2019

UPPER THERMAL LIMITS VARY AMONG AND WITHIN NATIVE BEE SPECIES IN RELATION TO SEASON, VOLTINISM, AND NEST TYPE

Kálmán K. Csigi XIV

“*If we die, we’re taking you with us”*

- The Bees

UPPER THERMAL LIMITS VARY AMONG AND WITHIN NATIVE BEE SPECIES IN RELATION TO SEASON, VOLTINISM, AND NEST TYPE

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

By

KÁLMÁN KÁROLY CSIGI XIV
B.S., University of Maine at Farmington, 2015

Co-Advisors:
KAREN M. KESTER, PH.D.
Associate Professor, Department of Biology

and

SALVATORE J. AGOSTA, PH.D.
Associate Professor, Center for Environmental Studies

Virginia Commonwealth University

Richmond, Virginia

May, 2019

Acknowledgements

I would like to thank my advisors Drs. Kester and Agosta for their continuous support and aid in this project. Additionally I would like to thank Dr. Mays for his help and patience with my statistical inquiries and Sam Droege for his constant help with identifying specimens and providing materials to improve my own skills. I would like to thank Dr. Turbeville for his aid as chair of the graduate program as well as generously donating his time. The undergraduate volunteers, Marina Williams, Juan Bonilla, Tyler Crossen, Erica Schulze, and Mary Clark Lind, were very helpful in collection of specimens as well as lab maintenance and leads on good forage locations. I would like to thank my fellow graduate students Aoife Mahaney, Allyson Richins, and Brittany Moretz for aiding in specimen collection as well as Jessica Johnston, Abby Walter, Rabecca Vareed, and Lauren Jurczak for their support. I would like to thank Maymont Estate and Gardens for allowing me to collect on their grounds as well as Tricycle and Broadfork Farms for allowing me to collect in their fields. Finally, I thank the VCU Rice River Center for allowing collection on premise and for their financial support. *Es végül szeretnék köszönetet mondani Apámnak a támogatásért: köszönet mindenért.*

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

UPPER THERMAL LIMITS VARY AMONG AND WITHIN NATIVE BEE SPECIES IN RELATION TO SEASON, VOLTINISM, AND NEST TYPE

By Kálmán Károly Csigi XIV

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University

Virginia Commonwealth University, 2019

Co-Advisers:
Karen M. Kester, Ph.D.
Associate Professor, Department of Biology

And

Salvatore J. Agosta, Ph.D.
Associate Professor, Center for Environmental Studies

Native bees are only recently gaining attention for the extent to which they aid in pollination and ecosystem services. These services are threatened by predictions of warming temperatures if bees are not able to respond. Voltinism - the number of generations produced annually- can strongly influence thermal conditions experienced by both developing and adult bees based on emergence strategies for each voltinism type. Differences in experienced thermal conditions brought on by climate change could therefore affect upper thermal limits (UTL) in bees. This study observes UTLs across a foraging season within and among native bee species to elucidate the potential response by bees to a warming climate. Bees were collected across the field season in Central Virginia and subjected to dynamic ramping trials to determine the fatal knockdown point (FKP). Results show in both univoltine and multivoltine bees an increase in upper thermal tolerance as the foraging season progresses (in quadratic and linear fashions respectively). Within multivoltine bees, FKP was related to nest type; with stem-nesting bees having the highest FKP. All average FKPs were higher than historic air temperatures of the study region, but within several degrees of the highest recorded maximum temperature. The diversity of responses in native bees provides evidence for both genetic and plastic responses to extreme temperature. While bees still face a potential myriad of other issues brought on by climate change, the observed increases in FKP across warming temperatures offer tentatively hopeful predictions for limited physiological responses in native bees to a warming climate.

INTRODUCTION

One of the greatest threats posed by climate change to ectotherms is extreme temperatures, which, even if infrequent, can have significant impacts on fitness, abundance and distribution (Hance et al. 2006; Kingsolver et al. 2013). Native bees are a highly diverse group of pollinators widespread across the globe that provide major agricultural and economic benefits. They contribute approximately $3 USD billion in agricultural services in the U.S annually (Koh et al. 2016) and are largely responsible for the pollination of crops grown for human consumption (comprising ~9.5% of the global value of agriculture production; (Gallai et al. 2009). Native bees emerge annually from early spring to late summer in temperate regions of the US. While most foraging adults live only a few weeks, many are specialized foragers, resulting in tightly-linked associations of bees and their floral hosts (Michener 2000; Wojcik et al. 2008). Therefore, any declines in native bee populations or reductions in their performance are concerning due to the services they provide to these associated floral hosts. Despite their importance, native bees are vastly understudied, especially regarding their tolerance to extreme temperatures.

Most research on North American bees has focused on pollination by the non-native European honey bee (*Apis mellifera*) and more recently, managed hives of native *Bombus* spp. (Dramstad and Fry 1995; Vanengelsdorp et al. 2009; Colla et al. 2012). This is primarily due to the economic importance of these species for agriculture (Cameron et al. 2011; Calderone 2012) as well as their ability to be successfully reared in laboratory settings. Declines in European honeybee populations have resulted in increases in native bee research. However, many native bees, unlike the heavily studied eusocial *Bombus*, encompass a wide range of characteristics and life history traits, which could result in a difference of thermal environments experienced.

Voltinism and Sociality in Native Bees

Voltinism - the number of generations produced annually - varies among and within native bee species, and has direct consequences for pollination services provided (O'Toole and Raw 1991). The majority of native bees in temperate regions are solitary and univoltine, producing a single brood per year (O'Toole 2013). These bees tend to be specialist feeders, forming close associations with many species of native plants (Minckley 2008). Bivoltine bees, producing two broods a year, are generally solitary and often facultatively bivoltine, only having a second generation annually if conditions are favorable; otherwise they are univoltine (Tepedino and Parker 1988; Forrest et al. 2019). Bivoltine bees tend to be more generalized in their foraging behaviors than univoltine bees, as forage composition shifts between generations (Milet-Pinheiro et al. 2016). Multivoltine bees vary in degree of sociality. Parasocial species share nesting sites or entrance holes, or display shared defense of nesting sites. Eusocial species produce overlapping generations and exhibit shared brood care (Michener 2000; O'Toole 2013). Due to the need to sustain multiple generations across multiple flowering periods, multivoltine bee species are generalist feeders (Michener 2000).

Native bee species vary in nesting site preferences, which determines their local abundance and distribution (O'Toole and Raw 1991). Some bee species have specific nesting requirements based on soil, pith, or wood type and are often seen in aggregations (i.e. "villages") (O'Toole and Raw 1991; Michener 2000) in areas that fulfill these requirements, thereby promoting parasocial behaviors. While univoltine bees exhibit a comparatively limited emergence period, they are often found at high densities in restricted geographic areas, whereas bivoltine and multivoltine species are more widely distributed both spatially and temporally due to less constricting nesting requirements. Compared to managed bees that are often situated in ideal nesting locations, provided with supplemental food, and are administered treatments against disease, native bees are subject to greater environmental pressures, including increasing temperatures (Willmer 2014; Pyke et al. 2016) and extreme temperature fluctuations (Vesterlund and Sorvari 2014). Therefore, increasing temperatures associated with global climate change could result in declines of native bee populations and the ecosystem services they provide.

Upper thermal limits of ectotherms

Only a few studies have investigated the upper thermal limit (UTL) of native bee species in North America, (Oyen et al. 2016; Hamblin et al. 2017; Oyen and Dillon 2018; Burdine and McCluney 2019). Due to the limited number of species investigated in these studies (less than two dozen), broader predictions for native bees must consider the general literature on ectotherms. High aerial temperatures nearing individual UTL limit active periods in insects (foraging, reproduction, etc.), *de facto* cause increased time and energy allocation towards behavioral regulation, an important defense against temperature extremes (Kearney et al. 2009). Previous research demonstrates less UTL variation among species compared to lower thermal limits (Addo-Bediako et al. 2000), suggesting UTL is highly evolutionarily constrained. Geographically, mid-latitude and tropical ectotherms appear to have the most evolutionarily constrained UTL compared to ectotherms both at higher latitudes and elevations (Sunday et al. 2011; Kellermann et al. 2012; Hoffmann et al. 2013). This potentially leaves terrestrial ectotherms from both tropical and mid-latitude regions more susceptible to heat stress. Therefore, the potentially difficult evolutionary adaptation to increasing temperatures compared to decreasing temperatures in ectotherms is troubling due to the predictions of increasing global temperatures and extreme weather events in the coming years (IPCC 2013). For native bees, this could be concerning due to the levels of diversity they display in tropical and temperate areas (O'Toole and Raw 1991). A lack of diversity in UTLs, either among or within species of native bees, would suggest their ability to respond to warming temperatures is highly constrained. The possibility that UTL could be constrained motivates the need for a greater understanding of the diversity of responses to extreme temperatures in native bees within the mid-latitudes: whether UTL is evolvable, how much variation among and/or within species is present, and whether this variation is based on climate experienced.

Variation in thermal limits

In ectotherms, variation in UTL has been shown to vary both within and among species. These differences may be the result of evolution in different thermal environments (Kellermann et al. 2012), plastic responses to temperatures experienced during larval development (Cavieres et al. 2019), and/or acclimation by adults post-eclosion (Bowler and Terblanche 2008). Because the majority of a bee's lifecycle is spent in the nest (O'Toole and Raw 1991), temperatures experienced within nests may influence UTL. Indeed, ectotherms have been noted to be the most sensitive to environmental conditions during development (Saxon et al. 2018) and different nesting environments in native bees have been shown to result in differing UTL (Hamblin et al. 2017). Aerial temperatures in the Mid-Atlantic shift from cool spring temperatures to warmer summer temperatures and decrease to cooler fall temperatures (Fig. 1). Lacking sufficient microclimate and nesting temperature data, aerial temperatures across the forage season can be utilized as a crude proxy both for nest temperatures experienced by developing bees as well as for temperatures experienced by adults when foraging. Observing UTLs across a foraging season within and among species could elucidate the potential response by bees to a warming climate. If variation is seen among species related to emergence period, this would suggest UTLs may evolve in response to a warming climate. If variation within species is observed, this would suggest a role for plasticity in this response. The goal of this study is to quantify UTL in native bees to address these issues.

Objectives

This study quantifies and compares UTL in an assemblage of native bee species common to central Virginia across a foraging season to address these specific hypotheses:

i) Native bees will vary in their tolerance to extreme temperatures with respect to emergence period

ii) Univoltine bee species that emerge in the early spring will have lower thermal tolerance than univoltine bee species that emerge later in the foraging season.

iii) First generation bivoltine bees will have a lower thermal tolerance than second generation bivoltine bees.

iv) Successive generations of multivoltine bees will exhibit increasing thermal tolerance over the foraging season.

