A major cause of amphibian declines worldwide is habitat destruction or alteration. Public green spaces, such as golf courses and parks, could serve as safe havens to curb the effects of habitat loss if managed in ways to bolster amphibian communities. In a series of experiments, I examined how terrestrial buffer zones affect the larvae of Blanchard’s cricket frogs and green frogs as well as juvenile and adult Blanchard’s cricket frogs. Larval survival of both species was affected by the presence of a buffer zone, with increased survival with buffer zones for cricket frogs and decreased survival for green frogs when reared in ponds with buffer zones compared to that reared in ponds without buffer zones. When given a choice, juvenile cricket frogs generally preferred unmown grass versus mown grass. My results suggest that small changes in land management could have large impacts for some amphibians.
EFFECTS OF TERRESTRIAL BUFFER ZONES ON AMPHIBIANS IN MANAGED GREEN SPACES

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Holly J. Puglis

Miami University

Oxford, Ohio

2010

Advisor__________________________

Michelle D. Boone

Reader_____________________________

Thomas O. Crist

Reader_____________________________

María J. González
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Introduction

Habitat loss is the number one cause of the biodiversity crisis (Wilcove et al. 1998). Humans destroy or alter the landscape for residential, agricultural, commercial, and recreational use. Since 1945, it is estimated that urban land area in the United States has nearly quadrupled from about 15 million to 60 million acres (Lubowski et al. 2006). Disturbance events can favor non-native invasive species over native species (Hobbs & Huenneke 1992). If wildlife declines are to be curbed, conservation efforts will need to focus on protecting habitat and also managing areas in ways that minimize conflict with wildlife.

At least 43% of known amphibian species are experiencing population declines (Stuart et al. 2004) and are, therefore, in desperate need of conservation. Amphibians play an important role in the ecosystems they inhabit. Some research has pointed to their ability to act as indicators of ecosystem stress because amphibians are thought to be sensitive to changes in the environment since they utilize both aquatic and terrestrial habitat (Welsh & Ollivier 1998). Amphibians are integral in their food webs by serving as both predator and prey. Through their diet amphibians can control populations of pest insects, such as mosquitoes (Brodman & Dorton 2006) and algae (Ranvestel et al. 2004). Amphibians can also assimilate a large amount of the energy they ingest (Bobka et al. 1981, Smith 1976, Beard et al. 2002) and convert their food resources to biomass, which serves as a prey base for higher trophic levels. Studies have indicated that amphibian biomass can exceed that of other taxonomic groups such as birds and small mammals (Burton & Likens 1975) and that removal of amphibians can depress plant production and alter nutrient cycling (Beard et al. 2002, Sin et al. 2008). By conserving amphibian populations, the services they provide in their ecosystems will also be preserved. Because the leading cause of amphibian declines worldwide is habitat destruction and alteration (Stuart et al. 2004), managed green spaces offer a unique opportunity for amphibian conservation to maintain these services in altered habitats.

Recreationally managed green spaces such as parks and golf courses may partly mitigate the effects of habitat loss. There are more than 17,000 golf courses in the United States comprising over half a million hectares of land (Kenna & Snow 2002). On a golf course, up to 70% of the course is considered “rough” or out of play (Colding & Folke 2009), leaving a large area of land that if managed in ways consistent with natural habitats, could provide habitat to
some native species of wildlife. In fact, Colding and Folke (2009) found golf courses to have a higher ecological value (based on measurements of species diversity, richness, abundance, and other measures of biota) in many cases than other land types, such as parkland, agricultural, residential, and highly urban. Also, Blair (1996) found a greater number of bird species and a higher diversity of birds on golf courses than at more natural sites in California. However, golf courses have also been found to harbor a greater number of nest predators than other land types (Solace & Visentin 2007), which may add stress to these systems. Golf courses could benefit from diverse populations of animals, such as amphibians, because amphibians could reduce the cost of maintaining aquatic sites on the course. As larvae, amphibians eat algae and insects, and as juveniles and adults they eat insects, such as mosquitoes. This may reduce the need to stock fish in ponds, use algaecides/herbicides to control algal growth, or spray pesticides to manage mosquitoes creating a win-win situation for managers and wildlife.

Golf courses often contain aquatic habitat, such as ponds or wetlands, which is integral to amphibians with complex life cycles. Previous research has shown that amphibians will use golf course ponds, but that most courses have lower amphibian biodiversity than reference sites because of a lack of hydroperiod variability (Scott et al. 2002). Boone et al. (2008) has also shown that amphibian survival on golf courses can exceed survival on reference sites in some cases, implicating lower macroinvertebrate predator abundance in golf course ponds as one reason for higher survival. These studies suggest that some amphibians can utilize golf courses for a portion of the life cycle. However, these studies do not address whether golf courses provide the necessary terrestrial habitat for the completion of amphibian life cycles, which is essential for population persistence.

Suitable adjacent or upland terrestrial habitat is required for amphibians with complex life cycles (Semlitsch 1998); however, terrestrial life stages are less studied. Golf courses have green space, but the quality is likely compromised by physical alteration such as mowing and by chemical management with pesticides and nutrient supplementation such as nitrogen and phosphorus applied as fertilizer (Beard 2002), which likely reduce suitability for amphibians. The effects of changes in the terrestrial habitat can potentially be minimized with terrestrial buffer zones.

