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MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Meghan J. McGill

A Project Presented in Partial Fulfillment
of the Requirements for the Degree
Masters of Science
in Environmental Biology

REGIS UNIVERSITY
May, 2018

MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Meghan J. McGill

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CHAPTER 1. LITERATURE REVIEW: HUMAN-INDUCED BIODIVERSITY LOSS IS THE MOST INFLUENTIAL FACTOR IN THE INDUCTION AND STRENGTH DETERMINATION OF TROPHIC CASCADES

Introduction

A trophic cascade is an indirect, multi-trophic-level ripple effect, induced by a significant change in the presence of an important member within a food web (Daskalov, Grishin, Rodionov, & Mihneva, 2007). Often these cascades are top-down, and stem from the addition or removal of an apex predator. Loss of these top species alters the population demographics of that predator's respective prey, and in turn, populations of the plant or animal that prey feeds on. These ripples have the potential to greatly alter mesopredator, herbivore, and vegetation composition within communities, and may even lead to entire regime shifts (i.e. abrupt changes in ecosystem function and structure) (Bergstrom et al., 2009; Daskalov et al., 2007; Österblom et al., 2007). These shifts may open niches for invasive species establishment as well as facilitate local extinction events.

Because trophic cascades can substantially change ecosystems, identifying the possible drivers that influence the strength of trophic cascades is important to better manage and mitigate their indirect influence on communities. Human-induced biodiversity loss is the most influential factor that determines when a trophic cascade will occur and how strong its ripples will be. Understanding the extensive influence humans have in ecosystems, both local and global, is vital in conserving biodiversity and preventing the ecosystem-wide negative consequences of trophic cascades.

Biodiversity and Ecosystem Health

High biodiversity stabilizes ecosystems by acting as an “insurance policy” that provides redundant species to fill niches when another species is removed (Cleland, 2011). This increases the likelihood that communities will contain species in different trophic levels that are resilient to food web perturbations (Cleland, 2011; Tilman, Reich, & Knops, 2006; Yachi & Loreau, 1999). Biodiversity loss can be particularly impactful to community composition when apex predators and keystone species are lost. Apex predators fill the food web’s highest trophic levels and control the population dynamics of lower trophic levels in a top-down fashion (Estes, Tinker, Williams, & Doak, 1998; Hollings, Jones, Mooney, & Mccallum, 2014). Apex predators are often also keystone species, because they disproportionately influence the presence of lower trophic levels compared to their overall biomass within the ecosystem (Byrnes et al., 2005; Estes et al., 1998; Hollings et al., 2014).

When these important predators are eliminated, their prey populations (mesopredators or herbivores) increase. This leads to an associated decrease in the populations (plants and animals) this prey feeds on, and forms a multi-trophic-level cascade with the potential to affect many different branches within a food web, thereby destabilizing the overall community (Albins & Hixon, 2013; Bergstrom et al., 2009; Worm & Duffy, 2003). Finke and Denno (2004) demonstrate that by maintaining greater species richness of predators, specifically intraguild predators that prey on each other as well as lower trophic levels, the effects of predator loss can be mitigated by dampening the trophic cascade strength. The redundant predator species maintain enough top-down control on herbivores to prevent their populations from increasing substantially, and prevent severe plant population decline.

Disease and Biodiversity

One driver of apex-predator loss and trophic cascade induction is disease. Pathogens that are quick spreading with a high mortality rate are the most detrimental. One such example is the decline of the Tasmanian devil, *Sarcophilius harrisii*, in response to a communicable cancer, Devil Face Tumor Disease (DFTD) (Hollings et al., 2014). In a little less than 20 years, Tasmanian devil populations have decreased by 95% over 80% of their range (Hollings et al., 2014). This population decline released the invasive mesopredator, *Felis catus*, from top-down control. In contrast, populations declined in another mesopredator, *Dasyurus maculatus*, because other mesopredator species preyed upon it in the absence of the Tasmanian devil (Hollings et al., 2014). This cascade provides evidence that the loss of an apex predator extends not just to lower trophic levels, but also ripples across food web branches within the same trophic level, thus disrupting more species than a linear food-chain model would suggest. Similarly, after the outbreak of an unknown pathogen, the mass die-offs of the herbivorous urchin, *Diadema antilarum*, in Caribbean coral reefs released macroalgae, the prey of choice of *D. antilarum*, from predation. This caused populations of this species to flourish, facilitated greater competition with coral for space, and limited coral colony establishment (Lessios, Robertson, & Cubit, 1984; Mumby et al., 2006).

Interestingly, disease-driven trophic cascades also have the potential to alter disturbance regimes. The eradication of rinderpest, a common pathogen of water buffalo in Africa, released the buffalo from this top-down disease control and allowed the buffalo to increase grazing pressure on native savannah grasses (Holdo et al., 2009). A decrease in grass biomass reduced fire occurrence by limiting the amount of fuel for fire disturbances, and ultimately led to an increase in the biomass and canopy cover of savannah tree species by promoting seedling survival (Holdo et al., 2009). Release from top-down control actually *increased* biodiversity in this case by facilitating not only herbivore species growth, but also the recruitment of tree species. This provides additional evidence that trophic cascades can affect multiple species both between trophic levels (herbivores and plants) and within trophic levels (grasses and trees).

Extirpation by Humans and Trophic Cascades

Human-induced extirpation (i.e. local extinction) is biased towards species occupying higher trophic levels, many of which are also apex predators and keystone species (Byrnes et al., 2005). Examples of trophic cascades from overfishing and hunting are abundant and affect many aquatic species including large predatory fish, seals, porpoises, and sea otters (Estes et al., 1998; 2013; Österblom et al., 2007). In all these aquatic communities, the loss of predator diversity released lower trophic levels and destabilized the entire community. One well-known terrestrial example of similar predatory release is the extirpation of wolves from Yellowstone National Park.

Wolves, *Canis lupus*, were hunted to extinction in Yellowstone in the mid-1920's, and were not reintroduced until 1995 (Ripple & Beschta, 2012). In their absence, populations of elk, *Cervus elaphus*, drastically increased, and so did browsing intensity on willow (*Salix spp.*) and aspen (*Populus tremuloides*) saplings (Painter, Beschta, Larsen, & Ripple, 2015; Ripple & Beschta, 2012; Ripple, Larsen, Renkin, & Smith, 2001). This browsing reduced the recruitment success of these plants, and tree height and abundance both declined (Painter et al., 2015; Ripple & Beschta, 2012; Ripple et al., 2001). In addition, this cascade extended beyond the linear food chain model and affected multiple food web branches including grizzly bear, another apex predator, and beavers, an herbivore species (Ripple, Beschta, Fortin, & Robbins, 2014; Smith & Tyers, 2012). Grizzly bears experienced increased foraging competition with elk for berries, an important part of their pre-hibernation diet (Ripple et al., 2014), and beaver populations declined as they abandoned riparian zones that no longer supported the willows they used for food (Smith & Tyers, 2012). This example shows how the loss of a single keystone species can have a massive impact on the ecosystem's total biodiversity through the loss or decline of other species at multiple trophic levels.

Because such human-induced species loss affects higher trophic levels at a much greater rate than lower levels (Byrnes et al., 2005), apex predators, the species whose presence is so imperative in maintaining ecosystem health and stability, may be at the greatest risk for extinction in the near future. Understanding species loss on a global scale is especially prevalent because the Earth is currently experiencing its sixth period of mass extinction, distinguished from others by the fact that the event is human-induced (Ceballos et al., 2015; Rockström et al., 2009). Humans are found on every continent, and with the global population increasing at an exponential rate, their influence on ecosystems will likely increase (Bongaarts, 2009). As humans increase their ranges to accommodate growing populations, local extinctions are expected (Vitousek, D'Antonio, Loope, Rejmanek, & Westbrooks, 1997). Increased overexploitation of species for food may also extirpate keystone species or remove prey species also needed by other apex predators. Consequently, food webs will be disrupted and destabilized.

Invasive Species and Biodiversity Loss

Invasive species pose a major threat to ecosystem health and the potential for trophic cascade induction, as their introduction may lead to the decline or extinction of native species (Gurevitch & Padilla, 2004). When these introduced species are predators, the food chain may be significantly disrupted by the addition of a new trophic level and/or increased predation pressure on mesopredators and herbivores (Tronstad, Hall, Koel & Gerow, 2010). In addition, the spread and introduction of many alien species is further exacerbated by anthropogenic interference as human populations increase (Vitousek et al., 1997).

One such example is the human-facilitated introduction of feral cats, *Felis catus*, to World Heritage Macquerie Island in the late 1820s. When cats became a new apex predator in the island's ecosystem, they rendered at least two native bird species extinct (Bergstrom et al., 2009). With no natural predators, cat populations grew unchecked. Sixty years later, rabbits, *Oryctolagus cuniculus*, were also unintentionally introduced, and became the prey of choice of the cats. However, rabbit feeding diminished native vegetation abundance, and thus the first trophic cascade was induced (Bergstrom et al., 2009).

To mitigate the effects of rabbit grazing, management crews released a pathogen, the *Myxoma* virus, to reduce rabbit populations (Bergstrom et al., 2009). While this strategy did decrease rabbit abundance and released vegetation from herbivory (the second trophic cascade, a reversal of the first one), it also caused cats to prey upon the native birds as rabbits became harder to find. This shift prompted management crews to exterminate cats from the island (Bergstrom et al., 2009). With no predators to impose top-down control, rabbit populations grew again, herbivory intensified, and the islands vegetative composition experienced a dramatic shift from long-lived, slow-growing native species, to fast-growing, often invasive, species (the third cascade, a strengthened version of the first cascade) (Bergstrom et al., 2009).

This is an extreme example, but certainly not the only one, of how the introduction of non-native species destabilizes ecosystem dynamics and leads to the extinction of native species, the introduction of additional alien species, and the loss of overall biodiversity. A similar ecosystem-wide disruption is seen in studies on invasive whelks and crabs prompting the loss of oyster habitat in California (Kimbro et al., 2009), and the human-facilitated introduction of an invasive comb jelly, *M. leidyi*, into an ecosystem already experiencing a trophic cascade caused by overfishing (leading to a strengthening of this cascade) (Österblom et al., 2007). These studies suggest that invasive species, once established, may be nearly impossible to remove without further upsetting the community.

Climate-Induced Cascades and Biodiversity Loss

Because of its global span and the multiplicative effect it has on other drivers of trophic cascades, human-induced climate change may threaten the world's biodiversity. The global climate is changing at an alarming rate in response to increases in atmospheric CO_2 produced through human activities (namely, the burning of fossil fuels and deforestation) (Mitchell, Lowe, Wood, & Vellinga, 2006). While evidence of direct climate-induced trophic cascades is scarce, external climatic fluctuations have the potential to alter endogenous community aspects. As global temperatures rise, many terrestrial species are experiencing range shifts to higher elevations and more northern latitudes to find suitable habitats (Chen, 2012). Along with the loss of biodiversity in the original ecosystems, there is the potential that the moving species may become invasive within their new range, thus disrupting the new ecosystem, increasing interspecific competition, and leading to the loss of native species (Hellmann, Byers, Bierwagen, & Dukes, 2008). Additionally, for northern species, or species with range sizes too large to be shifted, the inability to move to more habitable areas may lead to their extinction, and further biodiversity loss (Thomas et al., 2004). When these extinctions are of apex predators and keystone species, the initiation of trophic cascades is highly probable.

Climatic fluctuations also pose a risk for future disease outbreaks by imposing exogenous factors on food webs. One such outbreak occurred in 1993 during an El Niño event (Yates et al., 2002). Unusually high precipitation in the American southwest led to a large increase in vegetative biomass. This abundance in plants provided food to support an increased population of deer mice, *Peromyscus maniculatus*, the vector of hantavirus (Yates et al., 2002). The large populations of deer mice living in close proximity to residential areas led to the first outbreak and the discovery of this particular strain, and killed 10 people (Yates et al., 2002). This disease had always existed within the population, but it took external climatic factors to trigger its outbreak.

Current global climate increase estimates of 2-6 °C are predicted to not only prompt further species range shifts and invasive species introduction, but also increase extreme weather events and El Niño frequency (Chen, 2012; Mitchell et al., 2006; Timmerman et al., 1999; Yeh et al., 2009). More frequent El Niño events may provoke future trophic-cascade-induced disease outbreaks of not only hantavirus, but other vector-borne diseases like dengue, yellow fever, malaria, zika virus, and West Nile virus (Reiter, 2001).

