ANALYSIS OF BIRD BIODIVERSITY WITHIN AND AMONG ISOLATED WETLANDS ACROSS SPACE AND TIME

A proposal for research
submitted in partial fulfillment
of the requirements for the
degree of Doctor of Philosophy

by

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1. Project Summary

Geographically isolated wetlands, or GIWs, are a unique class of wetlands which occur around the globe and have periodic or permanent, hydrologic isolation from permanent water bodies for all or part of the year. They are biodiversity hotspots supporting many at-risk plant and animal species. Current research on bird biodiversity in these wetlands has focused on regional studies that compare species richness in wetlands of the same typology across small spatial scales. I plan to utilize a mixed methods approach to synthesize existing research to identify overlaps, and gaps in research on these systems from across the conterminous United States. I also plan to use a multiscale approach, looking at bird biodiversity in these systems from the macro-ecological scale, across ecoregions and wetland typologies, to better understand the impacts of human altered land at the local and regional scale. I will investigate how biodiversity in these systems across the US capture both taxonomic and functional richness, to better evaluate the contributions of these systems from a functional community perspective. I will use big data from eBird to analyze beta diversity metrics at GIWs to understand if these systems capture a larger proportion of the regional species pool than expected based on biodiversity estimates at nearby eBird hotspots. Finally, I'll use traditional field methodologies to investigate temporal and spatial turnover at multiple scales along the rural-urban gradient to understand if these systems experience habitual use by different species for all our part of the day, and year. Together, utilizing synthesis, macro-scale biodiversity assessments, land cover impacts, and local scale turnover; I will better contribute to our scientific understanding of bird biodiversity in the underappreciated, yet numerous geographically isolated wetland landscape.

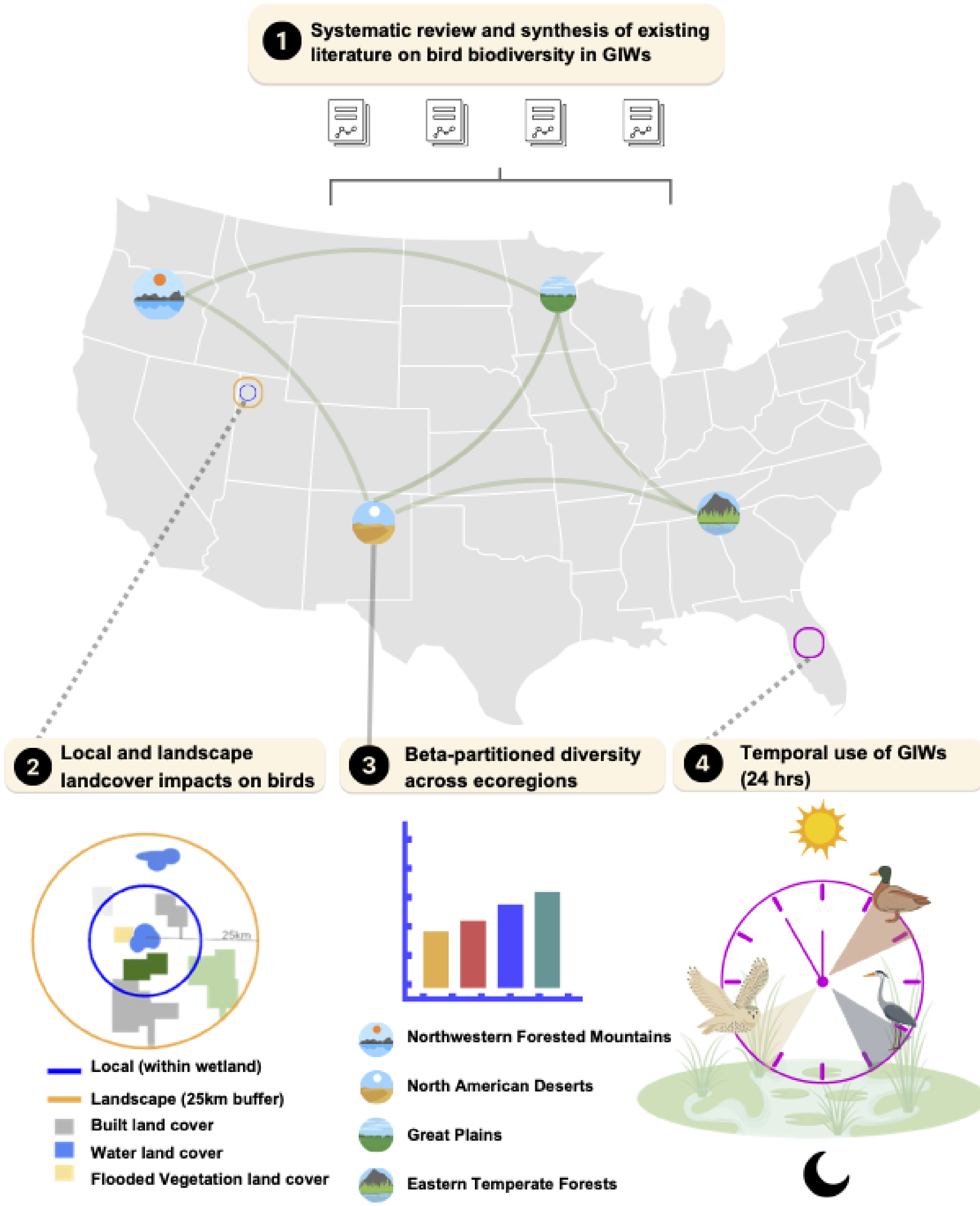

Figure 1. Graphical abstract of 4 research objectives. Overall objectives of this dissertation project are to (1) conduct a synthesis of bird biodiversity in isolated wetlands, (2-3) two different macro-scale analyses of diversity across the United States, and (4) a local investigation of temporal turnover in the 24-hour period of the day.

2. Specific Aims

The goal of my dissertation is to quantify and document patterns of isolated wetland use by birds across different spatial and temporal grains. Specifically, I aim to understand how isolated wetlands contribute to bird biodiversity both taxonomically and functionally, as well as understand critical components of temporal and spatial turnover that contribute to especially unique bird community composition and enhance ecosystem resilience and conservation of rare and threatened species. This project will help determine the isolated wetland land cover attributes that increase biodiversity, how conservationists should value these systems in both rural and urban contexts, and how they are being used seasonally and throughout the 24-hour period of the day. Using multiple methodologies across spatial scales from Gainesville to the entire conterminous United States, I've organized my research into four overarching research objectives:

Research objective 1: Review the state of current knowledge on bird biodiversity in isolated wetlands.

Research objective 2: Determine how local and landscape land cover attributes impact both overall taxonomic diversity as well as diversity of specific functional groups in GIWs across the United States.

Research objective 3: Assess the relative contributions of isolated wetlands to bird biodiversity regionally, for example their beta-partitioned diversity estimates, and determine whether different regions support higher beta-partitioned diversity.

Research objective 4: Measure the degree of temporal species turnover throughout the day in isolated wetlands along a rural-urban gradient.

