# Exploring breeding season phenology of the Eastern Gray Treefrog (*Dryophytes versicolor*)

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# Abstract

Altered phenological cycles due to climate changes may affect species' geographical distributions as the new timing of important life-history events may allow species to expand or shift their range. Insight into these processes can be gained through analyzing relationships between environmental conditions and breeding phenology of the same species at different latitudes. As climate change continues to impact species ranges, especially in variable environments, exploring what influences breeding season will help to understand ecosystem dynamics and how species can account for variable environments. I analyzed how breeding season varies in timing and duration between the northern range edge and range core of *Dryophytes versicolor* (Eastern Gray Treefrog, Hylidae). I found that populations at the northern range edge had a significantly shorter breeding season than the range core. This included a later onset of calling for the northern range edge and an earlier end to the calling season. Populations at the northern range edge also began calling at colder temperatures compared to those at the range core. I explored life-history trade-offs within *D. versicolor* to understand if reproductive investment may cause the end of breeding season. I analyzed the amount of mass lost by *D. versicolor* males across a breeding season in relation to when calling ended. I found that there was not a threshold mass loss in males that triggered the end of breeding season. I did confirm the presence of a reproduction-survival trade-off, as males that invested less the previous season were more likely to be recaptured in the following season. There are multiple environmental and biological factors at play that determine the timing of breeding season for *D. versicolor*. Further work would contribute to understanding their life history and at what scale or severity they could be impacted by future climate change.

# Acknowledgements

Throughout the last two years, there have been many people involved in this project that I am extremely grateful for and without them, I may not have completed this thesis on time, if at all.

To begin I would like to thank my supervisor Dr. Adam Algar. I am very appreciative of your guidance and expertise throughout this project. You encouraged me out of my comfort zone when needed (making me code) and granted me autonomy in areas I knew best (fieldwork). I am grateful to have completed this project under your supervision.

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I have been lucky enough to gain an incredible community in Thunder Bay, each of you supporting and pushing me along the way. Colin, you have listened to all my frustrations while still pushing me to do my best. Without question, you agreed to a slightly rogue

field adventure, contributing to my project. Thank you for all your encouragement and love.

I would not be completing this degree if it weren’t for my parents. Dad, you sparked the love for nature so young as we looked for bugs under rocks, and frogs in the pond, creating such a joy to be outside. Mum, you constantly remind me I can do hard things and are always there for me when I need you. Your visits to Thunder Bay over the last two years are always so fulfilling. Thank you both for the endless love.

Finally, the one who tests my patience the most, but is always worth it. Thank you to my sweet companion Billie, for providing emotional support and constant company, you taught me what unconditional love is.

## Land Acknowledgement

I would like to respectfully acknowledge that the fieldwork for this project took place on ancestral and traditional lands of a diverse set of Indigenous peoples. Through this acknowledgement, I seek to honour the Indigenous people of the various places I conducted fieldwork. I look to recognize how we have inadvertently benefited from the history of colonization of Indigenous people and that land acknowledgements are only a starting point and should not be the only way to support and recognize Indigenous communities.

Lakehead University along with the fieldwork that I completed in the Thunder Bay area, is located on the traditional lands of the Fort William First Nation, Signatory to the Robinson Superior Treaty of 1850.

Collaborations in the United States involved fieldwork in Chicago, Illinois and St. Louis, Missouri. Chicago is located on the ancestral homelands of the Council of the Three Fires (the Ojibwa, Ottawa, and Potawatomi tribes) and a place of trade with other tribes including the Ho-Chunk, Miami, Menominee, Sauk, and Meskwaki. St. Louis occupies the ancestral, traditional, and contemporary lands of the Osage Nation, Otoe-Missouria, Illinois Confederacy, Quapaw, Ho-Chunk, Miami and many other tribes.

In offering this land acknowledgement, I look to affirm and support the various Indigenous peoples and land, on which I am honoured to have completed fieldwork.

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# Chapter 1: General Introduction

## 1.1 Background

Changes in species phenology, i.e., the timing of recurring biological events, are occurring alongside changes in climate (Bradley et al. 1999, Brown et al. 2016, Ettinger et al. 2021). Altered phenological cycles due to climate changes may affect species' geographical distributions as the new timing of important life-history events may allow species to access new areas (Gomi et al. 2007, Duan et al. 2016). These shifts occur more often in springtime phenological events than in later seasonal cycles (Bradley et al. 1999, Ettinger et al. 2021). If phenological shifts are too great, they can lead to the desynchronization of phenology amongst interacting species, causing changes to the ecosystem (Thackeray et al. 2016). Understanding the drivers and impacts of shifting phenology is best achieved through long-term datasets. However, in systems where such data are unavailable, insight into these processes can gained through analyzing variation relationships between environmental conditions and breeding phenology of the same species at different latitudes (Morrison and Hero 2003, Duan et al. 2016), i.e., making a space-for-time assumption.

## 1.2 Anuran Breeding Phenology

Most phenological activity has some biotic or abiotic cues; determining what these cues are is important for understanding ecosystem interactions (Gomi et al. 2007, Fuentes-de La Rosa et al. 2021). For breeding seasons in variable seasonal environments, temperature is often the main cue (Berven et al. 1979, Hoffmann and Mitchell 2022). However, for anurans (frogs and toads) it is still unclear what abiotic or biotic cues initiate the breeding season and, in addition, which cues may trigger its end. Breeding activity and cycles vary largely between and within species, as does the duration of breeding activity (Heinermann et al. 2015). Anurans are often categorized as either explosive breeders or prolonged breeders (Hartel et al. 2007, Heinermann et al. 2015). Wells (1977) defines explosive breeding as reproduction that occurs during a single night, while prolonged breeding is reproduction occurring over several months (Wells and Bevier 1997, Hartel et al. 2007, Heinermann et al. 2015). Anurans are sensitive to climate as their reproduction is often tied to the availability of water in their environment, making rainfall, temperature, and humidity the common environmental cues considered when studying anuran breeding season (Madelaire and Gomes 2016, Hoffmann and Mitchell 2022, Forti et al. 2022). It is unclear if multiple cues are working together or if it is a single environmental cue that triggers the onset and end of breeding season in anurans. Below, I review the existing literature to identify gaps in our current understanding of what environmental cues may initiate or cease breeding season in anurans.

Following Cahill et al., (2014), I performed a systematic literature search using a set of predetermined keywords. The initial search used the keyword ‘breeding season’, with 37,553 articles retrieved. This was then refined with the keyword ‘anurans’, retrieving 200 articles. Next, I used ‘start* OR begin* OR initiation* OR onset*’ to refine the search to 24 articles for the onset of breeding season, with 12 of actual relevance. For the cessation of breeding season, I used ‘End* OR cessation* OR stop*’ along with ‘breeding season’ and ‘anurans’. This retrieved 35 articles, with 11 of relevance. Lastly, to increase the number of articles included in this review, the keywords

"Length* OR Duration*' were included as a sub-search after 'breeding season' and 'anurans'. This search produced 34 articles, and of these 13 were relevant.

I identified 10 studies discussing the onset of breeding season, ranging from the years 1996 to 2022. From these 10 articles, seven of the studies were located in tropical areas and three were in low temperate areas, indicating more focus on areas of tropical weather and less variable seasonality.  Five of the 10 articles studied systems in South America (Lampo and Medialdea 1996, Vaira 2005, Canelas and Bertoluci 2007, Madelaire and Gomes 2016, Forti et al. 2022), three in Europe (Eggert and Guyétant 2003, Hartel et al. 2007, Llusia et al. 2013) one in North America (Fuentes-de La Rosa et al. 2021) and one in Australia (Yasumiba et al. 2016). With a small representation from North America, further investigation is required to understand the breeding onset of anurans in more variable climates, such as Canada, with long winters and short breeding opportunities in the spring. Regarding the environmental cue that initiates the onset of breeding in anurans, rainfall was a common cue across the 10 papers analyzed (Vaira 2005, Canelas and Bertoluci 2007, Yasumiba et al. 2016), however, in most of these experiments, there was rarely a single factor contributing to the initiation of breeding (Lampo and Medialdea 1996, Llusia et al. 2013, Fuentes-de La Rosa et al. 2021, Forti et al. 2022). It can be concluded that breeding onset is triggered by multiple cues depending on the location and species being studied.

My literature review also evaluated the length of breeding season in anurans, including five papers from 2008 to 2019. Of these five papers, one study was completed in North America (Wheeler and Welsh 2008), one in Asia (Ngo and Ngo 2013) and three in South America (Cairo et al. 2013, Ximenez and Tozetti 2015, Torres-Cervantes et al.

2019). Thus, there is still an underrepresentation of North America, specifically areas with more variability in seasons, which in turn have more reproductive pressure for anurans. Three of these five studies concluded that rainfall was indicative of the length of breeding season for anurans (Wheeler and Welsh 2008, Cairo et al. 2013, Torres-Cervantes et al. 2019).

The literature for the cessation of breeding season resulted in eight articles ranging from 2005 to 2020. Of these, a variety of environmental factors were considered in various places. The lunar cycle and moonlight were included in two articles, both of which were situated in Europe (Grant et al. 2009, Vignoli et al. 2014). Central America (Lynch and Wilczynski 2005) and South America (Schalk and Saenz 2016, Cassiano-Lima et al. 2020, Bonnefond et al. 2020) were the study locations for four articles, and Africa (Heinermann et al. 2015) and India (Joshi et al. 2017) accounted for the other two. Once again, North America was underrepresented, as were areas with more variable seasons. In terms of factors considered to influence breeding season, the lunar cycle was analyzed for three studies (Grant et al. 2009, Vignoli et al. 2014, Schalk and Saenz 2016), hormone level relationships were discussed for two studies (Lynch and Wilczynski 2005, Joshi et al. 2017), and humidity was considered for the remaining three (Heinermann et al. 2015, Cassiano-Lima et al. 2020, Bonnefond et al. 2020). Overall, most of these studies mentioned that more than one factor is influencing breeding season phenology, and multiple should be included when analyzing breeding season in anurans.

My review found that most studies conducted their studies in areas with little variability among seasons, with underrepresentation of higher latitude environments. These areas of higher latitudes will place greater seasonal pressures on anuran breeding seasons, making it an important topic to study further. Additionally, there were slightly fewer studies on cessation of breeding season (eight compared to 10 on onset). In highly seasonal environments, cues influencing cessation of breeding may be especially important to give both parents and offspring sufficient time to gather resources for winter. Furthering our understanding of the processes that influence breeding season onset and cessation is especially important as, if species move northward with climate change, it is possible they will experience shorter activity periods and in turn shorter breeding seasons (Morrison and Hero 2003, Klaus and Lougheed 2013). As climate change continues to impact species ranges, especially in variable environments, exploring what influences the end of breeding season will help to further understand the ecosystem dynamics and how species are able to account for shorter seasons in these variable environments.

### 1.3 Study Species

My study species is the Eastern Gray Treefrog, *Dryophytes versicolor* (Hylidae), previously known as *Hyla versicolor* (from here on referred to as *D. versicolor*). *D. versicolor* is a tetraploid which differentiates it from its nearly morphologically indistinguishable, but diploid, sister species, *D. chrysoscelis* (Dodd 2023). Additionally, these two species have different call rates. *D. versicolor* has a trill rate of 17-35 notes/second and *H. chrysoscelis* trills faster at 34-69 notes/second, though both are affected by temperature (Dodd 2023). *D. versicolor* has a distinct lichen-like pattern and is capable of changing colour depending on the temperature and light conditions of its environment (Harding and Mifsud 2017, Dodd 2023). *D. versicolor* prefers forested areas with ponds within 200m of the forested area for breeding (Dodd 2023). Amplexus between a male and female can last for hours. Females lay eggs in breeding ponds that are nearby wooded areas, with immediate fertilization by the males during amplexus (Dodd 2023). These eggs hatch within 5-7 days and then the tadpoles metamorphose between 40-60 days after hatching. *D. versicolor* males begin calling in spring and will continue throughout part of the summer, approximately May until July and in some locations March until August, depending on the latitudinal location (Harding and Mifsud 2017, Dodd 2023). However, calling is very energetically costly for males so they will decrease the number of hours spent calling later in the season as it becomes more costly as time goes on (Taigen and Wells 1985, Runkle et al. 1994, Dodd 2023). Females may not be present on every night of calling, with Dodd (2023) reporting female presence being correlated to chorus size and density. It has also been noted that females will have a preference for males with a higher pulse rate and longer calls (Klump and Gerhardt 1987, Dodd 2023). Whether females prefer larger males has mixed results (Gatz 1981, Dodd 2023, Fellers 1979b).

The environmental triggers of calling onset in spring are unknown for *D. versicolor*. It has been suggested that males will begin calling when evening temperatures reach 15 degrees Celsius (Harding and Mifsud 2017, Dodd 2023), but published data and tests of this claim are lacking. Furthermore, the range of *D. versicolor* spans from Thunder Bay, Ontario Canada at the northern range edge, south into Texas, United States (Dodd 2023) (Figure 1.1), resulting in substantial differences in seasonality and timing of spring between the northern range edge and southern areas, raising the possibility of local adaptation and or plasticity in the temperature-calling onset relationship, making it a strong study system for evaluating environmental cues of spring phenology in variable environments (Bonnefond et al. 2020).

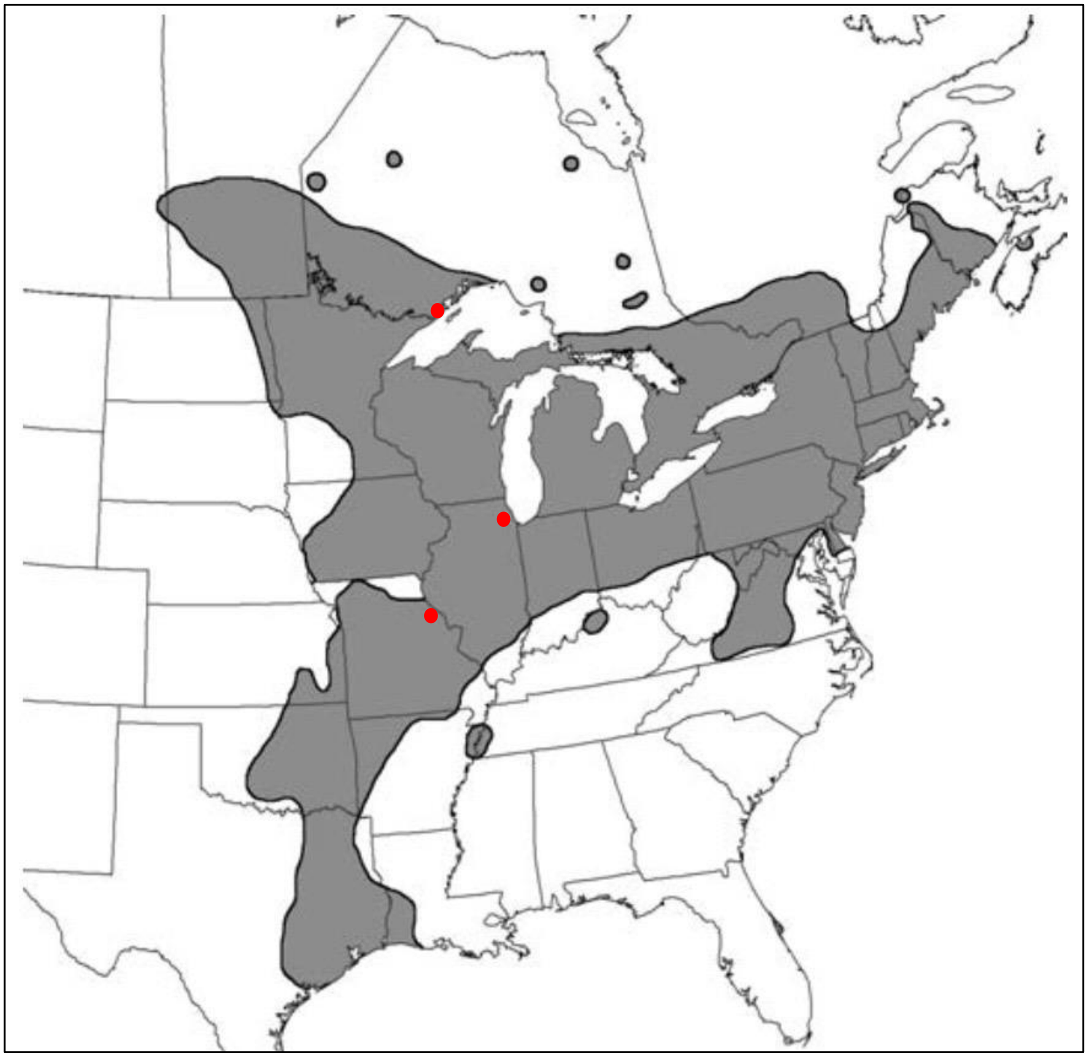

Figure 1.1. Distribution map of *D. versicolor* (modified from (Dodd 2023). Red points indicate study regions with Thunder Bay the most northern and St. Louis at the range core.

## 1.4 Aims

For Chapter 2 of this project, I aim to understand how breeding season varies in timing and duration between the northern range edge and range core of the distribution of *Dryophytes versicolor* (Eastern Gray Treefrog). Furthermore, I will test three hypotheses for how the temperature at which calling commences (onset calling temperature) changes from the northern edge to core. In Chapter 3, I will examine the role biotic factors play on the end of breeding season, focusing on the northern range limit. I will test three hypotheses to investigate the costs and potential benefits of investing, to understand what might determine the end of male advertisement at the northern range edge of *D. versicolor*.

# Chapter 2: Breeding season initiation and length of *D. versicolor* from northern range edge to range core

## 2.1 Introduction

The phenology of breeding season can be greatly affected by changes in climate (Hoffmann and Mitchell 2022). For species that do not breed year-round, breeding season is a crucial time of year. Yearly breeding seasons are dependent on favourable environmental conditions (Visser and Both 2005); these favourable environmental conditions can be more important in areas with highly variable seasons. Highly variable seasons limit the time species have to breed and that their young have to develop (Visser and Both 2005). While comparisons of breeding season phenology in stable and seasonal environments have been made, we still have a limited understanding of how species account for variable environments in their timing of important phenological events (Morrison and Hero 2003, Bauer et al. 2020). Such comparisons are possible along a geographical gradient where seasonality changes with latitude and can provide insight into environmental drivers and phenological cues, as well as how plasticity and local adaptation have shaped phenological responses to seasonality.

All phenological activity has some biotic or abiotic cues (Gomi et al. 2007). For breeding seasons in variable seasonal environments, temperature is often the main environmental cue (Berven et al. 1979, Hoffmann and Mitchell 2022). Such cues may change, either due to plasticity or local adaptation, along seasonality gradients, to ensure favourable environmental conditions are present when anurans begin their breeding season, and when their young are developing (Visser and Both 2005, Hoffmann and Mitchell 2022). While temperature is regularly identified as a key trigger for breeding activity in anurans, other potential cues have been identified, particularly photoperiod

(Huang, Jian-Guo 2020). Canavero and Arim (2009) evaluated mean monthly temperature, monthly rainfall, and photoperiod as potential cues initiating amphibian phenological cycles. They found that photoperiod was most often used by amphibians to predict a window of favourable conditions for phenological cycles (Canavero and Arim 2009). It has been suggested that photoperiod may be a better proxy of favourable breeding conditions across years than temperature itself (Huang, Jian-Guo 2020, Ettinger et al. 2021). However, relationships between temperature and photoperiod vary greatly across latitudes, with longer photoperiods accompanying colder springtime conditions at high latitudes while at lower latitudes, warmer conditions accompany shorter photoperiods. Thus, while photoperiod may be a proximal breeding cue at a particular latitude, variation in breeding phenology across a latitudinal gradient is more likely to reflect underlying climatic variation.

