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Sustained warming alters Bombus impatiens foraging, colony performance, and pollination of zucchini (Cucurbita pepo)

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Abstract

Climate change is intensifying heat exposure for managed pollinators, yet most bumble bee heat stress studies use short-term heatwave designs. We exposed *Bombus impatiens* micro-colonies and *Cucurbita pepo* plants to three sustained temperature treatments (control (29.5°C day/21°C night), + 1.5°C, and + 3°C) in growth chambers for approximately ten weeks, with three colonies per treatment. Queen weight was significantly lower at + 3°C; adult colony size did not differ. Fanning rate increased approximately 20x at + 1.5°C, while brood-care declined significantly under both warming levels. Within-hive spatial clustering was disrupted at + 3°C, with significantly reduced aggregation in brood-adjacent and entrance-adjacent zones. Nectar volume nearly doubled at + 3°C, and bee visitation was approximately 70% higher, yet zucchini fruit set per female flower was lowest under moderate warming. Colony responses were non-linear: moderate warming elicited active behavioral compensation, while extreme warming exceeded compensatory capacity and disrupted spatial organization. Elevated visitation reflected plant-mediated nectar increases rather than improved colony condition and did not translate into proportional yield gains. These results indicate that sustained warming can decouple pollinator activity from crop production, and that caste-specific physiological costs and floral reward conditions require monitoring in commercially managed bumble bee systems under climate change.

Introduction

Rising temperatures and the increasing frequency of extreme heat events in temperate and subtropical agricultural regions are accelerating due to climate change [1]. Pollinators can experience heat stress directly through physiological limits on flight, foraging, and reproduction, and indirectly through changes in the floral resources they rely on [2, 3]. Bumble bees (*Bombus* spp.) are particularly vulnerable because their large body size and high metabolic heat production make thermoregulation difficult at elevated temperatures [3, 4]. Population declines of bumble bees reaching approximately 46% have been documented in Europe and North America, with the steepest reductions in regions experiencing warmer-than-average climates [5]. Some North American bumble bee species have declined most steeply in regions that have warmed by more than 1°C since 1980, with species ranges shifting northward and to higher elevations [6]. Continental-scale evidence further indicates that warming summer temperatures are already restructuring North American bumble bee communities, with measurable shifts in species occurrence and abundance occurring in real time rather than as projected future change [7]. A 0.6°C rise in global temperature has also produced shifting plant phenology and mismatches between plants and pollinators; pollinator-dependent crops are consequently more vulnerable to resource limitation [8].

Previous research has shown that elevated temperatures may reduce foraging activity, flight performance, and survival across pollinator taxa [3, 4, 9]. For bumble bees specifically, threshold internal temperatures for flight function lie near 30°C, and air temperatures above 35°C make heat dissipation difficult until a maximum heat stupor occurs at approximately 40–55°C [3]. In addition to reducing flight capacity, elevated temperatures can impair colony development by increasing energetic demands for

thermoregulation and reducing worker allocation to brood care and foraging tasks [3, 4, 9]. Bumble bee declines also reflect the combined effects of climate change, pesticides, and land use change, as recent multi-stressor analyses of the western bumble bee have shown [10], so colony-level heat stress findings may need to be interpreted alongside other concurrent stressors operating in agricultural systems.

Floral resources may also alter their reproductive morphology under sustained heat. Previous research has shown that nectar volume and sugar concentration can rise under moderate warming, possibly through accelerated metabolic activity in nectary tissues up to a thermal optimum [11]. We chose *Cucurbita pepo* (zucchini squash) for this study because the flowers stay open for only a single morning, the crop depends entirely on insect pollinators, and male and female flowers appear on the same plant, making them an ideal system for measuring how warming alters floral reward, pollinator visitation, and fruit set [12].

The commercial eastern bumble bee, *Bombus impatiens*, is a buzz-pollinating species native to eastern North America and the primary managed pollinator used in open-field and greenhouse cucurbit production throughout the eastern and southeastern United States. In Florida, commercial *B. impatiens* deployment for squash production is concentrated in Collier County and northward, where *Cucurbita* crops are grown during the cooler months (September through March in South Florida)[13], conditions more thermally suitable for *B. impatiens* than the hot summer season. Miami-Dade County, though among Florida's most productive agricultural regions, is generally too warm for commercial *B. impatiens* use even during the winter months. Colonies are commercially produced and shipped to growers in small starter units (micro-colonies) containing a queen and at least 70 workers each. We selected *B. impatiens* as our focal species because of this direct commercial relevance to Florida cucurbit systems and because the species has documented thermal tolerance limits[3, 4] that fall within the warming range currently projected for the southern United States.

Most experimental tests of bumble bee heat stress have used acute heatwave designs in which colonies are exposed to elevated temperatures for hours or days [2, 9]. Heatwave studies have shown that workers fan and thermoregulate at higher rates under elevated temperatures and that direct heat exposure may reduce flight bout duration and flower visitation. To our knowledge, however, no prior study has tested how sustained warming over the active lifespan of a *B. impatiens* colony affects queen weight, worker behavior, within-hive spatial organization, floral reward, bee visitation, and fruit yield simultaneously. Recent reviews have specifically called for explicit measurement of sub-lethal heat effects, carry-over effects across colony stages, and colony-level compensation in bumble bee thermal biology [14]. Our chronic warming design addresses these gaps by tracking morphological, behavioral, and spatial responses simultaneously within the same experimental framework.

Climate projections for the southern United States anticipate a rise of approximately 1.5°C within the next two decades and approximately 3°C by 2100 [1]; future commercial pollination systems will therefore operate under sustained temperature elevations rather than discrete heatwave episodes alone.

Hand pollination of crops at an industrial scale is not economically feasible (an estimated $18 million for 1,600 ha), which has led to the worldwide adoption of buzz-pollinating bumble bees in commercial greenhouse production[15]. Native populations of *B. impatiens* and other *Bombus* species also pollinate cucurbit and other agricultural crops in the eastern U.S., so colony-level responses to warming may have implications for wild pollinator service as well as for commercial pollination. However, wild bumble bee colonies nest underground, where soil buffering substantially moderates external air temperature; the thermal conditions experienced by commercial above-ground hives in this study should not be extrapolated directly to wild colony responses without accounting for that buffering. In south Florida, the specialist squash bee *Peponapis pruinosa* is absent[12], potentially making managed bumble bees the primary viable buzz-pollination option for regional cucurbit production.

We tested six predictions. First, queen weight, colony population size, and individual bee biomass will decline with increasing temperature, because chronic heat may impose energetic and developmental costs on the queen and on overall colony growth. Second, fanning rate per visible worker will increase under warming, while brood-care and grooming rate will decline, as thermoregulation may divert worker effort away from brood maintenance. Third, within-hive spatial clustering in brood, brood-adjacent, and entrance-adjacent zones will decline under warming, as workers may disperse to dissipate heat at the cost of colony coordination. Fourth, nectar volume will increase under moderate warming, possibly through accelerated metabolic activity in nectary tissues up to a thermal optimum. Fifth, higher nectar volume in warmed flowers will attract more bee visits, such that visitation scales with nectar availability across treatments. Sixth, fruit set per female flower will drop under warming because heat may impair reproductive efficiency in *C. pepo* even when pollinator visit rates remain high. We expected the + 3°C treatment to produce the largest absolute effects and tested whether responses scaled linearly with temperature or whether thresholds emerged at the higher exposure that were not predicted from the + 1.5°C response.

Results

A total of nine *B. impatiens* micro-colonies (three per temperature treatment) were maintained continuously in their assigned growth chambers for ten weeks each, corresponding to the active lifespan of each experimental colony. Dissection at the end of each colony yielded data on queen weight, individual worker and male weights, total adult counts, brood and honeycomb structure, and within-hive spatial organization quantified from weekly video recordings (n ≈ 90 video sessions total across the experiment).

Colony morphology and reproductive traits

Queen weight differed significantly among temperature treatments (ANOVA $F(2,6) = 12.07$, $p = 0.0038$). The + 3°C treatment yielded queens with the lowest average weight (≈ 0.26 g), significantly lighter than those in both the control (≈ 0.63 g) and the + 1.5°C treatment (≈ 0.75 g) (Fig. 1). Male bee weight per

individual showed no significant differences among treatments, although mean male weight was lowest in the warmest treatment.

Other morphological variables, including dissected honeycomb weight, pupa and larvae count and weight, total bee biomass, queen width, and queen length did not differ significantly across treatments (Table 1).

Table 1
Effects of temperature treatment on additional bumble bee colony characteristics at hive dissection.

Variable	ANOVA F	p-value
Pupa weight (g)	F(2, 6) = 2.685	0.136 (NS)
Total bee weight (g)	F(2, 6) = 0.098	0.907 (NS)
Queen width (mm)	F(2, 6) = 1.496	0.281 (NS)
Queen length (mm)	F(2, 6) = 0.828	0.471 (NS)
Honeycomb weight (g)	F(2, 5) = 0.144	0.869 (NS)
Note: p-values from one-way ANOVA. NS = not significant ($p > 0.05$). No post hoc comparisons were applied.		

Colony population size

Total adult bee count per hive did not differ significantly among temperature treatments (Kruskal–Wallis $H = 0.472$, $p = 0.790$). Although colony totals varied numerically among hives, no consistent effect of warming on final bee count was detected.

Worker behavioral responses

Across the ten-minute observation videos, worker behavior was quantified from 30-second intervals and analyzed as counts with an offset for the total number of visible workers per interval. Relative to control colonies, colonies exposed to + 1.5°C showed a substantially higher fanning rate per visible bee (incidence rate ratio [IRR] = 20.01, $p < 0.001$) and a significantly lower brood-care rate (IRR = 0.57, $p < 0.001$) (Fig. 2; Table 2). Colonies at + 3°C showed the same directional shifts but only brood-care rate declined significantly (fanning $p = 0.071$; brood-care IRR = 0.70, $p < 0.001$).

Table 2
Worker behavioral rates and within-hive spatial clustering indices across the three temperature treatments.

Response	Control	+ 1.5°C	+ 3°C	Statistic and p-value
Fanning rate (per visible bee)	Baseline	Much higher	Higher (NS)	IRR = 20.01, p < 0.001 (+ 1.5°C); p = 0.071 (+ 3°C)
Brood-care rate (per visible bee)	Baseline	Much lower	Much lower	IRR = 0.57, p < 0.001 (+ 1.5°C); IRR = 0.70, p < 0.001 (+ 3°C)
Grooming rate (per visible bee)	Baseline	Lower (p = 0.074)	Much lower	IRR = 0.23, p = 0.074 (+ 1.5°C); IRR = 0.09, p = 0.004 (+ 3°C)
Colony clustering score (1–5)	Mean = 2.1 ± 1.0	All scores = 1	All scores = 1	Descriptive only (invariant under warming)
Brood-zone clustering index	1.87 ± 0.31	1.83 ± 0.38	1.58 ± 0.14	Treatment effect NS
Brood-adjacent zone clustering	Baseline	Similar to control	Lower	p = 0.014 (+ 3°C); +1.5°C NS
Entrance-adjacent zone clustering	Baseline	Similar to control	Much lower	p < 0.001 (+ 3°C); +1.5°C NS

Note: Behavioral rates analyzed using negative binomial or Poisson GLMs with visible worker count as offset. Clustering indices analyzed using linear mixed-effects models with hive as random intercept. NS = not significant (p > 0.05).

Grooming rates per visible bee differed significantly among treatments (Wald $\chi^2(2) = 10.04$, p = 0.007). Relative to control colonies, grooming was significantly lower at + 3°C (IRR = 0.09, p = 0.004), but the reduction at + 1.5°C provided weak evidence for an effect (IRR = 0.23, p = 0.074). Walking, flying, and resting rates did not differ significantly among temperature treatments (all p > 0.05).

Within-hive spatial clustering

Colony-level clustering scores varied with treatment, with the strongest effects at + 3°C (Table 2). Under + 3°C, clustering in the brood-adjacent zone (p = 0.014) and the entrance-adjacent zone (p < 0.001) dropped significantly below control. Workers in the + 3°C hives were less aggregated in zones critical for brood access and for traffic in and out of the entrance. Moderate warming (+ 1.5°C) did not significantly affect clustering in these zones.

Although brood-zone clustering indices were numerically lower under + 3°C, this effect was not statistically significant, and overall colony-level clustering scores could not be analyzed statistically because scores were invariant within the warming treatments. Descriptively, however, aggregation decreased with increasing temperature. Together, these results suggest that while core brood aggregation may be maintained under heat stress, spatial organization in surrounding functional regions becomes disrupted under more extreme temperatures.

