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Journal

ISSN

Authors

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Publication Date

DOI

ORIGINAL PAPER

First field-based estimates of bumblebee diapause survival rates showcase high survivorship in the wild

Genevieve Pugesek[1,2] · Jessie A. Thuma[2] · Elizabeth E. Crone[3]

Abstract

Bumblebee (*Bombus* spp.) queens overwintered in artificial settings tend to have low survival rates, raising concerns that diapause may be a particularly sensitive life cycle stage for this ecologically and economically valuable group of pollinators. However, it remains unclear whether lab-based estimates of diapause survival are comparable to survival rates of natural populations. In this study, we monitored the survival of *Bombus impatiens* queens overwintering in the field in Ipswich, MA, and conducted a meta-analysis of studies that estimate queen diapause survival in the lab to compare our field-based estimates of survival to those of lab-based studies. We found that queen *B. impatiens* had relatively high rates of overwintering survival after about six months ($> 60\%$), especially when compared to estimates of six-month survival from lab studies ($< 10\%$). We also observed a trend that broadly corroborates many lab studies of bumblebees, in that overwinter survival of queens was related to colony origin. In addition to providing the first estimate of diapause survival for bumblebee queens in nature, our study emphasizes the need to verify patterns observed in the lab to field-based studies.

Implications for insect conservation Although protecting target species during sensitive life cycle stages is a fundamental goal of conservation ecology, it is first necessary to identify at what stages of the life cycle populations are most vulnerable. Our results suggest that, at least in some study systems, diapause survival of queen bumblebees in the field may be higher than suggested by lab studies.

Keywords *Bombus* · Bumblebee · Diapause · Overwinter · Survival · Vital rates

Introduction

Diapause is a strategy that many species of insects rely upon to avoid adverse environmental conditions. For example, in temperate regions, insects typically enter diapause (a period of arrested development) to better cope with freezing temperatures (Denlinger 1986; Gill et al. 2017). Although bypassing environmental stressors via dormancy has obvious fitness benefits, diapause can still be

an energetically and physiologically taxing life cycle stage (Hahn and Denlinger 2011; Sinclair 2015). Because they are unable to replenish energy stores, insects face potential damage from cold shock, desiccation, and depletion of fat reserves during diapause. These kinds of damage can result in reduced survival and post-diapause fitness (Hahn and Denlinger 2007). There is concern that some insect species may be particularly vulnerable to mortality during diapause, and that low overwintering survival rates may lead to "demographic bottlenecks" (cf. Treanore and Amsalem 2020), where conditions experienced during the winter disproportionately impact population dynamics. Though many species of insects spend most of their lifespan in diapause (Gill et al. 2017), it is often unclear how sensitive natural populations are to overwintering mortality. To help address this knowledge gap, we conducted a field-based study to estimate diapause survival of queen bumblebees (*Bombus impatiens*).

Many studies that address insect diapause are carried out entirely in the lab, often with the goal of characterizing

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the physiological mechanisms that underpin survival (Leather et al. 1993). Researchers may rely on lab-based methodologies, at least in part, because studying the ecology of overwintering insects in the field can be incredibly challenging. Many insect species spend the winter buried in the soil or under leaf litter or woody debris, and are thus difficult to locate and monitor. Monitoring vital rates in the lab also allows researchers to better control sources of environmental variation. However, the conditions experienced by animals in artificial settings are not always ecologically realistic. For this reason, it is often unclear if discoveries about dormancy from lab-based studies can be extrapolated to natural populations (c.f., Woodard et al. 2019).

Perhaps unsurprisingly, lab-based methodologies also play an essential role in studies of the biology of hibernating bumblebees. Bumblebees are some of the most important native pollinators of crops in the United States (Kleijn et al. 2015), and recently there has been great interest in understanding their biology throughout the life cycle (Liczner and Colla 2019; Williams et al. 2019a). Multiple studies (> 10) have monitored diapausing bumblebees in the lab, relating survival rates to environmental factors like temperature or relative humidity (Gretenkord and Drescher 1997; Yoon et al. 2013; Lindsay 2020) or to intrinsic physiological factors like body mass (Woodard et al. 2019; Treanore and Amsalem 2020). Many of the studies that target this lifecycle stage are focused on improving methods of husbandry or commercial rearing (Gretenkord and Drescher 1997; Beekman et al. 1998; Yoon et al. 2013; Lindsay 2020). Conditions experienced by queens in the lab are not always ecologically realistic (e.g., Beekman et al. 1998). For example, researchers may monitor overwintering queens for only 1–4 months (Woodard et al. 2019), even though bumblebee queens in temperate regions generally spend 6–9 months in diapause (Alford 1969; Pouvreau 1970; but c.f., Stelzer et al. 2010). From lab-based studies, we know that maintaining queen bumblebees during diapause can pose a significant hurdle in rearing captive populations in the lab (Beekman et al. 1998; Gosterit and Gurel 2009). However, we do not know whether low rates of diapause survival observed in lab studies are typical in nature (c.f. Woodard et al. 2019).