A terrestrial buffer zone, or vegetative filter strip, is a tract of land with vegetation such as grass, trees, herbaceous plants, or shrubs adjacent to roadways, cropland, or ponds. Taller,
unmown grass around ponds may provide essential habitat for juvenile and adult amphibians because it may harbor more insects, thus more food, suitable overwintering sites, and protection from desiccation and predators. If juvenile and adult amphibians use this habitat, it could increase the probability of survival and slow population declines, but more research is needed to understand the effect of changes in the terrestrial environment on amphibians. In addition to providing habitat for juvenile and adult amphibians, terrestrial buffer zones could also reduce contaminants such as pesticide, nutrient, and sediment loads from runoff (Barfield et al. 1979; Chaubey et al. 1994; Rankins et al. 2001). Contaminants have the potential to hinder amphibians’ ability to complete their life cycle (Hayes et al. 2003; McCallum & Tauth 2007); however, some contaminants may also have indirect positive effects on amphibian communities by releasing them from predation (Boone & Semlitsch 2003) or increasing food resources (Boone & Semlitsch 2001; Boone et al. 2004). Terrestrial buffer zones filter sediment-bound nutrients and pesticides by slowing the velocity of the runoff to allow for deposition and also to allow soluble materials to be adsorbed into plants and soil (Patty et al. 1997; Srivastava et al. 1998; Rankins et al. 2001). The United States Department of Agriculture’s Natural Resources Conservation Service already considers buffer zones a best management practice for reducing nonpoint source pollution (Krutz et al. 2005).

To investigate whether unmown, terrestrial buffer zones around golf course ponds can be used to support the life cycle of amphibian populations, I used two species of amphibians, Blanchard’s cricket frog (Acris blanchardii) and green frogs (Rana clamitans). Blanchard’s cricket frog is a widespread grassland species experiencing enigmatic population declines in parts of its range (Brodman & Kilmurry 1998; Hay 1998; Mierzwa 1998; Lehtinen & Skinner 2006). This species is well suited for this study because it is associated with permanent water bodies (Wright & Wright 1995), the most common type of aquatic habitat created by humans for recreation or aesthetics. Permanent water bodies often have fish; therefore, it is likely that cricket frogs coexists with fish (Porej & Hetherington 2005), which many golf course superintendents stock to control algae and vegetation in aquatic sites. Both juvenile and adult cricket frogs utilize the perimeter of ponds (Smith et al. 2003), which makes this species an ideal candidate for examining the effects of a terrestrial buffer zone. Blanchard’s cricket frogs reach reproductive maturity within one year; therefore, they are an ideal species on which to test the effects of terrestrial buffer zones on the full life cycle of an amphibian. Green frogs are also widespread
and are considered habitat generalists. They are found along ponds and lakes, streams, and even ditches. These frogs are commonly associated with human dominated landscapes such as mitigation ponds, parks, and golf courses (Scott et al. 2002; Vredenburg 2004; Porej & Hetherington 2005). Golf course ponds are typically permanent which makes them suitable for green frog larvae as these animals can spend up to two years in the pond as tadpoles before metamorphosing. However, because green frogs overwinter as larvae, I only used green frog tadpoles in the larval study and not in any of the terrestrial experiments.

I examined the effects of terrestrial buffer zones on the full life cycle of amphibians by conducting a series of studies. First, I assessed the effects of buffer zones on larval survival and development using both cricket frogs and green frogs reared separately in enclosures within golf course ponds with and without terrestrial buffer zones surrounding the pond. Second, to examine the effects of buffer zones on the terrestrial phase of the life cycle, I followed Blanchard’s cricket frogs reared in golf course ponds with and without buffers through overwintering with a mark-recapture study. Third, I assessed juvenile cricket frog preference for mown versus unmown grass as well as the effects of mown versus unmown grass on the overwintering survival and mass of juvenile/adult cricket frogs.

Due to the filtering nature of the terrestrial buffer zones, I expected greater survival and mass for both larval green frogs and metamorphosed cricket frogs reared in ponds with buffer zones than those reared in ponds without buffer zones because buffer zones should reduce mortality from direct toxicity of contaminants to amphibians. Ultimately, the impact of contaminants is a balance between the direct and indirect effects on amphibians. In the terrestrial environment, I anticipated greater survival and mass at maturity for those cricket frogs reared and released at ponds with buffer zones than those that were reared and released in ponds without buffer zones because of the potential positive effects of greater food abundance and lower desiccation in buffer zones. For these same reasons, I predicted both juvenile and adult cricket frogs would have a greater affinity for unmown grass over mown grass when given a choice. I also anticipated cricket frogs overwintered in terrestrial enclosures with unmown grass would experience greater survival to and mass at spring emergence compared to those overwintered in mown grass. The main objectives of this research were to evaluate how common practices on managed areas can affect amphibians and to develop simple management strategies.
that can be implemented to improve the possibility that amphibians would use golf course ponds and other managed green spaces.