Conclusion

To preserve global biodiversity and reduce the occurrence of trophic cascades, humans must recognize their complex influence on ecosystem health. Conservation plans and better management efforts are needed to stop the spread of invasive species, remove established invasive species (if possible), and avoid local extirpation of important predator species. Furthermore, policies to reduce CO_2 emissions and switch to more renewable energy sources are required to prevent further increases in global temperature. To ensure the resiliency of food webs and provide an “insurance policy” in the event of perturbations, maintaining high biodiversity is imperative. In addition, a shift from looking at trophic cascades as linear, food-chain-altering models, to a more comprehensive food web model, would provide a more comprehensive view of how communities as a whole respond to disturbances.

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CHAPTER 2. GRANT PROPOSAL: UNDERSTANDING BIODIVERSITY’S ROLE IN TROPHIC CASCADE STRENGTH

Abstract

This study aims to provide a deeper understanding of the role of competition in food webs, and more specifically, in trophic cascades induced by biodiversity loss. Trophic cascades are food web disturbances that result from the removal of an important species, often a predator, and lead to dramatic changes in herbivore and plant populations. It is critical to understand the mechanisms that drive and mitigate trophic cascades, because global biodiversity loss is increasing. Previous research suggests that biodiversity, specifically intraguild biodiversity with members in the same trophic level, is an important factor in reducing the negative effects of trophic cascades. High biodiversity increases competition, which limits population growth of individual species. Research on competition in relation to trophic cascades is scarce, prompting the need for more direct study.

I plan to test the hypothesis that increased herbivorous insect biodiversity will decrease typical trophic cascade strength, by increasing competition between the herbivores and reducing plant loss. Using microcosm ecosystems in fish tanks, I will study a three-trophic-level food chain where a predator is present, and a two-trophic-level cascade where the predator has been removed. By manipulating herbivore diversity and predator presence in the tanks, I will investigate how herbivore diversity influences competition, and how the effect of changing herbivore diversity differs in food webs with and without predators. I expect that higher herbivore diversity will yield higher average plant biomass compared to herbivore monoculture treatments, and that plant biomass will be greater in tanks where the predator is present. Maintaining high biodiversity within ecosystems ensures that another mechanism, competition, maintains stable levels of herbivores and protects against major plant loss. My study will provide information to aid in management practices to help sustain ecosystems experiencing biodiversity loss.

Background/Rationale/Significance

Understanding biodiversity loss on a global scale is especially important as the Earth undergoes its sixth period of mass extinction, distinguished from others by the fact that it is caused by a single species, humans (Ceballos et al., 2015; Rockström et al., 2009). High biodiversity stabilizes ecosystems by acting as an "insurance policy" that provides redundant species to fill community roles when another species is removed (Cleland, 2011). This increases the likelihood that communities will possess different species in the same trophic levels, that is species that eat the same kind of food, that are resilient to food web disruptions (Cleland, 2011; Tilman, Reich, & Knops, 2006; Yachi & Loreau, 1999). Trophic cascades are perturbations with the potential to ripple across multiple branches of a food web. Trophic cascades are indirect, multi-trophic-level fluctuations, that are induced by a significant change in the presence of an important member within a food web (Daskalov, Grishin, Rodionov, & Mihneva, 2007). They may greatly alter populations of less dominant predators, called mesopredators, as well as herbivores and vegetation composition within communities, and may even lead to entire regime shifts (i.e. abrupt changes in ecosystem function and structure) (Bergstrom et al., 2009; Daskalov et al., 2007; Österblom et al., 2007). Biodiversity buffers cascade strength after predator loss, and is therefore critical within ecosystems. By maintaining high biodiversity, we can reduce the negative effects of trophic cascades and prevent further loss of species.

Biodiversity loss can be particularly impactful to community composition when apex predators and keystone species are removed. Apex predators fill the food web's highest trophic levels and control the population dynamics of lower trophic levels in a top-down fashion (Estes, Tinker, Williams, & Doak, 1998; Hollings, Jones, Mooney, & Mccallum, 2014). Keystone species disproportionately influence the population dynamics of lower trophic levels compared to their overall biomass within the ecosystem (Byrnes et al., 2005; Estes et al., 1998; Hollings et al., 2014). Apex predators are also often keystone species. When these important predators are eliminated, their prey populations (mesopredators or herbivores) increase. This leads to an associated decrease in the populations of plants and animals this prey feeds on. These sudden changes in populations create a multi-trophic-level cascade with the potential to affect many different branches within a food web, thereby destabilizing the overall community (Albins & Hixon, 2013; Bergstrom et al., 2009; Worm & Duffy, 2003). In addition, apex predators are at a greater risk of being lost because human-induced extirpation (i.e. local extinction) is biased towards species occupying higher trophic levels (Byrnes et al., 2005, Vitousek, D'Antonio, Loope, Rejmanek, & Westbrooks, 1997). As a result, apex predators, the species whose presence is so imperative in maintaining ecosystem health and stability, may be at the greatest risk for extinction in the future.

In a three-level trophic cascade, the release of lower trophic levels after apex predator loss impacts vegetative composition, and may depress native plant biomass and facilitate invasive species introduction (Bergstrom et al., 2009). Loss of plant biomass poses a threat to other species within food webs that may use these plants for shelter or food. For example, after wolves were hunted to extinction in Yellowstone National Park, lack of predation allowed elk populations to increase dramatically (Painter, Beschta, Larsen, & Ripple, 2015). Consequently, more intensive elk browsing on aspen and willow saplings led to an overall decrease in the abundance of these plant species (Ripple, Beschta, Fortin, & Robbins, 2014). Beaver populations in Yellowstone also declined as they abandoned riparian zones that no longer supported the willows they used for food and building materials (Smith & Tyers, 2012). Preventing sudden upsets in communities that cascade across multiple food web branches is important for biodiversity maintenance at all trophic levels.

Finke and Denno (2004) used insects to demonstrate that higher biodiversity of intraguild predators (i.e. species within the same trophic level) that prey on each other and lower trophic levels, dampens trophic cascade strength and mitigates apex predator loss. Greater intraguild predator diversity balances both herbivore diversity and plant growth. The redundant predator species maintain enough top-down control on herbivores to prevent herbivore populations from increasing substantially and averts severe plant population decline (Finke & Denno, 2004). However, because predator loss is so prevalent, it is imperative to identify other means of mitigating trophic cascades other than managing predator diversity.

Following Finke and Denno (2004), I plan to test whether high intraguild herbivore diversity dampens three-level trophic cascades involving a predator, herbivore, and primary producer. The niche overlap theory suggests that by increasing biodiversity of herbivores who utilize the same food source, competition for that resource increases (Pianka, 1974; Medina, Bonnaud, Vidal & Nogales, 2014; Zeilinger, Olsn & Andow, 2011). This competition reduces individual species fitness and maximum population size (Pianka, 1981). By inhibiting dramatic population increases when an apex predator is removed, competition among herbivores may reduce the strength of trophic cascades, and increase a food web's resistance to predator loss. Smaller herbivore population sizes may exert less grazing pressure on vegetation, and maintain greater plant biomass. Preserving high levels of herbivore diversity ensures that another mechanism, competition, maintains sustainable population sizes under low predation pressure. In this way, biodiversity provides redundancy in both trophic and competitive roles of species. Research examining this mechanism in direct relation to trophic cascades is scarce, prompting the need for further examination of competition within food webs experiencing this disturbance.

Because trophic cascades can substantially change ecosystems, understanding the processes that influence their strength is important to better manage and mitigate their indirect influence on communities. Conserving biodiversity will not only prevent trophic cascades by providing multiple species to fill functional niches, but also lessen the negative effects of cascades by strengthening other limiting mechanisms. It is vital that adequate research be available for management of ecosystems experiencing species loss, as disturbance in the overall food web is likely. My study will provide necessary information to address the current gap in knowledge of trophic cascade mechanisms, and focus our understanding of biodiversity and food webs.

In addition to pursuing knowledge, Regis University encourages students to live thoughtfully by addressing the question "How ought we to live?". This study will increase our understanding of ecological communities and how human interference alters community dynamics. This allows us to not only minimize future negative, anthropogenic impacts on ecosystems, but also mitigate those issues humans have already created. This directly addresses Regis' core question by forcing us, as humans, to acknowledge our influence in nature and encourage us to make a change.

Purpose and Specific Aims

The purpose of this project is to understand the effects of changing food web diversity on the strength of a trophic cascade. With this knowledge, we should tailor management plans towards maintenance of high biodiversity within the whole community, rather than focusing solely on the preservation of individual species. I will test the hypothesis that increased herbivorous insect biodiversity will decrease typical trophic cascade strength by increasing competition and reducing plant loss. I plan to use microcosms containing the predacious hunting spider *Pardosa amentata*, three species of generalist, herbivorous leafhoppers from the suborder Auchenorrhyncha (*Arthaldeus pascuellus, Deltocephalus pulicaris, Streptanus sordidus*), and the perennial grass, *Festuca rubra*.

Prediction 1: Increased herbivore diversity will increase interspecific competition and limit herbivore population increases after a predator is removed. This will result in smaller herbivore populations and higher aboveground plant biomass in microcosms with higher diversity than monocultures that contain only one herbivore species at similar densities. I predict this effect of biodiversity will be stronger in microcosms without predators than in microcosms where predators are present and already controlling herbivores. Because interspecific competition among herbivores has the potential to dampen trophic cascade strength by asserting a new control-pressure on herbivore populations, I will not see the dramatic decrease in plant biomass that would be expected with a typical trophic cascade.

Prediction 2: In microcosms with increased herbivore diversity and no predator, one species will emerge as dominant over other species. This should result in one species having significantly higher abundance than the other herbivore species. In addition, microcosms without predators will have more variation in population sizes than microcosms that are being controlled by predators. Since the removal of apex predators releases lower trophic levels from top-down control, previously subordinate species, such as mesopredators, will increase dominance within a food web and assert greater predation pressure than would be expected under normal circumstances (Medina, Bonnaud, Vidal & Nogales, 2014; Hollings, Jones, Mooney & McCallum, 2014; Bergstrom et al., 2009). When their predator is removed, herbivores are likely to compete more fiercely, and the greatest competitor will maintain the highest population abundance.

Methods

Animal Study Species

Leafhoppers are small sapsuckers that feed on the sap transporting part of plants, phloem (Biedermann, Achtziger, Nickel & Stewart, 2005). Multiple species are known to reside on the same plant and engage in interspecific competition (Denno, Raupp & Tallammy, 1981). To reduce this competition, species disperse, utilize different regions of the plant, or rely on predator-induced mortality to reduce competitor populations (Stiling, 1994). However, my microcosm experiment is a closed system in which predation is controlled, prohibiting dispersal and regulating predation mortality, and thus I predict competition will increase. The system will also contain only a limited amount of plant resources (and space on each plant), meaning the insects must alter their population dynamics to adapt.

I will use *Arthaldeus pascuellus, Deltocephalus pulicaris,* and *Streptanus sordidus* as study species because they all feed on the same grass species, *Festuca rubra*, are roughly the same size, can be found in the same grassland habitat, and all produce an average of two generations annually (Nickel & Remane, 2002). Leafhoppers are very diverse. Their populations react quickly to disturbances and they serve as important prey sources, making them model organisms for studying changes in community and food web dynamics (Biedermann, Achtziger, Nickel & Stewart, 2005; Everwand, Rösch, Tscharntke & Scherber, 2014).

The hunting spider, *Pardosa amentata*, is a generalist predator known to feed on leafhoppers and occupy the same habitat (Virant-Doberlet, King, Polajnar & Symondson, 2011; Komposch & Holzinger, 2005). *P. amentata* is found in a variety of ecosystems around the world, including grasslands (Rushton & Eyre, 1992; Jocqué & Alderweireldt, 2005; Clough, Kruess, Kleijn & Tscharntke, 2005). This species has been used in previous studies using leafhoppers as the main prey (Virant-Doberlet, King, Polajnar & Symondson, 2011).

Lab Procedure

This will be a 12-month experiment, occurring between January 3, 2018 and January 2, 2019. I will create microcosms using a total of 24 10-gallon fish tanks, measuring 56 × 30.5 × 34 cm and covered with 0.6mm mesh (Finke & Denno, 2004). Each tank will be filled 10-cm deep with all-purpose potting soil. I will place two individual *Festuca rubra* stems into each tank. Each plant will be roughly 12 cm tall and have the same number of tillers (i.e. shoots). I will then add the animal treatments and place the tanks under grow lights. The grow lights will be turned on at sunrise and turned off at sunset to mimic natural light cycles.

This study follows a design looking at a three-trophic-level food chain where a predator is present, and a two-trophic-level cascade where the predator has been removed (Figure 1). One factor investigates how changing herbivore diversity effects competition. A second factor looks at the effect of this changing diversity in food chains with and without predators.

