# Wetland birds and invasive plant management

by

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## Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

## Statement of Contributions

This thesis work is my intellectual property and I take sole responsibility for it. However, this thesis could not have been completed without help. To recognize this, I use “we” throughout the document. I intend to publish Chapters Two and Three as co-authored manuscripts with my supervisor Dr. Rooney.

# Abstract

The Great Lakes coastal wetlands are some of the most diverse ecosystems in Ontario. However, their ecological integrity is continually threatened by development, nutrient pollution, and invasive species. Over the past two decades, marsh-nesting birds in the southern portion of the Great Lakes have experienced a substantial decline; approximately eight of 18 species have lower abundances now than they did in the mid-90s. Invasive Common Reed (*Phragmites australis* subsp. *australis*) is a grass that has been displacing native coastal wetland habitat for several decades, and it is a contributing factor to the decline in marsh-nesting species, particularly those of conservation concern. Long Point, ON, is a UNESCO Biosphere Reserve located Lake Erie, which is comprised of 13,465 ha of ecologically significant habitat experiencing invasion. To reverse damage from *P. australis* invasion and restore habitat quality for marsh-nesting birds and other wildlife, two invaded National Wildlife Areas in Long Point were treated with a glyphosate-based herbicide, followed by mechanical rolling to flatten dead *P. australis* beginning in 2019. The long-term outcomes of *P. australis* management are expected to positively impact the wetland bird community, but there have been limited studies investigating the short-term impacts that could arise from habitat alteration following treatment. Therefore, we undertook two studies to monitor the short-term response of marsh bird communities 1-2 years following *P. australis* management. First, we undertook a Before-After-Control-Impact study to monitor birds before and after treatment. Throughout the 2019 marsh bird breeding season, autonomous recording units (ARUs) were used to record bird vocalizations in areas where herbicide treatment of *P. australis* was planned for fall 2019 and in *P. australis*-invaded areas where no treatment was planned (control sites). These sites were resurveyed in 2021 to compare to 2019 baseline recordings. We determined that ARU recordings should be transcribed on one survey date in the middle of the breeding season, comprised of three 15 min segments split across the dawn chorus, to maximize avian richness estimates by capturing both early and late-morning vocalizing species. Second, we undertook a space-for-time substitution design. ARUs were deployed in 2021 to survey birds in invaded control sites, 1 or 2-year post-treatment sites, and uninvaded reference sites. For both studies, we assessed how avian species richness (both total and marsh-user) and community composition differed among vegetation type. We found small-scale effects of *P. australis* management on bird richness and community composition, but such effects are insignificant when compared to the natural variation in bird community composition in Long Point. Birds displaced by *P. australis* management tended to be non-marsh affiliated birds that can find refuge in surrounding habitats. Notably, the provincially and federally Threatened Least Bittern (*Ixobrychus exilis*) occurred infrequently in herbicide-treated sites, but it is expected that it will use the increase in hemi-marsh arrangement as time progresses. We conclude that two years post-management is too short of a timeframe to see the materialization of considerable positive effects on the avian community. However, we did find evidence of positive trends occurring to birds most impacted by *P. australis* invasion, as they were observed using, or have the potential to use, the increase in open water and hemi-marsh arrangement remaining after herbicide treatment. We recommend continued monitoring to assess the long-term consequences of *P. australis* control for the avian community.

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…

As my sister once said, "ah, the dawn chorus…it sounds dreamy". It truly is.

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# List of Abbreviations

ANOVA - Analysis of variance

ARU - Autonomous recording unit

BACI - Before-after-control-impact

CWS-ON - Canadian Wildlife Service - Ontario Region

ECCC - Environment and Climate Change Canada

ISA - Indicator species analysis

IV - Indicator value

MRPP - Multiple response permutation procedure

NMS - Non-metric multidimensional scaling

NWA - National Wildlife Area

perMANOVA - Permutational analysis of variance

SAR - Species at risk

SOCC - Species of conservation concern

Std - Standard deviation

# *1. General introduction*

## *1.1 Overview*

Wetlands in southern Ontario have been lost at an alarming rate; at least 72% since pre-colonial time (Ducks Unlimited Canada, 2010). Thus, remaining, intact wetlands are vital in providing ecosystem services and functions, such as flood storage and wildlife habitat. The Long Point peninsula is home to 70% of the intact coastal wetland area on the north shore of Lake Erie (Ball et al., 2003). It is designated as a Wetland of International Importance as it plays an important role in harbouring wildlife and their ecologically significant habitat. However, an invasive grass, *Phragmites australis* subsp. *australis*, has been spreading through the coastal wetlands in Long Point, jeopardizing their ecological integrity. Stands of *P. australis* are tall and dense, which displace native vegetation communities and ultimately alter habitat for wildlife inhabitants, including species at risk (Wilcox et al., 2003; Robichaud & Rooney, 2017). Wetland birds are one group of species that is losing critical habitat to *P. australis* invasion in Long Point (Robichaud & Rooney, 2017). To reverse damage from *P. australis* invasion and restore habitat quality for wildlife, the invasive grass can be managed through chemical, mechanical, or biological methods (Hazelton et al., 2014). The outcome of *P. australis* management for the avian community is expected to be positive. Indeed, studies focusing on the long-term efficacy of *P. australis* management find support for avian community recovery following treatment (e.g., Tozer & Mackenzie, 2019). However, there have been limited studies assessing the potential for short-term impacts of *P. australis* management on avian communities. Thus, some land managers in the Great Lakes region have been reluctant to engage in *P. australis* control, voicing concerns that suppression could trigger negative short-term effects on wildlife, including birds (e.g., due to habitat alteration). In this thesis, we use autonomous recordings units to investigate if any short-term impacts of *P. australis* management on wetland birds arise in two National Wildlife Areas in the Long Point Biosphere Reserve, which will help inform how to strategically proceed with management, while minimizing any risks to avian communities. Autonomous recording units can increase spatial and temporal surveying of bird communities, but their ability to collect large amounts of data can be a double-edged sword, as these large amounts of data are laborious to analyze. We investigated the optimal duration and time within the dawn chorus to survey breeding birds to capture accurate estimates of avian diversity in the Long Point coastal wetlands.

### *1.2 Great Lakes coastal wetlands*

Great Lakes coastal wetlands are diverse and productive ecosystems that provide numerous ecosystem services and functions such as water filtration, wildlife habitat, and areas for recreation (Sierszen et al., 2012). The hydrology and geomorphology of a wetland are important controls of wetland services and functions (Albert et al., 2005). These controls will influence abiotic factors such as water chemistry and soil type, which give rise to unique vegetation and wildlife inhabitants (Brinson, 1993). Water level fluctuations are a main driver in shaping vegetation communities in coastal wetlands (Keddy & Reznicek, 1986; Mortsch et al., 2006).

Great Lakes Erie, Michigan and Huron do not have regulated water levels, and therefore experience both short- and long-term water level fluctuations (Quinn, 2002). Daily water levels are influenced by seiches and storm surges, while long-term fluctuations are influenced by seasonal, annual, and decadal changes in factors such as precipitation, runoff, and ice-melt (Keddy & Reznicek, 1986; Herdendorf, 1992; Quinn, 2002). The persistence of wetland vegetation communities is closely related to the hydrology of a wetland (Mortsch et al., 2006). Wetland plants that share similar environmental tolerances (e.g., substrate and moisture needs) grow at similar elevations (Mortsch et al., 2006). Wetland plants are classified into five main communities: 1) woody (trees and shrubs); 2) wet meadow; 3) emergent macrophytes; 4) floating macrophytes; and 5) submerged macrophytes (Wilcox et al., 2002). Coastal wetland vegetation communities can be displaced either landward or lakeward as water levels rise or recede (Wilcox et al., 2002). In periods of low water levels, mudflats are exposed, which causes a lakeward expansion of communities; the emergent zone is replaced with shrubs and sedges, while submerged aquatic vegetation is replaced with emergent vegetation as seeds germinate in the mudflats (Keddy & Reznicek 1986; Mortsch et al., 2006). During periods of high-water levels, the woody and emergent vegetation dies back, and there is an increase in floating and submerged aquatic vegetation (Keddy & Reznicek, 1986). Fluctuating water levels act as a natural disturbance that leads to continual change of vegetation communities, which in turn maintains structurally complex and diverse habitats (Keddy & Reznicek, 1986; Wilcox et al., 2002).

Anthropogenic disturbances, such as pollution, fragmentation, shoreline hardening, and invasive species introduction threaten the ecological integrity of Great Lakes coastal wetlands (Smith et al., 2015).

Invasive species introduction is one of the top environmental stressors for Great Lakes wetlands (Smith et al., 2015; Escobar et al., 2018). There are at least 184 non-native species reported in the Great Lakes, spanning numerous taxonomic groups, including bacteria, viruses, protozoa, diatoms, arthropods, mollusks, fish, and plants (NOAA, 2016; Escobar et al., 2018). Biological invasions can be costly; in the Great Lakes region, invasive aquatic species can cause over $100 million in damages per year (Rothlisberger et al., 2012), and tens of millions of dollars are spent on prevention and management (Rosaen et al., 2012; MNDNR, 2015). Lakes Erie and Ontario may be more susceptible to biological invasions and other anthropogenic stressors due to the amount of anthropogenic activity located around the lakes (Trebitz & Taylor, 2007). For example, Long Point, ON contains a vast and diverse coastal wetland complex that is located on Lake Erie and has been impacted particularly by invasive wetland plants (Wilcox et al., 2003).

### *1.2.1 Long Point coastal wetlands*

The Long Point peninsula is a 32 km sand-spit that is located on the north shore of Lake Erie. Sand-spits create protected, shallow embayments on their landward side, and often have a high diversity of vegetation, invertebrates, fish, and birds (Albert et al., 2005).

Long Point is a Wetland of International Importance (designated under the Ramsar Convention on Wetlands), UNESCO World Biosphere Reserve, and a globally significant Important Bird Area (designated by BirdLife International). The area is also home to two of Ontario's 10 National Wildlife Areas – elements of Environment and Climate Change Canada's protected areas network that are managed to conserve essential habitats for migratory birds and other wildlife under the Canada Wildlife Act (Government of Canada, 2022). Long Point is located in a relatively developed region of Ontario; approximately 72% of wetlands in Southern Ontario have been lost since pre-settlement time, and 65-85% of wetlands have been lost in the county Long Point resides in (Ducks Unlimited Canada, 2010). The peninsula contains approximately 70% of the intact coastal wetland area on the north shore of Lake Erie (Ball et al., 2003). It also lies in Canada's Carolinian vegetation zone – a biodiversity hotspot containing over 2,000 plant species including 65% of all Ontario's rare plants (Argus et al., 1982) and nearly 400 species of birds comprising 50% of all the bird species in Canada (Carolinian Canada, 2006). Therefore, this area is exceptionally important for harbouring wildlife, including herptiles, birds and plants, as well as many species at risk and their ecologically significant habitat (Ball et al., 2003; Sierszen et al., 2012; Government of Canada, 2021b). One of the largest threats the coastal wetlands in Long Point face is biological invasion, specifically invasive Common Reed (*Phragmites australis* ssp. *australis*) (Bickerton, 2015).

### *1.3 Invasive* Phragmites australis *subsp.* australis

*Phragmites australis* subsp. *australis* is a perennial grass that originated in Europe (Saltonstall, 2002). It was likely introduced to North America in the late 1700s or early 1800s in ballast material (Saltonstall, 2002; Swearingen & Saltonstall, 2010). In North America, *P. australis* subsp. *australis* is considered a cryptic invader, as it resembles the native *P. australis* subsp. *americanus.* (Saltonstall, 2002). Invasive *Phragmites australis*, hereafter *P. australis*, can tolerate a wider range of environmental conditions than the native subspecies, as well as produce a higher amount of above-ground biomass and have a greater relative growth rate (Mozdzer & Megonigal, 2012). Such traits make it an aggressive competitor (Ailstock et al., 2001).

*Phragmites australis* often invades wetlands, recently disturbed areas, or ditches along the side of roadways (Catling & Carbyn, 2006; Baldwin et al., 2010). It can grow up to 5 m tall and form dense, monotypic stands (> 200 stems/$m^2$; Government of Ontario, 2012). *Phragmites australis* can reproduce sexually and asexually, allowing it to spread vigorously. Sexual reproduction occurs through seeds, which are primarily dispersed via wind (Haslam, 1972), and can remain in the seed bank until growing conditions are suitable (Kettenring & Whigham, 2009; Wilcox, 2012). Asexual reproduction occurs through rhizomes (horizontal underground stems) and stolons (horizontal aboveground stems) that establish themselves on exposed mudflats, usually during periods of low water levels (Tulbure et al., 2007). Rhizomes extend several meters into the ground and can spread up to 3 m horizontally (Swearingen & Saltonstall, 2010), and they can also continue to grow if cut off from the parent plant (Derr, 2008). Thus, tall, dense stands of *P. australis* can crowd and shade-out native plants below (Robichaud & Rooney, 2022a) and outcompete them for limiting nutrients (Meyerson et al., 2002).

### *1.3.1* Phragmites australis *in Long Point, ON*

*Phragmites australis* became established at Long Point between the late 1990s and early 2000s during a prolonged period of low water levels in Lake Erie (Wilcox et al., 2003). Since then, *P. australis* has expanded in Long Point exponentially (Wilcox et al., 2003), and it is reducing the diversity of vegetation within coastal wetlands by displacing native vegetation with dense, monotypic stands (Robichaud & Rooney, 2017, 2022b). *Phragmites australis'* alteration of wetland vegetation and structure has consequently impacted the habitat quality of wildlife inhabitants, such as birds (Robichaud & Rooney, 2017; Tozer & Beck, 2018; Robichaud & Rooney, 2022b).

## *1.4 Wetland birds and consequences of* P. australis *invasion*

### *1.4.1 Wetland birds in the southern Great Lakes*

The Long Point peninsula's coastal wetlands are of regional and global significance to avifauna (McCraken et al., 1981; Government of Canada, 2021b). Its location along the Atlantic Flyway makes the wetlands important stop-over grounds for birds during spring and fall migration (McCracken et al., 1981; Knapton & Petrie, 1999). Long Point is also of regional importance for local breeding marsh bird populations (McCracken et al., 1981). As mentioned previously, the expansive and sheltered sand-spit bays along the peninsula are some of the most pristine coastal wetlands remaining in Southern Ontario, making them ideal habitat for local marsh bird populations (Hebb et al., 2013; Government of Canada, 2021b).

Marsh bird populations in the southern Great Lakes region have experienced substantial declines (Tozer 2013, 2016, 2020). Tozer (2013, 2016) demonstrated that 10 marsh-using species (i.e., those that regularly or exclusively nest in marshes) have declined since 1995 by 0.5-10.5% per year based on abundance and 1.2-4.9% based on occupancy. Furthermore, a recent report from Birds Canada summarizing trends from the Great Lakes Marsh Monitoring Program over the past two decades found that there were substantial declines in five out of seven elusive marsh birds (Tozer, 2020). Several factors have likely contributed to this decline (e.g., habitat loss and fragmentation), but recent research indicates that one of the main culprits is the expansion of *P. australis* and its homogenization of breeding bird habitat in wetlands (Tozer, 2016; Robichaud & Rooney, 2017; Tozer & Beck 2018; Tozer & Mackenzie, 2019; Robichaud & Rooney, 2022b).

Many of the bird species in decline are rails, bitterns and grebes (Tozer 2016; Tozer et al., 2020). These birds are habitat specialists, which exclusively breed in marshes and have specific habitat requirements regarding water depth, vegetation type, and vegetation structure (Chin et al., 2014; Grand et al., 2020). They tend to be more sensitive to changes in habitat conditions than habitat generalists that breed and forage in either marsh or upland habitat (Chin et al., 2014; Grand et al., 2020). Several marsh birds in Ontario are listed as either 1) species of conservation concern under the Lower Great Lakes/St. Lawrence regional bird conservation strategy (ECCC, 2014), 2) at-risk under the Ontario Endangered Species Act (Government of Ontario, 2022), or 3) at-risk under the federal Species at Risk Act (Government of Canada, 2021a).

Marsh birds select habitat based on both landscape features (e.g., surrounding urban land use) and finer-grained, local features (e.g., plant assemblage; Fairbairn & Dinsmore, 2001; Lor & Malecki, 2006; Glisson et al., 2015). Marsh birds use certain plant assemblages for breeding and foraging (Lor & Malecki, 2006), and the expansion of invasive plant species can adversely impact birds' abilities to do so (Glisson et al., 2015). The best quality of habitat for many marsh birds includes a heterogeneous cover of emergent vegetation interspersed with open water, which is often called "hemi-marsh" (Lor & Malecki, 2006; Rehm & Baldassarre, 2007; Bolenbaugh et al., 2011). Marsh birds use emergent plants such as cattail (*Typha* spp.) for material to build and conceal nests, hide from predators, or as a matrix for foraging (Johnson & Dinsmore, 1986; Lor & Malecki, 2006; Melvin & Gibbs, 2012). Furthermore, vegetation interspersed with open-water pools and channels provides feeding areas for many marsh birds, as this hemi-marsh arrangement provides access to fish, macroinvertebrates, and floating plants (seeds and tubers) while providing nearby vegetation for cover (Rehm & Baldassarre, 2007).

Some marsh birds avoid areas of dense emergent plants, whether it be dense cattail or *P. australis* (Rehm & Baldassarre, 2007; Lishawa et al., 2020). For example, large patches of *P. australis* may decrease roosting habitat for larger-bodied birds such as the Sandhill Crane (*Grus canadensis*) (Kessler et al., 2011). The litter accumulation of *P. australis* is greater than most native plants and it tends to increase sediment accretion, which serves to fill in water channels and pools, leading to the loss of high-value hemi-marsh habitat and a reduction in marsh bird access to feeding grounds (Windham & Lathrop, 1999; Meyerson et al., 2000). Furthermore, *P.*

*australis* may not provide high-quality nesting material due to its rigidity, particularly for ground-nesting birds such as waterfowl or rails (Meyer et al., 2010). The resulting change in vegetation and vertical structure from *P. australis* invasion can impact habitat quality for marsh birds (Whyte et al., 2015; Robichaud & Rooney, 2017; Tozer & Beck, 2018).

### *1.4.2 Impacts of* P. australis *invasion on wetland birds*

The impacts of *P. australis* invasion on avian communities have been well documented (e.g., Benoit & Askins, 1999; Meyer et al., 2010; Gagnon-Lupien et al., 2015; Whyte et al., 2015; Robichaud & Rooney, 2017) and two main trends have emerged from these studies. First, there may be a "lag effect" whereby bird communities evidence a delayed response to *P. australis* invasion; early stages of invasion may seem benign or have positive effects on avian communities, because low densities of *P. australis* (e.g., less than 100 live stems/$m^2$; Yuckin & Rooney, 2019) may increase habitat heterogeneity in vegetation assemblages and add structural diversity (e.g., new nesting locations; Meyer et al., 2010; Gagnon-Lupien et al., 2015). However, as *P. australis* expands exponentially and becomes denser, it homogenizes wetland habitat and no longer contributes to the heterogeneity of the habitat (Robichaud & Rooney, 2017, 2022b). This homogenization of the habitat leads to losses in avian diversity: a phenomenon termed "biotic homogenization" (Robichaud & Rooney, 2022b).

Second, there are "winners and losers" with the invasion of *P. australis* in wetlands. Habitat generalists (i.e., those that don't exclusively rely on wetland habitat) and marsh-users (i.e., those that rely on wetland habitat for breeding, foraging, or loafing), specifically small-bodied, may benefit from *P. australis* invasion, while larger-bodied marsh-users and aerial foragers may suffer (Gagnon-Lupien et al., 2015; Whyte et al., 2015; Robichaud & Rooney, 2017). Several studies looking at the impact of *P. australis* invasion on bird communities found an increase in bird abundance in *P. australis* habitat compared to uninvaded, 'reference' habitat, but this was often attributed to increases in habitat generalist species or small-bodied marsh-users such as Red-winged Blackbird (*Agelaius phoeniceus*) and Common Yellowthroat (*Geothlypis trichas*) (Wells et al., 2008; Meyer et al., 2010; Whyte et al., 2015). The dense and dry habitat of *P. australis* may be suitable for generalist species that are not sensitive to vegetation type or water levels, or for small-bodied marsh-users that prefer shrubby vegetation (Robichaud, 2016).

In contrast, larger-bodied marsh-users, such as Least Bittern (*Ixobrychus exilis*), American Bittern (*Botaurus lentiginosus*), and Virginia Rail (*Rallus limicola*), all of which are marsh bird species of conservation concern, may avoid dense patches of *P. australis* (Robichaud & Rooney, 2017), possibly due to its impenetrability or unsuitable foraging or roosting sites (Rehm & Baldassarre, 2007; Kessler et al., 2011). Furthermore, it has been determined that populations of certain large-bodied marsh bird species of conservation concern, such as Common Gallinule (*Gallinula galeata*), American Coot (*Fulica americana*) and Virginia Rail, have declined in Lake Erie coastal wetlands over the past two decades due to, at least in part, the expansion of *P. australis*, and that the increase in *P. australis* percent cover in Lake Erie coastal marshes could lead to the local extinction of American Bittern in areas where *P. australis* takes over entirely (Tozer & Beck, 2018). Furthermore, aerial insectivores, including at-risk swallows, have been found to avoid foraging over *P. australis* invaded areas (Robichaud & Rooney, 2017). Presumably, controlling *P. australis* invasion in coastal wetlands would help restore the avian community to pre-invasion conditions and benefit those birds most impacted by invasion.

### *1.5* Phragmites australis *control*

There are many methods for *P. australis* control, including chemical (herbicide-based), mechanical (e.g., burning, rolling, cutting, flooding), biological (e.g., herbivory, biocontrol), or a combination of methods (Hazelton et al., 2014). The most common method in North America is the use of either glyphosate or imazapyr-based herbicide (e.g., Martin & Blossey, 2013; Hazelton et al., 2014; Hunt et al., 2017; Robichaud & Rooney, 2021a). The efficacy of herbicide-based control (i.e., *P. australis* stem density suppression) is variable; studies have reported lows of 50-60% (e.g., Farnsworth & Meyerson, 1999; Ailstock et al., 2001) or highs of >90% suppression (e.g., Derr, 2008; Zimmerman et al., 2018; Robichaud & Rooney, 2021a).

Glyphosate and imazapyr-based herbicides are classified as "non-selective", meaning they will kill any plants sprayed (Hazelton et al., 2014). It is recommended that herbicide application occur in the fall when flora and fauna activity has declined (e.g., due to migration, senescence, etc.; OMNR, 2011), and it is often best practice to apply herbicide on large, dense patches of *P. australis*, or apply by spot-treatment, to reduce 'non-target' effects (e.g., overspray onto native plant communities, or other sensitive habitats).

Glyphosate and imazapyr-based herbicides are relatively non-toxic to birds because they act through the inhibition of an enzymatic pathway that is not present in birds (Wu et al., 2006; Gill et al., 2018). The impacts of glyphosate application on aquatic biota in Long Point, ON was assessed between 2016 and 2018, after it was applied to control *P. australis* in 1000 ha of marsh (Robichaud & Rooney, 2020b). Glyphosate and its primary breakdown product never exceeded the threshold of toxicological concern, and concentrations in the water returned to pre-treatment levels 20-30 days after application (Robichaud & Rooney, 2021b). Glyphosate and its breakdown product remained in the sediment up to two years after application, but in low concentrations that were well below the short-term and long-term threshold of concern for aquatic biota in freshwater (CCME, 2012; Robichaud & Rooney, 2021b). Glyphosate residue can also accumulate in plant litter (Sesin et al., 2021), but glyphosate bound to organic matter it is not easily biologically available (Hagner et al., 2019), so the likelihood of it impacting birds is very low. Therefore, acute toxicity from glyphosate exposure on birds is unlikely, however, there is some concern regarding indirect effects of herbicide application that may impact wetland birds.

Initially, herbicide application causes a dramatic change in emergent vegetation availability, which can impact roosting, nesting, and foraging sites for certain wetland birds (Linz et al., 1996; Lazaran et al., 2013). There have been few studies looking at the initial impacts of *P. australis* management on wetland bird communities, and there is some concern regarding the immediate change in habitat (e.g., Lazaran et al., 2013). The changes to wetland habitat due to herbicide application and the subsequent impacts on wetland birds are reviewed in Chapter Two of this thesis. Secondly, herbicide application and the subsequent dieback of emergent vegetation can affect macroinvertebrate communities, which are key sources of prey for many wetland-dependent birds (All About Birds, 2022). Studies have found that chironomid emergence significantly increased in herbicide-treated sites (Linz et al., 1999; Baker et al., 2014; Robichaud et al., 2021), which may benefit wetland birds like Virginia Rail and Swamp Sparrow (*Melospiza gerogiana*) that forage for macroinvertebrates (Cornell Lab of Ornithology, 2022). Herbicide application is often coupled with mechanical treatment to increase efficacy because rolling or burning herbicide-treated *P. australis* can remove standing dead biomass to better assist regrowth of native vegetation (Kettenring et al., 2011; Lombard et al., 2012; Hazelton et al., 2014), but this can also come with challenges.

Mechanical treatment alone has relatively low efficacy, as it does not target the belowground biomass like herbicide application does (Hazelton et al., 2014). Mechanical methods such as mowing can also increase *P. australis* shoot production (Derr, 2008), and several other mechanical treatments are quite labour-intensive that require multiple treatments each year to suppress *P. australis* (Hazelton et al., 2014). There is concern that indirect effects on birds may occur from repeated use of heavy machinery and/or boats for *P. australis* control, as the machinery could harass birds or compact wetland soil, which may impact nesting sites or food availability. Biological control may be a low-cost strategy that could replace the need for herbicide application or mechanical treatment, but application in the field has been limited thus far (Blossey et al., 2020).

Several factors can influence the efficacy of *P. australis* control, such as water levels and patch size, causing outcomes of management that may vary each time (Rohal et al., 2019). Repeat treatments over many years are needed to control *P. australis* and complete eradication of the invasive plant is unlikely to be achieved (Martin & Blossey, 2013; Hazelton et al., 2014; Quirion et al., 2017). Therefore, continued *P. australis* management is costly and time-consuming. For example, the Great Lakes Restoration Initiative spent over $25 million on *P. australis* management between 2010 and 2014 in the Great Lakes region (GLRI, 2015). Furthermore, repeated disturbances to wetland habitat may negatively impact biota. All in all, the potential costs and impacts of *P. australis* management must be weighed against the risks of unabated invasion

### *1.6 Surveying wetland birds*

Wetland birds are valuable bioindicators of wetland health (Amat & Green, 2010; Grand et al., 2020). Many are reliant on wetlands for at least one portion of their life cycle, and many are particularly sensitive to changes in their habitat (Amat & Green, 2010; Grand et al., 2020). Therefore, changes in wetland habitat due to human activities or natural causes are tracked by wetland birds and reflected in their population trends (Glisson et al., 2017; Grand et al, 2020). Birds are also ideal subjects to survey because they are common taxa that regularly vocalize and can be visually identified. Thus, surveying birds allows for reliable and repeatable methods for assessing their communities and the habitat they use (Birds Canada, 2009). For example, the Marsh Monitoring Program is a long-term monitoring program that assesses wetland-associated species, specifically birds and anurans, to monitor the ecological integrity of wetlands across Canada.

In-person point counts have traditionally been used to survey bird communities (Shonefield & Bayne, 2017). Point count surveys involve 1-2 people stationed at a set location for a set amount of time to visually and aurally identify birds. In the past 20 years, technological advancements have led to the use of autonomous recording units (ARUs) to supplement or replace in-person surveys of birds (Darras et al., 2019). ARUs are devices that are deployed in the field and programmed to record sound. Once retrieved, recordings are reviewed to aurally identify bird species. There are advantages and disadvantages to using either in-person observers or ARUs for surveying bird communities (summarized in Table 1.1). Specific advantages and disadvantages of using in-person observers or ARUs to survey wetland bird communities are reviewed in Chapter Three of this thesis. A project's research goals will help inform which survey method should be employed to capture target diversity metrics and/or suite of birds.

*Table 1.1*. Comparing the use of in-person point counts and autonomous recording units (ARUs) to survey bird communities (Shonefield & Bayne, 2017; Darras et al., 2019).

| **Survey Method** | **Advantages** | **Disadvantages** |
| --- | --- | --- |
| In-person point count | ➢ Secondary identification via visual observation may facilitate the detection of quiet vocalizers<br><br>➢ Can visually identify bird behaviour and therefore collect additional information<br><br>➢ Can estimate the distance to bird vocalizations or sightings (useful for identifying if birds are in the target habitat being surveyed, or can be used to calculate survey area for population estimates and | ➢ Point counts situated in remote or difficult to access locations make it challenging to revisit sites multiple times. This may miss variation in bird occurrence across the breeding season and underestimate species richness at a site<br><br>➢ Limit to how many sites can be surveyed in one day (e.g., during the dawn chorus). Multiple teams can be used to increase sample size, but this introduces interobserver |

| Survey Method | Advantages | Disadvantages |
| --- | --- | --- |
| | detection probability estimation)<br><br>➢ Visual sightings and triangulation of species positions enables estimates of abundance | bias that is challenging to control<br><br>➢ Often short survey duration (e.g., 5-15 mins), which may not capture infrequent vocalizing species<br><br>➢ Hard to complete longer-duration surveys as they require continuous attention. For example, surveying a 2-hour dawn chorus would be nearly impossible for an observer. This makes it difficult to capture variation across the dawn chorus, and risks missing infrequent vocalizing species, thus underestimating species richness<br><br>➢ Human presence can alter bird behaviour<br><br>➢ Requires trained personnel to accurately identify species in real-time, which can be expensive both in terms of salary and travel costs |
| ARU | ➢ Enables longer surveys without additional survey effort – can program to record at any time of day for any duration (e.g., beneficial for detecting species that call | ➢ Expensive (e.g., $1000 CAD for Wildlife Acoustics SM4 unit, and ongoing maintenance costs for batteries, SD cards and microphones) |

| Survey Method | Advantages | Disadvantages |
|---|---|---|
| | at night, or vocalize infrequently)<br><br>➢ Permanent record of survey that additional experts can QA/QC<br><br>➢ Can pause and replay to better identify vocalizations, which is not possible in person<br><br>➢ Can analyze longer duration recordings than what could be achieved with in-person surveys (i.e., can pause recordings, take breaks, etc.)<br><br>➢ Audio recordings can be analyzed by automated species recognizers to reduce human effort<br><br>➢ Reduced field time (visit a site once to set up and once to take down, which is beneficial for remote locations)<br><br>➢ Ability to simultaneously record at multiple sites | ➢ Increasing spatial coverage requires either a large amount of ARUs or moving ARUs among sites during the breeding season. However, this still enables more simultaneous stations to be surveyed without interobserver bias than is possible with in-person surveys<br><br>➢ Loss of visual identification of birds may lead to underestimates<br><br>➢ Can produce a large amount of data, which is laborious to analyze. Whereas in-person surveys yield data immediately without additional transcription effort<br><br>➢ Data corruption can occur and may not be noticed for a long time until retrieved from the field<br><br>➢ Can be difficult or not possible to estimate bird abundance from ARU recordings as birds may move and call from multiple locations<br><br>➢ The recording range is often unknown or roughly estimated (difficult to |

| Survey Method | Advantages | Disadvantages |
|---|---|---|
| | | estimate area sampled) which limits population size estimates and detection probability assessments |

*1.7 Research objectives*

In 2005, *P. australis* was deemed Canada's worst invasive plant by Agriculture and Agri-food Canada (Gabby, 2020). Almost 20 years later, *P. australis* invasions across Ontario and Canada have not slowed, which has justified more intensive and disruptive control practices. Assessing the impacts of invasive species control on native species is important to ensure that such measures do not impose more harm on wildlife and their habitat than the invasion itself. Furthermore, it is important that survey methods balance effort and ability to capture accurate estimates of diversity to accurately assess the response of native species to management actions.

In Chapter Two, we used two field studies – a Before-After-Control-Impact design and a space-for-time substitution design – to investigate the short-term impacts of *P. australis* management on wetland bird communities in two National Wildlife Areas in Long Point, ON. We used ARUs to survey birds during the dawn chorus in the breeding season to investigate whether diversity metrics (species richness, community composition, and functional traits) differed between control (*P. australis*), 1-or 2-years post-herbicide-rolling treated *P. australis*, and uninvaded reference vegetation. We conclude that there are minor changes to wetland bird communities two years after *P. australis* management, as non-wetland affiliated birds experienced more change following treatment.

In Chapter Three, we investigated how to optimize the use of ARUs to survey breeding wetland birds during the dawn chorus. We also investigated if ARUs and in-person observers can detect certain wetland birds at comparable distances in different wetland vegetation types. We conclude that a longer duration survey on one day within the breeding season captures comparable avian diversity metrics as many short duration surveys across the breeding season. But it may be more economical to employ the one longer duration survey, which permits more sites to be surveyed by moving the ARUs around during the breeding season. We also found that the detection distances of ARUs and in-person observers are relatively comparable in the three vegetation types, except in open areas where background noise may have a greater influence on ARUs and reduce their detection ability. Importantly, there was no difference in avian detection distances between cattail and invasive *P. australis* vegetation types, regardless of the survey method.

In Chapter Four, we summarize our findings, review management implications regarding *P. australis* control in wetlands, and provide recommendations for how to optimize ARUs to monitor wetland bird communities.

# *2. Short-term effects of Phragmites australis management on avian species diversity in Long Point coastal wetlands*

## *2.1 Introduction*

Marsh bird populations in the southern Great Lakes region have experienced substantial declines since the mid-90s (Tozer, 2013, 2016, 2020). One main cause for this decline is the expansion of the invasive grass species, *Phragmites australis* subsp. *australis* (*P. australis*) and its homogenization of breeding bird habitat in wetlands (Tozer, 2016; Robichaud & Rooney, 2017; Tozer & Beck 2018; Tozer & Mackenzie, 2019; Robichaud & Rooney, 2022b). *Phragmites australis* invasion has exponentially expanded in the coastal wetlands of Long Point, Ontario, which are of both regional and global significance to avifauna (Wilcox et al., 2003; Government of Canada, 2021).

*Phragmites australis* invasion alters the vegetation structure and composition within wetlands and can displace native vegetation preferred by many marsh birds for breeding and foraging (Whyte et al., 2015; Robichaud & Rooney, 2017; Tozer & Beck, 2018). The tall and dense stands of *P. australis* fill in water channels and pools, leading to the loss of high-value hemi-marsh habitat and a reduction in marsh bird access to preferred feeding and breeding grounds (Windham & Lathrop, 1999; Meyerson et al., 2000). *Phragmites australis* may also lack high-quality nesting material due to its rigidity, particularly for ground-nesting birds such as waterfowl or rails (Meyer et al., 2010).

Indeed, the impacts of *P. australis* invasion on avian communities have been well studied (e.g., Benoit & Askins, 1999; Meyer et al., 2010; Gagnon-Lupien et al., 2015; Whyte et al., 2015; Robichaud & Rooney, 2017, 2022). A main trend that has emerged from these studies is that there are "winners and losers" with the invasion of *P. australis* in wetlands: habitat generalists and small-bodied marsh-users may benefit, while larger-bodied marsh-users and aerial foragers may suffer (Gagnon-Lupien et al., 2015; Whyte et al., 2015; Robichaud & Rooney, 2017). Expansion of the dry and dense habitat of *P. australis* may be utilized by both habitat generalists, as they are not sensitive to vegetation type or water levels, and small-bodied marsh-users that prefer shrubby vegetation (Robichaud, 2016). In contrast, larger-bodied marsh-users, such as the provincially and federally Threatened Least Bittern (*Ixobrychus exilis*), and marsh bird species of conservation concern, such as the Virginia Rail (*Rallus limicola*), may avoid dense patches of *P. australis* (Robichaud & Rooney, 2017), possibly due to its impenetrability or unsuitable foraging or roosting sites (Rehm & Baldassarre, 2007; Kessler et al., 2011). Furthermore, provincially and federally at-risk swallows may avoid foraging over *P. australis* invaded areas (Robichaud & Rooney, 2017). Presumably, controlling *P. australis* invasion in coastal wetlands would help restore the avian community to pre-invasion conditions and benefit those birds most impacted by invasion.

To reverse the ecological degradation caused by *P. australis* invasion and recover habitat value and wetland floral and faunal diversity, many jurisdictions around the Great Lakes are engaged in *P. australis* control efforts (Braun et al., 2016). In most cases, this entails herbicide-based treatment of *P. australis* and some form of secondary treatment with amphibious vehicles to flatten or remove the resulting litter (Martin & Blossey, 2013; Hazelton et al., 2014). For example, in 2016 the Ministry of Natural Resources and Forestry initiated *P. australis* control efforts in the Long Point peninsula, specifically in the Crown Marsh Waterfowl Management Area and the Long Point Provincial Park. Surrounding land managers quickly joined the project, culminating in 2019, when Environment and Climate Change Canada – Canadian Wildlife Service joined the peninsula-wide effort to eliminate *P. australis*. The Canadian Wildlife Service – Ontario region (CWS-ON) maintains two National Wildlife Areas (NWAs) along the Long Point peninsula. The purpose of an NWA is to conserve ecologically significant habitat for migratory birds and other wildlife, as well as habitat for species at risk (ECCC, 2020). Because there have been few studies investigating potential harms to birds or other wildlife arising from *P. australis* suppression activity, CWS-ON were concerned about the potential for unanticipated harms. In the Long Point Walsingham Forest, which is Ontario's Priority Place for species at risk conservation, the CWS-ON has published an Integrated Conservation Action Plan, which sets the goal that 90% of the vegetation in wetlands and dunes now dominated by *P. australis* will be native by 2025 (MacLeod, 2019). This goal aims to reduce *P. australis* extent and maintain cover at less than 10% of its 2018 extent across the Long Point coastal wetland complex (ECCC 2020b; MacLeod, 2019). Long Point supports critical habitat for over 50 species listed under the Species at Risk Act, including at least 28 at-risk bird species (ECCC, 2020b). The ultimate aim of this conservation action is to suppress *P. australis* to encourage the recovery of native vegetation and the re-establishment of ecologically significant habitat (ECCC, 2020 a,b). As part of the conservation action plan, CWS-ON must evaluate the effects of treatment on wetland biota, including species at risk and marsh birds (MacLeod, 2019). However, for CWS-ON to engage with the broader peninsula-wide efforts, they wanted to monitor the short-term effects of *P. australis* suppression activity on wetland birds, as distinct from the growing body of evidence of long-term improvements in habitat quality for wetland birds that ultimately result from *P. australis* suppression.

*Phragmites australis* management can be challenging and costly, and it may require long-term repeated control measures to sustain *P. australis* removal, and there is concern that the associated recurrent habitat alteration can have unintended consequences to wetland biota (Martin & Blossey, 2013; Hazelton et al., 2014; Quirion et al., 2017, Angoh et al., 2021). For example, the heavy machinery used for mechanical treatment may pose a risk of injury to at-risk turtles using *P. australis* as habitat (Angoh et al., 2021). Furthermore, the goal of *P. australis* restoration is to promote the recovery of native vegetation, but this recovery can be context-dependent and is not guaranteed, as the environmental conditions at a site (e.g., soil moisture levels, water levels) can greatly influence what vegetation returns after *P. australis* management (Rohal et al., 2019).

For example, a study monitored the response of vegetation communities after *P. australis* was treated with an herbicide in two coastal wetland complexes on Lake Erie, including the Long Point region (Robichaud & Rooney, 2021a). They found that two years after treatment, over half of the treated plots had vegetation communities that diverged significantly from the control plots (i.e., where *P. australis* remained), but nonetheless remained dissimilar from the reference condition (i.e., uninvaded emergent and meadow marsh habitat). Instead, these treated plots were a novel community composed of floating and submerged aquatic vegetation and dominated by the invasive species European Frogbit (*Hydrocharis morsus-ranae*). This highlights that the removal of one invasive species can lead to secondary invasion by other invasive species. Indeed, several factors regarding *P. australis* management may influence whether marsh birds will use and benefit from the restored habitat, not least of which is how the vegetation communities will respond to *P. australis* removal. Few studies have looked at the immediate response of bird communities to the removal of *P. australis* in coastal wetlands (see Lazaran et al., 2013).

Lazaran et al. (2013) determined that the immediate effects of *P. australis* management in Lake Erie coastal marshes may harm certain breeding marsh birds, such as the Marsh Wren (*Cistothorus palustris*). They determined that in 1-year post-herbicide-treated sites, Marsh Wren singing territory and nest density was significantly lower, and initiation of nest of nests was significantly later, compared to pre-treatment conditions. It is likely that the removal of dense, vertical structure provided by *P. australis,* as well as the delay in the regeneration of vegetation one year after treatment, reduced the breeding habitat required by the Marsh Wren (Lazaran et al., 2013).

The ultimate outcome of *P. australis* management for the avian community is expected to be positive. For example, Tozer & Mackenzie (2019) looked at a longer-term response of marsh birds to *P. australis* management and found positive effects on marsh birds. They found that species richness and abundance of marsh birds of conservation concern significantly increased five years after treatment, and that three out of four of the common marsh breeding birds experienced no significant change in occurrence after treatment, except for Marsh Wren, which experienced a significant increase (Tozer & Mackenzie, 2019). This finding may seem to contradict the results of Lazaran et al. (2013) but recall that Tozer and Mackenzie (2019) found this positive effect of *P. australis* management on Marsh Wren five years after treatment, by which time vegetation should have recovered. Therefore, we anticipate that the short-term effects of suppression activity may not agree with the longer-term outcome of *P. australis* removal and expect that it will take time for the avian community to positively respond to *P. australis* management as the vegetation communities equilibrate post-treatment.

Another avian functional group that is sensitive to *P. australis* invasion and therefore may benefit from management are aerial insectivores, which catch insects in flight (Robichaud & Rooney, 2017). Aerial insectivores have been experiencing steep population declines in Canada, losing approximately 59% of their population since the 1980s (North American Bird Conservation Initiative Canada, 2019). In the years immediately following *P. australis* management, treated areas were found to support a high density of emergent chironomid macroinvertebrates, which are a crucial prey item for aerial insectivores (Robichaud et al., 2021). The Barn Swallow, which is provincially designated as Threatened (Heagy et al., 2014) and federally designated as Special Concern (COSEWIC, 2021) and the Bank Swallow, which is provincially and federally designated as Threatened (Falconer et al., 2016; ECCC, 2021) are aerial insectivores that use marsh habitat and may benefit from the increased access to foraging grounds in the years immediately following *P. australis* control.

Overall, there are both benefits and costs to managing *P. australis* invasion in wetlands. Removing *P. australis* may benefit certain marsh birds that have been most impacted by *P. australis* invasion, including species of conservation concern, and those that are habitat specialists that rely on marsh habitat (Robichaud & Rooney, 2017; Tozer & Mackenzie 2019). The effects of *P. australis* removal on marsh birds seem to vary with time since management, as it takes time for the vegetation communities to rejuvenate after treatment (Lazaran et al., 2013; Tozer & Mackenzie, 2019; Robichaud & Rooney, 2021a). The recovery of the avian community is going to be context-dependent and vary with the response of the vegetation community (e.g., Rohal et al. 2019, Robichaud & Rooney, 2021). Any harm, even short-term harms, caused by *P. australis* suppression are cause for concern given that most monitoring studies suggest that *P. australis* suppression activities require frequent follow-up treatments that can cause repeated disturbance to wetland habitat over the long term (Martin & Blossey, 2013; Hazelton et al., 2014; Quirion et al., 2017, Angoh et al., 2021). These potential harms need to be understood to inform responsible land management decisions and to enable land managers to strategize how best to control invasive *P. australis* while mitigating any risk to birds and other wildlife.

To meet the conservation goal of CWS-ON's Integrated Conservation Action Plan (MacLeod, 2019), extensive *P. australis* management is occurring in the Long Point Walsingham Forest within the Big Creek NWA and the Long Point NWA to reduce the extent of *P. australis* to 10% of its 2018 extent by 2025. *Phragmites australis* is being treated with a glyphosate-based herbicide via aerial and ground application, followed by cutting or rolling of standing dead litter via an amphibious Marsh Master™. The motivating objective behind this work is the conservation of species at risk, including at least 28 avian species considered threatened by *P. australis* invasion-drive habitat loss (ECCC, 2020b). The purpose of our study is to assess the short-term effects of *P. australis* control on the avian community in Long Point to determine if any consequences arise from treatment.

In this chapter, we had two main objectives: 1) use a Before-After-Control-Impact design to assess the effects of *P. australis* control on avian species richness (total, marsh-users, species at-risk, and species of conservation concern) and community composition (total and marsh-user), and 2) use a space-for-time substitution design to compare avian species richness (same metrics), community composition (same metrics), and functional trait composition among herbicide-rolling treated sites, uninvaded 'reference' sites, and untreated 'control' sites (*P. australis*-dominated).

We hypothesize that total species richness will be similar between control, reference, and herbicide-treated sites because species richness can be an insensitive metric for determining changes in avian diversity in different wetland habitats (Robichaud & Rooney, 2017). *Phragmites australis* invaded habitat supports similar richness to reference wetland habitat (Gagnon-Lupien et al., 2015; Whyte et al., 2015; Robichaud & Rooney, 2017). *Phragmites australis* may support a different composition of birds than reference habitat due to structural differences in vegetation, but this may not alter total site-level richness if there is turnover in community composition (i.e., a loss of habitat specialists, but gain of habitat generalists; Robichaud & Rooney, 2017). We predict that total species richness will not be different among control, herbicide-treated, and reference habitat, because birds preferring the tall and dense habitat of *P. australis* may be replaced by those who prefer the open-water habitat remaining after treatment. We hypothesize that the richness of marsh-users and species of conservation concern will be similar in reference and treated habitat and be greater than invaded *P. australis* control sites. Several marsh-users and species of conservation concern are waterfowl and wading birds that use hemi-marsh habitat for breeding and foraging and will likely use the increase in open-water and hemi-marsh habitat remaining after treatment (Lor & Malecki, 2006; Rehm & Baldassarre, 2007; Schummer et al., 2012). We predict that herbicide-treated and reference habitat will support greater richness of marsh-users and species of conservation concern than invaded *P. australis* habitat. We hypothesize that the richness of species will be lower in *P. australis* sites than treated or reference sites. There is a mix of species at risk observed in Long Point (e.g., swallows, bitterns, terns) which have different habitat requirements. However, the expansion of invasive *P. australis* has likely displaced their preferred habitat type, shifting them to other vegetation within the wetlands. We predict that herbicide-treated and reference sites will have a greater richness of species at risk than invaded *P. australis* sites.

We hypothesize that avian community composition will differ among control, reference, and herbicide-treated sites. *Phragmites australis* supports species that use dense, vertically structured vegetation, which are often small-bodied species or habitat generalists (Gagnon-Lupien et al., 2015; Rooney & Robichaud, 2017). The more open habitat resulting from herbicide treatment will likely support species that prefer to forage and nest in areas with greater interspersion of emergent vegetation and open water than in dense vegetation (Rehm & Baldassarre, 2007; Schummer et al., 2012). We predict that the community composition will differ between *P. australis* invaded habitat and herbicide-treated habitat. We further predict that community composition in treated sites will lie somewhere between reference habitat and *P. australis* invaded habitat. We hypothesize that herbicide-treated habitat will not support novel avian species with novel functional traits, but instead a subset of birds with functional traits found in reference habitat. The recently treated habitat will likely resemble reference habitat (more open water and hemi-marsh arrangement), which may support waterbirds that often use hemi-marsh for breeding and foraging (Rehm & Baldassarre, 2007; Baschuck et al., 2012). We predict that functional trait composition will be similar in reference and treated habitat, but distinct from *P. australis* invaded habitat.

## *2.2 Methods*

### *2.2.1 Study area*

In the spring of 2019 and 2021, we conducted avian surveys in coastal wetlands in the Big Creek and Long Point National Wildlife Areas located in Long Point, Ontario, Canada. The NWAs are separated into management units, and our study surveyed the Big Creek unit within the Big Creek NWA, and the Thoroughfare, Squire's Ridge, and Long Pond units within the Long Point NWA.

### *2.2.2 ARU deployment*

We used a Before-After-Control-Impact (BACI) design (*sensu* Underwood, 1992) to determine the response of marsh birds two years after *P. australis* removal. This is a spatially replicated design which we used to compare bird diversity in control and herbicide-treated sites to themselves over time. In spring 2019, CWS-ON deployed eight ARUs (Song Meter SM4s units; Wildlife Acoustics, 2021) across the Big Creek and Long Pond management units in non-native *P. australis*-dominated areas to record baseline conditions of bird communities prior to *P. australis* treatment (Figure 2.1). Four ARU sites would remain as untreated *P. australis* (statistical controls) and four ARU sites would be treated in fall of 2019. Control and treatment sites were paired by water depth and clustered by management unit; four ARUs were placed in Big Creek and four in Long Pond. An Emergency Registration (no. 32356) was obtained, and in fall 2019, a glyphosate-based herbicide (Roundup® Custom for Aquatic & Terrestrial Use Liquid Herbicide, Bayer CropScience Inc., Canada) combined with a nonionic alcohol ethoxylate surfactant (Aquasurf®, registration no. 32152, Brandt Consolidated, Springfield, IL, USA) was used to treated approximately 10 ha of wetland. This herbicide application was followed up by mechanical treatment by cutting and rolling dead *P. australis* via an amphibious Marsh Master™. In spring 2021, we worked with CWS-ON to deploy ARUs in the same control and treatment locations across the Big Creek and Long Pond management units. These 2021 ARU sites were surveyed approximately 20 months after treatment, but for simplicity, we will refer to this as 2-years post-herbicide-rolling treatment.

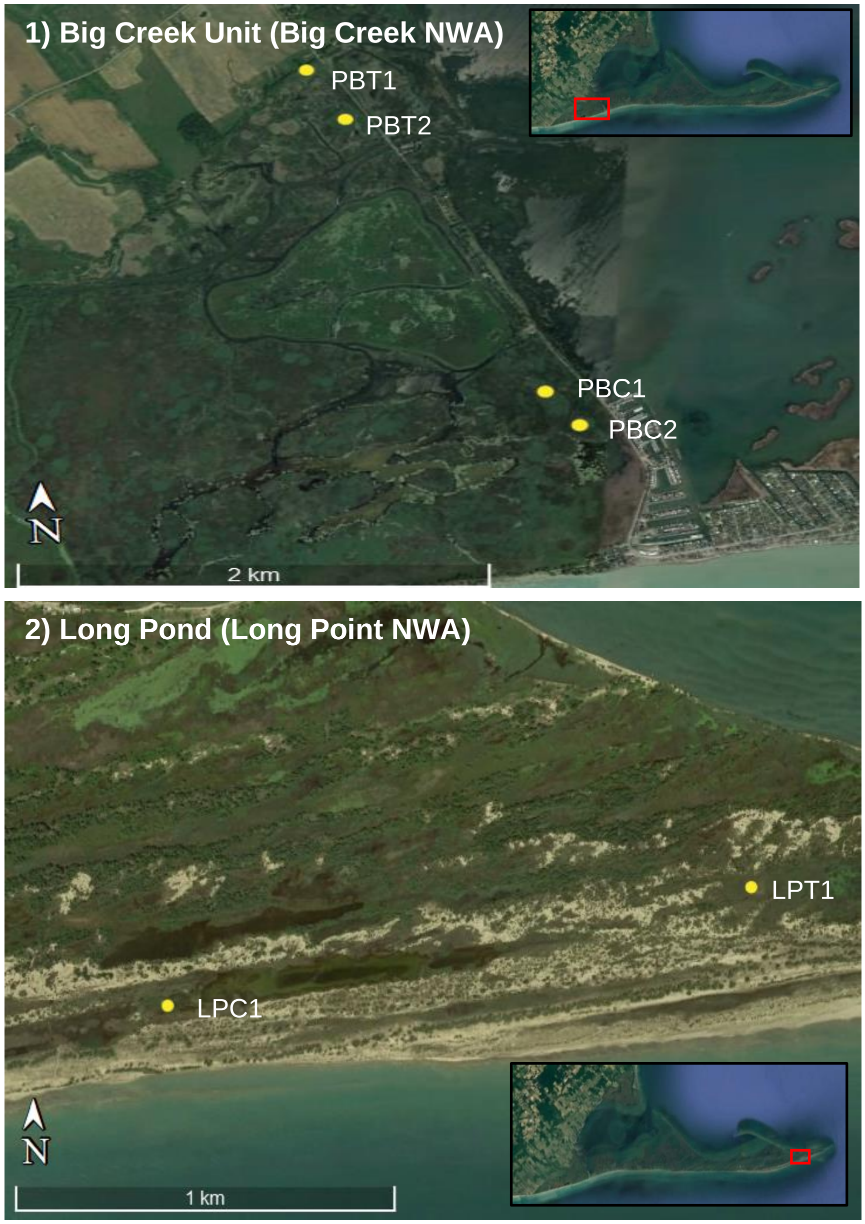
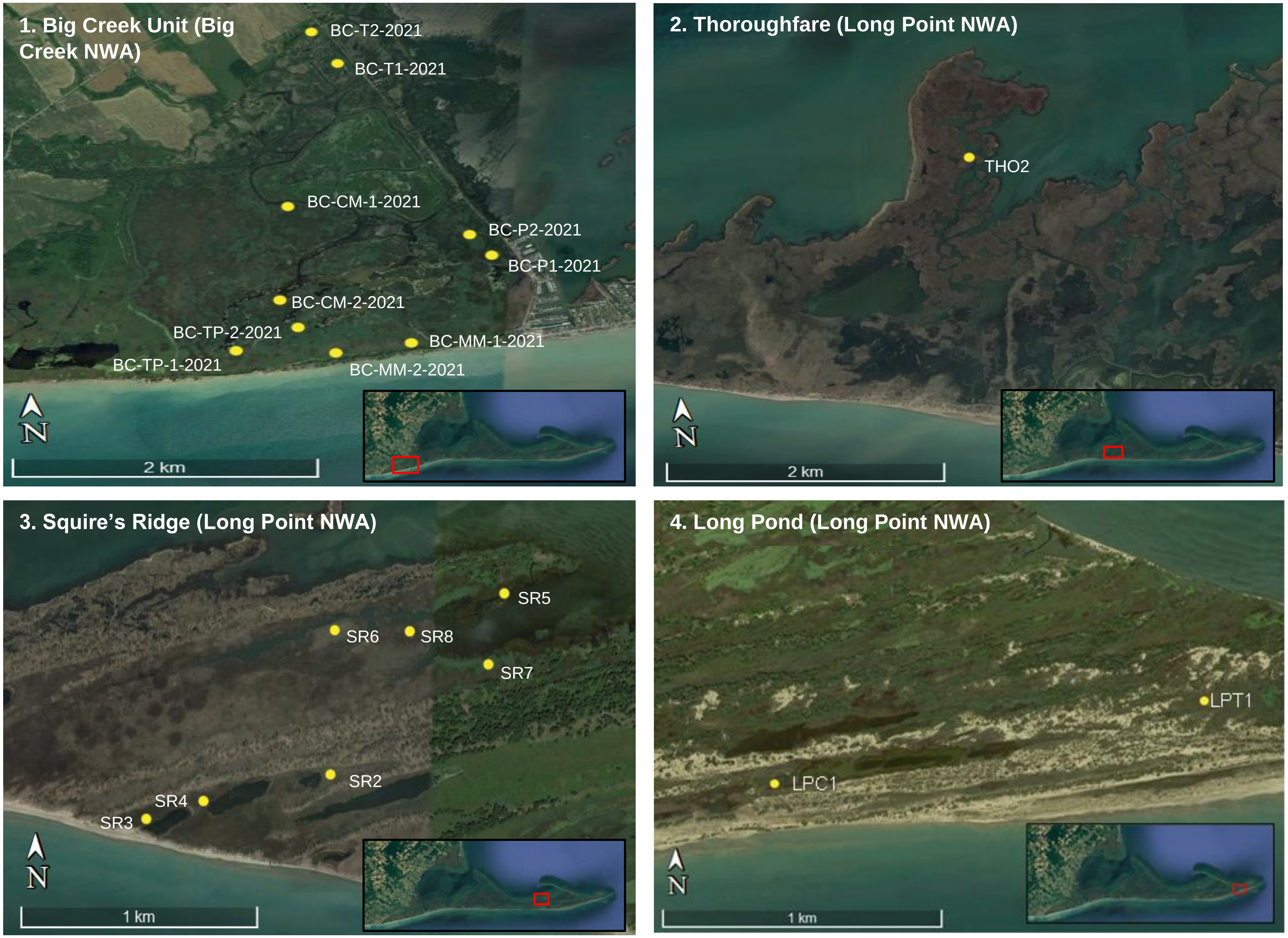



*Figure 2.1.* Map of non-corrupted autonomous recording unit locations in the Big Creek National Wildlife Area (1) and the Long Point National Wildlife Area (2) on the Long Point peninsula located on the north shore of Lake Erie. Sites were sampled in June 2019 and June 2021 for the Before-After-Control-Impact design. Site names with "T" indicate treatment (glyphosate-based herbicide application followed by mechanical rolling of litter) and "C" indicate control (untreated *P. australis*). In 2021, technical difficulties occurred with the ARUs due to a firmware update which resulted in corrupted audio files on several units.

We originally planned to repeat the BACI experiment in other areas of the wetland where treatment was planned for fall 2020, but could not implement this plan due to COVID-19 restrictions. Instead, in spring 2021, we used a space-for-time substitution design to determine how marsh bird diversity and composition compared among herbicide-rolling treated sites, reference sites that had never been invaded by *P. australis*, and untreated control sites that remain dominated by *P. australis*. A space-for-time substitution design is used to collect data with large spatial extent over a short duration of time to determine relationships between predictor and response variables without having to wait several years to collect the data (Pickett, 1989). We included reference sites to determine if bird communities in herbicide-treated sites are starting to resemble bird communities found in reference habitat.

In fall 2020, approximately 110 ha of wetland received herbicide application and rolling treatment across the Big Creek management unit and the Thoroughfare management unit. In spring 2021, we worked with CWS-ON to deploy ARUs across the Big Creek, Thoroughfare, Squire's Ridge, and Long Pond management units to record bird communities in 1-or 2-year-post-treatment sites, untreated control sites (*P. australis*-dominated) and uninvaded reference sites (Figure 2.2). In 2021, ARUs placed in sites that were treated in fall 2020 were surveyed approximately 8 months after treatment, but for simplicity, we will refer to this as 1-year post-herbicide-rolling treatment. Reference sites included cattail marsh (*Typha* spp.), meadow marsh, and hemi-marsh (50% open water, 50% emergent vegetation). ARUs were clustered by management unit to spatially represent the Big Creek and Long Point NWAs. The four management units were broken into four directional quadrants – northeast, southeast, southwest, northwest – and we attempted to equally distribute the number of ARUs for each vegetation type in each quadrant, to the extent possible. Prior to deployment, all ARU microphones were calibrated.

*Figure 2.2.* Map of non-corrupted autonomous recording unit locations in the Big Creek National Wildlife Area (1), Thoroughfare management unit (Long Point NWA) (2), Squire's Ridge management unit (Long Point NWA) (3), and Long Pond management unit (Long Point NWA) (4), on the Long Point peninsula located on the north shore of Lake Erie. Sites were sampled in June 2021 for the space-for-time substitution design. "P" indicates *P. australis*, "T" and "TP" indicate treated *P. australis* (herbicide application followed by rolling), "CM" indicates cattail marsh and "MM" indicates meadow marsh. THO2 is a reference (hemi-marsh) site. SR 2, 5 and 6 are reference sites (meadow marsh, hemi-marsh, and cattail marsh, respectively), and SR 3, 4, 7 and 8 are control sites (*P. australis*). In 2021, technical difficulties occurred with the ARUs due to a firmware update which resulted in corrupted audio files on several units.


1. Big Creek Unit (Big Creek NWA)
BC-T2-2021
BC-T1-2021
BC-CM-1-2021
BC-P2-2021
BC-P1-2021
BC-CM-2-2021
BC-TP-2-2021
BC-MM-1-2021
BC-TP-1-2021
BC-MM-2-2021
N
2 km
2. Thoroughfare (Long Point NWA)
THO2
N
2 km
3. Squire's Ridge (Long Point NWA)
SR5
SR6
SR8
SR7
SR2
SR4
SR3
N
1 km
4. Long Pond (Long Point NWA)
LPT1
LPC1
N
1 km

We programmed ARUs to record during the dawn chorus within the marsh bird breeding season (mid-May to early July) in 2019 and 2021. ARUs began recording a half-hour before dawn and continued for two hours and did so for 4 – 7 consecutive days across mid-late June in 2019 and 2021. ARUs were deployed following CWS's 2021 ARU deployment protocol. We deployed ARUs in homogenous patches of target vegetation that were at least 25 m in radius and 25 m from open water. We positioned ARUs to be 1) perpendicular to the depth gradient, with the front of the ARU facing open water and the back facing shallow water or shoreline, and 2) installed to have microphones 1.5 m above the water level. All ARUs were at least 250 m apart to prevent their estimated 125 m recording radii from overlapping.

In 2021, technical difficulties occurred with the ARUs due to a firmware update which resulted in corrupted audio files on several units. This reduced the 2019-2021 BACI sample size to three control and three treatment sites, and it reduced the 2021 space-for-time substitution experiment from 30 ARUs to 20 (Table 2.1). To improve statistical power for the space-for-time substitution experiment, the three reference vegetation types (meadow marsh, cattail marsh and hemi-marsh) were grouped together as 'reference', and the 1-or 2-year post-herbicide treatment sites were grouped together as 'treated'.

