The life history of *Anax junius* (Drury) in Minnesota: Determining instars, growth development pathways, emergence phenology, and the effect of temperature on development (Odonata: Aeshnidae)

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Acknowledgements

A good friend recently told me that earning a PhD demonstrates one’s tenacity more than intelligence. This observation rings as a bright and clear truth for me. Pursuing this degree has been both a joyful and agonizing journey with a relentless series of intellectual, emotional, and physical trials. The product of enduring these tribulations is more than this dissertation; I am emerging from this experience a stronger, more capable, and confident scientist. I have only been able to leap (or stumble, or crawl, or be lifted) through all of the flaming hoops thanks to vital infusions of generosity, support, and grace from my mentors, friends, and family.

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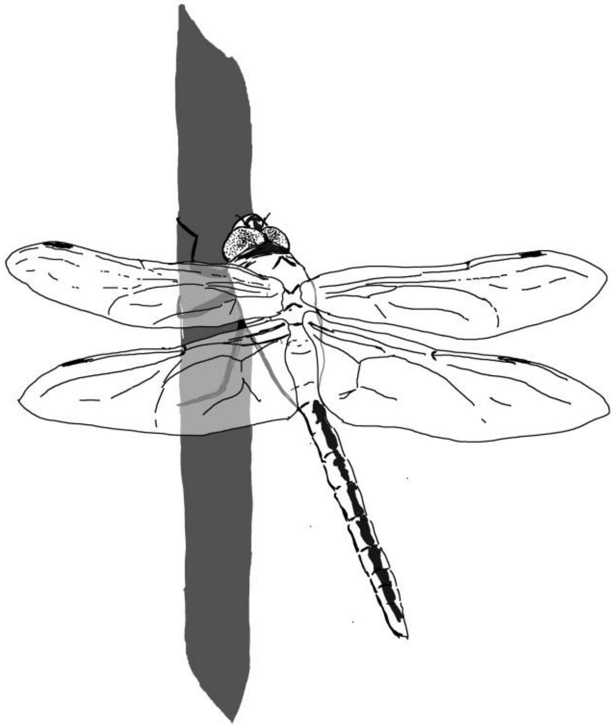
Finally, a truly heartfelt “thank you” to all of my friends. If you think this means you, it does. I have noticed and am grateful for every iota of support that you have sent my way. I share this accomplishment with all of you.

Dedication

This dissertation is dedicated to anyone who has ever felt that science is not for them. You can be a scientist if you want to be. My advisors opened the door for me to become a biologist, and I am propping it open for you. I hope you rip the door off its hinges when you walk through.



Anax junius illustration by Nora Pennarola

Abstract

This dissertation is a report based on careful observations of *Anax junius*, the common green darner dragonfly. Odonatology, the study of dragonflies and damselflies, is presently at a delightful stage of maturity. The basics of odonate life history are well understood but the details of how different species express variations in their development and reproduction, in response to different evolutionary drivers, is ripe for exploration. These variations can only be discovered through field observations executed with thoughtful experimental design and then the observational data collected must be interpreted with appropriate statistical and analytical tools. For a naturalist with a patient and inquisitive mind this kind of research is exceptionally rewarding.

Chapters 1, 2, and 3 focus on these goals. Observational data on *A. junius* growth and emergence were collected over a period of two years at Crow-Hassan Park Reserve, near Minneapolis, Minnesota. Severe winter conditions are the largest constraint influencing dragonfly life histories in Minnesota. Odonata must both survive the winter, and time their emergence and reproduction so that they occur during the short growing season. This research was designed to explore how northern *A. junius* have adapted to survive winter. Chapter 1 demonstrates the application of a statistical tool that identifies the number of instars in a field-collected sample of nymphs: a need for interpreting observed growth and development data. This mixed distribution analysis has been used in other fields of entomology, but this is the first time (to the author's knowledge) that it has been applied to dragonflies. Chapter 2 maps nymph growth over time and identifies different *A. junius* growth pathways that are associated with two different overwintering strategies. Chapter 3 summarizes observations of *A. junius* emergence phenology via exhaustive exuviae collection, and reveals that cold temperatures and stochastic events are the greatest constraints on emergence duration.

Northern ectoderms, like dragonflies, with life histories that are constrained by severe winters are impacted by climate change. The implications of the changing climate on dragonfly natural history are of increasing conservation interest. Rare and threatened niche species of Odonata could be at risk of extinction if their habitat conditions are altered beyond to what they can adapt to. However, *A. junius* is a common and abundant dragonfly and is of conservation interest for different reasons. Extremely common species are the skeletons of ecosystems; they make up most of the biomass and provide structure and support for all the other components. A change in the geography or abundance of *A. junius* could have wide-ranging and cascading effects.

Models based on known temperature thresholds are required to predict ectoderm response to the changing climate. Chapter 4 describes an experiment that defined these threshold temperatures for the northern winter growth pathway of *A. junius.* Development duration and rate were measured for nymphs reared in different temperature-controlled chambers, and the base and optimum growth temperatures were calculated for the last two nymph development stages. These values can now be used in models to predict the impact of climate change on the phenology of *A. junius* development and emergence.

Note: Some materials and methods descriptions, figures, and tables are repeated in this dissertation because each chapter was written with the intent to be published individually.

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Chapter 1

Determining *Anax junius* (Drury) instars with mixed distribution analysis (Odonata: Aeshnidae)

SYNOPSIS

More observational dragonfly and damselfly life history studies are needed to build foundational knowledge about the order. However, determining the instars of field-collected dragonfly nymphs is difficult and this challenge has limited the interpretation of field-collected data. This paper builds on previous methods for identifying odonate nymph instars, quantifies the likelihood of misclassifying nymphs when using different morphological parameters, and provides guidance on choosing the most efficient instar determination process depending on researcher goals and needs. I collected *Anax junius* nymphs weekly from five ponds over two years from a reserve in central Minnesota, USA, and measured and recorded nearly 5,000 nymph head widths and hind wing sheaths. Mixed distribution analysis of these data identified the number of instars present in the sample and calculated the likelihood of misclassifying a nymph, using both head width and the ratio of hind wing sheath by head width distributions. A method to assign nymphs to instars that minimized the potential of misclassifying by assigning larger instars with ratio parameters and smaller instars with head width parameters is presented. This analysis confirmed that instar boundaries can be determined through a visual assessment of the dips between the peaks within morphological histograms. However, mixed distribution analysis is required to statistically determine the number of instars present and to calculate the likelihood of assigning a nymph to the wrong instar. A departure from normality in a head width histogram instar distribution may indicate that multiple growth pathways are present in a sample. Researchers should be careful not to interpret the presence of instars in a sample as a map of nymph growth because development can vary with growth pathway, temperature, and season. Efficiently collecting and classifying the smallest nymphs remains a challenge and would benefit from future attention.

INTRODUCTION

Observational life history studies of dragonflies and damselflies have been a neglected aspect of odonate research. Before we can understand their ecological interactions or predict how they may respond to invasive species, degrading habitats, or climate change we must first understand the basics of how they grow (Lutz 1968; Benke 1970; Tauber & Tauber 1976; Corbet 2002). Growth and development knowledge has been gathered from experiments rearing nymphs in labs where researchers can observe and count the number of molts and know the growth stage of each nymph precisely (Dunkle 1985; Velasquez et al. 2009). However, this experimental work does not reflect the complexity of natural systems and may obscure seasonal patterns or interactions that affect dragonfly growth and behavior (Benke 1970; Danks 2002).

Conducting dragonfly natural history observational field studies is challenging because it is extremely labor intensive. Aquatic nymphs and their exuviae need to be collected, measured, and counted frequently (daily or weekly) for at least one year (ideally over many years) in order to understand their phenology (Tauber & Tauber 1976; Corbet 1999). In addition to requiring a great deal of time and effort to collect, the data can be hard to interpret because most dragonfly nymphs are not easily assigned to their growth stages (Benke 1970; Corbet 2002). Here I build on previous methods for identifying odonate nymph instars, quantify the likelihood of misclassifying nymphs when using different morphological parameters, and provide guidance on choosing the most efficient instar determination process depending on researcher goals and needs.

Dragonflies go through a variable number of molts, even within a single species (Corbet 1999). For example, *A. junius* nymphs can experience between 10–14 instars (12 on average) including the pronymph (Beesley 1972). Instar nomenclature standardizes the final instar and counts backwards. The ultimate instar is named “final-minus-zero” or “F-0”, the penultimate “final-minus-one” or “F-1”, then “F-2”, and so on (Lutz 1968). Naming the older and larger instars is a priority because they are more frequently encountered, better studied, easier to differentiate from other instars, and more readily identifiable to species (Tennessen 2017a). Corbet (2002) argued that because of the variable number of molts, it is only helpful to give ordinal number names to the first and last three odonate instars. Instead, he suggests middle-sized instars should be described by a physical parameter, such as head width. Our approach uses both ordinal names and size measurements to describe instars.

Unlike the smooth continuous growth of vertebrates, dragonflies (and other insects) exhibit discontinuous leaps in growth after each molt (Corbet 1999; Wigglesworth 2012). These size jumps are observable in dragonfly nymphs as peaks within a histogram of a morphological parameter such as head width. The valleys between the peaks have been used to mark the size boundaries for assigning a dragonfly nymph to a particular instar (Benke 1970; Wissinger 1988; Aoki 1999; Lamelas-Lopez et al. 2017; Velasco-Villanueva et al. 2018).

If we think of these histograms as being composed of overlapping normally distributed curves, one for each instar, we can tease additional and more precise information out of a body-size data set. This method can statistically determine how many instars are represented in a histogram, calculate exactly where the distributions overlap to identify lower and upper size limits for each instar, and determine the probability of incorrectly assigning an individual nymph to a size-class (Christianson et al. n.d.; McClellan & Logan 1994; Delbac et al. 2010).

There are many potential morphological parameters to use to distinguish instars: hind femur length, head width, wing sheath length, total length, or labium length (Di Giovanni et al. 2000). Head width has the advantages of being the least variable, increasing isometrically, generally exhibiting a smooth declining growth rate, and being easy to measure nondestructively (Calvert 1934; Paulson & Jenner 1971; Folsom 1979; Corbet 2002). Head width histograms alone are commonly used to assign immature insects to a growth stage (Aoki 1999; Lamelas-Lopez et al. 2017; Velasco-Villanueva et al. 2018). However, the distributions of head width sizes overlap, meaning that a percentage of nymphs will be wrongly assigned to a larger or smaller instar depending on how much the distributions mix (Christianson et al. n.d.; McClellan & Logan 1994; Ferreras-Romero 1997; Delbac et al. 2010). Unlike head width, nymph wing sheath growth rates increase dramatically between the last few instars. Dividing a parameter that grows allometrically, like nymph wing sheath, by a parameter that grows isometrically, like head width, creates data for a ratio histogram in which the larger-sized distributions become independent or overlap much less (Ferreras-Romero 1997; Di Giovanni et al. 2000; Delbac et al. 2010; Tennessen 2017a). This ratio is often alternatively visualized to identify odonate instars by graphing the head width against wing sheath length on a scatter plot (Ferreras-Romero 1997; Di Giovanni et al. 2000; Goretti et al. 2001; Velasquez et al. 2009; Lamelas-Lopez et al. 2017; Velasco-Villanueva et al. 2018).

Here, I apply mixed distribution analysis to both head width and ratio distributions to identify the number of instars represented in the data set and calculate the likelihood of misclassifying a nymph. Finally, I present a method to assign nymphs to instars that minimizes the potential of misclassification by assigning larger instars with ratio parameters and smaller instars with head width parameters.

MATERIALS AND METHODS

Field research was conducted at Crow-Hassan Park Reserve in central Minnesota, USA within 3.4 km^2 of restored prairie pothole habitat. *Anax junius* nymphs were collected at five pothole ponds. Two of the ponds contained fish and three did not. The ponds were shallow and did not experience seasonal turn-over. Surface area ranged from 2,090 m^2– 53,860 m^2, and maximum depth varied from 0.61 m – 1.56 m. All of the ponds had small watersheds entirely contained within the reserve.

Anax junius nymph surveys were conducted weekly in spring, summer, and fall and periodically during the winter beginning in the summer of 2016 through the summer of 2018. Surveys were not conducted in the two potholes with fish in 2017 and 2018 because nymph abundance was not high enough to justify collection efforts.

Nymphs were collected by sweeping heavy-duty 38 x 23 cm recreational fishing nets with 0.5 cm mesh through the pond vegetation and along the benthic substrate. *Anax junius* nymphs were identified in the field by looking for three lateral spines on abdominal segments 7, 8, and 9; an elongate prementum with pointed labial palps, and eyes taller than they were wide (Calvert 1934; Walker 1958; Needham et al. 2000; Tennessen 2019). Nymphs were separated by size, to prevent cannibalism, and transported to the lab in a cooler for measurement. Nymphs about to eclose, indicated by their large size and the presence of swollen wing sheaths, were tallied in the field as F-0 and released directly back into the pond at the end of each survey day. From spring through fall nymphs were collected while wading in the ponds from the shoreline to as deep as the waders would allow. Nymphs were collected in winter through holes that were cut in the ice with a chainsaw.

Nymphs were measured within 24 hours of collection and released back into their ponds within 48 hours, except for winter-collected nymphs, which were not released. Maximum head width and the length of the right hind wing sheath (if present) was measured with an ocular micrometer on a Leica MZ7.5 dissecting scope to an accuracy of 0.1 mm as described in Tennessen (2017). The four nymph-measuring research technicians were all instructed to use the same measuring techniques by the same trainer. They practiced and collaborated during training and then periodically checked with each other to calibrate their accuracy to within 0.1 mm.

Nymph instars were determined with mixed distribution analysis using the *mixdist* package (Macdonald & Du 2018) in R version 3.3.2 GUI 1.68 Mavericks build (R Development Core Team 2011). Mixed distributions of both head width (mm) and the ratio of hind wing sheath length (mm) divided by head width (mm) were separately used to determine the number of instars present in the sample and to define the parameters of size distributions (mean and standard deviation) (Christianson et al. n.d.; McClellan & Logan 1994). Starting estimates for distribution parameters were determined by visually observing the histograms. The smaller instar distributions in the ratio histogram overlap considerably so starting estimates were additionally informed by *A. junius* instar ratios reported in Tennessen (2017). The four smallest nymphs (less than 2.1 mm in head width) were determined to be outliers and removed from the dataset before running the mixed distribution analysis. The F-0 distribution (n=865) in the ratio histogram did not overlap with any other distributions so it was not included in the ratio mixed distribution analysis. Twenty supernumerary nymphs larger than F-1 but smaller than F-0 were also not included in the ratio analysis.

The intersections of the distributions, marking the lower and upper limits of each instar, were calculated with the quadratic formula. Areas under the tails of overlapping adjacent distributions were calculated and summed to determine the probability of incorrectly assigning a nymph to a larger or smaller instar (Figure 1-1) (Christianson et al. n.d.; McClellan & Logan 1994).

The probability of misclassifying was totaled for each instar for each mixed distribution. Thresholds from both the head width and ratio distributions were used to assign nymphs to instar categories in a way that minimized the probability of misclassifying. First, instars were assigned using the thresholds as determined by the ratio distributions. Then, a portion of same nymph data set was re-assigned to the smaller instars F-4 through F-7 based on the head width distributions. In this way, F-0, F-1, F-2, and the upper threshold of F-3 were defined by the ratio distribution and the lower threshold of F-3, and F-4, F-5, F-6, and F-7 instars were defined by the head width distributions. Growth ratios for each instar were calculated by dividing average head width for that instar by the average head width of the next smaller instar (Corbet 2002; Wigglesworth 2012).

Instar parameter values were assigned through F-10 for a total of 11 instars, the average number of molts experienced by *A. junius* (excluding the pronymph) (Beesley 1972). Nymphs younger than F-7 were generally too small to be collected with the 5 cm mesh nets, so the head width size thresholds for F-8 through F-11 were calculated by dividing the next larger instar's head width parameters by the F-6 growth rate value of 1.24 (the smallest instar's growth rate that could be calculated directly from the field collected data) (Corbet 2002; Wigglesworth 2012). Final assigned instar values were visualized on a scatter plot of wing sheath length against head width.

RESULTS

A total of 5570 nymphs were collected; of these, 547 had visibly swollen wing sheaths and were identified as F-0 and released in the field. Of the nymphs that were brought into the lab, 4995 had measurable wing sheaths (range 0.08-13.3 mm). The same number (4995) had measurable heads widths (range 1.3-10.6 mm). Four thousand nine hundred ninety individuals had both measurable wing sheaths and heads. Damaged wing sheaths and head widths were not measured. Instars smaller than F-7 had not yet developed wing sheaths.

Mixed distribution analysis determined that the head width data could be described by eight distributions ($\chi^2 = 526.82$, df = 59, $p < 2.2e^{-16}$) (Figure 1-2). Head width parameters defining each instar are listed in Table 1-1 along with the probability of misclassifying an individual nymph as one instar too large (*i* as *i* +1) or as one instar too small (*i* as *i* -1).

The total probability of classifying a nymph to the wrong instar using head width parameters was ~37%.

Mixed distribution analysis determined that the ratio data [wing sheath length(mm)/head width(mm)] could be described by seven distributions ($\chi^2 = 5865.8$, d.f. = 126, $p < 2.2e^{-16}$) (Figure 1-3). The F-0 distribution did not overlap with the F-1 distribution, so it was not included in the analysis. Ratio parameters defining each instar are given in Table 1-2 along with the probability of misclassifying an individual nymph as one instar too large (i as $i +1$), or one instar too small (i as $i -1$). The total probability of classifying a nymph to the wrong instar using ratio parameters was ~52%.

Ratio parameters generate the lowest probabilities of misclassifying for F-0 (0%), F-1 (<1%), and F-2 (2%) whereas head width parameters provide the lowest likelihood of error for F-3 (4%), F-4 (6%), F-5 (9%), and F-6 (7%). The total probability of misclassifying F-7 and smaller instars is unknown because I did not have data to model those distributions. Using the combination of ratio parameters to assign larger instars and head width to assign smaller instars minimized the total probability of classifying a nymph to the wrong instar to ~28% (Table 1-3). Instars assigned with this method were visualized on a wing sheath by head width scatter plot (Figure 1-4).

Head width growth ratios roughly followed the predicted trend of gradually decreasing over time (Table 1-4) (Corbet 1999, 2002).

DISCUSSION

Mixed distribution analysis builds on current methods for identifying dragonfly nymph instars by enabling researchers to statistically determine the number of instars present in a sample, precisely determine the parameter values that define an instar, and quantify the likelihood of misclassifying a nymph to the wrong instar.

With the exception of the final stage, it will likely never be possible to identify a field-collected nymph to its instar with total certainty. Variability in field environments and nymph growth manifests in parameter distributions that mix. Therefore, knowing the likelihood of misclassifying a nymph to an instar that is too large or too small is valuable in order to quantify classification error. Furthermore, summing the total likelihood of error for all observed instars is a useful tool for comparing the accuracy of models based on different morphological parameters.

The low *p*-values indicate some mismatch between the normal curves and the empirical data. One reason for this discrepancy could be that the distributions have some skew, reflecting progression to subsequent life stages that is not perfectly symmetric. For example, Odonata emergence curves often exhibit a pulse of development and then progression tails off (Willey 1973; Purse & Thompson 2003; May et al. 2017). Another, not mutually exclusive reason, could be a mixing of distributions from putative summer and winter growth pathways (Chapter 2). This is explored in Appendix C, but also did not produce better fits. Despite the lack of absolute statistical congruity between the empirical distribution and theoretical curves, the technique remains a useful tool for categorizing instars for field practitioners.

MODEL SELECTION

Overall, the head width model defines *A. junius* instars more accurately (total model error of ~37%) than the model based on the ratio of hind wing sheath divided by the head width (total error of ~52%). This result is not surprising because using ratio parameters has been utilized as a way to improve the accuracy of determining larger instars and was never intended as a tool for separating smaller nymph classes (Tennessen 2017a). Head width parameters are best for determining F-3 and younger instars. In this sample, the benefit of using ratio instar parameters to differentiate larger instars, in combination with head width, to categorize smaller instars, yields an increase of ~9% total model accuracy over using head width alone.

This information can be used to determine if it is worth the extra effort to measure wing sheath length in addition to head width based on the needs of their study. If assigning later instars with precision is critical, then ratio parameters should be used. In this case, ratio parameters generated nearly 100% accuracy in assigning F-0 and F-1 instars and about 98% accuracy in assigning F-2. If time or resources are limited, then researchers may opt to use only head width parameters. In this study, estimates using head widths were ~99%, 97%, and 93% accurate for F-0, F-1, and F-2 respectively. Note that only head width measurements are useful for preserved odonate nymphs because the preservatives can alter wing sheath lengths (Paulson & Jenner 1971).

Most odonate species have similar overall head width and hind wing sheath growth patterns. Visually assessing histograms for determining instars has been used with many dragonfly species (Corbet 2002; Suhling et al. 2015), so the general model accuracy trends should apply to other odonates. However, it would be valuable to repeat this analysis with a variety of species to confirm this assumption.

GROWTH PATHWAYS

Instar morphological size distributions within a dragonfly cohort were expected to be normally distributed. However, the head width histogram from this data set shows a right-skewed F-0 and a bi-modal F-1 distribution. This deviation from normality is a clue that more than one cohort was present in the data. In this sample, *A. junius* nymphs from two growth pathways were present (Chapter 2). Summer pathway *A. junius* nymphs go through one fewer instar, and the F-1 and F-0 instars are slightly smaller than nymphs following the winter growth pathway. These differences in cohort growth manifested in non-normal instar head width distributions. The ratio histogram obscures the cohort differences because, regardless of size, the ratio of hind wing sheath divided by head width in larger instars were similar. In any case, researchers using mixed distribution analysis on field-collected dragonfly nymph data should be cautious not to assume that the instars that are present in the sample represent a timeline of growth. For example, the presence of seven instars in a sample does not indicate that a nymph will go through those seven instars as it matures. To understand the progression of nymph development, including determining if multiple development pathways are present in a sample, nymph size parameters need to be graphed over time and interpreted within an ecological context (Chapter 2).

The instar size parameters from this paper should be applied to other *A. junius* populations with awareness that the number of instars present, head widths, hind wing sheath lengths, and growth ratios can vary with latitude, temperature, and habitat

(Corbet 2002). Our analysis is based on a large observational data set spanning multiple ponds and years; it captures natural variation but only from one park reserve in Minnesota, USA. That said, the instar parameters established by this data set generally match those observed across the United States by Tennessen (2017) (Alabama, Colorado, California, Florida; n=35), Kime (1974) (Washington; n=276), and Macklin (1961) (Indiana; n=2).

SUPPORT FOR SIMPLE VISUAL INSPECTION

Instar parameters estimated from visual inspection of the head width histogram were close to the values that were calculated with mixed distribution analysis; the modeled distributions crossed at about the same locations as the valleys in the histogram. This result provides quantitative support for the traditional (and simpler) method of visually inspecting the histogram to determine instar-classifying parameters. Mixed distribution analysis is still required if researchers need to statistically determine how many instars are present, and to quantify assignment error.

SUPERNUMERARY INSTAR

Nymphs can enter an extra supernumerary instar later in their immature development, often between F-1 and F-0, potentially triggered by the combination of warm temperatures and short photoperiods (Sawchyn 1972; Ingram & Jenner 1976; Corbet et al. 1989; Aoki 1999; Corbet 1999). Indeed, most of the 20 supernumerary nymphs from this study were collected during an unusually warm fall. They can be observed as the data points between the F-0 and F-1 clusters in Figure 1-4. This phenomenon is discussed in Chapter 2.

DETERMINING SMALL NYMPH INSTARS

Head width size parameters for *A. junius* nymphs younger than F-7 (head widths smaller than 2.1 mm) were extrapolated. They were not readily collected from the field, so their size parameters could not be estimated directly from the sample. However the extrapolated sizes generally matched those of nymphs reared, measured, and reported by Calvert (1934) (location unknown; n = 1), Kime (1974) (Washington, USA; n= 276) and Macklin (1964) nymphs (Indiana, USA; n=2). The tiny nymphs were difficult to spot among the vegetation, and could slip through the 5 cm gaps in the net mesh. Dip nets with finer mesh were tested in the field but quickly clogged with organic matter. Methods for collecting smaller nymphs are described in Corbet (1999) in section 7.2.4, but are effort intensive, and were not practical for this study.

Growth ratio in odonates is not constant throughout larval development, and can be well above or below the expected 1.26 ratio for hemimetabolous insects. Growth ratios can also vary by development pathway (Corbet 1999; Danks 2002; Wigglesworth 2012). While variable, *A. junius* head width growth ratios are expected to roughly exhibit a gentle decline (Folsom 1979; Corbet 2002). Therefore, the largest observed growth ratio of 1.24 (for the smallest instar that was able to be calculated from the sample) was used to predict the lower head width thresholds to define F-7 through F-11 instars.

Additional techniques to determine and classify young *Anax* instars can be found in Calvert's (1934) detailed observations. He described the number and length of antennal segments (instars gain segment number and length over time), the shape of the labial palps (they change from blunt to pointed), and shifts in color patterns (from banded to solid). While precise, these characteristics for differentiating instars are subtle, difficult to observe without a microscope, and may be impractical if categorizing a large quantity of nymphs. Paulson & Jenner (1971) state that total body length, while an inconsistent parameter in larger nymphs, was helpful in distinguishing very young instars.

Developing methods for easily identifying the instars of field collected nymphs is a prerequisite for further insights into dragonfly ecology (Corbet 2002; Velasco-Villanueva et al. 2018). This chapter made good progress towards this goal but it is still a challenge, particularly with the smallest instars, and more accurate methods require more effort. Knowing the amount of error in each method, as calculated with mixed distribution analysis, will assist researchers in choosing the most efficient method to meet their needs.

Figure 1-1. Areas of distribution overlap determine the probability of misclassifying. The grey area under the right side of the F-3 distribution represents the likelihood of incorrectly assigning a nymph to instar F-2 when it is in fact in instar F-3 (misclassifying i as i +1). The grey area on the left side under the F-3 distribution represents the likelihood of incorrectly assigning a nymph to instar F-4 when it is in fact in instar F-3 (misclassifying i as i -1).