In this study, we estimate diapause survival rates of queen bumblebees (*B. impatiens*) in the field by monitoring queens overwintering in large aggregations. To compare our estimates of diapause survival to those produced by lab-based studies, we conducted a meta-analysis of past research, using Web of Science and OATD (Open Access Theses and Dissertations) to build a data set using 32 different studies of bumblebee survival in laboratory conditions. In addition to providing general information regarding the locations of overwintering *B. impatiens* queens, our study is the first to estimate overwintering mortality of natural bumblebee populations (see Szabo and Pengelly 1973 for the most closely related past study).

Methods

Study species

Bombus impatiens is one of the most common species of bumblebee in the Northeastern United States (Richardson et al. 2019), including in our study region (Ipwich, MA, USA). The primary reason we chose to work with *B. impatiens* is because the overwintering ecology of this species is well suited to field-based monitoring. Most bumblebees overwinter below the ground (Alford 1969), and like most other social Hymenoptera, overwinter solitarily (Leather et al. 1993). However, there are a handful of social bee species (including *B. impatiens*) that overwinter in large aggregations in the soil around natal nest sites (Plath 1927; Sakagami et al. 1984). Thus, although locating queen bumblebees in sufficient numbers has posed a major hurdle to studying diapause for other bumblebee species in the field (Williams et al. 2019a), it is feasible to monitor large numbers of overwintering queens in *B. impatiens* as long as nest sites can be found. *B. impatiens* is also one of the only species of bee native to the United States that is reared commercially. In the Northeast, commercial *B. impatiens* colonies are used by farmers to enhance pollination of greenhouse tomatoes (*Solanum lycopersicum*) as well as a number of other crops, including blueberries (Stubbs and Drummond 2001) and pumpkin (Artz and Nault 2011). As commercial colonies are also available to researchers, *B. impatiens* has been used in research targeting diapause (Lindsay 2020; Treanore and Amsalem 2020), allowing us to compare field based studies to lab studies using the same species.

Study area

Field work was performed at Appleton Farms (42°38′52.09"N, 70°51′1.01"W) and Appleton Grassrides (42°38′33.26"N, 70°51′57.12"W), two adjacent properties managed by the Trustees of Reservations, a non-profit land stewardship organization (for a detailed description of these study sites, see Pugesek and Crone 2021). Both properties are located in Essex County, Massachusetts. According to the Köppen–Geiger climate classification climate system, our study area falls within a warm-summer humid continental climate (Bryant et al. 2017), with the coldest month of the year averaging below − 3 °C, all months with average temperatures below 22 °C, and at least four months averaging above 10 °C. In both years of our study, average monthly minimum and maximum air temperatures were comparable (Table 1).

Table 1 Average monthly minimum and maximum temperatures (°C), from NOAA National Centers for Environmental information, Climate at a Glance: County Mapping (NOAA National Centers for Environmental information 2023)

Year	Month	Average	Average minimum	Average maximum
2019–2020	Oct	11.3	6.6	16.1
	Nov	3.2	− 1.7	8.2
	Dec	− 0.3	-4.9	4.2
	Jan	0.3	− 4.3	5.1
	Feb	0.3	− 4.3°	5.0
	Mar	5.0	− 0.3	10.3
2020–2021	Oct	11.6	6.5	16.7
	Nov	7.4	1.7°	13.2
	Dec	1.0	− 3.5	5.6
	Jan	− 1.3	− 5.4°	2.7
	Feb	− 2.7	− 7.0	1.6
	Mar	3.8	− 2.3	9.9

Locating overwintering queens

In the fall of 2019 and 2020, we performed systematic searches around the entrances of *B. impatiens* nests to look for evidence of overwintering queens (e.g., queens actively excavating overwintering sites or patches of soil disturbed by queens; Fig. 1a, b). We searched for evidence of overwintering queens around four nest sites: three nests were located in 2019 during other field work (Pugesek and Crone 2021) and one nest was located in 2020 during a meandering search for bumblebee nests at Appleton Farms and Grassrides. Although we did not confirm that these queens were genetically related, we assume that each aggregation largely consisted of siblings. All nests monitored for overwinter survival were located in woodlands or forests. Although we have observed queens excavating hibernacula around nest entrances in woodlands, forests, and grasslands (Pugesek and Crone 2021; G. Pugesek pers. obs.), we did not search for overwintering queens around nest sites in grasslands because it was far more difficult to find evidence of hibernacula under dense grassland vegetation than in leaf litter.

