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Increased overwintering temperature reduces reproductive success of the solitary bee species *Osmia bicornis*

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Solitary wild bees play a key role as pollinators of wild plants and crops, but they are increasingly at risk from anthropogenic global change, such as climate warming. However, how warmer temperature during overwintering affects reproductive success of those bees remains largely unknown. In a semi-field experiment we assessed individual life-long reproductive success of 144 females of the solitary bee species *Osmia bicornis* that had been wintered at three different temperatures. Overwintering mortality of bees was on average 32% higher at winter temperatures of 8 °C compared to 4.5 °C–0 °C, at which almost all bees successfully emerged. After wintering at 4.5 °C and 8 °C females produced less offspring than after overwintering at 0 °C (26% or 36% less offspring, respectively). Although longevity and daily offspring production rate were not significantly affected, nesting duration of females wintered at 0 °C tended to be longer (+ 2.5 days) than that of bees wintered at 4.5 °C, which likely contributed to the higher offspring production at colder overwintering temperatures. Mortality and sex ratio of offspring was not significantly affected. While future studies should also consider climatic variation during winter, these findings indicate that increasing mean overwintering temperatures could threaten *O. bicornis* and potentially other solitary bee populations.

Keywords Climate change, Fitness, Global change, Reproductive success, Survival, Wild bee pollinator populations

Wild bees play a crucial role as pollinators of wild flowering plants and crops[1–3]. However, declines of wild bee abundance and diversity in several regions of the world have been reported e.g.[4,5]. Land use intensification, associated loss of habitats, and exposure to pesticides and climate change are considered important drivers of this decline[3,6,7].

Several aspects of climate change have been shown to affect insect pollinators, including phenological shifts and prolonged activity periods[8–10], more frequent extreme events, such as heat waves or droughts[11,12], or asynchronous changes in pollinator and flower phenology, resulting in disruptions of plant-pollinator interactions[13–15]. A poorly explored potential pathway of climate change impacts on insect pollinators is warmer temperatures during overwintering due to climate change. For example, warmer temperature during overwintering can increase fat body depletion and weight loss of solitary bees[16–18], resulting in enhanced mortality e.g. [19]. Solitary bees that overwinter as adults, such as *Osmia* sp., can be particularly vulnerable to suboptimal overwintering conditions compared other solitary bee genera, such as *Megachile*, overwintering in other life stages (e.g., pre-pupae)[8,20]. For bees that survive, increased temperature during winter might also result in reduced performance, longevity and reproductive success, with ultimate consequences for population dynamics[21]. But this remains, to our best knowledge, largely unexplored.

Thus, we hypothesize that temperatures above the optimal overwintering conditions of *Osmia* result in decreased longevity and/or offspring provisioning performance of these solitary bees as a consequence of high energy losses, associated with increased loss of weight and depleted fat body reserves during overwintering[22–24]. This could further result in reduced investment in terms of provisioned food resources per produced offspring, and consequently increased offspring mortality or male-biased sex ratio (as the considerably smaller males require less investment in terms of food provisioning[22,25]). Alternatively, it is conceivable that foundress bees

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that overwintered under sub-optimal conditions quickly replenish lost reserves after eclosion, thereby mitigating any impacts on their performance and reproductive success, as hypothesized for longer pre-wintering periods[23].

Here, we combine laboratory and semi-field experiments to test how warmer overwintering temperatures affect key determinants of reproductive success of the solitary bee species *Osmia bicornis* (L.). We address important knowledge gaps by quantifying impacts of elevated winter temperatures on a multitude of population-level reproductive variables across generations by tracking the same bees through overwintering all the way to the production of their own progeny and exploring potential underlying mechanisms. *Osmia bicornis* is a univoltine, generalist and widely distributed species in the Palearctic region with an important function as pollinator of a range of wild plant species and crops in Europe e.g. [26–29]. Despite its widespread distribution and adaptation to a relatively broad range of climatic conditions, certain life-history traits *O. bicornis* shares with other functionally important solitary bee species could make this model species particularly vulnerable to warmer overwintering temperature: (i) it overwinters as adults and (ii) emerges relative early in spring[30]. Both traits are predicted to lead to higher sensitivity to warmer overwintering temperatures, for example due to an expected lesser extent of metabolic depression during diapause and shorter time period of strongly decreased respiration compared to summer-active species that overwinter as prepupa[20]. It is also an important pollinator of many crops and wild plants in Europe[2,31] and is commercially available, polylectic and readily uses pre-built nesting cavities of nesting aids, making it a suitable model species for this study e.g[32,33]. Specifically, we address the following research questions:

(1) Do warmer temperatures increase mortality during overwintering of female *O. bicornis*

(2) How do warmer temperatures during overwintering affect longevity, nesting period, offspring provisioning performance and offspring production of hatched female bees?

(3) How do different overwintering temperatures of foundress bees affect mortality and sex ratio of their offspring?

Materials and methods

Study species

Osmia bicornis is a univoltine solitary bee species native to large parts of Europe that overwinters as adults[26]. After emerging in spring (typically beginning of April to mid-May in Central Europe), female bees mate with males that have emerged a few days earlier[30] and start nesting, readily using pre-built cavities of nesting aids. Females typically construct several nests with each several brood cells, separated from each other by a wall of mud (Fig. 1). Each brood cell contains an egg laid onto pollen-nectar provisions. After the developing larvae have consumed the food resources, they spin a cocoon and pupate after about two months and develop into adults about three to four months after egg hatching[26].

Overwintering experiment

Osmia bicornis females purchased from a local producer (Wildbiene & Partner AG, Switzerland) were overwintered under controlled conditions at three different temperature treatments: 0, 4.5 and 8 °C. As body size of bees could affect their sensitivity to different overwintering temperatures, emergence success and fitness consequences[8], the size of each cocoon was measured and it was ensured that cocoon sizes were not significantly different across overwintering treatments. Cocoon size (length) was measured with a digital slide gauge. To avoid an abrupt sharp decrease of temperature and to mimic natural conditions during fall, temperature was decreased slowly in 14-16-day steps to the final overwintering temperatures. On 1 October 2020, all bees were put to 15 °C, and on 16 October temperature was further reduced to 8 °C. The bees of the 8 °C treatment were kept under these conditions until the following spring. On 30 October, the 4.5 and 0 °C bees were transferred to 4.5 °C, and on 13 November the bees wintered at 0 °C reached their final wintering temperature. Humidity was kept at around 70% RH during the overwintering period. Temperatures were monitored throughout the whole overwintering period using HOBO UA-001-64 pendant temperature loggers. Bees of each overwintering treatment were overwintered in individual plastic containers allowing air circulation.