MATERIALS AND METHODS

Specimen Collection

A subset of native bees common to central Virginia was collected using aerial sweep netting and direct vial capture of foraging adults. Sampling dates ranged from late-March through October 2018 across 45 sites around Richmond, VA comprised of both urban and non-urban habitats. A prior studied revealed no significant difference in upper thermal limits between bees from urban and non-urban sites at similar elevations in Toledo, OH (Burdine and McCluney 2019), therefore no quantification of urban vs non-urban sites was attempted. Bees were collected during all weather conditions, with the exception of excessive wind and rain, between 10:00 and 18:00 at forage-hosts; several farms were sampled between 06:30 and 09:00 for cucurbit specialists due to their extreme scarcity during later parts of the day. Collected specimens were stored in ventilated vials and transported to the laboratory in a dark cooler to minimize stress. To reduce the need for feeding and to provide a standardized start-temperature, freshly caught specimens were held overnight in a refrigerator (~6.5°C) prior to testing.

Quantifying Upper Thermal Limits

There are two procedures to determine thermal limits: 1) static method, which utilizes exposure to a single temperature over a given time, and 2) dynamic method, which incorporates an increasing temperature rate to a given endpoint (Lutterschmidt and Hutchison 1997b). Of the two, the dynamic method has gained popularity in recent studies focusing on insect thermal maxima (Terblanche et al. 2005; Bowler and Terblanche 2008), including UTL in native bees (Oyen et al. 2016; Hamblin et al. 2017; Oyen et al 2018; Burdine and McCluney 2019). One of the most popular metrics for quantifying UTL is critical thermal maximum (CT_{max}). CT_{max} is when the individual reaches a temperature where muscular control is lost and self-removal from the conditions are impossible; therefore is considered the fatal point for organisms (Lutterschmidt and Hutchison 1997a). The issue with CT_{max} as it is defined when looking at hard-bodied organisms (e.g. bees) is that it can be difficult to objectively determine due to the often rapid and agitated movement in test conditions (personal observation). Due to these issues, UTL were quantified using fatal knockdown point (FKP) via a dynamic ramping method. FKP is here defined as the point at which the ability to self-right is lost along with the visible loss of motor coordination (i.e. visible violent muscle spasms). Preliminary trials for quantifying FKP generally led to mortality in individuals. Those that were not expired prior to the end of a ramp would be unlikely to survive more than 24 hours (Oyen and Dillon 2018).

Prior to ramping, specimens caught the previous day were removed from the refrigerator and allowed 20 minutes for individuals to adjust to room temperature (~24°C). Specimens were then placed in clear glass vials, which were then weighted down and placed into a circulating water bath (Huber, Kältemaschinenbau AG, Germany) preheated to 35°C. Specimens were then given 15 minutes for body temperature to equilibrate with the start temperature as suggested by previous studies (Klok and Chown 1997; Terblanche et al. 2006; Terblanche et al. 2007; Mitchell and Hoffmann 2010; Agosta et al. 2018). Afterwards, a dynamic heat ramp was initiated from 35°C to 55°C at a rate of 0.25°C min^{-1} (Lighton 2004; Terblanche et al. 2007; Terblanche et al. 2011; Agosta et al. 2018; Oyen and Dillon 2018). The temperature of the water bath at which FKP occurred was noted for each specimen and then individuals were removed from the trial. After removal from the water bath, individuals were given several minutes to cool down as to prevent the explosion of exoskeletons brought on by rapid temperature shifts (personal observation) and then frozen. Specimens were kept frozen until they could be pinned and preserved. All specimen identifications were conducted using the literature along with expert opinion (Sam Droege, USGS-BIML).

Thermal Lag Adjustments in Measuring FKP

Because water temperatures within the circulating water bath are used to measure body temperatures of vialed specimens, an assumption is made that body temperatures (T_{body}), vial air temperatures (T_{air}), and water temperatures (T_{water}) are near-synchronous. Additionally, the likelihood of a lag in these temperatures becomes greater as body size increases, which can cause overestimation of thermal limits (Agosta et al. 2018). To determine if any significant thermal lag was present in collected specimens due to size differences between body temperatures (T_{body}), vial air temperatures (T_{air}), and water temperatures (T_{water}), calibration tests were conducted on four species of bees that varied in mass (0.03g - 0.57g; n=8). Trial ramps were run using a modified empty vial with a bare tip T-type thermocouple probe (Cooper-Atkins model 39138 T, Cincinnati OH, USA) inserted and secured into place with silicone sealant. This same process was repeated with a bare tip 26-gauge T-type thermocouple (Physitemp Instruments model W-TW-26, Clifton NJ, USA) inserted into the central thorax of a frozen-then-thawed bee. Both thermocouples were connected to the same digital thermometer (Amprobe model TMD-52, Everett WA, USA) and T_{body} temperatures were allowed to equilibrate to match T_{air} and T_{water} temperatures. The same ramping procedure for live specimens as described above was followed but with temperatures of T_{body}, T_{air}, and T_{water} recorded every two minutes for the duration of each ramping session (from 35°C to 55°C at a rate of 0.25°C min^{-1}) to determine any lag between T_{body}, T_{air}, and T_{water}.

Classifying Voltinism Type

There is a dearth of established literature on voltinism assignments for multiple species collected during the sampling season. Additionally, some voltinism strategies varied within species when local distributions were compared to different geographic locations based on latitudinal variation. For example, *Ceratina calcarata* exhibits bivoltinism in southern regions of the US (Rau 1928) but exhibits univoltinism in northern regions of the US; (Rehan and Richards 2010). To attempt to determine reliable voltinism classifications for bees collected, long-term data from the USGS Bee Inventory and Monitoring Lab focusing on phenology of bees in the Mid-Atlantic (Boo, 2018) as well local species distribution and phenology data from the North American Native Bee Collaborative (2017) were utilized to provide voltinism assignments for each species collected. Voltinism types were classified in species by determining abundance throughout the annual emergence period(s) as well as with expert opinion (Sam Droege, USGS-BIML). Species with a single distinct emergence pulse in the year were identified as univoltine, whereas two distinct emergence pulses were categorized as bivoltine and an extended emergence across several months was categorized as multivoltine.

Nesting type comparisons for species level subset

Nesting types have been compared to determine differences in CT_{max} (Hamblin et al 2017) but not FKP, which by definition occurs at a higher temperature. To determine whether nesting type could be used to predict FKP, the aforementioned subset of six multivoltine species were categorized by the literature and expert opinion (Sam Droege, USGS-BIML) into stem, cavity, or ground nesting types.

Maximum Air Temperatures

To compare FKP of bee species sampled to aerial temperatures, daily maximum air temperatures from a subset of 118 years compiled between two stations in the greater Richmond, VA area were obtained from the Global Historical Climate Network-Daily Summaries (GHCN-Daily) database from NOAA's Center for Environmental Information (NCEI) (Menne et al. 2012). This was compared to mean FKP of each voltinism type as a crude predictor of overall sensitivity concerning increases in climatic temperatures.

Data Analysis

FKP data approximated a normal distribution based on normal quantile plots and plotted residuals were homogenous. All species collected with recorded FKP were included in analyses. An ANCOVA analysis was used to test for differences in FKP between voltinism types across date along with their interaction. Collection date was included as a covariate. To determine whether the pattern of increasing FKP across the season was present at a species level, a subset of six multivoltine species (*Bombus impatiens*, *Augochlora pura*, *Agapostemon virescens*, *Halictus ligatus/poeyi*, *Lasioglossum imitatum*, and *Ceratina calcarata*) with sufficient sample sizes ($n \geq 30$) distributed across the duration of the season were isolated for analysis. Within univoltine bees, only one species (*Ptilothrix bombiformis*) had a sufficient enough distribution to test for increases across emergence period. Simple linear regression was used to test for increases in FKP across time for each multivoltine and univoltine bee. Univoltine bees were also analyzed by average species FKP and collection date to determine whether an increase across the season based on species rather than individuals was present. This was tested using simple linear regression. Nesting habits of the multivoltine species subset was analyzed with a one-way ANOVA. All statistical analyses were run in R (v 3.5.3). *Post hoc* Tukey pairwise comparisons for nesting type ANOVA was run using the *multcomp* package. All tests were considered significant at $p \leq 0.05$. Means are reported with ± 1 standard error.

RESULTS

Specimen Collection

A total of 1,508 specimens representing five families, 28 genera and 98 species were collected across the study period (Table 1). This represents approximately 16-20% of known bee diversity in the Mid-Atlantic (Sam Droege, Personal Communication). Apidae and Halictidae were the most common families collected with the majority of these two families comprised of *Bombus*, *Halictus,* and *Lasioglossum* spp. Two species collected had not been previously reported in Virginia. *Svastra petulca* (one specimen) was collected in an urban area (VCU campus). *Hylaeus georgicus* (two specimens) was collected in a non-urban area (Belle Isle, James River Park System).

Thermal Lag Adjustments

All thermal lag temperatures recorded across the vast majority of bee sizes were within the error rate of the temperature of the circulating water bath used in experimentation (~±0.25˚C). Therefore no appreciable thermal lag was present between T_{body}, T_{air}, and T_{water} and no adjustments of fatal knockdown point (FKP) values were required prior to analysis.

Upper Thermal Limits: Assemblage Level

FKP varied across time and with respect to voltinism. ANCOVA revealed a strong interaction between collection date and voltinism type (voltinism*date: $F_{2,1232}$=6.44;P=0.001).

Therefore, voltinism types were analyzed separately. For univoltine bees, FKP increased over the season in a quadratic fashion ($F_{2,346}$= 21.59; r^2= 0.111; P< 0.0218; Fig. 2). In contrast, for bivoltine bees, FKP did not differ significantly over the season (P= 0.33; Fig. 3). For multivoltine bees, FKP increased linearly over the season ($F_{1,762}$= 23.69; r^2= 0.030; P< 0.0001; Fig. 4).

Upper Thermal Limits: Comparisons at a Species Level

All multivoltine species with the exception of *H. ligatus/poeyi* were found to have a significant increase in FKP across the season (Fig. 5; Table 2). *P. bombiformis* had a significant increase in FKP across emergence period (P=0.045; R^2=0.15; n=30; Fig. 6). Univoltine speices FKP was marginally non-significant (P=0.057) but clearly illustrated a positive trend of increasing FKP as the season progressed (Fig. 7).

Nesting Type Comparisons of FKP

There was a significant difference in average FKP with respect to nest type. Stem nesters had the highest FKP (46.4 ± 0.19˚C), followed by ground nesters (44.7 ± 0.15˚C) and cavity nesters (43.6 ± 0.13˚C; Fig. 8).