3. Background and Significance

3.1 Geographically isolated wetlands serve multiple ecosystem functions and services

Wetlands are globally important habitats, providing important habitat and ecosystem services, yet globally, we have lost almost 21% of inland wetlands since 1700 (Fluet-Chouinard et al. 2023). Geographically isolated wetlands, or GIWs, are a specific type of wetland which are surrounded by upland habitat (Tiner 2003), and they provide an assortment of ecosystem services including biochemical processing, flood protection, and habitat for flora and fauna. GIWs represent an estimated 29% of all wetland types in the United States and may represent up to 16% of total wetland area in the United States (Lane and D'Amico 2016, Cohen et al. 2016). GIWs are incredibly important hydrologically for their contributions to flood control, water storage, and chemical processing (Golden et al. 2017, Lane et al. 2023). Although numerous, these wetlands have been relatively understudied for their contributions to biodiversity, and currently lack federal protections (Leibowitz and Nadeau 2003), even though they are important habitats for many different species, including birds (Semlitsch and Bodie 1998, Snodgrass et al. 2000, Czapka and Kilgo 2011).

3.2 Moving past small-scale wetland site studies to big data and synthesis

GIWs have a wide distribution across the United States, but due to varying typology, and changes in nomenclature, synthesizing biodiversity studies in these systems has historically been difficult (Leibowitz and Nadeau 2003, Mushet et al. 2015, Leibowitz 2015). Systematic reviews, bibliometric studies, and meta-analysis have all become important synthesis tools in ecology to review literature on topics of interest that require formal synthesis of analyses, to identify common patterns across spatial and temporal scales (Zhang et al. 2012, Guan et al. 2019). Historically, logistical constraints in how GIWs are referenced in the literature have hampered synthesis efforts (Zedler 2003, Mushet et al. 2015, Leibowitz 2015). GIWs have different names depending on their ecology and occurrence; for example, there are vernal pools in the Northeast, prairie potholes in the Midwest, pocosins in the Southeast, and playas in the Southwest–all of which are geographically isolated wetlands (Cowardin 1979, Tiner 2003, Comer et al. 2005). Additionally, prior to the term “geographically isolated wetland” offered by Leibowitz and Nadeau in 2003, they were not referenced under a common larger umbrella categorization; instead, they utilized the natural community typing, or a variation of “isolated wetland”, “temporary pond”, as well as many other terms (Leibowitz and Nadeau 2003). Synthesis, leveraging modern technologies, computational methods, and overall breadth will help to overcome research silos in GIW systems to understand their combined contribution to bird biodiversity across greater spatial scope.

Ecologists use synthesis and review papers to achieve generality in results which can help integrate multiple field studies with limited spatial or temporal scope (Spake et al. 2022). There is currently no research that documents the contribution of GIWs to bird biodiversity across large spatial scales, or between the various typologies of GIWs in the United States. Furthermore, Tiner (Tiner 2003) outlines upwards of 20 GIW types, some with overlapping ranges, others that are confined to specialized geographies. Analyzing these GIW types together allows us to either suggest these systems act similarly across time and space or are especially unique and functionally valuable depending on the type, size, general vegetation characteristics, or geographic position they occupy. By analyzing literature using both bibliometric analyses of citations, authors, and publication countries, as well as conceptual mapping that targets areas of research overlap, or disjointed areas of research; we can achieve research weaving, with more impactful synthesis findings (Nakagawa et al. 2019, Spake et al. 2022). Lastly, macro-scale synthesis can help clarify biodiversity value of these systems to policymakers and decision-makers who may be more likely to act on generalizable scientific phenomena to inform policy decisions, rather than small, local scale studies that lack explanatory power at national or international scales.

In addition to synthesis and meta-analysis techniques, another commonly employed methodology that can be used to understand GIWs is citizen or community science data. This data is becoming an increasingly useful tool for researchers to conduct research across larger spatial scales by harnessing checklists or observations recorded by individuals into databases such as eBird or iNaturalist (Bonney et al. 2009, Fink et al. 2021). Data submitted by community scientists can help monitor biodiversity at scales (temporal and spatial) not accessible through traditional field methodology (Callaghan and Gawlik 2015, Callaghan et al. 2018). Birds are an easily monitored taxa with both traditional field monitoring, and big data from platforms like eBird and iNaturalist. However, without contextualizing the biodiversity values of these systems through beta-partitioned diversity, and species turnover, we cannot identify priority areas for conservation. Using community science data, we can achieve explanatory and statistical power, not achievable through traditional field methodologies due to financial constraints of carrying out continent-wide studies. I will use data collected from eBird to determine the value of GIWs across the conterminous United States to understand if their biodiversity value changes depending on their geographic position and ecoregion.

3.3 Leveraging data across large geographic extents with local-scale relevance

The value of GIWs to biodiversity is substantial for many rare and endangered species that specialize on these habitats for all, or part of their life-history (Semlitsch and Bodie 1998). GIWs have also been shown to support higher richness and abundance of birds, especially waterfowl in the Midwest, compared to surrounding upland habitats, making their projected future loss a serious conservation priority (Uden et al. 2015, Cerda-Peña and Rau 2023). Land development, urbanization, and agriculture have destroyed many of these wetlands, resulting in the construction, restoration, and sometimes preservation of GIWs for their ecosystem service benefits (Brown and Bedford 1997, Marty 2015, Fluet-Chouinard et al. 2023). Through restoration activities, GIWs now occur both naturally, and as restored/constructed habitats across the landscape and rural-urban gradient (Tiner 2003, Lane and D'Amico 2016). However, most biodiversity studies have focused on a single type of GIW (e.g., Carolina Bay [Czapka and Kilgo 2011]), or GIWs in a single state or region, utilizing traditional field methodology to test hypotheses (Riffell et al. 2006, Uden et al. 2015, Herteux et al. 2020).

Understanding how changes in land cover, in the form of human-altered land, agriculture, and tree cover, is not well understood in the context of GIWs, which is incredibly important given the rapidly changing environment we live in, and the spatial extent over which GIWs occur. It is vital for landscape ecologists and land managers alike to know which kinds of vegetation or land cover positively influences biodiversity to promote certain management strategies. For example, is it important to have high amounts of variation in land cover, for instance, tree, shrub, and flooded vegetation together in a single local site, or is it more important to just have higher flooded vegetation cover to increase biodiversity. If single land cover attributes are important for increasing biodiversity, we can make conservation prescriptions for managers who are creating/preserving these systems at the local level, and if we find regional land cover attributes are driving biodiversity, we can identify priority regions for conservation. The aim of comparing the local and landscape attributes of GIWs across the US is to try and understand generalizable phenomena which make them important, and to understand where preservation/conservation should be prioritized along both the rural-urban gradient and in specific ecoregions of interest.

GIWs also come in a variety of shapes and sizes, depending on their geographic position, whether they were constructed or occur naturally, and if they have been impacted by development activities (Tiner 2003). Testing the species-area relationship is a critical biodiversity concept from Island Biogeography Theory, which stipulates that larger areas will support more species (MacArthur and Wilson 2001). GIWs across the entire spatial extent of the United States, can vary quite dramatically in area, depending on the hydrology, soil conditions, climate, and precipitation. Managers or engineers constructing wetlands may take these conditions into account when evaluating the biodiversity value of these systems (Golden et al. 2017). Therefore, it is critical to know if these systems follow the typical species-area relationship, or, if the relative biodiversity value is not linear, where area maximizes biodiversity value along the species-area curve.