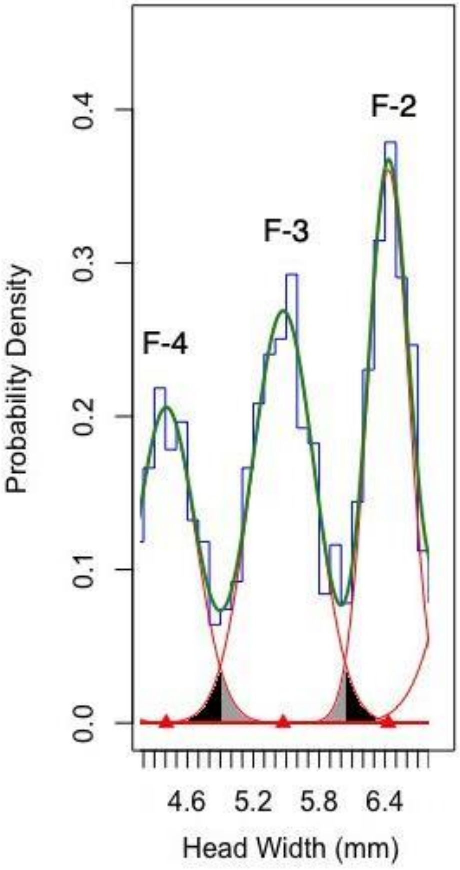


Figure 1-2. Head width mixed distribution analysis for identifying instars. Individual distributions (red lines) were fitted to the original data (blue bars). Red triangles indicate distribution means. The green line represents the combined fitted distributions.

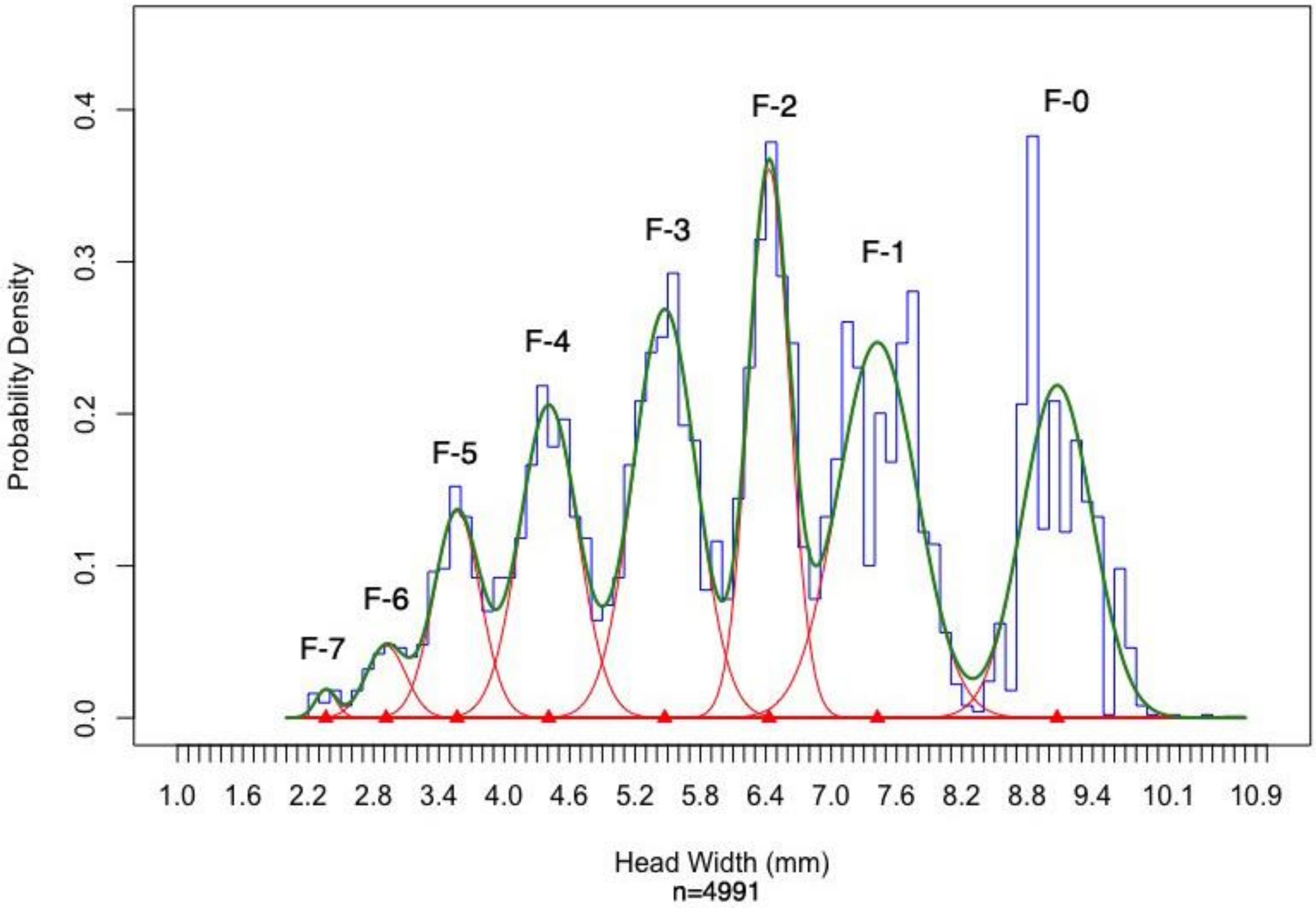

Probability Density
0.0
0.1
0.2
0.3
0.4
F-7
F-6
F-5
F-4
F-3
F-2
F-1
F-0
1.0
1.6
2.2
2.8
3.4
4.0
4.6
5.2
5.8
6.4
7.0
7.6
8.2
8.8
9.4
10.1
10.9
Head Width (mm)
n=4991

Table 1-1. Instars assigned using head width mixed distribution analysis alone and the corresponding probability of assigning a nymph to the wrong instar. Four nymphs with width head widths smaller than 2.1 mm were not assigned an instar.

Instar (*i*)	Head Width (mm)	n	Mean Head Width (SD) (mm)	Probability of Misclassification		
				i as *i* - 1 (smaller)	*i* as *i* +1 (larger)	*i* as another instar (total)
F-0	>8.29	888	9.08 (0.32)	0.0075	NA	0.0075
F-1	6.80 - 8.28	1089	7.43 (0.36)	0.0206	0.0086	0.0292
F-2	6.03 - 6.79	896	6.43 (0.19)	0.0269	0.0459	0.0728
F-3	4.92 - 6.02	948	5.47 (0.29)	0.0254	0.0163	0.0417
F-4	3.96 - 4.91	678	4.41 (0.26)	0.0333	0.0281	0.0614
F-5	3.22 - 3.95	353	3.57 (0.21)	0.0438	0.0432	0.0870
F-6	2.57 - 3.21	116	2.92 (0.18)	0.0138	0.0532	0.0670
F-7	2.10[a] - 2.56	23	2.36 (0.09)	-[b]	0.0293	-
				Total Probability of Misclassification		0.3666

[a] The lower head width boundary for F-7 was estimated to be where the left side of the F-7 distribution approaches zero.

[b] The probability of misclassifying *i* as *i* - 1 for F-7 is unknown because smaller instar distributions were not modeled.

Figure 1-3. Ratio mixed distribution analysis for assigning instars. Individual distributions (red lines) fitted to the original data (blue bars). Red triangles indicate distribution means. The green line represents the combined fitted distributions. The F-0 distribution was independent (see inset) and not included in the analysis.

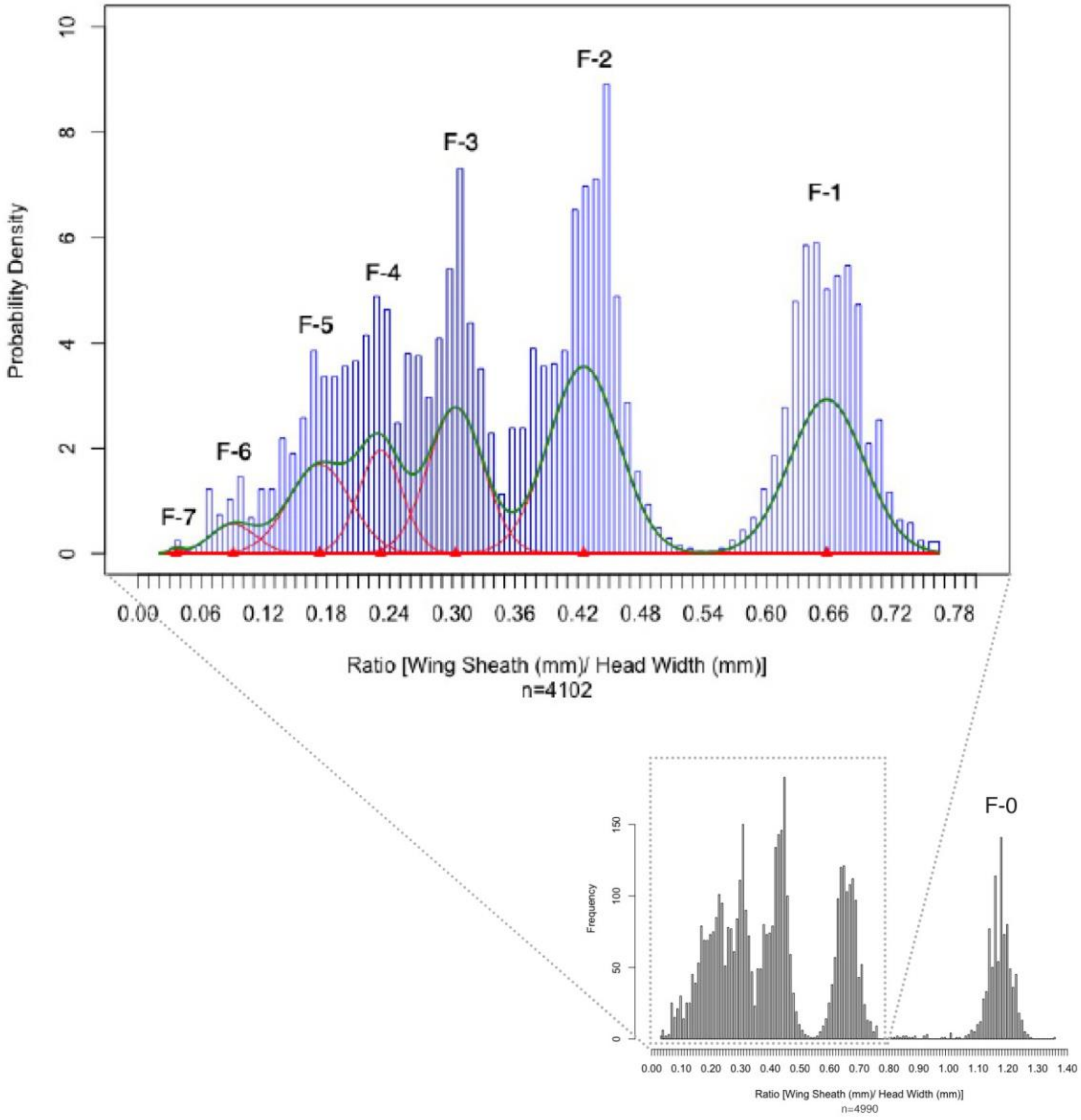


Table 1-2. Instars assigned using ratio mixed distribution analysis alone, and the corresponding probability of assigning a nymph to the wrong instar. Twenty supernumerary nymphs were not assigned an instar value because their ratio was equal to or between 0.79 and 0.99.

Instar (*i*)	Ratio[a]	n	Mean Ratio (SD)	Probability of Misclassifying		
				i as *i* - 1 (smaller)	*i* as *i* +1 (larger)	*i* as another instar (total)
F-0	>1.00	865	1.17 (0.04)	0.0000	NA	0.0000
F-1	0.54 - 0.78[b]	1069	0.66 (0.04)	0.0004	0.0000	0.0004
F-2	0.36 - 0.53	1241	0.43 (0.03)	0.0175	0.0004	0.0179
F-3	0.26 - 0.35	793	0.30 (0.03)	0.054	0.0232	0.0772
F-4	0.20 - 0.25	480	0.23 (0.02)	0.1501	0.0733	0.2234
F-5	0.13 - 0.19	379	0.17 (0.03)	0.0418	0.0915	0.1333
F-6	0.05 - 0.12	135	0.09 (0.02)	0.0073	0.0618	0.0691
F-7	0.02[b] - 0.04	8	0.04 (0.01)	-[c]	0.0328	-
			Total Probability of Misclassifying			0.5213

[a] Ratio values were calculated by dividing the hind wing sheath length (mm) by the head width (mm).

[b] The upper ratio boundary for F-1 and the lower boundary for F-7 were estimated to be where the modeled distributions approached zero.

[c] The probability of misclassifying *i* as *i* - 1 for F-7 is unknown because smaller instar distributions were not modeled.

Table 1-3. Instar thresholds with a minimized probability of misclassifying. Instar size ranges were determined using ratio mixed distribution analysis (for instars F-0, F-1, F-2, and the upper threshold of F-3) and head width mixed distribution analysis (from the lower F-3 threshold through F-7) and the corresponding probability of assigning a nymph to the wrong instar. Twenty supernumerary nymphs were not assigned an instar value because their ratio was equal to or between 0.79 and 0.99.

Instar (*i*)	Thresholds	n	Probability of Misclassifying		
			i as *i* - 1 (smaller)	*i* as *i* +1 (larger)	*i* as another instar (total)
	Ratio[a]				
F-0	>1.00	865	0.0000	NA	0.0000
F-1	0.54 - 0.78	1069	0.0004	0.0000	0.0004
F-2	0.36 - 0.53	1241	0.0175	0.0004	0.0179
F-3	0.26 - 0.35	793	0.0254[b]	0.0232	0.0486
	Head Width (mm)				
F-4	3.96 - 4.91	678	0.0333	0.0281	0.0614
F-5	3.22 - 3.95	353	0.0438	0.0432	0.0870
F-6	2.57 - 3.21	116	0.0138	0.0532	0.0670
F-7	2.10[c] - 2.56	23	-[d]	0.0293	-
F-8	1.69[e] - 2.09	3	-	-	-
F-9	1.37 - 1.68	0	-	-	-
F-10	1.10 - 1.36	1	-	-	-
			Total Probability of Misclassifying		0.2823

[a] Ratio values were calculated by dividing the hind wing sheath length (mm) by the head width (mm).

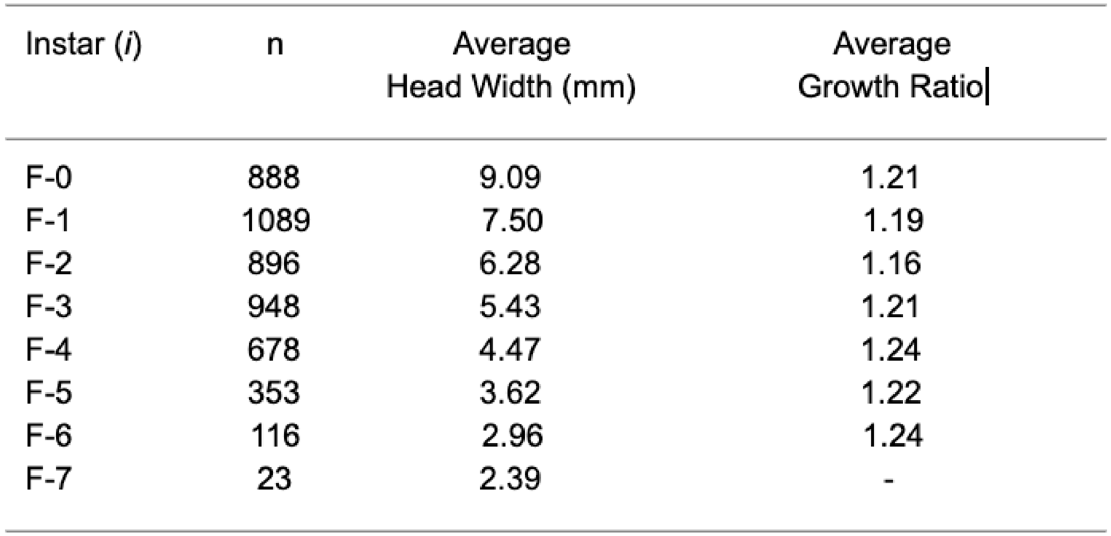
[b] Probability of misclassifying *i* as *i* - 1 for F-3 was determined using the head width distribution because the F-3 classification is defined on the left by the intersections of the head with distributions and on the right by the ratio distributions.

[c] The lower boundary for F-7 was estimated to be where the distributions approached zero.

[d] The probability of misclassifying *i* as *i* - 1 for F-7 is unknown because smaller instar distributions were not modeled.

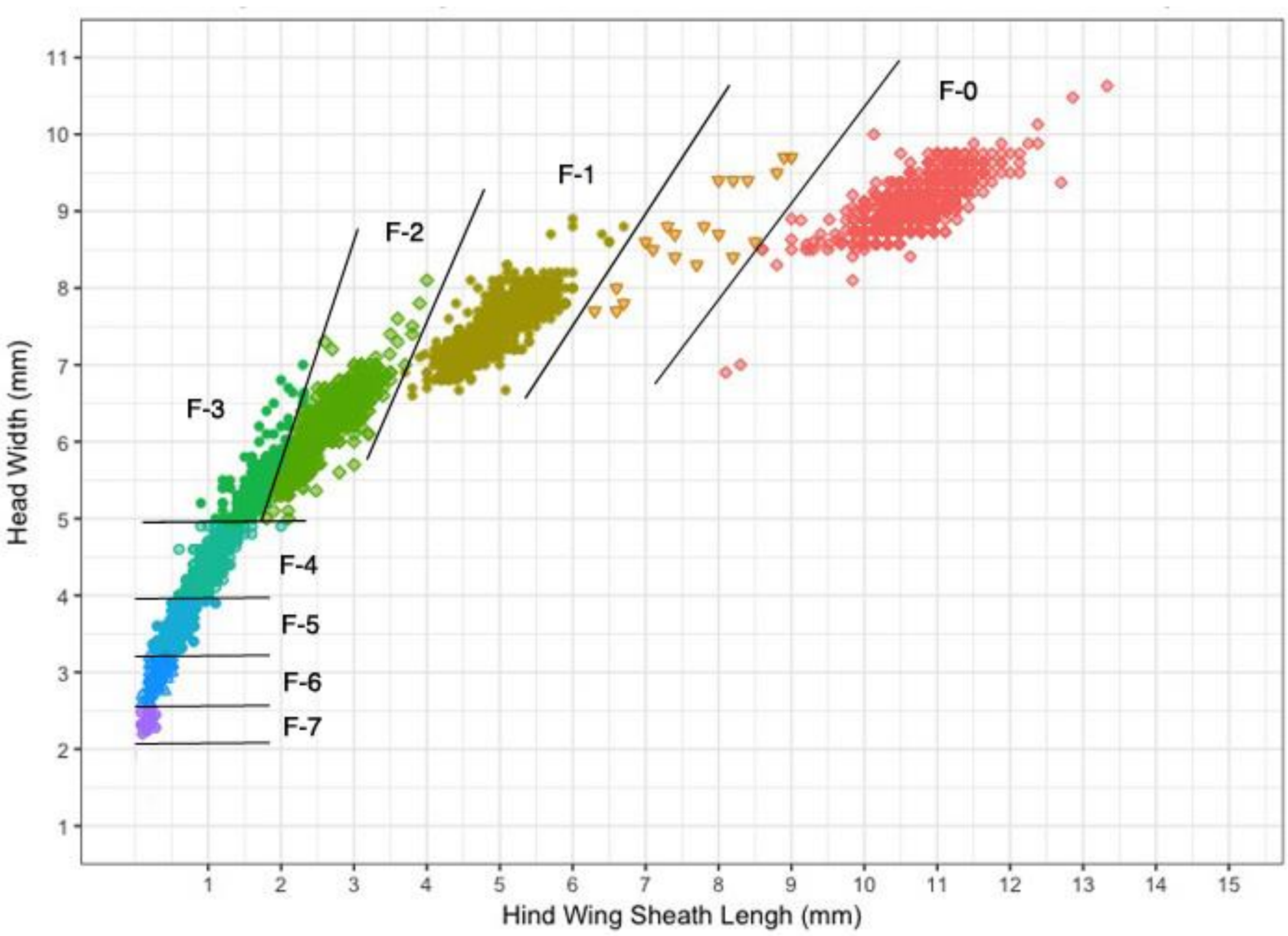
[e] F-8, F-9, and F-10 thresholds were calculated by dividing the next larger instar's lower-boundary head width by the F-6 growth rate value of 1.24.

Table 1-4. Average growth ratios for instars F-0 through F-6. Ratios were calculated for each instar by dividing the average head width of that instar by the average head width of the next smaller instar.



Instar (*i*)	n	Average Head Width (mm)	Average Growth Ratio
F-0	888	9.09	1.21
F-1	1089	7.50	1.19
F-2	896	6.28	1.16
F-3	948	5.43	1.21
F-4	678	4.47	1.24
F-5	353	3.62	1.22
F-6	116	2.96	1.24
F-7	23	2.39	-

Figure 1-4. Instars with thresholds that minimized the probability of misclassifying visualized with a scatter plot of wing sheath length against head width (n = 4990). F-0, F-1, F-2 and upper threshold of F-3 were determined using the ratio of the wing sheath length divided by head width and the lower threshold of F-3 and instars F-4 through F-7 were determined using head width. There are 20 unassigned points between F-0 and F-1 indicating nymphs in a supernumerary F-1 instar.


F-0
F-1
F-2
F-3
F-4
F-5
F-6
F-7
Head Width (mm)
1
2
3
4
5
6
7
8
9
10
11
Hind Wing Sheath Lengh (mm)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Chapter 2

Anax junius (Drury) growth development pathways (Odonata: Aeshnidae)

SYNOPSIS

Climate change is altering ecosystems and ecologists are interested in determining how the biotic components will adapt. Observing how northern insect species seasonally modify their development can shed light on the physiological tools and breadth of plasticity that they can utilize to adjust to climate change. Nearly 5,000 *Anax junius*, common green darner dragonfly, nymphs were collected, measured, and released weekly during the growing season for two years from five pothole ponds in central Minnesota, USA. Plotting nymph head width over time revealed two growth development pathways. The pathways were associated with both season and nymph overwintering strategy. Summer pathway nymphs developed underwater in the ponds for three months during summer, metamorphosed into adults in fall, and then migrated south. Winter pathway nymphs developed in the ponds for 11 months, overwintered underwater as nymphs, and metamorphosed into adults the following spring. Evidence for an ontogenetic behavioral shift from primarily prey to predator was observed at a head width threshold >5 mm. Above this threshold, nymphs were rarely disruptively colored and exhibited an elevated growth rate between instars. Experiments to determine what biotic or abiotic triggers determine a nymph's development pathway and testing for what time during ontogeny the insect is sensitive to these cues would be enlightening.

INTRODUCTION

Climate change is altering the abiotic components of ecosystems, and determining how the biotic components will respond to these changes is of great interest to ecologists (Parmesan & Yohe 2003; Parmesan 2006). Ectotherm growth and phenology are governed in part by environmental temperature, so insects and other poikilotherms can be directly affected by an altered climate (Hassall et al. 2007; Zipkin et al. 2012; Scranton & Amarasekare 2017). Insects change the rate of their development to avoid environmental dangers, maximize access to resources, and to synchronize their growth (Tauber & Tauber 1976; Jenkins et al. 2001; Danks 2002; Śniegula et al. 2012; Stoks et al. 2012; Bybee et al. 2016). Observing how and when insects modify their growth will shed light on the physiological tools and breadth of plasticity they can utilize to adapt to climate change (Reed et al. 2011; Stoks et al. 2012, 2014). Insect development can be slowed by diapause, quiescence, or by adding an additional molt. Alternatively, development can accelerated up by skipping molts, decreasing instar size, and increasing growth rate (Tauber & Tauber 1976; Danks 2002). The methods and reasons for modifying development pathways are layered and complex, but are affected broadly by the environment, genetics, and life-cycle requirements and constraints (Tauber & Tauber 1976; Danks 2002). Dragonflies are good candidates for studying the spectrum of biotic response to altered climate because they are i) aquatic as nymphs and terrestrial as adults, ii) regulated by temperature in both growth and distribution, iii) sometimes are very abundant, iv) accompanied by robust historical citizen science phenology data sets for many species (Hassall 2015).

Winter is the largest abiotic constraint on odonates in temperate climates. Adult dragonflies cannot survive winter conditions so they synchronize their aerial phase of life with spring, summer, or fall (Pritchard 1982; Corbet et al. 2006). Most temperate dragonflies species complete their reproductive cycle before winter arrives. Their eggs or nymphs suspend their development below the snow or ice until spring (Corbet 1999). The adults of a few northern dragonfly species migrate south in fall, complete one to two generations during the winter, and then their offspring return north in spring (Corbet 1999). *Anax junius*, a widespread North American generalist species, does both.

Anax junius in northern latitudes either metamorphose into adults in fall and conspicuously migrate south in swarms or remain as nymphs and enter quiescence under the ice (Corbet 1999; May 2012; May et al. 2017; Hallworth et al. 2018). The factors that determine whether an individual nymph will metamorphose and migrate or enter into quiescence are unclear (May 2012; May et al. 2017), but they must be tied to ontological development. Nymphs that over winter are probably not mature enough to metamorphose into adults by fall.

Beyond their unique dual overwintering strategy, *A. junius* is a valuable species to study because it is a widespread keystone species (Rudolf & Rasmussen 2013). *Anax junius* nymphs can be found in nearly any water body between southern Canada and Central America and thrive in shallow fishless ponds (Abbott 2006; Paulson 2018). Nymphs form unregulated size-structured populations with changing ontological functional roles as they age (Wissinger 1988; Crumrine 2005, 2010a; Rudolf & Rasmussen 2013). Small nymphs are both predator and prey whereas large nymphs are top predators in fishless ponds (Crumrine 2005, 2010a). Changes in the nymph size-class abundances can contribute to cascading trophic effects (Crumrine 2010a; Start & Gilbert 2017).