From the first week of September until the end of October, we visited nest sites once per week to mark the locations of overwintering queens (visits ceased when colonies expired). During each visit, we searched 7–9 quadrats (each 16 m²) around each nest site for evidence of *B. impatiens* hibernacula (Fig. 1a, b). Quadrats were laid out at varying distances from nest site entrances, with the furthest 10 m from the colony entrance (most queens were found within two meters of nest entrances [see Table 2]). The same
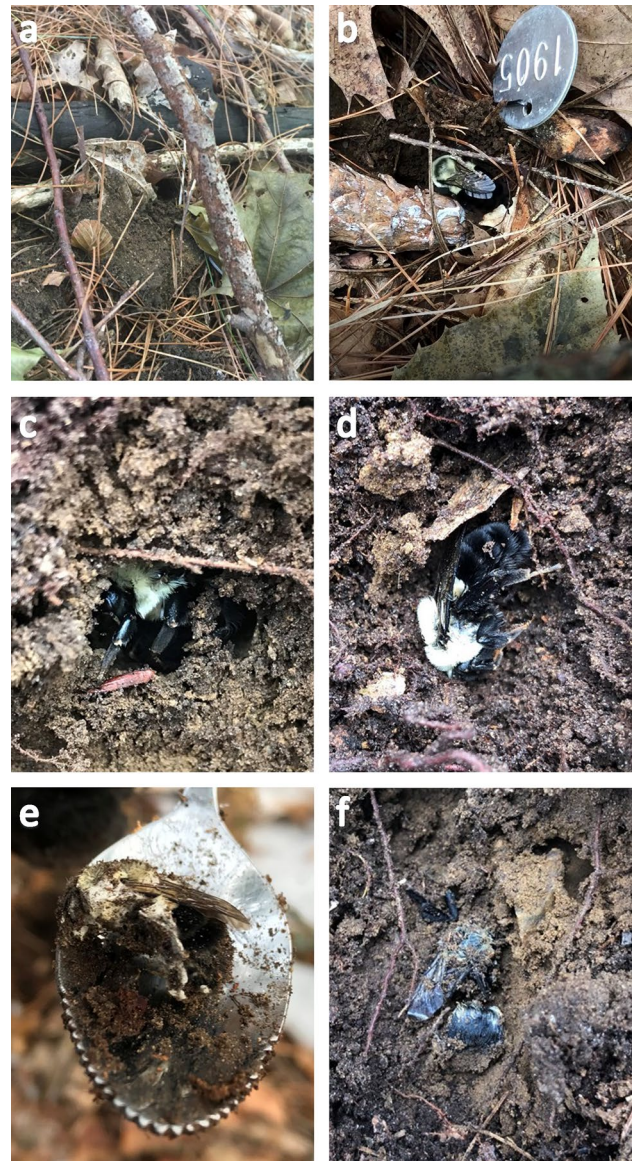

Fig. 1 a Soil displaced by an overwintering queen (*B. impatiens*) excavating her hibernaculum, **b** a queen excavating her hibernaculum, **c** a live queen within her hibernaculum, **d** a live queen with a pristine coat, **e** the remains of a dead queen recovered in the fall, and **f** the remains of a dead queen recovered in the spring

Table 2 The number of hibernacula located and excavated at each aggregation

Aggregation ID	Year	Number of sites found	Number of sites excavated by researchers	Median distance of sites from nest entrances (m)
1	2019	92	36	1.42
2	2019	103	38	1.44
3	2019	101	33	1.53
4	2020	39	34	1.45

quadrats were searched each week for approximately 10 min per visit. All potential overwintering sites were marked with uniquely numbered aluminum tags.

Recovering overwintering queens

To confirm the presence of overwintering queens in the soil, we excavated 141 marked overwintering sites in late November and early December across the four nesting aggregations; approximately 35 overwintering sites, selected randomly, were excavated from each aggregation (Table 2). To excavate queens, we gently dug around the marked entrance of overwintering sites. Many queens were found just a few centimeters below the surface, in distinct hibernacula (Fig. 1c). However, if queens were not immediately located, we dug approximately 10 cm below the soil surface before ceasing our search.

