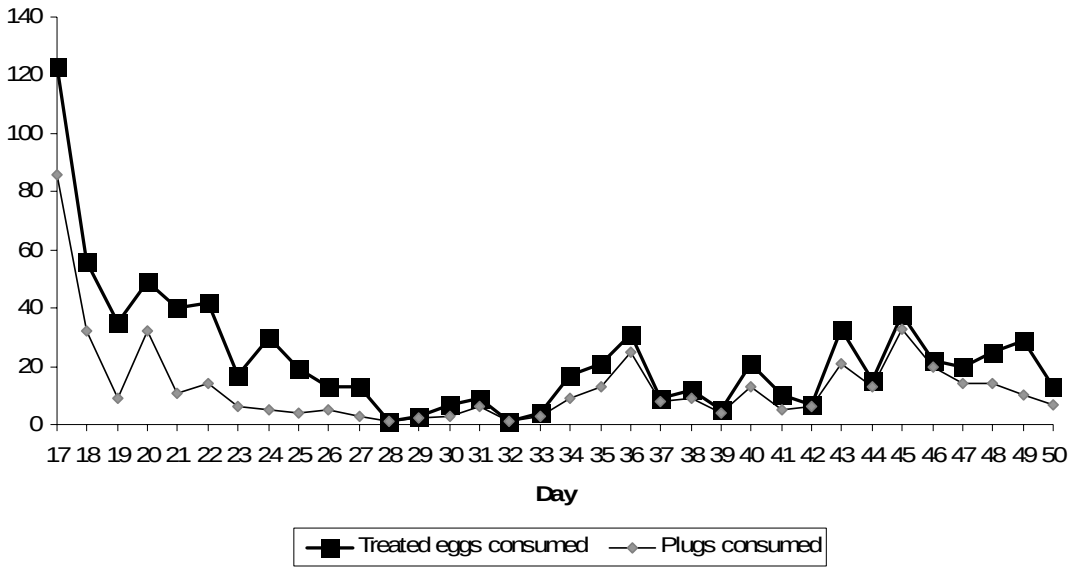

Figure 40. Total number of treated eggs and estrogen plugs consumed daily by raccoons in artificial colonies 1-6 (2006).

Discussion

Alternate foods.—There is evidence that large clusters of eggs, such as those found in breeding bird colonies, prompt predators to sample rather than completely consume each egg (Hartman et al. 1997). This propensity causes colony nesters to suffer much greater damage from even small numbers of raccoons (Hartman et al. 1997) and decreases the likelihood that treated plugs will be consumed (Semel and Nicolaus 1992).

This experiment did not address the effect that the presence of nesting adult birds could have on egg consumption, although previous studies have shown nest site selection to be a larger factor in nest predation rates than adult activity prior to the nestling stage (Martin et al. 2000). It also did not address the importance of alternative food sources (Semel and Nicolaus 1992), nutrient sufficiency of alternate food sources (F. D. Provenza, Utah State University,

personal communication), or sensory satiation (tired of eating the same foods) on the effectiveness of conditioned aversion (C. D. Cheney, Utah State University, personal communication). Lack of an alternate food source is an obvious reason for the failure of an aversion because extreme hunger is a powerful motivator. The raccoons on Skidmore Island had constant access to various species of fiddler crabs (*Uca* spp.), ghost crabs (*Ocypode quadrata*), blue crabs (*Callinectes sapidus*), several families of clams, and other types of fish and marine organisms that frequently wash up on the beach. They also had access to insects and various plant foods throughout the spring and summer.

