INVESTIGATING METAPOPULATION RESPONSES TO LANDSCAPE-LEVEL HABITAT CHANGES

by

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Dedicated to my mother, father, brother, and sister. I couldn't have done this without you.

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ABSTRACT

The study of landscape structure and configuration is firmly established as integral to the continued advancement of ecology. The configuration of resource patches can have far-reaching implications for biodiversity, metapopulation dynamics, community structure, and habitat quality. Human activities, such as forestry, agriculture, and residential construction alter patch configuration by breaking larger patches into smaller fragments. This frequently results in pronounced, unforeseen consequences for species. The fragmentation and shrinking of habitat patches can lead to changes in the environmental conditions within the remaining patches (e.g., degradation), prompting responses from local populations. These responses can, in turn, cause changes to the metapopulation structure on large spatial scale.

I examined the relationship between the degree of habitat fragmentation (edge density), and forewing lengths of the ebony jewelwing damselfly (*Calopteryx maculata* Beauvois, Odonata: Calopterygidae). I used correlated random walks to determine the biologically relevant landscape area over which forest fragmentation was calculated. Then, I used Moran's I to determine the spatial scale of wing length response to fragmentation. I found that wing lengths increased with edge density. I also found that wing lengths were spatially autocorrelated at distances below 5 Km. These findings suggest that damselflies adapt to changes in forest fragmentation at a relatively small spatial scale.

Next, I assessed the slime mold *Physarum polycephalum*'s usefulness as a microcosm of dispersal in fragmented landscapes. Slime mold plasmodia were placed in dishes with oat patches of varying sizes and distances. The probability of each patch type being colonized first was compared to predictions of patch occupancy based on *C. maculata.* Patches that were nearer or larger were likely to be colonized before patches that were more distant, or smaller. Observed patch occupancy matched model predictions when only patch distance was varied, but not when patch size was varied. These results suggest that *P. polycephalum* has the potential to serve as a useful microcosm of dispersal in patchy landscapes. However, more testing is needed to develop the microcosm system.

Finally, a lesson plan was developed to teach high school students about the concepts of landscape ecology and connectivity. An emphasis was placed on using active learning techniques, which have been demonstrated to result in greater understanding than traditional lecture formats.

The lesson plan incorporates an education boardgame, Humans & Habitats, that I developed to illustrate how the conflicting goals of resource managers impact habitat connectivity. It also incorporates a scientific inquiry activity that uses *P. polycephalum* to test predictions about the effect of altered connectivity. The lesson plan and materials will be available to members of the public, free of charge.

CHAPTER 1.   GENERAL INTRODUCTION

Landscape ecology can provide avenues for disentangling the far-reaching effects of habitat change caused by human activities. The field has important applications in conservation and in developing sustainable land use practices. This chapter consists of a brief introduction to topics explored in subsequent research chapters. Specifically, introductions to local adaptation, landscape configuration and habitat fragmentation, and metapopulations will be presented. Additionally, brief introductions to the study insect, *Calopteryx maculata* (Beauvois, Odonata: Calopterygidae), and the slime mold *Physarum polycephalum* are provided. Finally, the chapter is concluded with a description of the importance of educating the public on landscape ecology, the objectives of this work, and an outline of the research chapters of the dissertation.

1.1   Local Adaptation

A population of organisms is said to be locally adapted if its individual members possess a phenotype unique to the population that provides greater fitness in their home environment. It is often assumed that gene flow between local populations limits the capacity of species with continuous distributions to develop localized adaptations (Richardson et al. 2014). Nonetheless, numerous studies describe adaptive divergence within continuous distributions, or at small spatial scales (e.g. Reznick and Endler 1982; Willi and Hoffmann 2012; Richardson and Urban 2013; Lindestad et al. 2019; Endo and Watanabe 2020). The existence of these results suggests that gene flow does not always suppress the effect of highly localized selective pressures. Although locally adapted populations may possess limited genetic diversity, they may act as sources of new phenotypes that are suited to unique niches (Leimu and Fischer 2008; Gavrilets 2003). Locally adapted populations may contribute to survival following widespread ecological change, or even to eventual speciation. Therefore, understanding the spatial scale at which local adaptations occur is important to fully appreciating the risks presented by habitat degradation and loss to biodiversity.

1.2   Habitat Fragmentation

Changing a habitat's size can cause it to become either more or less fragmented, changing its suitability, and influencing the way dispersers move through the landscape (Collinge and

Forman 1998; Schumaker 1996). Successful dispersal between local populations is important to the dynamics of metapopulations (Hanski 1998; Hunter 2002; and Schtickzelle et al. 2006). Fragmentation exerts selective pressure on populations by making resources more difficult to access, adding or removing resource patches, or altering the configuration of resource patches (Van Dyck and Matthysen 1999; Hunter 2002; Jonsen and Taylor 2000a; Wilcove et al. 1986; Taylor et al. 1993). If distant patches contain fewer competitors, increased dispersal capacity may increase fitness (Heino and Hanski 2001). Conversely, if there are high costs or risks associated with crossing unsuitable habitat, increased dispersal may be selected against (Olivieri and Gouyon 1997).

1.3   Patch Connectivity

Patch connectivity is an index of the potential for a species to travel between habitat patches (Merriam, 1984, Taylor et al., 1993). Typically, it is a measure of physical distance coupled with survivability in the intervening habitat and dispersal capacity of the species of interest. As habitats change in size, they can become either more or less fragmented, altering connectivity (Collinge & Forman, 1998). Changes in connectivity influence the way dispersers move through the landscape (Schumaker 1996). Successful dispersal in subpopulations is a key factor in the dynamics of metapopulations (Hanski, 1998, Hunter 2002, and Schtickzelle et al., 2006).

Traditionally, habitat corridors connecting isolated fragments are thought to increase local richness and abundance (Hunter, 2002). However, Collinge (2000) suggested a weak relationship between presence of corridors and extinction and recolonization of remote patches. Patch quality, as opposed to area or connectivity, may have a significant effect on patch occupancy (Gathmann et al., 1994).

1.4   Metapopulations

Metapopulations are groups of geographically distinct populations that interact to some extent (Hanksi and Gilpin, 1991). This idea was based on the work of Richard Levins (1969), who proposed that species exist in networks of dynamic local populations. Metapopulations exist in a balance between extinction and re-colonization events within available habitat patches (Levins 1969, Hanski, 1998). Local populations must produce enough new populations in order ensure the

metapopulation persists (Gyllenberg et al., 1997). Typically, this condition is met as a result of asynchrony between extinction of and colonization by local populations (Lande 1993). However, local population growth may be diminished by numerous factors including increased emigration, and habitat loss (Thomas and Hanski 1997, Hanski 1998). Broadly, the scale of a species' metapopulation dynamics must exceed the rate of habitat loss if the species is to persist (DeWoody et al. 2005). These extinction – colonization dynamics are the focus of numerous metapopulation studies.

While the viability of extant populations in habitat patches is important, migration from one local population to another is often, although not always, a driving force behind the maintenance of metapopulations (Hanski 1998, Govindan et al. 2015). Successful migration of individuals is influenced by the isolation, or fragmentation, of habitat patches (Moilanen and Hanski, 1998). Migration mortality tends to correlate positively with degree of habitat fragmentation (Schtickzelle et al. 2006).

Landscapes typically display a greater degree of spatial variation in patch arrangements than is assumed by metapopulation models, such as the Levins model (Hanski 1994, Hanski 1998, Tilman & Karevia 1997). However, mathematical models of metapopulation dynamics have been improved. The incidence function model takes fragmentation and isolation into account when quantifying average probability of long-term patch occupancy, presenting a more realistic view of metapopulations (Hanski 1994). Using metapopulation models, it is possible to gain understanding of how species move through networks of habitat patches, which can be used to identify critical habitat, and guide conservation efforts

1.5 *Calopteryx maculata*

The ebony jewelwing damselfly (*Calopteryx maculata*) is a large and immediately identifiable insect, inhabiting streams throughout eastern North America. Adults emerge from the water in early summer, dispersing from their natal streams for a period of about 11 days to complete development (Waage 1972). When they return to the stream, males establish and defend territories containing emergent vegetation for oviposition. Prior studies propose that *C. maculata* wing lengths vary in different habitats (Taylor and Merriam 1995). Although their preferred reproductive habitat is forested streams, *C. maculata* can be found under a wide variety of canopy conditions, including open meadows and fields

1.6 *Physarum polycephalum*

Physarum polycephalum is a protist known as a slime mold that is found in damp forest undergrowth throughout the northern hemisphere. It can detect local concentration gradients and direct its growth towards nutrient-rich resources (Jones 2009). It consumes bacteria and other microbes, and displays activity reminiscent of foraging behavior in animals (Wu et al. 2015, Latty and Beekman 2006). It is a popular model system in network analysis and biological computing due to its ease of use, and dendritic plasmodium phase (Shirakawa et al 2008). When placed in petri dishes with an arrangement of oats, *P. polycephalum* will eventually develop a network of veins that connect it to the multiple sources of nutrients. It has even been observed solving mazes by shortest path (Nakagaki and Toth 2000).

1.7 Education

In 2019, the National Academies of Science Engineering and Medicine (NASEM) released a report in which listedlisting the organization's grand challenges in environmental engineering for the 21st century (Board). Among the list of challenges were: 1) Sustainably supply food, water, and energy, 2) create efficient, healthy, and resilient cities, and 3) foster informed decisions and actions. These challenges are incredibly far-reaching in their scope, and will require the efforts of many different people and approaches. In order tTo meet these grand challenges, researchers must find ways to communicate concepts of landscape ecology to the public. Introducing people to topics at younger ages has been demonstrated to improve not only understanding, but attitudes towards the topiccis (Lopatto 2007, Wijesinghe et al. 2016, Schmitz and Da Rocha 2018). Fortunately, the demand for STEM-educated people remains high, providing inroads for researchers eager to share their knowledge (NRC 1989, Sanders 2008). While outreach and extension events offer excellent pathways for communicating research, there is a unique opportunity presented by designing lesson plans for K-12 education (Goldner et al. 2020). K-12 educators routinely cite lack of expertise with current science as a key reason they don't incorporate it into their lessons (Kim and Fortner 2007, Kelley et al. 2020). Researchers can assist with surmounting of this barrier by creating lesson plans for teaching students about their area of expertise. However, if researchers are willing to invest the time and effort required to create such

lesson plans, they should focus on using active learning techniques, that which have been demonstrated to improve learning outcomes over traditional lecture formats (Freeman et al. 2014).

1.8   Aim of Work and Chapter Outlines

The objective of this dissertation is to study local adaptation, fragmentation, and metapopulation dispersal in the patchy landscape of Indiana, US, and develop a lesson plan to teach K-12 students concepts of landscape ecology. The dissertation contains four major chapters, and a general conclusion. Following is an outline of the dissertation research chapters, along with the hypotheses tested, the associated predictions, and brief notes on the methodology used for each chapter.

In chapter two, I seek to confirm and explain the relationship between *C. maculata* wing length measurements and forest cover documented by Taylor and Merriam (1995), and determine the spatial scale at which the response occurs. In this chapter, I hypothesized that *C. maculata* wing length would increase with the degree of forest fragmentation, and that variance in wing length would increase with distance between collection sites. I collected 1686 specimens, from 21 sites across the state of Indiana. I used a correlated random walk model, based on empirical movement and mortality data, to estimate dispersal capacity for this species, which I used to determine the relevant landscape area. I used edge density to represent forest fragmentation.

In chapter three, I assess suitability of *P. polycephalum*'s suitability as a microcosm system for testing metapopulation dispersal hypotheses. I tested hypotheses concerning the probability that certain patch types were more likely to be colonized before others. Specifically, I hypothesized that near patches had a higher probability of being colonized before far patches, that large patches had a higher probability of being colonized before small patches, and that near, large patches had a higher probability of being colonized before any other combination of distance and size. I tested these hypotheses by placing *P. polycephalum* plasmodia in large dishes, with patches of rolled oats placed in appropriate arrangements, and observing at regular intervals for the first patch colonized. Additionally, I assessed dispersal capacity of *P. polycephalum*'s dispersal capacity, in landscapes devoid of resources. I placed *P. polycephalum* plasmodia in large plates of agar, and observed their movements at regular intervals to generate distributions of dispersal distances. These distributions were used to relate the microcosm experiments to patch connectivity estimates, calculated for *C. maculata* in proportionally sized landscapes.

Finally, in chapter four, I discuss the value of researchers creating lesson plans for K-12 educators, and describe the development of one on the topic of landscape connectivity. I emphasize the use of evidence-based, active learning techniques to make efficient use of class time. I also target state-required teaching standards, to facilitate educators' implementation of the lesson plan. At the end of the dissertation, a general conclusion summarizes the major findings of this research, and gives insight on the possible applications, and future directions of each chapter.

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1.10   Document Accessibility Statement

According to Purdue University Policy, Electronic Information, Communication and Technology Accessibility (S-5): As a public university and federal contractor, Purdue University is required to adhere to Sections 504 and 508 of the Rehabilitation Act of 1973 and Title II of the Americans with Disabilities Act. This standard specifies the means by which the University ensures compliance with these laws.

CHAPTER 2.   HOW LOCAL IS ADAPTATION? THE SPATIAL SCALE OF *CALOPTERYX MACULATA*'S MORPHOLOGICAL RESPONSE TO FOREST FRAGMENTATION

2.1   Abstract

Context Slight environmental differences between locations can lead to subpopulations adapted to these unique conditions. These local adaptations can contribute to the types of divergences that lead to new species. Previous work has shown that jewelwing damselflies had different wing sizes in different types of habitat within the same region.

Objectives Our aim was to understand how space affects the process of local adaptation by (i) describing the relationship between damselfly wing lengths and a gradient of forest fragmentation, and (ii) determining the spatial scale at which this adaptation occurs.

Methods I collected jewelwing damselflies from across Indiana, US, in habitats across a gradient of forest fragmentation. I examined the link between differences in forest edge density at three landscape sizes, and differences in wing length between populations using linear models. Then I examined the distance to which wing length variance was autocorrelated using Moran's I.

Results I found positive linear or unimodal relationships between wing length and edge density, in both males and females, at all three landscape scales. Spatial autocorrelation in wing length indicated that adaptation of dispersal morphology occurred at a small scale, with local adaptations emerging within the span of 1 km.

Conclusions I demonstrate a way to quantify the spatial scale of a local adaptation. Adaptations to habitat fragmentation can occur at relatively fine spatial scales, well within the limits of a species dispersal capacity, even in an abundant species with a wide range.

Keywords

Fragmentation; dispersal capacity; deforestation; local adaptation; spatial scale; landscape ecology

2.2   Introduction

It is often assumed that gene flow between local populations limits the capacity of species with continuous distributions to develop localized adaptations (Richardson et al. 2014). However,

the list of studies describing adaptive divergence within continuous distributions, or at small spatial scales, is extensive (e.g., Reznick and Endler 1982; Willi and Hoffmann 2012; Richardson and Urban 2013; Lindestad et al. 2019; Endo and Watanabe 2020). This indicates that gene flow does not always suppress the effect of highly localized selective pressures. While locally adapted populations themselves can possess limited genetic diversity, they can provide to the metapopulation new phenotypes that are suited to unique niches (Leimu and Fischer 2008; Gavrilets 2003). These local populations may help contribute to survival in the face of widespread ecological change, or even to eventual speciation. Therefore, it is important to understand the spatial scale at which local adaptations occur to fully appreciate the risks that habitat degradation and loss present to biodiversity. Sutherland et al. (2013) identified quantifying the spatial scale of local adaptation as one of 100 fundamental ecological questions that should be thoroughly explored over the next few decades.

As the scope of the environment that impacts a population varies tremendously across species, the question of spatial scaling of adaptation can have no single answer. Local adaptation occurs as populations adapt to environmental conditions on a small spatial scale (Blanquart et al. 2013). Adaptation is a fusion of environmental selective pressure, phenotypic plasticity, and adaptive genetic variation (Kawecki and Ebert 2004). This integrated nature makes deconstructing local adaptation a challenging and exciting pursuit. It also makes quantifying local adaptations within a population difficult.

The level of interaction between local populations is driven by connectivity, an index of the potential for a species to travel between habitat patches (Merriam 1984; Taylor et al. 1993). Typically, it is a measure of physical distance coupled with survivability in the intervening habitat and dispersal capacity of the species of interest. As habitats change in size, they can become either more or less fragmented, altering connectivity, and influencing the way dispersers move through the landscape (Collinge and Forman 1998; Schumaker 1996). Successful dispersal between local populations is a key factor in the dynamics of metapopulations (Hanski 1998; Hunter 2002; and Schtickzelle et al. 2006).

Fragmentation exerts selective pressure on populations by making resources more difficult to access by surrounding them in matrices of less suitable habitat, adding or removing resource patches, or altering the configuration of resource patches (Van Dyck and Matthysen 1999; Hunter 2002; Jonsen and Taylor 2000a; Wilcove et al. 1986; Taylor et al.; 1993). These factors can create

opposing pressures. If distant patches contain fewer competitors, increased dispersal capacity may increase fitness (Heino and Hanski 2001). Conversely, if there are high costs or risks associated with crossing unsuitable habitat, increased dispersal may be selected against (Olivieri and Gouyon 1997). For example, while Jonsen and Taylor (2000a) found evidence that dispersal capacity of a damselfly increased with fragmentation, Schtickzelle et al. (2006) found that dispersal capacity of the butterfly *Proclossiana eunomia* decreased with increasing fragmentation.

In this study, I present one process for addressing the question, "How local is adaptation?" as applied to an insect inhabiting the highly fragmented forests of Indiana, the ebony jewelwing damselfly (*Calopteryx maculata* Beauvois, Odonata: Calopterygidae). *Calopteryx maculata* is a large and immediately identifiable damselfly, inhabiting streams throughout eastern North America. Adults emerge from the water in early summer, dispersing from their natal streams for a period of about 11 days to complete development (Waage 1972). When they return to the stream, males establish and defend territories containing emergent vegetation for oviposition. Prior studies observed that *C. maculata* wing lengths vary in different habitats (Taylor and Merriam 1995). Although their preferred reproductive habitat is forested streams, *C. maculata* can be found under a wide variety of canopy conditions, including open meadows and fields. Individuals found at streams in open pastureland in Canada, 200 – 500 m distant from forest, had longer and wider wings than those found at forest-covered streams. Furthermore, *C. maculata* is more likely to make longer movements in partially forested landscapes (Jonsen and Taylor 2000b).

Generally, insects occupying more isolated habitats have increased wingedness (Wagner and Liebherr 1992). I hypothesized that the fragmented forest stream habitats of Indiana USA are exerting selective pressure on the wing morphology of *C. maculata*, leading to local adaptation of wings at a sub-state scale. I predicted that I would find differences in damselfly wing lengths along a gradient of forest fragmentation. Additionally, I predicted that wing lengths would show a pattern of autocorrelation with a range detectable within the boundaries of Indiana, US, consistent with the hypothesis that wing length is a result of local adaptation.

2.3 Methods

Study Area

Calopteryx maculata samples were collected from 21 sites across the state of Indiana, located in the Great Lakes Region of the United States. Indiana measures 94,320 km^2, with an

approximate extent of 37° 46′ N to 41° 46′ N, 84° 47′ W to 88° 6′ W. It has a mean elevation of 230 m above sea level, with a maximum elevation of 383 m, and a minimum of 97 m. It has a Köppen climate classification of hot summer humid continental in the northern half, and humid subtropical in the southern half. The Central USA Plains, and Southeastern USA Plains make up its Omernik level II ecoregions, and its level III ecoregions are Central Corn Belt Plains, Eastern Corn Belt Plains, Southern Michigan/Northern Indiana Drift Plains, Huron/Erie Lake Plains, Interior Plateau, Interior River Valleys and Hills (Omernik 1987). The area, once comprised of widespread forests and wetlands, experienced extensive deforestation during the 19th century (Carman 2013). Current landcover consists mainly of agricultural land (65%), fragmented temperate deciduous forests (17%), and urban development (11%) (NRCS 2017).

Study Sites and Specimen Collection

To assemble a representative sample of insects, collection sites were selected across the state. To address the question of spatial scale, 21 collection sites were selected at a range of inter-site distances ranging from 400 m to 360 km (Figure 1). Collection sites were selected based on the presence of at least 1000 meters of suitable stream within the property boundary. Access to sites was granted by several public and private organizations. Sites included seven Purdue University Agricultural Centers, six Purdue Forestry and Natural Resources properties, four public parks, two Division of Natural Resources properties, and two privately owned areas.

Collection sites consisted of a single 1000 m stretch of stream. Each stretch was divided into ten continuous, 100 m sample units. One site only encompassed a 500 m stretch, which was divided into five sample units. Two sites contained open stretches >100 m in length that adult *C. maculata* eschewed in the presence of alternative habitat. These stretches were excluded from the 1000 m stretch. Unit numbering for these ceased at one end of the open stretch, and commenced at the other.

Up to ten adult *C. maculata* were collected from each sample unit, for a maximum total of 100 *C. maculata* collected per site. If it was impossible to collect at least 50% of the maximum sample at each unit or site due to weather, nightfall, or abundance, the site was visited again until at least 50% of the maximum sample was reached. Adult *C. maculata* specimens within 5 m of the stream were collected using a 46 cm (18 inch) diameter aerial net with a 1.5 m handle (Bioquip Products Inc., Rancho Dominquez USA). Recently emerged, teneral adults, identified by their brown eyes and glossy wings, were excluded, as their exoskeletons were not fully hardened and

hence they may change in size or shape slightly. All specimens from a sample unit were placed into a single zip top bag, labeled with the date, collection site, and sample unit number. Upon returning from the field, bags were placed in a freezer to preserve specimens.

Specimen Preparation and Wing Measurement

Specimens were identified as male or female using the presence of the white stigma, and translucent coloration of female wings. Next, the wings from the right side of the body (forewing and hindwing) were removed using a pair of stainless-steel dissecting scissors. Wings were clipped as close to the thorax as possible. If the right wings were damaged, the most intact pair (mesothoracic and metathoracic wing on same side) of wings were removed. Removed wings were placed inside labeled glassine envelopes. Each specimen was then placed into a transparent envelope, along with the wing envelope, and a label, following the common method for odonates.

Wings were measured following the methods outlined by The Odonate Wing Digitization Project (National Science Foundation award #1611642 and #1564386), using the software Odomatic (The Odonate Wing Digitization Project 2020). A paper frame including a ruler and color standardization card (CameraTrax) was prepared and fixed to the surface of a high-resolution flatbed scanner (Seiko Epson Corp., Suwa, Nagano, Japan). This frame allowed the software to identify regions of the image as specific parts of the specimen. Specimens, wings, and labels were placed in designated locations within the frame and covered with a white paper backdrop. Scans were taken at 1200 dpi resolution and uploaded to the Bisque database on Cyverse. Odomatic accesses image files through Bisque, and automatically produces 25 wing measurements, including forewing length, the length outside the wing contour (Fig 2). I restricted analysis to wing length, as it was the feature that showed variation in response to habitat by Merriam and Taylor (1995).

Head width and hind femur length were used to quantify body size. These measurements were collected from the same high-resolution scans, using ImageJ (Schneider et al. 2012). Head width was defined as the maximum distance between the outer edges of left and right eyes. Femur length was defined as the distance between the proximal and distal ends of the femur on the right metathoracic leg. If this leg was not intact, the femur of the left metathoracic leg was used.

Landscape Extent and Habitat Fragmentation Gradient

It is vital to select biologically relevant units of area when quantifying landscape metrics. If the unit is too small, it is possible to commit a type I error. If the unit is to large, the possibility of committing a type II error increases. Three circles of differing radii around each sample unit

were used to delineate the extent of the relevant landscapes for specimens (Fig 3). These radii were defined as the 95th percentile of dispersal distances (200 m), 50% of the maximum dispersal distance (800 m) (Jackson and Fahrig 2012), and 500 m (Jonsen and Taylor 2000b). Because small body size and high mortality rates make tracking dispersal of many insects prohibitively difficult, the radii of these landscapes were based on the distribution of dispersal distances produced by a correlated random walk (CRW).

CRWs simulate the movement of animals by randomly drawing step parameters from biologically relevant probability distributions (Koh et al. 2013). Lifespans, daily dispersal distances, and turning angle frequencies of *C. maculata* were collected from preexisting studies (Appendix A) (Waage 1972; Jonsen and Taylor 2000a). These were used to create probability distributions of number of steps, step length, and step angle, respectively. Total lifetime movement was simulated for 10000 individuals and used to create the distribution of dispersal distances used to determine relevant landscape size.