In spring 2021, eclosion of overwintered *O. bicornis* was induced (see below), the emergence rate determined, and emerged bees were weighed before transferring them to flight cages (see detailed description below). To minimize the potential influence of parasitism as a cause of mortality during overwintering rather than temperature effects, cocoons used for the experiment were screened for signs of parasitism, and cocoons considered parasitized were not used for the experiment. On 30 June 2021 all remaining cocoons of which no bee had emerged were opened. If parasitoids were found in a cocoon, it was excluded from the analysis of emergence rate (because death was not directly due to the wintering treatments). However, in total, only 6 out of 1032 cocoons were parasitised (0.6%), and parasitism rate did not differ across overwintering temperature treatments (LRT = 0.99, $P = 0.609$).

Semi-field experiment: experimental design and setup

To study consequences of overwintering temperatures on fitness related parameters, a semi-field experiment using flight cages was conducted at Agroscope near Zürich. The experimental field site was sown with field mustard (*Sinapis arvensis*, 1 g /m^2) as forage plants for the bees at the beginning of March (Fig. 1). *Sinapis arvensis* is an early flowering plant species suitable to feed *O. bicornis* by providing abundant floral resources during several weeks[34]. Soon after sowing, six large flight cages (steel frame covered with transparent nylon netting of ca. 1.15 (0.95–1.35) mm mesh size; Howitec Netting b.v, Netherlands; 9 m x 6 m; height: 2 m) were established at a minimum distance of 5 m to each other (Fig. 1). The flight cages were additionally covered with a nylon net of mesh width = 0.8 mm x 0.8 mm (Kulturschutznetz Rantai S48; Hortima AG, Switzerland) to prevent attack of mustard plants by pollen beetles. Each flight cage contained a nesting aid facing east and installed at a height of 1 m above the ground, consisting of 12 wooden plates (mdf) with 10 drilled holes each (120 nesting

Fig. 1. *Upper left*: flight cage (9 m × 6 m; height: 2 m) sown with blooming *Sinapis arvensis* as forage; *upper right*: video camera setup in front of a nesting unit; *lower left*: individually marked *O. bicornis* female (colour-digit marking); *lower middle*: snapshot of a visualized output of the automatic identification of nesting female bees according to their ID tags and their nests (cavity ID in which female constructs a nest) through the video-analysis software "BeeTracker" (see methods section); *lower right*: layer of the nesting unit with constructed nests containing brood cells covered with an acetate sheet allowing to track nesting and offspring production progress.

cavities per cage; depth: 8 cm; diameter: 0.9 cm) (Fig. 1). Nesting aids were protected against rain and excessive heat from direct sun exposure with a wooden box (Fig. 1),

After the onset of *S. arvensis* flowering, emergence of the differently overwintered *O. bicornis* was induced by transferring them into emergence boxes that allowed air circulation in a temperature-controlled room at 23 °C. Exclusively female bees emerged from cocoon sizes of 11.1 –13.0 mm length (representing the median sized bees (68.4% of measured cocoons) were considered for the semi-field experiment, in order to control for any influence of body size on fitness related measures[22]. From those bees, a total of 144 females, 48 of each overwintering treatment (0 , 4.5 and 8 °C) were individually marked, weighted and randomly assigned in equal numbers per overwintering treatment to each of the 6 flight cages (8 females of each overwintering treatment per flight cage). The used *O. bicornis* density is thus within the range of densities recommended by the Non-*Apis* working group of the International Commission for Plant-Pollinator Relationships (ICPPR) for semi-field studies with *O. bicornis* (up to one female per m^2 crop area[32]). Thus, the experimental study consisted of a block design in which each block (flight cage) contained the same number of females from each of the three overwintering treatments. This ensured overall equal floral resource availability across differently overwintered bees. Females were marked with a digit from 1 to 8 in three different colours (yellow, white, green; marking kit for honey bee queens, Imkereibedarf Wespi GmbH, Switzerland) (Fig. 1). Each colour-digit combination was represented once per cage and allowed individual recognition of nesting females.

Emergence and introduction of females into the flight cages was synchronized across overwintering treatments, to exclude any effects of weather conditions on fitness related variables see [25,35]. As overwintering temperature can slightly alter pre-emergence period (i.e., the duration of the time from induction of eclosion to eclosion[20]), induction of hatching (by transferring bees to 23 °C) slightly differed across overwintering treatments: induction of the 0 °C females was done on 25 May 2021, that of females wintered at 4.5 °C three days later (on 28 May 2021) and that of the females wintered at 8 °C again three days later (on 31 May 2021). These time periods of the staggered induction were identified by a previously performed emergence experiment designed for this purpose. As a result, female bees of the three overwintering treatments used in the semi-field experiment emerged highly synchronized (31 May to 2 June 2021). Similar numbers across wintering treatments were stored at 4 °C after emergence before introducing them into the flight cages on 2 June 2021. The slight difference in eclosion induction compared to the long overall overwintering period of more than 220 days is very unlikely to have significantly impacted the measured response variables. In fact, studies that assessed impacts of marked differences in overwintering periods of 30 days found no effect on longevity of female *Osmia* bees[36]. To allow mating, male *O. bicornis* were introduced four days prior to females (males also naturally emerge slightly before females[30]) at a rate of 1.5 males per female to ensure enough mating partners for females[23] in each flight cage (36 males per cage). Males were all overwintered at 4.5 °C and incubated on 28 May 2021.