In seasonal environments, activities that determine fitness, such as resource gathering and reproduction, are restricted to a brief period of the year when resources and environmental conditions are favourable (Bauer et al. 2020). Selection is thus expected to favour individuals who can maximize activity during this favourable period and/or extend this period of activity (Visser and Both 2005). For example, individuals that migrate to breeding grounds, or emerge from winter dormancy earlier, would be more likely to find a mate and reproduce quickly, thereby providing offspring more time to develop prior to the next dormancy period (Shutt et al. 2019, Bauer et al. 2020). However, phenological shifts, such as earlier migration or emergence, also come with a cost (Ha, Renne 2010, Botero et al. 2015) and organisms must balance the cost and benefits of timing these phenological events. For example, Korner et al. (2016)

discussed a trade-off for tree species that need to balance the timing of budding to avoid freezing while still having an adequate season length. Tree species at the poleward range limit, delay the spring flush to avoid the potential of freezing with the risk of being damaged by the later freezing events. This delay comes with the trade-off that they will have a shorter growing season but avoid freeze damage. Thus, species must account for the seasonal environmental pressures in terms of temperature and peak time for breeding, with the cost of food and predatory synchrony (Reed et al. 2013, Shutt et al. 2019).

Amphibians are potentially greatly impacted by the effects of climate change on their phenological cycles as they are potentially highly sensitive to environmental changes due to their reproductive requirements (Klaus and Lougheed 2013). In addition, climate change will have an impact on the spatial patterns and distribution of amphibians (Duan et al. 2016, Benard and Greenwald 2023). As amphibians expand their latitudinal range poleward with climate change, there may be a trade-off of shorter activity periods and therefore potentially shorter breeding seasons (Morrison and Hero 2003, Klaus and Lougheed 2013). Warmer temperatures my also mean more heat in the in the shorter timeframe, or possibly extend the activity period. Amphibians may expand poleward with climate change because ongoing changes in climate allow for adequate conditions for phenological cycles to occur. However, if such climatic changes are not sufficient, range expansion into poleward areas will be accompanied by the pressure of more variable seasons. In response, species may shift their phenological cycles to account for environmental pressures. Shifts in their phenological cycles may include changes to their environmental cues for the onset of breeding season, such as photoperiod and temperature (Morrison and Hero 2003, Klaus and Lougheed 2013, Duan et al. 2016). While observing such changes through time requires detailed long-term datasets on phenology, range expansion and climate change, insight into these processes can be gained, at least in part, through a space-for-time assumption and evaluating relationships between environmental conditions and breeding phenology of the same species at different latitudes (Morrison and Hero 2003, Duan et al. 2016).

Geographically widespread species generally experience a large range of environmental conditions and thus are exposed to different selection pressures across their range. Range edge populations often have distinct phenotypes, either due to local adaptation or plasticity, compared to populations at the range core, though such adaptations may be insufficient to maintain high fitness, leading to range limitation (Angert et al. 2020). Phenological events can vary among populations across large geographical ranges (Morrison, C and Hero, J.M. 2003). For example, in North America, amphibian populations farther south in a species range are well known to call earlier in the year than populations farther north (Dodd 2023). However, we know far less about how the end of breeding season varies across the range. Moreover, because spring arrives later at higher latitudes, later calling may not reflect shifts in environmental conditions under which calling commences.

Here, I use bioacoustic monitoring to evaluate how the timing of breeding season and the temperature at which it commences differs between populations at the range core and northern edge of Eastern Gray Treefrogs (*Dryophytes versicolor*). First, I ask whether breeding season start, end, and duration differ between the range core and edge.

Next, I test three hypotheses for how calling onset temperature varies amongst these populations:

Hypothesis 2.1: The onset calling temperature is fixed across *D. versicolor*'s range, i.e.,, no local adaptation or plasticity. Prediction: calling should begin at the same temperature across the entire range (Figure 2.1b).

Hypothesis 2.2: Seasonality favours early calling to maximize breeding time. Prediction: calling onset temperatures will decrease with increasing latitude (Figure 2.1 c).

Hypothesis 2.3: Risk mitigation against costs of extreme events in variable environments favours later calling. Prediction: calling onset temperatures will increase with increasing latitude (Figure 2.1 d).

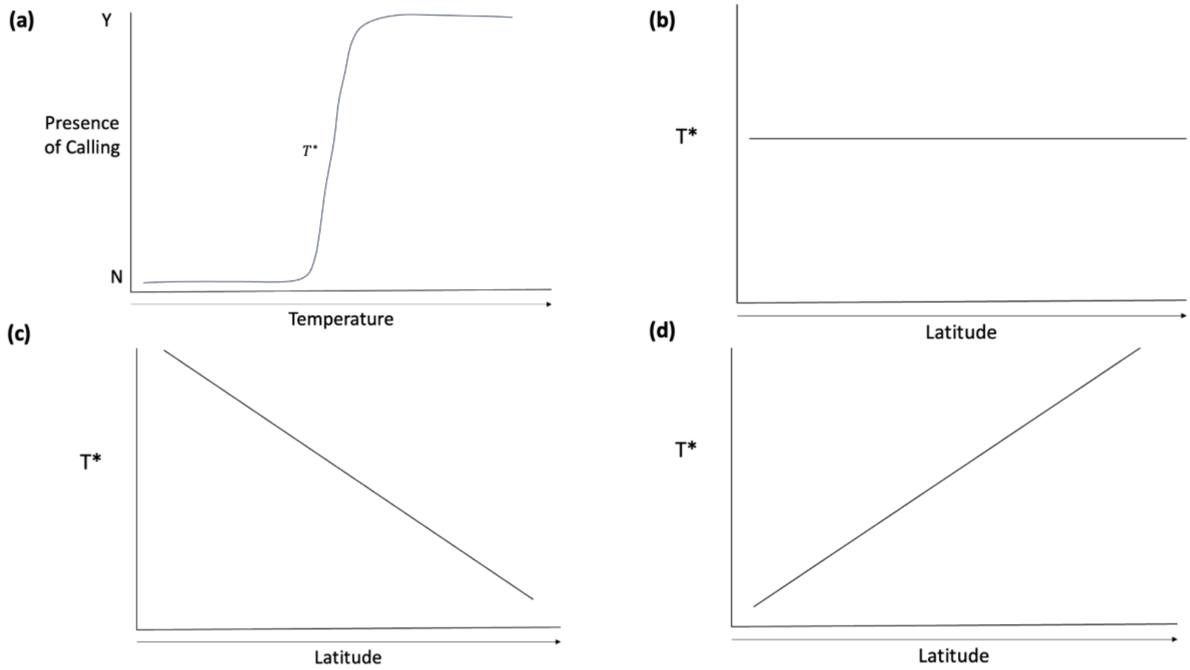

Figure 2.1. Predictions of three hypotheses for how the temperature at calling onset of *D. versicolor* will vary across latitude (Hypotheses 2.1 – 2.3 Panel a) demonstrates a general threshold temperature (T*) for *D. versicolor* to begin calling at a single site. Panel b) represents Hypothesis 2.1, where calling should begin at the same T* across the entire range. Panel c) represents Hypothesis 2.2, where species at high latitude start calling at lower temperatures. Panel d) represents Hypothesis 2.3, where the temperature of calling onset increases with latitude.

## 2.2 Methods

### *2.2.1 Study Sites*

I selected three regions from *D. versicolor*'s range core to northern edge. This included 1) Thunder Bay, Ontario, Canada (northern range edge); St. Louis, Missouri, United States (approximate latitudinal range centre); and Chicago, Illinois, United States (approximately midway between Thunder Bay and St. Louis) (Figure 1.1). Seasonality varies greatly among these three regions. Thunder Bay, for 2023, had an average temperature of 0.8 °C in April and 9.9 °C in May (Government of Canada 2024). Chicago was warmer with an average of 9.83°C and 15.89°C for April and May respectively. St.

Louis, the most southern region, had an average temperature of 14.16℃ and 19.72℃ for April and May (National Weather Service 2024).

Within each region, ponds were selected where *D. versicolor* may potentially breed, with 10 ponds around each Thunder Bay and Chicago, and 11 around St. Louis (Table 2.1 and Figure 2.2, Figure 2.3, and Figure 2.4). Ponds were selected based on previous knowledge of *D. versicolor* breeding locations, or in likely habitats, such as ponds near or within deciduous-mixed forests.

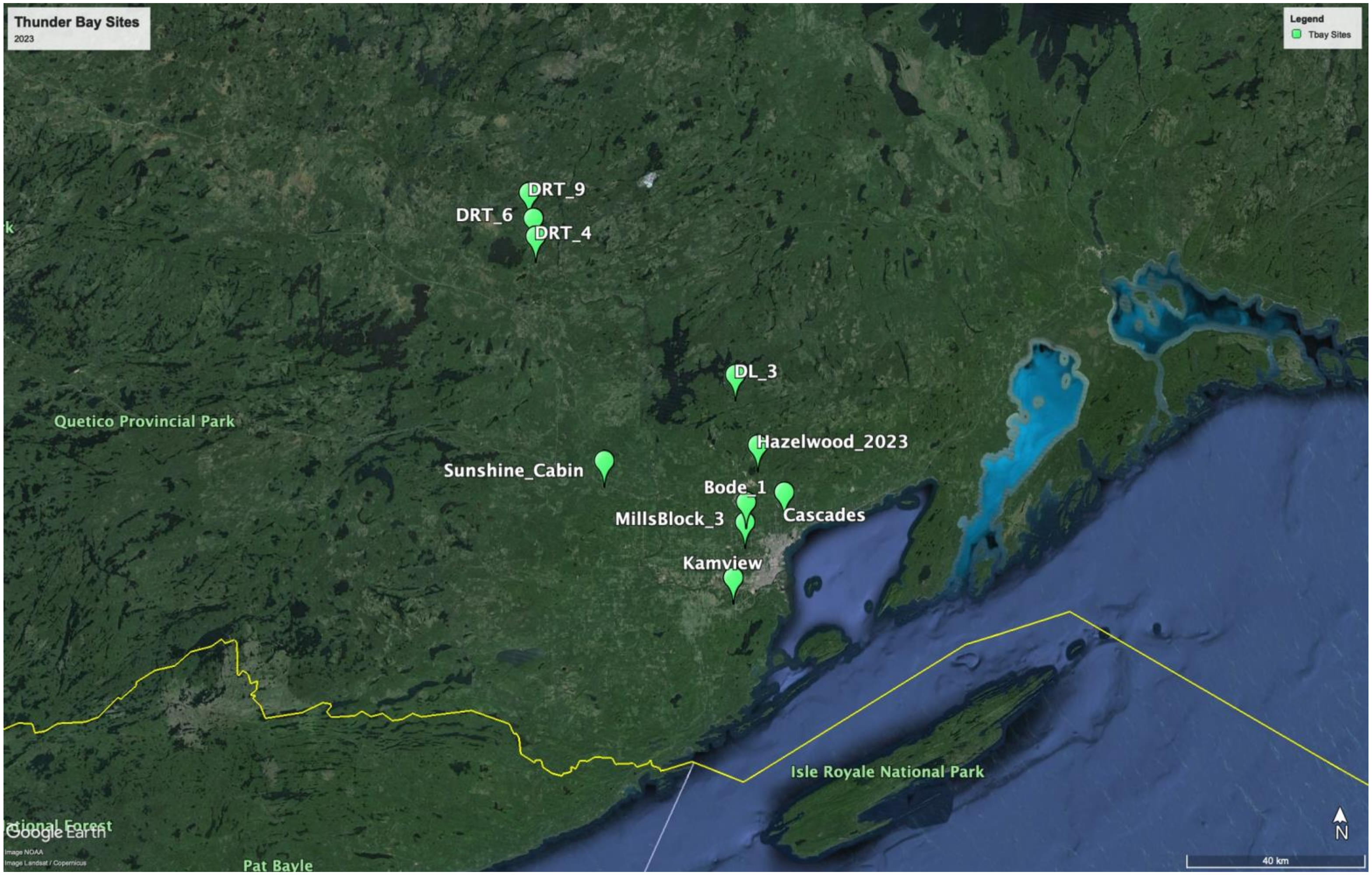

Figure 2.2. Audiomoth (with Hobo temperature logger) site locations for Thunder Bay (northern range edge) as potential breeding ponds for *D. versicolor*.

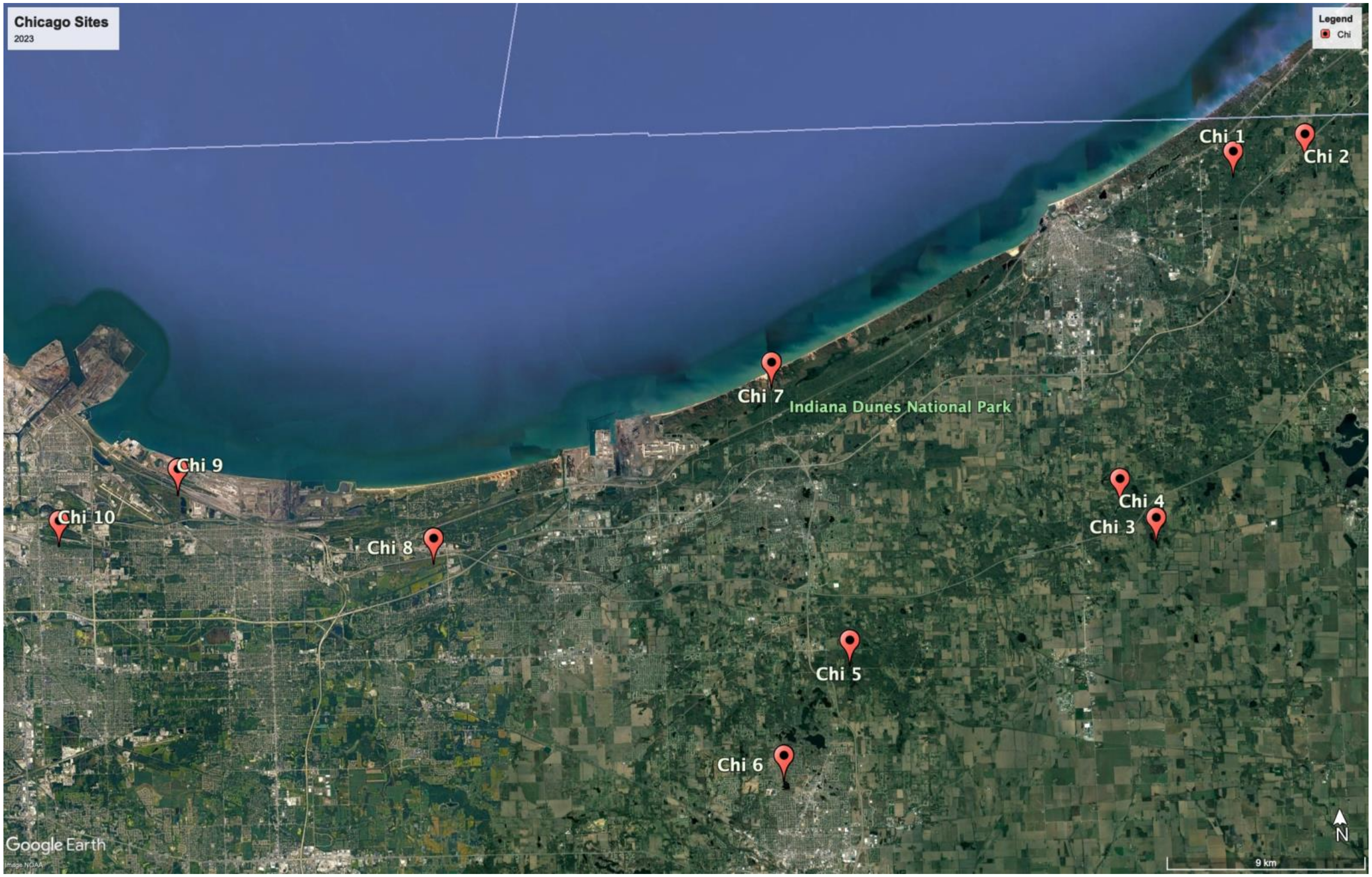

Figure 2.3. Audiomoth (with Hobo temperature logger) site locations for Chicago, Illinois at potential breeding ponds for *D. versicolor*

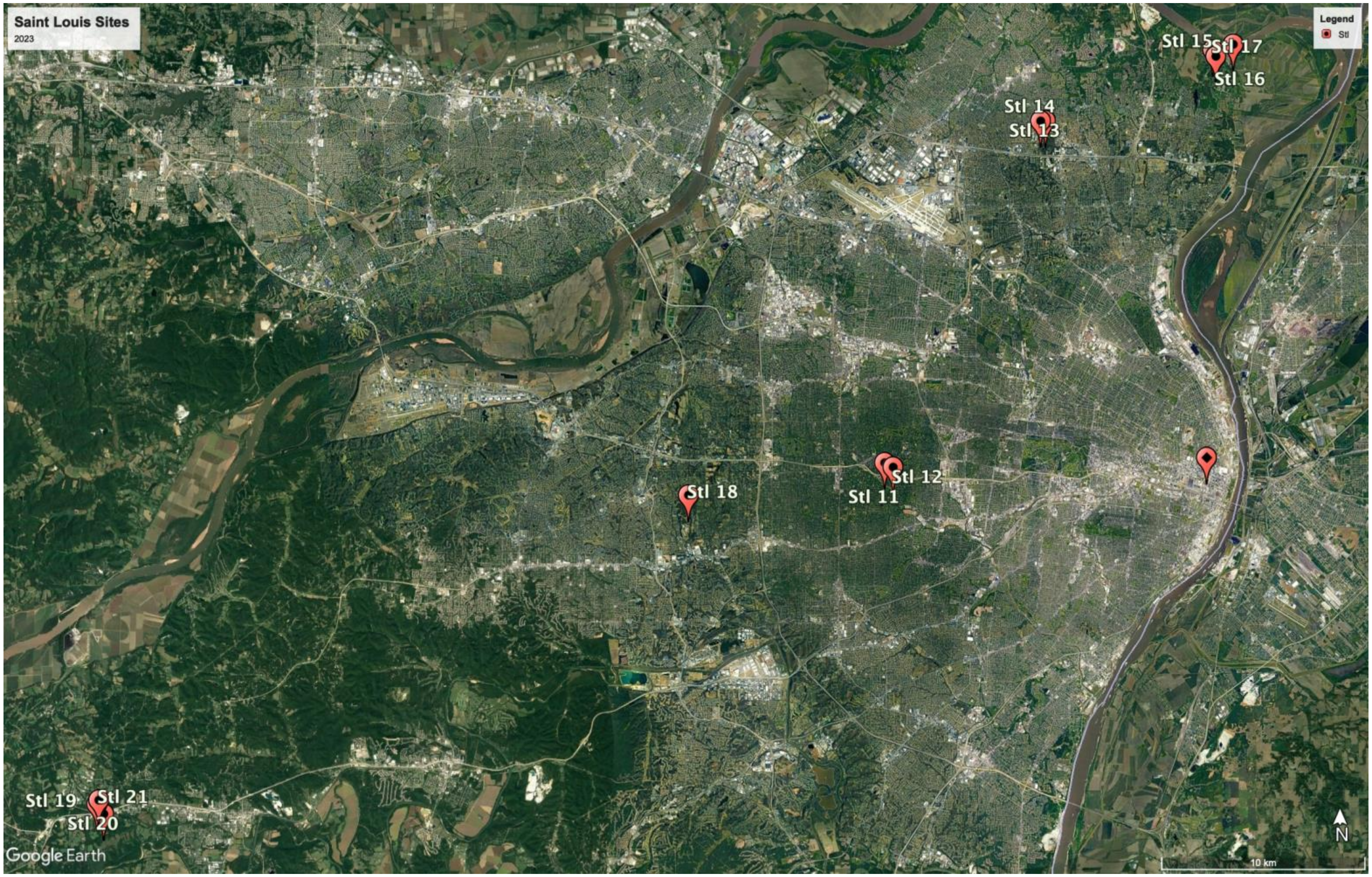

Figure 2.4 Audiomoth (with Hobo temperature logger) site locations for St. Louis, Missouri (range core) at potential breeding ponds for *D. versicolor*.