Common species are often overlooked because they are not facing immediate threats, but commonness does not guarantee protection from extinction (Gaston & Fuller 2007; Gaston 2010, 2011); the passenger pigeon (*Ectopistes migratorius*) and rocky mountain grasshopper (*Melanoplus spretus*) both had populations exceeding one billion before they went extinct (Lockwood & DeBrey 1990; Hung et al. 2014). Conserving common species should also be a priority because they make up most of an ecosystem's biomass, and are essential to its function (Grime 1998; Smith et al. 2004; Gaston & Fuller 2007; Gaston 2010). If rarity is what makes a species worthy of conservation, then it is interesting to note that common species are relatively rare. To quote Gaston (2010): "the state of being common is actually very rare."

Scientists who choose to study abundant species are rewarded with relatively easy collection, and ultimately, large sample sizes. This is particularly true with insects. Species like *A. junius* can be collected in large enough numbers across space and time to quantify the natural variation in their growth, abundance, and habitats with small margins of error. As experimental subjects, they can be used in large enough sample sizes to generate both statistically and biologically significant results. Although the results of studies involving a common species may not be directly transferable to rare species, they can help biologists understand which variables should become research priorities for more difficult-to-study species of conservation need (Kunin & Gaston 2012; Siqueira et al. 2012).

Identifying the adaptations that allow a species like *A. junius* to be so successful across a large and heterogeneous geographic range requires multi-year field-based surveys of growth and development (Kormondy & Gower 1965; Danks 2002). The objective of this study was to observe and map the growth patterns of field populations of northern *A. junius* as a preliminary requirement for studying other aspects of odonate ecology, including responses to climate change (Kormondy & Gower 1965; Benke 1970).

MATERIALS AND METHODS

Anax junius nymphs were collected from five pothole ponds within Crow-Hassan Park Reserve in central Minnesota, USA (Figure 2-1). The entire reserve is 10.5 km^2, and contains 3.4 km^2 of restored prairie pothole habitat.

The pothole ponds were small and shallow, and did not undergo seasonal turnovers. Two of the sampled ponds contained fishes (*Culaea sp., Lepomis sp.,* and *Ameiurus sp.)* and three did not (Table 2-1). The pond watersheds were completely within the reserve, and were not exposed to anthropogenic wastewater runoff or pollution. Mosquito control chemicals were not applied within the reserve. *Phalaris arundinacea, Sagittaria latifolia, Carex lacustris, Carex stricta, Lycopus americanus, Cyperus erythrorhizos, Typha* sp., *Bolboschoenus fluviatilis, Bidens cernua, Ceratophyllum demersum, Spirodela polyrrhiza, Lemna minor,* and *Potamogeton foliosus* were the dominant aquatic plants in the ponds (Appendix A).

Anax junius nymph surveys were conducted weekly from summer 2016 through summer 2018 when the water was open and periodically during the frozen winter months. Nymph surveys in 2017 and 2018 focused only on the fishless potholes in which nymph abundance was high enough to justify collection efforts.

Aluminum-edged, 38 × 23 cm minnow nets with 0.5 cm mesh were swept through the pond vegetation and along the benthic substrate to collect nymphs. Most nymphs were identified as *A. junius* in the field by the presence of three lateral spines only on abdominal segments 7, 8, and 9; an elongate prementum with pointed labial palps, and “crescent moon-shaped” eyes (Calvert 1934; Walker 1958; Needham et al. 2000; Tennessen 2019). Small Aeshnidae nymphs were brought into the lab for species identification. To minimize mortality and cannibalism, the nymphs were kept cool and separated by size during transport. Final instar *A. junius* nymphs with swollen wing sheaths were tallied in the field and released directly back into the ponds to prevent them from emerging in the lab and altering field emergence phenology observations (Chapter 3). Nymphs were collected from spring through fall by wading in the ponds from the shoreline to as deep as the waders would allow. Nymphs were collected one to three times during winter from each pond through large holes cut into the ice.

Sampling was done with even effort at different depths during summer 2017 and 2018 to control for potential size-based depth preferences. The number of net dips was standardized for each depth category: the shallow water adjacent to shore, the middle-depth area among the emergent vegetation, and the deep water with predominantly submerged vegetation. This methodology was not feasible in winter because dip netting was done through a limited number of holes that were cut into the ice.

Nymphs were measured within 24 hours of collection and released back into their ponds within 48 hours (winter-collected nymphs were not released). Maximum head width and the length of the right hind wing sheath (if present) was measured with an ocular micrometer on a Leica MZ7.5 dissecting scope to an accuracy of 0.1 mm as described in Tennessen (2017). Body color and sex were also recorded for a subset of nymphs.

We utilized the instar nomenclature established by Lutz (1968), which counts backward from the final instar. The last instar before metamorphosis is named “final-minus-zero” or F-0, the penultimate instar is called “final-minus-one” or F-1, the next younger instar F-2, and so on.

Nymph head widths were plotted against Julian day for each pond to visualize growth patterns and phenology. Multiple years of data were combined in each plot. Nymphs were categorized as being part of either the summer or winter development pathway based on visual inspection of the plots. Nymphs that grew rapidly during midsummer; experienced one fewer instar; were smaller in F-2, F-1, and F-0 instars; and emerged later in summer were assigned to the summer pathway. Nymphs that overwintered and emerged in spring or early summer were determined to be on the winter pathway. See Chapter 3 for emergence data. Most nymphs clearly belonged to one pathway, but small nymphs observed during late summer were more difficult to assign (Figure 2-2) because the development pathways temporally overlapped and cohort spitting may have occurred (Danks 2002).

Each collected nymph was assigned to an instar based on Table 1-3 (Chapter 1), utilizing the morphological parameters that minimize the probability of instar misclassification. Summer development nymphs underwent one fewer instar, making original instar assignments based on Chapter 1, which analyzed the dataset assuming one development pathway incorrect. This classification error was corrected by re-assigning summer nymphs originally labeled as F-4 or younger to the next older instar.

The sex ratio of summer and winter nymphs per pathway was calculated for F-1 through F-4 instars following Corbet & Hoess (1998). Sex was determined by looking for morphological indicators of developing reproductive organs in F-4 instars and larger as described in Tennessen (2019) (Table 2-2). Percentages of nymphs with black and white banded disruptive coloration were calculated for instars F-1 through F-6 (Table 2-3).

RESULTS

Of the 5570 nymphs collected, 547 were F-0 instars with swollen wing sheaths so they were not measured and released in the field, 4995 had undamaged measurable heads, and 4995 (the same quantity) had measurable right hind wing sheaths. The ponds with fish (Island and Betty) required substantially more survey effort to find and collect nymphs, presumably because fewer nymphs resided in those ponds. Final instar nymphs were never observed in Betty Lake.

Plotting head width by Julian day revealed two nymph growth development pathways (Figure 2-2). Summer pathway dragonflies completed their nymph stage in one summer. Winter pathway nymphs overwintered and emerged in the spring the year after they hatched. All of the surveyed ponds contained *A. junius* nymphs from both development pathways during all years surveyed (Appendix B).

Summer pathway nymphs were first observed in a given year during early June (ca Julian day 155) as F-6 and F-7 instars. These summer nymphs grew rapidly, and began entering F-0 within 30 days (ca Julian day 180). The population of summer nymphs reached F-0 by the middle of September (ca Julian day 255).

Young-of-the-year winter pathway nymphs were first observed in early to mid-August (ca Julian day 220). These nymphs grew more slowly, the first observation of a winter pathway F-0 nymph occurred ~50 days later. Most winter pathway nymphs did not reach F-0 in fall and instead overwintered in a range of smaller instars: F-1, F-2, F-3, and F-4 were most commonly collected in late fall, winter, and early spring. A few F-0, F-5, F-6 instars were also observed. Little or no nymph growth was observed from October (ca Julian day 270) through April of the following year (ca Julian day 120). Most overwintered nymphs entered their final instar in May or June (between ca Julian days 120 and 180).

Summer development pathway nymphs utilized one fewer instar than those in the winter pathway. Summer and winter growth patterns were similar when nymph head widths were smaller than 5 mm. Above this threshold, summer and winter nymphs underwent three and four more instars, respectively. During these last molts, summer nymphs had larger growth ratios between instars, but the F-2, F-1, F-0 head widths were slightly smaller, on average, than winter pathway nymphs.

Sex ratio per instar by growth pathway did not significantly differ from 1:1 when assessed with a χ^2 test at $\alpha = 0.05$ (Table 2-2). There was no significant difference in size (head width in mm) between male and female F-0 nymphs (*t*-value =197.84, 667 df, *p*-value = 0.344).

The percentage of nymphs that exhibited light and dark transverse banding was calculated for F-6 and larger instars by growth pathway and combined. Most F-6 and F-5 instars in both development pathways were disruptively colored with banding. The percentage decreased as nymphs aged. Fifty-six percent of summer pathway nymphs retained the disruptive coloration during F-4 and 13% in F-3, while only 11% and 0.002% of winter nymphs, respectively, were banded. The banding essentially disappears when nymphs reach head widths of 5 mm and larger. This size threshold is passed by F-2 and larger instars in the summer growth pathway, and by F-3 and larger instars in the winter growth pathway (Table 2-3).

Thirteen (0.3%) of *A. junius* nymphs had a small lateral spine on one or both sides of segment 6. This characteristic is notable because the presence of lateral spines only on segments 7, 8, and 9 is an attribute used to key Anisoptera nymphs to the genus *Anax* (Tennessen 2019). These individuals with spines on segment 6 represent a rare departure from that standard.

DISCUSSION

This study demonstrates that the number of *A. junius* nymph instars is associated with growth pathways (in one park reserve in Minnesota, USA). Furthermore, the growth pathways are associated with time of year, have some overlap in late summer, and determine the insect's overwintering strategy. These observations were consistent across ponds and years. These data begin to untangle a long standing mystery about what conditions cause individuals within an odonate species to undergo different numbers of instars (Corbet 1999).

WINTER AND SUMMER DEVELOPMENT PATHWAYS

Anax junius were thought to only be summer migrants in northern latitudes until Trottier (1971) observed overwintering nymphs in a pond near Ontario, Canada in 1967. He observed two distinct populations, which he called the winter (or resident) and the summer (or migratory) groups. Trottier's nymph growth data match those observed here. The summer group nymphs exhibited a three-month growth period from June through August, and the winter group nymphs an 11-month growth period from the middle of July to the end of June. Kime (1974) and May et al. (2017) report similar *A. junius* nymph growth phenologies from Washington, Maryland, and New York.

Trottier (1971) reported a temporal break of more than a month between the emergence of the winter group in spring and the later emergence of the summer group. This observation was the basis of his suggestion that they were two separate populations. More recently collected phenological data show no gap in timing between groups at similar northern latitudes, with *A. junius* adults emerging and F-0 nymphs present continuously from early June to well into fall (Kime 1974; Catling 2003; May 2012; May et al. 2017). I observed nymphs with swollen wing sheaths, which would emerge within days, present continually from mid-May until mid-October during 2017 (see Chapter 3).

The temporal gap Trottier observed between the emergence of the two groups could have been a singularity, or indicate that a change in emergence phenology has occurred in the intervening 50 years as a result of climate change. Warmer water temperatures increase *A. junius* nymph growth rates and can alter intraspecific interactions in size-structured populations which could impact emergence phenology (Start & Gilbert 2017).

While Kime (1974) did not observe a time gap between emergence of the summer and winter dragonflies, she did report that mid-summer nymphs “omitted one or two instars” foreshadowing our finding that summer pathway nymphs go through one fewer instar during the second half of their ontogeny. She suggested that the summer and winter growth pathway nymphs might be genetically different. However, preliminary genetic testing (Freeland et al. 2003), the temporal overlap of the development pathways in late summer, and tendency of female *A. junius* to hedge-bet by mating at and laying eggs in many ponds are evidence that the species is genetically similar (May et al. 2017). This suggests that North American *A. junius* are comprised of one population with an individual's development pathway being determined by external variables and that migration has long been facultative.

Growth development pathways were associated with Julian day, so abiotic conditions that are known phenological drivers, like temperature or photoperiod, may determine which development pathway a nymph follows (Danks 2002). Cool temperatures and lengthening photoperiod during spring could trigger eggs to express genes for the summer development pathway, and the warm temperatures combined with the shortening days of late summer could trigger winter pathway development (Franchini et al. 1984; Corbet 1999). Future experiments should determine if the offspring from a single female express their genes differently when exposed to different temperatures and photoperiods.

Anax junius nymph response to temperature and photoperiod could be maladaptive in the fall when nymphs are exposed to unusually warm temperatures along with short photoperiods: new conditions being observed in our modern, altered climate (Corbet 1999; Linderholm 2006). Twenty unusually-colored yellow *A. junius* nymphs were collected during fall (2016 and 2017). They all had head width by wing sheath ratios between 0.79–0.99 indicating there were in a supernumerary (i.e., intermediate) instar between F-1 and F-0 (Chapter 1). All of these nymphs died during an attempt to rear them in the lab. Mortality for other *A. junius* nymphs reared with the same methods at room temperature (20ºC) was only 21% (Chapter 4). Entering this supernumerary instar appears to be a fatal path for *A. junius* and at least a few other odonates (Sawchyn 1972; Ingram & Jenner 1976; Corbet et al. 1989; Aoki 1999; Corbet 1999). Further research on supernumerary nymphs would be valuable given our changing climate and the increased likelihood of temperate regions experiencing warmer falls.

OVERWINTERING

Catling (2003) wonders why experts before Trottier had not observed resident nymphs during the previous decades of dragonfly research in the province. Walker (1958), Matthews (2004), and Trottier (1971) all reported difficulty finding ponds with overwintered nymphs, and Catling speculated that Ontario might be at the edge of their overwintering range. This study suggests that these researchers struggled because they sampled in spring when overwintering nymphs are best observed in fall.

All of the ponds surveyed in this study contained winter pathway nymphs each fall, but severe winter conditions and/or fish predation effectively eliminated some of those populations by spring. Entire populations of *A. junius* expired during the harsh 2017–2018 winter, when the pond water froze nearly solid and became anoxic. In February and March of 2018, I sawed holes through more than 20-inches of ice to attempt to collect nymphs. The disturbed substrate smelled strongly of sulfur (typical of anoxic conditions), and carcasses of dead *A. junius* nymphs floated to the surface. Dead nymphs were also frozen to the underside of the ice; evidence that they had died earlier in winter. No living *A. junius* nymphs were observed in 2018 until the eggs of the spring migrating adults hatched in June.

The winterkill in 2017-2018 suggests that *A. junius* may be nearing its northern limit for successfully overwintering in ponds in Minnesota and Ontario. (Crow-Hassan Park Reserve in Minnesota, USA and Trottier's research ponds near Toronto, Ontario in

Canada are at similar latitudes: 45.2 ^{o}N and 43.5 ^{o}N respectively.) However, the consistent presence of nymphs in fall indicates that they do attempt to overwinter at these latitudes.

Collecting nymphs through holes cut in the ice was difficult and time consuming, so fewer nymphs were collected during winter and no nymphs smaller than F-4 were observed when ice covered the ponds. Nymphs younger than F-4 were collected when the water was open in fall and spring, so they were part of the overwintering community but the winter sampling method did not detect them. These resident nymphs were in water below their base temperature during winter, and therefore not growing (Chapter 4). The collected nymph size structure did not change from October through May, which supports the hypothesis that nymphs did not grow in winter.

Living *A. junius* nymphs collected during the winter of 2016–17 appeared to be in a temperature-induced quiescence. When these nymphs were brought to room temperature in the lab, they ate immediately when fed, and many ecdysed within a day. These observations suggest nymphs were not in a physiologically induced diapause, but it is possible that, by late winter, the intensity of a fall diapause was waning (Tauber & Tauber 1976). Entomologists have developed a precise lexicon to define the diversity of ways that an insect can survive winter. These terms should become familiar to odontologists. For example, whereas “diapause” refers to the hormonally-induced physiological changes in body function that take time to induce and release, “quiescence” indicates the immediate response that an ectotherm has when it surpasses an environmental threshold (like halting feeding and growth below a critical temperature), but is not experiencing an endocrinological diapause (Denlinger 1991; Kostal 2006).

Repeated sampling and testing throughout a year is required to determine the presence and duration of diapause in a field population of insects because the time and duration of diapause can vary within a population and the intensity of diapause decreases over time (Tauber and Tauber 1976). These testing requirements were fulfilled by the study’s sampling design. Nearly all of the weekly samples that were brought into the lab for 24 hours contained nymphs that went through ecdysis or cannibalized other nymphs as evidenced by fresh exuviae and partially eaten nymphs in the collection containers. If there had been a seasonal diapause, then it would have been observed by the lack ecdysis in these samples (Tauber & Tauber 1976). Furthermore, nymphs collected in fall could be reared to adulthood at room temperature indicating that they do not have an obligatory diapause (Chapter 4). It is possible that *A. junius* could enter a facultative diapause that was not triggered by the field conditions during this study: drought or a combination of photoperiod and temperature that was not present at northern latitudes. Kime (1974) observed the slower growth rate of the overwintering nymphs, compared to summer, and proposed that *A. junius* may enter a “weak, short-day diapause.” Our data suggest that nymphs on the winter growth development pathway utilize a lower growth rate, but do not enter a diapause. This hypothesis should be determined definitively with lab experimentation.

More cold tolerant northern specialist Odonata nymphs, like those in the genus *Somatochlora,* do likely undergo a true diapause, and can therefore can survive multiple winters before eclosing. Nymphs in the genera *Lestes*, *Luecorrhinia*, *Epitheca*, and *Aeshna* were collected in spring of 2018, and had survived the winter when *Anax* did not. *Anax junius* is a tropically evolved, generalist species (Corbet 1999) and this lack of cold adaptation likely makes it less tolerant of winter conditions and more vulnerable to winterkill.

PRESENCE OF FISH

Ponds with fish can be population sinks for dragonflies including *A. junius* (McPeek 1990; Šigutová et al. 2015). Breeding adults do not avoid ponds with fish, despite their offspring having no fish predation avoidance behaviors (Kime 1974; May et al. 2017). I witnessed *A. junius* patrolling, breeding, and ovipositing at Betty Lake in large numbers during the mid-summer months, but no F-0 nymphs or exuviae were collected from the lake during the entire survey period. Winter pathway nymphs were observed in fall of 2016 in Betty Lake, but no nymphs were collected during winter or spring of 2017. Fish presumably consumed all of the overwintering nymphs (nymphs in the fishless ponds did not experience a winterkill in 2016–2017). *Anax junius* abundance in the ponds with fish appeared to be smaller based on comparing the survey effort required to collect nymphs. For example, a field technician in People Pothole would often collect 2–4 nymphs in one swoop of a net, whereas a technician in Betty Lake could look for an hour or more before finding a single nymph.

PATHWAY TRADE-OFFS

Summer pathway nymphs traded off fast development for a smaller final size; skipping an instar in order to metamorphose in time for migration. Fast growth requires active foraging, and likely put these nymphs at greater risk of predation (Kime 1974; Crumrine 2010a, 2010b; Crumrine & Crowley 2016). Predators in shallow, fishless ponds were primarily larger conspecifics. Smaller nymphs are most vulnerable to intraspecific predation. That may be why summer pathway nymphs did not increase their growth ratios until head widths exceeded 5 mm. The evolutionary rewards for fast growth include avoiding a vulnerable period of molting and the opportunity for migrators to parent two successive generations during the winter, while the winter nymphs are in quiescence (Kime 1974; Hallworth et al. 2018).

Winter pathway nymphs do not become mature before winter arrives. Their only alternative is to overwinter under the ice. Paulson & Jenner (1971), Kime (1974), and Beesley (1972) observed a wide range of nymph instars (F-7 through F-0) overwintering, which corroborates our findings. *Anax junius* nymphs do not appear to attempt to synchronize or regulate their growth before winter, but simply pause development when the water is below their base temperature (Chapter 3, Chapter 4). The overwintered nymphs resume growth in spring. Their final instars are slightly bigger than F-0 summer nymphs, which may increase their fecundity (Preziosi et al. 1996). Their slower growth also likely reduce their foraging needs, and lowered their predation risk (Crumrine 2010b). These nymphs risk winterkill, but also avoid the dangers of migration.

A. junius nymphs use two development pathways to maximizing their temporal and spatial resources. Their generalist adaptations enable them to hedge-bet over space and time, thereby increasing their likelihood of survival in highly variable environments such as northern, fishless ponds (May et al. 2017).

MICROHABITATS

Microhabitats are the main factor driving odonate niche partitioning (Johnson & Crowley 1980; Corbet 1999). Nymph use of microhabitats has been associated with aquatic plant phenology, season, nymph size, and the presence of other species of dragonflies (Lawton 1970; Kime 1974; Corbet 1999).

Anax junius nymphs were found clumped together in the ponds. These "hot spots" were generally in the same locations, even during winter. The nymphs were nearly always collected among vegetation (except in midwinter) with an apparent preference for *Carex* sp. and *Sagitarria* sp. over *Typha* sp. However, nymph clusters were not always found in locations with these plants. Experienced research technicians were able to look at pond vegetation and identify likely *A. junius* microhabitats, but only sampling revealed whether or not the nymphs were utilizing those areas of the pond. Small nymphs might have been taking refuge in the aquatic plants near their hatching location, and this food source attracted larger nymphs to the same areas. Kormondy and Grower (1965) also observed clumped odonate nymph distribution associated with vegetation. Aquatic plants are important for the stability of size-structured communities in fishless ponds: increased vegetation increases the density of prey that can exist among predators (Crowder & Cooper 1982; Thompson 1987; Corbet 1999).

Anecdotally, F-0 nymphs were predominantly collected in the shallow areas near shore, often with swollen wing sheaths and likely staging for emergence. When water levels dropped during dry hot periods, *A. junius* nymphs appeared to move towards the center of the ponds at a depth of ~0.5 m. In fall, the nymphs were found nearly exclusively in the middle to deeper areas of the ponds, still among vegetation. *Aeshna canadensis* nymphs were found in the shallowest areas near shore. Technicians observed that collecting *A. canadensis* nymphs indicated that they were unlikely to find *A. junius* in the same microhabitat. During winter, *A. junius* nymphs were pushed deeper into the ponds as the ice froze solid in the shallows. This was the only time of year that nymphs were collected in the deepest areas of the ponds with no vegetation. Kime (1974) also observed *A. junius* nymphs moving to deeper water in fall, and final instars staging for emergence in the shallows.

SEX RATIOS

Observed sex ratios from this study provide no evidence of mortality based on sex-specific growth strategies. Odonata sex at hatching is genetically determined and expected to be 1:1; therefore a change in sex ratio as nymphs grow can be evidence for sex-specific growth strategies (Lawton 1972; Corbet & Hoess 1998). Theory predicts that larger males of patrolling species are able to claim higher quality habitats and attract more females (Serrano-Meneses et al. 2007, 2008). To become larger, male nymphs would need to forage more and be more vulnerable to predators. This could tip the sex ratio towards female as nymphs age (Johansson et al. 2005; Fuselier et al. 2007). Fuselier et. al. (2007) experimentally observed that male *A. junius* nymphs were more actively foraging and growing larger than females but, like in this study, they did not find that field collected F-0 sex ratios varied from unity. Large dragonfly nymphs in fishless ponds are top predators and may not be affected enough by predation pressure to alter sex ratios (Crumrine 2006). The sex ratio of Odonata instars younger than F-4 is unknown because they have not yet developed morphologically observable sexual differences (Corbet 1999; Tennessen 2019).

Corbet and Hoess (1998) suggested that sex ratios at emergence (using exuviae) be assessed per season, instar, pond, and development pathway with a minimum sample size of 99 per instar. The data from this study (using nymphs) used these criteria as a guide, but ponds were pooled per development pathway to increase sample sizes. The data do not show a shift in sex ratios. There is also no evidence that spending more time as a nymph disproportionally affects the mortality of either sex because both the 3 month (summer) and 11 month (winter) development pathways ratios did not significantly differ from 1:1 (Table 2-2).

The percent of F-0 male nymphs in this study ranged from 41% to 58%. Trottier observed *A. junius* exuviae to be 47–48% male (1966, 1971). Beesley (1972) reported no significant difference between the number male and female nymphs in his studies

(Riverside, California). Fuselier et al. (2007) observed exuviae *A. junius* sex ratios between 48% and 55% (Clay County, Minnesota). Generally, Anisoptera exhibit a female-dominated sex ratio at emergence (Lawton 1972); the data from this study trend towards male dominated at F-0, but did not significantly differ from 1:1 (Table 2-2). Note that comparing F-0 instar sex ratios to exuviae sex ratios in this discussion assumes that there is no sex biased mortality during emergence.

DISRUPTIVE COLORATION

Smaller nymphs exhibited a light and dark laterally striped disruptive coloration body pattern, which may mimic light rays in shallow water (Williams 1936; Corbet 1957; Van Buskirk 1992). The stripes were most often black and white. Occasionally, the lighter stripes were a pale green, and black stripes had a greenish hue. This disruptive coloration has been observed in other small Aeshnidae nymphs, most often in unsynchronized, summer species in size-structured populations with high rates of predation and/or cannibalism (Kime 1974; Corbet 1999). As the nymphs from our sample aged, their body color and pattern changed to solid or mottled greens and browns, which may minimize their detection by prey while they are hunting (Kime 1974).

Most banded nymphs were observed from ca Julian day 195–300 (mid-July – October) when predation pressure from larger conspecifics is at its most intense (Crumrine 2010b). Two of the three banded nymphs with head widths larger than 5 mm were collected from Island Pond, which contains fish. The presence of these larger, camouflaged nymphs suggests that fish predation pressure may cause nymphs to retain disruptive coloration in later instars. Kime (1974) observed a similar pattern. The majority of her field collected F-6 and F-5 *A. junius* nymphs were banded; the percentage of nymphs with this coloration dropped to zero with F-2 instars and larger.