**Methods**

*Effects of Buffer Zones on the Aquatic and Terrestrial Life Stages of Amphibians on Golf Courses*

I collected 16 pairs of Blanchard’s cricket frogs in amplexus between 5 May 2008 and 14 June 2008 and 18 pairs between 25 June 2008 and 16 July 2008 from an uncontaminated pond at Miami University’s Ecology Research Center in Oxford, Ohio (U.S.A.). Each pair was kept in a 2 L plastic container with 5 centimeters of pond water overnight. The adults were returned to the pond the following morning after laying eggs. I collected 3 egg masses of green frogs from a forested pond in Miami University’s Natural Areas in Oxford, Ohio (U.S.A.). I kept Blanchard’s cricket frog and green frog eggs in the laboratory until use in the study. After hatching, tadpoles were fed TetraMin fish flakes ad libitum and water was changed daily until added to the field enclosures.

I placed tadpoles in enclosures at three local golf courses: Hueston Woods Golf Course (College Corner, Ohio, Butler County), Oxford Country Club Golf Course (Oxford, Ohio, Butler County) and Twin Run Golf Course (Hamilton, Ohio, Butler County). At each golf course, I used two ponds, one with an approximately 1 m grass buffer zone and one without. To create the buffer zone, golf course staff did not mow 1 m from the pond and allowed the grass to grow taller while ponds without a buffer zone were mowed all the way to the ponds edge.

On 1 July 2008, I added five green frog field enclosures to each of the ponds and on 22 July 2008, I added five Blanchard’s cricket frog field enclosures to each of the ponds. Enclosures were cylindrical and made of fiberglass screening (with a 1 mm X 2 mm mesh size). They were approximately 0.5 X 2 m and I placed a 1 x 3 m piece of hardware netting (approximately 4 cm X 4 cm mesh size) inside the enclosure to maintain the cylindrical structure. One meter from the bottom of the enclosure, I attached two flotation devices (i.e., pool noodles). Each enclosure was secured to a post in the water and the tops were rolled down and pinned to the posts with binder clips. I added 0.5 kg of deciduous leaf litter for refuge to each enclosure the same day they were placed in the ponds.
On 2 July 2008, we added 40 green frog tadpoles to each enclosure. Blanchard’s cricket frog tadpoles were added on two days because I was not able to collect enough individuals at once and to minimize differences in amount of time that tadpoles spent in the lab. Therefore, on 23 July 2008, I added 20 Blanchard’s cricket frog tadpoles to each of the cricket frog field enclosures and on 4 August 2008 I added another 20 cricket frog tadpoles to those same enclosures for a total of 40 tadpoles in each enclosure. This is within the range of natural densities for larval amphibians (Morin 1983).

I monitored enclosures daily for metamorphosed amphibians. I collected the metamorphs with a net and placed all individuals from the same enclosures into a plastic container with some pond water. Metamorphs were brought to the lab and I recorded their mass and time to metamorphosis. Each metamorph was given a permanent mark by toe-clipping, but no more than one toe on each foot. Toe clipping is a widely used method of marking amphibians (Smith 1987, Morris & Maret 2007, Johnson et al. 2008). I returned metamorphs to terrestrial habitat surrounding the golf course pond where they were reared in enclosures on the following day. Only Blanchard’s cricket frogs metamorphosed because green frogs typically overwinter in the pond as tadpoles and do not emerge as metamorphs until the following year. On 9 October 2008, I removed all enclosures from the pond and collected surviving tadpoles. Tadpoles were brought back to the lab and I weighed and developmentally staged (Gosner 1960) each tadpole. I returned the green frog tadpoles to their natal pond.

On 9 July, 23 July, 20 August, and 4 September I collected water samples from all green frog enclosures in each pond and on 30 July, 14 August, and 4 September I collected water samples from all Blanchard’s cricket frog enclosures in each pond. From each sample, I took 100 ml of water and filtered it onto glass filter paper. The filters were placed in buffered acetone and refrigerated for 24 hours. I then analyzed the sample by fluorometry to measure phytoplankton, which I used as an estimate of algal abundance. On these collection dates, I also measured temperature, pH, and dissolved oxygen (DO) in each pond.

On 18 September 2008, I sampled macroinvertebrates by doing three to six 2 m sweeps in each pond outside of the enclosures with a dip net. If anything was collected in the first three sweeps, I only sampled three times. However, if nothing was collected in the first three sweeps, I sampled an additional three times. Individuals were identified to taxonomic group in the field and released.
For Blanchard’s cricket frogs, I determined date at metamorphosis, mass at metamorphosis, and percent survival to metamorphosis. The percent survival to metamorphosis was the same as total survival for the cricket frogs as all surviving tadpoles had reached metamorphosis when I terminated the experiment. For green frogs, I calculated percent tadpole survival, mass, and developmental stage (Gosner 1960) at the termination of the study. Survival data was angularly transformed while mass, developmental stage, and date at metamorphosis data were log transformed prior to analysis.