3.4 Incorporating scale into measurements of biodiversity by quantifying beta diversity

Biodiversity monitoring is important for determining priority areas for conservation, as well as identifying the macroscale processes which may be driving extinction of species (Donald et al. 2019). Biodiversity is multi-faceted, and scale-dependent, comprising functional, genetic, and taxonomic diversity in any system. To best monitor biodiversity it is important to incorporate multiple measurements of diversity, including species richness, species turnover, dissimilarity, and other abundance corrected diversity metrics, to best capture the ecological diversity within a given system (Chase et al. 2018, Hillebrand et al. 2018, McGlinn et al. 2018). Bird biodiversity studies in GIWs to date have focused on examining only raw species richness, and abundance, without taking into consideration the size of the regional species pool, or landscape metrics like land cover and Landsat Enhanced Vegetation Index (EVI) (Czapka and Kilgo 2011, Herteux et al. 2020). Furthermore, beta-diversity provides insight on uniqueness of species composition between sites and can inform priority GIWs for especially rare communities (Socolar et al. 2016). Species turnover, or beta-diversity, can also indicate how different species utilize a habitat across space and time. Redundant habitats support the same species, temporally and spatially, like your bird feeder. While dynamic wetland systems like the Okavango delta support everything from large mammals to wetland birds during the rainy season, and specialist drought tolerant plants during the dry season (Junk et al. 2006). GIW systems occur along a dynamic hydrologic regime during the year and may support a multitude of species that can’t be captured solely during 5-minute point counts during the morning hours of the breeding season, but rather require data from the full-day and full-year.

3.5 Temporal beta-diversity in local GIWs across a rural-urban gradient

In addition to the necessary macro-scale studies to determine priority areas for conservation and land cover attributes that contribute to functional and taxonomic bird diversity, we currently lack studies on both spatial and temporal species turnover (beta-diversity). Previous studies aiming to quantify beta-diversity in these systems have focused on the breeding season for birds, not considering changes that occur seasonally. Daniel et al. (2019) found higher diversity in dynamic GIWs with shorter hydroperiods, while others have indicated that GIWs are quite homogeneous spatially regardless of water permanence (Daniel et al. 2019, Barratt Heitmann et al. 2024a). However, the impacts of species turnover in wetlands that do not support breeding populations have not been targeted for analysis. It is critical to know if wetlands of different areas, along a rural-urban gradient experience varying amounts of turnover throughout the day, as these may act as satellite habitats used by a variety of species to meet a life history need for only a short period of time throughout the day, before returning to a core habitat. If turnover is higher in smaller wetlands than larger ones, this could indicate greater functional use of smaller GIWs. Higher turnover in rural wetlands would indicate redundancy in urban ponds, making them less functionally useful for birds. For example, in Rhode Island urban ponds supported higher species richness than rural ponds, but it was mostly due to the presence of urban tolerant species like the American Robin and Gray Catbird, that are functionally similar (McKinney and Paton 2009, McKinney et al. 2011).

4. Research Design & Preliminary Data

4.1 Research Objective 1: To review the state of current knowledge on bird biodiversity in isolated wetlands.

The objective of this research is 1) to synthesize all the existing literature on bird biodiversity in isolated wetlands into 1 review paper, since the last isolated wetland review conducted was in 2003 and included no discussion of biodiversity in these systems; 2) to analyze differences in species richness of birds in different GIW habitats across the global distribution of these systems according to the different dichotomies identified in the review, including rural v. urban, permanent v. ephemeral, natural v. agricultural, and constructed v. natural. This research objective is a systematic review/bibliometric study, and as such, I will not be testing any formal hypotheses. I am instead interested in reviewing all the current literature on bird biodiversity in GIWs to generate summary statistics on publications per year, contextualize the number of papers published in GIWs compared to other wetland complexes, and review how biodiversity comparisons are estimated in these systems across the conterminous United States.

4.1.1 Research design

This **study design** is a systematic/bibliometric review of bird biodiversity in GIWs across the United States. I will review published peer-review literature from a variety of ecotones in the conterminous United States, and my **scope of inference** will be all isolated wetlands within the lower 48 states. The **observational units** of this paper will be individual peer-reviewed publications on bird biodiversity in GIWs. I will perform a systematic search of the Web of Science, Scopus, Science Direct, and Google Scholar databases to collect and download all possible publications. I will then review each article individually to ensure it meets 3 criteria: 1) that the study was conducted on a GIW, 2) that the study utilized birds as the study taxa, and 3) that the study attempted to analyze bird biodiversity for more than 1 species (i.e., species richness, abundance, community composition, etc.) I will achieve **independence** by collating results from all 3 databases and removing duplicates. I will use a literature search to identify all possible papers and then proceed with a systematic review to ensure papers meet the 3 above criteria before analyzing their contents.

4.1.2 Proposed methods

I will identify search terms and the necessary Boolean operators to ensure every piece of literature related to bird biodiversity in GIWs is collected. I will utilize the search criteria listed below to do so. Search criteria must appear in the title, or abstract of the paper. I will conduct the literature search on 3 databases: Google Scholar, Scopus, and Web of Science.

Literature search criteria for GIWs and bird biodiversity
Criteria 1: (must contain 1 of the following)
“Isolated” OR “ephemeral” OR “geographically isolated” OR “temporary”

AND

“Pond” OR “wetland” OR “playa” OR [W*etland type listed in* Tiner 2003]

Criteria 2: (must contain 1 of the following)

“Bird” OR “avifauna” OR “waterfowl” OR “passerine” OR “species richness” OR “abundance” OR “biodiversity” OR “diversity” OR “community composition”

I will include a table of search results produced from the search listed above and those not including the GIW search term. I will do this to compare the existing literature on GIWs, to wetlands generally, and contextualize this with the % of land cover they occupy.
I will systematically review each paper once downloaded to ensure it meets all 3 criteria listed above. Following this cleaning process, once I have a final list of papers, I will collect the following data from each paper (see Table 1).

Table 1. Synthesis/review paper variables of interest, including bibliometric data and systematic review variables.

Bibliometric variables	**Systematic review variables**
Journal	Abundance measurement (Y/N)
# of citations	Species richness measurement (Y/N)
Author(s)	Evenness measurement (Y/N)
Study location (state, country)	Study context (rural-urban, hydroperiod, agriculture, wetland area, etc)
Study level (whole community or specific guild)	

4.1.3 Data analysis

I will produce summary statistics for the total number of publications, publications per year, publications per state, using the *bibliometrix* package in R (Aria and Cuccurullo 2017). I will visualize summary statistics of study level, study location, abundance measurement, species richness measurement, evenness measurement, and study context in *ggplot2* in R (Wickham 2016). Depending on the sample size of papers identified through the review process, I will decide the efficacy of running a traditional meta-analysis with effect sizes. If enough papers are collated, then we will follow a synthesis weaving framework developed by Nakagawa et al. (2019), where I will conduct both a bibliometric analysis, of citations, authors, and publication countries as well as a content mapping approach to identify critical areas of research overlap, disjoint areas of research, and prime opportunity areas for future research (Viechtbauer 2010, Nakagawa et al. 2019). To perform a meta-analysis, I will utilize the *metafor* package in R (Viechtbauer 2010).

