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Pond-breeding amphibian responses to wetland creation and reforestation on a legacy surface mine in the Monongahela National Forest

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POND-BREEDING AMPHIBIAN RESPONSES TO WETLAND CREATION AND REFORESTATION ON A LEGACY SURFACE MINE IN THE MONONGAHELA NATIONAL FOREST

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture, Food, and Environment at the University of Kentucky

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Abstract

Surface mine reclamation has been an evolving practice since the Surface Mining Control and Reclamation Act was passed in 1977, holding mining companies accountable for returning ecological function to areas directly impacted by mining activities. One recent method of reclamation, the Forestry Reclamation Approach (FRA), aims to enhance reforestation and ecosystem function through the creation of wetlands, as opposed to traditional methods that often revert land to grasslands. However, wildlife response to FRA has rarely been investigated. The goal of this project was to analyze the effects of the four treatment types, FRA in two chronosequences, natural regeneration, and unmined mature forest, on amphibian occupancy, species richness and abundance. The results of this project show that FRA wetlands are able to support most pond-breeding amphibian species in the region. I found that species had similar occupancy and species richness estimates values across the four treatments. Abundance estimates were lower in young FRA sites for forest-associated species, such as *L. sylvaticus* and *A. maculatum*, but higher abundance estimates in older FRA sites suggest that these species will be able to successfully utilize these wetlands in the near future.

KEYWORDS: Forestry Reclamation Approach, Amphibian, Coal Mining, Occupancy, Abundance, Wetlands

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DEDICATION

For Phil Sarver, who instilled within me a deep love of science that transformed me from a kid who spent her free time catching frogs into a researcher. Your kindness, persistence, and blind faith in my ability to do anything will stay with me always.

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Chapter 1. Introduction

Natural resource extraction practices have always been challenged with the task of repairing environmental conditions and mitigating long-term damage post extraction. This is especially true for surface coal mining practices in the Central Appalachians, where, prior to the 1970s, there were no federal regulations on how mining companies were expected to leave the land after the resource extraction process (Zipper, 2000). The lack of regulation led to steep high walls and benches, completely restructuring the physical environment (Bugosh, 2009). In 1977, the Surface Mining Control and Reclamation Act (SMCRA) mandated mining companies to make a concerted effort to restore the ecosystem or convert the land to other land-uses (i.e., agriculture, pasture, wildlife habitat). SMCRA also required mining companies to obtain a permit that detailed environmental controls that would be in place during the extraction process and outline reclamation plans prior to any soil disturbance to combat problems associated with reclamation practices (Skousen & Zipper, 2014). The wording of SMCRA, however, has allowed wide interpretations of how reclamation should be approached, and these interpretations have been met with disparate levels of relative success in restoring the previous ecosystem function.

Under SMCRA, reclamation often resulted in the conversion of reclaimed lands into grasslands and shrublands, as reclamation was commonly interpreted as simply recreating a naturalized slope to stabilize the soil, preventing erosion, and maintaining water quality (Zipper et al., 2011; Haynes, 1986). Heavy machinery was used to compact the soil after post-mining and non-native grasses were planted to inhibit erosion and

landslides. These activities, known collectively as conventional reclamation, prevented the establishment of native tree species due to limited root growth in compacted soils (Burger, 2011; Williamson & Gray, 1996). Consequently, conventional reclamation resulted in decreased native plant biodiversity, poor hydrologic function, forest loss, and low-quality, homogenous landscapes which lack the complexity needed to support forest-dependent wildlife (Bugosh, 2009; Rhodes, 2022; US Forest Service, 2016). These lands are referred to as legacy mines since they are no longer the responsibility of the mine operators and are commonly converted to different land uses including grazing lands or commercial real estate. These conventional reclamation methods are not necessarily implemented with the goal of restoring native ecosystems, which can negatively impact local wildlife.

An alternative to conventional reclamation, the Forestry Reclamation Approach (FRA), was developed in 2005 to restore ecological function through the establishment of native forests post-mining activities. Now widely applied in coal fields of the Appalachian region, this method creates suitable substrate for tree growth (deeper than 1.22 m) by leaving the topsoil loosely graded, planting of ground cover vegetation to reduce erosion, and planting early successional and commercially desired tree species to anchor the soil and create wildlife habitat (Burger & Graves, 2005). In this way, the FRA has been cited as beneficial to both the economic interests of the region and the health and function of the environment (Angel et al., 2009; Burger & Graves, 2005; Burger et al., 2013). The FRA has also been used to restore legacy mines through soil decompaction, non-native species removal, planting of native trees and shrubs, and with wetland creation, which can serve as both habitat for aquatic and semi-aquatic wildlife

and carbon sinks (Rosli et al., 2017). The FRA may result in restored minelands that have ecological conditions that are more similar to the native forest in the Appalachian region than those resulting from conventional reclamation. Not only does this provide habitat to wildlife in a time of climate uncertainty, but it also contributes to a more resilient ecosystem that is more suited to withstand the increasing impacts of climate change (Andres et al., 2023).

Wildlife responses to conventional (grassland) mine reclamation have been thoroughly examined, with most studies indicating reductions in abundances and species richness or changes in community composition in conventionally reclaimed sites compared to unmined forests (i.e., Lacki et al. 2004; Williams et al. 2017; Margenau et al. 2019). In particular, aquatic and semi-aquatic species on conventionally reclaimed minelands are greatly reduced compared to nearby forested sites (Bernhardt et al., 2012; Cianciolo et al., 2020; Gingerich, 2009; Matter & Ney, 1981; McAuley & Kozar, 2006; Wei et al., 2011, Lindberg et al., 2011, Merovich et al., 2021, Petty et al., 2013, Pond et al., 2008). A meta-analysis of freshwater biodiversity surveys in the Appalachian region showed significant differences in water quality between unmined streams and post-reclamation streams, showing that even carefully "restored" ecosystems have not recovered from mining activities (Giam et al., 2018). In addition to water chemistry, forest and wetland loss on conventionally reclaimed mined lands may also contribute to the reduction of aquatic and semi-aquatic species. These impacts on freshwater stream communities provide evidence that traditional reclamation practices have failed to return the landscape to its previous, unmined function.

In general, few investigations have examined the response of wildlife to the FRA on legacy surface mines. One study has been conducted on herbivore exclusion practices as a method of promoting tree growth on FRA sites that found such practices are beneficial at reducing deer browsing on seedlings (Hackworth et al., 2018). Another study found that FRA wetlands are utilized by bat populations and that there is comparable insect abundance and biomass at these sites for bat predation (Snyder, 2022). This study shows that FRA sites can have similar productivity to regenerated sites and even mature, unmined sites. Avian communities can also benefit from FRA practices, as shown by Davenport (2023), which reported usage of FRA habitat by many bird species, particularly those that prefer shrublands and/or forest edges, though these sites are expected to become suitable habitat for forest-associated species as the planted trees continue to develop.

Amphibians have been shown to be particularly impacted by surface mining. Stream-inhabiting amphibians show reductions in occupancy, abundance and species richness on conventionally reclaimed mined lands compared to forest sites (Williams, 2003; Muncy et al., 2014; Price et al., 2016; Price et al., 2018) and elevated levels of specific conductance, metals, and ions may be the mechanism responsible for these changes (Hutton et al. 2020). However, many wetland-associated amphibian species have high occupancy and abundances on surface mines. For example, Lambert et al. (2021) found that constructed wetlands on surface mines serve as suitable habitat for a variety of species. This study examined several age classes of constructed wetlands on FRA sites and found that even wetlands as young as 2 years old were able to be supported by multiple species. The constructed wetlands studied by Lambert et al. (2021) had

relatively good water quality (i.e. low specific conductance, etc.) as most were precipitation-fed. Lambert et al. (2021) provided evidence that amphibians can occupy constructed wetlands on FRA sites, but the impact of the FRA on occupancy, species richness, and abundance of amphibian species has yet to be directly compared to sites that have had no reclamation and sites that have never been mined.

The objectives of this study are as follows: 1. Evaluate differences in water chemistry and environmental/landscape variables across treatment types (two age classes of FRA sites, naturally regenerated sites, and mature, unmined sites) and, 2. Determine how amphibian species occupancy, species richness, and species abundance varies across treatment types. Addressing these objectives will allow us to better understand the effectiveness of current FRA reclamation practices and learn which environmental factors may be driving any present ecological differences.

Chapter 2. Materials and Methods

2.1. Study Sites

The Monongahela National Forest (MNF) encompasses approximately 372,000 ha in the Allegheny Mountains of eastern West Virginia. At higher elevations in West Virginia, red spruce (*Picea rubens*) dominated forests were historically common and occupied over 200,000 ha. These forests were characterized by relatively poor soils, scattered wetlands, and a thick layer of peat (Byers et al. 2010). Timber harvest and the burning of the peat layer in the early 1900s, followed by surface coal mining in the 1980s, reduced red spruce forests from over 200,000 ha to less than 12,000 ha (Branduzzi

et al., 2020; Trani 2002). After disturbance, high elevation forests regenerated as primarily a mix of northern hardwood species (i.e., sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), black cherry (*Prunus serotina*)); however, red spruce dominated forests remain common in some areas, particularly at the highest elevations (Rentch et al. 2007) and within my study area.

The majority of my study sites were located on the Mower Tract, which covers approximately 16,000 ha of the MNF on Cheat Mountain (1478 m elevation) in Randolph County, WV (Figure 2.1.1.). Like much of the surrounding area, the Mower Tract experienced significant logging in the early 20th century and extensive mining spanning about 810 ha in the 1980s (Lambert et al., 2021). While the land was recontoured following mining activities and replanted, native species such as the red spruce struggled to survive and reproduce, leaving the landscape in a state of arrested succession (Burger et al., 2017). Following this initial reclamation process, the land was sold to the US Forest Service as part of the MNF and more recent reclamation activities have since been undertaken (see below). I had additional study sites on Sharp's Knob (1382 m elevation, Pocahontas County, Figure 2.1.1.), which historically was dominated by red spruce forests and experienced disturbances (i.e., logging and coal mining) and conventional reclamation similar to those on the Mower Tract.

FRA sites were reclaimed as part of a collaboration between the US Forest Service, the nonprofit group Green Forests Work, and the U.S. Office of Surface Mining Reclamation and Enforcement's Appalachian Regional Reforestation Initiative (ARRI) (Lambert et al., 2021). As part of the reclamation plans, these groups established over

800 wetlands between 2010 and 2016 alone, constructing them at the same time the land was ripped to alleviate soil compaction (Lambert et al., 2021).

I selected a total of 32 wetlands located on or near legacy surface mines on the Mower Tract (n=29) and Sharp's Knob (n=3) for this study. All wetlands chosen for this study were seasonal wetlands, which often dry during late summer or fall. Thus, these wetlands were devoid of fish, and considered preferred breeding habitat for amphibians. The four treatment categories that the wetlands were divided into were young FRA (YFRA) sites (1-9 years old), older FRA (OFRA) sites (10-20 years old), naturally regenerated (REGEN) sites (> 40 years old), and unmined, mature (MAT) sites (Figure 2.1.2.).

All YFRA sites were replanted with a mix of red spruce and a dozen other native hardwoods between 2013 and 2021, and therefore have lower average canopy densities compared to sites of other treatments. These sites tended to have well established ground cover, with a mix of non-competitive upland, herbaceous species across the site, and wetland shrubs and other plants such as swamp milkweed (*Asclepias incarnata*) and boneset (*Eupatorium perfoliatum*) around the constructed wetlands. Old FRA sites were replanted between 2002 and 2013 and showed a noticeably larger amount of developed vegetation over the young FRA sites. These sites were replanted with red spruce, aspen (*Populus spp.*), serviceberry (*Amelanchier arborea*), and black cherry (*Prunus serotina*) (Snyder, 2022). At the time of surveying in 2022, the majority of the seedlings stood between 2-4 m high with a mix of native herbaceous cover filling in the ground level.

Most of the OFRA wetlands were constructed in forests directly adjacent to ripped land, while the newer YFRA sites are located in more open areas, directly on the

ripped land (Lambert, 2020). All FRA wetlands were constructed using the general guidelines of Thomas Biebighauser (2003), a biologist and wetlands expert, but each wetland was created based on the specific contour and soil properties of the land (US Forest Service, 2016). They were originally created as 5-10 m long irregular shapes, described as kidney-shaped or amoeba-shaped. Downed trees and other woody debris were placed in the wetlands as habitat features where they were available.

Naturally regenerated sites (REGEN) were mined prior to SMCRA and reclaimed as grasslands. These sites are located on benches between steep highwalls on flat, narrow strips of land with native forest on either side contributing seeds. The compacted soils have limited the growth of native later-successional trees on these sites and left the land in a state of arrested succession, where non-native shrubs and grasses dominate the landscape (Branduzzi, 2020; Groninger et al., 2017). The trees are at these sites are a mixture of red spruce and non-native conifers such as red pine (*Pinus resinosa*) and Norway spruce (*Picea abies*) with the oldest trees being greater than 40 years old. Wetlands were mostly surrounded by mosses and small shrubs, including cranberry bushes (*Vaccinium spp.*) at two of the sites (REC2 and REC4).

Unmined, mature sites (MAT), which serve as the controls, were located in forested lands adjacent to the mined lands. These sites have never been mined and are not utilized in timber harvesting or active management of any kind. The forests surrounding these natural wetlands are comprised primarily of red spruce, yellow birch (*Betula alleghaniensis*), red maple (*Acer rubrum*), and beech (*Fagus grandifolia*) (Snyder, 2022). Wetland perimeters at these sites tended to have a variety of sphagnum mosses and other native wetland plants.

2.2. Dip Net Surveys

I conducted dip net surveys for both larval and adult amphibians from June 8th to June 30th. Sweeps were performed according to the protocol of Denton & Richter (2012), in which each sweep encompassed approximately 5 m of wetland perimeter (Figure 2.1.3.). To standardize the procedure, each wetland was measured to calculate the perimeter, which was then divided by 5 (Table 2.1.1.). A 40 by 23 cm D-frame dip net was dragged across the bottom of the wetland for about one meter per sweep (Lambert 2020). Contents of the net were transferred into sorting bins and all amphibian specimens were counted, identified, measured for snout-to-vent length (SVL) and total length (TL), and returned to the wetland at the approximate location of capture. Each site was surveyed four times throughout the season (~ once a week). I released all individuals at their capture location after counts were completed.

2.3. Wetland Characteristics and Environmental Variable Measurements

Prior to each dip net survey, I recorded air temperature, cloud cover, wind speed, day of year, and time of day. Air temperature was measured using Kestrel Weather Meter units (KestrelMeters). Cloud cover was estimated using the 8-point Okta scale, with 0 representing a perfectly clear day and 8 representing complete cloud cover (Stull, 2015). Wind speed was estimated using the Beaufort 12-point scale where 0 is characterized by no leaf movement and 5 by stronger gusts (World Meteorological Organization, 1970). Air temperature and day of year (date) have been positively correlated with species detection probabilities in at least one study (Petitot et al., 2014). Percent cloud cover has

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been positively linked to detection in *A. maculatum* and wind speed has been shown to have a negative relationship with *L. sylvaticus* and a positive relationship with *N. viridescens* (Curtis & Patton, 2010). I collected all data with possible connections to amphibian detection probabilities for use in occupancy and abundance modeling (see below).