Assessments of abdomen volume of overwintering females

To gain a better understanding of the mechanisms underlying potential impacts of overwintering temperatures on emergence rate and reproductive success of females, micro-CT examinations were performed to track potential changes in fat bodies and abdomen volumes during overwintering of 12 bees (cocoons) overwintered at both the 0 °C and 8 °C overwintering temperature treatment (Fig. S1). This number of specimens was a compromise between sufficient replication for statistical analysis and the ability to perform all scans in a reasonable time with sufficient resolution but without motion artefacts. Scans of all monitored cocoons were carried out at four measurement dates during the overwintering period on 1 October 2020, 1 December 2020, 1 February 2021 and 1 March 2021. Due to difficulties to unambiguously identify and quantify fat bodies, only changes in abdomen volumes considered as a proxy of changes in body mass[37,38] including fat bodies[39] were further analysed. Detailed protocols of micro-CT assessments and results are provided in the Supplementary Material (Appendix A).

Semi-field experiment: data collection

From the day of release until the last bee was observed in the flight cages (7 July 2021) the number of females roosting inside the nesting units during the night (after 8:30 pm) was counted as an estimate of females alive inside each cage[33]. Females spending the night outside the nesting units were rarely observed. This data was also used to estimate longevity (i.e., the time from hatching until the bee was last observed alive). Longevity data was complemented by analysis of recorded videos. Software analysis of video recordings was used to link individual nests with produced brood cells (offspring) to individual females. One week after introduction of bees into the flight cages when bees had started to nest (9 June 2021) a high-resolution video camera (Canon LEGRIA HF G50 4 K Camcorder) was set up at a distance of 1 m at a height of 1.5 m in front of each nesting unit using a tripod to film the nesting female bees (Fig. 1). Nesting units in each cage were filmed for at least 1 h daily (usually 1.5 h) during suitable foraging conditions between 10:00 and 17:00 h. Nesting units were filmed roughly simultaneously with separate cameras in each cage. Videos were analysed with the novel machine-learning based software “BeeTracker”[40]. The software is able to identify the ID-tags (colour-digit marking) of individual nesting female bees and their nests (the cavity ID a female bee is nesting in; Fig. 1). Software precision (proportion of correctly identified bees, nests and events such as leaving or entering the nest) is very high (96% in a similar study[40]). Additionally, if a cavity ID could not be assigned unambiguously to a bee ID, videos were manually checked to complete such missing information. In total, only 20 brood cells containing offspring could not be assigned to the female bee constructing them (1.4% of offspring produced) those cells and were not included in the analyses.

During the same period as the video recordings, progress in brood cell production was monitored, every day between 17:30 and 18:00 h. To this end, newly produced brood cells were photographed and marked daily with a permanent marker on the acetate foil covering the drilled nesting cavities of each wooden layer (Fig. 1). The photographs along with the markings were later used to determine the number of newly produced brood cells by each female.

After the semi-field experiment was finished in mid-October 2021, it was assessed for each brood cell whether the offspring reached the cocoon stage and metamorphosis was completed (i.e., adult stage reached in the analysis of offspring survival), whether it was still alive, and its sex.

Statistical analysis

To analyse effect of overwintering temperature (factor with three levels: 0 °C, 4.5 °C, 8 °C) on emergence rate (i.e., the proportion of emerging bees of the total overwintered bees; binary response variable with 1 = emerged, 0 = not emerged; sample size 1032 female *O. bicornis* cocoons with 0 °C: 345, 4.5 °C: 354, 8 °C: 333 cocoons) a generalized linear model (GLM) with a binomial error distribution was fitted. Three bees died while emerging (all overwintered at 8 °C); they were not included in the analysis of emergence rate. To test for the effects of overwintering temperature on emergence duration (i.e., the time between induction of eclosion and ecclosion of a bee; square-root transformed) of successfully emerged bees (0 °C: 326 female bees; 4.5 °C: 333 bees; 8 °C: 208 bees), and weight of bees after emergence used for the semi-field experiment (48 female bees of each of the three overwintering treatments) linear models (LMs) were fitted.

To test for effects of overwintering temperature on adult survival (i.e., longevity), a mixed-effects cox proportional hazards model with the random term cage ID was used. To test for effects of overwintering on pre-nesting duration and nesting duration (i.e., time from building of the first until building of the last brood cell; square-root transformed) (all bees having built at least one brood cell were included (0 °C: 34 bees, 4.5 °C: 31 bees, 8 °C: 25 bees), the number of brood cells produced and provisioning performance (i.e., the total number of brood cells produced divided by the nesting duration; including all bees having built at least one brood cell) were fitted with linear mixed effect models (LMMs), using cage ID as a random term. In order to evaluate the cumulative “population” level effect of overwintering temperature considering both number of offspring produced and emergence rate, we also calculated the expected total number of offspring by multiplying the number of offspring by the emergence rate observed under the different overwintering treatments.

To analyse variation in offspring survival (i.e. the proportion of offspring per bee having entered the adult stage before wintering of total number of offspring produced (total sample size: 0 °C: 510 offspring, 4.5 °C offspring: 341, 8 °C: 240 offspring; binary response 1 = success, 0 = failure) and sex ratio of offspring (all offspring having reached the adult stage were included (0 °C: 489 offspring, 4.5 °C: 313 offspring, 8 °C: 228 offspring; binary response variable: 1 = female, 0 = male) generalized linear mixed effect models (GLMMs) with binomial error distributions were fitted, including the random terms female adult bee ID nested in cage ID. To test for significant differences between treatment (overwintering temperature) levels, Tukey post-hoc tests were conducted using the "emmeans" function of the package "emmeans" (version 1.7.2[41]).

To further explore potential pathways on the observed effect of overwintering temperature on fecundity (i.e., the total number of produced offspring (brood cells)), its relationships with nesting duration, longevity and weight of nesting females was explored using Spearman rank correlation tests (allowing potentially non-monotonic relationships; results of analyses using Pearson correlation tests were qualitatively identical).

