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Songbirds, Pesticides, and Golf Courses: Exposure and Effects

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APPROVAL PAGE

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COMPLIANCE PAGE

Research approved by

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ABSTRACT PAGE

The seemingly straightforward question "Are golf courses good places for birds to nest?" remains unanswered, in part due to the unknown impacts of chemical management practices. Although prior research has established that some birds do readily utilize and breed on golf courses, birds have also been known to die from pesticide exposure on golf courses. The single empirical study on avian pesticide exposure on golf courses determined ~20% of the birds sampled had been recently exposed. Our research utilized the eastern bluebird (*Sialia sialis*) as an indicator species to further investigate exposure and effects of golf course pesticide applications on avian development and prey availability. To test the hypotheses that birds on golf courses are exposed to cholinesterase-inhibiting pesticides (the most common class of insecticides used on golf courses), I collected 292 blood and 461 prey samples from nestlings bluebirds reared in nest boxes on 8 golf courses and 9 reference sites (college and hospital campuses, non-commercial pastures, national, state and county parks). All reference sites had some development and regular human disturbance and were free from pesticide use. At the end of the field season, several participating golf courses provided me with detailed spray logs, including chemical names, purpose, dates and location of applications. This allowed me to identify high risk nests and sampling dates. Enzyme assays of day 7 and day 8 golf course nestlings indicated no cholinesterase inhibition compared to reference birds ($p>0.05$). Ligature sampling of prey delivered to nestlings by adult birds indicated that nestling diet consisted primarily of Lepidoptera (35.3%), Orthoptera (18.2%), Coleoptera (17.9%), Araneae (14.2%) and Hymenoptera (6.2%). Diet composition was similar between reference and golf course habitat types. No difference was found in the number of prey items or mean biomass fed to each nestling per hour ($p>0.05$). Although my results do not indicate nestling exposure or prey limitation associated with insecticide use at golf courses, recently fledged bluebirds or other species may be at higher risk of exposure due to the dates of pesticide applications or different foraging strategies.

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BACKGROUND

Golf Courses as Wildlife Habitat

Urban and suburban development has fragmented wildlife habitat throughout the world and replaced native habitats with human-created landscapes. Avian conservationists attempt to combat this habitat loss by establishing networks of parks and refuges to maintain breeding and wintering areas and migratory stopovers. Because of the desperate need for replacement habitat, golf courses have been investigated for use in these conservation efforts. More than 20,000 golf courses cover the American landscape (Worldgolf.com 2008) and although the construction of new courses in the United States has slowed in recent years, the sport continues to expand globally. With an average size of 150 acres, golf courses offer relatively large green spaces amid rapidly growing urban and suburban areas and may play a major role in local conservation efforts.

Golf industry organizations such as The United States Golf Association and Golf Course Superintendents Association of America openly tout golf courses as wildlife habitat, particularly for birds. More than just "greenwashing", these claims are, in fact, based on scientific literature, including evidence of high abundance and species diversity (Tanner and Gange 2004). However, many biologists stop short of openly advocating golf courses, instead emphasizing the dearth of species of conservation concern on golf courses and pointing out that variation in course design and management is a major determinant of wildlife

success (LeClerc and Cristol 2005, Merola-Zwartjes and DeLong 2005, Hodgkison et al. 2007).

It is these management practices that have prevented the uncritical acceptance of golf courses as suitable wildlife habitat. The most oft-cited obstacles to golf courses serving as part of preserve networks include: human disturbance, high rates of fragmentation and depredation, landscaping with non-native vegetation and intensive chemical management practices. It is this final concern that garners the most suspicion from the public. A 2007 survey conducted by *Golf Digest* magazine found that 41% of American adults believe golf courses use too many chemicals and 66% consider golf course pesticides to be a health concern (Golf Digest 2008).

Even those that call for integration of golf courses and bird conservation urge caution on the issue of pesticide effects upon wildlife. The Cornell Birdhouse Network website warns: "A word of caution: Golf courses...are potentially good habitats for (bluebird) nest boxes, but avoid areas where pesticides and herbicides are used" (Birds.cornell.edu 2008) and the North American Bluebird Society's states "...golf courses are good locations for a bluebird trail provided pesticides are not used" (NAbluebirdsociety.org 2008). Because golf courses do use pesticides, these ambiguous recommendations send a mixed message to the public and demonstrate the lack of empirical research on this contentious issue.

The present study was motivated by the concern over golf course pesticides and possible exposure to birds in an attempt to address this oft-mentioned but little-studied aspect of golf course habitats. With the current uncertainties of the impacts of pesticides, golf course managers, conservationists and the general public still cannot truly answer the seemingly straightforward question "Are golf courses good places for birds to nest?" This study was designed to get much closer to an answer, determining for the first time whether the pesticides that are currently used on golf courses are reaching and affecting nestling birds.

Birds on Golf Courses

Golf courses can clearly support certain wildlife species (Terman 1997), notably certain species of birds. Most golf course wildlife research has consisted of surveying avian species diversity and abundance in comparison with reference habitats. One comparison of golf courses to agricultural lands determined that golf courses may enhance local diversity (Tanner and Gange 2004). However, most studies comparing golf courses to surrounding areas have found that golf courses tend to support fewer avian species (Jones et al. 2005, LeClerc and Cristol 2005, Porter et al. 2005, White and Main 2005, Yasuda and Koike 2006). The species that are found on golf courses tend to be more common suburban-adaptable species rather than species of conservation concern (Blair 1996, LeClerc and Cristol 2005, Hodgkison et al. 2007). Researchers have detected the presence of some species of conservation concern on golf courses, fueling

the notion that courses can provide some habitat of conservation value for migrants, wintering and most importantly, breeding birds (LeClerc and Cristol 2005, Merola-Zwartjes and DeLong 2005, Rodewald et al. 2005, Smith et al. 2005, White and Main 2005).

Although surveys of species diversity and abundance are useful, knowing the reproductive fitness of birds that nest on golf courses is more critical in assessing their potential conservation value. It can be argued that human disturbance, fragmentation, non-native vegetation and general turf management practices may negatively affect breeding success of birds on golf courses. If this is, in fact, the case, then surveys of abundance or diversity do little to address questions about quality of habitat. In a prime example, one survey found an impressive 42 species of waterbirds using Florida golf course ponds during the breeding season. However, the researchers also noted a complete lack of nesting behavior by these birds (White and Main 2005). Without this observation, a fortuitous result of studying large birds with large nests, the survey alone may have suggested golf course ponds were providing quality habitat for breeding waterbirds. Instead, this lack of reproductive efforts indicates that golf course ponds can supply only a portion of the annual requirements of wetland birds, and illustrates the need for more thorough investigations.

To date, there have been few published studies of reproductive success of birds on golf courses. To get a better understanding of the success of birds

breeding on golf courses, two recent studies monitored reproductive output of eastern bluebirds (*Sialia sialis*). To do this, LeClerc et al. (2005) compared Williamsburg, Virginia golf course nests to nearby suburban reference sites with no pesticide input, including parks, college and hospital campuses and cemeteries. They found that bluebirds on golf courses actually produced more eggs and fledglings than reference sites and that nestlings on golf courses weighed no less than reference birds. A similar study in North Carolina (Stanback and Seifert 2005) comparing eastern bluebirds on golf courses to those at hayfields, pastures and power line rights-of-way also found comparable reproductive success between golf courses and reference sites. However, that study reported that nestlings were of lower mass on golf courses. Subsequent data collection by College of William and Mary researchers has also detected this difference in nestling body mass (Swaddle and Cristol unpublished data, see Appendix A). These two studies were both hindered by not knowing actual pesticide applications on the golf courses studied and like most before them, the authors were forced to simply assume that golf course birds were likely exposed to pesticides.

The current study addressed this weakness by obtaining data on pesticide use and directly monitoring exposure through sampling nestling birds on golf courses. I hypothesized that nestlings on golf courses are exposed to sub-lethal levels of pesticides, inducing the weight loss observed by both prior studies. Because food gathered near their nests is the only route of exposure to

pesticides for nestling bluebirds, they served as a proxy for other ages of bluebirds and for other species of insectivorous birds nesting in the area. I also tested a second hypothesis for poor condition in golf course nestlings, that they are fed fewer or smaller prey due to the indirect impact of pesticide use on golf courses.

Organophosphate and Carbamate Pesticides

The commonly employed insecticides on United States golf courses are organophosphate (OP) and carbamate (CA) compounds (Rainwater et al. 1995). These chemicals are neurotoxins originally developed in the early 20th century as chemical weapons. Their use as insecticides became commonplace following World War II, and following the phase out of organochlorines they became the most common type of insecticide used in North America. The use of organochlorines was banned in 1972 due to their environmental risks and long persistence in the environment (EPA 1972). OPs and CAs were adopted as alternatives to these. OPs and CAs do have lower residence time in the environment than organochlorines and do not bioaccumulate, but they are, in fact, much more acutely toxic to wildlife and therefore remain an ecological concern.

Both OP and CA insecticides function by inhibiting cholinesterase (ChE) activity following neurotransmission. This enzyme functions to hydrolyze the neurotransmitter acetylcholine and clear the synapse in preparation for the next

signal. Inhibition occurs when the OP/CA phosphorylates the enzyme molecule, blocking the active site and making it more resistant to hydrolysis than the normal acetylated derivative. This inhibition of AChE leads to accumulation of acetylcholine in the synapse, preventing a return to baseline and disrupting subsequent transmission. If hydrolysis is prevented, signal transmission is disturbed, leading to muscle failure and ultimately death (Grue 1997). Once an individual enzyme molecule is bound to an OP, its effect is essentially irreversible, although the pathway can recover from low doses through synthesis of new proteins.

OP/CA toxicity varies between pesticide formulations and is affected by the amount and frequency of exposure and the sensitivity of brain AChE to inhibition (Grue 1997). Mortality following exposure appears to be more frequently related to habitat and foraging strategies and physiological condition than a species' ability to deal with effects of the actual toxic exposure (Grue 1997).

Although this enzyme system is conserved across animal taxa, birds exhibit a reduced ability to metabolize cholinesterase inhibitors and are thus more sensitive to acute exposure to anti-AChE pesticides than mammals (Brealey et al. 1980, Walker 1983). Numerous avian die-offs have been attributed to OP/CA insecticides following improper applications and those in accordance with recommended guidelines (Stone and Gradoni 1985, Mineau and

Whiteside 2006). Between 1980 and 2000, more than 335 separate avian mortality events and 9,000 bird deaths were identified and associated with OP/CA pesticide exposure in the United States (Fleischli et al. 2004). These bird kills have been primarily the result of direct ingestion of granulated compounds and of insects tainted on their outer surface with lethal amounts of insecticide (McEwen et al. 1972, White et al. 1979, DeWeese et al. 1983, Stone and Gradoni 1985). In addition, incidents of exposure are assumed to be much higher given that many incidents likely go unreported, and many more birds likely recover from exposure and do not die (DeWeese et al. 1983, Busby et al. 1987).

Sub-lethal Effects

In addition to outright mortality, there are many documented sub-lethal effects associated with low-level acute and chronic exposure to anti-cholinesterase pesticides. The wide range of effects observed in various taxa, including humans, is attributable to the mode of action on the essential acetylcholine neural pathway. General effects observed in birds include altered vision and memory and a range of physiological and behavioral impairments.

Physiological Effects

Cholinesterase-inhibiting pesticides cause several physiological effects in lab experiments. Hypothermia is commonly observed in birds, rats and humans following acute OP/CA exposure. Symptoms of gastrointestinal stress, including diarrhea, vomiting, convulsions and nausea, have also been observed in birds

and mammals (Grue 1997). Reduction in food consumption is subsequently a common symptom of OP/CA exposure. Common grackles (*Quiscalus quiscula*) given acute OP exposure experienced a 76% reduction in consumption of "clean" food (Grue 1982). Avoidance of contaminated food has also been observed in northern bobwhite (*Colinus virginianus*) (Bennett 1989).

Body Mass and Growth

Several studies have demonstrated that exposure to anti-ChE pesticides affects body condition of adult and nestling birds due to gastrointestinal stress and subsequent behavioral adjustments. House sparrows (*Passer domesticus*) were observed in poorer body condition following exposure to anti-tick pesticides (Martinez-Haro et al. 2007). Pesticide-induced anorexia was observed in adult European starlings causing up to 40% loss in body mass when fed a diet of OP-laden food (Grue 1982). When a single-dose was provided, as opposed to a daily diet, the weight loss was diminished but still apparent, with adult starlings losing an average of 14% body mass 24 hours following dosing (Grue and Shipley 1984). Weight loss in nestlings may be a direct effect of nestling exposure, causing anorexia, diarrhea or reduced foraging/ begging (Grue and Shipley 1984). It could also be due to reduced parental provisioning, either as a result of reduced begging, parental intoxication or a dearth of available arthropods.

The effect on developing chicks tends be more pronounced. The same single-dose treatment elicited weight losses of up to 26% in 15-day chicks and 31% in 5-day chicks, more than two-fold that of adults. Five-day chicks recovered quickly from the weight loss and successfully fledged at comparable weights to control groups (Grue 1997). However, other studies detected reduced fledging weights in dosed birds. Four-day old white-throated sparrows (*Zonotrichia albicollis*) dosed with the OP fenitrothion fledged at lower weights than controls (Busby and Pearce 1980). Carbofuran-dosed mallard (*Anas platyrhynchos*) ducklings gained mass at slower rates than controls but did eventually reach equal mass 6-18 days later (Martin et al. 1991). Differences in post-fledging survival were not detected in starlings dosed with dicrotophos (OP) even though fledging weights in dosed birds were 4% below controls (Stromborg et al. 1988).

Findings in field studies have been variable yet do correspond to the lab dosing studies. A study on lesser kestrels (*Falco naumanni*) in Spain found that fledglings had smaller tarsi and thinner pectoral muscle when the OP malathion had been applied within the colony's foraging range (Ortega et al. 2007). Growth rates were lower in red-winged blackbirds (*Agelaius phoeniceus*) in fields treated with organophosphates (Powell 1984). Nestling great tits (*Parus major*) in hedgerows treated with primicarb (CA) and dimethoate (OP) exhibited ChE inhibition and a tendency for slower weight gain than great tits on control sites

(Cordi et al. 1997). Notably, a highly significant correlation between butyrlcholinesterase activity and nestling mass was also identified.

Few studies have assessed the effects of pesticides on avian prey base and foraging success. No difference was detected on provisioning rate by chestnut-collared longspurs (*Calcarius ornatus*) in agricultural areas sprayed with an OP but adults were observed to travel nearly twice as far to obtain food as conspecifics on control plots (Martin et al. 2000). In addition, a significant reduction in insect larvae was found following a single application of the OP fenthion, suggesting the possible indirect effects of pesticides on the food supply of nestling birds (Powell 1984).

Overall, there is a large body of evidence on the negative effects and observed exposure of birds to pesticides in both lab and field settings. The fact that adult and young birds on golf courses live in an environment in which these chemicals are routinely applied suggests the need for a more thorough investigation of their actual exposure risk.

Avian Exposure Routes

Birds are susceptible to pesticide exposure primarily through dermal, inhalation and oral routes. Dermal exposure may occur through direct contact with spray drift or contact with sprayed surfaces such as vegetation or water runoff and puddles. Oral exposure can occur through direct ingestion,

consumption of food items with pesticide residues, consumption of granulated compounds, drinking of contaminated water or preening of feathers. Waterfowl, blackbirds and thrushes have been noted foraging in agricultural land and turf following pesticide applications (Brewer et al. 1988).

Birds also are known to ingest dead or moribund prey following pesticide applications (Stone and Gradoni1985, Brewer et al. 1993). Beyond these observations, a combined lab and field study showed that blackbirds readily consumed freshly dead crickets and mealworms, although they did have a preference for live prey (Stafford et al. 2003). These birds were also observed ingesting desiccated prey, which, if coated in residues, would have a higher pesticide concentration than the same item when fresh. This experimental evidence of avian consumption of dead and desiccated insect prey strengthens the hypothesis that birds on golf courses may be at risk of exposure through ingestion of contaminated prey. Furthermore, it underscores the risk of young, developing birds being provisioned with contaminated prey in areas of insecticide use.