Table 2.1. Geographic coordinates for each breeding pond site in Thunder Bay and USA sites

| Location | Site Name | Latitude and Longitude | |
|---|---|---|---|
| Thunder Bay | Hazelwood_2023 | 48.58723797 | -89.29544999 |
| Thunder Bay | Kamview | 48.32606801 | -89.368163 |
| Thunder Bay | MillsBlock_3 | 48.43541102 | -89.33328297 |
| Thunder Bay | Sunshine_Cabin | 48.55651 | -89.74840703 |
| Thunder Bay | DL_3 | 48.72577599 | -89.36226499 |
| Thunder Bay | Cascades | 48.49433698 | -89.21696504 |
| Thunder Bay | Bode_1 | 48.47512901 | -89.330125 |
| Thunder Bay | DRT_4 | 48.99677197 | -89.96131903 |
| Thunder Bay | DRT_6 | 49.03206498 | -89.96871396 |
| Thunder Bay | DRT_9 | 49.08262999 | -89.98262398 |
| Chicago, Illinois | Chi_01 | 41.73604097 | -86.80851501 |
| Chicago, Illinois | Chi_02 | 41.74210704 | -86.76932501 |
| Chicago, Illinois | Chi_03 | 41.58878401 | -86.85706601 |
| Chicago, Illinois | Chi_04 | 41.60497097 | -86.87595204 |
| Chicago, Illinois | Chi_05 | 41.54298898 | -87.02496501 |
| Chicago, Illinois | Chi_06 | 41.49726603 | -87.06214403 |
| Chicago, Illinois | Chi_07 | 41.656402 | -87.06246799 |
| Chicago, Illinois | Chi_08 | 41.58893002 | -87.24844197 |
| Chicago, Illinois | Chi_09 | 41.61991197 | -87.38555197 |
| Chicago, Illinois | Chi_10 | 41.60012699 | -87.45084196 |
| St. Louis, Missouri | Stl_11 | 38.62557001 | -90.38052298 |
| St. Louis, Missouri | Stl_12 | 38.62362198 | -90.37578603 |
| St. Louis, Missouri | Stl_13 | 38.77532696 | -90.28902504 |
| St. Louis, Missouri | Stl_14 | 38.775276 | -90.29178998 |
| St. Louis, Missouri | Stl_15 | 38.80862902 | -90.18262499 |
| St. Louis, Missouri | Stl_16 | 38.80337298 | -90.19255796 |
| St. Louis, Missouri | Stl_17 | 38.80623003 | -90.19421901 |
| St. Louis, Missouri | Stl_18 | 38.61172898 | -90.49079999 |
| St. Louis, Missouri | Stl_19 | 38.47642 | -90.82337698 |
| St. Louis, Missouri | Stl_20 | 38.47203501 | -90.819541 |
| St. Louis, Missouri | Stl_21 | 38.478132 | -90.82225104 |

### *2.2.2 Bioacoustic and Temperature Monitoring*

Passive acoustic monitoring devices (audiomoths, Open Acoustic Devices) were used to record the calls of *D. versicolor* prior to, during and after the breeding season (Sugai et al. 2020, Desjonquères et al. 2022). Audiomoths were deployed at each pond within the three study regions, attached to a tree within 20 metres of the potential breeding pond, via zip ties (Figure 2.5). Thunder Bay sites were deployed between April 13 and April 28, 2023, while Chicago sites were deployed on March 6, 2023, and St. Louis sites were deployed on March 8 and 9, 2023. Thunder Bay sites had a delay in deployment compared to the southern areas, due to the amount of snow limiting access to sites. Audiomoths were scheduled to record for 2 minutes every 30 minutes, from 1930 to 0030 CST in USA locations while Thunder Bay was scheduled to record for 2 minutes every 30 minutes from 2100 to 0200 EST. The difference in audiomoth scheduling times is due to the two different time zones, and to account for sunset. The scheduling time was adjusted to each location so the amount of time recording after sunset was the same across the range. All audiomoths were set at a sample rate of 48000 Hz, with medium gain. At each site, a Hobo MX2201(Onset) temperature logger (Hobo hereafter) was also employed at the same location as the Audiomoth at a height of approximately 1.3m. Each Hobo was protected by a radiation shield (Holden et al. 2013) (see Figure 2.5) and recorded hourly air temperature.

Figure 2.5. Set up for passive acoustic monitoring device (audiomoth) and temperature logger (Hobo MX2201) with radiation shield (Holden et al. 2013).

### 2.2.3 *D. versicolor call identification*

My audiomoths accumulated 3915 hours of recording in 117,470 files. Thus, I used Lapp et al.'s (2021) Repeat Interval-Based Bioacoustic Identification Tool (RIBBIT) to automate the detection of *D. versicolor* calling. RIBBIT is an algorithm that can detect vocalizations containing periodic structures (Lapp et al. 2021). Anurans produce calls with a periodic structure referred to as the pulse repetition rate of their call (Ryan MJ 2001). RIBBIT can detect these pulse repetition rates within specified frequency ranges, allowing species identification (Lapp et al. 2021). RIBBIT calculates a maximum score for each audio file based on the positive contributions to the amplitude signal from signal bands of the desired frequency range, and negative contributions from noise bands (e.g., wind, microphone pops). The presence of periodic structure is measured by calculating the power spectral density of the summarized amplitude signal, and the final RIBBIT score is the maximum value of the power spectral density within the target range of pulse repetition rates. Maximum scores above a pre-determined threshold were deemed to contain the focal species calls. To parameterize RIBBIT and select the presence-threshold for *D. versicolor*, I generated a parameterization set of audio files by randomly choosing 100 audio files (clipped to 30s) from each region. Two listeners then independently determined gray treefrog presence and absence in each. Disagreements were blindly resolved by a third listener (Adam C. Algar). This procedure resulted in a very low prevalence of *D. versicolor* from Chicago (3 of 100 audio files had prevalence for Chicago, 32 of 100 for Thunder Bay, and 37 of 100 for St. Louis), so I carried out targeted listening to identify 35 additional presences in Chicago to replace randomly selected absences. Initial tests suggested this initial training set was too small, so I then randomly selected another 200 clipped (30s) files from each region, following the same procedure as above to determine gray treefrog presence–absence. The final parameterization dataset had a regional prevalence of 45 of 300, 45 of 300, and 81 of 297 for Thunder Bay, Chicago, and St. Louis respectively. These files were then divided into a training and testing set. 75 files from each region were selected for the testing set using stratified random sampling to ensure prevalence for each region in the testing set equalled the prevalence in the training set.

To parameterize RIBBIT, I set bounds for RIBBIT parameters (Table 2.2) and randomly selected 1000 sets from this parameter space. For each parameter set, I calculated the RIBBIT score for each file in the training set. I then used logistic regression of presence-absence on the RIBBIT score to determine the value at which probability of presence equalled 0.5 and used this value as the threshold. I then predicted presence and absence in the testing set. I repeated this procedure 1000 times and selected the parameters and threshold that maximized the product of sensitivity and specificity in the testing set (selection using Cohen's kappa or Matthew's Correlation Coefficient was nearly identical) for each region. This allowed the 'best' parameters to potentially differ between regions. If more than one parameter set produced maximum specificity or sensitivity values, I randomly selected one parameter set from these.

Initial tests using RIBBIT and the 'best' parameter set in Thunder Bay revealed high numbers of false positives, particularly when there were no frogs calling in audio files. The algorithm was identifying a species of toad’s call in the United States as a *D. versicolor* call, as well the audiomoth was picking up certain high-pitch wind as *D. versicolor*. Inspection of the scores for each time window in these files suggested that rare, rogue, RIBBIT scores could occur in a single window, which resulted in a high overall RIBBIT score for that file. To resolve rogue RIBBIT scores, I modified the RIBBIT algorithm to include a proportion filter. Under RIBBIT's original algorithm, presence is identified in a file when a single score from any time window surpasses a threshold. Under my proportion filter, a set proportion of individual time window scores had to exceed the score threshold for a presence to be detected. To determine this proportion, I used an independent set of 556 2-minute audio files from Thunder Bay in which *D. versicolor* presence-absence had been manually determined for another study by an independent listener. I tested proportion filters of 0.05 and 0.10 and compared these to the original RIBBIT algorithm on this second testing set. Results showed that specificity (true negative detection) was highest for the 0.10 filter, but that sensitivity (true positive detection) was low. However, I chose to use the high specificity approach because a low commission rate is more important than a low omission rate for my analysis. A low commission rate is more important because the aim is to detect presence for each night, and I have multiple audio files from each night at each location. Assuming that a gray treefrog is indeed present, failing to detect the gray treefrog in all files from that night, even with relatively low sensitivity, is highly unlikely. However, if treefrogs are not present, then it only requires a single false positive to generate a false positive for that night.

Using the 0.10 proportion filter and regional-specific best parameter set, I used RIBBIT to analyze all files from each region and summarize *D. versicolor* presence and absence for each night of the sampling period.

Table 2.2. RIBBIT parameter ranges and fixed values for determining best suited parameter values for training set.

| Parameter | Values | Fixed Or Range |
|---|---|---|
| spectrogram-window overlap percentage | 0% | fixed |
| minimum decibels | -100 | fixed |
| maximum decibels | -20 | fixed |
| analysis window length | 1 - 3 seconds | range |
| samples per spectrogram window | 128 - 1024 | range |
| widest signal band | 0.6 - 2.5 kHz | range |
| noise bands | 0 - 24 kHz | range |
| minimum pulse repetition rate | 14 - 22 | range |
| maximum pulse repetition rate | 25 - 32 | range |

### *2.2.4 Calling season*

I determined the length of the calling season at each site using a 3rd order polynomial generalized linear model with a logit link with calling (yes/no) as the response variable and day of year as the predictor (Zuur 2009). For each site, I delineated the start and end of calling season as the inflection points of the GLM function. This approach was used instead of a probability of calling of 0.5 since not all regressions inferred maximum probabilities of 1.0 and minimum probabilities of zero. Inspection of regression plots for start and end dates suggested that inferred dates from less than 5 days of presences were likely unreliable, and these sites were omitted, leaving 9, 3, and 9 sites from Thunder Bay, Chicago, and St. Louis respectively. A further two sites from Chicago had calling dates that clearly did not correspond to breeding, e.g., inferred last calling day of 243, so two sites were omitted. With only one site left for Chicago, the region was removed from subsequent analyses. I tested whether calling start dates, end dates, and calling season length differed between regions using generalized linear models with a log link.

### *2.2.5 Calling onset temperature*

To compare calling onset temperatures between regions, I first calculated the average temperature for each day at each site during the recording period. Then, to focus on calling onset, I trimmed the data for each site to remove all days after the final detected *D. versicolor* call. I tested for a difference in calling onset temperatures between sites by fitting a generalized linear mixed effects model with a logit link, where calling (yes/no) was the response, daily temperature and region were the fixed effects and site was a random effect (Zuur 2009). In this model, significantly different intercepts reflect differences in calling onset temperature between regions.

## **2.3 Results**

### *2.3.1 D. versicolor call identification*

The parameters for the top five performing parameter sets for each region are shown in Table 2.3, Table 2.4, and Table 2.5. Performance measures for each of these sets are given in Table 2.6, Table 2.7, and Table 2.8. Performance measures for proportion filters of 0.05 and 0.10 based on the additional Thunder Bay testing data are given in Table 2.9. I used the 0.10 proportion filter as the low commission rate was more important than a low omission rate for my analysis. Using this 0.10 filter resulted in 92.63% accuracy, 61.18% sensitivity, and 98.3% specificity in the use of the RIBBIT algorithm for detecting *D. versicolor* calls.

Table 2.3. Thunder Bay top five performing parameter values based on highest sensitivity*specificity values for Thunder Bay (see Table 2.6) with a 0.10 proportion filter. Bolded parameters are the set used for the analysis of Thunder Bay audio files (this was selected randomly from the parameter sets with sensitivity*specificity values equal to the maximum. PRR is pulse repetition rate.

| Spectrogram window length | signal band minimum | signal band maximum | lower noise band minimum | lower noise band maximum | higher noise band minimum | higher noise band maximum | minimum PRR | maximum PRR | window length | ribbit threshold (Intercept) |
|---|---|---|---|---|---|---|---|---|---|---|
| 326 | 2.02 | 2.11 | 0.37 | 0.45 | 8.26 | 16.57 | 16 | 33 | 1.50 | 193.71 |
| **614** | **1.82** | **2.42** | **0.30** | **0.33** | **2.94** | **15.15** | **15** | **30** | **1.58** | **303.57** |
| 722 | 1.94 | 2.34 | 0.35 | 0.43 | 12.87 | 19.18 | 15 | 31 | 1.85 | 635.37 |
| 214 | 1.64 | 2.47 | 0.04 | 0.43 | 15.08 | 18.40 | 14 | 32 | 1.04 | 45.89 |
| 254 | 1.77 | 2.17 | 0.05 | 0.24 | 7.32 | 20.82 | 14 | 30 | 1.76 | 70.73 |

Table 2.4. Chicago top five performing parameter values based on highest sensitivity*specificity values for Chicago (see Table 2.7) with a 0.10 proportion filter. Bolded parameters are the set used for the analysis of Chicago audio files. PRR is pulse repetition rate.

| spectrogram window length | signal band minimum | signal band maximum | lower noise band minimum | lower noise band maximum | higher noise band minimum | higher noise band maximum | minimum PRR | maximum PRR | window length | ribbit threshold (Intercept) |
|---|---|---|---|---|---|---|---|---|---|---|
| **172** | **1.93** | **2.17** | **0.13** | **0.29** | **5.58** | **5.93** | **17** | **30** | **1.10** | **73.19** |
| 326 | 2.02 | 2.11 | 0.37 | 0.46 | 8.26 | 16.57 | 16 | 32 | 1.50 | 193.71 |
| 614 | 1.82 | 2.42 | 0.30 | 0.33 | 2.94 | 15.15 | 15 | 30 | 1.58 | 303.57 |
| 834 | 1.95 | 2.12 | 0.03 | 0.12 | 6.77 | 22.56 | 15 | 28 | 1.50 | 1374.15 |
| 600 | 1.87 | 2.24 | 0.32 | 0.43 | 3.44 | 18.58 | 14 | 31 | 1.74 | 426.66 |

Table 2.5. St. Louis top five performing parameter values based on highest sensitivity*specificity values for St. Louis (see Table 2.8) with a 0.10 proportion filter. Bolded parameters are the set used for the analysis of St. Louis audio files. PRR is pulse repetition rate.

| spectrogram window length | signal band minimum | signal band maximum | lower noise band minimum | lower noise band maximum | higher noise band minimum | higher noise band maximum | minimum PRR | maximum PRR | window length | ribbit threshold (Intercept) |
|---|---|---|---|---|---|---|---|---|---|---|
| **704** | **2.33** | **2.49** | **0.20** | **0.37** | **9.30** | **14.30** | **17** | **27** | **1.06** | **787.70** |
| 620 | 2.20 | 2.50 | 0.08 | 0.37 | 22.63 | 23.05 | 20 | 29 | 1.75 | 250.81 |
| 574 | 2.24 | 2.47 | 0.13 | 0.48 | 17.79 | 17.90 | 14 | 28 | 1.35 | 435.27 |
| 165 | 2.20 | 2.39 | 0.35 | 0.50 | 10.58 | 14.10 | 20 | 31 | 1.17 | 27.57 |
| 326 | 2.17 | 2.46 | 0.13 | 0.22 | 4.57 | 12.32 | 17 | 32 | 1.29 | 114.05 |

Table 2.6. Thunder Bay top five performance measures based on highest sensitivity*specificity values.

| Accuracy | Sensitivity | Specificity | Sensitivity*Specificity | Kappa | MCC |
|---|---|---|---|---|---|
| 0.99 | 0.92 | 1 | 0.92 | 1.80 | 0.95 |
| 0.99 | 0.92 | 1 | 0.92 | 1.80 | 0.95 |
| 0.99 | 0.92 | 1 | 0.92 | 1.80 | 0.95 |
| 0.97 | 0.92 | 0.98 | 0.90 | 1.64 | 0.90 |
| 0.97 | 0.92 | 0.98 | 0.90 | 1.64 | 0.90 |

Table 2.7. Chicago top five performance measures based on highest sensitivity*specificity values.

| Accuracy | Sensitivity | Specificity | Sensitivity*Specificity | Kappa | MCC |
|---|---|---|---|---|---|
| 0.97 | 0.92 | 0.98 | 0.90 | 1.64 | 0.90 |
| 0.97 | 0.83 | 1 | 0.83 | 1.76 | 0.90 |
| 0.97 | 0.83 | 1 | 0.83 | 1.76 | 0.90 |
| 0.97 | 0.83 | 1 | 0.83 | 1.76 | 0.90 |
| 0.97 | 0.83 | 1 | 0.83 | 1.76 | 0.90 |

Table 2.8. St. Louis top five performance measures based on highest sensitivity*specificity values.

| Accuracy | Sensitivity | Specificity | Sensitivity*Specificity | Kappa | MCC |
|---|---|---|---|---|---|
| 0.96 | 0.86 | 1 | 0.86 | 1.79 | 0.90 |
| 0.95 | 0.86 | 0.98 | 0.84 | 1.67 | 0.87 |
| 0.93 | 0.86 | 0.96 | 0.83 | 1.56 | 0.83 |
| 0.95 | 0.81 | 1 | 0.81 | 1.74 | 0.87 |
| 0.93 | 0.81 | 0.98 | 0.79 | 1.62 | 0.83 |

Table 2.9. Model metrics results for proportion filters compared to original RIBBIT algorithm (0.00).

| Proportion Filter | Accuracy | Sensitivity | Specificity | Kappa | MCC |
|---|---|---|---|---|---|
| 0.10 | 0.93 | 0.61 | 0.98 | 1.51 | 0.69 |
| 0.05 | 0.93 | 0.68 | 0.98 | 1.50 | 0.72 |
| 0.00 | 0.94 | 0.78 | 0.96 | 1.44 | 0.75 |

### *2.3.2 Calling season*

Start and end dates were delineated from the inflection points of the regression (GLM with logit link) of calling (yes/no) to the day of year for each site (Figure 2.6 and Figure 2.7). Calling start dates varied from May 12 to May 29, 2023, in Thunder Bay (Table 2.10) and April 4 to May 15, 2023, in St. Louis (Table 2.11). Calling end dates varied from June 5 to July 1, 2023, and June 8 to July 31 in Thunder Bay and St. Louis respectively (Table 2.10 and Table 2.11). Calling season length varied from 9 to 40 days and 53 to 90 days long in Thunder Bay and St. Louis respectively (Figure 2.8). Based on my generalized linear models, calling started significantly earlier in St. Louis than in Thunder Bay (z=-3.75, P=0.0002) and ended significantly later (z=4.59, P=0.00001). The difference in calling start and end dates resulted in a significantly longer calling season in St. Louis (z=14.22, P = <<0.00001). These results are represented in Figure 2.8.