Assumptions of normality and homogeneity in LMs as well as normality of the random effect in LMMs[42] were visually checked[43]. The software R (version 4.0.3[44]) was used for data analysis. To obtain model parameters for the LMMs and for statistical inference the function "KRmodcomp" (package "pbkrtest"; version 0.5.1[45] was used. GLMs and GLMMs were tested for overdispersion and checked for model assumptions according to recommendations by[42]. Overdispersion was not detected in any models. Log-likelihood ratio tests were used for statistical inference for GLMs and GLMMs using the "drop1" function of the package "stats" (version 4.0.3[44]). For LMs and GLMs the package "stats" (version 4.0.3[44]), for LMMs the function "lmer" from the "lme4" package (version 1.1.27.1[46] for GLMMs the function "glmer" from the "lme4" package (version 1.1.27.1[46] and for the mixed-effects cox proportional hazards model the function "coxme" from the package "coxme" (version 2.2.16[47]) was used. Visualising the model outputs was done with the package "ggplot2" (version 3.3.5[48]).

Results

Development of abdomen volume during overwintering, emergence rate, emergence duration and weight of emerged bees

Overall, abdomen volume decreased during the overwintering period ($F_{1,71}$ = 24.87, $P<0.001$). However, it did not differ between wintering treatments ($F_{1,22}$ = 0.88, $P=0.427$) and also the slope of the decrease over time was not significantly different across temperature groups (non-significant interaction overwintering treatment × time: $F_{1,70}$ = 0.73, $P=0.395$; Fig S2). However, hatching rate of female *O. bicornis* was strongly affected by overwintering temperature (LRT = 159.88, $P<0.001$), and was markedly lower in the 8 °C treatment than in the other two overwintering temperature treatments (-32%), in which almost all bees hatched (Fig. 2). Of successfully hatched bees, hatching duration strongly varied across overwintering treatments ($F_{2,864}$ = 3704, $P<0.001$); it was longest for bees wintered at 0 °C (8.25 ± 0.09 days), intermediate for bees wintered at 4.5 °C (3.44 ± 0.06 days) and shortest for 8 °C bees (0.33 ± 0.03 days). Overall, overwintering temperature had no significant effect on the weight of successfully hatched female bees, despite a tendency of successfully hatched bees overwintering at warmer temperatures to be heavier ($F_{2,141}$ = 9.88, $P=0.065$; Fig. S3).

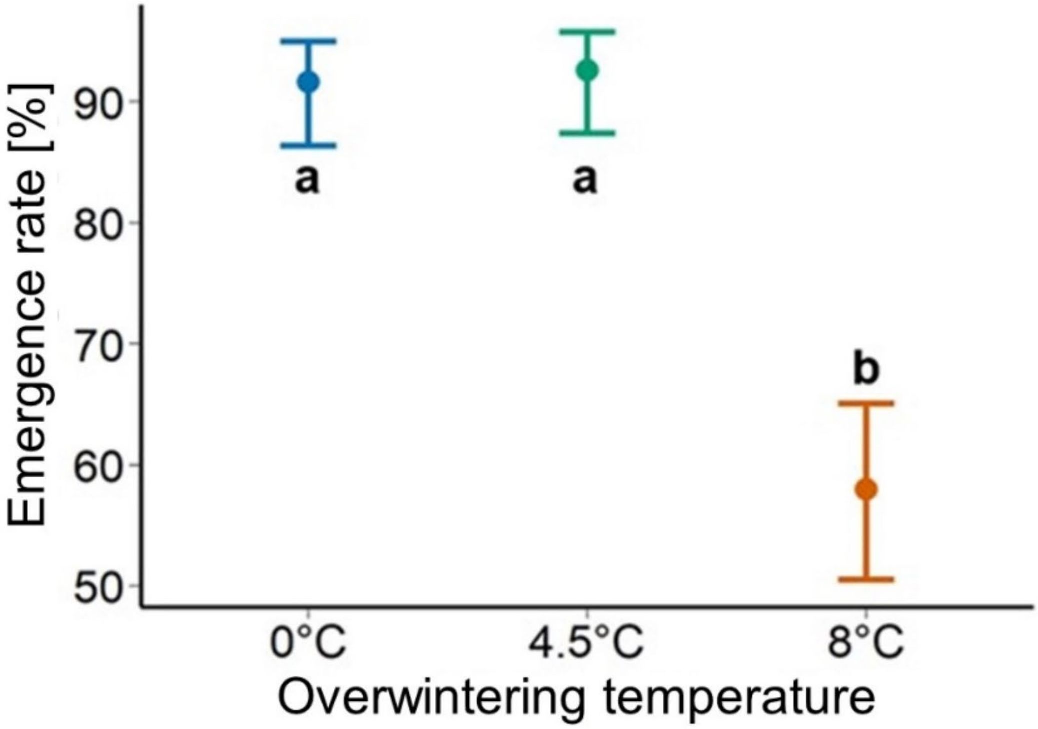

Fig. 2. Estimated mean (± 1 CI) emergence rate of female *O. bicornis* bees after overwintering at different temperatures. Different letters indicate significant differences ($P<0.05$) between overwintering temperatures according to Tukey's post-hoc tests.

Longevity, pre-nesting and nesting duration

Female *O. bicornis* lived for up to 35 days in the flight cages (Fig. 3; which is comparable to other semi-field experiments using *O. bicornis* as a model species[33], while under natural conditions females may typically live longer[28]). Longevity did not differ between the overwintering temperature treatments ($\chi2 = 4.32$, df = 3, $P = 0.229$; Fig. 3). However, overwintering temperature affected pre-nesting time ($F_{2,84.18} = 9.49$, $P < 0.001$), which ranged from 6 days to 13 days. It was shorter for *O. bicornis* females overwintering at 0 °C and 4.5 °C than for those overwintering at 8 °C (Fig. 4a). Nesting duration was affected by overwintering treatment ($F_{2,84.83} = 3.68$, $P = 0.029$); it was marginally significantly longer for bees overwintering at 0 °C than for those overwintering at 4.5 °C, but it did not differ between bees overwintering at 8 °C and 0 °C, or between 8 °C and 4.5 °C wintered bees (Fig. 4b).