I stopped prebaiting with dog food the day before the first treated eggs were set out because eggs represented at least as much of an attractant for raccoons as dog food (Semel and Nicolaus 1992), and its continued presence would have kept visitation artificially high in spite of a CFA to eggs. Raccoons maintained a constant high level of interest over the 16 days of baiting as long as there was the possibility of even a small snack. The precipitous decline in visitation during the treatment period indicated either a response to the treatment rather than the lack of dog food, or a complete indifference to eggs. Observations during concurrent pen trials also suggested that eggs represented just as much of a treat for raccoons as the dog food, if not more so. As long as the prospective food item is roughly equivalent in delectability, an animal's behavior toward it should be the same (C. D. Cheney, personal communication). Setting out eggs when the raccoons' interest in the colonies was high insured that all of them would consume treated eggs within a short

period and any changes in behavior due to the treatment would occur at essentially the same time.

CFA evidence.—Egg consumption was not simply correlated with the number of raccoons present. If egg consumption was only a factor of how many raccoons visited a colony, there would be some doubt whether they were actually developing an aversion to eggs. The raccoons probably lost interest in some of the colonies because once they averted to eggs, they stopped finding anything they wanted in them (C. D. Cheney, personal communication). Separating the data for the colonies that most resembled raccoon habitat illustrated this nicely with a high density of data from colonies 1 and 3 that showed no correlation between egg consumption and attendance.

The aversion may last long enough to cause treated raccoons to reject the eggs of the species of concern until the young hatch, usually 21-39 days (Sibley 2001). Egg consumption fell quickly after treatment and remained low unless new animals arrived or something changed in the environment to encourage sampling.

Location effect.—Swapping treated eggs with fresh eggs between nest and “guard” positions allowed me to differentiate between any aversion to a specific colony location and aversion to eggs in general. This provided evidence that raccoons were unable to detect the difference between treated and fresh eggs, and also demonstrated that there was some association of an aversion with location or context. It also indicated that at least some raccoons continued to sample in spite of an aversion to eating eggs. CFA can spread to the place

where the food was eaten under special conditions; olfactory and visual cues associated with the feeding place will elicit signs of disgust in animals; so weak place aversions can accompany strong taste aversions (Garcia et al. 1985). It is possible that certain raccoons used trial and error over a period of days to determine which eggs in which locations were less likely to cause distress. This would make it much more difficult to use conditioned aversion as a management tool because the raccoons would constantly be experimenting to figure out which eggs to avoid, while the manager would constantly have to alter the methodology of the application to keep them guessing.

Raccoons did not associate illness with detectable differences in the eggs themselves. Untreated eggs did not experience higher rates of predation regardless of whether they were in the colony nests or in the “guard” position (Figure 22). Raccoons ate colony nest eggs for several days after the switch with guard eggs, although untreated guard egg consumption slowly began to increase relative to colony nest egg consumption. So, the consumption of control eggs dropped relative to treated eggs depending on their location.

I did not attempt to quantify the ability of a raccoon to discriminate between chicken and quail eggs based upon taste, although I could not detect a difference in the taste of cooked chicken eggs and quail eggs. I used quail eggs as part of the experiment because they are very similar in appearance and size to the eggs of nesting shorebird species (Baicich and Harrison 1997). No doubt a surrogate egg must resemble the egg of a nesting bird in appearance and

flavor to a certain degree in order for a food aversion to generalize, but further testing is required in order to discover what that degree is.

The animals that I marked and released in 2006 were present on the island during the 2005 field season; therefore, it was likely that some or all of these individuals had been exposed to estrogen-treated eggs in 2005. This may have caused a faster response to treated eggs in some animals or even an unwillingness to try eggs at all in animals that retained an aversion to eggs from the previous year (Semel and Nicolaus 1992). Two animals in particular (#'s 7 and 8) appeared to make little or no change in their already low visitation rates as a result of treatment.

Photographs.—The setup in 2005 produced approximately 900 photos with 38 containing identifiable raccoons for a success rate of only 4%. The new setup in 2006 with drift fences and TrailMaster cameras produced 3,618 photos, of which 2,341 contained identifiable raccoons, for a success rate of 65%. This demonstrated the efficacy of drift fences in directing the movements of raccoons and increasing the effectiveness of trail monitors.