Using a circular areal unit does likely overestimate the area that an individual interacts with. In this case, *C. maculata* likely avoids large portions of the nonforested areas within the selected radii. However, attempting to restrict the areal unit to a more specific habitat, e.g. the riparian zone, would likely exclude areas that individuals do interact with. This would also make comparing edge densities between sites, as total area would vary for each location.

I used the edge density of forest patches (m/ha), i.e., the sum of all patch edge lengths (m) divided by the total landscape area (m^2), then multiplied by 10000, as a predictor variable for wing lengths. I measured the edge density of forest using land cover surrounding each sample unit collected from the National Land Cover Dataset (NLCD) (USGS 2016). These data were then reclassified as forest and nonforest using the raster package (Hijmans and van Etten 2014) in R version 3.6.2 (R Core Team 2021) before measuring edge density, using the ClassStat function in the SDMTOOLS package (VanDerWal et al. 2019).

ClassStat Geometry Challenges

I encountered several challenges while quantifying edge density, relating how the raster and SDMTOOLS packages interpret the habitat raster. If left unaddressed, these challenges can lead to overestimating the amount of edge, underestimating the area, and thus incorrectly measuring edge density. For each sample unit, the complete forest/nonforest map of Indiana was cropped to circles of the aforementioned radii. This resulted in rectangular images where cells

inside the circles possessed a value of either 0 (nonforest) or 1 (forest), while cells outside the circle were assigned a value of "NA." While our intention was to treat the circumference of each circle as the boundary of the landscape, disregarding the world beyond, ClassStat treated the forest cells that lay along this circumference as part of the true forest edge; a forest – NA interface, in conjunction with the forest – nonforest interface. In addition, the function only considers cells of the manually specified value[s]. Therefore, when applying it exclusively to forest cells, it only returns the total area of forest, rather than the area of the extent, undervaluing area when calculating edge density. Finally, when using a polygon shapefile to crop the raster image, the raster package must decide which cells are within or outside of the circle, creating a sawtooth edge, approximating a circle of the desired radius. This process frequently results in inconsistent perimeters and areas between extents that should be uniform in size.

It must be noted that these issues may be avoided if the raster is coded properly. The ClassStat function is based on the statistics calculated by FRAGSTATS (VanDerWal et al. 2019; McGarigal 2015). McGarigal discusses considerations for raster coding, including distinctions between background cells, border cells, and boundaries (2015). However, these considerations can become difficult to implement when dealing with a large number of sample units in close proximity. For example, one approach is to reclassify cells surrounding the study area, giving them negative values. While this approach can allow the ClassStat function to perform in the desired manner, it requires the raster to be reclassified, then reset, for each sample unit. In our case, doing this for 239 study areas, at three different landscape scales, became computationally taxing and required prohibitively long processing times. Therefore, I used following procedure to reduce processing time.

To calculate the true amount of forest edge, I applied the ClassStat function to both forest and nonforest cells in each circular landscape. Because the circumference of each landscape was known, I was able to use ClassStat's total edge metric for each cell type (forest and nonforest) to solve for the amount of the circumference occupied by forest cells (Equation 1).

$$X = \frac{F - N + C}{2} \ (\textbf{Equation 1})$$

The amount of landscape perimeter contributing to forest edge (X) is equal to ClassStat's total edge output for forest cells (F), minus ClassStat's total edge output for nonforest cells (N), plus the circumference of the circular landscape (C), divided by two. The value X is then subtracted from F, to yield the true amount of forest edge. In order to complete Equation 1, the circumference

of the landscape, or rather the sawtooth perimeter of the landscape, must be accurately measured. Since the rasterized landscapes were no longer perfectly uniform size, each had to be measured. To do this, I simply created a uniform landscape, corresponding to the forest/nonforest raster, and used ClassStat to calculate the total edge of each circular landscape. I used this same method to collect accurate landscape areas, which were used to calculate forest edge density.

Statistical Analysis

Habitat data

Edge density was selected from among the ClassStat fragmentation metrics as a predictor variable to represent habitat fragmentation. This decision was based on the results of a redundancy analysis (RDA) (Van Den Wollenberg 1977). RDA was performed using the wing measurements as response variables, and the ClassStat fragmentation metrics as explanatory variables. Edge density had the highest loading along the RDA1, indicating that it was a leading explanatory variable for the inertia in the model.

Damselfly Measurements

Linear regression was used to evaluate the correlation between head width and femur length as potential measures of body size. Because they were highly correlated ($F(1, 1636) = 1217$, $R^2 = 0.43$, $pF < 0.0001$, $n = 1638$), and because heads were more clearly visible in scans, head widths were used to scale wing measurements as a proportion of body size. Both the unscaled wing lengths, and scaled wing lengths (divided by head width) were used in subsequent analyses. Wing lengths, and scaled wing lengths were subjected to analysis of variance (ANOVA) by sex and by site in a preliminary analysis to ensure variation among sites and to determine whether the sexes should be analyzed separately. The prediction that wing length varies with forest edge density was tested using polynomial regression, i.e., wing length ~ edge density and then wing length ~ edge density + edge density^2. The preliminary analysis found that female wings were larger than male wings and thus sexes were analyzed separately. Linear models with and without edge density^2 were compared using ANOVA to determine if the more complex model was justified.

Quantifying the Spatial Scale of Adaptation

The spatial scale of local adaptation was determined by quantifying the spatial autocorrelation of wing length measurements within multiple distance bins. A matrix of Euclidean distances between all specimen collection locations was created. Specimens were initially recorded as being collected at the center of their sample unit. However, because this species is mobile this does not reflect their true collection location within the sample unit. Therefore, R package vegan (Oksanen et al. 2021) was used to reposition each specimen's location by a random distance of 1 – 50 m. Additionally, this correction prevented overrepresentation of 0 m distances. Using this distance matrix, an inverse distance weight (IDW) matrix was computed, using the inverse of the distance between samples to calculate a weighted average of the variable of each sample (Dale and Fortin 2014). This IDW matrix was used to calculate Moran's I across the complete dataset to test the assumption of spatial autocorrelation. Then, it was used to separate the wing data into bins of neighbor distances, spanning 18679 m each (e.g., bin 1: 0 – 18679 m, bin 2: 18680 – 37358 m). This bin size was used to create 10 bins with equal numbers of distances. Moran's I was calculated using the wing sizes in each bin. The distance bin in which Moran's I was not different than 0, indicating a loss of spatial autocorrelation, was then further subdivided into five bins, spanning 200 m each, to gain a more precise measure of the distance at which Moran's I approached 0.

2.4 Results

Specimens

A total of 1686 specimens were collected across 21 sites and 199 sample units. 45 specimens were too damaged for image analysis, and were excluded from the analyses. Head widths (5.20 ± 0.17 mm, mean $\pm$ sd) and femur lengths (6.89 ± 0.40 mm) were strongly correlated ($F_{(1, 1636)} = 1217$, $R^2 = 0.43$, $pF < 0.0001$). Wing lengths (29.96 ± 1.75 mm) varied between sites ($F_{(20)} = 131.6$, $p < 0.0001$) and sex ($F_{(1)} = 8958$, $pF < 0.0001$). Scaled wing lengths (5.76 ± 0.22 mm/mm) varied between sites ($F_{(20)} = 51.97$, $pF < 0.0001$), and sex ($F_{(1)} = 5382.49$, $pF < 0.0001$) as well.

Correlated Random Walk

The distribution of adult reproductive lifespans (6.82 ± 7.01 days), the distribution of teneral stage durations (10.27 ± 5.99 days), and the distribution of daily dispersal distances (50.63 ± 51.99 m) reported by Waage (1972) were fit to lognormal distributions (Appendix A). These were used in conjunction with the probability distribution of turning angles (157.5 ± 110.2 degrees), reported by Jonsen and Taylor (2000) for *C. maculata* over spans of 30 minutes, in the CRW to simulate lifetime dispersal events for 10000 adult *C. maculata* (51.1 ± 51.47 m) (Appendix A). The 98th percentile of all dispersal event distances was 200 m, and 50% of the maximum distance was 800 m (Appendix A). These distances were then used to quantify habitat fragmentation in landscapes biologically relevant to *C. maculata*.

Habitat Fragmentation

Edge density ranged from 0 – 190 m/ha (mean 52.21) in 200 m radius landscapes, 0 – 112 m/ha (mean 49.77) in 500 m landscapes, and 0 – 109 m/ha (mean 47.31) in 800 m landscapes. Note that 0 m/ha corresponds to both completely forested, and completely deforested habitats.

Wing lengths and scaled wing lengths were both found to vary with forest edge density in both sexes (Fig 4, 5, Table 1, 2). In 200 m landscapes, wing length varied as a function of forest edge density in both males ($F(1,1071) = 43.05$, $R^2 = 0.038$, $pF < 0.0001$) and females ($F(1,563) = 26.16$, $R^2 = 0.043$, $pF < 0.0001$), as did scaled wing length of females ($F(1,563) = 4.13$, $R^2 = 0.0055$, $pF = 0.043$). Scaled wing length of males ($F(1,1071) = 0.508$, $R^2 = -0.00046$, $pF = 0.48$) did not. In 500 m landscapes, wing length varied with forest edge density in males ($F(1, 1071) = 30.24$, $R^2 = 0.027$, $pF < 0.0001$), while it exhibited a nonlinear relationship with edge density in females ($F(2, 562) = 22.35$, $R^2 = 0.0703$, $pF < 0.0001$). Scaled wing lengths also varied with edge density in both males ($F(1, 1071) = 4.6$, $R^2 = 0.0033$, $pF = 0.032$), and females ($F(1, 563) = 8.8$, $R^2 = 0.014$, $pF = 0.0035$) in 500 m radius landscapes. In 800 m radius landscapes, wing length in males ($F(1,1071) = 26.1$, $R^2 = 0.023$, $pF < 0.0001$) varied as a function of edge density, while wing length in females ($F(1, 562) = 17.35$, $R^2 = 0.055$, $pF < 0.0001$) varied as a function of edge density and edge density squared. Scaled wing length in males was correlated with edge density non-linearly ($F(2, 1070) = 8.1$, $R^2 = 0.013$, $pF < 0.00032$), and linearly in females ($F(1, 563) = 6.56$, $R^2 = 0.0098$, $pF = 0.011$).

Autocorrelation

The mean distance between specimen collection locations was 122 km (sd = 81.4 km) (Appendix A). The maximum was 374 km, and the minimum was 0 m. The Moran's I of wing lengths became effectively zero (I = -0.0047, p = 0.91) at less than 37 km (Fig 6). Dividing the first two distance bins (distances < 37 Km) into smaller equal-width distance bins revealed that Moran's I of wing lengths reached 0 at 800 – 1000 m (I = 0.0068, p = 0.38) (Fig 6). The Moran's I of scaled wing lengths approached zero at less than 37 km as well (I = -0.00096, p = 0.47) (Fig 6). Splitting the first two distance bins reveals that Moran's I of scaled wing lengths reached 0 at 600 – 800 m (I = 0.012, p = 0.23).

2.5   Discussion

Results of the Moran's I spatial autocorrelation suggest that landscape features within relatively small areal extents are adequate predictors of variation in *C. maculata* wing length. I can see that spatial autocorrelation of wing lengths approaches randomness at a distance of 800 – 1000 m (Fig 6). While there is a lack of samples at lag distances from 1090 – 5096 m, significant Moran's I values display a sharp trend towards zero at lag distances less than 1000 m, and do not trend away from zero until lag distances are much greater, even though sample sizes increase at distances greater than 5200 m (Fig 6). The trend away from zero at high lag distances is likely a result of extremely distant sites bearing similar conditions. For example, two Purdue Agricultural Centers 130 Km apart had very similar edge density values. These findings indicate that *C. maculata* wing lengths respond readily to local environmental selective pressures and that adaptation to local conditions takes place at a spatial scale of approximately a kilometer. This scale indicates that local populations of *C. maculata* only a few kilometers apart can adapt to different fragmentation conditions. In the context of this study, this scale corresponds to individual sites as I defined them, suggesting that each suitable stream can potentially harbor a uniquely adapted local population. Additionally, this scale corresponds closely with the 800 m radius landscapes used to quantify habitat fragmentation for each sample unit. These represented 50% of the maximum dispersal distance produced by the CRW.

Adult *C. maculata* rarely disperse from the stream habitat once they return from their brief maturation period. While females make occasional foraging flights away from the stream, males

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devote the majority of their time to defending territories and courting females (Taylor 1994; Kirkton and Schultz 2001). This limited dispersal is further reduced in both forested and non-forested landscapes, and may explain why local adaptive divergence is not overwhelmed by intersite gene flow (Jonsen and Taylor 2000a; b). When the entire surrounding landscape is forest, there is no need for *C. maculata* to leave the stream, because foraging sites are immediately present. When the surrounding landscape is completely devoid of forest, there are no foraging sites to travel to. In these cases, *C. maculata* may remain at the stream and attempt to meet its life history requirements with the resources available there. This level of site fidelity may lead to the highly localized scale of adaptation that I observed. Since adults do not need to travel far from their natal stream to complete their life cycle, they could effectively isolate themselves from populations within reach of their flight abilities. However, these insects are highly abundant in suitable habitats. Therefore, it is likely that while individuals rarely travel more than a kilometer, a high volume of short distance dispersal along stream networks preserves genotypic, and phenotypic, diversity by maintaining a continuous gradient of diversity. These short-distance dispersers are likely females seeking favorable oviposition sites, and males being harried by other established males.

While interactions between *C. maculata* wing length and landscape composition have been documented, prior studies restricted to relatively small extents (i.e., < 100 km) showed a relatively weak effect (Taylor and Merriam 1995; Taylor and Merriam 1996; Jonsen and Taylor 2000a; b). It is possible then, that these observations were coincidental with some undetected environmental condition (e.g., water pollution in breeding streams). Caution is required when treating high degrees of variation in size as evidence of adaptation to a single variable (Hassall 2015; Pither and Taylor 1998). Therefore, I sought to reproduce these findings in this study, over a larger extent, using a gradient of forest edge density.

Linear models support the hypothesis that forest edge density accounts for variation in *C. maculata* wing length (Fig 4). Utilizing edge density for landscapes larger than 200 m radius predicts a negligibly higher amount of the variance in wing length. While variation in scaled wing length is also partially explained by edge density, the relationship is very weak (Fig 5). Therefore, it appears that smaller body size is selected against in landscapes with higher edge densities, rather than only shorter wings. Because larger wings require larger muscles to flap them, it is likely that selection simply favors larger individuals in more fragmented landscapes. This interpretation is

supported by Taylor and Merriam (1994), who documented higher thoracic dry weights in conjunction with longer, wider wings in pastureland.

While it is tempting to attribute this difference in size to spatial sorting, the relatively small spatial scale of variation in wing length, and the CRW lifetime dispersal estimate suggest that individuals rarely emigrate to new streams, and are rather subjected to natural selection in their natal landscapes. Larvae can be washed downstream during high flow events, and stee areas, such as waterfalls, can impede their ability to recolonize upper reaches. However, they can reoccupy these areas once the leave the water as adults. In fact, competition for oviposition sites should drive adults to fill in any empty areas in their natal streams. The fate of larvae washed into large streams is unknown. In their preferred habitat of forested streams, newly emerged, adult *C. maculata* fly away from the water's edge, seeking small light gaps where they roost, feed, and complete development (Kirkton and Schultz 2001). This dispersal event is thought to provide several benefits. First, prey density appears to be greater in light gaps, providing greater resources. Since males rarely leave established stream territories willingly, they must build fat reserves that can sustain them throughout the rest of their lifecycle (Waage 1979). Seeking these prey-rich light gaps probably allows males to develop their fat reserves more quickly, enabling them to establish their own territories sooner (Kirkton and Schultz 2001). Females travel between streams and forest gaps more often, likely to accumulate nutrients for egg production (Taylor 1994). Additionally, this dispersal event precludes harassment of newly emerged *C. maculata* by established males, as established males attack interloping males, and pursue females, even if they have yet to complete development (Kirckton and Schultz 2001).

When suitable streams exist in forested areas, *C. maculata* do not have to travel great distances to reach light gaps. In deforested areas, light gaps may be located several hundred meters away. Adult *C. maculata* will cross treeless areas, such as pastures, to reach forests, and have been observed travelling between streams and forest patches 500 m distant (Taylor 1994). However, *C. maculata* can persist in completely deforested areas as well. In these situations, adults rarely disperse from the stream, and apparently manage to fulfill their lifecycle requirements with the resources in the immediate area (Jonsen and Taylor 2000a). Thus, *C. maculata* make larger dispersal movements in partially forested habitats (Jonsen and Taylor 2000b). Since dispersal distance will generally be longer in more fragmented sites, it exposes individuals to greater risk of mortality. If an increase in the frequency of long-distance flights truly does select against shorter,

smaller wings, then I would expect to see greater wing lengths peak in very patchy landscapes, denoted in this study by high edge density.

2.6 Conclusion

Localized phenotypic variation has many causes and contributing factors. I do not propose that forest fragmentation alone is responsible for variation in *C. maculata* wing length. I do demonstrate how the spatial scale over which physiological variation affected by local environmental conditions can be quantified and in the case of *C. maculata* is approximately a kilometer in the fragmented forests of Indiana. By incorporating knowledge of a species' short-term dispersal, and selective pressures it faces, it is possible to determine how localized a local adaptation of interest is.