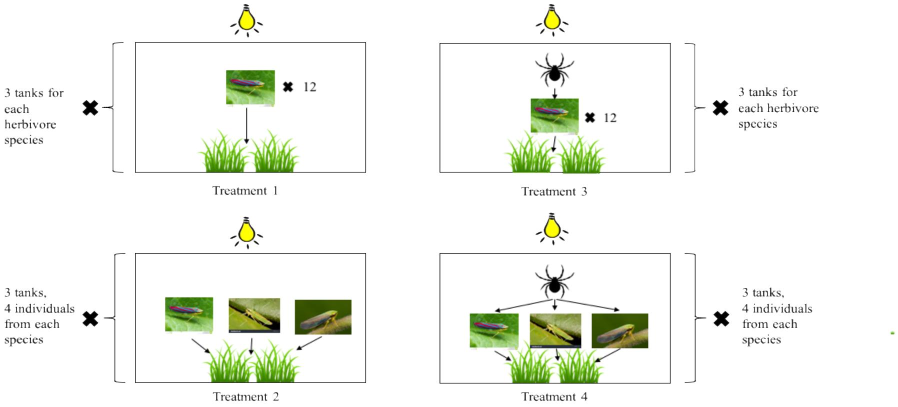

Figure 1: Tank treatments for my 12-month study. Each tank will contain an initial herbivore density of 12 individuals, regardless of diversity level. Each tank will be placed under grow lights to simulate natural light cycles. Treatment one contains one species of herbivore and the plant species, treatment two contains all three herbivore species and the plant species, treatment three has one herbivore species, one spider, and the plant species, and treatment four has all three herbivore species, one spider, and the plant species.

Initial herbivore density will be set at 12 individuals for every tank. Treatment one uses one species of herbivore and the primary producer. Each herbivore species will have three replicates for a total of nine tanks. Treatment two uses all three herbivore species and the primary producer. This treatment will have three replicates. Treatment three uses the spider, one herbivore species, and the primary producer. I will prepare three replicates for each herbivore species, for a total of nine tanks. Finally, treatment four uses all three herbivore species, the spider, and the primary producer, and will have three replicates.

At the end of the study, on January 3, 2018, I will count and record each insect individual from each tank, to calculate population totals. I will then remove all aboveground vegetation, dry the plants in an oven at 55°C for three days, and weigh them to calculate the aboveground biomass for each tank.

Statistical Analysis

I will adapt statistical methods used by Finke & Denno (2004) for analysis of my data in R (RStudio Team, 2016). Aboveground plant biomass and herbivore abundances from tanks of the same treatment will be pooled and averaged. The effect of herbivore diversity and predator presence on aboveground biomass will be analyzed using a mixed-model, two-way ANOVA with an interaction. Treatment type and herbivore species are the fixed-variables and treatment tank is the random variable. If biomass significantly differs between treatments, I will compare the treatments using individual t-tests with a Bonferroni correction for multiple comparisons. If needed, data will be log-transformed to normalize before analysis. In addition, I will compare final population sizes of all three herbivore species from the treatment two tanks using a one-way ANOVA with species as the independent variable. If this test shows that abundance differs between the species, then I will do individual t-tests with corrections to see which species is dominant. These data will also be compared to data from treatment four, in which the predator and all three herbivores are present, to see if population sizes differ with the presence of a predator.

I expect that higher herbivore diversity will yield higher average plant biomass compared to monoculture treatments. I also expect that plant biomass will be greater in tanks where the predator is present, than in tanks where the predator has been removed. In addition, I predict that the effect of herbivore biodiversity will differ based on predator presence or absence, indicating an interaction between predator status and biodiversity. Predator presence should have a greater effect on monoculture tanks than tanks with high herbivore diversity. Finally, in high herbivore diversity tanks with a predator present, I expect herbivore abundances to be lower overall and more even across species. However, when the predator is removed, I predict the overall herbivore abundances will be higher, but with more variation as the most well-adapted species outcompetes the others.

Work Plan

On January 3, 2018, I will prepare tank treatments and begin grow light cycles. Over the 12-month study period, I will observe the tanks every two days to ensure appropriate moisture levels. On January 3, 2019, I will conduct my abundance survey, begin drying plants, and start data entry. When the drying cycle is complete, I will weigh plants and dispose of all specimens. All analysis will be completed within one month of the study end date.

Budget

Table 1: Description and price of necessary study items.

Items ***(Please itemize amounts below)***	**Description**	**Funds requested from URSC**	**Funds requested from other sources**	**Source of other funds**
Supplies				
Equipment (non-consumables)				
Aqua Culture Aquarium, 10 gal	**$14.72 at walmart.com (24 needed)**	**353.28**		
4-foot, LED, Retrofit Grow Light	**$35.86 at beeslighting.com (7 needed)**		**251.02**	NSF Graduate Grant
Equipment (Consumable)				
14 Qt. All Purpose Potting Soil by Oldcastle	**$2.19 walmart.com (2 needed)**	**4.38**		
Tierra Garden 50-7000 Haxnicks Micromesh Prepack Blanket	**198"L x 70"W cut to size $25.35 at amazon.com (4 needed)**		**101.4**	NSF Graduate Grant
Pardosa amentata, Living, Pack of 6	**$38.50 at carolinabiological.com (2 needed)**	**77.00**		
Arthaldeus pascuellus, Living, Pack of 25	**$8.75 at carolinabiological.com (5 needed)**		**43.75**	NSF Graduate Grant
Deltocephalus pulicari, Living, Pack of 25	**$8.75 at carolinabiological.com (5 needed)**		**43.75**	NSF Graduate Grant
Streptanus sordidu, Living, Pack of 25	**$8.75 at carolinabiological.com (5 needed)**		**43.75**	NSF Graduate Grant
Festuca rubra, 4-inch pot	**$7.95 at anniesannuals.com (56 needed)**		**445.2**	NSF Graduate Grant
Total URSC Request		**434.66**		

URSC Budget Justification

Tank Setup: The fish tanks and mesh will house the microcosm and prevent bugs from escaping. The grow lights and potting soil provide the necessary habitat, and the insects, spiders, and plants make up the living portion of the ecosystem.

Other Supplies: Other supplies I need, but already have available for use, are a computer for data analysis, a scale to weigh dried plant matter, and tweezers to aid in insect counts. I have also obtained permission to use one of Regis University's biological science labs to house my treatment tanks for the duration of the study, and a drying oven for plant specimen preparation.

Application to Coursework

This study utilizes knowledge from my current Environmental Biostatistics and Research Design class, as well as my Advanced Ecology and Vegetation Management courses. I will specifically utilize my skills in statistical data analysis using R, organism ID, and study development. This experiment allows me to apply the theories and techniques honed through my Regis University graduate education to a project that informs the scientific community on the mechanisms and processes of trophic cascades, food webs, and biodiversity.

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CHAPTER 3. JOURNAL MANUSCRIPT: DETECTING SHORT-TERM SUCCESS IN A RIPARIAN ZONE RESTORATION PROJECT

Abstract

Riparian restoration aims to accelerate the recovery of disturbed or anthropogenically degraded areas by re-establishing stream geomorphology and functionality. In 2016, the Denver Botanic Gardens installed in-stream structures in several reaches of Deer Creek to help restore natural flow regimes and floodplains by encouraging bank overflow, raising water levels, and enhancing pooling. Annual post-restoration monitoring data was collected for vegetation relative abundance, water quality, stream morphology, and macroinvertebrate communities in both 2016 and 2017. Two years post-restoration, previously channelized stream reaches along Deer Creek with installed in-stream structures showed little improvement in vegetation, water quality, and macroinvertebrate communities. In fact, most changes observed reflected the disturbance caused by the actual restoration methods, indicating that the communities may get worse before they get better. These results are consistent with many other restoration projects that show recovery may take decades, and the extent of this recovery success is variable. Quantifying long-term success of projects is dependent on the ability to detect changes, and identify and correct inconsistencies in restoration monitoring protocols. Our results are evidence that analysis of short-term monitoring datasets can, and should, be employed to pinpoint metrics that require more information and find additional response variable that should be measured. In addition, frequent monitoring and analysis can detect the subtle declines and recuperation of biotic and abiotic stream characteristics that may be missed by studies that have large gaps in survey years.

Introduction

Riparian ecosystems act as important refuges and wildlife corridors in urban and suburban areas, and sustain a variety of ecological functions (Goodwin, Hawkins & Kershner, 1997; Rood et al., 2003). Riparian zones redistribute organic matter and nutrients, maintain biodiversity, and act as an ecotone between aquatic and terrestrial ecosystems (Bukaveckas, 2007; Nilsson & Svedmark, 2002). However, years of human influence and stream alterations have degraded many natural riparian settings, and impaired the ability of these ecosystems to function properly and support native biota (Goodwin, Hawkins & Kershner, 1997; Beechie et al., 2010; Rood & Mahoney, 2000). Pollution and nutrient enrichment, channelization of streams, floodplain loss, and invasive species introduction are all anthropogenic impacts common in riparian settings (Beechie et al., 2010; Goodwin, Hawkins & Kershner, 1997; McClain, Holl, & Wood, 2010). Ecological restoration aims to accelerate the return of riparian ecosystems to a less-disturbed state by re-establishing chemical, physical, and biological components of the ecosystem (Beechie et al., 2010; Nilsson et al., 2015). However, even projects that use the same restoration techniques may vary in their success, and significant recovery may take years to occur (Miller, Budy & Schmidt, 2009; Collins, Doscher, Rennie & Ross, 2013; Lennox et al., 2011; Louhi et al., 2011). For this reason, it is important not only to assess the effectiveness of restoration methods, but also to establish and maintain long-term monitoring protocols that assess whether the target ecosystem is improving.

Riparian restoration is imperative at sites that can no longer support native riparian vegetation. Riparian vegetation regulates microclimate by providing shade, which creates a more habitable environment for aquatic macroinvertebrates and fish (Knight & Bottorff, 1984; Miller, Budy & Schmidt, 2009). Riparian plants also influence allochthonous organic matter budgets and nutrient cycling through input of leaf litter into streams (Bukaveckas, 2007; Nilsson & Svedmark, 2002). Additionally, intact roots from vegetation stabilize streams by reducing erosion of stream banks and modulating water flow (Dosskey et al., 2010; Polluck et al., 2014; Saldi-Caromile, Bates, Skidmore, Barenti & Pineo, 2004). Consequently, the loss of riparian vegetation also leads to the loss of these essential ecosystem functions.

A common stressor in riparian zones is stream channelization. Stream channelization disconnects streamflow from its floodplain, and thereby reduces the abundance of vegetation like phreatophytes that rely on a permanent supply of groundwater (Bukaveckas, 2007). Without appropriate soil moisture, recruitment and maintenance of willow and cottonwood populations decrease. Reduction in this plant canopy cover harms aquatic fauna as well (Miller, Budy & Schmidt, 2009). In fact, restoration efforts to help macroinvertebrate and fish communities recover may fail in areas with little to no live, established riparian vegetation, and therefore only sparse shade to provide cooler microclimates (Miller, Budy & Schmidt, 2009; Polluck et al., 2014). By degrading riparian zones and reducing historic water flow, stream channelization not only disconnects terrestrial and aquatic communities, but also impairs water quality and damages aquatic habitats. Therefore, restoring both the physical components of streams (hydrogeomorphology) as well as the biological components (vegetation) is essential to reconstruct functioning riparian ecosystems.

Implementing several common strategies can aid in riparian restoration. One minimally invasive technique for directly restoring stream hydrology and morphology involves the installation of in-stream structures made from temporary sod plugs (TSPs). By fastening biodegradable bags into streams with wooden sticks, the TSP method mimics a natural beaver dam (Polluck et al., 2014). Like beaver dams, TSP structures alter channel flow by promoting overflow into floodplains, increasing stream heterogeneity and woody debris collection, slowing flow velocity, and creating patterns of pools and riffles necessary for diverse macroinvertebrate and fish communities (Bouwes et al., 2016; Polluck et al., 2014). Increased water levels and overflow frequency near TSPs indirectly sustain riparian vegetation by increasing the frequency of floodplain inundation, and maintaining a greater soil moisture content (Bouwes et al., 2016, Rood & Mahoney, 2000; Rood et al., 2003). Decreased stream velocity and increased pooling enhances nutrient retention and uptake in streams, reduces erosion and sediment transport, and improves habitat for aquatic fauna (Bukaveckas, 2007).

While TSPs restore vegetation by returning the stream geomorphology to a less disturbed state, planting and seeding directly restore riparian zones by accelerating successional changes in vegetation (Gonzalez, Sher, Tabacchi, Masip & Poulin, 2015; Beechie et al., 2010; McClain, Holl, & Wood, 2010). Planting prescriptions often add native riparian tree species within the genera *Salix* (willows) and *Populus* (cottonwoods). While encouraging native species growth, planting and seeding also reduces invasive species presence by increasing competition between native and non-native species (Gonzalez, Sher, Tabacchi, Masip & Poulin, 2015). However, in some projects, mechanical removal of invasive species prior to seeding treatments may also be necessary to ensure native plant establishment. In some cases, more than one instance of seeding and planting may be required to meet desired establishment rates (Gonzalez, Sher, Tabacchi, Masip & Poulin, 2015).