Pesticides and Birds on Golf Courses

Golf courses are sites of high chemical input in the form of fertilizers, growth regulators, herbicides, fungicides, and insecticides. The commonly applied insecticides on golf courses, organophosphates and carbamates, are known to have lethal and sub-lethal effects on non-target species, but research

on pesticide fate and exposure risk on golf courses has been limited. All pesticides sold in the United States, and thus employed by golf courses, must be deemed safe by the Environmental Protection Agency when applied in the manner described on product labels. However, a series of bird kills from 1975-1990 prompted a reevaluation of the chemicals used on golf courses. The largest such event saw the death of 700 brant (*Branta bernicula*) at a New York golf course (Stone and Gradoni 1985). In addition, 85 American wigeon (*Anas americana*) were killed at a Washington golf course in 1986 (Kendall et al. 1992). Both of these major incidents were attributed to the organophosphate diazinon. This compound was generally applied in a granulated form and was thus easily consumed by granivorous waterfowl foraging on the fairways. The use of diazinon on golf courses and turf farms was subsequently prohibited in 1986 (EPA 1986).

The lone study monitoring avian exposure on golf courses was conducted by Rainwater et al. (1995) at a South Carolina golf course. Researchers captured and sampled red-winged blackbirds, common grackles, and boat-tailed grackles (*Quiscalus mexicanus*) on golf courses and assayed cholinesterase activity following applications of the organophosphate bendiocarb. Further analysis confirmed OP exposure in 22 of 107 birds sampled. Researchers also observed a single laughing gull (*Larus atricilla*) lying on the turf exhibiting signs of intoxication. This bird was not sampled for pesticide exposure, but was seen eventually recovering and returning to its flock. This study also detected

pesticide residues on dead and moribund crickets collected on turf following pesticide application, further suggesting that birds could be exposed through contaminated prey items.

Although few exposures or acute effects were observed, the conclusion of this, the sole study of avian pesticide exposure on golf courses, was that the potential exists for avian exposure. A recommendation for future research was the use of nest box trails in monitoring avian pesticide exposure. Thus, the present study is a long-overdue follow-up of that recommendation.

OBJECTIVES

The study described herein aimed to fill the gap in our understanding of the risk of exposure and effects of golf course pesticides on birds. There are two lines of evidence suggesting that bluebirds hatched on golf courses have lower body mass (Stanback and Seifert 2005, Swaddle and Cristol unpublished data Appendix A). I propose two hypotheses to explain this finding: 1) bluebird nestlings on golf courses are exposed to pesticides, directly resulting in lower body mass, or 2) golf course nestlings are fed less or lower-quality prey by adult birds as a result of insecticide or herbicide effects.

I tested these hypotheses by analyzing blood samples from nestling birds to determine cholinesterase inhibition, a sensitive indicator of exposure to OP pesticides. This process involves the determination of total (TChE), acetyl-

(AChE) and butyryl- (BChE) cholinesterase activities within plasma samples by colormetrically assaying the enzymatic hydrolysis rate. To assess nestling food consumption I utilized esophageal ligatures to compare the food delivery rates and types of prey delivered on and off golf courses. Data were evaluated in light of timing of chemical applications provided by participating golf courses.

Pesticides used by golf course managers have long been known to cause both lethal and sub-lethal effects in birds and this study was needed in order to investigate possible exposure to nestling birds on golf courses. If pesticide exposure is implicated and sub-lethal effects detected, conservationists will then be able to apply this knowledge and work with golf course superintendents to minimize non-target pesticide contamination. Alternatively, if this study finds no evidence of pesticide exposure in nestling bluebirds, and additional studies confirm the result, golf courses could then be considered for integration into regional conservation efforts. In doing so, management for wildlife could be enhanced by inclusion of the more than one million hectares of green space on North American golf courses.

METHODS

Study Area

This study was conducted during the 2007 and 2008 breeding season in James City and York Counties and Williamsburg, Virginia. A system of 549 nest

boxes was erected by the College of William and Mary in 2003 and has since supported breeding populations of eastern bluebirds.

Nest boxes were opportunistically installed in microhabitats favored by bluebirds, consisting primarily of open or lightly wooded terrain along forest edges. Boxes were spaced at minimum 50 meters apart to maximize box occupancy as pairs will exclude one another within this distance (Gowaty and Plissner 1998). Nest boxes measured 16 x 16 x 23.8 cm, with a 3.8 cm diameter entrance and were mounted 1.5 m above ground on a pole equipped with an anti-predator collar. Four research sites already maintained nest box trails and were incorporated into this study. These boxes and associated predator guards were of various designs recommended by the North American Bluebird Society.

Golf Courses

Eight golf courses and 7 reference sites were used in this study. Golf course sites were chosen based on proximity and the willingness of the superintendent to allow installation and continued monitoring of nest boxes. Because some of these courses are integrated within neighborhoods in which homeowners could be applying pesticides, only those boxes >100 meters away from residential properties were included in analyses.

Upon initiation of this research, course superintendents were contacted to request access to chemical application logs. On the condition of anonymity, all

courses agreed to provide chemical application information at the completion of the field season. For the 2008 season, those courses applying organophosphate insecticides at pre-scheduled dates agreed to provide advanced notice of application dates.

Reference Sites

Reference sites were chosen on the basis of structural and functional similarities to golf courses, including human disturbance and fragmentation of habitat. All reference sites were confirmed, by verbal assurance from facilities managers, to be free of cholinesterase-inhibiting pesticide applications. Reference sites consisted of: New Quarter Park, Newport News Park, Eastern State Hospital, York River State Park, The College of William & Mary campus, South Henry Street roadside, open pastures and cemeteries.

Nest Box Monitoring

All nest boxes were cleaned of old nests by 1 March of each study year and were subsequently checked weekly for the presence of nesting material and/or eggs. Data collected included: species, nest stage (partial or complete nest) and number of eggs. Active nests were monitored for clutch initiation date, clutch size, hatch date and brood size. Old nests were removed from nest boxes after chicks fledged to facilitate subsequent monitoring efforts.

Morphological Measurements

Nestlings were aged with 1-day accuracy based on development appearance and estimated hatch date. Hatch day was numbered Day 1. Mass and wing chord were measured at approximately Day 7 and again at Day 12-13. In some cases the nestlings were measured a third time or at different ages, as noted. Mass was measured with a digital scale to 0.1 g. Wing chord, defined as the length from the bent wing to the tip of the first developing primary, was measured with dial calipers to 0.1millimeters.

Banding

Nestlings were tarsus-banded on Day 7 with size 1B United States Geologic Survey metal bands and on Day 12 with a unique combination of 3 plastic color leg-bands (Perler Bead Company, Reading, PA). Colors used were: Black, Blue, Brown, Orange, Pink, Red, Violet, Yellow and White.

Blood Sampling

Because date of pesticide applications was not known until after-the-fact in 2007, I attempted to sample all eligible nests during the 2007 breeding season. An approximately 110 ul blood sample was taken from the brachial vein of two randomly selected Day 7 nestlings in each brood. In broods of 3 or smaller, all chicks were sampled. The underside of the wing was swabbed with alcohol prior to piercing the vein with a sterile 26 ½ gauge PrecisionGlide® needle (Becton Dickinson, Franklin Lakes, NJ). Surface blood was collected using two 70ul

heparinized micro-hematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA). After sampling, cotton and pressure were applied to the puncture site and capillary tubes were sealed, placed in sterile vacutainers (Becton Dickinson) and immediately chilled in ice. All blood samples were collected between 0600 and 1100 hours. Maximum amount of blood taken was estimated as 1.1% of body mass.

Blood samples were transported to the lab within 1.5 hours, on average, for plasma separation and storage. Blood was transferred to 0.6 ml microcentrifuge tubes and spun at 12,000 rpm for 10 minutes to clearly separate the visually distinct plasma and red blood cells. Plasma supernatant was pipetted into a second microcentrifuge tube and stored at -80 degrees Celsius. Mean time between bleeding and freezer was 2 hours 12 minutes.

Ligature Sampling

Prey items brought to the nests and fed to nestlings were collected using the ligature method of esophageal restriction (Johnson et al. 1980). Four inch cable ties (GB Electrical) were constricted around the neck of 8-11 Day nestlings to restrict swallowing of food items, while still allowing the nestlings to breathe normally. If a chick appeared to have difficulty breathing the ligature was removed and the chick allowed to rest. In the event the chick continued to struggle after the second ligature was applied, it was placed back into the nest without a ligature. This was uncommon, occurring at approximately 5% of

sampled nests. Broods were sampled at least 1 day after blood sampling. Ligatures were not applied after Day 11 to ensure I did not induce premature fledging. No nests were sampled more than once.

Ligatures were applied for 1 hour, during which the parents were able to feed undisturbed. At completion of the 1 hour, chicks were removed from the nest and any prey items present were collected with round-tipped tweezers that did not injure the nestlings. Cable ties were then removed with wire-cutters. Collected prey items were placed in individual sterile vials and stored on ice until returning to the lab, where they were weighed, identified to Order (in many cases to Family), and stored at -25 degrees Celsius.

Turf Sampling

During the 2008 season I haphazardly collected arthropods on turf areas at two golf courses following treatments with chlorpyrifos. To do this, I searched putting greens for dead or living potential prey items by walking in concentric circles in from the border of the green. I searched six putting greens approximately 24 hours following treatments. Detected arthropods were collected with forceps and placed in chemically sterile jars and stored on ice until returning to the lab. Items were weighed and identified to Order and stored at -25 degrees Celsius.

Incidental Mortalities

During the 2008 field season a recently fledged bluebird was observed struggling in a drainage ditch on a golf course on 10 August 2008. As soon as the bird died, it was collected and stored at -40° Celsius for toxicology analysis.

Additional Research Efforts

During the 2007 field season other researchers carried out additional studies at some of the same sites. Thirty-seven adult birds were captured at their nest box for measurement of plumage color and presence of plumage microbes. These birds were measured, banded and plucked of two each of chest, rump and tail feathers. All birds were observed to return to regular nesting activities following release and therefore the research should not have affected parental provisioning or reproductive success.

During the 2008 field season, one nestling from each of 72 nests was affixed with 2.6 gram radio-transmitters prior to fledging. To minimize nest disturbance, transmitters were affixed in conjunction with chick banding and measuring and, therefore, should not have affected morphological measurements used in this study.

Lab Analyses

Plasma Cholinesterase

Plasma ChE assays were conducted at Texas Tech University in the method of Ellman et al. (1961) as modified by Hooper et al. (1989). Assay determined TChE, AChE and BChE activities within plasma samples by colormetrically measuring the breakdown rate of the substrate acetylthiocholine. Plasma TChE and AChE levels are highly correlated with brain ChE activity and although little is known of the function of BChE, it has also been shown as a sensitive endpoint when monitoring exposure to anti-cholinesterase pesticides.

Assay Overview

When the sulphur bond of acetylthiocholine (AThCh) is broken by cholinesterase, the thiocholine metabolite formed is a strong nucleophile. This thiocholine is attracted to and splits the sulphur-sulphur bond of the "Ellman reagent", dithionitrobenzene (DTNB). This results in a thiocholine-TNB conjugate as well as a molecule of TNB. The TNB molecule has many resonance forms and therefore absorbs a large amount of light and can be measured with a spectrophotometer. For each AThCh molecule hydrolyzed, one molecule of TNB is formed. Since AThCh is hydrolyzed by ChE at the same rate as AChE, a colorimetric determination of ChE activity is thus achieved through the use of a spectrophotometer to determine absorbance of TNB over time.

Sample Characterization

Prior to conducting cholinesterase assays, the sample enzymes were characterized to optimize the reagent concentrations for Day 7 eastern bluebird plasma. Performing this characterization was essential to account for age and species variation in plasma cholinesterase activity and ensured the activity in the assay was within a measurable and meaningful range. The characterization included three steps: Linearity of the assay with enzyme dilutions, separation of AChE and BChE using Iso-OMPA titration, and substrate affinity determinations for optimal assay and Iso-OMPA confirmation.

Assay Linearity

In order for the assay to accurately report the enzyme activity, the plasma samples required dilution in order to prevent activities too high for the assay to measure. To determine the appropriate dilution, assay activity was measured at plasma dilutions of 10^{-2}, 10^{-4}, 10^{-8}, 10^{-16}, 10^{-32} and 10^{-64}. These activities were plotted against the dilution to identify the linear areas of the curve. The linear range was determined as 10^{-8} to 10^{-32}. A dilution of 10^{-10} was used throughout the characterization and assay.

Iso-OMPA Titration

In order to determine AChE and BChE activity, the reagent iso-OMPA was added to selectively inhibit BChE activity. Iso-OMPA treated wells yielded AChE activity and BChE activity was determined as the difference between the total

and AChE activities. The optimal concentration of iso-OMPA (full inhibition of BChE with not AChE inhibition) was determined by measuring activity of samples incubated in iso-OMPA concentrations of 10^{-10}, 10^{-9}, 10^{-8}, $10^{-7.5}$, $10^{-6.5}$, 10^{-6}, $10^{-5.5}$, 10^{-5}, $10^{-4.5}$, 10^{-4}, $10^{-3.5,}$ 10^{-3}. Percent of total activity was plotted against the dilution to identify the plateau representing full BChE inhibition without AChE inhibition. Optimal concentration was identified as 10^{-3}.

Ellman Assay

Assays were performed in triplicate in 96-well plates, 3 total cholinesterase wells and 3 butyrlcholinesterase (with Iso-OMPA) for each plasma sample. Sample volumes below 30ul were run in duplicate. Each plate included blank control wells and horse serum standards (Sigma Chemical, St. Louis, MO) to assess plate validity.

Absorbance was measured at 410 nm in 10 second intervals using a SPECTROmax spectrophotometer (Molecular Devices Corporation, Palo Alto, CA) with an automated microplate reader and recorded directly into Softmax (Molecular Devices Corporation, Palo Alto, CA) computer program. Output included analysis of variation within replicate wells. If the coefficient of variation between replicates was above 5.0% the sample was run again.

Prey Residues

Collected prey items were sent to Southern Illinois University-Edwardsville for determination of pesticide residues. Prey items were minced and placed into 125 ml Erlenmeyer flasks and mixed with 50 ml acetonitrile on an orbital shaker. The extract was then filtered and dried using a vacuum rotary evaporator. Extracts were quantitatively transferred to 2 ml volumetric flasks, brought to volume with methanol and transferred to amber autosampler vials.

Organophosphate concentrations were determined by an HP 6890GC with a 0.5 ug/ml (1 ug of chemical) detection limit. All samples were analyzed with 4 standard concentrations. This assay screened for 13h OPs: Azinphos Methyl, Chlorpyrifos, Diazinon, Dimethoate, Ethyl Parathion, Malathion, Methamidophos, Naled, Phorate, Phosmet, Profenfos and Terbufos.

Forensic Toxicology

The deceased bluebird collected opportunistically after a scheduled spraying was analyzed in the toxicology laboratory at the Virginia-Maryland College of Veterinary Medicine. The bird was analyzed for total brain cholinesterase activity as well as for the presence of chlorpyrifos metabolites in the liver and kidney.

Statistical Analyses

Pesticide exposure was examined by comparing TChE, AChE and BChE activities from golf courses to reference sites using two-way ANOVA. Analyses were performed for Day 7 and Day 8 mean brood activities as well as for all Day 7 and Day 8 birds analyzed separately. Although siblings should not be considered independent data points, I did this alternative analysis to ensure that lumping broods did not mask any low-activity birds. Effect of site was analyzed using GLM. In addition, a diagnostic threshold of two standard deviations below the reference mean was used as a benchmark for identifying pesticide induced cholinesterase inhibition (Maul and Farris 2004). Linear regressions were performed on enzyme activities in samples collected following applications of OP/CA insecticides.

Two measures of parental feeding rate were calculated: mean number of prey items collected per chick per hour and the mean biomass per chick per hour. Rates were compared using two-way ANOVA.

Nestling body mass was analyzed using a factorial model with habitat type, brood size and hatch date as the main factors. Body mass was corrected for skeletal size by using the OLS residuals of mass regressed on the natural log-transformed wing chord.

RESULTS

Nesting Activity

2007

In 2007 I monitored 176 nests on 8 golf courses and 105 nests on 10 reference sites. Many of these nests were second nests in the same nest box, but I did not keep track of parents at all nests and thus cannot be certain of which were second nesting efforts versus late first nests. The earliest hatch date was 22 April, 2007. The median hatch date for first round nests on golf courses was 14 May 2007, as opposed to 1 July 2007 for second round nests. Median hatch date for first round nests on reference sites was 12 May and 5 July for second round nests.

2008

In 2008 I monitored 79 nests on 7 golf courses and 73 nests on 10 reference sites. The earliest hatch date was 14 April 2008. The median hatch date for first round nests on golf courses was 1 May 2008 and 1 July 2008 for second round nests. Median hatch for first round nests on reference sites was 3 May 2008 and 30 June 2008 for second round nests.

Chemicals Applications

Of the 8 golf courses sampled during 2007, 6 were confirmed to use organophosphate and/or carbamate insecticides as part of their turf management practices. Of the remaining 2 courses, 1 used pyrethroids and 1 used nicotinyl-

based insecticides. Neither of these types of insecticides are cholinesterase inhibitors so any nestling exposure to these chemicals would have gone undetected in the present study.