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2.8 Figures and Tables
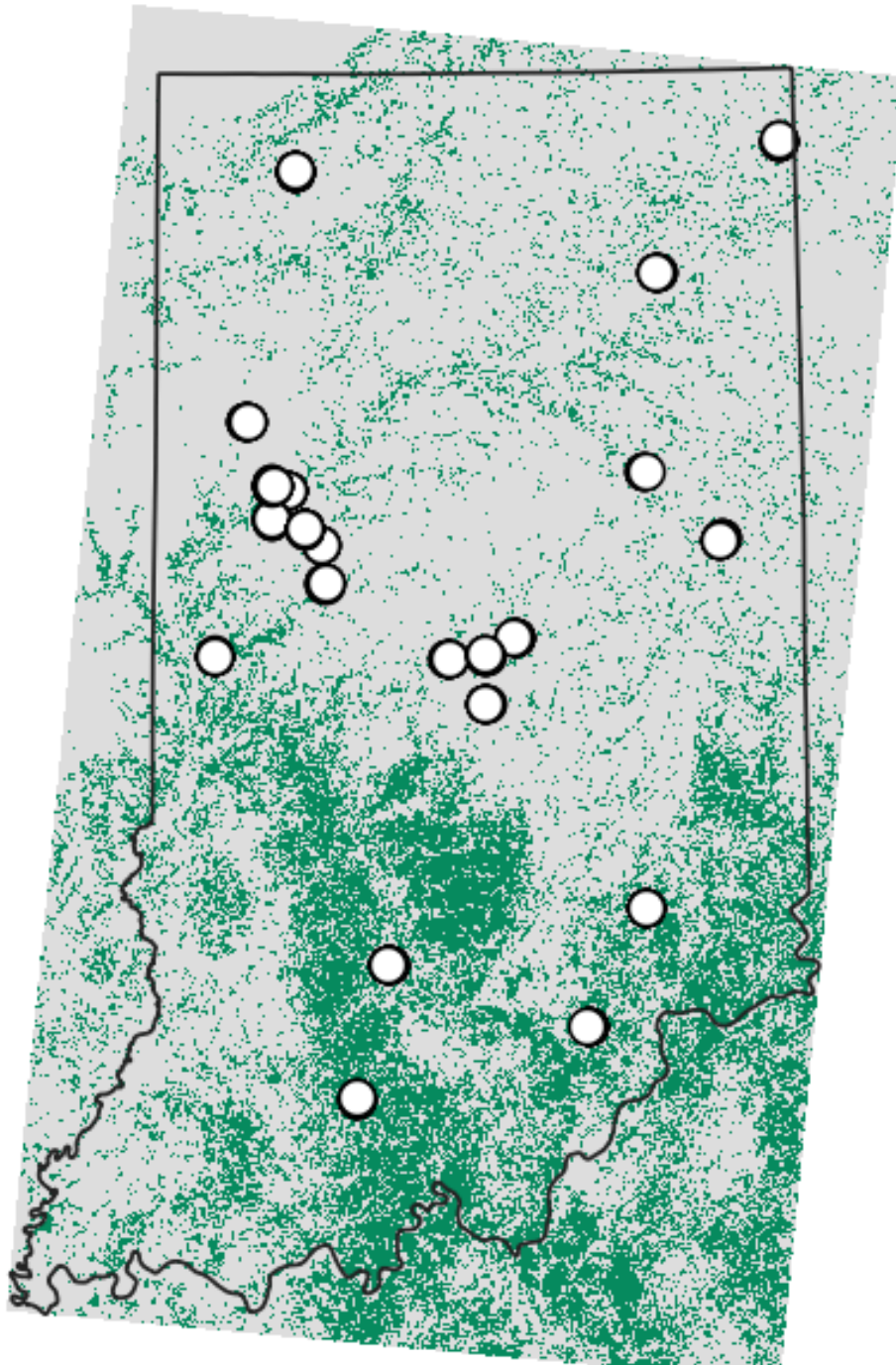
Fig. 2.1 Locations of the 21 sample sites.
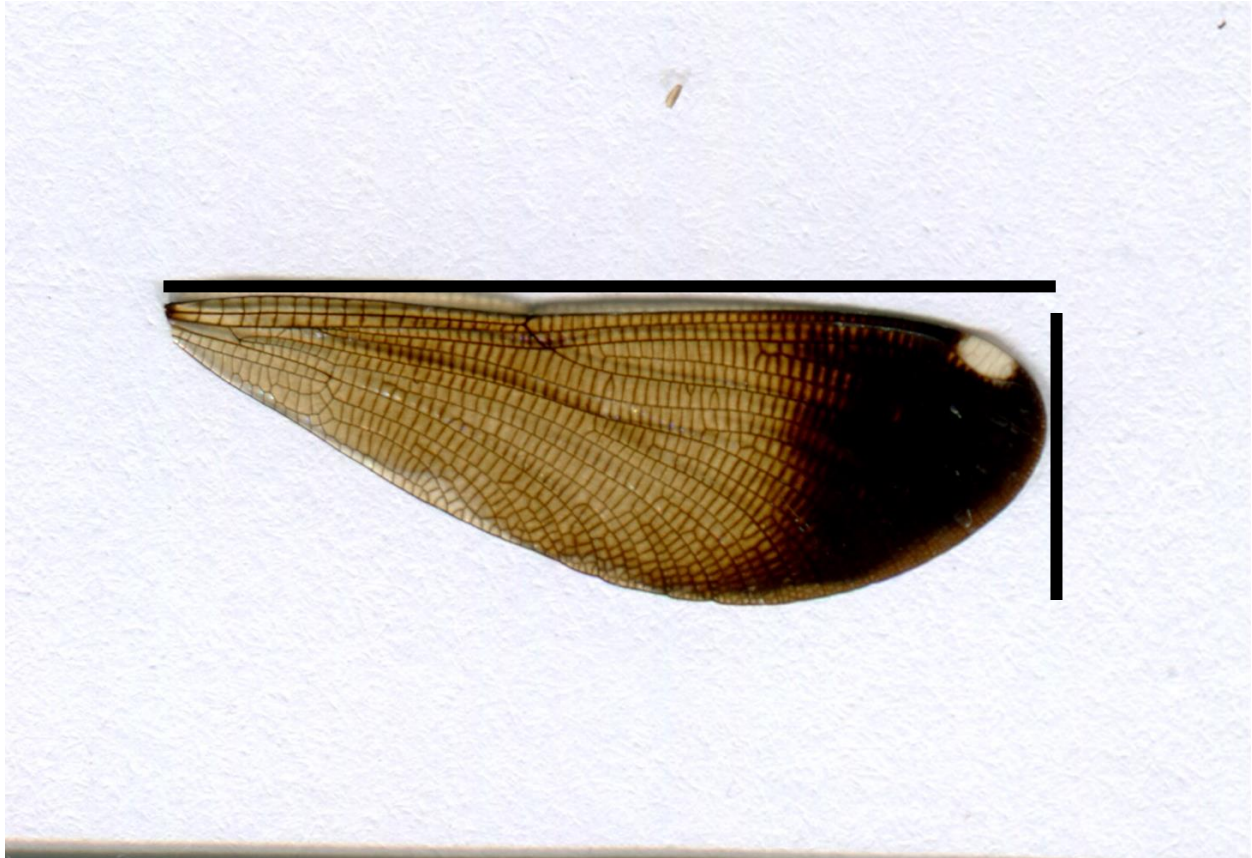
Fig. 2.2 Example of how Odomatic measures wing length and width using the outer wing contour. The black bars indicate approximate positions of wing length and width measurements. The horizontal bar represents approximate wing length. The vertical bar represents approximate wing width.
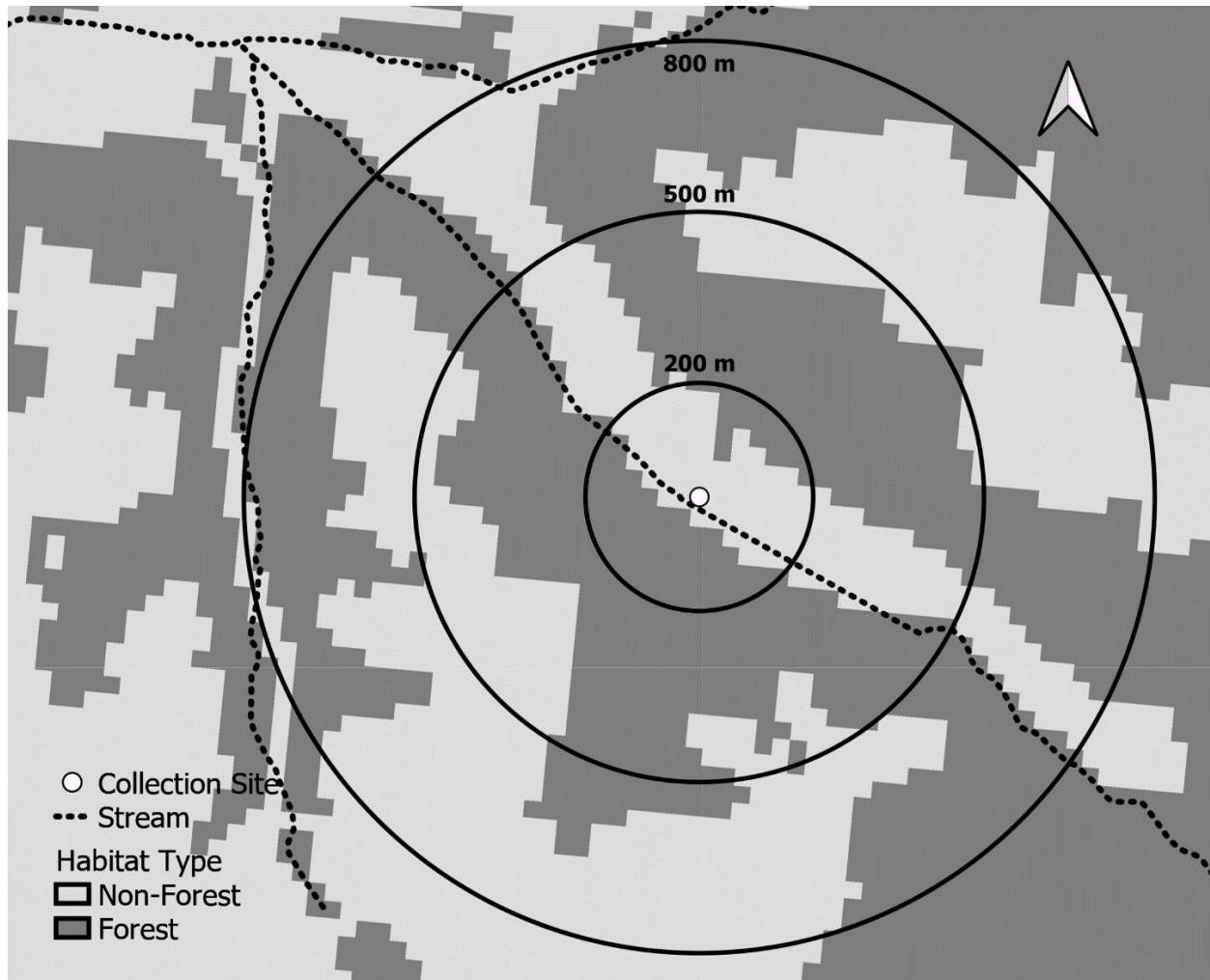
Fig. 2.3 Edge density was calculated for forest patches in landscapes of three distinct radii, based on movement data, surrounding each collection site: 200 m representing the 98th percentile of simulated dispersal distances, 500 m representing the distance used by Jonsen and Taylor (2000b), and 800 m, 50% of the maximum simulated dispersal distance.
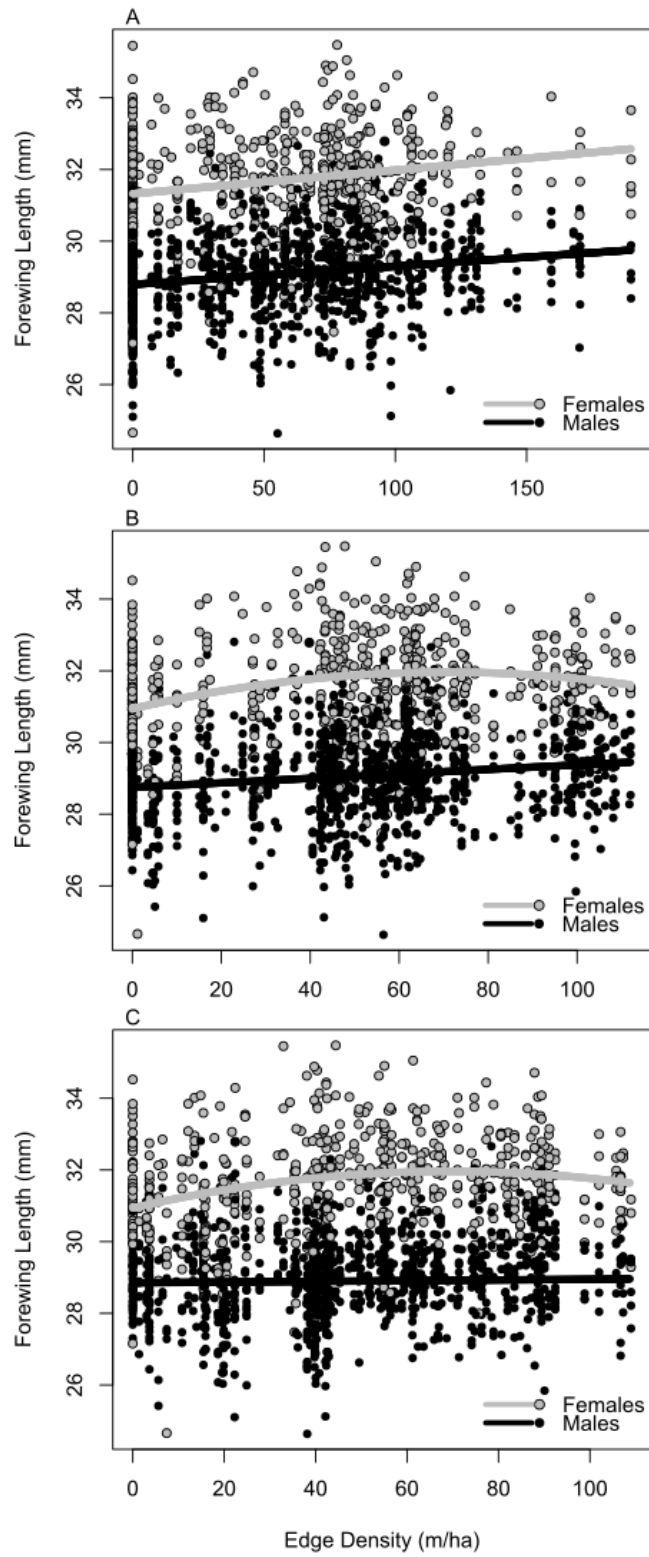
Fig. 2.4 Results of linear regressions of forewing length as a function of edge density, for males and females, at three landscape sizes: **A)** 200 m radius, **B)** 500 m radius, **C)** 800 m radius.
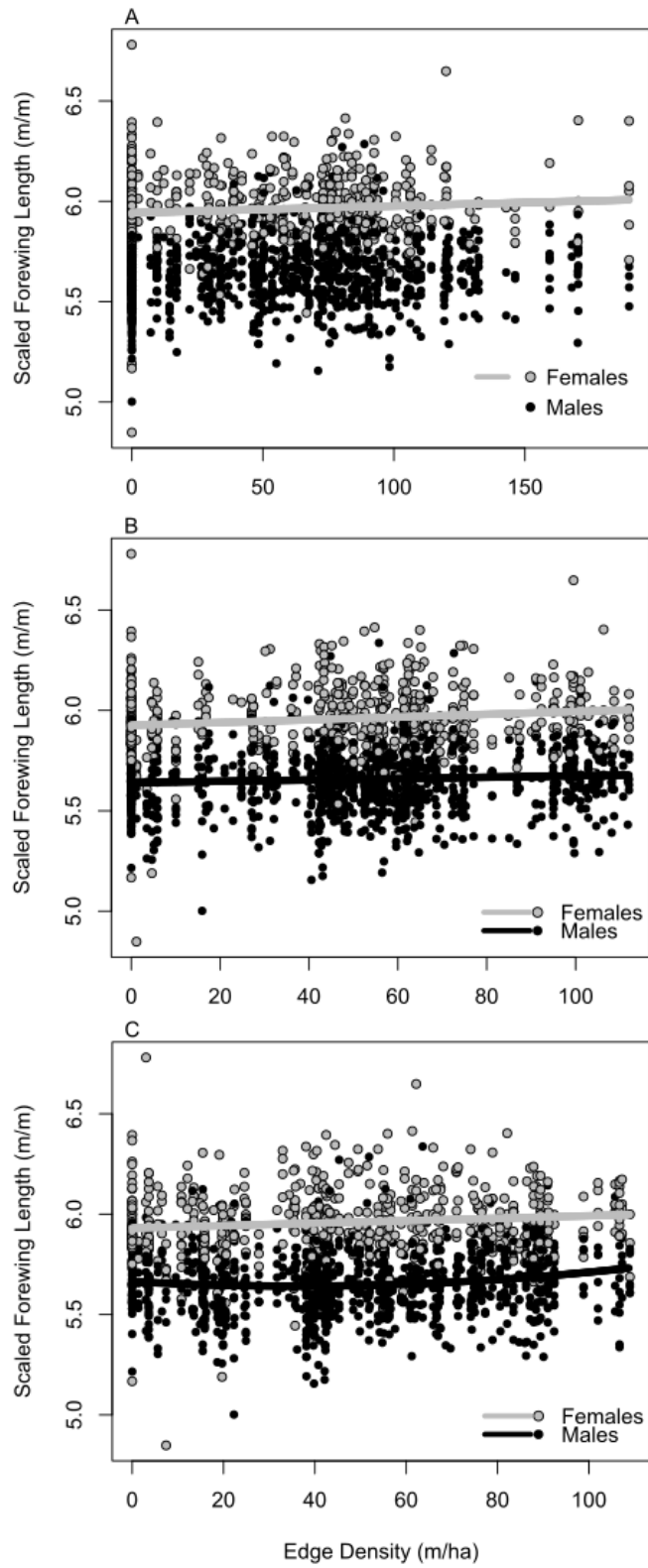
Fig. 2.5 Results of linear regressions of scaled forewing length as a function of edge density, for males and females, at three landscape sizes: **A)** 200 m radius, **B)** 500 m radius, **C)** 800 m radius.
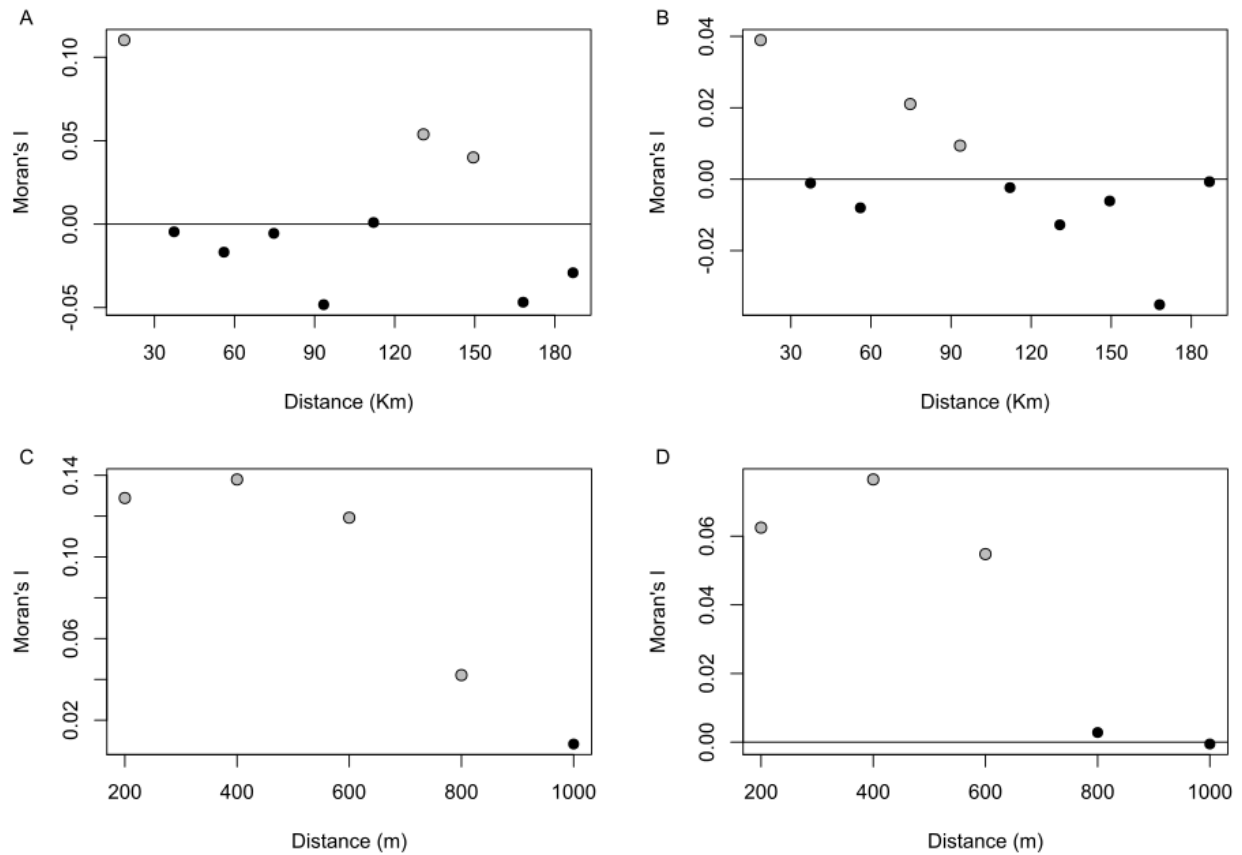
Fig. 2.6 Moran's I correlograms depicting the degree of spatial autocorrelation of: **A)** wing length over extent of the study area, **B)** scaled wing length over extent of the study area, **C)** wing length over a single site, **D)** scaled wing length over a single site. Moran's I values > 0 indicate positive spatial autocorrelation. Values < 0 indicate negative spatial autocorrelation. Values ≈ 0 indicate an absence of spatial autocorrelation. Grey circles indicate significant Moran's I values ($p < 0.05$). Black circles indicate insignificant Moran's I values ($p > 0.05$).

Table 2.1 Response of *C. maculata* wing length to forest edge density, in Indiana, US. Significant results are shown in bold.

Wing length	Spatial Scale	Sex	F statistic	D.F.	P value	Adjusted R^2
Edge Density + Edge Density²	**200 m radius**	**F**	**26.16**	**1, 563**	**< 0.0001**	**0.043**
Edge Density	**200 m radius**	**M**	**43.05**	**1, 1071**	**< 0.0001**	**0.038**
Edge Density + Edge Density²	**500 m radius**	**F**	**22.35**	**2, 562**	**< 0.0001**	**0.0703**
Edge Density	**500 m radius**	**M**	**30.24**	**1, 1071**	**< 0.0001**	**0.027**
Edge Density + Edge Denisty²	**800 m radius**	**F**	**17.35**	**2, 562**	**< 0.0001**	**0.055**
Edge Density	**800 m radius**	**M**	**26.1**	**1, 1071**	**< 0.0001**	**0.023**

Table 2.2 Response of *C. maculata* scaled wing length to forest edge density, in Indiana, US. Significant results are shown in bold.

Scaled wing length	Spatial scale	Sex	F statistic	D.F.	P value	Adjusted R^2
Edge Density	**200 m radius**	**F**	**4.13**	**1, 563**	**0.043**	**0.0055**
Edge Density	200 m radius	M	0.508	1, 1071	0.48	-0.00046
Edge Density	**500 m radius**	**F**	**8.8**	**2, 563**	**0.0035**	**0.014**
Edge Density	**500 m radius**	**M**	**4.6**	**1, 1071**	**0.032**	**0.0033**
Edge Density	**800 m radius**	**F**	**6.56**	**1, 563**	**0.011**	**0.0098**
Edge Density x Edge Density²	**800 m radius**	**M**	**8.1**	**2, 1070**	**0.00032**	**0.013**

CHAPTER 3. USING SLIME MOLDS TO ANSWER LANDSCAPE-LEVEL QUESTIONS AT TABLETOP SCALE

3.1 Abstract

Context Testing hypotheses of landscape-level metapopulation dynamics is time-consuming and expensive. It is virtually impossible to perform some experimental treatments, such as changing the location of a forest. The slime mold *Physarum polycephalum* exhibits unique qualities that give it the potential to act as a microcosm of populations occupying mosaics of resource and non-resource patches.

Objectives Examine *P. polycephalum*'s potential as a microcosm of damselfly population dispersal, by quantifying its dispersal ability, and testing the effects of patch distance and size on the probability that a focal patch will be colonized before others in the model landscape.

Methods Lifetime dispersal of *P. polycephalum* was quantified by placing plasmodia in large dishes of agar, and tracking their location until the end of their life cycle. Effects of patch distance and size were tested by placing plasmodia in model landscapes containing oat patches of differing distances and sizes. Suitability as a microcosm system was assessed by comparing the observations of patch colonization with calculations of patch connectivity for a damselfly species in a proportionally scaled landscape.

Results Lifetime dispersal of *P. polycephalum* was 62.2 mm ± 65.9. Patch distance and size were independent of one another. Near patches were more likely to be colonized before far patches ($p = 0.0056$), but large patches were not more likely to be colonized before small patches ($P = 0.17$). Estimates of patch connectivity for the damselfly were corroborated by *P. polycephalum* microcosms half of the time.

Conclusions *Physarum polycephalum* offers a unique system for testing hypotheses of landscape-level population dynamics. While further testing is needed to fully understand the strengths and limitations of this potential model, microcosm experiments aligned with current metapopulation theory on the effect of patch distance.

Keywords

Microcosm; metapopulation model; Physarum polycephalum; model landscape; landscape microcosm; patch connectivity

3.2   Introduction

The question of how population traits interact with landscape configuration to determine realized dispersal distances is fundamental to ecology (Sutherland et al. 2013). Currently, scientists test hypotheses of metapopulation dispersal using extensive tracking and observation of organisms in the field, or performing dramatic experimental manipulations of large plots of land. Virtual simulations are extremely useful in understanding how manipulations of resource patches will influence realized dispersal of populations, but outcomes can be deterministic. The objective of this project is to evaluate the use of the protist *Physarum polycephalum* as a model system for testing dispersal hypotheses in a microcosm setting. This would allow researchers to move beyond computer simulations, into a simplified, but still biologically realistic system.

The arrangement of elements in a landscape can influence the survival, distribution, and behavior of animal species (Wiens 1976). If I consider a landscape to be a matrix of habitat and non-habitat patches, it is apparent that landscape composition is an important feature for most species. Animals depend on the resources available in a habitat patch, thus population sizes are linked to the amount of available habitat in a landscape (Fahrig 2003, Prugh et al. 2008). The total area of a habitat patch must be large enough to sustain a minimum viable population for the species to persist, unless the population is being supplemented by immigrants from other populations (Traill et al. 2007, Levins 1969). As the size of a population approaches a habitat patch's carrying capacity, competition for resources increases, and some individuals are forced to disperse in search of new habitat patches (Taylor 1988).

The arrangement of habitat patches on the landscape, the habitat configuration, influences the likelihood that dispersers will succeed in locating new resources. Any number of habitat configurations is possible, but in general they range from uniform to clumped. For example, a forest may be comprised of a contiguous patch of trees 10 km^2 in area, or of five patches of trees measuring 2 km^2 each. The total area of forest remains the same, but individual patch sizes differ greatly. Most species have a minimum patch size that can sustain a population (Beier 1993).

Patch suitability can be due to factors such as energy availability, or microclimate conditions (Collinge 2009). While the total forest area may be enough to support the species in question, individual patch size may preclude its presence. This is not to say that the degree of brokenness, fragmentation, of a habitat has only negative effects on a population. Intermediately fragmented habitats may guard against environmental stochasticity, by harboring multiple local

populations of a species, which can repopulate a habitat patch that experienced an extinction event (Simberloff and Abele 1982, Wilcox and Murphy 1985, Govindan et al. 2015).

The orientation of habitat patches on the landscape has implications for individuals dispersing from their source patch. If the distance between a habitat patch and the nearest neighboring patch of the same type is too great, the probability of an individual disperser reaching it approaches zero (Holland 2009). Indeed, if a critical isolation distance between a source patch and the surrounding patches is reached, a population will become cut off from reaching new patches. However, this isolation can be one-way. Larger patches can support larger populations, which tend to have higher probabilities of reaching distant patches (Holland 2009). So, while one patch may be gaining immigrants, it may not be producing emigrants. Habitat connectivity is an index of the potential for a species to travel between habitat patches (Merriam 1984, Taylor et al., 1993). Typically, it is a measure of physical distance coupled with survivability in the intervening habitat and dispersal capacity of the species of interest. As habitats change in size, the degree of fragmentation can change, altering connectivity (Collinge and Forman 1998). This degree of change in fragmentation will influence the way populations move through the landscape (Schumaker 1996). Successful dispersal in local populations is a key factor in the dynamics of metapopulations (Hanski 1998, Geurry and Hunter 2002, and Schtickzelle et al. 2006).

Metapopulations are networks of geographically distinct, dynamic, local populations that interact with one another through immigration or emigration (Levins 1969, Hanski and Gilpin 1991). Metapopulations exist in a balance between extinction and re-colonization events within available habitat patches (Levins 1969, Hanski, 1998). Local populations must produce enough new populations to ensure the metapopulation persists (Gyllenberg and Hanski 1997). Typically, this condition is met as a result of asynchrony between extinction of and colonization by local populations (Lande 1993). However, local population growth may be diminished by factors including increased emigration, habitat loss, and changes in habitat suitability (Thomas and Hanski 1997, Hanski 1998, DeWoody et al. 2005). Migration from one local population to another is a driving force behind the maintenance of metapopulations (Hanski 1998). Successful migration of individuals is influenced by the isolation, or fragmentation, of habitat patches, since migration mortality tends to correlate positively with degree of habitat fragmentation (Moilanen & Hanski 1998, Schtickzelle et al. 2006).

The metapopulation concept is based on the work of Richard Levins (1969), who proposed a model describing the balance between local extinction and recolonization. In this model, patch occupation at a given time, *dN/dt*, is a function of migration rate (*m*), proportion of occupied patches (*N*), total number of sites that may support local populations (*T*), and extinction rate of local populations (*EN*).

$$\frac{dN}{dt} = mN\left(1 - \frac{N}{T}\right) - EN$$

The migration rate, *m*, represents the rate at which empty patches are colonized by dispersers from a local population. Levins describes *m* as:

$$m = m_o e^{-aD}$$

Where m_o is the migration rate to neighboring plots when distance is 0, *a* is a constant depending on the scale, and *D* is distance between patches. Following this equation, as distance between patches increases, the rate of migration decreases, and the time to recolonization increases.

The dispersal distance of animals can be estimated using random walks (Koh, et al. 2013, Jackson and Fahrig 2012). The maximum realized distance that individuals from a local population are likely to travel will scale with population size, since dispersal distances will be drawn randomly from a probability distribution, the dispersal kernel. Evidence supports the hypothesis that population size scales with habitat patch size (Wilson and MacArthur 1967, Hanski 1994). Therefore, it is reasonable to assume that more distant patches become more likely to receive dispersers in the presence of larger source patches. Likewise, larger habitat patches lying within the likely dispersal area will have a higher probability of being colonized, since they occupy a larger proportion of the available space (Wilson and MacArthur 1967). These principles form the basis for patch connectivity models that predict the probability of a patch receiving new colonists from a source population (Moilanen 2004).

