

**Phenological Shifts Detected for Zygoptera in the Context of an Ongoing Inventory
of Odonata**

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Abstract

The influence of long-term climate change on phenology, the timing of biological events, for Odonata (consisting of separate suborders for dragonflies and damselflies) has been well documented in several parts of the world, showing a negative correlation between emergence date and temperature. Previous studies have shown that spring-emerging species, with an overwintering diapause, are more susceptible to climate change than those species emerging later in the summer. The context of this study is an ongoing biodiversity inventory of Odonata on the campus of UVA-Wise in Wise, VA that began in 2015. Odonates collected in Spring 2015 experienced a much colder winter than the specimens collected in 2017 and allowed for the testing of links between any earlier emergences and warmer temperatures. If winter temperatures increased, then it would be expected that adult odonate emergences will occur earlier in the year. First capture dates for 24 species of spring-emerging odonates were compared for 2015 and 2017 and these first capture dates served as a proxy for emergence dates. The statistical analyses revealed that the 2017 first capture dates are significantly earlier than the 2015 dates ($p = 0.036$) for just the damselflies. This link observed here between temperatures and earlier emergences for damselflies, but not with dragonflies, is for a very limited scope and time frame, but is consistent with other research linking the effects of climate to larger-scale patterns specifically just for damselflies.

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Keywords biodiversity, damselflies, dragonflies, emergence, inventory

Introduction

The order Odonata, which contains the suborders Anisoptera (dragonflies) and Zygoptera (damselflies), is a group of carnivorous insects that spend their immature stages as naiads in aquatic ecosystems (Triplehorn & Johnson, 2005). These insects serve a valuable role in wetland ecosystems, as they feed upon other insects, such as mosquitos and mosquito larvae, in both the aquatic and aerial parts of their life cycle. This order of insects is widespread, inhabiting both tropical and temperate regions, and can be found on every continent except Antarctica. Odonata likely evolved in the tropical regions and then invaded temperate regions and uses diapause (an inactive resting stage) as a strategy to withstand cold winters (Pritchard & Leggott, 1987; reviewed by Hassall & Thompson, 2008 and references therein). Entering diapause provides a mechanism by which odonate larvae can survive the low energy and food availability during the winter season by lowering the metabolic needs of the insect (Hassall & Thompson, 2008). In the spring, following the overwintering diapause, the mature naiad emerges from the water, molts, and enters the more familiar winged adult form (Triplehorn & Johnson, 2005). These odonates then enter a flight period, a time span where the individual is actively flying and mating, which begins when they emerge from the naiad stage and lasts until the death of the individual. Odonates, like all insects, are ectothermic and rely on the temperature of their external environment to drive their activity (Colinet et al., 2015). Like many ectotherms, odonates have been observed to be sensitive to changes in temperature, particularly in the context of widespread climate change, as they must rely on behavioral changes to regulate body temperature (Hassall, 2015).

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In response to longterm increases in temperature, Odonata have been observed to respond over time with both poleward shifts of ranges and advances in phenology, the timing of crucial biological events (Hassall et al., 2007). A 44 year long study of British Odonata between the years 1960 and 2004 demonstrated this trend, revealing a 1.51 ± 0.060 day advance in Odonata flight periods per degree rise in annual mean temperatures (Hassall et al., 2007). This indicates that, as average temperatures rise, these insects can be expected to emerge earlier in the year than previously observed. The same conclusion was reached during a study of Dutch Odonata, which have been found to have advanced their mean flight dates in response to a gradual rise in temperature over the 10 year focal period (Dingemanse, et al., 2008). As a possible explanation for advanced flight dates, Hassall hypothesized that the developmental rates of Odonata eggs and larvae would increase as the environmental temperatures rose, resulting in the observed advancement of the leading edge of their flight period (Hassall et al., 2007); this flight period is marked when the adult emerges after completing development from the overwintering immature stage. Likewise, it has also been shown that the duration of the diapausing period decreases at higher temperatures (Bale & Hayward, 2010). Therefore, after the warmer winter, development could have resumed much earlier in the year for these larval odonates. This repeatedly observed sensitivity to climate change has resulted in Odonata being alluded to as “candidate macroecological barometers” for global climate change (Hassall, 2015).