Amphibian data were analyzed with one-way nested analyses of variance (ANOVA) with two error terms. To test for differences among golf courses I used the nested term buffer nested within golf course (i.e., buffer(golf)) as the error term. Also, to test for differences between buffered and non-buffered ponds, I used enclosure nested within buffer zone (i.e., encl(buffer)) as the error term to take into account that enclosures were nested within ponds. Survival was used as a covariate in the analyses for mass and time to metamorphosis for cricket frogs and for stage and mass at the end of the study for green frog tadpoles. Oxford Country Club was eliminated from analyses for cricket frogs because an animal chewed large holes in the enclosures and the tadpoles escaped.

Phytoplankton was angularly transformed prior to analysis and effects were tested with nested ANOVAs. To test for differences in phytoplankton abundance over time, the main effect of time was tested against the residual error (Time * Encl). Enclosures within pond (Encl[Buffer*Golf]) were tested against residual error, as were differences among golf courses over time (Golf * Time), and differences between buffered and non-buffered ponds among ponds over time (Buffer * Time[Golf]). The buffered effect was tested against enclosures within ponds (Encl[Buffer*Golf]).

DO, pH, and temperature were also analyzed with nested ANOVAs. To test for differences among golf courses, the golf course effect (Golf) was tested against pond (Buffer[Golf]). The buffer effect (Buffer[Golf]), or pond, was tested against the residual error, as were the effects of time and the interaction of the golf course effect over time.

I returned the following spring and summer to each golf course pond to locate adult Blanchard’s cricket frog survivors. I gave each juvenile a unique toe clip code the previous summer in order to identify them upon recapture. Each pond was visited at least three times in the early morning (between 6 and 7:00 a.m.). At this time, cricket frogs were calling at a
reference site and some of the golf course ponds, and I routinely found cricket frogs at these sites. I collected animals at some of the sites; however, none of the animals collected had any identifying toe clips. I was unable to locate any survivors from the enclosure experiment from the previous summer.

I was unable to locate any adult Blanchard’s cricket frog survivors on the golf courses in the summer of 2009. Therefore, I was unable to determine if the buffer zone had any effect on adult survival or reproduction. At Twin Run, 41 juveniles were released at the non-buffered pond and 17 were released at the buffered pond. At Hueston Woods Golf Course, 109 juveniles were released at the buffered pond and 48 were released at the non-buffered pond. At Oxford Country Club, 11 juveniles were released at both ponds. Eleven of the frogs released at each site were from a cattle tank study; the remainder was survivors from the larval study conducted on the golf courses in the summer of 2008. The number of cricket frogs released reflected the number collected from the enclosures at a given pond; so the number released varied at each pond.

Blanchard’s Cricket Frog Choice Between Mown and Unmown Habitat

On 23 July 2009, I collected 80 recently metamorphosed juvenile Blanchard’s cricket frogs from a pond on Miami University’s Ecology Research Center in Oxford, Ohio. I brought all the animals into the lab and held them in individual containers until tail resorption. After tail resorption, I obtained the animals’ mass and it was given an identifying toe clip by clipping no more than one toe on each foot. I held all animals in the lab and fed them small crickets ad libitum until the start of the study.

I constructed eight pens that were 3 m x 3 m with silt fencing that once buried, were approximately 1 m tall, in a grassy field at Miami University’s Ecology Research Center. In each pen, I mowed the grass in half of the pen and left the other half unmown. On 27 July 2009, I haphazardly assigned ten frogs to each of the eight pens and released them into the pens at 10:00 p.m. At that time, I also measured the soil moisture, relative humidity and temperature in each treatment, mown and unmown grass, in each pen. On 31 July at 1:00 p.m., I placed silt fence barriers between the mown and unmown grass in each pen. I collected the juvenile cricket frogs from each section and placed them in containers with damp paper towels together with all frogs caught from the same section in the same pen. I returned the animals to the lab and recorded their mass after which, they were released to the pond where I collected them. On 28 July I collected insects by sweep netting in a mown and unmown area adjacent to the pens. I sampled three
unmown transects and three mown transects with ten sweeps for each transect. After completing each transect, I collected insects in a large plastic Ziploc bag and placed each bag in the freezer. Each replicate was bagged separately. Insects were weighed and counted to determine potential food differences between mown and unmown habitat.

I determined that a cricket frog made a choice of one habitat over the other (mown vs. unmown grass) if it was located on one side of the barrier vs. the other. To test for the effects of cricket frog habitat on choice I analyzed the data with a Hotelling’s T-squared test using the proportion of individuals that were found on each side. I analyzed relative humidity, temperature, soil moisture, and insect biomass data with Hotelling’s T-squared test to test for differences between mown and unmown grass. I also calculated change in mass by subtracting the mass of each frog after the choice was made from its initial mass. To test for the effects of habitat choice on the change in mass I analyzed the change in mass with a one-way ANOVA.

