Sex ratios in Spring Peepers in Wetlands from Natural and Human-Dominated Landscapes

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Statement of Work

For over a decade now, there has been concern that human activities are contaminating wetlands with endocrine disrupting chemicals (EDCs; Reeder et al. 1998). These EDCs have hormone-like effects on wildlife, like frogs. These hormonal effects can include the production of eggs in male testes (testicular oocytes; Hayes et al. 2003) and the feminizing of sex ratios where proportionally more females are born into a cohort (Brande-Lavridsen et al. 2008, Langlois et al. 2010). While most field research has centered on how agricultural wetlands and associated pesticides (e.g., atrazine) affect frogs (Hayes et al. 2003, Murphy et al. 2006, Papoulias et al. 2013), there is recent evidence that suburban wetlands may also be problematic. Skelly et al. (2010) found that male green frogs (*Rana clamitans*) had higher frequencies of testicular oocytes in suburban and urban wetlands as compared to nearby agricultural or forested wetlands, indicating that everyday human living is affecting the reproductive health of wetland wildlife. My own master's research evaluated sex ratios of green frog metamorphs (the developmental stage where tadpoles become terrestrial frogs) in suburban and forested wetlands. I found that suburban wetlands produce proportionally more female metamorphs than forested wetlands; that suburbanization *feminizes* the sex ratio of baby frogs. Furthermore, I found that sex ratios were correlated with the amount of garden lawn cover surrounding wetlands, where increasing lawn cover was correlated with a higher proportion of females born. This project also found that the number of phytoestrogens in each wetland was correlated with the amount of lawn cover surrounding a wetland. Phytoestrogens are EDCs that are actually made by living plants (Benlhabib et al. 2002, Skipor et al. 2012) and so this result indicates that lawn and garden plants may potentially be potent sources of EDCs in suburban wetlands (Lambert et al *In Prep*). If seemingly innocuous activities, such as a homeowner's decision of which plants to garden, can have drastic effects on wetlands and the health of amphibians, then it will be important to take a closer look as to how human land use affects wetland wildlife. My next research goals are 1) to understand if the sex ratios of other amphibian species are also affected by human land use and 2) to find an amphibian species that will be a useful model for experiments related to endocrine disruption in wetlands.

Although many laboratory studies have shown that EDCs cause female-biased sex ratios in amphibians (e.g., Brande-Lavridsen et al. 2008, Langlois et al. 2010) few studies have evaluated sex ratios in wetlands affected by human land use. In fact, we have only a minimal understanding of amphibian sex ratios in "natural" wetlands (but see Berven 1990, Lambert et al *In Prep*). It is therefore important to understand the sex ratios of amphibians in natural, undisturbed habitats in addition to sex ratios in amphibians from wetlands affected by humans. While it is assumed that natural sex ratios should yield equal proportions of males and females (a theoretical 50:50 split), my master's research found green frog sex ratios in forested wetlands were 37% (± 4%) female, implying that the "natural" sex ratio in this species is actually dominated by males. Any study that examines amphibians in altered wetlands should therefore also evaluate amphibians in less-disturbed wetlands. However, this is rarely done and most endocrine disruption studies only use one control (natural) wetland (Hayes et al. 2003, Papoulias et al. 2013) and my master's project is one of the first, to my knowledge, to use multiple natural

wetlands. In 2014, I aim to understand how primary sex ratios of another amphibian species varies along gradients of both natural land use (forest types) as well as human land use (e.g., suburban and agricultural).

Although my prior work focused on green frogs in forested and suburban wetlands, this species is not a tractable animal for experiments. Green frog larvae take over a year to metamorphose (Klemens 1993). This means that both laboratory and field experiments are particularly challenging with this species. My future work will require experiments to understand which factors (e.g., particular suburban garden plants) affect wetlands and amphibian sex ratios, thereby necessitating a experimental species that metamorphoses more quickly. Therefore, my secondary aim is to find another amphibian species which has sex ratios that vary along environmental gradients and which metamorphoses within several months, making this species a more useful experimental model for understanding how human land use impacts wetland health.

To address both of these goals, I will target spring peepers (*Pseudacris crucifer*). Spring peepers are distributed in wetlands across eastern North American, from the Gulf of Mexico into Canada (Klemens 1993). This species can be found across a variety of natural gradients (e.g., tree canopy cover; Skelly et al. 2002) as well as human land uses like cities, suburbs, and golf courses (Klemens 1993, Bunnell and Zampella 1999, Gibbs et al. 2005). Furthermore, peepers metamorphose in just a few months (Klemens 1993). These characteristics make spring peepers an ideal species to study sex ratio dynamics in response to wetland alteration.