Maximum Air Temperatures

The maximum temperature recorded around the study site in the past 118 years 41.7˚C was observed once. Temperatures above 38.0˚C were observed 76 times. In general, for each voltinism type, mean FKP temperatures were above the highest recorded maximum temperature in the study area (Fig. 9).

DISCUSSION

These results indicate that there are increases in native bee fatal knockdown points (FKP) across a foraging season in relation to environmental temperature, both among and within species. The increase in FKP across emergence periods by individual univoltine bees suggests evolved differences based on periods of emergence. This is also supported by the trend of increasing FKP seen in univoltine species across the forage season. While seeing no changes in FKP across the season in bivoltine bees, the increase of FKP in multivoltine and univoltine bees, both at an assemblage and species level, illustrates diversity in response to increasing temperatures. Results support the initial hypothesis that native bees will vary in their tolerance to extreme temperatures with respect to emergence period, which differs based on voltinism type. Results also support the hypotheses that earlier emerging univoltine bees would have a lower FKP than later-emerging univoltine bees, and that FKP in multivoltine bees would increase across the foraging season. However, the hypothesis that the first generation of bivoltine bees would have a lower FKP than the second generation was not supported. Within the multivoltine bee subset, all species with the exception of *H. ligatus/poeyi,* exhibited an increase in FKP across the foraging season. While this suggests bees exhibit an increased FKP in response to warming temperatures, the mechanisms behind this response are unknown. However, the increase in FKP is presumably related to genetically and non-genetically based plastic responses to thermal environments experienced.

Differences in upper thermal limits between univoltine species may be genetically based, in that species emerging in the relatively cooler early summer had lower FKP values than species emerging later in the warmer part of season. In contrast, the increase of FKP in multivoltine bees across the season suggests either genetics or plasticity is determining UTL. Additionally, the increase in FKP of *P. bombiformis* across its emergence period suggests plasticity may be present in univoltine species as well. The increase in FKP across the year with subsequent generations of multivoltine bees both at the assemblage and species level lends support to emergence period affecting upper thermal limits via plasticity. The lack of significance in increasing FKP in *H. ligatus/poeyi* within the subset of multivoltine species analyzed is likely due to the inability to visually differentiate *H. ligatus* and *H. poeyi* as they are both cryptic but distinctly different species (Danforth et al. 1998). It is likely that this group contained both species in the analyses which could have masked any patterns that may have existed.

Evidence of thermal environments influencing FKP in native bees suggests that temperatures experienced within the nest during development could be linked to variance in FKP. This is assuming that nesting aboveground in stems would have less thermal buffering and experience greater temperature variance than nests at ground level or belowground. The finding of significantly different FKPs based on nesting type agrees with results seen in Hamblin et al (2017). This supports the idea that differences in experienced temperature could be altering UTL through developmental plasticity in native bees (Forrest et al. 2019). Differences in FKP based on nesting type also agree with Cavieres et al. (2019), where increased developmental temperatures resulted in increased UTL in ectotherms.

Comparing UTL to conditions found in nature

Due to a lack of microclimate data, a coarse but potentially insightful comparison of mean FKP temperatures and maximum recorded aerial temperatures near to the study sites can provide a crude estimate of thermal safety margins (the distance between maximum aerial temperatures and an organism's UTL; (Sunday et al. 2014)) for each voltinism type. While the FKP for each voltinism type is greater than the highest recorded temperature in the historical record, these FKP values are similar to those of two other hymenopterans and a lepidopteran within Central Virginia (Agosta et al. 2018), despite using different UTL metrics. Irrespective of voltinism type, the majority of native bee species sampled in the present study had FKPs that were either at or above historically maximum recorded temperatures, suggesting that aerial temperature, in part, is acting as a selection agent for upper thermal limits in bees. Comparison of maximum aerial temperatures and FKP values reveal that native bees have a relatively narrow thermal safety margin (univoltine bees, mean = 1.9˚C; bivoltine bees, mean = 5.7˚C; multivoltine bees, mean = 2.6˚C). These thermal safety margins are similar to those reported for other insects (Sunday et al. 2014) yet much narrower than those reported for two native bee species, *B. impatiens* and *Agopostemon sericeus,* and the European honeybee, *A. mellifera* (Burdine and McCluney 2019). However, the different experimental method employed in this study does not allow for a direct comparison of results. In any case, the narrow thermal safety margins found for native bees in the present study are within the minimum predicted increase of 2˚C over the next century (IPCC 2013), suggesting that univoltine and multivoltine bees in temperate regions will be at the greatest disadvantage and bivoltine bees will be slightly less disadvantaged. However, this coarse-scale utilization of macroclimate data probably underestimates realized local temperature, in that microclimate temperatures experienced by individuals often exceed macroclimate temperatures (Pincebourde and Casas 2015). It does, however, illustrate the susceptibility of native bees to warming temperatures without adaptation. The results presented here show a diverse response to increasing temperatures by native bees and point towards their overall response to a warming climate. This varied response by native bees to increasing temperatures suggests potential adaptation to climate change based on several possible genetic or non-genetic factors. However, even if native bees can adapt to increasing temperatures, other challenges associated with global climate change should be considered. These issues include phenological mismatches between bees and their forage hosts (Memmott et al. 2007), sub-lethal effects of increased temperatures on activity and behavior (Buckley and Huey 2016), increased potential for emerging infectious diseases (Epstein 2001), and reductions in fitness induced by heat-stress (Saxon et al. 2018).

Quantifying Ramp Rate

To date, few studies have looked at UTL of native bees in North America, and have typically focused on a small subset of species (Oyen et al. 2016; Hamblin et al. 2017; Oyen and Dillon 2018; Burdine and McCluney 2019) and utilized CT_{max} to determine UTL, not FKP. Therefore, specific temperature comparisons between studies cannot be made; however, general conclusions can still be drawn from these studies. The range of UTL temperatures seen in this study (36.17-50.91˚C) are similar but not identical to those reported by Hamblin et al (2017) (44.6 - 51.3˚C) and are drastically different from the narrow range of UTL temperatures reported by Burdine & McCluney (2019) (49.1 - 53.1˚C). These differences are likely due to an increased ramping speed (0.50˚C min^{-1}) and perforce increased thermal inertia during ramping trials. In the present study, FKP of *Colletes inaequalis* was 1.8˚C higher at a thermal ramp rate of 0.25˚C min^{-1} than at a ramp rate of 0.50˚C min^{-1} (see Appendix I). This indicates that the higher UTLs reported in other studies may have been overestimated due to thermal ramp rate.

Conclusions

Native bees differ in FKP over the foraging season with respect to voltinism type. Evidence is seen for both evolved and plastic differences between species of native bees based on emergence time. Plastic differences may be due to temperatures experienced during development or post-eclosion. Because thermally-derived plasticity is complex and often cannot be attributed to a single factor (Stillwell and Fox 2005), investigation of the mechanisms responsible for increased FKP across the foraging season is necessary and should consider potential interactions between genetic and non-genetic factors. Although the present study is the most exhaustive examination of UTLs in native bees to date, the need for additional research across a wider geographic area, especially at temperate mid-latitudes is indicated. Finally, the collection of several rare and previously uncollected species during the present study suggests that even in the Mid-Atlantic, which is likely one of the best-surveyed regions in the US, there is still much to be discovered about native bee diversity and distributions.

LITERATURE CITED

Addo-Bediako A, Chown SL, Gaston KJ. 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. B Biol. Sci. 267:739–745. doi:10.1098/rspb.2000.1065.

Agosta SJ, Joshi KA, Kester KM. 2018. Upper thermal limits differ among and within component species in a tritrophic host-parasitoid-hyperparasitoid system. Edwards OR, editor. PLoS One 13:e0198803. doi:10.1371/journal.pone.0198803.

Boo, W. 2018. Phenology of Common Bee Species: MidAtantic States. USGS-BIML.

Bowler K, Terblanche JS. 2008. Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? Biol. Rev. 83:339–355. doi:10.1111/j.1469-185X.2008.00046.x.

Buckley LB, Huey RB. 2016. How extreme temperatures impact organisms and the evolution of their thermal tolerance. In: Integrative and Comparative Biology. Vol. 56. Oxford University Press. p. 98–109.

Burdine JD, McCluney KE. 2019. Differential sensitivity of bees to urbanization-driven changes in body temperature and water content. Sci. Rep. 9. doi:10.1038/s41598-018-38338-0.

Calderone NW. 2012. Insect Pollinated Crops , Insect Pollinators and US Agriculture: Trend Analysis of Aggregate Data for the Period 1992 – 2009. 7:1–27. doi:10.1371/journal.pone.0037235.

Cameron S a, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL. 2011. Patterns of widespread decline in North American bumble bees. Proc. Natl. Acad. Sci. U. S. A. 108:662–7. doi:10.1073/pnas.1014743108.

Cavieres G, Alruiz JM, Medina NR, Bogdanovich JM, Bozinovic F. 2019. Transgenerational and within-generation plasticity shape thermal performance curves. Ecol. Evol. 9:2072–2082.

doi:10.1002/ece3.4900.

Colla SR, Gadallah F, Richardson L, Wagner D, Gall L. 2012. Assessing declines of North American bumble bees (*Bombus* spp.) using museum specimens. Biodivers. Conserv. 21:3585–3595. doi:10.1007/s10531-012-0383-2.

Danforth BN, Mitchell PL, Packer L. 1998. Mitochondrial DNA differentiation between two cryptic *Halictus* (Hymenoptera: Halictidae) species. Ann. Entomol. Soc. Am. 91:387–391. doi:10.1093/aesa/91.4.387.

Dramstad W, Fry G. 1995. Foraging activity of bumblebees (*Bombus*) in relation to flower resources on arable land. Agric. Ecosyst. Environ. 53:123–135.

Epstein PR. 2001. Climate change and emerging infectious diseases. Micro. Infec.747-754.

Forrest JRK, Cross R, CaraDonna PJ. 2019. Two-Year Bee, or Not Two-Year Bee? How Voltinism Is Affected by Temperature and Season Length in a High-Elevation Solitary Bee. Am. Nat. 193:560–573.

Gallai N, Salles JM, Settele J, Vaissière BE. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. 68:810–821. doi:10.1016/j.ecolecon.2008.06.014.

Hamblin AL, Youngsteadt E, López-Uribe MM, Frank SD. 2017. Physiological thermal limits predict differential responses of bees to urban heat-island effects. Biol. Lett. 13:20170125. doi:10.1098/rsbl.2017.0125.