When the new unmarked raccoons showed up in the photos on day 35, more treated guard eggs were consumed than untreated nest eggs. This was logical as the guard eggs were the first ones the new animals encountered. The situation reversed (day 40) when, after 2 of the 3 newly-arrived, unmarked raccoons had left the island, the quail eggs were placed in the nests. A possible reason for the reversal besides the exodus of the new animals is that the quail eggs baited all the raccoons to the nests within the colony.

Most or all of the raccoons were still visiting the colonies when the quail eggs were put out even though chicken egg consumption was very low. The instant interest in quail eggs, combined with the results of switching guard and colony nest eggs, suggested that any aversion has taste, appearance, and contextual components. The aversion did not generalize to eggs of markedly different appearance and size. The raccoons were not averted solely to the flavor of egg.

Unfortunately, the 3-day exposure to quail eggs prompted many of the marked raccoons to resume eating chicken eggs as well; suggesting that the CFA was somewhat tenuous when there was a sudden change in food availability. The only significant spike in consumption during the treatment and challenge phases was due to the appearance of unmarked animals, so no raccoon continued eating large numbers of eggs after as little as 2 days of treatment.

The use of guard eggs was an effective strategy because consumption spiked for 2 days then fell back to a low level after 3 new animals arrived. One of the new animals appeared in the photographs on days 35 and 36, the second animal only on day 35, and the third animal on days 36 through 50. Apparently, the consumption of treated eggs prompted 2 of the new arrivals to immediately reject Skidmore Island as suitable habitat. The third appeared to either like Skidmore Island or dislike the swim because it stayed. Nonetheless, it apparently developed a speedy aversion to eggs because egg consumption

dropped immediately on days 37-39 after the other 2 new animals disappeared (Figure 33).

I did not attempt to avoid depositing human scent in or around the colonies. This did not appear to be a problem since raccoons did not avoid areas with human scent before or after encountering treated eggs during the 2005 island trial. They also did not appear to be attracted to specifically human-disturbed areas although they did investigate any significant change in the environment.

Ratnaswamy et al. (1997) detected no response by raccoons to their treated chicken eggs and concluded that conditioned aversion did not work to protect the eggs of sea turtles from raccoons. In response to negative results of this and a very few other studies on conditioned aversion, the wildlife management community lost interest in what was once considered a major breakthrough in wildlife damage management technology. Both the conclusion that CFA was a major breakthrough in wildlife damage management technology and the conclusion that it does not work were perhaps premature. I found that there was a strong response and that my treatment did protect the eggs of chickens from raccoons; however, treated chicken eggs provided no protection for Japanese quail eggs.

Garcia (1989) found that when 2 successive flavors, one familiar and one novel, are paired with a single toxic dose, the novel flavor acquired much greater aversive strength regardless of the temporal order of the flavors. This suggests that inexperienced raccoons are more likely than older, experienced

ones to acquire and retain an aversion. So, if an aversion protocol could be maintained long enough to allow the older individuals to die out, the younger ones could be made to reject eggs.

My results mostly agreed with Nicolaus et al. (1989) in that 1) the raccoons did not discriminate between eggs containing 10 mg estrogen and untreated eggs, 2) at least some of the predators that visited the treatment sites developed an aversion to chicken eggs, 3) there was a reduction in predation on both treated and untreated eggs. Semel and Nicolaus (1992) concluded, after a second year of study, that some of the animals retained some aversion from the previous summer and they quickly reacquired faded aversions from the previous year. This appeared to be the case with free-range raccoons from the island trials as well. Semel and Nicolaus (1992) found that the aversion did not depend on location or surrounding scent cues; whereas, I found that introducing a different type of egg encouraged resampling, and that raccoons slowly reduced their consumption of treated eggs more than untreated eggs depending on location relative to the colony nests.