ONTOGENETIC BEHAVIORAL SHIFT

Small nymphs appear to be predominantly adapted for predator avoidance via their banded coloration and avoidance behaviors. Larger nymphs, once relieved of this predation pressure, alter their bodies and behaviors to become aggressive predators. (Kime 1974; Van Buskirk 1992; Crumrine 2010b; Hopkins et al. 2011). A head width of 5 mm was the ontogenetic turning point from prey to predator in our sample. Above this threshold, very few nymphs had disruptive coloration and the predation pressure must have been significantly released because summer pathway nymphs grew more rapidly and had much larger growth ratios. An experiment that manipulated the presence and abundance of large and small *A. junius* nymphs, and their different ecological functional roles, triggered trophic cascades that impacted primary producer abundance and basic ecosystem processes (Rudolf & Rasmussen 2013).

Additional observational research to determine which growth pathways are used by *A. junius* at different latitudes would be valuable, along with experimental research testing for what abiotic or biotic factors determine growth pathway selection at what period during ontogeny. Furthermore, future research examining fishless pond ecosystem function should take into account that macroinvertebrates with size-structured populations can have multiple functional roles, and their proportions can alter community structure.

Figure 2-1. A map of the five study ponds within Crow-Hassan Park Reserve in central Minnesota, USA. The three fishless research ponds are filled-in solid and the two ponds with fish are filled with lines. Ponds not surveyed are unfilled.

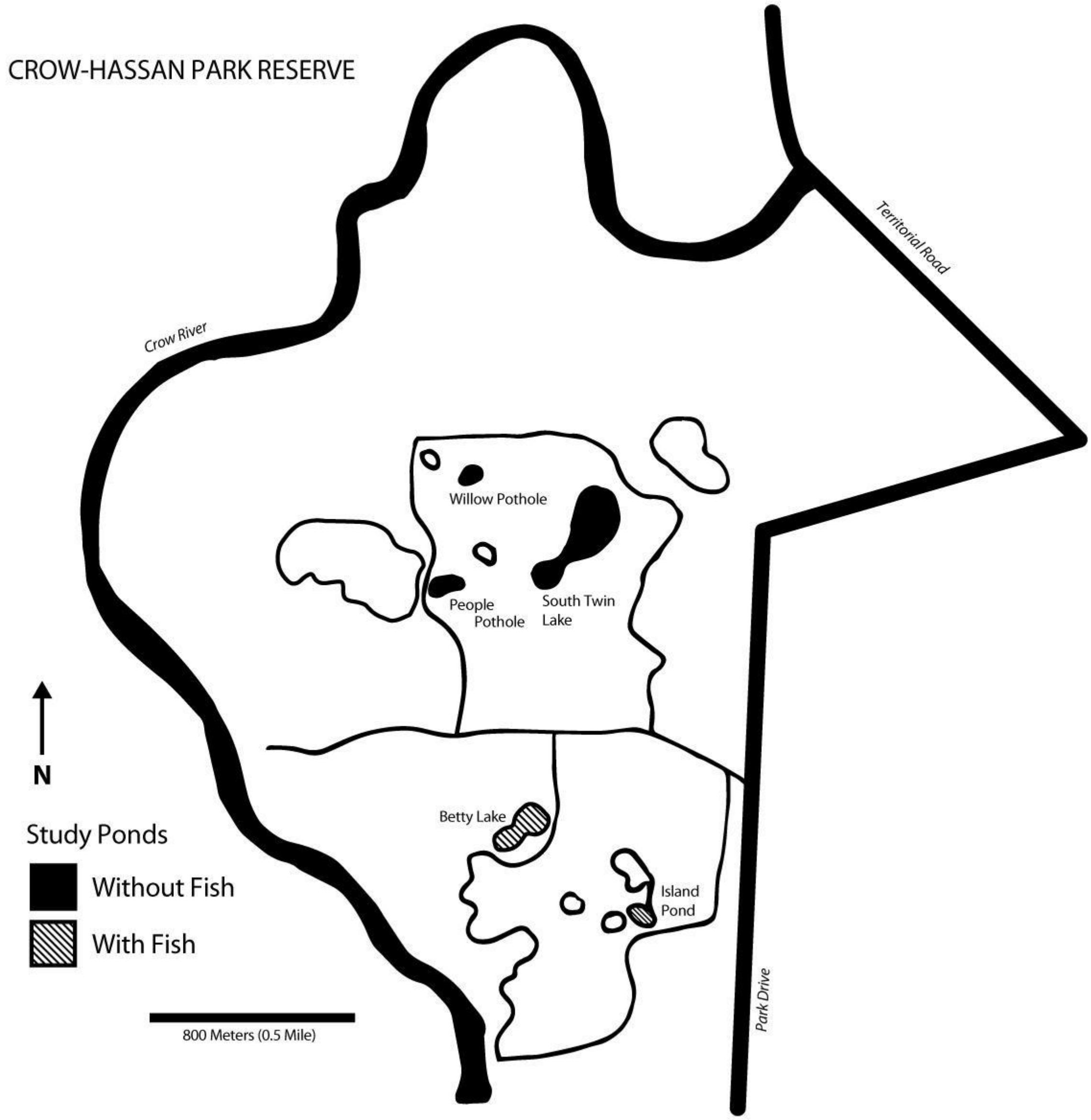


Table 2-1. Descriptions of the Crow-Hassan Park Reserve (Minnesota, USA) research ponds and the number of *A. junius* nymphs collected at each pond per year.

Pond	Coordinates	Lake Type	Area[a]	Maximum Depth[b]	Collection Dates	Year	n
Willow	45.194082° -93.639446°	Fishless	2,090 m²	0.61 m	June 2016 – June 2018	2016 2017 2018	850 509 34
People	45.190287° -93.640387°	Fishless	5,230 m²	0.74 m	June 2016 – Sept. 2018	2016 2017 2018	823 838 264
South Twin	45.192081° -93.633065°	Fishless	53,860 m²	1.56 m	June 2016 – June 2018	2016 2017 2018	1085 814 3
Betty	45.181587° -93.636097°	Fish	14,210 m²	0.76 m	June 2016 – Oct. 2016	2016	87
Island	45.178221° -93.630323°	Fish	2,760 m²	0.57 m	July 2016 – Jan. 2017	2016	259

[a] Water body area was calculated using the 2018 Google Earth polygon area measurement tool.

[b] Maximum Depth was measured at each pond on December 12, 2018 with a Humminbird Helix 5 Sonar Fish Finder. Area and maximum depth varied over time with precipitation.

Figure 2-2. *Anax junius* nymph growth visualized as head width (mm) over time (Julian day). This figure includes all nymphs collected over the entire collection period (2016–2018) from Minnesota, USA (n = 4,991). The solid arrow indicates the summer growth pathway where nymphs matured during the three summer months. The open arrows indicate the winter growth pathway where nymphs overwintered and matured after 11 months. The pathways overlap from ca Julian day 200 through 250 highlighting an opportunity for cohort splitting. F-1S nymphs were an uncommon supernumerary instar between F-1 and F-0.

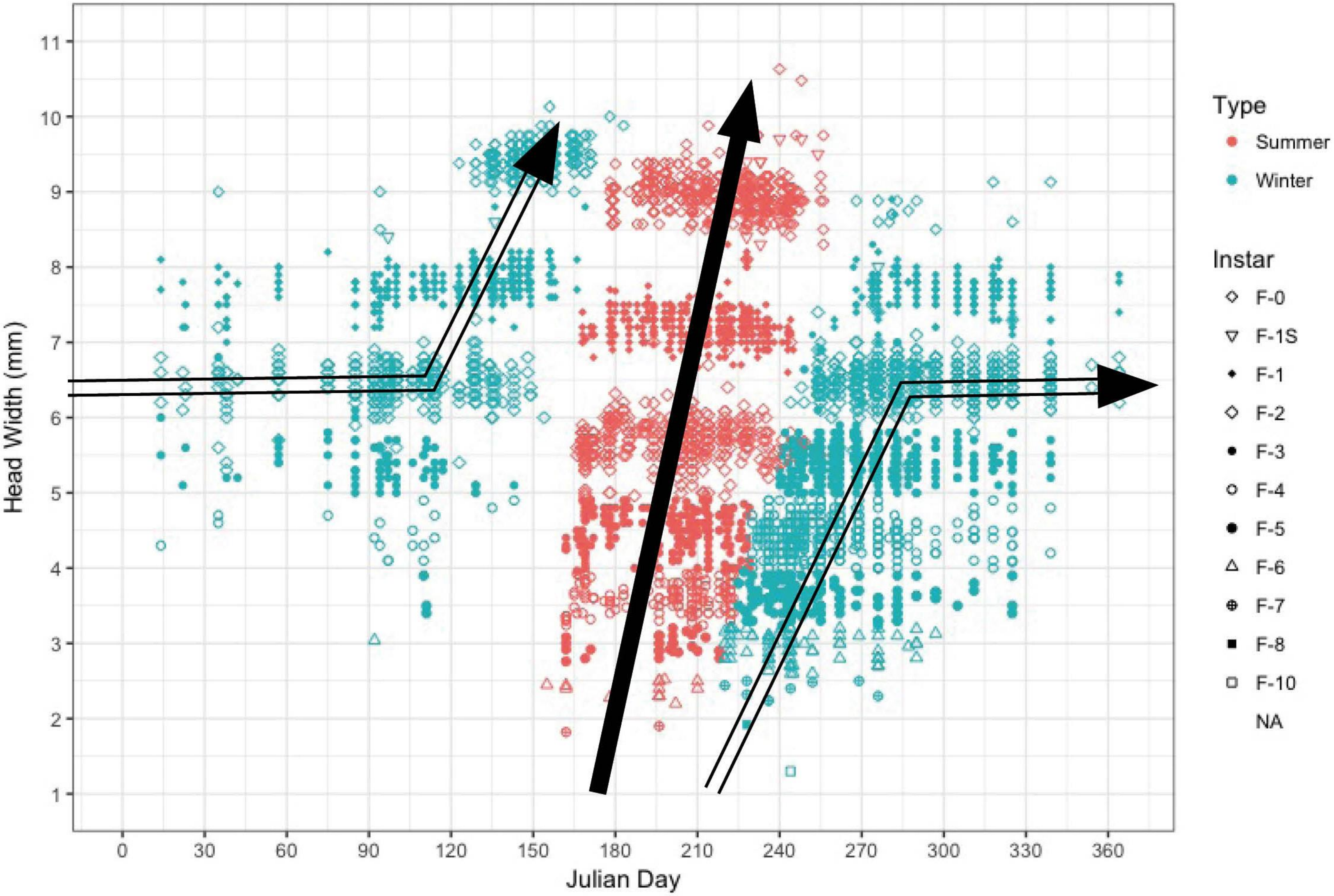
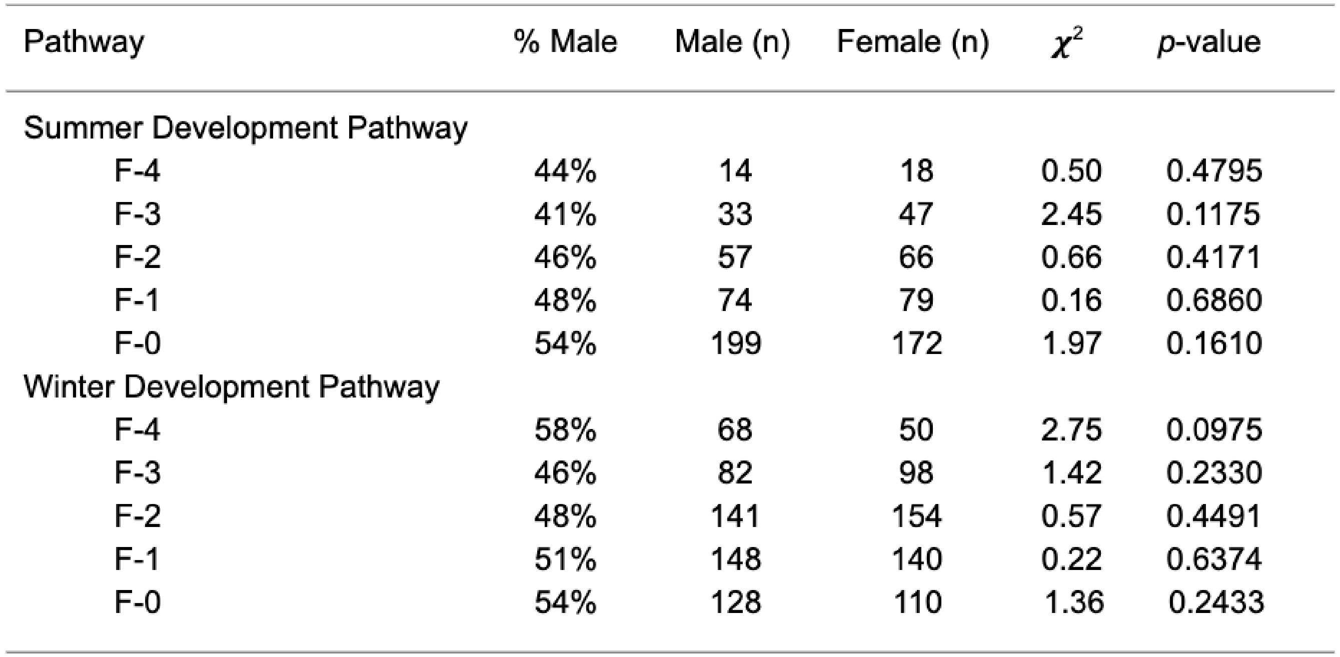
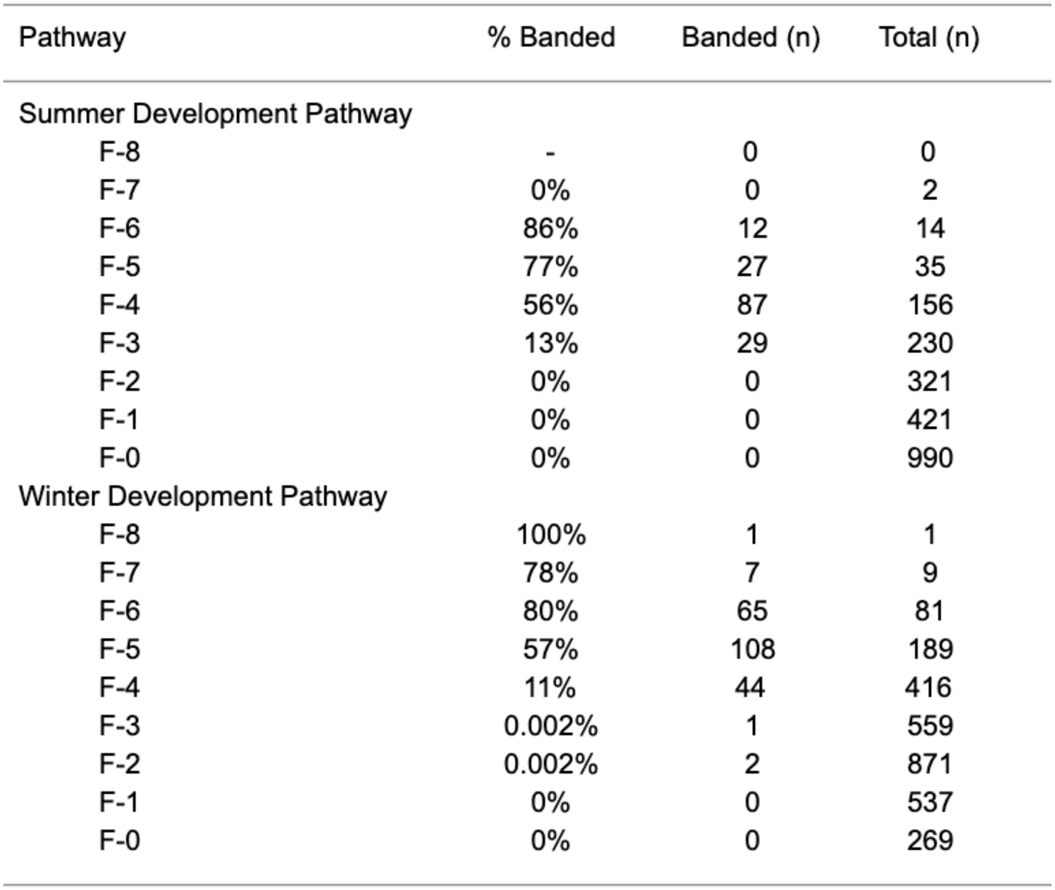

Head Width (mm)
11
10
9
8
7
6
5
4
3
2
1
0
30
60
90
120
150
180
210
240
270
300
330
360
Julian Day
Type
● Summer
● Winter
Instar
◇ F-0
▽ F-1S
✦ F-1
◇ F-2
• F-3
○ F-4
● F-5
△ F-6
⊕ F-7
■ F-8
□ F-10
NA

Table 2-2. Sex ratios (% male) of *A. junius* nymphs collected from Minnesota, USA in 2017 for each instar and separated by development pathway. None of the ratios varied significantly from 1:1 when assessed using a χ2 test with α = 0.05.



Pathway	% Male	Male (n)	Female (n)	χ^2	*p*-value
Summer Development Pathway					
F-4	44%	14	18	0.50	0.4795
F-3	41%	33	47	2.45	0.1175
F-2	46%	57	66	0.66	0.4171
F-1	48%	74	79	0.16	0.6860
F-0	54%	199	172	1.97	0.1610
Winter Development Pathway					
F-4	58%	68	50	2.75	0.0975
F-3	46%	82	98	1.42	0.2330
F-2	48%	141	154	0.57	0.4491
F-1	51%	148	140	0.22	0.6374
F-0	54%	128	110	1.36	0.2433

Table 2-3. Percentage of *A. junius* nymphs transversely banded with light and dark stripes by instar and separated by development pathway. The presence of banding was noted in nymphs collected from Minnesota, USA from 7/21/16 through 9/24/18.



Pathway	% Banded	Banded (n)	Total (n)
Summer Development Pathway			
F-8	-	0	0
F-7	0%	0	2
F-6	86%	12	14
F-5	77%	27	35
F-4	56%	87	156
F-3	13%	29	230
F-2	0%	0	321
F-1	0%	0	421
F-0	0%	0	990
Winter Development Pathway			
F-8	100%	1	1
F-7	78%	7	9
F-6	80%	65	81
F-5	57%	108	189
F-4	11%	44	416
F-3	0.002%	1	559
F-2	0.002%	2	871
F-1	0%	0	537
F-0	0%	0	269

Chapter 3

Anax junius (Drury) emergence curves illustrate variation in the phenology of a summer species (Odonata: Aeshnidae)

SYNOPSIS

Collecting odonate nymph skins, called exuviae, which are left behind after metamorphosis provides information about the timing, abundance, geography, and sex ratio of emerging dragonflies. Accumulating exuviae data over many years can demonstrate changes in populations, illuminate successional shifts, and inform habitat management. This life history knowledge is the foundation for research about the impacts of climate change and informs dragonfly conservation. For these reasons, the phenology of *Anax junius*, the common green darner dragonfly, has been of scientific interest for nearly 100 years. However, patterns of emergence phenology have been difficult to define. I studied the emergence phenology of *A. junius* by collecting their exuviae during 2016 and 2017 at ponds near Minneapolis, Minnesota, USA. The observed cumulative emergence curves matched what is expected for a generalist summer odonate: a gentle slope, a long duration, and a late date to reach 50% emergence. The seasonal emergence curves were unsynchronized, and exhibited a variable number of peaks between ponds and years. Years of exuviae surveys evaluated as a whole reveal that there is no predictable pattern in northern *A. junius* emergence curve peaks. Future studies that assess the impacts of temperature, barometric pressure, and water level on emergence phenology and success may be able to better define what mitigates the variation in *A. junius* emergence, and assist with predicting the impacts of anthropogenic ecological changes.

INTRODUCTION

The life history of *Anax junius* has been of scientific interest since the 1930s (Calvert 1934). Biologists have observed, asked questions about, and tested hypotheses regarding the growth, development, emergence, and migration of this charismatic and widespread generalist species. Despite this attention, patterns of *A. junius* emergence phenology have been difficult to define (Trottier 1966; Wissinger 1988; May & Corbet 2003; May 2012; May et al. 2017).

Dragonflies and damselflies (order Odonata) are hemimetabolous insects that develop directly from the aquatic nymphs into terrestrial adults. Nymphs that are ready to emerge crawl out of the water and latch onto vegetation, soil, or rocks. Adults emerge by pulling themselves out of the nymph skin, inflating the body and wings, and then flying away (Corbet 1999). The exuviae that are left behind are easily collected, sexed, and identified to species (Tennessen 2019). They also provide information about the timing, abundance, geography, and sex ratio of Odonata emergence (Foster & Soluk 2004; DuBois 2015). Collecting exuviae data over many years can demonstrate changes in populations, illuminate successional shifts, and inform habitat management (Corbet 1999). The timing of emergence is a major component of a dragonfly’s life history. Emergence needs to occur when the weather is hospitable, when conspecific adults are available for reproduction, and early enough in the growing season to allow offspring to reach a winter critical size (Corbet 1999).

Corbet (1999) identified two categories of dragonfly life histories. “Spring species” overwinter as nymphs in their last instar, and emerge during a short, synchronized period in spring. “Summer species” nymphs overwinter in a range of instars and emerge, unsynchronized, over a span of weeks or months (Corbet 1999, 2003). Although some *A. junius* individuals emerge in spring, their life history corresponds with what Corbet has defined as a summer species. *Anax junius* are unusual among odonates in that they have different overwintering strategies based on their life stage. Nymphs spend winter under the ice in quiescence, and adults migrate south in fall. The progeny of the migrators fly north in spring.

Dragonfly emergence can be visualized and quantified using seasonal and cumulative emergence curves. Seasonal curves illustrate the abundance of exuviae collected over time (Corbet 1999; Richter et al. 2008). Spring species usually have one, narrow seasonal emergence curve distribution early in the growing season. Summer species exhibit a variety of seasonal emergence curves, but often have multiple peaks and can span the duration of the growing season. Cumulative emergence curves quantify synchronicity, and allow one to visualize the rate of emergence over time (Corbet 1999; Richter et al. 2008). Spring species have steeply inclining cumulative emergence curves and reach 50%, of total emergence (EM50) within a few days (Tatarkiewicz 2012). The cumulative curves of summer species are less steep, and can take weeks to reach EM50 (Kormondy & Gower 1965; Wissinger 1988; Corbet 1999).

Here, I report on the emergence phenology of *A. junius* for two years at ponds near Minneapolis, Minnesota, USA. The observed phenological data are placed in the context of previous emergence studies, and can serve as a foundation for understanding and predicting the impacts of anthropogenic ecological changes (e.g., accelerated emergence, distribution shifts, freshwater trophic shifts) (Hassall et al. 2007; Richter et al. 2008).

MATERIALS AND METHODS

Anax junius exuviae were collected from shallow prairie pothole ponds within Crow-Hassan Park Reserve, near Minneapolis, Minnesota, USA. Surveys occurred weekly throughout the 2016 and 2017 emergence seasons. During 2016, surveys began at South Twin Lake, People Pothole, and Willow Pothole in June, and at Betty Lake and Island Pond in July. Betty and Island contained fish; the other ponds were fishless (Table 3-1, Figure 3-1). All surveys ended in November. Exuviae were collected by technicians who walked meandering surveys. All aquatic emergent vegetation and terrestrial vegetation within 0.5 meter of shore along the surveys were searched. Survey events ranged in length from 60 to 225 minutes. A survey event was defined as one survey on one day at one pond. Search time was calculated as the total survey event duration in minutes multiplied by the number of field technicians searching for exuviae. To allow calculation of absolute abundance I began collecting from transects in 2017.

Surveys during 2017 focused only on the fishless ponds in which exuviae abundance was high enough to justify collection efforts. Fieldwork began after ice-out in April and continued until into October. Exuviae were exhaustively collected from pre-established shoreline transects directly off of the vegetation, or if they were floating in the water and looked obviously “fresh.” Each transect was searched twice per survey event, and included all adjacent emergent aquatic vegetation and the terrestrial vegetation within 0.5 meter from shore. The transect for People Pothole included 102 meters of the north shoreline; South Twin Lake included 186 meters along the eastern shoreline; and Willow Pothole included the entire 250 meter pond perimeter.

All collected exuviae were confirmed in the lab to be *A. junius* by looking for lateral spines only on abdominal segments 7, 8, and 9; eyes that were longer than wide, and a long prementum with pointed labial palps (Tennessen 2019). Exuviae were counted, dried, and stored after each survey event. All of the specimens were sexed following Tennessen (2019) on days when fewer than 50 exuviae were collected. Otherwise, only a subset of 50 exuviae were sexed per collection event.

The number of exuviae collected per minute in 2016 was calculated by dividing the total number of exuviae obtained during a survey event by the total survey time. Seasonal emergence curves for all five ponds were created by graphing the date of collection by exuviae collected per minute (Figure 3-2). Total exuviae abundance in 2017 was plotted across time to visualize the seasonal emergence curves for People Pothole, South Twin Lake, and Willow Pothole (Figure 3-3).

Total duration was defined as the number of days spanned by each observed emergence season. The number of days required to reach 50% cumulative emergence (EM50) was calculated for the 2017 abundance data (Table 3-2). Cumulative curves were constructed comparing emergence between the ponds and sexes (Figures 3-4 and 3-5). The closest observed cumulative emergence value to 50% was selected as the EM50 date for each pond. Cumulative emergence was not calculated for the 2016 sample because exuviae abundance was not recorded.

Data were analyzed and visualized using the software R (R Core Team 2018), and the packages *scales* (Wickham 2018), *dplyr* (Wickham et al. 2019), and *ggplot2* (Wickham 2016).