Hance T, van Baaren J, Vernon P, Boivin G. 2006. Impact of Extreme Temperatures on Parasitoids in a Climate Change Perspective. Annu. Rev. Entomol. 52:107–126. doi:10.1146/annurev.ento.52.110405.091333.

Hoffmann AA, Chown SL, Clusella-Trullas S. 2013. Upper thermal limits in terrestrial ectotherms: How constrained are they? Funct. Ecol. 27:934–949. doi:10.1111/j.1365-2435.2012.02036.x.

IPCC. 2013. Climate change 2013: the physical science basis: Working Group I contribution to the fifth assessment report of the Intergovernmental Panel on Climate Change. Stocker TF, Plattner G-K, Tignor MMB, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, editors. Cambridge University Press.

Kearney M, Shine R, Porter WP. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. Proc. Natl. Acad. Sci. 106:3835–3840. doi:10.1073/pnas.0808913106.

Kellermann V, Overgaard J, Hoffmann AA, Flojgaard C, Svenning J-C, Loeschcke V. 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. Proc. Natl. Acad. Sci. 109:16228–16233. doi:10.1073/pnas.1207553109.

Kingsolver JG, Diamond SE, Buckley LB. 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Funct. Ecol. 27:1415–1423. doi:10.1111/1365-2435.12145.

Klok CJ, Chown SL. 1997. Critical thermal limits, temperature tolerance, and water balance of sub-antarctic caterpillar, *Pringleophaga marioni* (Lepidoptera: Tineidae). J. Insect Physiol. 43:685–694.

Koh I, Lonsdorf E V., Williams NM, Brittain C, Isaacs R, Gibbs J, Ricketts TH. 2016. Modeling the status, trends, and impacts of wild bee abundance in the United States. Proc. Natl. Acad. Sci. 113:140–145. doi:10.1073/pnas.1517685113.

Lighton JRB. 2004. Thermolimit respirometry: an objective assessment of critical thermal maxima in two sympatric desert harvester ants, *Pogonomyrmex rugosus* and *P. californicus*. J. Exp. Biol. 207:1903–1913. doi:10.1242/jeb.00970.

Lutterschmidt WI, Hutchison VH. 1997. The critical thermal maximum: data to support the onset of spasms as the definitive end point. Can. J. Zool. 75:1553–1560. doi:10.1139/z97-782.

Lutterschmidt WI, Hutchison VH. 1997. The critical thermal maximum: history and critique. Can. J. Zool. 75:1561–1574. doi:10.1139/z97-783.

Memmott J, Craze PG, Waser NM, Price M V. 2007. Global warming and the disruption of plant-pollinator interactions. Ecol. Lett. 10:710–717. doi:10.1111/j.1461-0248.2007.01061.x.

Menne MJ, Durre I, Korzeniewski B, McNeal S, THomas K, Yin X, et al. 2012. Global Historical Climatology Network - Daily (GHCN-Daily), Version 3. :Subset: CITY: US510015. doi:10.7289/V5D21VHZ.

Michener CD. 2000. The bees of the world. Baltimore: Johns Hopkins University Press.

Milet-Pinheiro P, Herz K, Dötterl S, Ayasse M. 2016. Host choice in a bivoltine bee: How sensory constraints shape innate foraging behaviors. BMC Ecol. 16. doi:10.1186/s12898-016-0074-z.

Minckley RL. 2008. Faunal composition and species richness differences of bees (Hymenoptera: Apiformes) from two north American regions. Apidologie 39:176–188. doi:10.1051/apido.

Mitchell KA, Hoffmann AA. 2010. Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. Funct. Ecol. 24:694–700. doi:10.1111/j.1365-2435.2009.01666.x.

North American Native Bee Collaborative. 2017. Bees of Maryland: A Field Guide. Washington

D.C: North American Bee Collaborative.

O’Toole C. 2013. Bees: a natural history. Buffalo: Firefly Books.

O’Toole C, Raw A. 1991. Bees of the world. New York: Facts On File.

Oyen KJ, Dillon ME. 2018. Critical thermal limits of bumble bees (*Bombus impatiens*) are marked by stereotypical behaviors and are unchanged by acclimation, age, or feeding status . J. Exp. Biol.:jeb.165589. doi:10.1242/jeb.165589.

Oyen KJ, Giri S, Dillon ME. 2016. Altitudinal variation in bumble bee (*Bombus*) critical thermal limits. J. Therm. Biol. 59:52–57. doi:10.1016/j.jtherbio.2016.04.015.

Pincebourde S, Casas J. 2015. Warming tolerance across insect ontogeny: Influence of joint shifts in microclimates and thermal limits. Ecology 96:986–997. doi:10.1890/14-0744.1.sm.

Pyke GH, Thomson JD, Inouye DW, Miller TJ. 2016. Effects of climate change on phenologies and distributions of bumble bees and the plants they visit. Ecosphere 7:1–19. doi:10.1002/ecs2.1267.

Rau P. 1928. The Nesting Habits of the Little Carpenterbee, *Ceratina Calcarata*. Ann. Entomol. Soc. Am. 21:380–397. doi:10.1093/aesa/21.3.380.

Rehan SM, Richards MH. 2010. Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). Can. Entomol. 142:65–74. doi:10.4039/n09-056.

Saxon AD, O’Brien EK, Bridle JR. 2018. Temperature fluctuations during development reduce male fitness and may limit adaptive potential in tropical rainforest Drosophila. J. Evol. Biol. 31:405–415. doi:10.1111/jeb.13231.

Stillwell RC, Fox CW. 2005. Complex Patterns of Phenotypic Plasticity : Interactive Effects of Temperature during Rearing and Oviposition. Ecology 86:924–934.

Sunday JM, Bates AE, Dulvy NK. 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proc. R. Soc. B Biol. Sci. 278:1823–1830. doi:10.1098/rspb.2010.1295.

Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc. Natl. Acad. Sci. 111:5610–5615. doi:10.1073/pnas.1316145111.

Tepedino VJ, Parker FD. 1988. Alternation of sex ratio in a partially bivoltine bee, *Megachile rotundata* (Hymenoptera: Megachilidae). Ann. - Entomol. Soc. Am. 81:467–476. doi:10.1093/aesa/81.3.467.

Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL. 2007a. Critical thermal limits depend on methodological context. Proc. R. Soc. B Biol. Sci. 274:2935–2943. doi:10.1098/rspb.2007.0985.

Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL. 2007b. Critical thermal limits depend on methodological context. Proc. R. Soc. B Biol. Sci. 274:2935–2943. doi:10.1098/rspb.2007.0985.

Terblanche JS, Hoffmann AA, Mitchell KA, Rako L, le Roux PC, Chown SL. 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. J. Exp. Biol. 214:3713–3725. doi:10.1242/jeb.061283.

Terblanche JS, Klok CJ, Krafsur ES, Chown SL. 2006. Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): Implications for distribution modelling. Am. J. Trop. Med. Hyg. 74:786–794. doi:74/5/786 [pii].

Terblanche JS, Sinclair BJ, Klok CJ, McFarlane ML, Chown SL. 2005. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica* *chalcoptera* (Coleoptera: Chrysomelidae). J. Insect Physiol. 51:1013–1023. doi:10.1016/j.jinsphys.2005.04.016.

Vanengelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, et al. 2009. Colony collapse disorder: a descriptive study. PLoS One 4:1–17. doi:10.1371/journal.pone.0006481.

Vesterlund S-R, Sorvari J. 2014. Longevity of starved bumblebee queens (Hymenoptera: Apidae) is shorter at high than low temperatures. Eur. J. Entomol. 111:217–220. doi:10.14411/eje.2014.035.

Willmer P. 2014. Climate change: Bees and orchids lose touch. Curr. Biol. 24:R1133–R1135. doi:10.1016/j.cub.2014.10.061.

Wojcik VA, Frankie GW, Thorp RW, Jennifer L, Wojcik VA, Frankie GW, Thorp RW, Hernandez JL. 2008. Seasonality in Bees and Their Floral Resource Plants at a Constructed Urban Bee Habitat. J. Kansas Entomol. Soc. 81:15–28.