Insecticides used at the other 6 courses were the organophosphate chlorpyrifos (trade name Dursban Pro) and the carbamate carbaryl (trade name Sevin). Two of these 6 courses regularly applied chlorpyrifos at monthly intervals to course greens to prevent outbreaks of cutworms. The remaining 4 courses treated turf areas in response to pest infestations, generally later in the summer months. As a result of this treatment practice, insecticide applications often occurred on dates when nestlings were older than Day 8. Therefore many blood samples were not collected at highest risk dates.

Blood Collection

During 2007 a total of 293 blood samples were collected: 202 birds in 74 golf course nests and 91 birds in 33 reference nests. Of these, 231 were Day 7 chicks and 34 were Day 8. No overt signs of poisoning were observed in any of the nestlings sampled.

Cholinesterase Activity

Day 7 Birds

There was no significant cholinesterase inhibition in Day 7 broods when comparing golf courses to reference sites (TChE: $t= -1.06$, $p= 0.291$, $df= 105$;

AChE: t= 0.68, p= 0.498, df= 104; BChE: t= -1.29, p= 0.201, df= 104; Figures 1-3). However, I also compared all individual Day 7 birds as independent data points and found a significant difference in TChE (t= -2.07, p= 0.040, df= 223) and BChE (t= -2.49, p= 0.013, df= 222) by habitat type, with golf courses having higher activities than reference sites, in contrast to the hypothesis that pesticide applications would suppress ChE activity (Figures 4-6). There was no effect of site among golf courses for Day 7 brood TChE (F= 1.71, p= 0.121), AChE (F= 0.88, p= 0.528) or BChE (F= 1.59, p= 0.154) activities (Figures 7-9).

A comparison of Day 7 golf course birds to the diagnostic threshold (DT = 2 standard deviations below the reference mean) yielded two golf course chicks below this level for AChE activity (Figure 5). Although these 2 birds' AChE levels were below the DT, they had both been sampled from golf courses that did not employ anti-ChE pesticides, suggesting exposure to golf course pesticides had not caused the lower activity. When examining data from pooled broods, I found one golf course nest for which mean brood BChE activity was below the DT (Figure 3). This nest was at a golf course employing the cholinesterase-inhibiting CA carbaryl, suggesting the possibility of exposure. These blood samples were collected prior to the first reported carbaryl application of the 2007 season.

Day 8 Birds

There were no significant differences in brood TChE (t= 0.18, p= 0.860, df= 14), AChE (t= -0.56, p= 0.587, df= 14), or BChE (t= 0.47, p= 0.648, df= 14)

activities for Day 8 birds (Figures 10-12). I also found no difference in individual TChE (t= 0.00, p= 1.00, df= 32), AChE (t= -0.68, p= 0.501, df= 32) or BChE (t= 0.27, p= 0.785, df= 32) activities (Figures 13-15). There were no Day 8 birds or broods below the diagnostic threshold.

ChE Activity Following OP Applications

There were slight increases in activities over time after spraying, as would be predicted with degradation and removal of the chemicals from the environment. Post-hoc analysis of nestling ChE showed weak positive correlations of higher TChE and AChE levels the later the sample was collected following a known pesticide application (TChE: r^2= 0.08, p= 0.114; AChE: r^2= 0.19, p= 0.029; Figures 16-18). However, all values were above the diagnostic threshold and were comparable to reference enzyme activities, suggesting that these trends were not related to pesticides.

Incidental Mortality

Toxicology on the bluebird found dead under suspicious circumstances determined total brain cholinesterase to be 14 umol per min per gram, slightly below the adult range of 20–40 umol per min per gram. No metabolites of chlorpyrifos were detected in liver and kidney samples.

Prey Sampling

2007 Ligature Sampling

Ligature sampling efforts occurred throughout the breeding season, beginning 1 May and continuing through 18 July 2007. A total of 490 prey samples were collected 97 bluebird nests. Of these 490 prey items, 295 items were from 66 golf course nests and 165 items were from 32 reference nests.

2008 Turf Sampling

Twenty arthropod samples were collected on golf course greens within 48 hours of OP pesticide applications during the 2008 breeding season. Samples were comprised of similar prey types to those collected from 2007 ligature sampling and did not contain any prey Families not present in ligature collections.

Prey Residues

Thirty arthropods from the 295 prey items collected at golf course nests in 2007 were analyzed for organophosphate pesticide residues, including those known to be used by participating golf courses. These 30 samples consisted of prey items collected within 7 days after known OP pesticide applications and were chosen as a preliminary sub-set to assess the likelihood of contamination. All 30 samples were below detection limit (0.5 ug chemical/mL) for all 12 OPs screened (Appendix B).

The 20 arthropod samples collected from golf course turfs were also analyzed for the same 12 OPs, including chlorpyrifos which had been applied within 24 hours of arthropod collection. All 20 samples were below detection limit (0.5 ug chemical/mL).

Nestling Diet

Nestling diet was composed primarily of 4 arthropod Orders: 35.32% Lepidoptera, 18.16% Orthoptera, 17.91% Coleoptera and 14.18% Araneae. Nearly 100% of the Lepidoptera prey were larvae and the Coleoptera were mainly adult scarab beetles. Hymenoptera (ants) comprised 6.22%. No other prey group comprised more than 2.0 percent (Figure 19). Diet was similar across habitat type, with slightly more Araneae (spiders) being consumed in reference areas and more ants (Hymenoptera) on golf courses (See Appendix C for complete prey list). There was no difference in either number of items fed per nestling per hour ($t = 0.69$, $p = 0.495$, Figure 20) or biomass per nestling per hour ($t = 0.20$, $p = 0.841$, Figure 21).

Nestling Body Mass

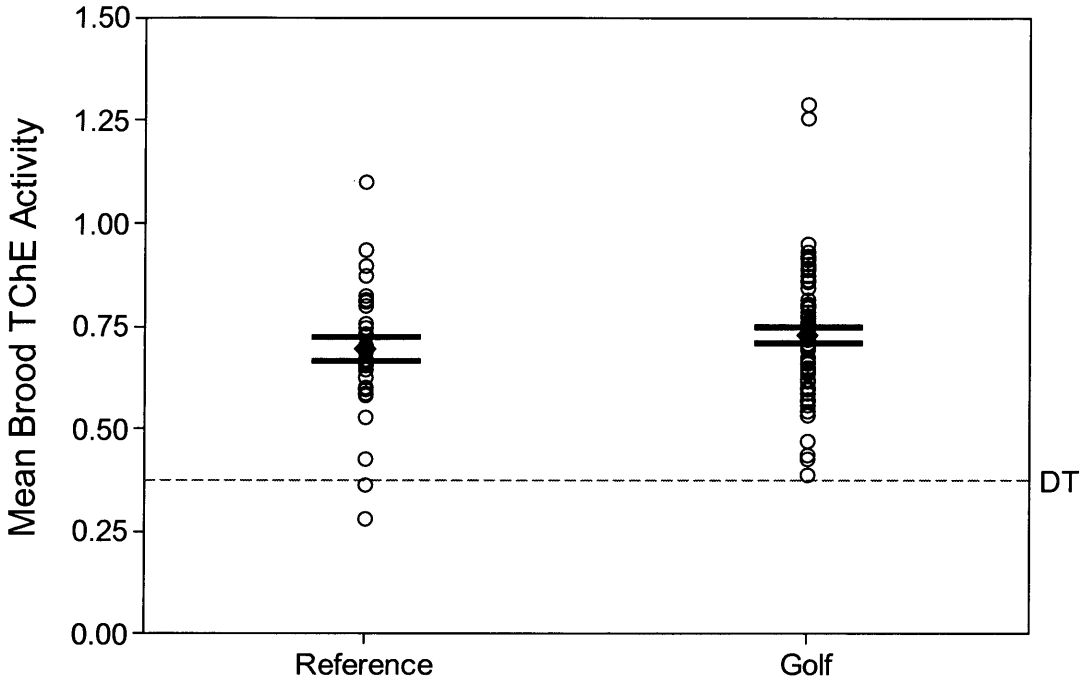
A total of 2,124 morphological measurements were taken from 1,121 chicks in 2007. Body mass and wing chord were measured from 698 nestlings at 8 golf courses and 423 nestlings at 9 reference sites. Relative body mass was calculated for all measurements of birds 5-14 days old by regressing mass (g) on the natural log of wing chord (mm) and storing the residuals (Figure 22).

Residual values were averaged for broods to obtain an independent relative body mass score for statistical analyses.

There was a significant effect of habitat type on relative brood body mass ($F= 4.257$, $p= 0.043$, Figure 23) with golf course broods having a lower size-corrected mass score than reference nests. There was also a significant difference for hatch date with later hatching nests having smaller mass broods ($F=1.588$, $p=0.026$) and a near significant difference with the interaction of brood size and hatch date ($F=1.488$, $p=0.058$).

FIGURE 1

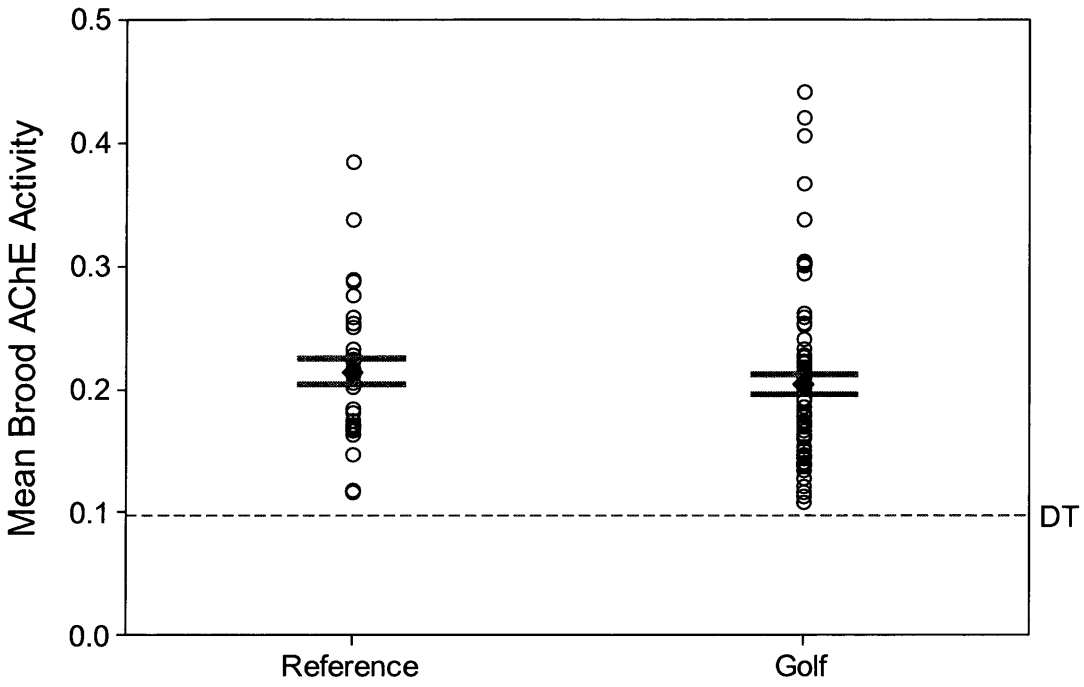
DAY 7 BROOD TChE BY HABITAT TYPE



Day 7 total cholinesterase activities shown by habitat type as brood averages (black diamonds) and broods (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 2

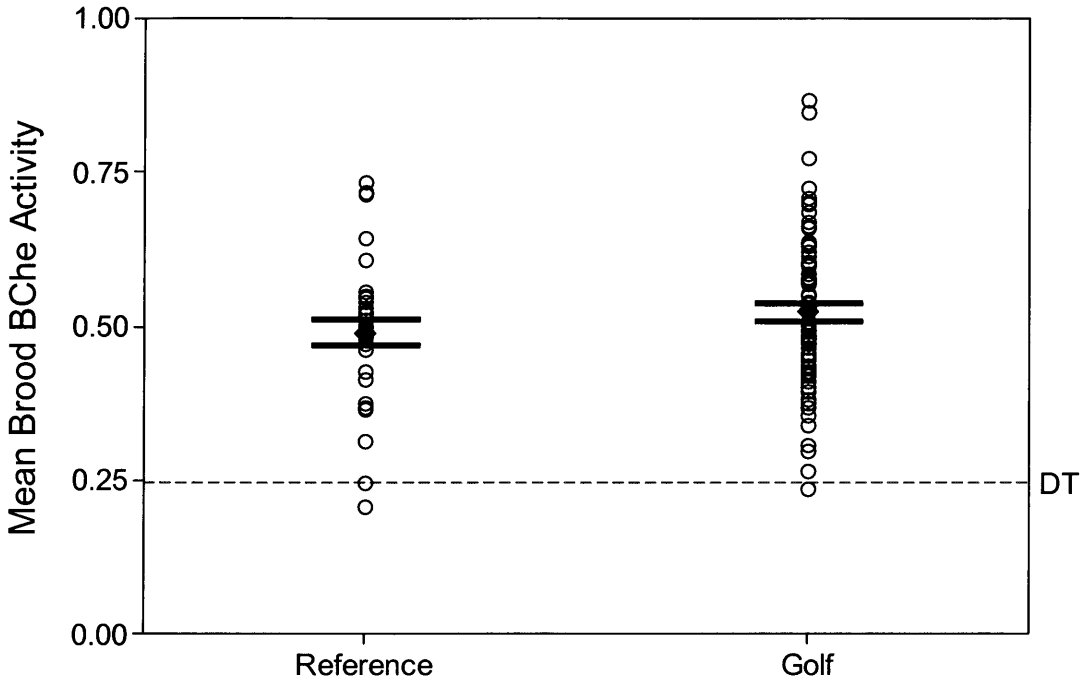
DAY 7 BROOD AChE BY HABITAT TYPE



Day 7 brood acetylcholinesterase activities shown by habitat type as brood averages (black diamonds) and broods (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 3

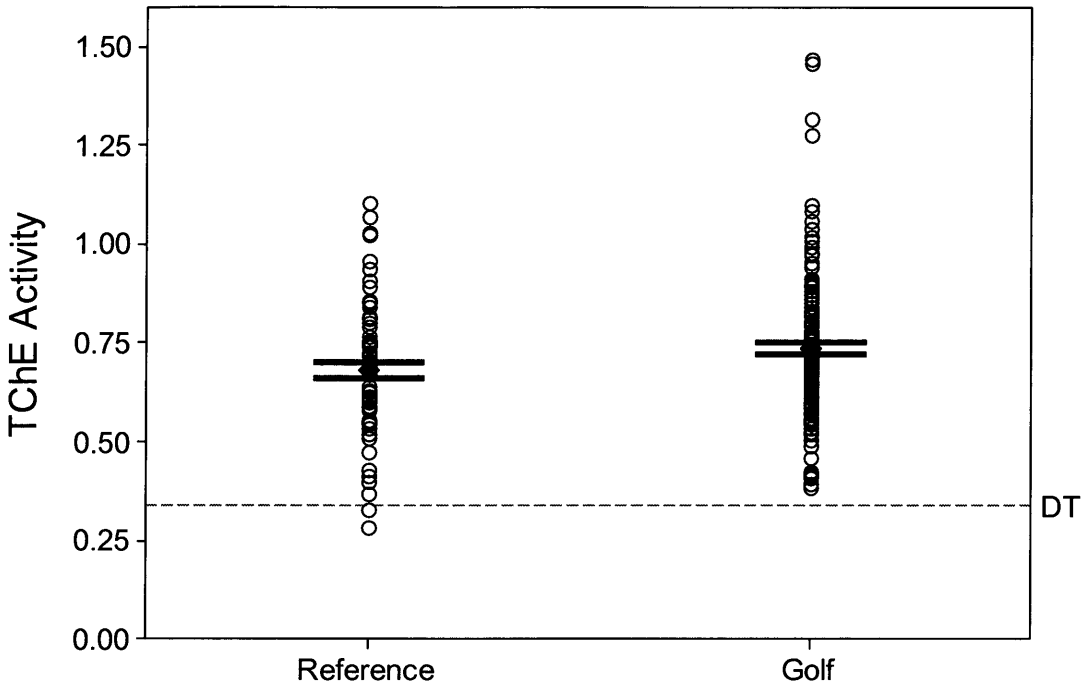
DAY 7 BROOD BChE BY HABITAT TYPE



Day 7 brood butyrylcholinesterase activities shown by habitat type as brood averages (black diamonds) and broods (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 4