Nectar volume and sugar concentration

Nectar volume differed significantly among temperature treatments ($F(2, 42.33) = 4.79$, $p = 0.013$) and declined over time across all treatments ($F(1, 141.34) = 21.19$, $p < 0.001$; Table 3). Pairwise comparisons showed that nectar volume was significantly higher at + 3°C (8.35 ± 1.15 µL) than in the control (4.26 ± 0.68 µL; difference = 4.30 ± 1.47 µL, $p = 0.018$; Fig. 3a). The + 1.5°C treatment (5.69 ± 0.79 µL) did not differ significantly from control. Nectar sugar concentration (°Brix) increased numerically from 32.14 ± 1.86 in the control to 36.46 ± 1.59 at + 1.5°C and 36.49 ± 1.13 at + 3°C, but the treatment effect was not significant ($F(2, 45.7) = 0.86$, $p = 0.432$; Fig. 3b). However, nectar sugar concentration declined significantly over time across treatments ($F(1, 189.3) = 31.06$, $p < 0.001$).

Table 3
Pollinator visitation, fruit production, and floral rewards in *Cucurbita pepo* across the three temperature treatments.

Trait	Control (mean ± SE)	+ 1.5°C (mean ± SE)	+ 3°C (mean ± SE)	Statistic and p-value
Bee visitation (count per flower)	4.000 ± 0.727; n = 28	3.594 ± 0.785; n = 32	7.000 ± 0.897; n = 32	$\chi^2(2) = 13.07$, $p = 0.001$ (Neg. binomial GEE)
Zucchini fruit count	1.727 ± 0.210; n = 22	1.111 ± 0.111; n = 9	1.211 ± 0.123; n = 19	$\chi^2(2) = 4.36$, $p = 0.113$ (Poisson GEE)
Fruit set per female flower	0.427 ± 0.073; n = 19	0.197 ± 0.080; n = 20	0.360 ± 0.077; n = 20	$H = 10.215$, $p = 0.006$ (Kruskal–Wallis)
Nectar sugar concentration (°Brix)	32.136 ± 1.858; n = 22	36.460 ± 1.592; n = 20	36.488 ± 1.134; n = 25	$F(2, 45.73) = 0.86$, $p = 0.432$ (LMM)
Nectar volume (µL)	4.26 ± 0.68; n = 21	5.69 ± 0.79; n = 16	8.35 ± 1.15; n = 21	$F(2, 42.33) = 4.79$, $p = 0.013$ (LMM)

Note: LMM = linear mixed model with pot as random intercept and time as covariate. GEE = generalized estimating equations with sampling day as time covariate. Kruskal–Wallis pairwise comparisons used Mann–Whitney U tests with Holm correction.

Bee visitation to flowers

Bee visitation differed significantly among temperature treatments (Wald $\chi^2 = 13.07$, $df = 2$, $p = 0.001$; Table 3). Compared to control, visitation was significantly higher at + 3°C (B = 0.532 ± 0.240; IRR = 1.70, 95% CI 1.06–2.72; $p = 0.026$), with + 3°C plants receiving approximately 70% more bee visits per flower than control plants across the experimental period (7.00 ± 0.90 vs 4.00 ± 0.73 visits per flower; Fig. 4). Visitation at + 1.5°C (3.59 ± 0.79 visits per flower) was statistically the same as control (IRR = 0.79, $p = 0.253$). The visitation increases at + 3°C tracked the elevated nectar volume in the same treatment.

Fruit count and fruit set per female flower

Raw zucchini fruit count per pot did not differ significantly among treatments (Wald $\chi^2 = 4.36$, $p = 0.113$; Table 3). Standardizing yield by female flower production revealed a clearer pattern: zucchini fruit produced per female flower differed significantly among treatments (Kruskal–Wallis $H = 10.22$, $df = 2$, $p = 0.006$). Pairwise Mann–Whitney tests with Holm correction showed that control plants produced significantly more zucchini per female flower (0.43 ± 0.07) than + 1.5°C plants (0.20 ± 0.08; $U = 80$, $p = 0.002$; Fig. 5). Control and + 3°C plants (0.36 ± 0.08) did not differ significantly ($U = 158$, $p = 0.365$), and the + 1.5°C and + 3°C treatments were not statistically different after Holm correction ($U = 123$, $p = 0.060$).

Discussion

B. impatiens colonies responded to sustained warming through physiological and behavioral changes whose magnitude did not scale linearly with temperature. Queen weight declined significantly at + 3°C, while total colony size and male weight did not differ statistically across treatments. Worker behavior shifted markedly under moderate warming, with elevated fanning and reduced brood-care; spatial organization within the hive became disrupted only under extreme warming. The non-linearity of those responses suggests that moderate warming elicited active colony compensation, whereas more extreme warming exceeded the capacity of those mechanisms.

Caste-specific responses to sustained warming

Queen weight declined significantly under sustained extreme warming (+ 3°C), a pattern in agreement with prior reports that chronic heat stress may reduce body size in *B. impatiens* [3, 4]. Total population size, in contrast, did not vary significantly across treatments; the highest colony numerical counts occurred in the + 1.5°C group, and the means at control and + 3°C overlapped. Male weight and the remaining hive-level metrics were unaffected. These results may therefore indicate a selective temperature response, with queen development and overall bee count more sensitive to warming than the other traits we measured. Over a longer warming window, queen-level costs of this kind may be expected to accumulate at the colony level.

The decline in queen weight together with maintained total bee biomass per colony may represent a decoupling between caste-specific and colony-level effects. Heat-stressed colonies may reallocate resources within the hive, maintaining total biomass at the cost of investment in specific castes or in reproduction. Total biomass alone is therefore insufficient as an indicator of colony health under thermal stress, because caste-specific metrics and behavioral responses varied despite stable overall biomass.

Worker behavior and spatial organization

Worker behavior and within-hive organization both responded to warming, but this response was not uniform across treatment levels. These patterns are consistent with prior experiments showing that heat exposure reduces bumble bee foraging performance once air temperature exceeds the thermal optimum for flight [2]. At + 1.5°C, workers fanned significantly more and reduced time spent on brood-care tasks;

this pattern is consistent with reallocation of effort from brood maintenance toward thermoregulation reported in other warming experiments [4]. At + 3°C, brood-care also declined significantly (IRR = 0.70, $p < 0.001$), although fanning at + 3°C provided only weak evidence for an effect ($p = 0.071$). The pattern at + 3°C may indicate that workers continue to reduce brood-care effort under sustained extreme warming, but that the fanning response may approach a physiological ceiling at higher temperatures, or that within-treatment variability in fanning at + 3°C limited statistical detection at this sample size.

Grooming behavior also declined significantly at + 3°C (IRR = 0.09, $p = 0.004$), with weak evidence for a reduction at + 1.5°C (IRR = 0.23, $p = 0.074$). Reduced grooming may reflect a broader reallocation of worker time toward thermoregulation and away from non-essential maintenance tasks, in addition to the reduction in brood-care reported above. Grooming behavior has been linked to colony hygiene and parasite resistance in bumble bees, so a sustained reduction in grooming under chronic warming may compound other stress effects on colony health.

Spatial organization within the hive responded to temperature unevenly across functional zones. The strongest effects were observed at + 3°C in the brood-adjacent and entrance-adjacent zones, where clustering values declined significantly relative to control hives. The same zones in the + 1.5°C hives did not differ from control. Brood-zone clustering remained above one across all three treatments, indicating that workers continued to aggregate over the brood. Colony-level clustering scores could not be tested statistically because the warming treatments lacked between-treatment variation.

These behavioral and spatial-organization results diverged. Moderate warming elevated fanning frequency and reduced brood-care time without a detectable shift in hive spatial organization. In contrast, under extreme warming, workers redistributed away from brood-adjacent and entrance-adjacent zones. Spatial dispersion under heat stress may be a thermoregulatory response that improves passive heat dissipation at the cost of colony coordination and brood-access pathways [22]. Crall and colleagues [21] observed similar disruption to spatial structure and task allocation in pesticide-exposed bumble bees [21], which suggests that heat stress may affect colony function through mechanisms shared with other stressors. Reduced clustering in brood-adjacent zones may compromise brood thermoregulation and developmental outcomes, while elevated fanning at the cost of brood care has been associated with reduced colony growth and worker production in prior work [3, 9].

Nectar production and bee visitation under sustained warming

Nectar volume nearly doubled at + 3°C relative to control plants (Fig. 3A), consistent with the hypothesis that sustained warming stimulates nectar production in *C. pepo* flowers through accelerated metabolic activity in nectary tissues up to a thermal optimum [11]. This contrasts with studies reporting reduced nectar secretion under heat stress in other plant species [26, 27]. Nectar sugar concentration did not differ significantly among treatments (Fig. 3B); sugar concentration declined significantly over time across all treatments.

Bee visitation was approximately 70% higher at + 3°C than at control, tracking the elevated nectar volume in the warmest treatment. This pattern contrasts with the acute-heatwave findings of Hemberger et al. [2], who reported that heat-developed *Brassica* flowers produced approximately 50% less nectar and that bumble bee foraging declined. The opposite pattern in our chronic warming *Cucurbita* system may reflect two interacting factors. First, our plants had four weeks of chronic warming before flower initiation and bees foraged on them, allowing flowers time to develop with elevated nectar production rather than reduced nectar as in Hemberger's acute design [2]. Second, the bees in our experiment were not exposed to acute heat during the foraging trial itself. The bumble bee foraging response to warming may therefore depend not only on whether the heat is acute or chronic, but also on how warming alters the floral reward available to the bees at the moment of foraging. The pollen-restricted hive design used here also concentrated bumble bee foraging on the warmed plants in each chamber. Because floral resource continuity may be a strong driver of colony performance [16], the colony-level responses we observed may partly reflect interactions between sustained warming and a continuous floral resource that did not vary in availability across the experiment.

Fruit yield and the limits of pollinator visitation under heat

Standardizing yield by female flower production gave a clearer picture of treatment effects than raw fruit counts. Control plants produced significantly more fruit set per female flower than + 1.5°C plants. Control and + 3°C plants did not differ significantly. This pattern suggests that moderate warming may impose a pollination or early-fruit-development bottleneck without a compensating gain in floral rewards or pollinator activity. At + 3°C, the higher nectar and visitation observed in the same treatment may have partly offset reductions in reproductive efficiency, leaving yield intermediate. Raw fruit counts alone may mask treatment effects, and accounting for female flower availability gives a more accurate read on plant–pollinator performance under thermal stress.

Per-female-flower yield at + 3°C was not significantly higher than at + 1.5°C, even though bee visits rose by about 70%. Pollinator visitation alone may not buffer plant reproductive output against direct heat stress. Bishop et al. [28] reported the opposite result in faba bean, where insect pollination reduced heat-induced yield loss. The contrast may reflect differences in crop species and in the magnitude of warming applied.

Implications for commercial pollination

The combination of reduced queen weight, altered worker behavior, disrupted spatial organization, and elevated bee visitation did not translate into proportional yield gains, suggesting that *B. impatiens* colonies and their plant partners may experience cumulative stress under sustained warming, even when total biomass appears stable, and visitation to flowers is maintained or increased. If warming trends continue at the projected pace (2.4–5.9°C rise by 2050; [1]), growers who rely on commercial *B. impatiens* hives may need to replace colonies more often, which could increase pollination costs. From a crop-side perspective, the + 3°C decoupling of bee visitation from proportional yield gains may also suggest that

pollinator inputs alone may not recover yield losses driven by direct heat stress on plant reproductive function.

Several aspects of the experimental design limit the strength of our inferences. The experiment was conducted over the active lifespan of a single *B. impatiens* micro-colony per chamber and at a single subtropical baseline temperature, which limits inference about long-term, multi-generational responses. Behavioral metrics were derived from visible surface activity and may underrepresent activity inside brood pots or internal nest structures. Future replications may benefit from automated tracking platforms such as the BumbleBox [29], which allows continuous and individually identified behavioral quantification and may resolve activity at finer temporal scales than the manual 30-second sampling used here.

The flight arena was not temperature-controlled to match chamber treatments, so the visitation, nectar, and fruit set results reflect responses of plants and bees that had been chronically exposed to warming, rather than responses during acute heat in the arena itself. Sample sizes for female flower yield comparisons were also small (n = 20 pots per treatment), which limits inference about the moderate-warming yield bottleneck.