Active riparian restoration techniques may require investment of additional time and money than originally budgeted, and project success may vary from site to site. The need for multiple planting treatments and frequent repair of TSPs means riparian restoration methods can be costly and time-consuming to implement (Gonzalez, Sher, Tabacchi, Masip & Poulin, 2015;

Ruwanza, Gaertner, Elser & Richardson, 2013). For this reason, restoration practitioners should tailor protocols to project goals, and include both pre- and post-project long-term monitoring to quantify success and identify areas in need of improvement (Ruwanza, Gaertner, Elser & Richardson, 2013; Louhi et al., 2011; Beechie et al., 2010; Lennox et al., 2011).

Even with monitoring, success of ecological restoration can be difficult to detect and quantify over short time frames (Collins, Doscher, Rennie & Ross, 2013; Nilsson et al., 2015). Studies suggest that recovery rates for macroinvertebrate and vegetative communities can vary widely from as little as a year, to several decades, before showing true signs of recovery after restoration efforts, if recovery occurs at all (Miller, Budy & Schmidt, 2009; Collins, Doscher, Rennie & Ross, 2013; Lennox et al., 2011; Louhi et al., 2011). This discrepancy further solidifies the need for continuous, annual monitoring of restoration sites, as well as project-specific assessment of restoration methods, to better predict the extent to which signs of restoration success can be quantified over different time frames.

The Deer Creek riparian restoration project at the Denver Botanic Gardens' Chatfield Farms location, provides an opportunity to assess both restoration success over short time periods, as well to assess the effectiveness of a combination of restoration techniques. In 2016, researchers from the Botanic Gardens installed TSPs into three highly channelized portions of Deer Creek to replicate historical floodplain inundation patterns. In 2017, these TSPs required repair and were re-installed. In addition, the restoration plan utilized planting techniques to restore *Salix* and *Populus* species to the adjacent stream banks and floodplain areas. Post-monitoring protocols for this project include annual surveys at the restored reaches, other reaches of Deer Creek downstream of the project area, and three reaches on Jefferson County Open

Space sites upstream of the TSP installation sites. The surveys consist of monitoring canopy and ground vegetation, macroinvertebrate communities, and water quality.

To statistically assess the extent of short-term success of the Chatfield Farms restoration project, I compared 2017 biological survey data from two years post-restoration, to 2016 baseline data. The goal of the restoration project is to improve in-stream and riparian endpoints, however, over such a short time frame, detecting significant changes is unlikely. In addition, the restored sites experienced multiple disturbances in both 2016 and 2017, so changes in vegetation may simply reflect these disruptions. However, due to the direct nature of the in-stream restoration method, water quality and macroinvertebrate communities are more likely to show changes from 2016 to 2017, and these changes have the potential to be seen farther downstream from the restoration sites. If restoration is successful, we would expect to see changes in the restored sites that make them more similar to reference sites, and less similar to disturbed sites. This effect should strengthen as post-restoration time increases.

I also evaluated the effectiveness of the restoration project's monitoring strategies to identify additional variables and techniques that should be included in future monitoring and analysis. Because of the expensive, time-consuming nature of restoration efforts, studies like this are important not only to make sure that time and resources are being used efficiently to maximize project success, but to also inform future restoration projects on Jefferson County Open Space land.

Methods

Site Description

The Denver Botanic Gardens at Chatfield Farms is located at 8500 W. Deer Creek Canyon Rd., in Littleton, Colorado (Figure 1). This site includes the Hildebrand Ranch historical site, a farm built in the late 1860s. Chatfield Farms has multiple walking trails and managed gardens, and is open to the public. Canopy vegetation along Deer Creek at Chatfield Farms is dominated by narrowleaf cottonwood (*Populus angustifolia*), boxelder (*Acer negundo*), and eastern cottonwood (*Populus deltoides*). Invasive grasses such as smooth brome (*Bromus inermis*) and reed canarygrass (*Phalaris arundinacea*) are common.

Just west of Chatfield Farms is Deer Creek Canyon in Jefferson County Open Space. North Fork Deer Creek flows through the center of Deer Creek Canyon and converges with South Fork Deer Creek upstream from the transect sites. Deer Creek Canyon sits at the base of the foothills and contains a high diversity of plants, animals, and habitat types. Vegetation includes Ponderosa Pine-Douglas Fir forest (*Pinus ponderosa/Pseudotsuga menziesii*) on north-facing mountain slopes, Gambel oak (*Quercus gambelii*) shrublands, grasslands, and cool canyons with quaking aspen (*Populus tremuloides*), bluestem willow (*Salix irrorata*), and Rocky Mountain maple (*Acer glabrum*) (Sovell et al., 2012).

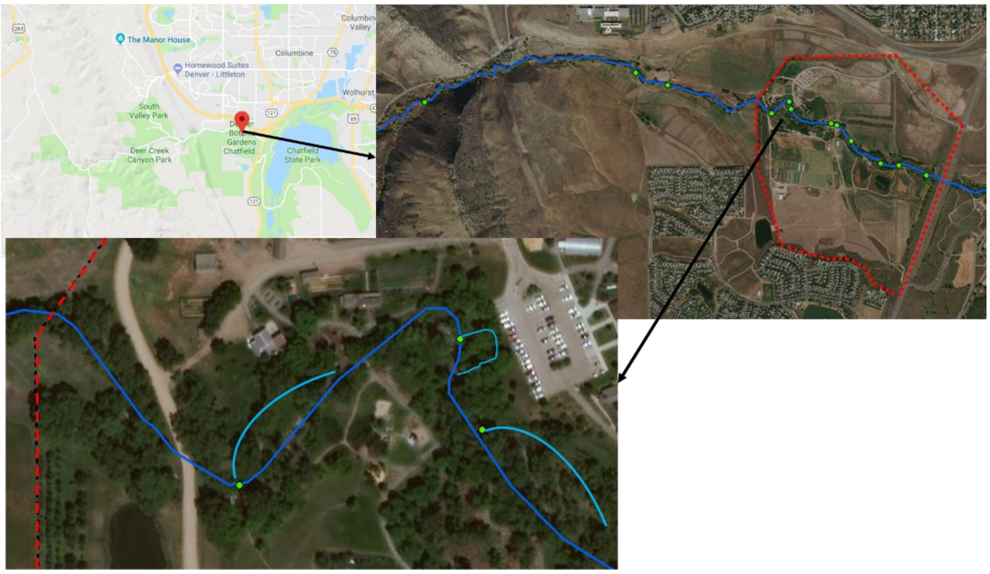

Figure 2: Map showing the 12 transect origins (green circles) in the restoration project. The dark blue line represents Deer Creek and the dotted red line is the Denver Botanic Gardens at Chatfield Farms boundary. The light blue lines are the predicted flow into flood plains (away from green dot) at restored transects.

Restoration Plan: In-stream

In 2016 three restoration sites with evidence of historical floodplains were identified along channelized reaches of Deer Creek, and in-stream TSP structures were installed (Figure 1). These structures, consisting of biodegradable coconut fiber bags filled with vegetation/ organic fibers and gravel filled bags, were held in place by wooden stakes (Figure 2). The TSPs aimed to raise the water levels in these parts of the stream, and promote overflow into historical floodplains. These structures also increase woody debris retention to further increase pooling and water overflow. Damage to the TSPs from high flows in 2016 necessitated their repair and reinstallation in 2017. To minimize in-stream disturbances, the repair occurred early in March 2017, before significant flows returned to Deer Creek.

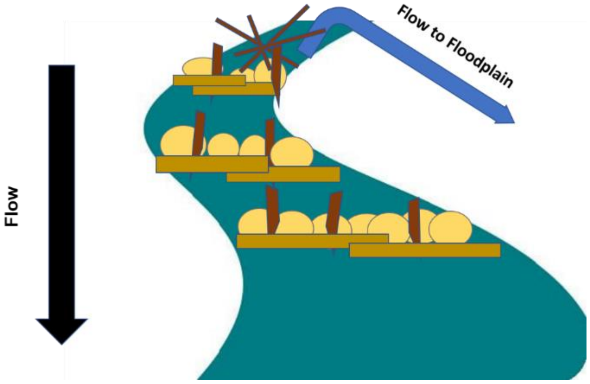

Figure 2: Front view diagram of instream TSP. This represents one TSP that has a tiered structure. Tan circles are natural fiber bags filled with rocks and vegetation, and held in place by wooden stakes. Large woody debris and sediment collect in from of the first tier, promoting pooling, and increasing overflow into the adjacent floodplain.

Restoration Plan: Vegetation

During the installation of TSPs in 2016, portions of the understory vegetation near each TSP installation site were removed to allow water flow into the floodplain and reduce invasive species. After TSP installation in 2016, willow stakes and cottonwood plugs were planted both in the floodplain and along the stream bank to promote regrowth of native riparian vegetation. Additional planting occurred to a lesser extent in 2017 after TSP repair.

Transect Locations and Monitoring

To monitor restoration success, I compared three transects adjacent to the restoration sites to 9 other transects along Deer Creek, for a total of 12, 25-m long transects (Figure 1). Three transects are located in Jefferson County Open Space land upstream of the restoration sites. The farthest upstream location, transect I, is off-limits to the public, while transects II and III receive frequent human visitation (personal observation by Dr. Rebecca Hufft). Transects IV, V, and VI begin at the TSP installation sites along channelized reaches on the Chatfield Farms property (Figure 1). Transects VII, VIII and IX are located downstream of the TSPs in a highly channelized area with buildings on both sides. Transects X, XI and XII are farther downstream, just west of Wadsworth Blvd., in a less channelized area that, until 2016, was colonized and altered by beavers (Figure 3).

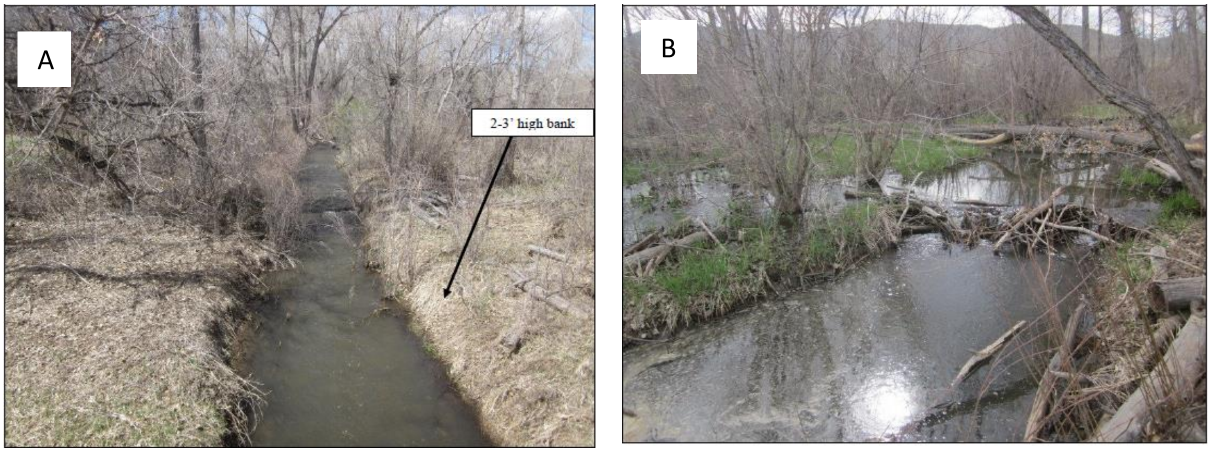

Figure 3: Site photographs of typical channelized stream portion (A) and natural stream structure with beaver activity typical of the three downstream transects (B). Notice that the channelized portion has a much taller bank and smaller width.

I consulted with researchers at the Denver Botanic Gardens to decide upon a stream type classification that would accurately contrast the restored sites from degraded, negative control sites, and natural, positive control sites that possess characteristics the restoration project aims to achieve (Bukaveckas, 2007). We decided on 3 final groups that best reflect the local conditions:

Reference, Restored, and *Disturbed*. Based on visual inspection of the sites' physical characteristics and vegetation, we placed transects I, X, XI, and XII into the *Reference* category, because they all contained stream reaches with minimal channelization, historical beaver presence and/or limited human disturbance. Transects II, III, VII, VIII, and IX were placed in the *Disturbed* category because all are found in areas with high human use, in close proximity to human structures, or where the stream has been channelized to reduce flooding impacts. Finally, we placed the three restoration transects, IV, V, and VI, into the *Restored* category to signify that active restoration efforts were employed there.