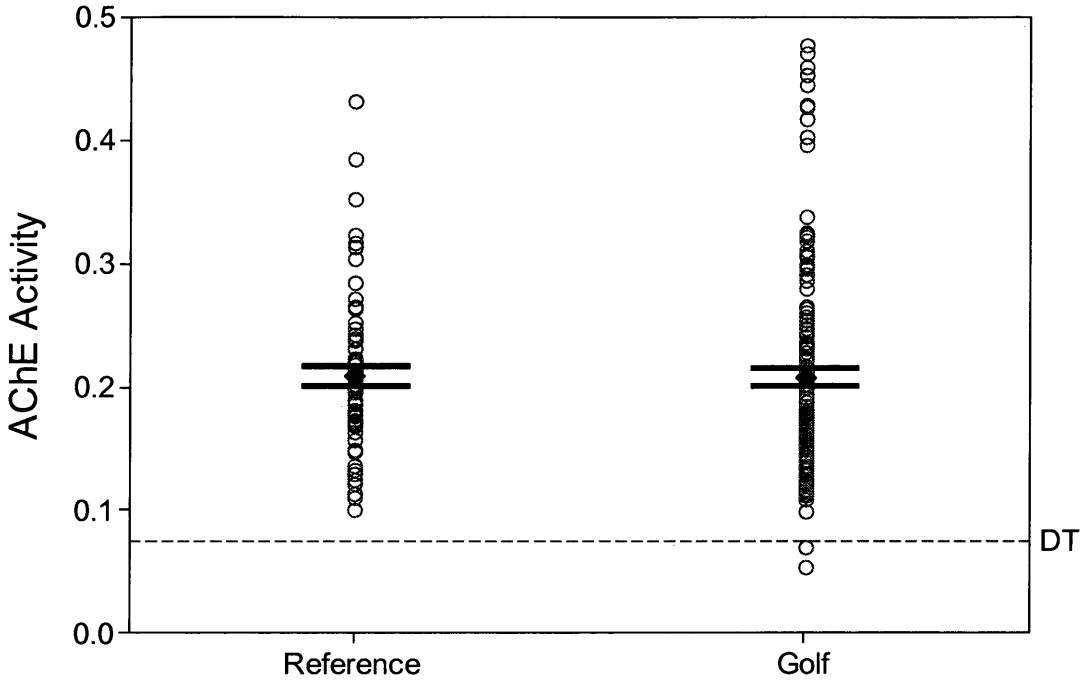
DAY 7 TChE BY HABITAT TYPE



Day 7 brood total cholinesterase activities shown by habitat type as brood averages (black diamonds) and individual birds (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 5

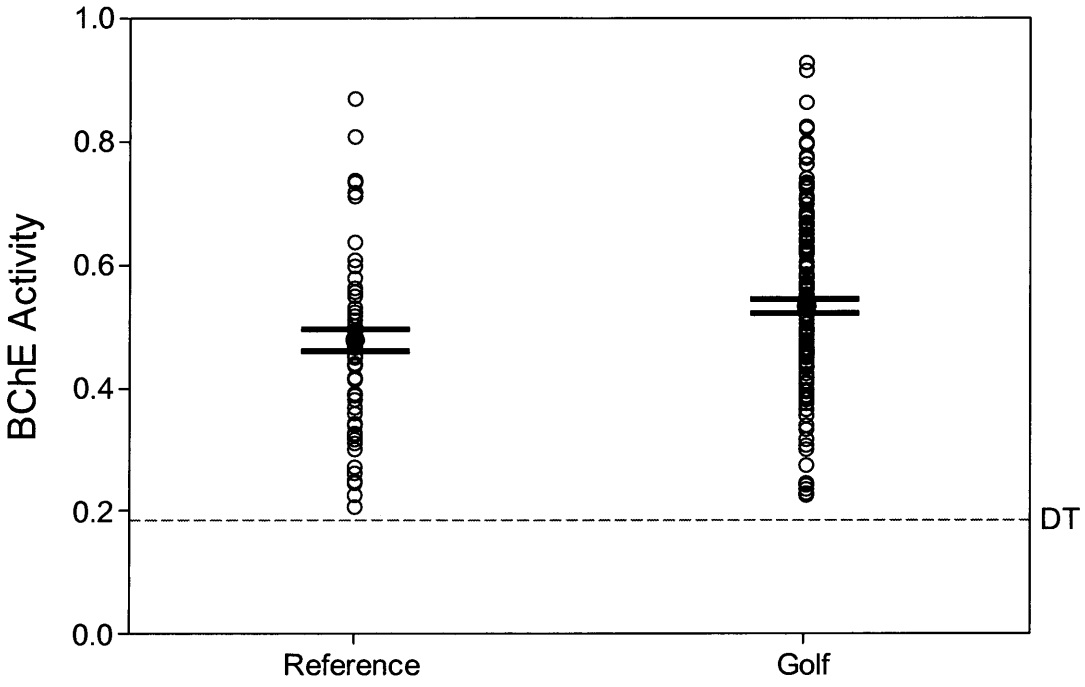
DAY 7 AChE BY HABITAT TYPE



Day 7 brood acetylcholinesterase activities shown by habitat type as brood averages (black diamonds) and individual birds (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 6

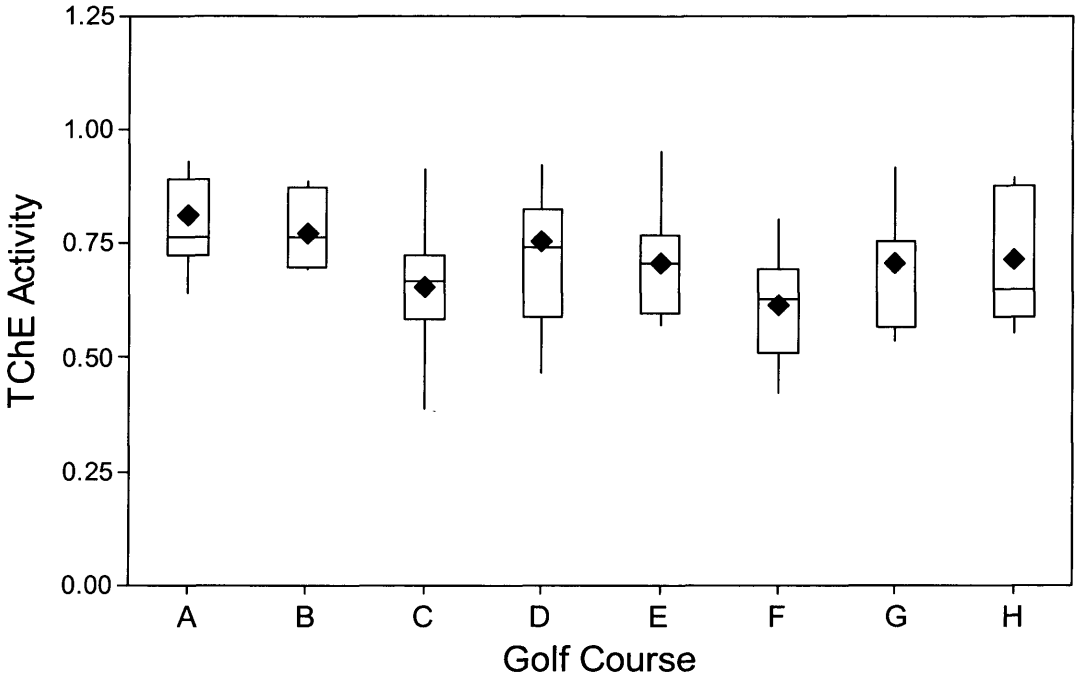
DAY 7 BChE BY HABITAT TYPE



Day 7 brood butyrylcholinesterase activities shown by habitat type as brood averages (black diamonds) and individual birds (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 7

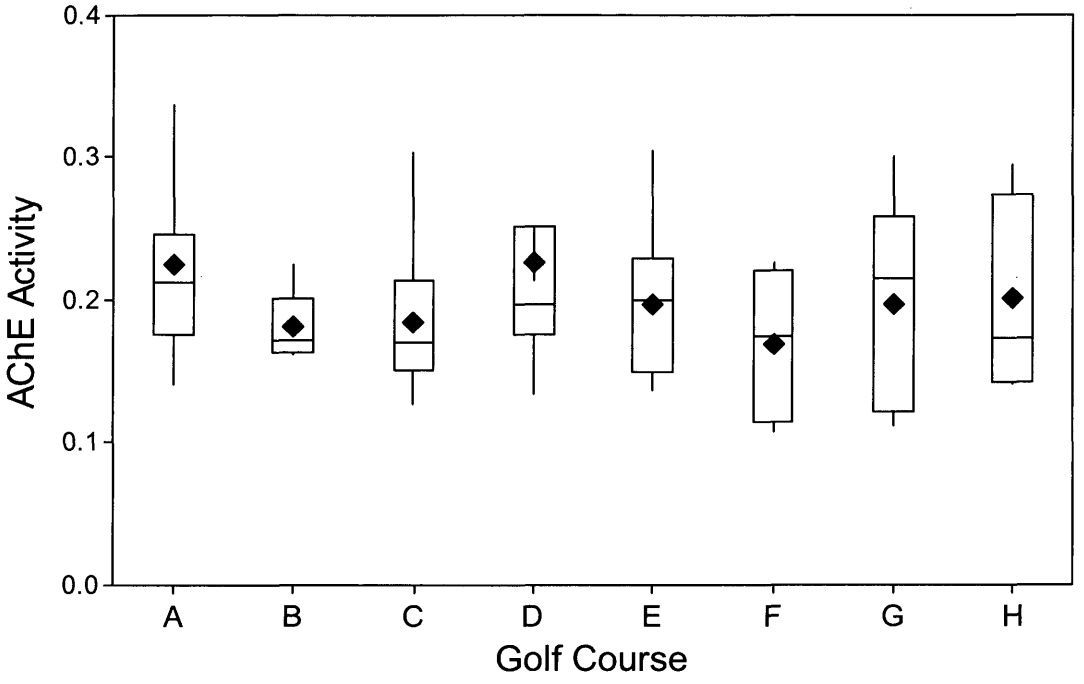
TChE BY GOLF COURSE



Box plots of TChE activities at the 8 golf course study sites. Means are shown as black diamonds.

FIGURE 8

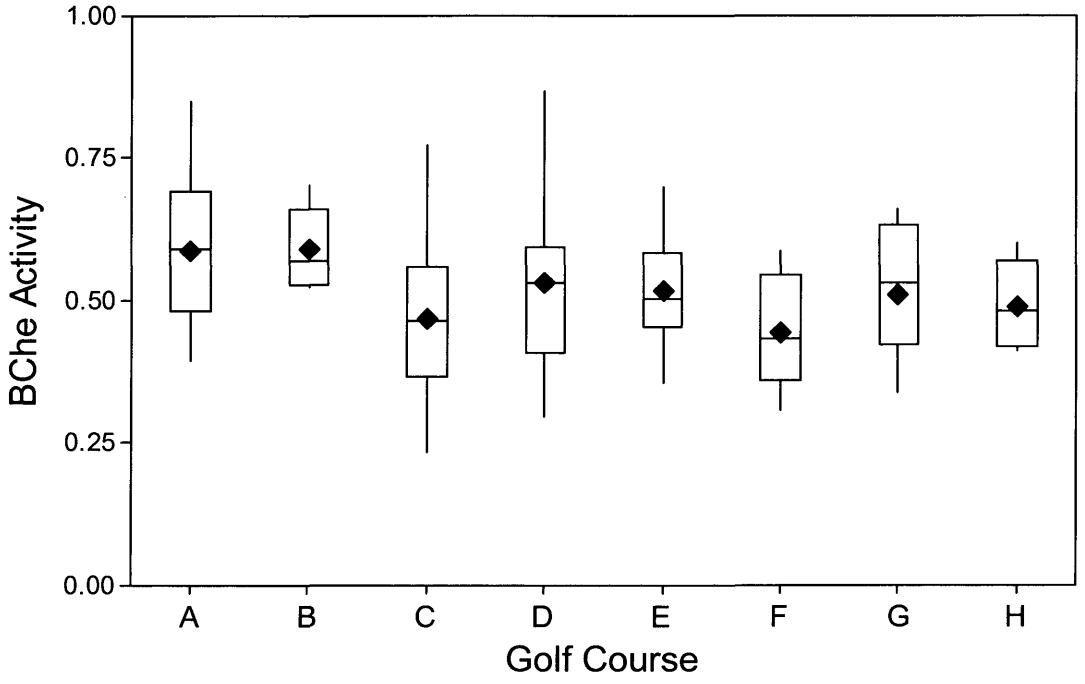
AChE BY GOLF COURSE



Box plots of AChE activities at the 8 golf course study sites. Means are shown as black diamonds.

FIGURE 9

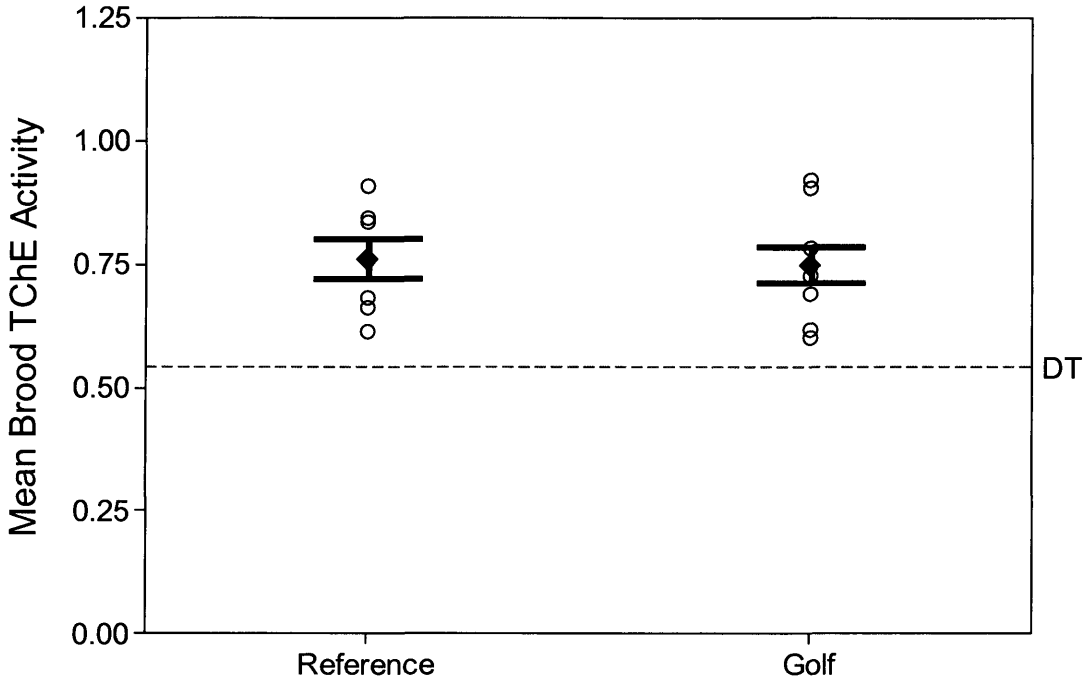
BChE BY GOLF COURSE



BChE activities at the 8 golf course study sites. Means are shown as black diamonds within box plots.