RESULTS

We collected 9,185 exuviae from all of the ponds over two years (Table 3-2). Exuviae were primarily found attached to vegetation at their location of emergence. This occurred at a variety of heights from near the surface the water level up to ~2 meters above the water, or ground. No adults appeared to have emerged on rocks or soil. Most *A. junius* exuviae were found on the underside of emergent *Sagittaria* sp., within clumps of *Carex* sp., on *Typha* sp., and less often on *Phalaris arundinacea*. There was no emergent vegetation available during a period of low water at South Twin Lake, so *A. junius* nymphs had to crawl more than a meter over moist soil to reach the shoreline vegetation (*Scirpus fluviatilis* and *P. arundinacea*).

Anax junius nymphs emerged continuously throughout the growing season (June through November). Emergence periods ranged from 61 to 151 days (Figure 3.2, Figure 3.3). Seasonal emergence curves exhibited one, two, or three peaks. The 2017 seasonal curves exhibited primarily bimodal distributions in which emergence peaked in spring and late summer (Figure 3.3). The 2016 data, constructed with exuviae collection rate, did not present a consistent pattern across ponds (Figure 3.2).

The 2017 exuviae sex ratios for South Twin Lake and Willow Pothole did not differ from unity. However, the 2017 People Pothole exuviae sex ratio was significantly female-biased (46% male and 54% female, $\chi^2 = 4.8869$, 1 df, $p = 0.027$) (Table 3-3).

The 2017 cumulative emergence curves were gently-sloped, as is typical for a non-synchronized summer species (Corbet 1999) (Figure 3-4). Fifty percent of the 2017 emergence (EM50) occurred between days 75–85. South Twin Lake reached EM50 two days before People Pothole, and 10 days before Willow Pothole (Table 3-2). There was little difference in the rate of emergence between males and females, with a zero to three day EM50 difference between the sexes (Table 3-3). Females reached EM50 slightly earlier in the season. Male and female cumulative emergence curves closely match except in Willow Pothole, where female emergence initially accumulated more rapidly (Figure 3-5).

DISCUSSION

EMERGENCE TIMING AND DURATION

Anax junius emergence was observed throughout the entire growing season. This observed duration in days (d) was long even for a “summer species,” as defined by Corbet (1999). I observed emergence durations of 61–151 days. A typical summer species of damselfly, *Ischnura verticalis*, has an emergence duration of 34–59 days, and reaches EM50 after 11 to 29 days (Kormondy & Gower 1965). The summer dragonfly species *Erythemis simplicicollis* and *Pachydiplax longipennis* emerged over 120 and 110 days, respectively (Wissinger 1988; Corbet 1999). In contrast, a spring synchronized species, *Libellula fulva,* emerges over 19–20 days, and reaches EM50 after 5–7 days (Tatarkiewicz 2012).

The short, 61-day emergence duration from Island Pothole is probably an incomplete measurement. Exuviae had already been collected from nearby pothole ponds by the time the first survey of Island Pothole occurred in July. The longest observed period at Willow Pothole, 151 days, was extended by a single outlier exuvia collected in November. Setting aside these two observations, the duration of *A. junius* emergence spanned ~130 days from early June into October.

John A. and Suzanne M. Gregoire collected *A. junius* exuviae daily during the growing season at Kestrel Haven Avian Migration Observatory in Burdett, New York, USA from 2004–2013 (May et al. 2017). Their observed emergence durations of 115–130 days were similar to our results (May et al. 2017). Young (1967) reports a short emergence duration (55 days) from a small pond in Northern Illinois, USA. He surveyed for *A. junius* exuviae from April to November, so the entire potential emergence period was included. Perhaps the previous winter was severe enough to cause the overwintering *A. junius* nymphs to winterkill. In this scenario, the first nymphs of the season to emerge would be the progeny of the spring migrators in July. I observed a winterkill event at our field sites during the winter of 2017–2018 (nymph sampling continued after the exuviae sampling portion of the field work was complete). No living *A. junius* nymphs were collected from People Pothole until early June, despite weekly sampling beginning in April (Chapter 2). Field technicians first anecdotally observed exuviae at People Pothole during 2018 in mid-July. Trottier (1966) observed a 30-day emergence duration (July 29–August 27, 1965) with EM50 by day 13 from a pond in Quebec, Canada. The late start of Trottier's emergence period, relative to our data, could also be the result of a winterkill. However, the early end of emergence in August is unexpected. Given that Trottier reports that the pond's "greatest depth varied seasonally from one meter to a few centimeters", I speculate that the water level in late summer could have been too low for *A. junius* nymphs to survive.

Anax junius can thrive in extremely variable habitats but, are vulnerable to fish predation (Kime 1974; Johnson & Crowley 1980). No exuviae were collected from Betty Lake despite weekly surveys throughout the 2016 growing season. Dozens of adult *A. junius* were observed ovipositing at Betty Lake, and small nymphs were collected, but none made it to adulthood. The nymphs were probably all consumed by fish. Betty was the only surveyed pond that contained fishes that were longer than 15 cm (*Ameiurus melas* and *Lepomis cyanellus*). Island Pothole contained smaller fishes (*Umbridae* sp., stickleback from the family Gasterosteidae, and small *Lepomis* sp.), but the 2016 exuviae collection data from Island were comparable to the fishless People and Willow Potholes.

SEASONAL EMERGENCE CURVE VARIATION

The first published *A. junius* seasonal emergence curves were bimodal with well separated peaks (Trottier 1971; Wissinger 1988). This was the basis of speculation that two phenologically separate, perhaps genetically distinct, groups of *A. junius* existed (Trottier 1971; Wissinger 1988; May 2012). An overwintered cohort was thought to emerge in late spring and a migrating cohort in late summer. More recent data now demonstrate that there is a great deal of variety in the shape of *A. junius* emergence curves (May et al. 2017), and that individuals from the two peaks are not genetically distinct (Freeland et al. 2003).

Data from Kestrel Haven (May et al. 2017) revealed highly variable *A. junius* emergence. The seasonal emergence curves in some years were clearly bimodal, but varied in relative size (i.e., 2006, 2011, and 2012). The early distribution was much larger in 2006, but the later distributions were larger in 2011 and 2012. Emergence peaks were not obvious in 2004 and 2005. Additionally, the total numbers of exuviae collected per year ranged widely from 87 (2007) to 1004 (2004). Our emergence curves were similarly unpredictable. Exuviae collected from South Twin Lake in 2016 indicated one large emergence curve, while the other ponds in that year produced smaller curves with two or three peaks. All three ponds in 2017 had fairly synchronized smaller early emergence peaks, and larger later peaks. Therefore, a single year of *A. junius* emergence phenology provides insufficient information to draw conclusions. A decade of exuviae surveys evaluated as a whole reveal that there may be no predictable pattern in northern *A. junius* emergence peaks. Corbet (1999) describes this kind of development as having “unmitigated variation.”

COLLECTION CHALLENGES

There are two challenges with using exuviae to quantify emergence over time. The first challenge is to develop and carry out collection protocols to ensure that abundance is not underestimated by missed exuviae. The second challenge is to confirm the age of exuviae; if age is underestimated, then abundance could be overestimated (Corbet 1999). I addressed the first concern in 2017 by abandoning the meandering walk surveys in favor of two collection passes along an established transect during a survey event (DuBois 2015). This method also allowed us to calculate and compare exuviae abundance between ponds. I assumed that all exuviae collected from vegetation were from dragonflies that emerged during the previous week. DuBois (2015) determined that two survey passes along a single transect generates an exuviae detection probability of ~87%, and that smaller exuviae are less likely to be detected. *Anax junius* exuviae are large and conspicuous, so may have had a higher detection rate. Aliberti Lubertazzi & Ginsberg (2009) reported that exuviae fall off of their emergence supports quickly. Wisssinger (1988) observed a 15% loss within three days. Given that the detection probability for the transect method is high, and that exuviae quickly disappear off of their emergence supports, I analyzed the data assuming that I did not collect exuviae that were older than our one-week survey frequency.

We executed a small field test to determine how long exuviae floating in the water remained detectable, and if they could be assumed to have originated within our one-week collection period. In August of 2016, ten dry intact *A. junius* exuviae were placed inside of an open-topped, 0.7 × 0.7 meter, mesh-sided box near the shore in Willow Pothole and observed daily for seven days. Exuviae looked limp and soggy, no longer crisp and fresh, after three days; progressively decomposed during days four through six; and were unobservable after seven days. Therefore, I was confident that fresh-looking exuviae floating in the water during summer were from dragonflies that had emerged within the previous week.

Corbet (1999) recommends daily exuviae surveys for measuring emergence phenology, given the quick loss of exuviae off of their perches. Daily exuviae collection data are needed to define the phenology of spring species with short emergence periods and steep cumulative emergence slopes; however, weekly collections were a practical use of field technician time, and sufficient to accurately record the slow and lengthy *A. junius* emergence. Some exuviae may have been lost in the seven days between our surveys, so the 2017 total abundance observations may be underestimates. However, the relative emergence phenology patterns over time and between ponds retain integrity.

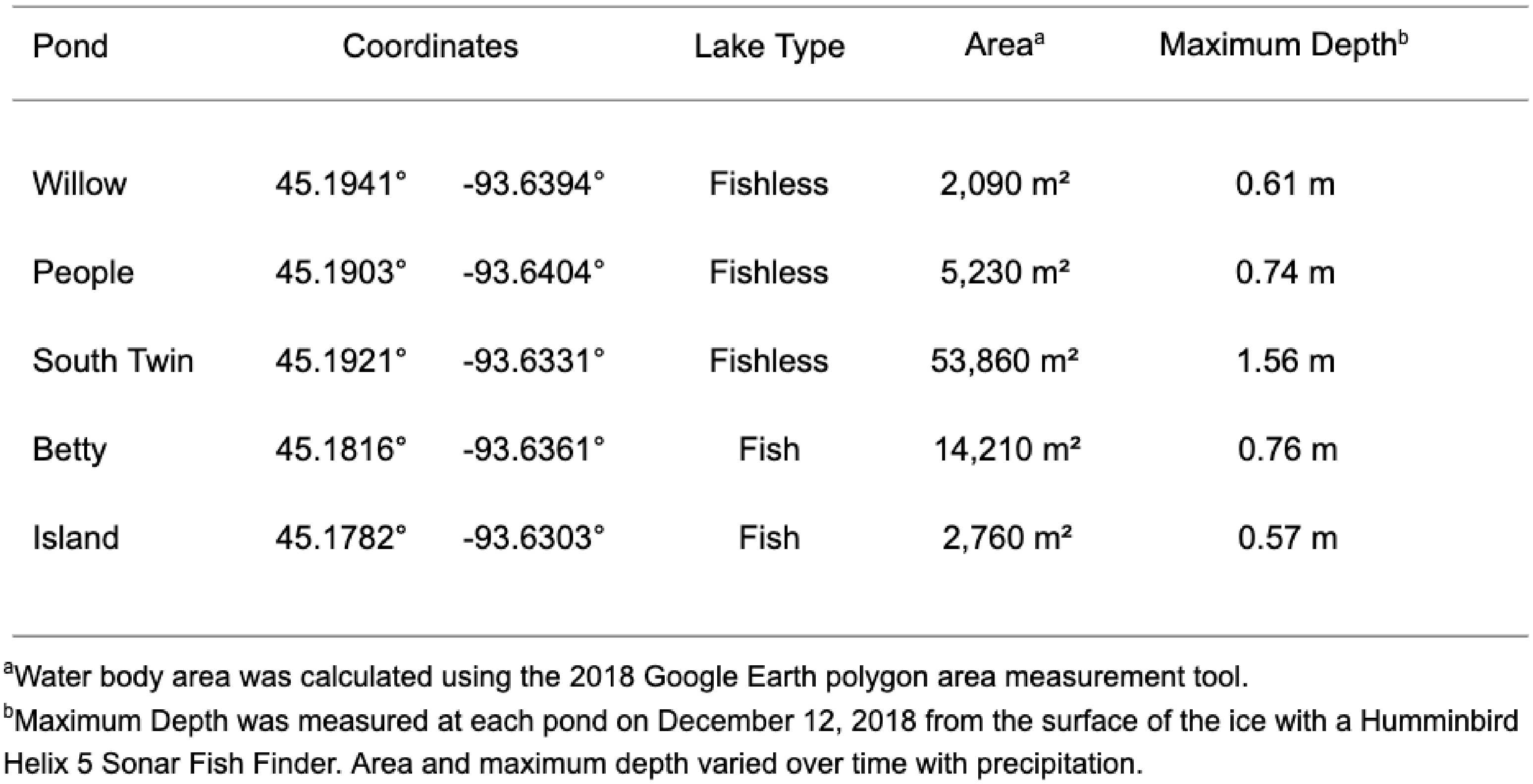
SUMMARY AND CONCLUSION

The long emergence duration and variable seasonal emergence peaks suggest that temperature is the primary constraint on the timing of northern *A. junius* emergence. This species does not require a short and synchronized emergence period to find mates because they are always present during the growing season (Paulson 2011; Mead 2017). A wide range of instars (F-0–F-6) can successfully overwinter, so the timing of *A. junius* emergence is also not limited by a requirement for their offspring to reach a winter critical size (Chapter 2).

The variability in the timing of seasonal emergence peaks could be a result of unpredictable events. A year with strong recruitment from spring migrators could increase the size of a late summer emergence peak; alternatively, a cold snap in spring could delay or prevent the arrival of migrating adults. A sudden influx of fish in fall, or a severe winter may kill over wintering nymphs and delay the first emergence date by a month or more. Drought in late summer may truncate the emergence season. Cohort splitting also contributes to the unpredictability of summer species phenology; it increases complexity at lower latitudes, and becomes more synchronized at higher latitudes and elevations (Corbet 1999).

If the ecology and phenology of *A. junius* are primarily influenced by seasonal temperature and stochastic events, then they are also directly impacted by climate change. Warmer temperatures are expected to cause odonate ranges to move towards the poles and increase the nymph growth rate of northern species (Suhling et al. 2015). The emergence and natural history knowledge from this study can serve as a foundation for understanding and predicting the future impacts of anthropogenic ecological changes (e.g., accelerated emergence, distribution shifts, freshwater trophic shifts) (Hassall et al. 2007; Richter et al. 2008). Common species, like *A. junius,* should be of conservation concern because they make up most of an ecosystem's biomass and are fundamental to its function (Grime 1998; Smith et al. 2004; Gaston & Fuller 2007; Gaston 2010). Long-term monitoring can detect changes in dragonfly populations, illuminate successional shifts, and inform habitat management (Corbet 1999). Future studies that assess the impacts of temperature, barometric pressure, and water level on emergence phenology and success may be able to better define what mitigates the variation in *A. junius* emergence, and assist with predicting the impacts of climate change.

Table 3-1. Descriptions of the ponds where *A. junius* exuviae were collected within Crow-Hassan Park Reserve in central Minnesota, USA.



Pond	Coordinates		Lake Type	Area[a]	Maximum Depth[b]
Willow	45.1941°	-93.6394°	Fishless	2,090 m²	0.61 m
People	45.1903°	-93.6404°	Fishless	5,230 m²	0.74 m
South Twin	45.1921°	-93.6331°	Fishless	53,860 m²	1.56 m
Betty	45.1816°	-93.6361°	Fish	14,210 m²	0.76 m
Island	45.1782°	-93.6303°	Fish	2,760 m²	0.57 m

[a] Water body area was calculated using the 2018 Google Earth polygon area measurement tool.

[b] Maximum Depth was measured at each pond on December 12, 2018 from the surface of the ice with a Humminbird Helix 5 Sonar Fish Finder. Area and maximum depth varied over time with precipitation.

Figure 3-1. A map of the five ponds in which *A. junius* exuviae were collected within Crow-Hassan Park Reserve in central Minnesota, USA.

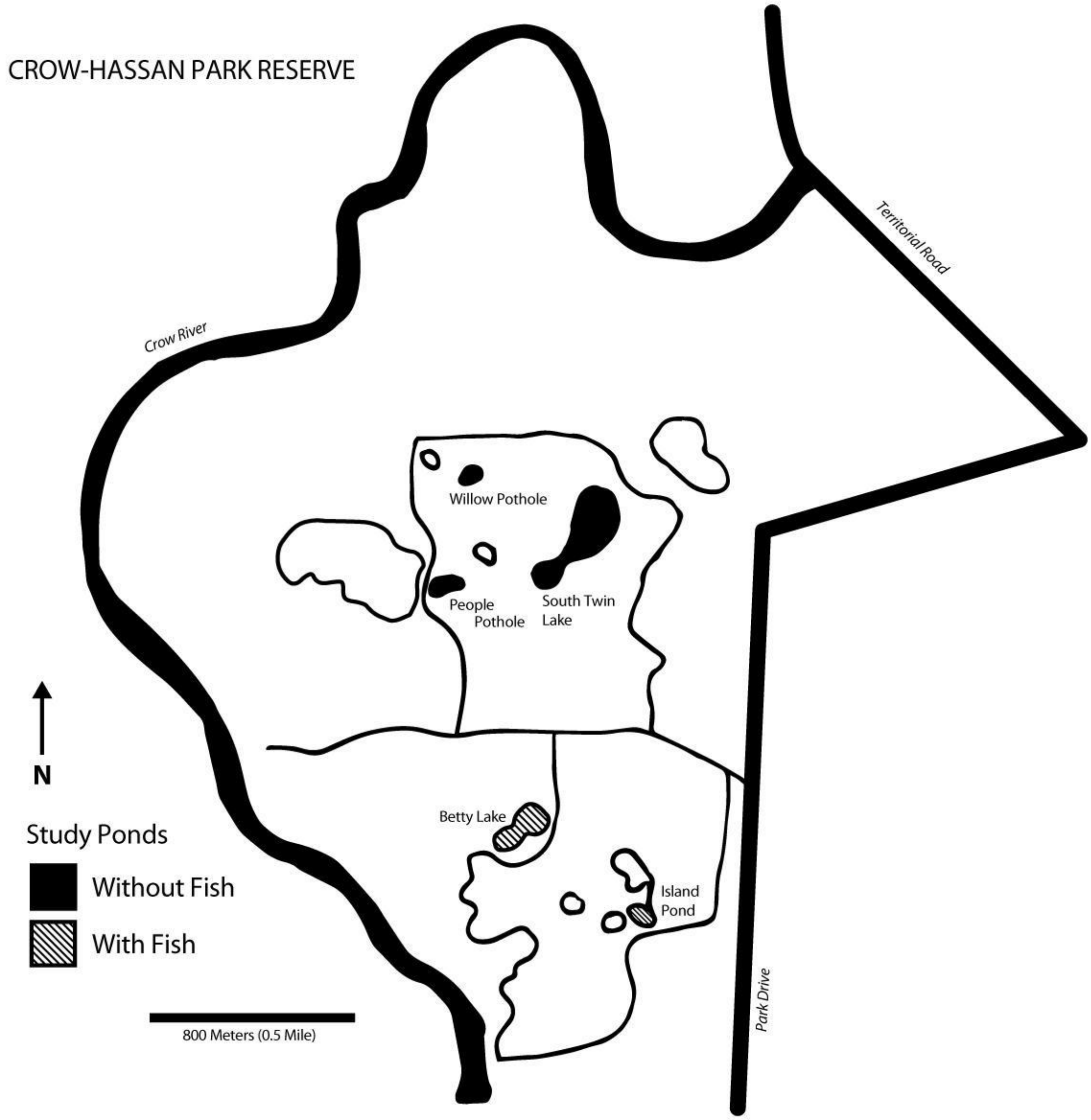


Table 3-2. Seasonal timing and duration of *A. junius* emergence by pond, from 2016 and 2017.

Pond	Year	Date of first observed emergence	Date of last observed emergence	Total duration of emergence in days	EM50[a] (% on observed date)	n[b]
Betty	2016	NA	NA	NA	NA	0
Island	2016	26 July[c]	25 Sept.	61	NA	280
People	2016	7 June	9 Oct.	124	NA	624
	2017	13 June	21 Oct.	130	77 (54%)	1320
South Twin	2016	2 June	9 Oct.	129	NA	2233
	2017	7 June	22 Oct.	137	75 (55%)	3423
Willow	2016	8 June	6 Nov.	151	NA	244
	2017	6 June	22 Oct.	138	85 (49%)	1015

[a] Empirical percent of cumulative emergence is reported for the day of observation nearest 50%.

[b] Total number of exuviae collected.

[c] Surveys began late at Island Pothole and missed observing the beginning of the emergence season.

Table 3-3. *Anax junius* exuviae sex ratio and days until 50% emergence during 2017 by pond.

Pond	% Male	Sex	EM50[a] (% on observed date)	Difference in EM50 between sexes	n[b]
People	46%[*]	Female	62 (52%)	0	337
		Male	62 (49%)		282
South Twin	51%	Female	63 (52%)	0	455
		Male	63 (50%)		473
Willow	50%	Female	70 (47%)	3	290
		Male	67 (53%)		292

[a] Empirical percent of cumulative emergence is reported for the day of observation nearest 50%.

[b] Total number of exuviae observed of that sex.

[*] A chi-squared test indicated the number of male and female exuviae was significantly different.

Figure 3-2. *Anax junius* seasonal emergence curves from 2016 by pond with exuviae collected per minute.

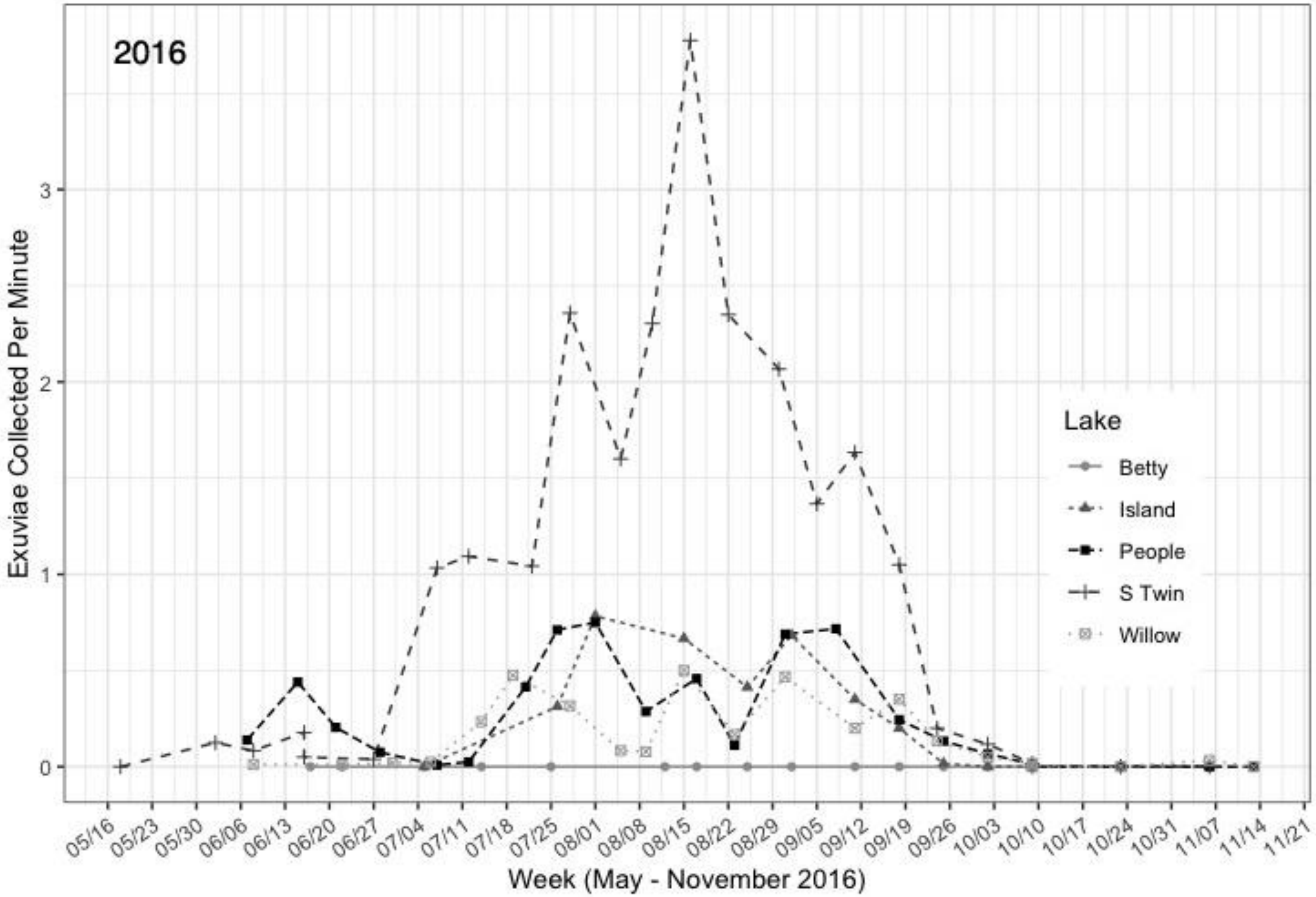

2016
Exuviae Collected Per Minute
0
1
2
3
Week (May - November 2016)
05/16
05/23
05/30
06/06
06/13
06/20
06/27
07/04
07/11
07/18
07/25
08/01
08/08
08/15
08/22
08/29
09/05
09/12
09/19
09/26
10/03
10/10
10/17
10/24
10/31
11/07
11/14
11/21
Lake
Betty
Island
People
S Twin
Willow

Figure 3-3. *Anax junius* seasonal emergence curves from 2017 by pond with total number of exuviae collected.