Offspring production and offspring provisioning performance

Offspring (brood cell) production of successfully hatched females was affected by overwintering temperature ($F_{2,85.13} = 7.38$, $P = 0.001$). It was higher for female bees wintered at 0 °C compared to bees wintered at 4.5 °C and 8 °C (-26% and – 36%, respectively; Fig. 4c). Moreover, the expected number of offspring considering both emergence rate and number of produced offspring differed across overwintering treatments. It was higher for bees wintered at 0 °C (14.3) than for bees wintered at 4.5 °C (10.5; -27%) and 8 °C (6.1; -57%), respectively. However, the rate at which offspring (brood cells) were produced and provisioned (provisioning performance) did not significantly differ between overwintering temperature treatments ($F_{2,83.21} = 1.63$, $P = 0.203$; Fig. 4d).

Offspring survival and sex ratio

Offspring survival was not significantly different across differently overwintered foundress bees and was on average over 90% ($\chi2 = 4.05$, df = 1, $P = 0.132$; Fig. 4e). Further, offspring sex ratio produced by differently wintered foundress bees did not significantly differ; all three estimated means were close to an equal sex ratio of 0.5 ($\chi2 = 1.97$, df = 1, $P = 0.374$; Fig. 4f).

A strong positive monotonic relationship was found between the nesting duration and the total number of offspring (brood cells) produced per bee (Fig. 5a; $r = 0.86$, $P < 0.001$). In contrast, pre-nesting duration had a

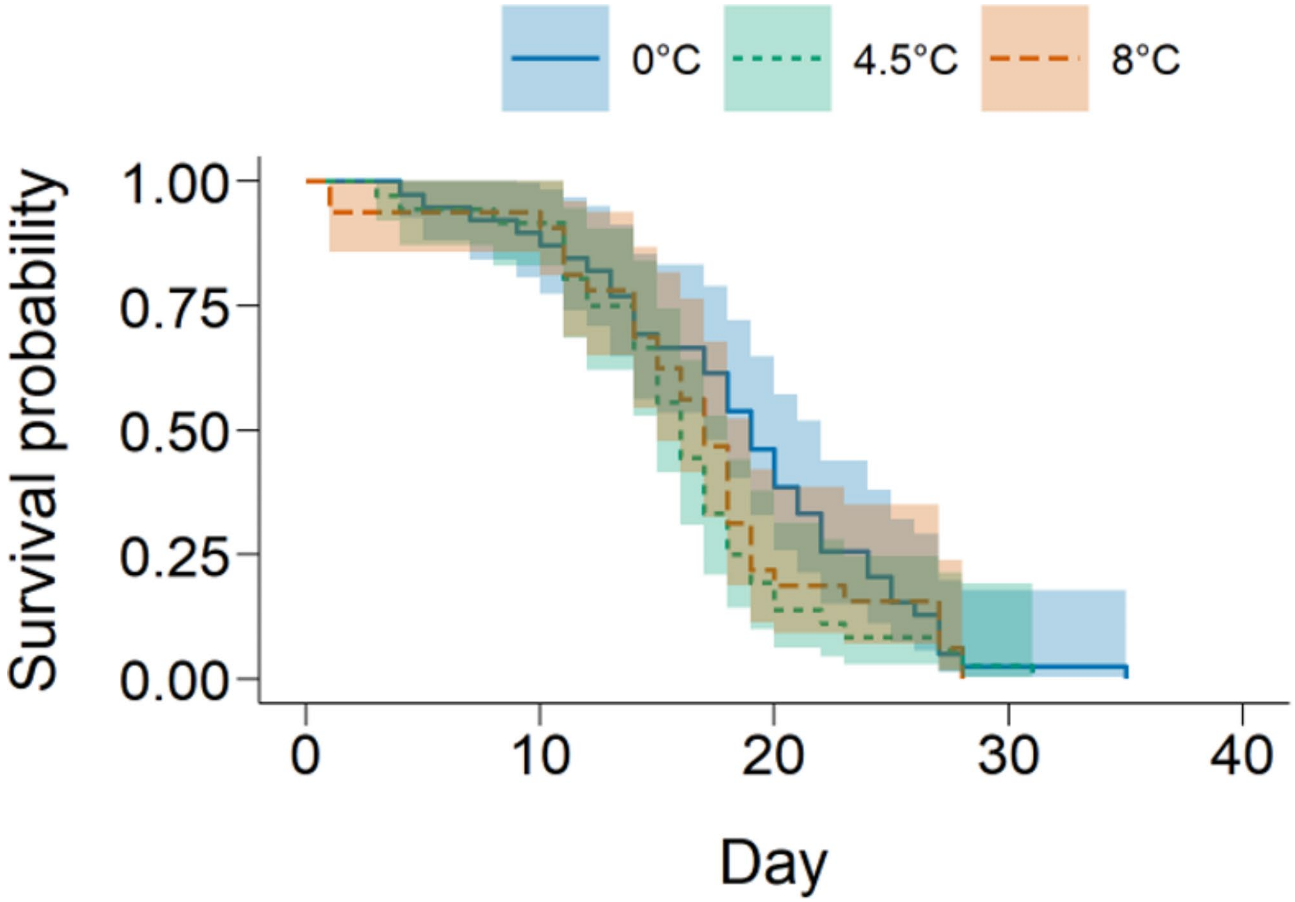

Fig. 3. Survival probability (Kaplan-Meier plot of the raw data) of adult female *O. bicornis* during the semi-field experiment (*O. bicornis* foraging and nesting in flight cages in the field sown with *Sinapis arvensis* as forage plant), after experimentally controlled overwintering at three different temperatures: 0 °C, 4.5 °C and 8 °C.