4.1.4 Preliminary data

I conducted several preliminary test searches with a variety of search terms to gauge the number of results based on different search terms. As indicated below, the number of publications involving isolated wetlands, birds, and biodiversity (*n*=3) is miniscule compared to the number of hits produced by just using the search term “wetland” instead of “isolated wetland” (n=1,143). This is important considering that ~20% of wetland cover are GIWs, yet they do not represent 20% of the published literature for bird biodiversity (Golden et al. 2017). When I added all the search terms from our criteria 1 above including a GIW search, assuming there is no overlap between the GIW term searches, I got 26 hits (note this does not include an exhaustive list of all possible search terms). If all 26 of these articles are included in the “wetland” AND “biodiversity” and “bird” search, this represents 2% of the published papers, for a wetland type that represents 20% of all wetland cover. I will include more search terms, as outlined above, to ensure I am capturing every possible paper. More nuanced analysis of the contents of these papers is necessary to identify what relationships have been investigated (agricultural context, rural-urban, silviculture), and where they have been conducted (Northeast, Midwest, Pacific Northwest, etc.).

Table 2. Preliminary search terms on *Web of Science*.

Wetland AND biodiversity AND bird	1,143
“Isolated wetland” AND biodiversity AND bird	3
“Ephemeral wetland” AND biodiversity AND bird	4
"Prairie pothole" AND biodiversity AND bird	14
"Isolated wetland" AND "species richness" AND bird	5

4.2 Research Objective 2: To determine how local and landscape land cover attributes impact both overall taxonomic and functional bird biodiversity as well as diversity of specific functional groups in GIWs across the United States.

This research objective has 3 specific objectives: A) to determine if bird species richness adheres to the species-area relationship in GIWs, B) to quantify how land cover variables within local and landscape contexts including vegetation cover, water cover, and human development, impact bird species richness in a variety of GIW wetland types across the US, C) to determine if these relationships remain the same for measures of functional diversity, including primary lifestyle and trophic level.

I hypothesize that bird biodiversity will follow the typical species-area relationship (MacArthur and Wilson 2001). I hypothesize that water cover and flooded vegetation cover at both the local and landscape scale, will increase species diversity in GIWs (Fairbairn and Dinsmore 2001). I also hypothesize that local land cover metrics will be more important predictors of bird biodiversity than landscape level land cover (Callaghan et al. 2018). Finally, I hypothesize that aquatic lifestyle birds (Tobias et al. 2022) will increase species richness estimates in GIWs as wetland area increases. I will control for the following confounding variables in model construction, detailed in the data analysis section below (4.2.4): the number of submitted eBird checklists at each GIW, the size of the regional species pool (higher in east versus west), and abundance (N-effect).

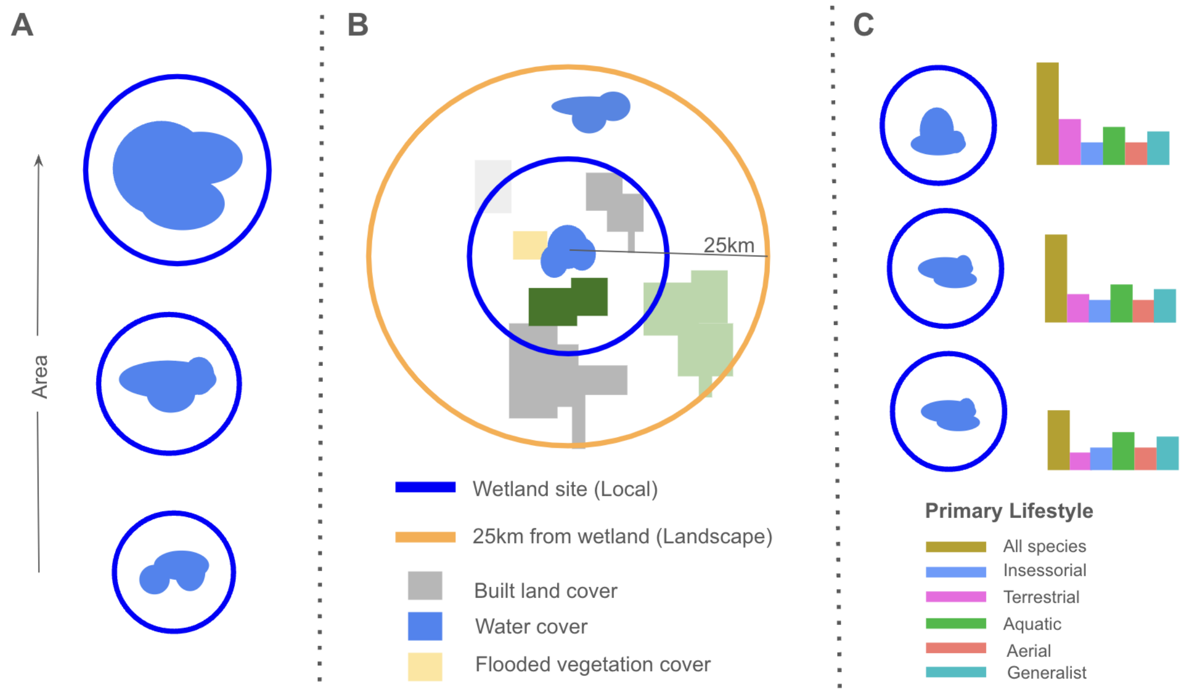

Figure 2. Graphical outline of research objective 2. A) Species-area relationship (SAR) for bird biodiversity in GIWs, B) Impact of local and landscape characteristics on bird biodiversity, C) contribution of different functional group diversity (Primary Lifestyle) to the SAR and impacts from local and landscape land cover.