CONCLUSIONS

In 2005, I trapped, marked, and released 22 raccoons on Skidmore Island; 10 of which I radio-collared. I deployed estrogen-injected eggs in 4 artificial colonies for 11 days, followed by untreated eggs for 10 days. Total consumption of eggs dropped from 75 to 20 eggs in the first 3 days.

In 2006, I trapped and marked 22 raccoons on Skidmore Island, 10 of which I radio-collared and re-released on Skidmore (plus 2 kits released with

their mother) as the study population. I deployed estrogen-injected eggs in 6 artificial colonies for 13 days followed by a mix of treated and untreated eggs for 19 days. The raccoons reduced their egg consumption from 123 eggs to 42 eggs over the first 6 days of treatment, and photos revealed a concurrent reduction in visitation in 4 out of 6 colonies as well. No radio-collared raccoons died during or within 4 months after the trials in either year.

The island trials confirmed the efficacy of estrogen as an aversive agent when deployed in eggs in that it induced quick aversion. The aversion appeared likely to last longer than 21 days under ideal circumstances. The trials demonstrated that the formation and effectiveness of an aversion involves taste, smell, appearance, and context. They also demonstrated that the raccoons did not discriminate among estrogen-injected eggs, carrier-only injected eggs, and fresh eggs of the same type. The aversion did not automatically generalize to eggs that were significantly different in appearance and size from the treated eggs. Nest cages did not restrict raccoons from foraging in the artificial colonies. Although some raccoons visited the colonies more than others, they not only reduced egg consumption, but apparently even altered their foraging pattern by reducing their visitation of areas with treated eggs. Taken together, these findings support the application of estrogen-induced aversive conditioning as a management tool, but also suggest that such field applications may be relatively complex in their design and execution.

I was unable to quantify some issues in these trials such as 1) the nature and persistence of illness caused by estrogen, 2) the health effects of estrogen

on raccoons, and 3) whether a natural propensity to “sample” might cause even the most carefully constructed CFA application to fail if used with real bird colonies. I did not address the following questions: 1) Exactly how similar must a treatment egg be to a target egg for the aversion to generalize? 2) Which, if not all, aspects of context must be similar, and to what degree, to prevent sampling or extinguishing the aversion? The answers to these questions are critical for designing a successful application of conditioned aversion as a management tool and subjects for further research.

Under the right circumstances CFA holds great promise as an effective tool to help limit predation on the eggs of ground-nesting birds and perhaps terrapins and sea turtles in locations with limited predator populations. At the same time, its usefulness is limited and best suited as an ancillary to other management techniques. The practicality of CFA in raccoons is limited by 1) their propensity to sample; 2) the dependence of the aversion on the appearance of the egg and the context in which the egg is found, as well as the taste or smell of the egg; and 3) the fact that it causes only moderate illness (see Chapter 2).

MANAGEMENT IMPLICATIONS

The U.S. National Wildlife Refuge System includes at least 110 islands along the Atlantic, Gulf, and Pacific coasts and in the Great Lakes (http://www.fws.gov/refuges/). Many of these islands have nesting bird populations that are declining due to mammalian predation, and would benefit from effective predation management (Blackburn et al. 2004). Although some of

these areas manage predators through shooting and trapping, the American public is demanding more humane methods than traditional trapping methods for the control of problem wildlife (Messmer et al. 1999). At the same time, the public is more tolerant of predator control for the preservation of endangered species (Messmer et al. 1999).

Given the near-universal need for the control of raccoon predation on the eggs of beach-nesting birds, diamondback terrapins, and sea turtles, the findings of this project may influence the future management of refuges, parks, and conservation areas all along the Atlantic coast, from New England down through Florida and possibly on the Pacific coast as well. The National Park Service, the U.S. Fish and Wildlife Service, The Nature Conservancy, and a host of state agencies already spend heavily on predation management programs. My research tested the use of CFA as a nonlethal management tool for use in island situations. CFA is probably not a “magic bullet” for managing predation, but for critically endangered species it may be worth developing as a tool to be used in concert with lethal control and habitat modification.