*Table 2.1.* Sample size in reference (comprising of three vegetation types), treated (1-or 2-years post-herbicide-rolling) and control (*P. australis*) sites across the Big Creek and Long Point NWAs in 2021.

| **Vegetation** | **Sample Size** |
|---|---|
| *Reference* | 8 |
| Hemi-marsh | 2 |
| Cattail (*Typha* spp.) | 3 |
| Meadow marsh | 3 |
| *Herbicide-treated* | 5 |
| 1-year post 2020 treatment | 2 |
| 2-years post 2019 treatment | 3 |
| *Control* | 7 |
| Total | 20 |

*2.2.3 ARU transcription*

Our research determined that ARUs within Long Point coastal wetlands should be transcribed for 45 min on one day in June, split across the dawn chorus, to capture at least 80% of the species estimated to be present using nonparametric “true” richness estimators, as well as capture species of interest such as marsh-users, species at risk, and species of conservation concern (see Chapter Three). Transcription effort was split into three 15 min windows to capture the early and late portion of the dawn chorus: 1) the 15 min immediately preceding dawn, 2) the 15 min immediately following dawn, and 3) the 15 minutes running between 1 h 15 min after dawn to 1 h 30 min after dawn.

Recordings were transcribed using the audio editing program Audacity ® (version 2.4.2; Audacity, n.d). Audacity displays audio as spectrograms, which are visualizations of bird vocalizations (Figure 2.3). Spectrogram settings were set to a logarithmic scale to show frequencies between 1000-10,000 Hz, the window type was set to Hann, and the window size was set to 1024, while the gain was 15 dB and range was 80 dB (Reynolds, 2020). The spectrogram color was set to grayscale for ease in visual interpretation. These settings were chosen to best identify vocalizations 1000 Hz or higher, which is the range of most diurnal avian species (Hu & Cardoso, 2009). Birds were identified by their audible vocalizations, and when possible, confirmed visually by analyzing the species’ unique spectrogram (Figure 2.3). The recordings were transcribed in 1 min intervals, and the presence of a species heard vocalizing within the interval was recorded.

Vocalization identifications with low certainty were reviewed by Dr. Doug Tozer of Birds Canada. Vocalizations that were too quiet, degraded in quality, or unidentifiable as a unique song or call were omitted from all subsequent analyses, but vocalizations that were possibly unique species were kept in subsequent analyses as “unknown species”.

The six ARUs in 2019 were transcribed on the same day on June 23$^{rd}$, and the 20 ARUs in 2021 were transcribed on either June 17$^{th}$ or 20$^{th}$, as one date for all recordings could not be chosen due to either poor weather conditions or technical difficulties leading to corruption of files. Dates were chosen in compliance with the Marsh Monitoring Program Protocol; light wind, no rain, and minimal background noise (Birds Canada, 2009). However, some background noise including nearby traffic and lake activity was unavoidable across all days for certain ARUs, but the duration of background noise interference was often short and did not significantly impact transcription.

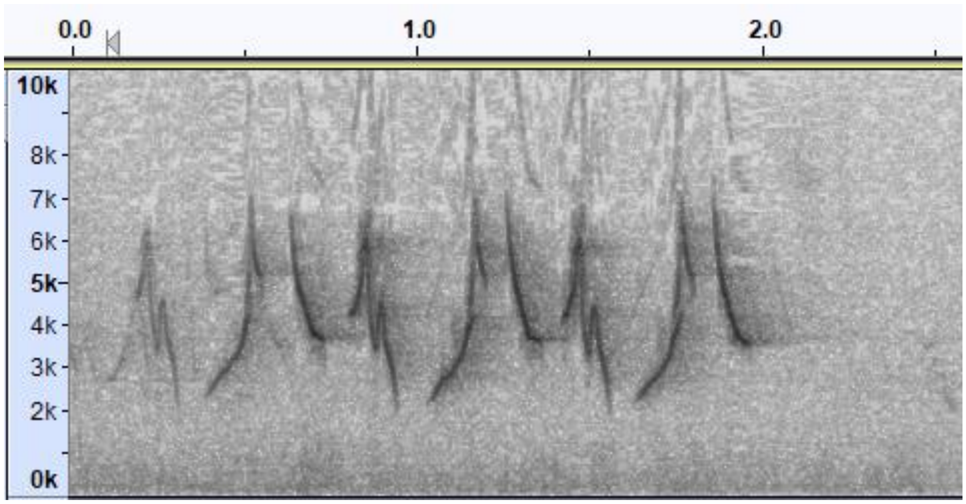



*Figure 2.3.* Spectrogram of a Common Yellowthroat's (*Geothlypis trichas*) "witchita witchita" song.

*2.2.4 ARU site characteristics*

To characterize bird habitat surrounding each ARU in 2021, we determined vegetation composition and vertical structure by completing vegetation contact profile surveys following a similar methodology outlined in Gagnon-Lupien et al. (2015). We completed contact profile surveys between June 5$^{th}$- 20$^{th}$ 2021 to reflect the habitat used by birds during the ARU transcription period of mid-June. To complete the surveys, a 1 m rod was placed horizontally on the ground or water's surface and a 4.5 m rod was placed vertically at one end of the 1 m rod. Each rod was 3 cm wide and marked with red and blue tape alternating every 20 cm. Starting at the bottom of the vertical rod, each plant species touching the rod was documented in 20 cm intervals. The vertical rod was moved in 20 cm intervals along the horizontal rod until the entire 1 m was assessed. Plants were grouped in categories similar to the Ontario Wetland Evaluation System (2014) vegetation classification: broad-leaved emergent, narrow-leaved emergent, robust emergent, floating, ground cover, shrub, *P. australis,* and standing dead litter. Sampling occurred at five locations at each ARU site: 1 m in front of the ARU, 40 m and 80 m left of the ARU, and 40 m and 80 m right of the ARU. Water depth was taken at each of the five sampling points and averaged for each site.

We plotted vegetation contact profiles by summing the total number of contacts for each vegetation type for each height class for each ARU. We also carried out an NMS ordination to visualize trends in ARU site characteristics.

*2.2.5 Statistical analyses*

For both the 2019-2021 BACI experiment and 2021 space-for-time substitution, we performed analyses on avian diversity and community composition. For the 2021 space-for-time substitution, we additionally investigated the composition of bird functional traits (i.e., how a bird forages, what it forages for, and its nesting preferences), which were retrieved from the Cornell Lab of Ornithology "All About Birds" online resource (Cornell Lab of Ornithology, 2022). For avian diversity, we analyzed total avian species richness and the richness of species at risk, marsh-users, and marsh bird species of conservation concern, reflecting the importance of the Big Creek and Long Point NWAs in avian biodiversity conservation. Birds that were designated as provincially and/or federally at-risk were included in the species at risk group. In consultation with Dr. Doug Tozer of Birds Canada, we created a list of marsh-user species, which were defined as species that rely on wetlands for breeding, foraging and/or loafing. This group included species designated as marsh-users in the Marsh Monitoring Program. We identified marsh bird species of conservation concern as those that the Marsh Monitoring Program designates as "focal" species that are often secretive in nature, require adequate habitat quality, and may be most sensitive to changes in their habitat (Birds Canada, 2009; Tozer, 2013a). They include the following: Least Bittern (*Ixobrychus exilis*), American Bittern (*Botaurus lentiginosus*), Virginia Rail (*Rallus limicola*), King Rail (*Rallus elegans*), Sora (*Porzana carolina*), Common Gallinule (*Gallinula galeata*), American Coot (*Fulica americana*), and Pied-billed Grebe (*Podilymbus podiceps*). For community composition, we analyzed both the total avian community and the marsh-user community. All univariate statistics were computed using SYSTAT v. 13.1 (SYSTAT, 2009) and all multivariate analyses were computed using PC-ORD v. 7 (McCune & Mefford, 2018). Assumptions of all statistical tests were reviewed.

### 2.2.6 Before-After-Control-Impact experiment

#### 2.2.6-a Avian species richness

We conducted a two-factor ANOVA (type III SS) to examine the effect of year (2019, 2021), treatment (control or herbicide-rolling treatment) and their interaction on total species richness and the richness of marsh-users, species of conservation concern, and species at risk. Both year and treatment were set as fixed factors. We assessed the normality of the residuals with an Anderson-Darling test, and homogeneity of variance with a Levene's test, as well as visual inspection of plots of residual vs fitted values. With a BACI design, we are most interested in the significance of the interaction term, which would indicate that bird diversity diverged over time between treatment and control plots following herbicide-rolling treatment.

#### 2.2.6-b Avian community composition

We conducted two two-factor multivariate permutational analysis of variance (perMANOVA) to investigate the effects of year (2019 vs. 2021), treatment type (control vs. treatment), and their interaction on the total avian community composition and the marsh-user community composition. Year and treatment type were set as fixed factors and our main interest was in whether a statistically significant interaction effect (i.e., $< 0.05$) was present, which would indicate that the community composition of birds diverged between treated and control sites following the herbicide-rolling treatment. We used the Sorenson distance measure calculated using presence-absence data to test if the herbicide-rolling treatment had an effect on bird community composition. For the total avian community dataset, fourteen species that occurred in only one of the 12 site-year combinations were excluded from the analysis to reduce sparsity in the dataset (Peck, 2010).

To visualize changes in community composition across year and treatment, we conducted a non-metric multidimensional scaling (NMS) ordination, using the same Sorensen dissimilarity matrix calculated from the total avian community presence-absence data. An NMS produces a gradient in which sites with similar species compositions are positioned closer together and sites with dissimilar species compositions are positioned farther apart (Kenkel & Orloci, 1986). To determine optimal dimensionality, we contrasted 1- 4 dimension solutions via a Monte Carlo test method, whereby the final stress values from 50 randomized runs were compared to 50 runs with real data from random starting configurations. The runs were permitted a maximum of 200 iterations and a solution was deemed stable if the stress had a maximum standard deviation of 0.00001 over the last 10 iterations. Twelve species that occurred in only one of the 12 site-year combinations were excluded from analysis to reduce sparsity in the dataset. Three species – Purple Martin (*Progne subis*), Mourning Dove (*Zenaida macroura*), and Red-winged Blackbird (*Agelaius phoeniceus*) – were present at every site, and subsequently not plotted in ordination space.

To determine if NWA location was a significant predictor of differences in community composition (both total and marsh-user), we conducted two multi-response permutation procedures (MRPP) with a Sorensen distance measure. We used an MRPP because it does not require a balanced design like the perMANOVA (Big Creek NWA N = 4, Long Point NWA N = 2).

We ran two more two-factor perMANOVAs with Sorenson distance measure to determine if treatment impacted total avian community composition and marsh-user community composition at the Big Creek NWA sites only. Year and treatment were set as fixed factors, and again, we sought to determine whether their interaction was statistically significant.

*2.2.7 2021 space-for-time substitution experiment*

*2.2.7-a Avian species richness*

We conducted a one-factor ANOVA (type III SS) to examine the effect of vegetation type (herbicide-rolling treated, untreated control, and uninvaded reference) on total avian species richness and the richness of marsh-users, species of conservation concern, and species at risk. We assessed the normality of the residuals with an Anderson-Darling test, and homogeneity of variance with a Levene's test, as well as visual inspection of plots of residual vs fitted values.

*2.2.7-b Avian community composition*

We conducted four MRPPs using the Sorenson distance measure calculated using presence-absence data to test if 1) vegetation type (treated, control, and reference) and 2) ARU location (Big Creek, Thoroughfare, Squire's Ridge, and Long Pond management units) were significant predictors of differences in total avian community composition and marsh-user community composition.

To visualize changes in community composition across the three vegetation types, we conducted an NMS ordination, using the same Sorensen dissimilarity matrix calculated from the total avian community presence-absence data. To determine optimal dimensionality, we contrasted 1– 4 dimension solutions via a Monte Carlo test method, whereby the final stress values from 50 randomized runs were compared to 50 runs with real data from random starting configurations. The runs were permitted a maximum of 200 iterations and a solution was deemed stable if the stress had a maximum standard deviation of 0.00001 over the last 10 iterations.

#### *2.2.7-c Functional trait composition*

We conducted two MRPPs using the Sorenson distance measure calculated using functional trait occurrence data to test if 1) vegetation type, and 2) ARU location were significant predictors of differences in functional trait composition.

To visualize changes in functional trait composition across the three vegetation types, we conducted an NMS ordination of weighted abundance, using the Sorensen dissimilarity matrix calculated from the functional trait relative occurrence data. Traits were abundance-weighted to reflect the number of species observed at an ARU site possessing a given trait. To identify the optimal dimensionality, the same parameters in community composition NMS ordination were used (see Section 2.2.7-b).

## *2.3 Results*

### *2.3.1 Before-After-Control-Impact experiment*

#### *2.3.1-a ARU transcription*

Fifty-two avian species were observed in 2019 and 46 avian species were observed in 2021 across the six ARUs in the BACI experiment (Table 2.2, 2.3).

*Table 2.2.* Species identified after transcribing twelve 45-minute recordings on one day in June in 2019 and 2021 across control and treatment sites in the Big Creek and Long Point NWAs. Treatment occurred at treatment sites in the fall of 2019, after avian surveys were complete. Hence 2019 data is pre-herbicide application, and 2021 data is post-herbicide application at the treatment locations. Marsh-user species are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

| **Common Name** | **Scientific Name** | **4-Letter Alpha Code** |
|---|---|---|
| *Birds observed only in 2019* | | |
| Belted Kingfisher• | *Megaceryle alcyon* | BEKI |
| Black-crowned Night Heron• | *Nycticorax nycticorax* | BCNH |
| Blue-gray Gnatcatcher | *Polioptila caerulea* | BGGN |
| Blue-winged Teal• | *Spatula discors* | BWTE |
| Brown Creeper | *Certhia americana* | BRCR |
| Brown-headed Cowbird• | *Molothrus ater* | BHCO |
| Chestnut-sided Warbler | *Setophaga pensylvanica* | CSWA |
| Common Loon• | *Gavia immer* | COLO |
| Common Tern• | *Sterna hirundo* | COTE |
| European Starling• | *Sturnus vulgaris* | EUST |
| Gray Catbird | *Dumetella carolinensis* | GRCA |
| Herring Gull• | *Larus argentatus* | HERG |
| Mute Swan• | *Cygnus olor* | MUSW |
| Willow Flycatcher• | *Empidonax traillii* | WIFL |
| *Birds observed only in 2021* | | |
| Carolina Wren | *Thryothorus ludovicianus* | CAWR |
| Eastern Wood-pewee° | *Contopus virens* | EAWP |
| Great Crested Flycatcher | *Myiarchus crinitus* | GCFL |
| Indigo Bunting | *Passerina cyanea* | INBU |
| Northern Flicker | *Colaptes auratus* | NOFL |
| Northern Rough-winged swallow• | *Stelgidopteryx serripennis* | NWRS |
| Orchard Oriole | *Icterus spurius* | OROR |
| Virginia Rail•* | *Rallus limicoladd* | VIRA |
| *Birds observed in 2019 & 2021* | | |
| American Bittern•* | *Botaurus lentiginosus* | AMBI |
| American Crow | *Corvus brachyrhynchos* | AMCR |
| American Goldfinch | *Spinus tristis* | AMGO |
| American Robin | *Turdus migratorius* | AMRO |
| Baltimore Oriole | *Icterus galbula* | BAOR |
| Bank Swallow•° | *Riparia riparia* | BANS |
| Barn Swallow•° | *Hirundo rustica* | BARS |

| Common Name | Scientific Name | 4-Letter Alpha Code |
|---|---|---|
| Black-capped Chickadee | *Poecile atricapillus* | BCCH |
| Blue Jay | *Cyanocitta cristata* | BLJA |
| Brown Thrasher | *Toxostoma rufum* | BRTH |
| Canada Goose• | *Branta canadensis* | CAGO |
| Cedar Waxwing | *Bombycilla cedrorum* | CEDW |
| Chipping Sparrow | *Spizella passerina* | CHSP |
| Common Gallinule•* | *Gallinula galeata* | COGA |
| Common Grackle• | *Quiscalus quiscula* | COGR |
| Common Yellowthroat• | *Geothlypis trichas* | COYE |
| Eastern Kingbird• | *Tyrannus tyrannus* | EAKI |
| Eastern Towhee | *Pipilo erythrophthalmus* | EATO |
| Field Sparrow | *Spizella pusilla* | FISP |
| Great Blue Heron• | *Ardea herodias* | GBHE |
| House Wren | *Troglodytes aedon* | HOWR |
| Killdeer• | *Charadrius vociferus* | KILL |
| Least Bittern•*° | *Ixobrychus exilis* | LEBI |
| Mallard• | *Anas platyrhynchos* | MALL |
| Marsh Wren• | *Cistothorus palustris* | MAWR |
| Mourning Dove | *Zenaida macroura* | MODO |
| Northern Cardinal | *Cardinalis cardinalis* | NOCA |
| Pied-billed Grebe•* | *Podilymbus podiceps* | PBGR |
| Purple Martin• | *Progne subis* | PUMA |
| Red-winged Blackbird• | *Agelaius phoeniceus* | RWBL |
| Sandhill Crane• | *Antigone canadensis* | SACR |
| Song Sparrow• | *Melospiza melodia* | SOSP |
| Swamp Sparrow• | *Melospiza georgiana* | SWSP |
| Tree Swallow• | *Tachycineta bicolor* | TRES |
| Warbling Vireo | *Vireo gilvus* | WAVI |
| Wood Duck• | *Aix sponsa* | WODU |
| Yellow Warbler• | *Setophaga petechia* | YEWA |
| Yellow-billed Cuckoo | *Coccyzus americanus* | YBCU |

*Table 2.3*. Cumulative richness of total species, marsh-users, species at risk (SAR), and species of conservation concern (SOCC) in control (N = 3) and treatment sites (N = 3) in 2019 and 2021 across the Big Creek and Long Point NWAs.

| | **2019** | | | **2021** | | |
|---|---|---|---|---|---|---|
| | Total | Control | Treatment* | Total | Control | Treatment* |
| Total species | 52 | 40 | 46 | 46 | 41 | 37 |
| Marsh-users | 32 | 26 | 27 | 24 | 24 | 19 |
| SAR | 3 | 3 | 3 | 4 | 3 | 3 |
| SOCC | 4 | 4 | 4 | 5 | 5 | 3 |

*Note: herbicide-rolling treatment occurred at treatment sites in the fall of 2019, after avian surveys were complete. Hence, 2019 data is pre-herbicide application, and 2021 data is post-herbicide application at the treatment locations.

*2.3.1-b Avian species richness*

Total species richness was greater in 2019 than in 2021 ($F_{1,8} = 13.59$, $p = 0.01$), and it was greater in treatment sites compared to control sites ($F_{1,8} = 7.85$, $p = 0.02$; Figure 2.4). The interaction of year and treatment was at the margin of statistical significance ($F_{1,8} = 3.67$, $p = 0.09$). There was no significant effect of year, treatment, or interaction of year and treatment on the richness of marsh-users, species at risk, or species of conservation concern ($p > 0.1$; Table 2.4).

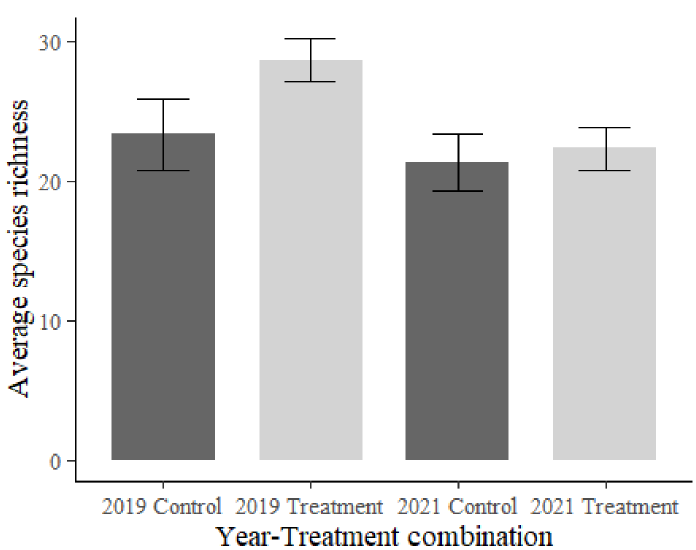


*Figure 2.4.* Plot of average avian species richness in control and treatment sites in 2019 and 2021 (N = 3 per year-treatment combination). Error bars indicate standard error. Note that the two-factor ANOVA with interaction concluded the interaction was not significant, but both year and treatment differed were ($p < 0.02$; Table 2.4). Also note that treatment occurred at treatment sites in the fall of 2019, after avian surveys were complete. Hence, 2019 data is pre-herbicide application, and 2021 data is post-herbicide application at the treatment locations.

*Table 2.4.* Two-factor ANOVA results comparing bird richness among year (2019, 2021), treatment (control or herbicide-rolling treatment) and their interaction. "SAR" represents species at risk and "SOCC" represents species of conservation concern.

| | **Total Species Richness** | | | **Marsh-user Richness** | | | **SAR Richness** | | | **SOCC Richness** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | df | p | F | df | p | F | df | p | F | df | p |
| Treatment | 7.85 | 1,8 | 0.02 | 0.02 | 1,8 | 0.90 | 0.06 | 1,8 | 0.81 | 0.02 | 1,8 | 0.89 |
| Year | 13.59 | 1,8 | 0.01 | 1.05 | 1,8 | 0.33 | 0.06 | 1,8 | 0.81 | 0.02 | 1,8 | 0.89 |
| Treatment x Year | 3.67 | 1,8 | 0.09 | 0.07 | 1,8 | 0.80 | 0.53 | 1,8 | 0.49 | 0.96 | 1,8 | 0.36 |

*2.3.1-c Avian community composition*

When we considered the Big Creek and Long Point NWAs ARU data combined, neither the composition of the total avian community or the marsh-user community exhibit a significant interaction between year (before and after) and treatment (control or treatment) ($p > 0.4$; Table 2.5). Neither were the main effects of year or treatment significant predictors of avian community composition ($p > 0.20$; Table 2.5).

To visualize the trends in avian community composition among the ARU locations, we carried out NMS ordination on the avian occurrence dataset. The optimal NMS ordination of community composition within the two NWAs had two dimensions ($p = 0.02$), with a final instability < 0.00001 and a final stress value of 9.58 after 69 iterations. Axis 1 explained 85.9% of the variance in community composition and axis 2 explained 3.5%. Correlations of species vectors with site scores can be found in Appendix 1A. Axis 1 – the axis that explains the greatest amount of variation – reflects a major differentiation between the bird community using the Big Creek NWA and the bird community using the Long Point NWA; the Big Creek sites group together at low axis 1 scores, while the Long Point sites group together with high axis 1 scores (Figures 2.5, 2.6). Species of conservation concern, such as American Bittern, Common

Gallinule, Least Bittern, and Pied-billed Grebe, as well as marsh-users such as Sandhill Crane (*Antigone canadensis*), Marsh Wren, and Tree Swallow (*Tachycineta bicolor*), occurred more frequently within the Big Creek sites (Figure 2.7). Whereas terrestrial species, such as Field Sparrow (*Spizella pusilla*), Black-capped Chickadee (*Poecile atricapillus*), and Eastern Towhee (*Pipilo erythrophthalmus*), occurred more frequently within the Long Point sites (Figure 2.7). The MRPPs confirmed that the area sampled in the two NWAs support distinct bird communities (total avian community: $A = 0.25$, $p < 0.01$; marsh bird community: $A = 0.20$, $p < 0.01$).

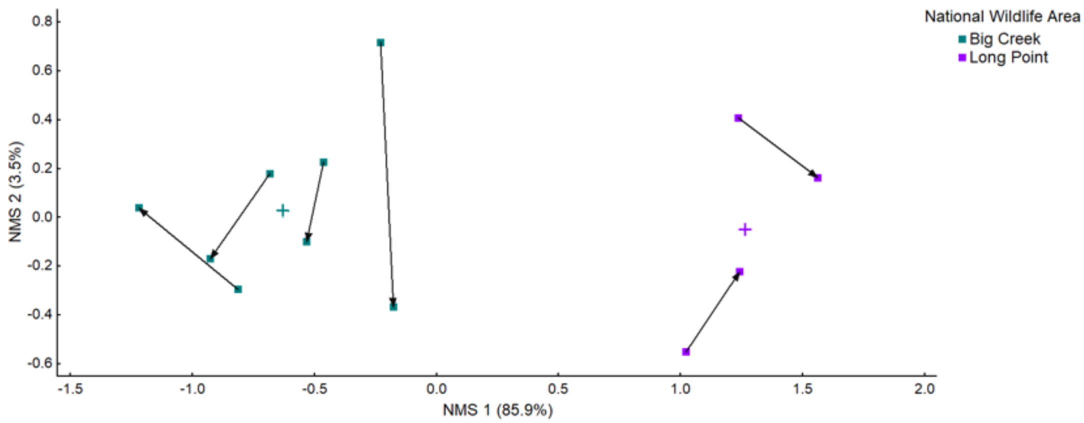



*Figure 2.5.* NMS ordination solution of bird community composition within the Big Creek and Long Point NWAs. Centroids (+) represent the geometric mean location (e.g., mean axis score of all Big Creek sites is indicated by teal centroid).

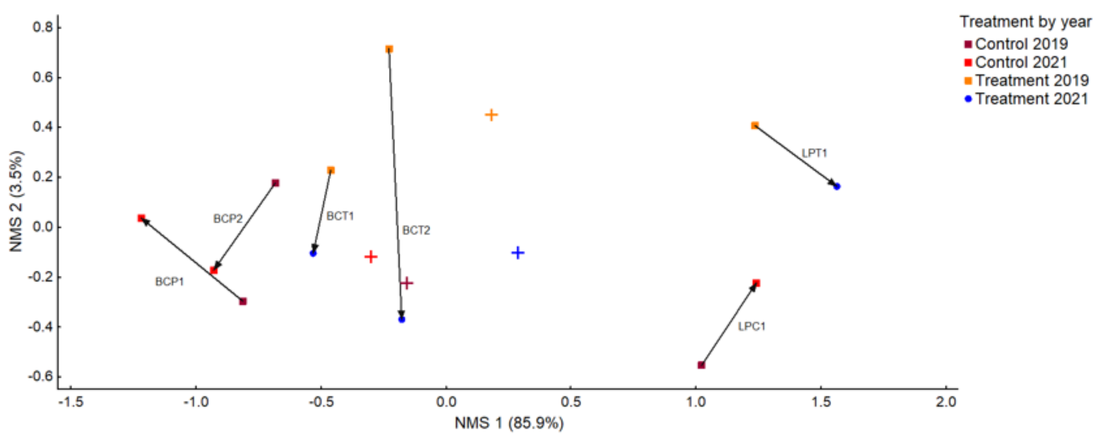



*Figure 2.6.* NMS ordination solution of bird community composition within the Big Creek and Long Point NWAs. Treatment occurred at treatment sites in the fall of 2019, after avian surveys were complete. Hence, 2019 data is pre-herbicide application, and 2021 data is post-herbicide

application at the treatment locations. Sites starting with "BC" are present in Big Creek NWA, and sites starting with "LP" are present in Long Point NWA. Centroids (+) represent the geometric mean location (e.g., mean axis scores of all Treatment 2021 sites are indicated by a blue centroid).

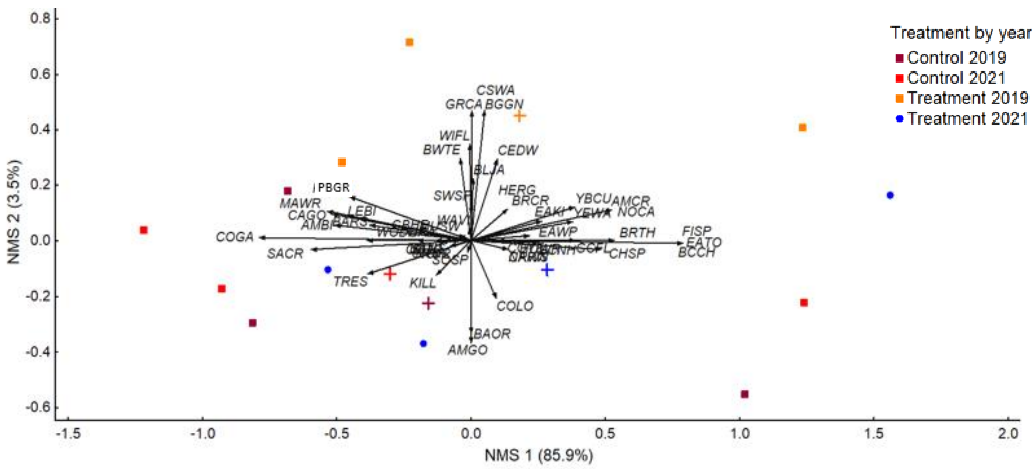



*Figure 2.7.* NMS ordination solution of bird community composition within the Big Creek and Long Point NWAs. Treatment occurred at treatment sites in the fall of 2019, after avian surveys were complete. Hence 2019 data is pre-herbicide application, and 2021 data is post-herbicide application at the treatment locations. Bird species are represented by the American Ornithologist Union four-letter alpha codes (see Table 2.2 for corresponding species names). Centroids (+) represent the geometric mean location (e.g., mean axis score of all treatment 2021 sites is indicated by a blue centroid). Black vectors represent how correlated the occurrence of a species is with NMS axis 1 and 2; species with an $r^2 \geq 0.05$ were considered reasonably correlated. Vectors were scaled to 50% to fit on the plot.

Due to the overwhelming influence of ARU location on avian community composition, we investigated the Big Creek NWA ARUs in isolation, to determine whether an effect of treatment might be observed without the masking effect of the difference between Big Creek and the Long Point NWAs. The two two-factor perMANOVAs carried out on Big Creek NWA ARUs alone did reveal a statistically significant interaction term for both total avian composition and marsh-user composition (Table 2.5). The interaction terms explained 44.1% of the variation in total avian community composition, and 25.2% of the variation in marsh-user community composition. This represents the proportion of the variation in community composition that can be explained by the interaction of year and treatment type.

*Table 2.5.* Two-factor perMANOVA results comparing total birds and marsh-user community composition among year (2019, 2021), treatment (control or herbicide-rolling treatment) and their interaction within all ARU sites (Big Creek and Long Point NWA) and within Big Creek sites alone.

| | **Big Creek & Long Point NWA** | | | | | | **Big Creek NWA** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total avian community | | | Marsh-user community | | | Total avian community | | | Marsh-user community | | |
| | F | df | p | F | df | p | F | df | p | F | df | p |
| Treatment | 0.56 | 1,8 | 0.70 | 0.96 | 1,8 | 0.45 | 2.30 | 1,4 | 0.02 | 2.36 | 1,4 | 0.05 |
| Year | 0.97 | 1,8 | 0.43 | 1.51 | 1,8 | 0.22 | 2.55 | 1,4 | 0.01 | 3.26 | 1,4 | 0.02 |
| Treatment x Year | 0.52 | 1,8 | 0.71 | 0.21 | 1,8 | 0.91 | 2.57 | 1,4 | 0.01 | 3.24 | 1,4 | 0.02 |

Graphs of each species' occurrence in control and treatment sites can be found in Appendix 1B**.** Given the limited number of ARU sites, it is inadvisable to place too much weight on the inferences regarding individual species. However, there are some trends that might inform future monitoring. As evidenced in Table 2.6, medium and larger-bodied marsh-user species like the Great Blue Heron (*Ardea herodias*), Mallard (*Anas platyrhynchos*), Sandhill Crane, and Killdeer (*Charadrius vociferus*), as well as species of conservation concern such as the Common Gallinule and American Bittern, occurred frequently in herbicide-treated sites. The Great Blue Heron was not present in the Long Point NWA until after herbicide-treatment occurred, and the Mallard was present in two herbicide-treated sites within Big Creek NWA that it was absent from prior to treatment. Other small-bodied marsh-users such as Tree Swallow, Barn Swallow (*Hirundo rustica*), Eastern Kingbird (*Tyrannus tyrannus*), and Yellow Warbler (*Setophaga petechia*) were observed frequently within herbicide-treated sites (Table 2.6).

In contrast, two large-bodied marsh-users may be avoiding recently herbicide-treated sites, including the federally and provincially Threatened Least Bittern and Canada Goose (*Branta canadensis*). The Least Bittern may be avoiding recently treated areas, as it was absent from two sites it was present in prior to treatment, while the Canada Goose occurred in pretreated *P. australis* and control sites, but not in post-herbicide-treated sites (Table 2.6). Other marsh-users that occurred less frequently in recently treated sites and more frequently in *P. australis* sites (both control and pre-treatment) included two small-bodied species, the Swamp Sparrow (*Melospiza georgiana*) and Willow Flycatcher (*Empidonax traillii*). Several non-marsh affiliated species that occurred less often in herbicide-treated sites tended to be small-bodied species, such as Cedar Waxwing (*Bombycilla cedrorum*), Gray Catbird (*Dumetella carolinensis*), Blue-gray Gnatcatcher (*Polioptila caerulea*) and Northern Cardinal (*Cardinalis cardinalis*) (Table 2.6).

Several species did not seem to be impacted by the presence or removal of *P. australis.* Red-winged Blackbird, Purple Martin, Mourning Dove, and Common Yellowthroat were present in either every or almost every site across the two study years (Table 2.6). Other species rarely occurred over the two study years, such as Virginia Rail, which is a species of conservation concern, and marsh-users such as Black-crowned Night Heron (*Nycticorax nycticorax*) and Blue-winged Teal (*Spatula discors*) (Table 2.6).

*Table 2.6.* Occurrence of species in control and treatment sites in 2019 and 2021. Species designated as "often" observed in 2021 herbicide-treated sites were present in at least two out of the three sites, and species that were designated as "infrequently" observed in 2021 herbicide-treated sites were present in ≤1 of the sites. Mean occurrence of birds in 2019 control, 2019 pre-treatment, and 2021 control sites is presented (Mean-U) for ease of comparison with 2021 herbicide-treated sites (two years post-treatment) (N = 3 for all four categories). Birds are ordered from greatest to fewest occurrences in 2021 treated sites. Marsh-user species are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°). Note that "T2019" is pre-treatment, as avian surveys took place in the spring prior to herbicide application in the fall.

| Common Name | C2019 | T2019 | C2021 | Mean-U | T2021 | Occurrence |
|---|---|---|---|---|---|---|
| American Goldfinch | 3 | 1 | 2 | 2.00 | 3 | Often |
| Common Grackle• | 1 | 2 | 1 | 1.33 | 3 | Often |
| Common Yellowthroat• | 3 | 3 | 2 | 2.67 | 3 | Often |
| Eastern Kingbird• | 0 | 2 | 1 | 1.00 | 3 | Often |
| Great Blue Heron• | 2 | 2 | 1 | 1.67 | 3 | Often |
| Mallard• | 0 | 1 | 3 | 1.33 | 3 | Often |
| Mourning Dove | 3 | 3 | 3 | 3.00 | 3 | Often |
| Purple Martin• | 3 | 3 | 3 | 3.00 | 3 | Often |
| Red-winged Blackbird• | 3 | 3 | 3 | 3.00 | 3 | Often |
| American Bittern•* | 1 | 2 | 2 | 1.67 | 2 | Often |
| American Robin | 3 | 3 | 3 | 3.00 | 2 | Often |

| Common Name | C2019 | T2019 | C2021 | Mean-U | T2021 | Occurrence |
|---|---|---|---|---|---|---|
| Barn Swallow•° | 3 | 3 | 2 | 2.67 | 2 | Often |
| Chipping Sparrow | 2 | 1 | 1 | 1.33 | 2 | Often |
| Common Gallinule•* | 2 | 2 | 2 | 2.00 | 2 | Often |
| House Wren | 1 | 2 | 1 | 1.33 | 2 | Often |
| Killdeer• | 2 | 0 | 2 | 1.33 | 2 | Often |
| Marsh Wren• | 2 | 3 | 2 | 2.33 | 2 | Often |
| Sandhill Crane• | 3 | 2 | 2 | 2.33 | 2 | Often |
| Song Sparrow• | 3 | 2 | 1 | 2.00 | 2 | Often |
| Tree Swallow• | 3 | 2 | 3 | 2.67 | 2 | Often |
| Yellow Warbler• | 2 | 3 | 1 | 2.00 | 2 | Often |
| American Crow | 1 | 3 | 1 | 1.67 | 1 | Infrequent |
| Baltimore Oriole | 1 | 0 | 1 | 0.67 | 1 | Infrequent |
| Bank Swallow•° | 1 | 1 | 2 | 1.33 | 1 | Infrequent |
| Black-capped Chickadee | 1 | 1 | 1 | 1.00 | 1 | Infrequent |
| Blue Jay | 1 | 2 | 1 | 1.33 | 1 | Infrequent |
| Brown Thrasher | 1 | 1 | 0 | 0.67 | 1 | Infrequent |
| Eastern Towhee | 1 | 1 | 1 | 1.00 | 1 | Infrequent |
| Eastern Wood-pewee° | 0 | 0 | 0 | 0.00 | 1 | Infrequent |
| Field Sparrow | 1 | 1 | 1 | 1.00 | 1 | Infrequent |
| Great-crested Flycatcher | 0 | 0 | 1 | 0.33 | 1 | Infrequent |
| Indigo Bunting | 0 | 0 | 0 | 0.00 | 1 | Infrequent |
| Northern Cardinal | 1 | 3 | 1 | 1.67 | 1 | Infrequent |
| Pied-billed Grebe•* | 1 | 2 | 2 | 1.67 | 1 | Infrequent |
| Warbling Vireo | 0 | 1 | 0 | 0.33 | 1 | Infrequent |
| Wood Duck• | 1 | 1 | 1 | 1.00 | 1 | Infrequent |
| Yellow-billed Cuckoo | 0 | 1 | 0 | 0.33 | 1 | Infrequent |
| Belted Kingfisher• | 2 | 0 | 0 | 0.67 | 0 | Infrequent |
| Black-crowned Night Heron• | 1 | 1 | 0 | 0.67 | 0 | Infrequent |
| Blue-gray Gnatcatcher | 0 | 2 | 0 | 0.67 | 0 | Infrequent |
| Blue-winged Teal• | 1 | 0 | 1 | 0.67 | 0 | Infrequent |
| Brown Creeper | 0 | 1 | 0 | 0.33 | 0 | Infrequent |
| Brown-headed Cowbird• | 0 | 1 | 0 | 0.33 | 0 | Infrequent |
| Canada Goose• | 2 | 2 | 2 | 2.00 | 0 | Infrequent |
| Carolina Wren | 0 | 0 | 1 | 0.33 | 0 | Infrequent |
| Cedar Waxwing | 0 | 3 | 1 | 1.33 | 0 | Infrequent |
| Chestnut-sided Warbler | 0 | 2 | 0 | 0.67 | 0 | Infrequent |
| Common Loon• | 1 | 0 | 0 | 0.33 | 0 | Infrequent |
| Common Tern• | 2 | 0 | 0 | 0.67 | 0 | Infrequent |
| European Starling• | 0 | 1 | 0 | 0.33 | 0 | Infrequent |
| Gray Catbird | 1 | 2 | 0 | 1.00 | 0 | Infrequent |
| Herring Gull• | 0 | 1 | 0 | 0.33 | 0 | Infrequent |
| Least Bittern•*° | 1 | 2 | 2 | 1.67 | 0 | Infrequent |
| Mute Swan• | 1 | 0 | 0 | 0.33 | 0 | Infrequent |
| Northern Flicker | 0 | 0 | 1 | 0.33 | 0 | Infrequent |

| Common Name | C2019 | T2019 | C2021 | Mean-U | T2021 | Occurrence |
|---|---|---|---|---|---|---|
| Northern-rough Winged Swallow• | 0 | 0 | 1 | 0.33 | 0 | Infrequent |
| Orchard Oriole | 0 | 0 | 1 | 0.33 | 0 | Infrequent |
| Swamp Sparrow• | 2 | 3 | 1 | 2.00 | 0 | Infrequent |
| Virginia Rail•* | 0 | 0 | 1 | 0.33 | 0 | Infrequent |
| Willow Flycatcher• | 1 | 0 | 0 | 0.33 | 0 | Infrequent |

*2.3.2 2021 space-for-time substitution experiment*

*2.3.2-a ARU site characteristics*

Graphs of vegetation contact profiles can be found in Appendix 1C. Differences in vegetation structure and composition within control, reference, and herbicide-treated sites are evident. Control sites were primarily dominated by *P. australis* and standing dead litter (Appendix 1C-Figure 5.1). The tallest height class across the three vegetation types was found in control sites, with *P. australis* reaching the height class of 360-379 cm. One control site, BC-P1, appeared to have more emergent vegetation and floating vegetation than *P. australis* or standing dead litter. However, these surveys were not all-encompassing of the habitat an ARU would survey, because the vegetation was only characterized on the left and right of an ARU. Reference sites were the most diverse, as each of the eight defined vegetation classes were found in at least one reference site. Cattail marsh sites were dominated by robust emergent vegetation and standing dead litter, meadow marsh sites were dominated by narrow-leaved emergent, broad-leaved emergent, and floating vegetation, and hemi-marsh sites tended to have less vegetation, but typically had emergent vegetation and standing dead litter (Appendix 1C-Figures 5.2, 5.3). Vegetation contact heights within reference sites tended to be most numerous between 0-200 cm, and contacts reached a maximum height of 259 cm. One- and two-year post-herbicide treatment sites had little vegetation present (Appendix 1C-Figure 5.4). Floating vegetation and standing dead litter were the most frequently found vegetation classes. Contact heights were typically below 100 cm, and they reached a maximum height class of 280-299 cm.

Control, reference, and herbicide-treated sites had average water depths of 34.2 ± 18.7 cm (mean ± standard deviation), 51.3 ± 37.3 cm, and 35.4 ± 14.6 cm, respectively. Reference sites were deeper due to the inclusion of hemi-marsh habitat, which is a mix of cattail and open water, often which was greater than 70 cm in depth. Average water depths in cattail, meadow marsh, and hemi-marsh reference sites were 35.3 ± 8.0 cm, 30.4 ± 13.0 cm, and 112.7 ± 25.9 cm, respectively.

We also visualized trends in ARU site characteristics by carrying out an NMS ordination, which confirmed patterns observed in Appendix 1C. Floating and robust emergent contacts, as well as deeper water, were more associated with reference sites (Figure 2.8-D). *Phragmites australis*, standing dead litter, broad-leaf emergent, ground cover, and shrub contacts were more associated with control sites. Herbicide-treated sites lacked vegetation (Figure 2.8-D).

*2.3.2-b Avian species richness*

A total of 56 avian species, plus an additional three unknown species, were identified in 2021 across the 20 ARUs (Table 2.7). A total of 26 marsh-users, six species of conservation concern, and five species at risk were observed (Table 2.8). Fifty-two species were identified in control sites, 44 in reference, and 42 in herbicide-treated (Table 2.8).

*Table 2.7.* Avian species identified after transcribing twenty 45-minute recordings on one day in June 2021 across reference, control (*P. australis*) and treated (1- or 2-year post-herbicide-rolling) sites in the Big Creek and Long Point NWAs. Marsh-user species are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

| **Common Name** | **Scientific Name** | **4-Letter Alpha Code** |
|---|---|---|
| American Bittern•* | *Botaurus lentiginosus* | AMBI |
| American Crow | *Corvus brachyrhynchos* | AMCR |
| American Goldfinch | *Spinus tristis* | AMGO |
| American Robin | *Turdus migratorius* | AMRO |
| Baltimore Oriole | *Icterus galbula* | BAOR |
| Bank Swallow•° | *Riparia riparia* | BANS |
| Barn Swallow•° | *Hirundo rustica* | BARS |
| Belted Kingfisher | *Megaceryle alcyon* | BEKI |
| Black-billed Cuckoo | *Coccyzus erythropthalmus* | BBCU |
| Black-capped Chickadee | *Poecile atricapillus* | BCCH |
| Black Tern•° | *Chlidonias niger* | BLTE |
| Blue-gray Gnatcatcher | *Polioptila caerulea* | BGGN |
| Blue Jay | *Cyanocitta cristata* | BLJA |
| Brown Thrasher | *Toxostoma rufum* | BRTH |
| Canada Goose• | *Branta canadensis* | CAGO |
| Carolina Wren | *Thryothorus ludovicianus* | CAWR |

| Common Name | Scientific Name | 4-Letter Alpha Code |
|---|---|---|
| Cedar Waxwing | *Bombycilla cedrorum* | CEDW |
| Chipping Sparrow | *Spizella passerina* | CHSP |
| Common Gallinule•* | *Gallinula galeata* | COGA |
| Common Grackle• | *Quiscalus quiscula* | COGR |
| Common Nighthawk° | *Chordeiles minor* | CONI |
| Common Raven | *Corvus corax* | CORA |
| Common Yellowthroat• | *Geothlypis trichas* | COYE |
| Eastern Kingbird• | *Tyrannus tyrannus* | EAKI |
| Eastern Towhee | *Pipilo erythrophthalmus* | EATO |
| Eastern Wood-pewee° | *Contopus virens* | EAWP |
| Field Sparrow | *Spizella pusilla* | FISP |
| Forster's Tern• | *Sterna forsteri* | FOTE |
| Gray Catbird | *Dumetella carolinensis* | GRCA |
| Great Blue Heron• | *Ardea herodias* | GBHE |
| Great Crested Flycatcher | *Myiarchus crinitus* | GCFL |
| House Wren | *Troglodytes aedon* | HOWR |
| Indigo Bunting | *Passerina cyanea* | INBU |
| Killdeer• | *Charadrius vociferus* | KILL |
| Least Bittern•*° | *Ixobrychus exilis* | LEBI |
| Mallard• | *Anas platyrhynchos* | MALL |
| Marsh Wren• | *Cistothorus palustris* | MAWR |
| Mourning Dove | *Zenaida macroura* | MODO |
| Northern Cardinal | *Cardinalis cardinalis* | NOCA |
| Northern Flicker | *Colaptes auratus* | NOFL |
| Northern Rough-winged swallow• | *Stelgidopteryx serripennis* | NRWS |
| Orchard Oriole | *Icterus spurius* | OROR |
| Pied-billed Grebe•* | *Podilymbus podiceps* | PBGR |
| Purple Martin• | *Progne subis* | PUMA |
| Red-bellied Woodpecker | *Melanerpes carolinus* | RBWO |
| Red-winged Blackbird• | *Agelaius phoeniceus* | RWBL |
| Sandhill Crane• | *Antigone canadensis* | SACR |
| Song Sparrow• | *Melospiza melodia* | SOSP |
| Sora•* | *Porzana carolina* | SORA |
| Swamp Sparrow• | *Melospiza georgiana* | SWSP |
| Tree Swallow• | *Tachycineta bicolor* | TRES |
| Unknown Species 1 (#22) | - | - |
| Unknown Species 2 (#24) | - | - |
| Unknowns Species 3 (#26) | - | - |
| Virginia Rail•* | *Rallus limicola* | VIRA |

| Common Name | Scientific Name | 4-Letter Alpha Code |
|---|---|---|
| Warbling Vireo | *Vireo gilvus* | WAVI |
| Wood Duck• | *Aix sponsa* | WODU |
| Yellow Warbler• | *Setophaga petechia* | YEWA |
| Yellow-billed Cuckoo | *Coccyzus americanus* | YBCU |

There was no statistically significant effect of vegetation type on the richness of total species, marsh-users, species of conservation concern or species at risk ($p > 0.1$, Table 2.8). Control sites had the greatest total species richness (Table 2.9). Control and reference sites had the same number of marsh-users, species of conservation concern, and species at risk. Herbicide-treated sites had marginally (but not statistically significantly) lower richness of total species, marsh-users, and species of conservation concern, but had the same number of species at risk as control and reference sites (Table 2.9). Importantly, the number of ARUs differed by vegetation type due to the firmware errors, and consequently herbicide-treated sites were not sampled as intensively as the reference and control sites. This, coupled with the already limited statistical power, likely contributed to these minor and non-significant differences.

*Table 2.8.* One-factor ANOVA results comparing avian species richness among reference (cattail marsh, hemi marsh, meadow marsh), treated (1- or 2-year post-herbicide-rolling), and control (*P. australis*) sites in 2021.

| | F | df | p |
|---|---|---|---|
| Total species richness | 1.16 | 2,17 | 0.33 |
| Marsh-user richness | 0.68 | 2,17 | 0.52 |
| Species at risk richness | 1.11 | 2,17 | 0.35 |
| Species of conservation concern richness | 0.50 | 2,17 | 0.62 |

*Table 2.9.* Cumulative and mean avian species richness in reference (N = 8), control (N = 7), and herbicide-treated (N = 5) sites in 2021 across the Big Creek and Long Point NWAs. “SOCC” represents species of conservation concern, and “SAR" represents species at risk.

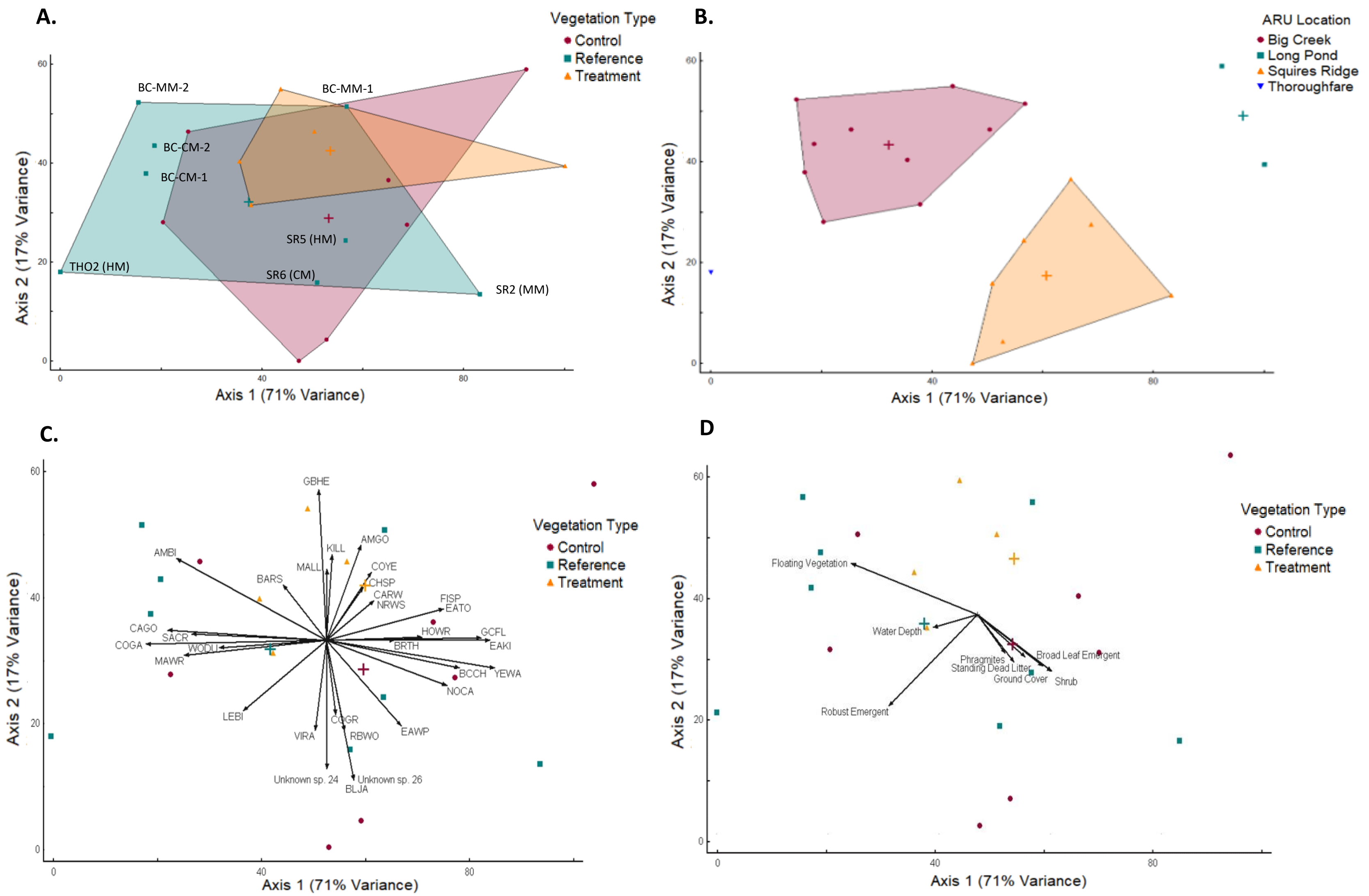
| | Control | | Reference | | Treatment | |
|---|---|---|---|---|---|---|
| | Cumulative | μ ± SD | Cumulative | μ ± SD | Cumulative | μ ± SD |
| Total | 52 | 21.8 ± 2.8 | 44 | 19.5 ± 4.1 | 42 | 22.3 ± 1.2 |
| Marsh-users | 26 | 14.7 ± 2.8 | 26 | 14.3 ± 2.5 | 22 | 12.8 ± 3.5 |
| SOCC | 6 | 2.7 ± 2.1 | 6 | 3 ± 1.8 | 4 | 2 ± 1.2 |
| SAR | 5 | 2.1 ± 1.3 | 5 | 1.4 ± 0.7 | 5 | 2.0 ± 1.0 |

*2.3.2-c Avian community composition*

To visualize the trends in avian community composition among the three vegetation types, we carried out NMS ordination on the avian occurrence dataset. The NMS ordination of avian community composition had two dimensions ($p = 0.02$), with a final instability of < 0.00001 and a final stress value of 12.13 after 54 iterations. Axis 1 explained a total of 71% of the variance in community composition and axis 2 explained a total of 17%. Correlations of species occurrences and environmental variables with site scores can be found in Appendix 1D and 1E, respectively. The species and environmental variables with reasonably strong correlations ($r^2 > 0.2$ for species and $r^2 > 0.05$ for environmental variables) are depicted in Figure 2.8 as vectors (panels C and D, respectively).

Community composition within control and reference sites overlap considerably (Figure 2.8-A). The 1- or 2-year post-herbicide-treated sites form a nested subset within the ordination space encompassed by control and reference sites, indicating that they support lower beta diversity or dispersion (Figure 2.8-A). The MRPPs confirmed that both total avian community composition and marsh-user composition did not differ between the three vegetation types ($p > 0.34$; Table 2.10), however, there are some trends that might inform future monitoring. Graphs of the occurrence of each species in control, reference, and herbicide-treated sites can be found in Appendix 1F.

*Figure 2.8.* NMS ordination solution of bird community composition in Big Creek and Long Point NWAs. A and B depict variation in community composition among vegetation type and ARU location, respectively. A depicts each of the three habitat types grouped as "reference" ("HM", "MM" and "CM" are hemi-marsh, meadow marsh and cattail marsh, respectively). Centroids (+) represent the geometric mean of each group. C depicts species occurrence in relation to ordination axes as vectors for species where $r^2 \geq 0.20$ on at least one axis. Species vectors were scaled to 100%. Bird species are represented by American Ornithologist Union four-letter alpha codes (see Table 2.7 for common names). D depicts the relationships between environmental variables and ordination axes for variables with $r^2 \geq 0.05$ on at least one axis. Environmental variables included the number of contact points of different vegetation classes measured with the horizontal contact profiling as well as water depth at the location the ARU was deployed. These environmental vectors were scaled to 200%.



A.
Vegetation Type
Control
Reference
Treatment
BC-MM-2
BC-MM-1
BC-CM-2
BC-CM-1
SR5 (HM)
THO2 (HM)
SR6 (CM)
SR2 (MM)
Axis 1 (71% Variance)
Axis 2 (17% Variance)
B.
ARU Location
Big Creek
Long Pond
Squires Ridge
Thoroughfare
Axis 1 (71% Variance)
Axis 2 (17% Variance)
C.
Vegetation Type
Control
Reference
Treatment
GBHE
KILL
AMGO
MALL
COYE
BARS
CHSP
CARW
NRWS
AMBI
FISP
EATO
CAGO
SACR
HOWR
GCFL
COGA
WODU
BRTH
EAKI
MAWR
BCCH
YEWA
NOCA
LEBI
COGR
VIRA
RBWO
EAWP
Unknown sp. 24
Unknown sp. 26
BLJA
Axis 1 (71% Variance)
Axis 2 (17% Variance)
D
Vegetation Type
Control
Reference
Treatment
Floating Vegetation
Water Depth
Broad Leaf Emergent
Phragmites
Standing Dead Litter
Ground Cover
Shrub
Robust Emergent
Axis 1 (71% Variance)
Axis 2 (17% Variance)

*Table 2.10.* MRPP results comparing total bird community composition and marsh-user community composition in 2021 control (*P. australis*), reference (cattail marsh, hemi-marsh, and meadow marsh), and herbicide-treated sites (1- or 2-year post-treatment).

| | **Total Avian Community** | | **Marsh-user Community** | |
|---|---|---|---|---|
| | A | p | A | p |
| All vegetation types | -0.01 | 0.59 | 0.01 | 0.35 |
| Control vs Reference | -0.01 | 0.63 | -0.01 | 0.55 |
| Control vs Treated | -0.02 | 0.71 | < 0.01 | 0.41 |
| Reference vs Treated | 0.01 | 0.27 | 0.03 | 0.16 |

As seen in Table 2.11, species that were most frequently found in 1- or 2-year post-herbicide-treated sites tended to be marsh-users. Both small-bodied marsh-users, such as species at risk Barn Swallow and Bank Swallow, and large-bodied marsh users such as the Mallard and Great Blue Heron, and marsh bird species of conservation concern including the Common Gallinule, American Bittern, and Least Bittern were found in herbicide-treated sites. Species that occurred infrequently or not at all in herbicide-treated sites tended to be small-bodied species. Out of 36 species that occurred in ≤ 2 of the five herbicide-treated sites, nine species were larger-bodied birds, including those that are of conservation concern; the Sora, Virginia Rail, and Pied-billed Grebe. The remaining species in ≤ 2 herbicide-treated sites were small-bodied species such as the Cedar Waxwing and Gray Catbird (Table 2.11).

Species that were most frequently found in reference sites were a mix of large-and-small-bodied birds, a majority of which were marsh-users (Table 2.11; Figure 2.8-C). Several species of conservation concern were frequently found within reference sites. Some species were absent from reference sites, such as Field Sparrow, Brown Thrasher (*Toxostoma rufum*), and Eastern Towhee.

Community composition within *P. australis* sites tended to be comprised of small-bodied species such as House Wren (*Troglodytes aedon*) and Northern Cardinal (*Cardinalis cardinalis*) (Figure 2.8-C). However, almost all birds observed in this study were found in *P. australis* sites at least once. Only seven species were not found in *P. australis*, which included Forster's Tern (*Sterna forsteri*) and Common Nighthawk (*Chordeiles minor*; Table 2.11). All six species of conservation concern observed in this study were observed in *P. australis* sites at least once, and some were observed frequently, such as the Least Bittern.

Several species were found frequently in control, reference, and herbicide-treated sites, such as the Purple Martin, Red-winged Blackbird, Common Yellowthroat, and Mourning Dove (Table 2.11).

*Table 2.11*. Fifty-six avian species detected across treated (herbicide-rolling; N = 5), reference (N = 8) and control sites (N = 7) in 2021, with differing degrees of occurrence by vegetation type. Species are ordered from greatest to fewest occurrences in herbicide-treated sites. Marsh-user species are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

| **Common Name** | **Occurrences in Treated Sites** | **Occurrences in Reference Sites** | **Occurrences in Control Sites** |
|---|---|---|---|
| Purple Martin• | 5 | 8 | 7 |
| Red-winged Blackbird• | 5 | 8 | 7 |
| Common Yellowthroat• | 5 | 7 | 5 |
| Mourning Dove | 5 | 7 | 7 |
| Mallard• | 5 | 2 | 3 |
| Sandhill Crane• | 4 | 7 | 5 |
| Tree Swallow• | 4 | 7 | 7 |
| Marsh Wren• | 4 | 6 | 6 |
| Common Gallinule•* | 4 | 5 | 4 |
| Common Grackle• | 4 | 5 | 5 |
| American Robin | 4 | 4 | 5 |
| Eastern Kingbird• | 4 | 4 | 4 |
| Great Blue Heron• | 4 | 4 | 2 |
| Yellow Warbler• | 4 | 4 | 5 |
| Barn Swallow•° | 4 | 3 | 3 |
| Wood Duck• | 3 | 6 | 3 |
| American Bittern•* | 3 | 5 | 2 |
| Northern Cardinal | 3 | 4 | 4 |
| Killdeer• | 3 | 1 | 3 |
| American Goldfinch | 3 | 0 | 2 |
| Least Bittern•*° | 2 | 5 | 5 |
| Swamp Sparrow• | 2 | 4 | 1 |
| Warbling Vireo | 2 | 4 | 3 |
| House Wren | 2 | 3 | 4 |
| Chipping Sparrow | 2 | 1 | 1 |
| Song Sparrow• | 2 | 1 | 1 |
| Bank Swallow•° | 2 | 0 | 3 |
| Canada Goose• | 1 | 6 | 2 |
| Pied-billed Grebe• | 1 | 5 | 4 |
| Black-capped Chickadee | 1 | 3 | 3 |

| Common Name | Occurrences in Treated Sites | Occurrences in Reference Sites | Occurrences in Control Sites |
|---|---|---|---|
| American Crow | 1 | 2 | 1 |
| Blue Jay | 1 | 2 | 3 |
| Baltimore Oriole | 1 | 1 | 2 |
| Common Nighthawk° | 1 | 1 | 0 |
| Eastern Wood-pewee° | 1 | 1 | 3 |
| Great-crested Flycatcher | 1 | 1 | 1 |
| Yellow-billed Cuckoo | 1 | 1 | 2 |
| Brown Thrasher | 1 | 0 | 1 |
| Common Raven | 1 | 0 | 0 |
| Eastern Towhee | 1 | 0 | 1 |
| Field Sparrow | 1 | 0 | 1 |
| Indigo Bunting | 1 | 0 | 0 |
| Northern Flicker | 0 | 3 | 2 |
| Orchard Oriole | 0 | 2 | 1 |
| Red-bellied Woodpecker | 0 | 2 | 1 |
| Sora•* | 0 | 2 | 1 |
| Virginia Rail•* | 0 | 2 | 3 |
| Black-billed Cuckoo | 0 | 1 | 0 |
| Belted Kingfisher• | 0 | 1 | 0 |
| Blue-gray Gnatcatcher | 0 | 1 | 0 |
| Black Tern•° | 0 | 1 | 1 |
| Cedar Waxwing | 0 | 1 | 1 |
| Forster's Tern• | 0 | 1 | 0 |
| Gray Catbird | 0 | 1 | 1 |
| Carolina Wren | 0 | 0 | 1 |
| Northern Rough-winged Swallow• | 0 | 0 | 1 |

Looking at bird community composition within each management unit that the ARUs were deployed in (Big Creek, Long Pond, Squire's Ridge, and Thoroughfare), the NMS ordination reflects a differentiation between the bird communities using each management unit, as each unit groups together (Figure 2.8-B). Marsh-users such as American Bittern, Common Gallinule, and Great Blue Heron occurred most frequently in sites at Big Creek, whereas terrestrial species such as Northern Cardinal, Blue Jay (*Cyanocitta cristata*), and Black-capped Chickadee occurred most frequently in sites at Squire's Ridge (Appendix 1F). The MRPPs confirmed that almost all of the four management units within the two NWAs support distinct bird communities; Long Pond and Squire's Ridge (both in Long Point NWA) supported similar marsh-user communities (Table 2.12).