FIGURE 10

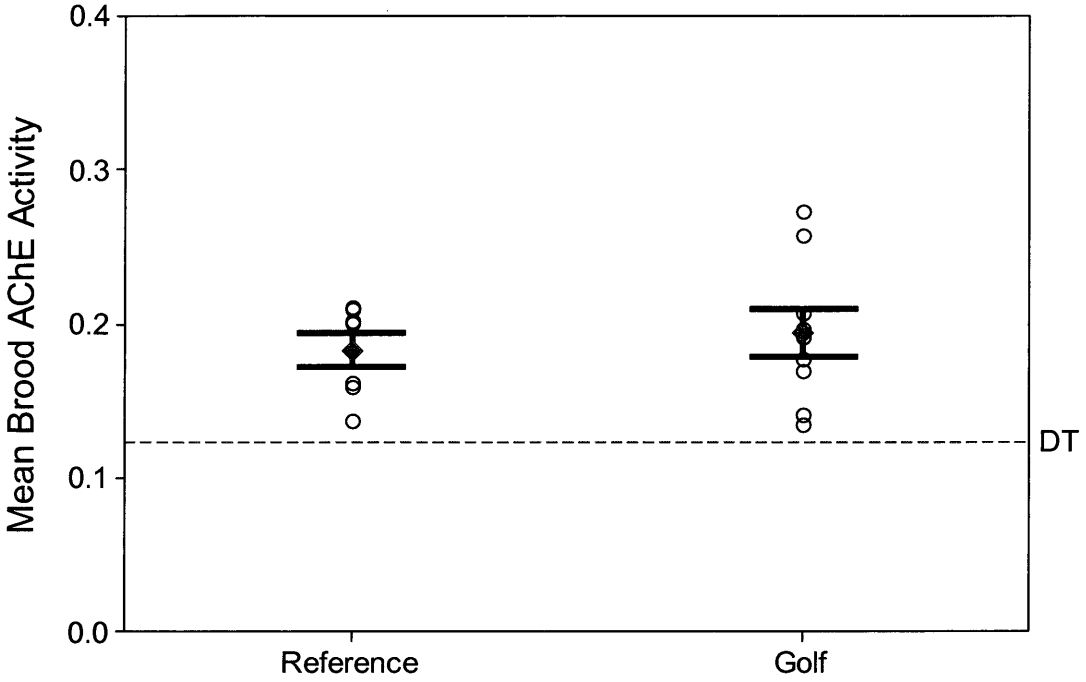
DAY 8 BROOD TChE BY HABITAT TYPE



Day 8 total cholinesterase activities shown by habitat type as brood averages (black diamonds) and broods (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 11

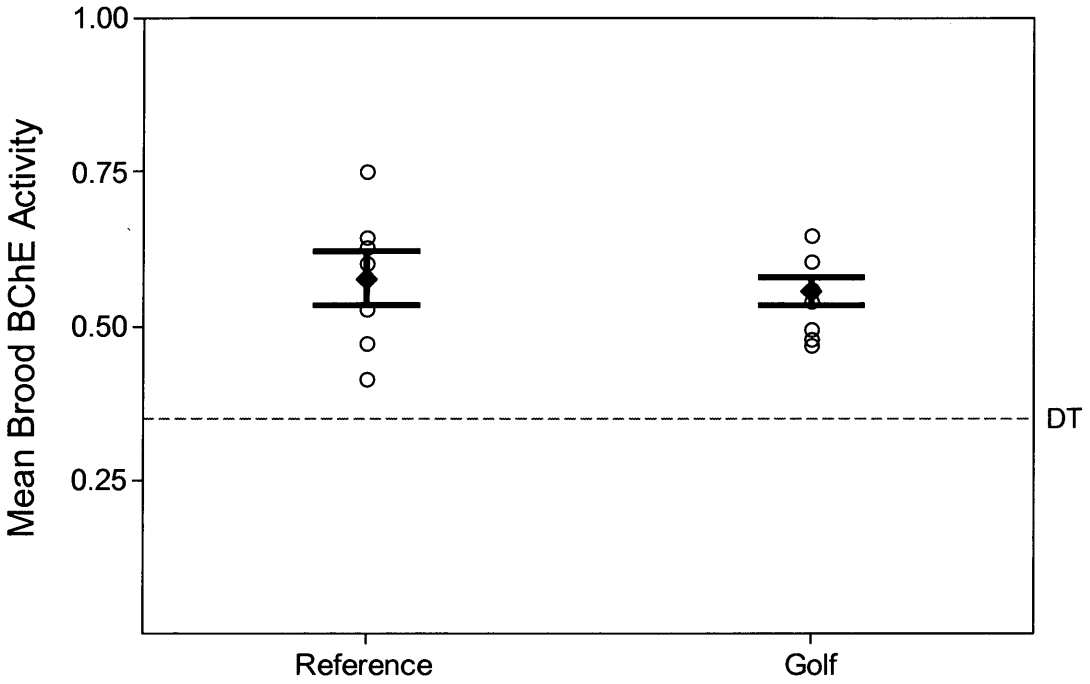
DAY 8 BROOD AChE BY HABITAT TYPE



Day 8 acetylcholinesterase activities shown by habitat type as brood averages (black diamonds) and broods (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 12

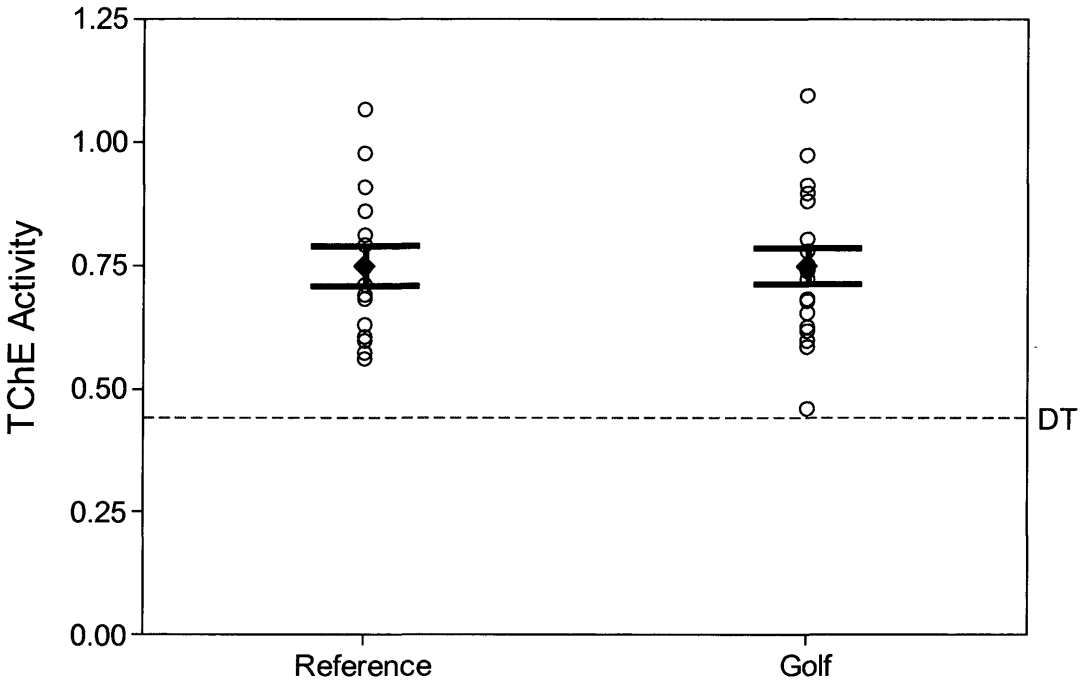
DAY 8 BROOD BChE BY HABITAT TYPE



Day 8 butyrylcholinesterase activities shown by habitat type as brood averages (black diamonds) and individual birds (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 13

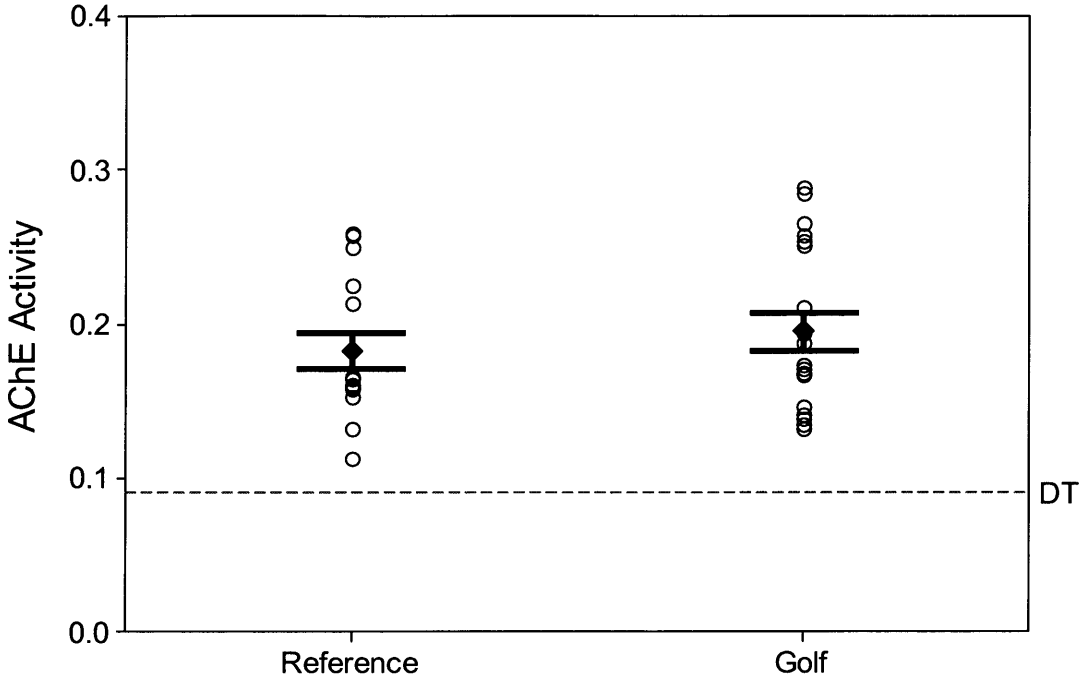
DAY 8 TChE BY HABITAT TYPE



Day 8 total cholinesterase activities shown by habitat type as brood averages (black diamonds) and individual birds (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 14

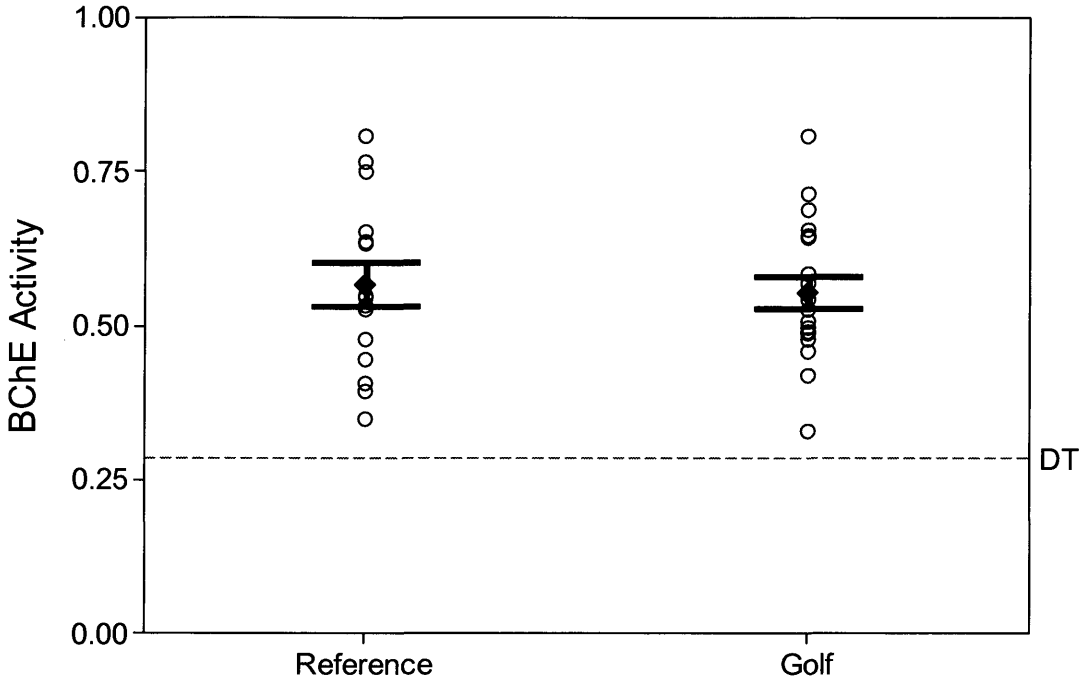
DAY 8 AChE BY HABITAT TYPE



Day 8 acetylcholinesterase activities shown by habitat type as brood averages (black diamonds) and individual birds (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 15

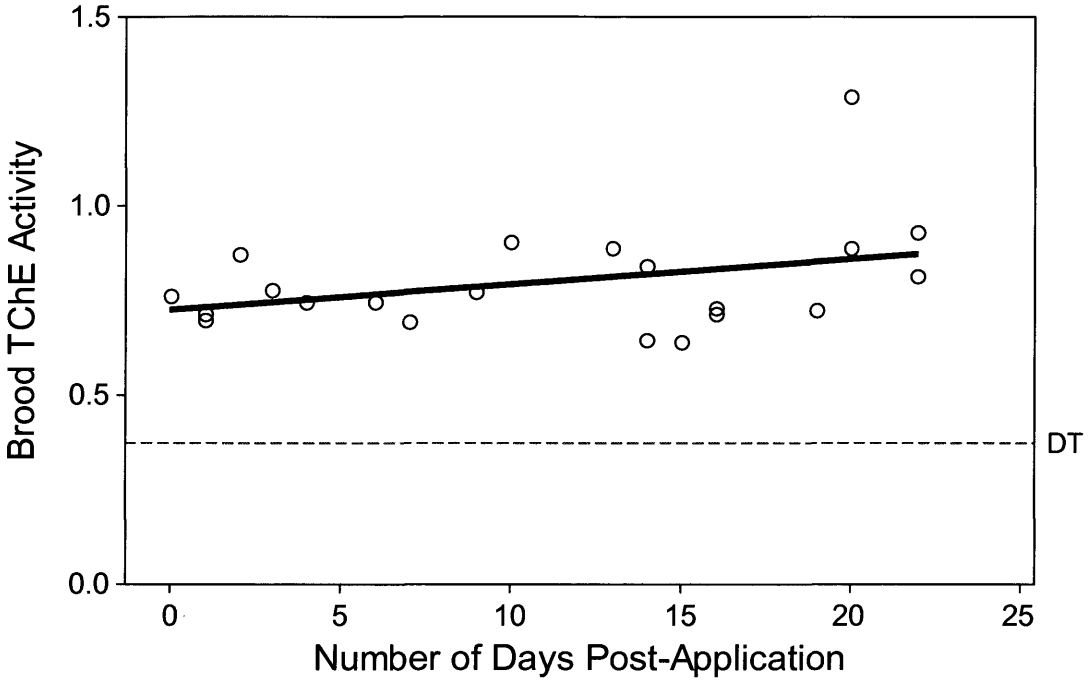
DAY 8 BChE BY HABITAT TYPE



Day 8 brood butyrylcholinesterase activities shown by habitat type as brood averages (black diamonds) and individual birds (open circles). Error bars represent the Standard Error. The diagnostic threshold (2 standard deviations below the reference mean) is shown as the dashed line.

FIGURE 16

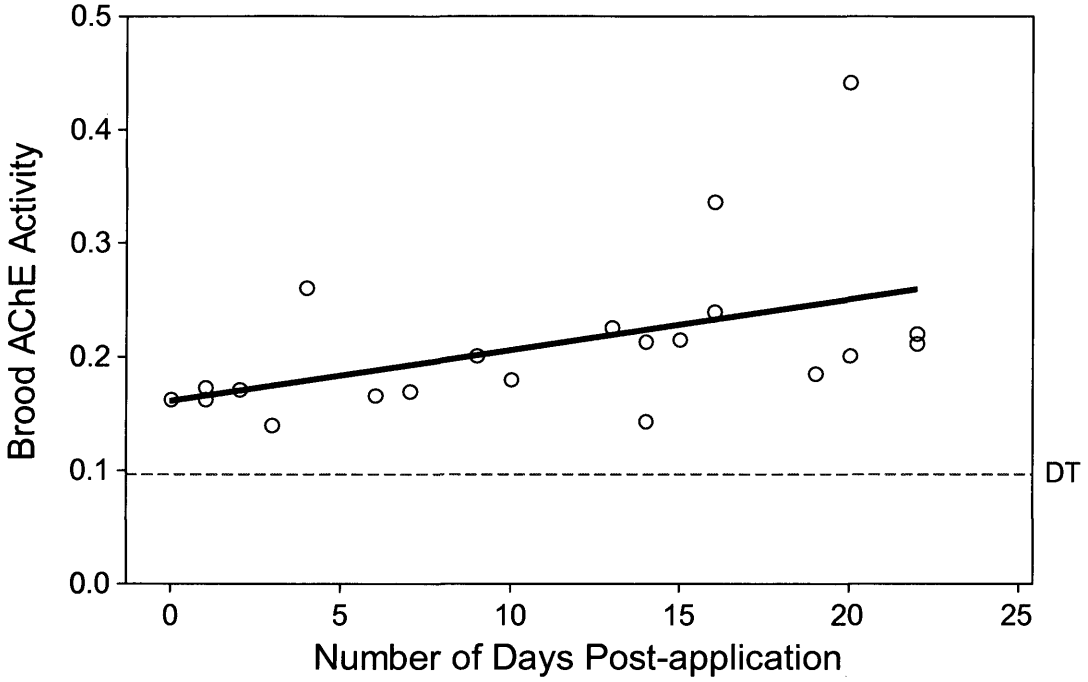
DAY 7 BROOD TChE VS. DAYS SINCE LAST APPLICATION



Day 7 brood TChE plotted versus the number of days since the last reported application of an anti-ChE pesticide.