LITERATURE CITED

Baicich, P. J., and J. O. Harrison. 1997. A guide to the nests, eggs, and nestlings of North American birds. Academic Press / Natural World, San Diego, California, USA.

Blackburn, T. M., P. Cassey, R. P. Duncan, K. L. Evans, and K. J. Gaston. 2004. Avian extinction and mammalian introductions on oceanic islands. Science 305:1955-1957.

Conover, M. R. 1997. Behavioral principles governing conditioned food aversions based on deception. Pages 29-41 *in* J. R. Mason, editor. Repellents in wildlife management. Colorado State University, Fort Collins, Colorado, USA.

Garcia, J., 1989. Food for Tolman: Cognition and cathexis in concert. Pages 45-85 *in* T. Archer and L. Nilsson, editors. Aversion, avoidance and anxiety. Lawrence Erlbaum Associates, Hillsdale, New Jersey, USA.

Garcia, J., P. A. Lasiter, F. Bermudez-Rattoni, and D. A. Deems. 1985. A general theory of aversion learning. Pages 8-21 *in* N. S. Braveman and P. Bronstein, editors. Experimental assessments and clinical applications of conditioned food aversions. New York Academy of Science, New York, New York, USA.

Hanlon, C. L., D. E. Hayes, A. N. Hamir, D. E. Snyder, S. Jenkins, C. P. Hable, and C. E. Rupprecht. 1989. Proposed field evaluation of a rabies recombinant vaccine for raccoons (*Procyon lotor*): Site selection, target species characteristics, and placebo baiting trials. Journal of Wildlife Diseases 25:555-567.

Hartman, L. H., A. J. Gaston, and D. S. Eastman. 1997. Raccoon predation on Ancient murrelets on East Limestone Island, British Columbia. Journal of Wildlife Management 61:377-388.

Kreeger, T. J., J. M. Arnemo, and J. P. Raath. 2002. Page 241 *in* Handbook of Wildlife Chemical Immobilization, International Edition. Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA.

Martin, T. E., J. Scott, and C. Menge. 2000. Nest predation increases with parental activity: Separating nest site and parental activity effects. Proceedings of the Royal Society of London 267:2287-2293.

Messmer, T. A., M. W. Brunson, D. Reiter, and D. G. Hewitt. 1999. United States public attitudes regarding predators and their management to enhance avian recruitment. Wildlife Society Bulletin 27:75-85.

Nicolaus, L. K., J. Herrera, J. C. Nicolaus, and C. R. Gustavson. 1989. Ethinyl estradiol and generalized aversions to eggs among free-ranging predators. Applied Animal Behaviour Science 24:313-324.

Ratnaswamy, M. J., R. J. Warren, M. T. Kramer, and M. D. Adam. 1997. Comparisons of lethal and nonlethal techniques to reduce raccoon depredation of sea turtle nests. Journal of Wildlife Management 61:368-376.

SAS Institute. 2006. Version 9.1.3 (Service Pack 4). SAS Institute, Cary, North Carolina, USA.

Semel, B., and L. K. Nicolaus. 1992. Estrogen-based aversion to eggs among free-ranging raccoons. Ecological Applications 2: 439-449.

Sibley, D. A. 2001. Plovers and Lapwings, and Oystercatchers. Pages 257-267 *in* Elphick, C., J. B. Dunning, Jr., and D. A. Sibley, editors. The Sibley guide to bird life and behavior. National Audubon Society, Alfred A. Knopf, New York, New York, USA.