*Table 2.12.* MRPP results comparing total avian community composition and marsh-user community composition in three out of the four management units ARUs were placed in across the Big Creek and Long Point NWAs in 2021. The Thoroughfare sub-area was excluded due to having only one ARU site (i.e., no replication).

| | **Total Avian Community** | | **Marsh-user Community** | |
|---|---|---|---|---|
| | A | p | A | p |
| NWA location | 0.22 | < 0.01 | 0.24 | < 0.01 |
| Big Creek vs Long Pond | 0.20 | < 0.01 | 0.20 | 0.02 |
| Big Creek vs Squire's Ridge | 0.16 | < 0.01 | 0.17 | < 0.01 |
| Long Pond vs Squire's Ridge | 0.17 | 0.01 | 0.12 | 0.11 |

*2.3.2-d Functional trait composition*

To visualize the trends in functional trait composition among the three vegetation types, we carried out NMS ordination on the species-occurrence weighted functional trait dataset. The optimal NMS ordination of functional trait composition had two dimensions ($p = 0.02$), with a final instability of $< 0.00001$ and a final stress value of 8.04 after 58 iterations. Axis 1 explained a total of 91.2% of the variance in functional trait composition and axis 2 explained a total of 4.8%. Correlations of functional trait and environmental variable vectors with site scores can be found in Appendix 1G and 1H, respectively.

Functional trait composition within control, reference, and herbicide-treated sites do show considerable overlap (Figure 2.9-A). The MRPP confirmed that trait composition did not differ among the three vegetation types ($p = 0.45$; Table 2.13). That said, we did observe some trends in avian trait distributions among the three vegetation types that warrant continued surveillance. Birds that had the following traits were most associated with reference sites: 1) forage by stalking, dabbling, surface diving or probing, 2) consume fish, aquatic invertebrates, or plants and 3) have floating nests or ground nests (Figure 2.9-C). Birds that had the following traits were most associated with control sites: 1) forage by foliage gleaning, ground foraging or flycatching, 2) consume insects, and 3) nest in trees, shrubs or cavities. Birds that consume seeds and nest by burrowing were associated with herbicide-treated sites (Figure 2.9-C).

*Figure 2.9.* NMS ordination solution of functional trait composition in Big Creek and Long Point NWAs. A and B depict differences in functional trait composition among vegetation types and management units, respectively. A depicts each of the three habitat types grouped as “reference” (“HM”, “MM” and “CM” are hemi-marsh, meadow marsh and cattail marsh, respectively). Centroids (+) represent the geometric mean of each group. C depicts the relationship between the species-occurrence weighted frequencies of different functional traits where the traits were reasonable ($r^2 \geq 0.20$) correlated with at least one axis. Vectors are scaled to 50%. D depicts the correlation between environmental covariates and ordination axes, where such correlations were reasonably strong ($r^2 \geq 0.05$).

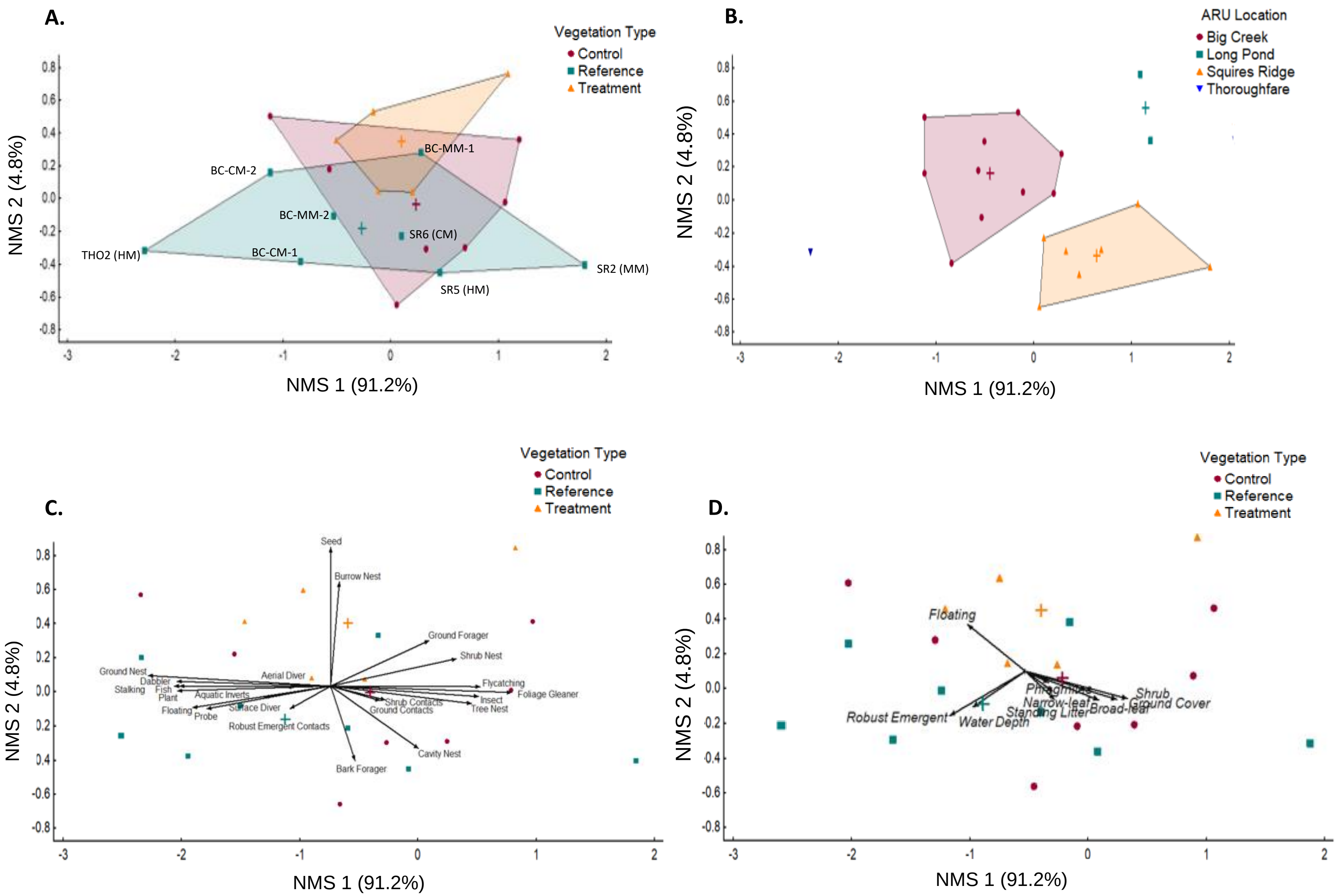

A.
Vegetation Type
Control
Reference
Treatment
BC-MM-1
BC-CM-2
BC-MM-2
SR6 (CM)
THO2 (HM)
BC-CM-1
SR2 (MM)
SR5 (HM)
NMS 1 (91.2%)
NMS 2 (4.8%)
B.
ARU Location
Big Creek
Long Pond
Squires Ridge
Thoroughfare
NMS 1 (91.2%)
NMS 2 (4.8%)
C.
Vegetation Type
Control
Reference
Treatment
Seed
Burrow Nest
Ground Forager
Shrub Nest
Flycatching
Foliage Gleaner
Insect
Tree Nest
Shrub Contacts
Ground Contacts
Cavity Nest
Bark Forager
Robust Emergent Contacts
Surface Diver
Aerial Diver
Ground Nest
Dabbler
Fish
Stalking
Plant
Aquatic Inverts
Floating
Probe
NMS 1 (91.2%)
NMS 2 (4.8%)
D.
Vegetation Type
Control
Reference
Treatment
Floating
Robust Emergent
Water Depth
Phragmites
Narrow-leaf
Standing Litter
Broad-leaf
Shrub
Ground Cover
NMS 1 (91.2%)
NMS 2 (4.8%)

*Table 2.13.* MRPP results comparing avian functional trait composition in reference (cattail marsh, hemi-marsh, meadow marsh), control (*P. australis*), and 1- or 2-year post-herbicide herbicide-treated sites in 2021.

| | **A** | **p** |
|---|---|---|
| All three vegetation types | < -0.01 | 0.45 |
| Control vs Reference | -0.01 | 0.57 |
| Control vs Treated | < 0.00 | 0.39 |
| Reference vs Treated | 0.01 | 0.27 |

As evidenced in Table 2.14, many functional traits occurred evenly across herbicide-treated, reference, and control sites.

*Table 2.14.* Occurrence of bird functional traits (diet, foraging technique, and nesting preferences) across herbicide-treated (N = 5), reference (N = 8) and control sites (N = 7) in 2021.

| | **Treated** | **Reference** | **Control** |
|---|---|---|---|
| *Diet* | | | |
| Insect | 24 | 29 | 30 |
| Omnivore | 7 | 4 | 6 |
| Seed | 6 | 6 | 7 |
| Fish | 3 | 5 | 3 |
| Aquatic invertebrates | 1 | 1 | 1 |
| Plant | 1 | 1 | 1 |
| Fruit | 0 | 1 | 1 |
| *Foraging technique* | | | |
| Ground forage | 15 | 17 | 19 |
| Foliage gleaner | 8 | 10 | 10 |
| Aerial forage | 5 | 5 | 6 |
| Flycatching | 3 | 3 | 3 |
| Stalking | 3 | 3 | 3 |
| Dabbler | 3 | 3 | 3 |
| Probe | 2 | 3 | 3 |
| Aerial dive | 1 | 2 | 0 |
| Surface dive | 1 | 1 | 1 |
| Bark forage | 0 | 1 | 1 |
| *Nesting preferences* | | | |
| Tree | 11 | 14 | 12 |
| Shrub | 10 | 10 | 10 |
| Ground | 10 | 9 | 10 |

| | Treated | Reference | Control |
|---|---|---|---|
| Cavity | 6 | 8 | 9 |
| Floating | 2 | 5 | 4 |
| Burrow | 1 | 1 | 2 |
| Cliff | 1 | 0 | 0 |
| Build | 1 | 1 | 1 |

Looking at functional trait composition within each ARU management unit, the NMS ordination reflects a differentiation between trait composition in each area. The MRPP confirmed that the four management units within the two NWAs have bird communities with distinct functional traits ($p < 0.01$; Table 2.15).

*Table 2.15.* MRPP results comparing functional trait composition in three out of the four management units that the ARUs were deployed in across the Big Creek and Long Point NWAs in 2021. The Thoroughfare management unit was excluded due to having only one ARU site (i.e., no replication).

| | A | p |
|---|---|---|
| All three management units | 0.22 | < 0.01 |
| Big Creek vs Long Pond | 0.19 | < 0.01 |
| Big Creek vs Squire's Ridge | 0.18 | < 0.01 |
| Long Pond vs Squire's Ridge | 0.11 | 0.03 |

*2.4 Discussion*

The coastal wetlands in Long Point, Ontario are designated as a Globally Important Bird Area, Ramsar Wetland of International Significance, and a World Biosphere Reserve. Provincially, they are designated as Ontario's Priority Place for species at risk conservation (MacLeod, 2019). Yet, *P. australis* invasion is homogenizing these once diverse coastal wetlands and reducing the habitat quality for many marsh breeding birds (Robichaud & Rooney, 2022). Recent efforts by CWS-ON to manage *P. australis* and promote the recovery of native flora and fauna in Long Point involved a glyphosate-based herbicide application followed by mechanical flattening or mowing of remaining litter via a Marsh Master™. This management action was motivated by the goal of species at risk recovery, but there is concern that habitat alteration may impact birds directly, at least in the short term.

We investigated the short-term effects of *P. australis* management on the avian community in Long Point (i.e., 1-2 years post-herbicide-rolling treatment). We assessed the effects of *P. australis* control on avian species richness and community composition by using a spatially replicated Before-After-Control-Impact design, and we compared avian species richness, community composition, and functional trait composition among herbicide-rolling treated sites, uninvaded reference sites (cattail marsh, meadow marsh, hemi-marsh), and untreated control sites (*P. australis*-dominated) using a space-for-time substitution design.

Briefly, we observed minimal impacts on avian species richness following *P. australis* management. Total avian richness exhibited a marginally significant decline after treatment in the BACI experiment ($p = 0.09$), but marsh-user richness did not, likely indicating that non-marsh affiliated birds are using *P. australis* habitat over the herbicide-treated habitat, at least in the short term. In terms of community composition, ARU location had a substantial influence on this diversity metric. When we restricted the BACI analysis to a subset of sites in Big Creek, we found that community composition differed among control and 2-year post-herbicide-treated sites. Birds displaced by *P. australis* treatment tended to be small-bodied, non-marsh affiliated species that use terrestrial habitats or are habitat generalists. Changes in marsh-user composition post-treatment came from the displacement of a few large-bodied and small-bodied species and the gain of a few large-bodied species. The space-for-time substitution did not detect a change in community composition but observed similar trends as the BACI experiment regarding large-bodied marsh-users beginning to use the recently treated habitat. In terms of functional trait composition, there was no difference among control, reference, and herbicide-treated sites, indicating that habitats where the herbicide-rolling treatment occurred are less heterogeneous, offering reduced niche space or more limited resource diversity compared to the reference and control locations. Overall, minor impacts were observed on marsh birds 1-2 years following herbicide-rolling management of *P. australis*.

### *2.4.1 Avian species richness*

Species richness can be an insensitive metric for determining changes in avian communities in wetlands (Robichaud & Rooney, 2017). For example, *P. australis* invaded habitat has been shown to support similar total species richness as noninvaded habitat (Gagnon-

Lupien et al., 2015; Whyte et al., 2015; Robichaud & Rooney, 2017), although the different habitats can support different species (Robichaud & Rooney, 2017). Species preferring *P. australis* displace those intolerant of *P. australis* invasions, resulting in community turnover without systematic alteration of site-level richness (Robichaud & Rooney, 2017).

We therefore anticipated that total species richness would not differ among invaded control sites (*P. australis*), reference sites (uninvaded habitat), and treated sites (1-or 2-years post-management), because species preferring the shallow open-water habitat remaining after treatment may replace those requiring tall and dense vegetation provided by *P. australis*. In contrast, we anticipated that the richness of marsh-users and species of conservation concern (i.e., those found to be most impacted by *P. australis* invasion), such as waterfowl and wading birds like herons and bitterns, would be similar in herbicide-treated and uninvaded “reference” sites but greater than in invaded “control” sites, as these birds may favor the increase in open-water and hemi-marsh habitats in the years immediately following management. Species at risk observed in Long Point coastal marsh are primarily aerial insectivores like Barn Swallow and Bank Swallow, or bitterns like Least Bittern, and consequently, we expected that the richness of species at risk might also be lower in *P. australis* habitat. Though we recognize that the ultimate effects of *P. australis* management will take several years to materialize, we anticipated that avian diversity could respond quickly to newly created open-water habitat.

In the 2019-2021 BACI experiment, the herbicide-rolling application to *P. australis* had no significant statistical effect on total avian species richness, as expected. But contrary to expectations, we also found no difference in the richness of marsh-users, species of conservation concern, or species at risk between control and treatment sites. Crucially, the interaction between treatment and year in predicting the four biodiversity response variables (total avian species richness, the richness of marsh-users, species of conservation concern, and species at risk) was never statistically significant. In a spatially replicated BACI monitoring design, it is this interaction term that reveals whether the management action caused a change in the avian community to occur vs whether pre-existing differences between control and treatment locations such as temporal trends affecting both locations might be responsible for observed patterns in the avian community (Gotelli & Ellison, 2004).

Total avian richness was higher, on average, in control and pre-treatment sites in the baseline year, 2019 than in control and post-treatment sites in 2021. Although efforts were made by CWS-ON in 2019 to pair control and treatment locations based on the vegetation and water depth (Graham Howell, CWS-ON biologist, pers. comm. January 27th, 2022), technicians did not have pre-existing data on the avian community at these candidate sites. This emphasizes the importance of using a BACI design in monitoring (*sensu* Underwood, 1992): in situations lacking pilot data, if the 'before' response variable values are not statistically equivalent, pre-existing differences between control and treatment sites can be accounted for and an effect of treatment can still be distinguished from pre-treatment differences in site character. Overall, avian species richness declined in both treatment and control sites between 2019 and 2021, possibly due to changes in annual climate and water levels on Lake Erie. For example, mean water levels in Lake Erie were 43 cm lower in June 2021 than in June 2019 (NOAA-GLERL, 2022), though the mean water depths in 2021 at herbicide-treated ARU sites (35.7 cm, std = 17.2 cm) were not different from mean water levels at 2021 control sites (42.7 cm, std = 20.4 cm) ($N_1 = N_2 = 3$, $U = 107.5$, $p = 0.85$).

Because of the technical difficulties with the ARUs that resulted in a small sample size (three replicates each of control and treatment), our power to detect a significant interaction term is limited. If we adopt a weight-of-evidence approach, given the small sample size and relatively low p-value for total species richness (0.09), these results do warrant continued monitoring of total avian species richness after *P. australis* management in Long Point. However, the lack of evidence supporting either main effects or an interaction effect in our analyses of the richness of marsh-users, species of conservation concern, and species at risk suggests that even a larger sample size would not reveal an immediate effect of herbicide-rolling treatment on these response variables. Given that marsh-user richness was not impacted by treatment over time, but there was a marginal decline in total species richness, this may have been facilitated by a decline in non-marsh affiliated birds following treatment. Untreated *P. australis* provides habitat for more non-marsh species compared to treated *P. australis*, at least in the short term.

In the 2021 space-for-time substitution, vegetation type (control, reference, herbicide-treated) had no detectable effect on total avian richness or the richness of marsh-users, species of conservation concern, or species at risk. However, all richness variables, except species at risk, were marginally, but consistently, lower in herbicide-treated sites. It is possible that species richness was marginally lower in treated habitat because it had a smaller sample size than the other two vegetation types; five sites in comparison to eight reference and seven control. Although ARUs on average recorded a similar number of species per site (herbicide-treated = 22 [std = 1.2], control = 21.2 [std = 2.8], reference = 19.5 [std = 4.1]), the number of total species recorded was higher at control sites (52), than at reference (44) and herbicide-treated (42) sites, as control sites captured more unique species.

As stated previously, our study focused on the immediate effects of *P. australis* management on avian diversity during the dawn chorus to determine whether these management actions might cause harm to birds. The ultimate effects of *P. australis* management on avian communities are expected to be positive – indeed, invasive plant management is predicated on the goal of species at risk recovery (MacLeod, 2019). Yet, these ultimate effects will not be evident immediately; continued long-term monitoring is necessary to track changes in avian species richness following *P. australis* management to determine how marsh-users and species of conservation respond to an increase in potentially favourable habitat. Overall, we observed minimal effects of *P. australis* control on avian richness, as we did not observe a statistically significant change in the richness of total avian species, marsh-users, species of conservation concern, or species at risk in the 2019-2021 BACI experiment, or the 2021 space-for-time substitution design. We did observe a marginal decline in total species richness in post-treated sites in the BACI experiment but given that we did not observe a decline in the richness of marsh-users, *P. australis* is likely providing habitat for more non-marsh affiliated birds compared to areas where *P. australis* was transformed into open-water habitat by herbicide treatment.

### *2.4.2 Avian community composition and functional traits*

Many studies have documented the impacts of *P. australis* invasion on avian community composition in wetlands (e.g., Benoit & Askins, 1999; Meyer et al., 2010; Whyte et al., 2015; Robichaud & Rooney, 2017). Principle changes in avian community driven by *P. australis* invasion that are documented in the literature include displacement of larger-bodied marsh-users, particularly those that forage by stalking or dabbling, or are ground nesters, displacement of aerial insectivores, particularly swallows, and an increase in small-bodied species (both generalist species and marsh-users), particularly foliage gleaners, ground foragers, and/or shrub nesters (Whyte et al., 2015; Robichaud & Rooney, 2017; Tozer & Beck, 2018). *Phragmites australis* invasion fills in open-water pools and replaces hemi-marsh habitat with dense monocultures of emergent reeds, reducing access to shallow open-water habitat that is crucial foraging grounds for waterfowl and wading birds (Perry & Deller, 1996; Lantz & Cook, 2011). As such, *P. australis* invasion can cause a shift in avian community composition by excluding larger-bodied species such as Bitterns, Herons, and waterfowl, but be utilized by smaller-bodied species that are not dependent on open water or prefer shrubby vegetation to nest in, such as Sparrows, Warblers, and Blackbirds (Whyte et al., 2015; Robichaud & Rooney, 2017; Tozer & Beck, 2018). Thus, we expected changes in community composition to be evident among the vegetation types we surveyed, even if no differences in avian richness occurred.

In contrast to the many studies on the effects of *P. australis* invasion on avian communities, relatively few studies have documented how avian communities respond to *P. australis* suppression activities (see Lazaran et al., 2013 and Tozer & Mackenzie, 2019). These studies have looked at either the immediate or the longer-term response of avian communities to *P. australis* management. Further, the few studies there are do not agree.

Lazaran et al., (2013) looked at the immediate impacts of *P. australis* management on a marsh-user species, the Marsh Wren, in a Lake Erie coastal marsh. They concluded that the removal of *P. australis* and the subsequent delay in vegetation regeneration one year after treatment reduced Marsh Wren breeding habitat. The Marsh Wren requires adequate vertical vegetation structure to build nests using vegetation such as *Typha* spp. or *P. australis*, and *P. australis* control likely removed favourable breeding habitat. In contrast, Tozer & Mackenzie (2019) looked at the occurrence of marsh birds 1-5 years before and 1-5 years after *P. australis* removal in several Lake Erie coastal marshes. They determined that the occurrence and abundance of five marsh birds of conservation concern (including Rails and Bitterns) increased in herbicide-treated sites. In contrast to Lazaran et al., (2013), this study found that shrub-nesters including the Marsh Wren occurred as frequently within sites 1-5 years after *P. australis* was removed as they did in *P. australis* invaded sites, likely because vegetation had time to recover and regrow after *P. australis* treatment. Our study looked at the short-term response of the avian community to *P. australis* control, and we anticipate that the removal of *P. australis* and its replacement with shallow open water, thus increasing the availability of hemi-marsh habitat, will eventually lead to an increase in the occurrence of larger-bodied waterfowl and wading birds, and a decrease in the occurrence of smaller-bodied species, specifically those that are shrub nesters, foliage gleaners, and ground foragers.

We anticipated that the recently treated habitat would not support novel avian species with novel functional traits, but instead a subset of species and functional traits found in reference habitat. Water birds (i.e., waterfowl, Herons, Bitterns) often use cattail marsh and hemi-marsh for breeding and foraging, and we anticipated that these birds would use the open-water habitat remaining after *P. australis* treatment (Baschuck et al., 2012). From our NMS ordinations, we determined that beta diversity differed in the 2021 space-for-time substitution design. In contrast to what we anticipated, the community composition and functional trait composition in herbicide-treated sites present a nested subset of the bird community and functional traits present in both reference and *P. australis* control sites. It is likely that treated habitat and invaded control habitat shared the presence of birds that have a wide distribution within the marsh, such as habitat generalists like the Red-winged Blackbird (Robichaud, 2016). These results suggest that during the first year or two post-treatment, the habitats where the herbicide-rolling treatment occurred are less heterogeneous, offering reduced niche space or more limited resource diversity compared to the reference and control locations (MacArthur & MacArthur, 1961). The horizontal contact profiles substantiate this, as one to two years after the herbicide-rolling treatment was applied to ARU locations, the habitat is mainly characterized as shallow open water (avg. depth 35.4 cm, std = 14.6 cm) with sparse floating vegetation and standing dead litter (Appendix 1C). However, we anticipate that as the emergent vegetation recolonizes these treated areas and habitat heterogeneity tied to vegetation structure is re-established, the avian beta diversity will also return to reference levels.

When further analyzing the community composition from our spatially replicated BACI design and our 2021 space-for-time substitution study, large differences in community composition associated with geographic location were revealed. The avian community using the Big Creek unit in the Big Creek NWA, which is located at the western base of the Long Point peninsula, was especially distinctive from the avian community using the Long Pond and Squire's Ridge units in the Long Point NWA, which is located at the eastern tip of the peninsula.

For example, in the 2021 space-for-time substitution, we observed that the four management units within the two NWAs support distinct bird communities with distinct functional traits. This is likely because the vegetation within the Long Point peninsula is a complex mosaic due to the varying topography and moisture regimes, and the dynamic action of Lake Erie shaping the surrounding landscape (Reznicek & Catling, 1989). The Big Creek unit within the Big Creek NWA is separated from Lake Erie by a barrier beach, and the habitat primarily consists of marsh with small areas of upland vegetation (ECCC, 2020a). Thoroughfare and Squire's Ridge are both adjacent to Lake Erie, and we observed that Thoroughfare is predominately marsh and swamp habitat, whereas Squire's ridge is a mixture of marsh, swamp, forest and dune habitat. Long Pond is separate from Lake Erie, and we observed that it is comprised of wetland swales situated between dunes. Since ARUs were placed within interdunal wetlands in the Long Point NWA, specifically Squire's Ridge and Long Pond, this allowed for more terrestrial species to be recorded. In Chapter Three of this thesis, we determined that SM4 ARUs can detect certain marsh birds 350 m away in wetland vegetation. CWS-ON's current ARU deployment protocol is to deploy ARUs within a 25 m radius of the target vegetation. At interdunal sites, a minimum recording radius of just 100 m would survey the wetlands between dunes but also extend to cover the dunes and upland vegetation growing on them (Figure 2.10). Consequently, it is not surprising that birds like Eastern Towhee and Field Sparrow (*Spizella pusilla*) were more common occurrences, as these species are all typically terrestrial (Cornell Lab of Ornithology, 2022). In contrast, the Big Creek NWA is a more homogenous expanse of marsh, hemi-marsh, and open-water habitat, and the same recording radius at these locations would not have included as much terrestrial habitat, except for two treatment sites that border a farm field and a treeline (Figure 2.10). Consequently, it is not surprising that marsh-user species including American Bittern and Common Gallinule were more frequently observed at the Big Creek ARU locations.

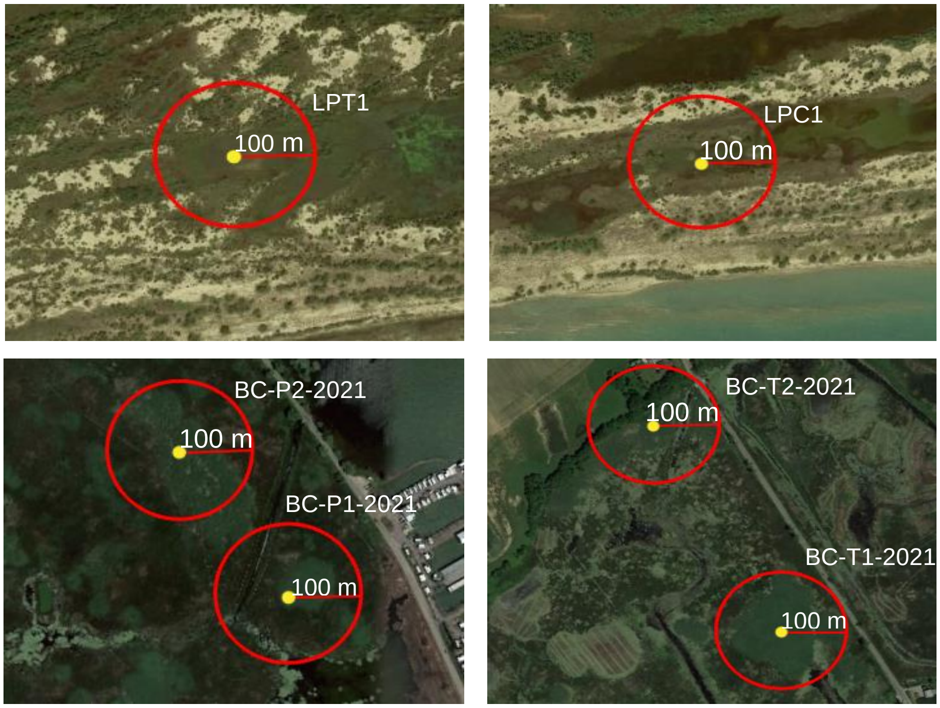



*Figure 2.10.* Examples of ARUs placed within the Long Pond management unit (Long Point NWA; top row) and in the Big Creek unit (Big Creek NWA; bottom row). Red circles highlight a 100 m radius around each ARU. ARUs in Long Pond were placed in wetlands (green coloured areas) that tended to be surrounded by dunes (lighter coloured areas), whereas ARUs in Big Creek tend to be surrounded by marsh and open water (green and black coloured areas).

These important habitat differences between the two NWAs masked our ability to detect divergence in avian community composition emerging as a consequence of herbicide application in our two studies. This conclusion was substantiated when we reanalyzed a dataset restricted to the Big Creek unit of the Big Creek NWA in the BACI experiment.

When restricting the analysis to this subset of the BACI site data ($N_{treatment} = 2$, $N_{control} = 2$), we observed a statistically significant interaction between year and treatment in predicting both total avian community composition and marsh-user community composition in Big Creek. The interaction reveals a significant effect of herbicide-rolling treatment on the bird community composition above and beyond any pre-existing differences between treatment and control sites or any effect of changing water levels between the two survey years. The less powerful 2021 space-for-time substitution design did not reveal a difference in total avian community composition or marsh-user composition among control, reference, and herbicide-treated sites, but this should not be interpreted as contradicting the results of the more powerful BACI design. Rather, the two analyses should be interpreted as indicating that there was a minor effect of herbicide application on the avian community composition in the years immediately following management, but that the consequences of treatment were negligible compared to the natural variation in avian community composition in accordance with habitat heterogeneity in Long Point (Reznicek & Catling, 1989; ECCC, 2020 a,b). Knowing that the avian community can respond sensitively to *P. australis* invasion in our study area (Robichaud & Rooney, 2017), we consequently conclude that there are small-scale effects on birds following *P. australis* suppression activities in the NWAs. There is some indication that certain groups of avian species may be utilizing the recently treated areas, specifically marsh-users that were documented to be most impacted by *P. australis* invasion.

We have reason to expect that *P. australis* management in Long Point will benefit the avian community over time based on longer-term studies of avian community response to *P. australis* removal (e.g., Tozer & Mackenzie, 2019). Given adequate time for vegetation recovery (e.g., 4-6 years; Jordan & Rooney, unpublished data), we anticipate that marsh-nesting species of conservation concern and species at-risk avifauna will benefit. Our study already provides some evidence that *P. australis* removal is benefiting avifauna of two types: 1) waterfowl and large-bodied wading birds that forage by stalking and dabbling (including several marsh species of conservation concern), and 2) aerial foragers that hunt insect prey on the wing (including at-risk swallows).

We observed waterfowl and wading birds generally occurring in sites where *P. australis* was removed. For example, we observed dabbling species like the Common Gallinule (species of conservation concern), Mallard (marsh-user and species of recreation value in Long Point) and Wood Duck (marsh-user). We also observed two stalking foragers, the Great Blue Heron and American Bittern, both of which are marsh-users, and the latter being a species of conservation concern, in recently treated sites. Interestingly, the Least Bittern, which is federally and provincially designated as Threatened (Environment Canada, 2014; OMNRF, 2016), is a stalking forager that was absent from all three treated sites in the BACI experiment, and infrequently found in treated sites in the 2021 space-for-time substitution.

Least Bitterns prefer to nest in emergent vegetation with nearby access to open-water pools, as they forage at the interface of emergent vegetation and open water (Gibbs et al., 1992; Bogner & Baldassarre, 2002). Least Bitterns were found in two of the five 2021 treated sites, specifically at the south end of the Big Creek management unit, where the wetland was not as homogenously treated with herbicide as it was in the north end of Big Creek, where the BACI treatment sites were located. The treatment sites in the south end were in closer proximity to more expansive emergent vegetation, which would have likely been favorable for the bittern to forage. The proximity of herbicide-rolling treated areas to vegetation may be important for determining if the at-risk bittern will use recently treated areas. This highlights that *P. australis* treatment should be done in stages and spread over several years to leave habitat refugia for Least Bittern. Overall, it seems as though several larger-bodied marsh-user species that may be most impacted by *P. australis* invasion are either using the open-water habitat that remains in the first years after treatment, or there is potential for them to use it. Other groups of birds that were found to be most impacted by *P. australis*, such as aerial insectivores, were also found in 1- or 2-year post-herbicide-rolling sites.

Aerial insectivores, particularly swallows, have been found to avoid foraging over *P. australis* invaded areas (Robichaud & Rooney, 2017). Therefore, *P. australis* suppression may benefit species like the threatened swallows: Barn Swallow (Heagy et al., 2014) and Bank Swallow (Falconer et al., 2016; ECCC, 2021). For example, it has been reported that the availability of swallow prey can be dramatically enhanced by herbicide-based *P. australis* control in Long Point (Robichaud et al., 2021), and unpublished results confirmed greater foraging activity by Barn Swallows over herbicide-treated regions of Long Point (Robichaud, unpublished data). In both studies, we observed that Barn Swallows occurred fairly frequently within 1- or 2-year post-treatment sites, while Bank Swallows occurred slightly less frequently. Other aerial insectivore marsh-users including Tree Swallows and Purple Martins were observed at almost every 1- or 2-year post-treatment site. Our study may not have detected the same significant effects of *P. australis* suppression on swallows as Robichaud (unpublished data), but our study had limited statistical power, and our focus was on avian habitat use indicated by vocalizations recorded during the dawn chorus. Swallows primarily forage early in the morning or later in the day, and foraging activity may not be well captured by ARU recordings, as foraging birds are not vocalizing in the same way breeding birds are during the dawn chorus (Dreelin et al., 2018). Therefore, we may have missed the increase in foraging by Bank Swallows and Barn Swallows because of the nature of our dawn chorus surveys. While swallows are small-bodied species that were found to use the recently treated *P. australis* areas, many other small-bodied species were infrequently found within treated *P. australis*.

Within the BACI experiment and 2021 space-for-time substitution, we observed that species that appeared less frequently in herbicide-rolled treated sites tended to be small-bodied species. Although functional trait composition did not differ among the three vegetation types, we observed a trend that small-bodied birds that were foliage gleaners, such as Cedar Waxwing, Chestnut-sided Warbler (*Setophaga pensylvanica*), and Blue-gray Gnatcatcher (*Polioptila caerulea*), ground foragers such as Swamp Sparrow (marsh-user), and/or shrub-nesters such as Willow Flycatcher (*Empidonax traillii*) tended to occur less frequently within treated sites. The reduced occurrence of small-bodied species and/or those with these life-history traits likely drove the change in total avian community composition within the herbicide-treated sites in the Big Creek NWA in the BACI experiment. Birds with these traits have been found to prefer *P. australis* habitat, and it is speculated that the dense vertical structure of *P. australis* provides insect prey for insectivorous foliage gleaners and ground foragers, and habitat for shrub-nesters (Robichaud & Rooney, 2017). The removal of *P. australis* in the BACI experiment seems to support this interpretation. In contrast, the change in marsh-user composition in herbicide-treated sites the BACI experiment arose from the displacement of both smaller-bodied species including the Swamp Sparrow and Willow Flycatcher and larger-bodied species including the Least Bittern and Canada Goose, but also due to gaining larger-bodied birds including Mallard and Killdeer.

We anticipated that the occurrence of shrub-nesting species in herbicide-treated *P. australis* would decline due to the removal of breeding and foraging habitat, however, many still frequently occurred in treated sites, such as the Red-winged Blackbird and Marsh Wren. In both NWAs, we see that treated habitat remains as open water with little vegetation that could be used as shrub-nesting habitat. Again, ARUs may be recording beyond the target treated habitat and be capturing shrub-nesting species in upland vegetation. Although many smaller-bodied species were those to occur less frequently in herbicide-treated sites, several larger-bodied species were also found infrequently in treated *P. australis* habitat, including those of conservation concern.

Virginia Rail and Sora are large-bodied species of conservation concern that were not found in 1- or 2-year post-treatment sites. Both birds require dense vegetation to place their nests in and may have not found recently treated habitat favorable to do so (Lor & Malecki 2007). This also highlights the need to retain habitat refugia during *P. australis* treatment. However, both Virginia Rail and Sora often forage in shallow water; Sora forages for seeds from emergent vegetation or aquatic invertebrates by pecking the substrate or water's surface, and Virginia Rail uses its long bill to probe the substrate for aquatic invertebrates (Sayre & Rundle, 1984; Johnson & Dinsmore, 1986). Therefore, there is potential for these species to forage at the edge of treated areas if they are near dense emergent vegetation such as cattail. Similarly, Pied-billed Grebe is a large-bodied species of conservation concern that was infrequently found within treated sites. The grebe is a surface diver that forages in deep open water, and it has been reported to dive 2-3 m deep for fish (Bleich, 1975). The shallow water within the treated sites (35.4 cm (std = 14.6 cm) in 2021) may be unfavourable foraging habitat for the grebe (Melvin & Gibbs, 1992).

*2.4.3 Recommendations and conclusions*

Recognizing the limitations of our small sample size and regional difference between management units, we found minimal evidence to support that *P. australis* control activities negatively impact wetland birds in the short term. We found no differences in avian diversity (total species richness, marsh-user richness, marsh species of conservation concern richness, or species at risk richness) attributable to the herbicide-rolling treatment. However, we did find a difference in community composition attributable to the herbicide-rolling treatment when analyzing a subset of the BACI site data, but not when analyzing the less powerful 2021 space-for-time substitution. We contend that these results do not contradict one another. Rather, the two analyses indicate that there was a minor effect of herbicide application on the avian community composition in the years immediately following management, but that the consequences of management were relatively small compared to the natural variation in avian community composition in accordance with habitat heterogeneity in Long Point.

We observed some trends which might comprise early indications of avian community recovery following *P. australis* suppression. For example, larger-bodied marsh-users used the recently treated marsh habitat frequently. Such species were previously recognized as those most negatively impacted by *P. australis* invasion (Robichaud & Rooney, 2017; Tozer & Beck, 2018). Conversely, we see evidence that species avoiding *P. australis* after herbicide application tend to be small-bodied, which prior research found were advantaged by *P. australis* invasion (Robichaud & Rooney, 2017). Many of these small-bodied species are habitat generalists that do not rely on the marsh for breeding and foraging and can find refuge in the surrounding forest or interdunal habitat. One provincially and federally threatened marsh-user, the Least Bittern, may avoid recently treated areas. However, as native vegetation recovers and the open-water habitat transitions to more hemi-marsh habitat, Least Bittern will likely return to these treated areas. We recommend that *P. australis* management be completed in stages rather than all at once, so habitat refugia remain for Least Bittern and other marsh birds to allow for their populations to persist through the early post-treatment period.

Marsh bird occurrence has been shown to be tightly linked to the vegetation composition present in wetlands (e.g., Lor & Malecki, 2006; Valente et al., 2011; Glisson et al., 2015; Chin et al., 2014). Therefore, long-term monitoring is essential to evaluate how both the vegetation and avian communities continue to respond to *P. australis* control to obtain a clearer assessment of the ultimate effects of this conservation action. We recommend increasing the sample size for future monitoring to capture the regional distinctness of bird communities within the Big Creek and Long Point NWAs, and to compensate for data corruption in 2021. Previous monitoring work in Long Point on the vegetation recovery following herbicide application to control *P. australis* concluded that 4-6 years is necessary for the vegetation to passively recovery following herbicide application (Jordan & Rooney, unpublished data). To accurately assess the efficacy of *P. australis* suppression on avian wildlife, additional monitoring efforts should extend the period of monitoring to match the 4–6-year time frame reported as necessary to track changes in vegetation and avian communities after *P. australis* control (Jordan & Rooney, unpublished data; Tozer & Mackenzie, 2019).

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# *3. Optimizing the use of autonomous recording units to survey wetland bird communities*

## *3.1 Introduction*

In-person point count surveys have traditionally been used to monitor avian species and determine population trends, habitat preferences, and breeding phenology (Shonfield & Bayne, 2017). Point counts occur when 1-2 observers survey birds aurally and visually at a set location for a set period of time (Drake et al., 2021). Within the last 20 years, technological advancements have led to the use of autonomous recording units (ARUs) to supplement or replace in-person surveys (Darras et al., 2019). ARUs are installed at a survey site and programmed to record sound, which is then later reviewed to aurally identify avian species, often with the help of audio spectrographic imaging software.

In surveys of wetland-dependent birds specifically, there are several advantages to ARU-recorded avian surveys. For example, wetland environments may be difficult to access and traverse for repeated point count surveys, which limits our ability to assess detection probabilities or to capture variation across the survey season. (Shonfield & Bayne, 2017; Stewart et al., 2020). Furthermore, bird vocalizations may be underestimated by in-person surveys if the presence of humans alters bird behavior (Bye et al., 2001), and species with short or infrequent calls can be misclassified or missed (Farmer et al., 2012). Many wetland-dependent birds are elusive (Conway & Gibbs, 2011), vocalize infrequently (Conway & Gibbs, 2011), vocalize at times of day that are not frequently surveyed by in-person observers (Sidie-Slettedahl et al., 2015), or are reluctant to vocalize with human presence (Conway & Gibbs, 2011; Bobay et al., 2018). Consequently, wetland birds can have low detection rates (Podoliak et al., 2022), which can make statistical modelling difficult and can lead to exclusion of species from analyses (Tozer et al., 2006; Tozer, 2016).

The use of ARUs can enable longer, more frequent surveys by reducing the need for trained personnel and the frequent site visits, as sites are visited once to deploy and once to retrieve ARUs, and as such, can record many times between these human disturbances (Shonfield & Bayne, 2017). More, short or quiet calls can be replayed, and spectrographs of recorded audio can be used to help identify species who call quietly or infrequently. ARUs can therefore increase spatial and temporal efficiency of monitoring wetland birds and allow for larger sample sizes (Castro et al., 2018; Stewart et al., 2020), and greater quality control assessment of auditory survey data.

One consequence of ARUs recording longer and more frequent surveys than is practically achievable with in-person surveys, is that ARUs generate a large amount of data, which can be time-consuming and laborious to analyze (Shonfield & Bayne, 2017). Therefore, standard recording methods (i.e., programming when an ARU records) and transcription methods (i.e., listening to the ARU recordings and identifying what avian species are vocalizing) must optimize the effort to collect avian diversity data of sufficient accuracy to meet monitoring and management objectives without wasting limited resources. The effort required for an ARU to record is less than the effort required to transcribe it; it is not much additional cost to record longer, more frequent surveys once an ARU is purchased and deployed, however, it is more work to transcribe the large amounts of data collected. Therefore, it is more important to optimize ARU transcription effort.

The probability of observing wetland bird vocalizations varies with time of day, date, and by location, which makes it important to determine the optimal time to program an ARU to record at a study location to maximize the chance of detecting species (Nadeau et al., 2008; Conway & Gibbs, 2011). ARUs are commonly programed to record during the breeding season's dawn chorus due to a high diversity of avian vocalizations (Brown & Handford, 2003; Gil & Llusia, 2020). For example, the Canadian Wildlife Service – Ontario region (CWS-ON) programs ARUs to record 30 min before sunrise for 2 hours for 5-7 consecutive days each month from May to July. Yet, it is common to transcribe only a 5-15 min section of a longer ARU recording for any one survey date (e.g., Tegeler et al., 2012; Sidie-Slettedahl et al., 2015; Frommolt, 2017; Symes et al., 2022). CWS-ON presently transcribes the first 15 min of one recording each month from May to July to estimate breeding bird diversity in wetlands. However, such short surveys positioned at the start of the dawn chorus may miss species that vocalize later in the morning or miss rare species that vocalize infrequently, thus underestimating species richness in a particular area (La & Nudds, 2016; Shirkey et al., 2017). One way to optimize the use of ARUs to survey bird communities is to determine the duration in which transcription should occur to maximize accurate estimates of diversity, while balancing effort.

Few field studies have examined how to optimize ARU transcription effort to capture accurate estimates of avian species richness during the dawn chorus in wetland habitats (La & Nudds, 2016; Reynolds, 2020). La & Nudds (2017) used ARUs to survey inland wetlands in Nipissing District and Algonquin Park, Ontario and determined that traditional morning surveys (10 min long) consistently and substantially underestimated species richness when compared to 720 min of data collected over three days. Reynolds (2020) studied avian diversity in mountain peatlands in Alberta and determined that transcribing a total time of 60 min across four days (240 min) in the breeding season was sufficient to determine accurate estimates of species richness for at least 75% of sites. She also determined that if 10 min surveys were used instead of the full 240 min, the richness detected at a site would decline to be 73% of the total richness. Stiffler et al., (2018) used ARUs to survey rails in tidal marshes in Virginia, US, and determined that a 10 min survey captured 19.1% of rails detected within a 60 min sampling period whereas a 45 min survey captured 76.6%. These studies highlight that standard survey lengths (i.e., less than 15 mins) may underestimate species richness at single survey point in wetland environments. Avian vocalizations exhibit a typical species accumulation curve, with short surveys underestimating the total number of species present at a survey point (Chao et al., 2013). Longer duration surveys will more fully capture the true richness of birds at a given point but increasing the duration of surveys will yield diminishing returns as the species discovery rate plateaus (Reynolds, 2020). Furthermore, surveying for longer periods may mean fewer sites can be sampled, in comparison to short duration surveys which allows for more sites to be surveyed in the same amount of time as one longer duration survey (Steidl et al., 2013). Longer duration surveys may be more beneficial for capturing accurate estimates of total species richness, whereas short surveys may gain better estimates of abundance and have the ability to collect more samples to increase statistical power for modelling (Tozer et al., 2017). Thus, determining the duration of transcription effort is important for optimizing ARU use.

A second way to optimize ARU use is to determine where transcription effort should be allocated within the dawn chorus. For example, Wheelhouse et al., (2022) studied forest bird communities in British Columbia and determined the optimal duration and time of day to transcribe ARUs. After centering 2-hour ARU recordings at dawn and dusk, they determined that the peak in avian vocal activity occurred within 30 min centered at dawn, as this longer duration captured early and late vocalizers, as well as infrequent vocalizers. Certain avian species may vocalize early in the dawn chorus, while others may vocalize later due to factors such as light availability (Thomas et al., 2002; Gil & Llusia, 2020) or to prevent overlap of vocalizations (Brumm & Naguib, 2009; Suzuki et al., 2012; Hart et al., 2021). This study's 30 min survey effort was spread across the breeding season by sampling every third day from the end of May to mid-July (Wheelhouse et al., 2022). A third factor warranting consideration in optimizing ARU transcription is whether effort should be spread across the breeding season or centered on a few days within the breeding season.

Centering effort around a few days within the breeding season could conserve valuable conservation resources; fewer ARUs could be purchased and rotated among sites to record a few days a week instead of purchasing many ARUs and stationing them at sites to record all season. For example, Reynolds et al. (2022) rotated a limited set of ARUs among a larger set of peatlands, allowing them to sample twice as many wetlands as they could have if ARUs were deployed at a single site all season. Since some species may call earlier in the morning or earlier in the breeding season than others to partition the avian breeding niche (Rehm & Baldassarre, 2007; La & Nudds, 2017; Wheelhouse et al., 2022), the distribution of transcription effort across the dawn chorus or across the breeding season may influence species richness estimates. Detection rates of certain marsh birds can vary with season (Rehm & Baldassarre, 2007; Harms & Dinsmore 2014), and geographic location (Rehm & Baldassarre, 2007). Bayne (2018) used ARUs to survey coastal wetlands across southern Ontario during the breeding season by collecting data every half hour throughout the day and transcribing the first minute of each recording. He used occupancy modelling to determine the probability of species occurrence across the breeding season, which revealed that the optimal time to survey wetlands in southern Ontario is during the dawn chorus between mid-May to mid-June. Furthermore, he determined that the probability of detecting marsh birds between mid-May to mid-June stayed relatively constant throughout the breeding season, with only a few species dropping off by late June to early July. Consequently, efficiencies in transcription could be gained by optimizing the distribution of transcription effort through time.

A fourth consideration for optimizing ARU use is determining what size of area or point count footprint is covered by an ARU recording in a given wetland habitat. Studies using ARUs to survey bird communities often either ignore or roughly estimate the distance at which their ARU model can detect avian vocalizations (Tegeler et al., 2012; Leach et al., 2016). Thus, ARUs often conduct unlimited distance sampling. It is important to determine the range at which ARUs can record avian vocalizations to determine if diversity estimates are accurately reflecting the habitat researchers are specifically targeting (Darras et al., 2016; Thomas et al., 2020). In contrast to ARUs, in-person observers can control the area of habitat they sample by conducting fixed-distance point counts and estimating the distance at which birds are heard or seen (Yip et al., 2017a). If researchers want to combine data surveyed by both ARUs and in-person observers in a single analysis, it is important to determine if their survey distances are comparable, or else diversity estimations will be biased by unequal sampling areas (Yip et al., 2017a). Alternatively, researchers could apply correction factors to make in-person and ARU point count data comparable prior to analysis (e.g., Van Wilgenburg et al. 2017, Stewart et al. 2020).

Research in the past decade has begun investigating the detection distances of ARUs and whether it is comparable to those of in-person observers. However, it can be difficult to accurately estimate the detection distance of ARUs and in-person observers due to variation in site-specific biological aspects such as presence or absence of vegetation, ambient noise level, direction of avian vocalization, and weather parameters such as wind (Yip et al., 2017a; Darras et al., 2016; Pérez-Granados & Traba, 2021). Furthermore, the detection range of an ARU can depend on several technical aspects such as signal-to-noise ratio, recording unit model, number of microphones, and microphone height (Yip et al., 2017a; Darras et al., 2018a; Thomas et al., 2020).

Methods for sampling the detection distances of ARUs and in-person observers vary among studies, but often involve either broadcasting recordings of bird vocalizations from known distances (e.g., Yip et al., 2017a; Darras et al., 2018b; Wheelhouse et al., 2022) or measuring the distance to live birds in the field (e.g., Rosenberg & Blancher, 2005; Stiffler et al., 2018; Schroeder & McRae, 2020). Studies have often investigated detection distance of songbirds for in-person surveys (e.g., Alldredge et al., 2007; Yip et al., 2017a) and ARU surveys (e.g., Rempel et al., 2013; Yip et al., 2017a; Van Wilgenburg et al., 2017; Furnas & Callas, 2015; Thomas et al., 2020), but fewer have determined the detection distance of marsh birds in wetland environments (e.g., Meyer, 2003; Schroder & McRae, 2020; Stiffler et al., 2018; Stewart et al., 2020). One study determined that the King Rail (*Rallus elegans*), an endangered marsh bird, had a maximum detection distance of 300 m for in-person observers and 200 m for Wildlife Acoustics SM4 ARUs in a wetland complex (Schroder & McRae, 2020). Furthermore, in an appendix to his thesis, Meyer (2003) reported how the maximum detection distances of select marsh bird vocalization broadcasts differed among vegetation types in Long Point, ON. He found that bird vocalizations travelled farthest in meadow marsh, followed by *Typha* spp., and travelled least far in *P. australis*. These studies highlight that avian vocalization detection distances may differ depending on both survey method and habitat type. CWS-ON deploys ARUs at least 250 m apart in wetlands. Therefore, this roughly estimates that an ARU has 125 m of non-overalpping recording radii. Consequently, it is important to characterize the sound detection space of an ARU to determine the area of habitat being surveyed.

If sound detection distances vary between survey methods or vegetation types, this can bias estimates of diversity, which can impact understanding of habitat selection trends and population statuses (Yip et al., 2017a). Scientists and managers are often interested in comparing biodiversity among habitats. Specifically for ecosystem restoration work, managers are often interested in differences in biodiversity between the restored area, reference conditions, and/or control areas to determine the success of the restoration action. If avian detection probabilities differ among habitats due to differences in detection distances, this could confound our ability to detect the influence of restoration actions on avian diversity using ARU survey methods.

As a case study, in 2019, CWS-ON undertook a pilot project to control invasive *P. australis* in the Big Creek and Long Point National Wildlife Areas (NWAs) in Long Point, ON. Because conservation of migratory birds and their habitat was a major motivation behind the pilot project, CWS-ON deployed 10 ARUs to monitor the effects of invasive *P. australis* management on marsh birds. It is important that avian diversity estimations are accurate, as it will help determine if diversity estimates differ in areas where *P. australis* has been removed compared to areas that remain invaded. This will ultimately help determine if and what changes are occurring to marsh birds following *P. australis* management.

In this chapter, our goal was to optimize the use of ARUs to survey wetland birds in the Big Creek and Long Point NWAs. We had four main objectives: 1) determine the optimal transcription duration within the dawn chorus to maximize estimates of avian species richness, 2) determine the timing within the dawn chorus where transcription effort should be allocated to capture our species of interest, 3) determine if transcription effort should be allocated to one long duration recording on one survey date within the breeding season or many short duration recordings across the breeding season to best capture accurate estimates of avian diversity, and 4) determine the maximum detection distance of select marsh birds of conservation concern by SM4 ARUs and in-person observers in different wetland vegetation types (*Typha* spp., *P. australis*, and herbicide-treated *P. australis*).

For objective one, we hypothesize that longer surveys will more fully capture the true richness of birds in a given point count location but that increasing the duration of surveys will yield diminishing returns as the species discovery rate plateaus (e.g., Reynolds, 2020). We predict that the optimal transcription duration to capture at least 80% of bird species at an ARU station will be greater than the 15 min typically transcribed by the CWS-ON standard operating procedure, but less than the 4 hours of recordings transcribed by Reynolds (2020) to characterize avian richness in peatlands in Alberta's Upper Bow River Basin.

For objective two, we hypothesize that not all species will have an equal probability of vocalizing at any point during the dawn chorus because certain avian species may sing early in the dawn chorus, while other species may sing later due to factors such as light availability (Thomas et al., 2002; Gil & Llusia, 2020), levels of competition from other birds (Foote et al., 2011), or to partition acoustic space and prevent overlap of vocalizations (Suzuki et al., 2012; Hart et al., 2021). We predict that avian species richness will be the highest in at least one of the 15 min intervals positioned in the middle of the 2-hour dawn chorus recording because this will capture both early and late vocalizing species (La & Nudds, 2016; Wheelhouse et al., 2022).

For objective three, we hypothesize that allocating transcription effort to one day within the breeding season for a longer duration will yield greater diversity metrics than allocating effort across the breeding season in shorter durations, because the probability of detecting marsh birds in southern Ontario wetlands stays relatively constant throughout the breeding season until end of June (Bayne, 2018). Therefore, if there is relatively little change in the probability of detecting species as the season progresses, surveying for longer durations on one day may capture more infrequent vocalizers. We predict that avian species richness will be greater, community composition will be more diverse, and more unique species will be captured when transcribing one longer duration recording on one day compared to transcribing many (N = 30) much shorter segments (e.g., 1 min) spread across the breeding season.

For objective four, we hypothesize that tall, dense vegetation (*P. australis*) will reduce the detection of avian vocalizations for an SM4 ARU and in-person observer compared to open habitat (e.g., herbicide-treated *P. australis*), because sound transmission can be dampened by vegetation obstruction and therefore travel farther in open habitat (Pacifici et al., 2008; Yip et al., 2017a). Based on the work by Meyer (2003), we predict that the avian detection zone of an SM4 ARU and in-person observer carried out in *P. australis* vegetation will be smallest, with surveys in native vegetation (*Typha* spp.) being an intermediate detection zone, and surveys in areas where *P. australis* was treated with herbicide and rolled to flatted dead *P. australis* to create open-water marsh will have the largest avian detection zones.

## *3.2 Methods*

### *3.2.1 Experimental design*

Our first objective was to determine the optimal duration of transcription to capture accurate estimates of avian species richness (both total and marsh-user richness) from ARUs in Long Point coastal wetlands. To address this objective, we transcribed 10 2-hour long recordings from ARUs in *P. australis*-dominated habitat and used species accumulation curves and non-parametric richness estimators to estimate the proportion of species we could expect to detect during certain break points in transcription duration. Our second objective was to determine where to allocate the determined transcription duration effort within the dawn chorus. We determined if there was a peak in species richness across the 2-hour dawn chorus and plotted when each observed species vocalized to determine early/late and rare/common vocalizers. We targeted when marsh-users, species of conservation concern, and species at risk most commonly vocalized (refer to Chapter Two for definitions of categories). For our third objective, we determined if transcribing a longer duration recording on one survey date within the breeding season captured similar avian diversity metrics as transcribing multiple shorter duration recordings across the breeding season. We transcribed 10 ARU recordings from *P. australis,* herbicide-treated *P. australis,* and uninvaded reference sites using the two transcription methodologies to determine if certain diversity metrics were comparable. Our fourth objective was to determine if the maximum detection distance of SM4 ARUs and in-person observers were comparable in different wetland vegetations. We conducted surveys along transects in *P. australis,* herbicide-treated *P. australis,* and cattail (*Typha* spp.) to determine how far 13 marsh bird vocalizations could be detected by the two survey methods.

### *3.2.2 Objective One: Optimal ARU transcription duration*

#### *3.2.2-a Field methods*

CWS-ON deployed 10 ARUs in *P. australis*-dominated habitat in Big Creek and Long Point NWAs in 2019. The two NWAs are separated into management units: the ARUs were deployed in the Big Creek unit within the Big Creek NWA, and in the Long Pond and Otter Pond units within the Long Point NWA (Figures 3.1, 3.2). ARUs were deployed in homogenous patches of *P. australis* that were at least 25 m in radius and 25 m from open water. ARUs were positioned to be 1) with the front of the ARU facing open water and the back facing shallow water or shoreline, 2) installed to have microphones 1.5 m above the water level, and 3) at least 250 m apart to prevent their estimated 125 m recording radii from overlapping. The ARUs were programmed to record 30 min before sunrise for 2 hours for seven consecutive days each month from May to July.


*Figure 3.1*. Locations of the four autonomous recording units deployed in *P. australis* habitat in the Big Creek National Wildlife Area in 2019.


*Figure 3.2*. Locations of the six autonomous recording units deployed in *P. australis* habitat in the Long Point National Wildlife Area in 2019.

*3.2.2-b Autonomous recording unit transcription*

We selected the dawn chorus from the June 23rd survey date, as this date had suitable 2-hour recordings from all 10 ARUs with light wind, no rain, and minimal background noise (in compliance with the Marsh Monitoring Program Protocol; Birds Canada, 2009). ARUs recorded a 2-channel stereo recording at 44100 Hz.

Recordings were transcribed using the audio editing program Audacity® (version 2.4.2; Audacity Team 2021). Audacity displays audio as spectrograms, which are visualizations of bird vocalizations. Spectrogram settings were set to a logarithmic scale to show frequencies between 1000-10,000 Hz, the window type was set to Hann and the window size was set to 1024, while the gain was 15 dB, and the range was 80 dB (Reynolds, 2020). The spectrogram colour was set to grayscale for ease of visual interpretation. These settings were chosen to best identify vocalizations 1000 Hz or higher, which is the range of most diurnal avian species (Hu & Cardoso, 2009). Birds were identified by their audible vocalizations, and when possible, confirmed visually by analyzing the species' unique spectrogram. The recordings were transcribed in 1 min intervals, and the presence of any species heard vocalizing within each interval was recorded. Vocalization identifications with low certainty were reviewed by Dr. Doug Tozer of Birds Canada. Vocalizations that were too quiet, degraded in quality, or unidentifiable as a unique song or call were omitted from analyses, but vocalizations that were possibly unique species were kept in subsequent analyses as "unknown species."

*3.2.2-c Statistical analyses*

To determine the optimal transcription duration during the dawn chorus, we created species accumulation curves (*sensu* Chao et al., 2013) to plot the cumulative number of both total species and marsh-users against survey effort, represented by transcription duration up to 2 hours. Curves were made in RStudio (RStudio Team, 2022) using the vegan package (Oksanen et al., 2019) and the ggplot package (Wickham, 2016). This process creates a species accumulation curve by calculating the cumulative richness for each minute increment of the 2-hour transcription by repeated randomizations (permutations = 999, seed = 412) that shuffled the order of the minute-long transcriptions to remove the effect of any ordering of species in terms of their preferred vocalizing times. The resulting mean cumulative richness (S-mean) is plotted against time (Oksanen et al., 2020). Because mean cumulative species (S-mean) factors out the influence of sample order (i.e., minutes transcribed) and takes an average of cumulative richness in each minute, we decided to use this value instead of observed richness (S-obs) to determine an accurate transcription duration. Curves were visually inspected to determine if and when richness plateaued for each ARU. We deemed an ARU to have an accurate estimate of total species richness and marsh-user richness if the curves plateaued within the 2-hour period. From the output of the species accumulation curve function, we determined the mean cumulative species richness (S-mean) in intervals increasing by 15 min (e.g., 15 min, 30 min, 45 min). We chose to analyze in 15 min increments to determine if and how richness estimates captured by CWS-ON's current standard operating procedure (transcribe 15 mins starting half an hour before dawn) are underestimating richness at any one ARU site. We also determined the time required to capture 80%, 85%, 90% and 95% of the mean cumulative species richness for total species and marsh-users in each recording to determine how long transcription should be to maximize richness estimates.

