

## Original Articles

## Temperature in overwintering honey bee colonies reveals brood status and predicts colony mortality



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## ARTICLE INFO

## ABSTRACT

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Winter losses in honey bee colonies, *Apis mellifera*, raise concerns both for their critical role in crop pollination and for the sustainability of beekeeping. Beekeepers struggle to limit losses due to a lack of indicators to measure colony health and predict mortality in winter under field conditions. Due to the critical role of social thermoregulation for winter colony survival and brood production, this study aims to assess whether monitoring nest temperature during the winter may provide indicators of the state of health and survival of honey bee colonies. Under field conditions, we monitored the nest temperature of 31 hives distributed along a thermal gradient spanning over different European climates (including France, Germany, and Greece) over the winter 2022–2023. We installed 1,083 temperature sensors at multiple points inside the hives, with an average of  $35 \pm 1.1$  sensors per hive (mean  $\pm$  SD). We collected a total of 26,322,085 temperature data measurements for which we computed the time series of minimum, median, maximum temperature, and thermal amplitude. We found that the thermal amplitude within the nest is an indicator of colony health, effectively distinguishing between surviving and dying colonies with an accuracy of 96.8 %. Moreover, we found that nest temperature enables the detection of collapsing phases with an accuracy of 83.9 % and up to one month before the death of the colony. Finally, we showed that monitoring nest temperature in winter can help detect brood presence and size, an important health indicator for beekeeping. We suggest that monitoring nest temperature could represent a relevant field indicator of honey bee colony health for understanding the success or failure of overwintering. This indicator could help beekeepers anticipate colony mortalities to limit the winter colony losses observed worldwide.

## 1. Introduction

The Western honey bee, *Apis mellifera*, is the most frequently managed species for crop pollination worldwide (Garibaldi et al., 2017) and forms the heart of the beekeeping economy (e.g., honey production). Over the last 30 years, winter mortality rates of honey bee colonies were recorded to have reached up to 50 % of beekeepers' operations in

many regions of the world, such as in North America (Bruckner et al., 2023), Latin America (Requier et al., 2024), and Europe (Gray et al., 2023). These winter losses are estimated to cost between 3 and 32 million euros per winter in several countries (e.g., in Austria, Czech Republic, Macedonia, or New-Zealand; (Popovska Stojanov et al., 2021; Stahlmann-Brown et al., 2023), raising concerns about the sustainability of beekeeping. Winter colony losses result from the weakening of honey

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bee colonies during the season due to multiple stress factors (Goulson et al., 2015; Henry et al., 2017; Potts et al., 2010; Steinhauer et al., 2018), including parasites and pathogens (Desai and Currie, 2015; van Dooremalen et al., 2012), predators (Laurino et al., 2019; Requier et al., 2019b), lack of flowers (Clermont et al., 2015; Kuchling et al., 2018; Requier et al., 2017), exposure to pesticides (Insolia et al., 2022; Van Der Zee et al., 2015), and climate change (Insolia et al., 2022; Overturf et al., 2022; Switanek et al., 2017; Zapata-Hernández et al., 2024). However, despite a massive scientific effort to identify stress factors, beekeepers struggle to limit winter losses due to a lack of indicators to measure colony health and predict mortality under field conditions.

Winter monitoring of colony health under field conditions is a technical challenge, as conventional assessments imply opening the hives and endanger the colony health by disrupting social thermoregulation (Requier, 2019; Requier et al., 2017). However, advancements in technology—including miniaturization, cost reduction, and diversification—have extended the application of electronics in beekeeping (known as precision beekeeping), offering various new tools for assessing colony health (Alleri et al., 2023; Hadjur et al., 2022; Vardakas et al., 2024; Zacepins et al., 2015). The use of electronics can be extended to the winter period for monitoring key health metrics such as temperature (Cook et al., 2022; Meikle et al., 2017, 2016; Senger et al., 2024; Zacepins et al., 2020; Zacepins and Karasha, 2013), which is crucial as the absence of social thermoregulation may indicate that colonies are weakened (Minaud et al., 2024a).

Temperature sensors have been used since the beginning of the 20th century to explore time series of minimum, average, and maximum temperatures in nests, using a single sensor per hive (Dunham, 1931; Gates, 1914). Nevertheless, during winter, bees produce heat in a moving cluster (Owens, 1971; Szabo, 1985), and using multiple temperature sensors in the nest could help accurately track the nest temperature of honey bees during the winter (Minaud et al., 2024a). The use of multiple temperature sensors was initiated in laboratory and semi-controlled areas using thermocouples (Owens, 1967, 1971; Szabo, 1985) and, more recently, using frames integrating sensors within the wax (Barmak et al., 2024, 2023; Kviesis and Zacepins, 2016; McVeigh et al., 2022). However, monitoring nest temperature of honey bee colonies with multiple sensors during the winter has not yet been applied in the field, although this could help beekeepers to track social thermoregulation as a potential indicator of colony health and winter survival.

Performing social thermoregulation is critical for honey bees to survive winter conditions (Heinrich, 1993). For that, honey bees form a cluster within the nest to maintain temperature above the critical threshold of 10 °C, below which bees enter a state of “chill-coma” (Free and Spencer-Booth, 1960). Heat is generated passively by the resting metabolism of the large number of individuals and actively by the temporary heat production of a group of central bees (Stabentheiner et al., 2003). The center of the bee cluster averages 27–35 °C, with temperatures decreasing towards the cluster surface to 18 °C–29 °C for an overall mean temperature of 21.3 °C (Barmak et al., 2023; Corkins and Gilbert, 1932; Fahrenholz et al., 1989; Owens, 1971; Southwick and Heldmaier, 1987; Szabo, 1985). The bee cluster can change shape and move over time, allowing bees to access honey reserves, or to change thermal characteristics in response to weather conditions (Owens, 1971; Severson and Erickson, 1990; Simpson, 1961; Szabo, 1985). In addition to supporting winter colony survival, social thermoregulation is involved in brood-rearing—a key point of colony health—by maintaining brood area at the optimum temperature of 34 ± 1.5 °C (Bujok et al., 2002; Kronenberg and Heller, 1982; Southwick and Heldmaier, 1987).

Winter colony dynamics and survival are thus linked to the outside temperature and therefore to winter conditions. However, winter conditions vary significantly across climates, and the impact of these variations on overwintering colony dynamic remains poorly understood. Before winter, colonies in warmer climates show fewer individuals but more brood than colonies in colder climates (Hatjina et al., 2014), a difference that could be linked to the influence of outside temperatures

on winter brood production. As winter approaches, the pollen supply, temperature, and photoperiod decrease, inducing a gradual decline in brood rearing (Döke et al., 2015). The queen usually stops egg-laying when the outside temperature is too low but must restart when temperature and photoperiod rise again (Medrzycki et al., 2010; Nürnberg et al., 2018) to prepare the colony for rapid growth in spring (Seeley and Visscher, 1985). Therefore, egg-laying is expected to decline (or stop) later and restart earlier in warmer climates and is expected to be progressive along a climate thermal gradient.

Due to the critical role of social thermoregulation in colony survival and winter brood production, this study aims to assess whether monitoring nest temperature of honey bee colonies with multiple sensors during winter may provide indicators of health and survival of honey bee colonies. We assumed that a surviving colony needs to maintain high temperature inside the cluster (27–35 °C, Barmak et al., 2023; Corkins and Gilbert, 1932; Fahrenholz et al., 1989; Owens, 1971; Southwick and Heldmaier, 1987; Szabo, 1985), which should translate into a high thermal amplitude within the nest (i.e., high difference between the hottest and coldest point of the nest). Conversely, a dead colony should present no or low thermal amplitude, reflecting the reduced thermal heterogeneity of a nest devoid of living bees. Detecting decreasing thermal amplitude during winter could therefore reflect difficulties in maintaining colony thermoregulation and may indicate an ongoing colony collapse, a potential anticipatory indicator of mortality. Furthermore, we explored the thermoregulation dynamics of collapsing colonies to provide clues to the pattern(s) of overwintering failure. On the other hand, we aimed to use nest temperature as an indicator of winter brood production. Since the brood is maintained within a narrow range of temperature (34 ± 1.5 °C, Bujok et al., 2002; Kronenberg and Heller, 1982; Southwick and Heldmaier, 1987), stable maximum temperatures should indicate the presence of brood. In addition, we tested whether the distribution of high temperatures in the nest may be an indicator of brood size, assuming a positive correlation between brood surface and the thermoregulated volume in the nest (McVeigh et al., 2022). We explored whether the temperature-based brood size indicator would depend on winter climate by monitoring colonies along a climate thermal gradient including Mediterranean, oceanic, and continental European climates. Finally, we aimed to estimate the realism and the accuracy of temperature-based indicators by performing validation tests with visual field-based colony inspections.