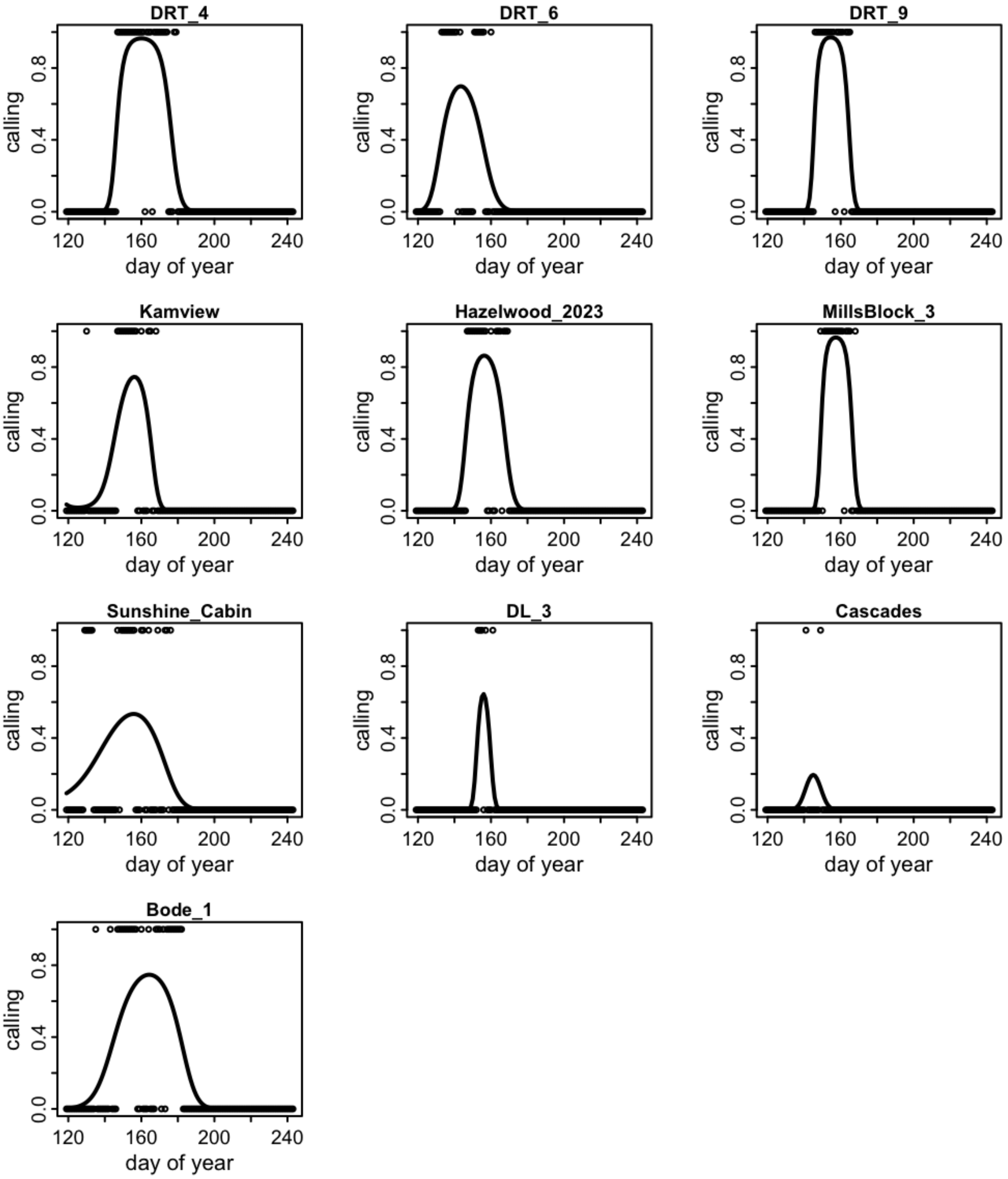

Figure 2.6. Thunder Bay individual site data for the relationship of probability of calling (y-axis) with calling season length (x-axis) for *D. versicolor*, using 3rd order polynomial logistic regression with logit link.

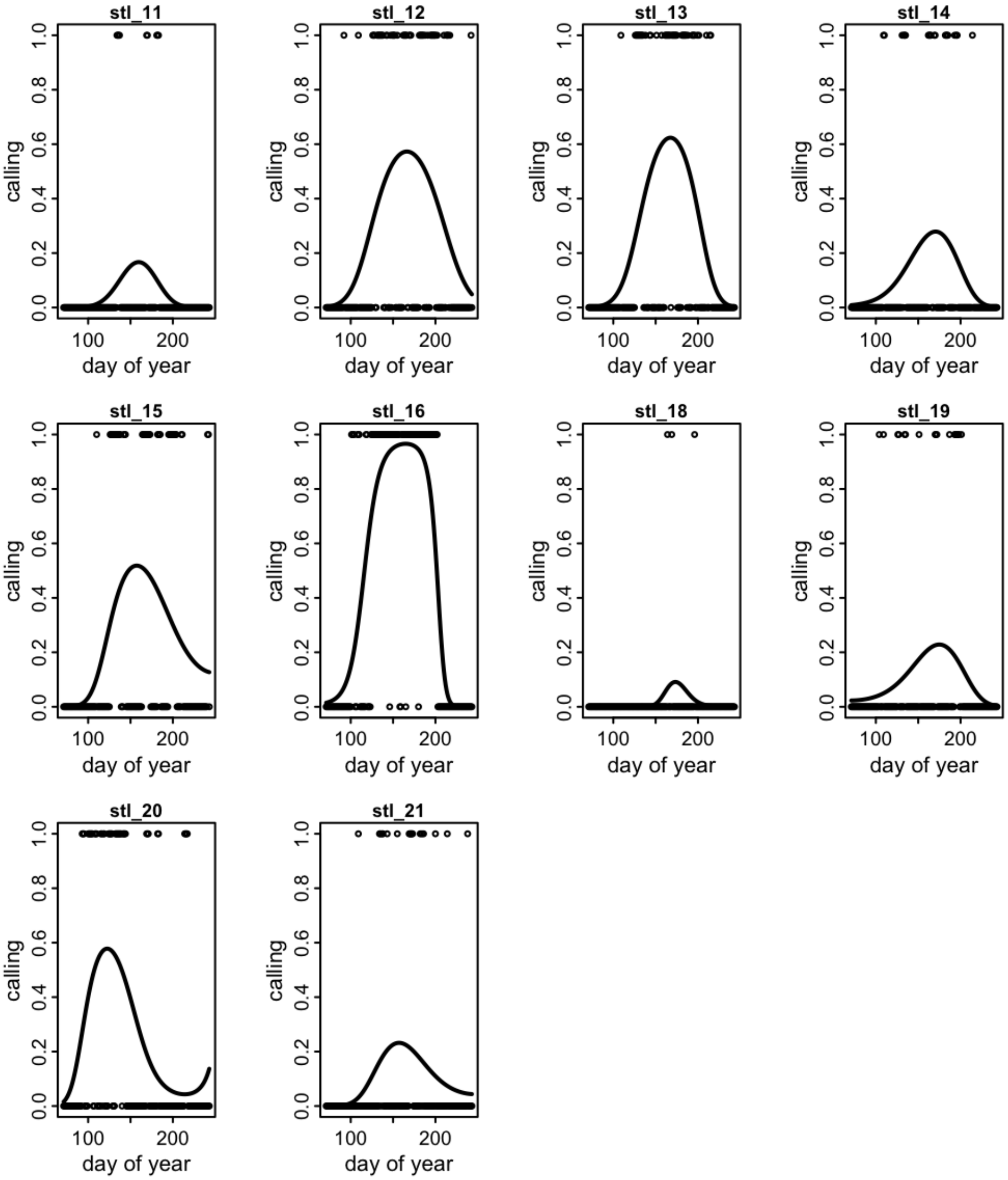

Figure 2.7. St. Louis individual site data for the relationship of the probability of calling (y-axis) with calling season length (x-axis) for *D. versicolor*, using 3rd order polynomial logistic regression with logit link.

Table 2.10. Calling season start and end days of the year and calling season length for Thunder Bay sites.

| Site | Calling start | Calling end | Calling season length |
|---|---|---|---|
| DRT_4 | 147 | 176 | 30 |
| DRT_6 | 132 | 156 | 25 |
| DRT_9 | 146 | 164 | 19 |
| Kamview | 145 | 165 | 21 |
| Hazelwood_2023 | 146 | 167 | 22 |
| MillsBlock_3 | 149 | 166 | 18 |
| Sunshine_Cabin | 134 | 173 | 40 |
| DL_3 | 152 | 160 | 9 |
| Bode_1 | 145 | 182 | 38 |

Table 2.11. Calling season start and end dates in days of the year and calling season length for St. Louis sites.

| Site | Calling start | Calling end | Calling season length |
|---|---|---|---|
| stl_11 | 133 | 185 | 53 |
| stl_12 | 123 | 212 | 90 |
| stl_13 | 130 | 203 | 74 |
| stl_14 | 134 | 201 | 68 |
| stl_15 | 122 | 206 | 85 |
| stl_16 | 116 | 202 | 87 |
| stl_19 | 135 | 206 | 72 |
| stl_20 | 94 | 159 | 66 |
| stl_21 | 126 | 198 | 73 |

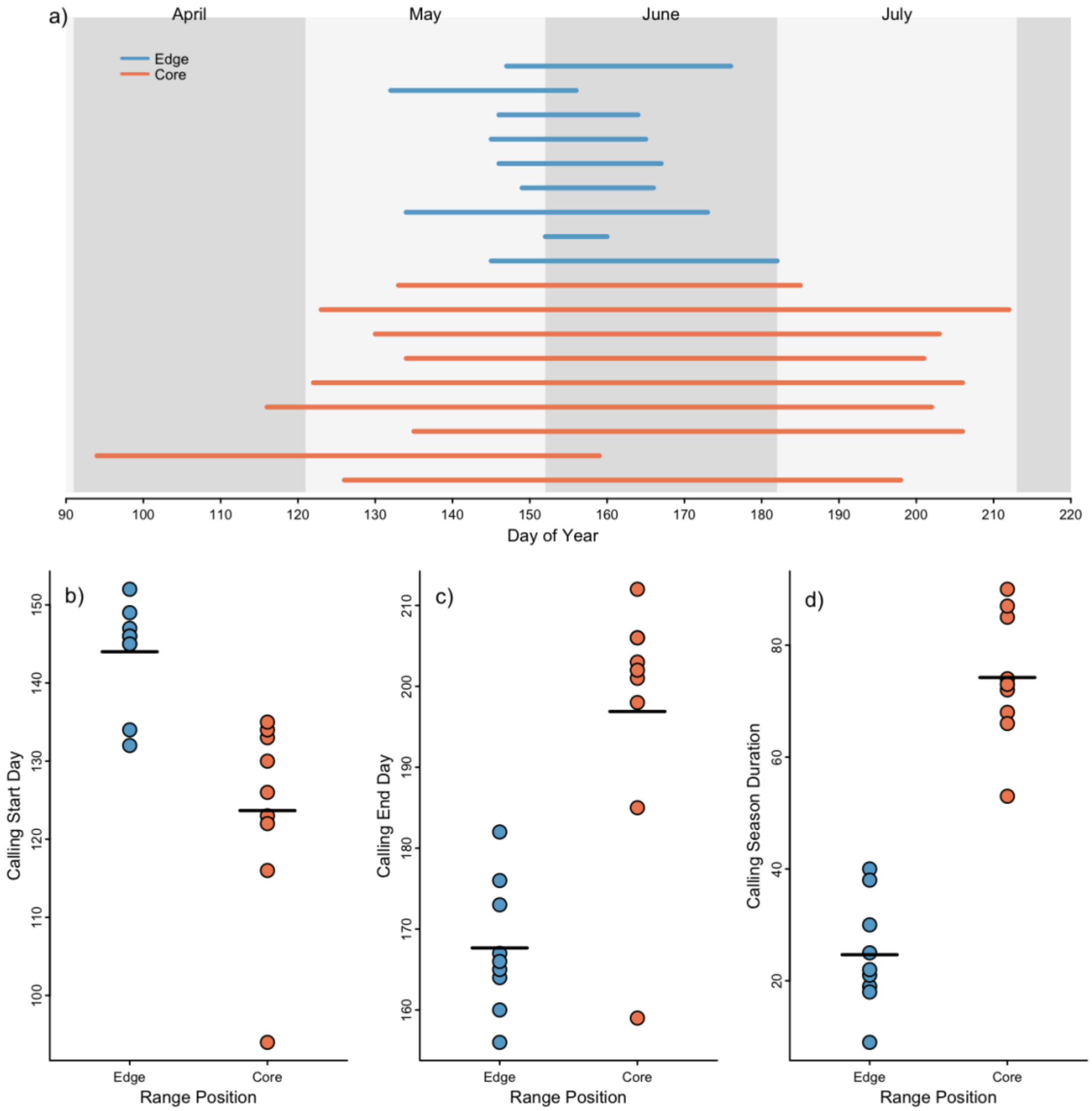

Figure 2.8. Breeding season timing at the range core and edge. a) Horizontal lines show start, end and duration of calling at each site in edge and core regions; b) the relationship between the calling start date at each site and range position (GLM with log link, z=3.75, P=0.0002); c) differences in calling end date (GLM with log link, z=4.59, P<0.00001); and d) differences in calling season duration (GLM with log link, z=14.22, P=<<0.00001). Black horizontal lines depict means.

### *2.3.3 Onset temperature*

The relationships between calling and daily temperature at each site, based on GLMs with logit link are shown in Figure 2.9 and Figure 2.10 and inferred calling onset temperatures in Table 2.12 and Table 2.13. The generalized linear mixed model (region as fixed effect, site as random) for calling onset temperature analysis for the northern range edge to range core showed a significant calling probability in relation to daily mean temperature ($z = 14.6$, $p \ll 0.00001$, see Figure 2.11). Additionally, the calling onset commenced at significantly lower temperatures at the northern range edge ($z = -5.321$, $p \ll 0.00001$). The mean calling onset temperature (± se) for the northern edge was 14.77± 0.49°C and the range core was 21.31± 0.98°C (Figure 2.11). For visualization purposes, panel b) in Figure 2.11 shows the difference in calling onset temperatures for each site at each region.

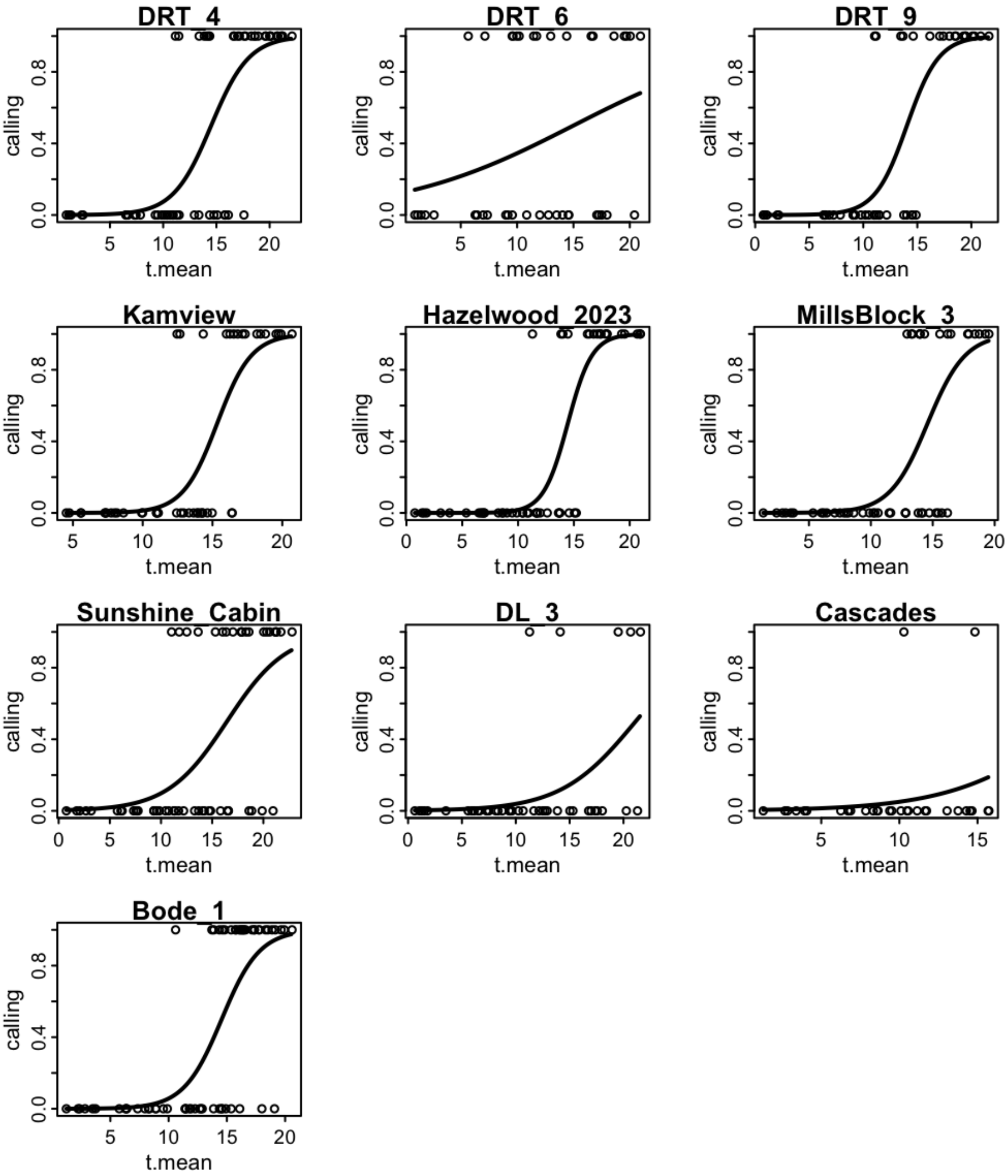

Figure 2.9. Thunder Bay individual site data for onset mean daily temperature (x-axis) in relation to onset of calling (y-axis) using a generalized linear mixed effects model with a logit link.