Additionally, we used the online SpadeR tool (Chao et al., 2019) to calculate non-parametric richness estimators to estimate the "true" richness at each site to compare to the observed richness. Non-parametric estimators use the frequency of rare species to determine true richness at a site, whereas parametric estimators rely on mathematical assumptions of species abundance distributions (Chao & Chiu, 2016). We calculated incidence-based richness estimators Chao 2, 1$^{st}$ order Jackknife (Jack 1), 2$^{nd}$ order Jackknife (Jack 2) and Incidence-based Coverage Estimator (ICE). We calculated a 95% confidence interval for Chao 2 because previous work concluded it was the optimal estimator of "true" richness with ARU-based avian monitoring in wetland environments (Reynolds, 2020). We deemed that an ARU detected an accurate estimate of species richness if the observed richness (S-obs) was at least 80% of the lowest "true" richness estimator, and if the observed richness was within the 95% confidence interval of the Chao 2 estimator (Thompson & Thompson, 2010; Reynolds, 2020).

### *3.2.3 Objective Two: Placement of transcription effort within the dawn chorus*

Once we determined how long to transcribe the 2-hour dawn chorus recording to capture accurate estimates of total species richness and marsh-user richness, we sought to determine where this effort should be allocated within the chorus. We determined when the peak in total species richness occurred within the 2-hour recording and plotted when species vocalized across the chorus to determine if there are early or late vocalizers, which would indicate if effort should be spilt across the start and end of the chorus.

*3.2.3-a Field methods*

Field methods for this objective followed the same procedure as section 3.2.2-a.

*3.2.3-b Autonomous recording unit transcription*

ARU transcription for this objective followed the same procedure as section 3.2.2-b.

*3.2.3-c Statistical analyses*

To determine when within the 2-hour dawn chorus the highest species richness occurred, we first averaged observed species richness (S-obs) across eight 15 min intervals in the 2-hour recordings (e.g., 1-15 min, 16-30 min, etc.) across the 10 ARUs. Intervals were grouped by 15 min to compare how well CWS-ON's current transcription protocol (15 min starting half an hour before dawn) is capturing a peak in avian vocalizations, if a peak exists. We used observed richness values to maintain the order of minutes transcribed to determine when within the chorus peak richness occurs. After determining that no clear pattern was seen by averaging values, we normalized the observed richness values for each ARU in each 15 min increment to the minimum richness detected by that ARU in any of the eight 15 min intervals. We then plotted the results to graphically compare the relative avian richness among time intervals for each ARU.

To determine when birds vocalize within the dawn chorus, we counted the number of minutes each species vocalized at least once within the eight 15 min intervals across the 10 ARUs. We also determined the total number of minutes each species vocalized at least once across the 1200 min (10 ARUs x 2 hours) to determine which birds were infrequent vocalizers. We classified a species that was not of conservation concern as an "infrequent vocalizer" if it had a lower total number of vocalizing incidences than the species of conservation concern with the highest number of vocalizing incidences. Species of conservation concern are considered secretive marsh birds because they do not vocalize as often as other species (Bolenbaugh et al., 2011), therefore we used their number of vocalizing incidences as a threshold of rareness.

### *3.2.4 Objective Three: Transcription effort on one day vs across the breeding season*

We sought to determine if transcribing ARUs during one longer duration recording on one day within the breeding season yields similar diversity metrics as transcribing many shorter duration recordings across the breeding season.

#### *3.2.4-a Field methods*

In the spring of 2021, we worked with CWS-ON to deploy 10 ARUs across the Big Creek and Long Point NWAs to record avian communities. Six ARUs were placed in the Big Creek NWA (Figure 3.3), and four ARUs were placed in the Squire's Ridge management unit in the Long Point NWA (Figure 3.4). Five ARUs were located in *P. australis*-dominated habitat, three in cattail (*Typha* spp.), and two in treated *P. australis* (1-year-post glyphosate-based herbicide application followed by mechanical rolling).

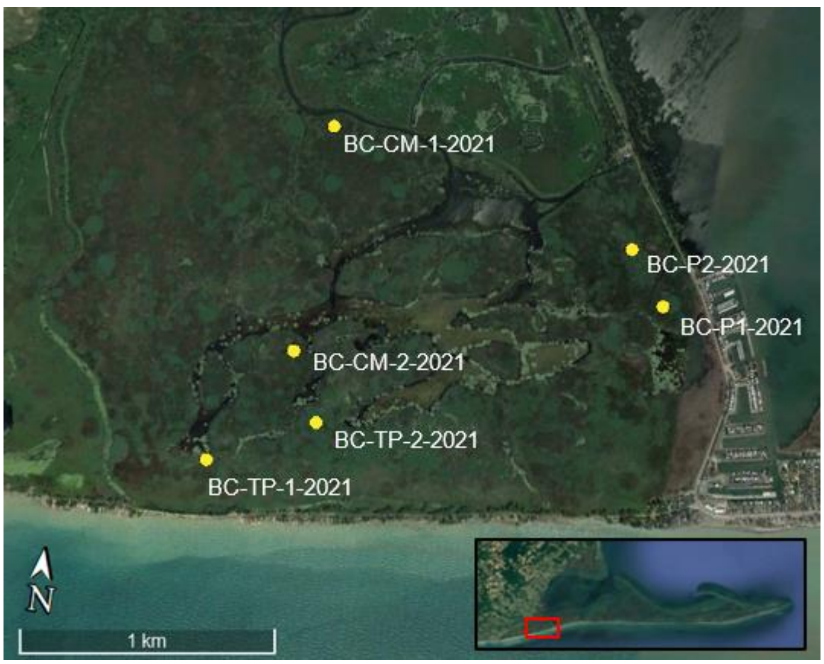


*Figure 3.3.* Locations of the six autonomous recording units deployed in the Big Creek NWA in 2021. "P" indicates *P. australis*, "TP" indicates treated *P. australis* (1-year-post glyphosate-based herbicide application followed by mechanical rolling), and "CM" indicates cattail marsh (*Typha* spp.).


*Figure 3.4.* Locations of the four autonomous recording units deployed in the Squire's Ridge management unit of the Long Point NWA in 2021. SR 4, 7, and 8 are *P. australis,* and SR 6 is cattail marsh (*Typha* spp.).

*3.2.4-b Autonomous recording unit transcription*

In consultation with Dr. Doug Tozer of Birds Canada, we defined the breeding season within Long Point, ON as May 20$^{th}$ – July 5$^{th}$ (+/- 5 days). We transcribed the 10 ARUs with two methods: 1) "one day"; 30 mins within the dawn chorus (15 mins immediately preceding 6 am, and 15 mins immediately following 7 am) on either June 17$^{th}$ or June 20$^{th}$, 2021, and 2) "season"; 1 min a day across 30 days within the breeding season (15 days transcribed at 6 am, 15 days transcribed at 7 am). Because we determined that there is variation in avian vocalizations across the dawn chorus (see section 3.3.1.4), we chose to transcribe the two times of 6 am and 7 am.

Transcription procedures (i.e., regarding suitable weather conditions, Audacity settings, etc.) followed the those outlined in section 3.2.2-b.

*3.2.4-c Statistical analyses*

First, we created species accumulation curves to determine if there was an interaction between transcription method (one-day and across-season) and vegetation type (*P. australis*, *Typha* spp., and herbicide-treated *P. australis*). Once we determined that no interaction existed, we conducted paired t-tests to determine if total species richness and the richness of marsh-users, species of conservation concern, and species at risk differed between the two transcription methods. We assessed the normality of the residuals with an Anderson-Darling test, and homogeneity of variance with a Levene's test, as well as visual inspection of plots of residual vs fitted values. T-tests were computed in RStudio v. 4.0.3 (RStudio Team, 2020).

To determine if birds had a preferred time within the season to vocalize (e.g., identify early season or late season breeders), we summed the number of days each species vocalized at least once in across the 10 ARUs from May 15$^{th}$ – July 10$^{th}$, 2021, and plotted the results.

We conducted a one-factor multivariate permutational analysis of variance (perMANOVA) using a Sorensen distance measure calculated using presence-absence data to investigate if avian community composition differed among the two transcription methods. To visualize trends in avian community composition between the transcription methods, we carried out a non-metric multidimensional scaling (NMS) ordination, using the same Sorensen dissimilarity matrix calculated from the presence-absence data. To determine optimal dimensionality, we contrasted 1– 4 dimension solutions via a Monte Carlo test method, whereby the final stress values from 50 randomized runs were compared to 50 runs with real data from random starting configurations. The runs were permitted a maximum of 200 iterations and a solution was deemed stable if the stress had a maximum standard deviation of 0.00001 over the last 10 iterations. All multivariate analyses were computed using PC-ORD v. 7 (McCune & Mefford, 2018).

We conducted an indicator species analysis (ISA) to determine if certain species were associated with one of the two transcription methods. An ISA determines the relative abundance and frequency of a species to a particular transcription method, producing an indicator value (IV) for each species for each method. IVs range from 0 (no preference for transcription method; the bird will be identified by either method) to 100 (largest preference for a transcription method; the bird will only be identified by one method). We conducted a randomized test for the sum of $IV_{Max}$, which is analogous to a perMANOVA, to determine whether there is a difference in birds captured by the two transcription methods. Analyses were computed in PC-ORD v. 7 (McCune & Mefford, 2018).

### *3.2.5 Objective Four: Detection distance of ARUs and in-person observers*

We sought to determine the maximum detection distances for vocalizations of eight marsh bird species of conservation concern, including relatively loud and quiet calls of certain species in three vegetation types: cattail (*Typha* spp.), treated *P. australis* (glyphosate-based herbicide application followed by mechanical rolling) and *P. australis.* We compared simultaneous surveys of avian broadcasts by an in-person observer and via transcription of an SM4 ARU recording.

#### *3.2.5-a Field methods*

In June 2021, we conducted detection distance surveys to coincide with the period in which ARUs were transcribed for the 2019-2021 Before-After-Control-Impact study and the 2021 space-for-time study (see Chapter Two). Surveys were conducted in the Big Creek NWA. We used Google Earth imagery to select three transects in each of the target vegetation types (Figure 3.5). These transects extended up to 550 m from the point count location through vegetation as homogenous as possible, though natural variation in vegetation community structure and stem density was unavoidable. We conducted surveys between 8:30-17:00, outside the peak of the dawn chorus and evening chorus to reduce the chance of confusing broadcasted avian vocalizations with real vocalizations. Surveys were completed in favourable weather conditions (no precipitation, good visibility, temperatures > 16°C, wind < 22 km/hr; Birds Canada, 2009).


*Figure 3.5.*Transects surveyed to determine the detection distance of an SM4 ARU and in-person observer in the Big Creek NWA. Treated *P. australis* entailed a glyphosate-based treatment followed by mechanical rolling via a Marsh Master$^{TM}$.

We retrieved marsh bird species of conservation concern vocalizations from the Marsh Monitoring Program (Birds Canada, n.d.). A total of eight species were used, with four species having two types of vocalizations (Table 3.1). All bird vocalizations were broadcast at 90 dB, and two were broadcast at 50 dB to determine if the effect of vocalization volume affects detection distance. Several studies investigating the detection distance of ARUs or in-person observers use 90 dB as the broadcast volume, citing it is within the natural range of vocalizing birds (e.g., Alldredge et al., 2007; Simons et al., 2007; Pacifici et al., 2008; Yip et al., 2017a). Vocalizations were normalized using the amplify function in Audacity® (version 2.4.2; Audacity Team, n.d) and calibrated using an R8050 dual-range handheld sound level meter 1 m from a handheld EcoXGear-EcoCarbon speaker (based on slow-time A-weighting).

*Table 3.1.* Marsh bird vocalizations that were broadcasted to determine the maximum detection distance of an SM4 ARU and in-person observer in three wetland vegetation types.

| Common Name | Scientific Name | 4-Letter Alpha Code | Vocalization(s) |
|---|---|---|---|
| American Bittern | *Botaurus lentiginosus* | AMBI | Loud (90dB) & Quiet (50dB) |
| American Coot | *Fulica americana* | AMCO | Single call type & volume |
| Common Gallinule | *Gallinula galeata* | COGA | Single call type & volume |
| King Rail | *Rallus elegans* | KIRA | “Mump” & “Kick” |
| Least Bittern | *Ixobrychus exilis* | LEBI | Loud (90dB) & Quiet (50 dB) |
| Pied-billed Grebe | *Podilymbus podiceps* | PBGR | Single call type & volume |
| Sora | *Porzana carolina* | SORA | “Kerwe” & “Whinny” |
| Virginia Rail | *Rallus limicola* | VIRA | “Kidick” & “Whacka” |

We set up an SM4 ARU at the start of a transect, with microphones 1.5 m above the water level and facing the transect with the in-person observer stationed 1 m away from it. While the in-person observer remained at the ARU, another technician progressively moved away along the transect in 50 m increments. At each 50 m interval, the technician played the 13 recordings of avian vocalizations directed at the ARU, which was continuously recording. The in-person observer (>5 years’ experience conducting wetland avian surveys) completed a survey datasheet, noting the identity of any species heard vocalizing.

Prior to each broadcast, the in-person observer used a handheld Kestrel 4000 weather meter to measure average temperature (°C), average wind speed (m/s) and maximum relative humidity (%), and a handheld sound level meter to measure ambient noise at the ARU. Ambient noise is therefore the decibel level of sound evident at the ARU location just prior to the broadcast procedure. These factors are known to impact sound attenuation and therefore may influence the detectability of avian calls by ARUs and humans (Padgham et al., 2004; Pacifici et al., 2008; Yip et al., 2017a; Morelli et al., 2022). Technicians used a handheld Garmin GPS (+/- 5 m) to navigate to each 50 m point along each transect. At each 50 m, a handheld speaker was placed at water level facing the ARU and in-person observer, because rails, coots, and grebes are known to call exclusively from this level (Cosens & Falls, 1984). We broadcasted bird vocalizations for 5 seconds, with at least 10 seconds in between, and played them in a randomized order each time. The in-person observer noted the occurrence of any vocalization that they could detect. When the in-person observer could no longer hear any bird vocalizations, technicians walking the transect would either go to the next 50 m or 100 m increment to ensure they had reached the limits of in-person detection and to provide for the possibility that the ARU might detect vocalizations at greater distances than the in-person observer.

To characterize the extent of vegetation that could interfere with the propagation of sound along the transects, we measured vegetation stem density in each of the three vegetation types. Every 8 m along a transect, we positioned a meter stick 1 m above the water's surface and recorded the number of stems in contact with the meter stick (i.e., vegetation density per 1 m).

*3.2.5-b Autonomous recording unit transcription*

ARU recordings were randomized before transcription occurred. Transcription followed the same procedure as section 3.2.2-b.

We completed a post-hoc analysis to determine if there was a difference in background noise levels on ARU recordings, specifically investigating wave-noise interference, between the three vegetation types. Background noise level was extracted from the ARU recordings within a period between broadcasted calls, which differed from ambient noise level, which was the loudness of sound in the marsh in situ before broadcasted calls measured with an R8050 dual-range handheld sound level meter. We used the plot spectrogram function on Audacity to perform a frequency analysis on the background noise clips. For each of the nine transects (three replicates/three vegetation types), one recording was randomly chosen to take a 1-3 second clip of background noise. Background noise clips were chosen where no birds vocalized (real or broadcasted) or human-made interference occurred (e.g., rustling or speaking at the ARU). The spectrograms from each transect were analyzed in ImageJ (Rasband, 1997) to determine the area under the curve. Each spectrogram had the same x and y-axis range and was saved to be the same size. In ImageJ, the scale was calibrated each time before measuring area.

*3.2.5-c Statistical analyses*

We conducted two two-factor ANOVAs (type III SS) to examine the effect of vegetation type (*Typha* spp., herbicide-treated *P. australis*, and *P. australis*), avian call type (N = 13, see Table 3.1), and their interaction on the maximum detection distance of 1) ARUs, and 2) in-person observers. We assessed the normality of the residuals with an Anderson-Darling test, and homogeneity of variance with a Levene's test, as well as visual inspection of plots of residual vs fitted values. If a main effect was statistically significant, we followed up with a Tukey's HSD post-hoc test to assess where differences among levels of the factor occurred. ANOVAs were computed in RStudio v. 4.0.3 (RStudio Team, 2020).

We conducted linear mixed-effects models with a hierarchical structure to determine if factors that are known to impact sound attenuation, including average wind speed, temperature, relative humidity, ambient decibel level, and stem contacts differed among the three vegetation types. Since we were only interested in differences between vegetation type and not within transects, we set the fixed factor as vegetation type, and the random factor as time of measurement nested in transect (e.g., average wind speed ~ vegetation + time[transect]). If a factor was significant, we completed a Tukey's HSD post-hoc test to assess where differences among levels of the factor occurred. To meet the assumptions of the test, response variables humidity and stem contacts underwent log and square root transformations, respectively. Data were analyzed in SYSTAT v 13.1 (SYSTAT, 2009).

To determine if temperature and humidity impacted the transmission of broadcasted avian vocalizations, we calculated atmospheric absorption of sound (dB/m) in each vegetation type. We used average temperature and humidity values recorded from each transect, set an average frequency of bird vocalization to 4,000 Hz, and set atmospheric pressure to 101 kPa. Many birds' frequencies range is typically between 1,000 – 8,000 Hz (Cornell Lab of Ornithology, 2009), therefore we chose an average value. Furthermore, the peak frequency of several King Rail (*Rallus elegans*) vocalizations has been determined to range from 2,000 Hz – 4,000 Hz (Schroeder & McRae, 2019).

To determine if background noise levels on ARU recordings differed among the three vegetation types, we conducted a one-factor ANOVA to determine if there was a difference in the area under the curve of spectrographs produced by ARU recordings in each vegetation type.

*3.3 Results*

*3.3.1 Optimal ARU transcription effort*

*3.3.1.1 2019 and 2021 ARU transcription results*

A total of 75 avian species, 38 marsh-users, five species of conservation concern, six species at risk, and five unknowns, were identified by transcribing both the ten 2-hour dawn chorus recordings from June 23rd, 2019, in *P. australis*-dominated habitat, and from the 10 ARUs transcribed by two methods (30 min on one day in June, and 30 mins across the breeding season) from 2021 in *P. australis*, herbicide-treated *P. australis,* and cattail habitat (Table 3.2).

*Table 3.2.* Seventy-five species identified after transcribing both the ten 2-hour dawn chorus recordings from June 23rd, 2019, in *P. australis*-dominated habitat (control), and from the 10 ARUs transcribed by two methods (30 min on one day in June and 30 mins across the breeding season) in 2021 deployed in *P. australis* (control), treated *P. australis* (1-year-post herbicide-rolling), and cattail (reference) habitat. NWA location indicated as Big Creek (BC) or Long Point (LP). Marsh-users are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

| **Common Name** | **Scientific Name** | **Vegetation** | **NWA** |
|---|---|---|---|
| *Both 2019 and 2021* | | | |
| American Bittern•* | *Botaurus lentiginosus* | Control, reference, treated | BC, LP |
| American Crow | *Corvus brachyrhynchos* | Control, reference, treated | BC, LP |
| American Goldfinch | *Spinus tristis* | Control | BC, LP |
| American Robin | *Turdus migratorius* | Control, reference, treated | BC, LP |
| Baltimore Oriole | *Icterus galbula* | Control, treated | BC, LP |
| Bank Swallow•° | *Riparia riparia* | Control, reference, treated | BC, LP |
| Barn Swallow•° | *Hirundo rustica* | Control, reference, treated | BC, LP |
| Belted Kingfisher• | *Megaceryle alcyon* | Control | BC, LP |
| Black-billed Cuckoo | *Coccyzus erythropthalmus* | Control | LP |
| Black-capped Chickadee | *Poecile atricapillus* | Control, reference | BC, LP |
| Black-crowned Night Heron• | *Nycticorax nycticorax* | Control, treated | BC, LP |
| Blue Jay | *Cyanocitta cristata* | Control, reference | BC, LP |
| Canada Goose• | *Branta canadensis* | Control, reference, treated | BC, LP |
| Chipping Sparrow | *Spizella passerina* | Control, reference | BC, LP |
| Common Gallinule•* | *Gallinula galeata* | Control, reference, treated | BC, LP |
| Common Grackle• | *Quiscalus quiscula* | Control, reference, treated | BC, LP |
| Common Yellowthroat • | *Geothlypis trichas* | Control, reference, treated | BC, LP |
| Eastern Kingbird• | *Tyrannus tyrannus* | Control, reference, treated | BC, LP |

| Common Name | Scientific Name | Vegetation | NWA |
|---|---|---|---|
| European Starling | *Sturnus vulgaris* | Control | BC |
| Gray Catbird | *Dumetella carolinensis* | Control, reference | BC, LP |
| Great Blue Heron• | *Ardea herodias* | Control, reference, treated | BC, LP |
| Great Crested Flycatcher | *Myiarchus crinitus* | Control | LP |
| Herring Gull• | *Larus argentatus* | Control, Reference | BC, LP |
| House Wren | *Troglodytes aedon* | Control, reference | BC, LP |
| Killdeer | *Charadrius vociferus* | Control, reference, treated | BC, LP |
| Least Bittern•* | *Ixobrychus exilis* | Control, reference, treated | BC, LP |
| Mallard• | *Anas platyrhynchos* | Control, reference, treated | BC, LP |
| Marsh Wren• | *Cistothorus palustris* | Control, reference, treated | BC, LP |
| Mourning Dove | *Zenaida macroura* | Control, reference, treated | BC, LP |
| Northern Cardinal | *Cardinalis cardinalis* | Control, reference, treated | BC, LP |
| Northern Flicker | *Colaptes auratus* | Control, reference | LP |
| Pied-billed Grebe•* | *Podilymbus podiceps* | Control, reference, treated | BC, LP |
| Purple Martin• | *Progne subis* | Control, reference, treated | BC, LP |
| Red-winged Blackbird• | *Agelaius phoeniceus* | Control, reference, treated | BC, LP |
| Sandhill Crane• | *Antigone canadensis* | Control, reference, treated | BC, LP |
| Swamp Sparrow • | *Melospiza georgiana* | Control, reference, treated | BC, LP |
| Tree Swallow• | *Tachycineta bicolor* | Control, reference, treated | BC, LP |
| Warbling Vireo | *Vireo gilvus* | Control, reference, treated | BC, LP |
| Wood Duck• | *Aix sponsa* | Control, reference, treated | BC, LP |
| Yellow Warbler• | *Setophaga petechia* | Control, reference, treated | BC, LP |
| Yellow-billed Cuckoo | *Coccyzus americanus* | Control, reference | BC, LP |
| *2019 only* | | | |
| Blue Winged-teal• | *Spatula discors* | Control | BC, LP |
| Blue-gray Gnatcatcher | *Polioptila caerulea* | Control | BC, LP |
| Brown Creeper | *Certhia americana* | Control | LP |
| Brown Thrasher | *Toxostoma rufum* | Control | LP |
| Brown-headed cowbird• | *Molothrus ater* | Control | BC, LP |
| Cedar Waxwing | *Bombycilla cedrorum* | Control | BC, LP |
| Chestnut-sided Warbler | *Setophaga pensylvanica* | Control | BC, LP |
| Common Loon• | *Gavia immer* | Control | LP |
| Common Tern• | *Sterna hirundo* | Control | BC |
| Eastern Towhee | *Pipilo erythrophthalmus* | Control | LP |
| Eastern Whippoorwill° | *Caprimulgus vociferus* | Control | LP |
| Field Sparrow | *Spizella pusilla* | Control | LP |
| Green Heron• | *Butorides virescens* | Control | BC, LP |
| Mute Swan• | *Cygnus olor* | Control | BC |
| Northern Rough-winged Swallow• | *Stelgidopteryx serripennis* | Control | LP |
| Red-eyed Vireo | *Vireo olivaceus* | Control | BC, LP |

| Common Name | Scientific Name | Vegetation | NWA |
|---|---|---|---|
| Song Sparrow• | *Melospiza melodia* | Control | BC, LP |
| Willow Flycatcher• | *Empidonax traillii* | Control | BC, LP |
| Wood Thrush | *Hylocichla mustelina* | Control | LP |
| *2021 only* | | | |
| Black Tern•° | *Chlidonias niger* | Control, treated | BC, LP |
| Common Nighthawk° | *Chordeiles minor* | Reference, treated | BC, LP |
| Common Raven | *Corvus corax* | Treated | BC |
| Eastern Wood-pewee° | *Contopus virens* | Control, reference | LP |
| Orchard Oriole | *Icterus spurius* | Control | BC |
| Red-bellied Woodpecker | *Melanerpes carolinus* | Control | LP |
| Ring-billed Gull• | *Larus delawarensis* | Control, reference, treated | BC |
| Sedge Wren• | *Cistothorus stellaris* | Reference | BC |
| Sora• | *Porzana Carolina* | Control | LP |
| Unknown species 1 | - | Control | LP |
| Unknown species 2 | - | Control | LP |
| Unknown species 3 | - | Control | LP |
| Unknown Species 4 | - | Control | LP |
| Virginia Rail•* | *Rallus limicola* | Control, reference | BC, LP |
| Woodpecker species | - | Control, reference | LP |

*3.3.1.2 Optimal transcription duration for capturing accurate richness estimates of all birds and marsh-users*

For the analysis of total species richness, a 2-hour survey was an ample duration to capture accurate estimates of richness. For all but one ARU, species accumulation curves plateaued within the 2-hours (Figure 3.6) and were within 80% of the Chao-2 estimated "true" richness (Appendix 2A- Table 5.6). None of the ARU observed richness values fell within the Chao 2 95% confidence interval, but 9 out of 10 ARU observed richness values fell within one species of it (Appendix 2A- Table 5.6). The outlier ARU 3656 had the highest total richness of the 10 ARUs, at 40 species. It had eight unique species that only vocalized once, which would increase the Chao-2 estimator, due to its sensitivity to the frequency of rare observations. This ARU is likely not representative of the target vegetation type because it was deployed in sparser *P. australis* than the other 9 ARUs. The eight species that vocalized only once through the recording were not rare species, except the American Bittern (*Botaurus lentiginosus*), which is a marsh bird of conservation concern typical of hemi-marsh or cattail (*Typha* spp.) habitat.

From the species accumulation curves, we determined that approximately 61-73% (mean = 67%, std = 5 %) of all species would be detected in a recording if it is transcribed for only 15 min (Appendix 2A - Table 5.7). We also determined that the duration of transcription time to capture 80%, 85%, 90% and 95% of observed species richness (S-obs) from mean species richness (S-mean) is 25-40 min, 34-53 min, 45-69 min, and 70-91 min, respectively (Appendix 2A – Table 5.8).

For the marsh-user richness analysis, a 2-hour survey was also an ample duration to capture accurate estimates of richness. For all 10 ARUs, species accumulation curves plateaued within the 2-hour survey period (Figure 3.7) and were within 80% of the Chao-2 estimated "true" richness (Appendix 2A - Table 5.9). None of the ARU observed richness fell within the Chao 2 95% confidence interval, but 9 out of 10 ARUs observed richness fell within one species of it (Appendix 2A- Table 5.9). Because a consistent plateauing trend emerged across species accumulation curves for almost all ARUs for total richness and all ARUs for marsh-user richness, we did not proceed to transcribe additional sample dates for each ARU (e.g., May or July). Notably, ARU 3656, which had the highest observed richness (S-obs) for total birds had one of the lowest observed richness for marsh-users (Appendix 2A – Figure 5.5). The highest richness of marsh-users was detected at ARUs 3647 and 3636, which had the lowest total avian richness.

From the species accumulation curves, we determined that approximately 53-75% (mean = 62.5%, std = 7%) of marsh-users would be detected from a 2-hour recording if it is transcribed for 15 min (Appendix 2A - Table 5.10). We also determined that the duration of transcription time to capture at least 80%, 85%, 90% and 95% of marsh-users in any recording is 19-55 min, 25-66 min, 34-82 min, and 58-101 min, respectively (Appendix 2A - Table 5.11).

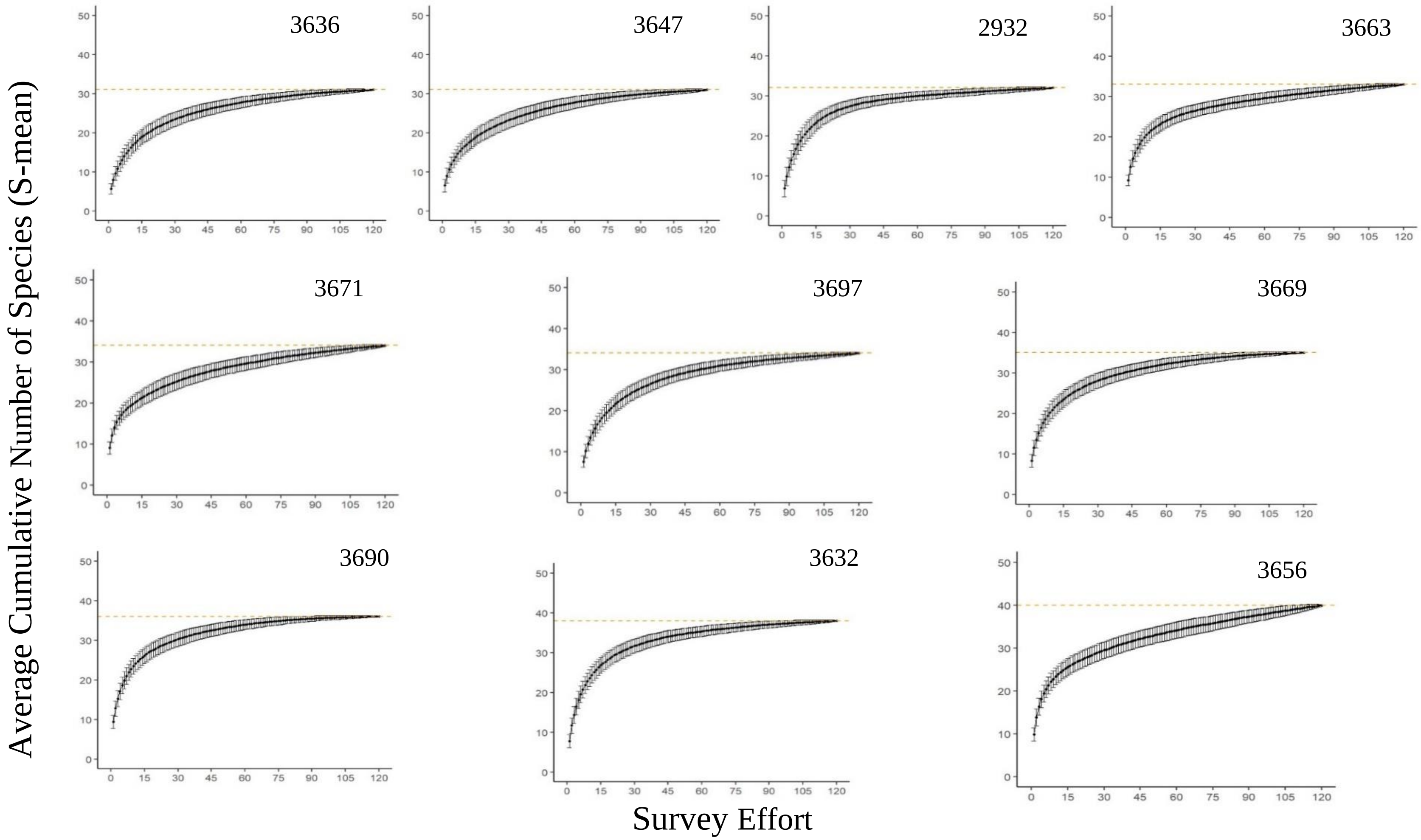


*Figure 3.6.* Total species accumulation curves for 10 autonomous recording units deployed in the Big Creek and Long Point National Wildlife Areas in 2019 (ARU unit number is indicated in the top right-hand corner). Curves show mean cumulative species richness (S-mean) plotted against survey effort (minutes transcribed), and dashed yellow lines indicate the total richness observed in each ARU. All ARUs except 3656 reached a plateau within the 120 mins (2 hour) transcription time. Curves are ordered from lowest S-mean to highest.

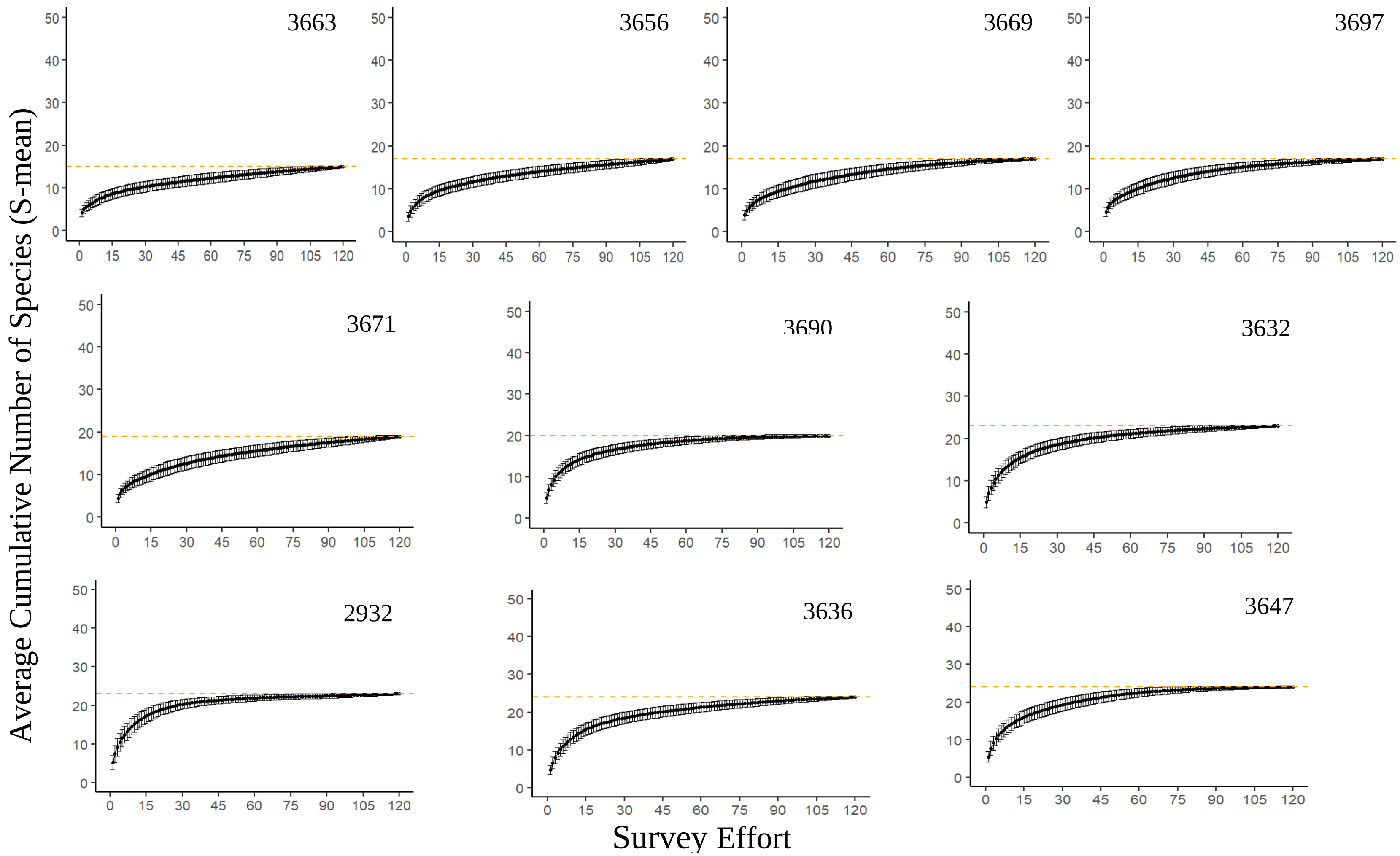


*Figure 3.7.* Marsh-user species accumulation curves for 10 autonomous recording units deployed in the Big Creek and Long Point National Wildlife Areas in 2019 (ARU unit number is indicated in the top right-hand corner). Curves show mean cumulative marsh-user species richness (S-mean) plotted against survey effort (minutes transcribed), and dashed yellow lines indicate the total richness observed in each ARU. All ARUs a plateau within the 120 mins (2-hour) transcription time. Curves are ordered from lowest S-mean to highest.

*3.3.1.3 Peak in species richness within the dawn chorus*

We normalized the observed richness values for each ARU in each 15 min increment to the minimum richness detected by that ARU in any of the eight 15 min intervals (Figure 3.8). There is no consistent trend regarding whether there are periods of higher or lower richness within the dawn chorus; no 15 min interval had a consistently greater number of species vocalizing than any other interval. If the diversity of bird vocalizations was greatest during the beginning of the dawn chorus and trailed off as the morning progressed, we would expect that each ARU's normalized total richness values should be highest within the first intervals and decline as time progressed. In contrast, if early and late vocalizers overlapped during the middle of the dawn chorus, we would expect a peak in normalized total richness values in the middle of the dawn chorus, creating a unimodal hump shape. However, we see no clear trend of when a higher or lower diversity of vocalizations occur.

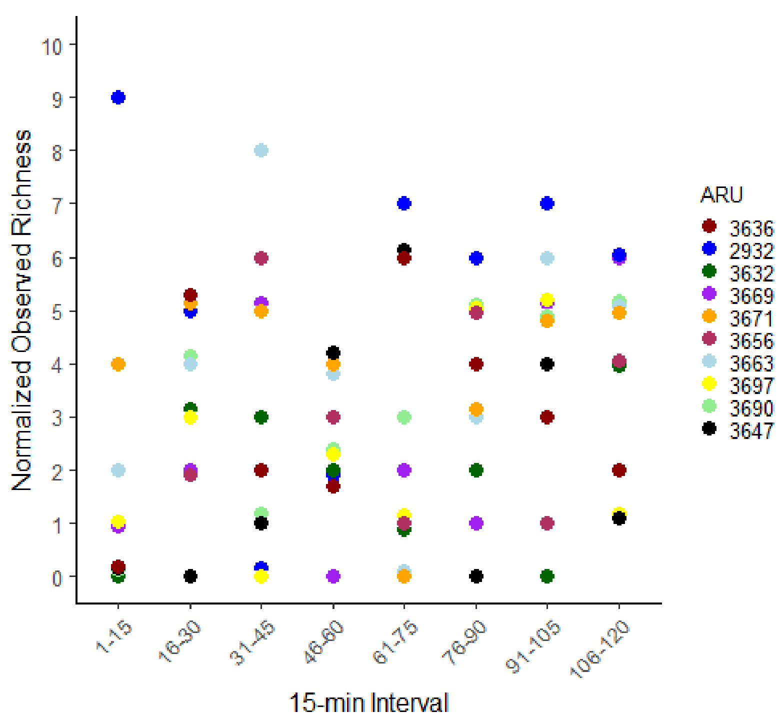


*Figure 3.8.* Normalized values of observed species richness (S-obs) in each 15 min interval for each of the 10 ARUs transcribed during the 2-hour dawn chorus. Richness was normalized for each ARU in each 15 sequential increment to the minimum richness detected by that ARU in any of the eight 15 min intervals.

*3.3.1.4 Timing of bird vocalizations within the dawn chorus*

Transcribing the 10 2-hour ARU recordings revealed that certain birds do have a preferred time to vocalize within the dawn chorus, while others either consistently, randomly, or rarely vocalize (Table 3.3). Twelve species preferred to vocalize in the early portion of the dawn chorus (i.e., within the first two or three 15 min intervals), two species preferred to vocalize in the middle of the chorus, four species preferred to vocalize at the end of the chorus (i.e., "late"), and eight species vocalized in either the early and late sections within the chorus or in the middle and late sections (Table 3.3). Seven species vocalized randomly throughout the chorus (i.e., had no pattern, but vocalized consistently), 12 species vocalized continuously (i.e., had a high frequency of vocalizations across all 15 min intervals), and 15 species rarely vocalized (i.e., had five or less occurrences per 15 min interval) (Table 3.3). These trends are visualized in Appendix 2B.

Considering the subset of all birds that are classified as marsh-users (N = 33), 14 species had a preferred window to vocalize in: eight species preferred to vocalize in the early portion of the dawn chorus, two species preferred to vocalize at the end of the chorus, and four species vocalized in either the early and late sections within the chorus or in the middle and late sections. Three species vocalized randomly throughout the chorus, six species vocalized continuously, and 10 species rarely vocalized (Table 3.3; Appendix 2B). In terms of marsh birds of conservation concern, four out of the eight were detected after transcribing the 10 ARU recordings: American Bittern, Least Bittern (*Ixobrychus exilis*), Pied-billed Grebe (*Podilymbus podiceps*), and Common Gallinule (*Gallinula galeata*). All species were heard vocalizing at least once in each of the eight 15 min intervals, except for the American Bittern, which did not vocalize in interval 91-105 mins (Appendix 2B). It appears that both the American Bittern and Pied-billed Grebe vocalize more frequently within the first hour of the dawn chorus compared to the second hour (Appendix 2B). The Common Gallinule and Least Bittern do not have as apparent trends, meaning they may not prefer a certain time within the dawn chorus to vocalize. However, they did consistently call throughout the chorus. In terms of marsh-users that are species at risk (either federally or provincially designated), the Bank Swallow (*Riparia riparia*) preferred to vocalize early within the dawn chorus and the Barn Swallow (*Hirundo rustica*) had no preferred time to vocalize (Table 3.3, Appendix 2B).

*Table 3.3.* Trends in vocalization activity by bird species detected in Long Point throughout the 2-hour dawn chorus transcription period, broken into 15 min intervals. Peak observation times (i.e., early, mid, late, rare, random, or continuous) are indicated. Ten ARUs were transcribed for 2-hours from the survey date June 23rd, 2019. Marsh-users are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

| **Bird Species Detected** | **Activity Trend** | **Peak Observation Time (minutes into recording)** |
|---|---|---|
| American Bittern•* | Early | 1-60 |
| American Crow | Random | NA |
| American Goldfinch | Mid & Late | 31-120 |
| American Robin | Continuous | NA |
| Baltimore Oriole | Mid | 61-90 |
| Bank Swallow•° | Early | 1-30 |
| Barn Swallow•° | Continuous | NA |
| Black-billed Cuckoo | Rare | NA |
| Black-capped Chickadee | Early | 1-60 |
| Black-crowned Night Heron• | Mid & Late | 61-120 |
| Belted Kingfisher• | Rare | NA |
| Blue-gray Gnatcatcher | Early | 1-30 |
| Brown-headed cowbird• | Rare | NA |
| Blue Jay | Random | NA |
| Brown Creeper | Rare | NA |
| Brown Thrasher | Continuous | NA |
| Blue Winged-teal• | Rare | NA |
| Canada Goose• | Random | NA |
| Cedar Waxwing | Mid & Late | 61-120 |
| Chestnut-sided Warbler | Early | 1-15 |
| Chipping Sparrow | Continuous | NA |
| Common Gallinule•* | Random | NA |
| Common Grackle• | Early | 31-45 |
| Common Loon• | Rare | NA |
| Common Tern• | Rare | NA |
| Common Yellowthroat • | Continuous | NA |
| Eastern Kingbird• | Early | 1-15 |
| Eastern Towhee | Random | NA |
| European Starling• | Early | 1-45 |
| Eastern Whippoorwill° | Early | 1-15 |
| Field Sparrow | Continuous | NA |
| Great Crested Flycatcher | Mid | 31-75 |
| Great Blue Heron• | Early & Late | 1-45, 91-120 |
| Gray Catbird | Mid & Late | 46-120 |

| Bird Species Detected | Activity Trend | Peak Observation Time (minutes into recording) |
|---|---|---|
| Green Heron• | Rare | NA |
| Herring Gull• | Late | 76-105 |
| House Wren | Continuous | NA |
| Killdeer | Late | 106-120 |
| Least Bittern•* | Random | NA |
| Mallard• | Rare | NA |
| Marsh Wren• | Continuous | NA |
| Mourning Dove | Continuous | NA |
| Mute Swan• | Rare | NA |
| Northern Cardinal | Mid & Late | 46-120 |
| Northern Flicker | Rare | NA |
| Northern Rough-winged Swallow• | Rare | NA |
| Pied-billed Grebe•* | Early | 1-60 |
| Purple Martin• | Early | 1-30 |
| Red-eyed Vireo | Rare | NA |
| Red-winged Blackbird• | Continuous | NA |
| Sandhill Crane• | Early & Late | 1-45, 76-120 |
| Song Sparrow• | Mid & Late | 31-120 |
| Swamp Sparrow • | Continuous | NA |
| Tree Swallow• | Early | 1-45 |
| Warbling Vireo | Random | NA |
| Willow Flycatcher• | Late | 61-120 |
| Wood Duck• | Rare | NA |
| Wood Thrush | Rare | NA |
| Yellow Warbler• | Continuous | NA |
| Yellow-billed Cuckoo | Late | 76-120 |

We determined that the frequency of vocalizations from each of the 60 detected species within dawn chorus varied considerably (Table 3.4). Out of the four species of conservation concern detected, the Least Bittern had the greatest number of total calling incidences across all ARUs. Based on our threshold for infrequent vocalizations, 36 species had fewer calling incidences than the Least Bittern (not including the three other species of conservation concern) and were therefore considered infrequent vocalizers. Of the 36 infrequent vocalizers, 21 are marsh-users (Table 3.4). The five least common species to vocalize were Black Billed-cuckoo (*Coccyzus erythropthalmus*), Mute Swan (*Cygnus olor*), Northern Flicker (*Colaptes auratus*), Red-eyed Vireo (*Vireo olivaceus*), and Wood Thrush (*Hylocichla mustelina*) (Table 3.4). The five most frequent vocalizing birds were Red-winged Blackbird (*Agelaius phoeniceus*), Mourning Dove (*Zenaida macroura*), Common Yellowthroat (*Geothlypis trichas*), Yellow Warbler (*Setophaga petechia*), and House Wren (*Troglodytes aedon*) (Table 3.4).

*Table 3.4.* The number of minutes each bird vocalized at least once across the 1200 minutes (10 ARUs deployed in *P. australis*-dominated habitat and transcribed for 2 hours each). Ordered from the species who vocalized the least (rare) to the most (common). Asterisks indicate a species of conservation concern.

| **Common Name** | **Number of Minutes at Least One Vocalization Occurred** |
|---|---|
| Black-billed Cuckoo | 1 |
| Mute Swan | 1 |
| Northern Flicker | 2 |
| Red-eyed Vireo | 2 |
| Wood Thrush | 4 |
| Common Tern | 5 |
| Green Heron | 5 |
| Northern Rough-winged Swallow | 5 |
| Brown Creeper | 10 |
| Common Loon | 10 |
| Eastern Whippoorwill | 10 |
| Mallard | 10 |
| Blue-winged Teal | 11 |
| Belted Kingfisher | 13 |
| Brown-headed Cowbird | 20 |
| Common Grackle | 20 |
| Wood Duck | 24 |
| Herring Gull | 26 |
| Killdeer | 31 |
| Bank Swallow | 34 |
| European Starling | 37 |
| Great Blue Heron | 38 |
| Baltimore Oriole | 43 |
| Warbling Vireo | 44 |
| Canada Goose | 58 |
| Pied-billed Grebe* | 59 |
| Blue Jay | 62 |
| Great Crested Flycatcher | 66 |
| Chestnut-sided Warbler | 69 |

| Common Name | Number of Minutes at Least One Vocalization Occurred |
|---|---|
| American Bittern* | 70 |
| Sandhill Crane | 79 |
| American Goldfinch | 80 |
| Willow Flycatcher | 84 |
| Common Gallinule* | 85 |
| Eastern Kingbird | 89 |
| Yellow-billed Cuckoo | 89 |
| Cedar Waxwing | 90 |
| Blue-gray Gnatcatcher | 97 |
| Least Bittern* | 104 |
| Black-capped Chickadee | 114 |
| American Crow | 118 |
| Gray Catbird | 124 |
| Barn Swallow | 133 |
| Eastern Towhee | 137 |
| Black-capped Night Heron | 158 |
| Northern Cardinal | 185 |
| Chipping Sparrow | 217 |
| Tree Swallow | 256 |
| Purple Martin | 270 |
| Song Sparrow | 334 |
| Brown Thrasher | 362 |
| American Robin | 408 |
| Field Sparrow | 450 |
| Marsh Wren | 455 |
| Swamp Sparrow | 475 |
| House Wren | 497 |
| Yellow Warbler | 673 |
| Common Yellowthroat | 676 |
| Mourning Dove | 777 |
| Red-winged Blackbird | 1197 |

*3.3.1.5 Transcription effort: one day vs across the season*

*3.3.1.5-a Species richness*

The one-day method captured a total of 45 species, including three unknowns, while the across-breeding season method captured 46 species, including two unknowns (Table 3.5).

*Table 3.5.* Species captured after transcribing 10 ARUs by two methods: 1) "one day"; within the dawn chorus on one day in June (30 mins), and 2) "season"; within the dawn chorus across the breeding season (1 min/ 30 days across mid-May to early July).

| Richness | Both Methods Combined | One Day | Season |
|---|---|---|---|
| Total species | 56 | 45 | 46 |
| Marsh-user | 31 | 24 | 29 |
| Species of conservation concern | 6 | 6 | 5 |
| Species at risk | 6 | 6 | 5 |

The one-day transcription method captured 10 unique species (including unknowns), while the across-season method captured 11 unique species (including unknowns) (Table 3.6).

*Table 3.6.* Unique species captured after transcribing 10 ARUs by two methods: 1) "one day"; within the dawn chorus on one day in June (30 mins), and 2) "season"; within the dawn chorus across the breeding season (1 min/ 30 days across mid-May to early July). ARUs were deployed in *P. australis, Typha* spp., and treated *P. australis* (1-year post-herbicide-rolling) habitat in the Big Creek and Long Point NWAs in 2021. Marsh-users are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°). Transcription method indicates which method a species was captured by.

| Common Name | Scientific Name | 4-Letter Alpha Code | Transcription Method |
|---|---|---|---|
| American Goldfinch | *Spinus tristis* | AMGO | One day |
| Chipping Sparrow | *Spizella passerina* | CHSP | One day |
| Common Nighthawk° | *Chordeiles minor* | CONI | One day |
| Common Raven | *Corvus corax* | CORA | One day |
| Orchard Oriole | *Icterus spurius* | OROR | One day |
| Red-bellied Woodpecker | *Melanerpes carolinus* | RBWO | One day |
| Sora• | *Porzana carolina* | SORA | One day |
| Unknown species 1 | - | - | One day |
| Unknown species 2 | - | - | One day |
| Unknown species 3 | - | - | One day |
| American Crow | *Corvus brachyrhynchos* | AMCR | Season |
| Belted Kingfisher• | *Megaceryle alcyon* | BEKI | Season |
| Black-billed Cuckoo | *Coccyzus erythropthalmus* | BBCU | Season |
| Black-crowned Night Heron• | *Nycticorax nycticorax* | BCNH | Season |

| Common Name | Scientific Name | 4-Letter Alpha Code | Transcription Method |
|---|---|---|---|
| European Starling• | *Sturnus vulgaris* | EUST | Season |
| Great-crested Flycatcher | *Myiarchus crinitus* | GCFL | Season |
| Herring Gull• | *Larus argentatus* | HERG | Season |
| Ring-billed Gull• | *Larus delawarensis* | RBGU | Season |
| Sedge Wren• | *Cistothorus stellaris* | SEDW | Season |
| Unknown Species 4 | - | - | Season |
| Woodpecker species | - | - | Season |

There are differences in species richness metrics when comparing the two transcription methods. There is a difference in total species ($p = 0.01$; Table 3.7) and marsh-user richness richness ($p = 0.01$; Table 3.7) captured by the two transcription methods. On average, the across-season method captured 3.2 more total species (std = 3.1) and 2.7 more marsh-users (std = 2.4) than the one-day method (Table 3.7). There is no difference in the richness of species of conservation concern ($p = 0.66$) or species at risk ($p = 0.11$) captured by the two transcription methods (Table 3.7).

*Table 3.7.* Paired t-test results comparing total species richness and the richness of marsh-users, species of conservation concern, and species at risk between two ARU transcription methods: 1) "one day"; within the dawn chorus on one day in June (30 mins), and 2) "season"; within the dawn chorus across the breeding season (1 min/ 30 days across mid-May to early July).

| | t | df | p | $\text{Mean}_{\text{One day}}$ ± std | $\text{Mean}_{\text{Season}}$ ± std |
|---|---|---|---|---|---|
| Total species | 3.28 | 9 | 0.01 | 18.7 ± 2.6 | 21.9 ± 3.1 |
| Marsh-user | 3.62 | 9 | 0.01 | 13.2 ± 1.4 | 15.9 ± 2.6 |
| Species of conservation concern | 0.45 | 9 | 0.66 | 3.2 ± 1.2 | 3.4 ± 0.7 |
| Species at risk | 1.77 | 9 | 0.11 | 1.6 ± 0.9 | 2.2 ± 0.8 |

*3.3.1.5-b Trends in species occurrences across the breeding season*

By visually inspecting plots of species detection over the breeding season, we determined that few birds have a preference for vocalizing during a specific section of the breeding season (i.e., early, middle or late), and that a majority either vocalize consistently or rarely throughout the season (Appendix 2C). An unidentified Woodpecker species preferred to vocalize early in the season, the Common Gallinule preferred to vocalize early to mid season, and the House Wren, Eastern Wood-pewee (*Contopus virens*), Mallard (*Anas platyrhynchos*), and Purple Martin (*Progne subis*) preferred to vocalize mid to late season. Notably, the American Bittern and Canada Goose (*Branta canadensis*) preferred to vocalize during early to mid season, as they had a large number of detections from mid-May to mid-June but experienced a large decline from mid-June to early July. Nine species vocalized consistently across the breeding season, including American Robin (*Turdus migratorius*), Common Grackle (*Quiscalus quiscula*), Common Yellowthroat, Least Bittern, Mourning Dove, Red-winged Blackbird, Swamp Sparrow (*Melospiza georgiana*), Sandhill Crane (*Antigone canadensis*), and Warbling Vireo (*Vireo gilvus*). The remaining 31 species infrequently vocalized across the breeding season. Although the across-season method captured 11 unique species that the one-day method did not, all of such species infrequently vocalized across the season and did not appear to have a preferred section to vocalize in (Appendix 2C).

*3.3.1.5-c Community composition and indicator species analysis*

Although total richness and marsh user richness were significantly higher with the across-season transcription method, the one-way perMANOVA revealed that avian community composition does not differ between the two transcription methods ($F_{1,19} = 1.70$, $p = 0.13$).

To visualize the trends in avian community composition between transcription methods, we carried out an NMS ordination on the avian occurrence dataset. The optimal NMS ordination of community composition between the two transcription methods had two dimensions ($p = 0.02$), with a final instability < 0.00001 and a final stress value of 14.11 after 64 iterations. Axis 1 explained a total of 72.4% of the variance in community composition and axis 2 explained 12.0%. Correlations of species vectors can be found in Appendix 2D – Table 5.12.

Axis 1 – the axis that explains the greatest amount of variation – reflects differentiation between the bird community using the Big Creek NWA and the bird community using the Long Point NWA (Squire's Ridge management unit). The Big Creek ARUs group together at high axis 1 scores, while the Long Point ARUs group together at low axis 1 scores (Figure 3.9). Marsh-users such as Common Gallinule, Mallard, and Canada Goose occurred more frequently in Big Creek NWA, whereas terrestrial species like Eastern Wood-pewee (*Contopus virens*), Warbling Vireo (*Vireo gilvus*), and House Wren occurred more frequently in the Long Point NWA (Figure 3.9).

Axis 2 demonstrates some discrimination between the two transcription methods: the avian community observed by the one-day dawn chorus transcription method is positioned at high axis 2 scores, whereas the avian community observed by the across-season method is positioned at low axis 2 scores (Figure 3.9). The two transcription methods seem to be more comparable in Big Creek NWA, as there is some overlap between avian community composition, whereas the community composition captured by the two methods in the Long Point NWA is spread farther apart on both axis 1 and 2 (Figure 3.9).

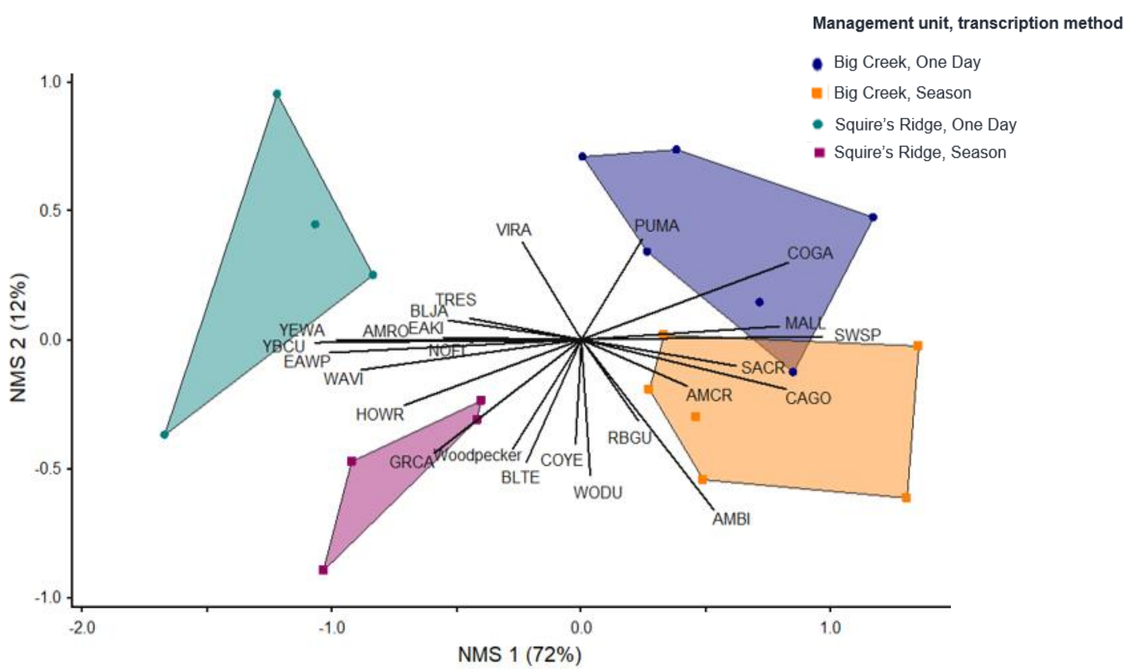


*Figure 3.9.* NMS ordination solution of bird community between two ARU transcription methods: 1) "one day" - within the dawn chorus on one day in June (30 mins), and 2) "season" - within the dawn chorus across the breeding season (1 min/ day across 30 days between mid-May to early July). ARUs were deployed in the Big Creek NWA (Big Creek management unit) and Long Point NWA (Squire's Ridge management unit). Bird species are represented by the four-letter American Ornithologist Union alpha codes (see Table 3.5 for corresponding species names).

The indicator species analysis's randomization test for the sum of IVmax revealed that there is some discrimination between species captured by the two transcription types, but it is at the margin of statistical significance ($p = 0.09$). Out of the 51 species detected (excluding unknown species), two birds are statistically significantly associated with one of the two transcription methods. The American Bittern ($p = 0.01$) and American Crow (*Corvus brachyrhynchos*) ($p = 0.03$) are statistically significant indicators of the across-season transcription method (Table 3.8).