Because the 20 *C. pepo* plants remained in their assigned chamber across all three sequential colony replacements, later colonies foraged on plants with cumulative heat exposure; any phenological or physiological carry-over in floral traits across colony cycles cannot be fully disentangled from treatment effects. Colony round (first, second, or third hive per chamber) was also not included as a factor in the behavioral models; the extent to which later-cycle colonies may have experienced treatment effects differently from earlier-cycle colonies — for instance, due to progressive changes in plant condition across the experiment — remains unquantified and represents a direction for future replications with sufficient chamber replication to model round-by-treatment interactions. Individual plants within each temperature treatment were not independently randomized across treatments, which limits their use as fully independent replicates; pot-level measurements should be interpreted as repeated observations within treatment rather than independent experimental units.

Conclusion

B. impatiens colonies under sustained warming showed physiological and behavioral responses that did not scale linearly with temperature. Moderate warming (+ 1.5°C) elicited active behavioral compensation such as elevated fanning and reduced brood-care, without disrupting colony size or spatial organization. Extreme warming (+ 3°C) reduced brood-care and grooming further and was associated with lower queen weight and significant disruption of spatial clustering in brood-adjacent and entrance-adjacent zones; these results indicate that the compensatory mechanisms expressed at + 1.5°C may be exceeded at higher temperatures. Nectar volume nearly doubled at + 3°C and bee visitation increased by approximately 70%, yet fruit set per female flower was lowest at + 1.5°C and did not increase proportionally with visitation at + 3°C — indicating that pollinator visitation alone may not protect crop

yield against sustained heat. Caste-specific physiological costs and the conditions under which commercial colonies forage therefore warrant closer monitoring in agricultural systems where warming is projected to intensify. Although the experimental colonies were commercially sourced, *B. impatiens* is native to eastern North America, and the colony-level responses documented here may extend to wild populations and related *Bombus* species with similar thermal tolerances. Whether these patterns generalize across additional *Bombus* species, longer warming windows, and field conditions involving combined heat, pesticide, and resource stressors remains to be tested.

Methods

Experimental design, replication, and growth conditions

We exposed *B. impatiens* micro-colonies and *Cucurbita pepo* zucchini plants together to three sustained temperature treatments representing present-day Florida baseline growing temperatures (29.5°C), moderate warming (+ 1.5°C), and extreme warming (+ 3°C). Colonies were maintained continuously in their assigned chambers and were provided weekly foraging access to flowering plants maintained under the same temperature regimes.

We used three Percival Plant Growth chambers (Model E-41L2) at the Jungle Lab, University of Miami, each set to one of three temperature treatments matched to the warming range projected for South Florida. Chambers operated on a 12/12-hour diurnal cycle calibrated to the average South Florida squash growing season. Daytime temperatures were 29.5°C (control), 31°C (+ 1.5°C warming), and 32.5°C (+ 3°C warming). Nighttime temperatures dropped to 21°C (control), 22.5°C (+ 1.5°C warming), and 24°C (+ 3°C warming). The warming offset (+ 1.5°C or + 3°C above control) was applied uniformly to both the day and night phases of the cycle, simulating a uniform climate shift rather than asymmetric daytime or nighttime warming. CO_2 concentration, light intensity, and relative humidity were standardized across chambers and held constant. The control temperature of 29.5°C reflects mean daytime temperatures during the South Florida squash production season (approximately October–May), not peak summer; we did not test responses to higher acute summer temperatures, and extrapolation beyond this range warrants caution. The Institutional Animal Care and Use Committee Protocol Exemption #IACUC-24-043 covered the invertebrate work.

Each chamber housed 20 *Cucurbita pepo* plants (zucchini, variety ‘Leopard’) and one active *B. impatiens* micro-colony at a time. The 20 plants remained in their assigned chamber throughout the experiment; new plants were not introduced between hive replacements. Temperature treatments started at seeding, before plant flower differentiation, so the bees later foraged on flowers that had developed entirely under the assigned regime. Plants received water at similar times across chambers throughout the experimental period.

Replication at the colony level came from sequential hive replacement within each chamber. When the active colony in a chamber ceased foraging for more than two consecutive days, we removed it and

introduced a new micro-colony into the same chamber. Three hives ran through each chamber over the course of the experiment, for a total of nine experimental colonies (n = 3 colonies per temperature treatment). Plants and chambers were therefore shared across the three colonies within a treatment, while colonies were the unit of replication for queen weight, worker behavior, within-hive spatial clustering, bee visitation, and fruit-set measurements. We acknowledge that this design shares plants and chamber identity across the three within-treatment colonies, and we accounted for this in our statistical analyses by including chamber as a random factor in mixed-effects models where appropriate.

For floral nectar sampling, we drew samples from ~ 5 freshly opened pistillate flowers per chamber per week, yielding total samples of n = 16–25 per treatment across the experiment (Table 3).

Bumble bee colonies

We sourced *B. impatiens* micro-colonies commercially ('Excel' hives, Koppert Biological Systems, Howell, MI, USA). At the start of each experimental run, every hive contained one queen and at least 70 worker bees. Three hives were kept under each chamber treatment across the course of the experiment. A transparent observation lid sat on top of every hive so we could record video and check the colony without opening it.

Inside each hive we installed nectar sacs of sugar solution and left these available ad libitum, so the bees had continuous carbohydrate access. Pollen was not provided in order to encourage natural foraging behavior on the *C. pepo* flowers available in each chamber and to ensure that observed colony differences would be attributable to the imposed temperature treatments rather than buffered by supplemental nutrition. This design choice also reflects prior evidence that floral resource continuity strongly affects bumble bee colony performance [16] and restricting hive-provided pollen ensured that any treatment-driven differences in foraging or colony outcomes would reflect access to the *C. pepo* flowers in each chamber rather than supplemental nutrition.

For weekly pollination trials, each colony and its associated plants were transferred together into indoor mesh flight arenas for 48-hour observation periods. Flight arenas were located under ambient indoor conditions and were not temperature-controlled to match chamber temperatures. Pollination behavior in the arenas therefore reflected the effects of prior chronic heat exposure on plants and bees, rather than acute temperature conditions during the trial itself. After each 48-hour interaction period, colonies and plants were returned to their assigned chamber, and bees and plants were never exchanged between temperature treatments. Flight cage interactions were repeated weekly for approximately ten weeks, corresponding to the active lifespan of each experimental colony.

Behavioral video analysis

To assess colony-level responses to sustained warming, we performed standardized behavioral observations using video analysis [3, 17]. Each colony was recorded once per week using a GoPro Hero 9 Black camera positioned approximately 30 cm above the observation hive [18]. Videos were recorded at consistent times across days and under stable temperature conditions in each treatment chamber.

Each session was ten minutes long. From each video, we scored ten 30-second blocks spaced evenly across the recording window. One trained observer reviewed and scored every video by hand using a standardized ethogram [19, 20]. For each 30-second block we counted bees engaged in fanning, grooming, walking, flying, resting, and brood care [21], assigned a colony-level clustering score on a 1–5 scale (where 1 = highly dispersed individuals and 5 = tight aggregation) [21, 22], and recorded the total number of visible workers. Workers often occupy brood pots or internal nest structures, so our behavioral metrics rely on visible surface activity and may underrepresent total colony behavior. We also noted queen presence whenever she was visible.

Spatial clustering analysis

We quantified within-hive spatial organization from still-image snapshots taken at 3, 6, and 9 minutes into each video. These three time points represented early, mid, and late phases of the observation period and did not overlap with the 30-second behavioral intervals. We then categorized each image into four spatial zones following Crall et al. [21]: the brood zone (areas with visible brood structures), brood-adjacent zone (surrounding the brood), entrance-adjacent zone (near the nest entrance of the Koppert Excel hive), and the peripheral zone (outer margins).

To account for differences in available space among zones, the proportional area of each zone was estimated for each hive and summed to equal one. Worker counts per zone were then used to calculate a zone-specific clustering index as the ratio of the proportion of workers occupying that zone to the proportion of hive area represented by the zone:

Clustering Index = Proportion of Bees in a Zone ÷ Proportion of Available Area in that Zone

Clustering index values of 1 indicate a random distribution relative to available area, values greater than 1 indicate aggregation, and values less than 1 indicate avoidance or under-utilization of a zone [23, 24]. Clustering indices were calculated separately for each zone and snapshot.

Colony dissection and morphology

Each colony's active period ended when we observed no active foraging for more than two consecutive days. At this point, we euthanized the micro-colonies by freezing and subsequently dissected in the laboratory. For each hive, adult bees were separated by caste, and the total number of workers and males was recorded. Queen presence was confirmed when applicable.

Adult bee biomass was quantified by weighing pooled individuals within each caste per hive. For worker, male, pupa, and larvae bees, total mass was divided by the number of individuals to obtain a mean individual weight per hive, which was used as the unit of replication in statistical analyses. Queen weight was measured individually. Honeycomb weight, brood, larvae, and pupa presence were also recorded. Colony size was quantified as the total number of adult bees present at dissection. This count served as a measure of cumulative colony performance under each temperature regime [18].

Nectar, bee visitation, and fruit yield

During every weekly 48-hour flight cage trial we counted bee visits to flowers and recorded nectar and fruit yield metrics from the *C. pepo* plants. Bee visitation was the total number of bee visits per plant, with male and female flowers tracked separately. Nectar volume and sugar concentration came from freshly opened pistillate flowers between 08:00 and 10:00, when nectar production tends to be highest. We gently bagged flowers before anthesis to prevent evaporative loss. Nectar volume (μL) was drawn using 15 μL graduated microcapillary tubes (Drummond Microcaps) and read to the nearest 0.25 μL. Immediately afterward, we read nectar sugar content in degrees Brix (°Brix) on a handheld refractometer (Atago PAL-1) calibrated with distilled water between samples. Marketable zucchinis were harvested at commercial size (> 12.5 cm long). We tracked female flower production per pot. For each replicate we calculated raw zucchini fruit count per pot and standardized yield as zucchini fruit produced per female flower across the experimental period.

Data analysis

All analyses were run in IBM SPSS Statistics (Version 30.0). Before analysis, we screened the data for normality and homogeneity of variance using Shapiro–Wilk and Levene's tests. We set significance at $\alpha = 0.05$ and adopted the language-of-evidence framework [25], where p-values fell between 0.05 and 0.10, we reported them as providing weak evidence for a difference rather than as 'trends' or 'marginal effects.' Queen weight, male weight per individual, weight, total bee biomass, queen length, queen width, and honeycomb weight were analyzed using one-way analysis of variance (ANOVA) with temperature treatment (Control, + 1.5°C, + 3°C) as a fixed effect. Tukey's Honest Significant Difference (HSD) post hoc tests were used to identify pairwise differences among treatments when ANOVA results were significant. Where parametric assumptions were not met, Kruskal–Wallis tests were used. Total adult bee count per hive, measured at final dissection, was analyzed using a Kruskal–Wallis test because hive-level sample size was small and colony totals were non-normally distributed.

Because the three colonies within each temperature treatment shared chamber identity and a common pool of 20 plants, we treated each colony as the unit of replication for colony-level metrics (queen weight, total bee count, worker behavior, spatial clustering). For plant-derived measurements (nectar volume, nectar Brix, bee visitation per flower, fruit count, fruit set per female flower), we sampled across

flowers and pots within each chamber and analyzed the data with chamber as a random effect in mixed-effects models, accounting for the shared plant resource.

Worker behaviors were quantified from 30-second video intervals by counting the number of bees engaged in each behavior, along with the total number of visible workers during each interval. Behavioral count data were analyzed using negative binomial generalized linear models (GLMs) with a log link and the total number of visible workers included as an offset to estimate per-bee behavioral rates. Negative binomial GLMs were used for overdispersed behaviors, while brood-care rate was analyzed with a Poisson GLM based on acceptable dispersion diagnostics. Temperature treatment was included as a fixed effect. Model coefficients were exponentiated to obtain incidence rate ratios (IRRs).

Clustering scores assigned on the 1–5 scale were summarized descriptively by treatment because scores were invariant in the warming treatments. Spatial clustering indices from snapshot data were log-transformed to improve normality and analyzed using linear mixed-effects models, with temperature treatment and snapshot time as fixed effects and hive as a random intercept. Bee visitation counts were analyzed with a Negative Binomial GEE because visitation counts were overdispersed; sampling day was included as a time covariate. Raw zucchini fruit counts were analyzed with a Poisson GEE. Standardized yield (zucchini fruit per female flower) was analyzed with a Kruskal–Wallis test followed by pairwise Mann–Whitney U tests with Holm correction. Nectar volume and nectar Brix were analyzed with linear mixed models (LMMs) with treatment and time as fixed effects and pot as a random intercept.