CHAPTER 4

CONCLUSIONS

When I began this research, I hoped to discover several things about the potential of using aversive conditioning as a tool for reducing mammalian predation on the eggs of ground-nesting wildlife: 1) Is conditioned food aversion still a promising technology for predation management? 2) Is estrogen still a particularly promising aversive agent? 3) What practical limitations apply to the use of estrogen-induced conditioned food aversion? 4) What conceptual issues might limit the application of estrogen-induced conditioned food aversion? 5) What are the limitations of pen trials in testing for the safety and efficacy of potential aversive agents, and are field trials really necessary?

The answers came as a result of answering yet another suite of questions: 1) Will the average raccoon reduce egg consumption, rather than food consumption in general, after consuming estrogen-treated eggs? 2) How quickly does an aversion form? 3) Does prior exposure to untreated eggs impede the formation of an egg aversion? 4) Will the aversion last long enough to cause treated raccoons to reject the eggs of the species of concern until the young hatch? 5) Can raccoons distinguish between estrogen-injected eggs and similar uninjected eggs? 6) Does an aversion to one type of egg generalize to avoidance of other types of eggs as well? 7) Is estrogen safe and effective for use with raccoons?

I found that most raccoons reduced egg consumption, rather than food consumption, after consuming estrogen-treated eggs. Even though there were

no conspicuous signs of aversion-inducing illness, an aversion formed generally within only a day or two of estrogen exposure. Averse animals not only reduced egg consumption, but apparently even altered their foraging pattern by reducing their visitation of areas with treated eggs. Prior exposure to untreated eggs impeded the formation of an egg aversion. The aversion appeared likely to last longer than 21 days under ideal circumstances. Raccoons did not distinguish between estrogen-injected eggs and similar uninjected eggs. An aversion to one type of egg did not appear to generalize to avoidance of other very different types of eggs as well. Estrogen appeared to be generally safe and effective for use with raccoons, with the possible exception of late-term pregnant females. No free-ranging animals died during the field trials.

Norbury et al. (2005) consider no-choice pen trials to be the standard procedure for food aversion tests and most likely to predict the success of the efficacy of aversion in field trials. However, Gustavson and Gustavson (1985: 355) asserted that "…isolated animal subjects in simple environmental surroundings have been used to formulate broad theories of behavior, including pathology, only to be met with frustration and insurmountable difficulty." I found that pen trials gave different results than field trials in testing aversions.

Several factors may have influenced the results in my pen trials: 1) Confinement may have altered behavior and caused stress, which suppressed immune function (Blecha 2000), which may have in turn facilitated the transmission of parasites and disease; 2) Boredom and lack of choice due to the inability to forage or leave the vicinity of treated eggs may have forced

unusual behaviors, such as eating treated eggs the animals knew would make them sick (Conover 1989); 3) The wide range of personalities and behaviors exhibited by wild-caught animals increased the expected variability and increased the number of replications required for meaningful statistical analysis; and 4) Using wild-caught animals made it impossible to distinguish between normal health problems, cage-stress induced problems, and the potential health consequences of estrogen ingestion. In spite of these limitations, the two pen trials were worthwhile in that they reasserted the efficacy of estrogen as an aversive agent when used in eggs, demonstrated that learned safety could delay or prevent the acquisition of a CFA, and demonstrated the inability of raccoons to discern the difference between treated and untreated eggs.

The island trials confirmed the efficacy of estrogen as an aversive agent when deployed in eggs. They demonstrated that the formation and effectiveness of an aversion involves taste, smell, appearance, and context. They also demonstrated raccoons' inability to discern the difference between fresh and injected eggs of the same type, and showed that an aversion does not automatically generalize to eggs that are significantly different in appearance and size from the treated eggs. Taken together, these findings support the application of estrogen-induced aversive conditioning as a management tool, but also suggest that such field applications may be relatively complex in their design and execution.