*Table 3.8.* Indicator species analysis results comparing species occurrence between two ARU transcription methods: 1) "one day"; within the dawn chorus on one day in June (30 mins), and 2) "season"; within the dawn chorus across the breeding season (1 min/ 30 days across mid-May to early July). "Transcription method" column indicates which method each bird was more associated with. Results are ordered from smallest to largest p-values. Marsh-users are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

| **Common Name** | **4-Letter Alpha Code** | **Transcription Method** | **P-value** | **Max IV** |
|---|---|---|---|---|
| American Bittern•* | AMBI | Season | 0.01 | 71.4 |
| American Crow | AMCR | Season | 0.03 | 50.0 |
| Wood Duck• | WODU | Season | 0.06 | 62.3 |
| Canada Goose• | CAGO | Season | 0.09 | 62.5 |
| Ring-billed Gull• | RBGU | Season | 0.09 | 40.0 |
| Virginia Rail•* | VIRA | One day | 0.14 | 41.7 |
| Sandhill Crane• | SACR | Season | 0.22 | 58.8 |
| Barn Swallow•° | BARS | Season | 0.30 | 32.0 |
| Common Nighthawk° | CONI | One day | 0.46 | 20.0 |
| Tree Swallow• | TRES | One day | 0.46 | 55.6 |
| Black-billed Cuckoo | BBCU | Season | 0.47 | 20.0 |
| Herring Gull• | HERG | Season | 0.48 | 20.0 |
| Least Bittern•*° | LEBI | Season | 0.48 | 55.6 |
| Purple Martin• | PUMA | One day | 0.48 | 55.6 |
| Bank Swallow•° | BANS | Season | 0.57 | 22.5 |
| Common Yellowthroat• | COYE | Season | 0.58 | 50.6 |
| Common Gallinule•* | COGA | One day | 0.61 | 45.7 |
| Common Grackle• | COGR | Season | 0.64 | 45.7 |
| Eastern Kingbird• | EAKI | One day | 0.64 | 26.7 |
| American Goldfinch | AMGO | One day | 1.00 | 10.0 |

| Common Name | 4-Letter Alpha Code | Transcription Method | P-value | Max IV |
|---|---|---|---|---|
| American Robin | AMRO | One day | 1.00 | 30.0 |
| Baltimore Oriole | BAOR | One day | 1.00 | 10.0 |
| Belted Kingfisher• | BEKI | Season | 1.00 | 10.0 |
| Black Tern•° | BLTE | Season | 1.00 | 13.3 |
| Black-capped Chickadee | BCCH | One day | 1.00 | 10.0 |
| Black-crowned Night Heron• | BCNH | Season | 1.00 | 10.0 |
| Blue Jay | BLJA | One day | 1.00 | 22.9 |
| Chipping Sparrow | CHSP | One day | 1.00 | 10.0 |
| Common Raven | CORA | One day | 1.00 | 10.0 |
| Eastern Wood-pewee° | EAWP | One day | 1.00 | 15.0 |
| European Starling• | EUST | Season | 1.00 | 10.0 |
| Gray Catbird | GRCA | Season | 1.00 | 13.3 |
| Great Blue Heron• | GBHE | One day | 1.00 | 20.0 |
| Great-crested Flycatcher | GCFL | Season | 1.00 | 10.0 |
| House Wren | HOWR | One day | 1.00 | 10.0 |
| Killdeer• | KILL | One day | 1.00 | 18.0 |
| Mallard• | MALL | Season | 1.00 | 32.7 |
| Marsh Wren• | MAWR | One day | 1.00 | 50.0 |
| Mourning Dove | MODO | One day | 1.00 | 50.0 |
| Northern Cardinal | NOCA | One day | 1.00 | 20.0 |
| Northern Flicker | NOFL | One day | 1.00 | 13.3 |
| Orchard Oriole | OROR | One day | 1.00 | 10.0 |
| Pied-billed Grebe•* | PBGR | Season | 1.00 | 37.7 |
| Red-bellied Woodpecker | RBWO | One day | 1.00 | 5.00 |
| Red-winged Blackbird• | RWBL | One day | 1.00 | 52.6 |
| Sedge Wren• | SEDW | Season | 1.00 | 10.0 |
| Sora• | SORA | One day | 1.00 | 10.0 |
| Swamp Sparrow• | SWSP | One day | 1.00 | 27.8 |
| Warbling Vireo | WAVI | One day | 1.00 | 20.0 |
| Yellow Warbler• | YEWA | Season | 1.00 | 37.7 |
| Yellow-billed Cuckoo | YBCU | One day | 1.00 | 18.0 |

### *3.3.2 Detection distance of ARUs and in-person observers*

#### *3.3.2-a Detection distance*

The maximum detection distance of an SM4 ARU differed by vegetation type ($F_{2,78}$ = 7.76, p = 0.01) and by call type ($F_{12,78}$ = 28.74, p = 0.00) (Table 3.9). Tukey's HSD post-hoc test revealed that ARUs can detect avian calls significantly farther in cattail (*Typha* spp.) compared to herbicide-treated *P. australis* (p = 0.02), and significantly farther in *P. australis* compared to treated *P. australis* (p = 0.01; Table 3.10). On average, ARUs can detect birds 38.46 m farther in cattail than in treated *P. australis*, and 53.85 m farther in *P. australis* than in treated *P. australis.*

*Table 3.9.* Two-factor ANOVA results comparing maximum detection distance of SM4 ARUs among vegetation type (cattail (*Typha* spp.), treated *P. australis* (herbicide-rolling), and *P. australis*) avian call type (see Table 3.1), and their interaction. Call type was included to statistically control for differences among observed distances.

| | **F** | **df** | **p** |
|---|---|---|---|
| Vegetation | 7.76 | 2,78 | 0.01 |
| Call type | 28.74 | 12,78 | < 0.01 |
| Vegetation x Call type | 0.91 | 24,78 | 0.59 |

*Table 3.10.* Tukey's HSD post-hoc results comparing maximum detection distances for an SM4 ARU in three vegetation types (cattail (*Typha* spp.), treated *P. australis* (herbicide-rolled), and *P. australis*). Herbicide-treated *P. australis* was used as the reference parameter to estimate differences in distance.

| **Vegetation** | **Difference** | **p** |
|---|---|---|
| Cattail x *P. australis* | -15.39 | 0.52 |
| Cattail x Treated *P. australis* | 38.46 | 0.02 |
| *P. australis* x Treated *P. australis* | 53.85 | 0.01 |

On average, the greatest distance at which a vocalization was detected by an ARU in cattail, *P. australis,* and herbicide-treated *P. australis* was 383.3 m (std = 28.9), 383.3 m (std = 28.9), and 366.7 m (std = 104.1), respectively. On average, the smallest distance at which a vocalization was detected by an ARU in cattail, *P. australis*, and herbicide-treated *P. australis* was 50 m (std = 50), 16.7 m (std = 28.9), and 33.3 m (std = 28.9), respectively.

The vocalizations of American Bittern “loud”, Least Bittern “loud”, Pied-billed Grebe, Common Gallinule, and King Rail “mump” were often detected at the greatest distances by an SM4 ARU (Figure 3.10). The vocalizations of American Bittern “quiet”, Least Bittern “quiet”, Virginia Rail “whacka” and Virginia Rail “kidick” were often detected at the smallest distances (Figure 3.10). Comparing the American Bittern and Least Bittern vocalizations broadcasted at 90 dB (“loud”) and 50 dB (“quiet”), those broadcasted at 90 dB were detected at significantly farther distances (Figure 3.10).

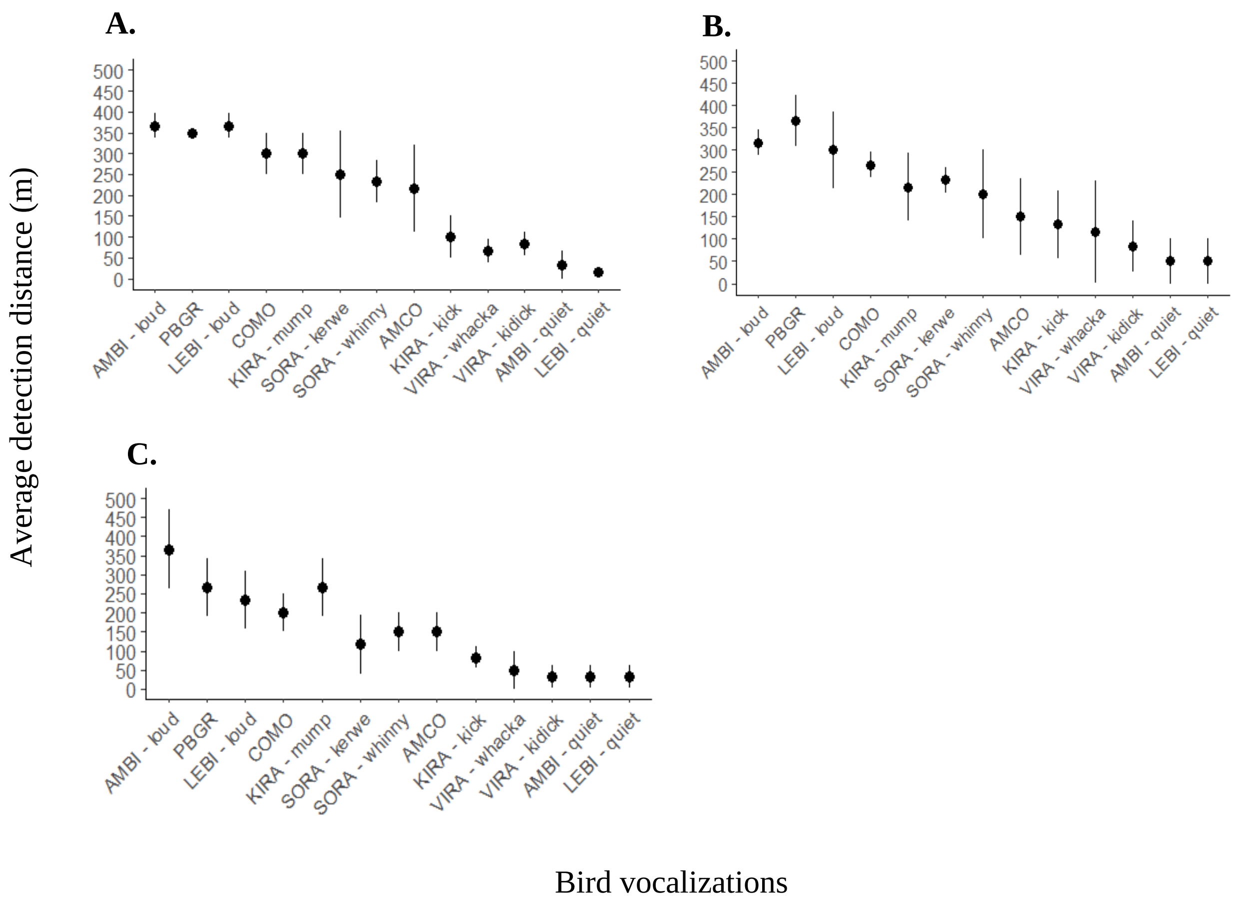


*Figure 3.10.* Average detection distances for SM4 ARUs in A) *P. australis*, B) cattail (*Typha* spp.), and C) treated *P. australis* (1 year post herbicide-rolling). Error bars represent standard deviation. Bird vocalizations are represented by the 4-letter American Ornithologist’s Union alpha codes, which can be found in Table 3.1.

The maximum detection distance of an in-person observer did not differ by vegetation type ($F_{2,78} = 1.76$, $p = 0.18$), but did differ by call type ($F_{12,78} = 30.52$, $p = 0.00$) (Table 3.11). On average, the greatest distance at which a vocalization was detected by an in-person observer in the three vegetation types was 416 m (std = 43.3). On average, the smallest distance at which a vocalization was detected by an in-person observer was 22.2 m (std = 26.4).

*Table 3.11.* Two-factor ANOVA results comparing maximum detection distance of an in-person observer among vegetation type (cattail (*Typha* spp.), treated *P. australis* (herbicide-rolling), and *P. australis*), avian call type (see Table 3.1), and their interaction. Call type was included to statistically control for differences among observed distances.

| | **F** | **df** | **p** |
|---|---|---|---|
| Vegetation | 1.76 | 2,78 | 0.18 |
| Call type | 30.52 | 12,78 | < 0.01 |
| Vegetation x Call type | 1.35 | 24,78 | 0.16 |

Similar to ARU detections, vocalizations of the American Bittern "loud", Pied-billed Grebe, King Rail "mump", and Common Gallinule were often detected at the greatest distances by an in-person observer (Figure 3.11). The vocalizations of the American Bittern "quiet" and Least Bittern "quiet" (50 dB) were often detected at the smallest distances and were detected at significantly shorter distances than "loud" bittern broadcasts (90 dB).

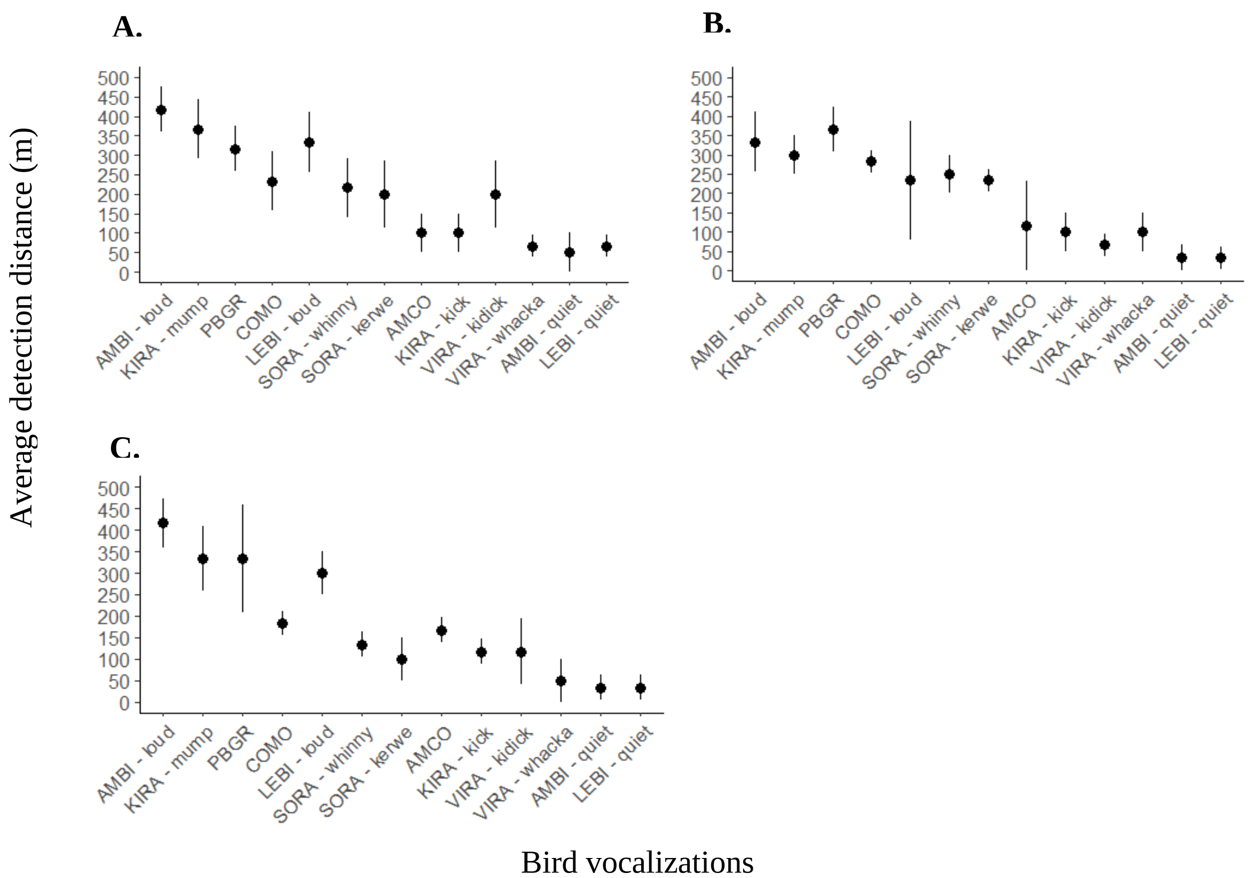


*Figure 3.11.* Average detection distances for in-person observers in A) *P. australis*, B) cattail (*Typha* spp.), and C) treated *P. australis* (1 year post herbicide-rolling). Error bars represent standard deviation. Bird vocalizations are represented by the 4-letter American Ornithologist's Union alpha codes, which can be found in Table 3.1.

*3.3.2-b Linear mixed-effects models with hierarchical structure*

All covariate data known to impact sound attenuation differed among vegetation types in some way (Table 3.12). Average wind speed was significantly lower in herbicide-treated *P. australis* than in the other two vegetation types. The average ambient decibel level was significantly higher in cattail than in the other two vegetation types. The average number of stem contacts differed among the three vegetation types, with herbicide-treated *P. australis* having the lowest, and cattail having the highest (Table 3.12).

*Table 3.12.* Summary statistics and results of linear mixed-effects models of factors expected to influence sound attenuation by vegetation type, with time nested in transect set as a random factor. Significant Tukey's HSD post-hoc test results are indicated by non-overlapping superscript letters ($p < 0.05$). Humidity and stem contacts underwent log and square root transformations, respectively.

| **Variable** | **ANOVA** | | | **Estimates Within Subjects** | **Model Fit** | | **Vegetation** | | |
|---|---|---|---|---|---|---|---|---|---|
| Fixed effects | Vegetation | | | Time(transect) | Measure | | Cattail | *P. australis* | Treated *P. australis* |
| | F | df | p | Variance parameter | -2log | AIC | μ ± SE | μ ± SE | μ ± SE |
| Avg. relative humidity (%) | 43.27 | 2,46 | < 0.01 | < 0.01 | -193.12 | -189.12 | $1.82 \pm 0.01^{ab}$ | $1.83 \pm 0.01^{ab}$ | $1.70 \pm 0.01^{c}$ |
| Avg. temperature (°C) | 22.01 | 2,46 | < 0.01 | < 0.01 | 318.41 | 322.41 | $26.24 \pm 0.46^{a}$ | $24.25 \pm 0.46^{b}$ | $21.90 \pm 0.46^{c}$ |
| Avg. wind (m/s) | 11.53 | 2,46 | < 0.01 | 0.50 | 252.03 | 256.03 | $5.07 \pm 0.29^{ab}$ | $4.79 \pm 0.29^{ab}$ | $3.44 \pm 0.29^{c}$ |
| Avg. decibel level (dB) | 10.51 | 2,46 | < 0.01 | < 0.01 | 471.44 | 474.44 | $44.17 \pm 1.40^{a}$ | $36.75 \pm 1.40^{bc}$ | $35.89 \pm 1.40^{bc}$ |
| Avg. stem contacts | 189.41 | 2,298 | < 0.01 | 0.02 | 1208.05 | 1212.05 | $2.49 \pm 0.08^{a}$ | $2.11 \pm 0.08^{b}$ | $0.57 \pm 0.08^{c}$ |

Average temperature was significantly higher in cattail and significantly lower in herbicide-treated *P. australis*, while average relative humidity was significantly lower in herbicide-treated *P. australis* than in the other two vegetation types. In terms of atmospheric absorption influenced by humidity and temperature, the average value, with a bird vocalization frequency set to 4000 Hz, in herbicide-treated *P. australis*, *P. australis*, and cattail was 0.028 dB/m (std = 0.003), 0.022 dB/m (std < 0.001) and 0.023 dB/m (std = 0.001), respectively.

*3.3.2-c ARU recordings background noise analysis*

Anecdotally, we noticed more wave sound in the recordings taken in herbicide-treated *P. australis*, and so we completed a post-hoc analysis to determine if there was a difference in background noise levels that might have interfered with detecting bird from the recordings in this vegetation type. Background noise level was extracted from the ARU recordings within a period between broadcasted calls. Background noise level is not to be confused with ambient noise level, which was the loudness of sound in the marsh measured in situ before vocalizations were broadcasted. Spectrogram plots from recordings can be found in Appendix 2E. A one-way ANOVA determined that the area under the curve ($cm^2$) for spectrogram plots of background noise differed among vegetation type ($F_{2,6} = 7.39$, $p = 0.02$). Tukey's HSD post-hoc test revealed that background noise level was significantly greater in herbicide-treated *P. australis* than in cattail or *P. australis* (Table 3.13).

*Table 3.13.* Tukey's HSD post-hoc results comparing the area under the curve ($cm^2$) for spectrogram plots of background noise in ARU recordings in cattail (*Typha* spp.), treated *P. australis* (herbicide-rolled), and *P. australis*. Treated *P. australis* is used as the reference parameter to estimate area differences.

| **Vegetation** | **Difference** | **p** |
|---|---|---|
| Cattail x *P. australis* | 3.55 | 0.86 |
| Cattail x Treated *P. australis* | -20.32 | 0.05 |
| *P. australis* x Treated *P. australis* | -23.87 | 0.03 |

*3.4 Discussion*

Using ARUs to survey avian communities can increase temporal and spatial efficiency, but this can be a double-edged sword, as the large amounts of data collected can be laborious to analyze (Shonfield & Bayne, 2017). Thus, researchers must balance the effort of transcribing ARUs with the ability to capture accurate estimates of avian diversity (La & Nudds, 2016). We sought to optimize ARU transcription during the dawn chorus to accurately assess avian richness and capture species of interest in a Great Lakes coastal wetland complex in Long Point, ON. We investigated what duration of ARU recordings must be transcribed to capture accurate species richness estimates during the dawn chorus, and how this transcription effort should be allocated within the dawn chorus on a single day and across dawn choruses spread throughout the breeding season. Briefly, we found that a 45 min survey was sufficient to capture at least 80% of the "true richness" expected to be present within a 2-hour recording beginning 30 mins before dawn. If recordings were made on a single survey date in mid-June, we determined that transcription effort was best divided into three 15 min segments situated at the start and end of the 2-hour dawn chorus. This allowed us to capture species that tend to call early in the morning, such as American Bittern (*Botaurus lentiginosus*), and late in the morning, such as Willow Flycatcher (*Empidonax traillii*), while constraining total transcription effort. In contrast, we found few species exhibited a preference to vocalize early or late within the breeding season, as many species either vocalized consistently all season or rarely at all. Two birds were more likely to be captured by transcribing across the season than transcribing one survey date. The across-season method did capture more total species richness and marsh-user richness than the one-day method, but differences were minor. Lastly, we wanted to compare the maximum detection distance of ARUs and in-person observers for marsh birds of conservation concern vocalizing in different wetland vegetation types, as differences in detection distances among vegetation types may confound efforts to study the effect of vegetation type on avian diversity. Contrary to what we expected, we found that ARUs detected avian vocalizations significantly farther in *P. australis* and cattail than in treated *P. australis* habitat (glyphosate-based herbicide application followed by mechanical rolling). Whereas there was no difference in detection distance for in-person observers among the three vegetation types. We determined that ARUs may be more influenced by the presence of background noise on recordings, specifically the sound of waves, than in-person observers.

### *3.4.1 Optimal ARU transcription duration*

ARUs are commonly transcribed in short segments (5-15 min) across several days within the breeding bird season to estimate avian diversity (e.g., Farina et al., 2011; Tegeler et al., 2012; Sidie-Slettedahl et al., 2015; Frommolt, 2017; Symes et al., 2022). CWS-ON presently transcribes the first 15 min of a recording beginning 30 min before dawn and does so on one day from May to July to estimate breeding bird diversity in Great Lakes coastal wetlands. However, short surveys positioned at the start of the dawn chorus may miss daily variation in vocal activity (i.e., species vocalize later than this defined time, or miss rare species that vocalize infrequently, such as marsh birds of conservation concern), thus underestimating species richness in a particular area (La & Nudds, 2016; Shirkey et al., 2017; Darras et al., 2019).

We analyzed a 2-hour dawn chorus recording on June 23rd, 2019, from each of 10 ARUs deployed in the Big Creek and Long Point NWAs to determine what the minimum duration of transcription should be to capture at least 80% of total species richness and marsh-users expected to be present from the 2-hour recording. We anticipated that optimal transcription duration would be greater than the 15 min typically transcribed by the CWS-ON standard operating procedure, as longer duration surveys will more fully capture the true richness of birds in a given location.

Based on the plateaus in species accumulation curves for total species richness and marsh-user richness we observed at almost all ARUs (Figures 3.6, 3.7), we concluded that a 2-hour recording is a sufficient amount of time to capture an accurate estimate of total and marsh-user species richness during the dawn chorus in coastal wetlands, and that this transcription duration can be reduced. We determined that to capture 80% of total species richness and 80% of marsh-user richness from the 2-hour recording, an ARU should be transcribed for a minimum of 40 minutes and 55 minutes, respectively, ignoring any variation in time of vocalization activity by different species (Appendix 2A). To balance effort and ability to detect an accurate representation of species, we recommend that 45 minutes is an optimal transcription duration. By reviewing species accumulation curves, we determined that transcribing an extra 10 min captures 1-2 more marsh-users, which may not be worth the extra effort. This conclusion highlights that transcribing only the first 15 min of each recording of the dawn chorus is likely underestimating the true species richness for any one date across the breeding season. For our 10 recordings, only an average of 67% (std = 5 %) of the species detected from the full 2-hour recording we observed to vocalize during any 15 min interval within it.

### *3.4.2 Placement of transcription effort within the dawn chorus*

Knowing that a 45 min transcription duration should capture approximately 80% of the species expected to be present, it does not address which interval within the 2-hour dawn chorus should be the focus of this transcription effort. We anticipated that avian species richness would be the highest in at least one of the 15 min increments positioned in the middle of the 2-hour dawn chorus because this would capture both early and late vocalizing species (La & Nudds, 2016; Wheelhouse et al., 2022).

Contrary to our expectations, we concluded that on aggregate, there is no single 15 min period within the 2-hour dawn chorus that species richness peaks (Figure 3.8). Although, we did identify that certain species are more likely to call early in the dawn chorus (e.g., American Bittern), whereas others are more likely to call late (e.g., Black-crowned Night Heron (*Nycticorax nycticorax*); Appendix 2B). The consequence is a turnover in the composition of vocalizations, such that the total avian richness of vocalizing bird species is relatively consistent across the dawn chorus. This may be explained by the fact that avian species partition acoustic space to prevent overlap with vocalizations of other birds (Suzuki et al., 2012; Hart et al., 2021). Other factors such as light availability (Thomas et al., 2002; Gil & Llusia, 2020), and levels of competition from other birds (Foote et al., 2011) may also influence variation in the onset of daily vocalizations. In contrast to our results, a study determining the optimal time to survey forest bird communities in British Columbia found that the highest diversity of vocalizations occurred 30 mins into the dawn chorus (Wheelhouse et al., 2022). Therefore, it is possible that different bird communities in different habitats/climate could vary in vocalization activity during the dawn chorus, highlighting that it is important to create a transcription protocol that is habitat-specific.

If we focused only on the four marsh bird species of conservation concern heard vocalizing and seek to maximize the likelihood of detecting them if they are present at an ARU, transcribing recordings 15-30 min before dawn would be best, but this would miss several later-vocalizing species, including multiple marsh-users, that contribute importantly to total avian richness. We consequently recommend that the 45 min transcription window be split between the start and the end of the dawn chorus period, to maximize the diversity of birds caught vocalizing and minimize errors of omission in future dawn chorus surveys of wetland birds with ARUs. We recommend three 15 min windows be established: 1) the 15 min immediately preceding dawn, 2) the 15 min immediately following dawn, and 3) the 15 min running between 1h 15 min after dawn to 1h 30 min after dawn. This maximizes the number of species detected by incorporating both early and late calling periods, ensures that the total duration of transcription is adequate to capture more of the infrequent vocalizing species, and is tailored to capture our priority species.

### *3.4.3 Transcription effort on one day vs across the breeding season*

From our second objective, we determined that there is variation in bird vocalizations across the dawn chorus. Because of this, CWS-ON's method of transcribing 15 mins on one day in each month from May – July (a total of 45 minutes) may underestimate dawn chorus richness because it is always positioned to start a half-hour before dawn. However, marsh bird detectability can vary seasonally (Harms & Dinsmore, 2014; Rehm & Baldassarre, 2007), and CWS's method may better capture this variation as it surveys one date each month from May to July. We sought to determine if transcribing one survey date for a longer duration captures similar avian species richness and community composition as transcribing multiple shorter segments spread across the breeding season. We anticipated that avian diversity would be greater when transcribing one survey date for a longer duration, because a prior study demonstrated that there is relatively little change in the probability of detecting marsh birds across the breeding season in southern Ontario coastal wetlands (Bayne, 2018), whereas a longer recording on a single day would capture more of the variation in vocalization activity across the dawn chorus. The one-day method would also have the benefit of allowing ARUs to be moved around to additional survey locations, increasing the spatial replication of any monitoring program compared to a protocol that required ARUs remain in a single place to record for the entire breeding season.

We determined that there are subtle differences between the one-day transcription method and the across-season transcription method. The across-season method captured more species all together and more marsh-user species than the one-day method. In particular, American Bittern and American Crow (*Corvus brachyrhynchos*) were more likely to be detected by the across-season method. No bird species were more likely to be detected by the one-day method. When graphically analyzing the plots of species occurrences over the breeding season, few species exhibited a preferred time (i.e., early or late in the season) to vocalize in; most were infrequent or continuous vocalizers (Appendix 2C). The main exception was American Bittern, which did exhibit a large decline in occurrence come late June to early July. Bayne (2018) similarly found that of 29 marsh-user species in southern Ontario wetlands, the detection probability of American Bittern was one of two species that declined over the breeding season, specifically exhibiting a strong decline from mid-June onward. However, our one day in June survey captured American bittern at multiple ARUs. Therefore, we are confident in the one-day method's ability to capture this species of conservation concern.

The differences in the species richness metrics detected by the two methods are small; on average, the across-season method observed 3.2 more total species (std = 3.1) and 2.7 more marsh-users (std = 2.4) than the one-day method. Furthermore, there was no difference in the richness of species of conservation concern or species at risk between the two methods. In terms of community composition, there was no difference between the transcription methods. Rather, the main effect on community composition was location; bird communities are distinct in Long Point NWA and Big Creek NWA (Figure 3.9). This supports the inference that maximizing the number of locations surveyed is more important than extending the survey at each location to capture the full breeding season, as spatial variation seems greater than temporal variation in avian vocalizations. Other studies have concluded similarly. For example, a study found that species accumulation curves for forest bird communities in New Hampshire, USA, saturated at 30 species when sampling one location, but 41 species when sampling effort was distributed across 10 locations (Symes et al., 2022). It is possible that if we had a balanced design between the Big Creek NWA (N = 6) and Long Point NWA (N = 4), we would have been able to nest transcription method by location and may have seen a difference in community composition between transcription methods.

ARUs are expensive to purchase; it is $1,090 CAD for a Wildlife Acoustic's SM4 unit in 2022. By using the across-season method, a large number of ARUs are required to be purchased and remain at each site for the entirety of the breeding season. By using the one-day method, fewer ARUs are needed, as they can be rotated between sites, which may be a more cost-effective solution for increasing sample size (e.g., as advocated by Reynolds, 2020).

We conclude that the gains in the across-season protocol are not worth the additional costs. In addition to the increase in spatial replication possible if ARUs can move between sites during a single breeding season, there are other disadvantages to the across-season protocol. For example, it may be difficult to find sufficient "good weather" days across the full breeding season. We initially planned to compare 45 mins on one survey date to 45 days across the breeding season, but we had to reduce this to 30 mins on one day and 30 days across the season, because we were unable to find 45 transcribable days for each ARU. By reducing this survey time, slightly fewer total species and marsh-users are captured. For example, by transcribing a 30 min segment of the 2-hour dawn chorus, on average you would detect 79% (std = 4%) of total richness and 76% of marsh-users (std = 7%). Compared to transcribing 45 mins, on average you would detect 86% of total richness (std =3%) and 83% of marsh-users (std = 6%).

Further, as detailed in the previous section, transcribing only 1 min of the recording within the dawn chorus is likely to miss the within-chorus temporal variation that spreading transcription effort across the chorus period on a single day would capture. It is possible that transcribing three days – 15 mins on one day in May, June, and July – and placing each 15 min interval at the start, middle, and end of a 2-hour dawn chorus could reduce the need to find 45 transcribable days across the breeding season but capture both variation across the breeding season and across the dawn chorus. Future research should investigate if species richness and community composition are comparable between these different transcription methods.

Overall, we conclude there are small differences in the richness and composition of birds detected when transcribing a longer duration of the dawn chorus on one survey day in June compared to transcribing shorter segments positioned at dawn across the breeding season. When considering other aspects of using ARUs, such as the cost of the devices and sample size, it may be beneficial to use the one-day method. Based on our study, we can conclude that transcribing ARUs for 45 minutes on one day in June, split between the early and late portions of the dawn chorus, can provide a cost-effective way to adequately estimate avian species richness and capture priority species (marsh-users, species of conservation concern, and species at risk) in the coastal wetlands in Long Point, ON.

### *3.4.4 Detection distance of ARUs and in-person observers*

Studies using ARUs to survey bird communities often either ignore or roughly estimate the distance at which their ARU model can detect avian vocalizations (Tegeler et al., 2012; Leach et al., 2016), thus, ARUs often conduct unlimited distance sampling. It is important to determine the range at which ARUs can record avian vocalizations to determine if diversity estimates are accurately reflecting the habitat researchers are specifically targeting. If differences in detection distances among vegetation types exist, this may confound efforts to study the effect of vegetation type on avian diversity (Yip et al., 2017a). In contrast, in-person observers can conduct fixed-distance point counts, allowing them to more accurately estimate the distance to birds observed within the target habitat. With the increasing use of ARUs, studies often now combine data collected from both ARUs and in-person point counts (Van Wilgenburg et al., 2017; Drake et al., 2021). If researchers want to incorporate data collected by both survey methods, it is important to determine if their survey distances are comparable, or else diversity estimations will be biased by unequal sampling areas (Yip et al., 2017a).

In 2021, we completed field surveys to determine if the maximum detection distance of select marsh birds for an SM4 ARU and in-person observer were comparable in three wetland vegetation types: cattail (*Typha* spp.), treated *P. australis* (herbicide-rolled) and *P. australis*. Contrary to our predictions and previous studies, we determined that 1) ARUs detect broadcasts of marsh birds significantly farther in cattail and *P. australis* than in herbicide-treated *P. australis*, and 2) there is no difference in detection distances for in-person observers among the three vegetation types.

We anticipated that herbicide-treated *P. australis* would have the largest detection distance for both ARUs and in-person observers, as this habitat was characterized as an open area with a low density of vegetation (remaining dead *P. australis* stems), in comparison to denser and taller habitat of cattail and *P. australis*. Sound transmission can vary between open and more dense environments (Fricke, 1984; Yip et al., 2017b), because vegetation can amplify the scattering and reverberation of sound and increase attenuation (Richards & Wiley, 1980; Yang et al., 2013). Studies investigating the detection distance of birds in different vegetation types have found that detection probabilities decreased more rapidly in closed/dense vegetation than in open or less-dense vegetation (Pacifici et al., 2008; Yip et al., 2017a, b). For example, broadcasts of boreal birds were detected at greater distances in open-roadside areas and forest edges than in interior coniferous and deciduous forests (Yip et al., 2017b). Vegetated areas may also experience increased levels of ambient noise due to the influence of wind, which causes rustling vegetation that can mask bird vocalizations, especially those of lower frequency (Yip et al., 2017b). Ambient noise can be any source of noise in a given environment, which can include biophony (i.e., other birds vocalizing), geophony (e.g., rain, wind, rustling vegetation), or anthrophony (e.g., boat traffic) (Priyadarshani et al., 2018). Masking of avian vocalizations can occur when there is an overlap in the frequencies of the vocalization and the noise source (Dooling & Blumenrath, 2013), which can cause detection issues for both in-person observers and on ARU recordings. Because herbicide-treated *P. australis* had the smallest avian detection distances, we anticipated that it would have the greatest average wind speed and ambient noise levels of the three vegetation types, thus masking the broadcasted vocalizations. However, we observed the opposite; average wind speed and average ambient noise level were significantly lower in herbicide-treated *P. australis* compared to the other two vegetation types.

Upon further investigation of background noise within ARU recordings, we identified that wave noise likely impacted our ability to detect birds in the herbicide-treated *P. australis* transects. This was surprising, because ambient noise levels in these transects were not significantly higher in *P. australis* or cattail vegetation. More, the wave noise did not greatly interfere with the ability to detect avian vocalizations in person. It was only identified as a problem for detections in the ARU transcription phase, where the recorded sound of waves obscured the more distant broadcasts of avian vocalizations. We determined post-hoc that background noise on a sample of the recordings in each vegetation type was significantly higher in herbicide-treated *P. australis*. Wave noise has a low frequency (30-500 Hz) that may have overlapped recorded avian vocalizations. The herbicide-treated *P. australis* transects were likely more impacted by wave noise than the other vegetation types because they were located at the south end of the Big Creek NWA, which was separated from Lake Erie by only a few hundred meters, where even on relatively calm days the waves make much noise as they break on the barrier beach (Figure 3.5). The *P. australis* and cattail transects were either located centrally or in the north end of the NWA or surveyed on a day that had no wave interference present. Future research should consider proximity to wave action and its potential to obscure vocalizations when situating ARUs and be cognizant of the potential for wave noise to confound detection distances among vegetation types if not controlled.

In addition to background noise levels, temperature and relative humidity can influence the detectability of bird vocalizations, as both parameters influence sound attenuation (Snell-Rood, 2012). We observed that average temperature and relative humidity were significantly lower in herbicide-treated *P. australis* compared to the other vegetation types. Temperature and relative humidity, coupled with sound frequency and atmospheric pressure, can influence the atmospheric attenuation of sound in a non-linear fashion (Attenborough, 2007; Goerlitz, 2018). Many birds' frequencies range is typically between 1,000 – 8,000 Hz (Cornell Lab of Ornithology, 2009). For frequencies under 10,000 Hz, the highest attenuation (i.e., poorest sound transmission) occurs at moderate to low temperatures and moderate humidity, while the lowest attenuation occurs at high temperatures and high humidity (Griffin, 1971), which means the lower temperature and humidity we measured in the herbicide-treated locations could have also contributed to the reduced detection distances we observed for broadcasts of avian vocalizations in treated sites. To test this, we calculated atmospheric absorption of sound (dB/m) in each transect to determine if temperature and humidity also had an influence on bird detectability in ARU recordings in herbicide-treated *P. australis*. We determined that average atmospheric absorption in the three vegetation types ranged from 0.022 dB/m to 0.028 dB/m. These values are insignificant (Griffin, 1971); therefore, it is unlikely that temperature and relative humidity differences among vegetation types were accountable for differences in the ARU's detection distances.

On average, the greatest distance at which a vocalization was detected from an ARU recording made in cattail, *P. australis*, and herbicide-treated *P. australis* was 383.3 m (std = 28.9), 383.3 m (std = 28.9), and 366.7 m (std = 104.1), respectively, which is comparable to results found by a few other studies. One study determined that real vocalizations of the King Rail had a maximum detection distance of 200 m for SM4 ARUs in a wetland complex (Schroder & McRae, 2020). Another study determined that two ground dwelling birds were detected by SM4 ARUs at least 300 m away in shrubland habitat (Thomas et al., 2020). In contrast, a study using SM4 ARUs determined that the detection distance of the Northern Cardinal (*Cardinalis cardinalis*) in clear-cut, retention, and forest plots, was 75-100 m (Wheelhouse et al., 2022). However, they broadcasted the vocalization at 75 dB (our study did 90 dB), which may account for this shorter distance.

Published studies have found that the detection distances of in-person observers and ARUs are relatively comparable, but in-person observers generally detect broadcasts at farther distances (Yip et al., 2017a; Schroeder & McRae, 2020; Stewart et al., 2020). On average, the greatest distance at which a vocalization was detected by an in-person observer was 416 m (std = 43.3) which is comparable to, but much larger than, another study in a wetland environment.

Schroder & McRae (2020) determined that in-person observers could detect real vocalizations of the King Rail up to 300 m away, and that this was greater than what SM4 ARUs could detect (up to 200 m). In contrast, in an appendix to his thesis, Meyer (2003) determined that the detection distance of an in-person observer in cattail marsh and *P. australis* habitat in Long Point, ON, was 53.25 m and 45.99 m, respectively, which is considerably smaller than what we observed. Similarly, he did not specify the decibel level at which birds were broadcasted, which may account for such large differences.

It can be difficult to compare detection distance results to other studies, because aside from already important site-specific influences such as weather, vegetation, and ambient noise, there are several differences in the methodologies that can cause differences among estimates for both ARUs and in-person observers. Differences in the methods used to estimate detection distance can include either measuring the distance to real birds (e.g., Stiffler et al., 2018; Schroeder & McRae, 2020) vs. broadcasted birds (e.g., Furnas & Callas, 2015; Yip et al., 2017a); sampling different bird communities, such as forest birds (e.g., Wheelhouse et al., 2022), boreal birds (e.g., Yip et al., 2017a), or wetland birds (e.g., Stiffler et al., 2018); using different recording devices (e.g., Wildlife Acoustics SM2, SM3 or SM4 units, or Zoom recorders; Yip et al., 2017; Thomas et al., 2020); and using different broadcast levels spanning a large range (e.g., 60-105 dB) (e.g., Furnas & Callas, 2015; Drake et al., 2021; Wheelhouse et al., 2022). Using different models of ARUs can especially contribute to differing detection ranges due to different microphone sensitivity levels (Yip et al., 2017a; Turgeon et al., 2017; Thomas et al., 2020). This highlights the complexity of determining and comparing detection distances of ARUs and in-person observers, and what should be taken into account when utilizing them to survey bird communities.

We anticipated that different levels of broadcast (50 dB vs 90 dB) would be detected at substantially different distances. We determined that vocalizations broadcasted at 50 dB were detected at significantly smaller distances than those broadcasted at 90 dB. Many studies investigating detection distances of ARUs and in-person observers have used 90 dB (measured 1 m from the speaker) as the broadcast volume, stating it is considered within the range of naturally calling birds (e.g., Alldredge et al., 2007; Simons et al., 2007; Pacifici et al., 2008; Drake et al., 2016; Yip et al., 2017a). This volume has been critiqued, stating that the decibel level of bird vocalizations is variable and still generally unknown (Van Wilgenburg et al., 2017; Thomas et al., 2020). However, it has been demonstrated that the Yellow Rail (*Coturnicops noveboracensis*), a marsh bird similar in size to the Virginia Rail (*Rallus limicola*), has an average call intensity of 90 dB (Drake et al., 2016). To accurately assess the detection of all marsh birds, more research is needed to determine the decibel level of their vocalizations.

We also anticipated that different species would be detected at different distances for both survey methods. We determined that certain species, such as American Bittern and Pied-billed Grebe (*Podilymbus podiceps*), were detected at significantly farther distances than species such as Virginia Rail in the three vegetation types for both ARUs and in-person observers. Studies have similarly found that either the maximum detection distance or effective detection radius varies for species (e.g., Rosenburg & Blancher, 2005; Yip et al., 2017a; Stewart et al., 2020). Effective detection radius is defined as the distance at which the number of birds detected outside such distance is equal to the number of birds missed within the distance (Pérez-Granados & Traba, 2021). Stewart et al., (2020) determined that the effective detection radius of certain marsh birds for in-person observers was largest for Pied-billed Grebe, followed by American Bittern, Sora (*Porzana carolina*), American Coot (*Fulica americana*), then Virginia Rail, which is similar to what we found in terms of maximum detection distances. Both effective detection radius and maximum detection distance can be used to calculate the area sampled by an ARU and therefore used to determine population estimates (Rosenburg & Blancher, 2005; Yip et al., 2017a,b), but effective detection radius is often preferred because it accounts for imperfect detection (Yip et al., 2017a; Pérez-Granados & Traba, 2021). However, rather than complete such area calculations for ARUs and in-person observers, our goal was to determine if the detection distance of avian vocalizations differs in general across certain vegetation types.

Overall, we determined that in-person observers detect select marsh birds at similar distances among three wetland vegetation types, while ARUs detect marsh birds at farther distances in cattail and *P. australis* than open herbicide-treated *P. australis,* likely due to interference of wave background noise. Importantly, there was no difference in avian detection distances between cattail and invasive *P. australis* vegetation types, regardless of survey method. In the absence of background noise, it is possible that detection distances for ARUs would be comparable among vegetation types, like with the in-person observer. Detection distances may be larger for in-person observers, but they are relatively comparable to ARU distances. Future research on ARU detection distance should acknowledge the effect of wave noise on bird detectability and position transects away from sources of wave noise. Our study may have other limitations in addition to surveying with wave-interference present.

First, it was difficult to find transect replicates in homogenous patches of each vegetation type in the Big Creek NWA, therefore several transects span into non-target vegetation or open-water patches, which may have influenced detection distances. Furthermore, it is possible that our maximum detection distances could be overestimates due to the decibel level chosen (90 dB) and the fact that vocalizations were broadcasted directly toward the ARU and in-person observer. The direction of vocalization can influence sound propagation (Titze & Palaparthi, 2018), and it has been demonstrated that the probability of detecting vocalizations facing away from a recording unit declines quicker as distance increases than vocalizations facing the recording unit (Perez-Granados et al., 2019). It has also been demonstrated that wind direction can impact detection distances; ARUs positioned down wind may better capture vocalizations, whereas ARUs positioned upwind will experience more distortion on recordings due to wind masking vocalizations (Priyadarshani et al., 2018; Thomas et al., 2020). Therefore, the direction of wind can influence the detection radius of an ARU and make it inconsistent through time (Thomas et al., 2020). Future work should measure wind direction and take it into account when determining detection distances of ARUs and in-person observers.

Our results may be used to inform ARU deployment protocols. Currently, CWS-ON's protocol is to deploy ARUs at least 250 m away from each other, meaning a 125 m recording radius does not overlap with other units. One study investigating forest harvesting methods on bird communities in British Columbia deployed SM4 ARUs 250 m apart and determined that the vocalizations of real marsh birds, including American Bittern, Canada Goose (*Branta canadensis*), and Sandhill Crane (*Grus canadensis*), were simultaneously detected on at least two ARUs. These results are similar to ours, demonstrating that certain marsh bird vocalizations can be detected at distances greater than 250 m by SM4 units, indicating that SM4 ARUs should be spaced farther apart in the field. However, we recommend that the appropriate distance to space ARUs apart should depend on a project's research or monitoring objectives. For example, if the goal is to maximize the probability of detecting a rare or endangered bird, then some overlap in recording radii may be beneficial to ensure you are thoroughly surveying an area and not missing any habitat. For this objective, it may be beneficial to deploy ARUs 600 m apart to have 300 m of independent recording radii and 300 m of overlapping recording radii. In contrast, if a project is trying to collect statistically independent replicates, then ARUs should be deployed at least 900 m apart to ensure their 450 m recording radii (i.e., the largest distance a bird was detected in our study) are not overlapping other units. Furthermore, the CWS-ON protocol states that ARUs should be placed in a 50 m diameter of target vegetation. If a project's research goal is to target a particular type of habitat, then a large, homogenous patch of vegetation greater than 400 m would be required to ensure the ARU is not recording mixed habitat. This can be difficult to find in small wetlands, or in large wetlands given their often heterogonous make-up of different vegetation communities. If it is not possible to find large patches of target vegetation, researchers could be selective during recording unit transcription and only include species thought to be calling within the defined vegetation patch (e.g., 100 m of cattail) and exclude distant calling species or loud species such as owls or loons that could be vocalizing far outside the target habitat (e.g., Wheelhouse et al., 2022). These detection distance results are specific to a Wildlife Acoustics SM4 unit in a wetland environment. Seeing as detection distance can vary by recording unit due to differing microphone sensitivity levels (e.g., Turgeon et al., 2017; Thomas et al., 2020) and by vegetation type (e.g., Yip et al., 2017), it is important for a project to complete detection distance surveys if their ARU unit's detection distance has not been established in the habitat they are studying.

### *3.5 Conclusion*

Using ARUs to survey wetland bird communities is an effective tool in enabling large-scaling monitoring across hard-to-reach locations, and it is an efficient way to gather data with limited conservation dollars. We determined that transcribing ARUs for 45 min, split between the early and late portions of the dawn chorus, on one survey date in the middle of the breeding season is an effective and economical way to capture marsh breeding birds in the coastal wetlands in Long Point, ON. Further, we determined that in-person observers can detect birds farther than SM4 ARUs, but overall their detection distances are comparable. SM4 ARUs detected bird vocalizations significantly farther in cattail and *P. australis* vegetation than treated *P. australis*, but this was likely influenced by the presence of wave interference on ARU recordings, whereas there was no difference in detection distances among the three vegetation types for the in-person observer.. We recommend that ARU deployment protocols be guided by project-specific objectives rather than following standard procedures such as deploying ARUs 250 m apart.

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## *4. Conclusion and recommendations*

### *4.1 Thesis overview*

The regionally and globally important coastal wetlands in Long Point, ON are being threatened by large-scale *P. australis* invasion, which is jeopardizing the ecologically significant habitat utilized by many taxa, including those that are at risk of extinction. Wetland birds are one group of species that are being negatively impacted by *P. australis* invasion. *Phragmites australis* alters the vegetation structure and composition of the wetland by displacing favourable native vegetation that may be preferred by many wetland birds for breeding and foraging (Robichaud & Rooney, 2017, 2022b). *Phragmites australis* management is occurring in Long Point to reduce *P. australis* to less than 10% of its 2018 extent to promote the recovery of native flora and fauna. *Phragmites australis* has been treated with a glyphosate-based herbicide via aerial and ground application, followed by mechanical treatment to cut and roll standing dead litter. The long-term effects of *P. australis* suppression are expected to be beneficial for birds, yet there were few studies addressing the concern from land managers that suppression activities could cause short-term harms. We addressed this gap, using autonomous recording units (ARUs) to monitor the short-term response of birds to *P. australis* suppression. ARUs collect large amounts of data; thus, survey protocols must balance transcription effort while still capturing accurate estimates of avian diversity. Our two main goals were to 1) determine the short-term response of wetland bird communities to *P. australis* management (1-2 years post-treatment) using a Before-After-Control-Impact design and space-for-time substitution design, and 2) optimize the use of ARUs to survey wetland birds by determining the optimal transcription effort and determine how far ARUs and in-person observers can detect birds.

### *4.2 Thesis summary*

In the first chapter, we provided an overview of the values and functions of Great Lakes coastal wetlands, as well as the threats they face. We then synthesized the impacts of *P. australis* invasions on wetland bird communities, as well as *P. australis* management options, challenges, and their potential impacts on birds. We also reviewed the advantages and disadvantages of using autonomous recording units and in-person observers to survey bird communities.

In the second chapter, we examined the immediate effects of *P. australis* management on avian diversity during the dawn chorus. We completed two studies: 1) a Before-After-Control-Impact design comparing species richness and community composition in untreated control sites and 2-years post-herbicide-rolling treated sites, and 2) a space-for-time substitution design comparing species richness, community composition, and functional trait composition in treated *P. australis* (1-2 years post-treatment), untreated control sites, and uninvaded reference sites. For species richness, the BACI design determined that the interaction of year and treatment had no statistically significant effects on total avian species richness, or the richness of marsh-users, species at risk, and species of conservation concern. However, there was a marginally significant interaction effect on total avian richness ($p = 0.09$), which warrants consideration given the limited sample size attainable in this study. Since total avian richness slightly declined after treatment in the BACI experiment, and marsh-user richness did not, non-marsh affiliated birds are likely using *P. australis* habitat over the treated habitat, at least in the short term. In the space-for-time substitution, none of the four species richness metrics differed among the three vegetation types. Overall, we observed small-scale impacts of *P. australis* management on avian richness 1-2 years following management.

In terms of functional trait composition, we did not find a difference among control, reference, and herbicide-treated sites. The functional traits of birds using recently treated habitat are a nested subset of functional traits present in both reference and *P. australis*-invaded control sites. In terms of community composition, no significant interaction between year and treatment was found in the BACI design. However, due to the overwhelming influence of ARU location on community composition, we restricted the analysis to Big Creek sites only. From this, we found a significant interaction, indicating that *P. australis* management caused a change in total avian community composition and marsh-user composition over the two-year study period. For the total avian community, birds displaced by *P. australis* treatment tended to be small-bodied birds that typically use terrestrial habitats or are habitat generalists. Changes in marsh-user composition following treatment came from the displacement of a few larger-bodied and small-bodied species, but also the gain of a few large-bodied species. Notably, the provincially and federally Threatened Least Bittern occurred infrequently in herbicide-treated sites, but we expect they will use the increase in favourable open water and hemi-marsh habitat remaining after treatment as time progresses. We did not find the same difference in community composition among the three vegetation types in the 2021 space-for-time design as in the BACI experiment, but we did find similar trends regarding the use of herbicide-treated *P. australis* by birds most impacted by *P. australis* invasion. For example, waterbirds are either beginning to use the recently treated habitat due to the increase in open water and hemi-marsh arrangement, or there is potential for them to use it, and those displaced by treatment tend to be small-bodied birds, typically non-marsh affiliated. Even though we found a difference in community composition attributable to the herbicide-rolling treatment when analyzing a subset of the BACI data, but not when analyzing the less powerful space-for-time design, we contend that these results do not contradict one another. Rather, the two analyses indicate that there was a minor effect of herbicide application on the avian community composition in the years immediately following management. The effects of *P. australis* management on wetland bird communities have not had time to fully materialize, and these results do warrant continued monitoring. Long-term monitoring will be essential for determining how vegetation communities respond to treatment and how birds will continue to track these changes in their habitat.

In the third chapter, we examined how to optimize the use of ARUs for surveying wetland bird communities. We had four objectives: 1) determine the optimal transcription duration to capture accurate estimates of avian diversity during the dawn chorus, 2) determine where this effort should be allocated within the dawn chorus to capture species of interest, 3) determine if transcription effort allocated to one day within the breeding season captures similar diversity estimates as transcription effort spread across the breeding season, and 4) determine the recording range of SM4 ARUs and in-person observers in three wetland vegetation types.

We determined that transcribing ARUs for 45 minutes during the dawn chorus on one day in the middle of the breeding season (i.e., mid-June) will capture at least 80% of the species expected to be present at any ARU site. We also determined that splitting this effort between the early and late portions of the dawn chorus will capture birds that vocalize early, late, or infrequently, which captures a suite of marsh-users, species of conservation concern, and species at risk. Furthermore, transcribing one longer duration recording on one day in June captures comparable avian diversity metrics as transcribing many shorter duration segments across the breeding season, but it may be more economic to employ the one-day transcription method. In terms of detection distances, we determined that an in-person observer and SM4 ARU can detect birds at relatively comparable distances, but an in-person observer can detect birds farther in herbicide-rolled treated *P. australis*, likely due to being less impacted by wave noise interference than the ARU. The SM4 ARU detected birds significantly farther in cattail and *P. australis* habitat than treated *P. australis*, which was contrary to our predictions. The presence of wave background noise on recordings likely influenced the ability of the ARU to detect birds in this open habitat. There was no difference in detection distances among the three vegetation types for in-person observers. On average, the greatest distance an SM4 ARU and in-person observer could detect broadcasted bird vocalizations was 383 m and 416 m, respectively.

*4.3 Research implications and recommendations*

These results have important implications regarding the management of *P. australis* in coastal wetlands and the use of ARUs to survey wetland bird communities. As *P. australis* invasion continues to jeopardize the ecological integrity of Great Lakes coastal wetlands, many jurisdictions surrounding the lakes are taking steps to control such invasion. It is crucial to implement both short and long-term monitoring programs to track how wetland biota respond to management activities, as these results will inform recommendations for if and how *P. australis* treatment should proceed to reduce potential negative impacts. Recommendations for how to manage *P. australis* with minimal impacts on wetland bird communities, as well as how to optimize surveys for wetland bird communities, are summarized in Table 4.1.

*Table 4.1.* Recommendations for both *P. australis* management in coastal wetlands to reduce harm to wetland bird communities, and how to optimize survey methods for monitoring bird communities.

| **Recommendation** | **Rationale** |
|---|---|
| *Phragmites australis* management should be completed in stages, with treatment spread across different areas over time | Treating patches of *P. australis* will leave habitat refugia for birds that use (i.e., many small-bodied birds, either non-wetland affiliated birds or marsh-users). This is especially important if a wetland is small and mainly comprised of *P. australis.* Leaving some small stands of *P. australis* may contribute to habitat heterogeneity and provide vertical structure that is a habitat requirement of many birds. Spacing out treatments over a few years may allow time for |

| Recommendation | Rationale |
| --- | --- |
| | vegetation to recover and provide either foraging grounds or vegetated habitat. |
| Bird communities should be monitored for a minimum of 5 years after *P. australis* management occurs | We found minor short-term effects of herbicide application on the avian community, particularly on marsh-users or marsh birds of conservation concern. However, we did not examine long-term consequences for the avian community. Bird communities should be monitored until the vegetation has had time to equilibrate to *P. australis* management (4-6 years; Jordan & Rooney, unpublished data). It is also essential to have long-term monitoring if repeated treatments are occurring over time. Long-term monitoring will help determine if substantial positive effects of *P. australis* management materialize over time for the avian community. |
| Bird communities should be surveyed on one day within the middle of the breeding season (i.e., June) during the dawn chorus. A 45 min duration should be split into three segments: 1) the 15 min immediately preceding dawn, 2) the 15 min immediately following dawn, and 3) the 15 min running between 1h 15 min after dawn to 1h 30 min after dawn | We found that that avian diversity metrics are comparable when assigning the same amount of transcription effort to either one survey date or spreading it across many days within the breeding season. Transcribing ARUs on one survey date may be more economical, while still allowing for a large sample size. Rather than purchasing many expensive recording units and deploying them in one location all season, researchers can buy a few recording units and rotate them between sites over three to five days (+/- a few days depending on weather). Surveying in the middle of the breeding season (i.e., June) will prevent capturing non-target migrants in early May, and it occurs before a drop-off in breeding bird occurrences by July. Furthermore, if transcribing for only one day within the breeding season, our results show that 45 minutes in mid-June is an optimal duration to capture an accurate estimate of species richness, |

| Recommendation | Rationale |
| --- | --- |
| | as well as capture species of interest (marsh-users, species at risk, and marsh birds of conservation concern). Splitting this effort between the early and late portion of the dawn chorus captures early, late, and infrequent vocalizers, which may be missed by surveys that start at the same time each survey. When using ARUs in different environments (e.g., forests, mountains, etc.) it is important to tailor a transcription protocol that will capture the suite of birds you are interested in. |
| ARU sample size should be large enough to capture regional distinctness in bird communities | Due to the heterogeneous habitat across the Long Point peninsula, future surveys using ARUs in this location should consider increasing the sample size in each unique management area and habitat type to better represent the bird communities found there. We recommend a minimum of three ARUs per habitat type in each sub-management unit within the NWAs. This would allow for statistical analyses to better control for the impact of location on community composition. This recommendation is also relevant to any project that spans several different habitat types within a large geographical location. |
| ARU deployment protocols should be project specific | A common distance to separate ARUs in the field is 250 m. Our results show that Wildlife Acoustics SM4 ARUs can detect birds greater than 350 m away in different wetland vegetation types. We recommend that decisions regarding ARU deployment be project specific. For example, ARU deployment may differ if the goal is to have statistically independent ARU replicates, or if the goal is to survey rare species and ensure all habitat is being surveyed (e.g., allowing for ARUs' recording radii to overlap to ensure all |

| Recommendation | Rationale |
|---|---|
| | habitat is being captured). Furthermore, we recommend that a project complete detection distance surveys if their ARU unit's detection distance has not been established in the habitat they are studying. |
| The detection distance of SM4 ARUs and in-person observers are relatively comparable, but more research should be completed | Our results demonstrate that in-person observers are less impacted by certain background noise sources than SM4 ARUs in open environments (e.g., wave noise), and therefore have farther detection distances in these environments (treated *P. australis*). However, detection distances between these two survey methods are comparable in *P. australis* and cattail (*Typha* spp.). More research should be completed to parse the difference in detection with background noise sources between these two survey methods. |

*4.4 Future work*

There are several directions for future research. First, additional monitoring of avian communities in Long Point for at least several more years will be useful in determining the long-term response of wetland birds to *P. australis* management, which will guide future management recommendations. Second, future research could investigate the value of incorporating dusk and/or nighttime surveys for monitoring avian communities. Even though dawn is a preferred time to survey due to a high diversity of avian vocalizations (Brown & Handford, 2003; Gil & Llusia, 2020), several marsh-users, including species of conservation concern such as the King Rail, were not captured in our dawn chorus recordings, as they often vocalize at dusk or nighttime (Nadeau et al., 2008; Harms & Dinsmore, 2014; Schroeder & McRae, 2020). Research could investigate if the occurrence of such birds differs between dawn, dusk, and nighttime, which would determine if transcription effort should be split amongst the three time periods to better capture these species of interest. Third, more research is needed for determining what influences the detection distance of ARUs and in-person observers. Future work should occur without the influence of background wave noise to better parse how comparable the two survey methods are.

*4.5 Concluding remarks*

The ultimate goal of *P. australis* management is to promote the recovery of native flora and fauna. Our results demonstrate that two years post-management is too short of a timeframe to see the materialization of substantial positive effects on the avian community. However, we have reason to expect that positive effects will occur once more time has passed, and the vegetation has time to re-establish. Birds that are most impacted by *P. australis* invasion (e.g., waterbirds – including many marsh-users and species of conservation concern) will begin to benefit from the increase in open water and hemi-marsh arrangement following herbicide treatment, and our findings show an early indication that this trend is occurring. Furthermore, these results indicate that no major negative impacts on the wetland bird communities resulted from the invasive management techniques employed to control *P. australis*. There was a small decline in non-marsh affiliated bird richness within treated sites, and there was a shift in avian community composition. However, most birds displaced by treatment tended to be non-marsh affiliated birds that reside mainly in surrounding habitat. A few marsh-users were displaced from treated sites, such as the provincially and federally Threatened Least Bittern, but it is expected that the bittern will use the increase in hemi-marsh arrangement over time.

Using ARUs to survey avian communities can increase temporal and spatial efficiency, but this can be a double-edged sword, as the large amounts of data collected can be laborious to analyze. Therefore, determining how long to transcribe ARUs must balance effort and ability to collect avian diversity data of sufficient accuracy to meet monitoring and management objectives without wasting limited resources. Developing a transcription protocol that is tailored to specific management objectives or a suite of birds may be more beneficial than employing standard morning surveys (i.e., short 5-15 min surveys positioned at the same time of day). Furthermore, when using ARUs to survey bird communities, it is important to consider the ARU model's detection range to ensure it is recording the target habitat and capturing estimates of diversity that are reflective of such habitat. Despite some challenges associated with ARU transcription, noise interference, and detection radius varying by vegetation type, ARUs remain an important tool in enabling large-scale monitoring of the avian community across remote, hard-to-reach field sites and are an efficient means of gathering data with limited conservation dollars.