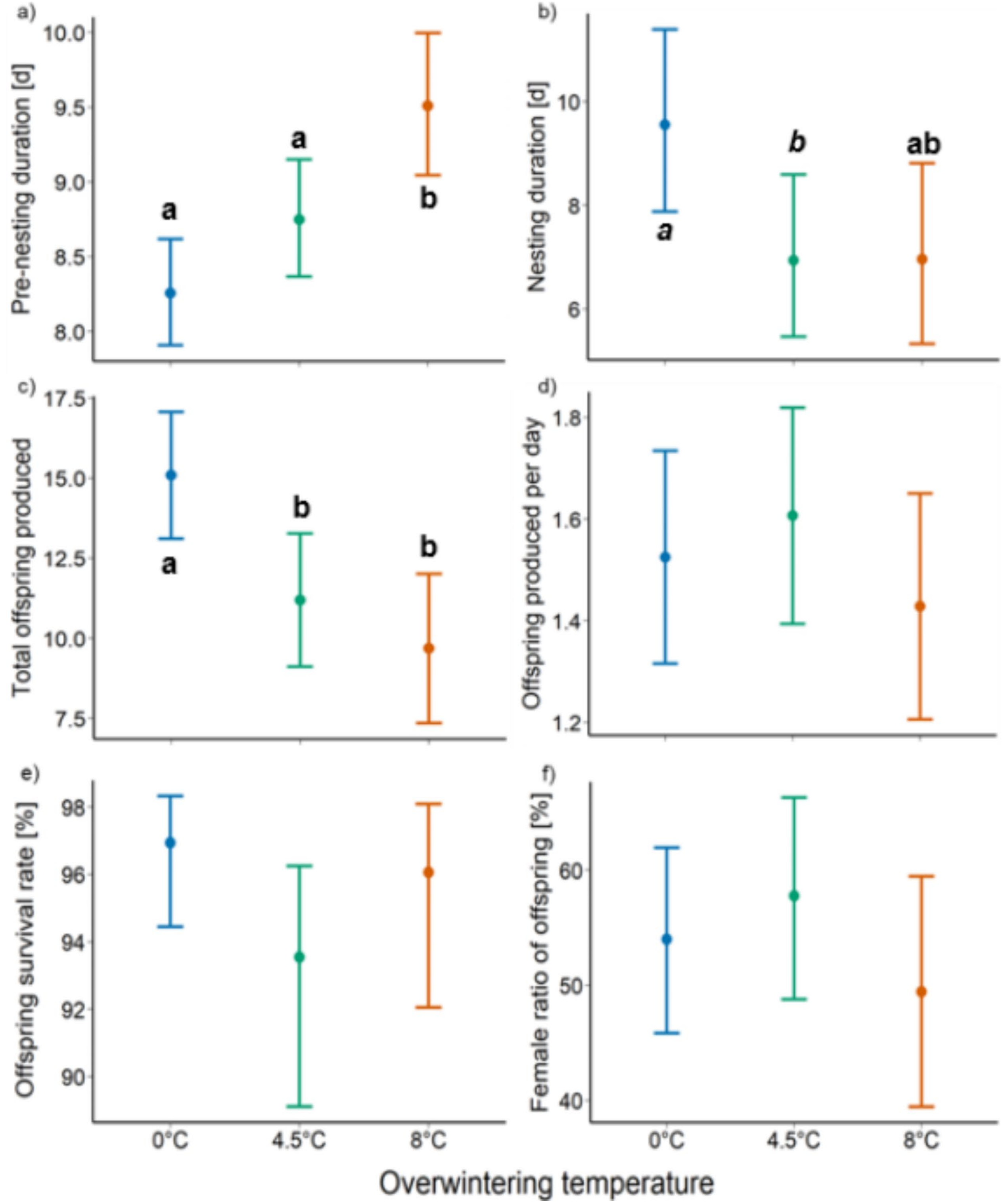

Fig. 4. Estimated means (± 1 CI) of (**a**) pre-nesting duration and (**b**) nesting duration, (**c**) total number of offspring produced, (**d**) daily offspring production, (**e**) offspring survival and (**f**) percentage of female offspring produced (offspring sex ratio) of *O. bicornis* studied under semi-field conditions (*O. bicornis* foraging and nesting in flight cages in the field sown with *Sinapis arvensis* as forage plant), after experimentally controlled overwintering at three different temperatures: 0 °C, 4.5 °C and 8 °C. Different letters indicate significant differences ($P < 0.05$) between overwintering temperatures according to Tukey's post-hoc tests.

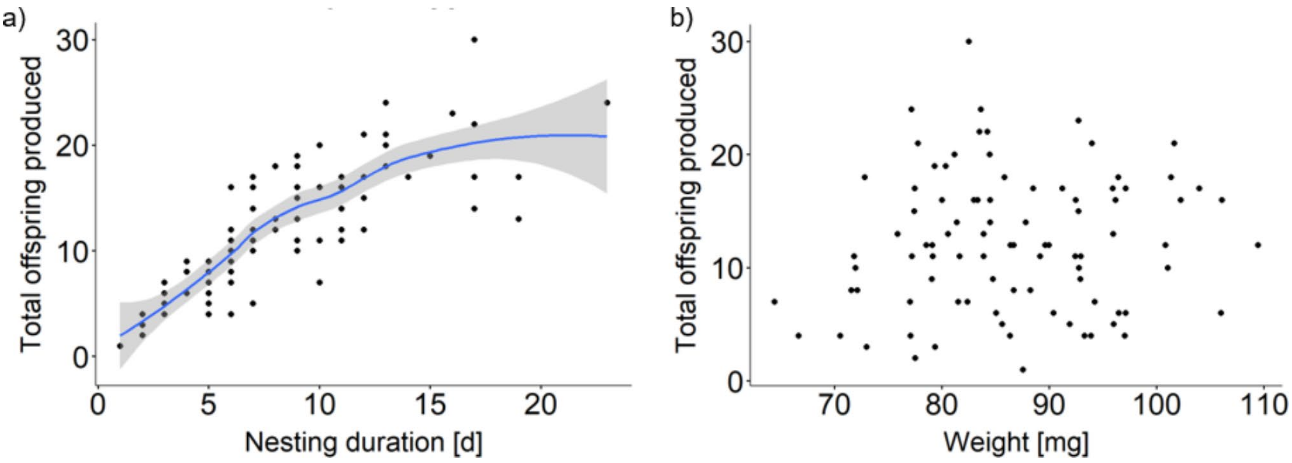

Fig. 5. Relationship between (**a**) nesting duration and (**b**) nesting female weight and the total number of offspring produced during the semi-field experiment. Raw data points (black dots) and a loess regression line with its CI (light grey area) are shown for the positive relationship of nesting duration and offspring production; no significant relationship was found between nesting female weight and offspring production.

negative relationship with the total number of brood cells produced (r = -0.33, P = 0.001). Furthermore, longevity and number of brood cells produced was positively correlated (r = 0.65, P < 0.001), but the slopes of these positive relationships did not significantly differ across overwintering temperature treatments (Fig S4). Adult female weight, however, was not significantly related to the number of brood cells produced (Fig. 5b; r = 0.08, P = 0.442), the pre-nesting duration (r = 0.05, P = 0.667) or the nesting duration (r = 0.07, P = 0.501). Longevity was also strongly positively correlated with nesting duration (r = 0.80, P < 0.001), but not with the weight of the bees after emergence (r = 0.04, P = 0.710).