Declarations

Acknowledgements

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Author Contributions

B.M.K. conceived and designed the study, conducted fieldwork and laboratory analyses, performed statistical analyses, and wrote the manuscript. K.J. contributed to the design and implementation of the study, as well as data analysis and writing. C.A.R. contributed to experimental design, intellectual contributions, and manuscript revisions. R.E.M. contributed expertise on pollinator–plant interactions, methodology, *Bombus impatiens*, and video behavioral analysis and reviewed the manuscript. S.F.O. co-

supervised the study and reviewed the manuscript. K.J.F. provided laboratory facilities, revised the manuscript, and co-supervised experimental design. S.T. assisted with data collection and manuscript revisions. All authors reviewed the manuscript.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Competing Interests

The authors declare no competing interests.

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Figures

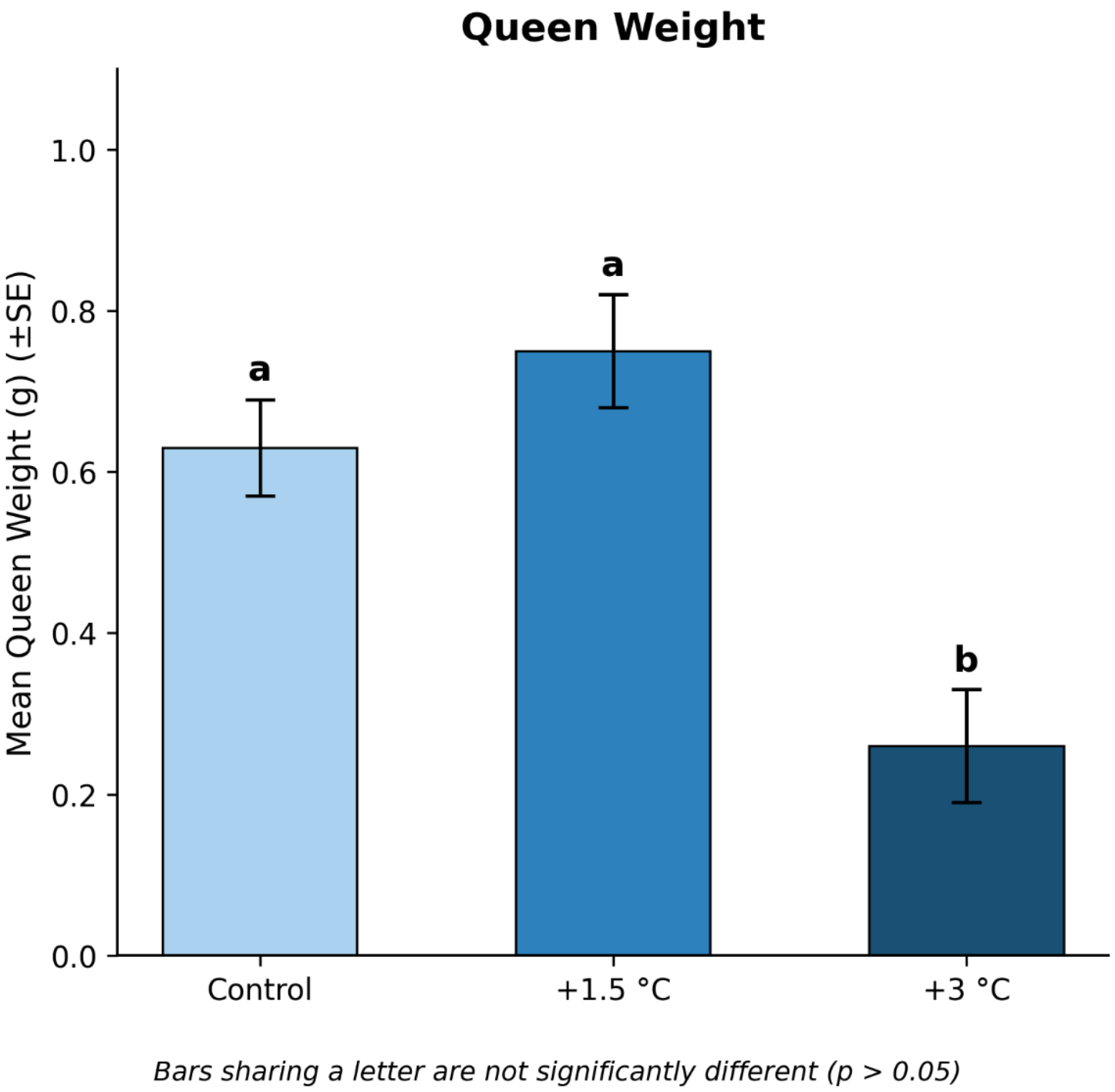


Figure 1

Effects of temperature treatment on *Bombus impatiens*queen weight. Queen weight differed significantly among treatments (ANOVA, F(2, 6) = 12.07, p = 0.004); queens from the +3°C treatment weighed significantly less than queens from both the control and +1.5°C treatments (Tukey HSD). Letters above bars indicate pairwise differences; bars sharing a letter are not significantly different (α = 0.05). Bars represent treatment means ± SE.

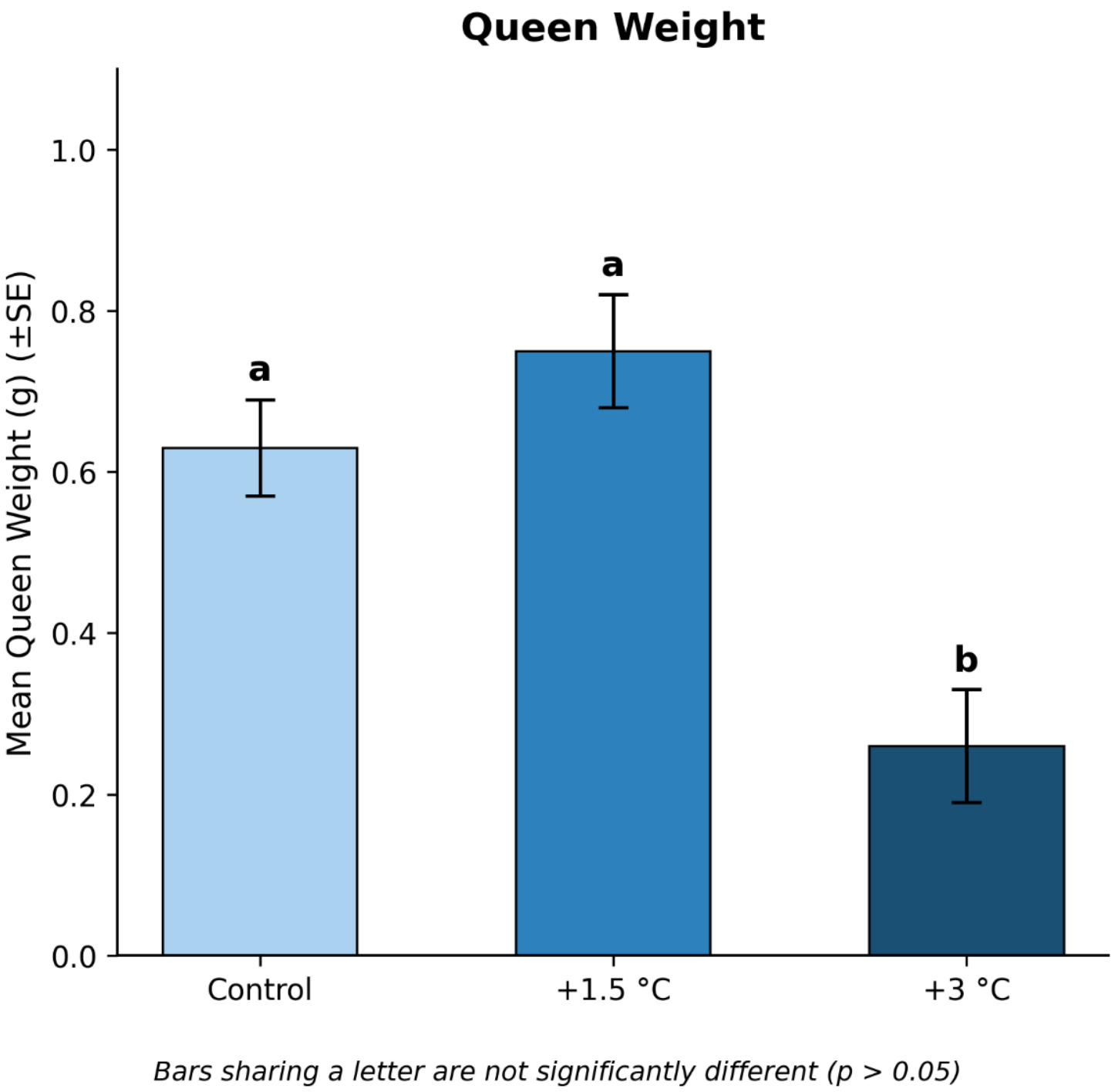


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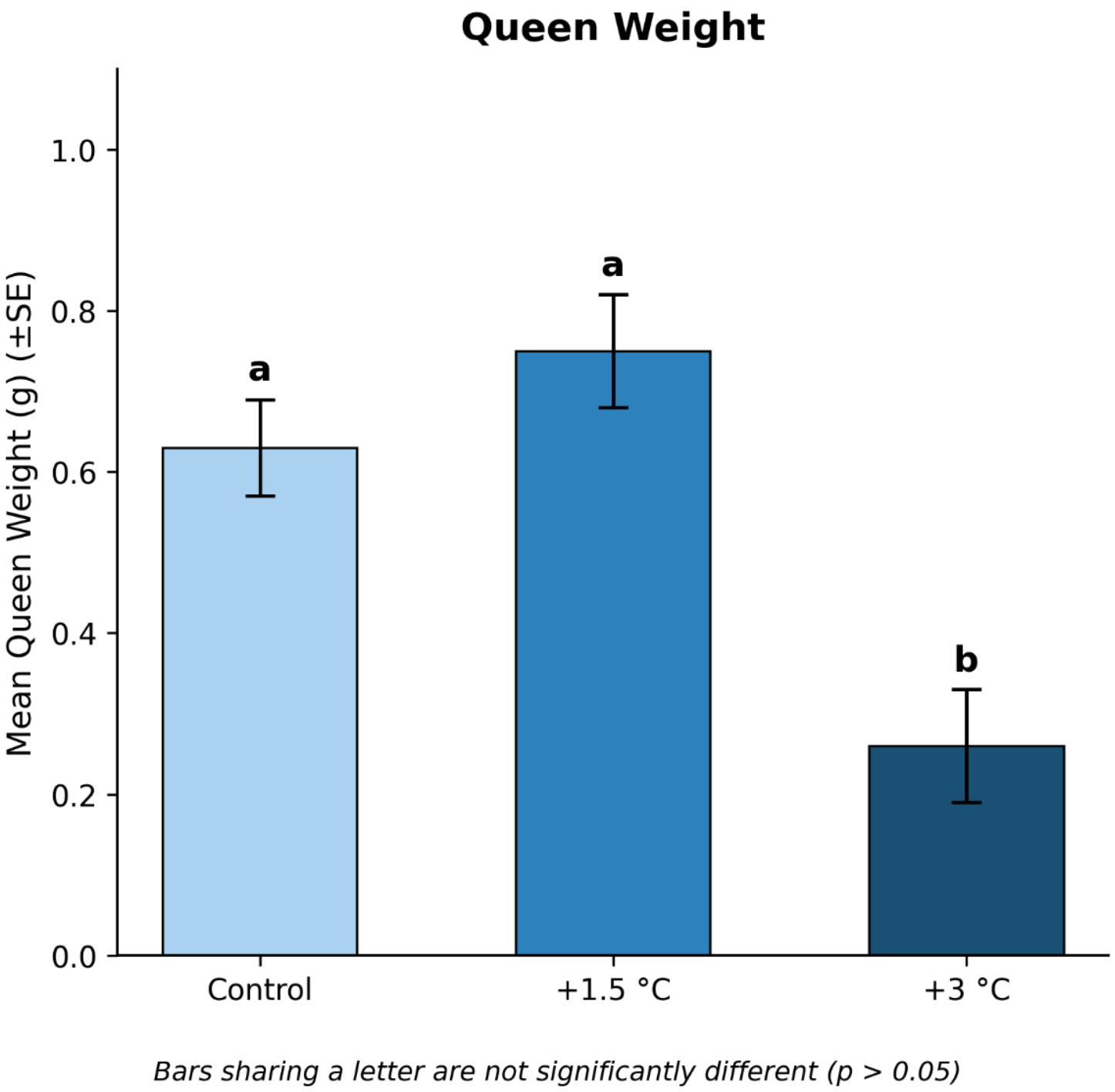