It is important to test metapopulation models for generalizability. To test predictive models of this scope in the field would require multiple years spent tracking individuals from multiple populations, and observing landscape changes. While some predictive models are tested using simulated species, it is more informative to the evaluation process to test the model in a biological system. Researchers have often balanced the rigorousness of field testing with the accessibility of model systems using microcosms (Collinge 2009). Microcosms allow researchers to test theoretical models in real biological systems, while being able to control for many environmental

variables, including patch arrangement, size, and spacing, competition, and resource abundance (Huffaker 1958). The small size of these experimental systems allows for large numbers of replications, resulting in robust statistical testing, and the nature of the organisms often allows the observation of multiple generations over a relatively short timeframe.

Physarum polycephalum is a protist known as a slime mold. It can detect local concentration gradients, and direct its growth towards nutrient-rich resources (Jones 2009). It consumes bacteria and other microbes, and displays activity reminiscent of foraging behavior in animals (Wu et al. 2015, Latty and Beekman 2006). It is a popular model system in network analysis and biological computing due to its ease of use, and dendritic plasmodium phase (Shirakawa et al 2008). When placed in petri dishes with an arrangement of oats, *P. polycephalum* will eventually develop a network of veins that connect it to the multiple sources of nutrients (Figure 1). It has even been observed solving mazes by shortest path (Nakagaki and Toth 2000). When *P. polycephalum* does not detect a local nutrient gradient the foraging activity resembles a reinforced random walk (Ma et al. 2013). This pattern suggests that while *P. polycephalum* is seeking resources, it behaves similarly to a population of animals dispersing across the landscape, suggesting that *P. polycephalum* could be a good model system to test hypotheses about population responses to habitat configuration (Holland 2009).

This project addresses the fundamental ecological question of how population traits interact with landscape configuration to determine realized dispersal distances as posed by Sutherland et al. (2013). Currently, scientists determine realistic dispersal distances by performing extensive tracking and observation of organisms in the field, or creating simulations based on empirical dispersal data. These findings will contribute to the field of landscape ecology by offering a way to account for different dispersal phenotypes in populations of the same species, and changes in landscape configuration. Ultimately, it will allow researchers to predict how the spatial distribution of local populations will change through time in the face of landscape changes, by allowing them to visualize their positions at all points in time simultaneously. This project tests hypotheses concerning the probability that certain patch types were more likely to be colonized before others. Specifically, I hypothesized that near patches had a higher probability of being colonized before far patches, that large patches had a higher probability of being colonized before small patches, and that near, large patches had a higher probability of being colonized before any other combination of distance and size. Additionally, I hypothesized that the results of the microcosm

experiments would match patch connectivity estimates, calculated for *C. maculata* in proportionally sized landscapes.

3.3   Methods

P. polycephalum Dispersal

Daily and lifetime dispersal of *P. polycephalum* were quantified by introducing small pieces of plasmodium in large trays of agar, and measuring step length and distance from origin every 24 hours. Stainless steel greenhouse watering trays, measuring 58.75 x 67.5 cm were painted black (Gloss Protective Enamel, Rust-Oleum) on the inside to improve visibility of plasmodium. The center of each tray was marked with a small dot of contrasting paint. Each tray was filled with 1.5 L of 2% agar (Telephone Brand) and 98% deionized water. Trays and agar solution were sterilized in an autoclave for 55 minutes at 121°C before filling. Trays of sterilized agar were allowed to cool under a protective sheet of plastic stretch wrap (76 cm wide, Pratt Retail Specialties) until solidified.

A flame-sterilized scalpel was used to transfer a 4 x 4 mm square of plasmodium from a *P. polycephalum* culture to the center of the cooled agar surface in each tray. Within each set of 10 replicates, all plasmodia were taken from the same culture. Since food can increase the size, and therefore the distance traveled, and life cycle duration of *P. polycephalum*, no food was added to trays.

Approximately 1.3 m of stretch wrap was wrapped tightly around each tray, so that it did not come into contact with the agar surface, and secured with duct tape to prevent contamination and drying. Holes in stretch wrap were patched using clear, heavy duty packaging tape (Scotch). Tape was also used to increase tension on any stretch wrap that began to sag by the following day. Trays were placed in a greenhouse maintained at approximately 21°C. To protect the plasmodia from direct light and extreme temperatures, a 3 x 3 m sheet of black, 40% shade cloth was suspended 0.5 m above trays, using short poles.

The position of each plasmodium was checked every 24 hours, until it either entered the sporangia or sclerotia phase, or otherwise remained in the same position for more than four days. The location of the leading edge of each plasmodium was marked and dated on the surface of the stretch wrap, using a permanent marker (Sharpie). The distance of each daily position from the origin, and the distance from the previous day's position were measured to the nearest mm using

a ruler. Occasionally, plasmodia split into multiple plasmodia. In these cases, each was denoted using a unique alphanumeric label added to its daily position mark. The extracellular slime trial (Fig 2) was used to track each plasmodium from the previous day's position, ensuring correct labeling. Lifetime dispersal was defined as the distance from origin when the slime mold ceased movement. Daily dispersal was defined as the distance the slime mold traveled from the previous day's measurement. Lifetime dispersal is thus not necessarily the sum of daily dispersal.

Effect of Patch Distance on Probability of Colonization

Twenty microcosms representing hypothetical landscape matrices were created by pouring 240 mL of 2% agar solution into 25 cm x 25 cm aluminum pie pans. Agar and the aluminum pie pans were sterilized in an autoclave for 30 minutes at 151°C. The plastic lids of the pie pans were sterilized by soaking in 70% ethanol for 30 minutes. After pouring the agar, I placed the lid on each pan, and allowed the agar to cool until solidified which took approximately 45 min.

After the agar solidified, four resource patches were created by placing rolled oats (The Kroger Co., Cincinnati, OH), in circular arrangements, directly on the surface in a North, East, South, West orientation, using a pair of flame-sterilized forceps. Each patch measured 1.5 cm in diameter, and consisted of five oats. The patches in the North and South positions were placed with their nearest edges 83 mm from the center of the pan. The patches in the East and West positions were placed with their nearest edges 31 mm from the center of the pan. Each pan was inoculated by placing a single oat, covered in *P. polycephalum* plasmodia, in the center, before replacing the plastic lid. To reduce any influence ambient light may have on the direction of the slime molds' foraging, each pan was randomly rotated 270°, 180°, 90°, or 0°, and covered in two sheets of newspaper.

Because *P. polycephalum* acquire most of their nutrition from bacteria, the oats cannot be autoclaved without greatly reducing their nutritional value to the organism. This inability to autoclave led to an issue with *Rhizopus* contamination in several packages of oats used throughout this project. To suppress *Rhizopus*, oats were placed in an 80°C oven for 60 minutes, and allowed to cool before using in an experiment (*Manners 1966).*

Microcosms were checked every 12 hours, until the *P. polycephalum* plasmodia made contact with one of the oat patches. Because *P. polycephalum* can increase in size and prolong its life cycle when it locates additional resources, all patches in a microcosm of this size are likely to be

colonized eventually. Therefore, the first patch type, "near" or "far", physically contacted was recorded.

Effect of Patch Size on Probability of Colonization

Forty microcosms were prepared as in the previous section, except when placing patches of rolled oats (Fig 3). Instead of four identically sized patches placed at different distances from the center, two large patches, measuring 4 cm in diameter (approx. 25 oats), were placed in the North and South position, and two small patches, measuring 1 cm in diameter (three oats), were placed in the East and West position. All four patches were placed with their nearest edge 58 mm from the center. A single oat containing slime mold plasmodium was transferred to the center of each pan, and the lids were replaced. Once again, pans were randomly rotated 270°, 180°, 90°, or 0°, covered in two sheets of newspaper, and checked every 12 hours. The first patch type, "large" or "small", physically contacted was recorded.

Effect of Patch Distance and Size on Colonization Probability

Twenty microcosms were prepared following the previous method, until patches were placed on the agar. In this experiment, oat patches were arranged in the North, East, South, West orientation. However, each patch was randomly assigned one of four possible combinations of distance and size without replacement: near and large, near and small, far and large, far and small. Near patches were placed with the nearest edge 31 mm from the center (Fig 4). Far patches were placed with the nearest edge 58 mm from the center. Large patches measured 4 cm in diameter, while small patches measured 1 cm in diameter.

Patch Connectivity Estimation

To assess *P. polycephalum*'s usefulness as a model of population dispersal, I compared the outcomes of the previous experiments to predictions of patch connectivity S_i, calculated for the damselfly *Calopteryx maculata*, in proportionally-scaled landscapes (Hanski 1994). Here, S_i represents connectivity of patch *i*. Patch connectivity is defined as:

$$S_i = \sum_{j \neq i} exp\left(-Dd_{ij}, \propto\right)A_j$$

Where A_j is the scaling of immigration as a function of area, $j = 1$ for occupied patches, and $j = 0$ for unoccupied ones, D represents the dispersal kernel with d_{ij} being the distance between patches i and j, and $\propto$ being the distribution of dispersal distances A_j is the scaling of emigration (Moilanen 2004, Prugh 2009).

S_i was calculated for each unique patch type in the patch distance, patch size, and patch distance and size experiments. Patch radii, and distances were scaled up to sizes relevant for *C. maculata*. The appropriate scaling factor was calculated by dividing the mean lifetime dispersal of *C. maculata* by the mean lifetime dispersal of *P. polycephalum*. Mean lifetime dispersal of *P. polycephalum* was calculated from the final distance from origin for each slime mold in the dispersal experiment, and acted as the denominator. Mean lifetime dispersal of *C. maculata* was calculated from a distribution of dispersal distances produced by a correlated random walk (CRW). CRWs simulate the movement of animals by randomly drawing step parameters from biologically relevant probability distributions (Koh et al. 2013). Lifespans, daily dispersal distances, and turning angle frequencies of *C. maculata* were collected from prior studies (Waage 1972; Jonsen and Taylor 2000a). These were used to create probability distributions of number of steps, step length, and step angle, respectively. Total lifetime movement was simulated for 10000 individuals. The final distance from origin for each of these 10000 individuals was used to calculate the mean, which acted as the numerator. The quotient of these values served as the scaling factor.

The S_i for each patch type was divided by the sum of the S_i's for each patch within its respective experiment. For example, the S_i of a large patch was divided by the sum of the S_i's of a large patch and a small patch in the patch size experiment. This resulted in an index representative of the proportion of patches of the same type that are likely to be colonized first. These indices were compared to the observed proportions of their respective patches that were colonized first in each of the experiments.

Statistical Analysis

Effects of Patch Distance, Patch Size, and Patch Distance and Size on Probability of Colonization

I examined whether near or far patches were more likely to be colonized first using a binomial test. I also examined whether large or small patches were more likely to be colonized first using a binomial test. For the microcosm experiment in which both patch size and distance

were varied, four models were compared to predict probability of first colonization as functions of distance and size: interactive and additive generalized linear mixed-effects models with random plate-specific intercepts and interactive and additive fixed-effects models. Because of convergence issues, models were fitted within a Bayesian framework using R package brms (Bürkner et al. 2021) and compared using the approximate leave-one-out information criterion (LOOIC) and the widely applicable information criterion (WAIC, Watanabe 2010, Vehtari et al. 2017). Default priors were used including flat priors for regression coefficients, a Student t (3 d.f., mean = 0, sd = 2.5) for the intercept, and a half-Student t with the same parameters for population- and group-level standard deviations. Models were fit with four chains totaling 9000 posterior draws following 2250 warmup draws and a thinning rate of one.

3.4 Results

P. polycephalum Dispersal

I used 40 replicates to quantify *P. polycephalum* dispersal. However, the plasmodia in 20 replicates split into at least two individuals, resulting in n = 50. Mean lifetime dispersal, the final distance from origin, was 62.2 mm ± 65.9 (mean ± sd) (Fig 5A). Mean daily dispersal, the distance from origin at each 24 hour mark, was 58.5mm ± 59.4. Mean daily step length was 11.9 mm ± 14.2 (Fig 5B). Lifespan ranged from 0 to 23 days, with a mean of 6.5 ± 6.2 days.

Effects of Patch Distance and Size on Colonization Probability

Of the 20 replicates used to examined whether near patches were more likely to be colonized first, 16 near patches, and four far patches were colonized first. The probability of near patches being colonized first was thus 0.8 (p = 0.0059, 95% ci = 0.6 – 1). One of the original 40 replicates used to examine whether large patches were more likely to be colonized first was discarded, because both a large and small patch were colonized at the same time. For 39 replicates, 23 large patches, and 16 small patches were the first to be colonized within their respective microcosms. The probability of a large patch being colonized first was 0.59. However, this was not significantly greater than 0.5 (p = 0.17, 95% ci = 0.45 – 1).

Effect of Patch Distance and Size

Models without random intercepts had greater support than random-intercepts models according to both WAIC and leave-one-out information criterion (LOOIC) (Table 1). The estimated standard deviation for the distribution of random intercepts was small (0.29) and imprecise (95% credible interval [0.01, 0.86]). The posterior distribution for the distance by size interaction term in the fixed-effect model overlapped zero (0.106 of posterior > 0), suggesting little evidence of an interactive effect. Fisher's exact probability test also indicated no interactive effect of patch distance and patch size on colonization (df $= 1$, p $= 1$). Based on the additive fixed-effects model, the odds of first colonization was 25.5 times greater for near patches than far patches (95% credible interval [6.89, 123.96], posterior probability < 0.001), and 7.0 times greater for large than for small patches (95% credible interval [2.39, 22.20], posterior probability < 0.001) (Table 2).

Patch Connectivity

Mean lifetime dispersal of *C. maculata* generated by the CRW simulation was 62.02 m, almost exactly 1000 times the mean lifetime dispersal for *P. polycephalum*. Accordingly, the patch distances and radii of the microcosms were multiplied by a factor of 997, to scale the microcosm to a relevant size for the insect. In the patch distance experiment, connectivity ($S_i / \sum S_{ij}$) was 0.7 for near patches, and 0.3 for far patches (Table 3). Large and small patches had an even more dramatic difference in connectivity, with 0.94 for large, and 0.05 for small. Pairing patch distance and size produced connectivity indices of 0.57 for near-large patches, 0.04 for near-small patches, 0.37 for far-large patches, and 0.02 for far-small patches.

3.5 Discussion

Physarum polycephalum met assumptions of patch occupancy based on distance and patch size. As expected, near patches were more likely to be colonized before far patches. When compared to indices of patch connectivity, *P. polycephalum* met our predictions, for some patch types, including near, far, near-large, and small-far (Table 3). There were some conflicting results about the effect of patch size. Large patches were not more likely to be colonized before small patches using binomial tests, or interactive generalized linear mixed-effects model. However, an additive generalized linear mixed-effects model did detect an effect of patch size, with larger patches being more likely to be colonized than small patches.

I found little evidence of interaction between patch distance and size using a generalized linear mixed-effects model. The random effects of plate ID exhibited little variation, suggesting that a fixed-effects model was sufficient.

The lack of difference between colonization probability of large and small patches may appear somewhat surprising, given *P. polycephalum*'s well-documented foraging activity (Nakagaki 2000). Here I propose two alternative explanations for this phenomenon. First, it is possible that *P. polycephalum* was responding to an external stimulus that signaled patch quality. It has been demonstrated that *P. polycephalum* is capable of detecting and altering its movement in response to chemical stimuli (Ueda et al. 1980). Typically, these stimuli have been concentration gradients of solutions, such as sugar, salt, and quinine (Boisseau et al. 2016, Kincaid et al. 1979). As my experiments only used dry, solid oats, on a relatively low-moisture agar surface, it seems unlikely that *P. polycephalum* was responding to a concentration gradient of dissolved oat nutrients. However, I did not test or control for this, or the prospect of other like stimuli. Indeed, many of the plasmodia appeared to travel directly towards a patch, in relatively straight lines (Fig 6). This would certainly appear to suggest some form of perceptive ability.

Alternatively, this phenomenon may simply be the result of *P. polycephalum*'s similarity to a dispersing population. The dispersal kernel of a species describes the probability density function from which dispersing individuals represent random draws (Okubo and Levin 2001, Holland 2009, Jackson and Fahrig 2012). Since populations consist of multiple individuals, the displacements of each individual over time overlap, forming an approximate ring, or brim, around the population, encompassing an area where colonization is likely (Ovaskainen 2004). Since that the target patches were placed within the dispersal kernel of the slime mold—mean lifetime dispersal for a smaller plasmodium exceeded this distance—I might not expect patch size to influence colonization probability in this system. Since I recorded the first instance of physical contact between the slime mold and an oat patch as a colonization event, *P. polycephalum* only needed to contact a single small point on the patch edge. Because the nearest edges of the large and small patches were equidistant from the center of the plate, i.e., the origin of the population, there were an infinite number of infinitely small points along those edges with essentially identical probabilities of colonization.

An individual disperser can only occupy a single point in space, and only travel in one direction at a time, so target patch size certainly plays an important role in the likelihood of

colonization by at least one individual (Hanski et al. 2000). If a smaller plasmodium were used, or if it were placed in a larger microcosm, with patches spaced farther apart, it is possible that an effect of patch size would become more pronounced. The slime mold tends to stretch its plasmodium into a more elongate configuration when traversing empty spaces (Fig 7). This may essentially coerce the plasmodium into performing more like an individual. However, *P. polycephalum*'s branching, plasmodial structure allows it to choose many paths simultaneously, like a source population generating emigrants. If this explanation withstands testing, the lack of difference between the colonization probabilities of large and small patches may actually be further evidence that *P. polycephalum* approximates the behavior of a population.

P. polycephalum presents a wealth of opportunity for testing landscape-level hypotheses. Patch distance changes connectivity for it in accordance with dispersal theory (Wilson and MacArthur 1967 Hanski et al. 2000). The slime mold plasmodium increases its size in proportion to the available resources, and this increase in size in turn increases the distance it can extend itself (Howard 1931). The microcosm performed well when applied to predictions of patch connectivity for *C. maculata*, concurring with half of the results. However, there are some limitations to the usefulness of the system presented here. Because *P. polycephalum* is capable of foraging in multiple directions at once, it should not be used as a model of an individual forager or disperser. Additionally, this system doesn't account for differences in habitat quality. Likewise, in this system, the only barrier to resource patches is distance. It does not account for additional obstacles, such as temperature, risk of predation, or physical barriers. However, it is likely that the system could be altered to include these.

Further testing will likely improve the accuracy of predictions. For example, using microcosm landscapes with more than one of each patch type, or more than two levels of each variable, or observing something other than first colonization event, such as time to colonization, could allow more detailed analyses of variable interactions. Of particular interest is the observation of *P. polycephalum* colonization of scale models of real-world landscapes. Comparing colonization of these microcosms to the known distribution of an animal species would substantiate this system's value for testing metapopulation dispersal hypotheses.

3.6 Conclusion

I assessed *P. polycephalum*'s suitability as a microcosm of landscape-level metapopulation dynamics. I found that 4 x 4 mm squares of *P. polycephalum* plasmodium had a mean lifetime dispersal of 62.2 mm, in a landscape devoid of resources. In landscapes containing resource patches, nearer patches had a higher probability of being colonized before more distant patches. Increasing patch size did not increase the probability that a patch would be colonized before other equidistant patches. Results of a generalized linear mixed-effects model, and a Fisher's exact probability test concurred that the effects of patch distance and size did not interact. An additive fixed-effects model demonstrated that both patch distance and size affected a patch's probability of being colonized first. The somewhat weak influence of patch size may be expected, if the nearest edge of each patch lies at the same distance along *P. polycephalum* dispersal kernel. Landscape microcosm experiments concurred with half of the patch connectivity estimates for the insect *C. maculata*. Discrepancies likely were due to the lack of a strong effect of patch size on probability of being colonized first. While further testing of *P. polycephalum*'s conformity to metapopulation theory is needed, I conclude that it offers a promising microcosm system for testing patch occupancy hypotheses.

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3.8 Figures and Tables

Fig. 3.1 Rolled oat flakes colonized by *P. polycephalum* plasmodium. Note the network of veins visible between the larger patches of oats.

Fig. 3.2 The faint trail of extracellular slime deposited by *P. polycephalum* as it forages was used to help determine where plasmodia had traveled.
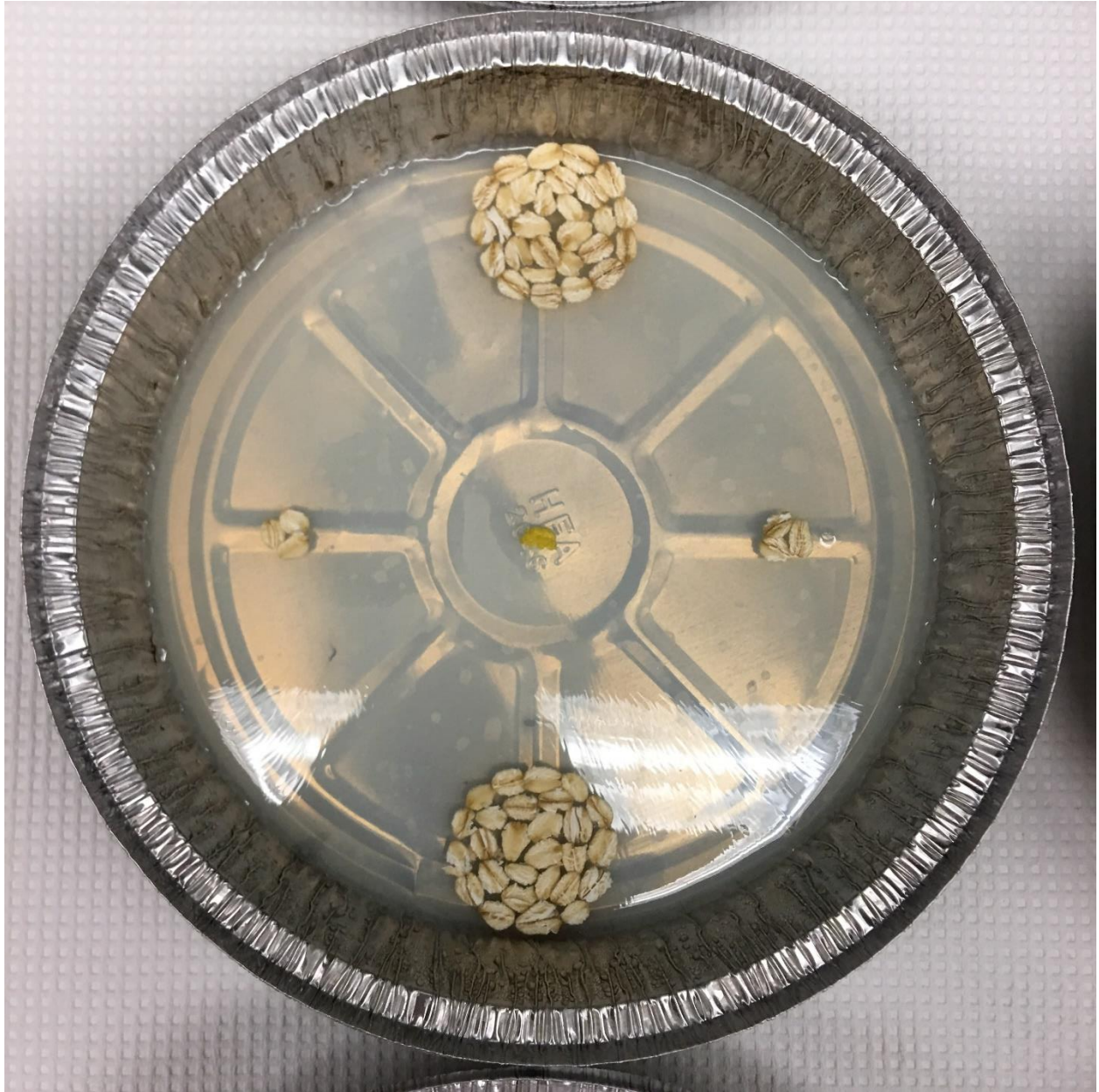
Fig. 3.3 Experimental microcosm for testing whether large patches were more likely to be colonized before small patches. Large patches measure 4 cm across, and small patches measure 1 cm across. Both large and small patches are 58 mm from the center of the plate.