Site covariates, such as canopy cover and wetland area are those that were expected to remain fairly constant for each wetland throughout the field season and were only measured once. Water chemistry metrics (pH, TOC, presence of metals, etc.) were measured three times throughout the season, but an average of the values for each sample was taken to account for small seasonal variations. Canopy cover was calculated using a spherical crown densiometer (Forestry Suppliers, Jackson, MS, USA) in the middle of June. Four measurements were taken facing each cardinal direction standing in the approximate center of the wetland and an average was used as the total canopy cover for each site. Wetland area was calculated prior to the first dip netting surveys to determine how many sweeps needed to be conducted for each site as a way of standardizing our field methods. Canopy cover, wetland area, and water chemistry have all been shown to have strong impacts on amphibian occupancy and abundance (Skelly et al., 2002; Shulse et al., 2010; Dale et al., 1985; Freda, 1991; Gascon, 1986).

Water chemistry data were analyzed in the UK Department of Forestry and Natural Resource Sciences' Hydrology Lab using an average of three samples taken from the field throughout the season. Samples of ~100 mL of water were collected in plastic vials, labeled by site and date, and placed on ice until they could be transported to a freezer. All water samples were tested for sixteen different metrics: turbidity (FTU),

conductivity (μmhos/L), pH (H+), TOC (mg/L), Cl (mg/L), SO_4 (mg/L), NO_3-N (mg/L),

NH_4-N (mg/L), Ca^{+2} (mg/L), Mg^{+2} (mg/L), K^+ (mg/L), Na^+ (mg/L), Mn (mg/L), Fe

(mg/L), Al (mg/L), and NO_2-N (mg/L). Turbidity was measured using a Hach

turbidimeter (Hach, Loveland, CO) and conductivity and measured with a YSI

conductivity bridge (YSI, Yellow Springs, OH). For measuring pH, I used a Thermo

Scientfic ™ Orion Star ™ benchtop pH meter (model 250A; Thermo Fisher Scientific,

Waltham, Massachusetts). I used a Shimadzu TOC-Vcsn analyzer (Shimadzu, Kyoto,

Japan) to measure total organic carbon. To measure Cl (chloride) and SO_4 (sulfate) I

used a Dionex™ Ion Chromatograph 2000 (Dionex Corporation, Sunnyvale, CA). To

measure NO_3-N (nitrate), NO_2-N (nitrite), and NH_4-N (ammonium) I used a Bran +

Luebbe™ auto analyzer (Bran + Luebbe Company, Norderstedt, Germany). Ca, Mg, K,

Na, Mn, Fe, and Al were measured with an Agilent™ Inductively Coupled Plasma

Optical Emission Spectroscopy (ICP-OES) (model 5110, Agilent, Santa Clara, CA).

Water quality sampling, preservation, and analytic protocols were performed in

accordance with standard methods (Greenberg et al., 1992).

Site covariates relating to land cover such as percent forest, distance to forest, and

distance to road were calculated using a supervised classification in the ArcMap

application of ESRI's ArcGIS (10.8.2; ESRI, Redlands, CA, USA). At least 10 training

samples of each land cover classification (forest, road, open field, and wetland) were

taken from a 2016 orthographic map of the study sites and used to create an updated land

cover map. For each wetland site a 100 m buffer was added, and percentages of land

cover were manually calculated within each buffer. Distance measurements were

calculated using the built in measurement tool in ArcMap and were taken from the GPS

point of each site to the nearest road or forested area. Percent forest and distance to forest were selected as metrics with a potential connection to amphibian occupancy and abundance due to several studies that point to forest cover as a factor influencing larval success in several amphibian species, including *L. sylvaticus, P. crucifer,* and *A. americanus* (Skelly et al., 2002; Werner & Glennemeier, 1999; Werner et al., 2007). Distance to road was selected as a possible covariate due to several studies that show connections between road fragmentation and decreases in amphibian populations (Hamer et al., 2021; Herrmann et al., 2005), though the roads through my study sites were mostly unpaved, Forest Service roads that see relatively little public traffic.

2.4. Analysis

I performed an analysis of variance (ANOVA) in R (Version 4.2.2.) to examine differences in site attributes (i.e., wetland area), water chemistry metrics, and environmental/landscape variables between treatments (i.e., YFRA, OFRA, REGEN, MAT). I then performed a Tukey's Honestly Significant Difference (HSD) test to identify which treatment types were responsible for those differences and compare across treatment types.

To examine the effect of treatment type, landscape parameters, water chemistry and sampling covariates on amphibian occupancy and species richness, I used a Bayesian multi-species occupancy model (MacKenzie et al., 2017; Zipkin et al., 2009). This model is able to generate occupancy estimates (Ψ) and detection probability (p) on a species-specific level as well as create species richness estimates (SpR) for each individual site (and site type). Site type, wetland area (zArea), and canopy cover (zCanopy) were the site

covariates for the occupancy model, and date was the sampling covariate used for the

detection model. Covariates were excluded from the model if they were strongly

correlated with another covariate in the model.

The occupancy model was based on the following equation:

$$\text{logit}(\varPsi_{ij}) = u_i + \alpha_{1i}(\text{YFRA})_j + \alpha_{2i}(\text{OFRA})_j + \alpha_{3i}(\text{REGEN})_j + \alpha_{4i}(\text{zArea})_j + \alpha_{5i}(\text{zCanopy})_j$$

where i represents the species, j represents the individual site, k represents the sampling

occasion, all as a function of the site covariates, represented here as α. The covariates in

this equation are being compared to another covariate (MAT) that serves as the reference

treatment type.

The detection model was based on the following equation:

$$\text{logit}(\rho_{ij}) = v_i + \beta_{1i}(\text{Date})_j$$

where i represents the species, j represents the individual site, k represents the sampling

occasion, all as a function of the sampling covariate, represented here as β.

In total, there were 5 site covariates and 1 sampling covariate used in this model. I

selected treatment type, wetland area, and canopy cover as the site covariates to keep the

model simple while still including area and canopy cover, which have been repeatedly

shown to impact amphibian occupancy (Babbitt, 2005; Chandler et al., 2017; Skelly et

al., 2002; Werner et al., 2007; Werner & Glennemeier, 1999). Date was included in the

detection model in order to account for variation in occupancy throughout the breeding

season. I chose to run all amphibian data together rather than separate data between

salamander species and frog species. Thus, the species I included in the model were

Anaxyrus americanus, *Ambystoma maculatum*, *Hemidactylium scutatum*, *Hyla versicolor, Lithobates clamitans*, *Lithobates sylvaticus*, *Notophthalmus viridescens*, and *Pseudacris crucifer*. This decision to include all species detected was based on their preference to breed in wetlands (Ray et al., 2022). All covariates were tested to ensure no two variables had correlations greater than 0.7 (Zuur et al. 2009). Continuous covariates were standardized using Z-scores. Parameters were determined to be significant to a species if the 95% confidence interval (CI) did not overlap with zero.

I used Markov chain Monte Carlo sampling within a Bayesian framework. Three parallel chains were run and convergence was assessed with the Gelman-Rubin statistic; all models were below the required value of 1.02, ensuring proper convergence (Gelman & Rubin, 1992). Each chain was run for 70,000 iterations with a burn-in at 20,000 samples and thinned every 3 samples. The output of this model resulted in 50,001 samples including the statistics mean, standard deviation, and 95% confidence interval (CI). The model was run through R package R2WinBUGS, which exports data into the program WinBUGS and imports the results from the model iterations.

To estimate species abundance, I used an N-mixture model modified from Royle (2004) to examine the effects of treatment types, site-specific covariates, and sampling covariates on species-specific abundance. For this analysis, I estimated abundances for the most common species, including *A. americanus*, *A. maculatum*, *H. versicolor*, *L. clamitans*, *L. sylvaticus*, *N. viridescens*, and *P. crucifer*. As with the occupancy and species richness model, all covariates were tested to ensure no two variables had correlations greater than 0.7 (Zuur et al. 2009). The same parameters from the occupancy and detection models were used in the abundance model. I used the same protocol for

determining a parameter's significance as for the occupancy model, where only

covariates with less than 2% overlap with 0 would be considered significant.

My abundance model was based on the following equation:

$$\text{logit}(\lambda_i) = \beta_1 + \beta_2\,(\text{YFRA})_i + \beta_3\,(\text{OFRA})_i + \beta_4\,(\text{REGEN})_i + \beta_5\,(\text{zArea})_i + \beta_6\,(\text{zCanopy})_i$$

where i represented the individual sites and site covariates were represented as β.

Per-individual detection probability was model using the following equation:

$$\text{logit}(p_j) = \alpha_1 + \alpha_2\,(\text{Date})_j$$

where j represented sampling events and sampling covariate was represented as α.

As with the occupancy model, I used Markov chain Monte Carlo sampling, and

three parallel chains were run for each species model. Each chain was run for 200,000

iterations with a burn-in at 100,000 samples and thinned every 50 samples. Convergence

was assessed with the Gelman-Rubin statistic; all models were below the required value

of 1.02, ensuring proper convergence (Gelman & Rubin, 1992). The output of this model

resulted in 6,000 samples including the statistics mean, standard deviation, and 95%

confidence interval.
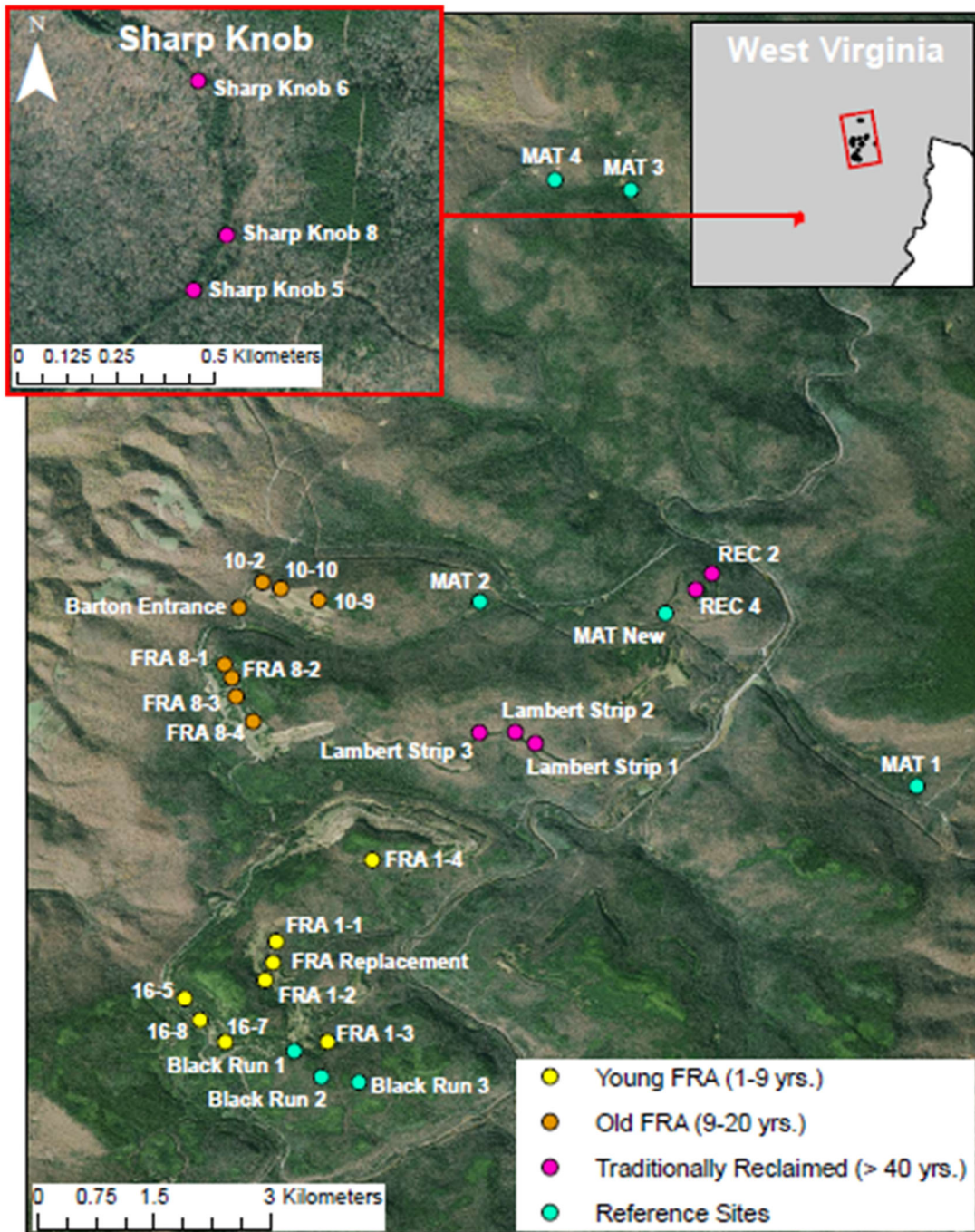
Figure 2.1.1. Study sites in the Monongahela National Forest (West Virginia). Larger map shows sites located in the Mower Basin in Randolph County and the inset map shows sites located at Sharp Knob in Pocahontas County.
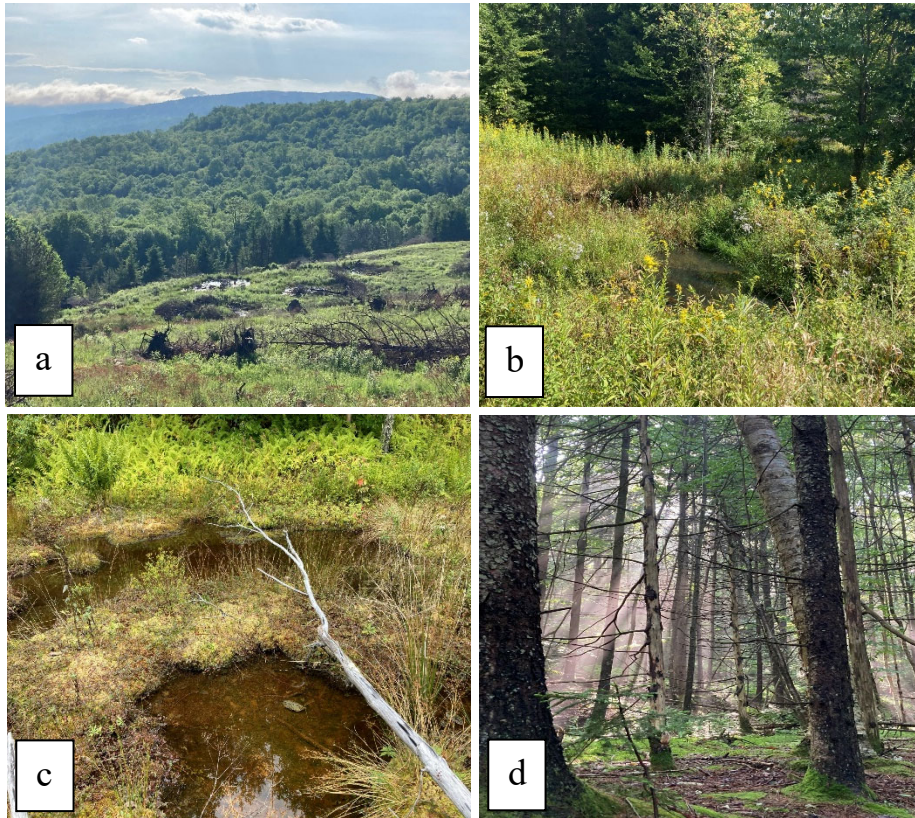
Figure 2.1.2. a) Young FRA (YFRA) site (1 – 9 years old) in the Mower Tract. b) Old FRA (OFRA) site (10 – 20 years old) in the Mower Tract. c) Naturally regenerated (REGEN) site (> 40 years old) in the Mower Tract. d) Mature, unmined forest (MAT) in the Mower Tract.