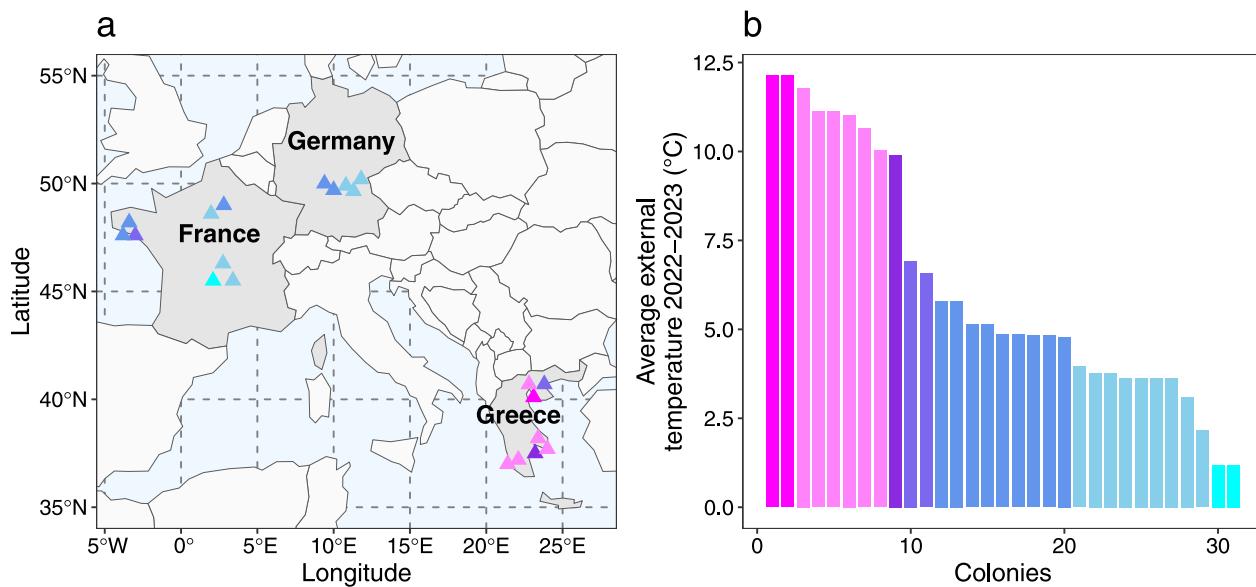
## 2. Material and methods

### 2.1. Study sites and honey bee colonies

We monitored 31 honey bee colonies, *Apis mellifera*, in France (n = 14), Germany (n = 7), and Greece (n = 10) over the 2022–2023 winter period (from 2022-10-31 to 2023-03-31). Colonies were maintained in 10-frame hives and were located in 21 sites (i.e., apiaries), each spaced at least 5 km apart and selected in different regions to represent the climate thermal gradient offered by these three countries (Fig. 1a). This includes European climates characterized as temperate with dry, hot summers (Mediterranean), temperate without a dry season and with hot summers (Oceanic), and cold with dry seasons and hot summers (Continental) (Peel et al., 2007). The honey bee subspecies in each of these countries are expected to differ due to these regional variations (Requier et al., 2019a). We monitored the winter climate at each site using five temperature sensors (Maxim Integrated™ DS18B20, 0.5 °C) placed below the colonies. Using these sensors, we recorded the external temperature every 20 min from 2022-12-22 to 2023-03-21, which we averaged for each site (Fig. 1b).

### 2.2. Monitoring nest temperature

We monitored the nest temperature of each hive with the use of multiple DS18B20Z+ temperature sensors (Maxim Integrated™, 9–12



**Fig. 1.** Experimental design with (a) geographical distribution of study sites; and (b) number of colonies monitored along the winter climate thermal gradient. Colors correspond to average outdoor winter temperatures for 2022–2023, classified by 2 °C intervals from 0–2 °C to 12–14 °C.

bits, set to 12 bits;  $-55^{\circ}\text{C}$  to  $+125^{\circ}\text{C}$ ,  $\pm 0.5^{\circ}\text{C}$ ). We followed the approach of frames integrating sensors (see also Barmak et al., 2024, 2023; Kviesis and Zacepins, 2016; McVeigh et al., 2022), embedding the sensors directly within the frame wax. We built a total of 93 frames integrating sensors, each composed of 12 temperature sensors. The 12 sensors per frame were placed in a matrix of three lines and four columns, with an average distance of  $10.5\text{ cm} \pm 0.6\text{ cm}$  between sensors (Supplementary material, Fig. S1). Three frames integrating sensors were introduced into each controlled hive, i.e. 36 temperature sensors per colony, for a total of 1,116 temperature sensors (12 sensors  $\times$  3 frames  $\times$  31 colonies). Looking at the hive from the back and numbering the frames from 1 to 10 from left to right, the frames integrating sensors were placed second, fifth, and eighth to cover the nest space (Fig. S1). We collected temperature data every 15 min with a microcontroller (Arduino<sup>TM</sup>) equipped with a real-time clock module. To minimize power consumption, the microcontroller enters in deep sleep mode between readings, which last a few seconds every fifteen minutes. It results in a few tens of milliamperes of power consumption on average, with an estimated operational time of up to two months. The devices were powered by the electrical sector or with an external power bank (5 V, 20,000 mAh), with a monthly rotation (note that field issues in the turnover led to some temporary interruptions in data collection). Due to failure in 0.03 % of the sensors (33 previously tested sensors stopped working), we collected data from 1,083 functional temperature sensors. On average, each hive contained  $35 \pm 1.1$  sensors (mean  $\pm$  SD) that successfully produced data, with 36 sensors in fourteen hives, 35 in seven hives, 34 in five hives, 33 in four hives, and 32 in one hive. This resulted in a total of 26,322,085 nest temperature data points recorded over the 2022–2023 winter period.

### 2.3. Assessment of nest temperature indicators

All statistical analyses were performed using R software version 4.1.1 (R Core Team, 2024). We first averaged the temperature data per hour and sensor to prevent temporal pseudo-replicates. We then collected nest temperature metrics for all the monitored colonies ( $n = 31$ ) with a timescale of one hour, including (i) the minimum temperature ( $T_{\min}$ ), (ii) the median temperature ( $T_{\text{median}}$ ), and (iii) the maximum temperature ( $T_{\max}$ ). To analyze time series and interpolate missing data, we ran Generalized Additive Models (GAMs) for each colony and each nest temperature metrics (i.e.,  $T_{\min}$ ,  $T_{\text{median}}$ ,  $T_{\max}$ ) using the *gam* function

from the *mgcv* R-package (Wood, 2017). We considered time as the smoothed term in the GAMs (with  $k = 10$ ). We then computed the thermal amplitude ( $T_{\text{ampli}}$ ) for each colony as the difference in GAM model predictions between  $T_{\min}$  and  $T_{\max}$  over time, allowing us to focus on long-term trends without considering the daily cycle of temperature variations. In this study, we did not account for the sensor uncertainty of  $\pm 0.5^{\circ}\text{C}$ , as it was considered negligible compared to variation observed in hive temperature, which often exceeds  $10^{\circ}\text{C}$  in winter (e.g., Owens, 1971; Szabo, 1985).

### 2.4. Predicting winter survival, collapse, and death of honey bee colonies

We continuously assessed the colony state throughout the overwintering period (i.e., alive, collapsing, or dead) based on nest temperature thresholds. First, we considered the threshold of  $30^{\circ}\text{C}$  to identify alive colonies, assuming that reaching this value in winter could only result from social thermoregulation. Second, the hourly thermal amplitudes of a 48-h window were statistically compared to temperature thresholds using one-sided Student conformity tests (*t.test* function in the *stats* R-package; R-core team, 2024). We tested a thermal amplitude threshold of  $9.5^{\circ}\text{C}$  to distinguish alive colonies (non-significant test: temperatures are not significantly below the  $9.5^{\circ}\text{C}$  threshold) from collapsing or dead colonies (significant test: temperatures are significantly below the  $9.5^{\circ}\text{C}$  threshold), considering that a colony surviving in winter maintains high temperatures in the cluster (see Fig. S2 for further details on the selection of different thresholds). For colonies detected as collapsing or dead, we tested a thermal amplitude threshold of  $4.4^{\circ}\text{C}$  to distinguish collapsing colonies (non-significant test: temperatures are not significantly below the  $4.4^{\circ}\text{C}$  threshold) from dead colonies (significant test: temperatures are significantly below the  $4.4^{\circ}\text{C}$  threshold). Focusing on mortality (colonies predicted as dead at the end of winter), we explored the possibility of a common winter collapse pattern. We estimated mortality dates by extracting the first transition date between the detection of a collapsing state to a death state. Then, we compared the thermal amplitude of the dead colonies on a timescale adjusted with respect to the first detection of collapsing.