Fig. 3.4 Experimental microcosm for testing whether patch size and distance have an interaction effect on which patch is more likely to be colonized first. Large patches measure 4 cm across, and small patches measure 1 cm across. Far patches are 83 mm from the center of the plate, and near patches are 31 mm from the center of the plate.
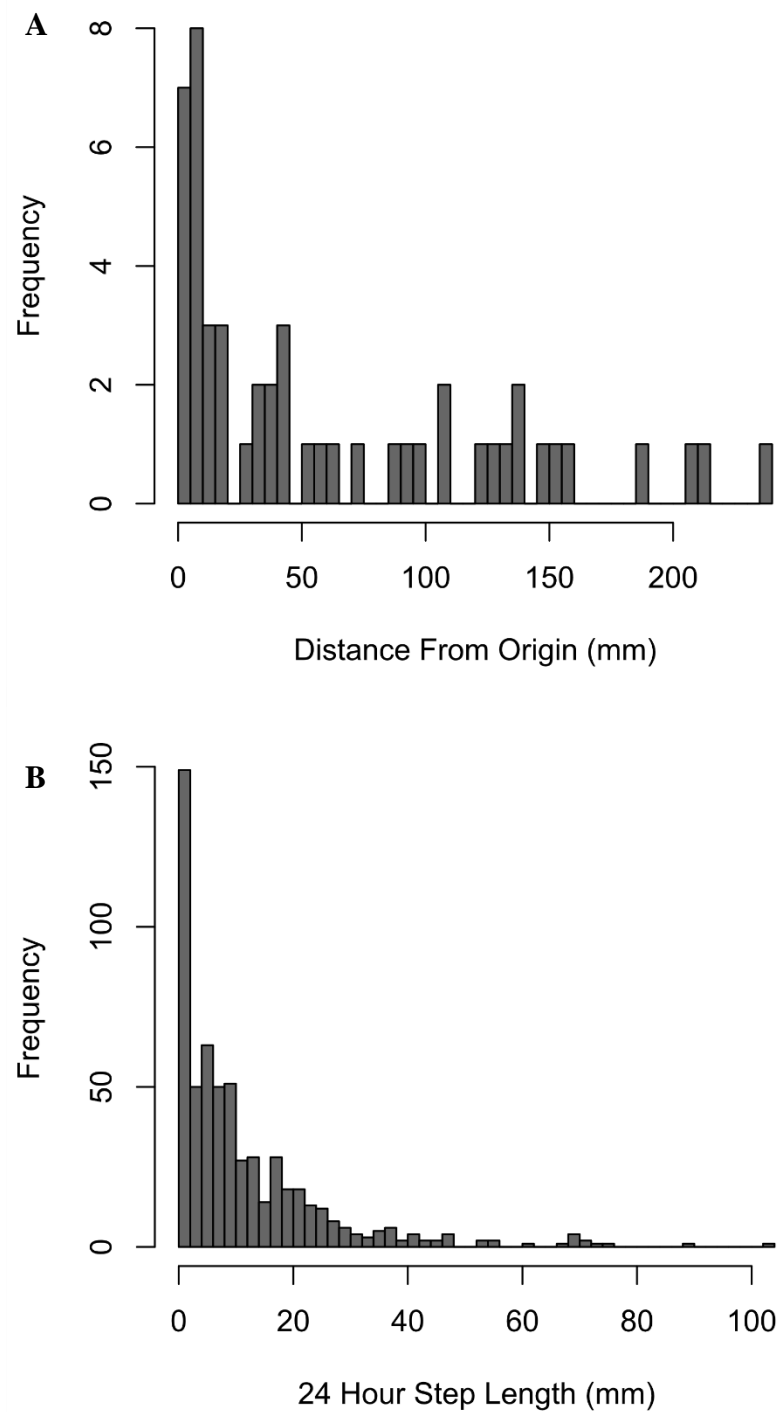
Fig. 3.5 Histograms of **A)** lifetime dispersal (distance from origin at the end of plasmodium's life cycle), and **B)** 24-hour step length (distance traveled by plasmodium in a 24 hour period).

Fig. 3.6 Some plasmodia took relatively straight approaches towards patches, suggesting that they may be responding to some external stimulus from the oats.
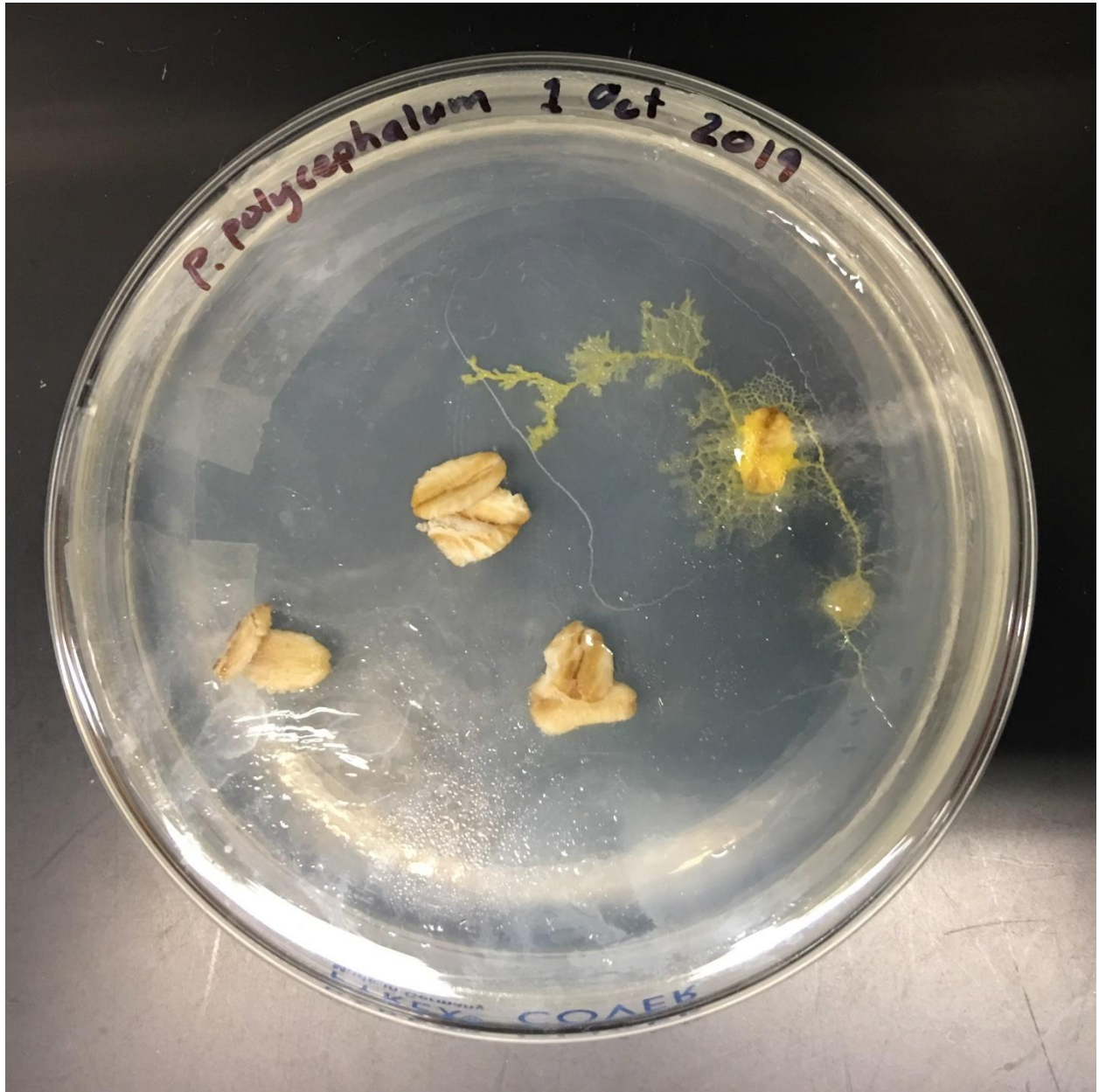
Fig. 3.7 When crossing open areas devoid of resources, plasmodia can adopt a more elongate shape.

Table 3.1 Comparison of four models to predict the dependent variable "first patch colonized" as a function of the independent variables patch size and patch distance. Plate ID was included as a grouping variable in random-intercepts models with and without an interaction term.

Model	WAIC	LOOIC
distance*size	68.532	68.695
distance + size	68.626	68.842
distance + size + random intercepts	70.964	71.173
distance*size + random intercepts	71.037	71.324

Table 3.2 Results of a binomial GLM fit in R package brms within a Bayesian framework using default, weakly informative priors, where the dependent variable was the binary variable "first patch colonized" and the independent variables were the additive effects of patch.

	First Patch Colonized		
Predictor	Odds Ratio	95% Credible Interval	Posterior probability
Intercept	12.3	3.86 – 49.90	**<0.001**
Patch Size	7.03	2.39 – 22.20	**<0.001**
Patch Distance	25.53	6.89 – 123.96	**<0.001**

Table 3.3 Connectivity indices and observed proportion that were colonized first of each patch type. Results in **bold** indicate nominal agreement between connectivity estimate and observed proportion. Probabilities significantly greater than 0.5 are marked with *

Patch Type	Connectivity	Observed proportion
Near	**0.7**	**0.8***
Far	**0.3**	**0.2***
Large	**0.94**	**0.59**
Small	**0.06**	**0.41**
Near/Large	**0.57**	**0.65***
Near/Small	0.04	0.25
Far/Large	0.38	0.1
Far/Small	**0.02**	**0***

CHAPTER 4.   A RESEARCH-BASED LESSON PLAN INTRODUCING STUDENTS TO LANDSCAPE ECOLOGY

4.1   Abstract

Grand challenges in sustainability require the efforts of many people, and numerous approaches to overcome. Landscape ecology will play an integral part in facing these challenges, but it is a difficult concept for the general public to appreciate. Improved education can improve public awareness and attitudes regarding issues of landscape ecology. Increased emphasis on STEM in K-12 education offers an excellent opportunity for scientists to educate young learners. Here I demonstrate an approach to creating a lesson plan for teaching the concept of landscape connectivity to high school students. I emphasize the use of evidence-based, active learning techniques to improve student outcomes, and state-mandated education standards to facilitate educators' ability to incorporate it into their coursework.

Keywords

Active learning; landscape ecology; connectivity, sustainability; education; ecology; biology; lesson plan; educational game

4.2   Introduction

In 2019, the National Academies of Science Engineering and Medicine (NASEM) released a report in which listed the organization's grand challenges in environmental engineering for the 21st century (Board 2019). Among the list of challenges were: 1) Sustainably supply food, water, and energy, 2) create efficient, healthy, and resilient cities, and 3) foster informed decisions and actions. These challenges are incredibly far-reaching in their scope, and will require the efforts of many different people and approaches. One approach to meeting these challenges is the application of landscape ecology. Where conventional ecology broadly examines the interactions between organisms and their more immediate, local surroundings, landscape ecology considers the interactions with the environment at larger spatial scales, with an emphasis on the heterogeneity of environmental patterns across the study area (Turner 1989). Human activities, such as forestry, agriculture, and urban development have major impacts on the world beyond their immediate boundaries (Fahrig 2003, Fohrer et al. 2005). These effects are not always intuitive, and can be

quite challenging to examine. Landscape ecologists often apply spatial analysis, mathematical techniques that analyze objects' locations in space relative to other objects, to understand how features of the landscape interact (Turner 2005, Collinge 2009).

One major element in the study of landscape ecology is connectivity (Taylor et al. 1993). Connectivity describes the potential for a species to travel from one point in a landscape to another (Merriam 1984). The dispersal of an organism is not solely dependent upon the organism's physiological traits. Different types of landscape features can increase or decrease an organism's resistance to passing through an area (Tischendorf and Fahrig 2000). Care must be taken to avoid confounding connectivity with habitat quality. A forest-dwelling animal may travel at a much faster rate if released in an open area than it would in its preferred habitat (Keeley et al. 2017). However, the forest-dwelling animal may also be less likely to enter the open area. While landscape ecology and connectivity are vital to the development of sustainable resource production, the complexities of construction of healthy cities and informed land management decisions can make them challenging subjects to communicate.

To meet the grand challenge of sustainable development, researchers must find ways to overcome these communication difficulties. Introducing people to topics at younger ages has been demonstrated to improve not only understanding, but attitudes towards the topcis (Lopatto 2007, Wijesinghe et al. 2016, Schmitz and Da Rocha 2018). Fortunately, the demand for STEM-educated people remains high, providing inroads for researchers eager to share their knowledge (NRC 1989, Sanders 2008). While outreach and extension events offer excellent pathways for communicating research, there is a unique opportunity presented by designing lesson plans for K-12 education (Goldner et al. 2020). K-12 educators routinely cite lack of expertise with current science as a key reason they don't incorporate it into their lessons (Kim and Fortner 2007, Kelley et al. 2020). Researchers can assist with this barrier by creating lesson plans for teaching students about their area of expertise. However, if researchers are willing to invest the time and effort required to create such lesson plans, they should focus on using using teaching techniques that enhance learning outcomes beyond those of traditional lecture formats. Here I describe the development of a lesson plan for teaching the concept of connectivity to high school students. This lesson plan targets Indiana Academic Standards (IAS), using teaching techniques from the active learning paradigm (Appendix B).

Educational Framework

Active learning is broadly defined as a collection of techniques that focus heavily on students' experiential participation in the learning process. More specifically, higher-order activities such as synthesis and evaluation should be used to engage students in active learning (Bonwell and Eison 1991). It is based on a philosophy of constructivism, which emphasizes using preexisting knowledge to build understanding of new concepts (Phillips 1995). Many organizations and agencies, from UNESCO to the President's Council of Advisors on Science and Technology, have called for increased active learning in formal education settings (Lima et al. 2017, Olson and Riordan 2012). These calls are based on the large body of evidence demonstrating the effectiveness of active learning techniques in STEM settings (Michael 2006, Abraham et al. 2012, Freeman et al. 2014, Hartikainen et al. 2019). In a meta-analysis of 225 studies, Freeman et al. found that students under traditional instruction were 50% more likely to fail than those under active learning, and that mean examination scores were 6% higher under active learning (2014).

While there is compelling evidence that suggests use of active learning improves outcomes, it is not without its limitations. Perhaps foremost, students can harbor biases against active learning techniques, believing that they learn better under traditional instruction, despite actual learning outcomes (Deslauriers et al. 2019). This can lead to poor reception of the lesson, and a perception that the instruction was less valuable to them. Perceptions of poorer learning may be attributable to greater cognitive effort required by active learning. While active learning should require significant cognitive effort, students should be properly prepared for problems and exercises. Since active learning is based on based on building upon known information, improper presentation can leave students feeling frustrated (de Novias et al. 2017). In the face of these challenges, it becomes increasingly important for the instructor to keep students on task, helping them work through difficulties (de Novias et al. 2017).

To mitigate these limitations, instructors must put considerable care into the preparations for the lesson. To avoid distractions, and delays, instructors should practice with materials themselves. By ensuring that they know how to operate equipment, use materials, or display media, instructors can make efficient use of class time, and demonstrate tasks clearly. They will also be able to provide enough background information to allow students to begin constructing new knowledge, without reverting to a traditional lecture. Furthermore, instructors that are familiarized with the lesson will be able to clearly define the goals and expected outcomes of each exercise,

and better prepared to guide students through difficult concepts, and reassure them that any difficulties they are experiencing are normal, and part of the exercise.

Ecology Background

Habitat loss is widely considered one of the dominant threats to biodiversity, and general environmental health (Fahrig 2003). Human activities now alter much of the earth's land area, driving changes in local environments such as reduced water availability, increased spread of disease, loss of biodiversity, and reduced habitat connectivity (Foley 2005, Fahrig et al. 2019, Richardson and Whittaker 2010). Reductions in connectivity can damage numerous ecological interactions that help maintain life as I understand it. If large patches of habitat become so divided that apex predators such as wolves or big cats can no longer travel between them, populations of herbivores can rapidly increase, leading to overgrazing, and malnutrition (Prugh et al. 2009). If migrators can no longer access their overwintering grounds, populations can experience collapses and genetic bottlenecks (Hanski 1998). In some cases, increased connectivity can also create issues. When two European lakes were connected through a channel, the downstream lake saw an increase in cyanobacteria that can potentially result in toxic blooms (Katsiapi 2020).

In the United States, thousands of dams and culverts prevent fish from reaching vital spawning areas, isolate populations from one another, and reduce genetic diversity (Martin 2019). Some initiatives are pushing for the removal or modification of these dams to mitigate these risks, but the expense and specialized labor required limit how many can be targeted. In New York and New Jersey, roadways and other development threaten to divide interrelated populations of tiger salamanders from breeding ponds (Titus et al. 2014). In this case, while a roadway may not directly harm salamander residence habitats, or their breeding ponds, the barrier to travel it creates may ultimately result in the decline of the populations. In Maine, suitable grey wolf habitat exists within the dispersal range of a population in Quebec, Canada. However, no breeding population has been established in the century following their extirpation from the area. It is hypothesized that the St. Lawrence River, a large swathe of agricultural land, and the continued harvest of wolves in Quebec have reduced the connectivity such that the unoccupied habitat is out of their reach (Wydeven et al. 1998).

4.3   Evidence-Based Curriculum Development

To use class time efficiently, researchers developing lesson plans should employ education techniques that have been demonstrated to improve understanding and retention. At least 14 education techniques associated with active learning have been demonstrated to improve performance (Olson and Riordan 2012). In this lesson plan, I utilize a combination of case studies, think-pair-share exercises, collaborative learning groups, an educational game, and problem-based learning to provide students with a variety of ways to experience the material.

Active learning is based on a constructivist philosophy, and therefore requires preexisting knowledge upon which to build. Since the concept of connectivity is likely new to most students, the instructor must provide them with an introduction to serve as a foundation. While it may be tempting and commonplace to simply provide an introductory lecture, it is not the only possibility. I employ a series of case studies to engage students in critical thinking, and the exploration of cause and effect, while also providing them with new information (Appendix C). Although the students remain reliant on the instructor at first, case studies provide an opportunity for them to contribute to the way the material is presented (Holley 2017). Students are instructed to develop explanations for the phenomena presented in the case study. Before presenting students with the explanation, they are asked to describe what information they think they would need in order to determine the explanation. In this lesson plan, the initial cases will have relatively simple explanations, and subsequent cases will become more complex.

Think-pair-share exercises make up a small, but crucial component of this lesson plan. In think-pair-share exercises, student pairs, or small groups, discuss interpretations and concepts before presenting their thoughts with the class (Prahl 2017). This exercise will take place at the beginning of each lesson, starting on day two. This gives students an opportunity to transition back to the subject, after being engaged in other classes. It also allows for class participation. Perhaps most importantly, it provides the instructor an opportunity to discover and correct misconceptions about the previous day's material. This and subsequent techniques all include the use of collaborative learning groups as an added active learning technique. Working in groups provides students with additional opportunities to discuss material, and compare their interpretations to others' (Armstrong et al. 2007).

Educational games are widely acknowledged as tools for triggering active learning (Sharp 2012). Young people in particular learn largely through play and games (Whitebread 2009). Games

used in the classroom range from simple spelling games, such as crossword puzzles, to custom-made video games, and provide novel ways of interacting with course materials (Sharp 2012). While traditional games such as chess, or Monopoly may have limited application, instructors and researchers have developed custom games, tailored to teach specialized topics (Annetta 2008, Hoy 2018). I designed a custom board game, Humans & Habitats, to teach high school students concepts of landscape connectivity, and illustrate the tradeoffs inherent in landscape management (Appendix D-E). The game is mechanically based on the commercially available Takenoko, where players take on the roles of gardeners and pandas, cultivating and collecting bamboo (Bauza 2011, Bombyx, Brittany, France). I modified the game to represent the conflicting needs of humans and nature.

In Humans & Habitats, players take on the role of a city planner, and place tiles representing forests, agricultural land, and urban areas to meet conflicting management goals, found on cards. Players must choose which arrangements of tiles can accomplish the most goals. Additionally, they can choose to either produce or consume resources, gaining additional points. The game concludes one round after the first player completes a predetermined number of objectives, based on the number of players.

This game was designed with the limitations of a public high school class in mind. The rules are streamlined, and occupy a single 8.5 x 11 inch sheet of paper (Times New Roman 12 pt font). Additionally, the end state of the game scales with the number of players, ensuring that a game can be completed within a single class period. Since public school teachers have limited extra time and funding, the pieces can be printed on paper, and cut out by students. This also allows them to be stored in envelopes, so they don't occupy a large amount of storage space. Most importantly, the game itself addresses state science education standards, so it can be used separate from the lesson plan, if instructors desire a shorter introduction to landscape ecology and connectivity.

Following the deployment of Humans & Habitats, the lesson plan culminates in a problem-based learning activity. When engaged in problem-based learning, students are presented with problems, or phenomena, and are asked to explain what's happening (Hutchinson 2002). This is rapidly becoming a favored active learning technique, as it incorporates thinking critically, applying knowledge to novel situations, group discussions, and often, real-world scenarios (Capon and Kuhn 2004, Kelley and Kellam 2009, de Novias et al. 2017). This method should be

particularly interesting to researchers designing lesson plans, as it offers an opportunity to incorporate scientific inquiry. When faced with a problem, students are asked to develop a solution for it. Then, they are asked how they will distinguish a success of their solution, or a failure. This is, in essence, hypothesis testing, a vital skill required in STEM fields. In this lesson plan, collaborative groups of students are tasked with developing hypotheses about connectivity. They will then develop experiments to test these hypotheses using the slime mold *Physarum polycephalum*.

Physarum polycephalum is a protist, like an amoeba, that is found in damp forest undergrowth throughout the world. It displays unique foraging activities, extending tendrils in multiple direction, and slowly pulsing across surfaces in search of bacteria and other food sources. In laboratory settings, it has been used to solve mazes and model roads and railways (Nakagaki et al. 2000, Shirakawa et al. 2009). These features make it an interesting model for traversal over a landscape. Other features make it an excellent organism to work with in a high school biology classroom. Its bright yellow color, and relatively large size make it easily visible without the use of magnification lenses. Additionally, it is readily available from science education suppliers for less than $20. It is readily cultured on plain agar gel, available from grocery stores, or any clean, damp surface, and can be fed with uncooked, rolled oats.

4.4   Practice: The Lesson Plan

This lesson plan consists of three to five 45 minute classroom periods. It is structured for a U.S. high school biology class, but is readily applied to environmental or earth science courses (Appendix B). It addresses IAS science standards for biology, earth science, and environmental science. The lesson plan and materials will be available for download, free of charge, on the Purdue education store (https://mdc.itap.purdue.edu/).

The initial lesson consists of an introductory exercise that provides students with foundational knowledge on which they will construct deeper understandings of landscape ecology and connectivity (Appendix C). The lesson begins with a brief explanation on why connectivity is being taught to the class. This is accompanied by an outline of the desired learning outcomes, and a list of the goals for this lesson.

The instructor will communicate the theory and concepts of connectivity by discussing case studies with the students. The initial case study describes a situation in which human activity

altered connectivity, producing a relatively intuitive result. Before revealing the result, the instructor will pause and instruct students to list the benefits gained from the human activity, and to imagine potential costs. The class will discuss the benefits and costs. Following this discussion, the instructor will reveal the result of the human activity. At this point, students will be asked to offer explanations of the mechanism behind the result. During this discussion, the instructor will remind the students that they are correct in feeling that they don't know the explanation, explain that the goal is for them to think about many different possible causes and effects. Following the discussion, the instructor will describe the mechanism that produced the environmental change. The class will be asked whether they feel the benefits were worth the cost, and to explain why.

Following the initial case study, the instructor will lead the students in more exercises of the same format, addressing progressively more complex case studies. In these subsequent discussions, if students are unable to determine the correct outcome, the instructor will ask the students to list any additional information they feel they would need to determine it. If students are able to determine the outcome, the instructor will ask them to list any knowledge, experiences, or information that helped them reach their conclusion. Each case study discussion will take approximately 10 minutes to complete, depending largely on how forthcoming students are with their replies. The prompts in this lesson are designed to draw on students' personal values and experiences, so that they feel more confident in their responses.

The second lesson begins with a think-pair-share exercise. Students will be instructed to discuss what new things they learned from the previous lesson, in pairs or as a group, for approximately one minute. After giving them time to organize their thoughts, the instructor will ask several students to list one new thing they learned from the previous lesson. The rest of the class will be asked if they concur with these items, and if they have any to add. This provides the instructor an opportunity to identify and address potential misconceptions of the prior material that the students may be developing. The instructor will then restate the topic, and highlight the students learning outcomes on the list of intended outcomes for the lesson, in order to reinforce the objectives, and reassure students that they are learning (Deslauriers 2019).

Next, the instructor will separate the class into groups of 2– 4 students. Students will be presented with a prompt to imagine themselves as Department of Natural Resources officials, charged with managing publicly-owned land, being approached by three groups with dramatically different interests in land use (Appendix D). Groups will be instructed to discuss how they will

resolve the issue, before reporting their decisions to the class. In addition to their final decisions, groups must list what was gained from the decision, and what was sacrificed. They must also explain whether the decision was unanimous, or divisive, and what personal values or experiences influenced the decision. Students should spend no more than 15 minutes on this exercise.