Figure 2.1.3. Each wetland was dip netted proportionately by the perimeter. This picture shows the contents of the net being transferred into a bin for sorting and measuring.

Table 2.1.1. Number of dip net sweeps per site based on the perimeter of the wetland.

Site Name	Site Type	Area (m^2)	Perimeter (m)	Number of Sweeps
REC2	REGEN	52	32	6
REC4	REGEN	115	40	8
Lambert Strip 1	REGEN	233	100	20
Lambert Strip 2	REGEN	50	25	5
Lambert Strip 3	REGEN	133	41	8
Sharp Knob 5	REGEN	5	8	2
Sharp Knob 6	REGEN	27	30	6
Sharp Knob 8	REGEN	39	27	5
FRA 1-1	YFRA	52	26	5
FRA 1-2	YFRA	43	23	5
FRA 1-3	YFRA	221	73	15
FRA 1-4	YFRA	19	15	3
FRA Replacement	YFRA	93	38	8
16 5	YFRA	5	9	2
16 7	YFRA	18	24	5
16 8	YFRA	14	17	3
10 2	OFRA	41	23	5
10 9	OFRA	61	29	6
10 10	OFRA	111	39	8
FRA 8-1	OFRA	64	30	6
FRA 8-2	OFRA	26	18	4
FRA 8-3	OFRA	29	19	4
FRA 8-4	OFRA	154	45	9
Barton Entrance	OFRA	86	33	7
MAT1	MAT	112	42	8
MAT2	MAT	27	29	6
MAT3	MAT	11	16	3
MAT4	MAT	48	25	5
MAT New	MAT	130	58	12
Black Run 1	MAT	14	18	4
Black Run 2	MAT	14	13	3
Black Run 3	MAT	41	31	6

Chapter 3. Results

3.1. Dip Net Surveys

In total, I detected nine species of amphibians during the dip net surveys. I detected 8 species at the YFRA sites: 296 total detections of *A. americanus*, 63 total detections of *A. maculatum*, 2 total detection of *H. scutatum*, 44 total detections of *H. versicolor*, 13 total detections of *L. clamitans*, 37 total detections of *L. sylvaticus*, 24 total detections of *N. viridescens*, and 214 total detections of *P. crucifer*, and 693 total detected individuals. I also detected 8 species at OFRA sites: 165 total detections of *A. maculatum*, 3 total detections of *H. scutatum*, 70 total detections of *H. versicolor*, 43 total detections of *L. clamitans*, 1 detection of *L. palustris*, 259 total detections of *L. sylvaticus*, 102 total detections of *N. viridescens*, and 8 total detections of *P. crucifer*, and 651 total detected individuals. I detected 7 species at the REGEN sites: 59 total detections of *A. maculatum*, 6 total detections of *H. scutatum*, 2 total detections of *H. versicolor*, 207 total detections of *L. clamitans*, 396 total detections of *L. sylvaticus*, 29 total detections of *N. viridescens*, and 1 detection of *P. crucifer*, and 700 total sampled individuals. Finally, I detected 6 species at the MAT sites: 81 total detections of *A. maculatum*, 8 detections of *H. scutatum*, 46 total detections of *L. clamitans*, 621 total detections of *L. sylvaticus*, 24 total detections of *N. viridescens*, and 1 detection of *P. crucifer*, and 781 total sampled individuals. Though many of the detected individuals of *N. viridescens* were gravid females, none of the detections of that species were in the larval stage. The species with the highest number of detections was *L. sylvaticus* with 1313 detections across all treatment types. *L. palustris* only had one detection during the

sampling season and *H. scutatum* only had 19 total detections, making them the most "data-poor" of the species in this study.

3.2. Objective 1: Site Covariates, Environmental/Landscape Variables, and Water Chemistry Data

Wetland area did not significantly differ ($p < 0.05$) between treatment types ($p = 0.73$, df = 3, F = 0.44) (Figure 3.2.1.) (Table 3.2.1.; Table 3.2.2.). Canopy cover percentages were different between the treatment types ($p = 0.004$, df = 3, F = 5.67), with REGEN ($p = 0.02$, df = 28, SE = 1.047) and MAT sites ($p = 0.02$, df = 28, SE = 1.057) having significantly more canopy cover than the YFRA sites (Figure 3.2.2.; Table 3.2.2).

Significant differences were found between treatments for distance to forest ($p = 0.008$, df = 3, F = 4.82) and percent forest ($p = 0.001$, df = 3, F = 6.95). Tukey's HSD divided treatment types into the same groups for both of these variables, with the REGEN ($p = 0.01$, df = 28, SE = 0.168) and MAT sites ($p = 0.01$, df = 28, SE = 0.168) having significantly lower distance to forest compared to the OFRA sites. REGEN ($p = 0.002$, df = 28, SE = 0.872) and MAT sites ($p = 0.004$, df = 28, SE = 0.845) also had a significantly higher percentage of forest than the OFRA sites, with YFRA sites overlapping with both groupings. Distance to road was not different among treatments ($p = 0.241$, df = 3, F = 1.48).

Several water chemistry attributes were significantly different across treatment types. First, pH was different among treatment type ($p = 0.001$, df = 3, F = 6.71), with YFRA wetlands ($p = 0.005$, df = 28, SE = 0.141) and OFRA wetlands ($p = 0.004$, df =

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28, SE = 0.144) having higher pH values than REGEN sites (Figure 3.2.2). I also found that Total Organic Carbon (TOC) had at least one significant difference between treatment types (p = 0.022, df = 3, F = 3.76). The HSD test showed that OFRA sites (p = 0.02, df = 28, SE = 0.180) had significantly higher TOC than REGEN sites, with FRA sites and MAT sites sharing overlap with both groupings. While not technically statistically significant, differences in aluminum concentrations between treatments were near significant levels (p = 0.054, df = 3, F = 2.87), though Tukey's HSD grouped all four treatments together. No other water chemistry statistics showed significant differences between treatment types (Table 3.2.1.). A correlation matrix for all site covariates, landscape/environmental variables, and water chemistry data can be found in Table 3.2.3.

3.3. Objective 2: Occupancy and Species Richness Estimates

The mean occupancy for all pond breeding amphibian species was estimated for each treatment type with MAT = 0.459 (CI = 0.148, 0.802); YFRA = 0.481 (CI = 0.122, 0.861); OFRA = 0.596 (CI = 0.208, 0.906); REGEN = 0.472 (CI = 0.124, 0.846) (Figure 3.3.1). None of the site covariates had a significant positive or negative relationship to the mean occupancy (all parameters overlapped with zero) (Figure 3.3.2.).

Species occupancy was estimated for all species detected at the study sites. *A. americanus* had relatively low occupancy estimates across treatment types with $\Psi_{YFRA} =$ 0.102 (CI = 0.011, 0.325), $\Psi_{OFRA} = 0.066$ (CI = 0.005, 0.225), $\Psi_{REGEN} = 0.048$ (CI = 0.003, 0.179), and $\Psi_{MAT} = 0.055$ (CI = 0.003, 0.258) (Figure 3.3.3.). I found no

significant relationships with occupancy of *A. americanus* and any of the site covariates (Table 3.3.1.). *A. maculatum* had very high occupancy estimates at all treatment types with $\Psi_{YFRA} = 0.860$ (CI = 0.552, 0.992), $\Psi_{OFRA} = 0.948$ (CI = 0.780, 0.997), $\Psi_{REGEN} = 0.903$ (CI = 0.638, 0.994), and $\Psi_{MAT} = 0.887$ (CI = 0.516, 0.995), but had no relationship with any site covariates (Figure 3.3.4.). Estimated occupancy by site type for *H. scutatum* was $\Psi_{YFRA} = 0.807$ (CI = 0.297, 0.996), $\Psi_{OFRA} = 0.861$ (CI = 0.386, 0.997), $\Psi_{REGEN} = 0.849$ (CI = 0.432, 0.995), and $\Psi_{MAT} = 0.775$ (CI = 0.205, 0.995) (Figure 3.3.5.) with no statistically significant relationships found between occupancy and the site covariates. *H. versicolor* occupancy estimates by treatment type were as follows, $\Psi_{YFRA} = 0.376$ (CI = 0.098, 0.770), $\Psi_{OFRA} = 0.426$ (CI = 0.134, 0.800), $\Psi_{REGEN} = 0.235$ (CI = 0.034, 0.635), and $\Psi_{MAT} = 0.246$ (CI = 0.023, 0.731) (Figure 3.3.6.). Wetland area was the only site covariate to significantly influence occupancy estimates for *H. versicolor*, showing a positive relationship ($\alpha_4 = 0.559$, sd = 0.367, CI = -0.073, 1.406). *L. clamitans* occupancy estimates had wide confidence intervals at all treatment types with $\Psi_{YFRA} = 0.498$ (CI = 0.176, 0.825), $\Psi_{OFRA} = 0.616$ (CI = 0.286, 0.892), $\Psi_{REGEN} = 0.587$ (CI = 0.271, 0.880), and $\Psi_{MAT} = 0.491$ (CI = 0.101, 0.900) (Figure 3.3.7.) and had a positive relationship to wetland area ($\alpha_4 = 0.559$, sd = 0.366, CI = -0.074, 1.404). Occupancy estimates for *L. palustris* had even wider confidence intervals with $\Psi_{YFRA} = 0.389$ (CI = 0.005, 0.993), $\Psi_{OFRA} = 0.471$ (CI = 0.019, 0.995), $\Psi_{REGEN} = 0.388$ (CI = 0.006, 0.992), and $\Psi_{MAT} = 0.381$ (CI = 0.004, 0.993) and had no significant relationships with any site covariates. *L. sylvaticus* had greater variability in occupancy estimates between treatment types with $\Psi_{YFRA} = 0.366$ (CI = 0.100, 0.730), $\Psi_{OFRA} = 0.398$ (CI = 0.116, 0.765), $\Psi_{REGEN} = 0.330$ (CI = 0.060, 0.732), and $\Psi_{MAT} = 0.262$ (CI = 0.024, 0.744), but no covariates showed a

significant relationship to occupancy estimates (Figure 3.3.9.) *N. viridescens* had the following treatment specific occupancy estimations, Ψ_{YFRA} = 0.830 (CI = 0.522, 0.991), Ψ_{OFRA} = 0.721 (CI = 0.361, 0.961), Ψ_{REGEN} = 0.755 (CI = 0.324, 0.980), and Ψ_{MAT} = 0.660 (CI = 0.169, 0.977) (Figure 3.3.10.). There was a positive relationship between *N. viridescens* occupancy and wetland area (α_4 = 0.589, sd = 0.421, CI = -0.083, 1.625), but there was no statistically significant relationship with any other covariate. Lastly, *P. crucifer* occupancy estimates varied by treatment type as follows, Ψ_{YFRA} = 0.239 (CI = 0.044, 0.614), Ψ_{OFRA} = 0.261 (CI = 0.061, 0.571), Ψ_{REGEN} = 0.162 (CI = 0.023, 0.435), and Ψ_{MAT} = 0.181 (CI = 0.015, 0.597) (Figure 3.3.11.). I found a positive relationship between *P. crucifer* occupancy estimates and wetland area (α_4 = 0.558, sd = 0.368, CI = -0.081, 1.410).

Mean detection probability for each species was as follows: *A. americanus* (p = 0.554, CI = 0.419, 0.674), *A. maculatum* (p = 0.559, CI = 0.479, 0.638), *H. scutatum* (p = 0.569, CI = 0.476, 0.673), *H. versicolor* (p = 0.553, CI = 0.443, 0.653), *L. clamitans* (p = 0.553, CI = 0.442, 0.653), *L. palustris* (p = 0.561, CI = 0.435, 0.694), *L. sylvaticus* (p = 0.534, CI = 0.408, 0.625), *N. viridescens* (p = 0.564, CI = 0.479, 0.654), and *P. crucifer* (p = 0.553, CI = 0.442, 0.653). Date had a positive relationship with *A. maculatum* detection probabilities (β_1 = 0.236, sd = 0.164, CI = -0.083, 0.568), and also with detection probabilities for *H. scutatum* (β_1 = 0.276, sd = 0.203, CI = -0.095, 0.722) and with N. *viridescens* (β_1 = 0.256, sd = 0.180, CI = -0.085, 0.635) (Table 3.3.2.).

Mean species richness was estimated for each treatment type using the same model that estimated occupancy with SpR$_{YFRA}$ = 4.27 (CI = 3.12, 5.88), SpR$_{OFRA}$ = 5.14

(CI = 4.00, 6.62), SpR_{REGEN} = 4.41 (CI = 3.50, 5.75), and SpR_{MAT} = 3.84 (CI = 2.88, 5.50). (Figure 3.3.12.).

3.4. Objective 2: Abundance Estimates

Abundance estimates were modeled for *A. maculatum*, *L. sylvaticus,* and *N. viridescens*, as these were the species with the greatest number of detections. These estimates varied across treatment types (Table 3.4.1.). *L. sylvaticus* (β_2 = -1.412, sd = 0.334, CI = -2.082, -0.777) abundance estimates were negatively associated with YFRA sites. The OFRA sites had a strong positive relationship with all three of the species' abundance estimates: *A. maculatum* (β_3 = 0.911, sd = 0.200, CI = 0.529, 1.311), *L. sylvaticus* (β_3 = 0.497, sd = 0.140, CI = 0.222, 0.771), and *N. viridescens* (β_3 = 0.642, sd = 0.314, CI = 0.048, 1.282). The REGEN sites had a negative influence on abundance estimates for *A. maculatum* (β_4 = -0.516, sd = 0.224, CI = -0.965, -0.079) but had no positive associations with any of the species. While not statistically significant, there were negative relationships between REGEN sites and both of the other species: *L. sylvaticus* (β_4 = -0.128, sd = 0.096, CI = -0.319, 0.057) and *N. viridescens* (β_4 = -0.482, sd = 0.362, CI = -1.166, 0.248). All three species had a significantly positive relationship with the MAT sites: *A. maculatum* (β_1 = 2.266, sd = 0.193, CI = 1.882, 2.636), *L. sylvaticus* (β_1 = 2.399, sd = 0.120, CI = 2.161, 2.632), and *N. viridescens* (β_1 = 1.874, sd = 0.472, CI = 1.043, 2.940).