FIGURE 17

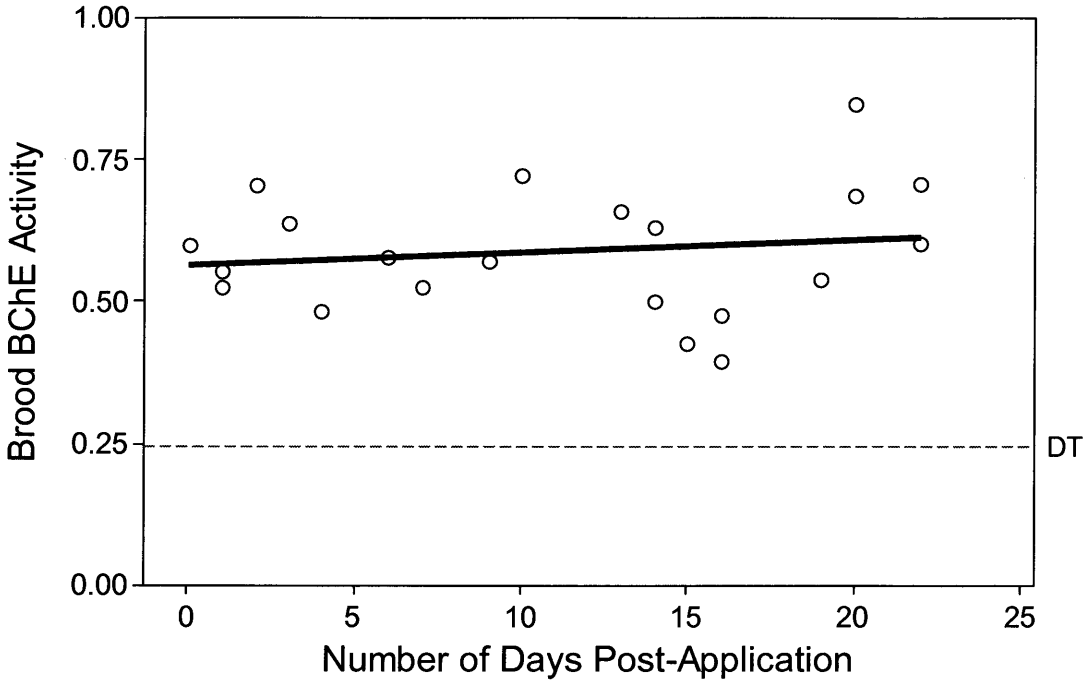
DAY 7 BROOD AChE VS. DAYS SINCE LAST APPLICATION



Day 7 brood AChE plotted versus the number of days since the last reported application of an anti-ChE pesticide.

FIGURE 18

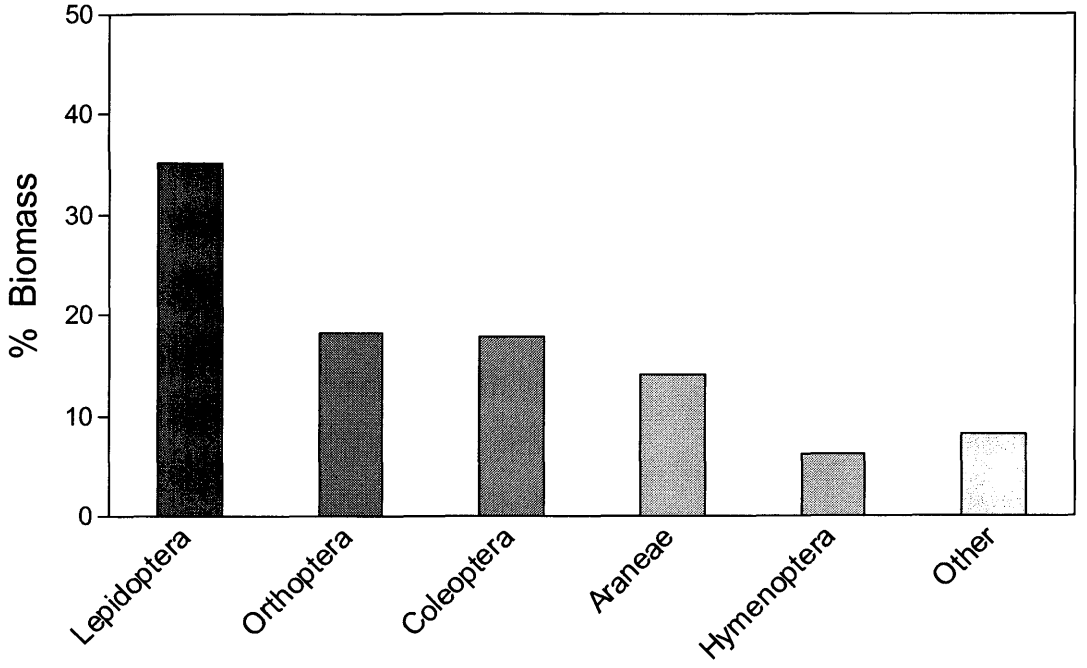
DAY 7 BROOD BChE VS. DAYS SINCE LAST APPLICATION



Day 7 brood BChE plotted versus the number of days since the last reported application of an anti-ChE pesticide.

FIGURE 19

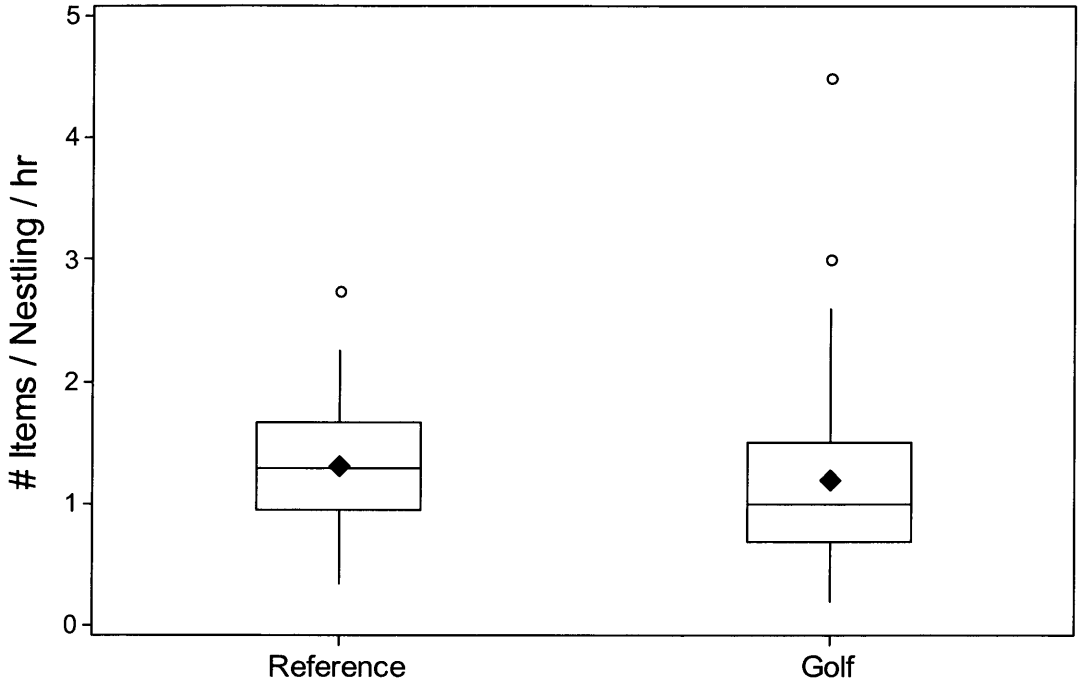
NESTLING DIET



Major prey groups collected from ligature sampling.

FIGURE 20

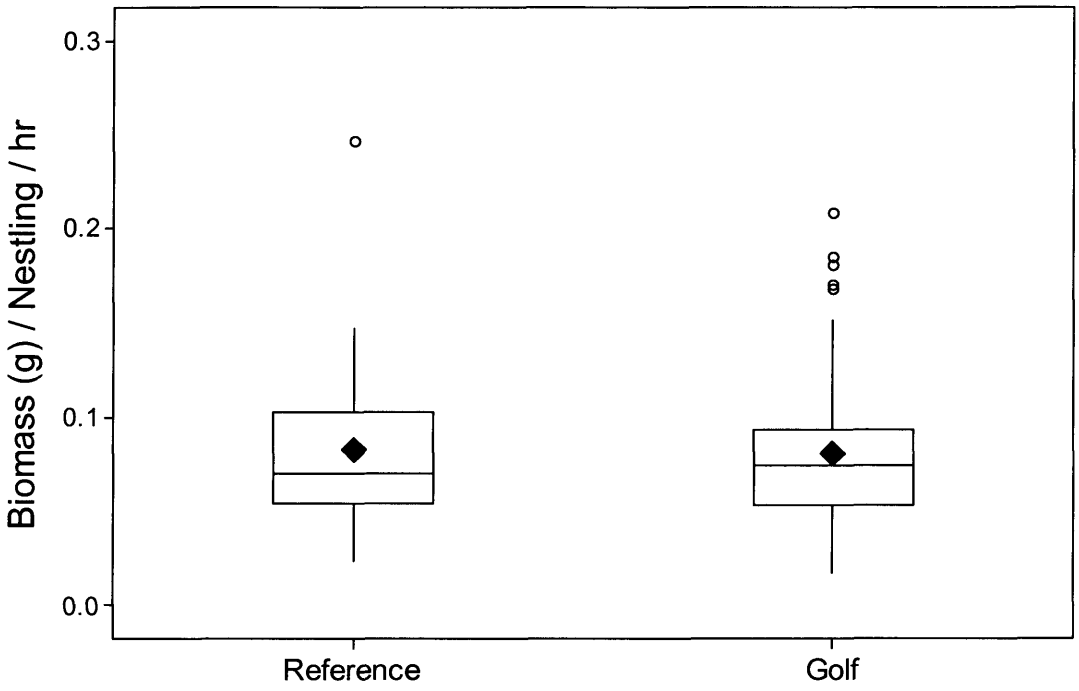
PREY ITEMS DELIVERED PER HOUR



Number of prey items collected per nestling per hour by habitat type.

FIGURE 21

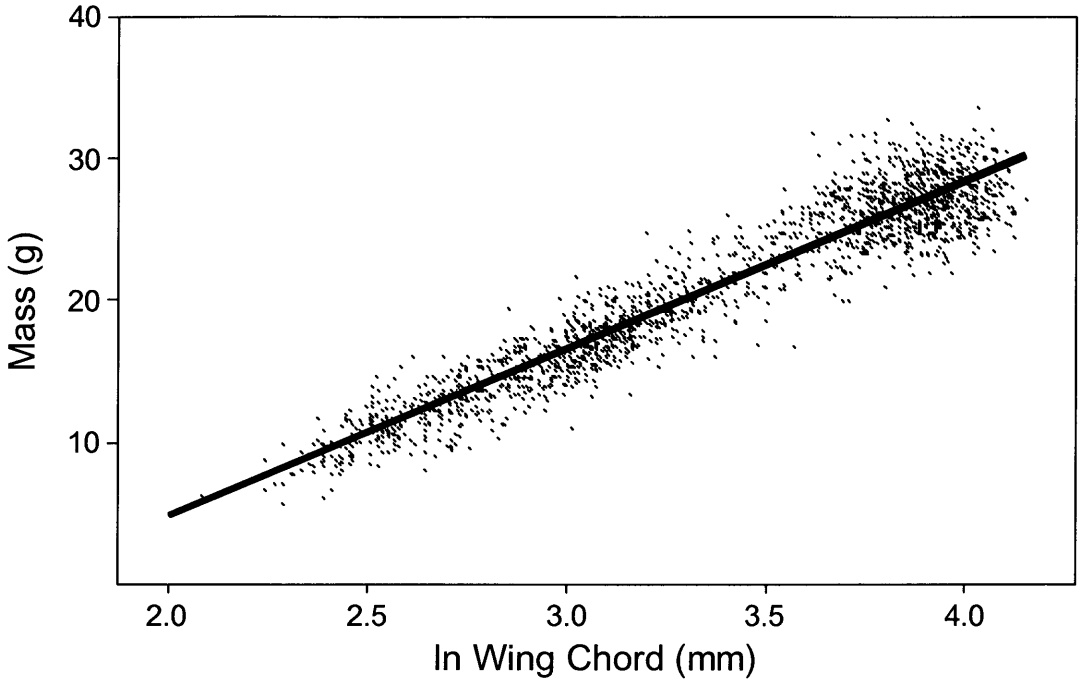
PREY BIOMASS DELIVERED PER HOUR



Biomass of prey items collected per nestling per hour by habitat type.

FIGURE 22

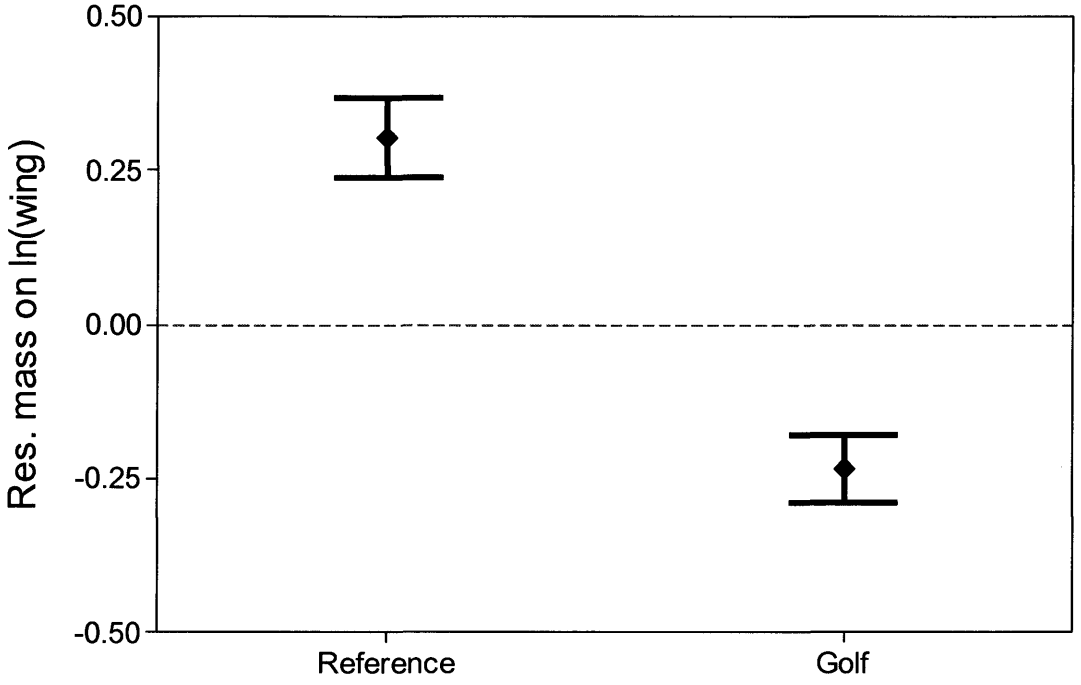
MASS VS. NATURAL LOG OF WING CHORD



Scatter plot of mass (g) vs. natural log of wing chord (mm) for nestlings measured between day 5 and 14. Residuals were stored and averaged for each brood.

FIGURE 23

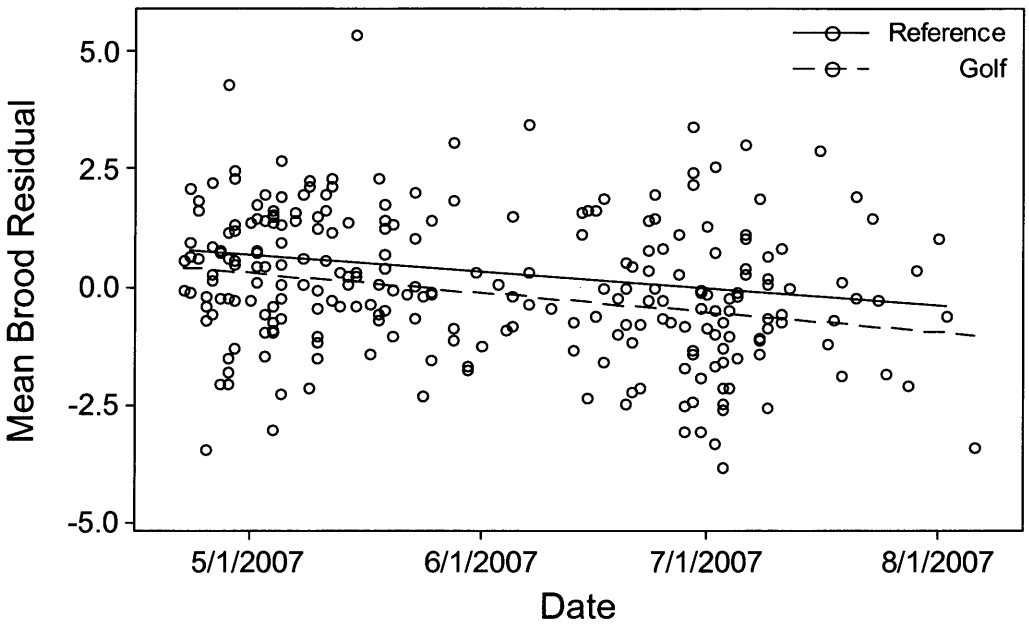
BROOD BODY MASS RESIDUALS BY HABITAT TYPE



Brood body mass residuals by habitat type. Error bars represent the Standard Error.