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## *Appendices*

*Appendix 1A.*

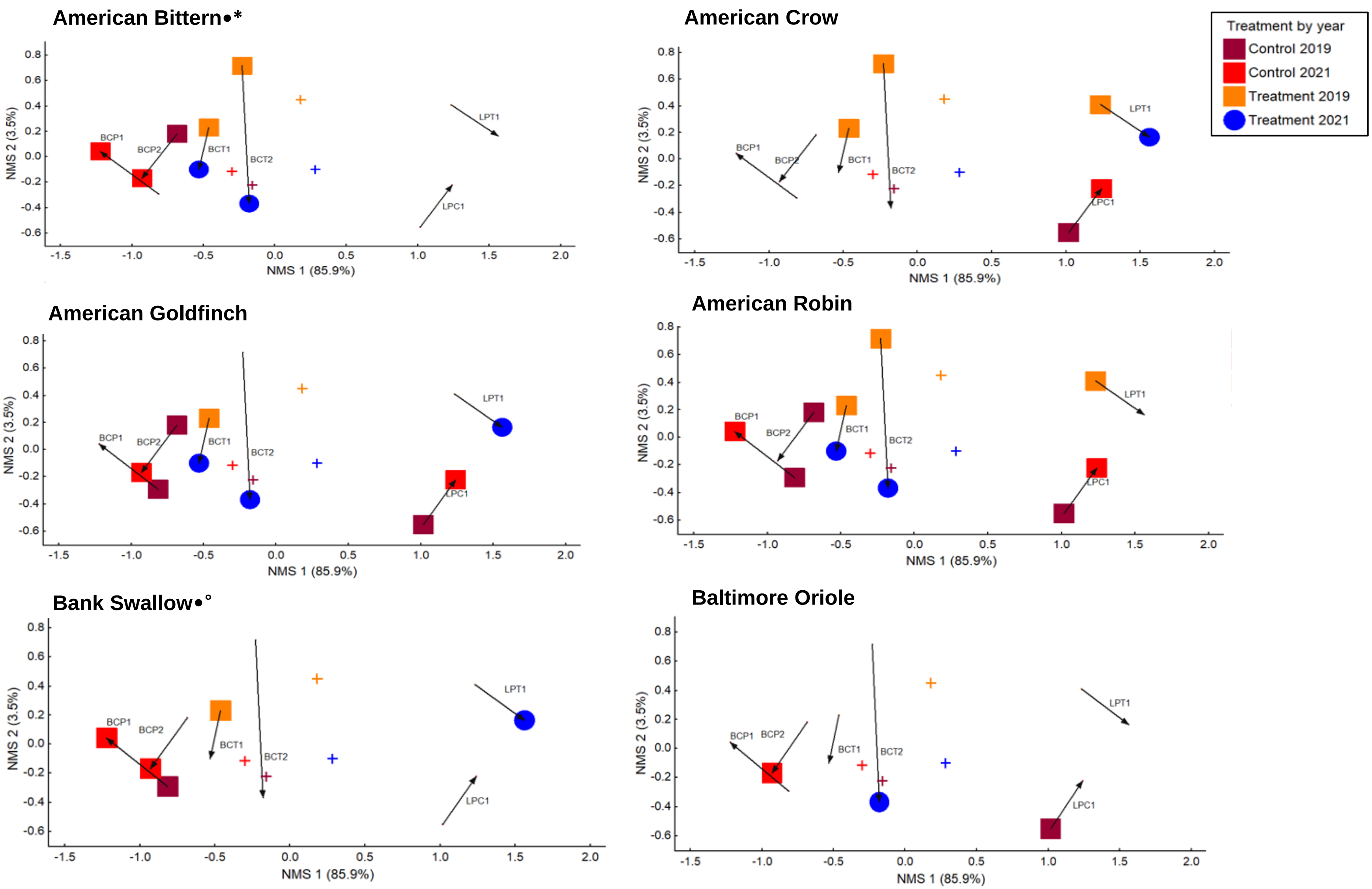
*Table 5.1*. Correlation coefficients (r) and coefficient of determination (r2) of vectors in the NMS ordination for bird community composition in control and treatment sites in the 2019-2021 BACI experiment. Species with an r2 ≥ 0.05 were considered reasonably correlated and included in Figure 2.7.

| | | **Axis 1** | | **Axis 2** | |
|---|---|---|---|---|---|
| **Common Name** | **4-letter Alpha Code** | **r** | **$r^2$** | **r** | **$r^2$** |
| American Bittern | AMBI | -0.762 | 0.581 | 0.250 | 0.062 |
| American Crow | AMCR | 0.773 | 0.597 | 0.355 | 0.126 |
| American Goldfinch | AMGO | 0.044 | 0.002 | -0.650 | 0.423 |
| American Robin | AMRO | -0.150 | 0.023 | 0.007 | 0.000 |
| Baltimore Oriole | BAOR | -0.018 | 0 | -0.616 | 0.38 |
| Bank Swallow | BANK | -0.336 | 0.113 | -0.022 | 0.000 |
| Barn Swallow | BARS | -0.666 | 0.444 | 0.041 | 0.002 |
| Belted Kingfisher | BEKI | -0.357 | 0.127 | -0.078 | 0.006 |
| Black-caped Chickadee | BCCH | 0.950 | 0.903 | -0.108 | 0.012 |
| Black-crowned Night Heron | BCNH | 0.536 | 0.287 | -0.096 | 0.009 |
| Blue Jay | BLJA | 0.095 | 0.009 | 0.505 | 0.255 |
| Blue-gray Gnatcatcher | BGGN | 0.239 | 0.057 | 0.731 | 0.535 |
| Blue-winged Teal | BWTE | -0.218 | 0.047 | 0.581 | 0.338 |
| Brown Creeper | BRCR | 0.396 | 0.157 | 0.358 | 0.128 |
| Brown Thrasher | BRTH | 0.781 | 0.61 | 0.008 | 0.000 |
| Brown-headed Cowbird | BHCO | -0.149 | 0.022 | 0.199 | 0.040 |
| Canada Goose | CAGO | -0.770 | 0.593 | 0.333 | 0.111 |
| Carolina Wren | CAWR | 0.398 | 0.158 | -0.197 | 0.039 |
| Cedar Waxwing | CEDW | 0.335 | 0.112 | 0.579 | 0.336 |
| Chestnut-sided Warbler | CSWA | 0.239 | 0.057 | 0.731 | 0.535 |
| Chipping Sparrow | CHSP | 0.743 | 0.552 | -0.196 | 0.038 |
| Common Gallinule | COGA | -0.950 | 0.903 | 0.108 | 0.012 |
| Common Grackle | COGR | -0.022 | 0 | -0.073 | 0.005 |
| Common Loon | COLO | 0.327 | 0.107 | -0.488 | 0.238 |
| Common Tern | COTE | -0.357 | 0.127 | -0.078 | 0.006 |
| Common Yellowthroat | COYE | 0.391 | 0.153 | -0.032 | 0.001 |
| Eastern Kingbird | EAKI | 0.548 | 0.301 | 0.284 | 0.081 |
| Eastern Towhee | EATO | 0.950 | 0.903 | -0.108 | 0.012 |
| Eastern Wood-pewee | EAWP | 0.501 | 0.251 | 0.143 | 0.020 |
| European Starling | EUST | -0.149 | 0.022 | 0.199 | 0.040 |

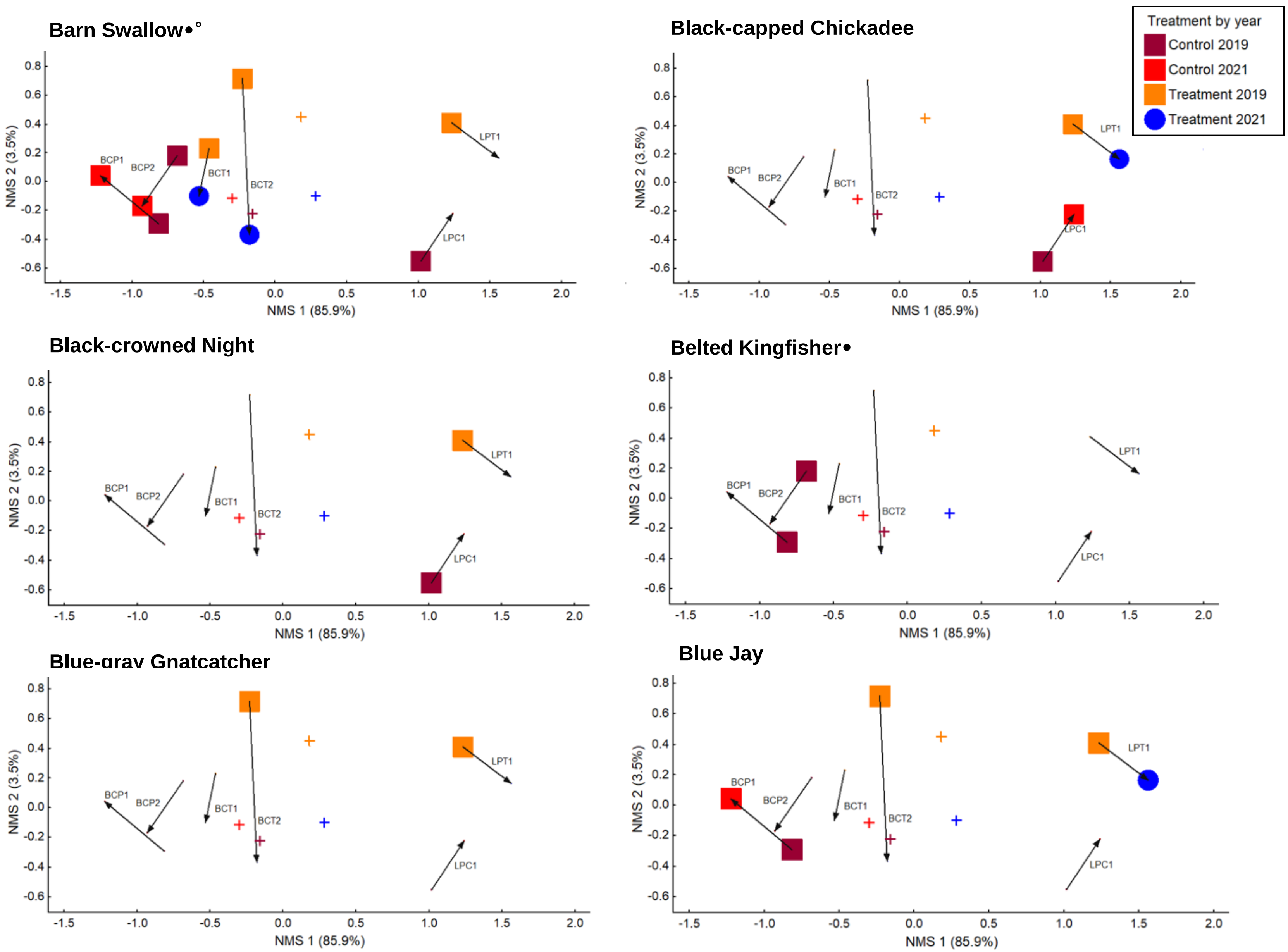
| | | Axis 1 | | Axis 2 | |
|---|---|---|---|---|---|
| **Common Name** | **4-letter Alpha Code** | **r** | **$r^2$** | **r** | **$r^2$** |
| Field Sparrow | FISP | 0.950 | 0.903 | -0.108 | 0.012 |
| Gray Catbird | GRCA | 0.065 | 0.004 | 0.729 | 0.532 |
| Great Blue Heron | GBHE | -0.427 | 0.183 | 0.173 | 0.030 |
| Great-crested Flycatcher | GCFL | 0.666 | 0.444 | -0.041 | 0.002 |
| Herring Gull | HERG | 0.396 | 0.157 | 0.358 | 0.128 |
| House Wren | HOWR | 0.452 | 0.205 | -0.064 | 0.004 |
| Indigo Bunting | INBU | -0.171 | 0.029 | -0.092 | 0.008 |
| Killdeer | KILL | -0.388 | 0.151 | -0.380 | 0.144 |
| Least Bittern | LEB | -0.658 | 0.433 | 0.250 | 0.063 |
| Mallard | MALL | 0.211 | 0.045 | -0.131 | 0.017 |
| Marsh Wren | MAWR | -0.782 | 0.612 | 0.347 | 0.120 |
| Mourning Dove | MODO | Present at every site | | | |
| Mute Swan | MUSW | -0.220 | 0.048 | 0.156 | 0.024 |
| Northern Cardinal | NOCA | 0.773 | 0.597 | 0.355 | 0.126 |
| Northern Flicker | NOFL | -0.298 | 0.089 | -0.152 | 0.023 |
| Northern Rough-winged Swallow | NRWS | 0.398 | 0.158 | -0.197 | 0.039 |
| Orchard Oriole | OROR | -0.298 | 0.089 | -0.152 | 0.023 |
| Pied-billed Grebe | PGBR | -0.720 | 0.518 | 0.426 | 0.182 |
| Purple Martin | PUMA | Present at every site | | | |
| Red-winged Blackbird | RWBL | Present at every site | | | |
| Sandhill Crane | SACR | -0.826 | 0.682 | -0.194 | 0.038 |
| Song Sparrow | SOSP | -0.121 | 0.015 | -0.223 | 0.05 |
| Swamp Sparrow | SWSP | -0.010 | 0 | 0.387 | 0.150 |
| Tree Swallow | TRES | -0.665 | 0.442 | -0.371 | 0.138 |
| Virginia Rail | VIRA | -0.391 | 0.153 | 0.032 | 0.001 |
| Warbling Vireo | WAVI | -0.097 | 0.009 | 0.224 | 0.050 |
| Willow Flycatcher | WIFL | -0.074 | 0.005 | 0.628 | 0.394 |
| Wood Duck | WODU | -0.479 | 0.229 | 0.036 | 0.001 |
| Yellow Warbler | YEWA | 0.658 | 0.433 | 0.277 | 0.077 |
| Yellow-billed Cuckoo | YBCU | 0.665 | 0.442 | 0.371 | 0.138 |

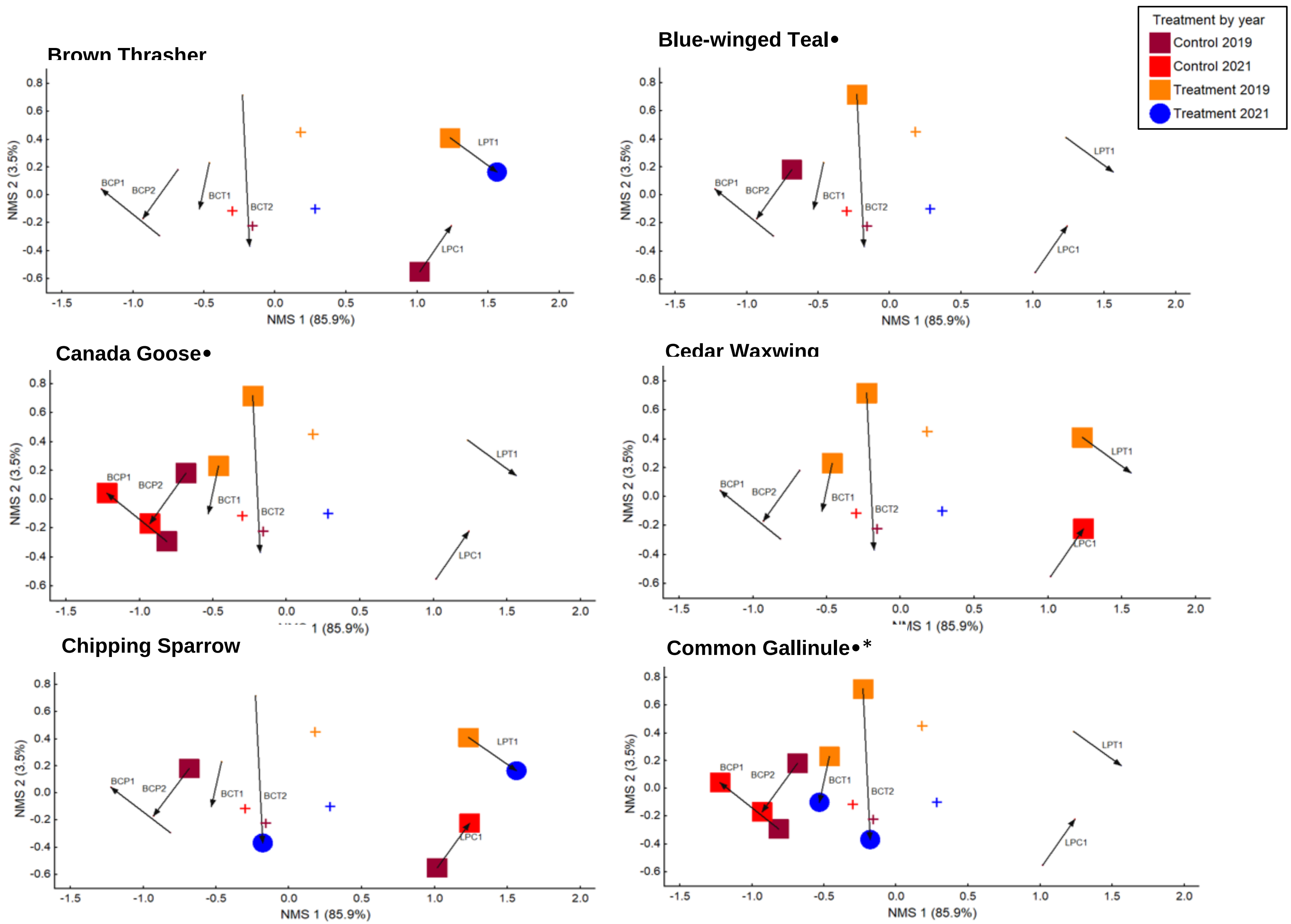
*Appendix 1B.*

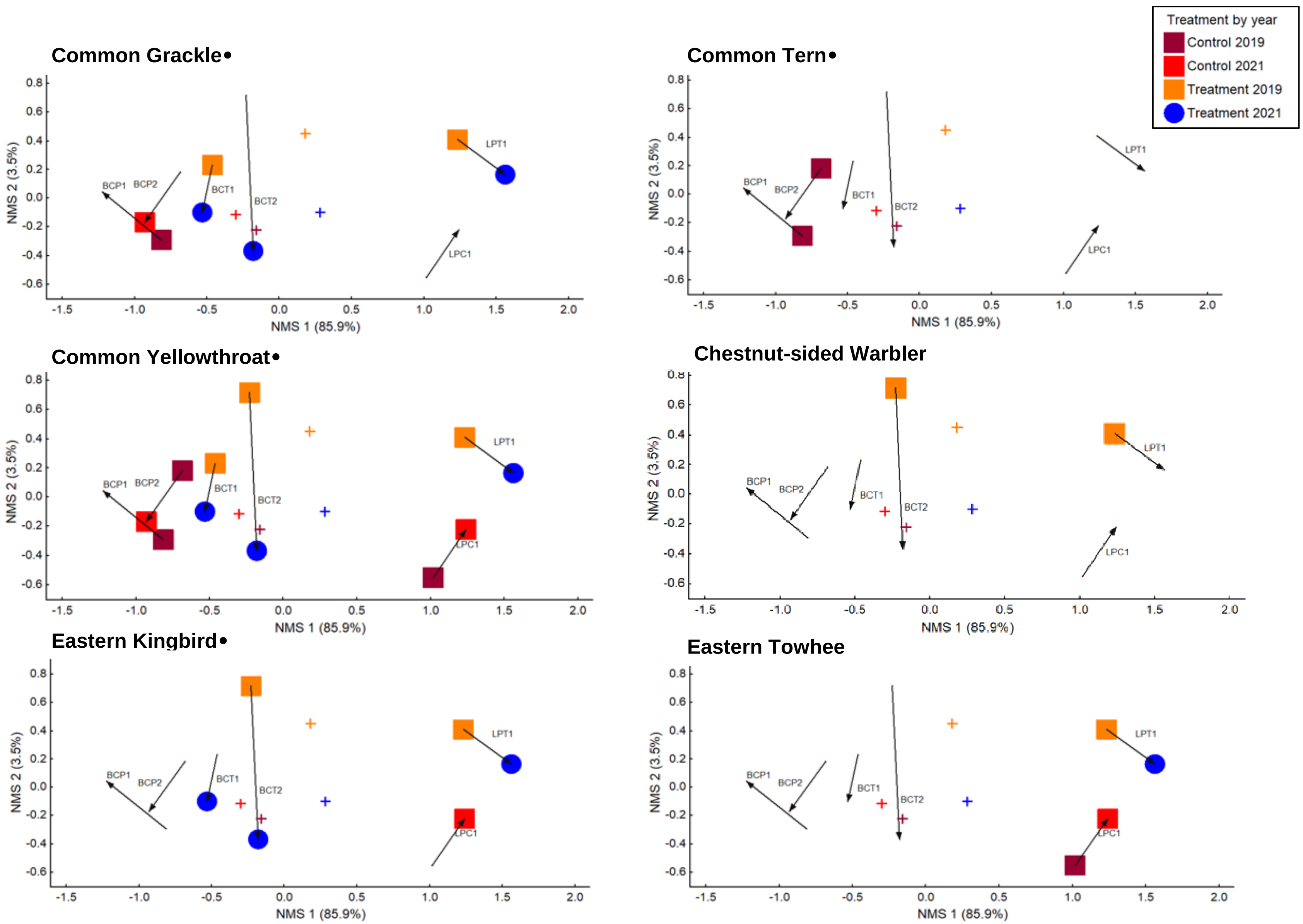
Individual species presence/absence plots in control and treatment sites between 2019 and 2021. The presence of a symbol indicates the species was present at that site-year combination. Species with only one occurrence across the two years were not plotted. Control and treatment sites were surveyed during the breeding season of 2019 prior to herbicide-rolling treatment in the fall of 2019 and the sites were surveyed again in 2021. “BC” and “LP” site names indicate Big Creek NWA and Long Point NWA, respectively. Marsh-user species are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

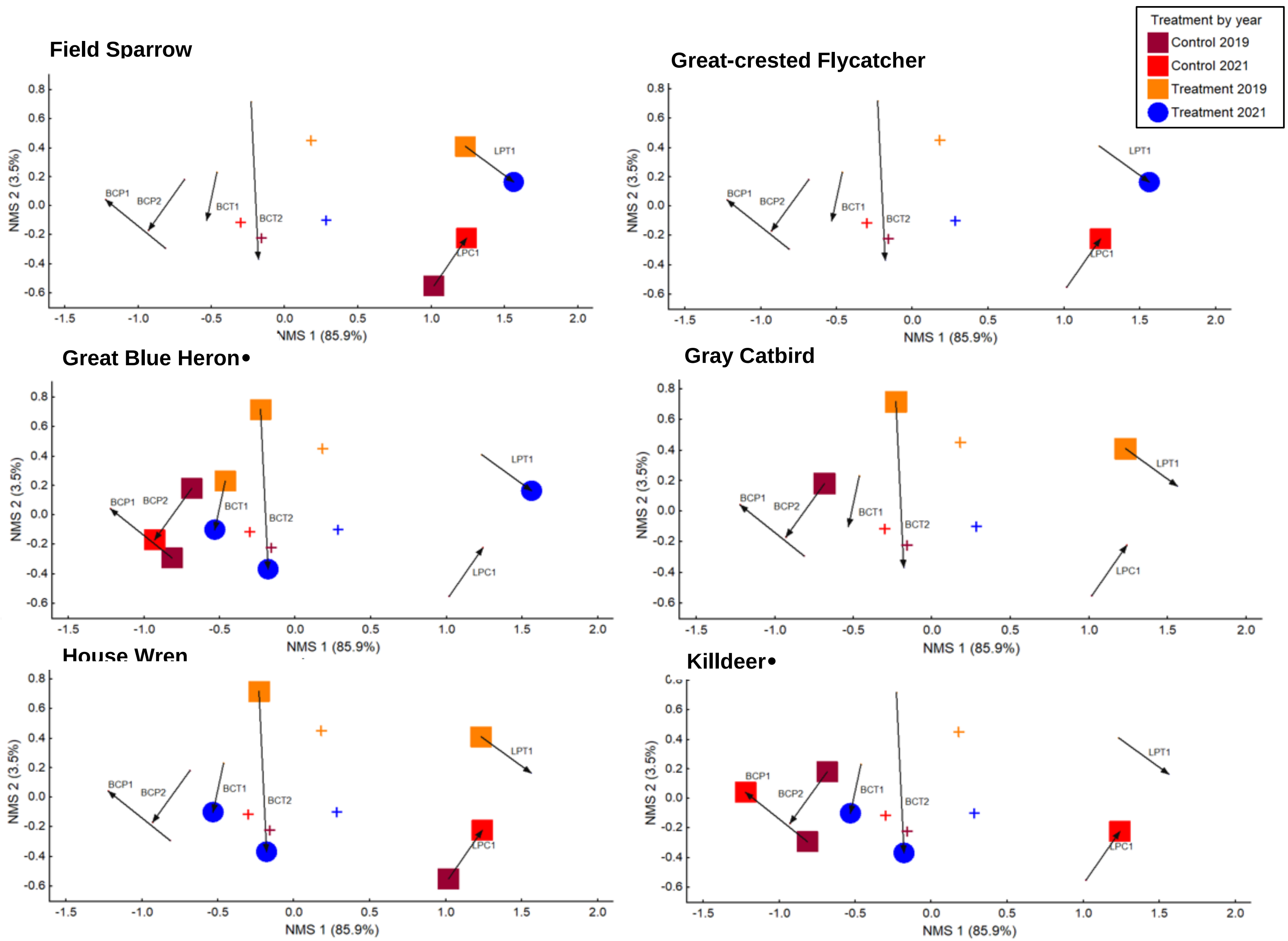


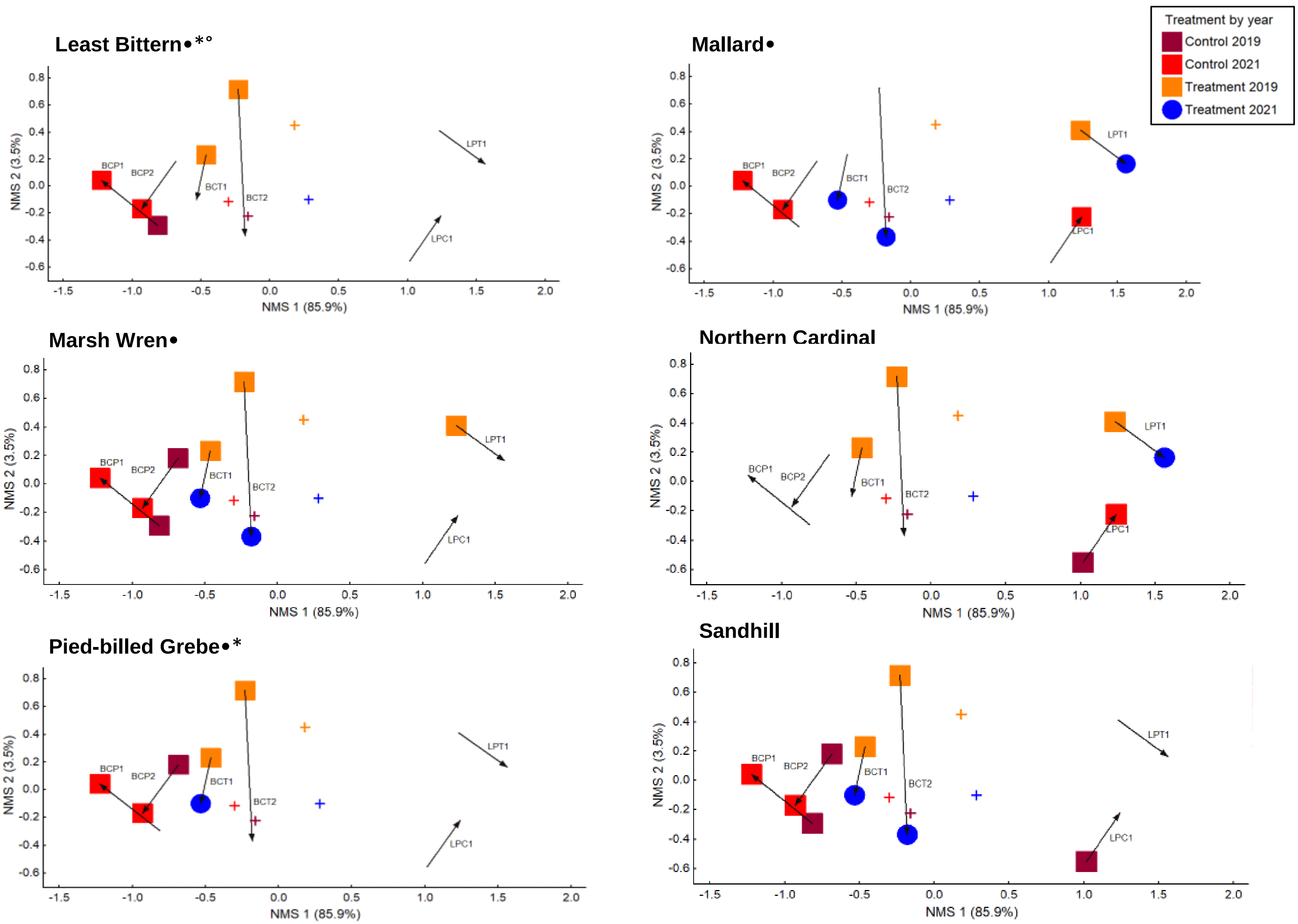
American Bittern•*
American Crow
American Goldfinch
American Robin
Bank Swallow•°
Baltimore Oriole
Treatment by year
Control 2019
Control 2021
Treatment 2019
Treatment 2021
BCP1
BCP2
BCT1
BCT2
LPT1
LPC1
NMS 1 (85.9%)
NMS 2 (3.5%)


Barn Swallow•°
Black-capped Chickadee
Black-crowned Night
Belted Kingfisher•
Blue-gray Gnatcatcher
Blue Jay
Treatment by year
Control 2019
Control 2021
Treatment 2019
Treatment 2021
NMS 1 (85.9%)
NMS 2 (3.5%)
BCP1
BCP2
BCT1
BCT2
LPT1
LPC1

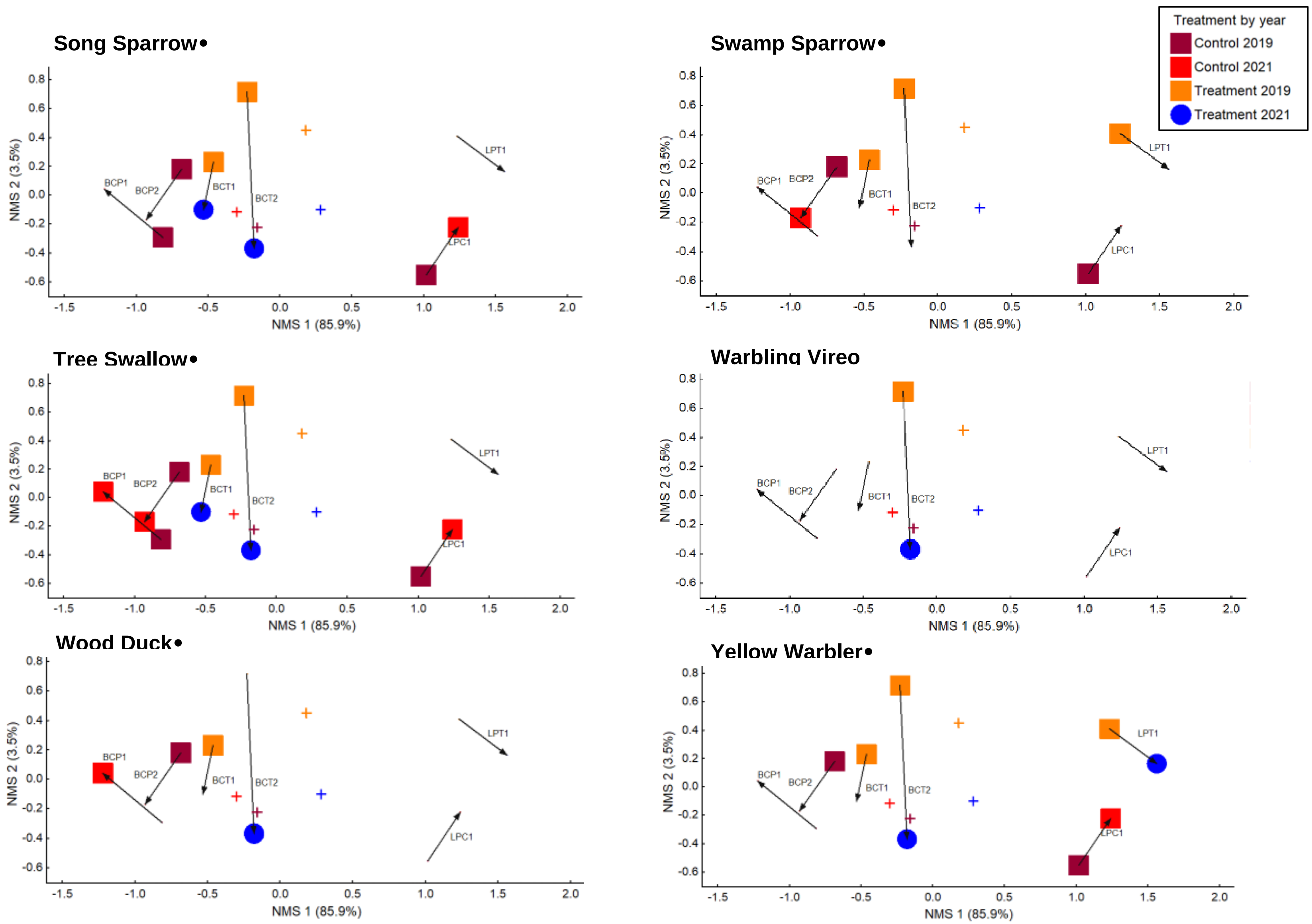

Treatment by year
Control 2019
Control 2021
Treatment 2019
Treatment 2021
Brown Thrasher
Blue-winged Teal•
Canada Goose•
Cedar Waxwing
Chipping Sparrow
Common Gallinule•*
NMS 2 (3.5%)
NMS 1 (85.9%)
BCP1
BCP2
BCT1
BCT2
LPT1
LPC1
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0

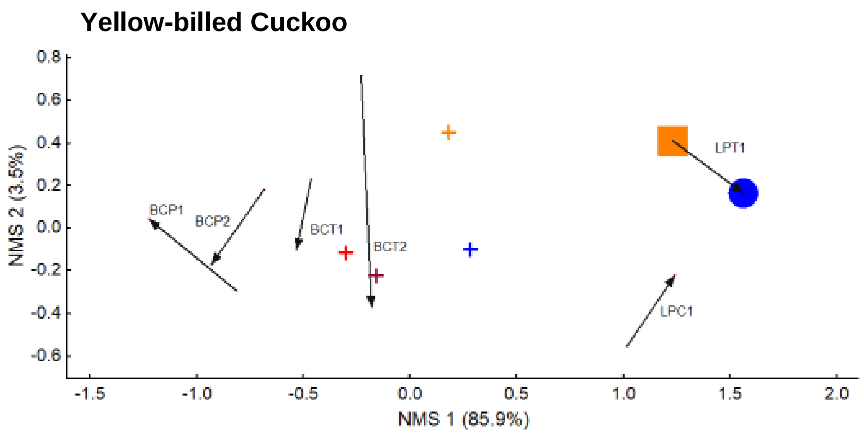

Treatment by year
Control 2019
Control 2021
Treatment 2019
Treatment 2021
Common Grackle•
Common Tern•
Common Yellowthroat•
Chestnut-sided Warbler
Eastern Kingbird•
Eastern Towhee
BCP1
BCP2
BCT1
BCT2
LPT1
LPC1
NMS 1 (85.9%)
NMS 2 (3.5%)


Field Sparrow
Great-crested Flycatcher
Great Blue Heron•
Gray Catbird
House Wren
Killdeer•
Treatment by year
Control 2019
Control 2021
Treatment 2019
Treatment 2021
NMS 1 (85.9%)
NMS 2 (3.5%)
BCP1
BCP2
BCT1
BCT2
LPT1
LPC1



Treatment by year
Control 2019
Control 2021
Treatment 2019
Treatment 2021
Least Bittern•*°
Mallard•
Marsh Wren•
Northern Cardinal
Pied-billed Grebe•*
Sandhill
BCP1
BCP2
BCT1
BCT2
LPT1
LPC1
NMS 1 (85.9%)
NMS 2 (3.5%)


Song Sparrow•
Swamp Sparrow•
Tree Swallow•
Warbling Vireo
Wood Duck•
Yellow Warbler•
Treatment by year
Control 2019
Control 2021
Treatment 2019
Treatment 2021
BCP1
BCP2
BCT1
BCT2
LPT1
LPC1
NMS 1 (85.9%)
NMS 2 (3.5%)


Yellow-billed Cuckoo
NMS 2 (3.5%)
NMS 1 (85.9%)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
BCP1
BCP2
BCT1
BCT2
LPT1
LPC1

*Appendix 1C.*

Vegetation contact profiles at each of the 30 ARUs surveyed between June $5^{th}$-$20^{th}$ 2021. Refer to section 2.2.4 “ARU site characteristics” for survey methodology. Sites are organized in three separate figures for control (*P. australis*; Figure 5.1), reference (cattail marsh, hemi-marsh, meadow marsh; Figures 5.2, 5.3) and 1 or 2-years post herbicide-rolling treatment (Figure 5.4). ARUs that experienced data corruption (and were therefore not transcribed) are indicated as “corrupted” in the site name.

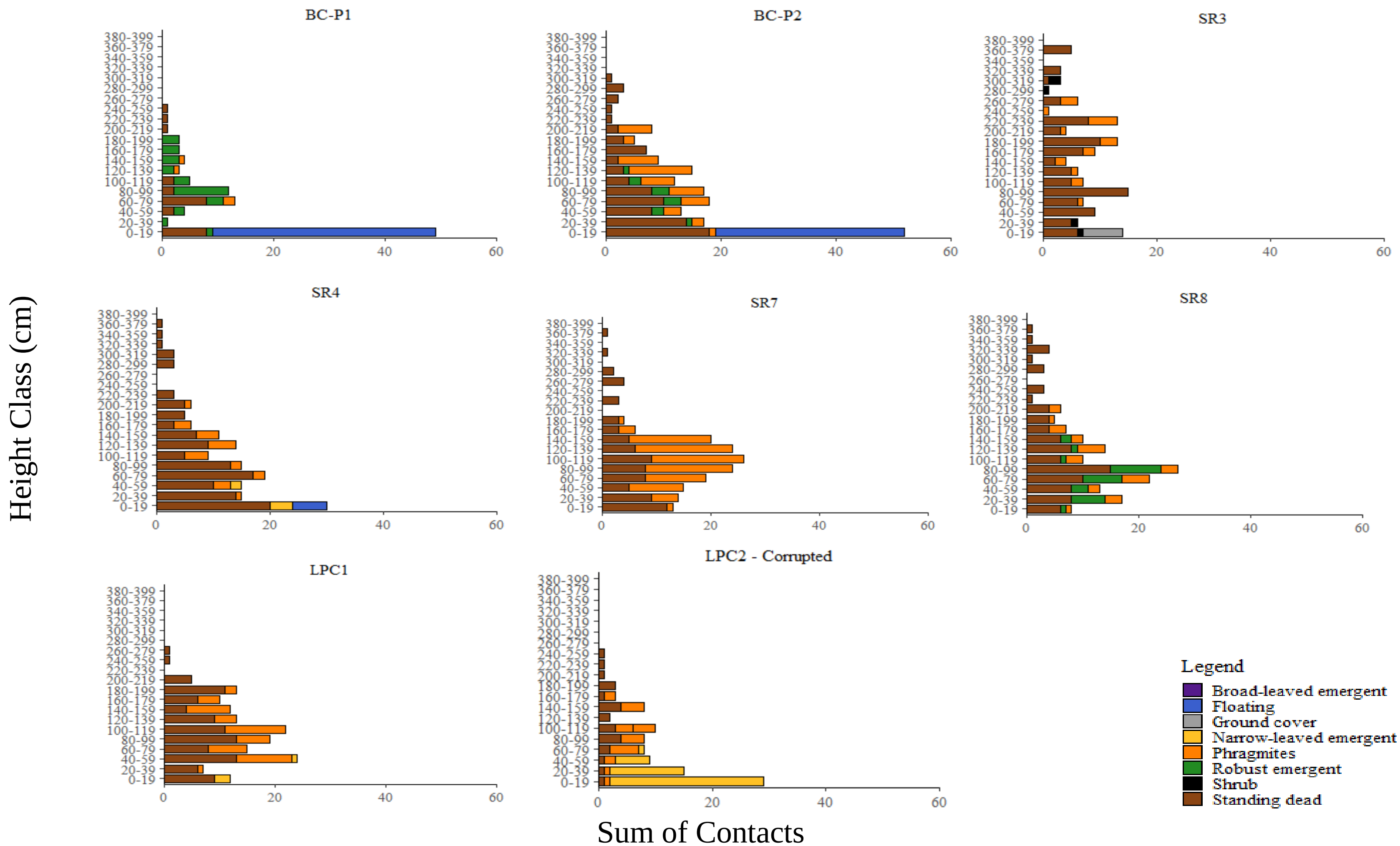



*Figure 5.1*. Horizontal contact profiles for control (*P. australis*) sites surveyed in 2021. “BC”, “SR” and “LP” indicate Big Creek, Squire’s Ridge and Long Pond, respectively.

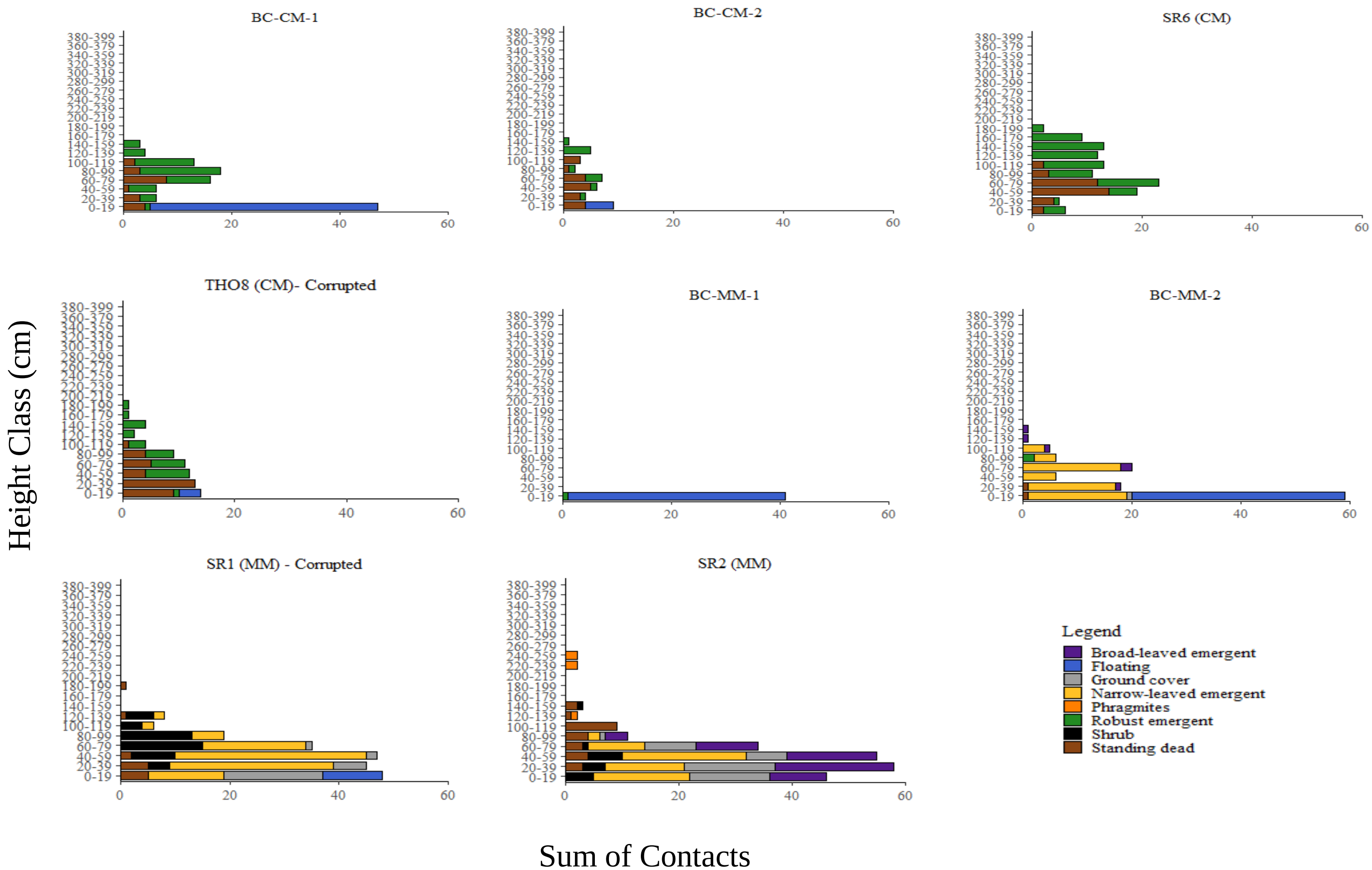



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*Figure 5.2.* Horizontal contact profiles for reference sites surveyed in 2021. "BC", "SR" and "LP" indicate Big Creek, Squire's Ridge and Long Pond, respectively. "CM", "HM" and "MM" indicate cattail marsh, meadow marsh and hemi-marsh, respectively.

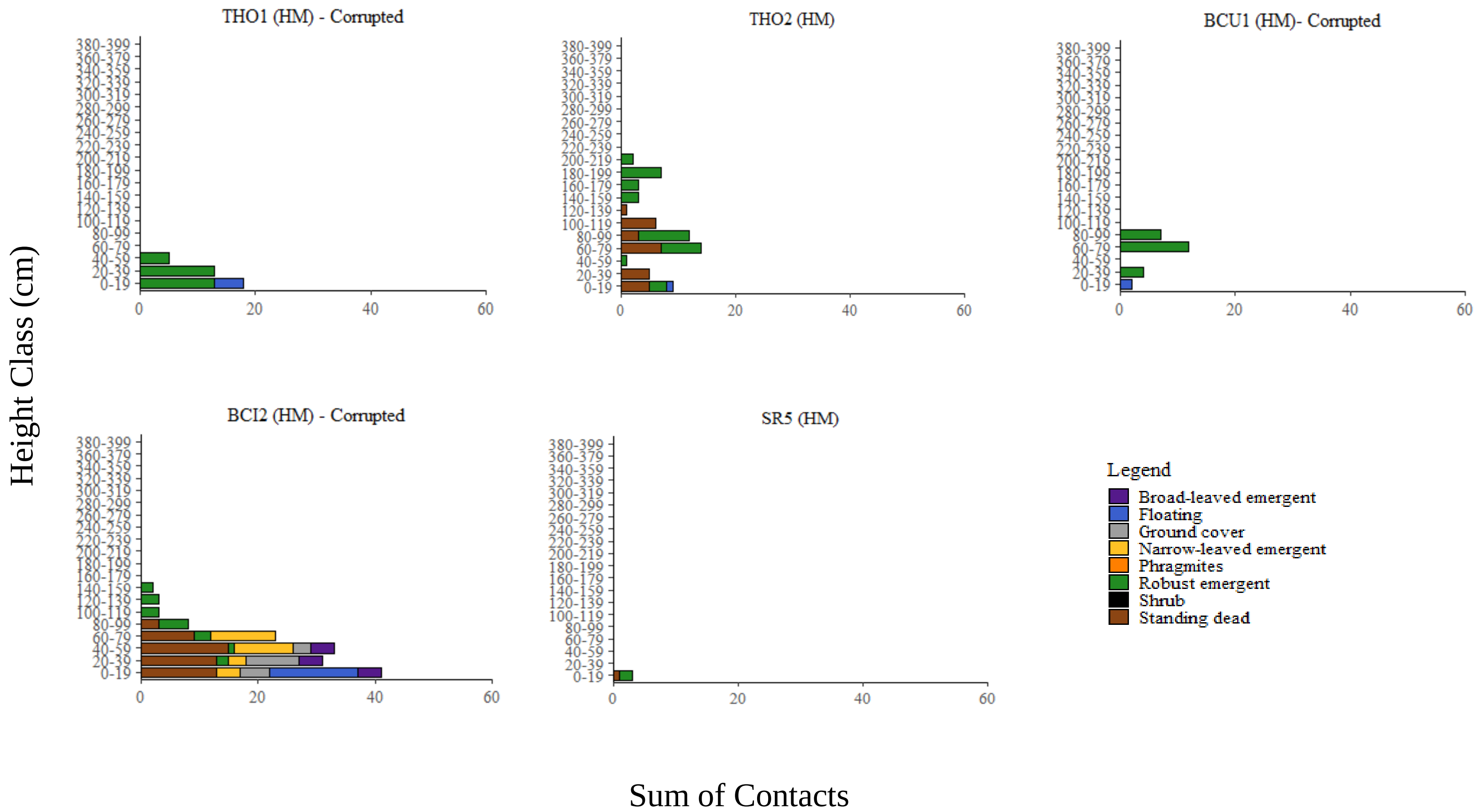



*Figure 5.3.* Horizontal contact profiles for reference sites surveyed in 2021. "BC", "SR" and "LP" indicate Big Creek, Squire's Ridge and Long Pond, respectively. "HM" indicates hemi-marsh.

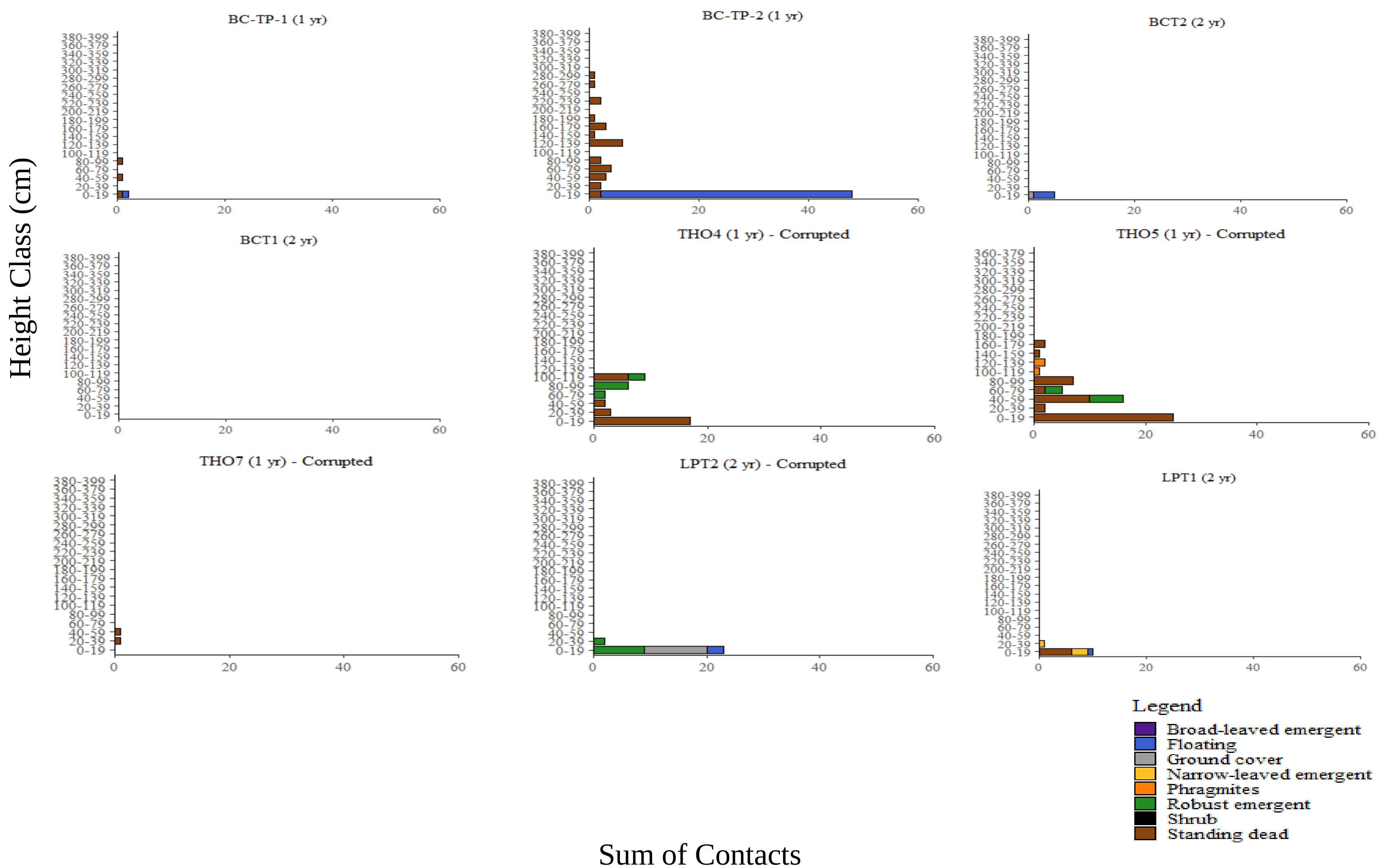



*Figure 5.4.* Horizontal contact profiles for treated *P. australis* sites (herbicide application and rolling) surveyed in 2021. Years indicate how many years after treatment. “BC”, “THO” and “LP” indicate Big Creek, Thoroughfare and Long Pond, respectively. No vegetation was present at BCT1.

*Appendix 1D.*

*Table 5.2.* Correlation coefficients (r) and coefficient of determination ($r^2$) of vectors in the optimal NMS ordination for bird community composition in 2021 control (*P. australis*), reference (cattail marsh, hemi-marsh, meadow marsh) and 1- or 2-year post-herbicide-rolling treatment sites. Species with an $r^2 \geq 0.20$ were considered reasonably correlated and included in Figure 2.8-C.

| | | **Axis 1** | | **Axis 2** | |
|---|---|---|---|---|---|
| **Common Name** | **4-letter Alpha Code** | **r** | **$r^2$** | **r** | **$r^2$** |
| American Bittern | AMBI | -0.714 | 0.51 | -0.609 | 0.51 |
| American Crow | AMCR | 0.179 | 0.032 | 0.036 | 0.412 |
| American Goldfinch | AMGO | 0.343 | 0.118 | 0.209 | 0.55 |
| American Robin | AMRO | 0.421 | 0.177 | 0.418 | -0.016 |
| Baltimore Oriole | BAOR | 0.116 | 0.013 | 0.109 | -0.18 |
| Bank Swallow | BANS | 0.079 | 0.006 | 0.059 | 0.036 |
| Barn Swallow | BARS | -0.385 | 0.148 | -0.334 | 0.418 |
| Belted Kingfisher | BEKI | 0.087 | 0.008 | 0.15 | 0.25 |
| Black Tern | BLTE | -0.184 | 0.034 | -0.097 | -0.127 |
| Black-billed Cuckoo | BBCU | 0.085 | 0.007 | 0.117 | -0.129 |
| Black-capped Chickadee | BCCH | 0.673 | 0.453 | 0.54 | -0.296 |
| Blue Jay | BLJA | 0.307 | 0.094 | 0.237 | -0.665 |
| Blue-gray Gnatcatcher | BGGN | 0.32 | 0.103 | 0.25 | -0.28 |
| Brown Thrasher | BRTH | 0.48 | 0.231 | 0.387 | -0.002 |
| Canada Goose | CAGO | -0.735 | 0.54 | -0.609 | 0.181 |
| Carolina Wren | CARW | 0.401 | 0.161 | 0.283 | 0.354 |
| Cedar Waxwing | CEDW | 0.09 | 0.008 | 0 | 0.447 |
| Chipping Sparrow | CHSP | 0.355 | 0.126 | 0.218 | 0.41 |
| Common Gallinule | COGA | -0.783 | 0.614 | -0.692 | -0.107 |
| Common Grackle | COGR | 0.177 | 0.031 | 0.174 | -0.486 |
| Common Nighthawk | CONI | -0.048 | 0.002 | -0.048 | -0.111 |
| Common Raven | CORA | -0.08 | 0.006 | -0.083 | -0.029 |
| Common Yellowthroat | COYE | 0.393 | 0.154 | 0.315 | 0.465 |
| Eastern Kingbird | EAKI | 0.745 | 0.556 | 0.637 | -0.014 |
| Eastern Towhee | EATO | 0.632 | 0.399 | 0.435 | 0.316 |
| Eastern Wood-pewee | EAWP | 0.506 | 0.256 | 0.41 | -0.521 |
| Field Sparrow | FISP | 0.632 | 0.399 | 0.435 | 0.316 |
| Forster’s Tern | FOTE | -0.413 | 0.171 | -0.316 | -0.216 |
| Gray Catbird | GRCA | 0.179 | 0.032 | 0.242 | 0.212 |
| Great Blue Heron | GBHE | -0.158 | 0.025 | -0.174 | 0.691 |
| Great-crested Flycatcher | GCFL | 0.726 | 0.527 | 0.518 | 0.095 |

| | | Axis 1 | | Axis 2 | |
|---|---|---|---|---|---|
| **Common Name** | **4-letter Alpha Code** | **r** | **$r^2$** | **r** | **$r^2$** |
| House Wren | HOWR | 0.57 | 0.325 | 0.547 | 0.097 |
| Indigo Bunting | INBU | -0.028 | 0.001 | -0.05 | 0.299 |
| Killdeer | KILL | 0.144 | 0.021 | 0.129 | 0.521 |
| Least Bittern | LEBI | -0.531 | 0.282 | -0.4 | -0.472 |
| Mallard | MALL | 0.045 | 0.002 | -0.029 | 0.475 |
| Marsh Wren | MAWR | -0.696 | 0.484 | -0.544 | -0.221 |
| Mourning Dove | MODO | 0.413 | 0.171 | 0.316 | 0.216 |
| Northern Cardinal | NOCA | 0.642 | 0.412 | 0.547 | -0.379 |
| Northern Flicker | NOFL | 0.206 | 0.043 | 0.226 | -0.219 |
| Northern Rough-winged Swallow | NRWS | 0.401 | 0.161 | 0.283 | 0.354 |
| Orchard Oriole | OROR | -0.216 | 0.047 | -0.152 | 0.115 |
| Pied-billed Grebe | PBGR | -0.371 | 0.138 | -0.232 | -0.296 |
| Purple Martin | PUMA | Present at every site | | | |
| Red-bellied Woodpecker | RBWO | 0.25 | 0.062 | 0.213 | -0.536 |
| Red-winged Blackbird | RWBL | Present at every site | | | |
| Sandhill Crane | SACR | -0.677 | 0.458 | -0.508 | 0.138 |
| Song Sparrow | SOSP | 0.394 | 0.155 | 0.272 | 0.301 |
| Sora | SORA | -0.002 | 0 | 0.051 | 0.032 |
| Swamp Sparrow | SWSP | -0.384 | 0.147 | -0.388 | 0.194 |
| Tree Swallow | TRES | -0.04 | 0.002 | 0 | 0.098 |
| Unknown Species 22 | - | 0.052 | 0.003 | 0.083 | -0.408 |
| Unknown Species 24 | - | 0.041 | 0.002 | 0.048 | -0.637 |
| Unknown Species 26 | - | 0.041 | 0.002 | 0.048 | -0.637 |
| Virginia Rail | VIRA | -0.197 | 0.039 | -0.142 | -0.534 |
| Warbling Vireo | WAVI | 0.378 | 0.143 | 0.416 | -0.255 |
| Wood Duck | WODU | -0.605 | 0.365 | -0.489 | -0.153 |
| Yellow Warbler | YEWA | 0.756 | 0.572 | 0.662 | -0.295 |
| Yellow-billed Cuckoo | YBCU | 0.364 | 0.132 | 0.29 | -0.323 |

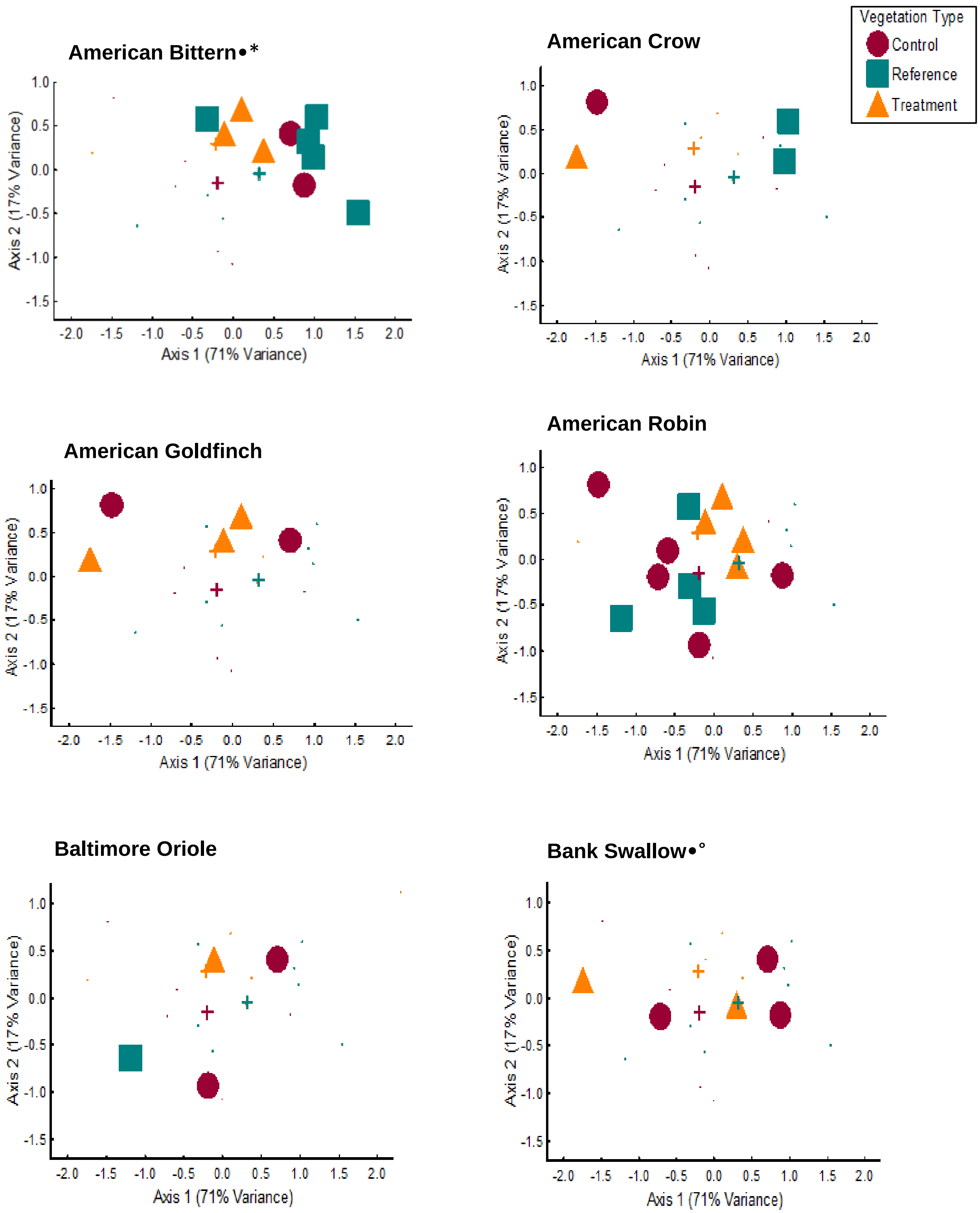
*Appendix 1E.*

*Table 5.3.* Correlation coefficients (r) and coefficient of determination ($r^2$) of vectors in the optimal NMS ordination for bird community composition in 2021 control (*P. australis*), reference (cattail marsh, hemi-marsh, meadow marsh) and 1- or 2-year post-herbicide-rolling treatment sites. Environmental variables (total vegetation contacts and average water depths) with an $r^2 \geq 0.05$ were considered reasonably correlated and included in Figure 2.8-D. Vectors were scaled to 200%.

| | **Axis 1** | | **Axis 2** | |
|---|---|---|---|---|
| **Environmental Variable** | **r** | **$r^2$** | **r** | **$r^2$** |
| Average water depth | -0.282 | 0.079 | -0.143 | 0.021 |
| Total broad-leaved emergent contacts | 0.294 | 0.086 | -0.255 | 0.065 |
| Total floating contacts | -0.478 | 0.228 | 0.284 | 0.081 |
| Total ground cover contacts | 0.343 | 0.118 | -0.283 | 0.08 |
| Total narrow-leaved emergent contacts | 0.078 | 0.006 | 0.011 | 0 |
| Total *Phragmites australis* contacts | 0.225 | 0.051 | -0.246 | 0.061 |
| Total robust Emergent contacts | -0.399 | 0.159 | -0.376 | 0.142 |
| Total shrub contacts | 0.367 | 0.135 | -0.297 | 0.088 |
| Total standing dead contacts | 0.255 | 0.065 | -0.27 | 0.073 |