Effects of Mown vs. Unmown Habitat on Blanchard’s Cricket Frog’s Overwinter Success

I reared larval anurans in mesocosms at the Ecology Research Center from hatching through metamorphosis in a separate experiment. Twenty-six clutches of Blanchard’s cricket frog eggs were collected using the same methodology as the aquatic and terrestrial stages on the golf course experiment. Clutches were mixed and added to 1000 L mesocosms and were reared in low or high density (20 or 60 tadpoles) in open canopy ponds. I used a hard wire netting with large mesh size that blocked 30% of incoming light to simulate open canopy ponds. Metamorphs were collected daily and held in the lab until tail resorption at which time I weighed the frog, assigned the animal to an enclosure, and gave it an identifying toe clip of no more than one toe on each foot. I mixed low and high-density animals in each enclosure. Animals were then held overnight and fed small crickets and fruit flies ad libitum. I released animals into their assigned enclosure the following night. Animals were released into the enclosures as they emerged from the mesocosms rather than held in the lab until all frogs metamorphosed in an effort to reduce stress on the animals.

Field enclosures were located in fields at Miami University’s Ecology Research Center. Each enclosure was 3 m x 3 m and constructed with aluminum flashing with a height of 0.63 m, buried 0.25 m in the ground. A pit, 48 cm deep and 33 cm in diameter, was dug in the center and a pine board, 39 cm X 54 cm, was placed over the top. I attached window screen perpendicularly
to the top edges of the enclosures to prevent escape. Enclosures were built in groups of four with four groups being constructed in replicated blocks at the Ecology Research Center. I used two of the enclosures in each group for this study. In each group, I mowed and maintained the grass in one enclosure at 4-5 cm. The other enclosure was mown in the early summer and left to grow all season, and the grass was approximately 30-38 cm tall when I started adding animals. Each pit was filled with 1 kg of leaf litter prior to the addition of animals to serve as a potential overwintering site. A small piece of plywood was placed over each pit. I released six recently metamorphosed animals into each of the pens. This density is below the maximum carrying capacity for similarly sized amphibians (Harper & Semlitsch 2007).

I added juveniles from 24 August-10 October 2009 as they reached metamorphosis and completed tail resorption. On 2 November 2009, I searched the enclosures for a pre-winter survival count. Animals were weighed and returned to the enclosures until the termination of the experiment. The following spring, I checked for animals in each pen once a week, starting 26 March 2010. When I found an animal, I brought it back to the lab and recorded the pen it was collected in, its toe clip code, and mass. The frog was then released at its natal pond. To test the effects of overwinter habitat (mown or unmown pen) and larval density on growth rate (change in mass/days in pen) and survival, I analyzed the data with two-way ANOVAs.

Results

Effects of Buffers on the Aquatic and Terrestrial Life Stages of Amphibians on Golf Courses

Green Frogs

There were significant differences in survival between buffered and non-buffered ponds among golf courses (buffer[golf]), with green frog tadpoles reared in buffered ponds on Hueston Woods and Twin Run having lower survival than those tadpoles reared in non-buffered ponds (Table 1; Fig. 1A). Also, green frog tadpole mass was significantly different between buffered and non-buffered ponds among golf courses with those animals reared in buffered ponds on Hueston Woods and Oxford Country Club having a greater mass at the end of the study than those tadpoles reared in non-buffered ponds on those courses (Table 1; Fig 2A). In contrast, green frogs at Twin Run reared in the buffered pond had a smaller mass than those animals reared in the non-buffered pond (Table 1; Fig. 2A). However, there were no significant differences in green frog tadpole survival (Table 1), tadpole mass (Table 1), or tadpole
developmental stage (Table 1) at the end of the study among animals reared in different golf courses. Also, there were no differences in developmental stage at the end of the study between buffered and non-buffered ponds (Table 2).

**Blanchard’s Cricket Frogs**

There were significant differences in survival to and mass at metamorphosis between cricket frogs reared in buffered and non-buffered ponds between golf courses (Table 1). Cricket frogs on Hueston Woods had greater survival (Fig. 1B) and were larger (Fig. 2B) when reared in buffered ponds than when reared in non-buffered ponds. In contrast, there were no differences in survival (Fig. 1B) or mass at metamorphosis (Fig. 2B) between frogs reared in buffered and non-buffered ponds on Twin Run Golf Course. Also, there were no significant differences in Blanchard’s cricket frog survival to, mass at, or date at metamorphosis (Table 1) among animals reared in different golf courses. There were also no significant differences in cricket frog date at metamorphosis between animals reared in buffered and non-buffered ponds (Table 1). Oxford Country Club was left out of these analyses because an animal chewed large holes in the enclosures in these ponds and the animals escaped.

**Water Quality**

Phytoplankton abundance was not significantly different among enclosures within ponds (Table 2). There were significant differences among golf courses with Oxford Country Club having the greatest abundance and Twin Run having the least (Table 2), between buffered and non-buffered ponds among golf courses (Table 2, Table 3), and also over time (Table 2). There were also significant interactions with golf course and time (Table 2), and between buffered and non-buffered ponds among golf courses over time (Table 2, Fig. 3). In general the buffered pond at Hueston Woods had greater phytoplankton abundance than the non-buffered pond, while the non-buffered pond at Oxford Country Club had greater phytoplankton abundance than the buffered pond. Also, phytoplankton abundance generally increased over time (Fig. 3).