Abundance estimates were also influenced by the other site covariates, wetland area and canopy cover. *A. maculatum* abundance estimates were positively influenced by

canopy cover (β_6 = 0.309, sd = 0.096, CI = 0.118, 0.500). *L. sylvaticus* abundance

estimates were positively influenced by wetland area (β_5 = 0.697, sd = 0.053, CI = 0.596,

0.803) and by canopy cover (β_6 = 1.331, sd = 0.069, CI = 1.195, 1.468). *N. viridescens*

abundance estimates were positively influenced by wetland area (β_5 = 0.517, sd = 0.096,

CI = 0.327, 0.700) and negatively influenced by canopy cover (β_6 = -0.689, sd = 0.192,

CI = -1.088, -0.325).

    A. maculatum abundance estimates by treatment type were Ψ_{YFRA} = 24.3 (CI =

17.9, 32.8), Ψ_{OFRA} = 5.92 (CI =3.72, 8.89), Ψ_{REGEN} = 10.9 (CI = 7.10, 16.2), and Ψ_{MAT} =

9.83 (CI = 6.55, 14.3) (Figure 3.4.1.). Abundance estimates for *L. sylvaticus* by treatment

type were Ψ_{YFRA} = 18.1 (CI = 14.6, 22.1), Ψ_{OFRA} = 9.72 (CI = 7.87, 11.8), Ψ_{REGEN} = 22.2

(CI = 17.9, 27.0), and Ψ_{MAT} = 11.1 (CI = 8.66, 13.8) (Figure 3.4.2.). Estimated

abundances for *N. viridescens* by treatment type were as follows: Ψ_{YFRA} = 13.202 (CI =

5.380, 33.449), Ψ_{OFRA} = 3.289 (CI = 1.220, 7.878), Ψ_{REGEN} = 11.902 (CI = 4.331,

30.705), and Ψ_{MAT} = 6.584 (CI = 2.479, 16.527) (Figure 3.4.3.).

    Per-individual detection probabilities for each species were as follows: *A.*

maculatum (*p* = 0.553, CI = 0.506, 0.600), *L. sylvaticus* (*p* = 0.476, CI = 0.439, 0.513),

and *N. viridescens* (*p* = 0.547, CI = 0.476, 0.618). *A. maculatum* per-individual detection

probability had a positive relationship with date (α_2 = 0.215, sd = 0.096, CI = 0.023,

0.405). Detection probabilities for *L. sylvaticus* and *N. viridescens* were not significantly

influenced by date.

Table 3.2.1. ANOVA p-values, F-statistics, and average values of all site covariates, environmental/landscape variables, and water chemistry data by treatment type. Superscript letters represent the Tukey's Honestly Significant Difference groupings.

Variable	p-value	F-stat	YFRA	OFRA	REGEN	MAT
Wetland Area (sq. m)	0.729	0.436	57.99 [a]	71.63 [a]	81.66 [a]	49.66 [a]
Canopy Cover (%)	0.004	5.67	0 [b]	10.45 [ab]	53.68 [a]	54.75 [a]
Distance to Road (m)	0.241	1.48	533.77 [a]	239.66 [a]	460.91 [a]	341.33 [a]
Distance to Forest (m)	0.008	4.82	4.82 [ab]	13.79 [a]	0 [b]	0 [b]
Percent Forest	0.001	6.95	80.67 [ab]	62.78 [a]	100 [b]	97.73 [b]
Average Cond.	0.316	1.23	44.4 [a]	52.09 [a]	23.24 [a]	33.39 [a]
Average pH	0.001	6.71	6.74 [a]	6.78 [a]	5.77 [b]	6.15 [ab]
Average NO3-N	0.407	1	0.002 [a]	0 [a]	0 [a]	0 [a]
Average Turbidity	0.328	1.2	8.57 [a]	4.02 [a]	6.35 [a]	2.88 [a]
Average TOC	0.022	3.76	3.75 [ab]	4.31 [a]	2.73 [b]	3.19 [ab]
Average Ca	0.47	0.87	4.77 [a]	6.78 [a]	1.78 [a]	5.14 [a]
Average Mg	0.131	2.04	3.04 [a]	2.78 [a]	1.03 [a]	0.49 [a]
Average Fe	0.397	1.02	1.74 [a]	3.9 [a]	7.81 [a]	1.74 [a]
Average NO2-N	0.179	1.76	0.29 [a]	0.2 [a]	0.42 [a]	0.39 [a]
Average Al	0.054	2.87	0.15 [a]	0.1 [a]	0.68 [a]	0.3 [a]
Average Na	0.505	0.8	0.75 [a]	0.56 [a]	0.55 [a]	0.83 [a]
Average Mn	0.261	1.410	0.31 [a]	1.02 [a]	0.99 [a]	0.13 [a]
Average K	0.117	2.15	1.39 [a]	1.39 [a]	0.76 [a]	0.67 [a]
Average Cl	0.172	1.790	0.67 [a]	0.7 [a]	0.71 [a]	1.04 [a]
Average NH4-N	0.569	0.685	0.02 [a]	0.01 [a]	0.1 [a]	0.01 [a]
Average SO4	0.188	1.71	4.77 [a]	2.23 [a]	2.74 [a]	2.42 [a]

Table 3.2.2. Tukey's Pairwise Comparison results for wetland characteristics found to significantly differ between treatment types (df = 28 for all test results in this table).

Wetland Attribute	YFRA ~ OFRA		YFRA ~ REGEN		YFRA ~ MAT		OFRA ~ REGEN		OFRA ~ MAT		REGEN ~ MAT	
	p-value	SE	p-value	SE	p-value	SE	p-value	SE	p-value	SE	p-value	SE
Canopy Cover (%)	0.93	0.462	0.02	1.047	0.02	1.057	0.07	0.939	0.07	0.951	1	0.148
Distance to Forest (m)	0.16	0.428	0.66	0.314	0.66	0.314	0.01	0.168	0.01	0.168	1	0.269
Percent Forest	0.24	0.604	0.18	0.628	0.28	0.590	0.002	0.872	0.004	0.845	1	0.215
Average pH	1	0.029	0.005	0.141	0.14	0.110	0.004	0.144	0.11	0.113	0.48	0.089
Average TOC	0.68	0.107	0.2	0.144	0.68	0.107	0.02	0.180	0.14	0.151	0.79	0.097

Table 3.2.3. Correlation matrix of all variable site related data.

	Area	CC	DistRoad	DistForest	PercForest	Ave.Cond	Ave.pH	Ave.NO3.N	Ave.Turb	Ave.TOC	Ave.Ca	Ave.Mg	Ave.Fe	Ave.NO2.N	Ave.Al	Ave.Na	Ave.Mn	Ave.K	Ave.Cl	Ave.NH4.N	Ave.SO4
Area	1.000	-0.282	0.215	0.073	-0.075	-0.022	0.073	0.480	-0.128	-0.216	-0.068	0.012	0.021	-0.121	-0.152	0.011	-0.023	-0.108	0.091	-0.078	-0.038
CC	-0.282	1.000	0.010	-0.313	0.280	-0.208	-0.338	-0.132	-0.104	-0.259	-0.042	-0.352	-0.119	0.068	0.360	-0.165	-0.212	-0.221	-0.116	-0.080	-0.145
DistRoad	0.215	0.010	1.000	-0.075	-0.074	0.078	0.206	0.240	0.299	0.170	0.008	0.115	0.215	0.125	-0.012	-0.113	0.134	0.229	-0.079	0.255	0.235
DistForest	0.073	-0.313	-0.075	1.000	-0.847	0.563	0.465	-0.087	-0.119	0.354	0.337	0.739	0.010	-0.395	-0.215	-0.006	0.206	0.368	-0.086	-0.092	0.475
PercForest	-0.075	0.280	-0.074	-0.847	1.000	-0.378	-0.437	0.104	0.006	-0.458	-0.185	-0.532	0.050	0.299	0.240	0.074	-0.053	-0.416	0.137	0.095	-0.439
Ave.Cond	-0.022	-0.208	0.078	0.563	-0.378	1.000	0.624	-0.018	0.036	0.462	0.889	0.858	0.205	-0.321	-0.163	0.165	0.375	0.513	0.064	0.113	0.544
Ave.pH	0.073	-0.338	0.206	0.465	-0.437	0.624	1.000	0.143	0.218	0.581	0.594	0.544	0.074	-0.416	-0.432	0.061	0.243	0.556	-0.103	0.013	0.180
Ave.NO3.N	0.480	-0.132	0.240	-0.087	0.104	-0.018	0.143	1.000	0.130	-0.039	-0.042	0.068	-0.071	-0.007	-0.066	-0.053	-0.080	0.047	-0.044	-0.058	-0.009
Ave.Turb	-0.128	-0.104	0.299	-0.119	0.006	0.036	0.218	0.130	1.000	0.336	0.017	0.108	0.542	0.232	0.389	-0.181	0.470	0.433	-0.014	0.645	0.185
Ave.TOC	-0.216	-0.259	0.170	0.354	-0.458	0.462	0.581	-0.039	0.336	1.000	0.503	0.383	0.438	-0.324	-0.168	0.103	0.486	0.695	0.003	0.429	0.100
Ave.Ca	-0.068	-0.042	0.008	0.337	-0.185	0.889	0.594	-0.042	0.017	0.503	1.000	0.591	0.160	-0.245	-0.140	0.158	0.290	0.389	0.014	0.065	0.313
Ave.Mg	0.012	-0.352	0.115	0.739	-0.532	0.858	0.544	0.068	0.108	0.383	0.591	1.000	0.233	-0.324	-0.130	0.092	0.455	0.575	-0.027	0.148	0.678
Ave.Fe	0.021	-0.119	0.215	0.010	0.050	0.205	0.074	-0.071	0.542	0.438	0.160	0.233	1.000	0.006	0.240	-0.008	0.890	0.549	0.138	0.946	-0.006
Ave.NO2.N	-0.121	0.068	0.125	-0.395	0.299	-0.321	-0.416	-0.007	0.232	-0.324	-0.245	-0.324	0.006	1.000	0.223	-0.285	-0.037	-0.403	-0.178	0.018	-0.079
Ave.Al	-0.152	0.360	-0.012	-0.215	0.240	-0.163	-0.432	-0.066	0.389	-0.168	-0.140	-0.130	0.240	0.223	1.000	-0.067	0.128	-0.067	0.120	0.306	0.024
Ave.Na	0.011	-0.165	-0.113	-0.006	0.074	0.165	0.061	-0.053	-0.181	0.103	0.158	0.092	-0.008	-0.285	-0.067	1.000	-0.053	0.060	0.672	0.084	0.120
Ave.Mn	-0.023	-0.212	0.134	0.206	-0.053	0.375	0.243	-0.080	0.470	0.486	0.290	0.455	0.890	-0.037	0.128	-0.053	1.000	0.564	0.036	0.780	-0.008
Ave.K	-0.108	-0.221	0.229	0.368	-0.416	0.513	0.556	0.047	0.433	0.695	0.389	0.575	0.549	-0.403	-0.067	0.060	0.564	1.000	-0.106	0.557	0.394
Ave.Cl	0.091	-0.116	-0.079	-0.086	0.137	0.064	-0.103	-0.044	-0.014	0.003	0.014	-0.027	0.138	-0.178	0.120	0.672	0.036	-0.106	1.000	0.251	0.017
Ave.NH4.N	-0.078	-0.080	0.255	-0.092	0.095	0.113	0.013	-0.058	0.645	0.429	0.065	0.148	0.946	0.018	0.306	0.084	0.780	0.557	0.251	1.000	0.032
Ave.SO4	-0.038	-0.145	0.235	0.475	-0.439	0.544	0.180	-0.009	0.185	0.100	0.313	0.678	-0.006	-0.079	0.024	0.120	-0.008	0.394	0.017	0.032	1.000