Differentiating between live and dead queens was fairly straightforward. Live queens had pristine coats (Fig. 1c, d) and became active upon disturbance, whereas dead queens were often partially decayed or covered in mold (Fig. 1e). We measured the depth at which each queen had been buried, and gently dislodged queens from their overwintering positions using either a bristled brush or a digging utensil. Live queens were immediately placed on ice to prevent complete disruption of overwintering. We measured the intertegular span (i.e., the distance between wing joints) of all live queens using digital calipers, and marked each queen with a uniquely numbered, fluorescent plastic tag (queen bee marking kit, Better Bee), after which we reburied queens in their original positions and replaced the numbered metal plant tag in the soil above each queen.

In order to ensure bees were collected before they normally emerge, we re-excavated queens in late March (March 24th, 2020, and March 21st, 2021), 3–4 weeks before *B. impatiens* queens are normally observed nest-searching in this study system. Ideally, we would have liked to collect queens at the beginning of April, 1–2 weeks before *B. impatiens* queens are normally observed; however, the timing of field work in 2020 had to be shifted due to the onset of Covid-19 pandemic restrictions to field work, and we chose to use the same timing in 2021. In the spring, we used similar methods to determine whether queens had survived over the winter, though dead queens were far more degraded in the spring (Fig. 1f) than they had been in the fall (Fig. 1e).

As queens began overwintering sometime between August and mid-October, there was some variability in the amount of time queens had spent overwintering. However, most queens had been overwintering for about two months when they were checked for the first time and for 6–7 months when they were checked for the second time. For simplicity, we will refer to bee fates at these time points as survival after "two" and "six" months.

Statistical analysis of field data

We estimated diapause survival rates using generalized linear models (binomial family, logit link). Statistical analyses were performed in R, version 4.0.2 (R Core Team 2019). Survival rates were estimated at two time points (after two and six months). At two months, survival was coded based on whether the queen was found live (=1) or dead (=0); we assumed that the site had been abandoned if no queen was recovered when the overwintering site was excavated, as queens may desert burrows if an obstruction is encountered while digging (Alford 1969). At six months, we analyzed survival in two ways. Our first analysis used only known-fate individuals, i.e., we coded survival as whether or not the queen was found live in the spring (=1) or dead either during the fall or the spring (=0); queens that were not recovered were treated as missing data. Our second analysis included all individuals, i.e., survival was coded as surviving (=1) if found live in spring or as dead (=0) if the queen was not recovered in the spring or was found dead in either the spring or fall. This second way of coding the data leads to a lower estimate of survival and was of interest due to the wide difference between field and lab estimates of survival (see *Results*). To estimate average survival, we fit an intercept-only model to each of the three response variables: survival to two months, survival to six months (known-fate individuals only) and survival to six months (all individuals). We tested whether survival differed among aggregations by fitting a model with aggregation ID included as a categorical predictor variable to each of the three response variables. We evaluated statistical significance using Wald chi-square tests implemented with the *Anova*() function in the package *car* (Fox and Weisberg 2019).

We tested if queen body condition (i.e., queen IT span and queen weight) and hibernaculum depth differed across aggregations using linear models. Linear models fit to each response variable (IT span, weight, hibernaculum depth) included only aggregation ID as a predictor. Similarly, we tested if rates of abandonment of hibernaculum differed across aggregations using a univariate generalized linear model (binomial distribution). We coded aggregation ID as a predictor variable and the presence/absence of queens during the first excavation of hibernacula in the fall as the response. We evaluated statistical significance using Wald chi-square tests implemented with the *Anova*() function in the package *car* (Fox and Weisberg 2019).

Data extraction and meta-analysis of published data

To obtain estimates of bumblebee overwintering survival from other studies, we conducted a systematic literature search on January 26th, 2022, using Web of Science and Open Access Theses and Dissertations. A total of 73 research articles and 2 theses/dissertations were obtained using the search terms: (Bombus OR "bumble bee" OR bumblebee) AND (overwinter* OR diapause* OR hibernat* OR dorman*) AND (survival). Of these, we retained 32 studies that provided direct estimates of bumblebee overwintering survival (30 research papers, 1 dissertation, and 1 thesis). We scanned the introduction and discussions of each of these retained manuscripts for additional relevant studies, locating an additional 9 papers. We excluded 6 of the studies we obtained that were not published in English. A few authors presented the same data sets in multiple publications; 2 studies were excluded as duplicate data. A final study was excluded because queen survival rates could not be calculated from the data as presented. Thus, a total of 32 studies were included in our literature review, nearly all of which were conducted in the lab.