Following the discussion prompt, groups will resume their roles as land managers, by playing the Humans & Habitat board game. Each group should have their own set of pieces and cards. This game is designed to be completed in 30– 45 minutes. Since this is the first time most students will have played, it is unlikely that all groups will have completed their playthroughs before the class period ends. The instructor can determine how much more class time they will spend on this game, but at least one full playthrough is recommended.

The third lesson begins with another think-pair-share exercise (Appendix F). Students are once again instructed to discuss and share new things they learned from the previous lessons. As before, the instructor will monitor these responses for misconceptions, and highlight responses on the list of intended learning outcomes. Next, the instructor will return the students to their groups of 2-4, and present the class with two maps, depicting an animal in its habitat. One map depicts the landscape, and animal's movement, before human activity, and one depicts the landscape following human activity. The instructor will ask the class how they would answer the question, "do the new gaps in the animal's habitat change the way the animal travels across the landscape?" Groups will be tasked with brainstorming ideas to answer this question. Once each group has developed an idea, have groups share their ideas with the class. Groups will then be asked to brainstorm how to answer the question at a small scale (e.g., how could they answer the question without extensive fieldwork?), and discuss their ideas.

The instructor will then introduce the students to the slime mold *P. polycephalum*. After describing the nature of the slime mold, and illustrating its activities with videos, the instructor will task groups with developing practicable ways to use *P. polycephalum* to answer the question, "do the new gaps in the animal's habitat change the way the animal travels across the landscape?" Once all groups have developed ideas, each will present them to the class. The instructor will then provide each group with the materials listed in the lesson plan (baking pan, acetate sheets, and oatmeal), and task each group with developing at least two other questions about connectivity they could answer using the slime mold and a way to answer each question using the provided materials. In addition to developing two questions, groups should make predictions about the answer to the

question, and describe how they will recognize if the answer is correct, or incorrect. Students will then use the remaining class time preparing and setting up a dry run of their chosen test.

The fourth lesson begins with the usual think-pair-share exercise, and restatement of the learning objectives. The instructor returns the students to their collaborative groups, where they will review their experiment once more. After reviewing, they set up the experiment with the instructor's assistance. Once the experiments are set up, students will make daily observations, according to their plans. As groups reach conclusions based on their results, they will make short, five-minute presentations of their questions, designs, and conclusions.

4.5 Discussion

Problems arising from altered landscape connectivity present major challenges for continued development of sustainable agriculture, resource production, and housing. Organizations such as NASEM identify them as some of the most important obstacles to come in the effort to create a sustainable future. Researchers can contribute to these efforts by developing lesson plans that leverage their expertise to improve perceptions of important science. These lesson plans must seriously consider teacher requirements, student engagement, and effective educational techniques.

Here, I present one approach to creating a lesson plan that meets these conditions. This lesson illustrates the importance of landscape connectivity, and the environmental tradeoffs humans make when acquiring resources for ourselves. It incorporates evidence-based educational techniques to create an active learning approach that engages students on a personal level. Crucially, the lesson targets IAS science standards. While these standards are required in Indiana, they are similar to the Next Generation Science Standards, adopted by most other states (NGSS Lead States 2013). Additionally, it requires relatively few material resources, and can be provided to a class of 24 (mean Indiana high school class size), for about $2.50 per student. These features make it accessible to teachers who have extremely limited time and funding.

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CHAPTER 5.   GENERAL CONCLUSION

The study of landscape structure and configuration is firmly established as integral to the continued advancement of ecology. The configuration of resource patches can have far-reaching implications for biodiversity, metapopulation dynamics, community structure, and habitat quality. Human activities, such as forestry, agriculture, and residential construction alter patch configuration by breaking larger patches into smaller fragments. This frequently results in pronounced, unforeseen consequences for species. The fragmentation and shrinking of habitat patches can lead to changes in the environmental conditions within the remaining patches (e.g., degradation), prompting responses from local populations. These responses can, in turn, cause changes to the metapopulation structure on large spatial scale.

As shown in chapter two, fragmentation, represented by the edge-length-to-area ratio, edge density, was associated with differences in flight morphology between insects at different sites. This was detectable using empirical dispersal data to determine how much of the overall landscape was relevant to this species. Increased fragmentation resulted in increased investment in flight morphology, suggesting increased movement in fragmented landscapes.

The spatial scale of variation in flight investment was relatively small, indicating that species adapt at highly localized scales, well within their maximum dispersal capacity. Here, I used wing length and edge density. I do not presume to claim that edge density alone is the cause of variation in wing length. Future analyses incorporating empirical data on variables known to influence insect body size, such as food quality, and temperature, should be used to understand the factors that result in realized wing length. Likewise, I do not propose that these findings demonstrate that wing length is adaptive. Rather, I hypothesize that it is, based on the detection of the relationship at sites my sites, ranging from 400 m to 360 km apart, and by other researchers in Canada, over 1000 km away.

The primary concern is that the observed relationship could potentially be the result of phenotypic plasticity, rather than selective pressure. This could be addressed by collecting nymphs from sites with different edge densities, and rearing them to adulthood in a lab setting, before measuring their wings. If wing lengths still follow the same relationship, it would suggest that phenotypic plasticity is not likely the cause.

While chapter two explores the spatial scale of the effects of patch structure on the dispersal morphology of a species, chapter three addresses the impacts of landscape configuration on metapopulation dispersal. Hypotheses of metapopulation dispersal can be challenging to test, considering the large spatial scales of many natural landscapes. Microcosm experiments offer methods for approaching these questions more economically.

The slime mold, *Physarum polycephalum* is already a popular organism for modelling networks and foraging. It also exhibits excellent potential as a microcosm for testing metapopulation dispersal hypotheses. In small-scale experiments, it meets patch connectivity predictions based on patch distance. It may also meet predictions based on patch size, and the interaction between distance and size, but further investigation is required. Placing *P. polycephalum* in larger microcosms, with more combinations of patch size and distance will clarify the effect, if any, of patch size on colonization. Future experiments should compare known distributions of species in patchy landscapes to *P. polycephalum* colonization of oat patches placed in scaled-down, but corresponding, arrangements.

Chapter two demonstrates one impact of landscape configuration on populations of an insect, that has potential ramifications for the species. Understanding the implications of altered landscape configuration is vital to mitigating the impacts of human activities on the natural world. Doing so is necessary for improving the sustainability of humanity's future. Therefore, more people should be educated on the concepts of landscape ecology, such as connectivity. Researchers with expertise in these fields can improve awareness and appreciation of these concepts' importance by providing K-12 educators with lesson plans that teach these concepts.

Efficient use of class time requires the adoption of evidence-based techniques that improve knowledge retention and comprehension, when compared to traditional lecture-based formats. Crucially, these lessons must address state-mandated education standards appropriate for the intended grade level. Doing so increases the utility of such lesson plans to educators, and gives them increased opportunity to incorporate them into their coursework. The lesson plan I developed here targets Indiana Academic Standards for biology, earth science, and environmental science. It is intended to introduce high school students to the relatively advanced ecological concept of landscape connectivity. It incorporates evidence-based, active learning techniques, intended to draw on students' personal experiences, and improve their comprehension of landscape connectivity. It will be made available for free download by any educator.

The results of this landscape ecology study may help improve the determination of relevant landscape sizes for species of interest. This study could aid in determining the spatial scale of local adaptations across large areas, or small areas with intensive sampling. I may also provide researchers with a way to test metapopulation-level dispersal hypotheses that are not feasible with current resources. The *P. polycephalum* microcosm system may be particularly useful for testing hypotheses about insects, which are too small to carry transmitters, and experience high mortality rates, making recapture difficult. The microcosm system may also be useful in exploring hypotheses that would ordinarily require dramatic reorganization of resource patches. The lesson plan presented may improve public knowledge of the intricacies of landscape connectivity, and enhance public consideration of connectivity when making land management decisions.

APPENDIX A. *CALOPTERYX MACULATA* DISPERSAL DATA
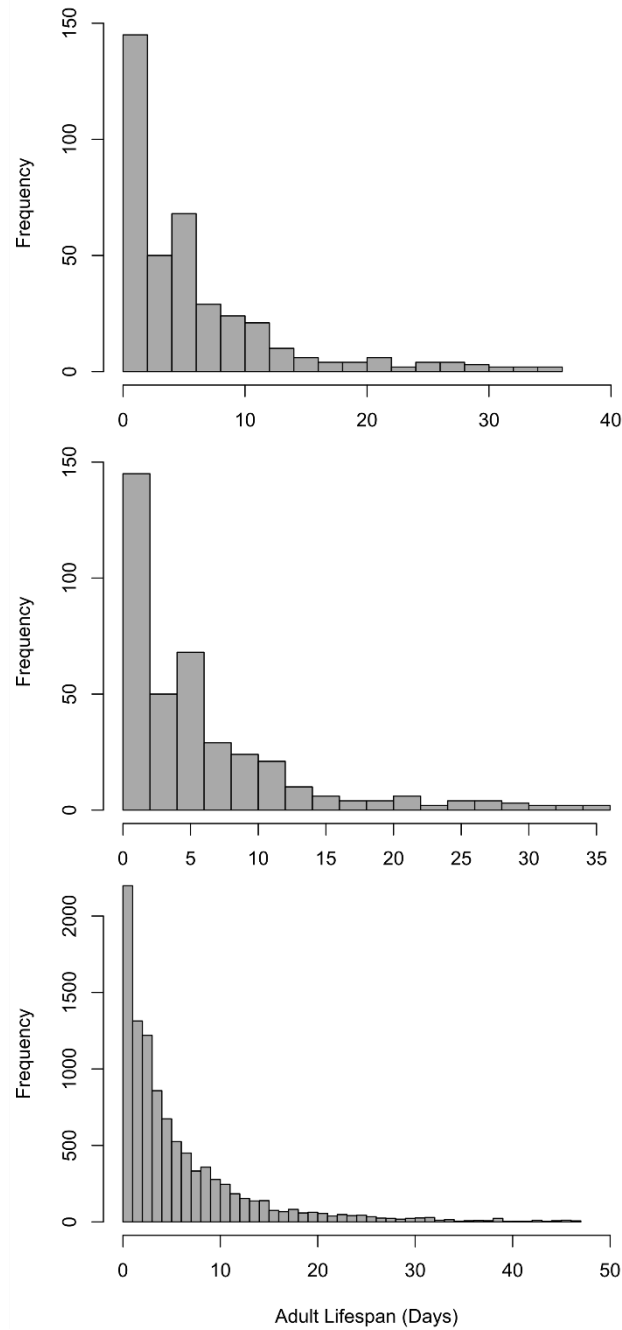
Fig. A.1 Histograms of *C. maculata* lifespans. A) Adult *C. maculata* longevity observations from a mark-recapture study (Waage 1972), B) Histogram of 10,000 random samples from the longevity observations, C) Histogram of 10,000 *C. maculata* adult lifespans randomly selected from random sample of longevity observations. This distribution was added to teneral period lengths to create a distribution of total adult lifespans, from which the correlated random walks simulation drew the number of steps for each simulated individual.
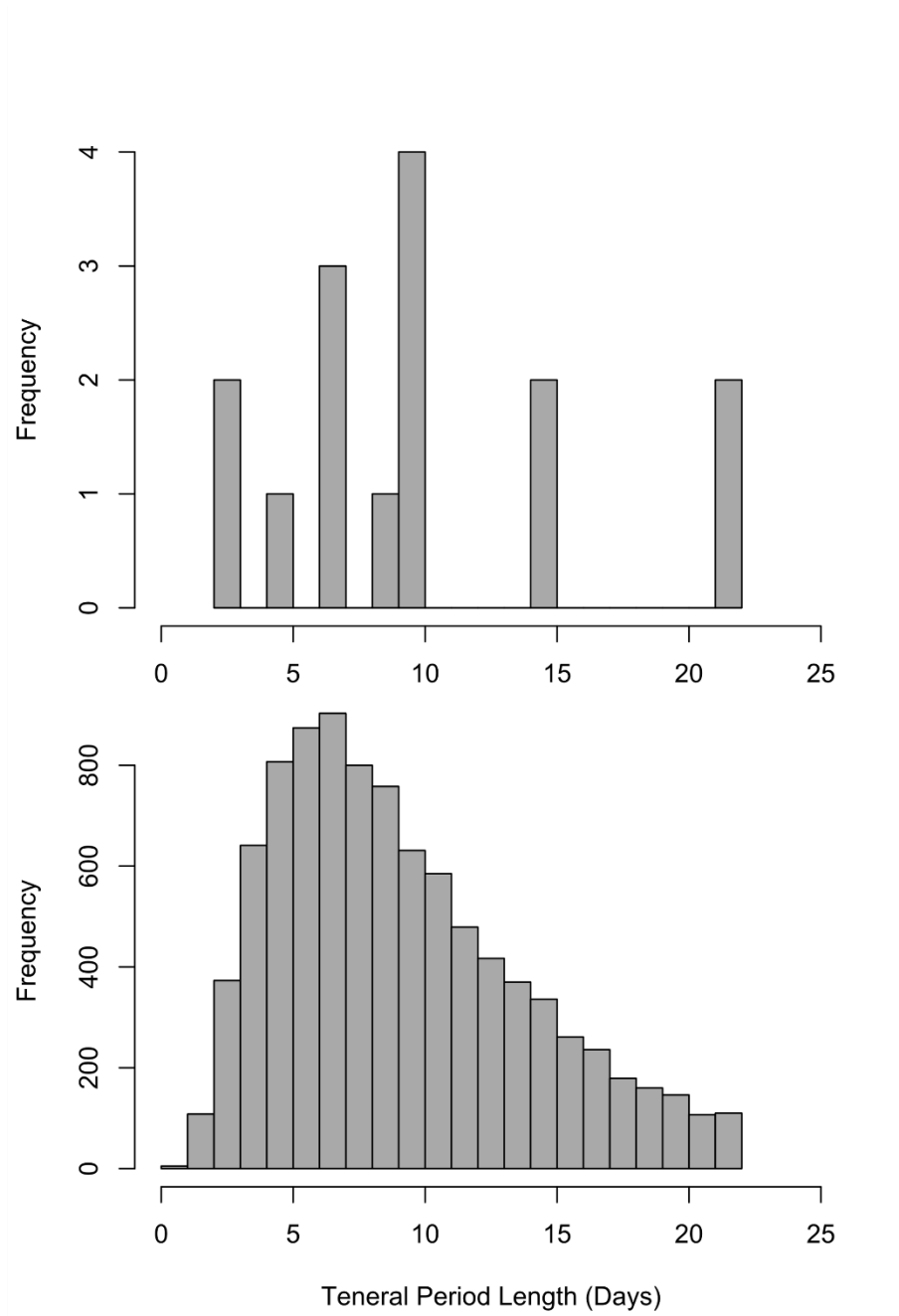
**Fig A.2** Histograms of A) Observed teneral period lengths (Waage 1972), and B) a random sample of 10,000 teneral period lengths drawn from the observations. These were added to adult lifespans to create a distribution of total adult lifespans, from which the correlated random walks simulation drew the number of steps for each simulated individual.
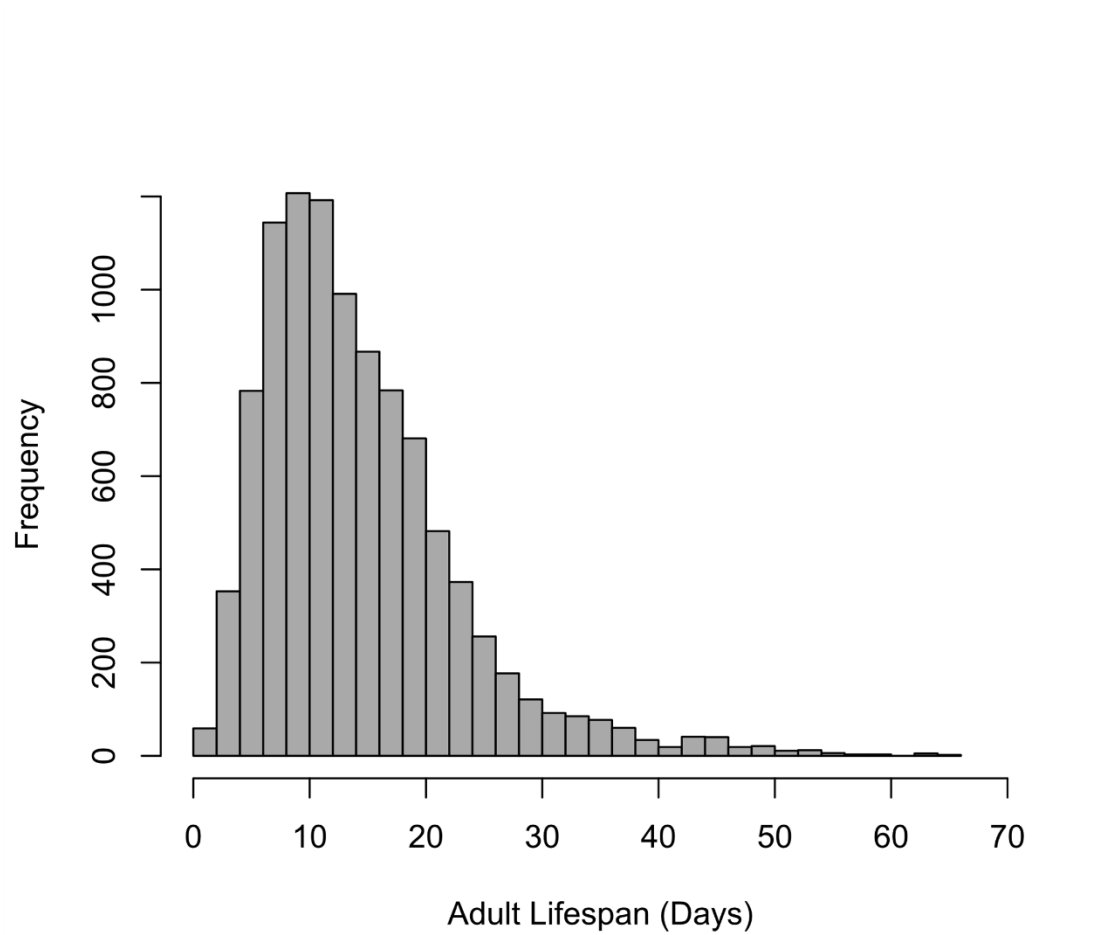
Fig A.3 Histogram of total adult lifespans, including teneral periods, from which the correlated random walks simulation drew the number of steps for each simulated individual.
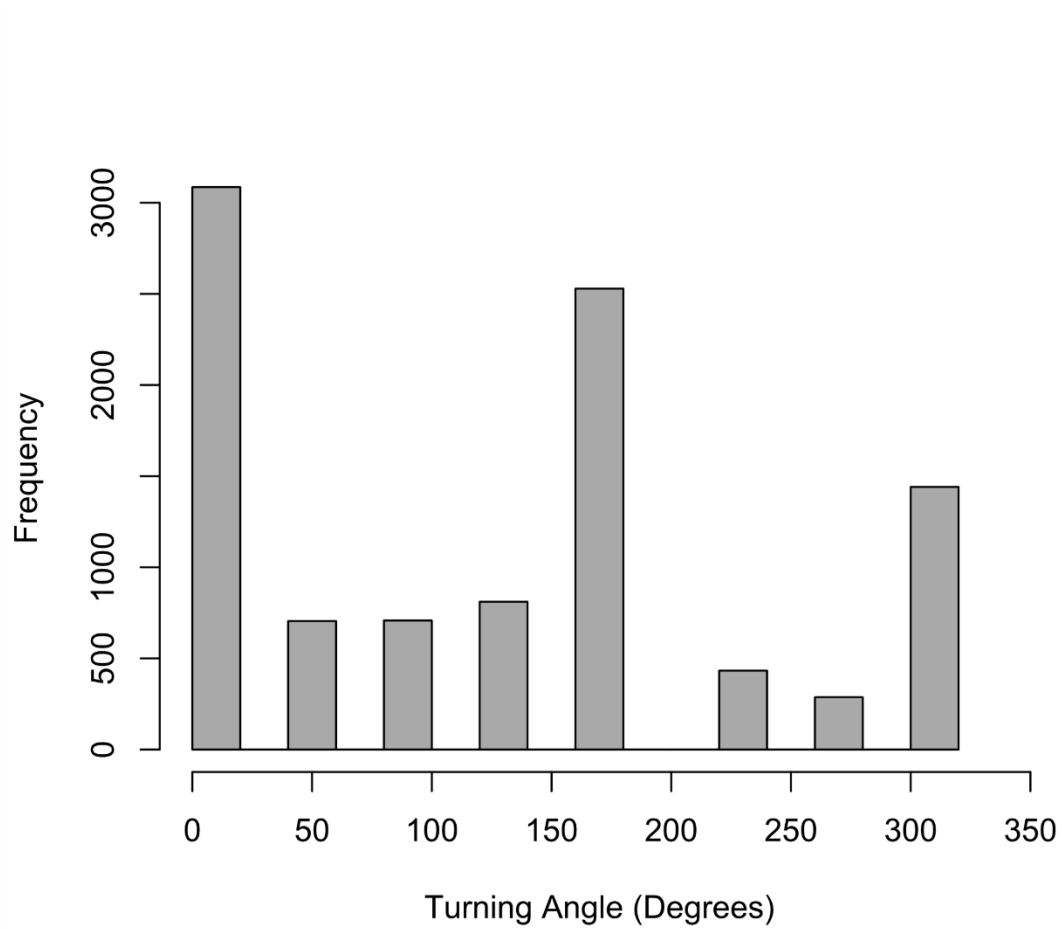
Fig A.4 Histogram of turning angle frequency observations, made my Jonsen and Taylor (2000a), from which the correlated random walks simulation drew the daily direction for each simulated individual.
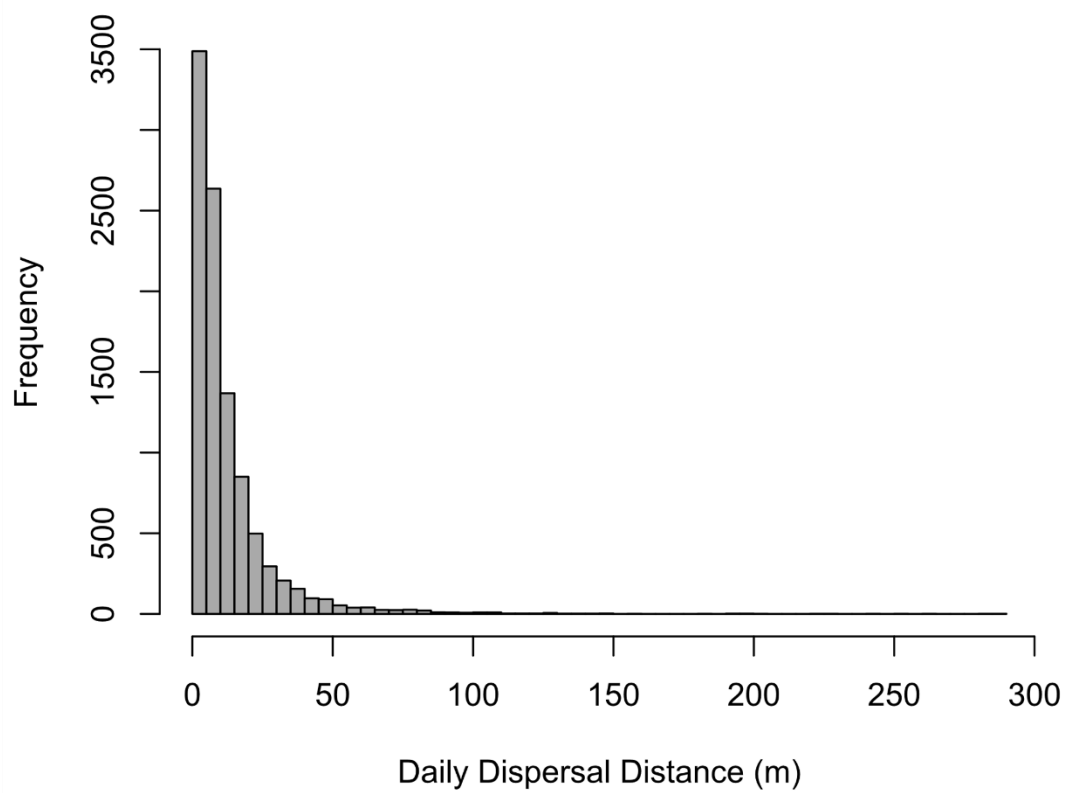
Fig A.5 Histogram of distances from which the correlated random walks simulation drew the length of each daily step for each simulated individual.
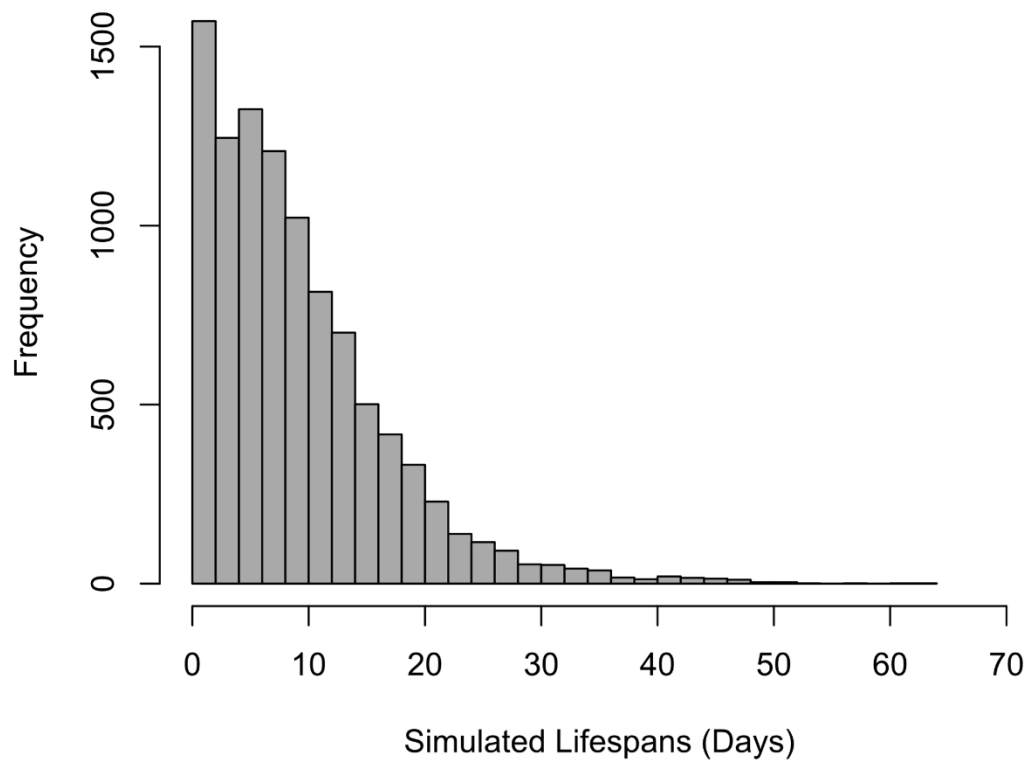
Fig A.6 Histogram of lifespans, dictating the number of steps, for each simulated individual.
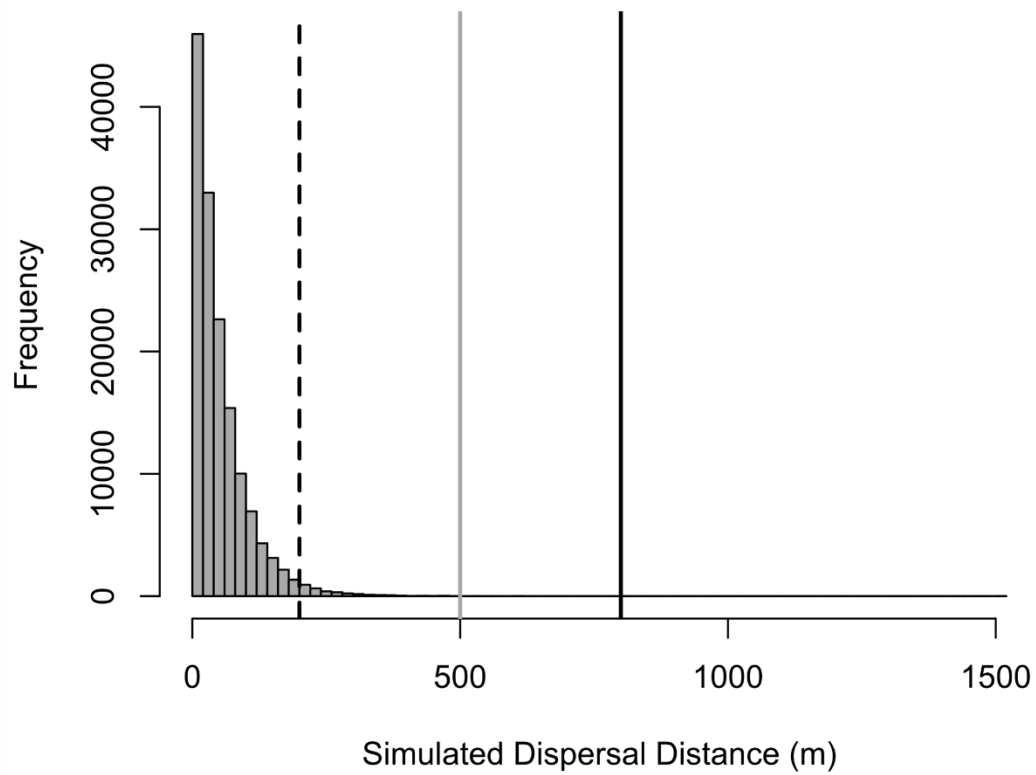
Fig 7 Histogram of simulated dispersal distances (Euclidean distance from origin) for all simulated individuals at each step. The dashed vertical line indicates 200 m, the 98th percentile distance. The grey vertical line indicates 500 m, the distance used by Jonsen and Taylor (2000b). The solid black vertical line indicates 800 m, 50% of the maximum dispersal distance.
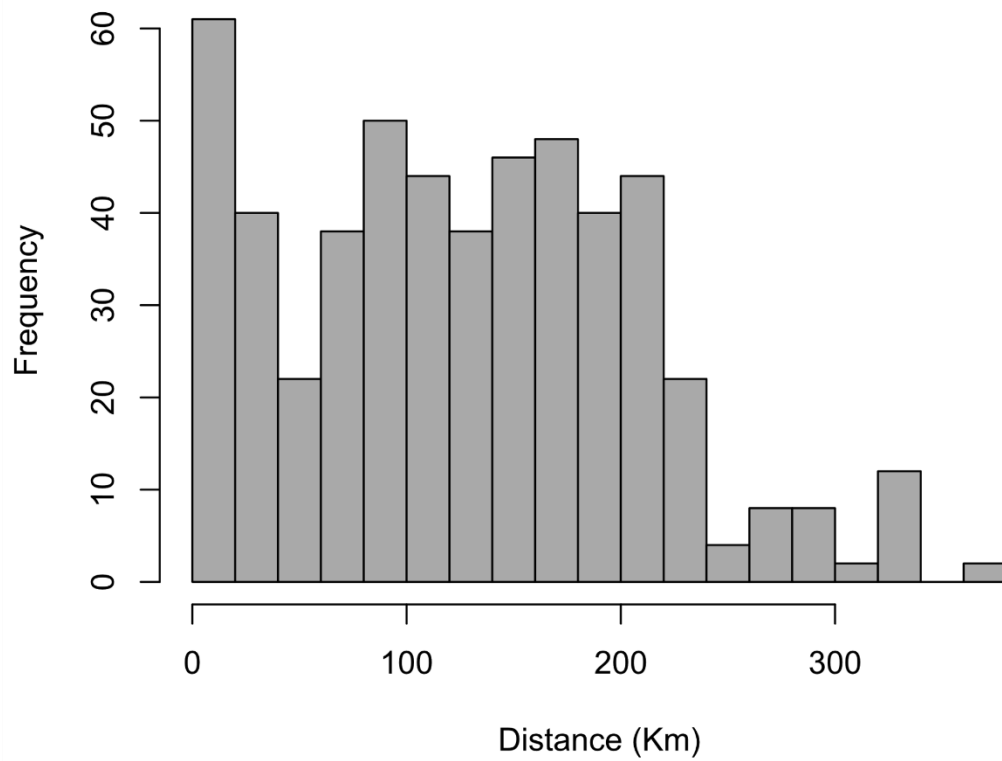
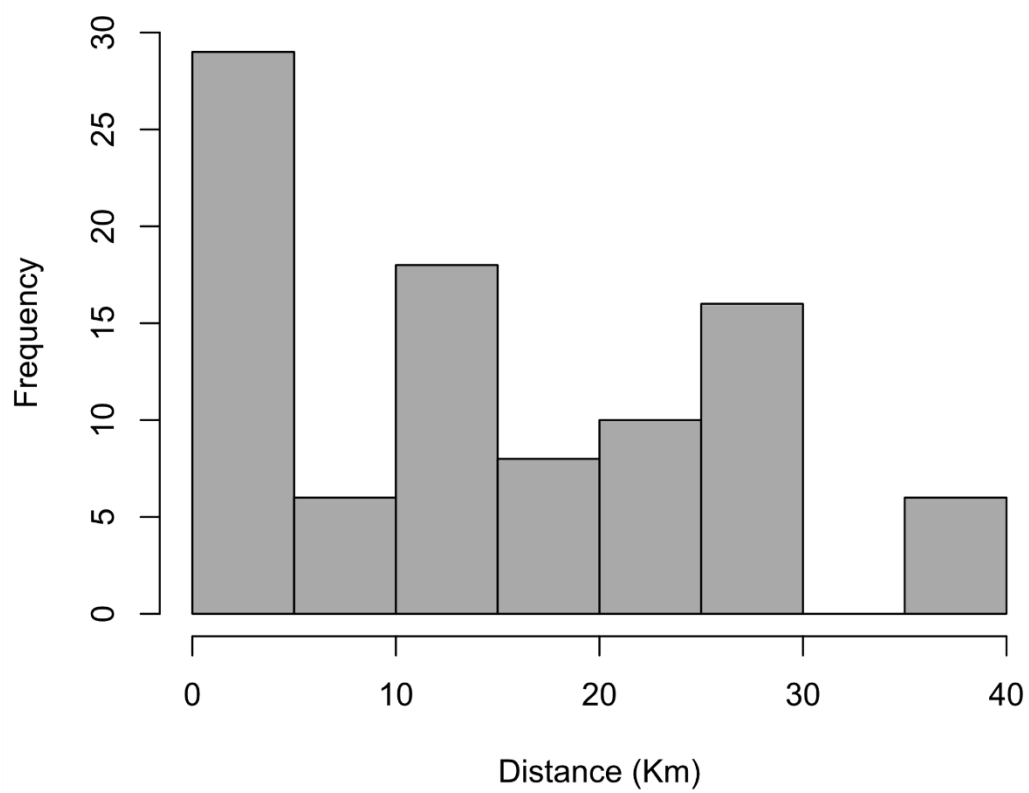**Fig A.8** Histogram of distances between each pair of field sites.