FAMILY	GENUS	SPECIES	VOLTINISM	KP	se	n
Andrenidae	Andrena	aliciae	Univoltine	42.75	0.03	2
		arabis	Univoltine	42.38	0.77	8
		atlantica	Univoltine	44.55	NA	1
		banksi	Univoltine	44.20	NA	1
		barbara	Univoltine	42.60	0.70	3
		carlini	Univoltine	42.82	0.30	4
		erigeniae	Univoltine	42.19	0.46	10
		fragilis	Univoltine	43.34	0.48	2
		illini	Univoltine	43.18	0.20	17
		imitatrix/morrisonella	Univoltine	43.60	0.14	22
		miserabilis	Univoltine	42.12	0.78	8
		nasonii	Univoltine	43.19	0.57	11
		nigrae	Univoltine	43.62	NA	1
		perplexa	Univoltine	42.28	0.43	3
		personata	Univoltine	42.62	0.65	12
		vicina	Univoltine	43.81	NA	1
	Calliopsis	andreniformis	Multivoltine	49.25	0.59	3
Apidae	Anthophora	abrupta	Univoltine	41.15	0.27	25
	Bombus	auricomus	Multivoltine	41.36	0.84	5
		bimaculatus	Multivoltine	40.90	0.49	17
		citrinus	Multivoltine	45.26	0.25	2
		griseocollis	Multivoltine	41.13	0.39	32
		impatiens	Multivoltine	43.36	0.15	169
		pensylvanicus	Multivoltine	40.92	0.79	8
		pensylvanicus/auricomus?	Multivoltine	45.06	NA	1
	Ceratina	calcarata	Multivoltine	46.46	0.20	70
		dupla	Multivoltine	46.67	0.94	3
		strenua	Multivoltine	48.27	0.37	11
	Habropoda	laboriosa	Univoltine	45.01	NA	1
	Melecta	pacifica	Univoltine	44.41	NA	1
	Melissodes	bimaculatus	Univoltine	44.63	0.33	53
		bimaculatus	Multivoltine	44.63	0.33	53
		comptoides	Univoltine	46.56	0.60	7
		denticulata	Univoltine	42.43	NA	1
		druriellus	Univoltine	44.28	NA	1
		trinodis	Univoltine	43.50	0.29	2
	Nomada	bidentate	Univoltine	43.06	NA	1
		cressonii	Univoltine	43.22	NA	1
		interesting	Univoltine	42.50	NA	1
		luteoloides	Univoltine	43.42	1.05	2
		pygmaea	Univoltine	43.55	0.38	8
	Peponapis	pruniosa	Univoltine	45.25	0.49	3
	Ptilothrix	bombiformis	Univoltine	46.65	0.28	30
	Svastra	obliqua	Univoltine	46.70	0.27	2
		petulca	Univoltine	46.45	NA	1
	Xenoglossa	strenua	Univoltine	44.55	1.37	2
	Xylocopa	virginica	Multivoltine	41.33	0.87	8
Colletidae	Colletes	inaequalis	Univoltine	42.94	0.24	8
		thoracicus	Univoltine	42.02	0.22	61
	Hylaeus	affinis/modestus	Multivoltine	44.69	0.62	12
		georgicus	Multivoltine	41.38	2.19	2
		leptocephalus	Multivoltine	46.09	0.29	42
		modestus	Multivoltine	43.35	1.32	4
Halictidae	Agapostemon	virescens	Multivoltine	43.32	0.19	56
	Augochlora	pura	Multivoltine	44.91	0.12	38
	Augochlorella	aurata	Multivoltine	43.77	0.46	17
	Halictus	confusus	Multivoltine	47.20	0.23	38
		ligatus/poeyi	Multivoltine	46.01	0.19	96
		parallelus	Multivoltine	42.92	NA	1
		rubicundus	Multivoltine	44.52	NA	1
	Lasioglossum	bruneri	Multivoltine	44.87	0.77	2
		callidum	Multivoltine	42.78	0.78	5
		cressonii	Multivoltine	44.34	NA	1
		fuscipenne	Multivoltine	41.65	0.48	3
		gotham	Multivoltine	43.04	NA	1
		hitchensi	Multivoltine	43.99	0.88	4
		illinoense	Multivoltine	43.09	0.40	8
		imitatum	Multivoltine	43.83	0.29	47
		lionotum	Multivoltine	44.93	NA	1
		near ethialtum	Multivoltine	43.66	0.80	2
		pilosum	Multivoltine	43.63	1.72	3
		subviridatum	Multivoltine	44.08	NA	1
		tegulare	Multivoltine	43.80	0.90	10
		trigeminum	Multivoltine	43.79	0.68	9
		weemsi	Multivoltine	41.97	2.77	2
		zephyrum	Multivoltine	43.28	0.41	28
	Sphecodes	antennaria	Univoltine	45.47	NA	1
		pimpinellae	Univoltine	44.67	NA	1
Megachilidae	Chelostoma	philadelphi	Univoltine	45.98	NA	1
	Coelioxys	modesta	Bivoltine	47.64	0.35	6
		sayi	Bivoltine	47.69	0.50	6
	Heriades	ceratina	Univoltine	48.50	NA	1
		leavitti/variolusus	Univoltine	48.86	NA	1
	Megachile	campanulae	Bivoltine	46.99	0.50	10
		concina	Bivoltine	47.93	0.51	18
		exilis	Bivoltine	46.90	0.24	22
		mendica	Bivoltine	47.22	0.39	21
		petulans	Bivoltine	46.24	0.71	3
		rotundata	Bivoltine	48.14	0.30	31
		texana	Bivoltine	46.31	1.68	2
		xylocopoides	Bivoltine	46.19	0.62	6
	Osmia	atriventris	Univoltine	47.83	0.52	5
		cornifrons	Univoltine	46.09	1.77	2
		georgica	Univoltine	45.38	NA	1
		lignaria	Univoltine	45.89	0.58	2
		pumila	Univoltine	44.60	2.49	3
		subfasciata	Univoltine	48.53	NA	1
		taurus	Univoltine	45.10	0.46	14

Table 2 Fatal knockdown points (FKP) from subset of multivoltine species across collection date (Linear regression: FKP~date collected)

Species	n	r^2	P-Value	Equation
Ceratina calcarata	70	0.12	**0.003****	FKP=0.0114*date-155.6
Bombus impatiens	169	0.07	**0.0003*****	FKP=0.0153*date-232.4
Lasioglossum imitatum	47	0.11	**0.024***	FKP=0.0131* date-187.7
Augochlora pura	37	0.10	**0.056.**	FKP=0.0053* date-48.47.6
Agapostemon virescens	56	0.15	**0.003****	FKP=0.0127* date-181.6
Halictus ligatus/poeyi	96	0.001	0.755	FKP=0.0012* date +17.336

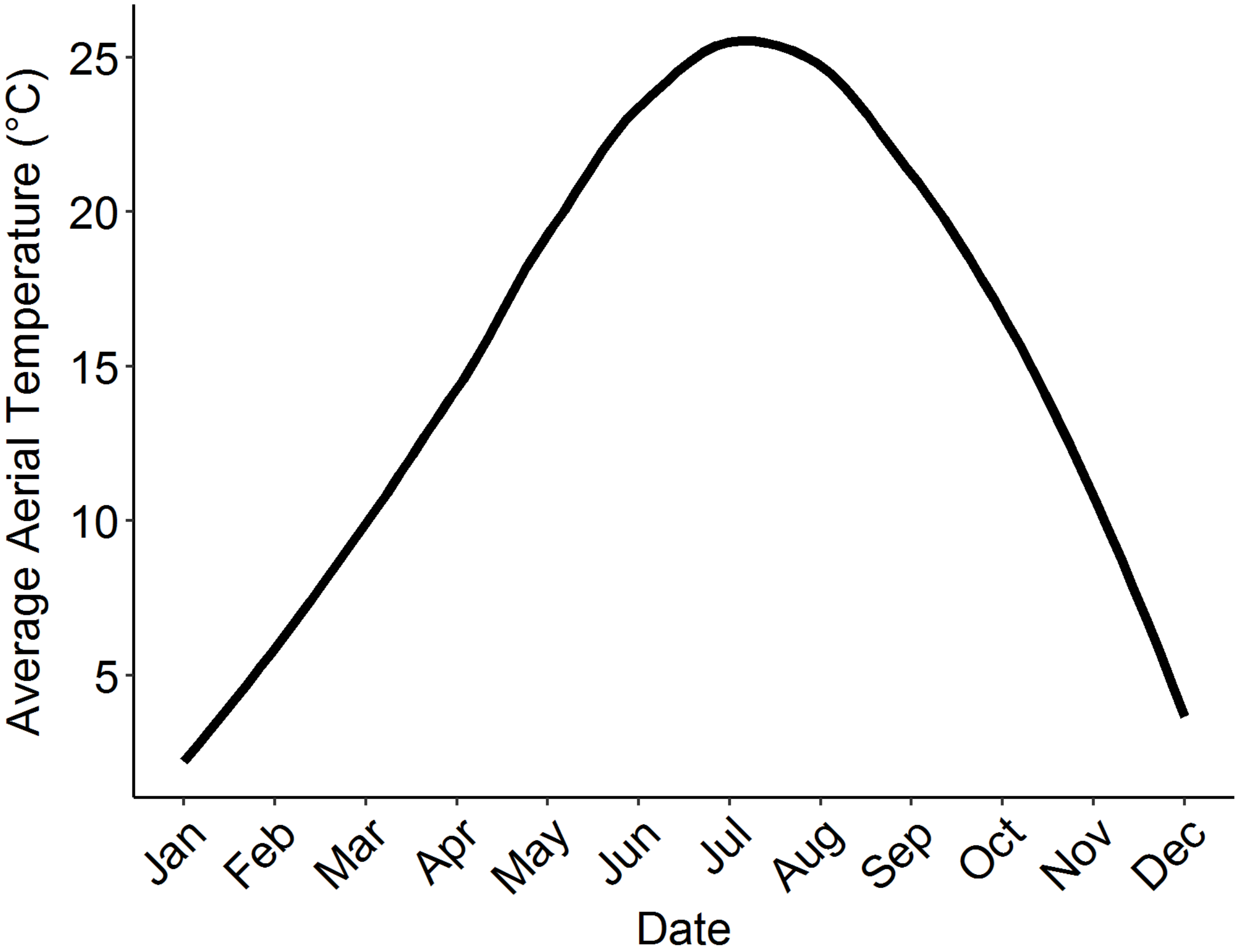

Figure 1 Mean monthly aerial temperatures for Richmond, VA study area across 118 years from NOAA GHCN-Daily database.

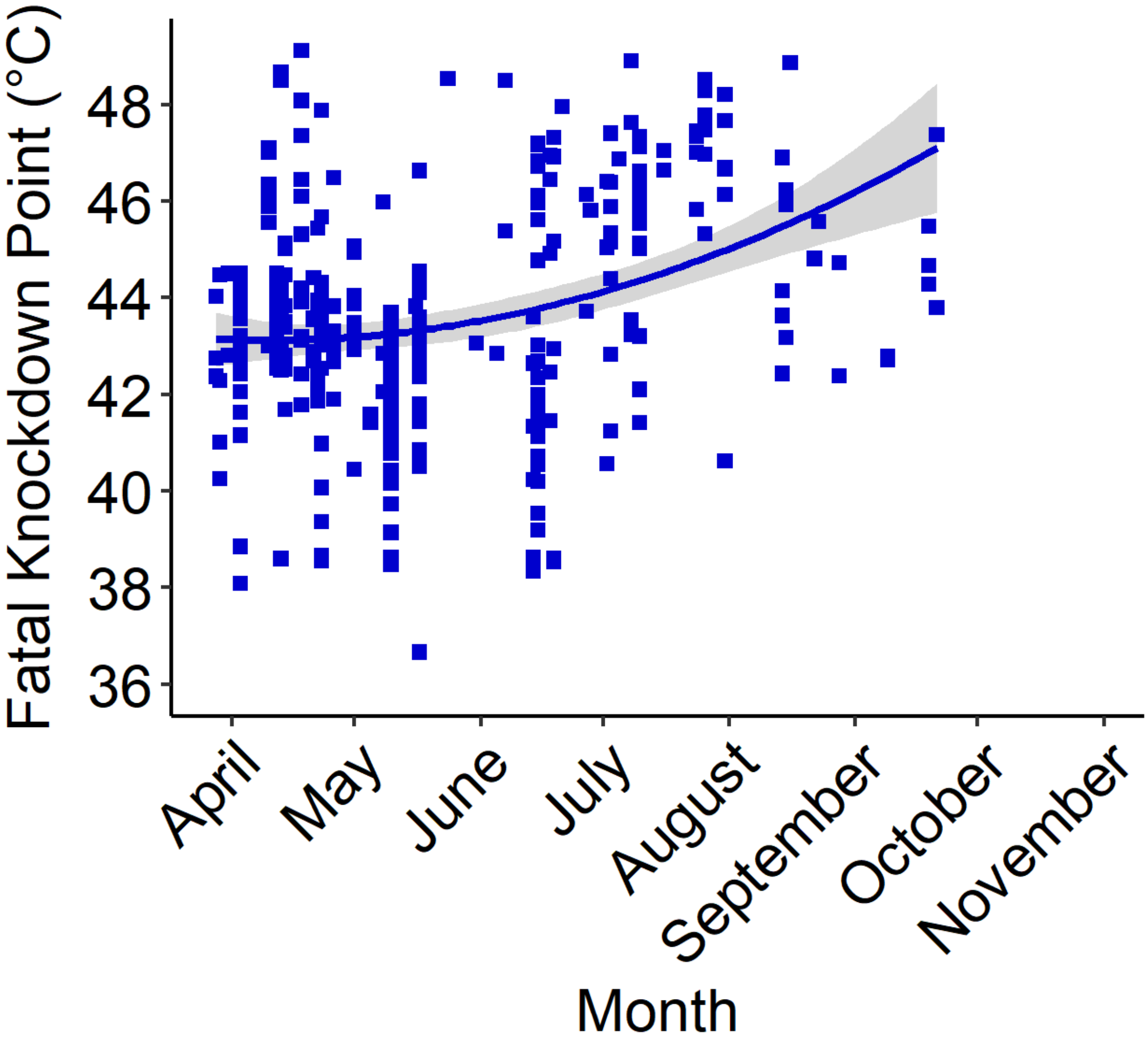

Figure 2 Fatal knockdown points across sampling season for all univoltine bees sampled. A significant quadratic increase was seen across the year across emergence groups (n=349; 15 genera, 48 species; P<0.0001). Note: Gray margins around quadratic line of best fit indicate standard error of slope.