Discussion

Our study is among the first assessing impacts of elevated temperatures during overwintering of a solitary bee species on multiple population-level reproductive variables by tracking the same bees through overwintering to the production of their own progeny. Findings show a strongly reduced emergence rate of female *Osmia bicornis* bees overwintered at 8 °C compared to bees wintered at 4.5 °C–0 °C. Moreover, warmer overwintering temperatures substantially reduced fecundity of successfully emerged females, with an average 36% and 26% fewer offspring produced by foundress bees wintered at 8 °C–4.5 °C, respectively, compared to those wintered at 0 °C. Considering the reduced fecundity together with the reduced emergence rate, the expected number of offspring per female wintered at 8 °C would be reduced by 57% compared to bees wintered at 0 °C. Our findings suggest that this impaired reproductive success was at least partly driven by shorter nesting periods of female *O. bicornis*, but not by reduced daily brood cell production or offspring provisioning rate. Offspring survival and sex ratio were not significantly affected by overwintering temperature.

The overwintering experiment demonstrates that while *O. bicornis* females wintered at cooler temperatures (0 °C and 4.5 °C) showed a low overwintering mortality (6% mortality in both treatments), females wintered at 8 °C had a strongly reduced emergence rate due to a higher overwintering mortality (38% mortality on average), reflecting a 32% increase in overwintering mortality. The mortality of bees overwintering at 8 °C is substantial, but in line with previous studies that found reduced survival of *Osmia lignaria* wintered over 7 °C, while an overwintering temperature of 4 °C was associated with a similarly low mortality[19].

Beyond mortality, the fecundity measured as the number of offspring produced by successfully emerged *O. bicornis* was strongly reduced if foundress bees had overwintered at warmer temperatures, and such impacts were already substantial at a relatively moderate increase from 0 °C to 4.5 °C. One hypothesized mechanism for the impacts on performance of *O. bicornis* overwintering at warmer temperatures is increased energy consumption. Higher metabolic rates and consequently a depletion of the fat reserves stored in the fat bodies of bees overwintering at warmer temperatures are predicted to result in less vigorous bees and reduced longevity[21] or offspring provisioning performance, and ultimately reproductive success [16,19,49]. In fact, some studies have observed increased fat body depletion in solitary bees, including *Osmia* bees, exposed to longer wintering periods and warmer overwintering temperatures, and especially for bees exposed to longer pre-wintering periods[16–20,49,50]. Such increased fat body depletion due to longer pre-wintering durations has also been proposed to account for reduced longevity of hatched *Osmia* bees[36,50]. Although we cannot assess the role of fat body depletion as an underlying mechanism of the observed effects as we were not able to unambiguously identify and quantify them, we could determine changes in abdominal volumes based on repeated micro-CT assessments. Despite an overall substantial decrease in abdominal volumes of overwintering bees, no evidence for stronger decreases under increased overwintering temperature was found. But these findings should be interpreted with caution considering that fat bodies could not be quantified directly and the relatively small samples size available for these analyses.

Interestingly, while bees across the temperature treatments were similar in size at the beginning, 8 °C bees tended to be heavier at the end of the overwintering period compared to bees wintered at cooler temperatures (Fig. S3). As high mortality rates occurred for the bees wintered at 8 °C, but not for bees wintered at cooler temperatures, it is conceivable that in the 8 °C treatment the larger bees, which were likely the heavier ones with more energy stores, survived and emerged. Yet, weight was not significantly related to any of the measured proxies of reproductive success in our study, and fecundity of females wintered at 8 °C was reduced rather than increased. This suggests that either fat body reserves of these slightly larger bees wintered at 8 °C were nonetheless more depleted than those of bees overwintering at cooler temperatures, or that other mechanisms contributed to the observed reduction in fecundity.

In fact, body weight of female *Osmia* bees has been found to be restored relatively quickly after eclosion[23,51], if adequate and readily accessible early spring floral resources are available. While in our study those resources were available, habitat fragmentation and degradation, or longer periods of unsuitable weather conditions might prevent emerged females from foraging under natural conditions. Indeed, there is likely a trade-off between recovering energy from fat bodies for the female herself and the provisioning of her offspring, that might be modulated by a shorter nesting duration observed for females that overwintered at warmer temperatures.

It has also been hypothesized that ovary maturation and the number of oocytes might be impaired by too warm temperatures during overwintering, due to reduced energy reserves available for ovary maturation through a depletion of fat bodies[17], or through impacts on protein content in ovaries (but see[52]), which could have contributed to the observed effects on nesting duration and offspring production associated with the different overwintering temperatures[23,52,53]. Differences in body size of female solitary bees, which have been found to be positively related to offspring provisioning performance and fecundity e.g. [22,54], can be ruled out in our study using bees of similar size across overwintering temperature treatments.

Irrespective of the exact physiological mechanism, our analyses indicate that in particular reduced nesting duration was an important pathway by which warmer overwintering temperatures negatively affected fecundity and reproductive success of *O. bicornis* females. Similar positive relationships between longevity, or nesting duration, and reproductive success have been observed in studies of impacts of altered pre-wintering periods[22–24]. Although nesting duration can be expected to be positively related to longevity, this is not necessarily the case, as bees can vary in their pre-nesting period, i.e., the time period from hatching to the start of nesting, as well as the period from the last offspring produced until the death of a female bee. In fact, the increased pre-nesting period of bees overwintered at warmer temperatures may at least partly explain the significantly negative effect on nesting duration but not longevity. Nesting duration was positively related to the total number of offspring produced and predicted offspring production better than longevity. Nesting duration of bees overwintering at 0 °C was on average 2.5 days longer than that of bees of the other two overwintering treatments, which is substantial considering that a female *O. bicornis* was predicted to produce on average approximately 1.5 offspring per day (0 °C: 1.52, 4.5°: 1.61, 8 °C: 1.43). In fact, this matches with the observed increase of four additional offspring produced by females wintered at 0 °C compared to those wintered at 4.5 °C. The observed difference in nesting duration was only partly explained by dissimilarities in pre-nesting duration, which was roughly 1.25 days longer for female bees wintered at 8 °C compared to those wintered at 0 °C.