Fig A.9 Histogram of distances between each pair of field sites less than 37 Km apart (the distance autocorrelation ceased at 19 Km bin sizes).
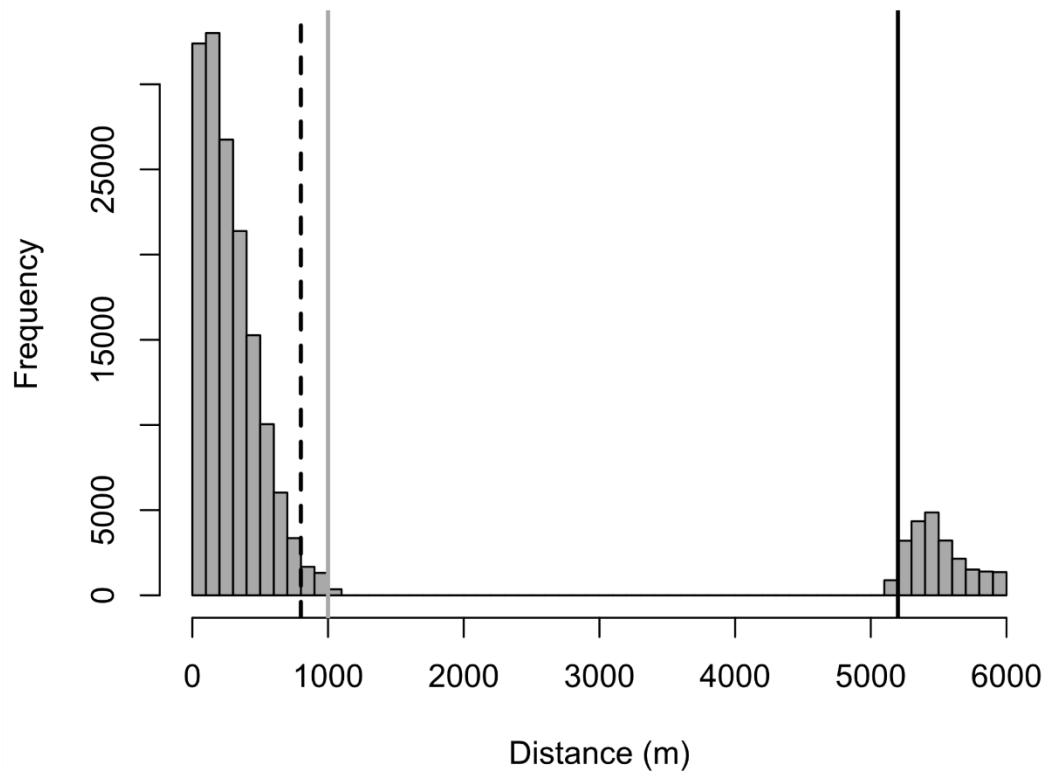
Fig A.10 Histogram of distances between individual *C. maculata* wing length measurements, less than 6000 m apart. The dashed vertical line indicates 800 m distance (distance at which spatial autocorrelation of wing lengths ceased), the grey vertical line indicates 1000 m distance, the solid black vertical line indicates 5200 m distance.
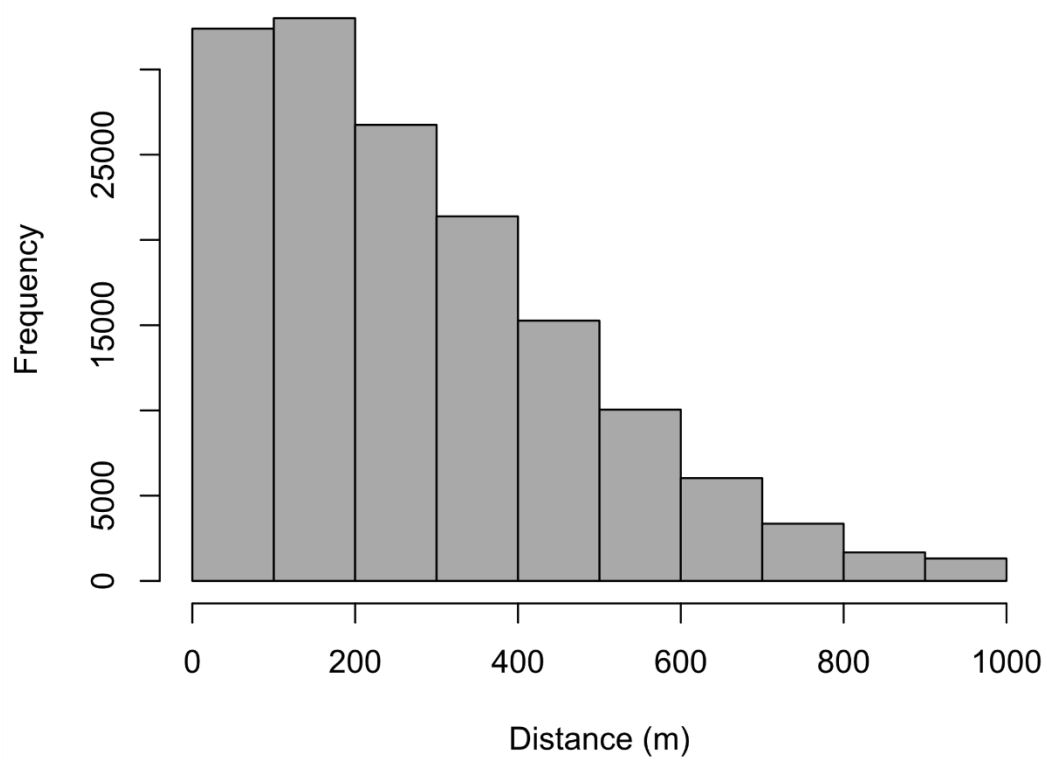
Fig A.11 Histogram of distances between individual *C. maculata* wing length measurements, less than 1000 m apart.

APPENDIX B. LANDSCAPE ECOLOGY AND CONNECTIVITY LESSON PLAN

l Landscape Ecology and Connectivity

Grade Level: 9 – 12

Total Time Required: 4 class periods (45 minutes)

Prepared by Jakob Goldner

Lesson Objectives:

1) Understand the importance of habitat connectivity to wildlife populations.

2) Understand that humans are part of Earth's ecosystems, and how human activities alter those ecosystems, deliberately, and inadvertently.

3) Explain the consequences of overuse, or increased consumption, of natural resources, and evaluate the benefits of researching, and designing sustainable resource use.

4) Design and evaluate a model that shows the effects of human activity on the movement of a species in a landscape.

Indiana Academic Standards

Earth Science 3.4 - Evaluate the use of sustainable versus nonrenewable resources. Explain the consequences of overuse and continued increased consumption of limited resources. Analyze and evaluate the benefits of researching, designing, and developing sustainable resources for private use and industry.

Biology 3.2 - Design, evaluate, and refine a model which shows how human activities and natural phenomena can change the flow of matter and energy in an ecosystem and how those changes impact the environment and biodiversity of populations in ecosystems of different scales, as well as how these human impacts can be reduced.

Environmental Science 1.2 - Understand and explain that human beings are part of Earth's ecosystems and give examples of how human activities can, deliberately or inadvertently, alter ecosystems.

Environmental Science 2.3 - Recognize and explain that the amount of life any environment can support is limited by the available energy, water, oxygen, nutrients, and minerals, and by the ability of ecosystems to recycle organic materials from the remains of dead organisms.

Environmental Science 2.11 - Recognize and describe the role of natural resources in providing the raw materials for an industrial society.

Environmental Science 4.2 - Understand that environmental policies/decisions have negative and positive impacts on people, societies, and the environment

Environmental Science 8.2 - Understand and describe the concept of integrated natural resource management and the values of managing natural resources as an ecological unit.

Environmental Science 8.4 - Describe how agricultural technology requires trade-offs between increased production and environmental harm and between efficient production and social values.

Recommended Instructor Preparation

Instructors should review material beforehand to make sure they know the principles behind the case studies, so that they can respond to questions confidently.

Lesson 1: Introductory Case Studies on Landscape Connectivity

   Lesson Focus: Introducing concept and principles of landscape connectivity

   Time required: 1 class period (45 minutes)

   Lesson Objectives:

   1) Recall and define the term "connectivity"

   2) Cite an example of the effects of reduced, or increased, connectivity

   3) List benefits and environmental costs of human activities

   Equipment and Materials:

   - Case studies slide show
   - Instructor's notes on case studies

   Lesson Procedures:

   1) Display introductory slide.

   2) Explain why this topic was chosen.

   3) Outline desired learning outcomes for entire unit.

      a. Highlight today's lesson objectives.

   4) Display and describe initial case study.

   5) Before revealing the result of the human activity, then pause.

      a. instruct students to list the benefits gained from the human activity, and to imagine potential costs.

b. Discuss the benefits and costs as a class.

6) Reveal the result of the human activity, then pause.

a. ask students will be asked to offer explanations of the mechanism behind the result.

b. During this discussion, remind the students that they are correct in feeling that they don't know the explanation.

c. explain that the goal is for them to think about possible causes and effects, rather than guess the "right" answer.

7) Following this discussion, describe what produced the environmental change.

8) Ask the class to state whether the feel the benefits were worth the cost, and to explain why.

9) Move to the next case study

10) Repeat steps 1 – 9

11) If students are unable to determine the correct outcome, ask the students to list any additional information they feel they would need to determine it.

12) If students can determine the outcome, ask them to list any knowledge, experiences, or information that helped them reach their conclusion.

13) Each case study discussion will take approximately 10 minutes to complete, depending largely on how forthcoming students are with their replies.

Lesson 2: Humans & Habitat

Lesson Focus: Students play the role of land managers, and assess costs and benefits of land management decisions

Time required: 1 class period (45 minutes)

Lesson Objectives:

1) Assess the benefits and costs associated with different land management practices

2) Identify personal experiences and prior conceptions that influence land management decisions

3) Balance the needs of a community while preserving connectivity in a model landscape

Equipment and Materials:

- Humans & Habitat lesson slide show
- 1 copy of Humans & Habitats per group

Lesson Procedures:

1) Begin with a think-pair-share exercise

    a. Instruct students to discuss what new things they learned from the previous lesson, in pairs or as a group, for approximately one minute.

    b. After giving them time to organize their thoughts, ask several students to list one new thing they learned from the previous lesson.

    c. The rest of the class will be asked if they concur with these items, and if they have any to add.

    d. Use this opportunity to identify and address potential misconceptions of the prior material that the students may be developing.

2) Restate the topic, and highlight the students learning outcomes on the list of intended outcomes for the lesson.

3) Separate the class into groups of 2-4 students.

4) Present students with the prompt from lesson 2.

5) Groups will discuss how they will resolve the issue, for no more than 10 minutes, before reporting their decisions to the class.

    a. In addition to their final decisions, groups must list what was gained from the decision, and what was sacrificed.

    b. They must also explain whether the decision was unanimous, or divisive, and what personal values or experiences influence the decision.

    c. Students should spend no more than 15 minutes on this exercise.

6) Have groups begin the Humans & Habitat board game.

    a. Each group should have their own set of pieces and cards.

    b. This game is designed to be completed in 30-45 minutes.

    c. Since this is the first time most students will have played, it is unlikely that all groups will have completed their playthroughs before the class period ends.

    d. The instructor can determine how much more class time they will spend on this game, but at least one full playthrough is recommended.

Lesson 3: Slime Time

Lesson Focus: Students play the role of land managers, and assess costs and benefits of land management decisions

Time required: 2 class periods (45 minutes each)

Lesson Objectives:

1) Develop research questions based on natural phenomena

2) Develop testable hypotheses on the effects of altered connectivity

3) Design and evaluate a model that shows how human activity changes the movement of a species in the landscape

Equipment and Materials:

- Slime Time slide show
- 1 aluminum baking sheet with plastic cover per group
- 1 small jar of rolled oats per group
- 1 sheet of acetate transparency per group
- Scissors
- Tape
- Agar powder and water
- Heat source and heat-proof vessel for preparing the agar
- Live *Physarum polycephalum* slime mold culture

Lesson Procedures:

Day 1

1) Begin with a think-pair-share exercise

   a. Instruct students to discuss what new things they learned from the previous lesson, in pairs or as a group, for approximately one minute.

   b. After giving them time to organize their thoughts, ask several students to list one new thing they learned from the previous lesson.

   c. The rest of the class will be asked if they concur with these items, and if they have any to add.

   d. Use this opportunity to identify and address potential misconceptions of the prior material that the students may be developing.

2) Restate the topic, and highlight the students learning outcomes on the list of intended outcomes for the lesson.

3) Separate the class into groups of 2-4 students.

4) Present map slide to class.

    a. Explain that one map depicts an animal's movement pathways in a landscape without human activity, and the other depicts the landscape after its been altered by human activity.

5) Instruct the groups to brainstorm ideas to answer the question **"do the new gaps in the animal's habitat change the way the animal travels across the landscape?"**

6) Once each group has developed an idea, discuss them as a class.

7) Instruct the groups to brainstorm ways to answer the question at a small scale

    a. e.g. how could they answer the question without doing extensive fieldwork?

8) Discuss these new ideas as a class.

9) Advance to the first slime mold slide.

10) Explain features of slime mold.

11) Play the slime mold videos for the class.

12) Instruct the groups to brainstorm ways to answer the previous question, **"do the new gaps in the animal's habitat change the way the animal travels across the landscape?"** that incorporate the slime mold.

13) Present each group with 1 pan, and 1 sheet of acetate, and a small jar of oats.

14) Task groups with creating at least two questions about connectivity that they can answer using the materials and the slime mold.

15) In addition to developing two questions, groups should make predictions about the answer to the question, and describe how they will recognize if the answer is correct, or incorrect.

16) Students will then use the remaining class time preparing and setting up a dry run of their chosen test.

Day 2

1) Begin with a think-pair-share exercise

    a. Instruct students to discuss what new things they learned from the previous lesson, in pairs or as a group, for approximately one minute.

    b. After giving them time to organize their thoughts, ask several students to list one new thing they learned from the previous lesson.

    c. The rest of the class will be asked if they concur with these items, and if they have any to add.

    d. Use this opportunity to identify and address potential misconceptions of the prior material that the students may be developing.