Monitoring along the 12 transects occurred once a year between June and July in both 2016 and 2017. At each transect, stream characteristics including microhabitat proportions (riffle, pool, undercut, woody debris), water appearance (murky, clear, foamy), and water quality (temperature, pH, TDS, velocity, and dissolved oxygen) were recorded. Water samples from the middle of each stream were also collected and sent to a laboratory to test for *E. coli,* the ratio of nitrate to nitrite, and total Kjeldahl nitrogen. In addition, macroinvertebrates were sampled in each stream. Sampling was distributed proportionally between microhabitats (i.e. riffles and pools) within each transect. Starting downstream and moving upstream, a combination of kick-netting (rocky substrate) and jabbing (soft substrate) with a D-frame net was used to collect macroinvertebrates. Samples were transferred to collection bottles filled with 70% ethanol and sent to a laboratory for identification.

Vegetation monitoring included both ground and understory vegetation, as well as an overstory survey. To assess ground vegetation, a point-intercept approach was employed every 0.25m along each 25m transect, for a total of 100 sampling points. Relative abundance was assessed using a long dowel rod placed on the tape at each 0.25m point. The first object the rod intercepted (live plant, bare soil, rock, plant litter, standing dead plant, or water) was recorded as a "first-hit." If the first-hit was a plant, that plant was identified to species level if possible. In addition, subsequent plants intercepted by the dowel were also identified and recorded as "second-hits." Less common species along the transect were tallied by recording the presence of all plant species within one meter on either side of the transect tape. Canopy cover was estimated using a GRS densiometer. Every 0.5m along the transect, canopy cover was recorded as open or covered. If canopy vegetation was present, the dominant species was recorded.

Seedling density was surveyed in June 2016 at each transect location. On the stream side of the transect tape, a 0.01m^2 frame was placed at each 1m mark. Within each frame, seedling density by species was recorded. Seeds were also collected for greenhouse germination. Seedling density was not measured in 2017 because of disturbance from repair of the TSPs.

Statistical Analysis

Vegetation

To support my site classification decision, I conducted a cluster analysis in R (R Core Team, 2014) on the twelve transects. I used physical stream and hydrology characteristics to classify the streams, including stream width at transect origin, stream depth at transect origin, thalweg to side bank distance, percent pools, and percent riffles, percent undercut bank, stream velocity (m/s). I used the hclust function to perform the cluster analysis using Ward's distance, plotted a dendrogram, and used the cutree function to prune the dendrogram into three groups (Maechler et al., 2017). I then used a chi-squared test on the original groupings and new clusters to see whether the two sets of categories were statistically associated.

To assess differences in restoration endpoints, I fit a series of mixed-effect models in R (R Core Team, 2014) to assess the effect of site category (Reference, Disturbed, and Restored) on five vegetative response variables: species richness, odds of finding *Bromus inermis*, odds of finding *Phalaris arundinacea*, odds of finding *Salix spp.*, and odds of finding *Populus spp.* In each model, site type was the fixed effect, and I added transect ID as a random effect to account for the correlation between the sets of paired transects for the two years. I used extra sum of squares F-tests to test whether year or the interaction of year and site type should also be added as a fixed-effect in the model for each metric.

Finally, I conducted a non-metric multidimensional scaling (NMDS) ordination on a Bray-Curtis distance matrix from log(x+1) transformed (to correct a heavy right skew) plant species relative abundances (McCune, Grace & Urban, 2002). I included 25 species that were present at more than three sites and made up more than 0.01% of the total species abundance. Using a stepdown procedure that assesses decreases in stress with the addition of dimensions, I chose a three-dimensional ordination. I fit my final three-dimensional ordination with 1000 random start values using the vegan package in R (Oksanen et al., 2018), and chose the solution with the lowest stress. I then rotated the final ordination with a principal component analysis (PCA) that assigned the largest amount of variation explained to the x-axis. I then fit environmental vectors to the ordination in a post hoc fashion to assess the effect of stream characteristics on plant community composition using the envfit function in the vegan package in R (Oksanen et al., 2018). I then used permutational analysis of variance (PERMANOVA) on the distance matrix to assess statistical differences in community structure based on site and stream characteristics using the adonis function in the vegan R package (Oksanen et al., 2018).

Water Quality, Macroinvertebrates and Hydrology

I assessed differences in water quality by analyzing the changes across site types for the two years using a similar process. Using a mixed-effect model with transect as a random effect, I investigated the effect of site type on water velocity, total dissolved solids (TDS in ppm), conductivity (microsiemens), in-stream pH, total dissolved oxygen (DO in mg/L), the ratio of nitrate to nitrite, and total kjeldahl nitrogen concentration (mg/L). Again, I used an extra sum of squares F-test to test whether year or the interaction of year and site type should also be added as a fixed-effect for each metric. In addition, I used the same procedure as vegetation and water quality metrics to analyze to analyze macroinvertebrate data. I fit mixed-effect models for total abundance (#/m^2), macroinvertebrate taxa richness, EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa richness, and Colorado MMI (multimetric index) score. Finally, to test for changes in hydrology over the two years, I conducted a student t-test to compare the mean thalweg water depth at all sites in 2016 and 2017.

Results

Site Cluster Analysis and Overview

Initial site classification based on expert visual assessments differed from the site groupings produced through stream cluster analysis based on stream habitat characteristics (Figure 4). Each cluster contains sites from at least two of our initial classification groups. Both reference and disturbed sites are found in every cluster. In addition, this dendrogram shows that upstream sites are grouped together (Cluster 1, far left), and downstream sites are generally also grouped together (Cluster 3, far right). Restored sites show similarities to both upstream and downstream sites (Cluster 2, middle). A chi-squared test showed no significant association between the initial classification groups and the new cluster groups (p=0.657). This cluster analysis suggests that stream physical characteristics such as microhabitat proportions and bank morphology have yet to change in two years at the restored sites. If these characteristics had changed, we would expect to see a stronger association between restored sites, with these areas clustered more closely together. The need to repair the TSPs and the continued disturbance this created likely delayed some of these physical changes.

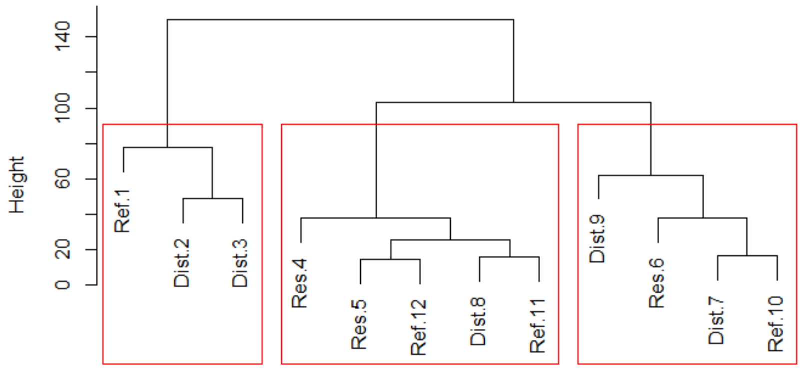

Figure 4: Transect cluster analysis based on stream characteristics. Each group contains multiple site types.

Overall, site metrics changed only minimally for vegetation, water quality, and macroinvertebrate communities in the two years post-restoration. Of the ten metrics examined, six showed significant differences (p≤0.05) at restored sites from 2016 to 2017 (Table 1). Very few site comparisons (4 out of 45) showed marginally significant changes (p<0.1), and the majority of comparisons (29 out of 45) showed no significant change (p>0.1). While all ten metrics showed response to site type, only six of these were significantly influenced by site type, year, and their interaction (Table 1).

Table 1: Summary of differences in metrics between sites in 2016 and 2017. The goal of restoration is for restored sites to look more like reference sites, and less like disturbed sites. This effect should look more pronounced as time goes on. In addition, we would expect to see a significant deviation between reference and disturbed sites. Bold values indicate p-values<0.05, italic values indicate marginal significance of p-values <0.1.

Response ~ Site+Year+Site*Year					
		Difference in Reference-Restored		**Difference in Restored-Disturbed**	
Response	**Difference in Restored from 2016-2017**	**2016**	**2017**	**2016**	**2017**
Log Odds of Finding *Phalaris arundinacea*	-0.71 95% CI: (-1.57-0.14)	*3.75* *95% CI:* *(0.34-7.15)*	2.05 95% CI: (-1.33-5.42)	-2.51 95% CI: (-5.79-0.78)	-0.78 95% CI: (-4.04-2.48)

Log Odds of Finding *Bromus inermis*	0.3115 95% CI: (-0.035-0.658)	-2.92 95% CI: (-6.23-0.38)	-0.656 95% CI: (-3.95-2.64)	0.58 95% CI: (-2.54-3.699)	0.24 95% CI: (-2.88-3.36)
Water Temperature (C)	**-3.93** **95% CI:** **(-6.66- -1.20)**	-0.35 95% CI: (-3.42 -2.72)	**-5.61** **95% CI:** **(-8.68- -2.54)**	0.72 95% CI: (-2.21 – 3.65)	**5.08** **95% CI:** **(2.03-8.14)**
CO MMI Score	**25** **95% CI:** **(11.7-38.297)**	-5.58 95% CI: (-22.48-11.32)	1.67 95% CI: (-15.23-18.57)	-3.67 95% CI: (-19.83-12.49)	-8.67 95% CI: (-24.83-7.49)
Log Total Macroinvertebrate Abundance (#/m^2)	**-2.08** **95% CI:** **(-2.15 - -2.02)**	**2.03** **95% CI:** **(1.5-2.57)**	0.33 95% CI: (-0.21- 0.86)	**-1.42** **95% CI:** **(-1.93- -0.91)**	-0.43 95% CI: (-0.94- 0.07)
Log EPT Taxa Richness	**1.912** **95% CI:** **(0.98-2.86)**	-0.23 95% CI: (-0.73 – 0.26)	**1.32** **95% CI:** **(0.35- 2.29)**	0.03 95% CI: (-0.42-0.48)	**-1.38** **95% CI:** **(-2.33- -0.42)**

Response ~ Site+Year

Response	**All sites difference of 2016-2017**	**Reference-Restored**	**Restored-Disturbed**	**Reference-Disturbed**
Log Odds of Finding *Salix spp.*	*0.36* *95% CI:* *(-0.06-0.77)*	0.66 95% CI: (-2.13-3.45)	0.3558 95% CI: (-2.53-3.24)	1.016 95% CI: (-1.61 - 3.64)
Log Vegetation Species Richness	**0.24** **95% CI:** **(0.05-0.42)**	0.14 95% CI: (-0.29-0.57)	0.09 95% CI: (-0.33-0.50)	0.27 95% CI: (-0.15-0.61)
Water pH	**0.67** **95% CI:** **(0.33-1.0)**	-0.42 95% CI: (-1.04-0.20)	0.50 95% CI: (-0.10-1.11)	0.08 95% CI: (-0.47-0.64)

Response ~ Site

Response	**Reference -Restored**	**Restored-Disturbed**	**Reference-Disturbed**
Log Macroinvertebrate Taxa Richness	*0.22* *95% CI:* *(0.005-0.44)*	*-0.22* *95% CI:* *(-0.42- -0.009)*	0.005 95% CI: (-0.17-0.18)

Vegetation

After controlling for site type and the random effect of transect, median vegetative species richness was 1.27x higher in 2016 than in 2017 (p=0.047, 95% CI: 1.05-1.52x). Species richness did not differ among site types after controlling for year (all comparisons yielded p-values >0.1, Figure 5 a). For the four species of interest, *Salix spp.* and *Populus spp.* (both native), and *Bromus inermis* and *Phalaris arundinacea* (both invasive), we generally saw very few significant differences in species presence between sites.

Although the odds of finding *Salix spp.* at all sites was 1.43x (95% CI: 0.94-2.16x) greater in 2016 than in 2017, after controlling for site type, this annual difference is likely due only to the greater number of willows at reference sites in 2016 than in 2017 (Figure 5 b). In 2016, the odds of reference sites containing *Phalaris arundinacea* were marginally higher than restored sites in 2016 (p=0.096). However, in 2017, the odds of finding *Phalaris arundinacea* at reference and restored sites were no longer significantly different, because the odds of finding this species at the reference sites decreased significantly in 2017 (p<0.001) (Figure 5 c). The odds of finding *Bromus inermis* did not significantly differ, except in reference sites in 2016 and 2017. In 2017, the odds of finding this grass species at reference sites were 7.06x higher than in 2016 (95% CI: 4.5-11x). Cottonwoods, *Populus spp.,* were present at all reference sites in both 2016 and 2017, and at every restored site in 2016. However, small sample size and lack of variation at some sites made fitting models of the odds of finding this species as a function of site and year challenging, so these models are not reported.