4.2.1 Research design

This **study design** is a comparative causal macroecological study of bird biodiversity in isolated wetlands across the conterminous United States, where I will quantify how human altered land cover impacts observed estimates of local richness and abundance within isolated wetland habitats. I will collect data from a variety of ecotones in the conterminous United States, and my **scope of inference** will be all isolated wetlands within the lower 48 states. This objective's **observational units** will be eBird sites that contain isolated wetlands. I will **randomly** sample from the available pool of checklists at each isolated wetland eBird site, 100 times without replacement. Main **sources of variability** in estimates of species richness will be related to ***alternative factors that I must measure and/or control to achieve explanatory surplus***. These factors include: effect of increased abundance on observed richness (I will utilize abundance corrected richness (Sn)); differences in vegetation productivity will impact number of available niches, and therefore richness estimates (I will calculate NDVI (proxy measure for productivity) to control for the effect of vegetation within a 25 km buffer from the isolated wetland); the regional species pool can change estimates of richness (I will utilize an evenness metric of diversity (SPIE) that measures diversity at the scale of 2 individuals, to account for relative differences in the size of the regional species pool); elevation will impact the species pool (I will collect elevation data at each site to incorporate into our data analysis model); area of the eBird site can impact richness estimate through the species-area relationship (I will measure area and incorporate it into the model to control for changes in area); whether the wetland is natural or constructed may impact, or be collinear with the amount of human altered land (I will include this as a variable in future modeling to account for this categorical difference). I will use a **stratified sampling design**, where I will map 250 eBird sites containing GIWs, and evaluate land cover characteristics to ensure I have variability in the predictor variable (human altered land). GIWs will be opportunistically sampled, based on the occurrence of the word *wetland* in the site name. I will randomly select 100 checklists at each eBird site containing a GIW, I will have **independence**, as each checklist will be selected without replacement. The land cover attributes impacting our estimates of species richness and abundance (**response variable**) will be measured at 2 scales, one will be at the local site scale, and additionally at the landscape scale, within a 25km buffer from the identified wetland. To estimate sample size, I utilized a **power function** that determined I needed 254 wetland samples to estimate a 0.1 effect size with 95% power and 0.05 alpha. To address collinearity with multiple predictor variables, I will use a generalized linear model approach to model fixed and random effects and ordination to test for collinearity between variables.

4.2.2 Proposed methods

I will select isolated wetlands using eBird by filtering registered sites that contain the word *wetland* or *Wetland* in the site name. I will only include eBird registered sites, to acquire sampling locations at well-established geographic positions. Personal site locations will not be included because they cannot be accessed by multiple users. I will only select sites within the conterminous United States and include sites that had ≥ 100 submitted checklists. Hereafter, eBird sites are denoted as site or site location.

I will confirm that sites contain isolated wetland by viewing locations in GeoJSON, overlaid with National Agriculture Imagery Program (NAIP) imagery from 2010-2022, and Google Earth Imagery from 1985-2022, to identify standing water, and relative hydrological connectivity. I will also utilize terrain maps from Google Earth, to determine presence of rivers, or other water bodies that would disqualify a wetland from being completely isolated, for at least part of the year. I will then delineate sites based on the coverage of the entire eBird site. For instance, I did not map *only* the wetlands, but rather the entire eBird site area (Fig 3). I will map eBird sites in public access locations to make delineation of entire wetland embedded sites most replicable. Public access locations will be delineated using a combination of aerial imagery and property boundaries obtained from each site location's respective websites based on a Google Search (i.e., Clark County Wetlands Park, (“Clark County, NV” n.d.)).

Figure 3. GIW wetland delineation using hotspot locations from eBird and NAIP aerial imagery in GeoJSON.

Geographically isolated wetlands are a suite of habitat types in the conterminous United States and delineating them can often be difficult given that some of these wetland types dry up seasonally, can be connected to other water bodies for short periods of the year, and may be forested, making it difficult to see the wetland boundaries. I will deal with these various issues by mapping wetlands where water is visible on aerial imagery at any point during the year (even if they are inundated for a short period of time). I will include constructed and restored wetlands that do not fit into the standard natural typologies outlined by Tiner (Tiner 2003), because these wetlands are still functionally isolated for birds, meaning they are still surrounded by upland habitat, and lack direct connectivity to larger water bodies like lakes and rivers in close proximity hydrologically. Additionally, GIWs have been created to treat wastewater across the United States, before being pumped back into lakes and/or other waterways underground up to 20 miles away ("Carl & Myrna Nygren Wetland Preserve" n.d.). These wetlands are also functionally isolated, because they do not occur in the immediate vicinity of a larger water body, or palustrine system for all or part of the year, much like typical GIWs. For the purposes of this study, typical geographically isolated wetlands and functionally isolated wetlands will both be incorporated because they act similarly on the macroecological scale, and best capture the present diversity of isolated wetlands found on the landscape, in both rural, and human dominated habitats.

4.2.3 Data analysis

I will calculate environmental variables at both the local scale (just the mapped site location), and at the landscape scale (site location + 25km buffer). I will use Google Earth Engine to calculate land cover percentages from the Google Dynamic World Dataset (10m resolution), including: human altered land cover (%), tree land cover (%), and wetland area (m^2). I will collect EVI data from Google Earth Engine to quantity the relative amounts of green vegetation (a proxy for productivity) within site locations and a 25km buffer around each wetland site.

I will use a generalized linear modeling approach (GLM) to model our response variables (abundance and richness) ~ our predictor variables (land cover type, NDVI, wetland area, wetland type, number of checklists). I will fit the model using a Poisson distribution, after assessing dispersion of the residuals in the response variables.

4.2.4 Preliminary data

We have conducted a preliminary analysis of bird biodiversity calculated as species richness from *n*=207 wetlands across the United States. We found that bird biodiversity conforms to the typical species-area relationship (SAR) in a variety of GIW habitats (Fig 4). We also found that flooded vegetation land cover was positively correlated with species richness at both the local and landscape scale (Fig 5). For more details, please see my recent poster presented at the *Forested Wetlands of the Upper Estuary Conference* (available here) describing our preliminary findings (Barratt Heitmann et al. 2024b).

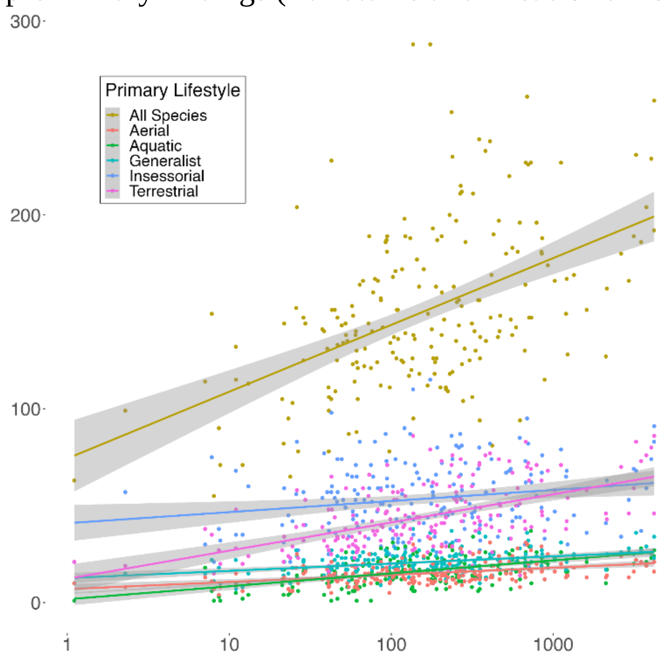

Figure 4. Species richness increases with area, due to increase in terrestrial primary lifestyle species.