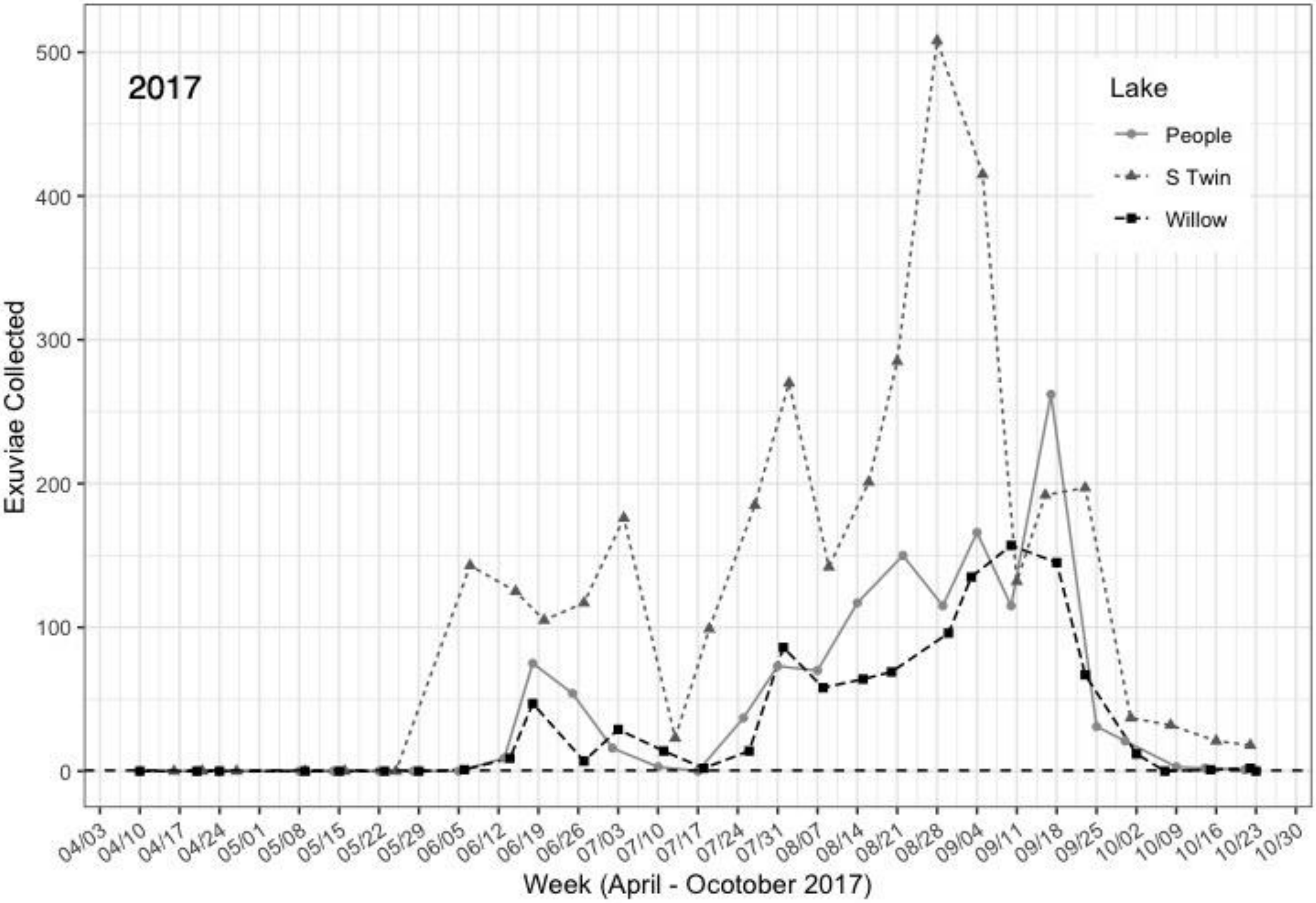
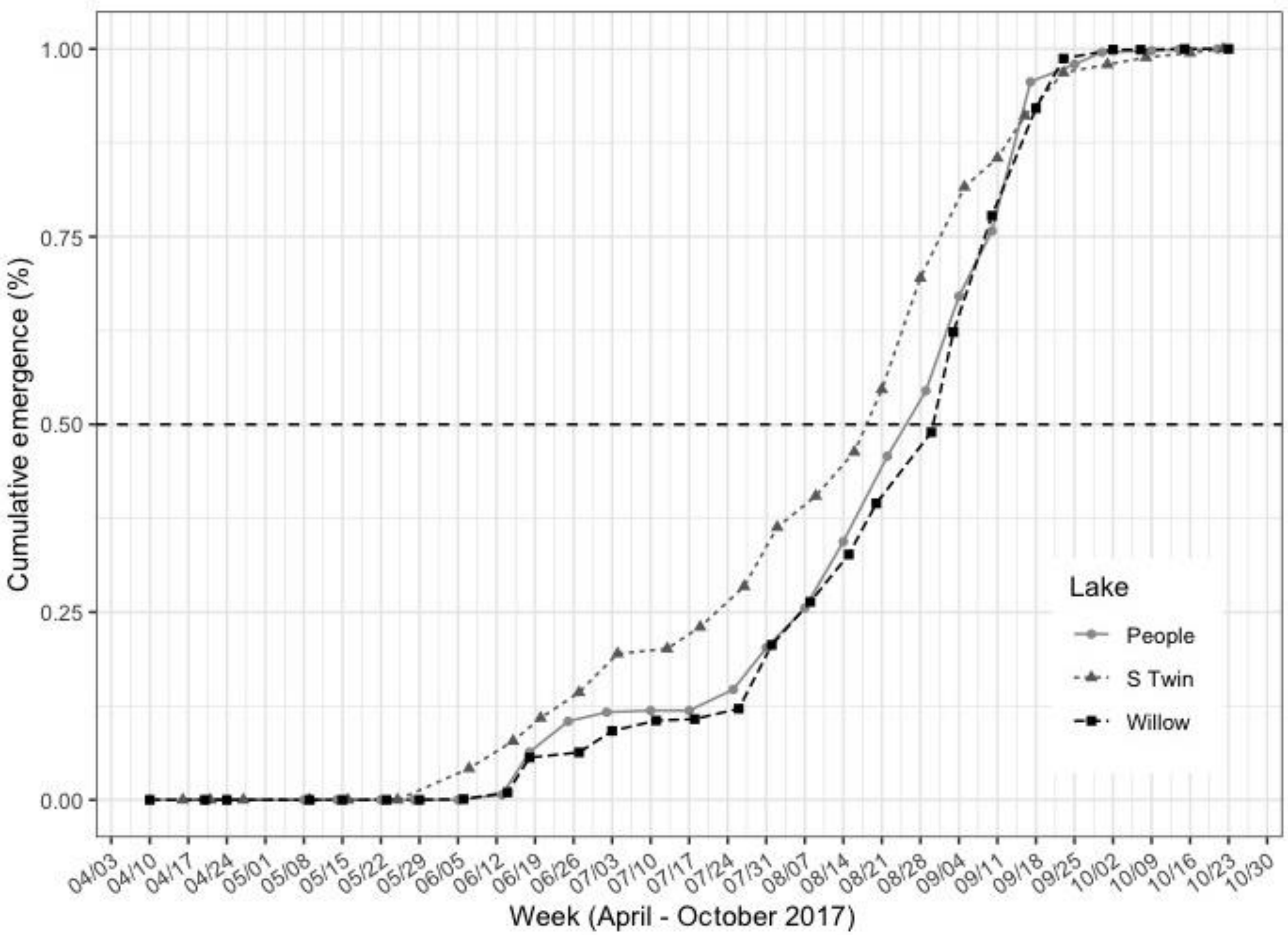

2017
Lake
People
S Twin
Willow
Exuviae Collected
0
100
200
300
400
500
04/03
04/10
04/17
04/24
05/01
05/08
05/15
05/22
05/29
06/05
06/12
06/19
06/26
07/03
07/10
07/17
07/24
07/31
08/07
08/14
08/21
08/28
09/04
09/11
09/18
09/25
10/02
10/09
10/16
10/23
10/30
Week (April - Ocotober 2017)

Figure 3-4. *Anax junius* cumulative emergence curves from 2017 by pond. The threshold of 50% of cumulative emergence (EM50) is noted with a dashed-black line.


Cumulative emergence (%)
1.00
0.75
0.50
0.25
0.00
Week (April - October 2017)
04/03
04/10
04/17
04/24
05/01
05/08
05/15
05/22
05/29
06/05
06/12
06/19
06/26
07/03
07/10
07/17
07/24
07/31
08/07
08/14
08/21
08/28
09/04
09/11
09/18
09/25
10/02
10/09
10/16
10/23
10/30
Lake
People
S Twin
Willow

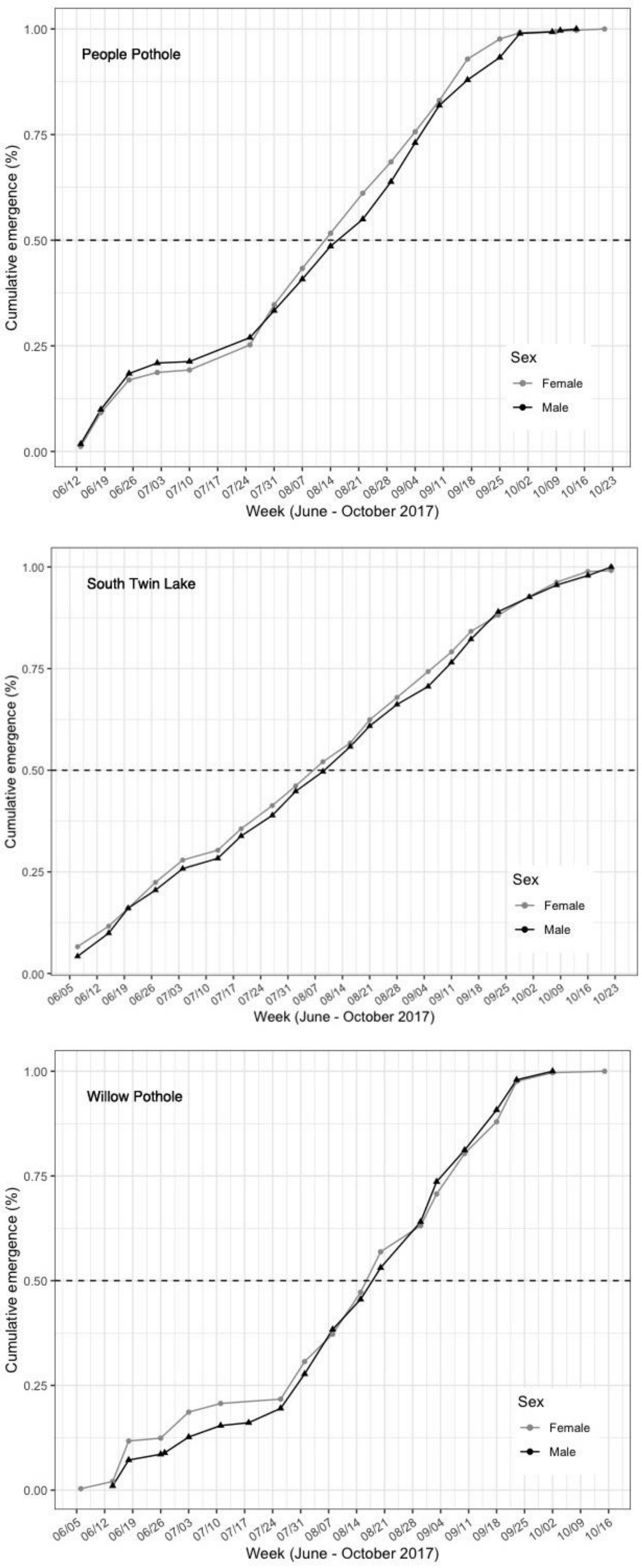


Figure 3-5. *Anax junius* cumulative emergence curves by sex and pond from 2017. The threshold of 50% of cumulative emergence (EM50) is noted with dashed-black lines.

Chapter 4

The effect of temperature on *Anax junius* (Drury) development (Odonata: Aeshnidae)

SYNOPSIS

Ecological thermal energy patterns are changing due to anthropogenic climate change, and the impacts are wide-ranging. Odonates can serve as indicators of temperature-driven ecological change, but information about their physiological and ecological responses to thermal change is lacking. The focal species of this study, *Anax junius*, is a large, abundant, and widespread dragonfly. A generalist species like *A. junius* with a high optimum temperature, wide range of effective temperatures, and strong dispersal ability is primed to quickly expand its range northward as the climate warms. Late instar nymphs were collected in fall from fishless ponds in central Minnesota, USA and reared at five temperatures: 16, 20, 24, 28, and 32°C. Of 105 nymphs, 16 died before eclosion, 18 died during emergence, 67 successfully emerged, and four were still nymphs when the experiment ended. The effect of temperature on nymph development was tested, analyzed, and described. The optimal development temperature for *A. junius* nymphs was ~32°C and the base temperature ~9°C. The average number of days needed for development within an instar generally decreased as temperature increased. On average, nymphs required 385 degree days to develop through F-0 and 294 DD through F-1. Nymphs reared at colder temperatures required more degree days. Understanding how temperature affects ectotherm growth is required for predicting the severity and direction of climate change-induced ecological shifts.

INTRODUCTION

Seasonal temperature patterns are shifting due to anthropogenic climate change (Parmesan 2006), with wide-ranging impacts (Gillooly et al. 2002). Ectotherms in particular are affected because temperature is their most important ecological driver (Huey & Stevenson 1979; Angilletta et al. 2002; Damos & Savopoulou-Soultani 2012; Suhling et al. 2015). Thermal responses of insect pests, invasive species, and agricultural crops have been well studied (Ascerno 1991; Trudgill et al. 2005; Damos & Savopoulou-Soultani 2012; Mckee & Aukema 2015; Ward et al. 2019) but insect species of conservation interest have received less attention (Richter et al. 2008; Faust & Weston 2009). Dragonflies and damselflies are a diverse order of insects that are well suited to be used as indicators of climate change impacts. They respond strongly in a variety of ways (e.g., geographical movement, phenological shifts, changes in body size); their ecology, genetics, and biology are of current research interest; and they are engaging and can inspire the general public to take conservation action (Córdoba-Aguilar 2008; Hassall & Thompson 2008; Hassall 2015).

The efficient use of odonates as indicators of temperature-driven ecological change hinges on observing and defining their physiological and ecological responses to a range of temperatures. Few studies have reared Odonata nymphs in different temperatures to measure their development or growth rates. Suhling et. al. (2015) found only 10 studies of odonate thermal growth in which nymphs were reared at a minimum of four different temperatures, with one above the temperature of optimal growth. Their results identified *A. junius* as a species of special interest related to climate change. Here, I expand our knowledge of *A. junius* thermal response research using modern temperature response modeling knowledge that has been acquired by recent entomological cold tolerance and insect community studies (Mckee & Aukema 2015; Ward et al. 2019).

Anax junius is a large, abundant, widespread, charismatic, generalist dragonfly in the family Aeshnidae (Corbet 1999; Paulson 2011). Its range includes all of the continental United States, southern Canada, stretches north into the southeast corner of Alaska, and south into Mexico, Cuba, the Dominican Republic, and Hawaii (Abbott 2006). Adults migrate, with northern spring movement following ice-out and in large migratory swarms in the autumn (Russell et al. 1998; Hallworth et al. 2018).

Anax junius nymphs in northern climates either mature completely in one summer and metamorphose in time for fall migration (summer growth pathway) or overwinter in the larval stage and emerge in spring (winter growth pathway) (Chapter 2). These pathways overlap in late summer, and there is an opportunity for cohort splitting (Crowley & Hopper 2015). Individual nymphs following different growth pathways may respond to environmental thermal inputs in different ways. Here, I reared winter pathway *A. junius* nymphs in growth chambers to define their optimal development temperature (T_{opt}) and ecological base temperature (T_b), and calculate the number of degree days (DD) required to progress through their last two instars. These parameters can be used as a foundation for future studies evaluating the impacts of climate change on freshwater ecosystems.

MATERIALS AND METHODS

SOURCE OF NYMPHS

Anax junius nymphs were collected from three fishless ponds within Crow Hassan Park Reserve, and from one fishless pond within the Lee and Rose Warner Nature Center (Chapter 2). Both collection sites were within 60 km of Minneapolis, Minnesota USA. Collection occurred in fall 2016 between October 13th and November 1st to ensure that all of the nymphs were from the overwintering growth development pathway (Chapter 2). Mid-sized nymphs, that would complete their final two instars during the experiment, were chosen based on visual inspection of head width relative to hind wing sheath length (Tennessen 2017b).

We refer to the instars using the nomenclature described by Lutz (1968). The aquatic nymphs of dragonflies undergo a series of molts, with a variable number of instars before metamorphosing into their adult stage (Corbet 1999). *Anax junius* matures through 10–13 instars (11.8 on average) (Beesley 1972; Corbet 2002). To adjust for this inconsistency, the instars are named in reference to the final instar working backwards. For example, the final instar before emergence is called “final minus zero,” or F-0, the penultimate instar is referred to as “final minus one,” or F-1, the next youngest instar is F-2, and so on (Lutz 1968).

TEMPERATURE TREATMENTS

Target rearing temperatures of 16, 20, 24, 28, and 32^{o}C were programmed into five growth chambers with a constant photoperiod of 16 hours of light and 8 hours dark. I abandoned a 34^{o}C treatment (~2^{o}C above optimum) after 17 of 20 nymphs died within three days; likely because this temperature was above the upper lethal limit for *A. junius*. A HOBO pendant temperature/light data logger (Onset Computer Corporation, Bourne, Massachusetts, USA) was placed into each growth chamber to record hourly air temperatures for the duration of the experiment.

EXPERIMENT SET-UP AND MAINTENANCE

Nymphs were brought into the lab on the day of their collection and placed into individual, 14 cm tall plastic deli containers filled with 5 cm of dechlorinated tap water. A small, igneous rock was placed into each container for perching. Water was dechlorinated via evaporation from buckets that sat out for at least 24 hours. Each nymph was randomly assigned to one of the five growth chambers. Twenty nymphs each were placed into the 16, 20, 24, and 28°C chambers. Twenty-five nymphs were placed into the 32°C chamber in anticipation of higher mortality at this temperature.

Nymphs were fed and their containers were cleaned daily. Frass and leftover food were removed with pipettes. Spoiled water was replaced as needed, but no less often than once a week, with dechlorinated water stored within each chamber. Nymphs were fed *ad libitum* with 0.62 ml of living *Lumbriculus variegatus* (aquatic black worms). Unconsumed living worms were present in most containers the following day, which confirmed *ad libitum* feeding. A strip of plastic window mesh was taped inside of the container after nymphs entered their final instar, as determined by Tennessen (2017). Nymphs climbed onto the window screen and out of the water to eclose. Final instar nymph containers were also covered with cheesecloth to prevent adults from escaping.

We checked for ecdysis daily from October 13, 2016 through May 25, 2017. The day of a nymph’s first ecdysis during the experiment was recorded as the first day in a fully observed instar. The number of days that a nymph spent in each instar was recorded until it died, eclosed, or the experiment ended. All nymphs spent at least three days acclimating in their growth chamber before undergoing their first ecdysis. Days-in-instar data were only used for analysis if the entirety of the stage, from ecdysis to ecdysis or eclosion, was observed.

ANALYSIS AND STATISTICS

We tallied development time (d, the number of days that a nymph spent in each instar), using the day of ecdysis as the first day and the day before the next ecdysis or eclosion as the last day. Development rate (1/d) was the inverse of the development time. Because chamber temperatures fluctuated slightly, I used the average temperature experienced by each nymph during each instar, calculated from the hourly temperature logger data.

The optimal development temperature (T_{opt}), the temperature at which nymphs are predicted to develop in the fewest number of days, was calculated separately for F-1 and F-0 instars following McKee & Aukema (2015). Mixed effect polynomial models predicting development time with average temperature as a fixed effect and pond as a random effect were used to model positive quadratic parabolas. The minimum vertex of each parabola was calculated algebraically using the quadratic equation to determine the optimal development temperature.

The base temperature (T_b), the temperature below which an ectotherm cannot grow, for each instar was also calculated separately for F-1 and F-0 instars using the linear method (Campbell et al. 1974; Zalucki 1982; Charnov & Gillooly 2003; Trudgill et al. 2005; Mckee & Aukema 2015). Mixed effect linear models predicting development rate using average temperature as a fixed effect and pond as a random effect were used to model the initial inclining portion of the temperature response curves. To exclude the non-linear portion of the curve, nymphs reared above an average temperature of 22°C were not included in this analysis. The modeled range includes the ecologically relevant temperatures that would be required for predicting the spring emergence of overwintered *A. junius* nymphs. Base temperature was determined by solving the modeled linear equations for x (average temperature) when y (development rate) was set to zero. The number of degree days (DD) required for the development of each nymph through each instar was calculated by subtracting the base temperature from the average development temperature and then multiplied by development time (Trudgill et al. 2005). Degrees days were averaged for each chamber and for each instar.

Variables were transformed as needed to meet model assumptions. Mixed effect models were selected based on AIC values, experiment design, and ecological relevance. Statistics were analyzed using the software R (R Core Team 2018) and the packages *nlme* (Pinheiro J, Bates D, DebRoy S, Sarkar D 2019) and *lmerTest* (Kuznetsova A, Brockhoff PB 2017), and *ggplot2* (Wickham 2016).

RESULTS

Of the 105 *A. junius* nymphs in the experiment, 16 died as nymphs, 18 died during emergence, 67 successfully emerged, and four were still nymphs when the experiment ended (Table 4-1). Nymph mortality was the highest in the 32°C chamber (32%) and lowest in the 28°C chamber (0%). Data on 83 nymphs in F-0 and 68 nymphs in F-1 were collected. In addition, 12 nymphs went through the entire F-2 instar during the experiment. Data from three nymphs were excluded from analysis because of data collection errors.

Hourly logger data indicated that chamber temperatures fluctuated slightly around their target temperatures (Table 4-2). The 28°C chamber malfunctioned more than halfway through the experiment and temperatures in that chamber briefly reached 38.1°C. As a result, nymphs still in the 28°C chamber at that point in the experiment experienced higher average temperatures than those that emerged earlier. Every nymph's average temperature exposure was calculated individually, so the results from the 28°C chamber include data points at a range of values between 28 and 32°C.

Nymphs spent more time in F-0 than in other instars, and F-1 and F-2 instars exhibited roughly equal durations at all temperatures (Table 4-2). The average number of days needed for development (d) within an instar decreased as temperature increased for F-0 and F-2 instars, ranging from 83.9–12.8 days and 63.3–8.0 days, respectively. F-1 instars experienced decreased development time as temperatures increased from 16°C to 28°C (59.1–8.4 days), but development time increased to 9.5 days at 32°C . The optimal development time temperature (T_{opt}) for F-0 was 31.82°C. The model predicted an F-0 instar could develop in 12.6 days at this temperature. The T_{opt} for F-1 was 33.07°C. The model predicted an F-1 instar could develop in 8.3 days at this temperature. The F-2 data were not analyzed because the sample size was too small.

The average development rate (1/d) within an instar increased as temperature increased for F-0 and F-2 instars ranging from 0.01200–0.0789 and 0.0177–0.125, respectively. F-1 instars experienced increased development rates as temperatures increased from 16^oC to 28^oC (0.0173–0.1215), but develop rate decreased to 0.1141 at 32^oC. Data from the linear portion of the development rate curves (average temperatures below 22ºC) were modeled to extrapolate the base temperature for each instar (Figure 4-2). The base temperatures (T_b) for F-0 and F-1 were 9.7ºC and 8.4ºC, respectively. The F-2 data were not analyzed because the sample size was too small. On average, nymphs required 385 degree days (DD) to develop through F-0 and 294 degrees days (DD) through F-1. Nymphs reared at colder temperatures required more thermal energy (Table 4-4).

DISCUSSION

A WARM-ADAPTED GENERALIST

The effect of temperature on winter pathway *A. junius* nymph development was tested, analyzed, and described by calculating optimal development temperature (T_{opt}), base temperature (T_b), and degree days (DD). The optimal development temperature for late instar *A. junius* nymphs is ~32°C. This temperature is on the warm end of what is expected for odonates. Conversely, the calculated base temperature of ~9°C is relatively low. Previous studies report optimal temperatures for Odonata between 21 and 31°C and base temperatures between 8 and 12°C (calculated using growth data) (Suhling et al. 2015). High or low optimal temperatures often indicate a warm or cold specialized species. However, *A. junus* is an undisputed generalist (Corbet 1999; Freeland et al. 2003; Paulson 2011; Rudolf & Rasmussen 2013). In their review paper, Suhling et al. (2015) corroborate these findings noting that *A. junius* was unusual for a generalist in that it exhibited a shallow slope on the leading edge of a temperature response curve (a low Q10), as expected, but had a high optimal temperature (calculated using growth rate). A steep slope demonstrates a large growth loss or benefit for each degree change from optimum and results in a narrower range of temperatures for robust growth. A gentle slope indicates a species in which growth is less impacted by temperature change, and that thrives over a wider range of temperatures. Perhaps being migratory has allowed *A. junius* to retain the tropically evolved high optimum temperature while successfully utilizing northern habitats. Analyzing the temperature response curves for other North American migrating dragonfly species would be a good way to explore this hypothesis.

A low base temperature may allow overwintering *A. junius* nymphs to begin growing early in spring. Reinitiating growth as early as possible in spring would be particularly valuable for nymphs overwintering in younger instars because they will require more thermal energy to emerge than older conspecifics. The T_b of the F-1 instar (8.4ºC) was less than the T_b of the F-0 instar (9.7ºC). A higher base temperature in autumn may prevent final instar nymphs from emerging too late for successful migration. In some species of insects that feed on trees, different instar base temperatures result in synchronized spring emergence (Powell & Jenkins 2000). The older instars of these species have higher base temperatures and stop growing earlier in fall, which gives smaller peers time to catch up. *Anax junius* is a summer species, as defined by Corbet 1999, and emerges all summer long (Chapter 3). Therefore, while it does not need adaptations to synchronize emergence, variations in instar base temperatures could be playing another ecological role. The fact that some individuals overwinter as nymphs might have resulted in a selective advantage to variations in instar base temperatures. Future experiments could determine if spring emerging dragonfly species, like those in the genus *Epitheca*, synchronize their emergence with methods similar to those used by forest insects.

Nymph mortality was highest in the warmest chamber that was held at a temperature near T_{opt} (Table 4-1). The temperatures that manifest peak physiological metabolizing can result in other costs that may slow growth and/or increase mortality (Martin & Huey 2008). Fast growth may be more efficient and sustainable at temperatures slightly below the calculated optimal, which would allow for some physiological reserves to weather unexpected events.