### 2.5. Predicting winter brood production

Excluding periods of collapsing or death (i.e., focusing on alive colonies), we considered that a colony produced brood when at least 95 % of

the  $T_{\max}$  measures of two consecutive days (48 h) fell within the interval of 48-h mean  $T_{\max} \pm 1.5^{\circ}\text{C}$  (i.e., the range of temperature for brood production). As only three frames were equipped with sensors in the 10-frame colonies, there is a notable probability that a small brood area can be distant from the sensors. To take that probability into account, we chose to focus on the stability of the temperature to detect brood presence rather than the temperature range for brood rearing of  $34 \pm 1.5^{\circ}\text{C}$ . We estimated the size of the brood by studying the nest temperature distribution. We considered that  $T_{\text{median}}$  tending towards  $T_{\max}$  reflected a greater proportion of the colony being positively thermoregulated and, therefore, a larger brood surface. We tested this hypothesis by computing the relative position of smoothed  $T_{\text{median}}$  ( $RP_{\text{median}}$ ) compared to smoothed  $T_{\text{ampl}}$  ( $RP_{\text{median}} = \frac{\text{GAM}(T_{\text{median}}) - \text{GAM}(T_{\min})}{\text{GAM}(T_{\max}) - \text{GAM}(T_{\min})}$ ). Based on preliminary analyses of the evolution of  $RP_{\text{median}}$  in a few colonies during the autumn transition where brood rearing slowed down and then stopped, we considered that when  $RP_{\text{median}}$  is below one-third, only a small part of the colony is thermoregulated, suggesting the colony may be in a survival strategy (i.e., presence of a bee cluster only). Conversely, if  $RP_{\text{median}}$  is greater than one-third, we considered that a large part of the colony is thermoregulated and that the colony may be in a growth strategy (i.e., the presence of a large brood surface).

## 2.6. Effects of a climate thermal gradient on winter brood production

We used two external temperature estimators to investigate whether brood size is affected by climate thermal gradients. First, we used the average external temperature over the winter period for each site. Second, we used the average  $T_{\min}$  over 48 h (2 days) as an estimator of the external temperature ( $T_{\min}$  is positively correlated with the external temperature (Fig. S3), and offers the advantage of being measured with the same sensor type and frequency for all colonies). Excluding periods identified as colony collapsing or death, we assessed the impact of these two external temperature estimators on winter brood production. For the presence of brood, we calculated the number of 2-day periods with or without brood. Two Generalized Linear Models (GLMs) with quasi-binomial error structure (*glm* function in the *stats* R-package; R-core team, 2024) were used to test whether the ratio of 2-day periods with brood to total 2-day periods (response variable) was affected by the average annual external temperature of each site in the first model, and the 48-h  $T_{\min}$  in the second. We ran two other GLMs with quasibinomial structures to test whether  $RP_{\text{median}}$  (response variable) was affected by the two external temperature estimators.

## 2.7. Field-based assessment of winter survival and brood size

To evaluate and test the accuracy of our temperature-based indicators, we performed validation tests between our temperature-based estimates (for survival state and brood) and traditional estimates obtained through visual inspections of hives in the field. We visually inspected each colony ( $n = 31$ ) by opening the hive and checking the content of the frames once at the beginning of the overwintering period (in October in France and Germany, and December in Greece) and once at the end of the overwintering period (in March in Greece, and May in France and Germany). For these two periods, we estimated the coverage of brood (open and closed) on the two sides of each frame ( $n = 10$  frames per hive) by visually dividing each side into ten equal sections, each representing 10 % of the total area. We then converted these percentage estimates into  $\text{cm}^2$  based on the method of Delaplane et al. (2013). Moreover, we recorded the survival (i.e., presence of a bee cluster) or winter death (i.e., absence of a bee cluster) of each colony at the end of the overwintering period. These post-winter assessments were used to define the selected thermal amplitude thresholds. We tested different thresholds and identified those that minimized both false positives and false negatives.

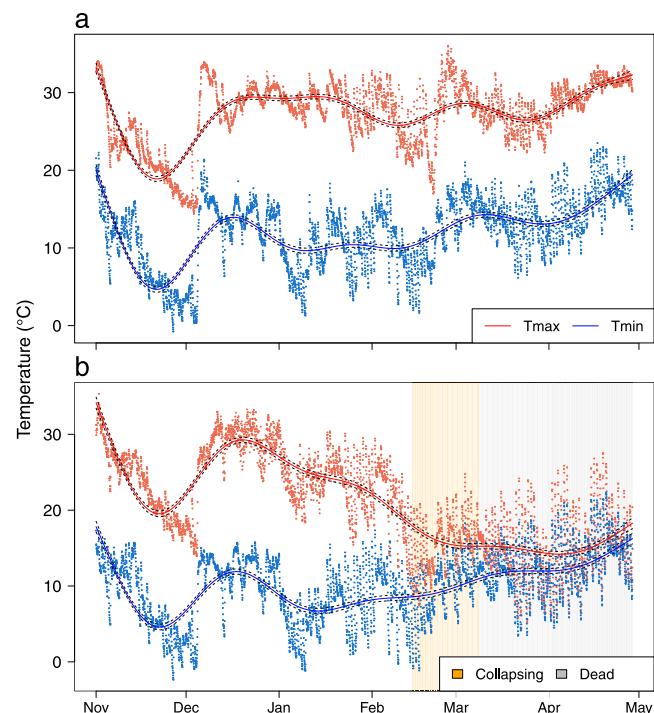
We performed a validation test between temperature-based

indicators of brood (presence and size) and visual inspections of brood area before and after winter. For brood presence, we used a GLM with binomial error structure and logit link function (*glm* function in the R *stats* package; R-core team, 2024). We tested the relationship between time with brood presence (response variable) and brood size estimated visually during the pre- and post-winter assessments (predicted variable). For brood size, we used a Linear Model (LM) (*lm* function in the R *stats* package; R-core team, 2024) to test the relationship between  $RP_{\text{median}}$  as estimator of brood size (response variable) and total brood size estimated visually during assessments (predicted variable). We used the temperature-based estimates of the second day before or after the pre- and post-winter assessment in order to avoid potential effects related to the hive inspection, assuming that brood area does not change drastically in two days.

## 3. Results

### 3.1. Predicting winter survival, collapse, and death of honey bee colony

Over the 2022–2023 monitoring of temperature of honey bee colonies ( $n = 31$ ), we found high nest thermal amplitude representing social thermoregulation of the bee cluster in the hives (Fig. 2a, Figs. S4a and S5), but also absence of such thermal amplitude suggesting death of the colonies (Fig. 2b, Figs. S4b and S5). Based on the nest thermal amplitude threshold of  $4.4^{\circ}\text{C}$ , we first estimated that a total of 23 colonies survived the winter (thermal amplitude  $>4.4^{\circ}\text{C}$ ), while 8 colonies were estimated dead (thermal amplitude  $<4.4^{\circ}\text{C}$ ). The validation inspection showed 96.8 % accuracy for the distinction between living and dead colonies (24 colonies were recorded alive and 7 dead with the post-winter field inspection). All estimated surviving colonies were confirmed as having survived in the field. However, we report one false-

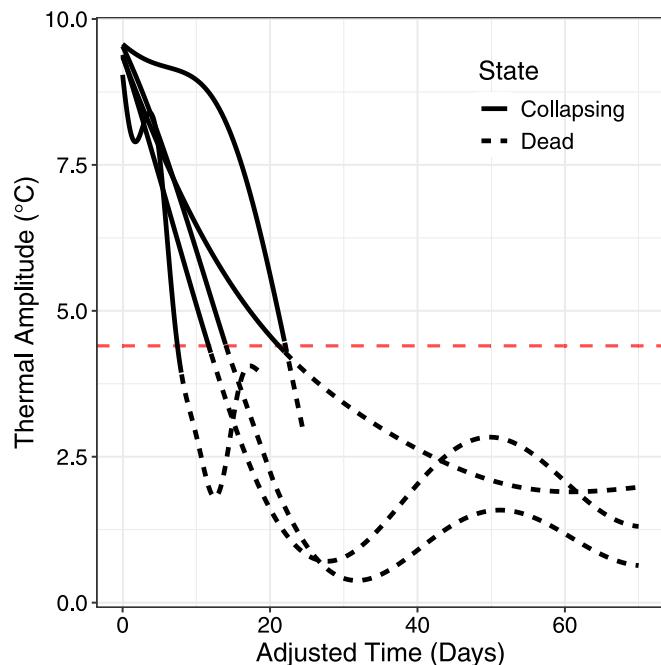


**Fig. 2.** Time series of maximum temperatures (red points), minimum temperatures (blue points) and their associated GAMs (solid lines, with dotted lines indicating 95 % confidence intervals) extracted from 36 temperature points for (a) a colony that survived winter, and (b) a colony that collapsed during winter. Period when the colony was identified as collapsing is represented in orange. Period when the colony was identified as dead is represented in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