My results mostly agreed with Nicolaus et al. (1989) in that 1) the raccoons did not discriminate between eggs containing 10 mg estrogen and

untreated eggs; 2) at least some of the predators that visited the treatment sites developed an aversion to chicken eggs; 3) there was a reduction in predation on both treated and untreated eggs; and 4) a few eggs containing 30 mg of estrogen were less effective in conditioning an egg aversion than many eggs containing 10 mg of estrogen. Semel and Nicolaus (1992) found that aversions persisted in treated raccoons that were present while untreated individuals consumed untreated eggs. My observations of penned animals were inconclusive on this account. Semel and Nicolaus (1992) concluded that estrogen dosages between 22.4 and 32.9 mg/kg per animal caused no obvious detrimental health effects. I found that much higher doses left many animals unaffected, but estrogen may be dangerous for pregnant animals or animals otherwise compromised by disease. Semel and Nicolaus (1992) concluded, after a second year of study, that some of the animals retained some aversion from the previous summer, and they quickly reacquired faded aversions from the previous year. This appeared to be the case with free-range raccoons from the island trials as well.

Most but not all of my results were consistent with the findings of Semel and Nicolaus (1992). They found that even raccoons with prior experience eating untreated eggs developed an aversion to them. In my 2005 pen trials, I found that it depends on the animal. Two of the animals that were pre-exposed to untreated eggs ate every treated egg they got, Semel and Nicolaus (1992) found that the aversion did not depend on location or surrounding scent cues; whereas, I found that introducing a different type of egg encouraged

resampling, and that raccoons slowly reduced their consumption of treated eggs more than untreated eggs in response to a change in location relative to the colony nests.

Ratnaswamy et al. (1997) detected no response by raccoons to their treated chicken eggs and concluded that conditioned aversion did not work to protect the eggs of sea turtles from raccoons. In response to negative results of this and a very few other studies on conditioned aversion, the wildlife management community lost interest in what was once considered a major breakthrough in wildlife damage management technology. Both the conclusion that CFA was a major breakthrough in wildlife damage management technology and the conclusion that it does not work were perhaps premature. I found that there was a strong response, and my treatment did protect the eggs of chickens from raccoons; however, treated chicken eggs provided no protection for Japanese quail eggs.

Garcia (1989) found that when two successive flavors, one familiar and one novel, are paired with a single toxic dose, the novel flavor acquired much greater aversive strength regardless of the temporal order of the flavors. This suggests that inexperienced raccoons are more likely than older experienced ones to acquire and retain an aversion. Therefore, if an aversion protocol could be maintained long enough to allow the older individuals to die out, the younger ones could be made to reject eggs.

I was unable to quantify some issues in these trials such as 1) the nature and persistence of illness caused by estrogen; 2) the health effects of estrogen

on raccoons; and 3) whether a natural propensity to “sample” might cause even the most carefully constructed CFA application to fail if used with real bird colonies. I did not address two questions: 1) Exactly how similar must a treatment egg be to a target egg for the aversion to generalize? 2) Which, if not all, aspects of context must be similar, and to what degree, to prevent sampling or extinguishing the aversion? The answers to these questions are critical for designing a successful application of conditioned aversion as a management tool and subjects for further research.

Under the right circumstances CFA holds great promise as an effective tool to help limit predation on the eggs of ground-nesting birds and perhaps terrapins and sea turtles in locations with limited predator populations. At the same time, its usefulness is limited and best suited as an ancillary to other management techniques. The practicality of CFA in raccoons is limited by 1) their propensity to sample; 2) the dependence of the aversion on the appearance of the egg and the context in which the egg is found, as well as the taste or smell of the egg; and 3) the fact that it causes only moderate illness.

MANAGEMENT IMPLICATIONS

The U.S. National Wildlife Refuge System includes at least 110 islands along the Atlantic, Gulf, and Pacific coasts and in the Great Lakes (http://www.fws.gov/refuges/). Many of these islands have nesting bird populations that are declining due to mammalian predation and would benefit from effective predation management (Blackburn et al. 2004). Although some of these areas manage predators through shooting and trapping, the American

public is demanding more humane methods than traditional trapping methods for the control of problem wildlife (Messmer et al. 1999). At the same time the public is more tolerant of predator control for the preservation of endangered species (Messmer et al. 1999).