FIGURE 24

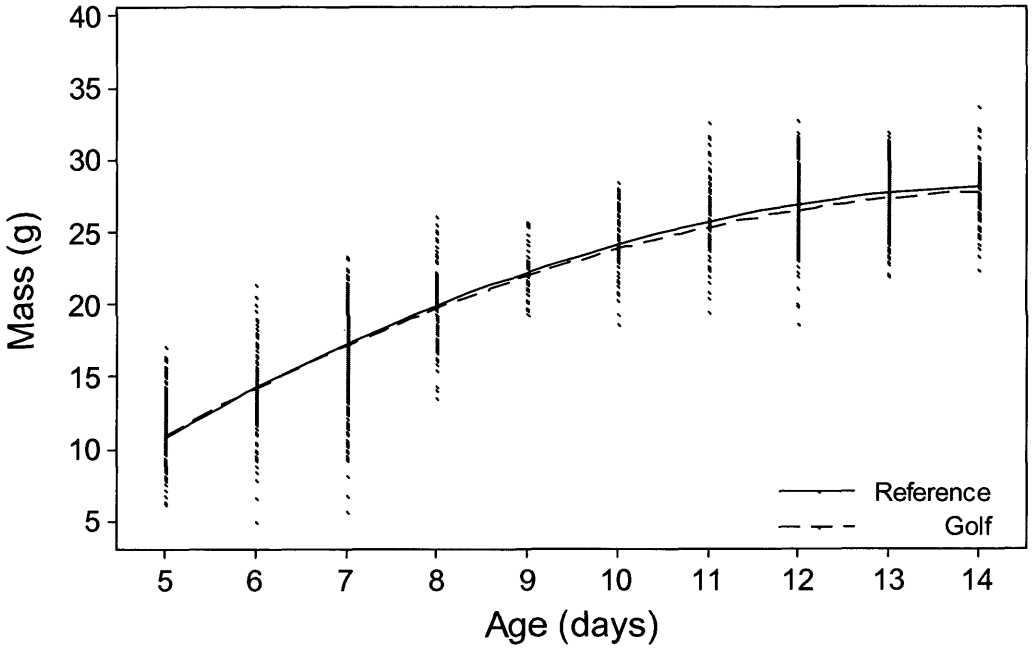
BODY MASS RESIDUALS VS. TIME



Brood body mass residuals plotted throughout the season. Open circles are mean brood residuals. Golf course nests are fitted to the green line and reference sites to the blue.

FIGURE 25

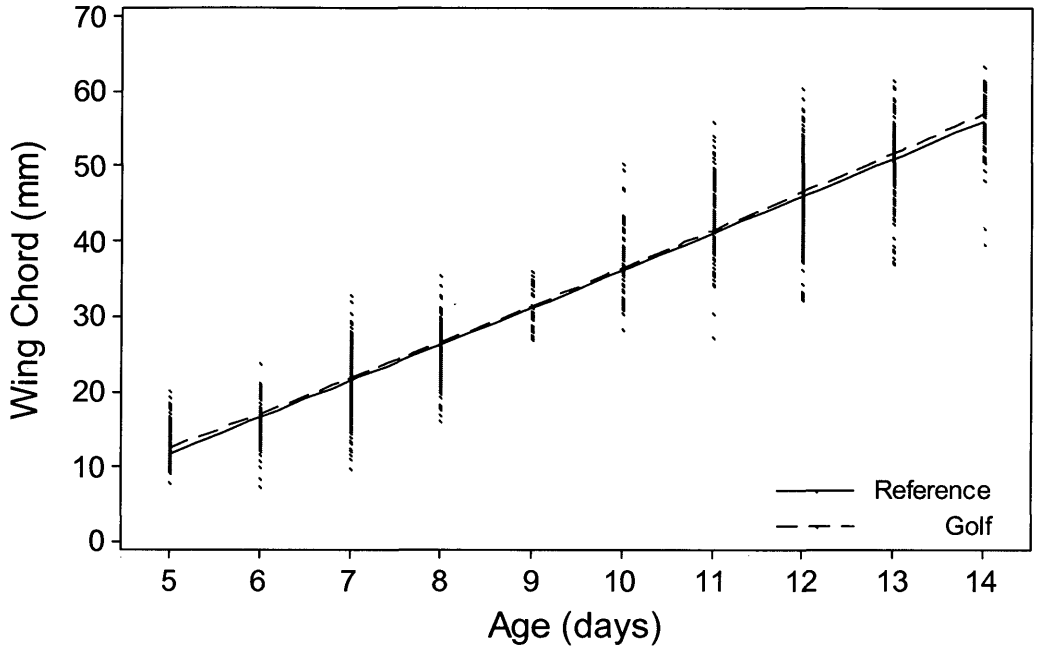
BODY MASS VS. AGE



Nestling mass plotted by age. Golf course birds are fitted to the dashed line and reference birds to the solid line.

FIGURE 26

WING CHORD VS. AGE



Nestling wing chord plotted by age. Golf course birds are fitted to the dashed line and reference birds to the solid line.

DISCUSSION

Nestling Exposure

Nestling eastern bluebirds on golf courses did not exhibit lower cholinesterase activities than same-age broods at reference sites without pesticide inputs. This lack of cholinesterase inhibition at golf course sites, including at sites confirmed to use anti-cholinesterase pesticides, indicates the sampled birds had either not been exposed to these chemicals or had been exposed but not enough to elicit detectable enzyme inhibition.

It is also possible that golf course nestlings were exposed to anti-cholinesterase pesticides and did suffer cholinesterase inhibition due to exposure to pesticides, but not at the time of sampling. Exposed birds my have been missed or sampled after exposure and resumption of normal cholinesterase. However, the lack of evidence of individual inhibitions suggests this is an unlikely explanation. There were two Day 7 golf course nestlings with AChE activities below the diagnostic threshold (DT=2 standard deviations below the reference mean). Pesticide exposure in these birds is unlikely because they were sampled at a golf course which had not reported a pesticide application prior to blood collection. In addition, the overall brood activity for this nest did not fall below the DT. A nest from a different golf course did show BChE below the DT but again, this nest was sampled at a golf course that did not report any recent pesticide applications. These data may have fallen below the DT due to inaccuracies in aging the birds or they could be naturally lower values associated with non-

pesticide related causes. For example, body condition has been shown to correlate with cholinesterase activity, including malnourishment or parasitic infection (Cordi et al. 1997).

If these birds' lower enzyme activities do represent pesticide induced inhibition, this would suggest less than 2% percent of the 192 golf course nestlings sampled had suffered enough exposure to lower enzyme activities. However, even this estimate is questionable given the uncertain value of applying the DT. The DT a useful comparison, but it is not necessarily indicative of pesticide exposure. For example, studies have shown cholinesterase inhibition in birds that did not fall below the DT as well as a lack of pesticide-induced inhibition in birds with enzyme activities below the DT (Maul and Farris 2005).

In the course of this research, several nestlings and entire broods were found dead within nests. These occurrences were not thoroughly documented but occurred in both golf course and reference habitats. It is possible the dead nestlings and broods in golf course nests could have been the result of pesticide exposure, but toxicological analyses were not conducted. The one bluebird found dead on a golf course, a newly independent juvenile, did not have chlorpyrifos metabolites in its gastrointestinal tract despite recent application of that pesticide. It did have below average brain cholinesterase activity for adult

levels, but this lower activity is likely the result of its age rather than pesticide exposure (Gard and Hooper 1993).

If bluebird nestlings had been exposed, the most likely pathway would have been through ingestion of contaminated prey items. However, no pesticide residues were retrieved from analysis of prey items fed to golf course nestlings. At the end of the season, after-the-fact review of 2007 chemical logs revealed that most of these prey items had not been collected following pesticide application dates. The 30 samples chosen from the 452 samples collected in 2007 were selected based on having been collected within 7 days of known pesticide applications and, therefore, represented the most likely items to contain pesticide residues. The lack of residues suggests that contaminated prey are either not present in abundance or adult bluebirds do not forage on contaminated dead or moribund prey.

In 2008 I opted to further assess potential ingestion risk by collecting arthropods directly from turf areas following pesticide applications. Although direct turf sampling is an estimate of potential prey, the lack of active nests with appropriate age nestling prevented extensive use of ligature sampling in 2008, despite getting forewarning of pesticide application events in that year. Analysis of these 20 items collected in 2008 again revealed no detectable organophosphates, suggesting low risk to bluebirds of ingesting contaminated prey.

The potential ingestion of intoxicated and weakened prey has been suggested as a route of exposure, but little empirical evidence exists regarding songbird consumption of dead or moribund arthropods (Stafford et al. 2003). Consumption of desiccated prey would increase exposure risk due to the increased concentration of contaminant. This behavior has been observed, including reports of birds gorging themselves at recently sprayed turf areas (Brewer et al.1988). In addition, there is clear evidence of fatal exposure of hundreds of Swainson's hawks (*Buteo swansoni*) due to the ingestion of crickets in South American agricultural fields recently sprayed with the OP monocrotophos (Goldstein et al. 1999). Bluebird foraging behavior may lessen the likelihood of selecting dead or moribund prey items from that of other species due to bluebirds' habit of visually searching for moving prey (Gowaty and Plissner 1998).

Although no pesticides were found on analyzed prey items, it is possible that newly independent birds are the most likely age class to consume contaminated prey, as opposed to the nestlings I studied. These inexperienced foragers may be unsuccessful at capturing and handling healthy, live prey and may take advantage of any dead or moribund prey items that are available. In addition, these birds are more likely to engage in terrestrial searching for prey as opposed to the perch-and-dive foraging of adult bluebirds. Finally, at several of the study golf courses pesticides were applied approximately 4 weeks after hatch dates, the same estimated dates at which adult birds cease provisioning of first-

clutch birds to begin second nesting efforts. This suggests that further attention should be paid to the vulnerable fledglings on golf courses that apply pesticides during their first few months of independence.

Pesticide Management Practices

The anti-cholinesterase compounds applied at participating golf courses are known to lower enzyme activities in birds. Pesticides in use during this research included chlorpyrifos and carbaryl. Several courses also employed non-cholinesterase inhibiting insecticides, including pyrethroids and nicotinil compounds. These types of pesticides are considered less toxic to wildlife than cholinesterase inhibitors. Golf courses also varied in their general management approach. Most superintendents waited to apply pesticides until noticeable evidence of pest outbreaks. This resulted in more late-season pesticide applications which should not have affected first round nesting efforts. In contrast, two golf courses applied pesticides once per month as a preventative measure, thus increasing the potential for exposure of nestlings.

Bluebird Diet

Direct sampling of prey items fed to nestling bluebirds indicated broods on golf course were fed the same number and biomass of prey as broods in non-golf course sites with no pesticide inputs. Nestlings were also fed similar types of prey. This evidence suggests adult bluebirds are equally capable of provisioning each nestling on golf courses as on reference sites. Parental ability to forage

successfully in a golf course environment is not surprising given adult bluebirds' ability to meet their own energy needs to be able to nest and lay eggs on golf courses. The data support this observation and further show their ability to successfully forage throughout the breeding season in spite of chemical management, and manicured turf.

Although some nestlings, and possibly entire broods, likely died from starvation, the cause of death of these birds was not determined. The data suggest that two provisioning adults are fully capable of meeting the dietary needs of nestlings on golf courses. However, because I ligatured only broods 8 -11 days old, it is possible I did not detect mortalities in the first week caused by an inability of parents to obtain appropriate sized prey items. However, given the high rate of nest success this seems unlikely and to the extent it may occur, unlikely caused by pesticide management.

It is possible that adult birds on and off golf courses spent significantly different amounts of time and energy to achieve comparable provisioning. Research in experimental plots sprayed with Decis™ and Furadan™ caused adult chestnut-collared longspurs (*Calcarius ornatus*) to forage twice to provision nestlings than in control plots, but these birds did achieve the same provisioning rate (Martin et al. 2000). Our bluebirds foraging range were not measured, but adults on golf courses did not appear to travel further than reference birds. In addition, several golf courses had pairs of bluebirds nesting within 100 meters of

each other, suggesting they do not require larger territories to obtain sufficient prey. In addition, any extra foraging effort did not appear to affect breeding success.

Body Mass

Broods on golf courses had lower relative mass than reference broods when correcting for skeletal size. This difference is partially explained by brood size and hatch date, but overall, golf courses produced chicks of lower mass during both first and second nests (Figure 24). My data suggest that the observed lower mass is not to the result of sub-lethal pesticide exposure nor was it due to lower feeding rates by provisioning adults. It is possible there was a difference in growth patterns not detected given my measuring schedule (Figure 25-26). A more thorough tracking of nestling growth on and off golf courses would provide a better indication of any differences in growth patterns.

It is unclear if the observed lower mass has an effect on survivorship in our birds, but it has been shown to be an indicator in other songbirds (Richner 1989, Brown and Roth 2004). This may be particularly important as golf course chicks fledge into a habitat potentially more demanding than reference sites. As discussed, adult bluebirds are capable of foraging successfully on golf courses, suggesting there is sufficient prey available in spite of pesticide use. However, it remains possible that less prey are available on golf courses but that adult birds are able to compensate. In contrast, young, inexperienced birds on golf courses

may not have the necessary skills to locate prey in golf courses. This would place these lower mass birds at a higher risk of starvation as well as higher likelihood of ingesting dead or moribund insects that may be present due to pesticide usage. Consumption of dead insects has been shown experimentally and observed on golf courses following pesticide applications (Brewer et al. 1988, Stafford et al. 2003). Therefore young, newly independent bluebirds may be at the highest risk of mortality related to golf course management practices.

It is possible the observed lower mass has no effect on survivorship and, in fact, does not represent mass at fledging. Eastern bluebirds tend to fledge between days 17 and 19 (Gowaty and Plissner 1998), but for my body mass analyses I only used measurements taken prior to day 15. In addition, my research efforts did not document fledge date, which could counter the observed lower mass of younger nestlings. If golf course broods spent more time in the nest and successfully fledged at a later date, then any difference in body mass would no longer matter.

Strength of Evidence

I chose to sample younger, developing birds because they are much more sensitive to exposure of pesticides than are adult birds (Gard and Hooper 1993). In addition, by controlling the location of nests I could be confident that any detected exposures would be confirmed from the golf courses and not surrounding areas. By sampling these birds and detecting exposure and

associated effects we intended to identify the risk to breeding birds on golf courses. However, in doing so we did not sample free-ranging adult birds which have several routes of exposure, including direct ingestion of granules or contaminated water, or dermal exposure from contact with sprays, sprayed vegetation or contaminated water during bathing. Although adult bluebirds, and other species, face these exposure risks, I opted to assess nestlings as a more sensitive indicator of risk. I assumed that since no negative reproductive effects had been detected in previous years' work (LeClerc et al. 2005), adults are facing fitness effects from exposure. On the other hand, exposure levels may be high enough to affect nestling growth and survival. Detecting no exposure in nestlings does not preclude adult exposure, but the lack of reproductive effects suggests this is not a major concern at the population viability level.

Are birds on golf courses at risk?

Although the eastern bluebird was a good study species for my objectives, no single species can serve as a proxy for all other species present on golf courses. It remains entirely possible that other species on the same golf courses from which I sampled bluebirds were exposed to pesticides and suffered detrimental effects. For example, behavioral and dietary differences between species could lead to differential exposure risk. By relying on a perch and dive foraging behavior, eastern bluebirds do not spend much time in contact with turf grass, likely lowering their risk of dermal exposure compared to terrestrial foragers. Perhaps more importantly, bluebirds' use of perches dictates where

they forage within a golf course. I generally observed adult birds foraging along the edge of fairways due to the high frequency of trees on which to perch. However, I also regularly observed adult birds foraging from perches near tee areas when no golfers were present. In contrast, the putting greens are rarely adjacent to perches from which bluebirds can forage. Since these putting greens are generally the highest chemical input areas, this structural feature common to all golf courses likely greatly reduces bluebirds' opportunity to ingest contaminated prey items.