Nearly all of the studies included in our meta-analysis tested several different diapause regimes (e.g., different temperatures, levels of humidity) and reported queen survival rates at monthly intervals. Thus, for each set of experimental conditions reported by each study, we recorded 1) the proportion of queens to survive, 2) the sample size, 3) the length of the diapause regime, 4) the species, mating status, origin (lab-reared, obtained from a commercial supplier), and approximate age of queens used in each study, 5) the temperature and relative humidity that the queens were exposed to during diapause, and 6) any other details related to the experimental design (for example, if queens were fed pesticides or exposed to infectious agents prior to treatment). We assumed that all queens were alive at the start of each experiment; thus, we recorded survival rates of 100% for each group of queens at month 0. For papers that did not report monthly survival estimates directly in the text of the manuscript, we used the *digitize* package in R (version 4.0.2) to extract data from figures. For studies that monitored diapausing queens continually and reported the length of time queens survived rather than monthly survival rates (Lindsay 2020; Treanore and Amsalem 2020), we estimated monthly survival rates manually. Prior to statistical analysis, we converted survival rates recorded from manuscripts to a binomial data set of the number of successes (survival, $=1$) and failures (death, $=0$) from sample sizes and percent survival at each time interval.

Many of the studies included in this literature review performed treatments on queens that we thought might lead to lowered rates of survival: inoculating queens with parasites, exposing queens to chemicals, starving queens, etc. We excluded all data from treatments we deemed obviously harmful, and included only measurements for queens that were overwintered in continuous darkness at constant temperatures between 1 and 5 °C (see AppendixS1, Table 2 for further detail). Queens from excluded treatments had lower survival than those included in our meta-analysis (Appendix S1, Fig. S1).

We used a generalized linear mixed-effects model (binomial family, logit-link), with queen survival coded as the response to estimate diapause mortality rates. GLMMs were fit using the command *glmer*() in R package *lme4* (Bates et al. 2015). Our model included diapause interval as a fixed effect and random slopes for both study species and paper ID (random intercepts were not included in our model, as survival is equal to 1 at the start of each study). As a basic check of model fit, we used a linear model to compare the observed and the predicted values, and estimated confidence intervals for the slope and the intercept of this model. (If the original model is a good fit for the data, the linear model fit to the predicted values should have a slope of ~ 1 and intercept of ~ 0.)