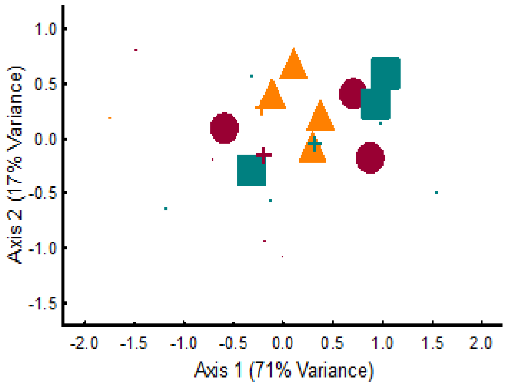
*Appendix 1F.*

Individual species presence/absence plots in control (*P. australis*; N = 7), reference (cattail marsh, hemi-marsh, meadow marsh; N = 8) and 1- or 2-year post-herbicide-rolling (N = 6) sites in 2021. Purple Martin and Red-winged Blackbird were observed at every site and subsequently not plotted. Note that the horizontal axis is reflected compared to NMS ordination plots for bird community composition within the document, as well as and r and $r^2$ values in Appendix 1D. Marsh-user species are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

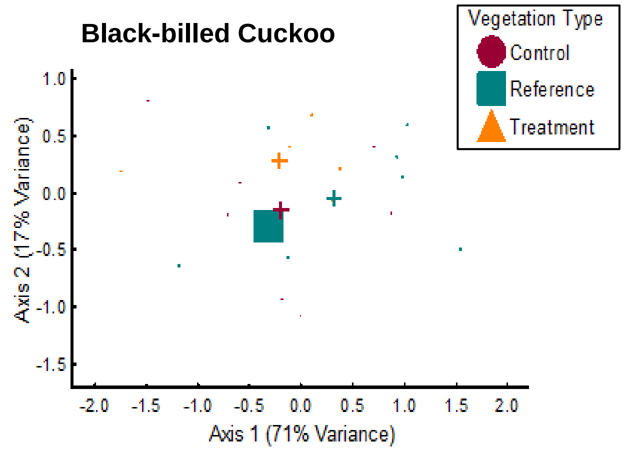


Vegetation Type
Control
Reference
Treatment
American Bittern•*
American Crow
American Goldfinch
American Robin
Baltimore Oriole
Bank Swallow•°
Axis 1 (71% Variance)
Axis 2 (17% Variance)
-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0
1.0 0.5 0.0 -0.5 -1.0 -1.5

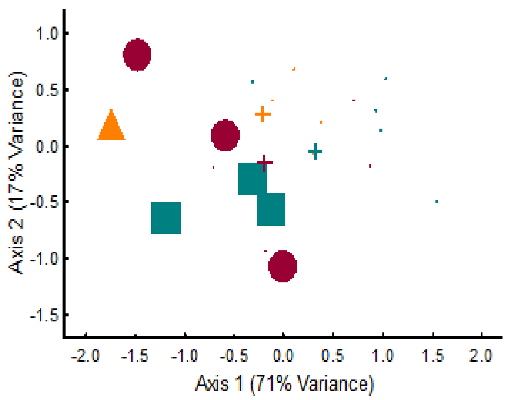
**Barn Swallow•°**



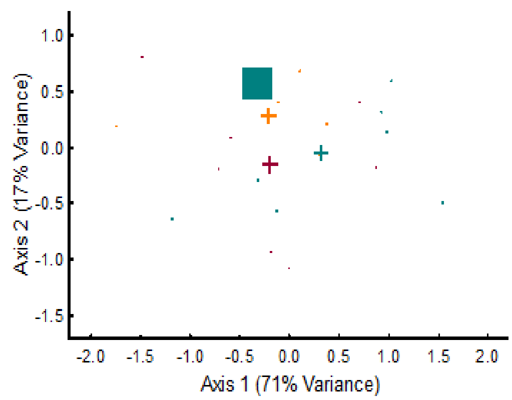

**Black-billed Cuckoo**



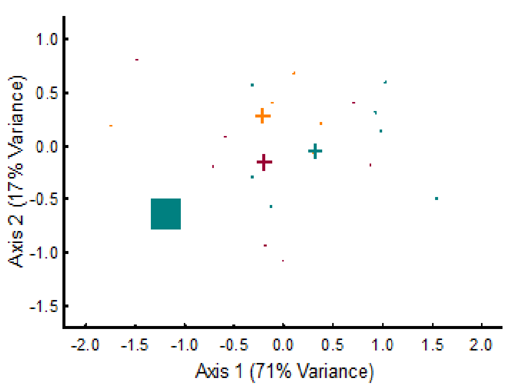

**Black-capped Chickadee**



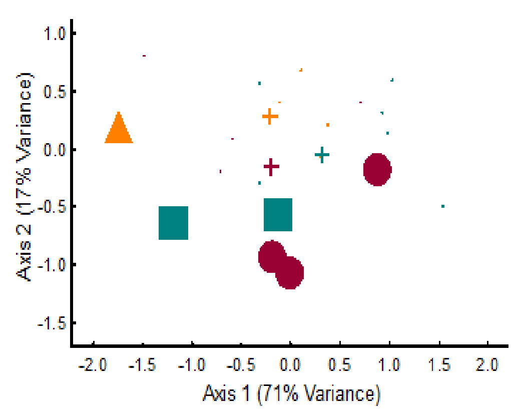

**Belted Kingfisher•**

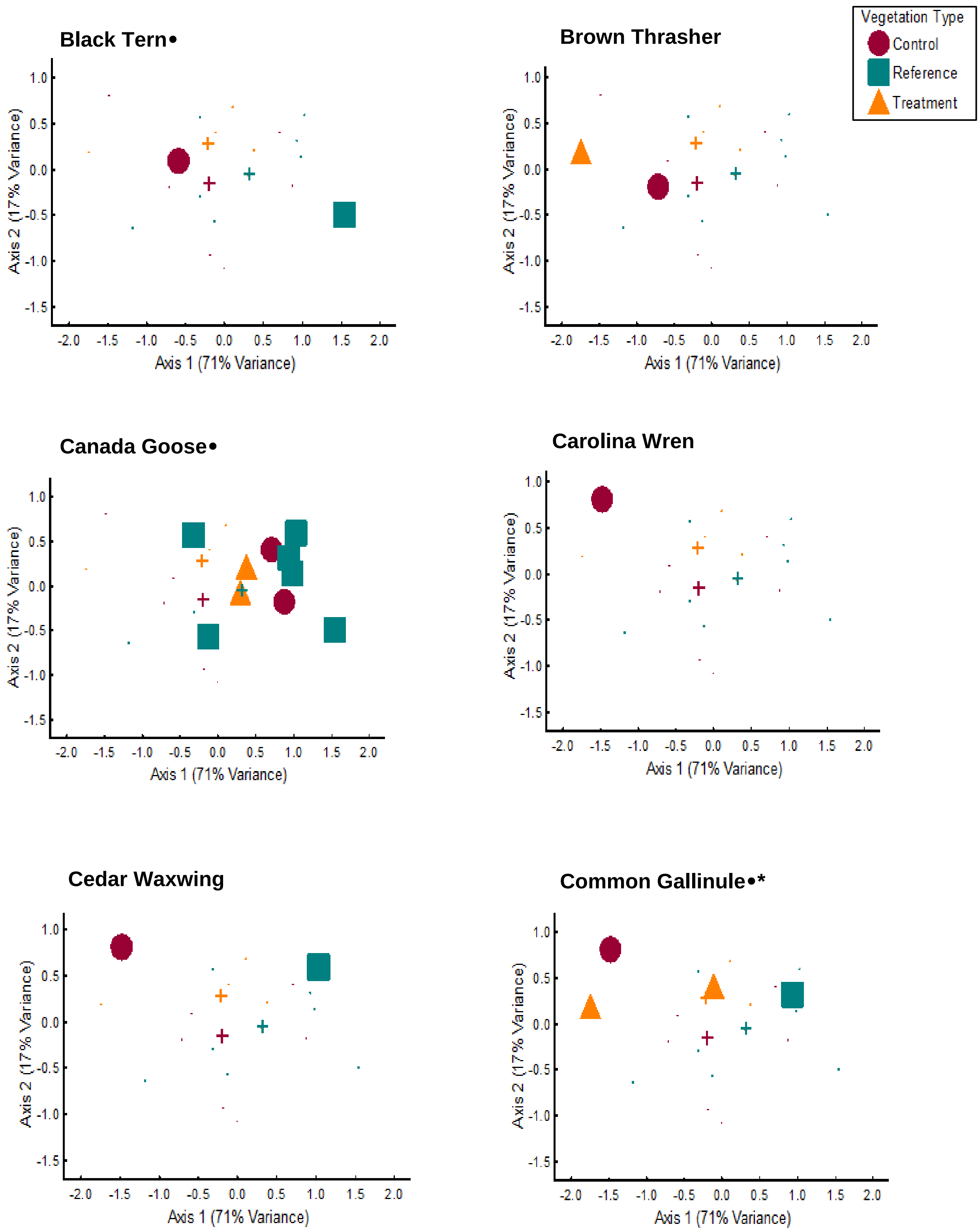



**Blue-gray Gnatcatcher**

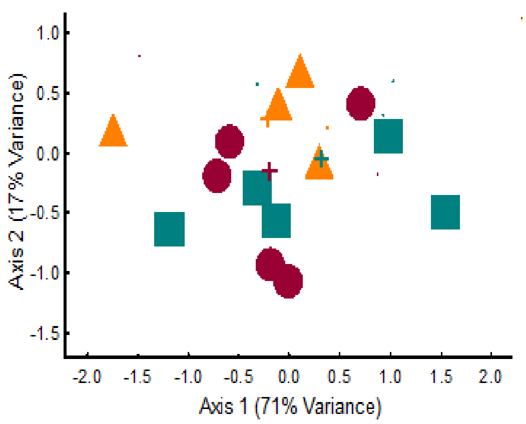



**Blue Jay**

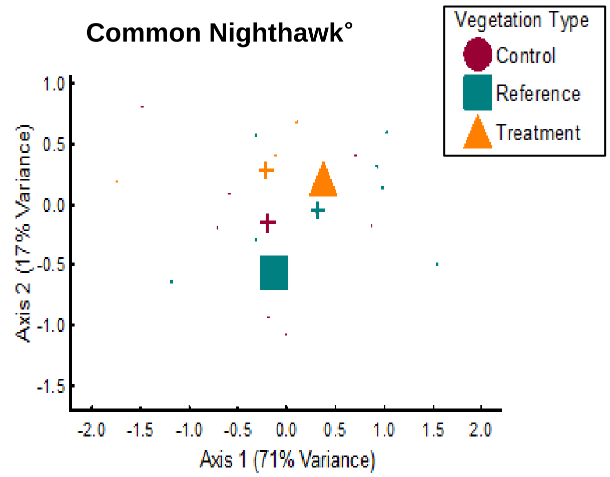



Black Tern•
Brown Thrasher
Vegetation Type
Control
Reference
Treatment
Canada Goose•
Carolina Wren
Cedar Waxwing
Common Gallinule•*
Axis 1 (71% Variance)
Axis 2 (17% Variance)

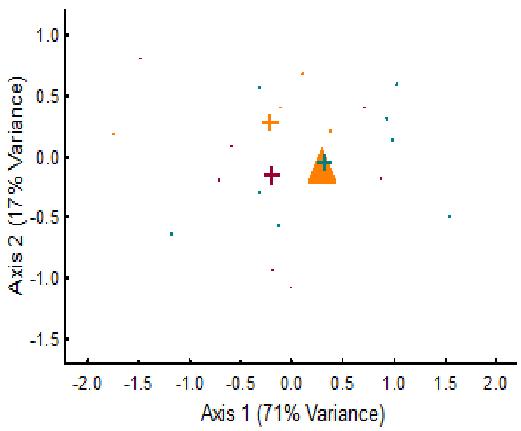
**Common Grackle•**



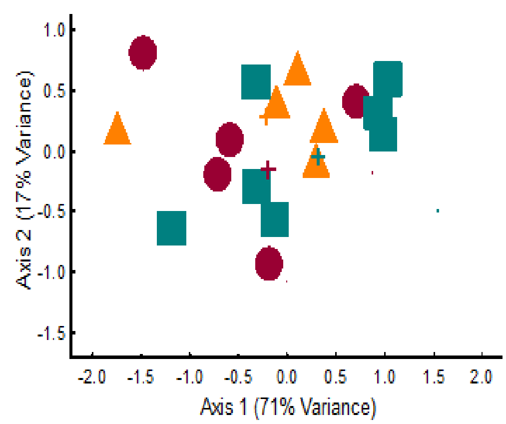

**Common Nighthawk°**



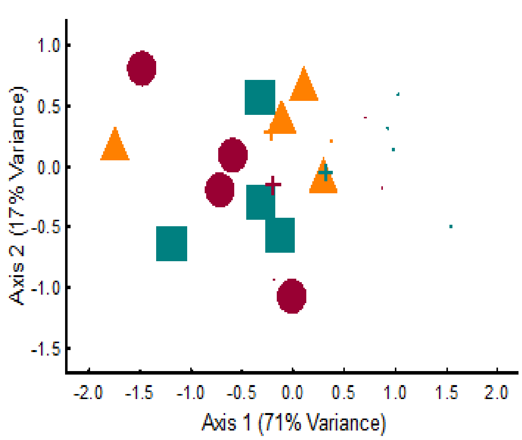

**Common Raven**



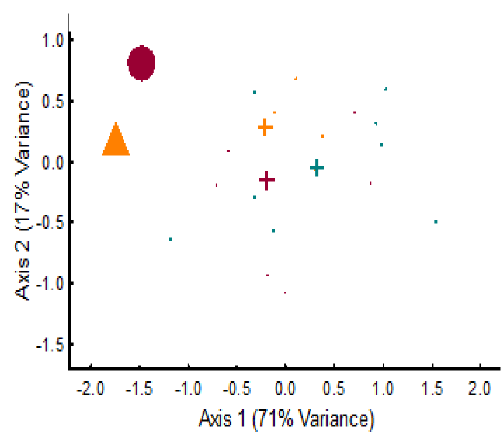

**Common Yellowthroat•**

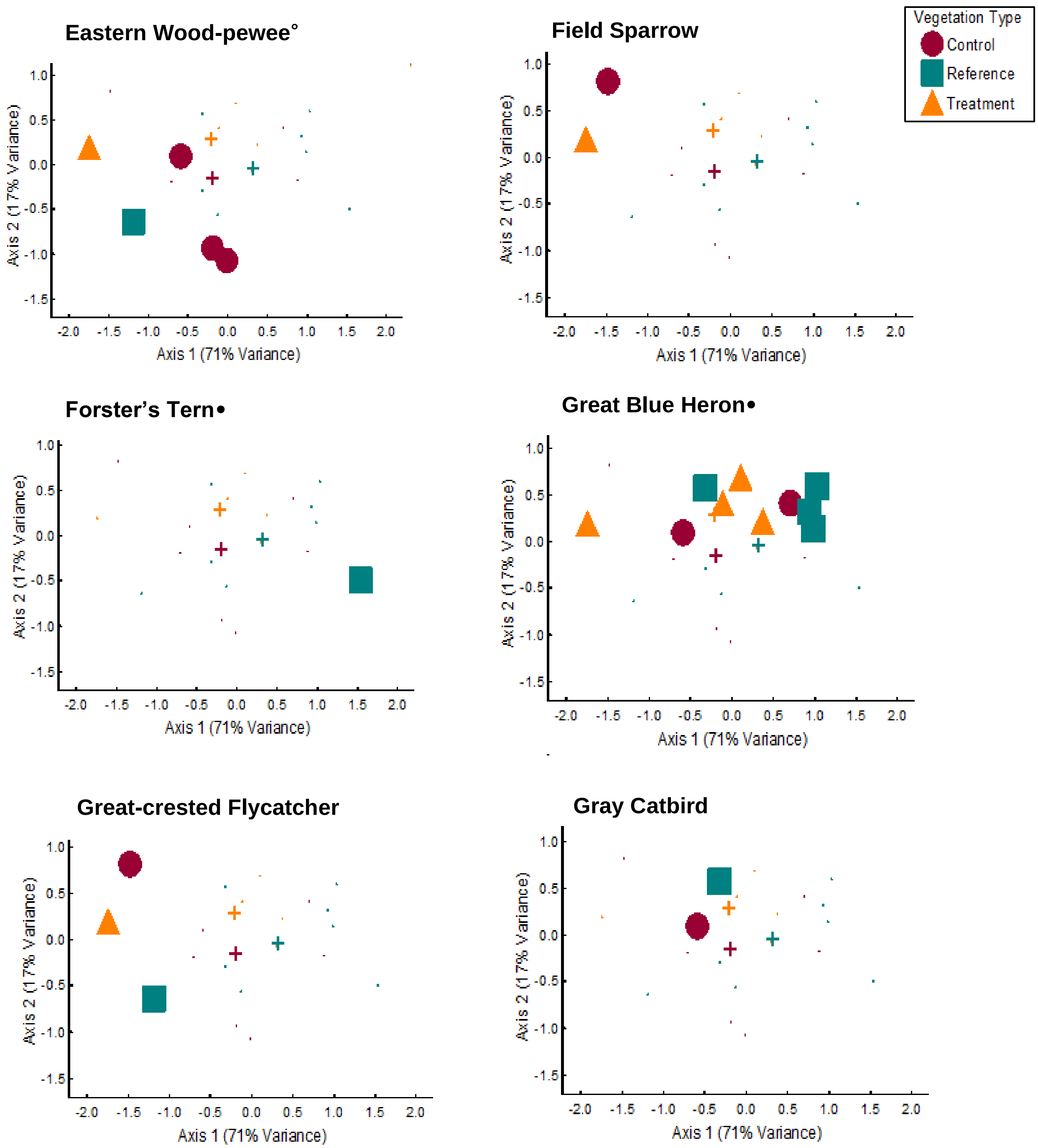


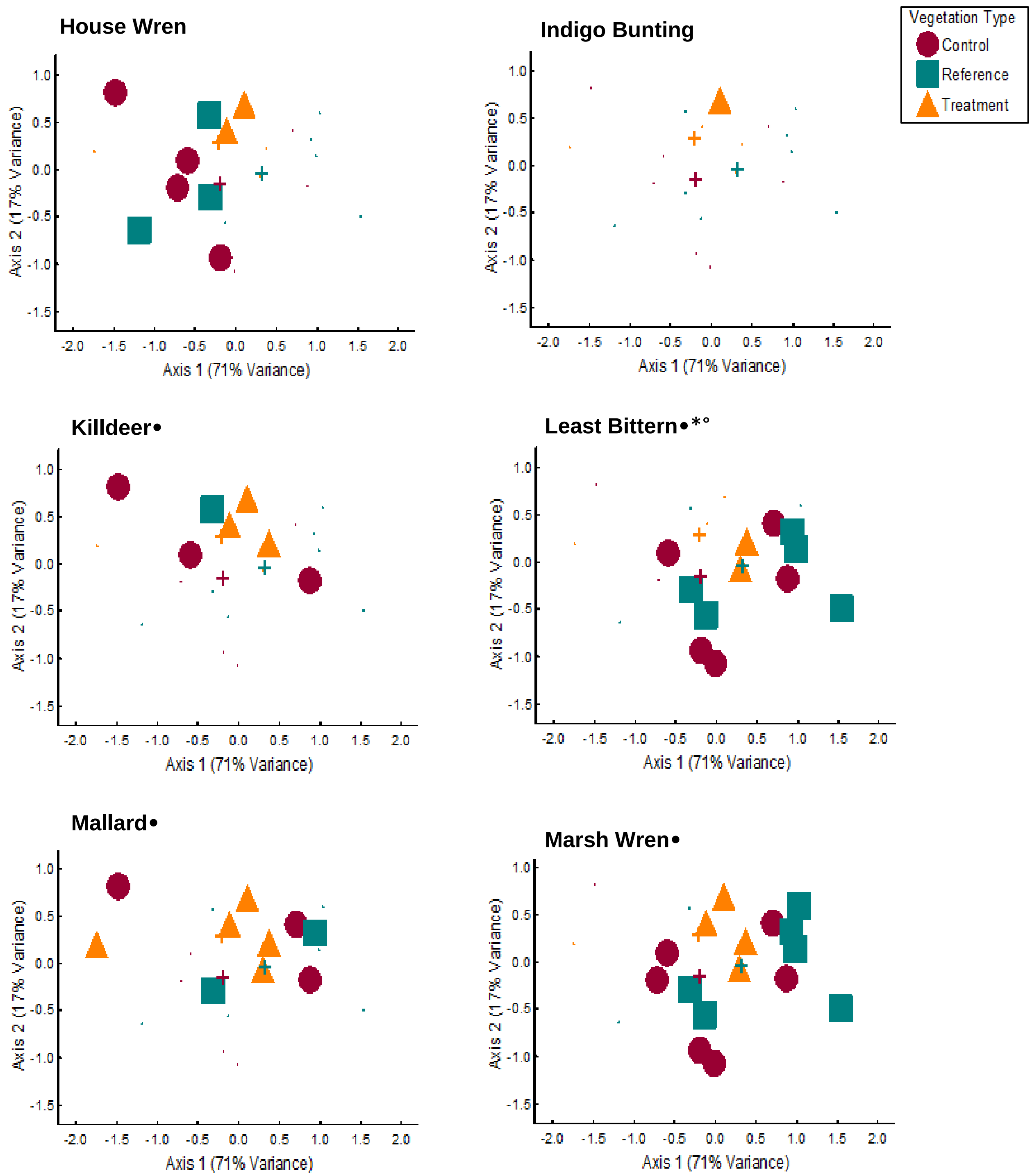

**Eastern Kingbird•**



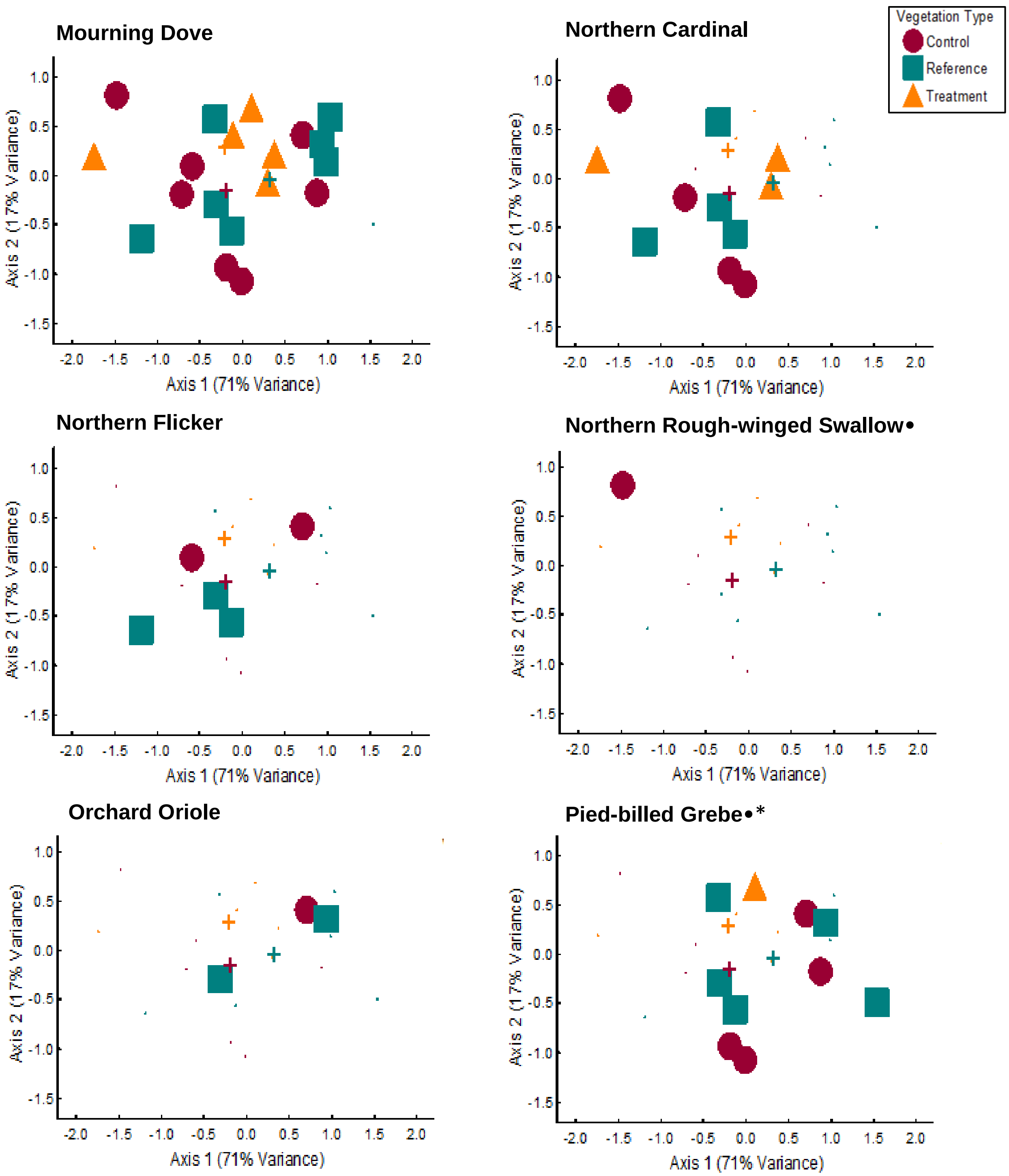

**Eastern Towhee**



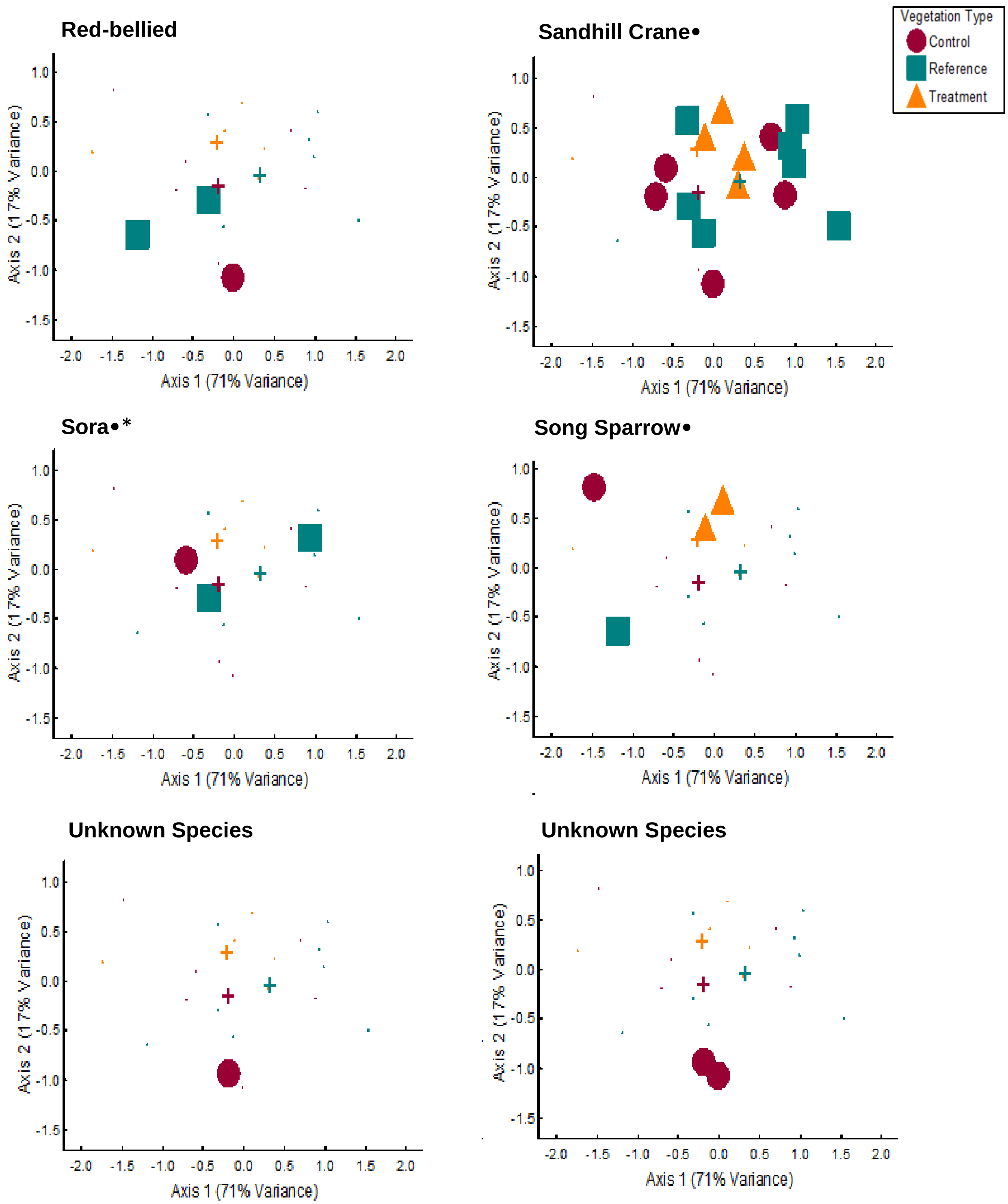

Eastern Wood-pewee°
Field Sparrow
Forster's Tern•
Great Blue Heron•
Great-crested Flycatcher
Gray Catbird
Vegetation Type
Control
Reference
Treatment
Axis 1 (71% Variance)
Axis 2 (17% Variance)

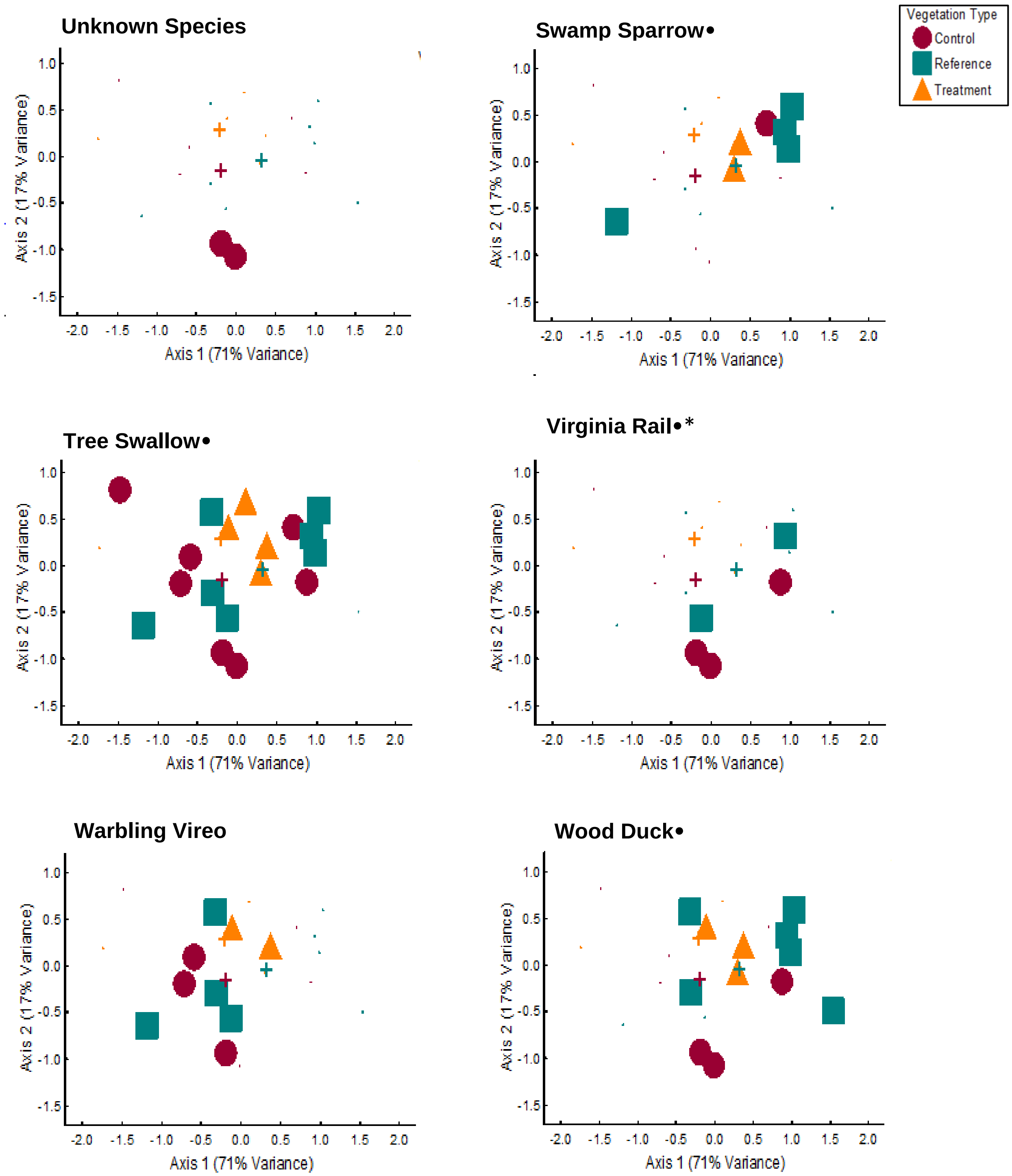


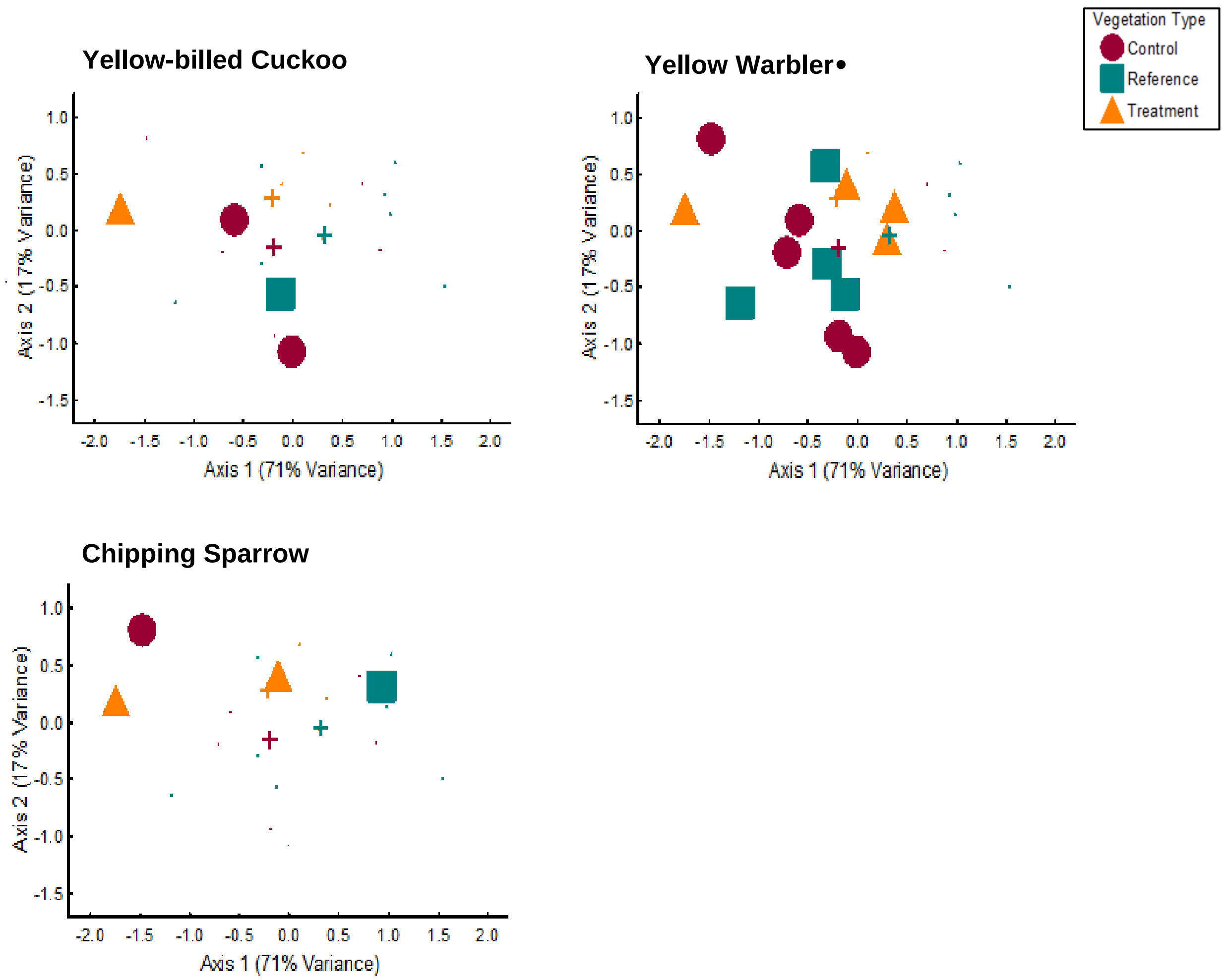
House Wren
Indigo Bunting
Vegetation Type
Control
Reference
Treatment
Killdeer•
Least Bittern•*°
Mallard•
Marsh Wren•
Axis 1 (71% Variance)
Axis 2 (17% Variance)
-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0
1.0 0.5 0.0 -0.5 -1.0 -1.5



Mourning Dove
Northern Cardinal
Vegetation Type
Control
Reference
Treatment
Northern Flicker
Northern Rough-winged Swallow•
Orchard Oriole
Pied-billed Grebe•*
Axis 1 (71% Variance)
Axis 2 (17% Variance)


Red-bellied
Sandhill Crane•
Vegetation Type
Control
Reference
Treatment
Sora•*
Song Sparrow•
Unknown Species
Unknown Species
Axis 1 (71% Variance)
Axis 2 (17% Variance)
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0


Unknown Species
Swamp Sparrow•
Vegetation Type
Control
Reference
Treatment
Tree Swallow•
Virginia Rail•*
Warbling Vireo
Wood Duck•
Axis 2 (17% Variance)
Axis 1 (71% Variance)
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0


Yellow-billed Cuckoo
Yellow Warbler•
Chipping Sparrow
Vegetation Type
Control
Reference
Treatment
Axis 1 (71% Variance)
Axis 2 (17% Variance)
-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0
1.0 0.5 0.0 -0.5 -1.0 -1.5

*Appendix 1G.*

*Table 5.4.* Correlation coefficients (r) and coefficient of determination ($r^2$) of vectors in the optimal NMS ordination for functional trait composition in 2021 control (*P. australis*), reference (cattail marsh, hemi-marsh, meadow marsh) and 1- or 2-year post-herbicide-rolling treatment sites. Functional traits (diet, foraging behavior, and nesting preference) with an $r^2 \geq 0.20$ were considered reasonably correlated and included in Figure 2.9-C.

| | **Axis 1** | | **Axis 2** | |
|---|---|---|---|---|
| | **r** | **$r^2$** | **r** | **$r^2$** |
| *Diet* | | | | |
| Aquatic invertebrates | -0.558 | 0.312 | -0.221 | 0.049 |
| Fish | -0.859 | 0.738 | -0.024 | 0.001 |
| Fruit | 0.055 | 0.003 | 0.067 | 0.005 |
| Insect | 0.835 | 0.698 | -0.216 | 0.047 |
| Omnivore | 0.006 | 0 | -0.028 | 0.001 |
| Plant | -0.85 | 0.723 | -0.147 | 0.022 |
| Seed | 0.007 | 0 | 0.78 | 0.608 |
| *Foraging Behavior* | | | | |
| Aerial dive | -0.457 | 0.209 | -0.08 | 0.006 |
| Aerial forage | -0.342 | 0.117 | 0.377 | 0.142 |
| Bark forage | 0.341 | 0.116 | -0.572 | 0.327 |
| Dabbler | -0.852 | 0.725 | 0.143 | 0.021 |
| Flycatching | 0.84 | 0.706 | -0.044 | 0.002 |
| Foliage gleaner | 0.923 | 0.852 | -0.171 | 0.029 |
| Ground forage | 0.681 | 0.463 | 0.447 | 0.2 |
| Probing | -0.764 | 0.584 | -0.316 | 0.1 |
| Stalking | -0.844 | 0.712 | 0.001 | 0 |
| Surface dive | -0.558 | 0.312 | -0.221 | 0.049 |
| *Nesting Preference* | | | | |
| Build | -0.345 | 0.119 | 0.275 | 0.076 |
| Burrow | 0.201 | 0.04 | 0.676 | 0.456 |
| Cavity | 0.643 | 0.413 | -0.523 | 0.274 |
| Cliff | -0.04 | 0.002 | 0.325 | 0.105 |
| Floating | -0.807 | 0.651 | -0.308 | 0.095 |
| Ground | -0.928 | 0.862 | 0.213 | 0.045 |
| Shrub | 0.771 | 0.594 | 0.346 | 0.12 |
| Tree | 0.815 | 0.664 | -0.279 | 0.078 |

*Appendix 1H.*

*Table 5.5.* Correlation coefficients (r) and coefficient of determination ($r^2$) of vectors in the optimal NMS ordination for functional trait composition in 2021 control (*P. australis*), reference (cattail marsh, hemi-marsh, meadow marsh) and 1- or 2-year post-herbicide-rolling treatment sites. Environmental variables with an $r^2 \geq 0.05$ were considered reasonably correlated and included in Figure 2.9-D.

| | **Axis 1** | | **Axis 2** | |
|---|---|---|---|---|
| | **r** | **$r^2$** | **r** | **$r^2$** |
| Average water depth | -0.363 | 0.132 | -0.285 | 0.081 |
| Total broad-leaved emergent contacts | 0.432 | 0.187 | -0.257 | 0.066 |
| Total floating contacts | -0.381 | 0.145 | 0.325 | 0.106 |
| Total ground cover contacts | 0.482 | 0.232 | -0.254 | 0.064 |
| Total narrow-leaved emergent contacts | 0.262 | 0.069 | -0.213 | 0.045 |
| Total *Phragmites australis* contacts | 0.243 | 0.059 | -0.16 | 0.025 |
| Total robust Emergent contacts | -0.438 | 0.192 | -0.318 | 0.101 |
| Total shrub contacts | 0.508 | 0.258 | -0.248 | 0.062 |
| Total standing dead contacts | 0.273 | 0.075 | -0.253 | 0.064 |

*Appendix 2A.*

*Table 5.6.* Observed total species richness (S-obs), non-parametric estimators of "true" species richness: Chao 2, 1st order Jackknife (Jack 1), 2nd order Jackknife (Jack 2), and Incidence-based Coverage Estimator (ICE), and 95% confidence interval for Chao 2 for each ARU. ARUs were deployed in *P. australis*-dominated habitat in the Big Creek and Long Point NWAs in 2019. Also indicated is whether the observed richness is 1) 80% of the lowest non-parametric estimator; 2) within the Chao 2 95% confidence interval, and 3) within one species of the confidence interval. ARUs are ordered from lowest S-obs to highest.

| ARU | S-obs | Chao 2 | 95% CI Lower | 95% CI Upper | Jack 1 | Jack 2 | ICE | S-obs 80% of lowest estimator? | S-obs within 1 species of 95% CI? |
|---|---|---|---|---|---|---|---|---|---|
| 3636 | 31.0 | 32.0 | 31.1 | 39.3 | 34.0 | 32.0 | 32.7 | Yes | Yes |
| 3647 | 31.0 | 35.0 | 31.6 | 59.5 | 35.0 | 37.0 | 32.9 | Yes | Yes |
| 2932 | 32.0 | 36.4 | 32.5 | 72.6 | 35.0 | 37.0 | 33.5 | Yes | Yes |
| 3663 | 33.0 | 37.1 | 33.7 | 58.6 | 38.0 | 40.0 | 38.2 | Yes | Yes |
| 3671 | 34.0 | 40.0 | 35.0 | 67.4 | 40.0 | 42.9 | 38.4 | Yes | No |
| 3697 | 34.0 | 38.0 | 34.6 | 62.5 | 38.0 | 40.0 | 36.0 | Yes | Yes |
| 3669 | 35.0 | 35.4 | 35.0 | 40.3 | 37.0 | 34.8 | 36.1 | Yes | Yes |
| 3690 | 36.0 | 36.1 | 36.0 | 38.9 | 37.0 | 34.1 | 36.4 | Yes | Yes |
| 3632 | 38.0 | 38.7 | 38.2 | 50.8 | 41.0 | 41.0 | 39.5 | Yes | Yes |
| 3656 | 40.0 | 89.6 | 47.9 | 53.0 | 50.0 | 58.8 | 54.1 | Yes | No |

*Table 5.7.* Mean cumulative species richness (S-mean) in 15 min intervals for each ARU. ARUs were deployed in *P. australis*-dominated habitat in the Big Creek and Long Point NWAs in 2019. S-mean is a hypothetical richness estimate from species accumulation curves that factors out the influence of sample order and takes an average of cumulative richness in each minute. ARUs are ordered from lowest S-mean to highest. Based on this table, transcribing only a 15 min segment of the dawn chorus recording detects only 61-73% (mean = 67%, standard deviation = 5%) of the species recorded vocalizing during the full 2-hour chorus.

| ARU | S-mean at 15 min | S-mean at 30 min | S-mean at 45 min | S-mean at 60 min | S-mean at 75 min | S-mean at 90 min | S-mean at 105 min | S-mean at 120 min |
|---|---|---|---|---|---|---|---|---|
| 3636 | 18.9 | 23.4 | 26.0 | 27.7 | 29.0 | 29.9 | 30.5 | 31.0 |
| 3647 | 18.9 | 23.2 | 25.9 | 27.7 | 29.0 | 29.9 | 30.5 | 31.0 |
| 3671 | 21.2 | 25.2 | 27.8 | 29.6 | 31.1 | 32.2 | 33.2 | 34.0 |
| 3697 | 21.7 | 26.5 | 29.2 | 30.9 | 32.0 | 32.8 | 33.4 | 34.0 |

| ARU | S-mean at 15 min | S-mean at 30 min | S-mean at 45 min | S-mean at 60 min | S-mean at 75 min | S-mean at 90 min | S-mean at 105 min | S-mean at 120 min |
|---|---|---|---|---|---|---|---|---|
| 3663 | 23.2 | 26.5 | 28.3 | 29.6 | 30.6 | 31.6 | 32.4 | 33.0 |
| 2932 | 23.3 | 27.6 | 29.1 | 30.0 | 30.6 | 31.1 | 31.6 | 32.0 |
| 3669 | 23.5 | 28.1 | 30.5 | 32.2 | 33.4 | 34.1 | 34.7 | 35.0 |
| 3656 | 25.6 | 29.5 | 32.2 | 34.1 | 35.8 | 37.3 | 38.7 | 40.0 |
| 3690 | 26.1 | 30.3 | 32.5 | 33.9 | 34.9 | 35.5 | 35.8 | 36.0 |
| 3632 | 26.8 | 31.7 | 34.0 | 35.3 | 36.3 | 37.1 | 37.6 | 38.0 |

*Table 5.8.* Time to capture 80%, 85%, 90% and 95% of observed species richness (S-obs) from mean species richness (S-mean) in each ARU. ARUs were deployed in *P. australis*-dominated habitat in the Big Creek and Long Point NWAs in 2019. S-mean is a hypothetical richness estimate from species accumulation curves that factors out the influence of sample order and takes an average of cumulative richness in each minute.

| ARU | Time to Capture 80% of S-obs from S-mean | Time to Capture 85% of S-obs from S-mean | Time to Capture 90% of S-obs from S-mean | Time to Capture 95% of S-obs from S-mean |
|---|---|---|---|---|
| 3636 | 37 min | 48 min | 62 min | 83 min |
| 3647 | 37 min | 48 min | 62 min | 83 min |
| 2932 | 22 min | 29 min | 42 min | 71 min |
| 3663 | 30 min | 44 min | 62 min | 87 min |
| 3671 | 42 min | 54 min | 71 min | 91 min |
| 3697 | 34 min | 43 min | 47 min | 80 min |
| 3669 | 30 min | 40 min | 53 min | 74 min |
| 3690 | 24 min | 32 min | 45 min | 64 min |
| 3632 | 25 min | 34 min | 47 min | 72 min |
| 3656 | 44 min | 59 min | 77 min | 98 min |
| Average time (± std) | 32.5 min (± 7.5 min) | 43.1 min (± 9.6 min) | 56.8 (± 11.8 min) | 80.3 (± 10.2 min) |
| Range | 25-40 min | 34-53 min | 45-69 min | 70-91 min |

*Table 5.9.* Observed marsh-user species richness (S-obs), non-parametric estimators of "true" species richness: Chao 2, 1st order Jackknife (Jack 1), 2nd order Jackknife (Jack 2), and Incidence-based Coverage Estimator (ICE), and 95% confidence interval for Chao 2 for each ARU. ARUs were deployed in *P. australis*-dominated habitat in the Big Creek and Long Point NWAs in 2019. Also indicated is whether the observed richness is 1) 80% of the lowest non-parametric estimator, 2) within the Chao 2 95% confidence interval, and 3) within one species of the confidence interval. ARUs are ordered from lowest S-obs to highest.

| ARU | S-obs | Chao 2 | 95% CI Lower | 95% CI Upper | Jack 1 | Jack 2 | ICE | S-obs 80% of lowest estimator? | S-obs within 1 species of 95% CI? |
|---|---|---|---|---|---|---|---|---|---|
| 3663 | 15.0 | 19.0 | 15.6 | 43.5 | 19.0 | 21.0 | 21.0 | Yes | Yes |
| 3697 | 17.0 | 18.0 | 17.1 | 28.0 | 19.0 | 19.0 | 17.8 | Yes | Yes |
| 3669 | 17.0 | 17.5 | 17.0 | 23.2 | 19.0 | 17.1 | 17.7 | Yes | Yes |
| 3656 | 17.0 | 26.9 | 18.9 | 69.3 | 22.0 | 26.9 | 24.4 | Yes | No |
| 3671 | 19.0 | 25.2 | 20.0 | 58.8 | 24.0 | 26.9 | 23.5 | Yes | Yes |
| 3690 | 20.0 | 20.5 | 20.0 | 28.4 | 21.0 | 21.0 | 20.4 | Yes | Yes |
| 2932 | 23.0 | 24.0 | 23.1 | 37.2 | 25.0 | 27.0 | 23.7 | Yes | Yes |
| 3632 | 23.0 | 24.0 | 23.1 | 34.0 | 25.0 | 25.0 | 23.7 | Yes | Yes |
| 3636 | 24.0 | 25.1 | 24.1 | 34.0 | 26.0 | 26.0 | 27.0 | Yes | Yes |
| 3647 | 24.0 | 24.5 | 24.0 | 32.4 | 25.0 | 25.0 | 24.3 | Yes | Yes |

*Table 5.10.* Mean cumulative marsh-user species richness (S-mean) in 15 min intervals for each ARU. ARUs were deployed in *P. australis*-dominated habitat in the Big Creek and Long Point NWAs in 2019. S-mean is a hypothetical richness estimate from species accumulation curves that factors out the influence of sample order and takes an average of cumulative richness in each minute. ARUs are ordered from lowest S-mean to highest. Based on this table, transcribing only a 15 min segment of the dawn chorus recording detects only 53-75% (mean = 62.5%, standard deviation = 7.3%) of the marsh-user species recorded vocalizing during the full 2-hour dawn chorus.

| ARU | S-mean at 15 min | S-mean at 30 min | S-mean at 45 min | S-mean at 60 min | S-mean at 75 min | S-mean at 90 min | S-mean at 105 min | S-mean at 120 min |
|---|---|---|---|---|---|---|---|---|
| 3663 | 8.6 | 10.3 | 11.4 | 12.3 | 13.1 | 13.8 | 14.5 | 15.0 |
| 3697 | 10.1 | 12.6 | 14.1 | 15.1 | 15.9 | 16.4 | 16.7 | 17.0 |
| 3669 | 9.5 | 11.8 | 13.4 | 14.6 | 15.6 | 16.2 | 16.7 | 17.0 |
| 3656 | 9.8 | 11.6 | 13.0 | 14.1 | 14.9 | 15.7 | 16.3 | 17.0 |
| 3671 | 10.1 | 12.7 | 14.4 | 15.6 | 16.7 | 17.6 | 18.3 | 19.0 |
| 3690 | 14.3 | 16.7 | 18.0 | 18.8 | 19.3 | 19.6 | 19.8 | 20.0 |

| ARU | S-mean at 15 min | S-mean at 30 min | S-mean at 45 min | S-mean at 60 min | S-mean at 75 min | S-mean at 90 min | S-mean at 105 min | S-mean at 120 min |
|---|---|---|---|---|---|---|---|---|
| 2932 | 17.3 | 20.3 | 21.4 | 21.9 | 22.3 | 22.5 | 22.8 | 23.0 |
| 3632 | 15.6 | 18.6 | 20.2 | 21.1 | 21.8 | 22.3 | 22.7 | 23.0 |
| 3636 | 15.5 | 18.5 | 20.2 | 21.3 | 22.2 | 23.0 | 23.6 | 24.0 |
| 3647 | 16.0 | 19.3 | 21.3 | 22.5 | 23.2 | 23.6 | 23.9 | 24.0 |

*Table 5.11.* Time to capture 80%, 85%, 90% and 95% of observed marsh-user species richness (S-obs) from mean marsh-user species richness (S-mean) in each ARU. ARUs were deployed in *P. australis*-dominated habitat in the Big Creek and Long Point NWAs in 2019. S-mean is a hypothetical richness estimate from species accumulation curves that factors out the influence of sample order and takes an average of cumulative richness in each minute.

| ARU | Time to Capture 80% of S-obs from S-mean | Time to Capture 85% of S-obs from S-mean | Time to Capture 90% of S-obs from S-mean | Time to Capture 95% of S-obs from S-mean |
|---|---|---|---|---|
| 3636-C | 36 | 48 | 65 | 86 |
| 3647-C | 30 | 38 | 49 | 65 |
| 2932-T | 19 | 25 | 34 | 58 |
| 3663-T | 55 | 68 | 83 | 99 |
| 3671-C | 55 | 68 | 82 | 100 |
| 3697-C | 40 | 50 | 63 | 84 |
| 3669-C | 48 | 58 | 71 | 89 |
| 3690-T | 25 | 34 | 46 | 66 |
| 3632-T | 29 | 39 | 53 | 76 |
| 3656-T | 53 | 66 | 82 | 101 |
| Average time (± std) | 39 min (± 12 min) | 49.4 min (± 14 min) | 62.8 min (± 16 min) | 82.4 min (± 14 min) |
| Range | 19-55 min | 25-66 min | 34-82 min | 58-101 min |

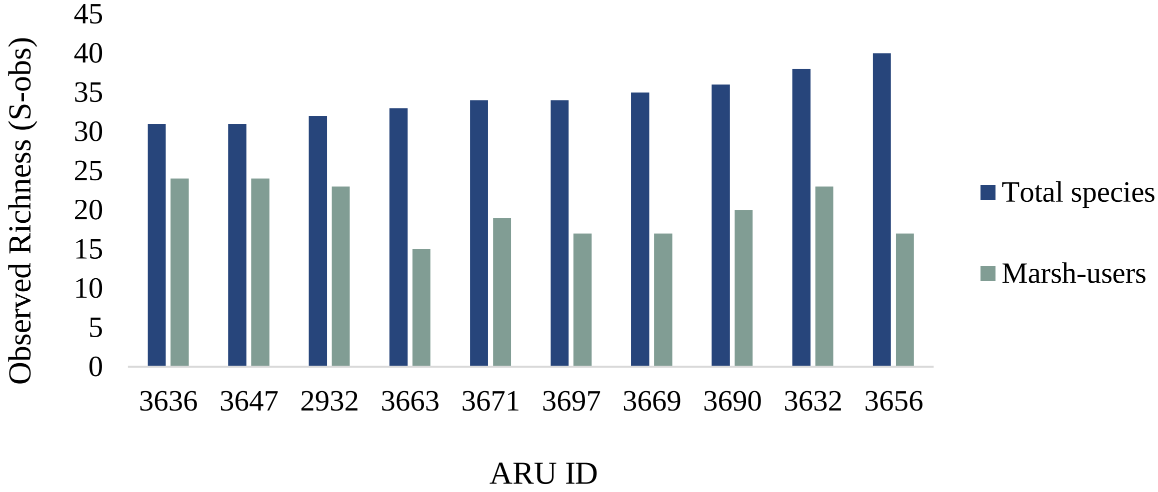
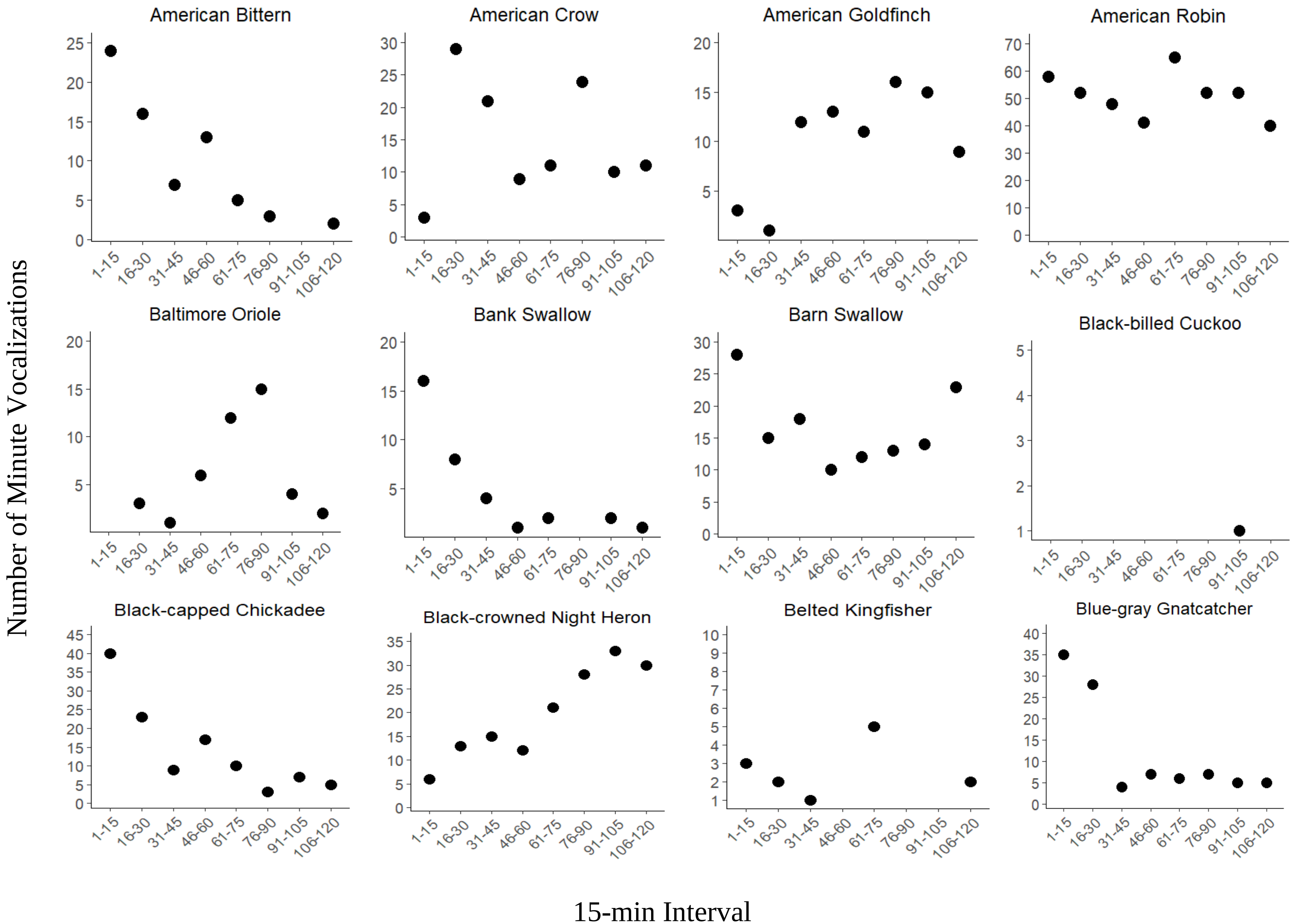
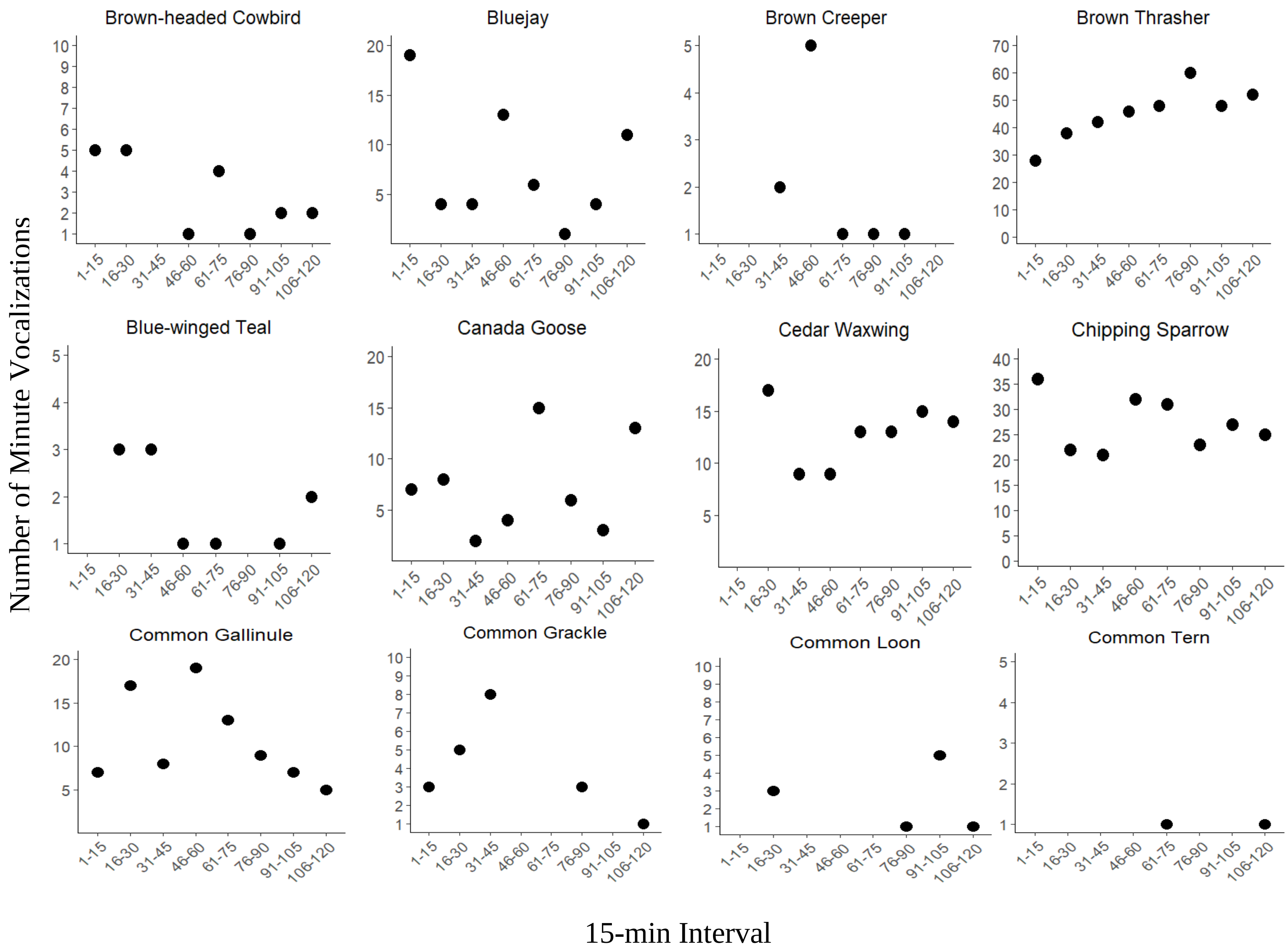


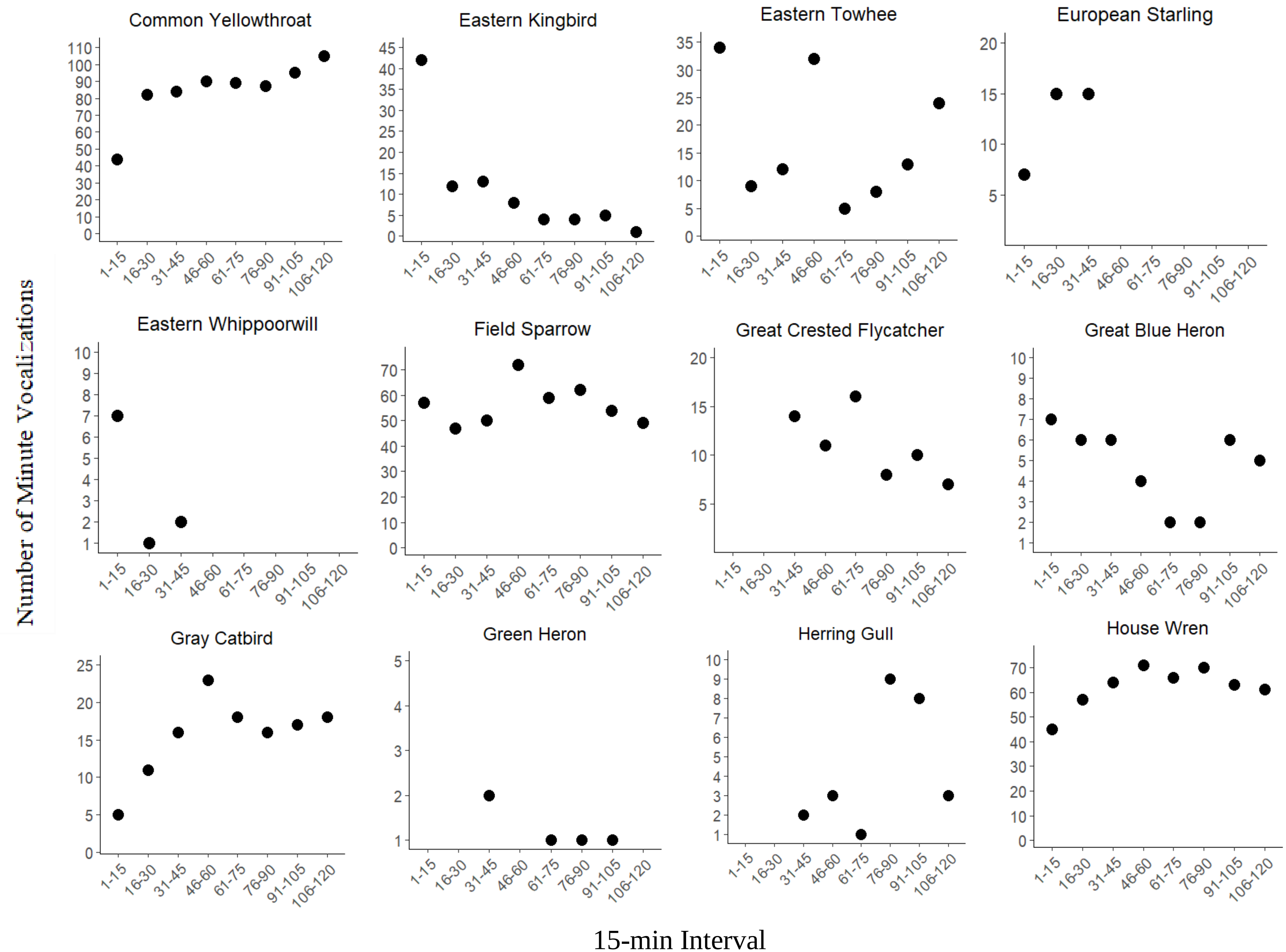

*Figure 5.5.* Observed richness (S-obs) for total species and marsh-users detected at the 10 ARUs deployed in *P. australis*-dominated habitat in the Big Creek and Long Point NWAs in 2019.