2) Restate the topic, and highlight the students learning outcomes on the list of intended outcomes for the lesson.

3) Instruct students to return to their groups, and begin preparing their experiments.

4) Instructor should assist with heating and pouring agar where appropriate.

5) Once the experiments are set up, students will make daily observations, according to their plans.

As groups reach conclusions based on their results, they will make short, five minute presentations of their questions, designs, and conclusions.

Introduction to Landscape Connectivity

Prepared by Jakob Goldner
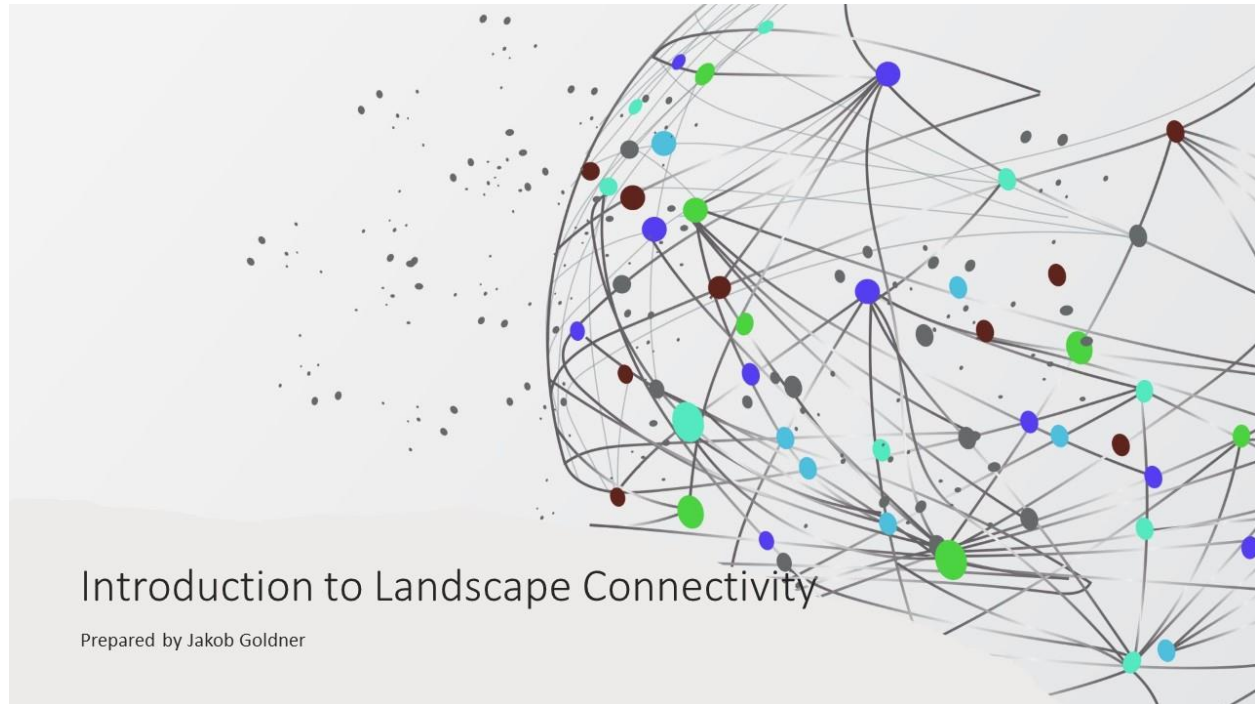
Landscape Connectivity

- Imagine you are a bobcat, making your way across this landscape to a hunting ground. What path would you take? Would you follow the roadways? Would you creep through the forest? Would you swim across the lakes?

- Now imagine you're a salamander, searching for a pond to lay your eggs in. You're very small, and must avoid drying out. Will you be able to access every pond? Or will some be harder, or even impossible, to reach?

Photo by Garth Lenz, 2011

Landscape Connectivity

- Animals make choices about how to move across the landscape, based on how easy or difficult it is for them to traverse.

- The description of how easy or difficult it is for a species to move between different parts of its habitat is called "**connectivity**".

Photo by Garth Lenz, 2011

Why is connectivity important?

- The effects of human activities are more complex than simple habitat destruction and pollution.

- A habitat can remain intact, but become inaccessible to animals that need it.

- The trade-offs to human activities are not always easy to predict.

Photo by Garth Lenz, 2011

Today's learning objectives

- You should be able to:
    1) Recall and define the term "connectivity"
    2) Name an example of the effects of reduced (or increased) connectiveity
    3) List the benefits and environmental costs of different human activities

Photo by Garth Lenz, 2011

Case Study 1: Erosion dams and white-spotted charr

- In the mountain rivers of Japan, the white-spotted charr (similar to the north American brook trout) is often the top predator. To protect residential areas, small, anti-erosion dams were constructed.

- Question: What are the benefits to building these dams?

- Answer: Dams slow the flow of rivers, making them safer, reduce erosion along riverbanks in residential areas, and lowers risk of landslides

- Question: How do the dams affect the white-spotted charr habitat?

- Answer: Connectivity was reduced, and Charr are unable to travel upstream, so upstream populations are slowly dying out.

- Question: Do you think the results are worth the environmental cost? Why?

Photo from Morita et al. 2019

Case Study 2: Lions in Ghana

- Ghana created a system of large national parks. Lions were protected in these areas. Outside of these parks, land has been converted to agriculture and residential areas.
- Question: What benefits do humans receive from this land use?
- Answer: More efficient agriculture, and increased economic development (Ghana has the 8th highest GDP among African countries)
- Question: How did these land use decisions affect lions?
- Answer: Connectivity was reduced, and Lions within the parks became more isolated. Their numbers declined, and they went extinct in some parks. With no predators, olive baboon populations grew, and ultimately resulted in baboons becoming major agricultural pests.
- Question: Do you Question: Do you think the results are worth the environmental cost? Why?

Photo by Michael Nichols 2012

Case Study 3: Ticks in New York

- There are hundreds of parks in New York City. Humans enjoy having access to more and bigger parks.
- Question: What benefits do humans get from having more and bigger parks?
- Answer: Increased opportunity for exercise, and recreation, which can improve physical and emotional wellbeing.
- Question: How do these land use decisions affect urban wild animals?
- Answer: Connectivity is increased with more or larger parks in an area of the city, but the number of ticks, and the infection rate of Lyme disease increase, because there are larger, denser populations of host animals.
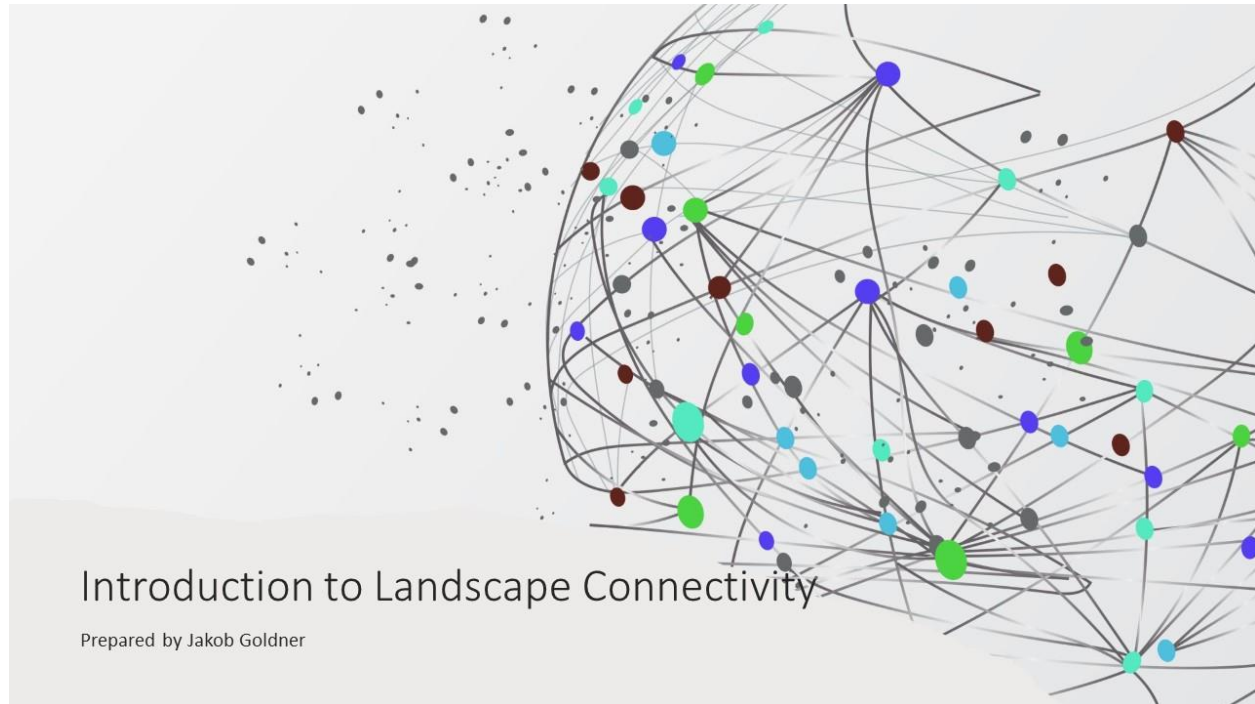

Photo by Andrew Savulich 2016

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References

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Introduction to Landscape Connectivity

Prepared by Jakob Goldner

Yesterday's learning objectives

- You should be able to:
    1) Recall and define the term "connectivity"
    2) Name an example of the effects of reduced (or increased) connectiveity
    3) List the benefits and environmental costs of different human activities

Photo by Garth Lenz, 2011

Why is connectivity important?

- The effects of human activities are more complex than simple habitat destruction and pollution.

- A habitat can remain intact, but become inaccessible to animals that need it.

- The trade-offs to human activities are not always easy to predict.

Photo by Garth Lenz, 2011

Today's learning objectives

- You should be able to:
    1) Assess the benefits and costs associated with different land management practices
    2) Identify personal experiences and prior conceptions that influence land management decisions
    3) Balance the needs of a community while preserving connectivity in a model landscape

Photo by Garth Lenz, 2011

Land management prompt

- Imagine your group is a committee of Department of Natural Resources officials, tasked with managing an area of public land. You are approached by:
    1. A timber company that wants to purchase and harvest trees.
    2. A large farm owner who wants to lease the land to expand their farm.
    3. A conservation group that wants to create a nature preserve.

- Discuss how you will resolve the issue within your group.

- List personal values and experiences that influence each members' opinions.

Photo by Ceres Barros

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APPENDIX E. LANDSCAPE ECOLOGY AND CONNECTIVITY LESSON 2 BOARDGAME RULES AND PIECES

Humans & Habitat Boardgame

How to Play

1. Shuffle the City Planner, Animal, and Human objective card decks (Fig I: 1, 10, and 11) separately, and place face down (Full set of game pieces available in Fig II).
2. Shuffle the hexagonal Nature, Urban, and Agriculture habitat tiles (Fig I: 2, 4, and 7) together, and place in a stack, face down.
3. Take one habitat tile from the top of the stack, and place it face up in the center of the table.
4. Each player draws one card from each objective, concealing them from the other players.
5. Beginning with the oldest player, and moving counter-clockwise with each turn, players may choose to take two of the following actions:
   a. Draw three habitat tiles, place one on the table, and return the other two to the bottom of the stack. The tile must share one edge with the first habitat tile, or two edges with other habitat tiles.
   b. Move the animal token (Fig I: 5) in a straight line in a direction of their choice, but may not cross 2 consecutive non-Nature tiles. When stopped on a Nature tile, grow one Resource token (Fig I: 6) on it, and one on each connected Nature tile.
   c. Move the human token (Fig: 9)) in a straight line in a direction of their choice to a:
      i. Nature tile to harvest one Resource token.
      ii. Agriculture tile to grow or harvest one Crop token (Fig I: 3).
      iii. Urban tile to build or take one Development token (Fig I: 8).
      iv. The Human token produces one token on each Urban or Agriculture tile connected to the tile they stop on.
   d. Draw one objective card from any category. Players may not do this when they have five cards in their hand.
6. Players should attempt to place habitat tiles in the arrangements depicted on the City Planner cards (e.g. Fig I: 1a and 1c). When a player possesses a card depicting a habitat arrangement on the table, they may remove that card from their hand and place it face up.
7. Players complete Animal objectives when they use the Animal token to grow the specified amount of Resource tokens on the tile they land on.
8. Players complete Human objectives when they collect all of the tokens specified on the card.
9. The final round begins when a player reaches the objective cap (2 players = 9 objectives, 3 p. = 8 obj., 4 p. = 7 obj.).
10. Upon completing the final round, players total the point values on their objective cards.
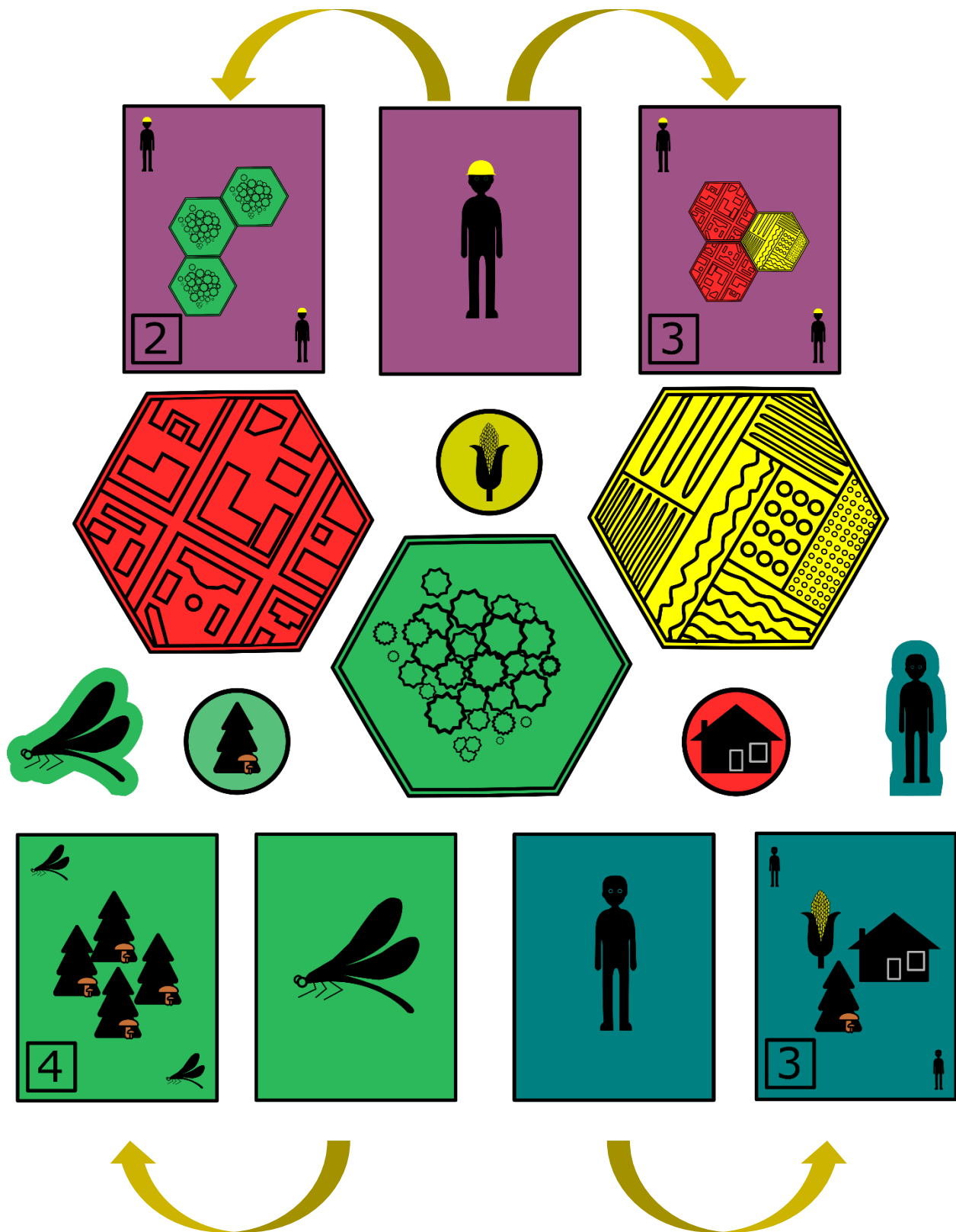11. Highest point value wins!

Fig E.1 Humans & Habitats game pieces. These will be available to print on paper or laser cut out of your material of choice.
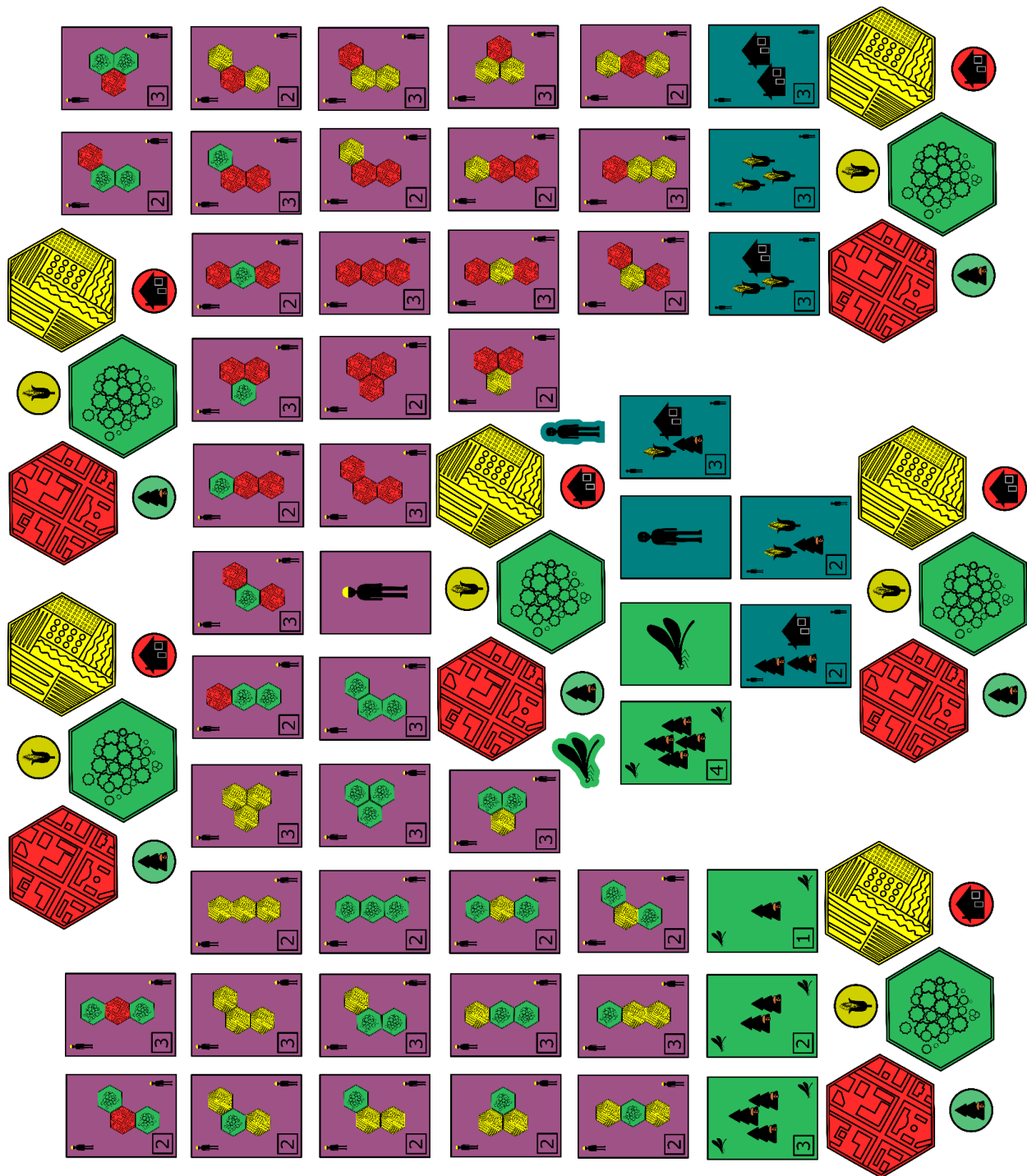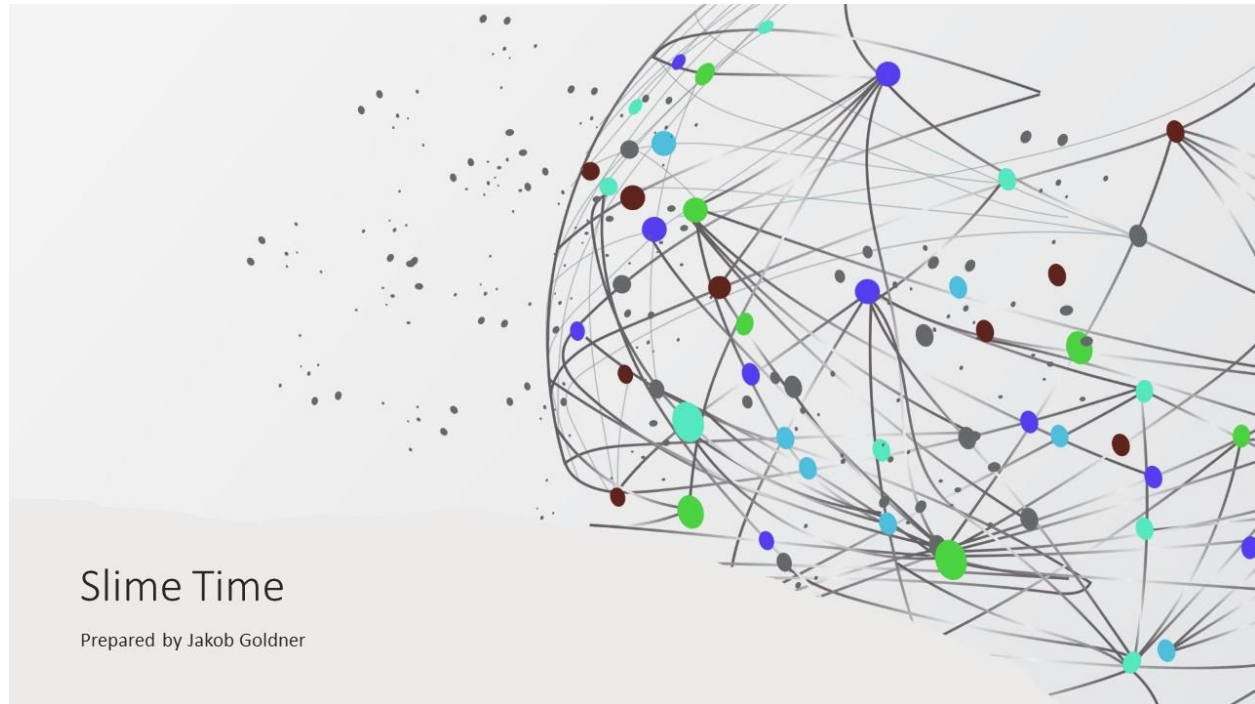
Fig E.2 Humans & Habitats complete game pieces

Slime Time

Prepared by Jakob Goldner

Yesterday's learning objectives

- You should be able to:
  1) Assess the benefits and costs associated with different land management practices
  2) Identify personal experiences and prior conceptions that influence land management decisions
  3) Balance the needs of a community while preserving connectivity in a model landscape

Photo by Garth Lenz, 2011

Why is connectivity important?

- The effects of human activities are more complex than simple habitat destruction and pollution.

- A habitat can remain intact, but become inaccessible to animals that need it.

- The trade-offs to human activities are not always easy to predict.
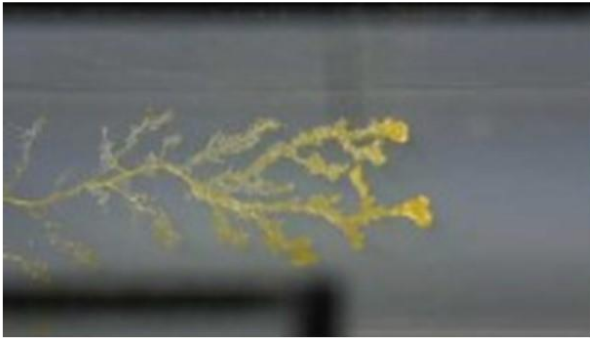
Photo by Garth Lenz, 2011

Today's learning objectives

- You should be able to:

    1) Develop research questions based on natural phenomena
    2) Develop testable hypotheses on the effects of altered connectivity
    3) Design and evaluate a model that shows how human activity changes the movement of a species in the landscape

Photo by Garth Lenz, 2011

Slime mold

PUBLICATION

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