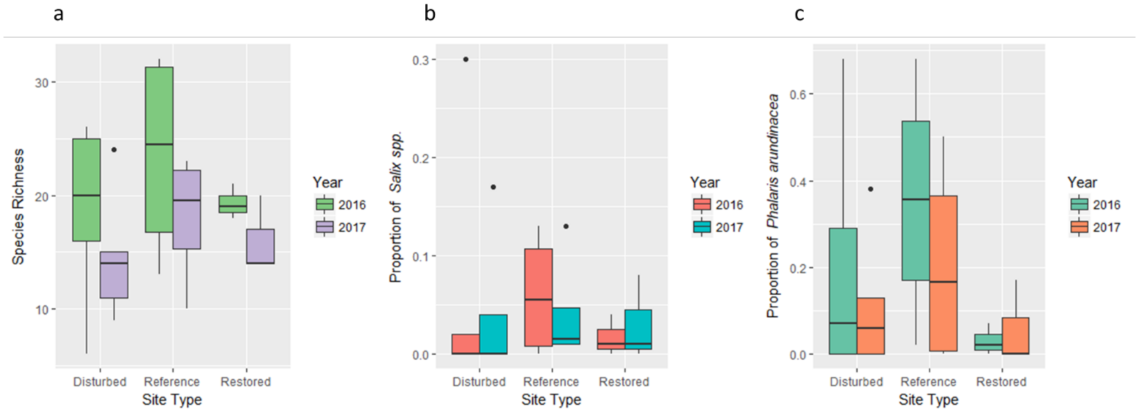

Figure 5: Across sites, metrics were generally higher in 2016 than in 2017. Response variables did not differ as a function of site type for a) species richness, or b) odds of finding *Salix spp*. c) In 2016, the odds of finding *Phalaris arundinacea* was higher in reference sites than in restored sites, but in 2017 the difference between these site types was no longer significant.

Although origin longitude was significantly correlated with plant species composition, this relationship was weak (permanova, $R^2=0.09$, $p=0.049$). Log stream width, log thalweg depth, log thalweg to sidebank distance, and number of flowmeter rotations showed no correlation with either of the NMDS axes (all $p>0.1$). Axis one captures 62.3 % of variation, followed by axis two with 9.6%, and axis three with 7.2 %. For ease of interpretation only the first two of the three axes are shown in the ordination. Figure 6 shows large within group variation, as evidenced by indistinct separation and large overlap between site type groups, indicating that these groups are not significantly different (permanova, all p-values>0.1). In addition, distribution of plant species by preferred habitat type shows riparian species dominate in nearly every transect, regardless of site type (Figure 7). Grouping of upland species around transects VIII (disturbed, both 2016 and 2017), IV (restored, both 2016), XII (reference, 2016), and I (reference, 2016), indicates that upland species may also be dominant in all site types. Lack of clear distinction between channelized areas and reference sites, as well as little to no correlation with stream characteristics, indicates that other variables not included in this analysis may be more important in determining species composition within transects.

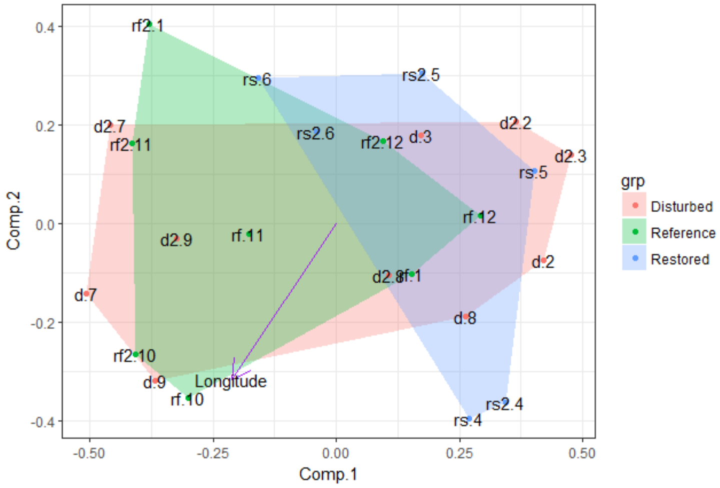

Figure 6: Results of our NMDS ordination for 2016 and 2017 indicate that the site type groups show large overlap. In addition, sites from all site type categories are present within all three group hulls. The arrow shows the correlation of sites with transect origin longitude. rf= reference 2016, rf2=reference 2017, d=disturbed 2016, d2=disturbed 2017, rs=restored 2016, rs2=restored 2017.

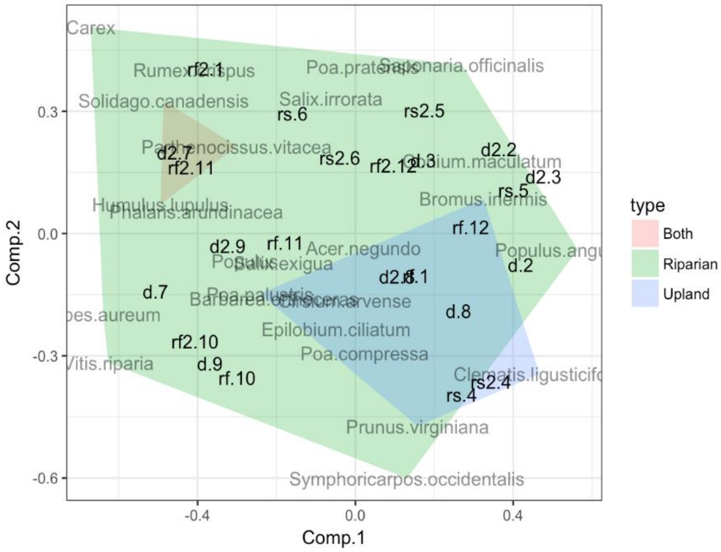

Figure 7: Upland and riparian/upland (both) adapted plants are distinct from each other. However, riparian plants encompass both of these groups, so true site distinction based on plant types is not clear. rf= reference 2016, rf2=reference 2017, d=disturbed 2016, d2=disturbed 2017, rs=restored 2016, rs2=restored 2017.

Water Quality

Due to unreported results from lab or field testing, and instances of dry transect sites, only two water quality metrics, pH and water temperature (°C), provided an appropriate sample size to conduct meaningful comparisons by site and year. DO, TDS, velocity, conductivity, nitrate:nitrite concentration, and concentration of total Kjeldahl nitrogen were either not measured in 2017, or were not reported for specific transects, and thus were excluded from analysis. Average water temperature in 2017 restored sites was 3.93 °C higher than in 2016 restored sites (p=0.02, 95% CI: 2.2-7.66) (Figure 8 a). In 2017, restored sites also had a significantly higher average water temperature than both reference and disturbed sites (p=0.002 and p=0.005, respectively). pH was marginally higher in 2017 for reference sites (p=0.045) and disturbed sites (p=0.10) (Figure 8b). However, due to a wide range in pH for restored sites in 2017 (from 6.5 to 8.5), there was no significant difference between restored sites in 2016 and 2017 (p=0.79). Average pH did not differ between restored and reference sites or disturbed and reference sites for either year (all p-values >0.1).

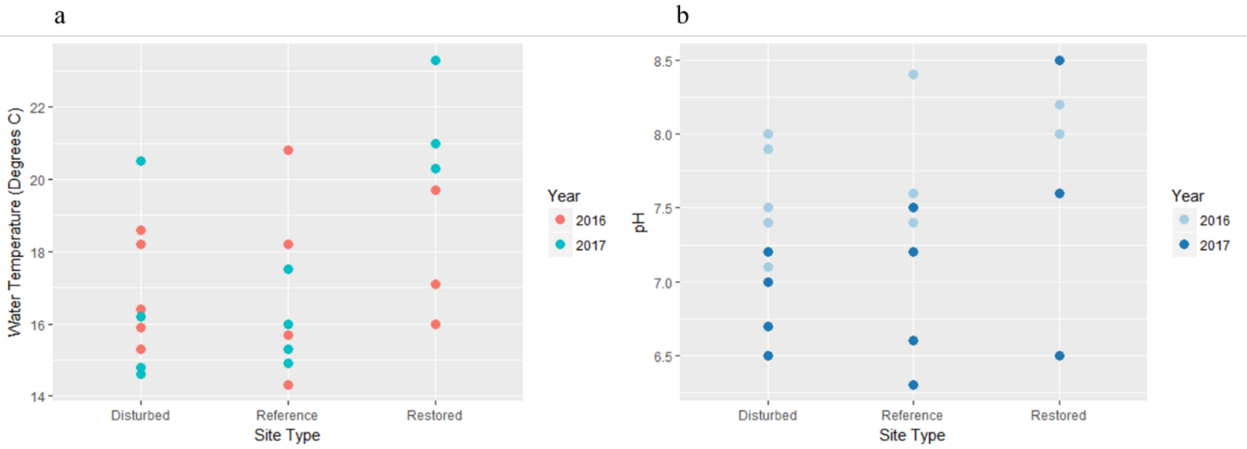

Figure 8: There was no significant difference between reference and disturbed sites for either water temperature or pH. a) However, in 2017, water temperature was significantly higher at restored sites than at either disturbed or reference sites (p=0.02). b) Sites in 2017 had lower pH than sites in 2016, but this effect did not differ between site type.

Macroinvertebrate metrics showed the most change over the two years. In 2016, total macroinvertebrate abundance was significantly higher on average for both reference and disturbed sites, compared to restored sites in 2016 ($p = 1x\ 10^{-4}$ for both comparisons). However, total macroinvertebrate abundance increased at all site types from 2016 to 2017 (all p-values >1e-04), resulting in no significant difference between restored sites and either reference or disturbed sites in 2017 (p=0.72 and 0.39, respectively) (Figure 9a). Conversely, mean EPT taxa richness was significantly higher in 2016 at restored sites than in 2017 (p>0.001). Mean EPT taxa richness was 85% lower in 2017 than in 2016 at restored sites (95% CI: 62-94%) (Figure 9b). EPT taxa richness was also lower at reference and disturbed sites in 2017, but not significantly so (p=0.59 and 0.11, respectively). EPT taxa richness in 2017 at both reference and disturbed sites was higher than at 2017 restored sites (p=0.044 and 0.027, respectively). All site types also saw a significant decrease in CO MMI score from 2016 to 2017 (all p-values <0.01). Restored sites had the largest drop in average MMI score in 2017 of 25 points (95% CI: 11.7-38.3). In 2016 and 2017, total macroinvertebrate taxa richness was marginally higher in both disturbed and reference sites compared to restored sites (p=0.1 for both comparisons).

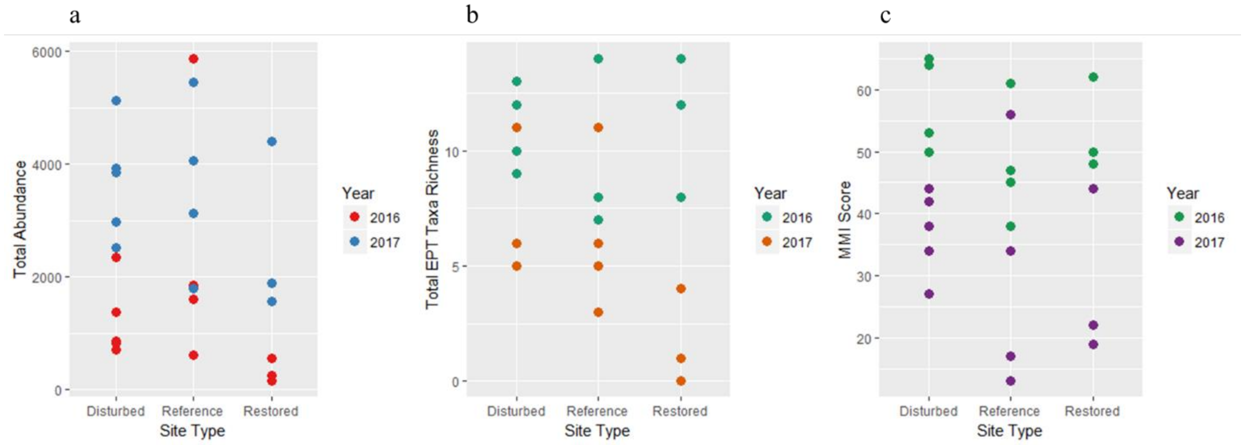

Figure 9: a) In 2016, both reference and disturbed sites showed a greater macroinvertebrate taxa abundance than restored sites ($p<0.05$), but in 2017 the difference is no longer significant ($p>0.1$). b) In 2016, there was no significant difference in EPT taxa richness between restored and reference and restored and disturbed sites ($p>0.1$). However, in 2017, both reference and disturbed sites had a significantly higher EPT richness than restored sites ($p<0.05$). c) MMI score dropped at all sites in 2017.

In 2017, stream morphology data was collected nearly a month earlier than in 2016. Across all sites, water depth was marginally higher in 2017 than water depth in 2016 ($p=0.065$; Figure 10). This change is likely due to higher surface water levels in the earlier part of the summer in 2017, rather than actual changes in streambed depth.