Results

Field study

Over the course of our study, we unearthed 111 diapausing queens from 141 potential overwintering sites (Table 1). Most of these queens survived the winter. At two months, $93.7 \pm 2.3\%$ of recovered queens were alive (Fig. 2; all estimates of survival rates reported with $\pm$ SE). Estimates of survival at two months did not differ between aggregations (binomial GLM, $\chi^2 = 5.16$, df = 3, P = 0.160), ranging between 88.8% and 100.0% (Fig. 3). At six months, $72.1 \pm 4.8\%$ of queens recovered were alive (though we failed to recover 12.2% of queens tagged in the fall). Thus, even by our more conservative measure, $63.9 \pm 4.8\%$ of queens survived the six-month diapause period (Fig. 3). Estimates of mortality at six months differed among aggregations; the effect of aggregation ID was significant for both the model fit to only known-fate queens (binomial GLM, $\chi^2 = 11.32$, df = 3, P = 0.010), and the more conservative model fit using all queens (binomial GLM, $\chi^2 = 12.74$, df = 3, P = 0.005). In the known-fate analysis, queens from aggregation 3 had the highest survival (90.5%), followed by aggregation 2 (82.1%), 4 (55.5%), and 1 (52.9%). In the model with all queens, queens from aggregation 3 had the highest survival (82.6%), followed by aggregation 2 (76.7%), 4 (50.0%), and 1 (40.9%).
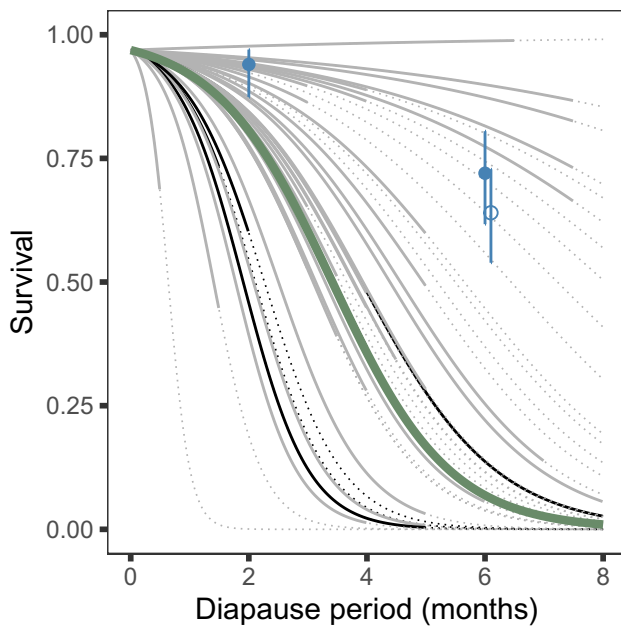
Fig. 2 Queen diapause survival as a function of time. Predictions generated from lab-based studies of queen diapause survival are represented by lines: the dark green line represents average estimates of diapause survival and each black or gray line represents the predicted estimate of diapause survival for a single study (black lines represent studies that used *B. impatiens*, whereas gray lines represent studies that used a different *Bombus* species). Estimates of survival from our field study are represented by dark blue points generated from pooled data (error bars represent 95% confidence intervals). The shape of each point indicates how survival was calculated (unfilled circles = queens that were not recovered during field work were included in estimates of mortality; filled circles = queens that were not recovered were treated as missing data)
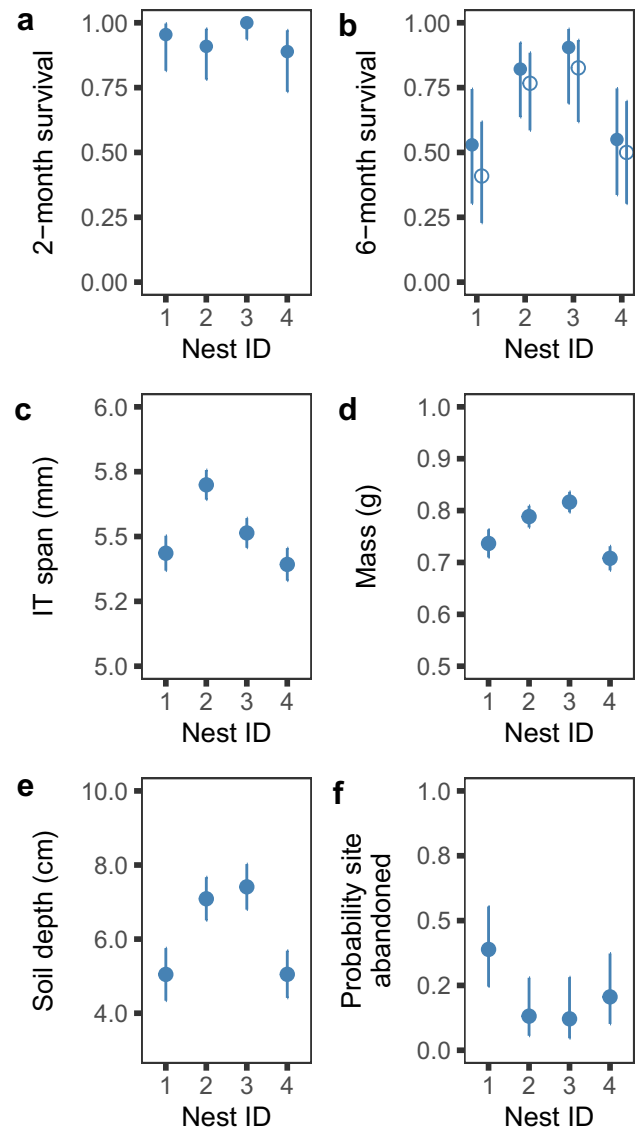

Fig. 3 Aggregation-specific estimates of **a** 2-month and **b** 6-month survival rates, **c** the IT span and **d** weight of recovered queens, **e** the depth at which queens were buried in the soil, and **f** the probability hibernacula were abandoned before being fully excavated. Error bars represent 95% confidence intervals, calculated using likelihood profiling (2-month survival rates) or the emmeans package in R (all else). For estimates of 6-month survival rates, the shape of each point indicates how survival was calculated (unfilled circles = queens that were not recovered during field work were included in estimates of mortality; filled circles = queens that were not recovered were treated as missing data)

There were differences in queen body size across aggregations (Fig. 3). Aggregation ID was a significant predictor of both IT span (LM; $F = 20.4$, $df = 3$, 99; $P < 0.0001$) and body mass (LM; $F = 19.6$, $df = 3$, 84; $P < 0.0001$). Rank orders of aggregations were similar for both metrics. Aggregation 2 produced the largest queens (IT span = 5.70 ± 0.03 mm), followed by aggregation 3 (5.51 ± 0.03 mm), aggregation 1 (5.44 ± 0.03 mm), and aggregation 4 (5.39 ± 0.03 mm). Aggregation 3 produced the heaviest queens (mass = 0.82 ± 0.01 g), followed by aggregation 2 (0.79 ± 0.01 g), aggregation 1 (0.74 ± 0.01 g), and aggregation 4 (0.71 ± 0.01 g).