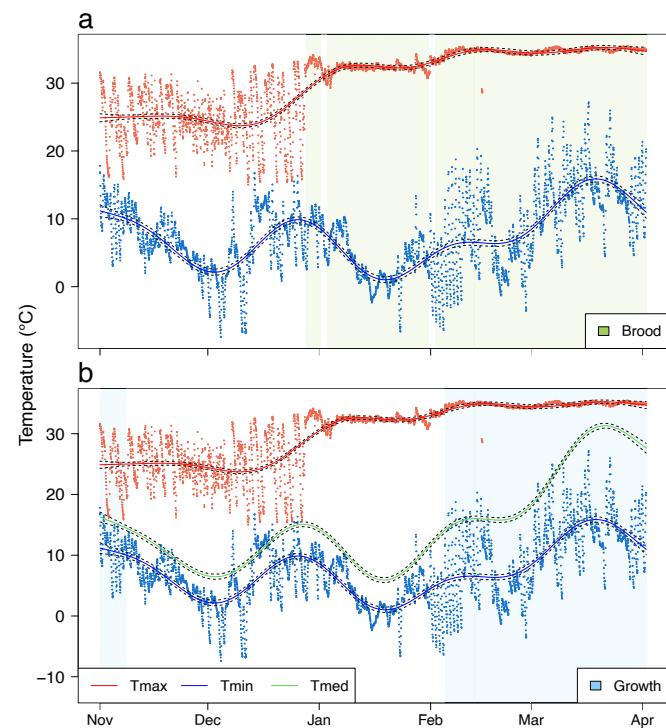
positive detection of mortality. Based on the nest thermal amplitude threshold of  $9.5^{\circ}\text{C}$ , we secondly estimated a total of 19 colonies surviving through the winter (thermal amplitude  $>9.5^{\circ}\text{C}$ ), and 12 colonies were estimated as collapsing (thermal amplitude  $<9.5^{\circ}\text{C}$ ). The validation test showed 83.9 % accuracy for predicting mortality by detecting collapsing, with seven true-positive detections of collapsing and five false-positive. Both thermal amplitude thresholds failed for the same colony, which was detected as dead or collapsing instead of surviving (this colony was ignored in the rest of the study). Lowering the thresholds reduced the false-positive rate but increased the number of false-negatives. Conversely, raising the thresholds enabled earlier detection of mortality, but increased the false positive rate (Fig. S2). We estimated the duration of the winter collapse of honey bee colonies as  $15.5 \pm 4.5$  days (from 8 to 22 days) based on five colonies (two collapses out of seven could not be fully tracked due to data interruptions), representing the time between the first and last dates of the collapsing period (Fig. 3). We did not identify a common pattern with regard to the dates of death, which occurred at the beginning (two colonies), middle (three colonies), or end of winter (two colonies). Nevertheless, we generally observed a continuous decrease in thermal amplitude during collapse.

### 3.2. Predicting winter brood production based on nest temperature

We found presence of brood during the winter for almost all colonies (29 of the 31 colonies, 93.5 %), based on the stability of  $T_{\text{max}}$  (Fig. 4a, Fig. S6). We found that surviving colonies had significantly more occurrence of brood than collapsing colonies (quasibinomial GLM,  $t = -3.778$ ,  $p < 0.001$ , Table S1a). Based on visual assessments, the validation tests confirmed the accuracy of the temperature-based estimates of brood presence (binomial GLM,  $t = 7.529$ ,  $p < 0.001$ , Table S1b; Fig. 5a), and brood size (LM,  $t = 5.658$ ,  $p < 0.001$ , Table S1c; Fig. 5d). In addition, we found that the brood detection indicator based on the maximum temperature stability accurately (95 % accuracy) detected the



**Fig. 3.** Time series of thermal amplitude during collapsing for five colonies dead during winter 2022–2023. Each line represents a colony. The solid lines represent thermal amplitude during the period of collapsing, and the dotted lines represent thermal amplitude of the dead colonies. The dotted red line represents the thermal amplitude threshold of  $4.4^{\circ}\text{C}$ , below which a colony is considered dead. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** Time series of maximum temperatures (red points), minimum temperatures (blue points) and their associated GAMs (solid lines, with dotted lines indicating 95 % confidence intervals) extracted from 34 temperature points for a colony that survived winter. (a) Periods identified with brood are represented in green. (b) Periods identified as growing are represented in cyan, and green lines correspond to the GAM of median temperatures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

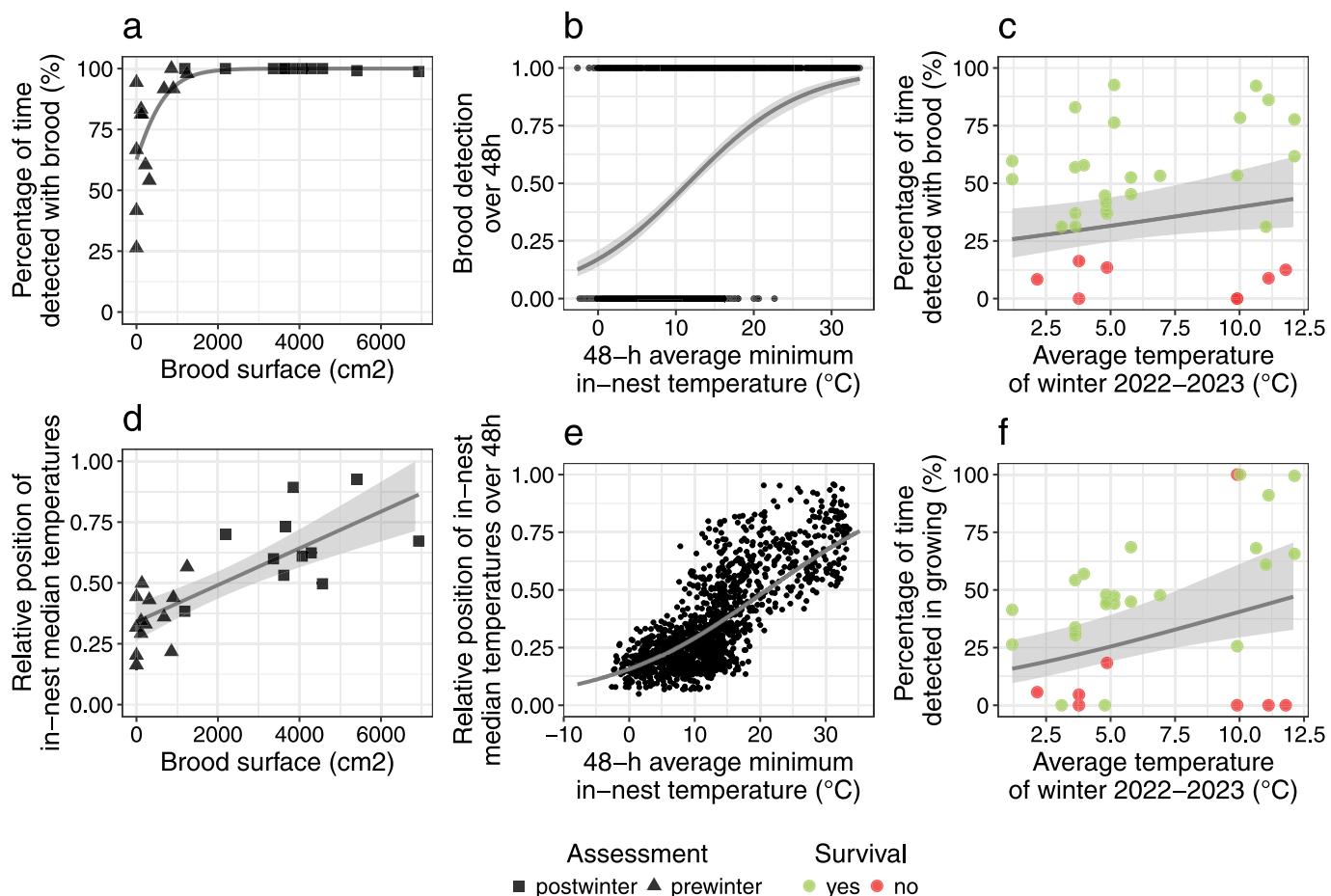
presence of brood from a total brood surface of  $1141 \text{ cm}^2$  (Fig. 5a). Moreover, we detected periods when we estimate that colonies are in a growth strategy ( $RP > 0.33$ ) using the relative position of the median temperature in relation to the thermal amplitude ( $RP_{\text{median}}$ ), in particular at the beginning of the winter period and at the end of the winter period (Fig. 4b). Finally, we found more growth strategy period occurrences in surviving colonies than in collapsing colonies (quasibinomial GLM,  $t = -3.141$ ,  $p = 0.004$ , Table S1d).

### 3.3. Effects of climate thermal gradient on winter brood production

We found a significant positive relationship between temperature-based estimates of brood presence and  $T_{\text{min}}$  over 48 h (quasibinomial GLM,  $z = 19.12$ ,  $p < 0.001$ , Table S1e; Fig. 5b), showing that we estimate brood presence in 50 % of honey bee colonies when  $T_{\text{min}}$  over 48 h reaches  $11.5^{\circ}\text{C}$  during the winter (while brood presence is estimated in 75 % of colonies at  $T_{\text{min}}$  over 48 h =  $19.6^{\circ}\text{C}$ ). Similarly, we found a positive relationship between brood size estimated by the  $RP_{\text{median}}$  and  $T_{\text{min}}$  over 48 h (quasibinomial GLM,  $z = 267.1$ ,  $p < 0.001$ , Table S1f; Fig. 5e), showing that colonies have a high probability of being in a growth strategy when  $T_{\text{min}}$  reaches  $12.1^{\circ}\text{C}$  over 48 h. We found a significant positive effect of temperature on the proportion of time spent producing brood (quasibinomial GLM,  $z = 2.500$ ,  $p = 0.018$ , Table S1a; Fig. 5c) and on the time spent on growth strategy (quasibinomial GLM,  $z = 4.012$ ,  $p < 0.001$ , Table S1d; Fig. 5f).