Dissolved oxygen was not significantly different among golf courses or over time (Table 2). There were significant differences between buffered and non-buffered ponds among golf courses with the non-buffered pond on Twin Run having higher DO than the buffered pond and the opposite effect between the ponds at Hueston Woods and Oxford Country Club (Table 2, Table 3). There were also significant differences among golf courses over time with DO
generally decreasing and then increasing over time at Twin Run, increasing over time at Hueston Woods, and decreasing over time at Oxford Country Club.

pH was not significantly different among golf courses or among golf courses over time (Table 2). There were significant differences in pH over time (Table 2) with pH increasing between the first two sampling dates and then holding steady, and also between buffered and non-buffered ponds among golf courses with the non-buffered pond at Twin Run having a higher pH than the buffered pond and the opposite effect between ponds at both Hueston Woods and Oxford Country Club (Table 2, Table 3).

Temperature was not significantly different among golf course (Table 2). There were significant differences in temperature over time with it fluctuating between 25-28.1°C as well as among golf courses over time (Table 2). There were also significant differences between buffered and non-buffered ponds among golf courses with the non-buffered pond at Twin Run having a higher temperature than the buffered pond and the opposite effect between ponds at Hueston Woods and Oxford Country Club (Table 2, Table 3).

**Habitat Choice**

Juvenile cricket frogs chose unmown habitat more than mown habitat when given a choice (Table 4; Fig. 3). Relative humidity was significantly higher in unmown grass (55.8% +/-0.901) than in mown grass (49.712% +/-1.44) (Table 4). Soil moisture did not differ significantly between mown and unmown grass (Table 4). There was significantly lower insect biomass (Table 4) in mown (0.1344 g +/- 0.0264) compared to unmown grass (0.4180 g +/- 0.0606).

**Overwinter Success**

During the pre-winter survival count, I located five animals in mown pens (25%), and zero animals in unmown pens (0%). At spring emergence, I located one animal in a mown pen and zero animals in unmown pens.

**Discussion**

The results of these studies indicate buffer zones may benefit some amphibians. Larvae of both Blanchard’s cricket frogs and green frogs were able to survive in golf course ponds. The buffer zone increased survival to metamorphosis for Blanchard’s cricket frogs and decreased the
survival of green frog tadpoles on some golf courses. Also, juvenile cricket frogs generally preferred unmown grass over mown grass, which may indicate a benefit to these animals in the unmown grass. Juvenile survival has been implicated as the most critical life stage for maintaining viable populations of other species of amphibians (Biek et al. 2002; Conroy & Brook 2003) because fecundity is relatively high, allowing for tolerance of increased larval mortality. If conservation efforts are geared to the more critical life stage of juvenile survival, rather than larval survival, the buffer zone may be important in providing terrestrial habitat regardless of the effects seen during the larval stage.

A lack of differences in larval survival between buffered and non-buffered ponds on some courses, such as Oxford Country Club (Fig. 1A), could imply the buffer zone was not wide enough to affect contaminant levels. Increasing width of buffer zones has been shown to reduce pesticides from runoff (Patty et al. 1997; Barfield et al. 1998; Krutz et al. 2005); however, a 2 m grass buffer zone reduced pesticide concentrations by at least 53% after application (Rankins et al. 2001). Therefore, I would expect that a >1 m buffer zone surrounding the golf course ponds in the present study would affect contaminant levels; however, I did not measure contaminant levels. Also, the buffer zones were implemented in late March/early April and the enclosures were placed in the ponds only a few months later. This may not have been enough time to establish buffer zones or prevent contaminated runoff from entering the ponds in the early spring. However, I did find some differences in survival and mass of tadpoles between buffered and non-buffered ponds, which suggests that this length of time was sufficient.

I expected some differences between golf course sites because each site is managed differently with varying types and levels of pesticides and fertilizers, mowing regimes, and pond uses. Some ponds were used simply as water hazards, an obstacle the golfers must avoid, while other ponds were used as a watering source for the course. Some of the ponds were very close to, or adjacent to greens, which are heavily managed, while others were in areas were lower management occurred. Hueston Woods used a more naturalistic approach in the management of the golf course, compared to the two other courses in this study, and was already leaving large tracts of land unmown. Twin Run seemed to use the most chemical control and in fact dosed the buffered pond with copper sulfate during the course of my study. Finally, Oxford Country Club used the ponds on the course mostly for irrigation and thus the water levels fluctuated regularly. These differences in management among the golf courses may explain some of the differences
seen in survival between buffered and non-buffered ponds on the golf courses. For example, on Twin Run both green frogs and cricket frogs experienced similar survival in buffered and non-buffered ponds most likely because the buffer zone was compromised with the addition of chemicals into the buffered pond.