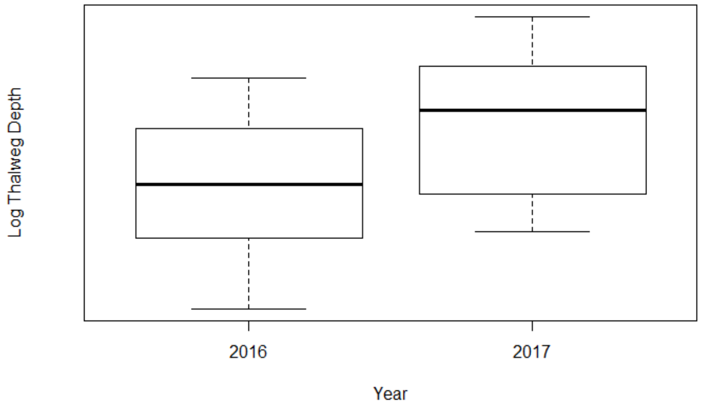

Figure 10: Stream depth was marginally higher in 2017 than in 2016, regardless of site type ($p=0.065$).

Discussion

Annual surveys of restored site vegetation and water quality along Deer Creek show little recovery between 2016 and 2017. Several response variables showed yearly shifts, but measures of in-stream and riparian restoration success did not differ between the site types, resulting in minimal differences between restored, reference, and disturbed sites. In addition, the metrics that did show changes at restored sites were often in the opposite direction than predicted, such as the loss of vegetative species richness. Macroinvertebrates showed greater response to site restoration, but again, this response was generally negative, and included the loss of sensitive taxa and a decrease in bioassessment score. If restoration is successful, we expect to see changes in restored sites that shift them towards reference conditions, and away from degraded sites. However, no response variables showed these expected changes. In fact, for most metrics, not even reference and disturbed sites differed significantly.

Several ecological and study design explanations exist for the lack of positive response in metrics at restored sites. One ecological driver behind slow response to restoration is the well-documented lag time associated with chemical, biological, and functional recovery of streams (Dosskey et al. 2010; Louhi et al., 2011; Meals, Dressing & Davenport, 2010; Hamilton, 2011). The length of this lag is dependent on several aspects of the restoration project, including the intensity and frequency of the disturbance and the restoration methods and techniques used (Dosskey et al. 2010). Many studies agree that monitoring for several decades is necessary to detect strong changes in metrics (Louhi et al., 2011; Feld et al., 2011; Hasselquist et al., 2015; Laasonen, Muotka & Kivijärvi, 1998). Our study uses a combination of active and passive restoration techniques that include the planting of woody riparian species and restoration of hydrogeological processes through placement of in-stream structures. Current extent of vegetative recovery is consistent with a previous study that showed little difference in vegetative metrics (specifically species richness and abundance) between channelized and restored reaches for 10-15 years after the initial restoration (Hasselquist et al., 2015).

However, Hasselquist et al. (2015) showed that changes in plant species composition between restored and channelized reaches may be detectable over shorter time periods (~5 years). So far, our results showed little support of this claim, but continued monitoring may detect these shifts in vegetation in the next few years. We would expect that unchannelized reaches would have a greater proportion of riparian plants than channelized reaches, however our ordination showed that riparian plants were found at nearly every site. This ordination also showed that upland vegetation tended to be found at transect VIII, one of the disturbed sites and a channelized reach (in both 2016 and 2017), but also at transect IV, one of the restored sites (in both 2016 and 2017). Lack of changes in species composition for this restored site suggests that the degradation was more significant there compared to the other restored sites (both of which contain mostly riparian species), and that recovery at transect IV may take longer. It is important to note that species composition at the other restored sites also did not differ over the two years.

Macroinvertebrate and water quality metrics also show lags in recovery after restoration. The decreases in EPT taxa and MMI score seen in our study are consistent with other studies that show macroinvertebrate communities in restored areas may get worse before they get better (Laasonen, Muotka & Kivijärvi, 1998; Carlson, Donadi & Sandin, 2018). These findings likely result from the disturbance caused by the actual restoration (i.e. hammering of stakes into riverbed, use of heavy machinery, laying of sod and rock bags). Carlson, Donadi & Sandin (2018) show that the effects of this restoration disturbance may dissipate after 15-20 months. However, since the in-stream structures in our study required installation and repair in both 2016 and 2017, the macroinvertebrate community will likely take much longer to recover from this repeated disturbance. Future analysis of annual surveys should incorporate NMDS ordination to track changes in the macroinvertebrate community as time passes, and to identify whether EPT taxa recolonize these restored reaches, and how long the process takes.

Our results reveal that limitations due to inappropriate initial clustering of sites into the three starting condition categories may have masked significant changes across sites. As evidenced by the cluster analysis, each group contains a mixture of site types, alluding to large variation in characteristics of sites in the initial categories. This variation is likely obscuring some of the annual changes at individual transects, because the data is averaged across the site types. These results indicate that visual surveys alone may not be sufficient to appropriately group positive and negative control sites. With this knowledge, we can better tailor future analysis to include further clustering methods, as well as incorporate additional, and likely more important, site characteristics to capture variation between transects and more accurately categorize transects into site type categories. Such variables include slope (Hasselquist et al., 2015) and soil type of the riparian zone, as well as hydrology measures to capture changes in flow and discharge. Additionally, clustering of sites based on vegetation and macroinvertebrate data would allow us to compare groupings based on biological variables to the site characteristic groupings to see how well the two correlate. Without a strong meter stick with which to compare our restored sites and differentiate between natural variation and restoration-induced changes, detecting success may be impossible.

In addition, this study brought to light inconsistencies in the data collection for nearly every group of response variables (Table 2). These short-term results allowed us to identify which metrics need additional information so that we can improve our monitoring protocol for the future. For example, lack of flow data for 2017 severely limited analytical opportunities. Water velocity, in particular, is a metric that we expect to decrease as a result of the type of direct stream restoration employed at the Chatfield sites (Bouwes et al., 2016; Polluck et al., 2014; Bukaveckas, 2007). However, with no data available for 2017, I was unable to test for any improvements (i.e. decreases in velocity) at restored and downstream sites. In addition, measuring velocity once a year may not provide a comprehensive view of river flow. Installation of level-loggers at sites, or multiple measurements of velocity and water depth over the spring and summer months, would provide a much more informative measure of both seasonal changes in the natural flow regime, and flow differences at the three site types (Poff et al., 1997). Level-logger records would also show frequency of bankful flows, which are important contributors to floodplain moisture and channel morphology. Sediment size and embeddedness should also be measured at sites, because these metrics can be used to infer both the flow regime and habitat quality of the stream (Poff et al., 1997; Buffington, Montgomery & Greenber, 2004).

Similarly, due to the absence of water quality data (or insufficient sample size) for conductivity, TDS, DO, the ratio of nitrite to nitrate, and total Kjeldahl nitrogen, I was unable to test for the expected changes in water chemistry that result from enhanced pooling and nutrient retention in restored stream reaches (Błędzki, Bubier, Moulton, & Kyker-Snowman, 2011). However, increases in water temperature at restored sites were consistent with other channelized stream restoration projects (Błędzki, Bubier, Moulton, & Kyker-Snowman, 2011; Bukaveckas, 2007). This temperature change is likely caused by enhanced pooling at restored sites and an increase of solar radiation caused by removal of understory vegetation (Larson & Larson, 1996). As with flow, water quality metrics show variation seasonally and after precipitation events, and therefore one-time measurements may not be sufficient (Yu, Zu, Wu & Zuo, 2016). Installation of level-loggers would also improve water quality monitoring because most of these devices can continuously record conductivity and temperature measures. In addition, multiple measures of water quality throughout the summer using DO probes or lab samples would help detect seasonal changes so we can better distinguish between natural fluctuation, and changes in response to TSP installation.

Finally, I predict that failure to measure key response variables left some important effects of TSP installation undetected. One such variable is soil moisture, as the goal of this project is to increase floodplain inundation. While stream morphology and vegetation types may act as proxies for floodplain inundation in the long-term, direct measurement of soil moisture is the most reliable short-term method for detecting stream overflow caused by the TSP structures. These measurements should be collected using a soil moisture probe several times throughout the late spring and summer, to see if floodplain inundation follows a seasonal pattern, and if site types differ in soil moisture (indicative of stream channelization). Inclusion of a complete physical habitat survey would also provide another measure of available fish and macroinvertebrate habitat within the stream reaches (Table 2) (Plafkin et al., 1989). Aspects of these surveys are already measured in this study (pool/riffle variation and bank characteristics), but estimates of substrate, overhanging vegetation, and in-stream woody debris should also be recorded (Kaufman et al., 1999). Results from the water depth data show that researchers should take care to collect data at the same time every year, as discrepancy may yield results showing seasonal changes in the stream rather than direct changes from the TSPs. General consistency in long-term measurement of response variables is necessary for use of early post-restoration data in place of baseline data for restoration recovery (Collins, Doscher, Rennie & Ross, 2013).

Perceived short-term changes are not always reliable for predicting long-term success (Herrick, Shuman & Rango, 2006).

Table 2: Restoration methods for 4/6 response variables exhibit varying levels of inconsistency. Long-term monitoring may fail and early changes may not be detected if appropriate baseline data is not available for comparison to later years.

Response Variable	Data Collected	Additional Data Needed	Method Comments
Flood Plain Inundation	None	Soil Moisture	Direct soil moisture data should be collected with a soil probe to test whether in-stream structures are encouraging overflow.
Vegetation	-Point-Intercept Density Survey -Canopy Cover -Cottonwood Seedling Density 2016	None	Due to continuous disturbance, seedling density was not estimated in 2017. Point-intercept surveys are inherently biased.
Water Quality	-pH -Temperature -TDS ppm -Conductivity -Dissolved Oxygen mg/L -Number of flowmeter rotations -*E. coli* mpn -nitrate/nitrite mg/L -Total Kjeldahl Nitrogen	None	Data collection is inconsistent. The only water quality data collected in 2017 were pH, temperature, *E. coli* mpn, nitrate:nitrite mg/L, and total Kjeldahl Nitrogen. In addition, data for the last 3 measurements is N/A for 7 out of the 12 sites for 2017. Should install level-loggers or measure at least 3 times throughout the summer
Macroinvertebrate	-Macroinvertebrate samples	None	Sampling is inconsistent and unclear from protocol. In addition, 2016 samples were pre-sorted before sending to the lab, in 2017 they were not.
Stream Morphology	-Estimated Stream Area m^2 -Percent Riffles/Runs/Pools/Undercut Bank/Other -Depth at Origin (m) -Width at Origin (m) -Depth at Origin Thalweg (m) -Thalweg to Bank Distance (m)	-Gradient -Soil Type -Sediment Size -Percent Embeddedness -In-Channel Cover and Woody Debris	Measurements of sediment size and sediment embeddedness provide an idea of flow and available habitat for macroinvertebrates. PHAB (Physical Habitat) surveys provide a comprehensive measurement of available fish and macroinvertebrate habitat based on stream characteristics.
Hydrology	-Velocity	-Discharge -Water level	Level-loggers can be installed to record constant water levels, conductivity, and water temperatures continuously at sites. Velocity should be measured at several locations along transect, at

			multiple times throughout the study season.

Formulating restoration plans around a clear idea of what "success" means for a project is imperative to ensure that the appropriate measures are monitored. For most projects, especially those in areas where complete removal of anthropogenic disturbance is not feasible, defining success as restoration to some "original" state before human interference is not appropriate (Goodwin, Hawkins & Hershner, 1997). Rather, success should be measured as restored functionality and connectivity of both the stream and riparian ecosystems through process-based restoration methods (Kondolf et al., 2006; Beechie et al., 2010). These methods aim to sustain chemical, hydrological, and biological processes that support ecosystem function, provide resiliency to disturbance, and promote self-sustainability so the need for continued restoration is minimal (Beechie et al., 2010; Palmer et al., 2005). By re-establishing the hydrogeomorphology of Deer Creek to encourage vegetation growth, macroinvertebrate diversity, and recovery of the riparian ecotone, our study follows this process-based methodology. Promoting riparian vegetation ensures long-term supplies of woody debris and leaf litter for in-stream carbon cycling, enhanced habitat heterogeneity, variation in velocity-depth regimes, and increases in water level for floodplain inundation (Buffington, Montgomery & Greenber, 2004, Webster & Meyer, 1997; Polluck et al., 2014). Vegetation also stabilizes banks and prevents further erosion, making this stream more resilient to damage from floods. Overtime, the TSP structures will biodegrade. The goal is that enough woody debris and sediment will have accumulated in restored reaches that the streambed will be raised and natural dams will have formed. If this is successful, further TSP installation will be unnecessary, thereby forming a healthy, self-sustaining ecosystem.