## 4. Discussion

By monitoring social thermoregulation during the winter with multiple nest temperature sensors, we identified indicators of honey bee



**Fig. 5.** Relationship tests between temperature indicators of brood (presence and size), visual inspections of brood area, and winter climate. Correlation of brood surface estimate by visual inspections compared to (a) brood detection by maximum temperature stability, and (b) brood size estimation by the relative position of the median temperature in relation to the thermal amplitude. Average 48-h minimum nest temperature (external temperature proxy) effects on (c) brood detection by maximum temperature stability, and (d) brood size estimation by the relative position of the median temperature in relation to the thermal amplitude. Average winter temperature of 2022–2023 effects on (e) relative time identified with brood, and (f) relative time identified in growth. Grey lines represent 95 % confidence intervals when the relationship is significant ( $p < 0.05$ ).

colony health state as a first step toward anticipating colony mortalities beekeepers suffer from each winter worldwide (Bruckner et al., 2023; Gray et al., 2023; Requier et al., 2024). We found that the nest thermal amplitude can be used to accurately distinguish surviving from dying honey bee colonies in winter. Moreover, we found that the thermo-regulated area in the nest is restricted, confirming that an overwintering colony concentrates high temperatures in an isolated bee cluster area to limit energy losses (Owens, 1971; Szabo, 1985). Furthermore, we found that the stability of maximum nest temperature can be used to estimate the presence of brood during winter, and the distribution of overall temperatures can be used to estimate its size. Monitoring these nest temperature indicators would help beekeepers limit colony losses.

We showed that decreasing thermal amplitude within the colony in winter represents an indicator of colony mortality. We successfully predicted mortality with a success rate of 83.9 % by using multiple nest temperature sensors under field conditions. However, we recorded false positives (colonies detected as collapsing that are not dead). These false positive detections of mortality may be related to the number of sensors used and the proximity between the center of the bee cluster (that can be restricted in space in winter) and a temperature sensor. Moreover, data interruptions and smoothing appear to be possible causes of errors. Thus, longer, uninterrupted monitoring (facilitated by access to a power grid) using a larger number of sensors could reduce the detection of false positives. Furthermore, collapsing detection followed by a return to a survival state can be a warning signal that may be linked to a real

weakening during the winter that some colonies managed to overcome. In addition, it is interesting to note that outside the winter period, a low thermal amplitude is associated with a surviving colony as it thermoregulates a large part of the nest for brood-rearing (with a higher average overall temperature; Cook et al., 2022). Nevertheless, the high maximum temperature of these strong colonies (around  $34 \pm 1.5$  °C) still allows them to be distinguished from dead colonies (with the nest temperature matching the outside temperature).

The detection of colony collapse allowed to explore the pattern of winter mortality. We estimated that the duration of colony collapse averaged two weeks, ranging from one week to one month before death occurred. The thermal amplitude continuously decreased during colony collapse, and, interestingly, two colonies with similar thermal amplitude decline patterns during their collapse were located on the same location (Paris countryside, France). This suggests that similar stress factor(s) could be related with their mortality. The duration of colony collapse we observed under field conditions exceeded that observed in semi-controlled conditions, where a 24-h collapse was accidentally induced by toxic shock from an oxalic acid treatment (Barmak et al., 2023). Furthermore, variation in colony profiles may be associated with initial population size. Smaller colonies are less thermally efficient, resulting in higher consumption per individual (Free and Racey, 1968) and higher overall weight loss during winter (Norström et al., 2021). Thus, the least populated colonies could have a shorter collapse time. However, in this study, we did not collect data to accurately estimate the size of the

adult population in the colonies. Further studies should investigate the possible relationship between the adult population in colonies and thermoregulatory performance, as well as other parameters such as reserve size, pathogen load or treatment effect.

We also detected brood production in winter using multiple nest temperature sensors, i.e., another key factor of colony health. We showed that tracking the stability in  $T_{\max}$  can be used to precisely detect the presence of brood in the field, as previously demonstrated in summer at the scale of the cells on a frame (Becher and Moritz, 2009), and as mentioned in winter under semi-controlled conditions (Nürnberger et al., 2018). In our study, we detected the presence of brood from a total brood surface of approximately 1141 cm<sup>2</sup>, i.e., approximately the surface of one side of a Dadant frame (Delaplane et al., 2013). However, temperature stability can be used to detect the presence of brood but not to estimate its size (Godeau et al., 2023). Nevertheless, we showed that tracking the temperature heterogeneity within the nest can be a good indicator of brood size in winter (see also McVeigh et al., 2022 for similar results in summer).

Using these two indicators to detect the presence and size of brood, we studied the dynamics of winter brood production. Our results confirm that producing brood in winter may be specific to surviving colonies, as observed in previous studies (Avitabile, 1978; Fukuda and Sekiguchi, 1966; Seeley and Visscher, 1985). Consistent with these findings (Avitabile, 1978; Fukuda and Sekiguchi, 1966; Seeley and Visscher, 1985), we found only production of small brood areas in winter, which could be related to the restricted duration of time with suitable weather for foraging. Indeed, brood rearing is protein-dependent (i.e., pollen-dependent), and after five days without foraging, young larvae may be cannibalized to limit energy loss (Schmickl and Crailsheim, 2001). However, we noticed during the pre-winter colony inspections that the colonies had pollen reserves in the comb, that may be used for brood rearing during winter. Additionally, honey bees may have flight activities during winter, especially when weather conditions are good, such as temperature above 12 °C (Joshi and Joshi, 2010; Minaud et al., 2024b) and the availability of flower resources, even sparse, that can be used for pollen intake. Nevertheless, while we have regularly detected brood in winter, this does not imply a general pattern. Only two colonies were placed in very cold conditions, which limits our ability to conclude that all colonies consistently produce brood during winter. In addition, we proposed a threshold ( $RP_{\text{median}} > 0.33$ ) to consider that the colony is in a growth strategy based on the distribution of temperatures in the nest. The timing of the return to growth is crucial, and synchronized development with better weather conditions and resources can offer a selective advantage (Seeley and Visscher, 1985). In spring, a return to growth is mandatory to prepare for the new season, but it can occur earlier, e.g. in winter (Avitabile, 1978). Colonies that have restarted egg-laying during the winter have more individuals in spring and summer and swarm earlier than colonies without winter brood (Nürnberger et al., 2019; Seeley and Visscher, 1985). However, a rapid resumption of egg-laying can also favor the development of parasites such as *Varroa destructor* (Nürnberger et al., 2019). It had already been demonstrated that one sensor above the nest could be used to estimate the start of the growth strategy after winter, but this method required data from the following months to identify a change in temperature profile (Stalidzans and Berzonis, 2013). We showed that using multiple sensors in the hive enables us to estimate the return of the growth strategy over a shorter period (in our case, 48 h). We find that colonies resume their growth strategy on average when the 48-h  $T_{\min}$  is 12 °C, which also corresponds to the time when there is a 50 % probability of finding brood in the colony. Furthermore, we showed that  $T_{\min}$  strongly correlates to external temperature with a positive buffer effect of around 4 °C in the hive. Therefore, as expected, the growth strategy is linked to outdoor temperature and would be implemented when outdoor temperature over two days are around 16 °C.

We found that weakened colonies no longer showed the same growth dynamics as healthy colonies before collapsing, with a shorter brood

period and smaller brood surface area. A hypothesis is that these weakened colonies could allocate energy to survival instead of brood rearing, given that thermoregulation for brood rearing is more energy-consuming than thermoregulation of only the bee cluster (Stabentheiner et al., 2010). In support of this hypothesis, the weight loss of colonies performing winter brood rearing is almost double (0.84 kg/week) compared to the weight loss of colonies performing thermoregulation for colony survival only (0.42 kg/week) (Seeley and Visscher, 1985). Furthermore, restarting egg-laying during winter triggers the onset of senescence in winter bees (Münch et al., 2013), which leads to an increased risk of colony mortality in spring, known as “spring dwindle” (Betti et al., 2016; Rajagopalan et al., 2024).

We found different winter colony dynamics according to the climate thermal gradient, with colonies in warmer winter climates showing extended brood periods and larger brood sizes. Given the temperature in Mediterranean sites, the winter period during which the colony is in winter survival seems shorter (one month) compared with at least three months in other countries. In the warmest climates of our gradient, the winter period is shortened, with several 48-h windows where external temperatures allow for growth strategies. However, shorter winter durations would not be systematically a factor in preventing winter mortality of honey bee colonies. Indeed, winter losses of managed honey bee colonies are common in Mediterranean countries (Gray et al., 2023). Moreover, in warmer climates, early winter flights are more frequent and are suspected of altering the age structure of the overwintering colony, unbalancing the population in favor of older bees, and increasing the risk of colony failure in spring (Rajagopalan et al., 2024). Additionally, the lifespan of winter bees in warm climates is shorter than in cold climates, which could be linked to a higher pathogen load and induce mortalities (Maes et al., 2021).

## 5. Concluding remarks

We proposed to use nest temperature monitoring with multiple sensors as indicators of colony health and survival of honey bees during winter. We suggest considering thermal amplitude as an indicator of colony winter survival. This indicator can help to detect the collapsing process in the thermoregulation of honey bees, in our study with an anticipation time of about 15 days. The proposed indicators are based exclusively on the internal temperature of the colony, eliminating the need for comparison with external sensors or between colonies. This allows the system to be applied to one or multiple colonies under any conditions. However, both the tool and analysis methods have limitations and could benefit from improvements to refine the proposed indicators. Enhancing the precision of the system, particularly its spatial resolution, could lead to more accurate measurements. Indeed, having sensors closer to the center of the cluster would provide more reliable thermal amplitude readings. This improvement could also reduce or eliminate false mortality detections, which seem to occur when the cluster is too far from the sensors. Our current system uses 36 sensors distributed across 3 frames in the hive, though networks of over 60 sensors embedded in frames have been proposed (Barmak et al., 2023; McVeigh et al., 2022). A simpler way to increase sensor numbers would be by adding more frames, allowing up to 120 temperature sensors to be placed across 10 frames.