I predicted both species would show positive effects on survival when reared in ponds with buffer zones versus being reared in ponds without buffer zones; however, green frog tadpoles had greater survival in non-buffered ponds (Fig. 1A) while cricket frogs had greater survival in buffered ponds on some golf courses (Fig. 1B). I did detect some differences in temperature, DO, and pH; however, these differences could be due to taking measurements at different times throughout the day and do not seem large enough to be biologically relevant. I anticipated a reduction in contaminant levels with buffer zones and thus those ponds may have returned to a more natural setting, with lower food resources and increased predator abundance as suggested in Boone et al. (2008). I did detect some differences in phytoplankton abundance; however, these differences do not match the differences seen in survival. For example, Oxford Country Club had the greatest abundance of phytoplankton (Fig. 3) but green frog tadpoles in the non-buffered pond had the lowest survival of the non-buffered ponds (Fig. 1A). Also, the buffered and non-buffered ponds at Hueston Woods had similar phytoplankton abundances (Fig. 3) yet cricket frogs had much greater survival to metamorphosis in the buffered pond than the non-buffered pond (Fig. 1B). Also macroinvertebrate predator densities were similar between buffered and non-buffered ponds on all three golf courses and therefore, do not explain the differences seen in survival. However, I did not measure fish or other predator abundance, which would have been excluded from enclosures, or periphyton, which is considered a major food source of anuran larvae (Johnson 1991, Kupferberg 1997, Hoff et al. 1999). Green frogs are commonly found in landscapes dominated by human activity (Scott et al. 2002; Vredenburg 2004; Porej & Hetherington 2005) and are potentially less sensitive to contaminants than other amphibian species (Bridges & Semlitsch 2000, Relyea 2004, Ade et al. 2010); therefore, green frogs may have experienced greater survival in non-buffered ponds because they have an advantage in a contaminated system. Ade et al. (2010) found cricket frogs to be more sensitive to both the insecticide imidaclorpid and aquatic predators than green frog larvae; therefore, increased survival of cricket frogs in the buffered ponds could indicate lower contaminant levels than in the non-buffered ponds. Regardless of the different effects of the buffer zone on the
survival of green frogs and cricket frogs, in natural ponds, survival to metamorphosis is typically 2-5% (Semlitsch 1987, Boone et al. 2004). Larval green frog survival and cricket frog survival to metamorphosis in my study was almost always well above this range (Fig. 1A, B), which supports the findings of Boone et al. (2008) that some amphibians can complete larval development in golf course ponds with higher survival than those reared in more natural ponds, and suggests that changes in the terrestrial environment can have effects on the larval stage.

As I predicted, both species had increased mass either at the end of the study (green frogs) or at metamorphosis (cricket frogs) when reared in ponds with buffer zones than when tadpoles were reared in ponds without buffer zones on some courses (Fig. 2A, B). The larger mass was not a result of differences in density between buffered and non-buffered ponds because survival was used as a covariate in the analysis. Cricket frogs reared in buffered ponds on Hueston Woods golf course experienced much larger growth than those reared in non-buffered ponds; however, there was no difference in mass between animals reared in buffered and non-buffered ponds on Twin Run. However, there were not major differences in phytoplankton between buffered and non-buffered ponds on Hueston Woods (Table 3, Fig. 3). Therefore, differences in phytoplankton do not explain the differences in mass, which suggests that tadpoles are eating periphyton rather than phytoplankton (Hoff et al. 1999, Altig et al. 2007). I did attempt to measure periphyton but was unable to scrape algae from the sides of the enclosures. However, during the study, copper sulfate was directly applied to the buffered pond on Twin Run, a contaminant known to affect amphibian growth (García-Muñoz et al. 2009). Copper sulfate application could explain the lack of a buffer response from cricket frogs at Twin Run, as the buffer was unable to filter the contaminant as it was directly applied to the water. Both species had greater mass either at the end of the study, or at metamorphosis, which could provide both species with fitness advantages later in life such as shorter time to reach sexual maturity (Smith 1987; Harper & Semlitsch 2007) or larger size at first breeding (Semlitsch et al. 1988; Berven 1990). Therefore, the buffer zone may have negative or neutral effects on survival in the larval stage of green frogs, but if greater mass at the end of the study leads to greater mass at or shorter time to metamorphosis, buffer zones could have positive implications for juvenile and adult green frogs. This supports previous research, which has found that the juvenile stage is the critical life stage for maintaining populations of some amphibians (Biek et al. 2002; Conroy & Brook 2003). However, green frogs in the present study were only reared in the ponds for a few
months, a fraction of their larval period. If the green frog tadpoles had been reared with and without buffer zones through metamorphosis, I may have seen different results. Also, because cricket frogs experienced increased survival and increased mass at metamorphosis on some golf courses when reared in ponds with buffer zones, I would expect to find more cricket frog populations in ponds with a terrestrial buffer zone.

Amphibians require terrestrial habitat to feed, grow, and overwinter, but terrestrial habitat is often overlooked when managing for amphibians (Semlitsch & Bodie 2003) and understanding how changes in the terrestrial environment affect amphibian populations was a major objective of this study, although the results were inconclusive. In my study, juvenile cricket frogs generally preferred unmown grass to mown grass when given a choice (Fig. 3), which suggests the unmown grass provides the cricket frogs with some advantage, most likely increased food resources and escape from desiccation (both of which I documented), over the mown grass. I may not have been able to detect a significant preference for the unmown grass because it may be more difficult to relocate animals in the unmown grass than in the mown grass. Therefore, those animals that I did not recover were probably in the unmown grass leading to an incidental bias toward capturing the frogs in the mown grass. However, there was a strong trend toward cricket frogs preferring unmown habitat vs. mown habitat. Birchfield & Deters (2005) found that adult green frogs traveled along the mow line between mown and taller, unmown grass and thought the animals would hop into unmown grass if a person walked near, suggesting that the animal was avoiding potential danger and seeking refuge in unmown grass. In the present study, the unmown grass was more humid and had more insects than the mown grass, indicating that the unmown grass would likely provide greater opportunities to forage and prevent desiccation than the mown grass.