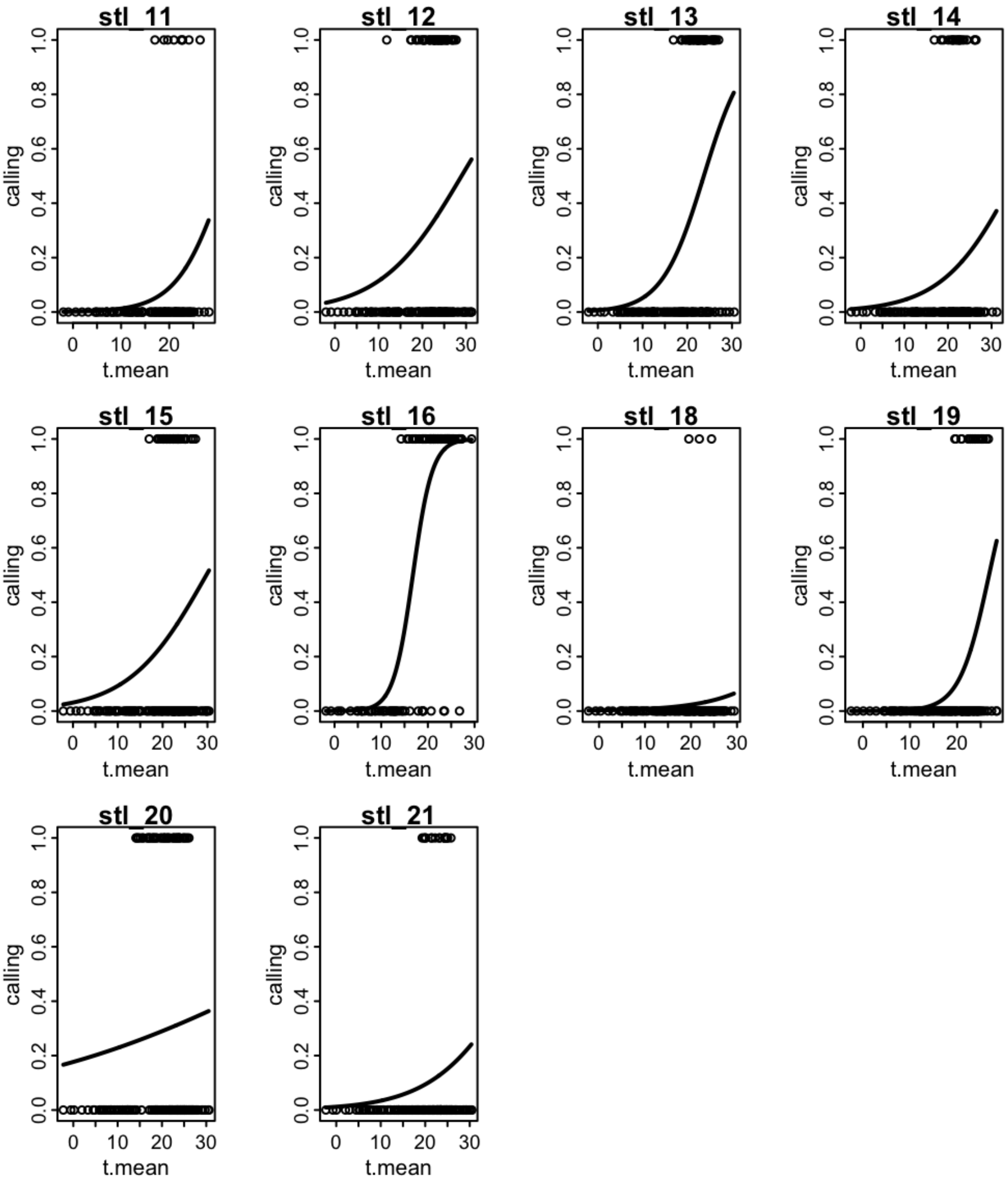

Figure 2.10. St. Louis individual site data for onset mean daily temperature (x-axis) in relation to onset of calling (y-axis) using a generalized linear mixed effects model with a logit link.

Table 2.12. Onset temperature data for each site at the northern range edge (Thunder Bay).

| Site | Temperature Threshold |
|---|---|
| DRT_4 | 14.46 |
| DRT_6 | 12.21 |
| DRT_9 | 14.02 |
| Kamview | 15.30 |
| Hazelwood_2023 | 14.45 |
| MillsBlock_3 | 14.47 |
| Sunshine_Cabin | 15.88 |
| DL_3 | 17.64 |
| Bode_1 | 14.48 |

Table 2.13. Onset temperature data for each site at the range core (St. Louis).

| Site | Temperature Threshold |
|---|---|
| stl_11 | 23.72 |
| stl_12 | 21.02 |
| stl_13 | 21.92 |
| stl_14 | 23.57 |
| stl_15 | 21.19 |
| stl_16 | 16.78 |
| stl_19 | 24.31 |
| stl_20 | 16.22 |
| stl_21 | 23.02 |

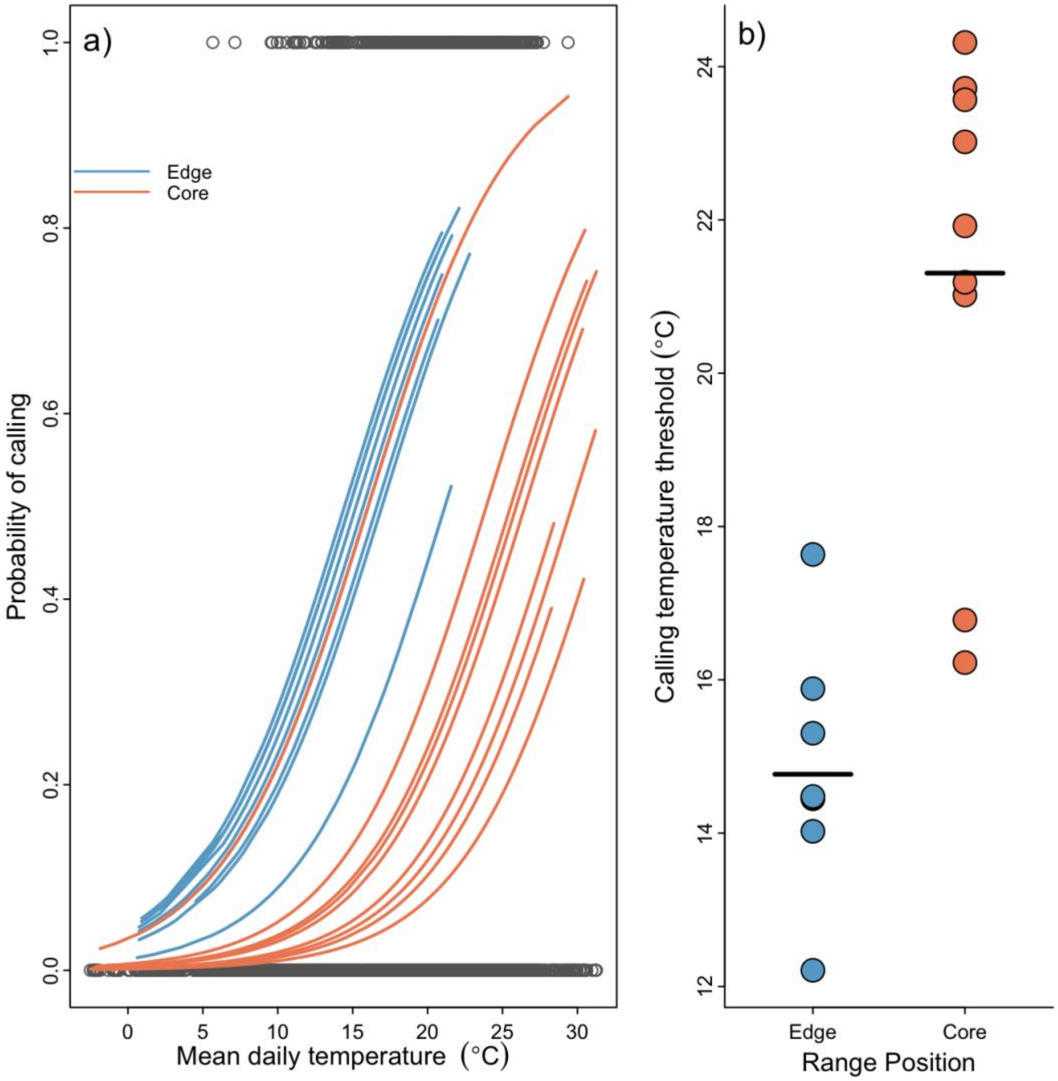

Figure 2.11. Differences in calling onset temperatures at the range core and edge. a) shows a generalized linear mixed model with region as a fixed effect and site as a random effect. Calling probability was significantly related to mean daily temperature (z=14.6, P<<0.00001), and commenced at significantly lower temperatures at the range edge (z=-5.321, P<0.00001). b) shows the relationship between calling onset temperatures at each site (inflection points from logistic regression) and range position. Black horizontal lines depict means. The mean (±se) onset calling temperature at the range edge was 14.77±0.49°C and at the range core it was 21.31±0.98°C.

## 2.4 Discussion

### *2.4.1 Summary*

The goal of this chapter was to evaluate if there are differences in the timing of breeding season, and the temperature at which it commences, between the northern range edge and range core of Eastern gray treefrogs (*Dryophytes versicolor*). I found that populations at the northern range edge, represented by the Thunder Bay region, had a significantly shorter breeding season than at the range core (St. Louis). This included a later onset of calling for the northern range edge and an earlier end to the calling season. Populations at the northern range edge also commenced calling at colder temperatures compared to those at the range core. In addition to determining these differences in breeding season across a geographical gradient, I demonstrated that the RIBBIT algorithm, adapted from Lapp et al. (2021), could be used to automate the detection of *D. versicolor* over 3915 hours of data recordings.

### *2.4.2 Calling season and temperature threshold*

Populations of *D. versicolor* at the northern range edge commenced calling at much lower temperatures than populations at the range core. Prior to my work, there was little existing data on the temperature at which *D. versicolor* initiates calling. Both Dodd (2013) and Harding and Mifsud (2017) suggested that males initiate calling when temperatures reach 15°C but provided no data to support this claim. Bertram and Berrill (1997) stated that males in Ontario (e.g., Thunder Bay) would not call at temperatures less than 8 °C. Furthermore, whether this temperature differed across geographical gradients was unknown. Based on just one season of data collection, I found that populations at the northern range edge commenced calling at 14.77 ± 0.49 (mean ± s.e.) degrees Celsius, which is highly consistent with the proposed 15°C level (Harding and

Mifsud 2017, Dodd 2023). In contrast, populations at the range core did not begin calling until temperatures were much higher (21.31±0.98°C). The differences in onset calling temperatures allows for rejection of hypotheses 2.1 and 2.3 and is consistent with my second hypothesis (H 2.2) that seasonality favours early calling to maximize breeding time. By commencing calling at a lower temperature at the northern range edge, which is highly seasonal, *D. versicolor* can breed earlier than would be possible if they waited until daily temperatures exceeded 21.31°C. Looking at the daily average temperature data I collected for Thunder Bay, some sites reached 21.31°C by June 22, while most sites did not reach this temperature threshold at all. If *D. versicolor* were to delay calling until 21.31°C, i.e., June 22 at the northern range edge, this would greatly limit the time available for their young to develop prior to autumn. Whether *D. versicolor* is reacting directly to temperature as the proximate cue, or some other short or long-term cue, such as photoperiod or rainfall (Canavero and Arim 2009, Huang, Jian-Guo 2020) is unknown but our results suggest that there are temperature thresholds that must be reached for species to begin calling (Llusia et al. 2013), and that these differ greatly with latitude.

Although my results are consistent with the hypothesis that selection has favoured early calling in more northern populations, this does not mean that *D. versicolor* actually called earlier in the year at the northern range edge than the range core. In Thunder Bay, they called earlier than expected based on the relationship of temperature and calling in the range core. Populations in St. Louis still started calling 38 days earlier in the year than in Thunder Bay. They also called until later in the year, suggesting a longer breeding season at the range core. These results are consistent with While and Uller's (2014) study on the effects of global warming on the breeding phenology of amphibians along latitudinal gradients. Their results explain that species at higher latitudes have a limited amount of time for breeding (compared to lower latitudes), causing these populations to begin breeding earlier (While and Uller 2014). While and Uller (2014) suggested that there is a stronger dependence on temperature as a phenological cue to breeding in northern latitudes because of these compressed breeding times and seasonal differences, compared to species in southern latitudes with more stable seasons, that may rely on other environmental cues, rather than temperature. Similarly, Morrison and Hero (2003) reviewed life-history traits of amphibian populations living at different latitudes and altitudes (i.e., along geographical gradients). They concluded that amphibian populations at higher latitudes have shorter breeding seasons due to the severity of seasonality at northern latitudes (Morrison and Hero 2003). *D. versicolor's* shorter breeding season at the northern range edge of its geographical distribution is consistent with this explanation. The compressed breeding season likely reflects their greater vulnerability at higher latitudes to the pressure of freeze risk at the onset of spring, and, simultaneously, the need to breed early to allow for sufficient offspring growth time before conditions degrade in the autumn. My results add further evidence that anuran populations in northern latitudes have more pressure on their breeding season compared to more southern populations.

The difference in onset calling temperature at the northern edge compared to the core of *D. versicolor*'s range could reflect local adaptation or plasticity. Difficulty in differentiating between these possibilities is common in studies of latitudinal variation in life history. For example, Blok et al. (2018) examined life history events of eelgrass populations across gradients of latitude and temperature. They analyzed the timing of flowering, seed maturation, and seedling emergence across a range of latitudes and annual mean air temperature. The timing of these events had significant changes across the latitudinal and temperature gradients, with a delay towards the northern regions. Early emergence was seen in the southern locations and emergence at colder temperatures was visible in northern locations. The authors concluded that increased latitude resulted in lower temperature thresholds for flowering, seed maturation and emergence of seedlings (Blok et al. 2018), but could not differentiate between local adaptation or acclimation. Variation in onset calling temperatures within regions, even at sites that are relatively close together suggest there is at least some plasticity in this threshold (though we can't discount genetic differences within regions as well since there is no information on the landscape genetics of *D. versicolor*), but whether this plasticity is sufficient to generate the large differences in calling onset temperature I observed is unknown. Differentiating between local adaptation and plasticity would require longer term studies to determine year to year variability in onset calling temperatures along with common garden, reciprocal transplant, and/or genetic analysis in this system.

### *2.4.3 RIBBIT*

I used an open-source algorithm based on pulse repetition rate of calls (RIBBIT; (Lapp et al. 2021) to identify *D. versicolor* in my recordings. Using RIBBIT allowed for an extensive set of data to be analyzed accurately and efficiently. Manual listening of the 117,470 audio files or 3915 hours of data recordings would have taken 163 (24 hour) days of listening; this does not include handling time of recordings (e.g., loading files and recording presence-absence). In contrast, once the parameters specific to *D. versicolor* were determined, this extensive set of audio files was analyzed in approximately six hours. The speed of this analysis is important because it allows for larger and more complete samples to be analysed, leading to a more efficient research process and more robust conclusions.

Since RIBBIT can be trained for other anuran species, the bioacoustic data I have collected have the potential to provide further insights into the calling behaviour, geographical distributions and community composition for a broad range of anuran species, and potentially other taxa that also vocalize and other characteristics of the soundscape (Pijanowski et al. 2011). However, there is potential for false positives and false negatives to occur with an automated algorithm. It is extremely important to test the specificity and sensitivity of RIBBIT for each target species, to ensure high accuracy. For example, I found that it was necessary to modify the RIBBIT algorithm using a proportion filter to avoid false positives. Even so, I found that while RIBBIT had high specificity (98.3%), i.e., it had few false positives, it had lower sensitivity (61.18%), i.e., it was prone to false negatives. However, because I was generating daily presence-absence data, false positives had a larger impact on the results than false negatives. This is because I analysed multiple recordings per night; in order to falsely record an absence for a night multiple false negatives (omission errors) would need to be made. However, a single false positive (commission error) in any file would result in a presence being falsely recorded.

### *2.4.4 Implication of results*

My results are important at a broader scale when thinking about climate change and range expansion and/or range shifts. Climate change has the potential to have indirect and direct impacts on a species' geographical range (Duan et al. 2016, Benard and Greenwald 2023). Amphibians are an important group to work towards conserving as they are sensitive to changes in climate due to their biphasic life cycles and highly permeable skin, and thus their numbers could drop quickly and substantially with climate change (Duan et al. 2016, Benard and Greenwald 2023). There are several studies that have created predictive models to understand range shifts of amphibians, as well as review papers on the effects of climate warming on the range of amphibian species (Duan et al. 2016, Benard and Greenwald 2023, Hsu et al. 2023). My results possibly support range expansion for *D. versicolor* as this species is capable of emerging at cooler temperatures compared to the range core, allowing for an expansion in suitable habitat. However, my study does not reach the extent of *D. versicolor's* southern range edge where temperatures are drastically higher compared to the north. As temperatures continue to rise there is a potential that the habitat will no longer be suitable in the south and result in a range shift (Duan et al. 2016). We currently do not have enough information to know for certain if *D. versicolor* will undergo range expansion or range shift, or if there are other environmental factors at play that may affect the range. However, we do have anecdotal evidence that *D. versicolor* is expanding its range, as there is presence data at increasing latitudes near the Thunder Bay northern range edge. Furthering this research in the form of a long-term data set would aid in understanding this thoroughly and knowing what to do in terms of conservation as climate change progresses.

Understanding the start and length of breeding season for *D. versicolor* is important for understanding temporal shifts in emergence and dates of first calling (Klaus and Lougheed 2013). Klaus and Lougheed (2013) analysed a 40-year long-term data set of southern Ontario frog species, including *D. versicolor*, analyzing spring emergence and calling phenology. The authors compared spring emergence and onset of calling to the local temperature of eight species of anurans (Klaus and Lougheed 2013). They concluded that as temperatures have increased (2.7-2.8 degrees Celsius increase over four decades) two species of anurans initiated calling earlier and a third species emerged earlier in the spring, due to increased local temperatures. My results reflect similarly to Klaus & Lougheed, (2013), specifically to *D. versicolor* and the observed earlier calling at higher latitudes. In terms of the increase in temperature and earlier calling I would expect to see similar patterns over time as temperature increases, i.e., earlier emergence and calling would occur for *D. versicolor*. Across the geographical gradient, this is visible as calling begins earlier in the season at warmer temperatures in the range core, compared to the range edge. Continuing my study would contribute to a similar data set to understand if similar patterns are occurring in northern Ontario anuran species, and more specifically to *D. versicolor*.

As mentioned, anurans are, in general, vulnerable to climate change and are at risk of decline and negative impacts due to climate change (Morrison and Hero 2003, Benard and Greenwald 2023). My results can contribute to further understanding the phenology of anurans and how they are currently being impacted by temperature and what may occur in the future in terms of their range shifts or expansion and how this will affect their conservation.

### *2.4.5 Limitations and Assumptions*

It would be valuable to have more sites and expand the number of regions included in the geographical gradient. Originally, I planned to have three regions (Thunder Bay, Chicago, and St. Louis). Chicago unfortunately did not have enough viable data (presences of *D. versicolor* at selected breeding ponds); this region would have represented the midpoint between the northern edge and core. Having a region included in the southern range edge would also have improved my results and allowed for a full north-south geographical range comparison, but this was logistically infeasible. Of course, having multiple years of data, and completing common garden experiments, along with reciprocal transplant experiments, would also strengthen my findings, allowing for greater insight into the extent of plasticity and whether temperature is a consistent cue.

There are multiple environmental factors that may have a role in triggering the onset of breeding phenology in anurans, including *D. versicolor* (Klaus and Lougheed 2013). It was most feasible to include only temperature as the environmental factor, and arguably the most important as there is a strong correlation between warming and latitude (While and Uller 2014). Being able to incorporate more environmental factors at a local scale (for individual breeding sites) would add to the understanding of what initiates calling and/or breeding season. This could be especially useful in the southern latitudes where temperature is more constant, and there are most likely other factors contributing (Morrison and Hero 2003, While and Uller 2014). Ideal factors to include would be rainfall and humidity at the local scale, rather than from existing weather stations which will not capture conditions specific to individual breeding ponds.

It is important to note that there were some assumptions made for my study. It was assumed that the beginning of the calling season for *D. versicolor* is the initiation of their breeding season, and I was unable to differentiate between advertisement and territorial calls. *D. versicolor* are known to call sporadically outside of the breeding season, as they approach and leave breeding ponds (Dodd 2023). Thus, it is possible that I have overestimated the breeding season. However, my approach of using logistic regression to identify the start of calling, rather than the first audible breeding call by a male from each site makes my measure of the start of breeding season less sensitive to sporadic calling by individual males. This also applies to the end of calling season. However, if males call outside of the breeding season more extensively in the range core than the edge, this could impact my results; further work to determine this is necessary. Another limitation is that, with the use of RIBBIT, there was potential in the southern latitudes for the sister species *Dryophytes chrysoscelis* to be erroneously detected as *D. versicolor*. These species calls differ in their pulse rate, with *D. versicolor*'s pulse rate ranging from 17 to 35 and *D. chrysoscelis*' from 34 to 69 (Dodd 2023). To limit the potential of detecting *D. chrysoscelis*, I set the maximum pulse repetition rate in RIBBIT to 32, which is below the minimum reported pulse rate for *D. chrysoscelis*. It was thus assumed that *D. chrysoscelis* would not be erroneously classified as *D. versicolor;* we detected no instances of *D. chrysoscelis* while listening to the training and testing sets of data, which suggests that its prevalence in our study areas was not high.