Furthermore, with the low cost of technologies (Rigakis et al., 2023; Senger et al., 2024), multiple nest deployments at a large scale could help investigate the impact of landscape composition and configuration on winter mortality processes in honey bees (Clermont et al., 2015). Moreover, such a large amount of data would help provide earlier warning indicators of colony mortality with, e.g., the use of machine learning techniques (Braga et al., 2021). Early indicators of mortality are only alert signals and do not, on their own, reduce the risk of mortality. In response to these alerts, beekeepers must intervene with a support intervention plan. These interventions can be of different kinds, such as supplementation (Branchiccela et al., 2023; Shumkova et al., 2021),

merging weak colonies, or indoor storage (Hopkins et al., 2023, 2021; Stalidzans et al., 2017). Recent studies have also equipped colonies with electronic heating systems to assist social thermoregulation (Barmak et al., 2024, 2023; Çakmak et al., 2023). Thus, monitoring nest temperature with multiple sensors offers a promising approach to predict and mitigate winter colony losses by tracking thermoregulation-based mortality processes, ultimately supporting beekeeping sustainability.

#### CRediT authorship contribution statement

**Etienne Minaud:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **François Rebaudo:** Writing – review & editing, Software, Methodology, Conceptualization. **Giulia Mainardi:** Writing – review & editing, Investigation. **Philippos Vardakas:** Writing – review & editing, Investigation, Conceptualization. **Fani Hatjina:** Writing – review & editing. **Ingolf Steffan-Dewenter:** Writing – review & editing. **Fabrice Requier:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Conceptualization.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2024.112961>.

#### Data availability

Data will be made available on request.

#### References

Alleri, M., Amoroso, S., Catania, P., Lo Verde, G., Orlando, S., Ragusa, E., Sinacori, M., Vallone, M., Vella, A., 2023. Recent developments on precision beekeeping: a systematic literature review. *J. Agric. Food Res.* 14, 100726. <https://doi.org/10.1016/j.jafr.2023.100726>.

Avitabile, A., 1978. Brood rearing in honeybee colonies from late autumn to early spring. *J. Apic. Res.* 17, 69–73. <https://doi.org/10.1080/00218839.1978.11099905>.

Barmak, R., Stefanec, M., Hofstädler, D.N., Piotet, L., Schönwetter-Fuchs-Schistek, S., Mondada, F., Schmidl, T., Mills, R., 2023. A robotic honeycomb for interaction with a honeybee colony. *Sci. Robot.* 8. <https://doi.org/10.1126/scirobotics.add7385>.

Barmak, R., Hofstädler, D.N., Stefanec, M., Piotet, L., Cherfan, R., Schmidl, T., Mondada, F., Mills, R., 2024. Biohybrid superorganisms—on the design of a robotic system for thermal interactions with honeybee colonies. *IEEE Access* 1–1. <https://doi.org/10.1109/ACCESS.2024.3385658>.

Becher, M.A., Moritz, R.F.A., 2009. A new device for continuous temperature measurement in brood cells of honeybees (*Apis mellifera*). *Apidologie* 40, 577–584. <https://doi.org/10.1051/apido/2009031>.

Betti, M.I., Wahl, L.M., Zamir, M., 2016. Age structure is critical to the population dynamics and survival of honeybee colonies. *R. Soc. Open Sci.* 3, 160444. <https://doi.org/10.1098/rsos.160444>.

Braga, A.R., Freitas, B.M., Gomes, D.G., Bezerra, A.D.M., Cazier, J.A., 2021. Forecasting sudden drops of temperature in pre-overwintering honeybee colonies. *Biosyst. Eng.* 209, 315–321. <https://doi.org/10.1016/j.biosystemseng.2021.07.009>.

Branchicella, B., Castelli, L., Diaz-Cetti, S., Invernizzi, C., Mendoza, Y., Santos, E., Silva, C., Zunino, P., Antúnez, K., 2023. Can pollen supplementation mitigate the impact of nutritional stress on honey bee colonies? *J. Apic. Res.* 62, 294–302. <https://doi.org/10.1080/00218839.2021.1888537>.

Bruckner, S., Wilson, M., Aurell, D., Rennich, K., vanEngelsdorp, D., Steinhauer, N., Williams, G.R., 2023. A national survey of managed honey bee colony losses in the USA: results from the Bee Informed Partnership for 2017–18, 2018–19, and 2019–20. *J. Apic. Res.* 62, 429–443. <https://doi.org/10.1080/00218839.2022.2158586>.

Bujok, B., Kleinhennz, M., Fuchs, S., Tautz, J., 2002. Hot spots in the bee hive. *Naturwissenschaften* 89, 299–301. <https://doi.org/10.1007/s00114-002-0338-7>.

Çakmak, I., Kul, B., Ben Abdelkader, F., Seven Çakmak, S., 2023. Effects of temperature adjustment with a heating device in weak honey bee colonies in cold seasons. *Int. J. Biometeorol.* 67, 1765–1774. <https://doi.org/10.1007/s00484-023-02537-w>.

Clermont, A., Eickermann, M., Kraus, F., Hoffmann, L., Beyer, M., 2015. Correlations between land covers and honey bee colony losses in a country with industrialized and rural regions. *Sci. Total Environ.* 532, 1–13. <https://doi.org/10.1016/j.scitotenv.2015.05.128>.

Cook, D., Tarlington, B., McGree, J.M., Blackler, A., Hauxwell, C., 2022. Temperature sensing and honey bee colony strength. *J. Econ. Entomol.* 115, 715–723. <https://doi.org/10.1093/jee/toac034>.

Corkins, C.L., Gilbert, C.S., 1932. *The Metabolism of Honeybees in Winter Cluster*. Univ. Wyo. Agric. Exp. Sta.

Delaplane, K.S., Van Der Steen, J., Guzman-Novoa, E., 2013. Standard methods for estimating strength parameters of *Apis mellifera* colonies. *J. Apic. Res.* 52. <https://doi.org/10.3896/IBRA/1.52.1.03>.

Desai, S.D., Currie, R.W., 2015. Genetic diversity within honey bee colonies affects pathogen load and relative virus levels in honey bees, *Apis mellifera* L. *Behav. Ecol. Sociobiol.* 69, 1527–1541. <https://doi.org/10.1007/s00265-015-1965-2>.

Döke, M.A., Frazier, M., Grozinger, C.M., 2015. Overwintering honey bees: biology and management. *Curr. Opin. Insect Sci.* 10, 185–193. <https://doi.org/10.1016/j.cois.2015.05.014>.

Dunham, W.E., 1931. *Hive temperatures for each hour of a day*. *Ohio J. Sci.* 31, 181–188.

Fahrenholz, L., Lamprecht, I., Schricker, B., 1989. Thermal investigations of a honey bee colony: thermoregulation of the hive during summer and winter and heat production of members of different bee castes. *J. Comp. Physiol. B* 159, 551–560. <https://doi.org/10.1007/BF00694379>.

Free, J.B., Racey, P.A., 1968. The effect of the size of honeybee colonies on food consumption, brood rearing and the longevity of the bees during winter. *Entomol. Exp. Appl.* 11, 241–249. <https://doi.org/10.1111/j.1570-7458.1968.tb02048.x>.

Free, J.B., Spencer-Booth, Y., 1960. Chill-coma and cold death temperatures of *Apis mellifera*. *Entomol. Exp. Appl.* 3, 222–230. <https://doi.org/10.1111/j.1570-7458.1960.tb00451.x>.

Fukuda, H., Sekiguchi, K., 1966. *Seasonal change of the honeybee worker longevity in Sapporo, North Japan, with notes on some factors affecting the life-span*. *Ecol. Soc. Jpn.* 16, 206–212.

Garibaldi, L.A., Requier, F., Rollin, O., Andersson, G.K., 2017. Towards an integrated species and habitat management of crop pollination. *Curr. Opin. Insect Sci.* 21, 105–114. <https://doi.org/10.1016/j.cois.2017.05.016>.

Gates, B., 1914. *The temperature of the bee colony*. *Bull. US Department Agric.* 96.

Godeau, U., Pioz, M., Martin, O., Rüger, C., Crauser, D., Le Conte, Y., Henry, M., Alaux, C., 2023. Brood thermoregulation effectiveness is positively linked to the amount of brood but not to the number of bees in honeybee colonies. *Peer Commun. J.* 3, e42. <https://doi.org/10.24072/pcjournal.270>.

Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347, 1255957. <https://doi.org/10.1126/science.1255957>.