Cricket frogs normally overwinter near the edges of ponds in crayfish burrows or cracks with moist soils (Irwin et al. 1999). Because unmown habitat maintains greater moisture than mown habitat, I expected cricket frogs would have greater survival and growth when reared in unmown terrestrial pens. My low recovery in the terrestrial pens from the overwintering study may be due to a lack of suitable overwintering sites and/or a result of escape. Also, cricket frogs do not tolerate hypoxic conditions (Irwin et al. 1999), and when I searched for survivors in the spring, I observed many of the pits in the terrestrial pens were half full of water. This water may have had very low oxygen levels; therefore, any cricket frogs in the pit may have died from
hypoxic stress. Cricket frogs in natural populations experience very high mortality prior to overwintering, 50-97% of the population in some places (Gray 1983, Burkett 1984). High mortality or escape may explain the lack of survivors on the golf courses in the spring of 2009 and the low pre-winter survival count in the fall of 2009. Cricket frog mortality could have been a result of predation as cricket frogs have many predators, such as larger frogs, birds, fish, snakes, and mammals (Gray et al. 2005), which may have been present on the golf courses. In fact Scott et al. (2002) found that most amphibians on golf courses were green frogs and bull frogs, which are known to prey on smaller amphibians, such as cricket frogs. Also, buffer zones may provide better habitat for other amphibian predators such as mammals and snakes. Even if other amphibians did not prey on the cricket frogs, Sams & Boone (2010) have shown that recently metamorphosed American toads altered their behavior in the presence of recently metamorphosed green frogs and this behavioral change resulted in reduced size and higher mortality of American toads. A reduction in size could negatively affect a cricket frog’s ability to overwinter and resulted in greater mortality, preventing me from finding animals the following spring. Cricket frogs may also have migrated to other ponds.

My study provides further evidence that larval requirements can be met by many managed wetlands, but the impact of changes in the terrestrial habitat on amphibians is still not well understood. My results provide some indication that unmown grass could provide suitable habitat based on cricket frog preference for this habitat. Future work should focus on whether or not amphibians can persist in the terrestrial environment on golf course and the minimum buffer zone necessary to sustain populations. Many golf course superintendents are not able to leave a buffer zone around the entire perimeter of a pond. Therefore, determining the amount of buffer zone needed around the perimeter of the pond would be useful, especially if buffer zones do not have a major impact on larval survival as my data suggests. This would provide golf course managers with more detailed guidelines on how to implement buffer zones on their golf courses. Also, studies evaluating if there is an optimum height for grasses to filter contaminants and also provide essential habitat for amphibians would be useful. Evaluating the effects of buffer zones on other grassland species of amphibian and wildlife would also provide more evidence to support the need for buffer zones on golf courses. Golf courses could serve as a model for other managed green spaces and this management strategy could be implemented on many types of aquatic sites. This strategy could provide site managers with an opportunity to reduce
environmental impacts and also slow or stop the decline of threatened species like the Blanchard’s cricket frog.
Literature Cited


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<td>F</td>
<td>p</td>
</tr>
<tr>
<td>-----------------------</td>
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<td>------</td>
</tr>
<tr>
<td>Choice</td>
<td>1,7</td>
<td>0.5629</td>
<td>5.49</td>
<td>0.0516</td>
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<tr>
<td>Relative Humidity</td>
<td>1, 7</td>
<td>0.2376</td>
<td>22.46</td>
<td>0.0021</td>
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<tr>
<td>Soil Moisture</td>
<td>1, 7</td>
<td>0.9635</td>
<td>0.26</td>
<td>0.6228</td>
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<tr>
<td>Insect Mass</td>
<td>1,2</td>
<td>0.0850</td>
<td>21.52</td>
<td>0.0435</td>
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<tr>
<td>Change in Mass</td>
<td>1</td>
<td>0.0001</td>
<td>0.11</td>
<td>0.7392</td>
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</tr>
<tr>
<td>Error</td>
<td>39</td>
<td>0.0004</td>
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</tr>
</tbody>
</table>
Figures

Figure 1.
Figure 2.

A. Mass (g) comparison among different conditions.

Buffer (Golf): $F_{3,19} = 9.96$, $P = 0.0004$
Surv covariate $P = 0.9392$

OCC: Hueston Woods: Twin Run

B. Survival to Metamorphosis comparison among different conditions.

Buffer (Golf): $F_{2,16} = 9.02$, $P = 0.0024$

Hueston Woods: Twin Run
Figure 3.

Buffer * Time (Golf)

$F_{15,120} = 7.68$

$P < 0.0001$

Phytoplankton Abundance (μg/L)

10-July 24-July 31-July 15-August 21-August 5-September

Time
Figure 4.

![Graph showing the proportion choosing habitat between mown and unmown areas.](image-url)

- P = 0.0525
- Wilks' λ = 0.5629