### *2.4.6 Conclusion*

In summary, I found that *D. versicolor* has a shorter breeding season and begins calling at a colder temperature threshold at its northern range edge, compared to the range core. These results move us one step closer to understanding if local adaptation or plasticity is occurring across *D. versicolor's* geographical range. Further work will need to be completed to compile a long-term data set, as well as considering additional environmental factors that may influence breeding phenology. It is very evident that RIBBIT is an important component of this study and can be used in multiple ways across different species in combination with passive acoustic monitoring, to expand research projects. Anurans are a very sensitive and vulnerable group to climate change and this work contributes to understanding their life history and at what scale or severity they could be impacted by future climate change.

# Chapter 3: A potential endogenous cue for the termination of breeding season of *D. versicolor*

## 3.1 Introduction

Life history evolution is fundamentally shaped by a trade-off between reproduction and adult survival (Morano et al. 2013). Semelparous species invest all their resources in a single reproductive event before death, while iteroparous species undergo multiple reproductive cycles throughout their lives (Morano et al. 2013, Becker et al. 2018). In iteroparous species, selection is expected to maximize lifetime reproductive output, rather than output in a single season of reproduction. When investing heavily in immediate reproduction there is a cost to future survival (or future reproduction), however, to maximize lifetime reproductive fitness, individuals may limit their immediate reproduction to maintain higher fitness in future years (Williams 1966). For example, Morano et al. (2013) found such a trade-off in North American elk, where reproduction during one season came at the cost of reduced probability of future reproduction. This variable reproduction allowed for more stable and higher adult survival (Morano et al. 2013). As reproduction can be an extremely costly life-history event, for both males and females of a species (Williams 1966, Bell 1980, Stearns 1989), the implications for fitness are high if organisms invest poorly. Exploring what limits individuals' reproductive investment, to allow for a higher chance of survival to the next breeding season, can aid in further understanding life history evolution (Becker et al. 2018).

Reproductive investment in anurans varies for a range of reasons, including environmental susceptibility (i.e., weather), amount of energy invested, and differences in annual life cycles (Taigen and Wells 1985, Lemckert and Shine 1993, Becker et al. 2018). An example of a reproduction-adult survival trade-off in an ectotherm is found in Rose's Mountain toadlet (*Capensibufo rosei*). Becker et al. (2018) found a breeding season trade-off due to the amount of rainfall in each particular year. When the breeding season was wet, these toads increased their reproductive output at the cost of their survival. In contrast, during dry breeding seasons, their survival increased at the cost of reproduction for that season (Becker et al. 2018). Although the authors stated more data was required, they could still conclude that this species adaptively responds to the annual amount of rainfall. An older study by Lemckert and Shine (1993) described the extreme reproductive investment by the common southeastern Australian frog (*Crinia signifera*). It was determined that males remain at breeding ponds for several weeks at a time, enduring high rates of body mass loss and exposure to high mortality rates due to predators. Females also invested extremely into reproduction with heavy clutches of eggs and reproducing more than once a year, despite the cost of energy (Lemckert and Shine 1993). The authors concluded that because a reproducing frog was unlikely to survive long enough to breed again, natural selection favoured extreme reproductive investment, making subsequent reproductive events even less likely (Lemckert and Shine 1993). *D. versicolor* also invest heavily in reproduction. The male call is one of the most energetically expensive activities of any anuran (Taigen and Wells 1985) and males lose mass during their breeding season due to this energetic cost of calling, like other frog species (Lemckert and Shine 1993, Dodd 2023). Males are also at high risk of predation due to the high exposure from calling (Lemckert and Shine 1993, Dodd 2023). Females will also invest a lot in egg production, despite not producing a call (Dodd 2023).

It is important to understand the extent to which *D. versicolor* will invest in reproduction in the context of a yearly breeding season, as this could be an endogenous factor to cue the end of breeding season. Based on Chapter 1, while recent years have seen considerable focus on environmental effects on the onset of breeding in a range of taxa, particularly in relation to climate change (Reed et al. 2013), it has proven difficult to determine any significant environmental factors that govern the cessation of breeding season, This may be because cessation of breeding reflects endogenous cues (i.e., related to the organism) rather than exogenous (e.g., environmental) cues. Given that male *D. versicolor* can potentially mate for multiple seasons, and that breeding calling behaviour is energetically expensive (Taigen and Wells 1985), the termination of breeding season may be related to the cost-benefit of reproductive investment in *D. versicolor*. If there is a trade-off between reproductive investment and survival to the subsequent breeding season, males may abandon breeding ponds when either benefits (reproductive opportunities) decline or costs (energy expenditure) rise.

To explore life-history trade-offs within *D. versicolor*, specifically in terms of reproductive investment, and potentially understand what may cause the cessation of breeding season, I propose the following hypotheses:

Hypothesis 3.1: The termination of male advertisement is determined by declining benefits, i.e., mating opportunities. Prediction: Males are more likely to abandon breeding ponds as the number of females in ponds decreases (Figure 3.1 a).

Hypothesis 3.2: The termination of male advertisement is determined by rising energetic costs. Prediction: There is a percent mass-loss threshold at which males abandon breeding ponds. (Figure 3.1b).

Hypothesis 3.3. Male advertisement termination is determined by balancing the benefits of breeding opportunities and the cost of calling. Prediction: If male mass loss is high but there is an abundance of females available, males should continue calling; alternatively, if there are few females available, males should stop calling even if the percent mass loss is low. i.e.,, the percent mass loss threshold where males stop advertising is a positive function of the number of females (Figure 3.1c)

Additionally, I tested for a reproduction-survival trade-off in *D. versicolor:*

Hypothesis 3.4. Individuals who invest less in reproduction are more likely to return the following breeding season. Prediction: Recaptured males will be individuals who lost less mass in the 2023 breeding season (Figure 3.1d).

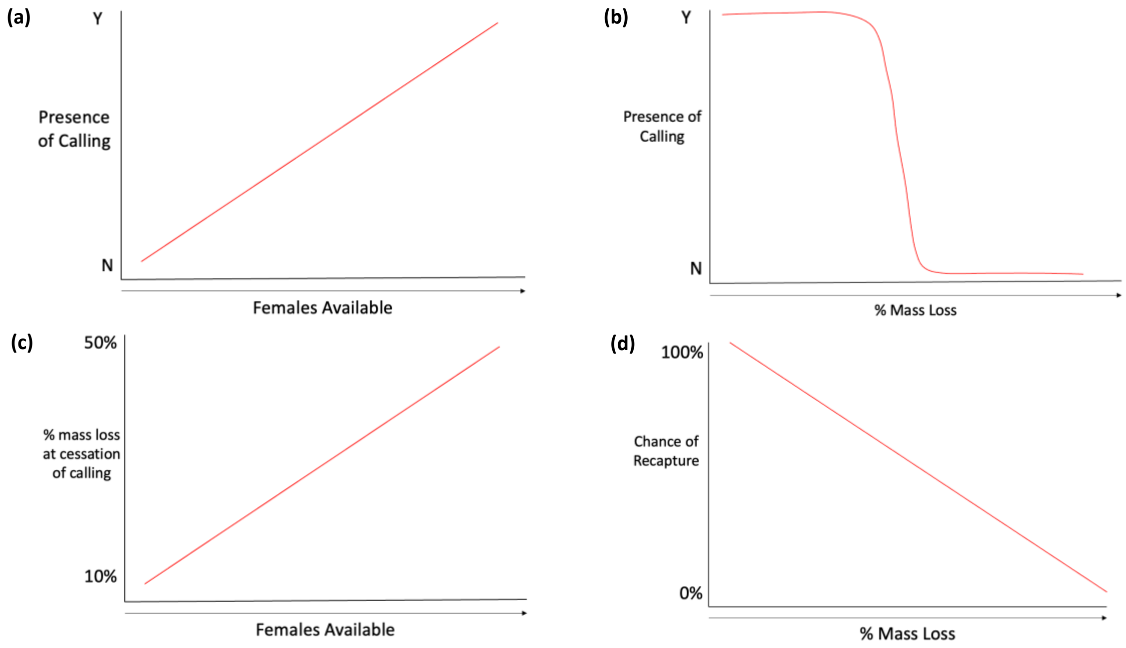

Figure 3.1. Predictions of three hypotheses to determine end of male advertisement at the northern range edge (Hypotheses 3.1, 3.2, and 3.3). (a) Hypothesis 3.1, where the cessation of calling will occur when less females are present. (b) Hypothesis 3.2 where there is a threshold mass loss in males, causing cessation of calling. (c) Hypothesis 3.3 where the mass loss threshold in males as a positive function of the number of females present. (d) Hypothesis 3.4, males that lose less mass have a higher chance of recapture the following breeding season.

## 3.2 Methods

### *3.2.1 Study Site*

Field surveys were conducted at Hazelwood Lake Conservation Area near Thunder Bay, Ontario, Canada (48.58723797, -89.29544999), a conservation area under the Lakehead Region Conservation Authority (LRCA). A small, semi-permanent pond at Hazelwood, known to be used by *D. versicolor* as a breeding site, was selected for surveys. The pond was approximately 33m long by 30m wide for an area of 990m$^2$. Before surveying, the pond was divided into six sections (Figure 3.2) with corners marked with reflective tape to allow for section boundaries to be visible at night using flashlights.

### *3.2.2 Male Surveys 2023*

Surveying occurred every second night, starting on May 26, 2023, until June 23, 2023, when males stopped calling. One exception was on June 17 when no survey occurred due to the low humidity and low temperature that night, which avoided unnecessary habitat disturbance. Surveys resumed June 18 followed by June 19 to resume the initial schedule. The end of calling season was determined by three consecutive survey nights of no calling, without any extreme weather events (i.e., no heavy rain or thunderstorms that limit *D. versicolor's* calling). Surveys were carried out by two people (me and my field assistant, Olivia Leach; OL) Each section of the divided pond was surveyed for 10 minutes using flashlights and headlamps by either me or OL. To avoid sampling bias the sections that OL or I surveyed were switched each sample night, additionally to avoid disturbance from the other sampler, we would begin sampling on opposite sides of the pond. For example, on the first survey night I sampled sections E, C, and A and OL sampled F, D, and B. The following sample night I sampled sections B, D, and F, and OL sampled A, C, and E. See Figure 3.2 for a diagram of the breeding pond and how each section was divided. Calling males were captured by hand and placed in ventilated plastic containers with wet paper towel at the bottom. All sections were surveyed before tagging or measuring individuals.

For each captured male, snout-vent length (svl) was measured with a ruler to the nearest 1.0 mm and weighed using a digital scale (PESOLA PPS200) to the nearest 0.01g. Before being weighed, each individual was lightly compressed to release any urine. Individual BioMark PIT tags were then implanted subcutaneously on the shoulder of each male. This was done using a disinfected needle and syringe, along with tweezers to lift the skin and inject the tag. Wounds were closed using 3M Vetbond, and animals were then placed back into their containers to rest. Each male was monitored for any signs of distress or injury, before being released. Measurements for svl and mass along with tag identification numbers were recorded before males were returned to place of capture in the pond. After the initial night of surveying, males were scanned using the HPR Lite PIT tag scanner to record recaptures. Mass and svl measurements were recorded for every night of capture.

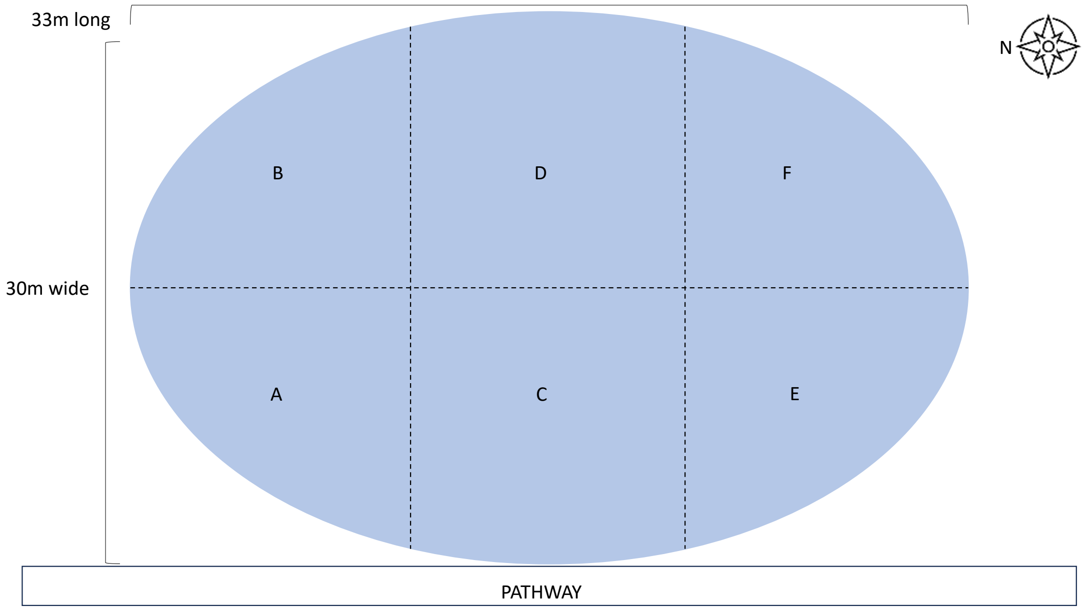

Figure 3.2. Diagram of Hazelwood *D. versicolor* breeding pond, with labelled sections.

### *3.2.3 Female Surveys 2023*

I intended to record the number of females observed in each pond section on each survey night, as well as the number of amplectic pairs. However, during initial surveys, it became clear that females could not be effectively surveyed in this way as none were observed and only three amplectic pairs were observed across all survey nights. Thus, I was unable to obtain sufficient data to evaluate hypotheses 3.1 and 3.3 so these were not considered further.

### *3.2.4 Mass Loss Threshold*

I used a null model to test whether there was a consistent mass loss threshold at which males abandoned the breeding pond. For each individual, I calculated departure mass loss as the mass difference between the final and initial capture and divided this by the individual's initial mass to get a measure of proportional mass change, where negative values indicate mass loss. I next calculated the standard deviation of departure mass loss across all males that were captured more than once. If there is a consistent mass loss threshold that induces males to abandon the breeding pond, then the standard deviation of proportion mass loss across individuals should be low (i.e., similar across individuals). To test this prediction, I constructed a null model by randomly selecting a departure day for each individual from their set of recapture mass data and computing the standard deviation of random departure mass losses across individuals. I repeated this 1000 times to generate a null distribution of expected variation in departure mass loss if individuals departed the pond for reasons unrelated to mass loss. I then computed the probability of observing a standard deviation of departure mass loss as low as I did by chance (P), using the following formula (Ruxton and Neuhäuser 2013):

$$P = \frac{1 + \sum_{i=1}^{N} I\left(s_{null,i} \leq s_{obs}\right)}{1 + N}$$

Where *I* is a function that takes a value of 1 when its argument is true and 0 otherwise, $s_{null}$ is a vector of length N composed of the null standard deviations of departure mass loss and $s_{obs}$ is the observed standard deviation of departure mass loss.

### *3.2.5 Male Surveys 2024*

During *D. versicolor's* breeding season of 2024, I carried out three additional nights of sampling (June 5, 12, and 13 2024) at Hazelwood Lake Conservation Area in the same pond as 2023. For these surveys I with OL (June 5 and 13) or Adam Algar (June 12) surveyed for one hour, collecting calling males and scanning them with the HPR Lite PIT tag scanner, to determine if they had a tag from the previous season. Males were then returned to their approximate place of capture. Males that had a tag were recorded, and a tally of un-tagged males was recorded as well. No further measurements were taken.

### *3.2.6 Recapture rate and 2023 departure mass loss*

I used a null model to determine whether males that lost less mass over the 2023 breeding season were more likely to be recaptured in 2024. I first ranked all captured males from the 2023 season based on their final departure mass loss, where a rank of 1 represented the individual that invested the most in reproduction, i.e., lost the most mass. I used ranks because the number of recaptures was small (n=6) and thus sensitive to single extreme values. I next computed the mean rank of the individuals recaptured in 2024 and compared this to a null distribution of mean ranks. The null distribution was constructed by randomly selecting 6 individuals from those tagged in 2023 and computing the mean rank; this was repeated 1000 times and a P-value was calculated as in section 3.2.4.

## **3.3 Results**

### *3.3.1 Male Surveys*

In 2023, I captured, and PIT tagged 29 individual males. Captures occurred from May 26, 2023, until June 18, 2023 (followed by three more survey days of no *D. versicolor* presences). Males had an average initial mass (±sd) of 8.74g ± 2.01g and had an average snout-vent length (svl) of 4.55cm ± 0.35cm. The heaviest male had an initial mass of 12.15g and the lightest male had an initial mass of 5.79g (Table 3.1). The greatest proportion of mass loss was 0.19 and the smallest proportion of mass loss was 0.04 (excluding single captures); one individual had a proportion mass gain of 0.06 (Table 3.2). All weights and capture dates for each captured male can be found in Table 3.1. The snout-vent length measurements were not utilized for my analysis but can be found in Appendix A. The number of times each male was captured varied between one and seven (Figure 3.3). Eleven individuals were captured only once. The median number of captures was three (four excluding single captures).