Gray, A., Adjlane, N., Arab, A., Ballis, A., Brusbardis, V., Bugeja Douglas, A., Cadahía, L., Charrière, J.-D., Chlebo, R., Coffey, M.F., Cornelissen, B., Costa, C.A.D., Danneels, E., Danihlič, J., Dobrescu, C., Evans, G., Fedoriak, M., Forsythe, I., Gregorc, A., Ilieva Arakelyan, I., Johannsen, J., Kauko, L., Kristiansen, P., Martikkala, M., Martín-Hernández, R., Mazur, E., Medina-Flores, C.A., Mutinelli, F., Omar, E.M., Patalano, S., Raudmets, A., San Martin, G., Soroker, V., Stahlmann-Brown, P., Stevanovic, J., Uzunov, A., Vejsnaes, F., Williams, A., Brodschneider, R., 2023. Honey bee colony loss rates in 37 countries using the COLOSS survey for winter 2019–2020: the combined effects of operation size, migration and queen replacement. *J. Apic. Res.* 62, 204–210. <https://doi.org/10.1080/00218839.2022.2113329>.

Hadjur, H., Ammar, D., Lefèvre, L., 2022. Toward an intelligent and efficient beehive: a survey of precision beekeeping systems and services. *Comput. Electron. Agric.* 192, 106604. <https://doi.org/10.1016/j.compag.2021.106604>.

Hatjina, F., Costa, C., Büchler, R., Uzunov, A., Drazic, M., Filipi, J., Charistos, L., Ruottinen, L., Andonov, S., Meixner, M.D., Bieńkowska, M., Dariusz, G., Panasiuk, B., Conte, Y.L., Wilde, J., Berg, S., Bouga, M., Dybba, W., Kipriyanovska, H., Korpela, S., Kryger, P., Lodesani, M., Pechhacker, H., Petrov, P., Kezic, N., 2014. Population dynamics of European honey bee genotypes under different environmental conditions. *J. Apic. Res.* 53, 233–247. <https://doi.org/10.3896/IBRA.1.53.2.05>.

Heinrich, B., 1993. In: *The Hot-Blooded Insects*. Springer Berlin Heidelberg, Berlin, Heidelberg. <https://doi.org/10.1007/978-3-662-10340-1>.

Henry, M., Becher, M.A., Osborne, J.L., Kennedy, P.J., Aupinel, P., Bretagnolle, V., Brun, F., Grimm, V., Horn, J., Requier, F., 2017. Predictive systems models can help elucidate bee declines driven by multiple combined stressors. *Apidologie* 48, 328–339. <https://doi.org/10.1007/s13592-016-0476-0>.

Hopkins, B.K., Chakrabarti, P., Lucas, H.M., Sagili, R.R., Sheppard, W.S., 2021. Impacts of different winter storage conditions on the physiology of diutinus honey bees (Hymenoptera: apidae). *J. Econ. Entomol.* 114, 409–414. <https://doi.org/10.1093/jee/toaa302>.

Hopkins, B.K., Long, J., Naeger, N.L., Sheppard, W.S., 2023. Comparison of indoor (refrigerated) versus outdoor winter storage of commercial honey bee, *Apis mellifera* (Hymenoptera: Apidae), colonies in the Western United States. *J. Econ. Entomol.* 116, 1063–1068. <https://doi.org/10.1093/jee/toad109>.

Insolia, L., Molinari, R., Rogers, S.R., Williams, G.R., Chiaromonte, F., Calovi, M., 2022. Honey bee colony loss linked to parasites, pesticides and extreme weather across the United States. *Sci. Rep.* 12, 20787. <https://doi.org/10.1038/s41598-022-24946-4>.

Joshi, N.C., Joshi, P.C., 2010. Foraging behaviour of *Apis* spp. on apple flowers in a subtropical environment. *N. Y. Sci. J.*

Kronenberg, F., Heller, H.C., 1982. Colonial thermoregulation in honey bees (*Apis mellifera*). *J. Comp. Physiol.* 148, 65–76. <https://doi.org/10.1007/BF00688889>.

Kuchling, S., Kopacka, I., Kalcher-Sommersguter, E., Schwarz, M., Crailsheim, K., Brodschneider, R., 2018. Investigating the role of landscape composition on honey bee colony winter mortality: a long-term analysis. *Sci. Rep.* 8, 12263. <https://doi.org/10.1038/s41598-018-30891-y>.

Kviesis, A., Zacepins, A., 2016. Application of neural networks for honey bee colony state identification. In: 17th Int. Carpathian Control Conf. ICCC. <https://doi.org/10.1109/CarpathianCC.2016.7501133>.

Laurino, D., Lioy, S., Carisio, L., Manino, A., Porporato, M., 2019. *Vespa velutina*: an alien driver of honey bee colony losses. *Diversity* 12, 5. <https://doi.org/10.3390/d1201005>.

Maes, P.W., Floyd, A.S., Mott, B.M., Anderson, K.E., 2021. Overwintering honey bee colonies: effect of worker age and climate on the hindgut microbiota. *Insects* 12, 224. <https://doi.org/10.3390/insects12030224>.

McVeigh, A., Newton, M.I., Tsakonas, C., Bencsik, M., 2022. A spatially resolved temperature measurement system for a honeybee colony brood box. In: ASEC 2022. Presented at the ASEC 2022, MDPI, p. 57. <https://doi.org/10.3390/ASEC2022-1375>.

Medrzycki, P., Sgolastra, F., Bortolotti, L., Bogo, G., Tosi, S., Padovani, E., Porrini, C., Sabatini, A.G., 2010. Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. *J. Apic. Res.* 49, 52–59. <https://doi.org/10.3896/IBRA.1.49.1.07>.

Meikle, W.G., Weiss, M., Stilwell, A.R., 2016. Monitoring colony phenology using within-day variability in continuous weight and temperature of honey bee hives. *Apidologie* 47, 1–14. <https://doi.org/10.1007/s13592-015-0370-1>.

Meikle, W.G., Weiss, M., Maes, P.W., Fitz, W., Snyder, L.A., Sheehan, T., Mott, B.M., Anderson, K.E., 2017. Internal hive temperature as a means of monitoring honey bee colony health in a migratory beekeeping operation before and during winter. *Apidologie* 48, 666–680. <https://doi.org/10.1007/s13592-017-0512-8>.

Minaud, É., Rebaudo, F., Davidson, P., Hatjina, F., Hotho, A., Mainardi, G., Steffan-Dewenter, I., Vardakas, P., Verrier, E., Requier, F., 2024a. How stressors disrupt honey bee biological traits and overwintering mechanisms. *Heliyon* 10, e34390. <https://doi.org/10.1016/j.heliyon.2024.e34390>.

Minaud, É., Rebaudo, F., Requier, F., 2024b. Long-lived winter honey bees show unexpectedly high levels of flight activity compared to short-lived summer bees. *Apidologie* Accept.

Münch, D., Kreibich, C.D., Amdam, G.V., 2013. Aging and its modulation in a long-lived worker caste of the honey bee. *J. Exp. Biol.* 216, 1638–1649. <https://doi.org/10.1242/jeb.078915>.

Norrström, N., Niklasson, M., Leidenberger, S., 2021. Winter weight loss of different subspecies of honey bee *Apis mellifera* colonies (Linnaeus, 1758) in southwestern Sweden. *PLOS ONE* 16. <https://doi.org/10.1371/journal.pone.0258398>.

Nürnberger, F., Härtel, S., Steffan-Dewenter, I., 2018. The influence of temperature and photoperiod on the timing of brood onset in hibernating honey bee colonies. *PeerJ* 6, e4801. <https://doi.org/10.7717/peerj.4801>.

Nürnberger, F., Härtel, S., Steffan-Dewenter, I., 2019. Seasonal timing in honey bee colonies: phenology shifts affect honey stores and varroa infestation levels. *Oecologia* 189, 1121–1131. <https://doi.org/10.1007/s00442-019-04377-1>.

Overturf, K.A., Steinhauer, N., Molinari, R., Wilson, M.E., Watt, A.C., Cross, R.M., vanEngelsdorp, D., Williams, G.R., Rogers, S.R., 2022. Winter weather predicts honey bee colony loss at the national scale. *Ecol. Indic.* 145, 109709. <https://doi.org/10.1016/j.ecolind.2022.109709>.

Owens, C.D., 1967. Electric Heating of Bee Honey Hives. *USDA Tech. Bull.*

Owens, C.D., 1971. The Thermology of Wintering Honey Bee Colonies. *U.S. Agricultural Research Service*.

Peel, M.C., Finlayson, B.L., McMahon, T.A., 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrocl. Earth Syst. Sci.* 11, 1633–1644. <https://doi.org/10.5194/hess-11-1633-2007>.

Popovska Stojanov, D., Dimitrov, L., Danihlič, J., Uzunov, A., Golubovski, M., Andonov, S., Brodschneider, R., 2021. Direct economic impact assessment of winter honeybee colony losses in three European countries. *Agriculture* 11, 398. <https://doi.org/10.3390/agriculture11050398>.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25, 345–353. <https://doi.org/10.1016/j.tree.2010.01.007>.

R Core Team, 2024. R: A Language and Environment for Statistical Computing.