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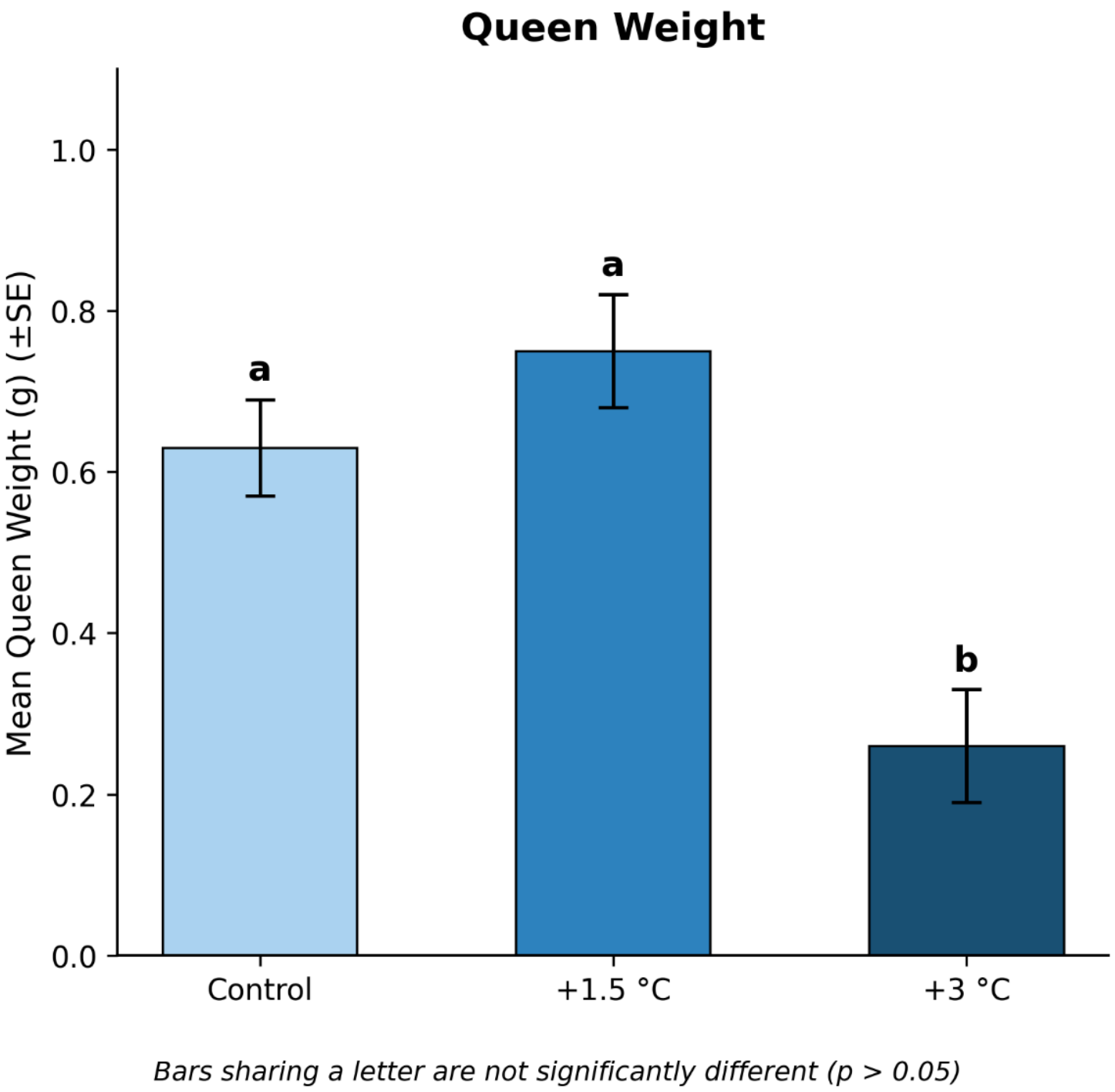


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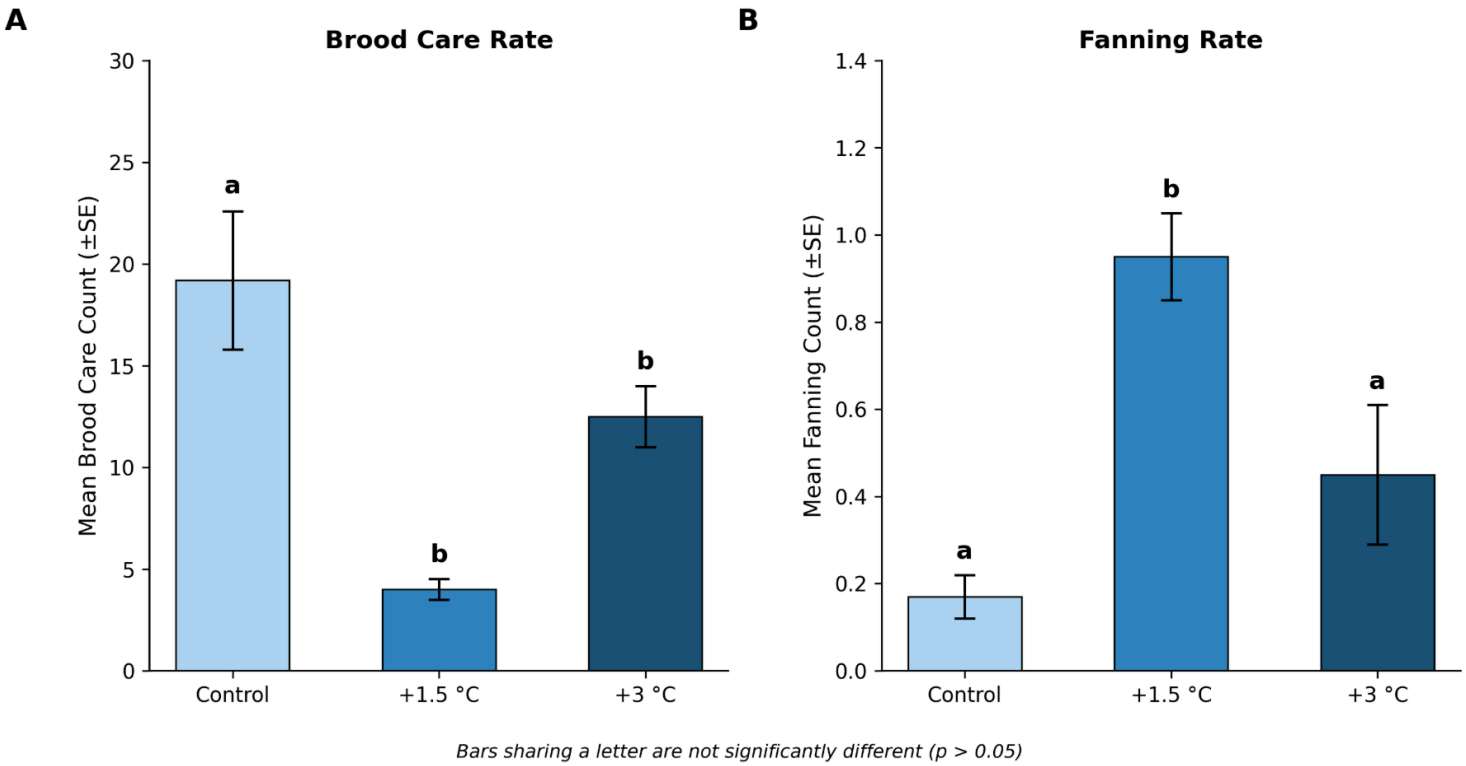


Figure 2

Effects of temperature treatment on worker thermoregulatory and brood-care behavior. (A) Fanning rate was significantly elevated under the +1.5°C treatment relative to both control (negative binomial GLM, IRR = 20.01, $p < 0.001$) and the +3°C treatment ($p < 0.001$); the +3°C treatment did not differ significantly from control ($p = 0.071$). (B) Brood-care rate declined significantly under both warming treatments relative to control (+1.5°C: IRR = 0.57; +3°C: IRR = 0.70; both $p < 0.001$); the two warming treatments did not differ from each other ($p = 0.108$). Letters above bars indicate pairwise differences; bars sharing a letter are not significantly different ($\alpha = 0.05$). Bars represent mean ± SE.