Figure 3.2.1. Average wetland area (m^2) by treatment type (YFRA = 57.99 m^2, OFRA = 71.63 m^2, REGEN = 81.66 m^2, MAT = 49.66 m^2). Dots represent data points classified as outliers.
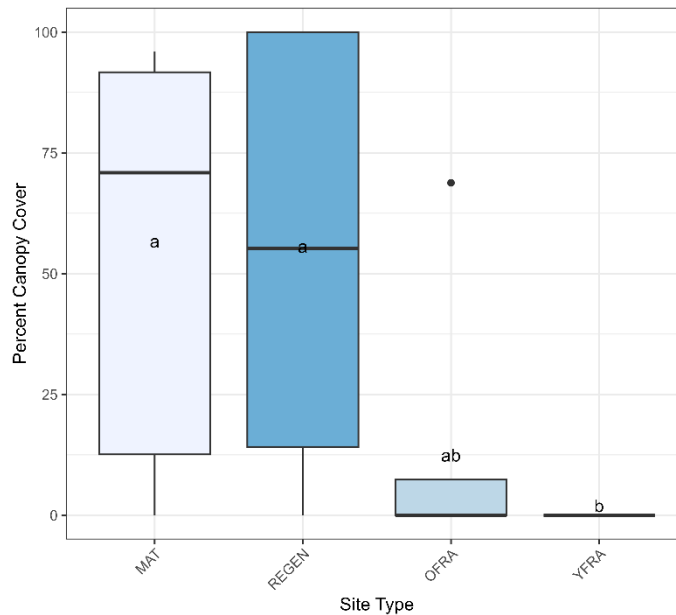
Figure 3.2.2. Average canopy cover percentages by treatment type (YFRA = 0%, OFRA = 10.45%, REGEN = 53.68%, MAT = 54.75%). Dots represent data points classified as outliers.
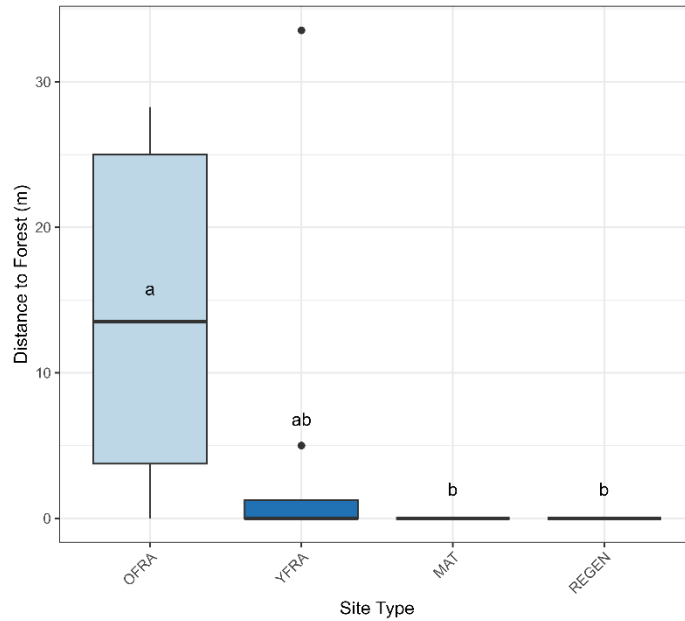
Figure 3.2.3. Average distances to nearest forested area by treatment type (YFRA = 4.82, OFRA = 13.79, REGEN = 0, MAT = 0). Dots represent data points classified as outliers.
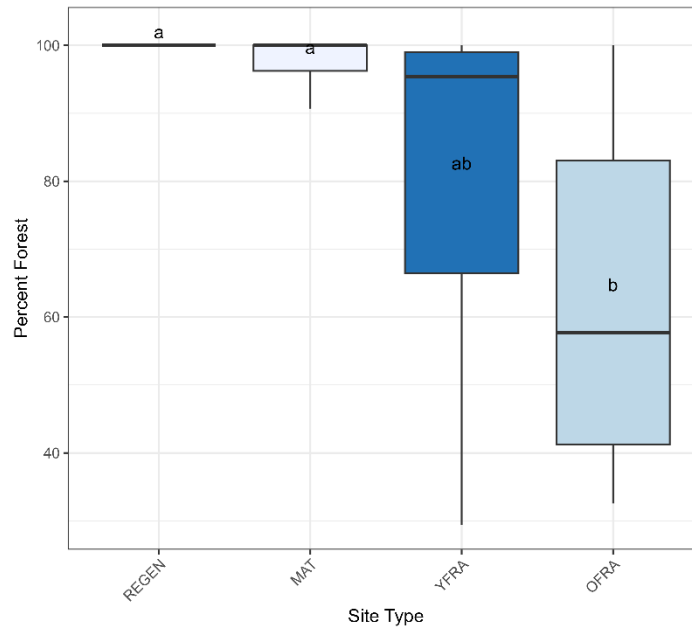
Figure 3.2.4. Average percentages of forested area within a 100 m buffer of each wetland by treatment type (YFRA = 80.67%, OFRA = 62.78%, REGEN = 100%, MAT = 97.73%).
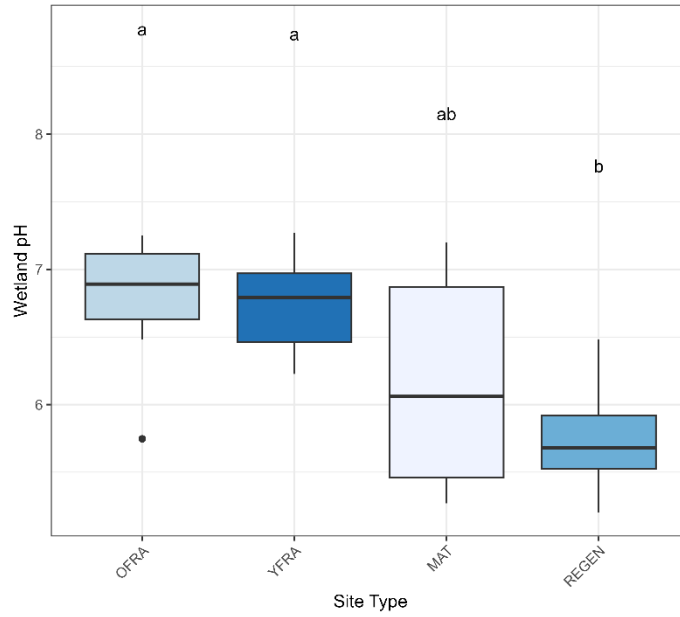
Figure 3.2.5. Average wetland pH values by treatment type (YFRA = 6.74, OFRA = 6.78, REGEN = 5.77, MAT = 6.15). Dots represent data points classified as outliers.
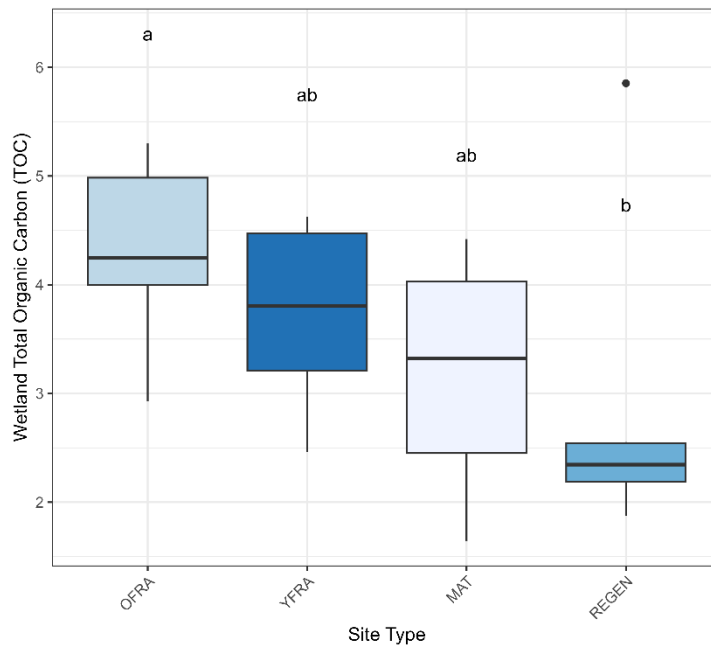
Figure 3.2.6. Average wetland TOC values by treatment type (YFRA = 3.75, OFRA = 4.31, REGEN = 2.73, MAT = 3.19). Dots represent data points classified as outliers.
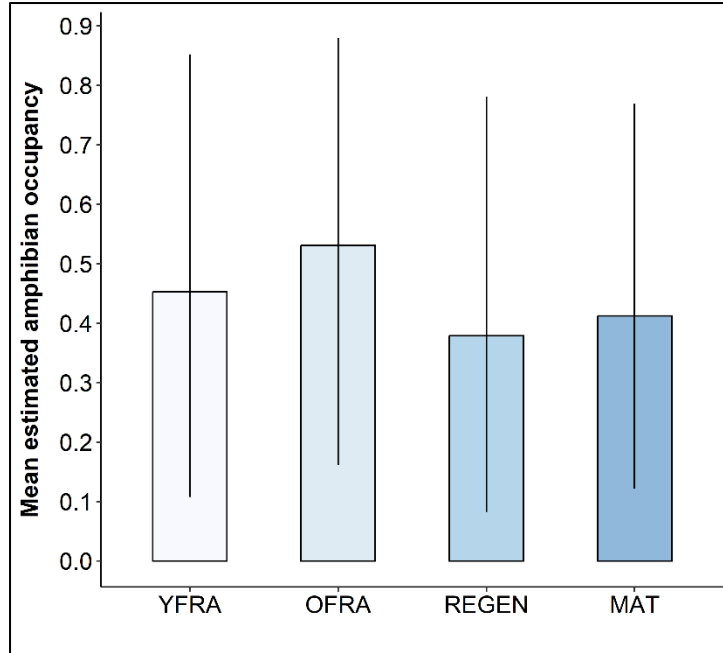
Figure 3.3.1. Mean estimated occupancy of all amphibian species (n = 9) in study wetlands by treatment type.
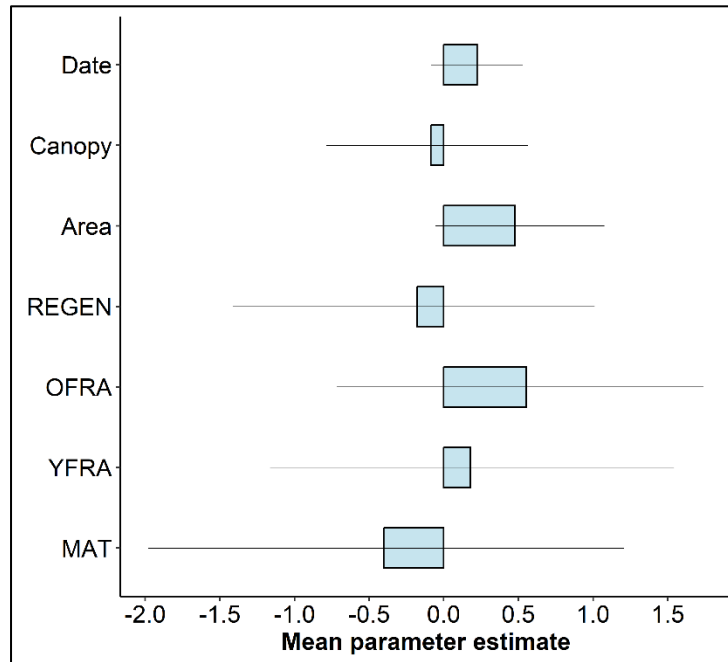
Figure 3.3.2. Mean parameter estimates relationship to overall occupancy and species richness of site wetlands.

Figure 3.3.3. a) Mean estimated occupancy of *A. americanus* across treatment types, $\Psi_{YFRA} = 0.102$ (CI = 0.011, 0.325), $\Psi_{OFRA} = 0.066$ (CI = 0.005, 0.225), $\Psi_{REGEN} = 0.048$ (CI = 0.003, 0.179), and $\Psi_{MAT} = 0.055$ (CI = 0.003, 0.258). b) Mean parameter estimates for *A. americanus.*

Figure 3.3.4. a) Mean estimated occupancy of *A. maculatum* across treatment types, $\Psi_{YFRA} = 0.860$ (CI = 0.552, 0.992), $\Psi_{OFRA} = 0.948$ (CI = 0.780, 0.997), $\Psi_{REGEN} = 0.903$ (CI = 0.638, 0.994), and $\Psi_{MAT} = 0.887$ (CI = 0.516, 0.995). b) Mean parameter estimates for *A. maculatum.*

Figure 3.3.5. a) Mean estimated occupancy of *H. scutatum* across treatment types, Ψ_{YFRA} = 0.807 (CI = 0.297, 0.996), Ψ_{OFRA} = 0.861 (CI = 0.386, 0.997), Ψ_{REGEN} = 0.849 (CI = 0.432, 0.995), and Ψ_{MAT} = 0.775 (CI = 0.205, 0.995). b) Mean parameter estimates for *H. scutatum*.

Figure 3.3.6. a) Mean estimated occupancy of *H. versicolor* across treatment types, Ψ_{YFRA} = 0.376 (CI = 0.098, 0.770), Ψ_{OFRA} = 0.426 (CI = 0.134, 0.800), Ψ_{REGEN} = 0.235 (CI = 0.034, 0.635), and Ψ_{MAT} = 0.246 (CI = 0.023, 0.731). b) Mean parameter estimates for *H. versicolor*.