Rajagopalan, K., DeGrandi-Hoffman, G., Pruitt, M., Jones, V.P., Corby-Harris, V., Pireaud, J., Curry, R., Hopkins, B., Northfield, T.D., 2024. Warmer autumns and winters could reduce honey bee overwintering survival with potential risks for pollination services. *Sci. Rep.* 14, 5410. <https://doi.org/10.1038/s41598-024-55327-8>.

Requier, F., 2019. Bee colony health indicators: synthesis and future directions. *CABI Rev.* 1–12. <https://doi.org/10.1079/PAVSNRR201914056>.

Requier, F., Odoux, J.-F., Henry, M., Bretagnolle, V., 2017. The carry-over effects of pollen shortage decrease the survival of honeybee colonies in farmlands. *J. Appl. Ecol.* 54, 1161–1170. <https://doi.org/10.1111/1365-2664.12836>.

Requier, F., Garnery, L., Kohl, P.L., Njouu, H.K., Pirk, C.W.W., Crewe, R.M., Steffan-Dewenter, I., 2019a. The conservation of native honey bees is crucial. *Trends Ecol. Evol.* 34, 789–798. <https://doi.org/10.1016/j.tree.2019.04.008>.

Requier, F., Rome, Q., Chiron, G., Decante, D., Marion, S., Menard, M., Muller, F., Villemant, C., Henry, M., 2019b. Predation of the invasive Asian hornet affects foraging activity and survival probability of honey bees in Western Europe. *J. Pest Sci.* 92, 567–578. <https://doi.org/10.1007/s10340-018-1063-0>.

Requier, F., Leyton, M.S., Morales, C.L., Garibaldi, L.A., Giacobino, A., Porrini, M.P., Rosso-Londoño, J.M., Velarde, R.A., Aignasse, A., Aldea-Sánchez, P., Allasino, M.L., Arredondo, D., Audisio, C., Cagnolo, N.B., Basualdo, M., Branchicella, B., Calderón, R.A., Castelli, L., Castilhos, D., Escareño, F.C., Correa-Benítez, A., Silva, F.O.D., Garnica, D.S., De Groot, G., Delgado-Cañedo, A., Fernández-Marín, H., Freitas, B.M., Cardona, A.G., García, N., Garrido, P.M., Giray, T., Gonçalves, L.S., Landi, L., Gonçalves, D.M., Martínez, S., Moja, P.J., Moliner, A., Müller, P.F., Nogueira, E., Pacini, A., Palacio, M.A., Parra, G.N., Parra-H, A., Gramacho, K.P., Pérez, E., Pires, C.S.S., Reynaldi, F.J., Luis, A.R., Rossini, C., Armijos, M.S., Santos, E., Scannapieco, A., Spina, Y.M., González, J.M.T., Fernández, A.M.V., García, C.A.Y., Viana, B.F., Vieli, L., Antúnez, K., 2024. First large-scale study reveals important losses of honey bee and stingless bee colonies in Latin America. *Sci. Rep.* 14, 1–15. <https://doi.org/10.1038/s41598-024-59513-6>.

Rigakis, I., Potamitis, I., Tatlas, N.-A., Psirofina, G., Tzagaraki, E., Alissandrakis, E., 2023. A low-cost, low-power, multisensory device and multivariable time series prediction for beehive health monitoring. *Sensors* 23, 1407. <https://doi.org/10.3390/s23031407>.

Schmickl, T., Crailsheim, K., 2001. Cannibalism and early capping: strategy of honeybee colonies in times of experimental pollen shortages. *J. Comp. Physiol.* 187, 541–547. <https://doi.org/10.1007/s003590100226>.

Seeley, T.D., Visscher, P.K., 1985. Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. *Ecol. Entomol.* 10, 81–88. <https://doi.org/10.1111/j.1365-2311.1985.tb00537.x>.

Senger, D., Gruber, C., Kluss, T., Johannsen, C., 2024. Weight, temperature and humidity sensor data of honey bee colonies in Germany, 2019–2022. *Data Brief* 52, 110015. <https://doi.org/10.1016/j.dib.2023.110015>.

Severson, D.W., Erickson, E.H., 1990. Quantification of cluster size and low ambient temperature relationships in the honey bee. *Apidologie* 21, 135–142. <https://doi.org/10.1051/apido:19900207>.

Shumkova, R., Balkanska, R., KoynarSKI, T., Hristov, P., 2021. Application of the natural products NOZEMAT HERB and NOZEMAT HERB PLUS can decrease honey bee colonies losses during the winter. *Diversity* 13, 228. <https://doi.org/10.3390/d13060228>.

Simpson, J., 1961. Nest climate regulation in honey bee colonies. *Science* 133, 1327–1333. <https://doi.org/10.1126/science.133.3461.1327>.

Southwick, E.E., Heldmaier, G., 1987. Temperature control in honey bee colonies. *BioScience* 37, 395–399. <https://doi.org/10.2307/1310562>.

Stabentheiner, A., Pressl, H., Papst, T., Hrassnigg, N., Crailsheim, K., 2003. Endothermic heat production in honeybee winter clusters. *J. Exp. Biol.* 206, 353–358. <https://doi.org/10.1242/jeb.000882>.

Stabentheiner, A., Kovac, H., Brodschneider, R., 2010. Honeybee colony thermoregulation – regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. *PLoS One* 5, e8967. <https://doi.org/10.1371/journal.pone.0008967>.

Stahlmann-Brown, P., Hall, R.J., Butt, R., McCall, B., Torres, G., Wright, T., 2023. Valuing over-winter colony losses for New Zealand's commercial beekeepers. *N. z. Econ. Pap.* 57, 184–190. <https://doi.org/10.1080/00779954.2022.2146527>.

Stalidzans, E., Berzonis, A., 2013. Temperature changes above the upper hive body reveal the annual development periods of honey bee colonies. *Comput. Electron. Agric.* 90, 1–6. <https://doi.org/10.1016/j.compag.2012.10.003>.

Stalidzans, E., Zacepins, A., Kviesis, A., Brusbardis, V., Meitalovs, J., Paura, L., Bulipopa, N., Liepniece, M., 2017. Dynamics of weight change and temperature of *Apis mellifera* (hymenoptera: apidae) colonies in a wintering building with controlled temperature. *J. Econ. Entomol.*, tow282 <https://doi.org/10.1093/jee/tow282>.

Steinhauer, N., Kulhanek, K., Antúnez, K., Human, H., Chantawannakul, P., Chauzat, M.-P., vanEngelsdorp, D., 2018. Drivers of colony losses. *Curr. Opin. Insect Sci.* 26, 142–148. <https://doi.org/10.1016/j.cois.2018.02.004>.

Switanek, M., Crailsheim, K., Truhetz, H., Brodschneider, R., 2017. Modelling seasonal effects of temperature and precipitation on honey bee winter mortality in a temperate climate. *Sci. Total Environ.* 579, 1581–1587. <https://doi.org/10.1016/j.scitotenv.2016.11.178>.

Szabo, T.I., 1985. The thermology of wintering honeybee colonies in 4-colony packs as affected by various hive entrances. *J. Apic. Res.* 24, 27–37. <https://doi.org/10.1080/00218839.1985.11100645>.

Van Der Zee, R., Gray, A., Pisa, L., De Rijk, T., 2015. An observational study of honey bee colony winter losses and their association with varroa destructor, neonicotinoids and other risk factors. *PLOS One* 10, e0131611. <https://doi.org/10.1371/journal.pone.0131611>.

van Dooremalen, C., Gerritsen, L., Cornelissen, B., van der Steen, J.J.M., van Langevelde, F., Blacquière, T., 2012. Winter survival of individual honey bees and honey bee colonies depends on level of varroa destructor infestation. *PLoS One* 7, e36285. <https://doi.org/10.1371/journal.pone.0036285>.

Vardakas, P., Mainardi, G., Minaud, E., Patalano, S., Rebaudo, F., Requier, F., Steffan-Dewenter, I., Hatjina, F., 2024. Unveiling beekeepers' use and preference of precision apiculture systems. *J. Apic. Res.* <https://doi.org/10.1080/00218839.2024.2355813>.

Wood, S.N., 2017. *Generalized Additive Models: An Introduction with R*, second ed. Chapman and Hall/CRC.

Zacepins, A., Brusbardis, V., Meitalovs, J., Stalidzans, E., 2015. Challenges in the development of precision beekeeping. *Biosyst. Eng.* 130, 60–71. <https://doi.org/10.1016/j.biosystemseng.2014.12.001>.

Zacepins, A., Karasha, T., 2013. Application of temperature measurements for bee colony monitoring: a review. In: *Conf. 12th Int. Sci. Conf. "engineering Rural Dev.", pp. 126–131*.

Zacepins, A., Kviesis, A., Komasilovs, V., Rido Muhammad, F., 2020. Monitoring system for remote bee colony state detection. *Balt. J. Mod. Comput.* 8, 461–470. <https://doi.org/10.2236/bjmc.2020.8.3.05>.

Zapata-Hernández, G., Gajardo-Rojas, M., Calderón-Seguel, M., Muñoz, A.A., Yáñez, K.P., Requier, F., Fontúbel, F.E., Ormeño-Arriagada, P.I., Arrieta, H., 2024. Advances and knowledge gaps on climate change impacts on honey bees and beekeeping: a systematic review. *Glob. Change Biol.* 30, e17219. <https://doi.org/10.1111/geb.17219>.