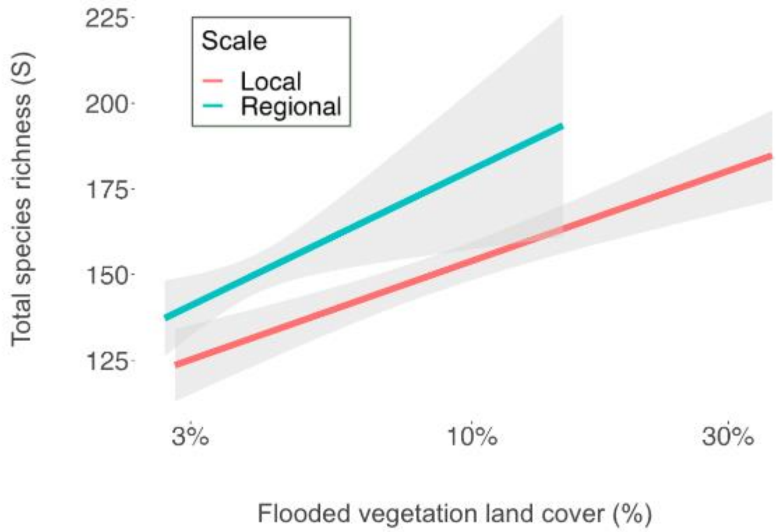

Figure 5. Flooded vegetation at the local (red) and regional (blue) scale, increase bird species richness in GIWs.

4.3 Research Objective 3: To assess the relative contributions of isolated wetlands to bird biodiversity regionally, for instance their beta-partitioned diversity estimates, and determine whether different regions support higher beta-partitioned diversity.

This research objective has 3 specific objectives (Figure 3): A) To determine the regional species pool (Beta partitioned diversity) captured by GIWs compared to 50 random eBird sites within 25km, B) to determine if local or landscape land cover impact estimates of beta-partitioned diversity, and C) to determine if GIWs differ in Beta partitioned diversity across different regions of the United States.

I hypothesize that GIWs will capture a larger amount of the regional species pool than expected at random because of the variety of landscape characteristics in GIW habitats that enable multiple functional groups to meet some life-history need (Scheffers et al. 2006, Riffell et al. 2006, Barratt Heitmann et al. 2024a). I also expect that GIWs in landscapes that have lower water cover will support higher beta-partitioned diversity. Finally, I expect arid regions like the North American Desert level I ecoregion, to have higher levels of beta-partitioned diversity than wetter habitats like the Eastern Temperate Forests, and Northwestern Forested Mountains. Lastly, I hypothesize that urban wetlands will have higher beta-partitioned diversity due to less habitat availability, and smaller regional species pool. I will control for the following confounding variables in model construction, detailed in the data analysis section from the previous research objective 2 above (4.2.4): the number of submitted eBird checklists at each GIW, abundance (N-effect), and wetland area (will be included/not included based on findings of objective 2 above).

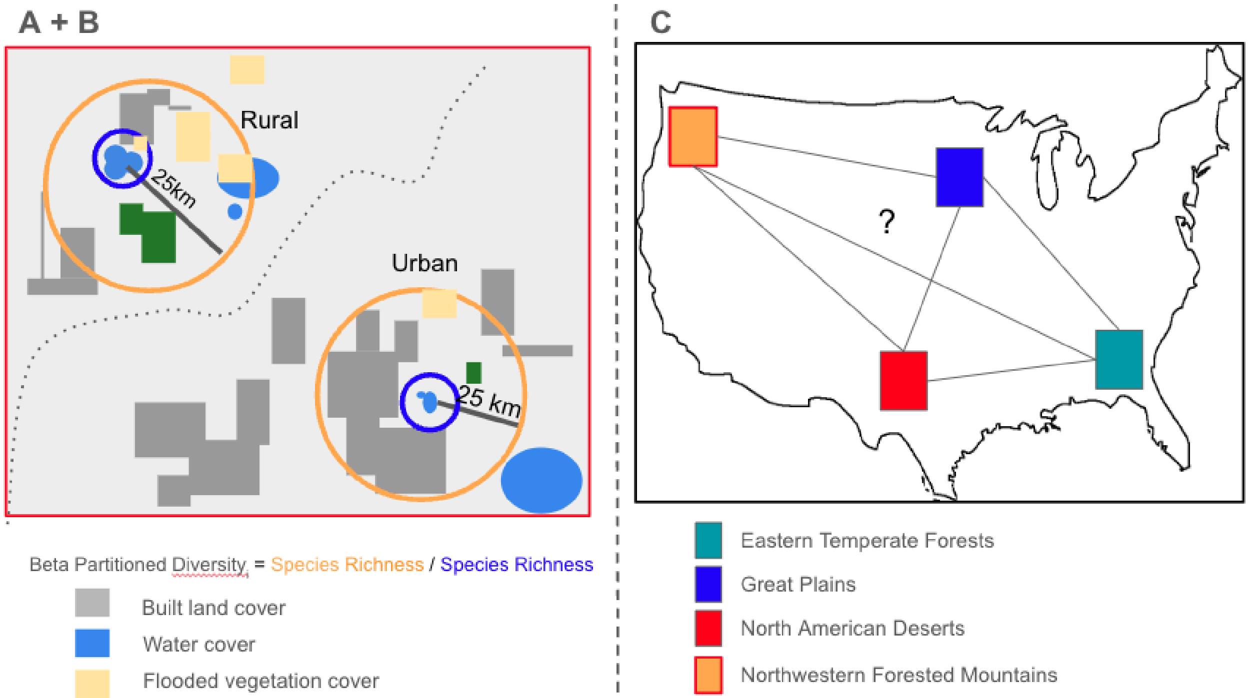

Figure 6. Graphical outline of research objective 3. A) How does species richness as beta-partitioned diversity at GIWs compared to 50 random eBird sites within 25km? B) Do local or landscape land cover metrics impact beta-partitioned diversity at GIWs? C) Is there regional variation in beta-partitioned diversity at GIWs?

4.3.1 Research Design

This **study design** is also a comparative causal study of bird biodiversity in isolated wetlands across the conterminous United States, where I will quantify how much of the regional species pool are held in isolated wetland eBird sites, compared to those in a 25km buffer. I will collect data from a variety of ecotones in the conterminous United States, and my **scope of inference** will be all isolated wetlands within the lower 48 states. This objective's **observational units** will be individual eBird sites, that I will **randomly** sample from the available pool of checklists at each isolated wetland eBird site (local/alpha diversity), 100 times without replacement, as well as 100 eBird sites randomly drawn from sites within 25km of the isolated wetland habitat (regional/gamma diversity). Main **sources of variability** in estimates of species richness will be related to ***alternative factors that I must measure and/or control to achieve explanatory surplus***. These factors include all those listed in the above objective, which will be incorporated in the exact same model structure (see section 4.2.1). I will use a **stratified sampling design**, where I will map 250 eBird sites containing GIWs, and then also collect a list of eBird sites within 25km of each individual site. I will then **randomly** sample each of those data sets separately, to calculate species richness and abundance. I will use a Monte-Carlo approach where I will shuffle the species-abundance matrix of 100 sites, 100 times, to control for sampling effort by those posting to eBird. This includes time duration of count, sampling method (traveling or stationary), and time of day. I will then calculate Beta-partitioned diversity, which is the regional/local richness) of the isolated wetland site, and 100 others, then shuffle the matrix and calculate the same values. This null model Monte-Carlo approach will then allow us to compare our observed Beta-partitioned results, compared to random chance. I will then use a generalized linear modeling approach to incorporate alternative factors, our main predictor variable (isolated wetland site versus random eBird site) and our response variable (Beta partitioned diversity). To estimate sample size, I used a **power function** that determined I needed 254 wetland samples to estimate a 0.1 effect size with 95% power and 0.05 alpha. To address **collinearity** with multiple predictor variables, I will utilize a generalized linear model approach to model fixed and random effects, as well as ordination to test for collinearity between variables.