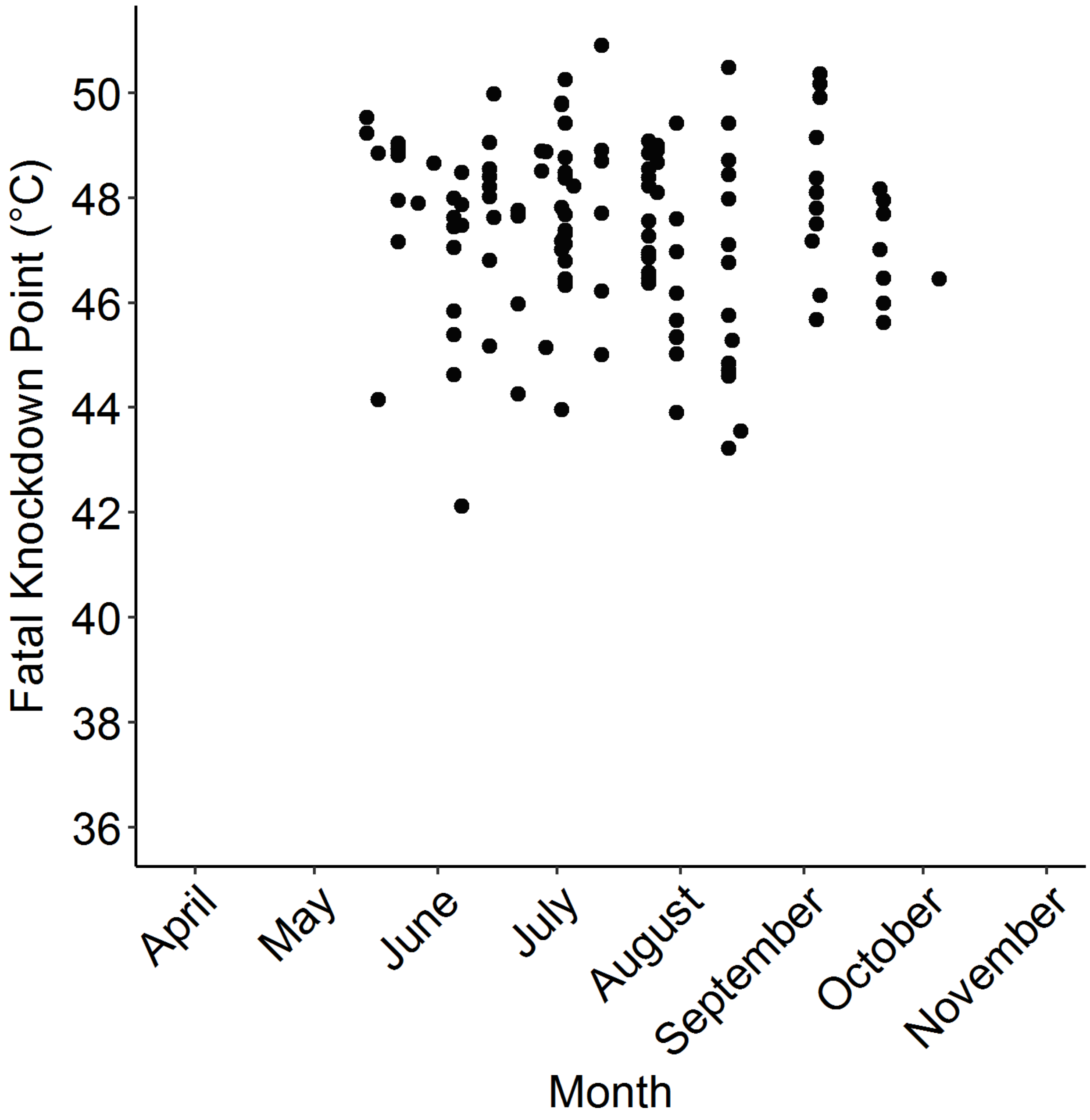

Figure 3 Fatal knockdown points across sampling season for all bivoltine bees sampled. Fatal knock down points did not change across the season (n = 125; 2 genera, 10 species; linear regression: P=0.33).

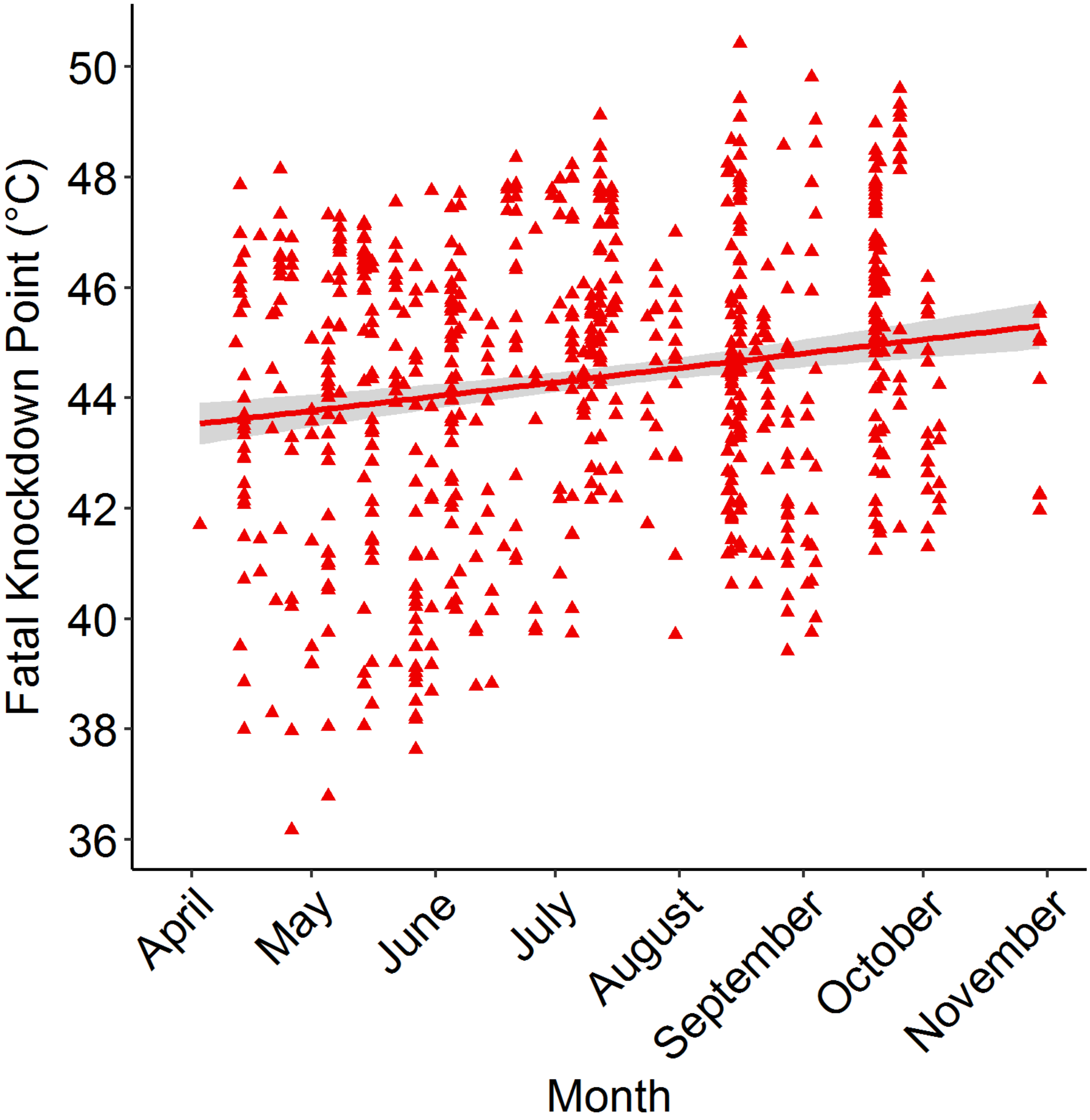

Figure 4 Fatal knockdown points across sampling season for all multivoltine bees sampled. A significant increase across the season was seen across generations (n=764; 11 genera, 40 species; P<0.0001). Note: Gray margin around line of best fit indicates standard error of slope.