Experimental nymphs required, on average for all temperature chambers, 385 degree days (DD) to develop through F-0 and 294 degrees days through F-1. Nymphs reared at colder temperatures required nearly double the amount of thermal energy than those in the warmer chambers (Table 4-4). Trottier (1971) calculated that field-observed overwintering *A. junius* nymphs required 20% more degree days to reach maturity than those that developed during the summer. It is difficult to assess whether the difference in degree day requirements between the growth pathways is a physiological difference between summer and winter pathways, or if *A. junius* nymphs in colder water universally need the same quantity of additional thermal input to mature (Taylor 1981). An experiment rearing summer pathway nymphs with the same experimental design could determine if their thermal requirements are different. Additional experiments testing nymphs from different latitudes would build an even more comprehensive picture of the ecological relationship between nymph growth, temperature, and geography.

Future work comparing these modeled degree day predictions to field observations of overwintered late instar *A. junius* development would assess model accuracy, and evaluate how much variation in spring emergence is due to temperature. If the modeled and observed degree day values match, then the model is correct and temperature explains most of the variation in spring *A. junius* emergence. Otherwise, a factor in addition to temperature (e.g., photoperiod) may affect the timing of emergence.

COMPARISON TO PREVIOUS WORK

To our knowledge, this is the first study to generate experimental data examining the effect of temperature on *A. junius* growth since the 1970s. The previous studies used small sample sizes and imprecise measurements of temperature exposure. They also varied in terms of the time of year and location from which study nymphs were collected (and thus their growth pathway), the response variable that was being evaluated (development time or growth), and the statistical method that was used to estimate key thermal thresholds. Our data are only directly comparable to those studies that used winter pathway nymphs from western hemisphere latitudes near 45°N, development time as a response variable, and linear regression to calculate the base temperature. Results from studies that utilized different parameters are discussed below, but should be used for comparison with caution.

Calvert (1929, 1934) reared one *A. junius* nymph, collected as an egg (Pennsylvania, USA; ~40° N) to an adult at an unspecified room temperature. This nymph spent 42 days in F-0, and 21 days in F-1. Calvert collected the eggs on July 13th, 1926. At that time of year and latitude, an egg had the potential to produce a nymph that followed either the winter or summer pathway. Calvert’s data fit within the range of our results if room temperature was between 20 and 24°C.

Macklin (1964) reared eight *A. junius* nymphs collected throughout 1960 (in January, June, and August) near Purdue University in Lafayette Indiana, USA (~40° N). One nymph was reared from an egg, and others were selected as the smallest nymphs from each outing. The sample would have contained a mix of summer and winter growth pathway nymphs, and he observed that variation in development times correlated with collection date. Nymphs raised between 20.0°C and 26.7°C, with 17 hours of light per day, spent 15–31 days in F-1 and 24–69 days in F-2. The reported development times at those temperatures coincide with those from this paper. When comparing his results to Calvert's winter pathway nymph, Macklin noted that some of his nymphs appeared to "skip instars." This observation is supported by evidence from this study (Chapter 2) that summer pathway nymphs go through one fewer instar than winter pathway nymphs. Trottier (1971) reared 20 F-0 *A. junius* at five temperatures (12.5, 15, 20, 25, and 30°C) with 14 hours of light. These nymphs were collected as F-1 instars in August (Toronto, Canada; ~44° N), so they were most likely following the summer growth development pathway. Trottier's nymphs took 14–86 days to develop, and he calculated the base temperature for F-0 instars at 8.7^{o}C. Our results roughly coincide with these values. Trottier used a "hyperbolic equation" to model the curve at the lower leading edge of temperature response curve slope, resulting in a base temperature lower than would have been calculated by the linear method. A review paper by Suhling et al. (2015) analyzed Trottier's (1971) data, using the O'Neil function, and calculated the optimum temperature for F-0 *A. junius* growth rate to be 29.3°C, which is just below our predicted development rate optimum temperature of 31.82°C.

Beesley (1972) raised *A. junius* from eggs collected near Riverside, California (~34° N) from July–September in 1970 and 1971. Given this wide seasonal spread of collecting and the more southern collection latitude, it is possible that the nymphs from those eggs could have taken the winter, summer, or another unknown growth pathway. However, Beesley immediately exposed the eggs to a warm (31°C) or cold (21°C) temperature treatment and noticed that the eggs in the warmer water developed using fewer instars than those in the colder water. This could be evidence that *A. junius* development pathways are determined by the water temperature at the time that eggs are laid. Future studies examining the gene expression and growth patterns of eggs from a single dragonfly reared at varying temperatures could explore this hypothesis.

Kime (1974) collected and reared *A. junius* nymphs from central Washington (~47° N) from April 1973 through March 1974. Nymphs were raised at 21°C and with 24 hours of light. Kime also observed that some nymphs collected in early to mid summer “skipped instars” as compared to those collected during late summer and fall. On average, Kime observed that nymphs spent ~25 days in F-0, and 10 days in F-1. These durations are much shorter than what I observed, but the 24 hours of light photoperiod could have increased the development speed.

CONSERVATION IMPLICATIONS

Understanding how temperature affects ectotherm growth is a vital to predicting the severity and direction of climate change-induced ecological shifts (Meyer et al. 1999; Winder & Schindler 2004; Hassall et al. 2007; Hassall & Thompson 2008). A species like *A. junius*, with a high optimum temperature, a generalist’s competitive strength within a wide range of temperatures, and a strong dispersal ability is primed to quickly expand its range northward as climate warms (Suhling et al. 2015). I hope that these findings are a foundation for future studies that assess the impacts of a rapid northward range expansion of *A. junius*. For example, it would be useful to study the effect of this range expansion on the phenological pairing of their migration with spring and fall birds, and the effect of an influx of this top-predator, competitor, or prey species (depending on the ecological context) into boreal ponds and lakes.

More temperature response studies need to be done with other species of Odonata and over a range of latitudes. *Anax junius* has a simple life history that is fairly easy to measure and predict because individuals do not synchronize emergence (Chapter 3) or experience a physiological diapause (Chapter 2). Many niche-specialized odonates are at greater conservation risk. Building temperature response models for these more derived odonate species with complicated life histories would need to define the impacts of other drivers (e.g., photoperiod or obligate exposure to periods of cold temperatures) in order to predict the effects of climate change.

Table 4-1. The fate of the *A. junius* nymphs within each temperature chamber. All chambers began the experiment with 20 nymphs, except for the warmest chamber which was stocked with 25. Four nymphs were still alive and immature when the experiment ended after 7 months: three from the 16°C chamber and one from the 20°C chamber. Two nymphs from the 16°C chamber and one from the 28°C chamber were not included in the analysis because of data collection errors.

Fate (n)	Temperature (°C)				
	16	20	24	28	32
Successful emergence	11	12	18	16	10
Failed emergence[a]	4	3	0	4	7
Died as nymph	2	4	2	0	8

[a] Died while eclosing

Table 4-2. The time in days (d) for the development of *A. junius* instars (F-0, F-1, and F-2) reared in growth chambers at five target temperatures.

Instar	(d)	Temperature (°C)[a]				
		16 (15.48 +/- 0.003)	20 (18.78 +/- 0.02)	24 (24.46+/- 0.01)	28 (28.36+/- 0.06)	32 (31.42+/- 0.03)
F-0	Range	73 - 96	46 - 67	19 - 26	12 - 22	11 - 15
	Mean	83.86	54.53	21.94	14.47	12.76
	SE	1.77	1.48	0.49	0.49	0.28
	n	14	15	18	19	17
F-1	Range	46 - 74	25 - 59	10 - 16	7 - 11	6 - 14
	Mean	59.14	41.44	12.50	8.40	9.45
	SE	2.51	2.43	0.51	0.34	0.87
	n	14	16	12	15	11
F-2[b]	Range	45 - 96	35 - 44	11 - 15	8 - 9	8
	Mean	63.33	39.67	13.00	8.50	8.00
	SE	16.37	2.60	1.15	0.50	NA
	n	3	3	3	2	1

[a] Target chamber temperature listed above. Empirical observed mean temperature +/- SE listed below, measured with a temperature logger in each chamber for duration of experiment.

[b] F-2 data not analyzed due to small sample size.

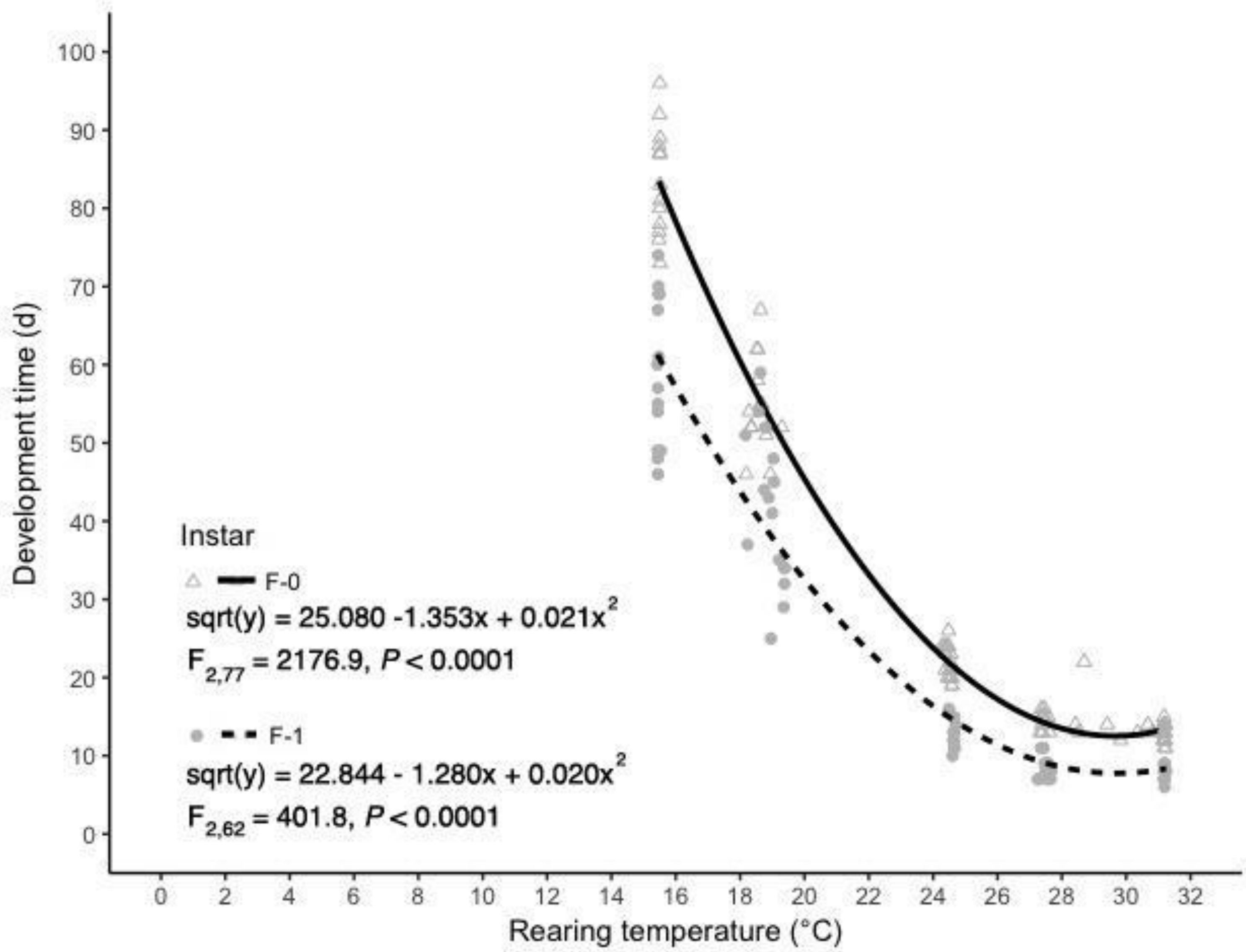
Table 4-3. The rate of development (1/d) of *A. junius* instars (F-0, F-1, and F-2) reared in growth chambers at five target temperatures.

Instar	(1/d)	Temperature (°C)[a]				
		16 (15.48 +/- 0.003)	20 (18.78 +/- 0.02)	24 (24.46+/- 0.01)	28 (28.36+/- 0.06)	32 (31.42+/- 0.03)
F-0	Range	0.0104 - 0.0140	0.0149 - 0.0217	0.0385 - 0.0526	0.0455 - 0.0833	0.0667 - 0.0909
	Mean	0.0173	0.0255	0.0814	0.1215	0.1141
	SE	0.0003	0.0005	0.0010	0.0019	0.0017
	n	14	15	18	19	17
F-1	Range	0.0135 - 0.0217	0.0169 - 0.0400	0.0625 - 0.1000	0.0909 - 0.1429	0.0714 - 0.1667
	Mean	0.0173	0.0255	0.0814	0.1215	0.1141
	SE	0.0007	0.0016	0.0032	0.0044	0.0094
	n	14	16	12	15	11
F-2[b]	Range	0.0104 - 0.0222	0.0227 - 0.0286	0.0667 - 0.0909	0.1111 - 0.1250	0.1250
	Mean	0.0177	0.0254	0.0782	0.1181	0.1250
	SE	0.0037	0.0017	0.0071	0.0069	NA
	n	3	3	3	2	1

[a] Target chamber temperature listed above. Empirical observed mean temperature +/- SE listed below, measured with a temperature logger in each chamber for duration of experiment.

[b] F-2 data not analyzed due to small sample size.

Figure 4-1. The number of days (d) needed for *A. junius* nymphs to develop during the final two instars over five target rearing temperatures. Each point represents the number of days spent in an instar by a nymph at the average rearing temperature that it individually experienced. The number of nymphs per instar per growth chamber (n) are listed in Table 4-2.



Development time (d)
100
90
80
70
60
50
40
30
20
10
0
Rearing temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Instar
△ F-0
$\mathrm{sqrt}(y) = 25.080 - 1.353x + 0.021x^2$
$F_{2,77} = 2176.9$, $P < 0.0001$
● F-1
$\mathrm{sqrt}(y) = 22.844 - 1.280x + 0.020x^2$
$F_{2,62} = 401.8$, $P < 0.0001$

Figure 4-2. Developmental rates (1/d) of *A. junius* nymphs during the final two instars at five target rearing temperatures. Temperatures below 22°C were modeled with linear regression (black lines), for each instar, to extrapolate the base temperatures at the x-intercepts. Each point represents the inverse of the number of days spent in an instar by a nymph at the average rearing temperature that it individually experienced. Grey curves were fitted to the data for each instar with loess smoothing. The numbers of nymphs per instar per growth chamber (n) are listed in Table 4-3.

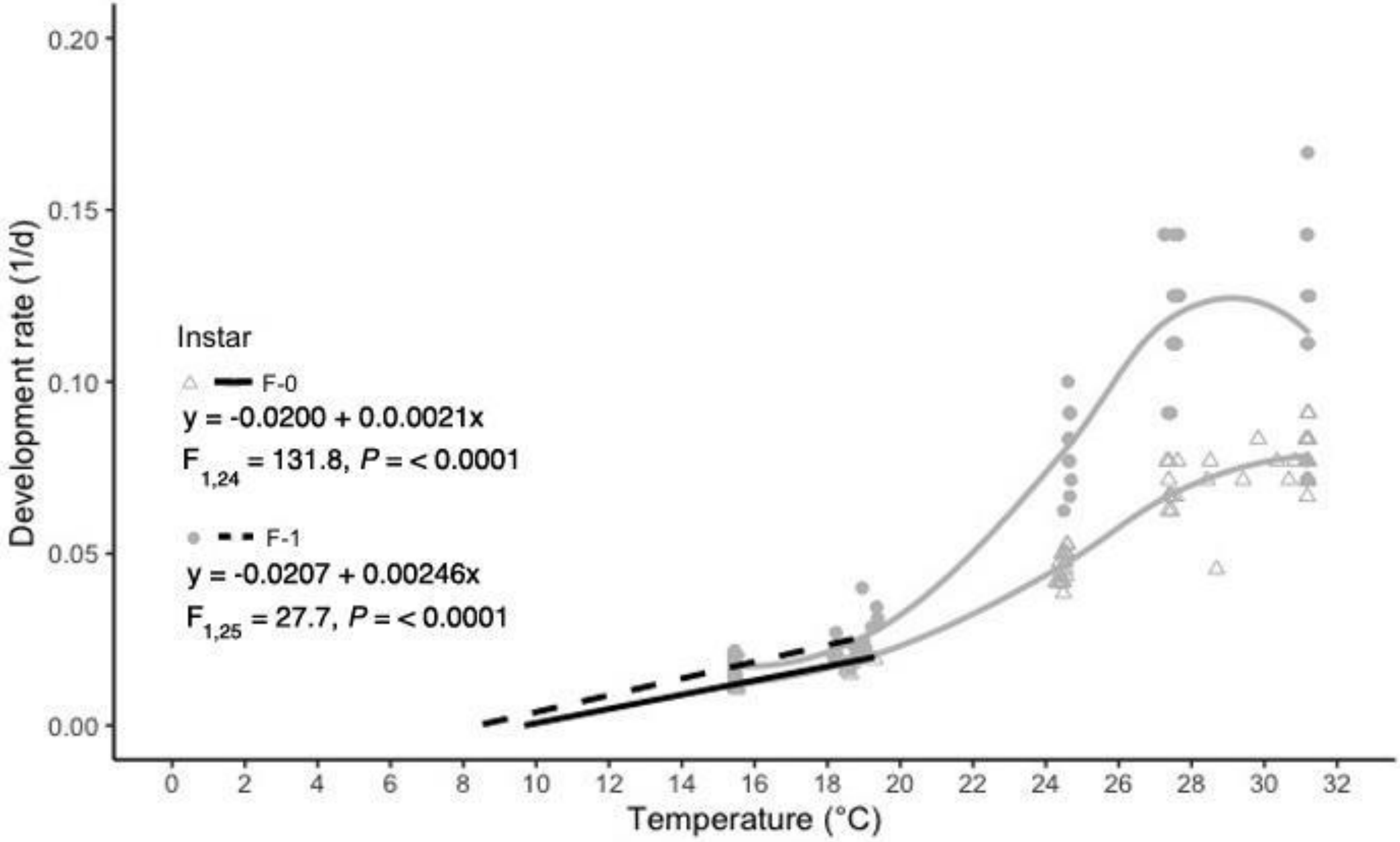
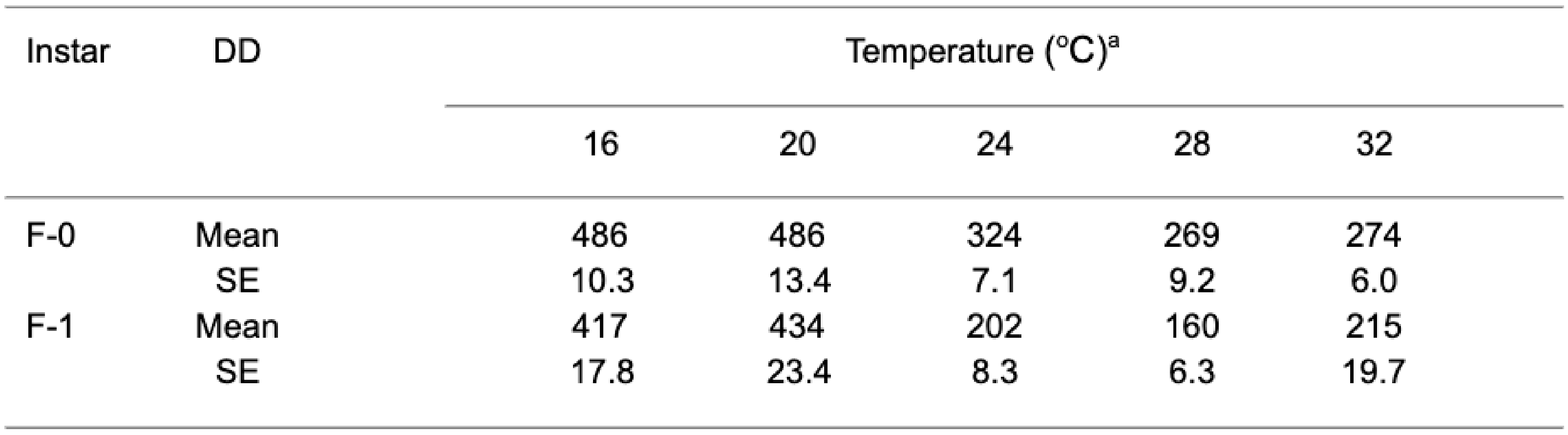

Development rate (1/d)
0.00
0.05
0.10
0.15
0.20
Temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Instar
△ F-0
y = -0.0200 + 0.0.0021x
F1,24 = 131.8, P = < 0.0001
● F-1
y = -0.0207 + 0.00246x
F1,25 = 27.7, P = < 0.0001

Table 4-4. The number of degree days (DD) required by F-0 and F-1 *A. junius* instars in each temperature chamber.



Instar	DD	Temperature (°C)[a]				
		16	20	24	28	32
F-0	Mean	486	486	324	269	274
	SE	10.3	13.4	7.1	9.2	6.0
F-1	Mean	417	434	202	160	215
	SE	17.8	23.4	8.3	6.3	19.7

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APPENDIX A

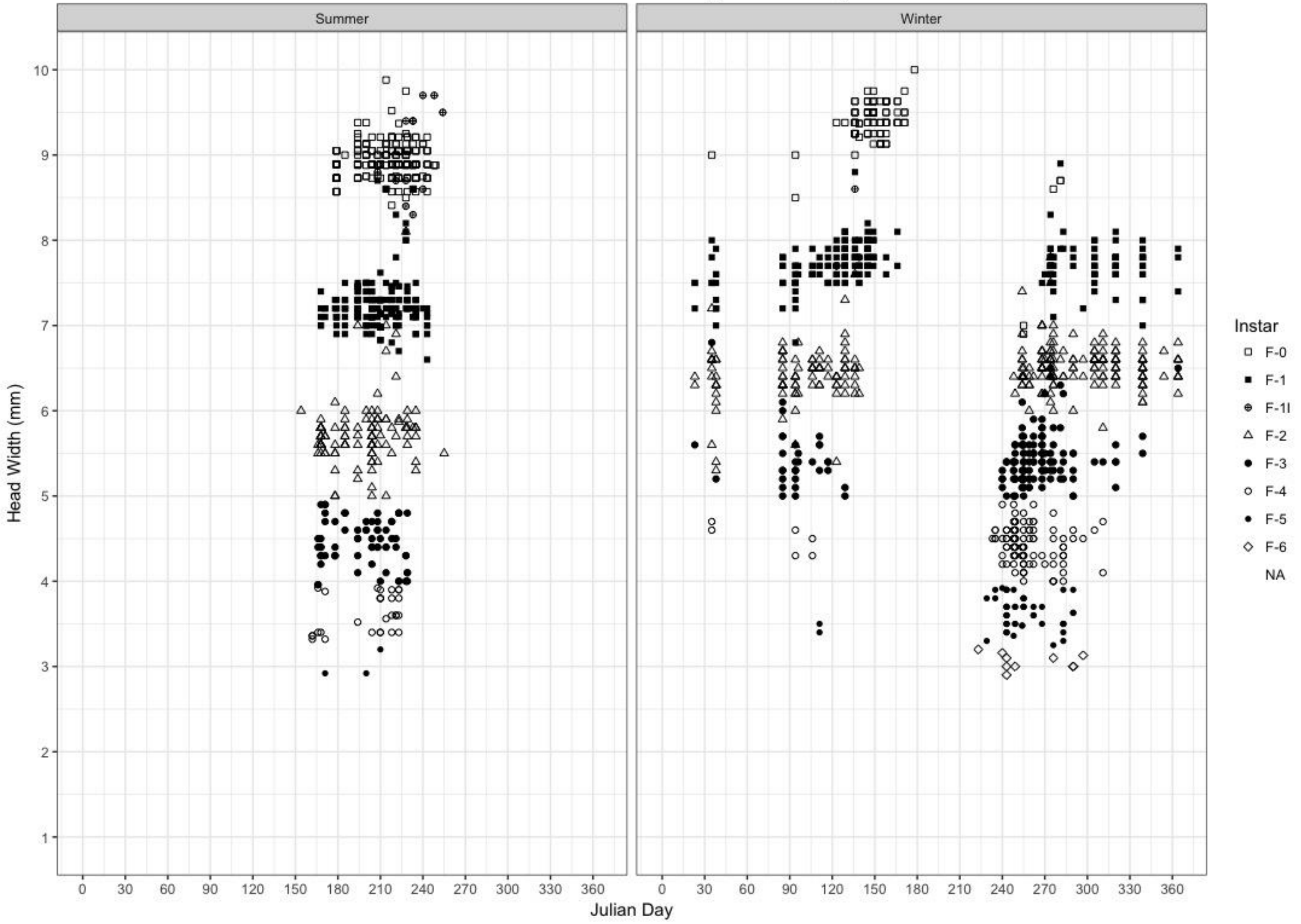
An aquatic plant survey was executed on August 12, 2018 at the three fishless prairie pothole research ponds within Crow-Hassan Park Reserve in Minnesota, USA: People Pothole, Willow Pothole, and South Twin Lake. Observed plant species are listed in alphabetical order and a check mark in the column of each pond indicates that the plant species was observed there. Michael Verhoeven and Wesley Glisson greatly assisted the authors with this plant survey.