Figure 3.3.7. a) Mean estimated occupancy of *L. clamitans* across treatment types, Ψ_{YFRA} = 0.498 (CI = 0.176, 0.825), Ψ_{OFRA} = 0.616 (CI = 0.286, 0.892), Ψ_{REGEN} = 0.587 (CI = 0.271, 0.880), and Ψ_{MAT} = 0.491 (CI = 0.101, 0.900). b) Mean parameter estimates for *L. clamitans*.
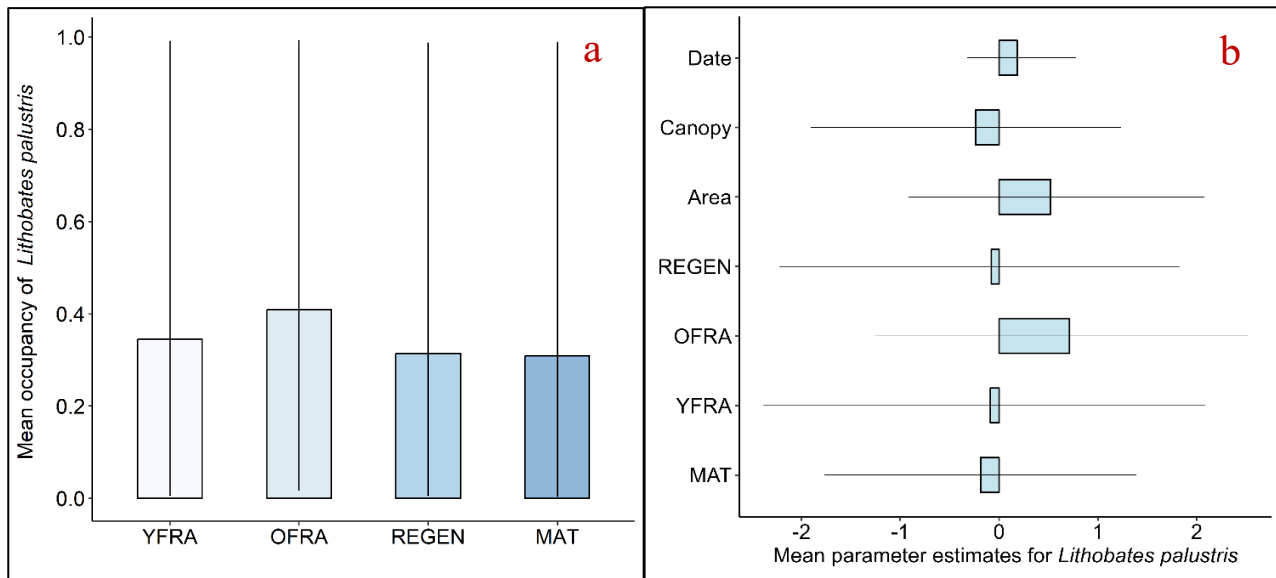
Figure 3.3.8. a) Mean estimated occupancy of *L. palustris* across treatment types, Ψ_{YFRA} = 0.394 (CI = 0.009, 0.993), Ψ_{OFRA} = 0.359 (CI = 0.006, 0.990), Ψ_{REGEN} = 0.424 (CI = 0.008, 0.995), and Ψ_{MAT} = 0.328 (CI = 0.002, 0.991). b) Mean parameter estimates for *L. palustris*.
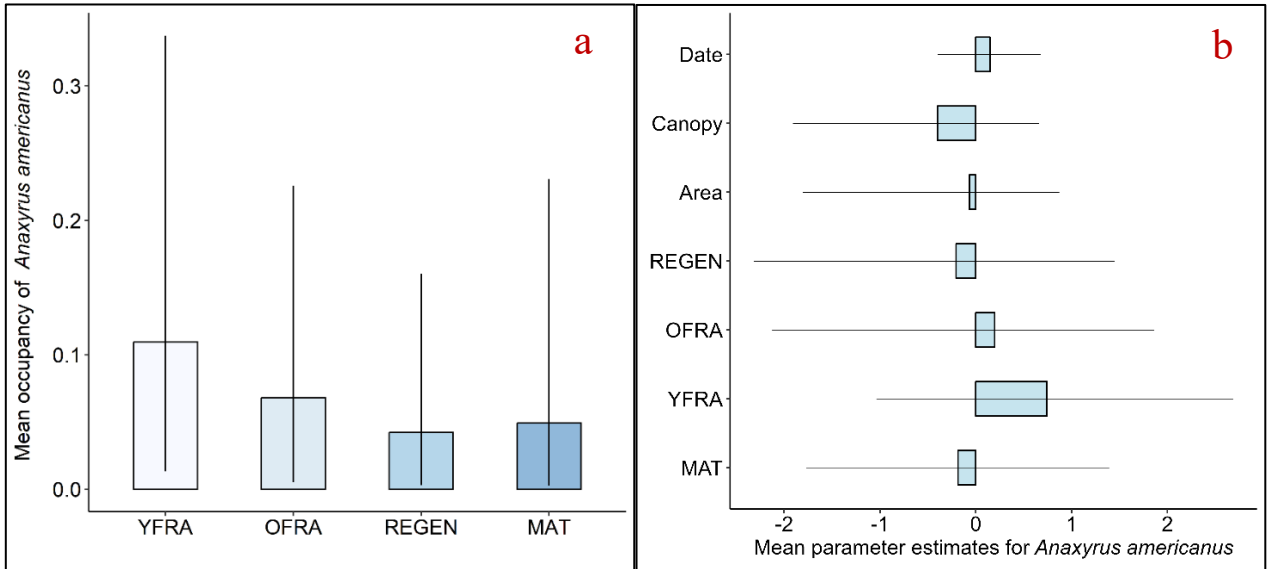
Figure 3.3.9. a) Mean estimated occupancy of *L. sylvaticus* across treatment types, Ψ_{YFRA} = 0.366 (CI = 0.100, 0.730), Ψ_{OFRA} = 0.398 (CI = 0.116, 0.765), Ψ_{REGEN} = 0.330 (CI = 0.060, 0.732), and Ψ_{MAT} = 0.262 (CI = 0.024, 0.744). b) Mean parameter estimates for *L. sylvaticus*.
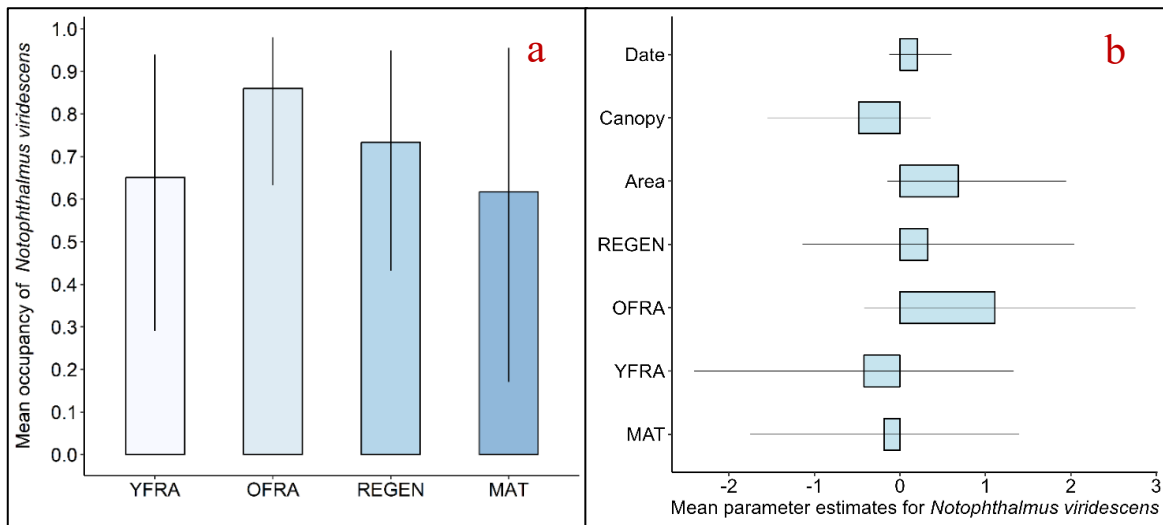
Figure 3.3.10. a) Mean estimated occupancy of *N. viridescens* across treatment types, Ψ_{YFRA} = 0.830 (CI = 0.522, 0.991), Ψ_{OFRA} = 0.721 (CI = 0.361, 0.961), Ψ_{REGEN} = 0.755 (CI = 0.324, 0.980), and Ψ_{MAT} = 0.660 (CI = 0.169, 0.977). b) Mean parameter estimates for *N. viridescens*.
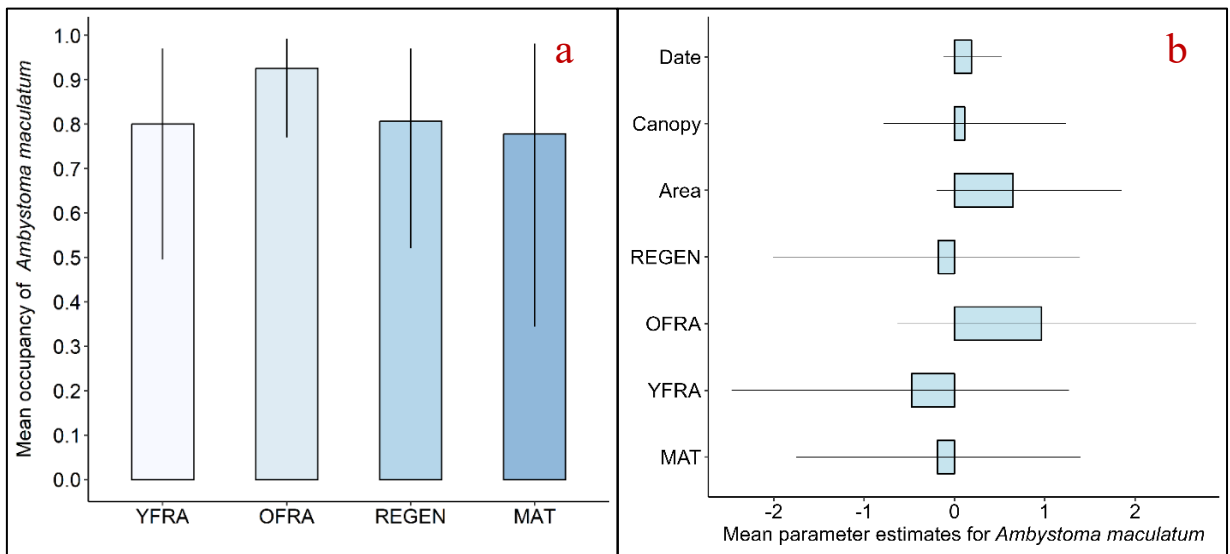
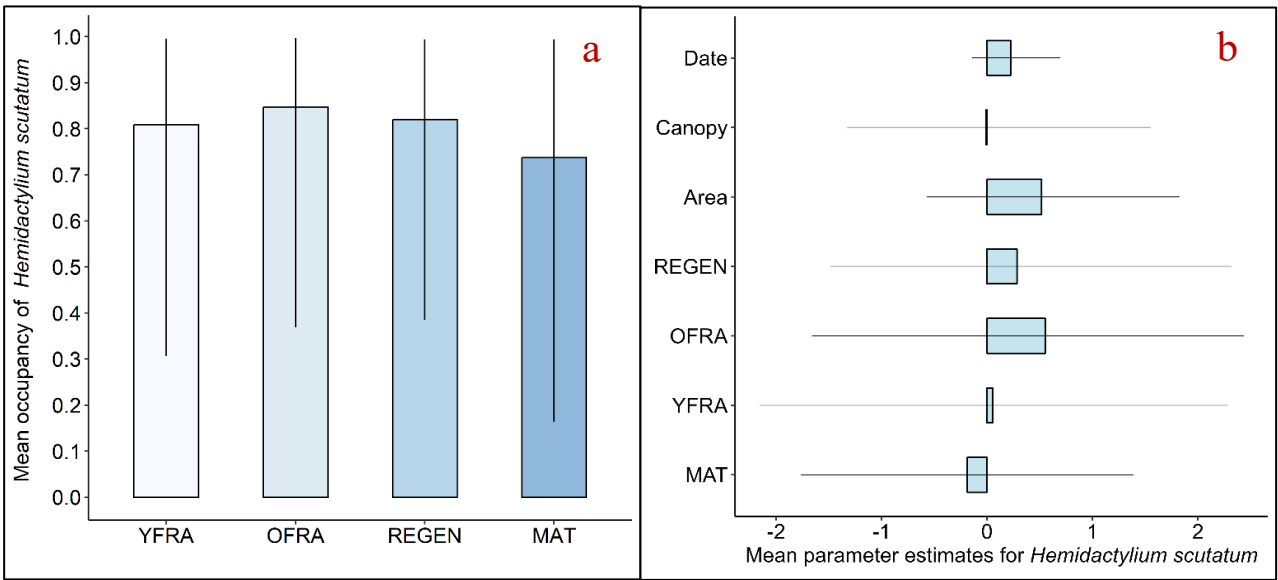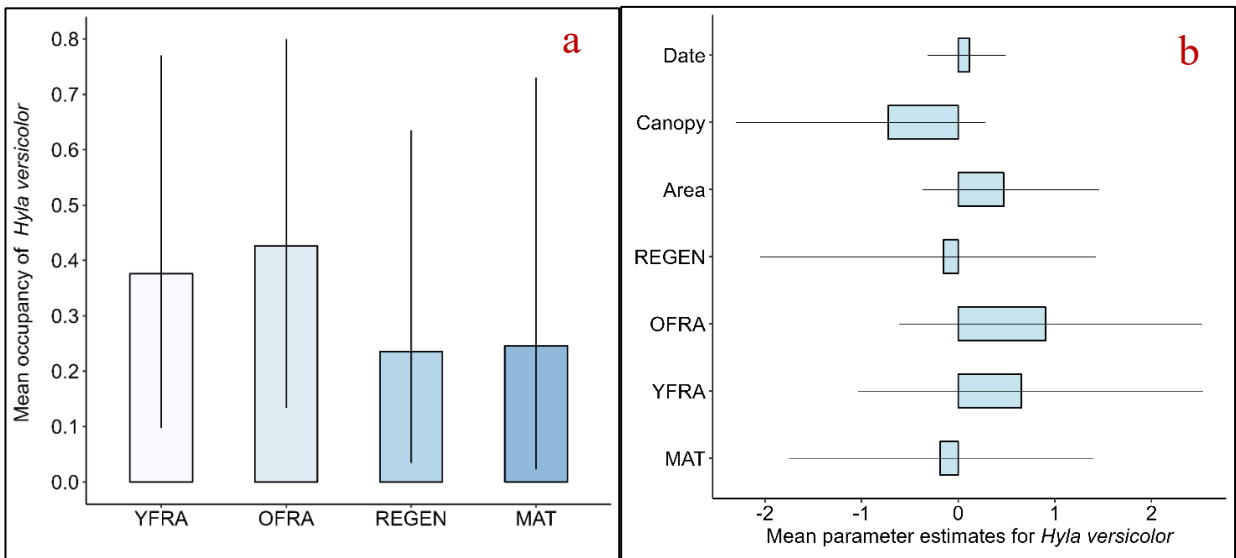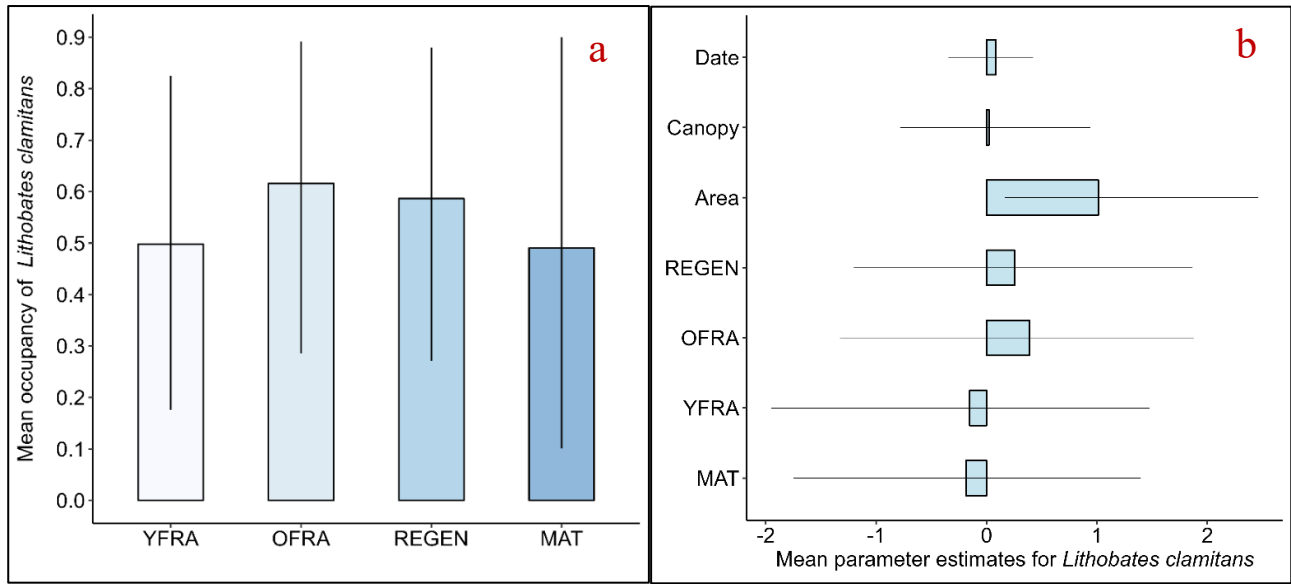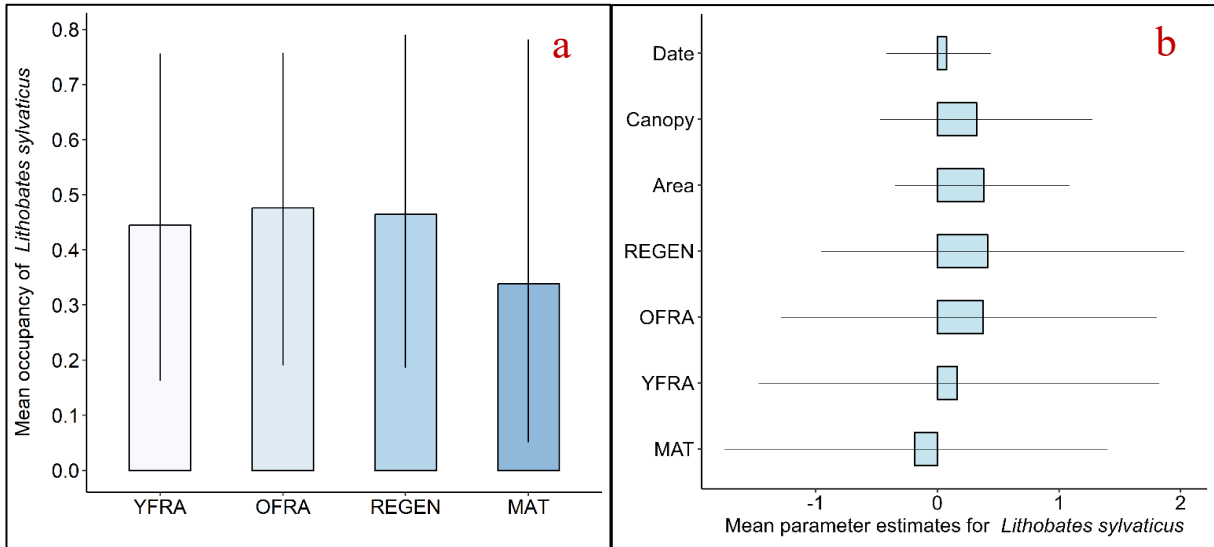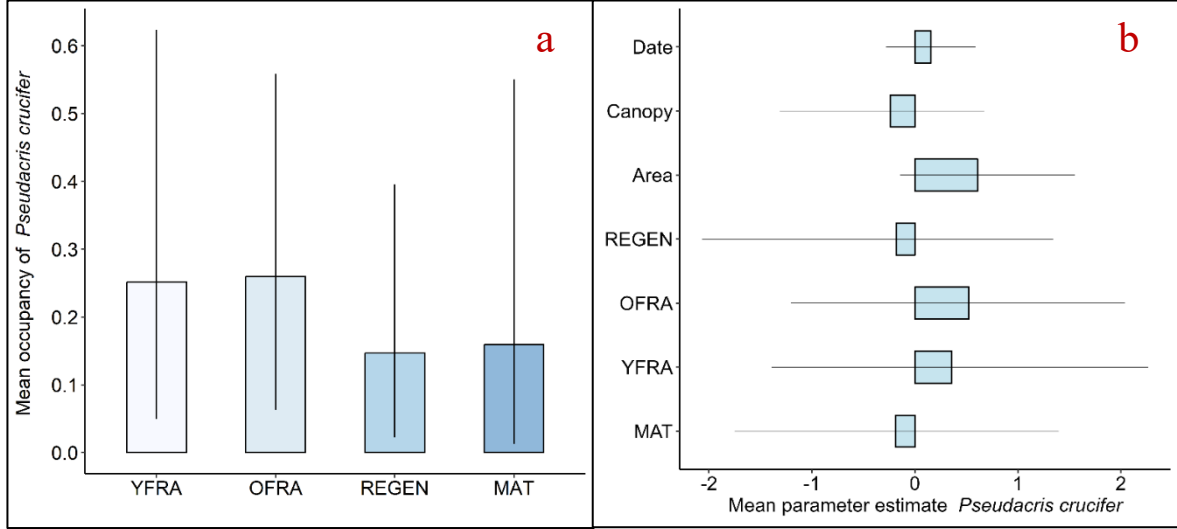**Figure 3.3.11.** a) Mean estimated occupancy of *P. crucifer* across treatment types, Ψ_{YFRA} = 0.156 (CI = 0.023, 0.475), Ψ_{OFRA} = 0.105 (CI = 0.012, 0.339), Ψ_{REGEN} = 0.228 (CI = 0.029, 0.645), and Ψ_{MAT} = 0.096 (CI = 0.004, 0.427). b) Mean parameter estimates for *P. crucifer*.