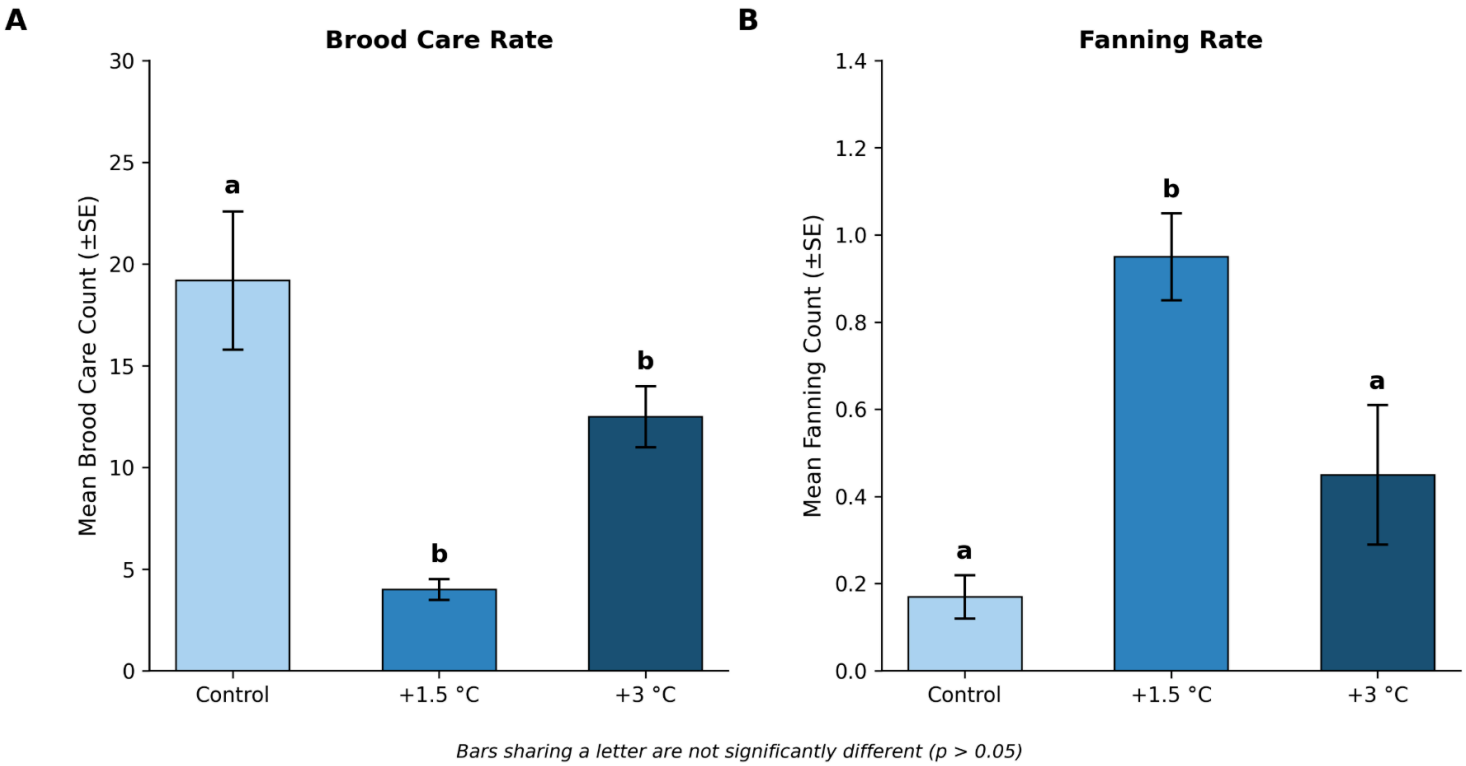


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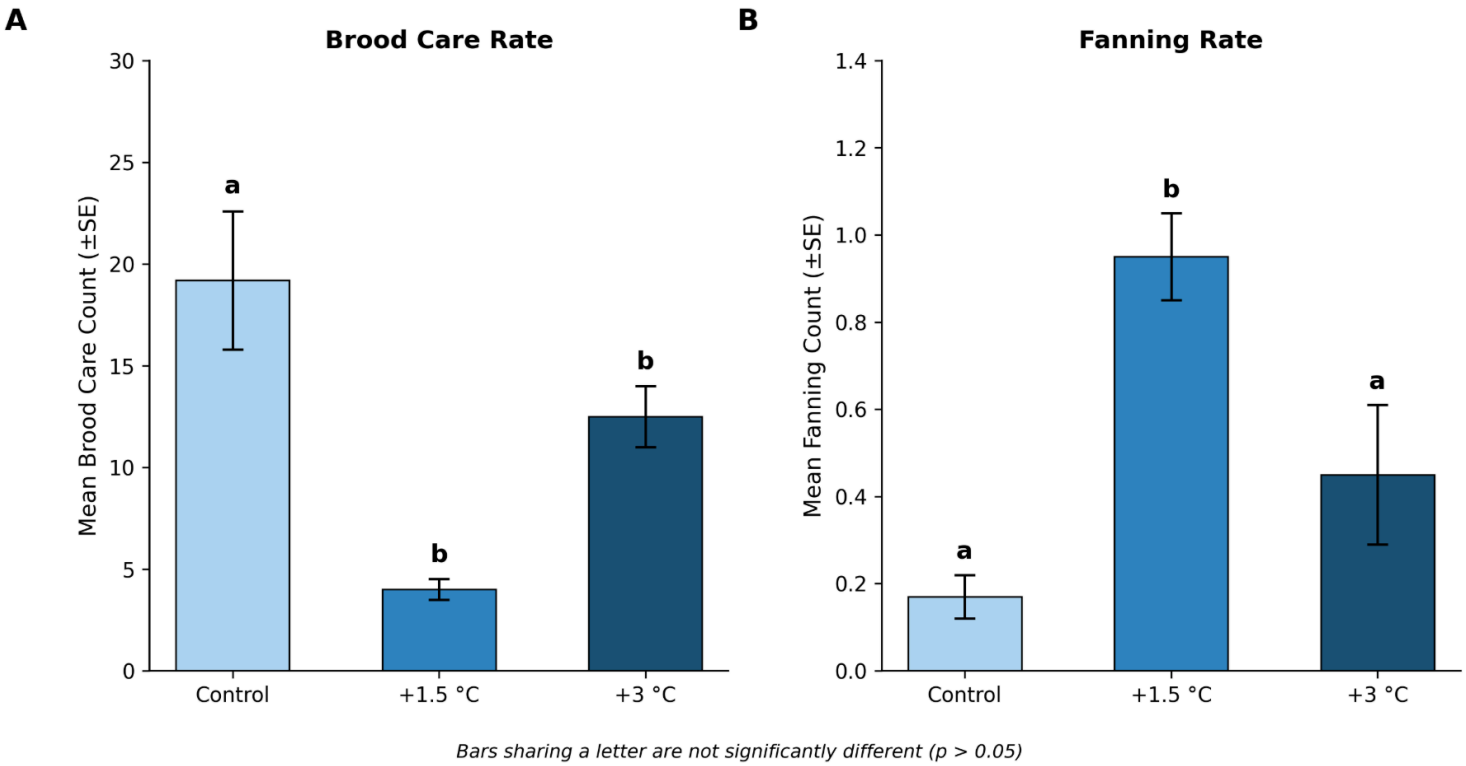


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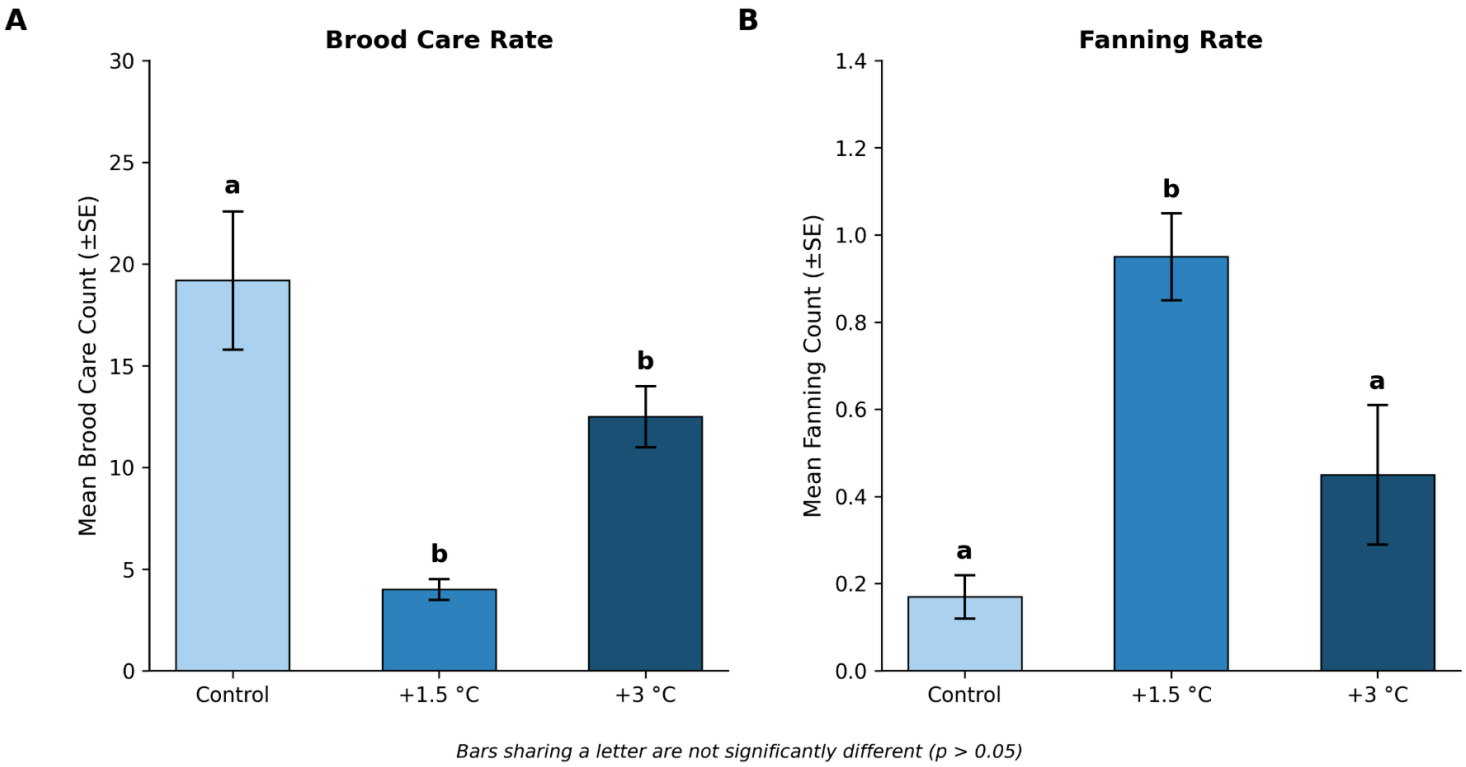


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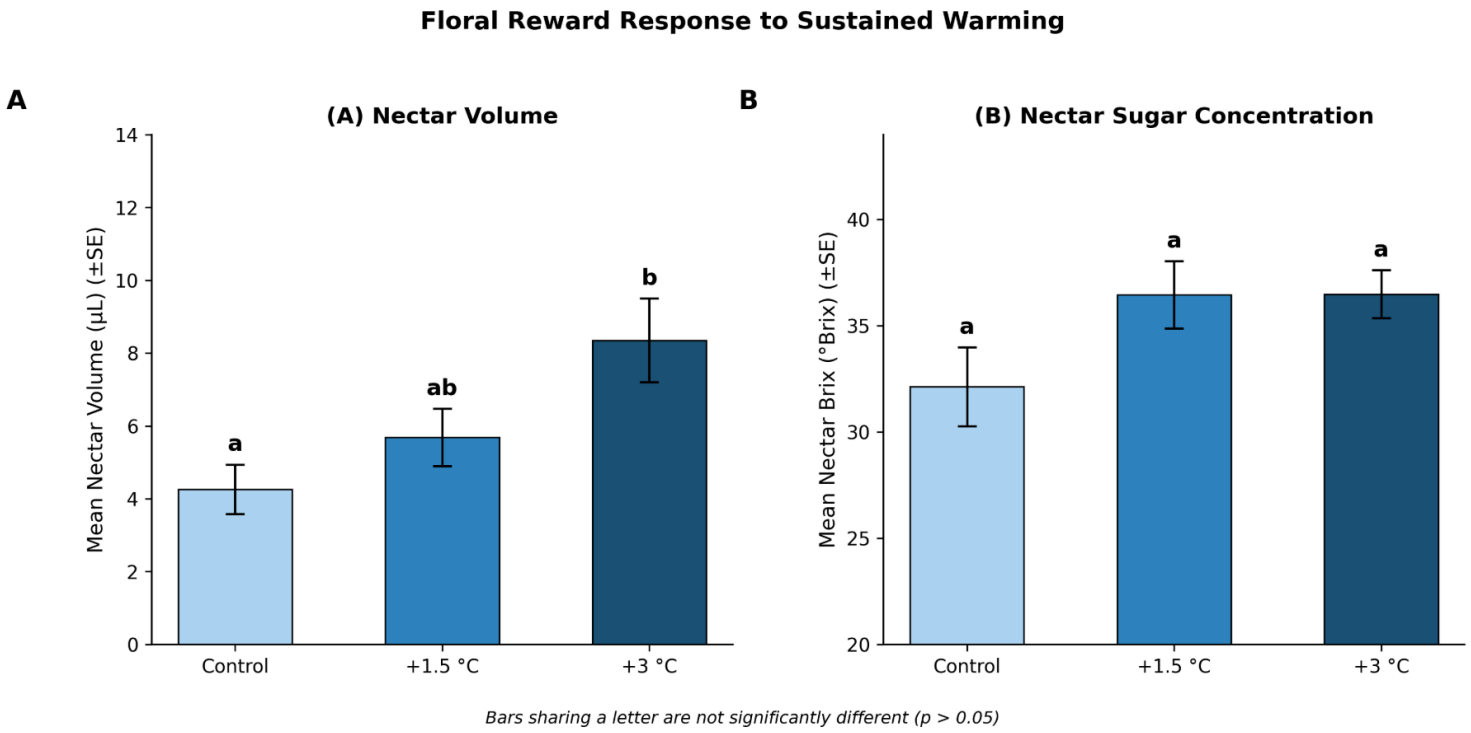


Figure 3

Floral reward response to sustained warming in *Cucurbita pepo*. (A) Nectar volume was significantly higher under the +3°C treatment than under control conditions (LMM, p = 0.018); the +1.5°C treatment was intermediate and did not differ significantly from either control (p = 0.734) or the +3°C treatment (p = 0.093). (B) Nectar sugar concentration did not differ significantly among temperature treatments (LMM, F(2, 45.7) = 0.86, p = 0.432). Letters above bars indicate pairwise differences; bars sharing a letter are not significantly different (α = 0.05). Error bars represent ± SE.