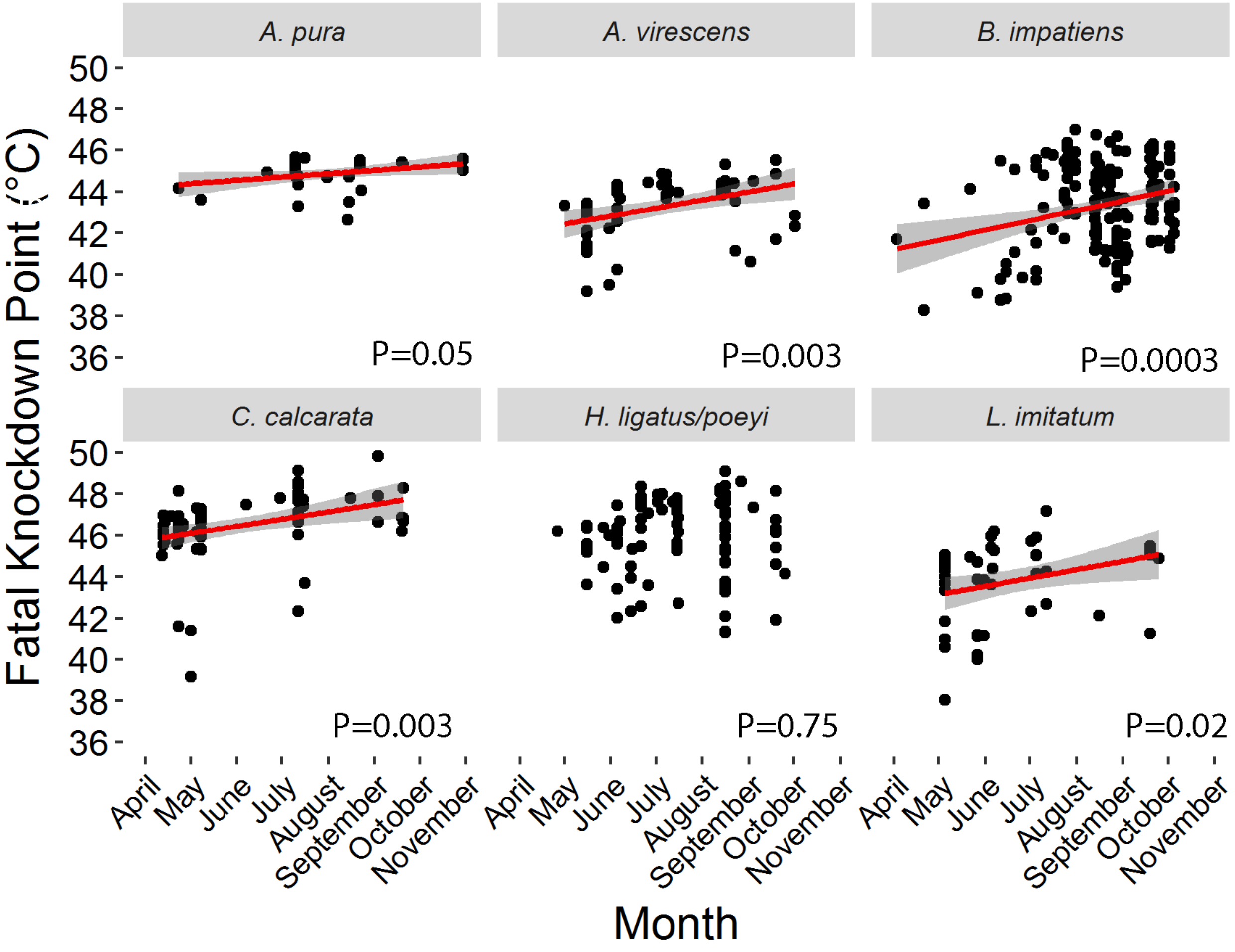

Figure 5 Fatal knockdown points of a subset of six multivoltine bees across the 2018 season. With the exception of *H. ligatus/poeyi*, all Fatal Knockdown Points increased over the season. Note: Gray margins around each best-fit line indicate standard error of slope.

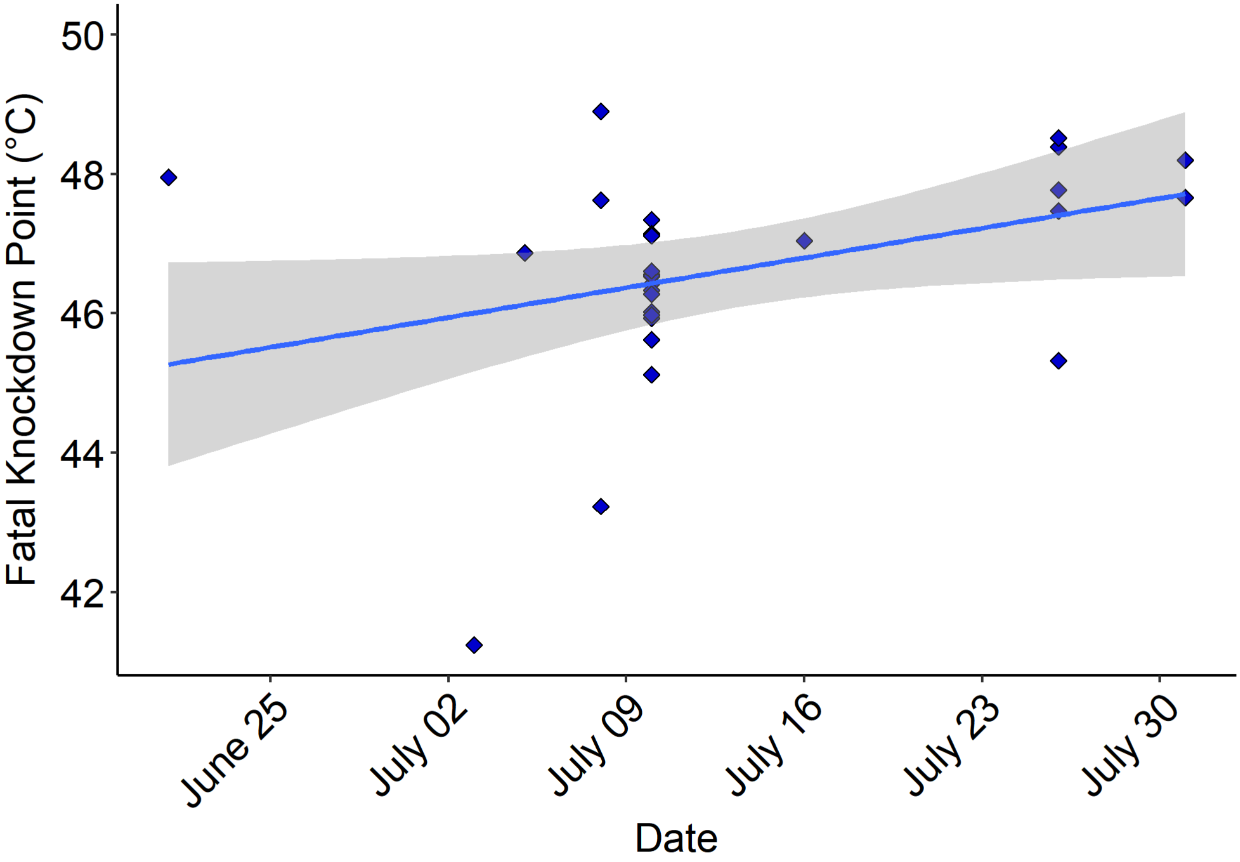

Figure 6 Fatal knockdown points of all sampled *Ptilothrix bombiformis* individuals collected across the 2018 season. A significant increase is seen across emergence period (n=30; P=0.045, r^2=0.10, FKP= 0.0611*date-103.7). Note: Gray margins around each best-fit line indicate standard error of slope.

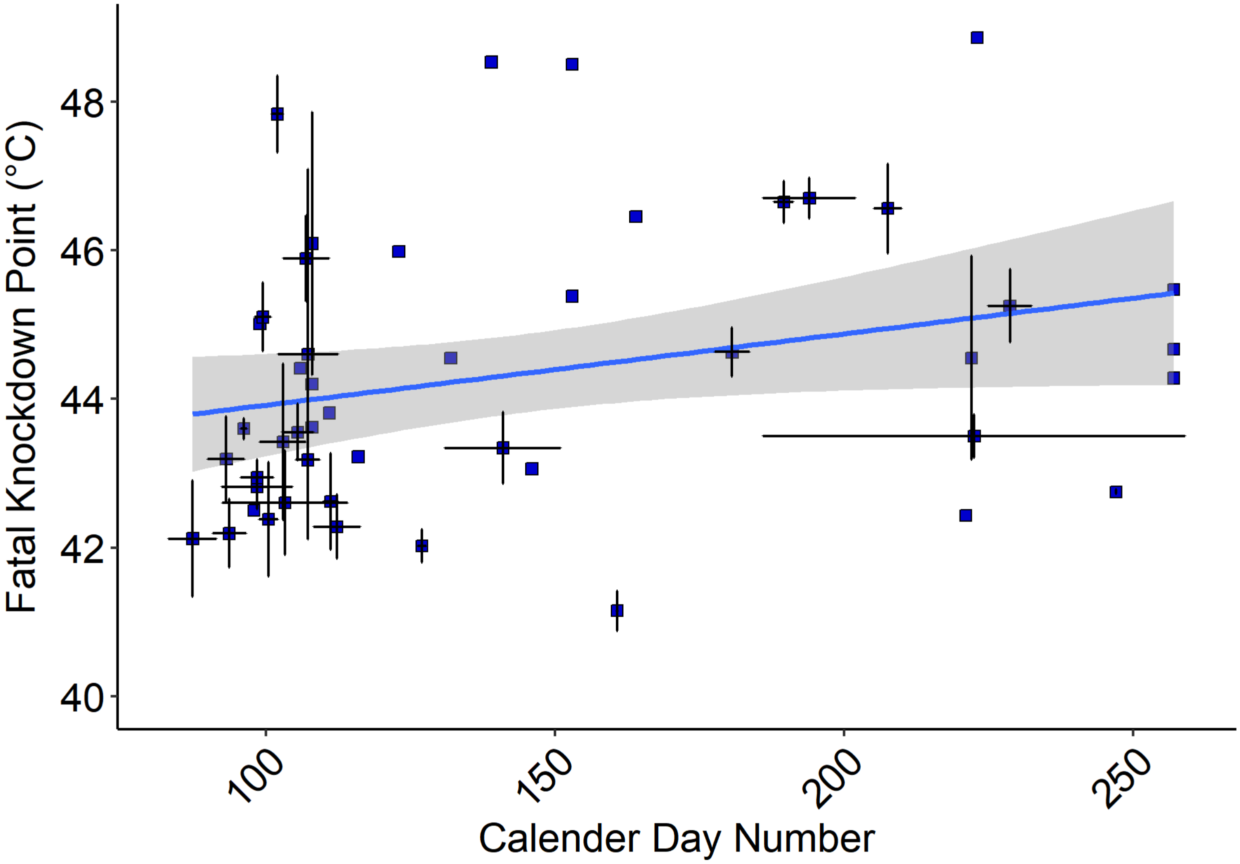

Figure 7 Average fatal knockdown points (FKP) of all univoltine species collected across the 2018 season. Note: Each point represents a specific species (n=349; 15 genera, 48 species; P=0.056). Horizontal lines indicate ± se in collection date. Vertical lines indicate ± se in FKP. Gray margins around each best-fit line indicate standard error of slope.