Table 3.3.1. Influence of site covariates on occupancy estimates for each species.

	YFRA	SD	95% CI	OFRA	SD	95% CI	REGEN	SD	95% CI	Area	SD	95% CI	Canopy	SD	95% CI
A. americanus	0.785	0.955	(-0.956, 2.692)	0.183	0.946	(-2.060, 1.797)	-0.400	0.894	(-2.392, 1.237)	0.135	0.540	(-1.301, 0.873)	-0.237	0.542	(-1.538, 0.683)
A. maculatum	-0.380	0.960	(-2.407, 1.390)	0.869	0.833	(-0.697, 2.620)	-0.349	0.812	(-2.074, 1.187)	0.556	0.409	(-0.139, 1.539)	0.097	0.441	(-0.695, 1.070)
H. scutatum	0.141	1.045	(-2.037, 2.278)	0.482	0.953	(-1.637, 2.333)	0.077	0.916	(-1.671, 2.149)	0.462	0.442	(-0.404, 1.446)	0.027	0.568	(-1.066, 1.310)
H. versicolor	0.390	0.887	(-1.132, 2.231)	0.474	0.780	(-1.166, 1.954)	-0.348	0.817	(-2.135, 1.160)	0.559	0.367	(-0.073, 1.406)	-0.156	0.441	(-1.129, 0.654)
L. clamitans	0.390	0.887	(-1.306, 2.243)	0.475	0.782	(-1.171, 1.955)	-0.346	0.816	(-2.134, 1.157)	0.559	0.366	(-0.074, 1.404)	-0.157	0.441	(-1.128, 0.651)
L. palustris	0.005	1.060	(-2.297, 2.094)	0.658	0.893	(-1.205, 2.446)	-0.291	0.947	(-2.346, 1.598)	0.475	0.512	(-0.560, 1.616)	-0.130	0.596	(-1.475, 1.021)
L. sylvaticus	0.171	0.814	(-1.446, 1.802)	0.310	0.764	(-1.324, 1.718)	0.266	0.770	(-1.131, 1.949)	0.387	0.317	(-0.267, 1.008)	0.268	0.418	(-0.456, 1.177)
N. viridescens	-0.285	0.936	(-2.274, 1.478)	1.026	0.826	(-0.484, 2.720)	0.129	0.792	(-1.350, 1.859)	0.589	0.421	(-0.083, 1.625)	-0.338	0.455	(-1.356, 0.438)
P. crucifer	0.391	0.888	(-1.312, 2.239)	0.478	0.781	(-1.167, 1.963)	-0.346	0.817	(-2.137, 1.167)	0.558	0.368	(-0.081, 1.410)	-0.158	0.442	(-1.134, 0.647)

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Table 3.3.2. Influence of sampling covariate β_1 (Date) on individual species detection probabilities. Neither of the covariates were statistically significant to detection probabilities for any amphibian species. Asterisks denote species that had statistically significant relationships to date.

Species	β_1 (SD)	95% CI
A. americanus	0.215 (0.256)	-0.325, 0.727
A. maculatum*	0.236 (0.164)	-0.083, 0.568
H. scutatum*	0.277 (0.203)	-0.095, 0.722
H. versicolor	0.213 (0.212)	-0.229, 0.632
L. clamitans	0.212 (0.213)	-0.233, 0.634
L. palustris	0.246 (0.267)	-0.263, 0.820
L. sylvaticus	0.137 (0.219)	-0.372, 0.509
N. viridescens*	0.256 (0.180)	-0.085, 0.635
P. crucifer	0.213 (0.213)	-0.233, 0.633
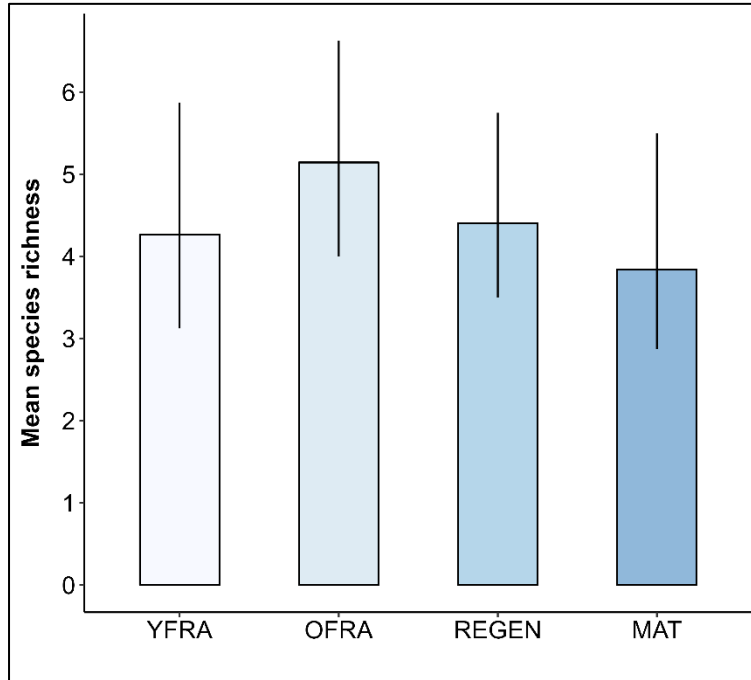
Figure 3.3.12. Mean species richness across treatment types, SpR_{YFRA} = 4.27 (CI = 3.12, 5.88), SpR_{OFRA} = 5.14 (CI = 4.00, 6.62), SpR_{REGEN} = 4.41 (CI = 3.50, 5.75), and SpR_{MAT} = 3.84 (CI = 2.88, 5.50).

Table 3.4.1. Influence of site covariates on abundance estimates for *A. maculatum*, *L. sylvaticus*, and *N. viridescens* including standard deviation and 95% confidence intervals.

	A. maculatum			*L. sylvaticus*			*N. viridescens*		
Covariate	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI
Date	0.215	0.096	(0.023, 0.405)	-0.098	0.076	(-0.245, 0.053)	0.189	0.148	(-0.096, 0.482)
MAT	2.266	0.193	(1.882, 2.636)	2.399	0.120	(2.161, 2.632)	1.874	0.472	(1.043, 2.940)
YFRA	0.007	0.267	(-0.508, 0.545)	-0.974	0.286	(-1.561, -0.424)	-0.668	0.395	(-1.455, 0.108)
OFRA	0.911	0.200	(0.529, 1.311)	0.497	0.140	(0.222, 0.771)	0.642	0.314	(0.048, 1.282)
REGEN	-0.516	0.224	(-0.965, -0.079)	-0.128	0.096	(-0.319, 0.057)	-0.482	0.362	(-1.166, 0.248)
Area	0.104	0.078	(-0.054, 0.255)	0.697	0.053	(0.596, 0.803)	0.517	0.096	(0.327, 0.700)
Canopy	0.309	0.096	(0.118, 0.500)	1.331	0.069	(1.195, 1.468)	-0.689	0.192	(-1.088, -0.325)
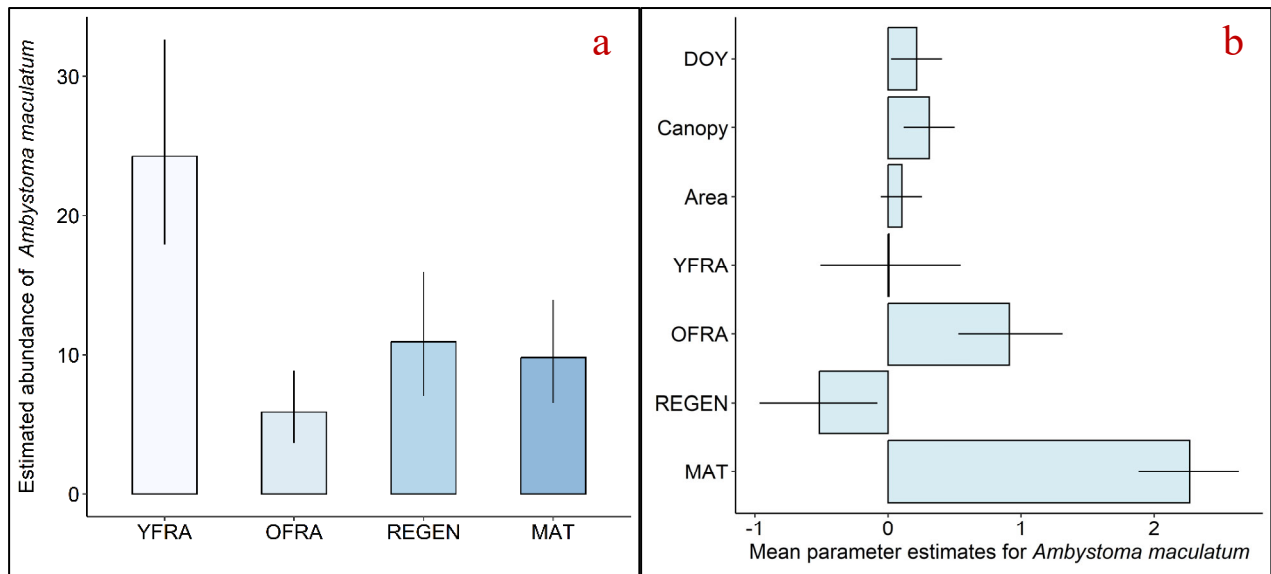
Figure 3.4.1. a) Estimated abundance of *A. maculatum* by treatment type, Ψ_{YFRA} = 24.3 (CI = 17.9, 32.8), Ψ_{OFRA} = 5.92 (CI = 3.72, 8.89), Ψ_{REGEN} = 10.9 (CI = 7.10, 16.2), and Ψ_{MAT} = 9.83 (CI = 6.55, 14.3). b) Mean parameter estimates for *A. maculatum*.
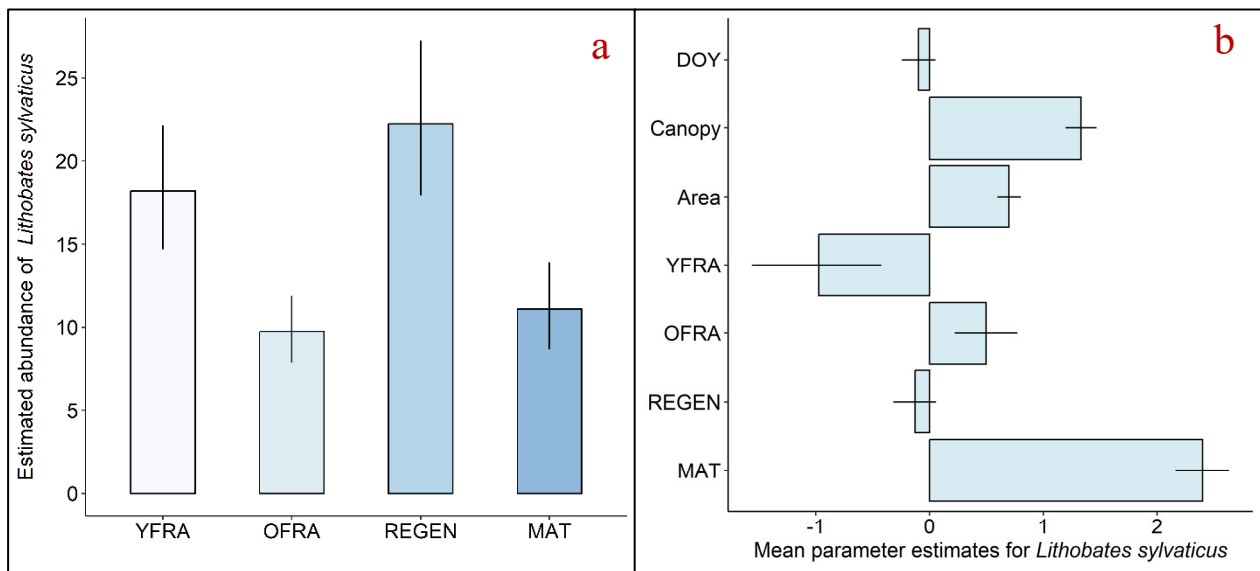
Figure 3.4.2. a) Estimated abundance of *L. sylvaticus* by treatment type, Ψ_{YFRA} = 18.1 (CI = 14.6, 22.1), Ψ_{OFRA} = 9.72 (CI = 7.87, 11.8), Ψ_{REGEN} = 22.2 (CI = 17.9, 27.0), and Ψ_{MAT} = 11.1 (CI = 8.66, 13.8). b) Mean parameter estimates for *L. sylvaticus*.