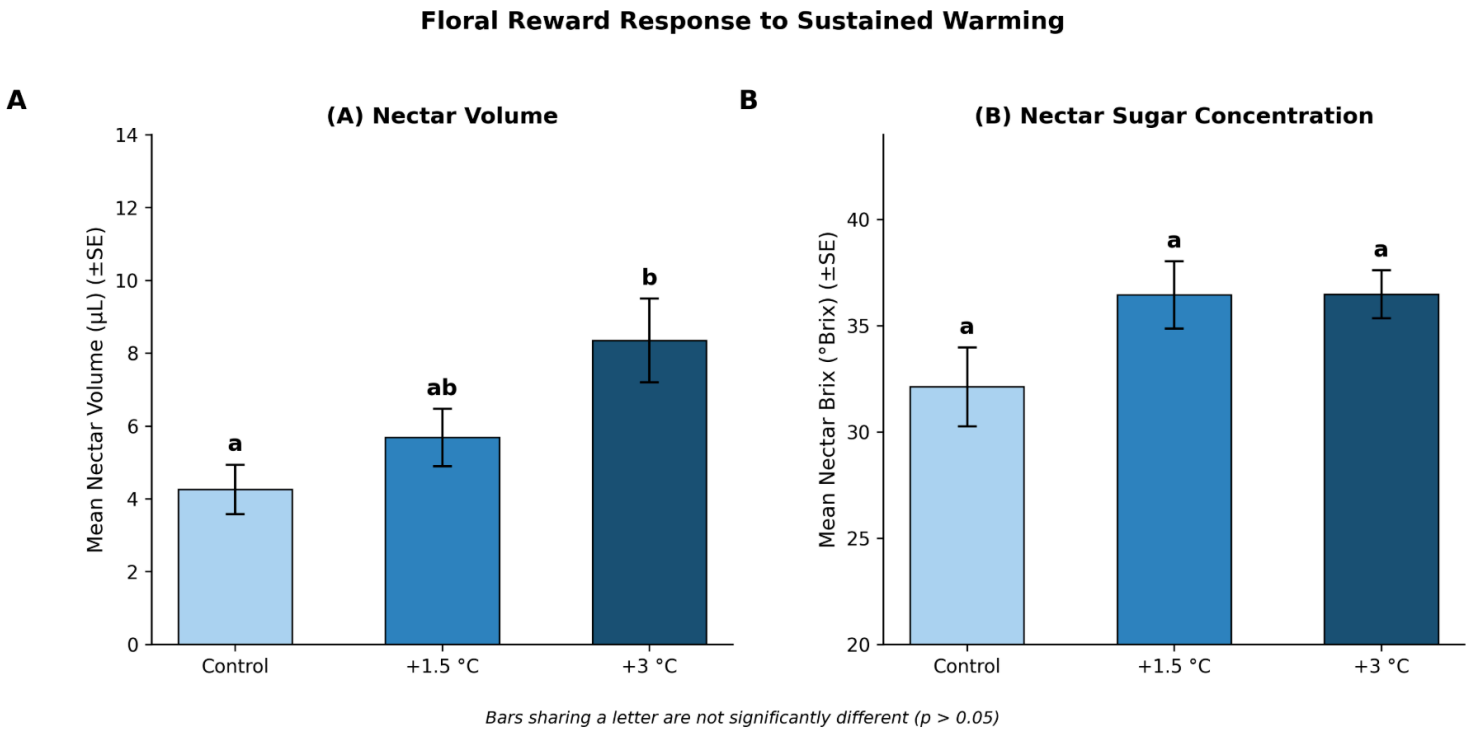


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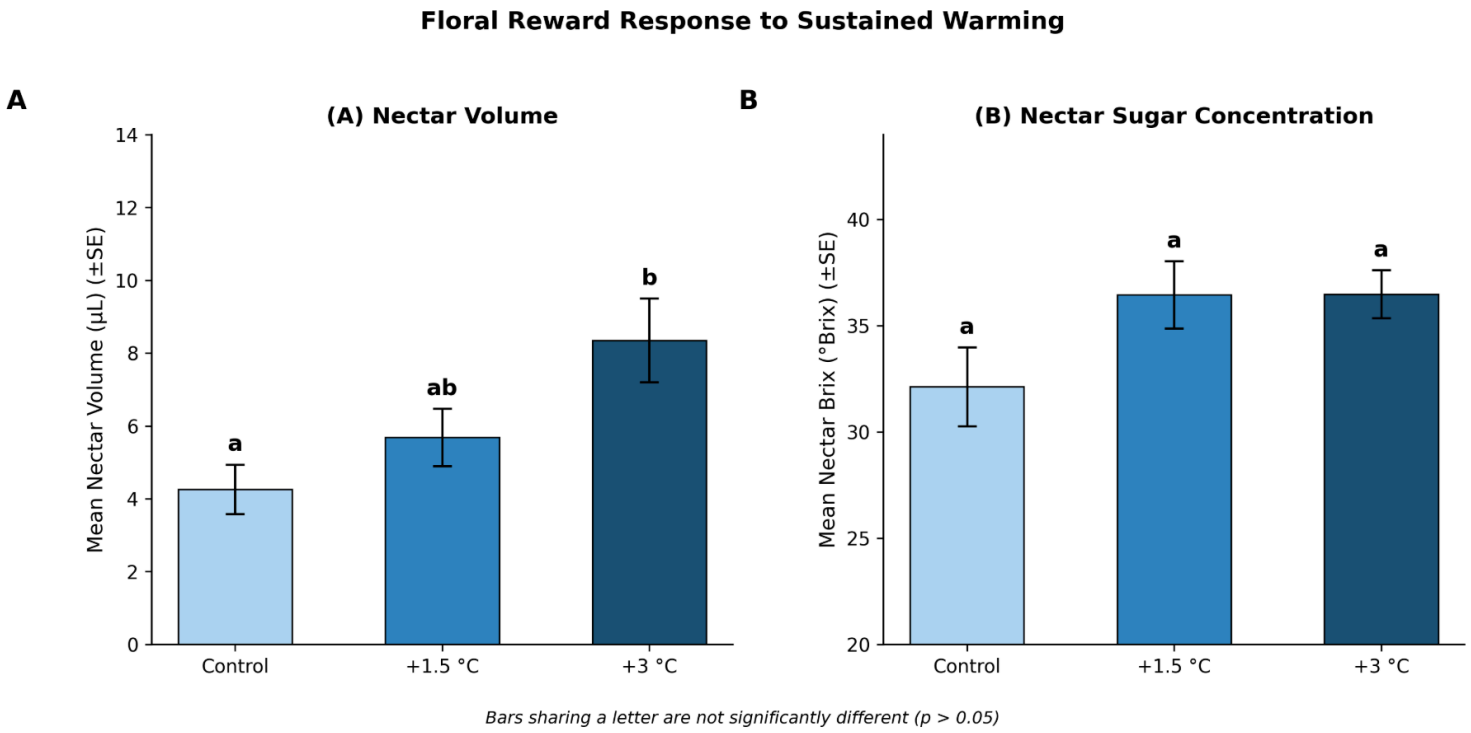


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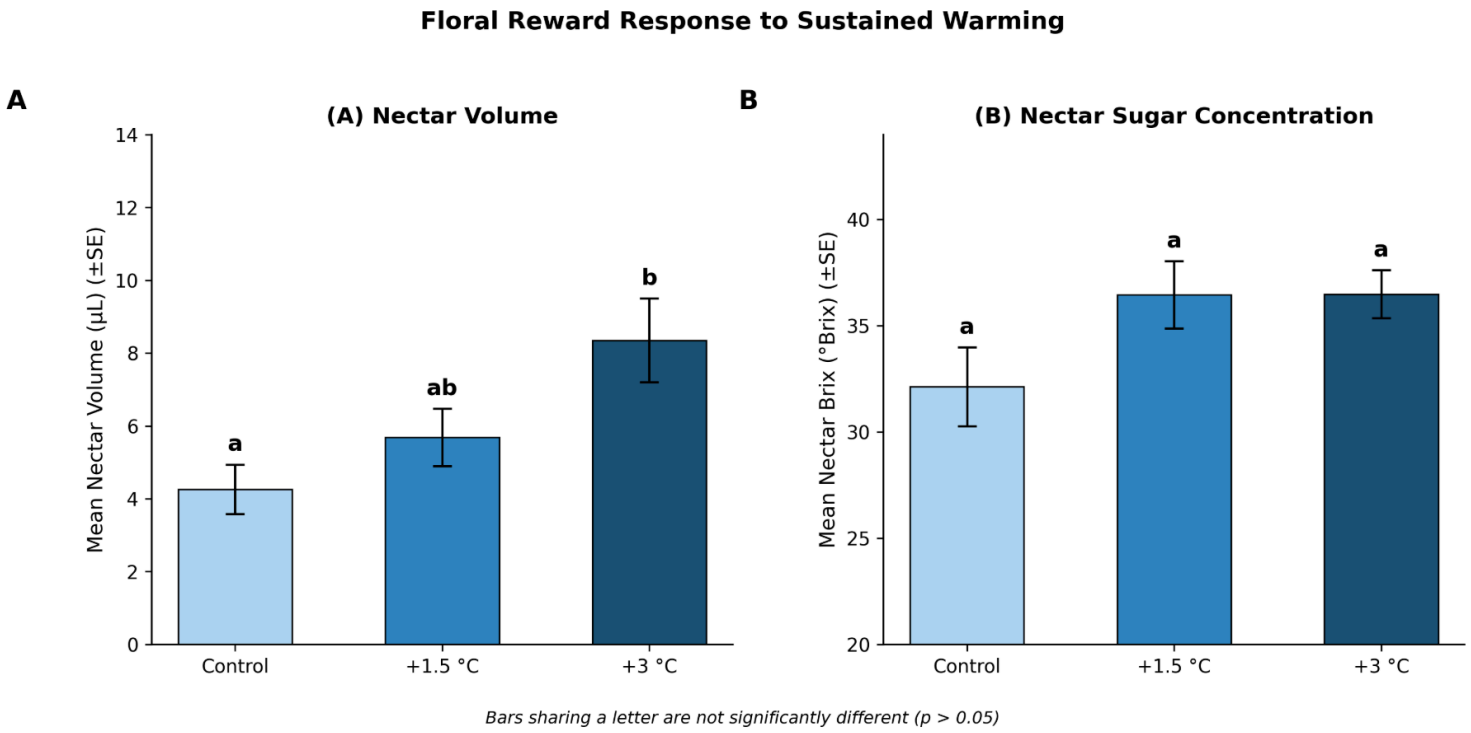


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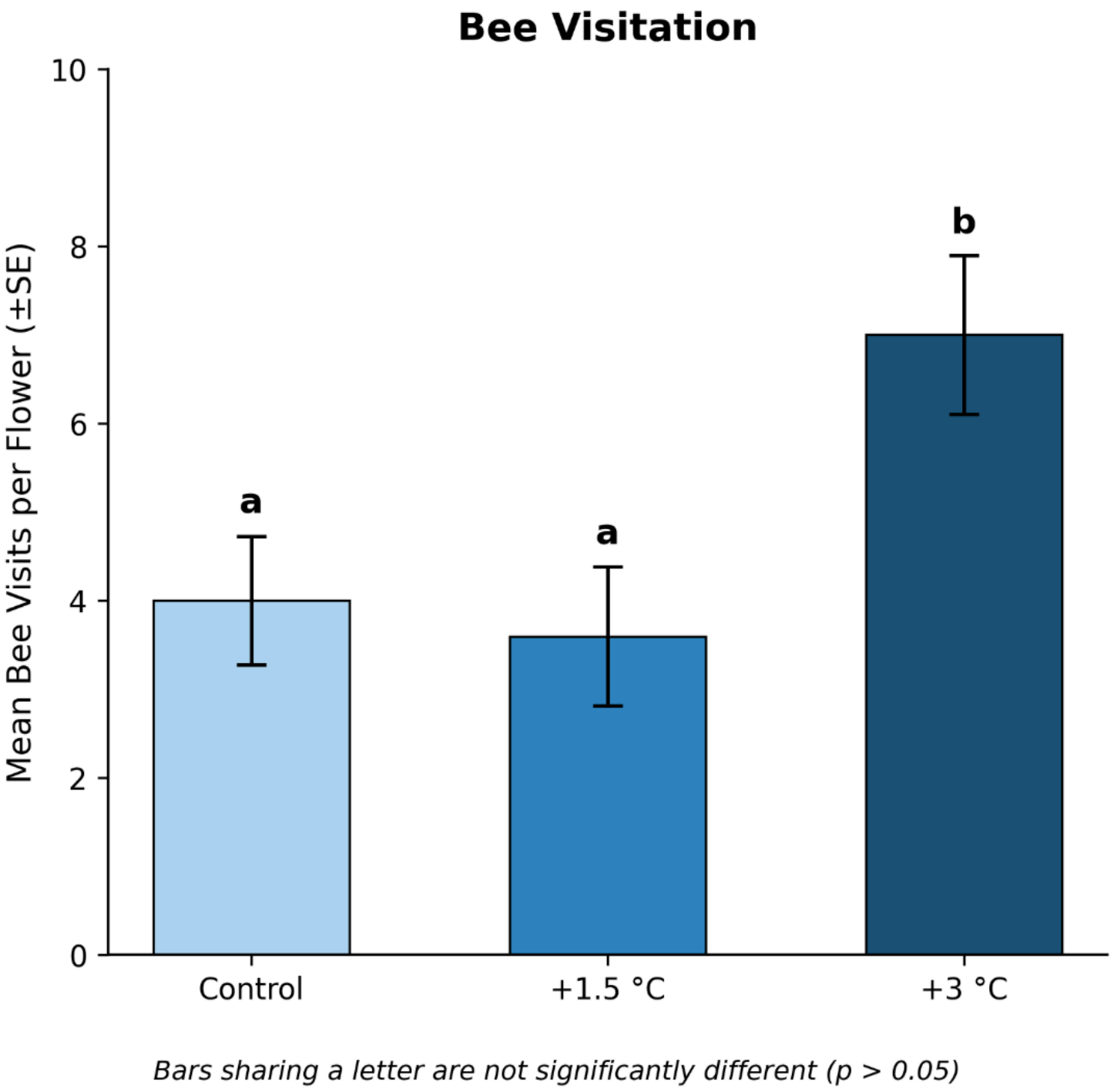


Figure 4

Bumble bee visitation rate per flower across temperature treatments. Visitation was significantly higher under the +3°C treatment than under control conditions (negative binomial GEE, p = 0.026); the +1.5°C treatment did not differ significantly from control (p = 0.253). Letters above bars indicate pairwise differences; bars sharing a letter are not significantly different (α = 0.05). Error bars represent ± SE.