Phenological shifts have both positive and negative consequences. Forward shifting of Odonata phenology allows for both the maximized utilization of the warmer seasons, and the reduced activity experienced by the naiad form during the colder winter seasons (Hassall, 2015). However, these shifted emergence times could have significant impacts for members of this order, as extremely shifted phenologies could result in a disruption in the synchrony between

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odonates and their prey items, amongst other unclear ecological disruptions (Winder & Schindler, 2004).

Traditionally, changes in phenology have been difficult to document, especially over extensive time frames. However, as can be seen with phenological studies in the United Kingdom and Netherlands, the use of long-term biodiversity datasets from inventories has made it possible to draw conclusions from long-term studies (Hassall et al., 2007; Dingemanse, et al., 2008). These long-term datasets allow for the modelling of complex systems that compare numerous environmental and behavioral factors, such as climate and emergence times, that would have been previously difficult to analyze. A short-term dataset on the UVA-Wise campus was established during a 2015 biodiversity study of Odonata (Marshall & Rodriguez, unpublished data), and continuation of this inventory in 2017 allows for the asking of questions regarding Odonata and temporal events, such as those regarding potential changes in phenology.

Local overwintering temperatures have greatly fluctuated between 2015 and 2017. The local winter of 2015 was abnormally cold, especially in comparison to the abnormally warmer and drier winter of 2017. Comparisons between the average monthly temperatures from the NOAA weather archives for the winter season (defined as the three winter months of December, January, and February) in Wise County revealed a large shift in temperature trends between 2015 and 2017 (Table 2) (NWS Internet Services Team, n.d.). While December temperatures remained fairly constant, with only a 2.9 °F (1.61°C) difference between the two years, January and February show a growing difference in monthly averages. The average January temperatures in 2017 were 7.4°F (4.11°C) warmer than the equivalent 2015 averages, while the average February temperatures in 2017 were 16.5°F (9.16°C) warmer than in 2015. This demonstrates

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that the overwintering conditions for 2017, especially in the later part of the winter season, were remarkably warmer than the 2015 season.

It can be expected that a drastic increase in local overwintering temperatures, even over such a short time frame, could impact local Odonata phenology. This leads to the question addressed in this study: Given the dramatic differences in the overwintering temperatures of 2015 and 2017, can shifts in emergence dates of the odonates be detected in a small dataset? Analyzing inventory data for these two years allowed for the testing of the hypothesis that, if subjected to warmer overwintering temperatures, then locally occurring species of Odonata can be expected to emerge earlier than species that endured a colder winter season.

Materials and Methods

Emergence Dates and Sampling Protocols for the Inventory

The UVA-Wise Odonata inventory serves as a foundation for our study and began with the 36 species of Odonata collected in 2015. This study used 2015 “first capture” dates for the odonates as a proxy for emergence dates and continued the inventory using similar protocols, as described below, in 2017.

Individuals were collected via insect net along the lakeside of the study sites and the immediate surrounding areas. Individuals captured were identified in the field, with individuals of interest being transferred to empty glassine Odonata envelopes. These Odonata envelopes were marked in-field with the date and site designation before being stored at the end of each day in a freezer. These stored individuals were then preserved in a voucher system that, in continuation of the 2015 inventory, pairs a single individual with a card bearing information relevant for long-term storage. This information, containing the species identification, date

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caught, and site designation for every caught individual, was also logged into an Excel datasheet, along with observations for duplicates of abundant species for future use.

As technical limitations prevented the direct observation of the naiad emergence, the first catch date of each included species served as a direct proxy for this emergence date for the purpose of statistical analysis. First observation dates, which began being recorded in the 2017 field season, could allow for the future comparisons of first occurrence dates, eliminating potential variability in catching ability.

Sampling

To determine 2017 emergence dates, odonates were collected along numerous sites on the UVA-Wise campus. In accordance with the 2015 survey procedure, collection occurred alongside the six man-made bodies resulting from past mining activity. Construction limited 2015 access to a seventh site (Figure 1, “G”), although access was restored by the 2017 field season. To remain consistent with the 2015 study, data from this additional site was not considered for final statistical analysis.

Sampling procedures followed the routine collecting pattern established during the 2015 study. Sites were visited at approximately the same time daily, such that each site was visited on the same day during a weekly rotation. Equal time, adjusted isometrically for the number of people, was spent at each locale, to ensure that overall effort (measured in person-hours) remained consistent with reported 2015 sampling efforts.