It has been suggested (Rainwater et al. 1995) that, because pesticides are more commonly applied early in the morning, prior to golfers being present, the early hours of the day (and spatially, the "back nine" of a golf course) are at greatest risk for wildlife exposure. For this reason, as well as for ease of access, I made extra effort to collect blood samples prior to any golfers being present. However, in doing so, I was unable to also collect prey items via ligatures during these early hours. Therefore, it is possible that adult birds do forage on contaminated prey from these high pesticide-input areas before golfers are present but not later in the day when golfers disturb their foraging efforts. However, if the prey fed to nestlings had in fact had significant amounts of pesticide residues I would have expected to detect cholinesterase inhibition in their blood samples. Furthermore, throughout the day I often observed flocks of larger terrestrial foragers such as American robins (*Turdus migratorius*), common grackles, American crows (*Corvus brachyrhynchos*) and brown-headed

cowbirds (*Molothrus ater*) foraging at putting greens, the presence of which may have additionally prevented bluebirds from collecting prey in high-input areas. Fledgling and newly independent first-year birds were more commonly observed walking and foraging on fairways and putting greens and, therefore, may be at higher risk of consuming contaminated prey.

CONCLUSION

This study aimed to fill the gap in our understanding of the risk of exposure and effects of golf course pesticides on birds. Two studies have indicated eastern bluebird chicks reared on golf courses have lower body mass than reference sites (Stanback and Seifert 2005 and Appendix A), a possible direct or indirect result of pesticide management practices. Overall my data suggest bluebirds are not detectably affected by the pesticides employed at participating golf courses. Extensive testing of nestling blood samples found no indication of pesticide exposure. In addition, I found no detectable traces of pesticide residues on prey items collected directly from bluebird provisioning. Comparison of prey delivery revealed golf course broods were not fed fewer or smaller prey items than reference nests.

This research represents only the second study performed on avian pesticide exposure on golf courses and the first in more than 10 years. Although my data do not point to high risk of pesticide exposure, it would be premature to say other species and taxa are equally safe. More work is needed, especially

since the only other study suggested other species may be at higher risk of exposure (Rainwater et al. 1995). Future work should continue to assess pesticide exposure in an array of species, particularly terrestrial foragers and waterfowl. In addition, research on young bluebirds should continue to close the gap on all stages of bluebird life history on golf courses.

Research related to non-pesticide concerns of golf courses, such as fragmentation and disturbance should also continue. These studies can now be better informed of the apparently low risk of pesticide exposure for certain species and be less concerned with pesticide use confounding their work. Notably, this and earlier studies focused on eastern bluebirds, but these birds nest in boxes which prevented nest predation. As pointed out in other studies, golf courses may host more predators than other suburban environments (Sorace 2007). Given their inherently fragmented structures, more work is needed to assess ecological obstacles before golf courses can be accepted as quality habitat.

In spite of common criticisms, golf courses may yet be of conservation value. With continued scientific study, superintendents will become better equipped to integrate environmentally friendly practices without sacrificing industry interests or playing surfaces. In doing so, more than 1 million hectares of existing North American golf courses will be enhanced for wildlife and provide an examples for golf courses worldwide.

REFERENCES

Bennett, R.S. 1989. Factors influencing discrimination between insecticide-treated and untreated foods by northern bobwhite. Archives of Environmental Contamination and Toxicology 18: 697-705.

Blair, R.B. 1996. Land use and avian species diversity along an urban gradient. Ecological Applications 6: 506-519.

Brealey, C.J., C.H. Walker and B.C. Baldwin. 1980. A-Esterase activities in relation to the differential toxicity of pirimiphos-methyl to birds and mammals. Pesticide Science 11: 546-554.

Brewer, L.W., C.J. Driver, R.J. Kendall, T.E. Lacher, J.C. Galindo, and G.W. Dickson. 1988. Avian response to a turf application of Triumph 4E. Environmental Toxicology and Chemistry 7: 391-401.

Brown, W.P. and R.R. Roth. 2004. Juvenile survival and recruitment of wood thrushes *Hylocichla mustelina* in a forest fragment. Journal of Avian Biology 35: 316-326.

Buerger, T.T., R.J. Kendall, B.S. Mueller, T. de Vos, and B.A. Williams. 1991. Effects of methyl parathion on northern bobwhite survivability. Environmental Toxicology and Chemistry 10: 527-532.

Busby, D.G., S.B. Holmes, P.A. Pearce, and R.A. Fleming. 1987. The effect of aerial application of Zectran I on brain cholinesterase activity in forest songbirds. Archives of Environmental Contamination and Toxicology 16: 623-629.

Busby, D.G. and P.A. Pearce. 1990. Effects of aerial spraying of fenitrothion on breeding white-throated sparrows. Journal of Applied Ecology 27: 743-755.

Cordi, B., C. Fossi and M. Depledge. 1997. Temporal biomarker responses in wild passerine birds exposed to pesticide spray drift. Environmental Toxicology and Chemistry 16: 2118-2124.

Cornell Nestwatcher's Resource Center. 2008. Available online at www.birds.cornell.edu/nestinginfo/nestboxref/placement. (accessed 4 September 2008).

Deweese, L.R., L.C. McEwen, L.A. Settimi, and R.D. Deblinger. 1983. Effects on birds of fenthion aerial application for mosquito control. Journal of Economic Entomology 76: 906-911.

Ellman, G.L., K.D. Courtney, V. Andres, and R.M. Featherstone. 1961. A new rapid colormetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7: 88-95.

Environmental Protection Agency. 1972. DDT ban takes effect. Available at www.epa.gov/history/topics/ddt/01.htm.

Environmental Protection Agency. 1986. Available at http://www.epa.gov/oppsrrd1/reregistration/REDs/diazinon_red.pdf.

Fleischli, M.A., J.C. Franson, N.J. Thomas, D.L. Finley, and W. Riley Jr. 2004. Avian mortality events in the United States caused by anticholinesterase pesticides: a retrospective summary of national wildlife health center records. Archives of Environmental Contamination and Toxicology 46: 542-550.

Gard, N.W. and M.J. Hooper. 1993. Age-dependent changes in plasma and brain cholinesterase activities of eastern bluebirds and European starlings. Journal of Wildlife Diseases 29: 1-7.

Goldstein M. I., TE. Lacher, M.E. Zaccagnini, M.L. Parker, and M.J. Hooper. 1999. Monitoring and assessment of Swainson's hawks in Argentina following restrictions on monocrotophos use, 1996-97. Ecotoxicology 8: 214-224.

Golf Digest Publications. 2008. Golf and the environment: golfer perceptions and attitudes concerning the game and its relationship with the environment. Available online at http://www.golfandtheenvironment.org/index.php?option=com_content&task=view&id=19&Itemid=40.

Gowaty, P. A. and J. H. Plissner. 1998. Eastern Bluebird (*Sialia sialis*). *In* The Birds of North America, No. 381 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia, PA.

Grue, C.E., G.V.N. Powell and M.J. McChesney. 1982. Care of nestlings by wild female starlings exposed to an organophosphate pesticide. Journal of Applied Ecology 19: 327-335.

Grue, C.E. and B.K. Shipley. 1984. Sensitivity of nestling and adult starlings to dicrotophos, an organophosphate pesticide. Environmental Research 35: 454-465.

Grue, C.E., P.L. Gibert and M.E. Seeley. 1997. Neurophysiological and behavioral changes in non-target wildlife exposed to organophosphate and carbamate pesticides: thermoregulation, food consumption, and reproduction. American Zoology 37: 369-388.

Hodgkison, S.C., J.M. Hero and J. Warnken. 2007. The conservation value of suburban golf courses in a rapidly urbanising region of Australia. Landscape and Urban Planning 79: 323-337.

Hooper, M.J., P.J. Detrich, C.P. Weisskopf, and B.W. Wilson. 1989. Organophosphorus insecticide exposure in hawks inhabiting orchards during winter dormant spraying. Bulletin of Environmental Contamination and Toxicology 42: 651-659.

Johnson, E.J., L.B. Best and P.A. Heagy. 1980. Food Sampling Biases Associated with the "Ligature Method". The Condor 82: 186-192.

Jones, S.G., D.H. Gordon, G.M. Phillips, and B.R.D. Richardson. 2005. Avian community response to a landscape unit gradient. Wildlife Society Bulletin 33: 422-434.

Kendall, R.J., L.W. Brewer, R.R. Hitchcock, and J.R. Mayer. 1992. American wigeon mortality associated with turf applications of diazinon AG500. Journal of Wildlife Disease 28: 263-267.

LeClerc, J.E. and D.A. Cristol. 2005. Are golf courses providing habitat for species of conservation concern in Virginia? Wildlife Society Bulletin 33: 463-471.

LeClerc, J.E., J.P. Che, J.P. Swaddle, and D.A. Cristol. 2005. Reproductive success and developmental stability of eastern bluebirds on golf courses: evidence that golf courses can be productive. Wildlife Society Bulletin 33: 483-493.

Martin, P.A., K.R. Solomon and H.J. Boermans. 1991. Effects of carbofuran ingestion on mallard ducklings. Journal of Wildlife Management 55: 103-111.

Martin, P.A., D.L Johnson, D.J. Forsyth, and B.D. Hill. 2000. Effects of two grasshopper control insecticides on food resources and reproductive success of two species of grassland songbirds. Environmental Toxicology and Chemistry 19: 2987-2996.

Martinez-Haro, M., J. Vinuela and R. Mateo. 2007. Exposure of birds to cholinesterase-inhibiting pesticides following a forest application for tick control. Environmental Toxicology and Pharmacology 23: 347-349.

Maul, J.D. and J.L. Farris. 2004. Monitoring exposure of passerines to acephate, dicrotophos, and malathion using cholinesterase reactivation. Bulletin of Environmental Contamination and Toxicology 73: 682-689.

Maul, J.D. and J.L Farris. 2005. Monitoring exposure of northern cardinals, *Cardinaliscardinalis*, to cholinesterase-inhibiting pesticides: enzyme activity, reactivations, and indicators of environmental stress. Environmental Toxicology and Chemistry 24: 1721-1730.

McEwen, L.C., C.E. Knittle and M.L. Richmond. 1972. Wildlife effects from grasshopper insecticides sprayed on short-grass range. Journal of Range Management 25l: 188-194.

Merola-Zwartjes, M. and J.P. DeLong. 2005. Avian species assemblages on New Mexico golf courses: surrogate riparian habitat for birds? Wildlife Society Bulletin 33: 435-448.

McInnes, P.F., D.E. Andersen, D.J. Hoff, M.J. Hooper, and L.L Kinkel. 1996. Monitoring exposure of nestling songbirds to agricultural applications of an organophosphorus insecticide using cholinesterase activity. Environmental Toxicology and Chemistry 15: 544-552.

Millikin R.L. and J.N.M. Smith. 1990. Sublethal effects of fenitrothion on forest passerines. Journal of Applied Ecology 27: 983-1000.

Mineau, P and M. Whiteside. 2006. Lethal risk to birds from insecticide use in the United States – a spatial and temporal analysis. Environmental Toxicology and Chemistry 25: 1214-1222.

North American Bluebird Society. 2008. Available online at www.nabluebirdsociety.org/gettingstarted. (accessed 4 September 2008).

Ortega J., J.M. Aparicio, A. Munoz, and R. Bonal. 2007. Malathion applied at standard rates reduces fledgling condition and adult male survival in a wild lesser kestrel population. Animal Conservation 10: 312-319.

Porter, E.E., J. Bulluck and R.B. Blair. 2005. Multiple spatial-scale assessment of the conservation value of golf courses for breeding birds in southwestern Ohio. Wildlife Society Bulletin 33: 494-506.

Powell, G.V.N. 1984. Reproduction by an altricial songbird, the red-winged blackbird, in fields treated with the organophosphate insecticide fenthion. Journal of Applied Ecology 21: 83-95.

Rainwater, T.R., V.A. Leopold, M.J. Hooper, and R.J. Kendall. 1995. Avian exposure to organophosphorus and carbamate pesticides on a coastal South Carolina golf course. Environmental Toxicology and Chemistry 14: 2155-2161.

Richner, H. 1989. Habitat-specific growth and fitness in carrion crows (*Corvus corone corone*). Journal of Animal Ecology 58: 427-440.

Rodewald, P.G., M.J. Santiago and A.D. Rodewald. 2005. Habitat use of breeding red headed woodpeckers on golf courses in Ohio. Wildlife Society Bulletin 33: 448-453.

Sorace, A. and M. Visentin. 2007. Avian diversity on golf courses and surrounding landscapes in Italy. Landscape and Urban Planning 81: 81-90.

Smith, M.D, C.J. Conway and L.A. Ellis. 2005. Burrowing owl nesting productivity: a comparison between artificial and natural burrows on and off golf courses. Wildlife Society Bulletin 33: 454-462.

Stafford, J.M., L.W. Brewer, and J.A. Gessaman. 2003. Avian food selection with application to pesticide risk assessment: are dead and desiccated insects a desirable food source? Environmental Toxicology and Chemistry 22: 1335-1339.

Stanback, M.T. and M.L. Seifert. 2005. A comparison of eastern bluebird reproductive parameters in golf and rural habitats. Wildlife Society Bulletin 33: 471-482.

Stone, W.B. and P.B. Gradoni. 1985. Wildlife mortality related to use of the pesticide diazinon. Northeastern Environmental Science 4: 30-38.

Stromborg, K.L, C.E. Grue and J.D. Nichols. 1988. Postfledging survival of European starlings exposed as nestlings to an organophosphorus pesticide. Ecology 69: 590-601.

Tanner, R.A. and A.C. Gange. 2005. Effects of golf courses on local biodiversity. Landscape and Urban Planning 71: 137-146.

Terman, M.R. 1997. Natural links: naturalistic golf courses as wildlife habitat. Landscape and Urban Planning 38:183-197.

Walker, C.H. 1983. Pesticides and birds – mechanisms of selective toxicity. Agriculture, Ecosystems and Environment 9: 211-226.

White, D.H., K.A. King, C.A. Mitchell, E.F. Hill, and T.G. Lamont. 1979. Parathion Causes secondary poisoning in a laughing gull breeding colony. Bulletin of Environmental Contamination and Toxicology 23: 281-284.

White, C.L and M.B. Main. 2005. Waterbird use of created wetlands in golf course landscapes. Wildlife Society Bulletin 33: 411-421.

World Golf Directory. 2008. Available online at http://www.worldgolf.com/courses/unitedstates/usa.html (accessed 12 November 2008).

Yasuda, M. and F. Koike. 2006. Do golf courses provide a refuge for flora and fauna in Japanese urban landscapes? Landscape and Urban Planning 75: 58-68.

APPENDIX A

2004-2006 BROOD BODY MASS

	Relative Body Mass (g / ln[mm])		Number of Nests	
Year	**Golf**	**Reference**	**Golf**	**Reference**
2004	0.63	-0.532	77	70
2005	0.286	0.256	45	44
2006	0.002	-0.055	95	75

APPENDIX B

PESTICIDE RESIDUE SCREENS

Sample Number	Azinphos Methyl	Chlorpyrifos	Diazinon	Dimethoate	Ethyl Parathion	Malathion	Methamidophos	Naled	Phorate	Phosmet	Profenfos	Terbufos
110	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
111	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
112	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
113	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
117	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
118	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
162	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
163	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
165	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
166	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
167	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
168	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
186	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
187	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
189	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
192	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
196	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
198	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
199	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
217	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
218	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
225	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
226	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
227	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
251	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
256	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
257	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
258	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
269	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
271	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
ALL UNITS ARE ug chemical/mL solution (or 1.0ug chemical)												

APPENDIX C

NESTLING PREY

Order	Percent Biomass
Lepidoptera	35.07
Orthoptera	18.16
Coleoptera	17.91
Araneae	14.18
Hymenoptera	6.22
Unidentified	2.49
Haplotaxid	1.99
Dictyoptera	1.24
Odonata	1.00
Diptera	0.75
Isopoda	0.75

VITA

Ryan B. Burdge was born in Kirkwood, Missouri on December 9, 1981. He attended Kirkwood High School in 2000 and went on to earn a B.A. in Environmental Studies from Macalester College in St. Paul, Minnesota in May 2004. After his undergraduate studies, Ryan completed an internship with the Bureau of Land Management in California and then moved to Washington, DC and worked as a grants manager with the National Fish and Wildlife Foundation. He married Zarina A. Morais in September 2006, shortly after beginning graduate school at the College of William and Mary. Ryan currently works as an Environmental Associate with the consulting firm Ecology and Economics Inc., conducting technical and policy analyses related to EPA's Superfund and underground storage tank programs. Ryan and his wife live in Charlottesville, VA, and they are anxiously awaiting the arrival of their first child.