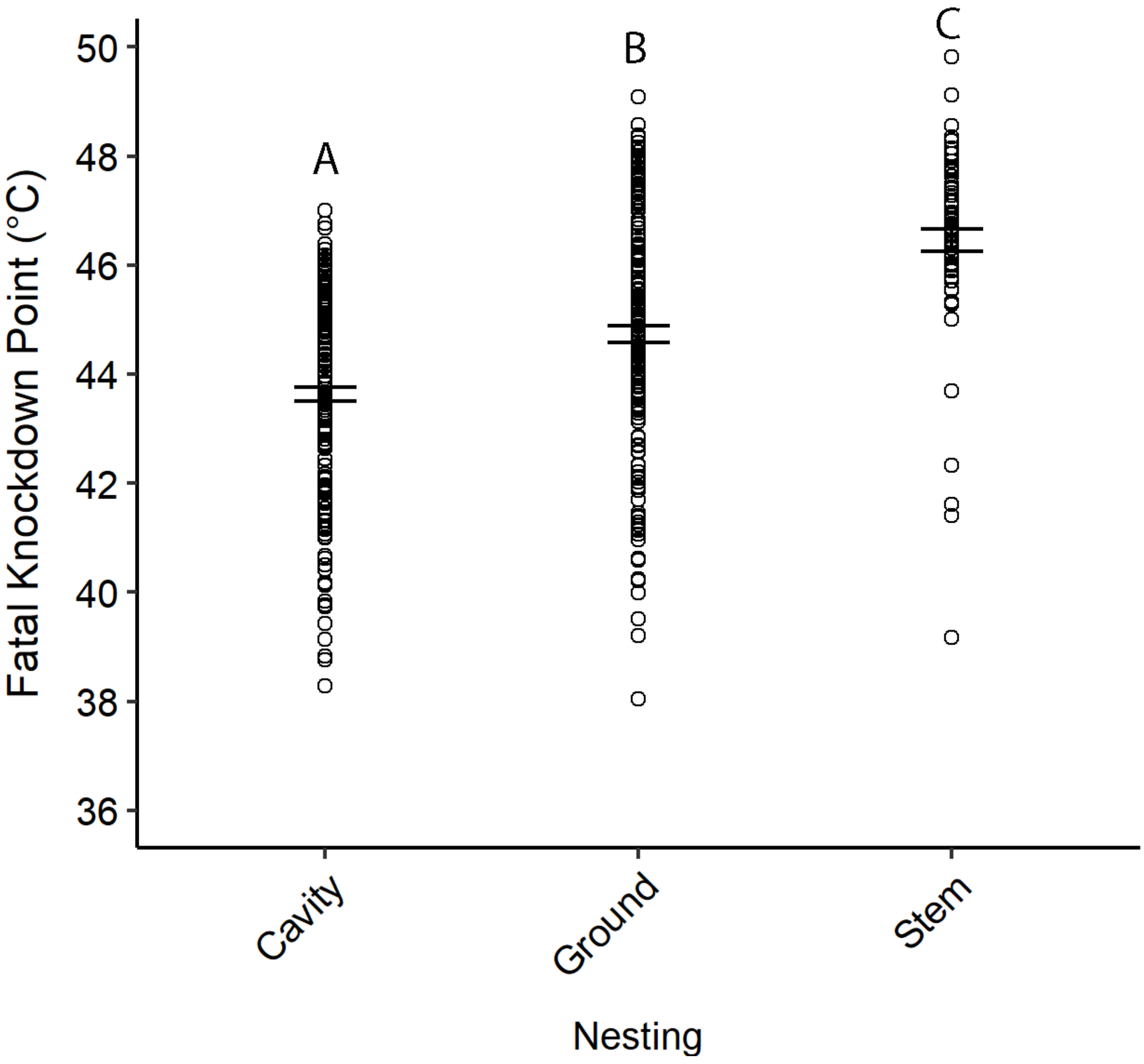

Figure 8 Fatal knockdown points of a subset of six species of multivoltine bees differ with respect to nesting type (cavity: 43.6 ± 0.13˚C, n=2; ground: 44.7 ± 0.15˚C , n=3; stem: 46.4 ± 0.19˚C, n=1). Note: whiskers are fitted mean ± se. Connecting letters report A-C indicates significantly different groups.

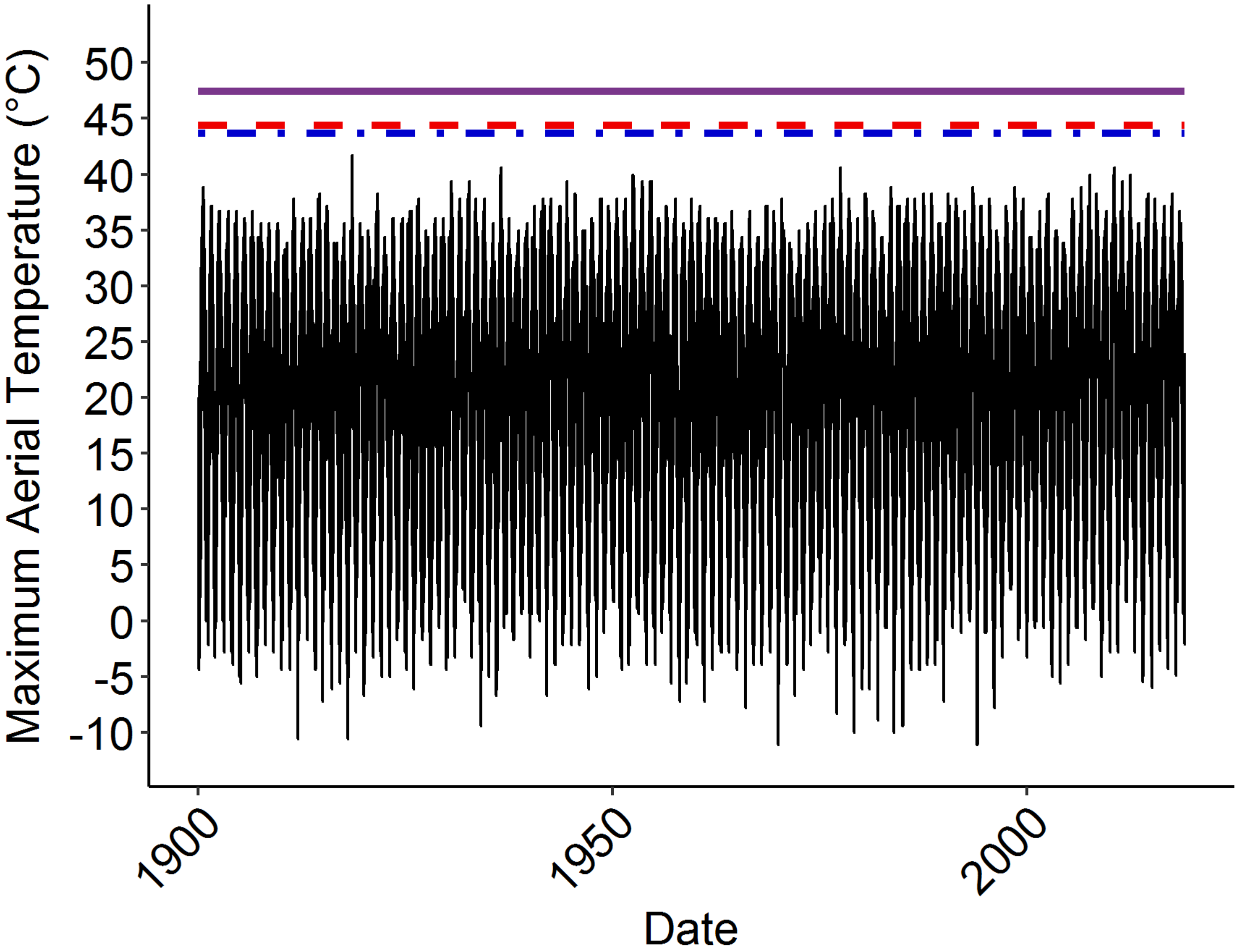

Figure 9 Maximum daily aerial temperatures for a subset of 118 years for the Richmond, VA study area. Red dashed line indicates average fatal knockdown point (FKP) of multivoltine bees (44.3 ˚C). The blue dot-dashed line indicates average FKP of univoltine bees (43.6 ˚C). The solid purple line indicates average FKP of bivoltine bees (47.4˚C). Weather data from NOAA GHCN-Daily (Menne et al. 2012).

APPENDIX

Comparison of UTL rates: 0.25°C min^{-1} vs 0.50°C min^{-1}

Experimental variation of ramp rate speeds in studies focusing on UTL has drastic variations in UTL temperatures (Terblanche et al 2007; Oyen & Dillon 2018). Yet, there is no comparison between 0.25°C min^{-1} and 0.50 °C min^{-1}, the most commonly used ramping rate observed in other upper thermal tolerance studies for wild bees (Oyen Giri & Dillon 2016; Hamblin et al 2017; Oyen & Dillon 2018; Burdine & McCurney 2019). However, As Oyen & Dillon (2016) have shown that not all ramp rates are significantly different.To permit direct comparison of UTLs reported in similar studies, a comparison of UTL for two of the most common ramp rates is necessary. To test this, a sample of *Colletes inaequalis* was collected at two nearby nesting sites in Richmond, VA over the period of one week using the aforementioned sampling schema. Individuals were randomly ramped either at 0.25°C min^{-1} or 0.50°C min^{-1} using the previously described dynamic ramping procedure. A two-sample t-test was used to test for differences in FKP between 0.25°C min^{-1} and 0.50°C min^{-1} ramp speeds for *C. inaequalis*. UTL in *C. inaequalis* ramped at 0.50 min^{-1} was a 1.8˚C higher compared to specimens ramped at 0.25 min^{-1} (44.9 ± 0.21˚C and 43.1 ± 0.14˚C respectively; df=75; T= 41.92; P=<0.0001). Therefore, studies utilizing a ramp rate of 0.50°C min^{-1} cannot be compared to studies utilizing a ramp rate of 0.25°C min^{-1}.

VITA

Kálmán (Kal/"Walkamolé!") Károly Csigi XIV was born January 25, 1994 in Nashua, NH. He grew up in the White Mountains of New Hampshire and graduated from White Mountain Regional High School in 2012 with Scholar's Honors. He graduated Summa Cum Laude from the University of Maine at Farmington in 2015 with a B.S in Environmental Science (Minor: Geology). While in college, he first discovered his joy for working with native pollinators while helping collect preliminary data for a citizen scientist project surveying *Bombus* species throughout Maine. He continued to work with this group for three more years along with aiding a local farm in managing their colonies of *Apis mellifera* as well as working several jobs in surficial geology before embarking on a northbound thru-hike of the Appalachian Trail in 2017. Upon completion, he joined the Insect Ecology and Behavior Lab at VCU in the fall of 2017. Kal plans on continuing a career working with pollinators as well as continuing to hike long-distance trails; in pursuit of the next trail marker.