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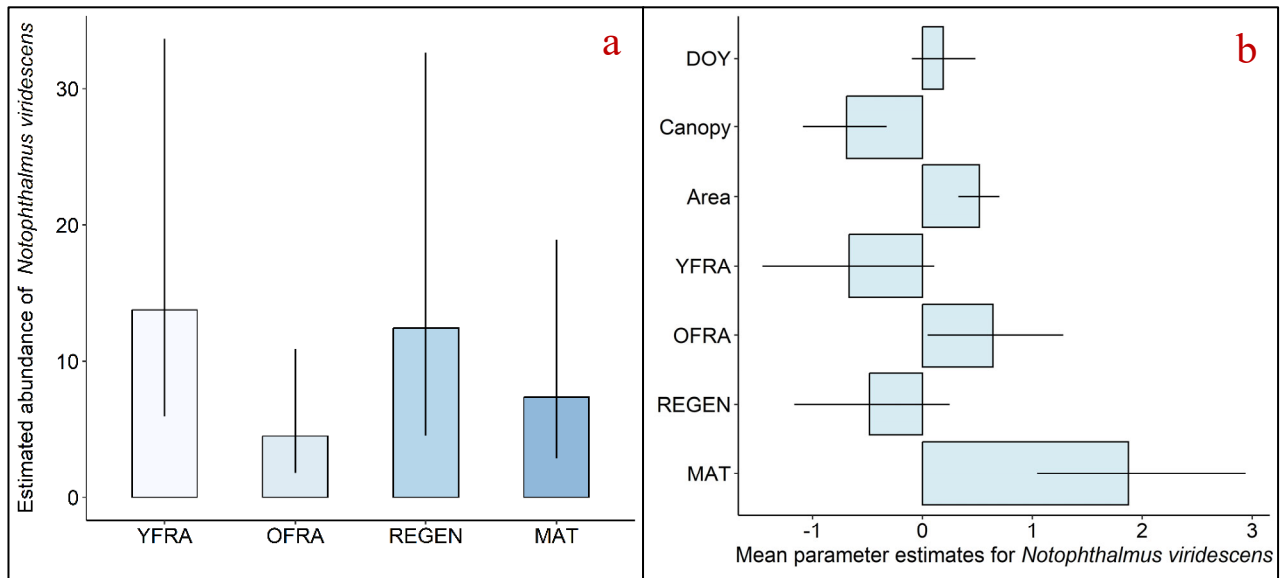
Figure 3.4.3. a) Estimated abundance for *N. viridescens* by treatment type, $\Psi_{YFRA} =$ 13.202 (CI = 5.380, 33.449), $\Psi_{OFRA} = 3.289$ (CI = 1.220, 7.878), $\Psi_{REGEN} = 11.902$ (CI = 4.331, 30.705), and $\Psi_{MAT} = 6.584$ (CI = 2.479, 16.527). b) Mean parameter estimates for *N. viridescens*.

Chapter 4. Discussion

It is important to understand not only the effects that reclamation practices have on populations, but also to gain insight on what environmental factors drive the effects of these practices. The results of my study provide some of that insight and address the question of how the FRA influences amphibian communities by yielding statistical evidence of the drivers behind species richness, occupancy, and abundance. The constructed wetlands of the FRA sites were comparable to REGEN and MAT sites in most water chemistry metrics and in amphibian occupancy, abundance, and species richness, showing that these sites have the potential to mimic ecological function of wetlands in undisturbed habitats. Overall, these results point to FRA wetlands being suitable habitat for many pond-breeding amphibian species.

4.1 Wetland Characteristics

While the majority of wetland characteristics did not significantly differ, I found several differences between wetland characteristics across treatment types. First, I found large differences in the canopy cover between the treatment types, with the YFRA sites having the least canopy due to being replanted 1-9 years prior to this study. Many of the REGEN sites and MAT sites had near complete canopy cover. Second, two landscape variables showed significant differences between treatment types, distance to forest and percent forest. These differences followed the same pattern as canopy cover, with REGEN and MAT sites having lesser distances to forest and higher percentages of

forested area compared to the FRA sites. As many of the REGEN and MAT sites had 100% canopy cover, they would also logically have 0 m as the distance to forest.

Several water chemistry metrics displayed significant differences between treatment types. The differences in pH between treatment types could be related to canopy cover, as the two parameters have been correlated in other wetland experiments (Simpson et al., 2021), though they were significantly correlated in my study. Mean pH of the FRA wetlands was higher than the REGEN sites, which has been documented in constructed wetlands in Kentucky (Drayer & Richter, 2016). OFRA sites had significantly higher levels of TOC than the REGEN sites based on the collected water samples. This result was unexpected, as REGEN sites have higher percentages of canopy cover and elevated TOC levels are often the result of decaying plant matter (Ahn & Jones, 2013). Aluminum content in the wetlands was another distinguishing feature between FRA sites and the REGEN and MAT sites. While the Tukey's HSD test grouped all treatment types together, the ANOVA p-value (0.054) suggests that there could still be significant differences between treatments. The sites with the highest aluminum concentrations were the REGEN and MAT sites, even though heightened aluminum presence in water is often associated with coal mining (Freda, 1991). This could provide some evidence that the FRA protocol of covering contaminated soil may be effective at keeping Al from leeching into the wetlands at these sites.

Compared to the findings of Lambert et al. (2021), which utilized many of the same sites, I found many differences, particularly in the water chemistry data. The average pH values found by Lambert et al. (2021) were dramatically more acidic than those from my study conducted just two years later. One possible explanation for the

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difference between Lambert et al. (2021) and my findings may be the US Forest Service's efforts to curb the effects of acid deposition inputs. These efforts may have been successful as the average pH of the OFRA sites (described in Lambert et al. (2021) as 8-year old wetlands) has risen from 3.20 to 6.78. Conductivity between Lambert et al. (2021)'s 8-year old wetlands and the OFRA wetlands of this study were also remarkably different, with Lambert et al. (2021)'s average conductivity at 23.22 μS cm-1 while the average from my findings was 52.09 μS cm-1. As conductivity is reliant on the amount of dissolved ions in the water, I also compared individual ion concentrations found in both studies. The average concentration of chlorides from Lambert et al. (2021)'s study (8-year old wetlands) was 0.29 mg/L and in my OFRA wetlands it was 0.70 mg/L. Lambert et al. (2021)'s average sodium concentration at 8-year old wetlands was 0.18 mg/L, while the OFRA average from my study was 0.75 mg/L. Potassium concentrations in Lambert et al. (2021)'s 8-year old wetlands averaged 0.59 mg/L while my OFRA sites averaged 1.39 mg/L. These differences could also be linked to the soil liming by the Forest Service which has been shown to increase conductivity in wetlands (Tran et al., 2014). Further information about the soil properties at these sites would be needed to fully understand the wide variation seen in a span of just two years.

Overall, there were more similarities between wetland characteristics across treatment types than differences. The water chemistry analyses showed similar water quality between constructed wetlands and wetlands that have been undisturbed for decades, which points to some degree of success for the FRA method returning the ecosystem to its approximate function prior to mining. With the extensive planting efforts on FRA sites, other characteristics that did show differences will likely change over time.

Canopy cover, distance to forest, and percentage of forested area are all variables that are expected to differ in the near future, closing some of the gap between constructed and natural wetland characteristics.

4.2 Species Overview

I detected all species had been previously detected in the region (Lambert et al., 2021; Barry et al., 2008). Some species that I did not find that could live in this region were *Lithobates catesbeianus* and *Scaphiopus holbrookii*. Similar studies have detected *L. catesbeianus* in constructed wetlands in West Virginia, though they are more commonly found in larger, permanent bodies of water due to their extended (i.e., ≥ 2 yr) larval period (Balcombe et al., 2005). Another previous study mentioned *S. holbrookii* as a species that likely resides in this area, but their irregular breeding patterns make them harder to detect than other species (Lannoo, 2005; Lambert et al., 2021). Every species that would be expected to be found breeding in our study sites was detected, meaning there are no species that have been excluded from this region purely due to mining and/or reclamation activities.

4.3 Occupancy and Species Richness

I found that species' occupancy and species richness did not differ across treatments. Several studies have been conducted on post-mining re-colonization of amphibian species, which could provide some insight into why there are no statistically significant differences in occupancy of wetlands between treatment types. Stiles et al.

(2017), looked at colonization after mining in Indiana and found that many species, including *N. viridescens*, *L. clamitans*, and *P. crucifer* were able to successfully colonize wetlands on reclaimed lands. Many of the individuals captured during in Stiles et al. (2017) were newly metamorphosed juveniles, suggesting that younger amphibians are driving the colonization of new wetlands (Stiles et al., 2017). Another study, conducted in North Carolina, looked at colonization of newly constructed wetlands compared to reference wetlands and reported that site philopatry is very weak among many amphibian species that will readily move to new, available wetlands (Petranka et al., 2003). Petranka et al. (2003) examined landscape variables as possible drivers of colonization, including distance to nearest wetland, distance to forest, and distance to nearest stream, but none of these factors were found to be important. Instead, hydroperiod, wetland depth, and wetland area were the most critical to amphibian colonization (Petranka et al., 2003). Though area was not statistically significant to occupancy in my study, it did have a strong positive trend, meaning this could still be a critical factor for colonization.

The lack of differences could also be attributed to my method of modeling, which generated occupancy estimates for multiple species, rather than having models for each species individually. There are benefits to both methods of modeling, with multi-species occupancy modeling filling in some data gaps for species with few detections by utilizing data from more prevalent species. While this creates fewer differences among species occupancy estimates (Pacifici et al., 2014; Sauer & Link, 2002; Zipkin et al., 2009; Zipkin et al., 2012), it also provides a better indication of community occupancy. It has been found to be more effective for creating management plans that accommodate the entire amphibian community, rather than excluding "data-poor" species like *H. scutatum*

and *H. versicolor* from consideration (Zipkin et al., 2009; Zipkin et al, 2012, Zipkin et al., 2020).

The parameter with the most consistent influence on species occupancy was wetland area. All significant relationships were positive, indicating that larger wetlands support greater amphibian occupancy. This has been found by other studies that show wetland size is an important indicator of amphibian occupancy and species richness (Drayer et al., 2020; Semlitsch et al., 2015; Lambert et al., 2021). No other parameters had any statistical significance to community occupancy or species richness. Overall, the lack of influence by treatment type indicates that FRA wetlands are able to be occupied by amphibian species just as well as natural wetlands and can host a wide variety of species.

4.4 Abundance Estimates

Estimations of abundance allow us to gauge the relative success of a species in a habitat and to examine how specific environmental parameters can influence that success. Forest-associated species, such as *A. maculatum* and *L. sylvaticus* (Gibbs, 1998) unsurprisingly showed preference for sites with more canopy cover. *N. viridescens* abundance estimates showed a strong negative relationship with canopy cover. Similar results were found by Drayer & Richter (2016) whose study showed that *N. viridecens* preferred constructed wetlands which had lower percentages of canopy cover over natural wetlands with closed canopies. The wetlands at the MAT sites and REGEN sites, which have mostly closed canopies, are more suitable for some of the amphibian species found in this region like *L. sylvaticus* (Skelly et al., 2002). There are other species that may

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benefit from the lack of canopy cover, however, such as the *P. crucifer*, that may not be able to grow and develop adequately in the conditions that a closed canopy wetland provide (Skelly et al., 2002). Canopy cover is indicative of many other environmental conditions that could influence larval growth such as water temperature and leaf litter that can provide nourishment for larvae, and therefore differences in canopy cover between treatment types could have cascading effects on ecological function (Werner & Glennemeier, 1999).

Lithobates sylvaticus and *N. viridescens* abundances had significant positive associations with wetland size, while *A. maculatum* showed a positive trend. The wetlands in my study ranged from 4.69 m^2 to 233.22 m^2 in area, providing habitat for species that prefer smaller wetlands as well as those that prefer larger wetlands. The wide range of wetland sizes suggests that creating a variety of wetland sizes across reclaimed minelands is the best solution for maximizing amphibian biodiversity and individual species abundance.

Only one species, *L. sylvaticus*, had a significant relationship to the YFRA sites and it was negative. The relationship between YFRA sites and *N. viridescens* abundance was not statistically significant, but there was a negative trend, suggesting that this species does not do well in YFRA wetlands. This was expected, considering both of these species are often associated with forested environments, though *N. viridescens* abundance also had a negative relationship with canopy cover. Salamanders, such as *A. maculatum*, breed in ponds but spend the majority of their adult lives in forested habitat (Pittman & Semlitsch, 2013). Significant differences between treatment types could therefore have ecological implications for amphibian species. Amphibians breeding in FRA site

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wetlands, which are still in early successional stages, would need to rely on nearby forest from unmined areas to provide habitat which could reduce colonization of these wetlands.

The results for the OFRA sites indicated that they are highly suitable for pond-breeding amphibians. All three species (*A. maculatum, L. sylvaticus,* and *N. viridescens*) had significant relationships with OFRA sites, and all were positive. This is a promising result suggesting that YFRA sites may become better habitat for these species as they mature.

Only one species, *A. maculatum*, had a statistically significant negative relationship with REGEN sites, but both other species had strong negative trends with this treatment type. Considering abundance was only estimated for the three most detected species, these negative relationships may not necessarily indicate that this treatment type has lower overall amphibian abundance.

Unlike the REGEN sites, which were negatively associated with the three species for which I estimated abundance, the MAT sites had positive associations with all three species for which I estimated abundance. This was unsurprising as these species are commonly associated with forested habitats and also due to the relatively undisturbed nature of these sites. As the references sites against which I compared the FRA sites and REGEN sites, it makes sense that the amphibian species native to this region would be found in greater abundances in these wetlands.

While most of the wetland characteristics were not incorporated into the models, I can still use these data to help interpret amphibian occupancy and abundance patterns.

Wetland pH is one characteristic that has been found to be linked to amphibian abundance estimates and also showed significant differences between treatment types. Constructed wetlands with higher pH values have been found to be better suited to *N. viridescens* and *L. clamitans*, both species that had high abundance estimates in FRA sites (Drayer & Richter, 2016). Other amphibian species, per Drayer & Richter (2016), found greater success in natural wetlands with higher pH levels. While my findings do not definitively suggest that the higher pH levels at the FRA sites have a dramatic impact on the species occupancy and abundance of these wetlands, this is still an important consideration when looking at overall wetland health between these sites and the more natural REGEN and MAT sites.

Another important aspect of wetland health, which can also have impacts on species abundance, is total organic carbons (TOCs). TOC levels are important specifically to amphibian health as increased TOCs can protect embryos from harmful UV rays that can prevent proper development and reduce the number of eggs that reach maturation (Calfee et al., 2006). Organic carbons can also bind with metals in wetlands, preventing them from solubilizing and, therefore, making them unavailable to aquatic organisms (Dodd, 2009).

High metal concentrations in wetlands have often been connected to low species abundances, particularly in regards to aluminum, since it becomes toxic when exposed to acid rain or acid mine deposition (Freda, 1991; Dodd, 2009). Although aluminum concentrations were elevated at some of the REGEN and MAT sites, values did not exceed 7,500 µg/L and the genus mean chronic value (GMCV), reported by the U.S. Environmental Protection Agency (EPA) for the genus *Lithobates* was over 10,684 µg/L

(U.S. EPA Office of Water, 2018). The REGEN and MAT sites are significantly more acidic than the FRA sites, though aluminum is not soluble in water above 5.5 which is lower than the pH values found at my sites (Lindsay & Walthall, 1996). Even though the aluminum levels are at a lethal level, this could still be a contributing factor to the relatively lower abundances of *L. sylvaticus* and *A. maculatum* and the lack of significant differences between occupancy at the different treatment types.

Chapter 5. Conclusions

Overall, FRA sites tended to host productive wetlands when compared against sites that were never actively managed and even sites that had never been mined. The OFRA sites seem to have developed into suitable habitat for most of the common amphibian species of the region, providing evidence that there is merit to the FRA, particularly when the wetlands are constructed near forested areas and have had some time for the canopy cover to develop. Potential future research that could further benefit amphibians on reclaimed lands might explore wetland connectivity through genetic analysis to see how forest fragmentation has influenced dispersal among sites or even conduct an extended study to see if any populations have developed site fidelity to the constructed wetlands. Further experimentation with wetland construction methods (depth, distance from forest, addition of logs/rocks, etc.) could also yield greater amphibian occupancy for future reclamation projects.

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