In summary, success of restoration projects depends not only on the degree and speed of recovery, but also on the use of appropriate restoration monitoring to detect signs of recovery. After just two years post initial restoration, our restored sites showed little improvement, but this is to be expected as recovery can take decades, if it occurs at all. Improvements in monitoring protocols, and the addition of new success measures, may not only increase the reliability and detection power of the project's long-term monitoring plan, but also highlight which processes are (or are not) recovering. The results of this study provide further evidence of the need for consistent, holistic monitoring practices to quantify restoration success, but also highlights the merit of short-term monitoring for assessing methodology. While this study showed that short-term monitoring may not reveal dramatic improvements at restoration sites, it was immensely helpful at identifying possible sources of error within our long-term study design. Future studies should incorporate analysis of short-term monitoring data to recognize sub-optimal restoration methods and areas of protocol improvement, identify sites that may require additional restoration work or longer recovery time, and detect more subtle changes in site characteristics and species composition that may otherwise be missed if analysis does not occur for decades after initial restoration.

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CHAPTER 4. ENVIRONMENTAL STAKEHOLDER ANALYSIS: PIPING PLOVER CONSERVATION, ARE REVISIONS REALLY JUSTIFIED?

The Atlantic Coast piping plover, *Charadrius melodus*, is a small, migratory shorebird that breeds on coastal dunes, sandflats, and mudflats on the Atlantic Coast of the United States and Canada (USFWS, 1996). Excessive hunting for feathers and habitat alteration led to the federal listing of piping plovers as threatened in 1986 (Gratto-Trevor & Abbott, 2011). Federally threatened or endangered species are subject to protection under federal and state-specific endangered species acts. These laws protect against the take of individuals of a listed species to reduce negative impacts on the species' populations. The term "take" refers to the killing, injuring, harassment, trapping, collecting, or general harming of a federally protected species (Endangered Species Act of 1973). However, take of some species is allowed under certain permits.

The Massachusetts Division of Fisheries and Wildlife (MADFW), under the supervision of the U.S. Fish and Wildlife Service (USFWS), applied for one such permit in January of 2016 to authorize the incidental take (i.e. the additional harm in lieu of additional activities) of piping plover. MADFW sought to alter previous state and federal guidelines for conservation of this species on nearly every Massachusetts coastal beach, to allow for increased recreation (USFWS, 2016). Such changes may result in the increased take of the piping plover during breeding season due to increased human activity in previously off-limits areas, and ultimately threaten the recovery of this species. While MADFW and the U.S. Fish and Wildlife Service legally followed all necessary steps to obtain this incidental take permit, including the preparation of an Environmental Assessment (EA) and the creation of a conservation plan, the projected increase in recreation is not worth the potential further decline of this threatened species. I propose that by allowing deviations from conservation guidelines in only the most human-populated and frequently used beaches, MADFW and other public stakeholders could increase recreation and revenue, while encouraging recovery of the piping plover populations in areas with more stringent management measures.

Piping plover breeding season lasts from late March to July or early August, and overlaps with peak summer recreation periods (USFWS, 2007). Nests and young piping plover chicks are camouflaged well to match the surrounding sand, so their hidden appearance makes then susceptible to being crushed by humans and vehicles traveling on the beach. Human and pet disturbance during nesting season may cause piping plover parents to abandon nests, and lead to the death of chicks (USFWS, 2007). In addition, human alteration of beaches reduces viable piping plover habitat, and limits reproductive success of breeding pairs (USFWS, 1996; Melvin, Hetcht, Griffin, 1994). These disturbances inhibit plover population growth throughout the Atlantic Coast region.

Since 1986, implementation of recovery and conservation plans has facilitated an increase in some Atlantic Coast plover populations. For this species to be removed from the threatened list, five criteria must be met: 1) maintain at least 2000 breeding pairs (distributed among 4 Atlantic sub-units) each year for five years, 2) verify genetic heterozygosity and long-term genetic diversity for these 2000 pairs, 3) achieve a 5-year average of 1.5 fledged chicks per pair in each of the 4 sub-regions (New England, NY-NJ, Southern, and eastern Canada), 4) assure long-term recovery of the species through management agreements, and 5) maintain wintering habitat sufficient to support the 2000 breeding pairs (USFWS, 1996). As of 2016, MADFW estimated total Atlantic Coast plover counts at 1,941 breeding pairs more than twice as many as the 1986 census (USFWS, 2017). The greatest recovery of piping plover populations is in the New England area, and these increases are attributed to stringent conservation management methods (USFWS, 2017).

Current management by the USFWS and MADFW includes the monitoring of breeding plovers and preservation/ restoration of natural piping plover habitat. Efforts aim to reduce human disturbance of breeding birds by limiting recreation in the vicinity of nests. This includes physical or symbolic fencing of areas around breeding plovers, restricting pets, prohibiting beach vehicles and beach raking, and in some cases, closing beaches all-together (USFWS, 1996; USFWS, 2016). Predator control is also enforced to limit top-down pressure on plovers through the extermination of problem fox, coyote and raccoon individuals, which mitigates impacts of human disturbance. In addition, presence of officers and trained volunteers helps to enforce these management efforts and provide educational outreach to the public. To maintain viable plover habitat, natural coastal processes are preserved by discouraging beach development, limiting beach stabilization, and by directly restoring degraded areas (USFWS, 1996). Additionally, annual census surveys and wintering habitat surveys ensure that population distribution and numbers are current, and critical winter habitat is identified and protected.

The USFWS also coordinates the Great Lakes Piping Plover Conservation Team. This team consists of federal and state land management agencies in the US and Canada, zoos, universities, private land owners, volunteers, and other non-governmental conservation organizations like the Audubon Society and the National Nature Conservancy. They seek to conserve the piping plover for use in captive-rearing programs, conservation research, and to promote general biodiversity out of love for wildlife and nature. These organizations have diverse values and goals that range from the intrinsic value of nature itself, to scientific inquiry, to legal obligation, but they all come together for one purpose: the long-term preservation and rehabilitation of this threatened species. The Great Lakes Piping Plover Conservation Team seeks to educate the public and involve them directly in plover conservation through citizen science projects and volunteer opportunities. Projects include keeping logs of plover sightings and breeding events, and monitoring nests during active breeding season. These efforts help to unite the interests of the public with the needs of the piping plover, by raising awareness and support for the conservation protocols.

However, not everyone is supportive of strict conservation guidelines, and the issue has sparked controversy over the years (Gratto-Trevor & Abbott, 2011). Local businesses, and those who value the recreational opportunities nature provides more than its intrinsic value, may suffer. Those who live in Massachusetts's coastal areas or visit from other places often value the natural beauty of these ecosystems, but they also appreciate the opportunity to interact with nature through recreational activities like swimming, picnicking, and hiking on beaches. Limits to recreation mean fewer people have access to the beach, even during peak summer seasons. In addition, if fenced or posted off-limits areas are near the entrance to beaches, entire stretches of the beach may be closed (USFWS, 2016). This applies to public parking lots and paved roads as well, because current guidelines require a 50m buffer zone around plover nests. For this reason, MADFW may see a decline in parking fee revenue in off-limit beach areas.

Reduced recreation also leads to a decline in tourism and loss of business for local stores and restaurants that rely on peak season visitors. Business owners, too, value these coastal areas for their beauty, but also recognize them as a profitable opportunity. Beach and coastal recreation increases foot traffic, and therefore may promote higher sales for these coastally-located businesses. The local economy would benefit from the relaxation of strict conservation guidelines which would allow more recreation and promote greater visitor numbers. However, it is also in the best interest of business owners to protect beaches and the ecosystems they support, as these natural settings and the chance to see unique flora and fauna are what initially draw some visitors to the area.

Conservation guidelines apply not only to the general public, but also to private landowners who live on or near the beach, as they may be required to apply for special permitting for activities in plover habitat areas on their land. This reduces the amount of freedom private landowners have to alter their land or partake in various activities that may cause direct or indirect harm to piping plovers. Private land owners and beach managers may apply for Certificate of Inclusion (COI) permits to relax these restrictions (MADFW, 2016). However, only a set number of permits can be issued, and not everyone may qualify (MADFW, 2016). Private landowners clearly appreciate the coastal environment for its natural aesthetic and high intrinsic and monetary value, however, being covered by the incidental take permit through COIs would allow them greater control over their land operations. Landowner outreach and stewardship programs coordinated by state organizations are an important part of conservation, because they can provide education and promote understanding between the state and landowners (Gratto-Trevor & Abbott, 2011). Nonetheless, some push-back is expected from individuals who care more about the ability to make decisions involving their private property than the conservation of a single species. Thus, a clear conflict exists between managing for the long-term survival and recovery of threatened biological resources, and management of a thriving tourism industry, local economic growth, and private land rights.

To assuage some of this conflict, MADFW and the USFWS propose to deviate from these stringent conservation guidelines to allow greater recreation, while still minimizing plover harm. The proposed deviations include a reduction in the fenced and symbolic buffer zones around eggs to a minimum of 10 meters in areas near beach entrances, and the re-opening of parking lots and roads near unfledged chicks (USFW, 2016). In the most extreme cases, nests may be moved if the minimum buffer zone can't be met without impacting recreation (USFWS, 2016). However, once nests are moved or disturbed, parents may abandon their eggs, leading to an unsuccessful breeding attempt. Increased signage, greater staff presence, and the inclusion of barriers in some areas may help to reduce additional take, but unfledged chicks are still at risk for injury and death because they are hard to see and can't fly away from potential threats (USFWS, 2016). Furthermore, MADFW and USFWS propose to allow increased beach recreation, foot traffic, and permitted over-sand vehicle (OSV) travel during mating season. Again, increased signage, training, and monitoring may help to minimize plover harm, but unfledged chicks are at greater risk of injury, and therefore successful reproduction may decline.

Managers seek to limit impacted areas to 10- 20% of available habitat (depending on the site), and only 15% of breeding pairs at each site (USFWS, 2016). However, for a species with less than 2,000 total breeding pairs across the whole Atlantic Coast region, losing up to 15% of breeding pairs at each Massachusetts site may have a large overall impact on this species. In addition, further reduction in protected habitat is inadvisable, as this species has already experienced substantial habitat disturbance from human development. As the spatial extent of these changes includes nearly every coastal beach in Massachusetts, these proposed changes have the potential to affect (to some extent) nearly all piping plover breeding habitat in Massachusetts. Leniency in the strict conservation guidelines may stunt the successful recovery of the piping plover in the New England sub-region (which includes Massachusetts). This sub-region supports the largest portion of breeding plovers on the Atlantic Coast, and shows the highest population growth of all regions, so declines in the New England population may pose an overall threat to the species' survival.

I propose that by reducing the extent of these deviations to include only the most heavily used recreational beaches in Massachusetts, conservation managers could balance the need to preserve some human-free breeding areas, with the economic and personal benefit of increased recreation. Deviation in conservation guidelines should be permitted only in areas where human presence and disturbance is consistently high, viable habitat is already limited, and plover colonization is low. Economic benefit will be highest in these human-populated areas. These areas are likely to be hotspots for restaurants and shops, and therefore would already sustain frequent human presence, but businesses would benefit from increased foot-traffic in peak seasons. In addition, the noise and constant human disturbance may discourage plover settlement on heavily used beaches, reducing the number of breeding pairs there that need protection.

Time and resources should instead be allocated for the preservation of more private, secluded, or intact coastal stretches, as well as federal lands. Because of the frequent human disturbance that comes with close proximity to human development, restoration of severely degraded habitat in populated areas is costly and time consuming. Therefore, resources are more successfully utilized, and the positive effects more long-term, if managers work to preserve already viable habitat on secluded beaches where the incidence of disturbance is lower. Coastal areas with a smaller human population may have larger areas of intact beach, and therefore may provide an overall larger habitable area. In addition, with fewer people utilizing the beach for recreation in general, there may be less push-back for the continued restriction of some recreational activities. These beaches may provide necessary refuges for breeding piping plovers, and allow the maintenance of this sub-region's population.

Now is a crucial time for piping plover population recovery. Climate change and human population growth are projected to facilitate further habitat loss, and increase the threat of losing this species. Positive changes in the New England populations of the piping plover indicate that the 1999 conservation plan is working, but more can be done to help this species. The other sub-regions have not seen the same level of recovery as New England. In fact, the Canadian sub-region shows the smallest population of breeding pairs to date (USFWS, 2017). For this reason, deviations in the conservation plan, especially in areas where it is proving successful, is inadvisable and should be limited as much as possible. By concentrating areas of recreation, managers can minimize further impacts over the greater New England area and maintain larger patches of suitable plover habitat. Conservation of this habitat and stabilization of plover population size promotes greater resiliency to future changes and increases the probability of long-term survival for this species.

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