4.3.2 Proposed methods

The proposed methods for this section will be similar to those mentioned above in section 4.2.2, as I will use the same list of mapped GIWs for this study. Local and landscape attributes will be collected using the exact same methods as section 4.2.2. The main difference will be our response variable, beta-partitioned diversity. I will calculate local scale species richness (alpha diversity) at each wetland site, as the total number of unique species from 50 random checklists, sampled 50 times, to match our sampling effort for the regional richness (gamma) listed below. I will calculate regional diversity (gamma diversity) as the total number of unique species from all checklists from all eBird sites within 25km of each wetland site location. I will then randomly sample 50 checklists, 50 times, from all the regional eBird sites and calculate their individual species richness (alpha diversity). Then I will calculate Beta-diversity as beta-partition (B = 1 - (y / a), which captures the number of shared species at our selected wetland site location with the regional species pool. I will calculate beta-partitioned diversity for the GIW and the additional 50 sampled checklists, 50 times. This will create a table of 50 beta-partitioned values for our GIW, and 50 beta-partitioned values randomly from the region. I will capture the EPA Level 1 ecoregion using the *sf* package in R, to analyze where the GIW centroid overlaps the ecoregion level shapefile (US EPA 2015).

4.3.3 Data analysis

I will use a generalized linear mixed modeling (GLMM) approach to analyze the first research question (A): Do GIWs capture more species in the regional pool compared to random habitats within the region (25km buffer). The response variable will be beta-partitioned diversity, and I will include wetland area and ecoregion as fixed effects in model construction, and likely employ a Poisson family distribution–depending on analysis of residuals and variance. The predictor variable will be a 2-level categorical variable named habitat type (GIW, Random).

β_p ~ habitat_type | wetland_area + Ecoregion

I will utilize a generalized linear mixed modeling (GLMM) approach to analyze our response variable (beta-partitioned diversity) by the local and landscape metrics from Google Earth Engine. I will include wetland area and ecoregion as fixed effects in model construction, and likely employ a Poisson family distribution–depending on analysis of residuals and variance.

β_p ~ local land cover variables + landscape variables | wetland_area + Ecoregion

4.3.4 Preliminary Data

This research objective will use the same *n*=207 wetlands mapped from the previous research objective (4.2). Below is a color-coded map of wetlands mapped by state, with image inlays of typical GIWs found in the different ecoregions (Fig 6). I have calculated alpha (wetland site) and gamma diversity (25km buffer) for all 207 wetland sites and will calculate beta-partitioned diversity.

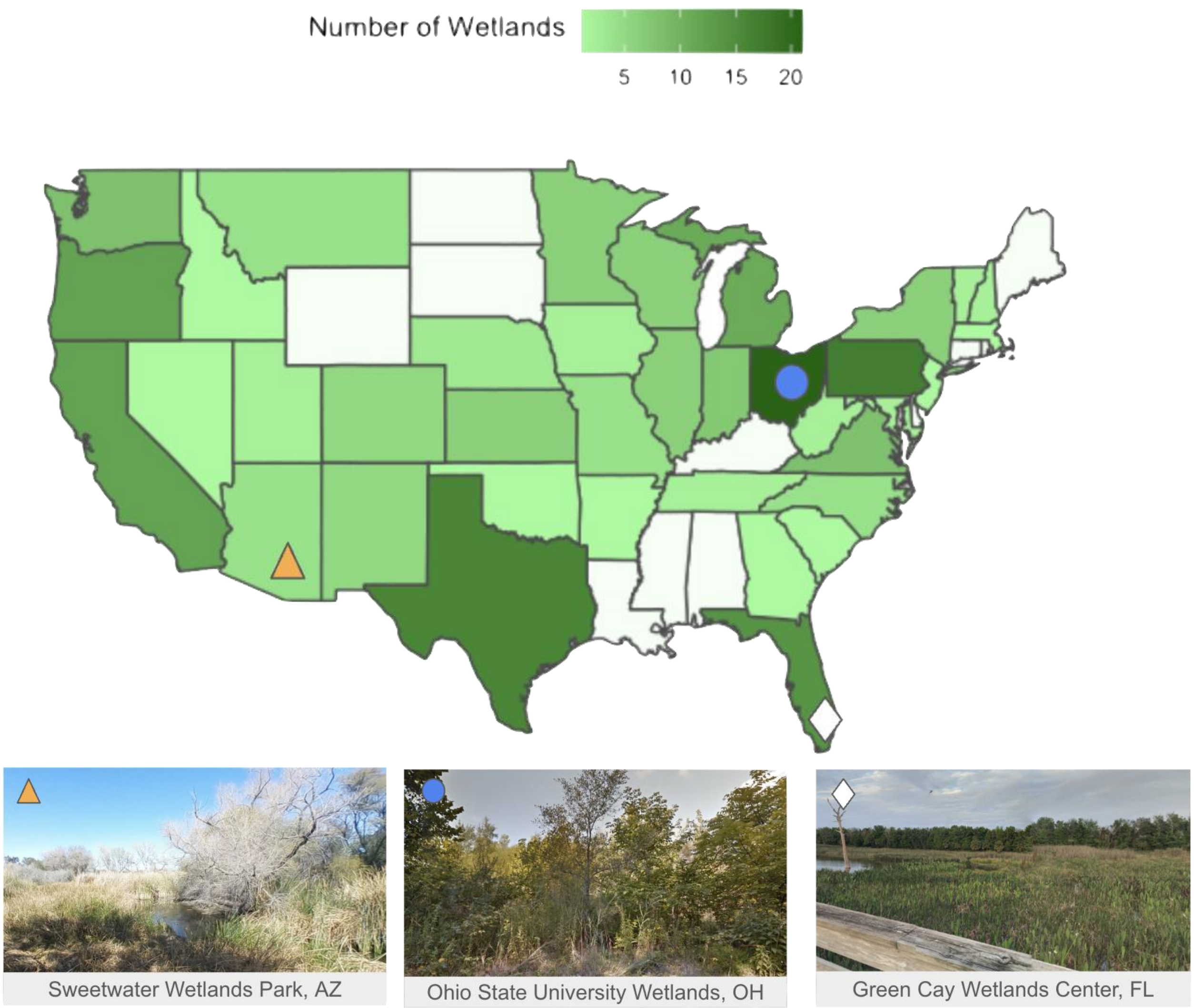

Figure 6. Number of mapped wetlands by state, with image inlays from 3 different ecoregions.

4.4 Research Objective 4: To measure the degree of species turnover, temporally over a 24-hour period, in isolated wetlands along a rural-urban gradient.

The goal of this objective is 1) to describe the species turnover (beta diversity) in habitat use of isolated wetlands in the 24-hour period of the day, and 2) to quantify how this variation changes along the rural and urban gradient.