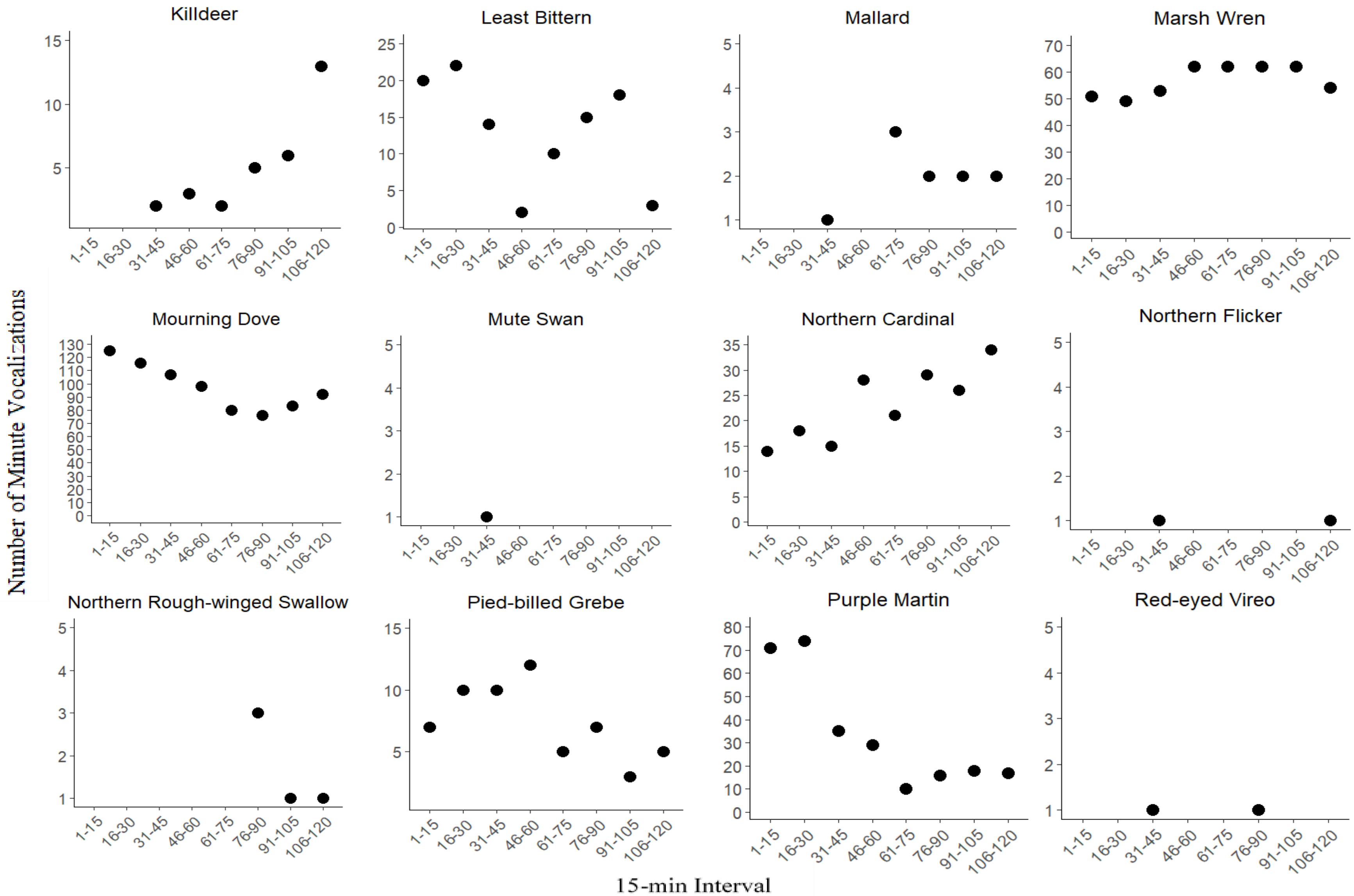
*Appendix 2B.*

The number of minutes summed across the 10 ARUs, during which each species of bird was detected vocalizing, broken down into 15 min intervals. ARUs were deployed in *P. australis* -dominated habitat in Big Creek and Long Point NWAs in 2019. The surveys commenced with minute “1” initiating 30 min prior to dawn and minute 120 occurring 90 min after dawn, on the survey date. For clarity, if a species was heard vocalizing continuously throughout a 15 min interval at all 10 ARUs, it would receive the maximum number of minute vocalizations of 150. If a bird species was heard vocalizing continuously across a 15 min interval at only one ARU, it would receive a number of minute vocalizations of 15. To be counted, the species must have been detected vocalizing at one ARU at least once within a 15 min interval, but multiple calls within a single minute would only count as 1-minute vocalization.

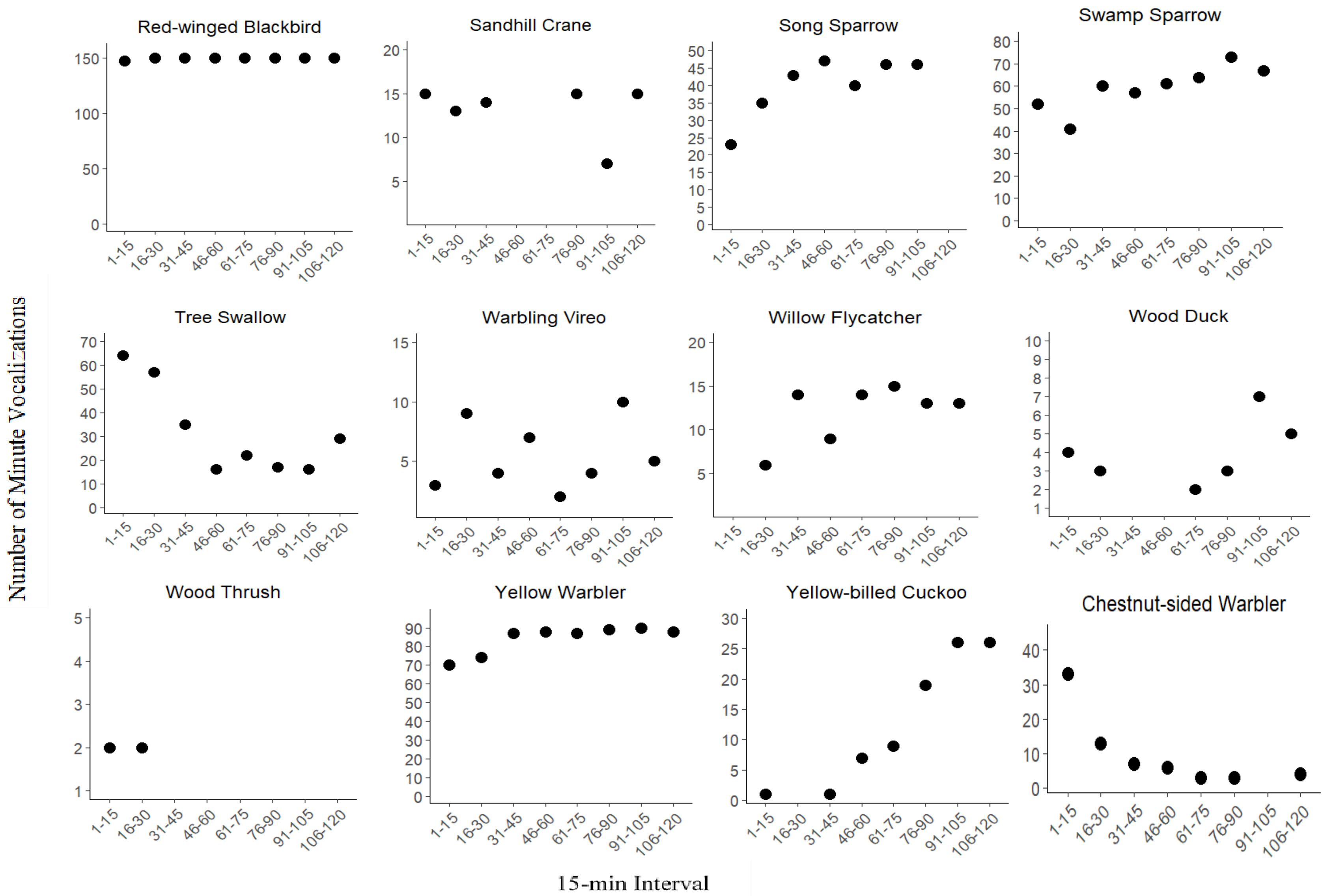

American Bittern
American Crow
American Goldfinch
American Robin
Baltimore Oriole
Bank Swallow
Barn Swallow
Black-billed Cuckoo
Black-capped Chickadee
Black-crowned Night Heron
Belted Kingfisher
Blue-gray Gnatcatcher
1-15
16-30
31-45
46-60
61-75
76-90
91-105
106-120
Number of Minute Vocalizations
15-min Interval


Brown-headed Cowbird
Bluejay
Brown Creeper
Brown Thrasher
Blue-winged Teal
Canada Goose
Cedar Waxwing
Chipping Sparrow
Common Gallinule
Common Grackle
Common Loon
Common Tern
Number of Minute Vocalizations
1-15
16-30
31-45
46-60
61-75
76-90
91-105
106-120
15-min Interval


Common Yellowthroat
Eastern Kingbird
Eastern Towhee
European Starling
Eastern Whippoorwill
Field Sparrow
Great Crested Flycatcher
Great Blue Heron
Gray Catbird
Green Heron
Herring Gull
House Wren
Number of Minute Vocalizations
1-15
16-30
31-45
46-60
61-75
76-90
91-105
106-120
15-min Interval

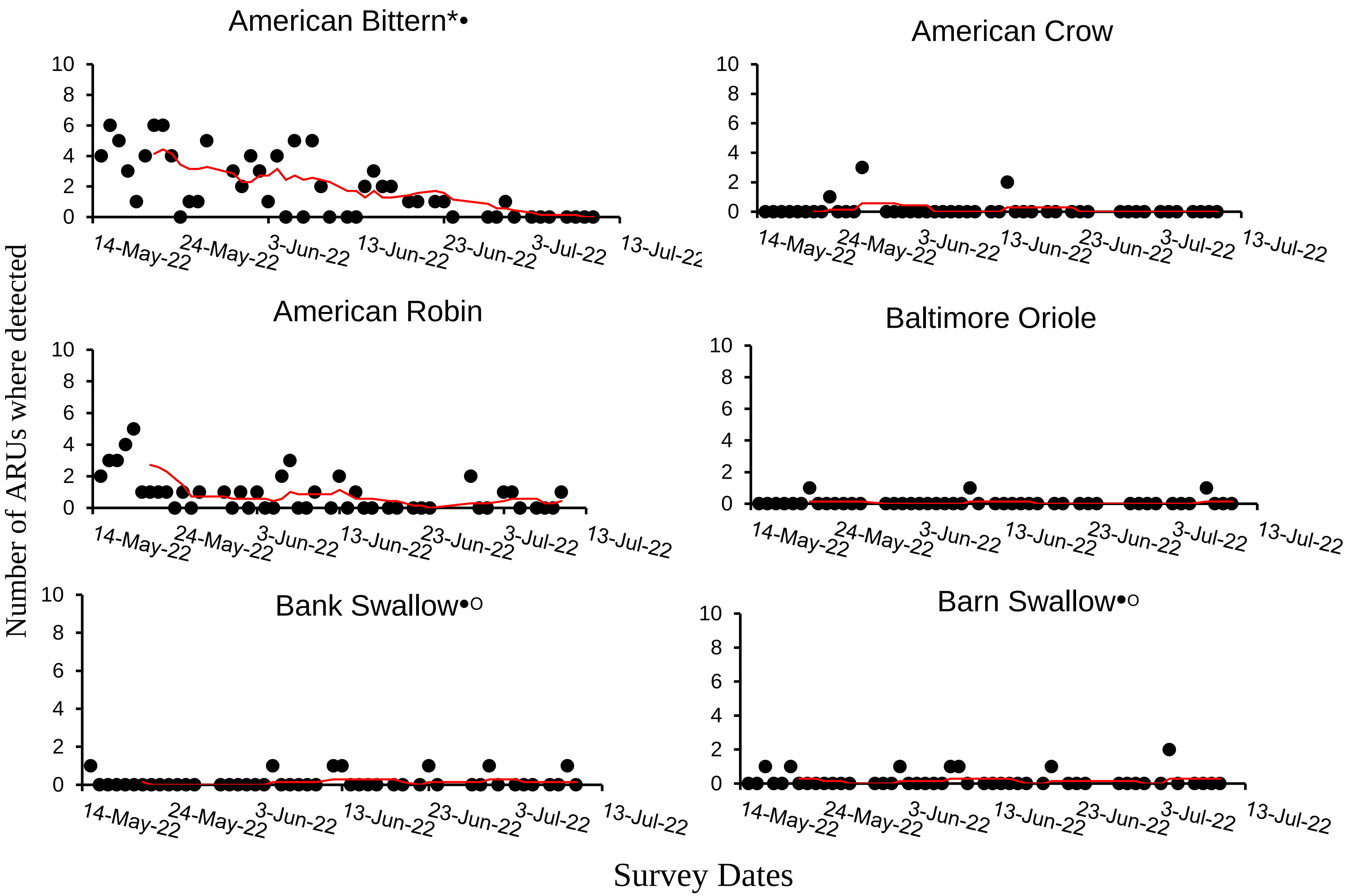


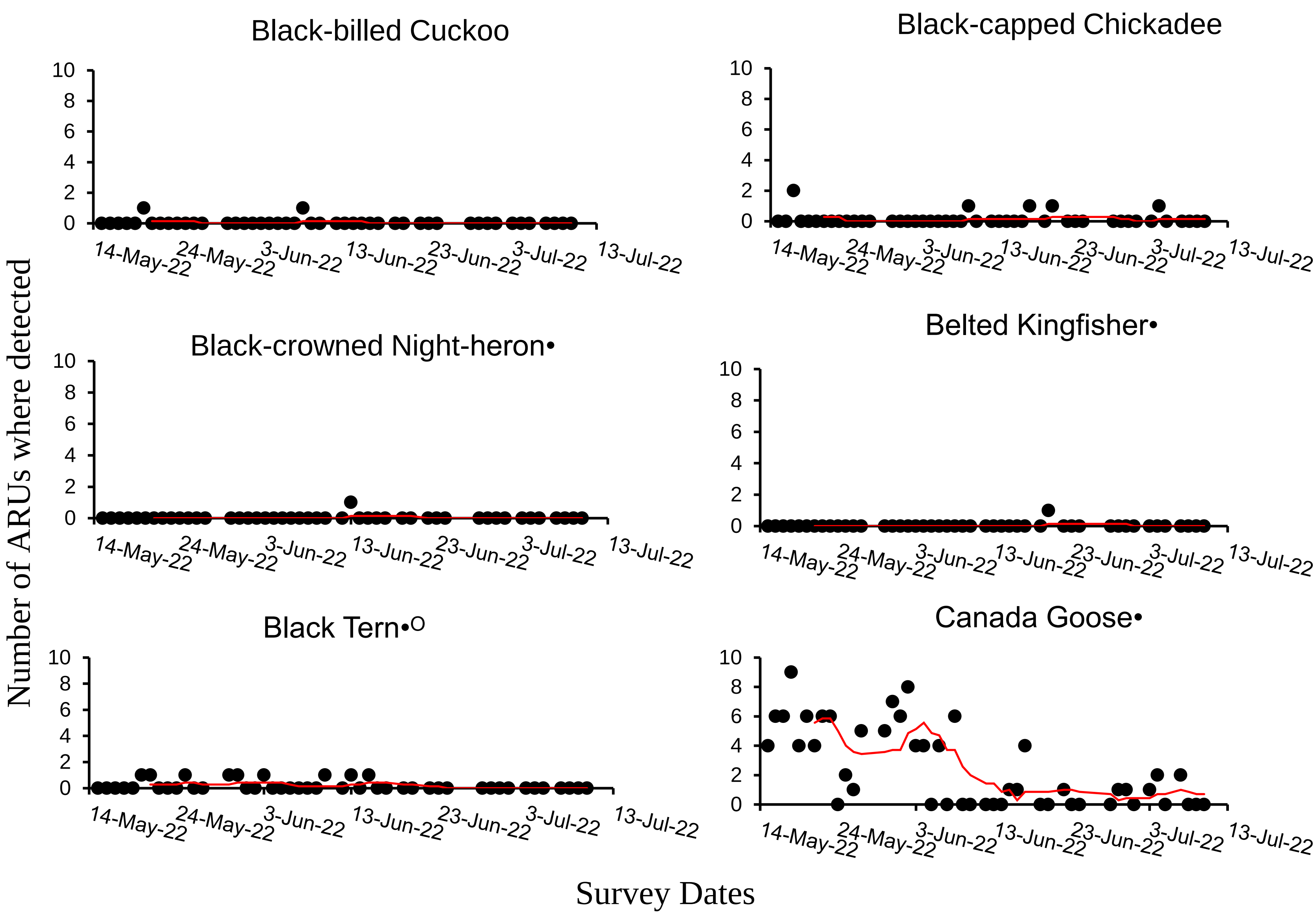
Number of Minute Vocalizations
Killdeer
Least Bittern
Mallard
Marsh Wren
Mourning Dove
Mute Swan
Northern Cardinal
Northern Flicker
Northern Rough-winged Swallow
Pied-billed Grebe
Purple Martin
Red-eyed Vireo
1-15
16-30
31-45
46-60
61-75
76-90
91-105
106-120
15-min Interval

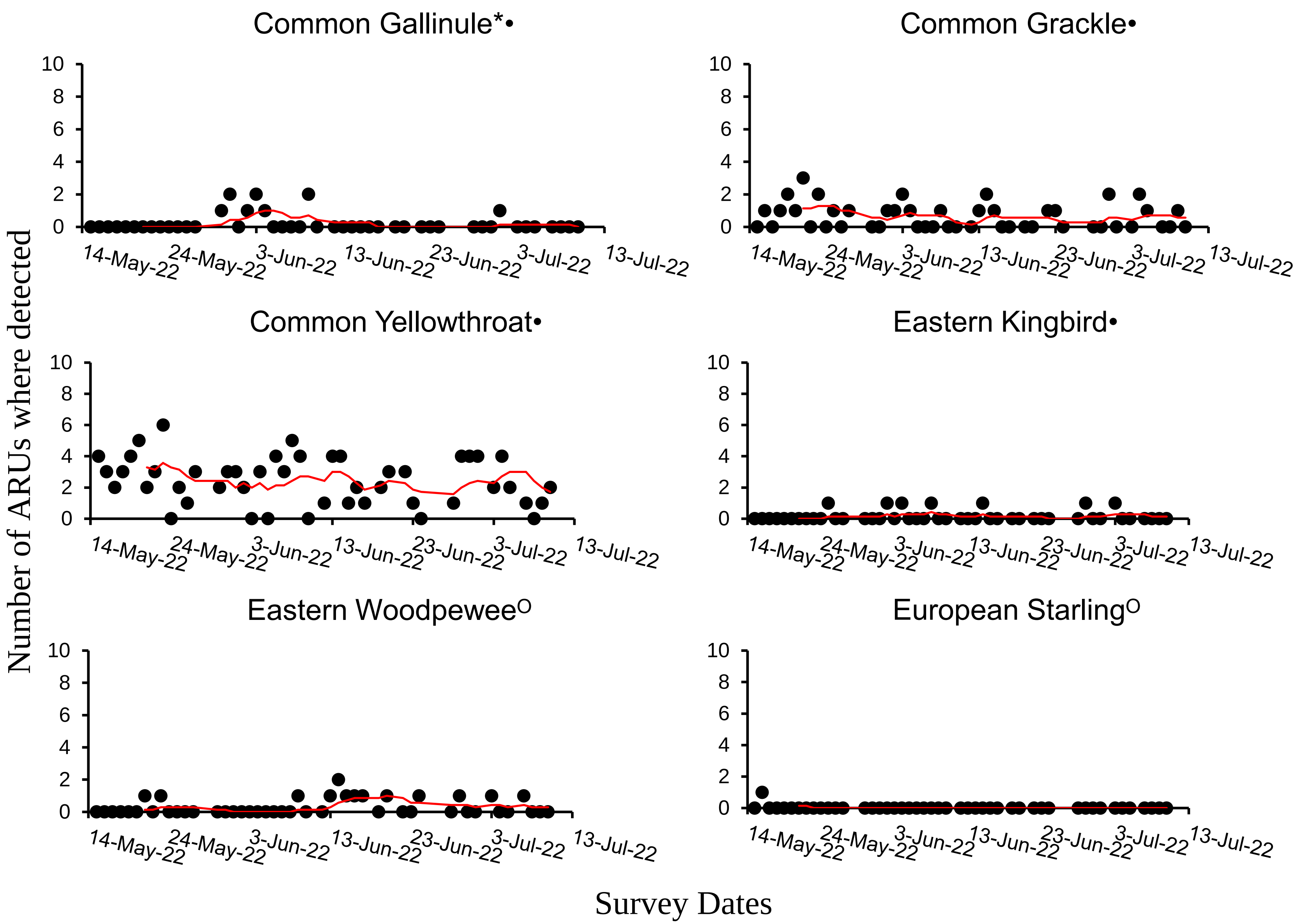

Red-winged Blackbird
Sandhill Crane
Song Sparrow
Swamp Sparrow
Tree Swallow
Warbling Vireo
Willow Flycatcher
Wood Duck
Wood Thrush
Yellow Warbler
Yellow-billed Cuckoo
Chestnut-sided Warbler
1-15
16-30
31-45
46-60
61-75
76-90
91-105
106-120
Number of Minute Vocalizations
15-min Interval

*Appendix 2C.*

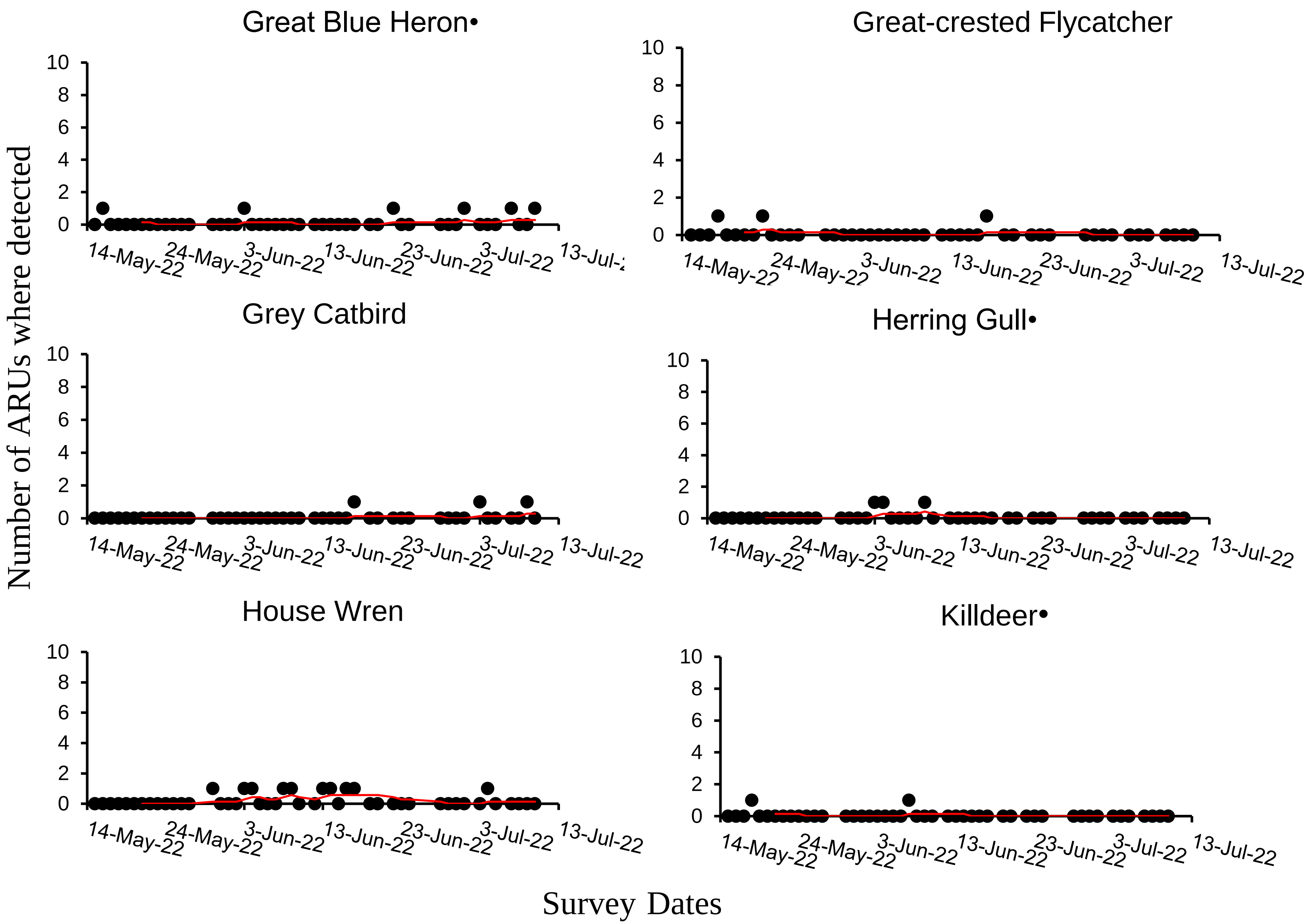
The number of days summed across the 10 ARUs during which each bird species was detected vocalizing in at least once. ARUs were deployed in *P. australis*, cattail, and treated *P. australis* habitat (1-year-post herbicide-rolling) in the Big Creek and Long Point NWAs in 2021. Each ARU was transcribed for 30 days out of the total 57 days across the breeding bird season (May 20$^{th}$ – July 5$^{th}$, 2021, +/- 5 days). The red trendline indicates a 7-day moving average. ARUs were transcribed 1 minute a day at either 6 am or 7 am. Marsh-user species are indicated with a filled dot (•), species of conservation concern are indicated with an asterisk (*) and species at risk are indicated with an open dot (°).

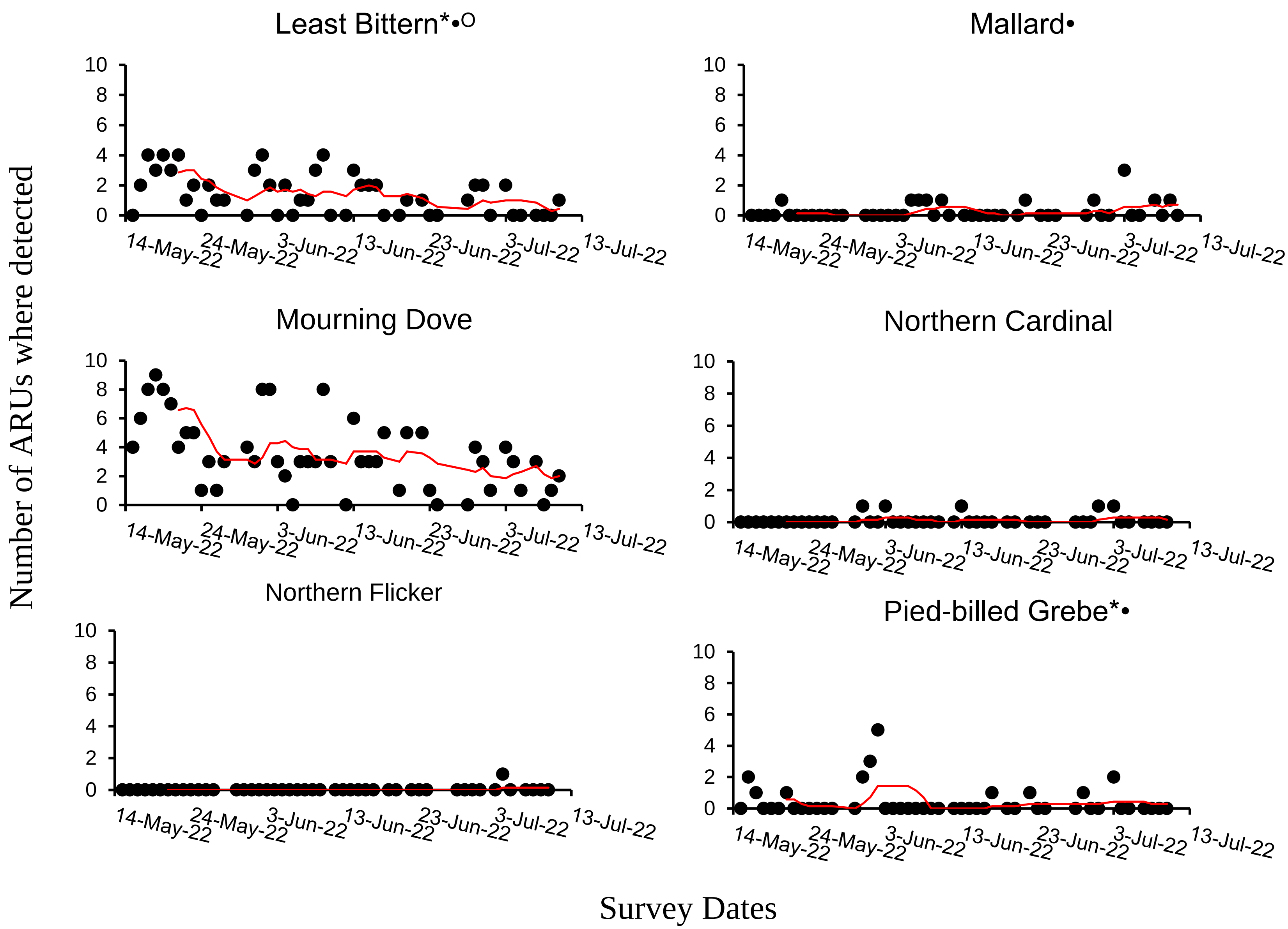

American Bittern*•
American Crow
American Robin
Baltimore Oriole
Bank Swallow•○
Barn Swallow•○
0
2
4
6
8
10
14-May-22
24-May-22
3-Jun-22
13-Jun-22
23-Jun-22
3-Jul-22
13-Jul-22
Number of ARUs where detected
Survey Dates


Black-billed Cuckoo
Black-capped Chickadee
Black-crowned Night-heron•
Belted Kingfisher•
Black Tern•O
Canada Goose•
0
2
4
6
8
10
14-May-22
24-May-22
3-Jun-22
13-Jun-22
23-Jun-22
3-Jul-22
13-Jul-22
Number of ARUs where detected
Survey Dates

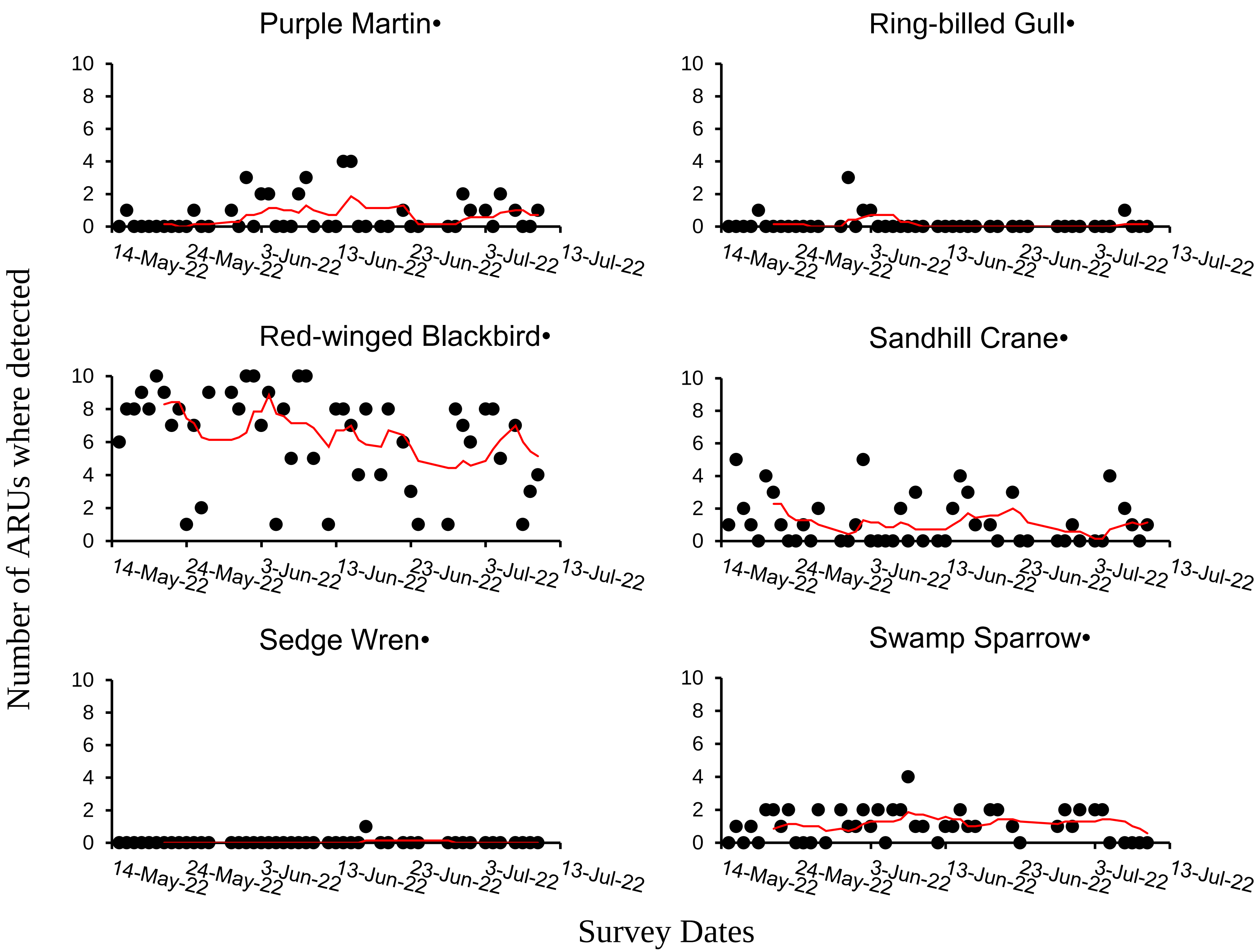


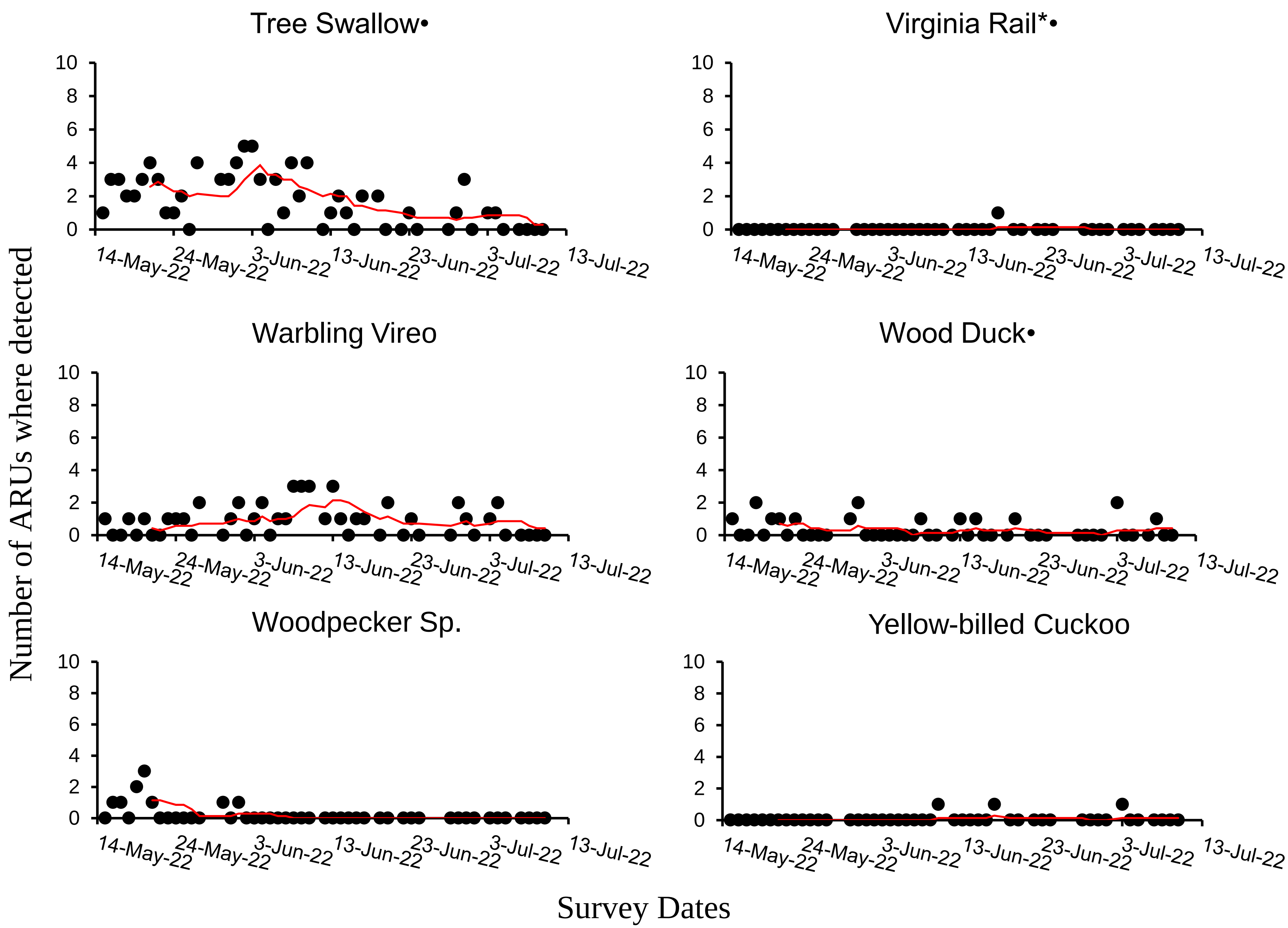
Common Gallinule*•
Common Grackle•
Common Yellowthroat•
Eastern Kingbird•
Eastern WoodpeweeO
European StarlingO
Number of ARUs where detected
0
2
4
6
8
10
14-May-22
24-May-22
3-Jun-22
13-Jun-22
23-Jun-22
3-Jul-22
13-Jul-22
Survey Dates


Great Blue Heron•
Great-crested Flycatcher
Grey Catbird
Herring Gull•
House Wren
Killdeer•
Number of ARUs where detected
0
2
4
6
8
10
14-May-22
24-May-22
3-Jun-22
13-Jun-22
23-Jun-22
3-Jul-22
13-Jul-22
Survey Dates

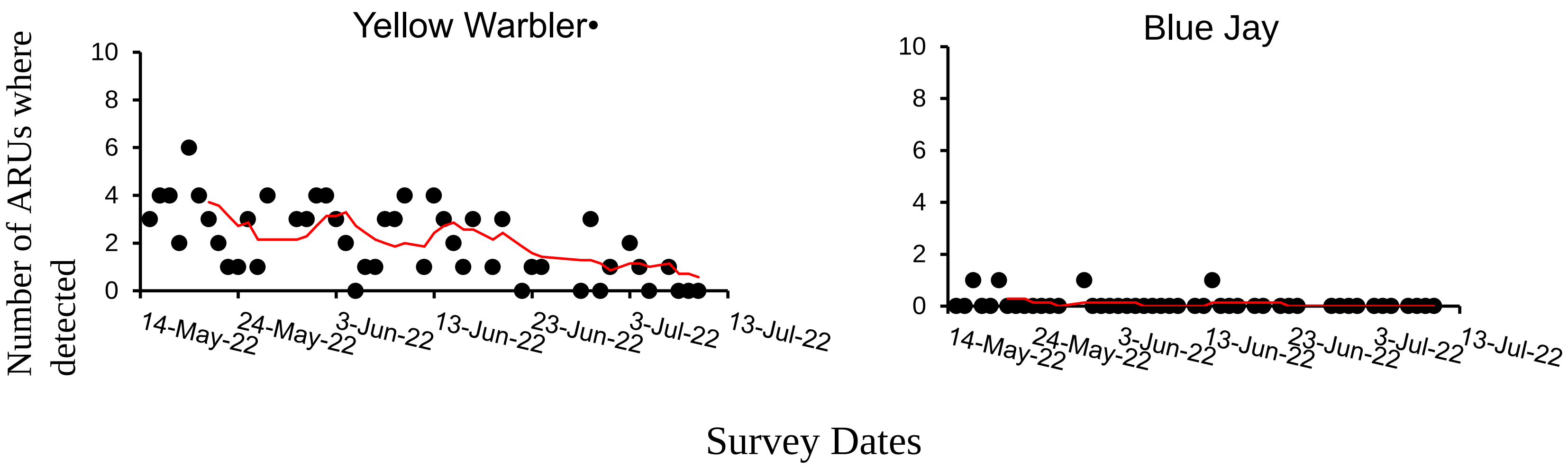


Least Bittern*•○
Mallard•
Mourning Dove
Northern Cardinal
Northern Flicker
Pied-billed Grebe*•
0
2
4
6
8
10
14-May-22
24-May-22
3-Jun-22
13-Jun-22
23-Jun-22
3-Jul-22
13-Jul-22
Number of ARUs where detected
Survey Dates


Purple Martin•
Ring-billed Gull•
Red-winged Blackbird•
Sandhill Crane•
Sedge Wren•
Swamp Sparrow•
Number of ARUs where detected
0
2
4
6
8
10
14-May-22
24-May-22
3-Jun-22
13-Jun-22
23-Jun-22
3-Jul-22
13-Jul-22
Survey Dates



Tree Swallow•
Virginia Rail*•
Warbling Vireo
Wood Duck•
Woodpecker Sp.
Yellow-billed Cuckoo
0
2
4
6
8
10
14-May-22
24-May-22
3-Jun-22
13-Jun-22
23-Jun-22
3-Jul-22
13-Jul-22
Number of ARUs where detected
Survey Dates


Yellow Warbler•
Blue Jay
Number of ARUs where detected
Survey Dates
0
2
4
6
8
10
14-May-22
24-May-22
3-Jun-22
13-Jun-22
23-Jun-22
3-Jul-22
13-Jul-22

*Appendix 2D.*

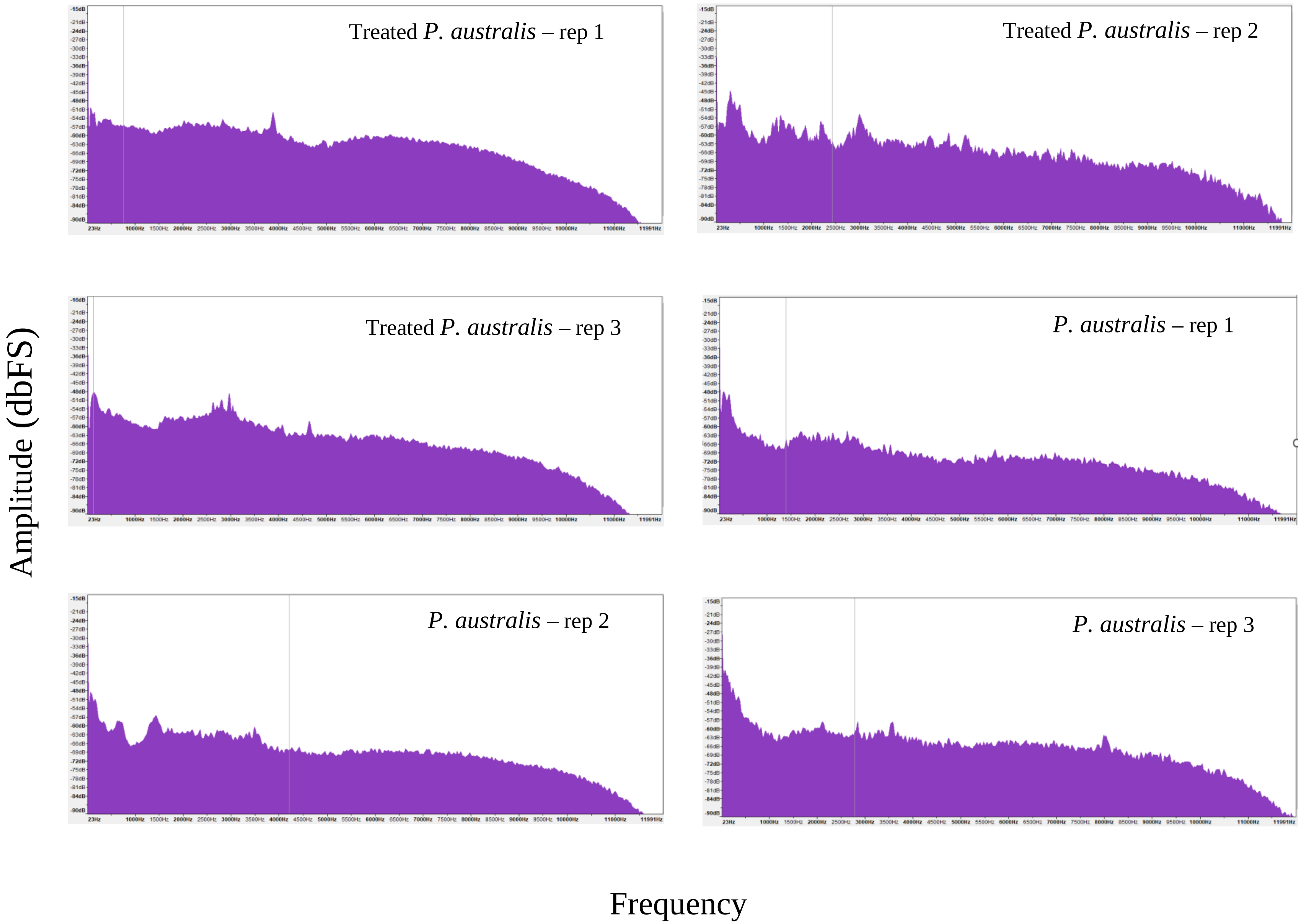
*Table 5.12.* Correlation coefficients (r) and coefficient of determination ($r^2$) of vectors in the optimal NMS ordination for bird community composition detected after transcribing 10 ARUs by two methods: 1) "one day"; transcribing 30 mins of the dawn chorus on one day in June, and 2) "season"; transcribing 30 days across the breeding season (mid-May to early July). Species with an $r^2 \geq 0.20$ were considered reasonably correlated and included in Figure 3.9. ARUs were deployed in *P. australis*, cattail, and treated *P. australis* habitat (1-year-post herbicide-rolling) in the Big Creek and Long Point NWAs in 2021.

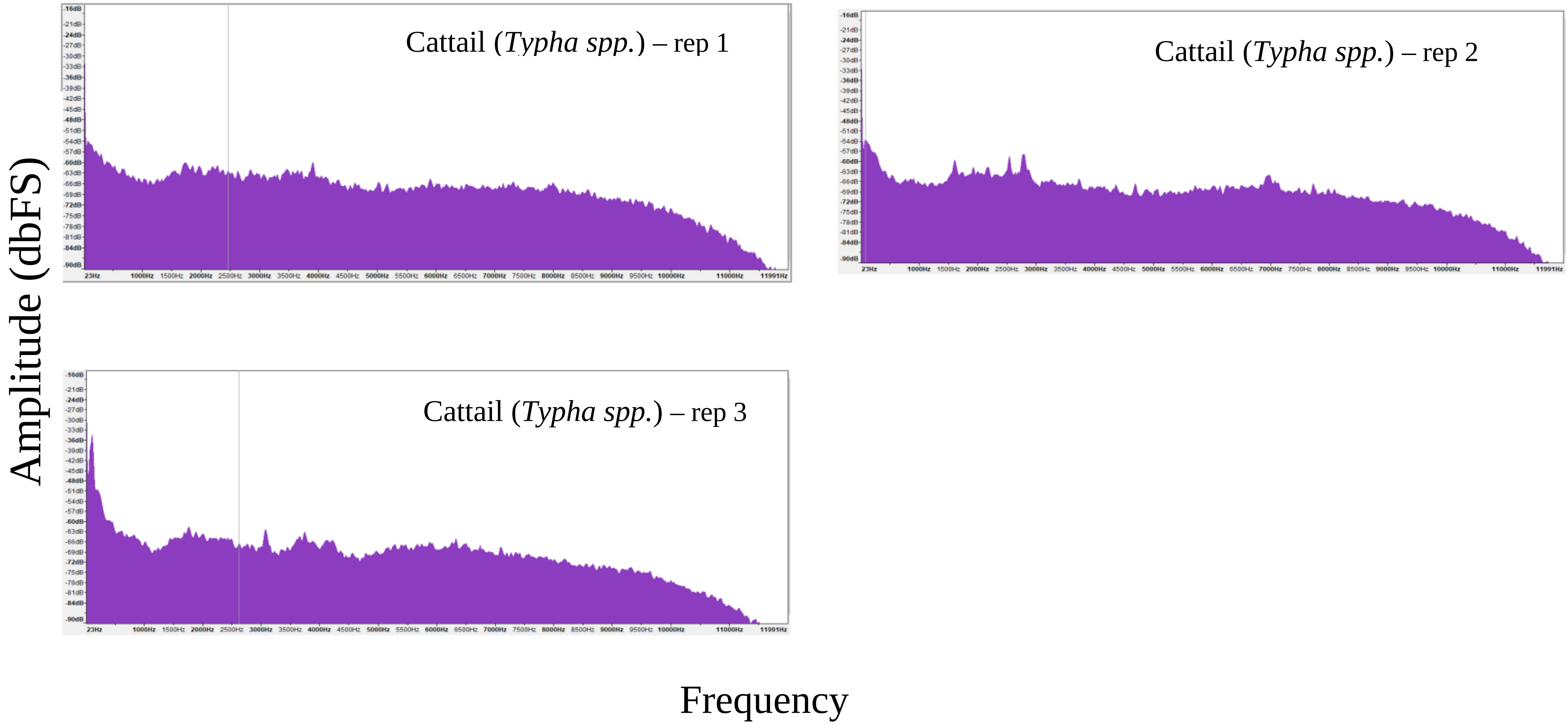
| | | **Axis 1** | | **Axis 2** | |
|---|---|---|---|---|---|
| **Common Name** | **4-Letter Alpha Code** | **r** | **$r^2$** | **r** | **$r^2$** |
| American Bittern | AMBI | 0.549 | 0.302 | -0.614 | 0.377 |
| American Crow | AMCR | 0.493 | 0.243 | -0.322 | 0.103 |
| American Goldfinch | AMGO | 0.307 | 0.094 | 0.224 | 0.05 |
| American Robin | AMRO | -0.595 | 0.355 | 0.007 | 0 |
| Baltimore Oriole | BAOR | -0.031 | 0.001 | -0.183 | 0.034 |
| Bank Swallow | BANS | 0.351 | 0.123 | 0.057 | 0.003 |
| Barn Swallow | BARS | 0.2 | 0.04 | -0.194 | 0.038 |
| Belted Kingfisher | BEKI | 0.341 | 0.116 | -0.29 | 0.084 |
| Black Tern | BLTE | -0.355 | 0.126 | -0.521 | 0.272 |
| Black-billed Cuckoo | BBCU | -0.277 | 0.077 | -0.414 | 0.171 |
| Black-capped Chickadee | BCCH | -0.411 | 0.169 | 0.245 | 0.06 |
| Black-crowned Night-heron | BCNH | 0.127 | 0.016 | -0.256 | 0.065 |
| Blue Jay | BLJA | -0.551 | 0.304 | 0.205 | 0.042 |
| Canada Goose | CAGO | 0.684 | 0.468 | -0.331 | 0.109 |
| Chipping Sparrow | CHSP | 0.187 | 0.035 | 0.068 | 0.005 |
| Common Gallinule | COGA | 0.688 | 0.473 | 0.413 | 0.17 |
| Common Grackle | COGR | -0.136 | 0.018 | -0.127 | 0.016 |
| Common Nighthawk | CONI | -0.153 | 0.023 | 0.271 | 0.073 |
| Common Raven | CORA | 0.001 | 0 | 0.336 | 0.113 |
| Common Yellowthroat | COYE | -0.112 | 0.013 | -0.481 | 0.231 |
| Eastern Kingbird | EAKI | -0.561 | 0.315 | 0.069 | 0.005 |
| Eastern Wood-pewee | EAWP | -0.758 | 0.575 | -0.173 | 0.03 |
| European Starling | EUST | 0.086 | 0.007 | 0.01 | 0 |
| Gray Catbird | GRCA | -0.58 | 0.337 | -0.502 | 0.252 |
| Great Blue Heron | GBHE | 0.24 | 0.058 | -0.395 | 0.156 |
| Great-crested Flycatcher | GCFL | -0.106 | 0.011 | -0.111 | 0.012 |
| Herring Gull | HERG | 0.081 | 0.007 | -0.171 | 0.029 |
| House Wren | HOWR | -0.637 | 0.406 | -0.383 | 0.147 |
| Killdeer | KILL | 0.08 | 0.006 | 0.027 | 0.001 |

| | | Axis 1 | | Axis 2 | |
|---|---|---|---|---|---|
| **Common Name** | **4-Letter Alpha Code** | **r** | **$r^2$** | **r** | **$r^2$** |
| Least Bittern | LEBI | -0.162 | 0.026 | -0.2 | 0.04 |
| Mallard | MALL | 0.673 | 0.453 | 0.17 | 0.029 |
| Marsh Wren | MAWR | Present at every ARU | | | |
| Mourning Dove | MODO | Present at every ARU | | | |
| Northern Cardinal | NOCA | -0.43 | 0.185 | 0.194 | 0.038 |
| Northern Flicker | NOFL | -0.505 | 0.255 | -0.067 | 0.004 |
| Orchard Oriole | OROR | 0.307 | 0.094 | 0.224 | 0.05 |
| Pied-billed Grebe | PBGR | -0.127 | 0.016 | 0.212 | 0.045 |
| Purple Martin | PUMA | 0.373 | 0.139 | 0.47 | 0.221 |
| Red-bellied Woodpecker | RBWO | 0.016 | 0 | 0.117 | 0.014 |
| Red-winged Blackbird | RWBL | -0.341 | 0.116 | 0.29 | 0.084 |
| Ring-billed Gull | RBGU | 0.359 | 0.129 | -0.424 | 0.18 |
| Sandhill Crane | SACR | 0.596 | 0.355 | -0.241 | 0.058 |
| Sedge Wren | SEDW | 0.353 | 0.125 | -0.012 | 0 |
| Sora | SORA | -0.438 | 0.191 | -0.175 | 0.031 |
| Swamp Sparrow | SWSP | 0.742 | 0.551 | 0.079 | 0.006 |
| Tree Swallow | TRES | -0.505 | 0.255 | 0.219 | 0.048 |
| Virginia Rail | VIRA | -0.368 | 0.135 | 0.464 | 0.215 |
| Warbling Vireo | WAVI | -0.71 | 0.504 | -0.261 | 0.068 |
| Wood Duck | WODU | 0.14 | 0.02 | -0.547 | 0.299 |
| Woodpecker Sp. | - | -0.397 | 0.158 | -0.493 | 0.243 |
| Yellow Warbler | YEWA | -0.748 | 0.56 | -0.01 | 0 |
| Yellow-billed Cuckoo | YBCU | -0.78 | 0.609 | -0.08 | 0.006 |

*Appendix 2E.*

Spectrogram plots of 1-3 second clips of background noise on SM4 ARU recordings in three vegetation types: cattail (*Typha* spp.), treated *P. australis* (1-year-post herbicide-rolling), and *P. australis*. Recordings were taken during 2021 detection radius surveys in the Big Creek NWA. The higher the purple curve (i.e., greater decibels), the louder the noise. Note – plots were reduced in size to fit on page and therefore were not used in the AUC calculations.


Treated *P. australis* – rep 1
Treated *P. australis* – rep 2
Treated *P. australis* – rep 3
*P. australis* – rep 1
*P. australis* – rep 2
*P. australis* – rep 3
Amplitude (dbFS)
Frequency


Cattail (*Typha spp.*) – rep 1
Cattail (*Typha spp.*) – rep 2
Cattail (*Typha spp.*) – rep 3
Amplitude (dBFS)
Frequency