In addition to changes in nesting duration, altered offspring provisioning performance of less vigorous female bees could influence the impact of increased overwintering temperatures on reproductive success[22,54]. For example, faster completion of brood cell construction and offspring provisioning with pollen and nectar resulted in increased offspring production by bigger females of *Megachile apicalis*[55]. Moreover, the amount of pollen and nectar provisioned by female solitary bees per foraging bout, and thus the number of flights required to provision a brood cell or the time needed per foraging trip can vary across female bees of different age or size[53]. Although we could not directly measure deposited total pollen-nectar provisions, collected amounts of provision per foraging bout or foraging bout duration, overall provisioning performance measured as daily brood cell completion did not differ between females from different overwintering temperature treatments. Additionally, neither offspring mortality, nor sex ratio nor body mass of female or male offspring were significantly affected by overwintering temperature of foundress bees, suggesting that food resources provisioned to offspring by foundress bees subjected to different overwintering temperatures did not differ substantially.

The studied warmer average winter temperature of 4.5 °C compared to 0 °C lies within ranges of current and predicted temperature changes during winter in Switzerland until 2085 compared to the norm-period ranging from 1981 to 2010: scenarios include predicted changes to up to 5.4 °C (scenario RCP8.5: no further measurements to reduce global greenhouse gas emissions are taken) compared current mean winter temperature (-1 °C to 1 °C;[56]). More optimistic models (under assumption of a drastic reduction of greenhouse gas emissions globally) predict a rise in mean winter temperature only up to 2 °C (RCP2.6) or under an intermediate scenario (RCP4.5) to about 3 °C[53]. However, the Swiss Federal Office of Meteorology and Climatology (MeteoSwiss) reported February 2024 as the warmest on record with 4.6 °C above the 1991–2020 long-term average for Switzerland[57,58]. Thus, the here tested increase of 4.5 °C in average winter temperature cannot be considered as a pessimistic long-term scenario for the distant future, but has already been realized. Nevertheless, it is important to further explore how *O. bicornis* and other solitary bee species respond to smaller average temperature increases during winter, and to explore also other aspects of predicted climatic changes during the overwintering period of solitary bees, such as the role of altered maximum (and minimum) daily temperatures and predicted stronger fluctuations in daily temperatures during the overwintering period [59], and to what extent they are capable to adapt to altered climatic conditions during their overwintering period, as such changes likely occur over very long time periods. For example, CaraDonna et al. [59] performed a neat field experiment across two years with differently coloured nest boxes used by the model solitary bee species *O. ribifloris* to simultaneously study the impact of warmer (up to 2.6 °C warmer average temperature in one experimental year) or cooler mean daily temperatures, as well as changes in minimum or maximum daily temperature (up to 6.6 °C increase in maximum daily temperature in one study year) inside nesting boxes. Warmer (but not cooler) mean and maximum daily temperature inside nest boxes resulted in delayed emergence phenology, reductions in body mass, fat content and substantial mortality of bees. More such studies and experiments that integrate more complex temperature regimes considering typical current and predicted daily and seasonal fluctuation of temperatures and extreme temperature events are needed that will provide important additional insights beyond those obtained here using a constant temperature regime towards an improved understanding of the impact of changes in climatic overwintering conditions on solitary bee populations under field conditions. Moreover, future studies should examine the role of life-history traits such as the life stage in which a bee overwinters and its phenology (e.g., how early in the season its activity period starts)[8,10,20]. Furthermore, the research should, also study bee species with more narrow climatic distributions than the here studied *O. bicornis*, for which impacts of warmer overwintering temperature could have even more severe consequences.

Conclusions and implications

Our findings indicate that already a relatively modestly warmer average overwintering temperature of 4.5 compared to 0 °C can markedly reduce fecundity and reproductive success of hatched *O. bicornis* females, with likely profound adverse impacts on population growth of this solitary bee species. These negative impacts on fitness and population development would not have been detected by considering overwintering mortality alone, as it was similar between female bees wintered at 0 and 4.5 °C. Under a more extreme scenario of an increase in mean temperature to 8 °C reproductive success of hatched females was further impaired, and also overwintering mortality strongly increased, which would have drastic negative long-term impacts on population development. Thus, climate warming induced warmer temperatures during the overwintering period might pose a *hitherto* underestimated threat to *O. bicornis* and potentially other solitary bee populations, which additively or synergistically exacerbate impacts of further anthropogenic stressors, such as habitat loss and fragmentation or land-use intensification.

However, more experiments and field studies are needed to further explore underlying mechanisms driving shorter nesting duration and impaired reproductive success, which consider how ongoing and predicted increased maximum daily temperature and temperature fluctuations during overwintering affect mortality, reproductive success and population development of solitary bee species, considering also the role of parasitism, to further improve our understanding of their impacts on solitary bee populations under field conditions. Finally, it is important to better understand how solitary bee species with distinct life-histories and overwintering stages respond to altered climatic conditions during the overwintering phase.

Data availability

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.3tx95 × 6pt.

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Author contributions

HR, RZ, JC and MA conceived the study; JC, SM and MA designed the overwintering experiment with contributions from HR and RZ; MA and SM designed the semi-field experiment with contributions from JC, HR, RZ; SM collected the experimental data; HR and RZ designed and performed the micro-CT analyses; SM and MA analysed the data with contributions of JC, HR and RZ; SM wrote the first draft of the manuscript with significant contributions of MA; all authors contributed to writing and gave final approval for publication.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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