I hypothesize that turnover will be higher in rural wetlands which are used by a variety of functionally unique species throughout the day. In contrast, I hypothesize that resident bird species will habitually utilize wetlands during less discrete time periods throughout the day and will be used by a functionally less unique community, rendering species turnover lower. Urban wetlands attract species that are urban-tolerant–which are fewer–and generally have been shown to have lower amounts of species richness (Oertli and Parris 2019, Neate-Clegg et al. 2023). Urban ponds also tend to have more stable hydrology due to stormwater inputs, whereas rural ponds in our surrounding area can fluctuate greatly based on precipitation events, potentially providing habitat to a greater number of species–a stochastic effect that has been shown to increase turnover in the Prairie Pothole region (Daniel et al. 2019, Oertli and Parris 2019). Greater turnover within the 24-hour period of the day in rural ponds would indicate, 1) that turnover occurs not only seasonally, but daily in isolated wetlands, potentially supporting a greater number of species than previously thought, 2) that we should manage urban systems to promote higher species richness, and 3) we should identify key rural isolated wetlands which harbor high amounts of beta diversity.

4.4.1 Research Design

This **study design** will be a comparative causal study design of temporal beta diversity in isolated wetlands along a rural-urban gradient in the Gainesville, FL area. The **scope of inference** for this study will be rural-urban isolated wetlands in sub-tropical climates. The **observational units** for this study will be individual isolated wetlands where I will **randomly conduct** point counts during 6 sampling periods in the 24-hour period of the day. I will conduct point counts at 6 total wetlands: 2 urban, 2 suburban, and 2 rural. The 6 sampling periods will be 4-7 AM, 7-10 AM, 10-1 PM, 1-4 PM, 4-7 PM, and 7-10 PM. In addition to these within-day sampling periods, we will also conduct point counts during the winter (Nov-Feb), spring (Mar-May), summer (Jun-Aug), and fall (Sep-Oct). Each wetland will have at least 1 count during each within-day sampling period, and each season, for a total of 24 point counts at each wetland, bringing the total number of counts to 144 over the course of the entire year. Main **sources of variability** in estimates of species richness and species turnover will need to be controlled to achieve explanatory surplus. We will conduct point counts under similar weather conditions (i.e., no rain). We will measure wetland area for all 6 wetlands and include it as a fixed effect in model construction. We will, however, attempt to control this change by selecting at least 1 wetland in each category (rural, suburban, and urban) that are similar in size. Hydroperiod will impact measurements of species richness, and so we will measure water permanence at each wetland during each point count and include this as a fixed effect during model construction. I will use a generalized linear mixed modeling (GLMM) approach to analyze the effect of our **main predictor** variables time, season, and rural-urban gradient. Fixed effects in model construction will include wetland area, and hydroperiod.

4.4.2 Proposed methods

I will conduct land cover analysis to identify potential wetlands in Alachua County using Google Earth Engine's Dynamic World dataset, previously collected data by Hess et al. (2022), and through field data collected by Lee et al. (2023) (Hess et al. 2022, Lee et al. 2023). Through this combination, I will identify 6 wetlands with similar hydrologic conditions, wetland areas, and vegetation characteristics, to control these confounding variables as much as possible.

I will collect wetland characteristics as land cover attributes using Google Earth Engine's Dynamic World dataset, which provides 10m resolution estimates of each of 9 land cover classes (Brown et al. 2022). I will calculate these estimates of each land cover class, with particular attention to water cover (%) as a proxy for hydroperiod. The standard deviation for water cover (%) to total area over the course of the calendar year will correlate with how much each wetland's water level fluctuates, for instance, the hydroperiod.

I will use a single-observer sampling design, where all birds heard or seen within 100m of each wetland site will be noted. We will exclude any flyovers from count observations. I will conduct 15-minute point counts to reduce the number of visits during each sampling period. I will also note whether birds are utilizing open water (OW), flooded vegetation (FV), or trees and shrubs (TS) within the wetland extent (high water mark) or outside of the wetland extent but within the 100m radius.

4.4.3 Data analysis

We will conduct 2 different analyses of temporal turnover and spatial turnover. For all 3 of these analyses, we will again use a generalized linear mixed modeling (GLMM) approach. The first model will test temporal turnover in the 24-hour period of the day. In this model, we will examine how beta diversity (Whittaker's species turnover: $\beta = y/a-1$) changes based on the sampling period (time) and rural-urban gradient, while also including the wetland site (6-level variable) as a random effect. In this beta diversity model, gamma will be calculated as the total number of species from every sampling time period in a single season (to control for seasonal variation). I will also include wetland area as a fixed effect in model construction–as necessary depending on final wetland delineation.

24H species turnover model:

β ~ sampling_period (time) + anthropogenic cover | wetland_site (random) + wetland_area (fixed)

In the seasonal temporal turnover model, I will again calculate beta diversity (Whittaker's species turnover: β = y/a-1), where gamma is equal to the total number of species seen at each site in a single year, and alpha is equal to number of species seen at the individual site across all sampling time periods. This model will use the same approach, only we will change the temporal scope to season and evaluate turnover differences due to temporal change in season and spatial change by season.

Seasonal species turnover model:
β ~ season + anthropogenic | wetland_site (random) + wetland_area (fixed)

4.4.4 Preliminary data

I've conducted preliminary counts at *n*=2 ponds in the urban landscape in Gainesville, FL. From limited observations, I've noticed habitat use by waterfowl (Pied-billed Grebe, Hooded Merganser) in the winter and early spring months (Dec-Feb), coinciding with waterfowl staging prior to migration. Consistent evening (4-7PM) use of urban wetlands by smaller wading birds (Little Blue Heron, Green Heron), who are absent from wetlands in early morning and mid-day periods. I have yet to conduct nighttime surveys, which may include habitat use by nocturnal predators. Community composition certainly changes over the course of the 24H period of the day in these wetlands, and it seems to be driven by waterfowl and wading birds, which use them habitually for only a short period in the day. Passerine birds (Yellow-rumped Warbler, Northern Cardinal) utilize these habitats fairly consistently throughout the day. This may mean that species richness is driven by passerine birds, which occur in higher density and richness, but that turnover is driven by habitual use for short periods in the day by waterfowl and wading birds, that have lower richness, but likely higher use of the actual wetland habitats (Open Water and Flooded Vegetation).

5. Synthesis and significance

The result of this study will be a continental-scale analysis of bird biodiversity in GIWs, in a variety of ecotones, habitat types, and along the rural-urban gradient. The findings of my first objective will help inform land managers at the local scale how to change the land cover in their wetlands to increase bird visitation. For instance, increase shrub cover, or decrease human developed land. Additionally, it will help inform macroscale ecologists about the relative importance of these systems in urban and rural contexts, which may vary in bird biodiversity estimates. My second and third objectives will help landscape ecologists and conservationists reckon with how regional variation in these systems may be important for biodiversity across the landscape. Coupled with the wealth of knowledge about hydrology and ecosystem services, this could paint an even more holistic picture of the importance these systems play in maintaining biodiversity, freshwater, flood protection, and biochemical processing. My study aims to inform and engage land management and conservation professionals, who may be designing or preserving GIWs in a variety of landscape contexts across spatial and temporal scales.

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