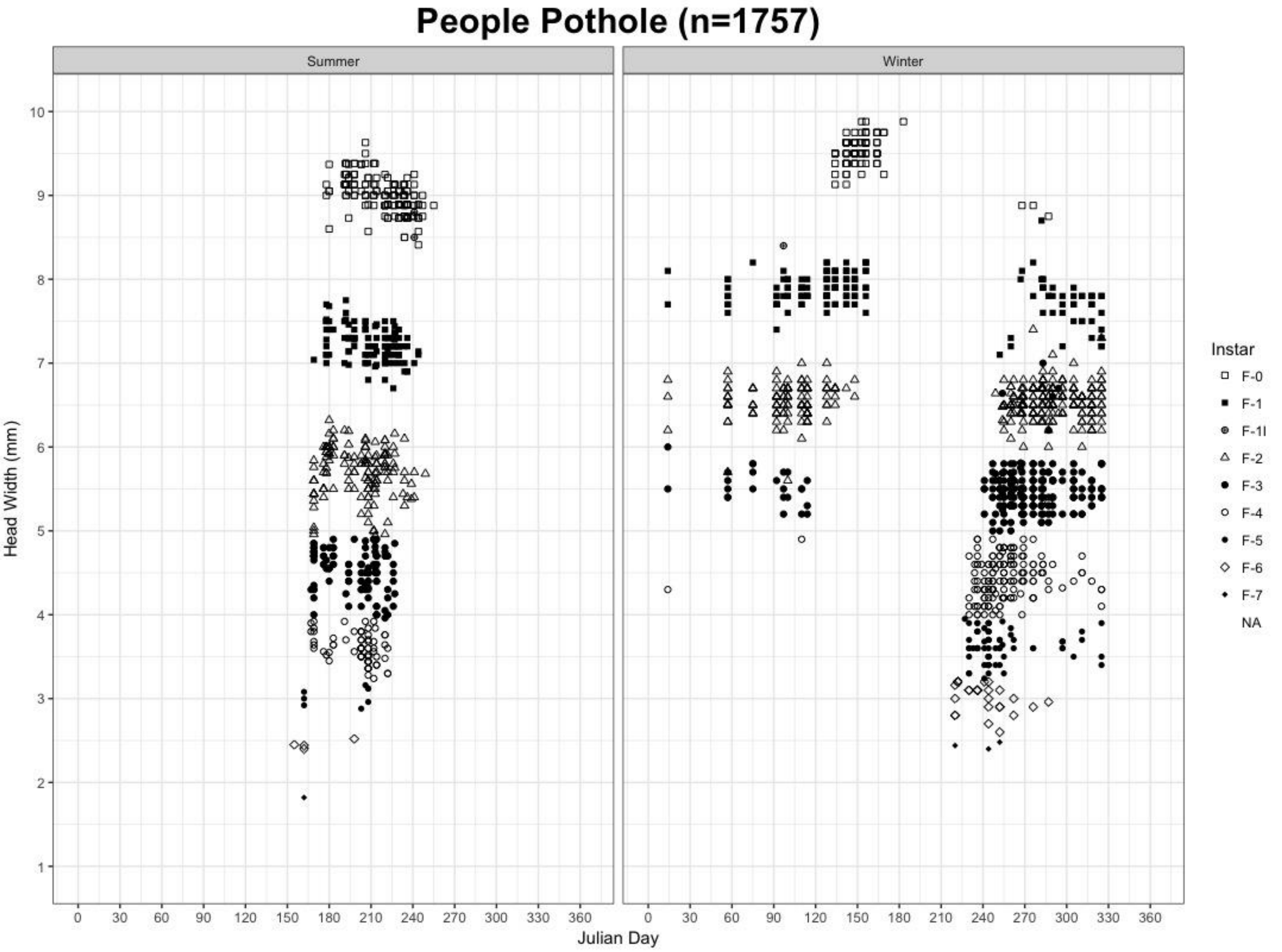
Observed Plant Species	People Pothole	Willow Pothole	South Twin Lake
Apocynum cannabinum	✓	✓	
Bidens cernua	✓	✓	✓
Bolboschoenus fluviatilis			✓
Carex lacustris		✓	✓
Carex stricta		✓	
Ceratophyllum demersum	✓	✓	✓
Ceratophyllum echinatum		✓	
Cicuta bulbifera		✓	
Convulvulus arvensis	✓	✓	
Cyperus erythrorhizos	✓	✓	✓
Eleocharis ovata	✓	✓	
Elodea canadensis			✓
Elodea nutallii		✓	✓
Galium tinctorium	✓		✓
Glyceria borealis or *grandis*	✓		
Lemna minor	✓	✓	✓
Lemna trisculca	✓		
Lycopus americanus	✓	✓	✓
Najas flexilis			✓
Nitella flexilis		✓	
Nuphar variagata	✓		
Phalaris arundinacea	✓	✓	✓
Polygonum amphibium	✓	✓	
Polygonum pensylvanicum		✓	✓
Potamogeton foliosus	✓	✓	✓
Potamogeton pusillis			✓
Potamogeton zosteriformis			✓
Riccia fluitans		✓	
Sagittaria graminea	✓		
Sagittaria latifolia	✓	✓	
Scirpus cyperinus	✓		
Scirpus/Scheonoplectus sp.	✓		
Scutellaria galericulata	✓		
Scutellaria lateriflora		✓	✓
Spartina pectinata	✓	✓	
Spirea alba		✓	
Spirodela polyrrhiza	✓	✓	✓
Teucrium canadense	✓		✓
Typha angustifolia or hybrid		✓	
Utricularia machrorhiza	✓		
Verbena hastata	✓	✓	✓
Wolffia sp.			✓

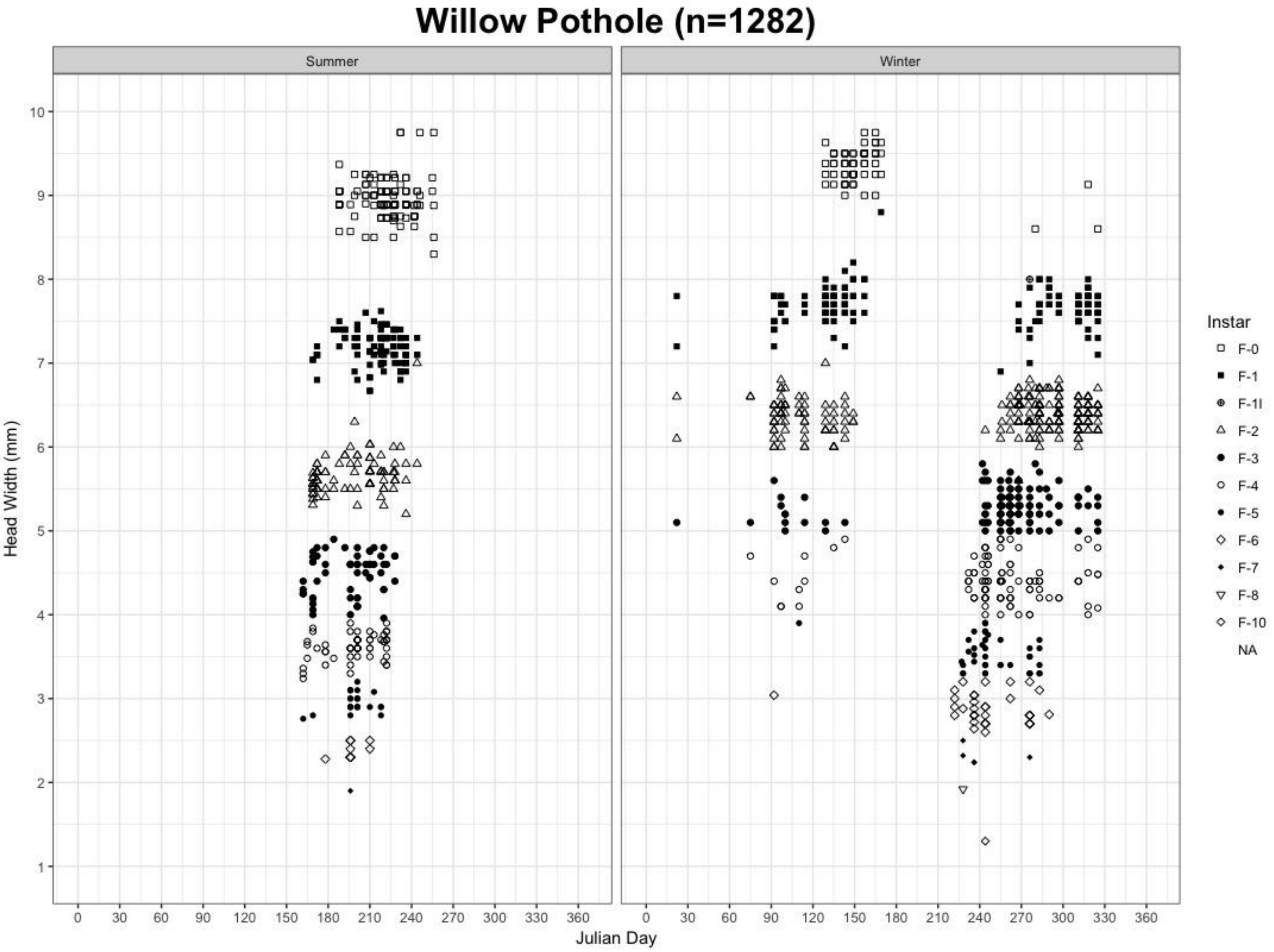
APPENDIX B

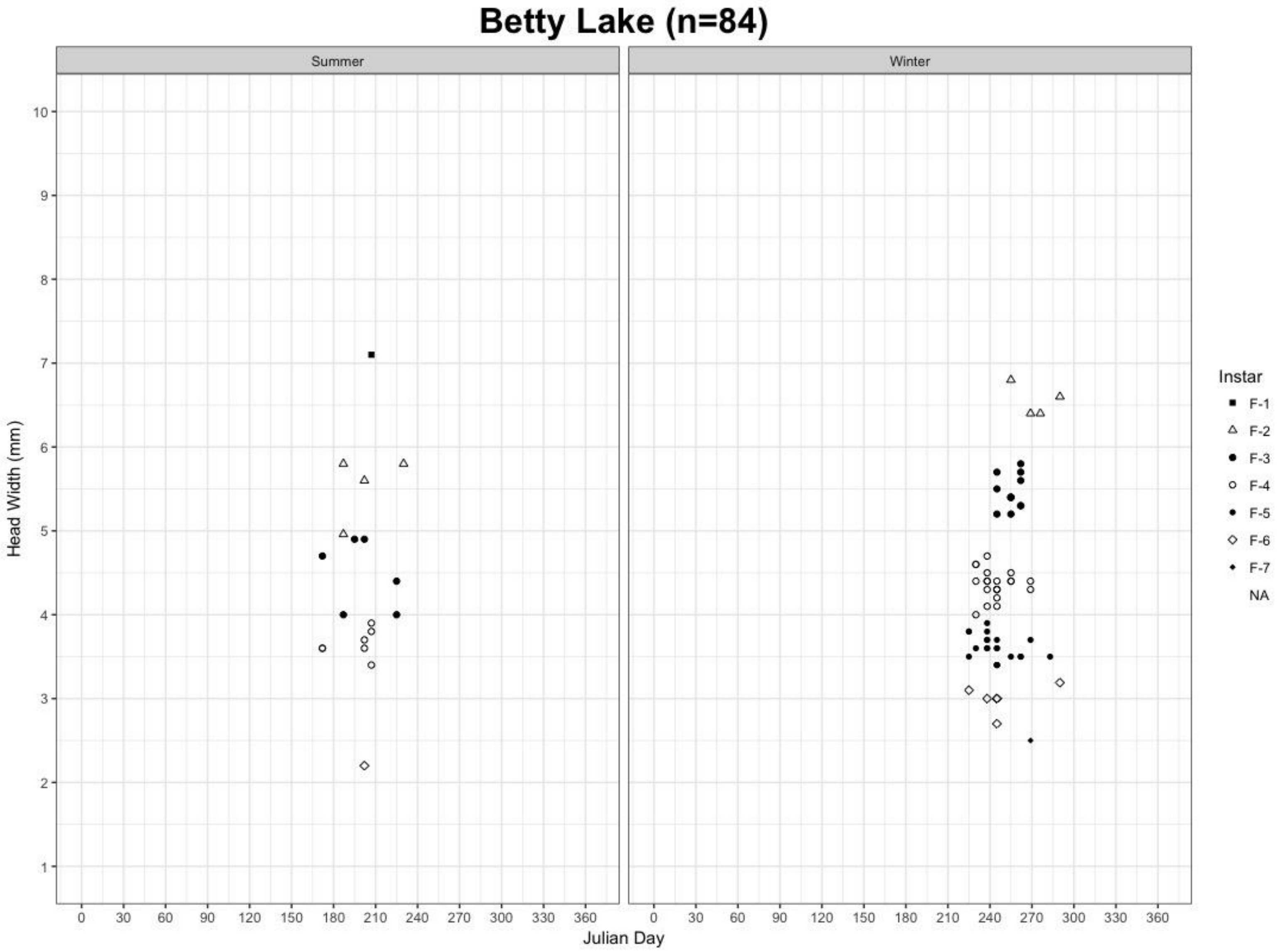
Plots of *A. junius* nymph development for each pond surveyed with the summer (left) and winter (right) pathways separated. Growth is visualized as head width (mm) over time (Julian day). Summer and winter development pathways where exhibited in all ponds (Minnesota, USA). Figures include all nymphs collected over the entire collection period (2016–2018) from Minnesota, USA.

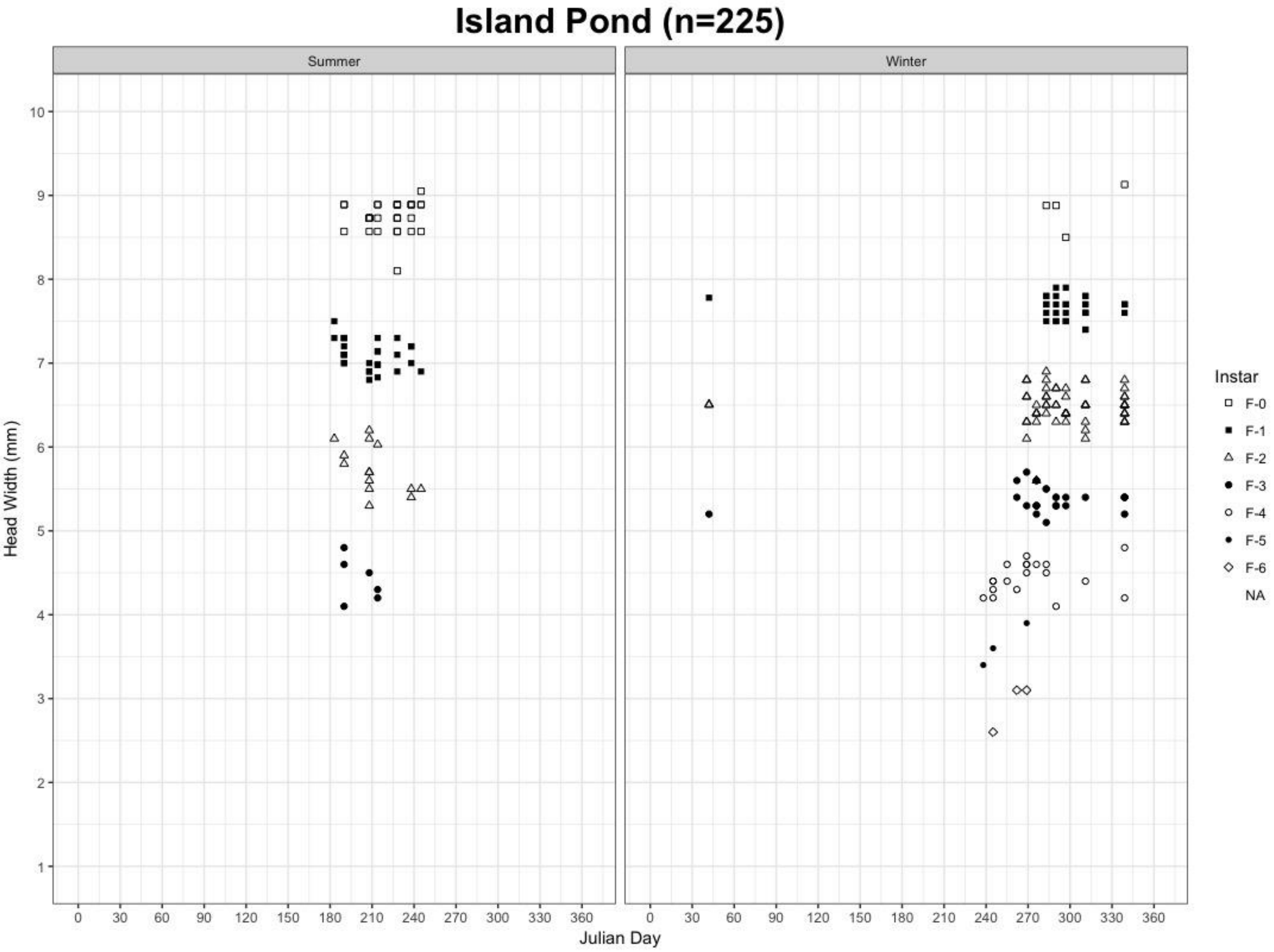
South Twin Lake (n=1643)




People Pothole (n=1757)
Summer
Winter
Head Width (mm)
1
2
3
4
5
6
7
8
9
10
Julian Day
0
30
60
90
120
150
180
210
240
270
300
330
360
Instar
F-0
F-1
F-1I
F-2
F-3
F-4
F-5
F-6
F-7
NA


Willow Pothole (n=1282)
Summer
Winter
Head Width (mm)
10
9
8
7
6
5
4
3
2
1
0
30
60
90
120
150
180
210
240
270
300
330
360
Julian Day
Instar
F-0
F-1
F-1I
F-2
F-3
F-4
F-5
F-6
F-7
F-8
F-10
NA

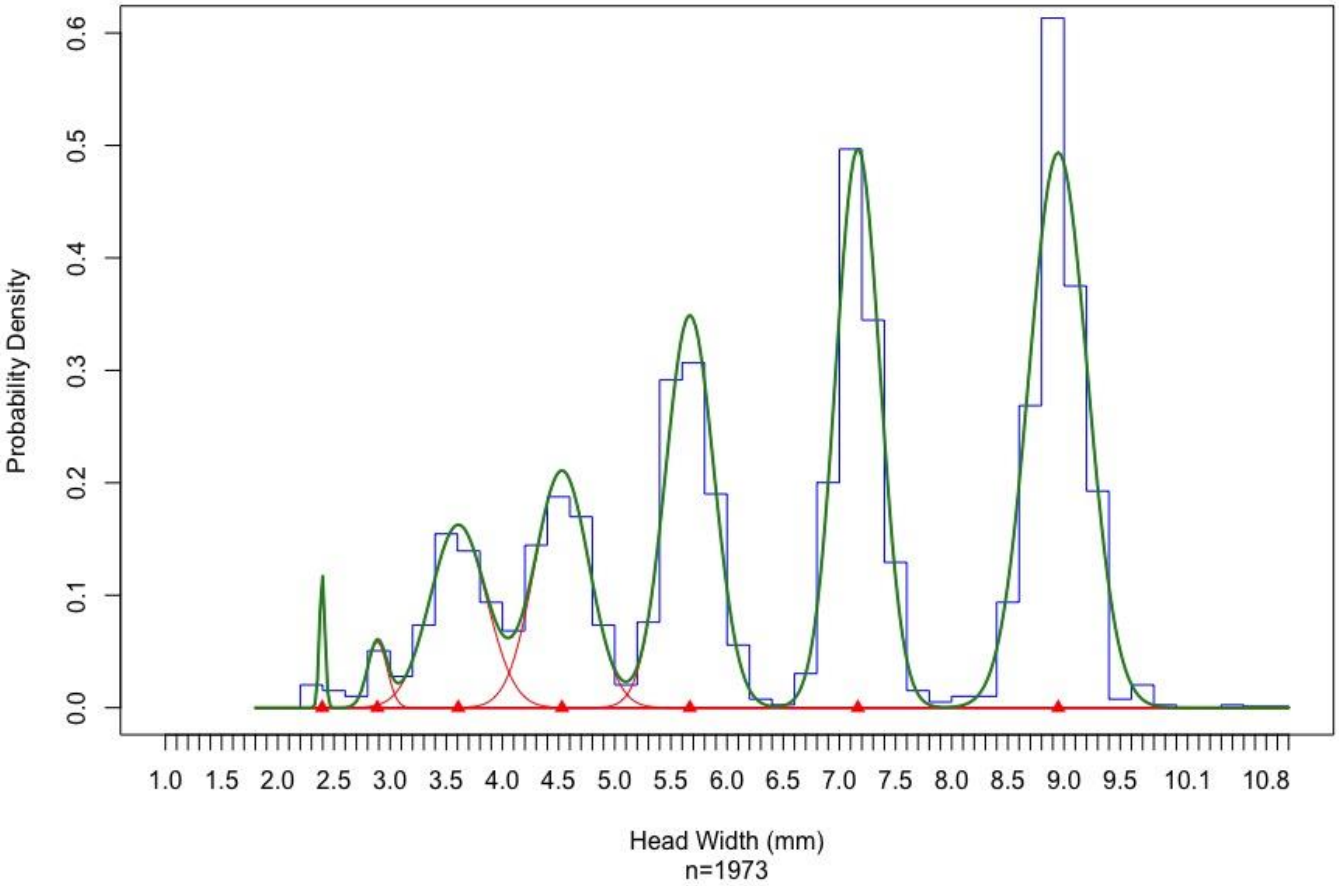

Betty Lake (n=84)
Summer
Winter
Head Width (mm)
Julian Day
1
2
3
4
5
6
7
8
9
10
0
30
60
90
120
150
180
210
240
270
300
330
360
Instar
F-1
F-2
F-3
F-4
F-5
F-6
F-7
NA


Island Pond (n=225)
Summer
Winter
Head Width (mm)
1
2
3
4
5
6
7
8
9
10
0
30
60
90
120
150
180
210
240
270
300
330
360
Julian Day
Instar
□ F-0
■ F-1
△ F-2
● F-3
○ F-4
• F-5
◇ F-6
NA

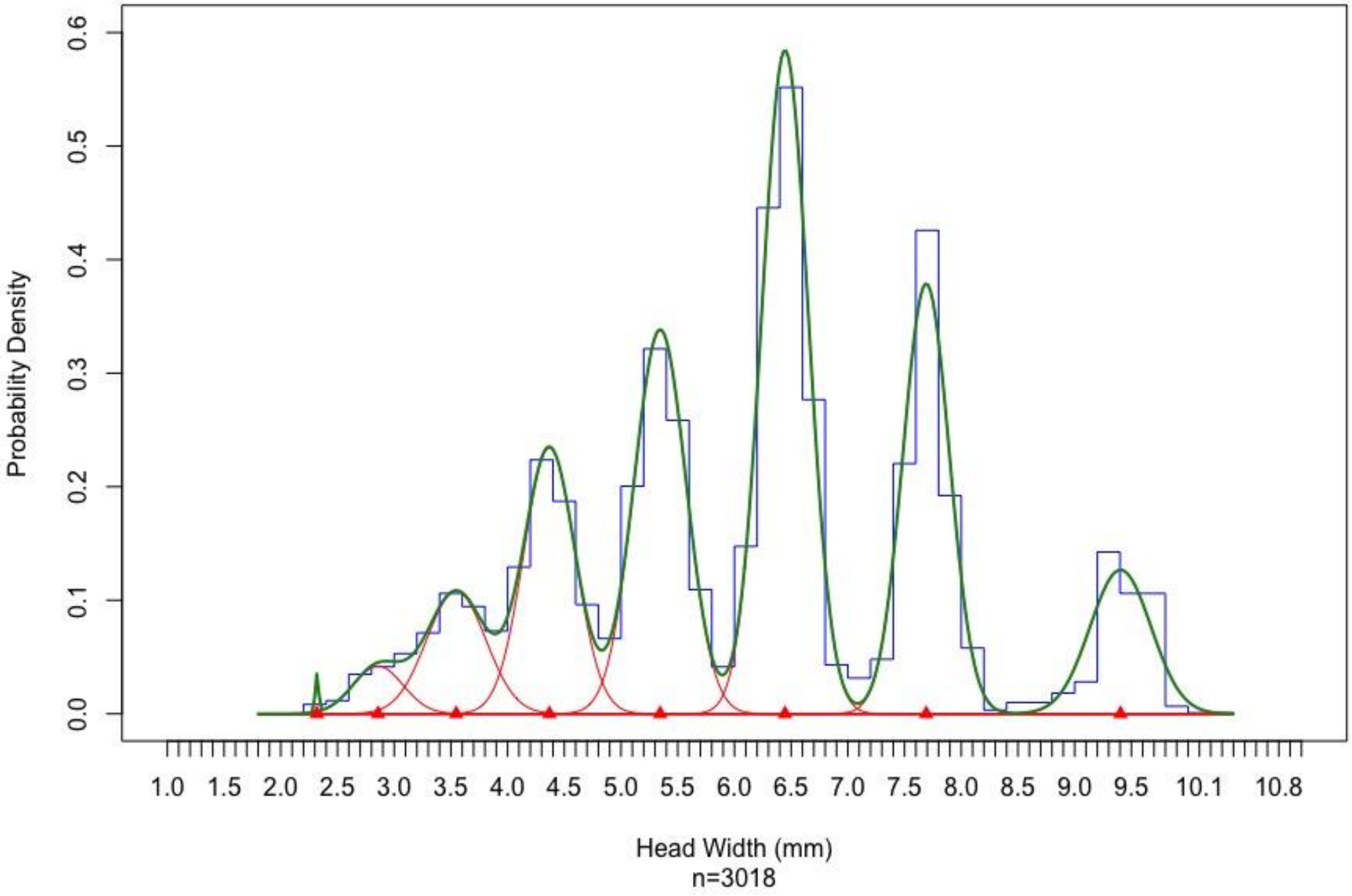
APPENDIX C

Mixed distribution analysis plots for the summer pathway nymphs only ($\chi^2 = 126.96$, df = 19, $p < 2.2e^{-16}$) (a) and winter pathway nymphs only ($\chi^2 = 99.3866$, df = 17, $p < 1.156e^{-13}$) (b).

a) Summer Pathway Nymphs


Probability Density
0.0
0.1
0.2
0.3
0.4
0.5
0.6
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.1
10.8
Head Width (mm)
n=1973

a) Winter Pathway Nymphs


Probability Density
0.0
0.1
0.2
0.3
0.4
0.5
0.6
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.1
10.8
Head Width (mm)
n=3018