This year I will study sex ratios in peepers from a variety of natural and altered wetlands. I will locate wetlands with spring peepers using published localities from Yale Peabody Museum specimens, from reports of spring peeper breeding activity through the online Amphibian Tracker hosted by Mark Urban's lab, and from sites that I am already familiar with from my previous research. Because some environmental factors, like pH and temperature, can alter sex ratios in natural populations of turtles and fish (Baroiller et al. 2009, McGaugh et al. 2011) it will be important to understand if natural environmental factors influence peeper sex ratios and how human land use might change sex ratios from this natural state. At each sampled wetland, I will measure tree canopy cover with a densiometer as well as pH, conductivity, and temperature. Because my previous work (Lambert et al *In Prep*) shows that frog sex ratios are increasingly feminized with increasing suburban lawn cover around a wetland, I will use a geographic information system (GIS) to measure the amount of different land covers (e.g., forest, lawn grass, crops, and impervious surfaces) surrounding each wetland. Using generalized linear models (GLMs) with a binomial distribution and a logit link function (*sensu* Hardy 2002) I will determine which field measurements and GIS land cover measurements best predict changes in spring peeper sex ratios. I hypothesize that, because other animal sex ratios are affected by pH or temperature (Baroiller et al. 2009, McGaugh et al. 2011) and because other frog species show sex ratio deviations in response to human land cover around wetlands (Lambert et al *In Prep*), spring peepers will exhibit sex ratio patterns that correlate with natural environmental factors as well as anthropogenic alteration of the landscape surrounding wetlands.

Completed Work/Prior Work

My master's research (Lambert et al *In Prep*) found that suburbanization feminizes green frog sex ratios. Using GIS analysis I found that the degree of sex ratio feminization increased with increasing lawn grass cover around wetlands, indicating that suburban gardens may be a source

of endocrine disrupting chemicals. Interestingly, I also found that green frog sex ratios were not equal between the sexes, but rather were consistently around 1/3 female. This indicates that natural sex ratios in amphibians may not always be 50:50 and that it is important to understand the natural, or background, sex ratio before we understand how human land use influences wetland wildlife. This prior work has led to my proposed research. Currently, I am studying spring peeper specimens at Yale's Peabody Museum of Natural History so that I familiar with the ovaries and testes in this new species before field sampling this spring and summer.

How the Study Benefits Coastal Wetlands

For over a decade, there has been concern of EDCs contaminating surface waters and affecting the reproductive health of wetland wildlife (Reeder et al. 1998, Hayes et al. 2003, Skelly et al. 2010). While there has been a strong focus on endocrine disruption in amphibians from agricultural wetlands (Hayes et al. 2003, Murphy et al. 2006, Papoulias et al. 2013) there is increasing evidence that suburban neighborhoods are also having an effects on wetlands and amphibian reproductive health (Skelly et al. 2010, Lambert et al *In Prep*). My research will be an important step in identifying what aspect of human land use affect the reproductive development of wetland wildlife. Amphibians are often called "canaries in a coal mine" as they can be used as indicators of wetland contamination (Kerby et al. 2009). Although EDCs are well-known to cause reproductive deformities in wetland wildlife (Skelly et al. 2010, Lambert et al *In* Prep), these chemicals can have an array of impacts ranging from altering methane production in wetland bacteria (Ruan et al. 2013) to affecting invertebrate reproductive viability (Oetken et al. 2004). Understanding how spring peeper sex ratios vary with human land use may thus be an important way of monitoring how contamination affects the biology and function of wetland ecosystems. Sex ratio dynamics are ecologically important for animal populations (Sakisaka et al. 2000, Monclus et al. 2014, Pryke et al. 2014) and are informative of EDC contamination in disturbed wetland systems (Vajda et al. 2008, Lambert et al *In Prep*); my research will therefore help understand how humans are affecting the health of wetlands and wetland wildlife.

How the Funds Will be Used

I will use these funds to purchase new waders and a dip net, amphibian sampling vials, a microscope camera to photograph and document amphibian gonads, transportation costs to study sites, as well as room and board at the Yale Myers Forest Research Camp.

Communicating Research with a Broader Audience

I plan to share my results through three avenues: educational lectures and wetland walks, through the Peabody Museum of Natural History, and by popular media. The Yale Myers Forest in northeastern Connecticut, one location for my field sampling, hosts events for local landowners and other New England residents to learn about land management and conservation. Previously (see CV), I have taken boy scouts and local Connecticut land owners on walks through the Yale Myers Forest. On these walks, I discussed amphibian and wetland ecology in the context of forest management practices, and how logging influences wetland ecology. This year, I will lead another walk but I will focus on how land use affects wetlands and amphibians. I am also heavily involved with the Yale Peabody Museum. For two years I have been preparing and maintaining amphibian, reptile, and fish specimens. Through the Peabody Museum, I will give a seminar on how human land use affects wetland ecology and amphibian development.

Furthermore, I am a participant in the Peabody EVOLUTIONS program which provides high school students the opportunity to participate in laboratory research. Last summer I trained two high school students how to curate museum specimens. In the summer of 2014, I will be working with two more high school students through EVOLUTIONS, but focusing this year both on laboratory work as well as wetland field sampling. Finally, I will use popular media to communicate my research. I will report about my research in *Sage Magazine*, an environmental publication originally started at Yale University and which has a global readership. My colleague Geoff Giller, former editor-in-chief of *Sage Magazine* as well as current journalist for *Scientific American*, and I have used multimedia to reach broader audiences. For example, in 2012 we used video footage to raise research funds (over $7,000, see CV) on a crowd-funding website. We also recently used time-lapse photography and Go Pro ® cameras to document the preparation of venomous king cobra and fer-de-lance snake specimens for the Yale Peabody Museum to use on its website as well as on display in the museum. I will continue to work with Geoff Giller to produce multimedia documenting my wetlands and amphibians. By combining educational walks, my affiliation with the Peabody Museum, and popular media I will be able to communicate my research to a wide audience.

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