Table 3.1. Mass (grams) for individual captured *D. versicolor* males throughout the breeding season at Hazelwood Conservation Area. Individuals are identified by the last four digits of their PIT tag number at top of each column.

| Date | 1082 | 1090 | 1091 | 1092 | 1093 | 1096 | 1104 | 1106 | 1109 | 1110 | 1128 | 1132 | 1134 | 1135 | 1136 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2023-05-26 | 9.03 | 5.88 | 11.97 | | | | | | | | | | | | |
| 2023-05-28 | | | 11.77 | | 11.25 | | | | 10.58 | | | | | | |
| 2023-05-30 | 7.85 | | 10.5 | | 10.39 | | | | 10.24 | 8.99 | | | | | |
| 2023-06-01 | 7.5 | | 9.96 | | 9.82 | 5.88 | | 9.52 | 9.18 | | | 7.29 | 5.79 | | |
| 2023-06-03 | 7.33 | | 10.03 | 7.53 | 9.8 | 6.09 | 7.03 | 9.87 | 9.52 | 8.42 | 7.69 | 6.65 | | | |
| 2023-06-05 | 7.29 | | | | 9.66 | | | | 9.12 | 8.09 | | 6.96 | | 5.39 | |
| 2023-06-07 | | | | | | | | | | | | | | | |
| 2023-06-09 | 7.32 | | | | | | | 9.1 | 9.41 | 7.79 | | | | | |
| 2023-06-11 | | | | | | | | | | | | | | | |
| 2023-06-13 | | | 10.83 | | | 6.27 | | 8.52 | 8.91 | | | | | | |
| 2023-06-15 | | | | | | | | | | | | | | | |
| 2023-06-18 | | | | | | | | | | | | | | | 9.00 |
| 2023-06-19 | | | | | | | | | | | | | | | |
| 2023-06-21 | | | | | | | | | | | | | | | |
| 2023-06-23 | | | | | | | | | | | | | | | |

Table 3.1. Continued. Mass (grams) for individual captured *D. versicolor* males throughout the breeding season at Hazelwood Conservation Area. Individuals are identified by the last four digits of their PIT tag number at top of each column.

| Date | 1137 | 1145 | 1150 | 1151 | 1154 | 1156 | 1160 | 1167 | 1170 | 1172 | 1174 | 1178 | 1179 | 1181 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2023-05-26 | | | | | | | | | | | | | | |
| 2023-05-28 | | | | | | | 6.33 | | | | | | 12.15 | |
| 2023-05-30 | | 10.21 | 10.16 | | 11.2 | 9.84 | 6.17 | 7.2 | | | 6.24 | | 11.86 | |
| 2023-06-01 | | | 9.71 | 9.93 | 10.28 | 8.84 | | | 10.86 | | | | | 9.14 |
| 2023-06-03 | | 8.75 | 9.06 | 9.28 | 10.25 | | 5.49 | | 10.21 | | 6.35 | 10.58 | | 8.36 |
| 2023-06-05 | 9.03 | 9.08 | 9.56 | 9.19 | 9.45 | | | | | 7.76 | 5.85 | | | 8.08 |
| 2023-06-07 | | | | | | | | | | | | | | |
| 2023-06-09 | | | | | | | | | 10.89 | | | | 10.8 | 7.96 |
| 2023-06-11 | | | | | | | | | | | | | | |
| 2023-06-13 | | | 8.82 | | | | 5.85 | | 10.12 | | | | 10.65 | 7.63 |
| 2023-06-15 | | | | | | | | | | | | | | |
| 2023-06-18 | | | | | | | 6.07 | | | | | | 11.31 | |
| 2023-06-19 | | | | | | | | | | | | | | |
| 2023-06-21 | | | | | | | | | | | | | | |
| 2023-06-23 | | | | | | | | | | | | | | |

Table 3.2. Proportion of mass remaining from initial mass, for individual captured *D. versicolor* males, at Hazelwood Conservation Area. Individuals are identified by the last four digits of their PIT tag number at top of each column.

| Date | Survey | 1082 | 1090 | 1091 | 1092 | 1093 | 1096 | 1104 | 1106 | 1109 | 1110 | 1128 | 1132 | 1134 | 1135 | 1136 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2023-05-26 | 0 | 1 | 1 | 1 | | | | | | | | | | | | |
| 2023-05-28 | 1 | | | 0.98 | | 1 | | | | 1 | | | | | | |
| 2023-05-30 | 2 | 0.87 | | 0.88 | | 0.92 | | | | 0.97 | 1 | | | | | |
| 2023-06-01 | 3 | 0.83 | | 0.83 | | 0.87 | 1 | 1 | 1 | 0.87 | | | | | | |
| 2023-06-03 | 4 | 0.81 | | 0.84 | 1 | 0.87 | 1.03 | | 1.04 | 0.90 | 0.94 | 1 | 1 | 1 | | |
| 2023-06-05 | 5 | 0.81 | | | | 0.86 | | | | 0.86 | 0.90 | | 0.91 | | | |
| 2023-06-07 | 6 | | | | | | | | | | | | 0.95 | | 1 | |
| 2023-06-09 | 7 | 0.81 | | | | | | | 0.96 | 0.89 | 0.87 | | | | | |
| 2023-06-11 | 8 | | | | | | | | | | | | | | | |
| 2023-06-13 | 9 | | | 0.90 | | | 1.07 | | | 0.84 | | | | | | |
| 2023-06-15 | 10 | | | | | | | | | | | | | | | |
| 2023-06-18 | 11 | | | | | | | | | | | | | | | 1 |
| 2023-06-19 | 12 | | | | | | | | | | | | | | | |
| 2023-06-21 | 13 | | | | | | | | | | | | | | | |
| 2023-06-23 | 14 | | | | | | | | | | | | | | | |

Table 3.2. Continued. Proportion of mass remaining from initial mass, for individual captured *D. versicolor* males, at Hazelwood Conservation Area. Individuals are identified by the last four digits of their PIT tag number at top of each column.

| Date | Survey | 1137 | 1145 | 1150 | 1151 | 1154 | 1156 | 1160 | 1167 | 1170 | 1172 | 1174 | 1178 | 1179 | 1181 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2023-05-26 | 0 | | | | | | | | | | | | | | |
| 2023-05-28 | 1 | | | | | | | 1 | | | | | | 1 | |
| 2023-05-30 | 2 | | 1 | 1 | | 1 | 1 | 0.97 | 1 | | | 1 | | 0.98 | |
| 2023-06-01 | 3 | | | 0.96 | 1 | 0.92 | 0.90 | | | 1 | | 1.02 | 1 | | 1 |
| 2023-06-03 | 4 | | 0.86 | 0.89 | 0.93 | 0.91 | | 0.87 | | 0.94 | | | | | 0.91 |
| 2023-06-05 | 5 | 1 | 0.89 | 0.94 | 0.93 | 0.84 | | | | | 1 | 0.94 | | | 0.88 |
| 2023-06-07 | 6 | | | | | | | | | | | | | | |
| 2023-06-09 | 7 | | | | | | | | | 1 | | | | 0.89 | 0.87 |
| 2023-06-11 | 8 | | | | | | | | | 0.93 | | | | | |
| 2023-06-13 | 9 | | | 0.87 | | | | 0.92 | | | | | | 0.88 | 0.83 |
| 2023-06-15 | 10 | | | | | | | | | | | | | | |
| 2023-06-18 | 11 | | | | | | | 0.96 | | | | | | 0.93 | |
| 2023-06-19 | 12 | | | | | | | | | | | | | | |
| 2023-06-21 | 13 | | | | | | | | | | | | | | |
| 2023-06-23 | 14 | | | | | | | | | | | | | | |

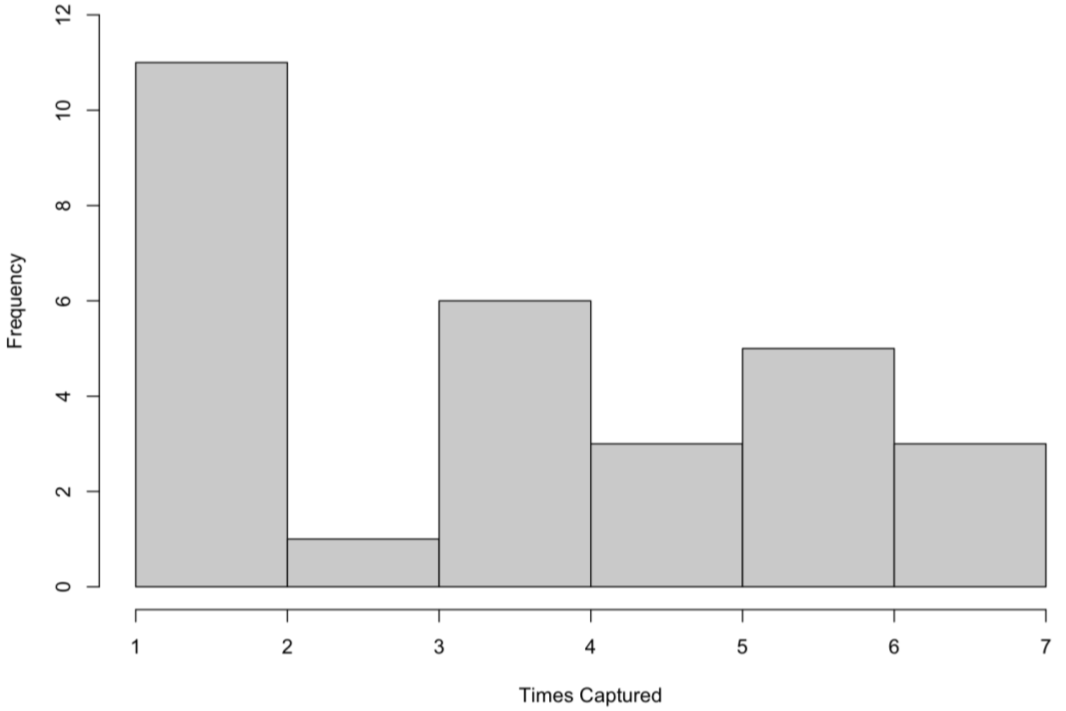

Figure 3.3. Distribution of capture number for *D. versicolor* males from Hazelwood Pond 2023.

### *3.3.2 Mass Loss at Departure*

Of the 18 individuals that were captured more than once, 17 had a lower weight on their final night of capture than on their initial night, i.e., they lost mass. A binomial test was used to test whether more males lost mass than expected by chance (null hypothesis: probability of mass loss = 0.5) with a resulting P value of 0.00015. The average (± sd) mass loss by the day of departure was 0.10 ± 0.05 of the initial mass (Figure 3.4). While over the whole breeding season, individuals tended to lose mass, mass changed positively and negatively between captures (Figure 3.5). The observed standard deviation of departure mass loss was 0.06, while the mean (±sd) of the null distribution was 0.06 ± 0.005 (Figure 3.6). The standard deviation of departure mass loss did not differ significantly from the null expectation (P=0.50).

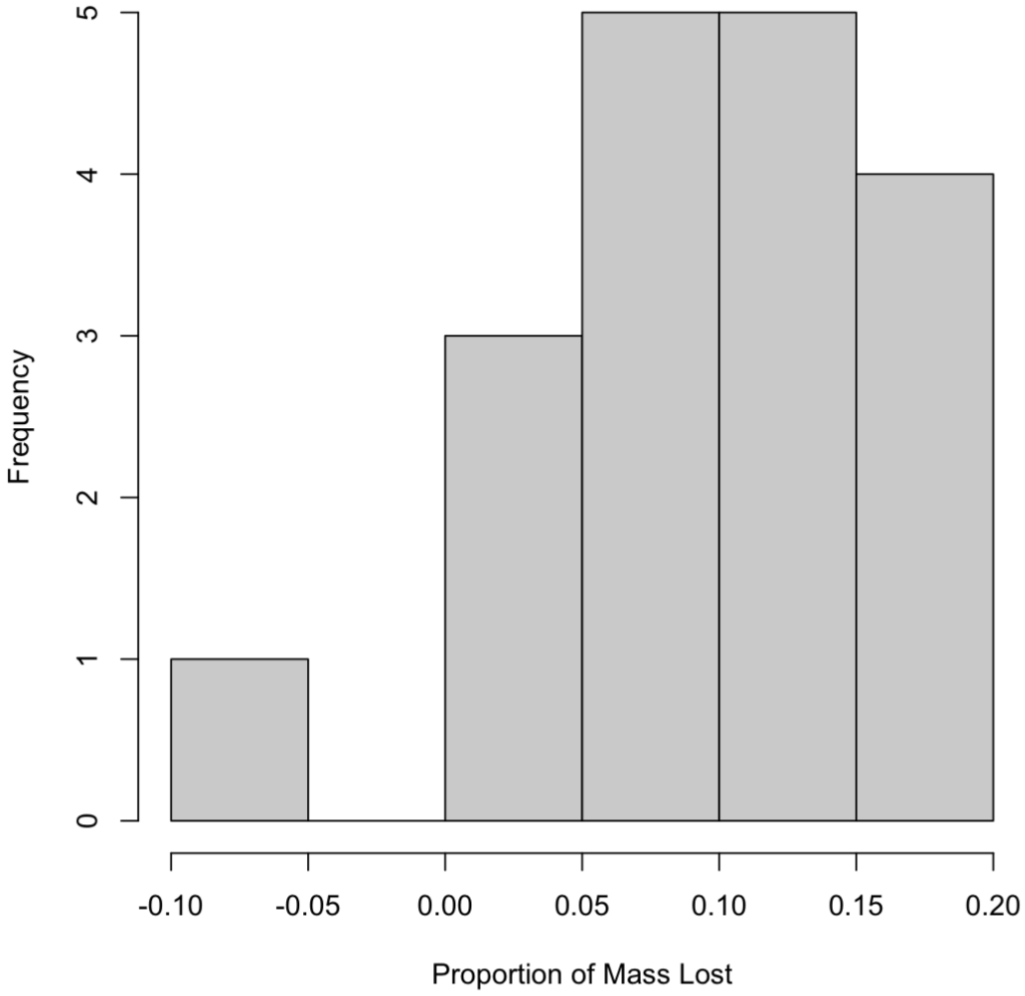

Figure 3.4. Distribution of proportion of mass lost at departure for *D. versicolor* males at Hazelwood Pond in 2023.

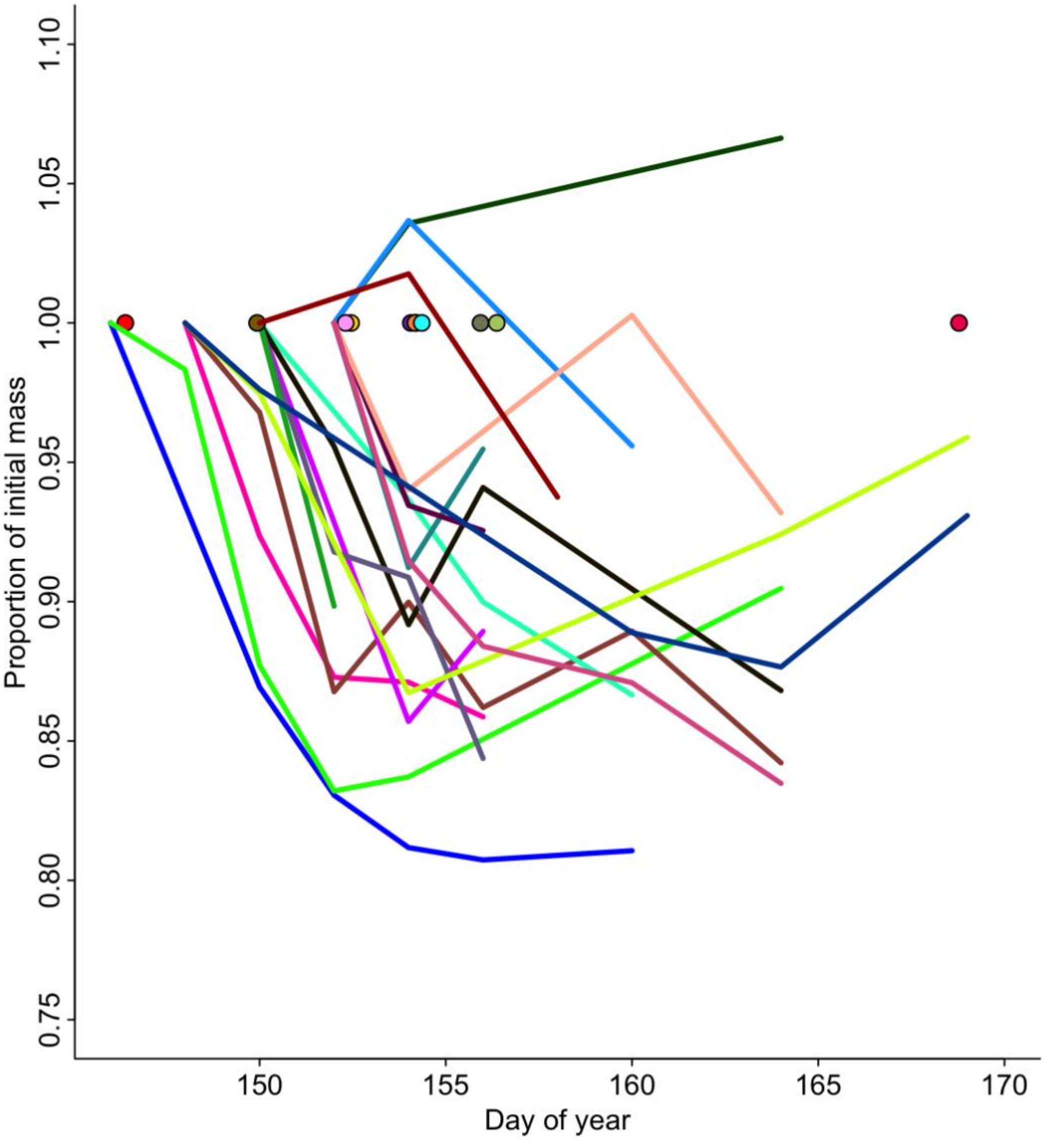

Figure 3.5.Proportion of initial mass through time of captured male *D. versicolor* from Hazelwood Conservation Area. Each colour represents a different male, with the circles indicating males that were captured a single time.

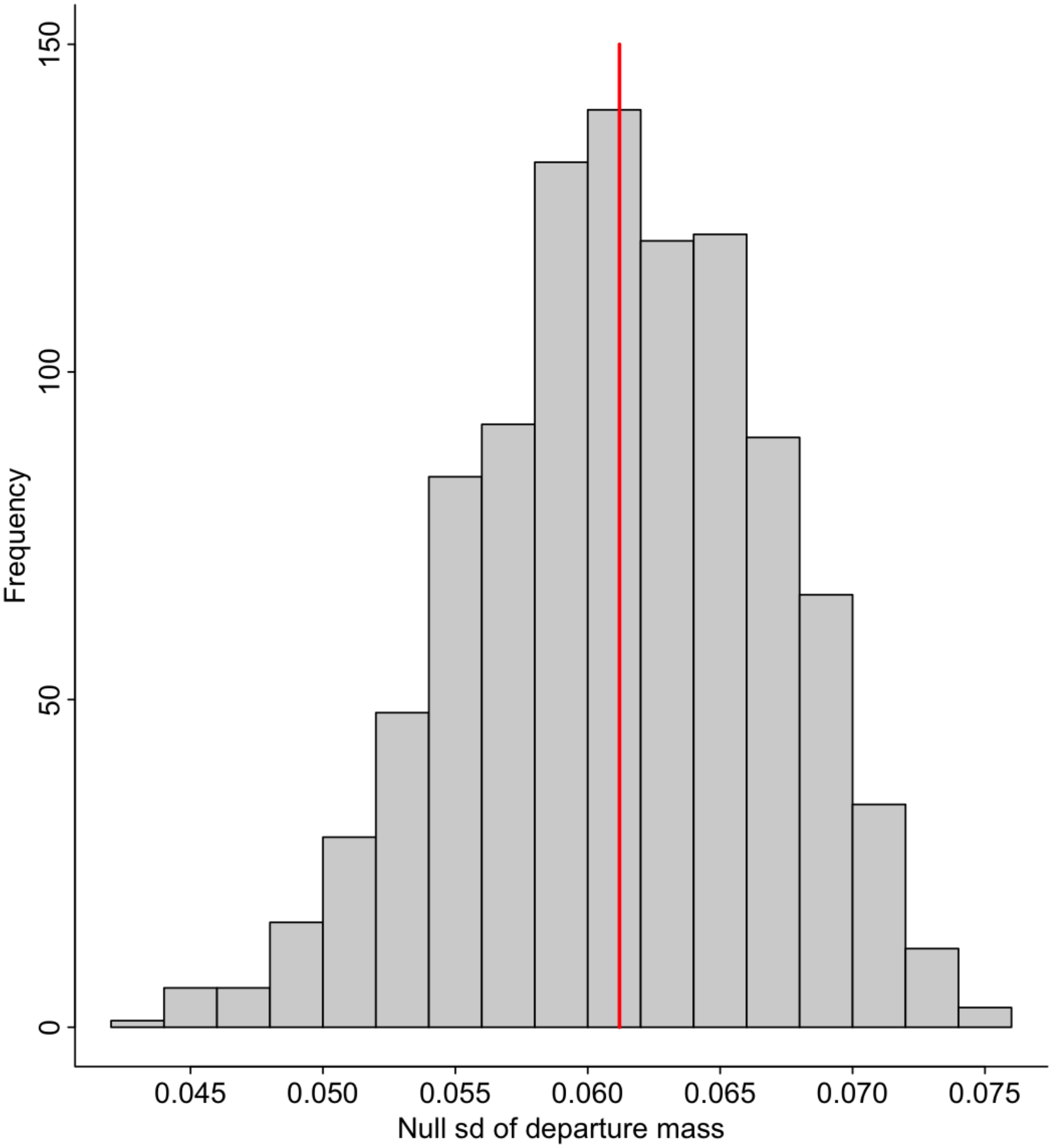

Figure 3.6. Null distribution of standard deviation of departure mass change for males of *D. versicolor* in 2023. Red line represents observed departure mass standard deviation (observed sd = 0.06, p-value = 0.50).