There were also differences in the depth of hibernaculum and the probability hibernaculum were abandoned across aggregations (Fig. 3). Aggregation ID was a significant predictor of depth ($F = 15.83$; $df = 3$, 102; $p < 0.0001$). Queens from aggregation 3 overwintered deepest in the soil (7.3 ± 0.3 cm), followed by queens from aggregation 2 (7.1 ± 0.3 cm), aggregation 4 (5.1 ± 0.3 cm)

and aggregation 1 (5.1 ± 0.4 cm). The probability that queens abandoned initiated hibernacula (i.e., no queen was found when the hibernacula was initially excavated) also varied across aggregations (binomial GLM, $\chi^2 = 9.3$, $df = 3$, $P = 0.025$). The probability of abandonment was highest for aggregation 1 (0.39 ± 0.08), followed by

aggregation 4 (0.21 ± 0.07), aggregation 2 (0.13 ± 0.05), and aggregation 3 (0.12 ± 0.06).

Meta-analysis

Diapause survival rates for eight species of bumblebee (*B. terrestris, B. lapidarius, B. impatiens, B. huntii, B. vosnesenskii, B. fervidus, B. lucorum, B. ignitus*) were extracted from the literature. *B. terrestris* was the most commonly studied species; 23 of the 32 studies from our systematic literature search used this species (Appendix S1, Table S1). The model used to predict survival as a function of diapause period seemed to be a good fit for the data. The intercept of the linear model fit to predicted values approximated 0 (0.009, 95% CI: -0.026, 0.045), and slope of the line approximated 1 (0.978, 95% CI: 0.933, 1.023).

On average, queen survival after two months was 80.7%, but dropped to 7.6% after six months (Fig. 2). Predictions varied considerably across studies (Fig. 2). Of the random effects included in the model, the random intercept of paper ID had the largest variance (0.926, SD = 0.962). In comparison, variance for species random effects was 0.032 (SD = 0.179). Note that standard deviations are calculated on a logit-scale and are therefore not comparable to survival on a back-transformed scale.

Discussion

Diapause is an important and often under-studied feature of insect populations. Our study suggests that estimates of bumblebee survival during diapause in the lab are lower than survival in the wild. Specifically, past laboratory studies of bumblebee diapause indicate that many queens are unable to survive the natural length of diapause (< 10% survival after 6 months). In contrast, we observed that more than 60% of *B. impatiens* queens monitored in the field survived a 6-month period of diapause. This estimate is higher than predicted for any laboratory study of *B. impatiens*, as well as most studies of other bumblebee species. There were a handful of studies that achieved high rates of survival after 6 months (Holm 1960, 1972; Milliron 1967), all of which obtained queens from field colonies. Unlike the other lab-based studies included in our review, Holm (1960) and (1972) allowed *B. terrestris* and *B. lapidarius* queens to dig themselves into containers of soil and other substrate placed in an outdoor greenhouse. Similarly, Milliron (1967) placed *B. fervidus* queens in large containers of heartwood, providing an opportunity for some queens to bury themselves in the substrate. Our field estimates of survival are also comparable to those of Pouvreau (1970), who used methods similar to Holm (1972) and reported similarly high survival rates (68–72%) for queens (*B. agrorum, B. hypnorum, B.*

lapidarius, B. pratorum, and *B. terrestris*) after the natural length of diapause. In combination with the findings of overwintering studies under semi-natural conditions, the results of our field study suggest that surviving diapause may be less of an ecological hurdle for queen bumblebees than previously indicated.

In many ways, it is surprising that we observed higher rates of diapause survival for overwintering queens in the field compared to the lab. Among other insect species, diapause can be a period of high risk (Hayes 1981). In temperate regions, bumblebees must cope with less-than-ideal temperatures in the winter; some queens may encounter additional environmental challenges during hibernation, including pesticide exposure (Rondeau et al. 2022) and pathogen infection (Rutrecht and Brown 2008). The fact that we observed generally high rates of diapause survival may be related to the location of our focal aggregations: Appleton Farms and Grassrides (Ipswich, MA) is located well within the geographic range of *B. impatiens*, which is found throughout the Eastern United States as well as parts of Southern Canada (Williams et al. 2014). We also measured survival only in forests, a habitat type in which colonies produced more new queens than in meadows (Pugesek and Crone 2021). It is possible that the environmental stressors experienced by bumblebees during diapause are heightened in marginal habitat types. For example, in areas near the edges of their climatic ranges, bumblebee populations are more vulnerable to decline (Kerr et al. 2015; Williams et al. 2019b). The northward expansion of at least one other *Bombus* species, *B. haematurus*, has been linked to warmer winter temperatures (Biella et al. 2021). Given that we monitored aggregations at one study site, using similar methods to monitor diapause survival rates of *B. impatiens* in other regions of the United States could be a valuable area of future research.