Given the near-universal need for the control of raccoon predation on the eggs of beach-nesting birds, diamondback terrapins, and sea turtles, the findings of this project may influence the future management of refuges, parks, and conservation areas all along the Atlantic coast, from New England down through Florida, and possibly on the Pacific coast as well. The National Park Service, the US Fish and Wildlife Service, The Nature Conservancy, and a host of state agencies already spend heavily on predation management programs. My research tested the use of conditioned food aversion as a nonlethal management tool for use in island situations. CFA is probably not a “magic bullet” for managing predation, but for critically endangered species it may be worth developing as a tool to be used in concert with lethal control and habitat modification. My research will hopefully stimulate further research on this and other nonlethal methods.

LITERATURE CITED

Blackburn, T. M., P. Cassey, R. P. Duncan, K. L. Evans, and K. J. Gaston. 2004. Avian extinction and mammalian introductions on oceanic islands. Science 305:1955-1957.

Blecha, F. 2000. Immune system response to stress. Pages 111-121 *in*

G. P. Moberg and J. A. Mench, editors. The biology of animal stress. CAB International, Wallingford, UK.

Conover, M. R. 1989. Potential compounds for establishing conditioned food aversions in raccoons. Wildlife Society Bulletin 17:430-435.

Garcia, J. 1989. Food for Tolman: Cognition and cathexis in concert. Pages 45-85 *in* T. Archer and L. Nilsson, editors. Aversion, avoidance, and anxiety. Lawrence Erlbaum Associates, Hillsdale, New Jersey, USA.

Gustavson, C. R., and J. C. Gustavson. 1985. Predation control using conditioned food aversion methodology: Theory, practice, and implications. Annals of the New York Academy of Sciences 443:348-356.

Messmer, T. A., M. W. Brunson, D. Reiter, and D. G. Hewitt. 1999. United States public attitudes regarding predators and their management to enhance avian recruitment. Wildlife Society Bulletin 27:75-85.

Nicolaus, L. K., J. Herrera, J. C. Nicolaus, and C. R. Gustavson. 1989. Ethinyl estradiol and generalized aversions to eggs among free-ranging predators. Applied Animal Behaviour Science 24:313-324.

Norbury, G., C. O'conner, and A. Byrom. 2005. Conditioned food aversion to eggs in captive-reared ferrets, *Mustela furo*: A test of seven potential compounds. Applied Animal Behaviour Science 93:111-121.

Ratnaswamy, M. J., R. J. Warren, M. T. Kramer, and M. D. Adam. 1997. Comparisons of lethal and nonlethal techniques to reduce raccoon depredation of sea turtle nests. Journal of Wildlife Management 61:368-376.

Semel, B., and L. K. Nicolaus. 1992. Estrogen-based aversion to eggs among free-ranging raccoons. Ecological Applications 2:439-449.

Appendix

Dear Joel,

Thanks for sending your Google Earth screen captures. Both appear to contain our imagery. Therefore, to the extent that it is our TruEarth 15-meter imagery displayed on the Google Earth frame captures, we hereby grant you permission for use of our imagery. Please note that your Google Earth screen captures may also contain imagery and other data from other providers. We cannot and do not extend any further permissions regarding the use of Google Earth's portrayal of our imagery or Google's or other parties' work, in general. We appreciate your display of our standard credit line and link:

Copyright 2007 TerraMetrics, Inc.
http://www.truearth.com

Best wishes on your project.

Best regards,
Julie Baxes
Customer Support
TerraMetrics, Inc.
8420 S. Continental Divide Rd., #110
Littleton, CO 80127
Phone: 1-303-979-5255
Fax: 1-603-696-8184
Visit us at www.truearth.com
----- Original Message -----
- Show quoted text -