### *3.3.3 Recapture Rate*

During the 2024 field season, six *D. versicolor* males were recaptured (Table 3.3). These were ranked 11, 15, 23 (x3) and 29 (Table 3.4) in terms of proportion mass lost in 2023, with a mean rank of 20.67. The null distribution, which assumed that recaptures were independent of 2023 mass loss, had an average mean (±sd) rank of 14.95 ± 3.13. Based on this null distribution, the probability of observing a mean rank of 20.67 or higher was 0.04 (Figure 3.7).

Table 3.3.Recapture data for male *D. versicolor* in 2024 at Hazelwood Conservation Area.

| Survey Date | PIT Tag ID of Recaptured Males | Number of Untagged Males |
|---|---|---|
| 2024-06-05 | 1128 | 3 |
| 2024-06-12 | 1096, 1128, 1137, 1132 | 10 |
| 2024-06-13 | 1096, 1128, 1136, 1151 | 7 |

Table 3.4. Ranking values and remaining mass proportion at departure for *D. versicolor* males from 2023 field surveying. Bolded individuals represent recaptures from 2024 surveying.

| *D. versicolor* Male Identification Number | Ranking Number | Proportion of Weight Remaining |
|---|---|---|
| 1082 | 1 | 0.81 |
| 1181 | 2 | 0.83 |
| 1109 | 3 | 0.84 |
| 1154 | 4 | 0.84 |
| 1093 | 5 | 0.86 |
| 1110 | 6 | 0.87 |
| 1150 | 7 | 0.87 |
| 1145 | 8 | 0.89 |
| 1156 | 9 | 0.90 |
| 1091 | 10 | 0.90 |
| **1151** | **11** | **0.93** |
| 1179 | 12 | 0.93 |
| 1170 | 13 | 0.93 |
| 1174 | 14 | 0.94 |
| **1132** | **15** | **0.95** |
| 1106 | 16 | 0.96 |
| 1160 | 17 | 0.96 |
| 1090 | 23 | 1.00 |
| 1092 | 23 | 1.00 |
| 1104 | 23 | 1.00 |
| **1128** | **23** | **1.00** |
| 1134 | 23 | 1.00 |
| 1135 | 23 | 1.00 |
| **1136** | **23** | **1.00** |
| **1137** | **23** | **1.00** |
| 1167 | 23 | 1.00 |
| 1172 | 23 | 1.00 |
| 1178 | 23 | 1.00 |
| **1096** | **29** | **1.07** |

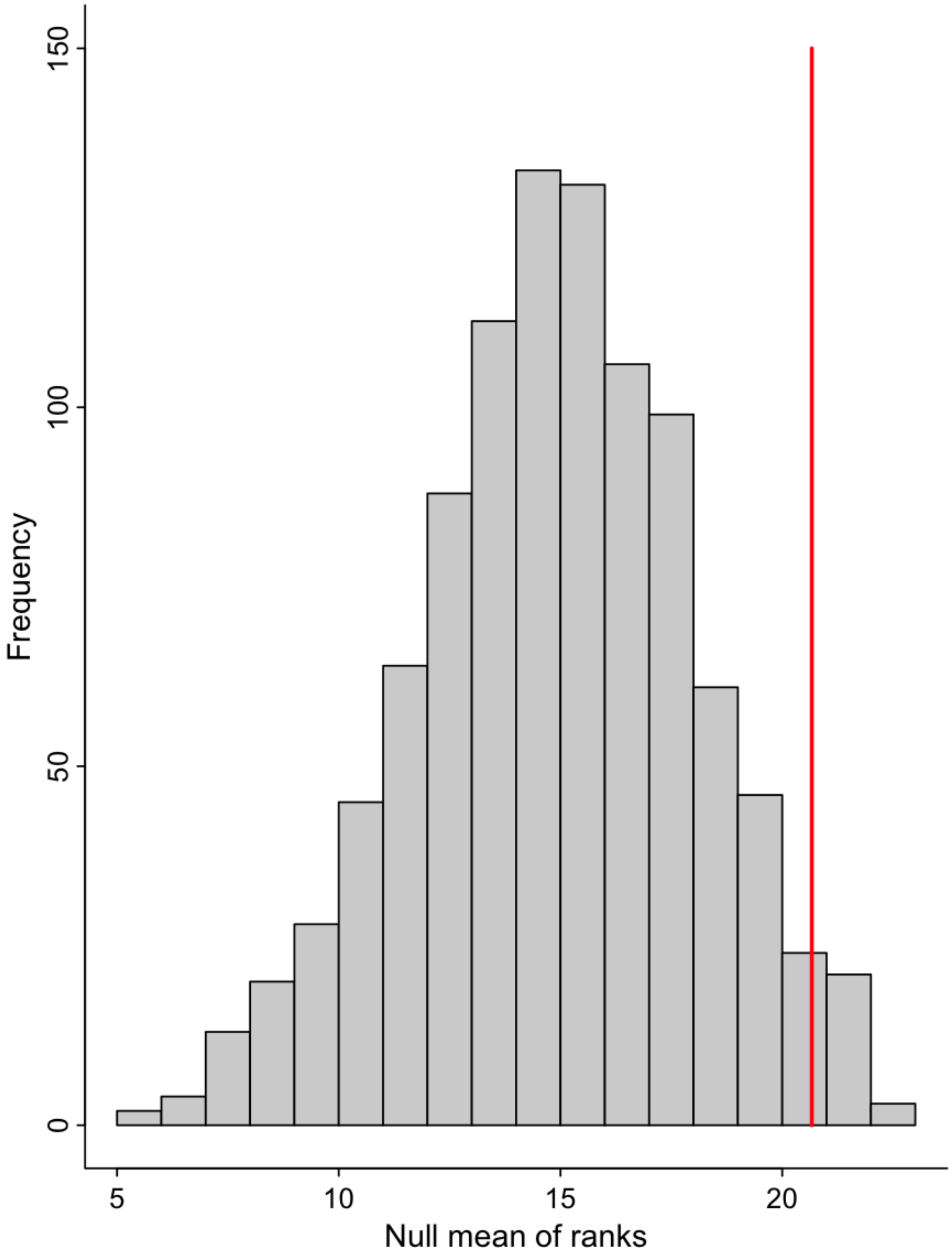

Figure 3.7. Null distribution model of mean ranked males based on total mass loss at the termination of breeding season, from the 2023 field season. The red line indicates the observed value of recaptured individuals from the 2024 season and their associated mean rank value (observed mean rank = 20.67, p-value = 0.04).

## 3.4 Discussion

### *3.4.1 Summary*

The goal of this chapter was to understand if life history trade-offs, particularly reproductive investment, in *D. versicolor* act as a cue to end the breeding season. I found that there is no consistent threshold mass loss in males that triggers the end of breeding season, rejecting hypothesis 2.2. Unfortunately, it was not possible to effectively survey females due to their low detection probability, so I could not determine the effect of female availability on the end of male breeding season (hypotheses 2.1 and 2.3). However, I did confirm the presence of a reproduction-survival trade-off in male *D. versicolor*, as males that invested less the previous season are more likely to be recaptured in the following breeding season.

### *3.4.2 Reproductive investment as a survival trade-off*

I found that male *D. versicolor*, on average, lost approximately 10% of their mass between their initial and final capture dates. Such losses match findings from other anuran species (Ryser 1989) and are consistent with the idea that calling consumes substantial energy (Taigen and Wells 1985) and reduces the time available for foraging. Life history theory suggests that the extent to which iteroparous organisms invest in reproduction depends on the fitness benefits in the current season versus costs to fitness in future seasons, via reduced survival or future reproduction (Williams 1966, Stearns 1989). I hypothesized that males would limit the cost of reproduction by abandoning the breeding pond when a mass loss threshold was reached. However, I found no evidence of a consistent threshold acting as the cue to end breeding. Instead, the variation in mass loss among males when they abandoned the breeding pond was no less than expected if they had abandoned the pond randomly.

One potential explanation for the lack of a consistent mass loss threshold is that reproductive investment in the current year had no effect on future fitness (e.g., survival). However, this was not the case. The six individuals recaptured in 2024 invested significantly less in reproduction in 2023 (as measured by breeding season mass loss) than expected based on my null model. This result not only indicates that there is a reproduction-survival trade-off, as posited by life history theory, but that males vary in their strategies in relation to this trade-off. McCauley et al. (2000) proposed a dynamic optimization model regarding anuran breeding phenology and energetic dynamics which concluded that resource availability could be shaping the amount of calling that occurs for some anurans. During the breeding season, male *D. versicolor* may forage during the day, or trade calling for foraging on some nights (Dodd 2023). My data are consistent with this as males were not captured on all nights between their first and last capture dates, and mass tended to fluctuate rather than decline monotonically during the breeding season (Figure 3.5). Males that are more effective foragers should be better able to maintain a higher condition (mass) during the breeding season. McCauley et al. (2000) also found that males may switch mating strategies (calling or satellite) based on their probability of surviving to another breeding season, which would in turn affect their energy consumption. Satellite males do not call, but rather remain silent near calling males in order to either steal a mating or take over a high quality perch if the calling male vacates it to breed (Ritke et al. 1991, Dodd 2023). The satellite strategy is less energetically intensive and thus would allow males to maintain greater energy reserves toward future survival and reproduction.

It is likely that male reproductive costs are not the only factor influencing the end of breeding season. Females may be the limiting factor to the breeding season in terms of their clutch size and egg production, along with their abundance at an individual pond (Lemckert and Shine 1993). Females may also be influenced and limited by environmental factors, contributing to their abundance and their reproductive investment within a given season (Lemckert and Shine 1993). Once females stop attending the breeding pond to mate, the benefits to males' decline, potentially leading them to depart the breeding pond. Thus, understanding the cues that lead to the end of breeding season will require information not just on males, but females as well. Unfortunately, I was unable to gain sufficient data on females to test my hypotheses 2.1 and 2.3. Focusing on survey methods that can effectively sample females (e.g., artificial arboreal refugia) is a crucial component to add to future studies (Johnson et al. 2008). This would allow for female mass and size analyses, as well as abundance, which can be compared to reproductive investment (i.e., time at the breeding pond) and ideally compared to when males stop calling.

There is a possibility that the end of breeding season is determined by cues unrelated to the reproduction-survival trade-off. In unpredictable environments, such as ephemeral ponds whose drying time depends on interannual variation in precipitation, the time available for tadpoles to metamorphose can vary greatly, as can the success rate of metamorphosis (Cogalniceanu et al. (2013). Cogalniceanu et al. (2013) argued that, in Eastern spadefoot toads (*Pelobates syracius*), this eliminated the negative trade-off between current reproductive effort and future reproductive output. While this trade-off was still detectable in *D. versicolor*, it could be of little importance as a predictor of breeding season's end. Instead, males may be under selection to breed early to maximize the chances of offspring metamorphosis and survival, with environmental factors affecting hydroperiod acting as the cue to end breeding. For example, Becker et al. (2018) found that, for Rose’s Mountain toadlet (*Capensibufo rosei*), calling was related to the amount of rainfall for that season and could limit the extent of breeding season. If exogenous cues like this have substantial impacts, it could have important implications for breeding season length, and potentially population dynamics under future climate change could affect the calling phenology and reproductive output for *D. versicolor* (Schalk and Saenz 2016, Becker et al. 2018).

Determining how exogenous (e.g., weather) and endogenous (life history trade-offs) interact to determine the end of breeding season would benefit from detailed data across multiple years. It would also be valuable to examine differences in the reproductive investment of *D. versicolor* across its geographical range. For example, Yu and Deng (2020) found that there are different environmental pressures across the geographical range that affect the life history traits of a toad species (*Bufo minshanicus*). Females produced higher fecundity and smaller egg sizes compared to lower altitudes of the same species, a trade-off in reproductive investment with no negative effect (Yu and Deng 2020). Such geographical variation in reproductive investment could be occurring for *D. versicolor*, potentially contributing to the differences in breeding seasons across the geographical gradient seen in Chapter 2.

Based on the literature, it seems there are other potential cues at play, potentially simultaneously, that are contributing to the end of breeding season for *D. versicolor*. Of course, this means that further work is required to understand what may be triggering the end of calling.

### *3.4.3 Conclusion*

While my results are inconclusive with respect to what determines the end of calling season, they suggest directions for future research. In summary, I determined that there is not a consistent mass loss threshold for *D. versicolor* males that would indicate the end of their breeding season, but that there is a trade-off between reproduction and survival which suggests other factors are influencing how males balance costs and benefits of calling. It seems likely that there are multiple environmental and biological factors at play that jointly determine the end of breeding season for *D. versicolor*. Further work needs to be completed to have a more in-depth understanding of the end of breeding season for *D. versicolor.* Data across multiple years and across the range can make valuable contributions to this understanding, but most crucial is increased focus and improved acquisition on the factors influencing reproductive costs and benefits of females.

# Chapter 4: General Summary and Conclusion

The aim of this thesis was to explore important phenological events of the Eastern Gray Treefrog (*Dryophytes versicolor*). This topic is of importance for determining if the timing of important life-history events will be impacted by climate change, potentially allowing for species to access new areas, i.e., range expansion or shift. I focused on the breeding season of *D. versicolor* as this is a crucial phenological event that has the potential to affect the range of this species. Attempting to understand what abiotic or biotic factors may influence the breeding season timing for *D. versicolor* led to insights into the life-history of this species and how it may be impacted by climate change in the future.

In Chapter 1, the literature was reviewed to understand the life history of anurans. I completed a systematic review on the breeding season of anurans, focusing on the initiation, length, and cessation of breeding season. This was completed to identify gaps in the current understanding of what environmental cues may initiate or cease breeding season in anurans. By using predetermined keywords, articles were retrieved focusing on the initiation, length, and cessation of breeding season. Ten studies were identified as relevant to the onset of breeding season, five studies for the length of breeding season, and eight for the cessation of breeding season. There was a common result across all papers that there are multiple cues involved whether exogenous (i.e., environmental) or endogenous (i.e., internal). Of all the studies retrieved, there was little representation of areas with highly variable seasonality and how this may impact the breeding season of anurans. Little information was found on the breeding season for *D. versicolor,* a species with an expansive range that includes areas with highly variable seasons.

For Chapter 2, I examined how the breeding season of *D. versicolor* varied in timing and duration between the northern range edge and range core of its distribution. I tested three hypotheses for how the temperature at which calling commences (onset calling temperature) changes from the northern edge to core. I found that populations at the northern range edge had a significantly shorter breeding season than at the range core. This included a later onset of calling for the northern range edge and an earlier end to the calling season. Populations at the northern range edge also commenced calling at colder temperatures compared to those at the range core. A large portion of Chapter 2 involved the use of the RIBBIT algorithm, adapted from Lapp et al. (2021), demonstrating that this algorithm can be used for extremely large data sets to automate the detection of *D. versicolor*.

Chapter 3 focused on the life-history trade-offs within *D. versicolor*, specifically in terms of reproductive investment and understanding what may cause the cessation of breeding season. I proposed three hypotheses pertaining to the termination of male advertisement due to a mass loss threshold, and a fourth hypothesis analyzing the reproductive investment and how this affected the return rate of *D. versicolor* males. I found that there is no consistent threshold mass loss in males that triggers the end of breeding season. Unfortunately, it was not possible to effectively survey females due to their low detection probability, so I could not determine the effect of female availability on the end of male breeding season. However, I did confirm the presence of a reproduction-survival trade-off, as males that invested less the previous season are more likely to be recaptured in the following breeding season.

Overall, this thesis provided insights into the breeding phenology of *D. versicolor* including: 1) the difference in breeding season temperature threshold and breeding season length across a geographical gradient 2) the use of the RIBBIT algorithm to automate 3915 hours of recordings 3) the presence of a reproduction-survival trade-off for *D. versicolor* males. Additional avenues have been presented through this work that requires further research to tease apart specific breeding season onset and cessation cues, whether exogenous or endogenous or a combination.

# Appendix A

Table 4.1. Snout-vent length measurements (cm) for *D. versicolor* males from Hazelwood Pond 2023.

| Date | 1082 | 1090 | 1091 | 1092 | 1093 | 1096 | 1104 | 1106 | 1109 | 1110 | 1128 | 1132 | 1134 | 1135 | 1136 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2023-05-26 | 4.6 | | 5.5 | | | | | | | | | | | | |
| 2023-05-28 | | | 5.2 | | 5.1 | | | | 5 | | | | | | |
| 2023-05-30 | 4.6 | | 5 | | 5 | | | | 5 | 4.7 | | | | | |
| 2023-06-01 | 4.7 | 4 | 4.2 | | 5 | 4.1 | | 4.7 | 4.6 | | | 4.5 | 4 | | |
| 2023-06-03 | 4.8 | | 5.2 | 4.5 | 4.6 | 4.1 | 4.2 | 4.7 | 4.7 | 4.4 | 4.4 | 4.4 | | | |
| 2023-06-05 | 4.6 | | | | 5 | | | | 4.7 | 4.5 | | 4.6 | | 3.8 | |
| 2023-06-07 | | | | | | | | | | | | | | | |
| 2023-06-09 | 4.4 | | | | | | | 4.8 | 4.6 | 4.5 | | | | | |
| 2023-06-11 | | | | | | | | | | | | | | | |
| 2023-06-13 | | | 5.5 | | | 4.2 | | 4.7 | 5 | | | | | | |
| 2023-06-15 | | | | | | | | | | | | | | | |
| 2023-06-18 | | | | | | | | | | | | | | | 4.6 |
| 2023-06-19 | | | | | | | | | | | | | | | |
| 2023-06-21 | | | | | | | | | | | | | | | |
| 2023-06-23 | | | | | | | | | | | | | | | |
| **Average** | 4.6 | 4 | 4.88 | 4.5 | 4.94 | 4.13 | 4.2 | 4.73 | 4.8 | 4.53 | 4.4 | 4.5 | 4 | 3.8 | 4.6 |

Table 4.1. continued. Snout-vent length measurements (cm) for *D. versicolor* males from Hazelwood Pond 2023.

| Date | 1137 | 1145 | 1150 | 1151 | 1154 | 1156 | 1160 | 1167 | 1170 | 1172 | 1174 | 1178 | 1179 | 1181 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2023-05-26 | | | | | | | | | | | | | | |
| 2023-05-28 | | | | | | | 4.5 | | | | | | 5 | |
| 2023-05-30 | | 4.9 | 5.1 | | 5 | 4.3 | 4.4 | 4.4 | | | 4.1 | | 5.2 | |
| 2023-06-01 | 4.9 | | 4.9 | 4.6 | 4.8 | 4.5 | | | 5.1 | | | | | 4.7 |
| 2023-06-03 | | 4.8 | 5 | 4.5 | 4.3 | | 4.1 | | 5 | | 4 | 5.1 | | 4.5 |
| 2023-06-05 | | 4.4 | 4.9 | 4.3 | 4.7 | | | | | 4.4 | 4.4 | | | 4.6 |
| 2023-06-07 | | | | | | | | | | | | | | |
| 2023-06-09 | | | | | | | | | | | | | 5 | 4.5 |
| 2023-06-11 | | | | | | | | | | | | | | |
| 2023-06-13 | | | 4.5 | | | | 4.2 | | 5.1 | | | | 5.3 | 4.7 |
| 2023-06-15 | | | | | | | | | | | | | | |
| 2023-06-18 | | | | | | | 4.1 | | | | | | 5 | |
| 2023-06-19 | | | | | | | | | | | | | | |
| 2023-06-21 | | | | | | | | | | | | | | |
| 2023-06-23 | | | | | | | | | | | | | | |
| **Average** | 4.9 | 4.7 | 4.88 | 4.47 | 4.7 | 4.4 | 4.26 | 4.4 | 5.07 | 4.4 | 4.17 | 5.1 | 5.1 | 4.6 |

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