At this point, it is unclear why survival rates of queens overwintered in the laboratory were generally lower than our field estimates. It may be that laboratory conditions are stressful for queen bumblebees; maintaining study organism in artificial settings can be challenging, and the ability of study subjects to thrive in the lab can depend greatly on methods of husbandry implemented by researchers (Yearian and Wilkinson 1965). Among-study variation in survival rates was high, suggesting that some researchers were better than others at maintaining queens in the artificial settings. It is also possible that study subjects used in lab and field experiments differ phenotypically. Insect populations maintained in the lab are known to adapt to artificial conditions (Hoffmann and Ross 2018); among other insect taxa (parasitoid wasps, lygus bugs), laboratory rearing has been observed to disrupt the ability of other insect species to enter diapause (Brent and Spurgeon 2011; Gariepy et al. 2014). Bumblebee queens used in diapause experiments are

typically obtained from colonies purchased from commercial suppliers (like Koppert Biological Systems or BioBest) or from colonies reared in the lab (Appendix S1; Table 2). As bumblebee queens can be induced to bypass diapause using carbon dioxide narcotization (Treanore and Amsalem 2022), it is possible that the selection on commercial bumblebees to undergo long periods of diapause has been relaxed. To date, no study has addressed the impacts of captive rearing on the ability of queen bumblebees to complete diapause. However, other authors have noted differences in the morphologies of wild and lab reared bumblebees (e.g., body size, Beekman et al. 2000; Pugesek et al. 2021), which may impact their ability to undergo diapause (Bogo et al. 2017; Fauser et al. 2017). Understanding how outcomes of artificial diapause are impacted by the origin of study subjects and by other husbandry strategies would be a valuable area of future research. Until best practices are better defined, we recommend that researchers leverage the methods of authors who have had relatively high rates of success in maintaining diapausing queens (Pouvreau 1970; Holm 1972), i.e., by obtaining queens from field colonies and providing queens an opportunity to excavate their own hibernacula.

Our field observations broadly corroborate a pattern observed by the many lab-based studies of queen diapause survival (Yourth et al. 2008; Whitehorn et al. 2009), in that survival rates of queens were dependent on colony origin/aggregation ID. For individuals that originate from the same colony, similarities in survival rates could be explained by a multitude of factors, including relatedness, environment during development, nutritional status, and body condition of adult queens (Yourth et al. 2008; Woodard et al. 2019; Treanore and Amsalem 2020). In the field, differences in environmental conditions around nests could have also contributed to differences in survival rates. Aggregations with lower survival rates (aggregations 1 and 4) were located less than a meter from walking paths. Compacted soils may have limited the ability of queens to dig their hibernacula, as queens dug themselves less deeply in the soil and greater number of hibernacula were abandoned by queens at these aggregations. These aggregations also had smaller queens, as measured by both IT span and body mass. Due to low sample sizes, we were unable to statistically separate effects of colony origin and the other ecological or environmental factors which might have influenced diapause survival (queen body size, depth of hibernacula, etc.). In this context, our research points to at least one major advantage of monitoring queen vital rates in the lab, in that extraneous sources of variation can be controlled in the lab (e.g. Nasir and Ata-Ul-Mohsan 2019), making identifying the environmental factors which impact queen vital rates in the field more straightforward.

Our study showcases the value of field-based research in understanding diapause as a potentially vulnerable or robust stage in an insect's life history. Diapause is an understudied phase of the life cycle for bumblebees and many other groups of insects. Lab-based studies that monitor bumblebee vital rates under controlled conditions will remain foundational in the effort to identify the environmental factors which impact diapause survival. However, lab-based studies cannot capture the real-world environmental pressures faced by diapausing insects, and lab-reared colonies cannot replicate the adaptive capacity of wild colonies to cope with these pressures. In demonstrating the utility of *B. impatiens* (the only *Bombus* species reported to overwinter in large aggregations) as a model system for exploring insect overwintering ecology, we are hopeful that our research will pave the way for future field-based studies targeting this life cycle stage.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Research involving human participants and/or animals Ethical approval was not required for this project.

Informed consent Not applicable.

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