Statistical Analysis

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To allow for comparisons between the 2015 and 2017 data sets, the date of first catch for every species recollected from the 2015 study was converted into a numerical day of year (DoY) (Table 1). This single number served as a numerical proxy representing the emergence date, and allowed for statistical analysis using pairwise t-testing in Excel. As preliminary analysis revealed that Anisoptera and Zygoptera differed in first catch date, statistical analysis was conducted to test for differences between the first catch date of 2015 and 2017 for each suborder separately. As warmer weather has been historically linked to earlier emergence times, these comparisons were made using a one-tailed testing model (Hassall, 2015). To account for the errors introduced by multiple testing, the p-values were adjusted in accordance with the False Discovery Rate method (Benjamini & Hochberg, 1995), using the p.adjust function in R (R Core Team, 2015).

Data and Results

From the 36 species caught in 2015, 24 recollected species met the criteria of being a spring-emerging species. Closer analysis between the responsiveness of the suborders Anisoptera and Zygoptera revealed mixed results. Members of the suborder Zygoptera were more phenologically responsive than Anisoptera, and displayed first capture dates that were significantly earlier in 2017 than in 2015 ($p = 0.018$, adjusted $p\text{-value} = 0.036$), with the mean first capture date for members of this suborder advancing approximately one week. However, members of the suborder Anisoptera showed a nonsignificant forward shift ($p = 0.453$, adjusted $p\text{-value} = 0.906$) corresponding with a mean first capture date that only advanced roughly 0.43 days.

A majority of odonates showing large shifts belonged to the suborder Zygoptera, the damselflies, with many members of this suborder displaying large shifts of greater than two

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weeks. For example, *Lestes vigilax* was caught 28 days earlier in 2017 than in the cooler 2015.

The single highly shifted Anisoptera, *Tramea lacerata*, is likely an outlier resulting from behavioral differences in dragonfly species.

Discussion

Despite just two collecting seasons, a shift in Odonata emergence times can be shown to have occurred in correspondence with drastic changes in overwintering temperature. Although attempts were made to standardize sampling efforts between 2015 and 2017, unavoidable variability between sampling years introduces the possibility of a sampling bias and remains as a limitation. Otherwise the odonates of 2017, which experienced a much warmer winter season than the odonates of 2015, were not significantly shifted as a whole, there was a significant difference in the response between the individual suborders. While the exact cause of this discrepancy remains unclear, the observed heightened responsiveness to overwintering temperatures displayed by the members of the suborder Zygoptera could potentially be caused by differences in physiology and behavior. Members of Zygoptera are known to be poor-flyers, especially in comparison to the strong, migratory fliers found in suborder Anisoptera (Sánchez-Herrera & Ware, 2012). A study of large-scale biogeographic patterns in Odonata revealed that climate was a stronger contributor to distribution patterns in the Zygoptera, but not the Anisoptera (Heiser & Schmitt, 2010). While the shifts observed in this suborder are in response to extreme fluctuations in local weather over a short-time span, these differences are consistent with long-term studies of phenological trends in Odonata (Hassall et al., 2007).

This same trend, which potentially signifies a forward shifted or hastened phenology in response to warming temperatures, was not observed in Anisoptera. Members of suborder

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Anisoptera are traditionally stronger fliers capable of migratory flights, and could instead be responding to warming overwintering conditions by displaying poleward shifts in range or changes in migratory behavior. This potential response is further evidenced by an occurrence of *Dythemis velox*, a tropical species whose North American range lies primarily in Alabama through North Carolina (Paulson, 2011), who was found polewards of its published range in the 2017 field season.

Future studies could address the hypothesis that the seemingly increased phenological responsiveness for the damselflies is potentially advantageous in competing against the stronger flying dragonflies, and that these earlier emergences can “compensate” for poorer dispersal and flying abilities in their environments.

Acknowledgments

We would like to recognize the Department of Natural Sciences at UVA-Wise for the support of this project. A special thanks goes to Austin Marshall (UVA-Wise) for the use of the 2015 Odonata emergence dates used in this analysis. We are grateful for a faculty reviewer for their very helpful comments, which greatly improved the manuscript and we also want to thank Dr. Liza Comita (Yale School of Forestry) for her help in running the `p.adjust` function in R.

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