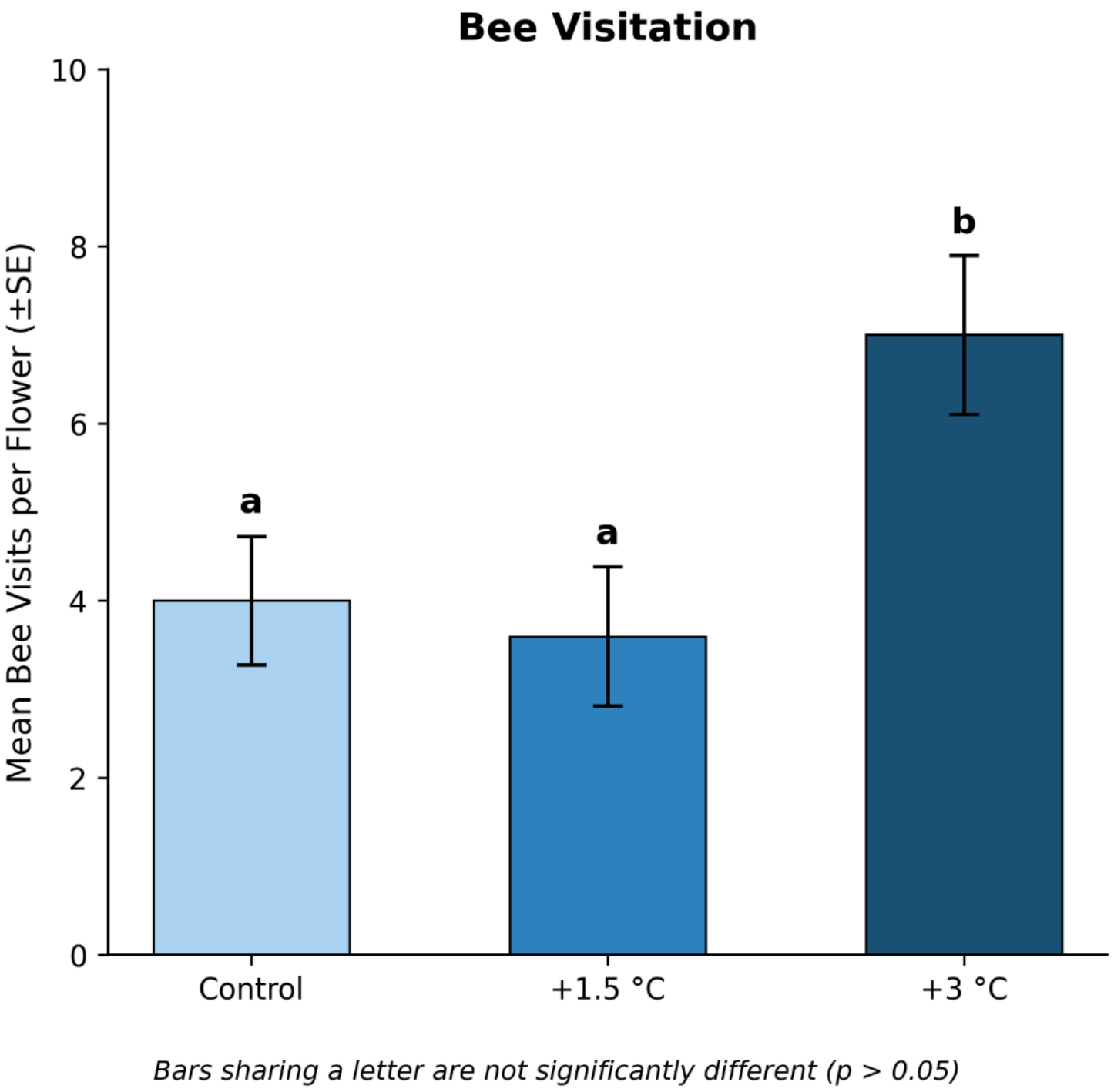


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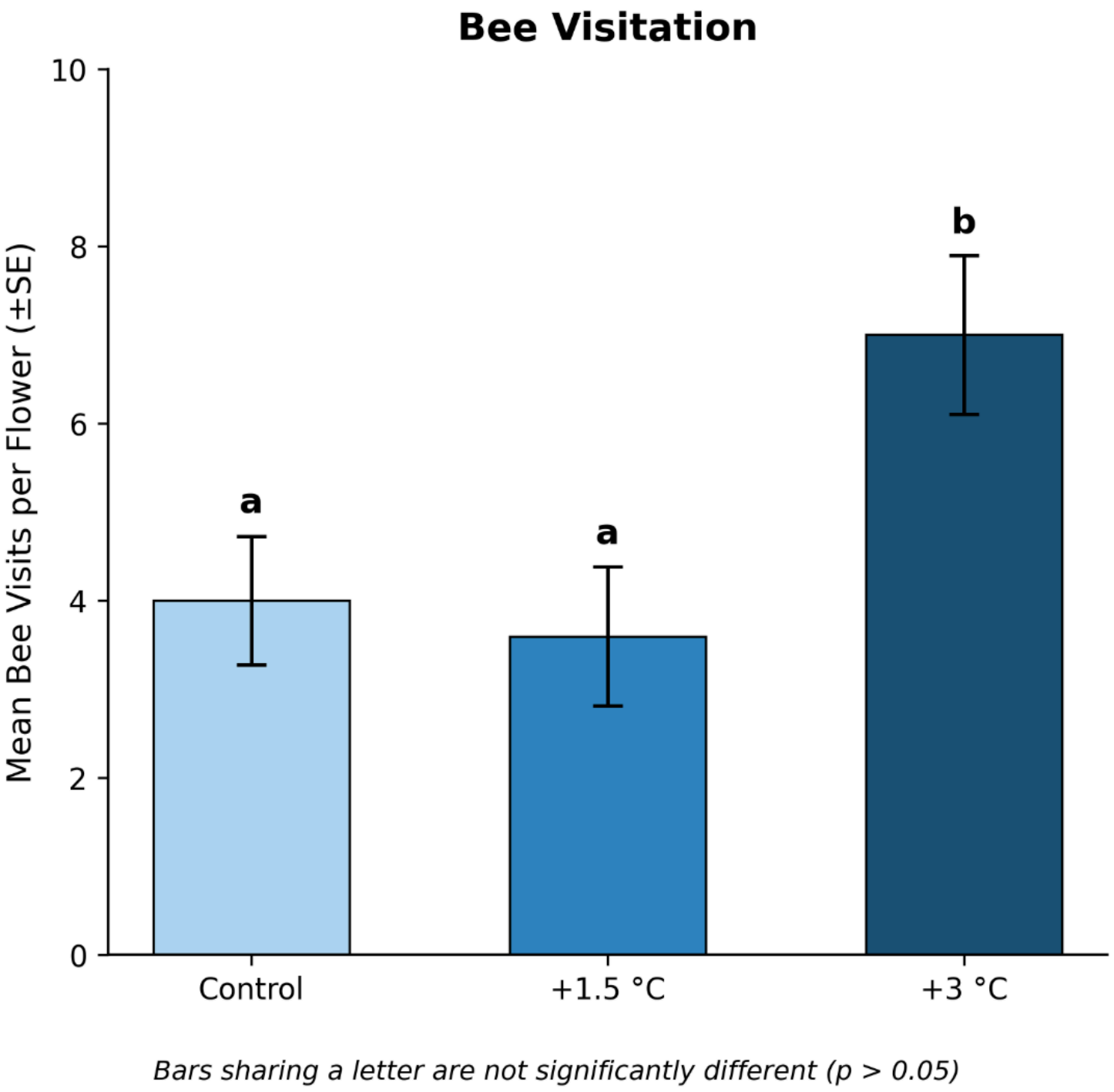


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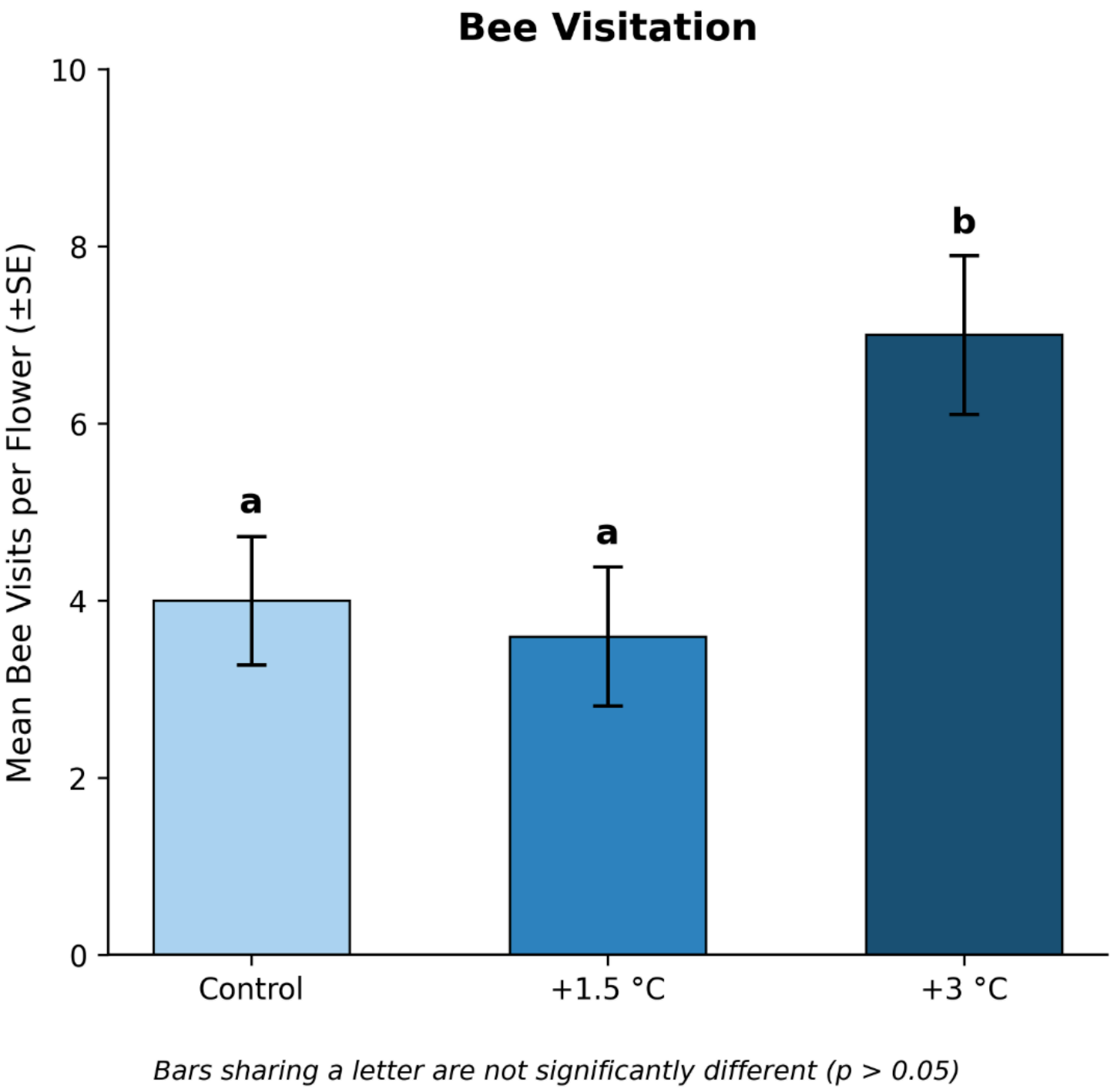


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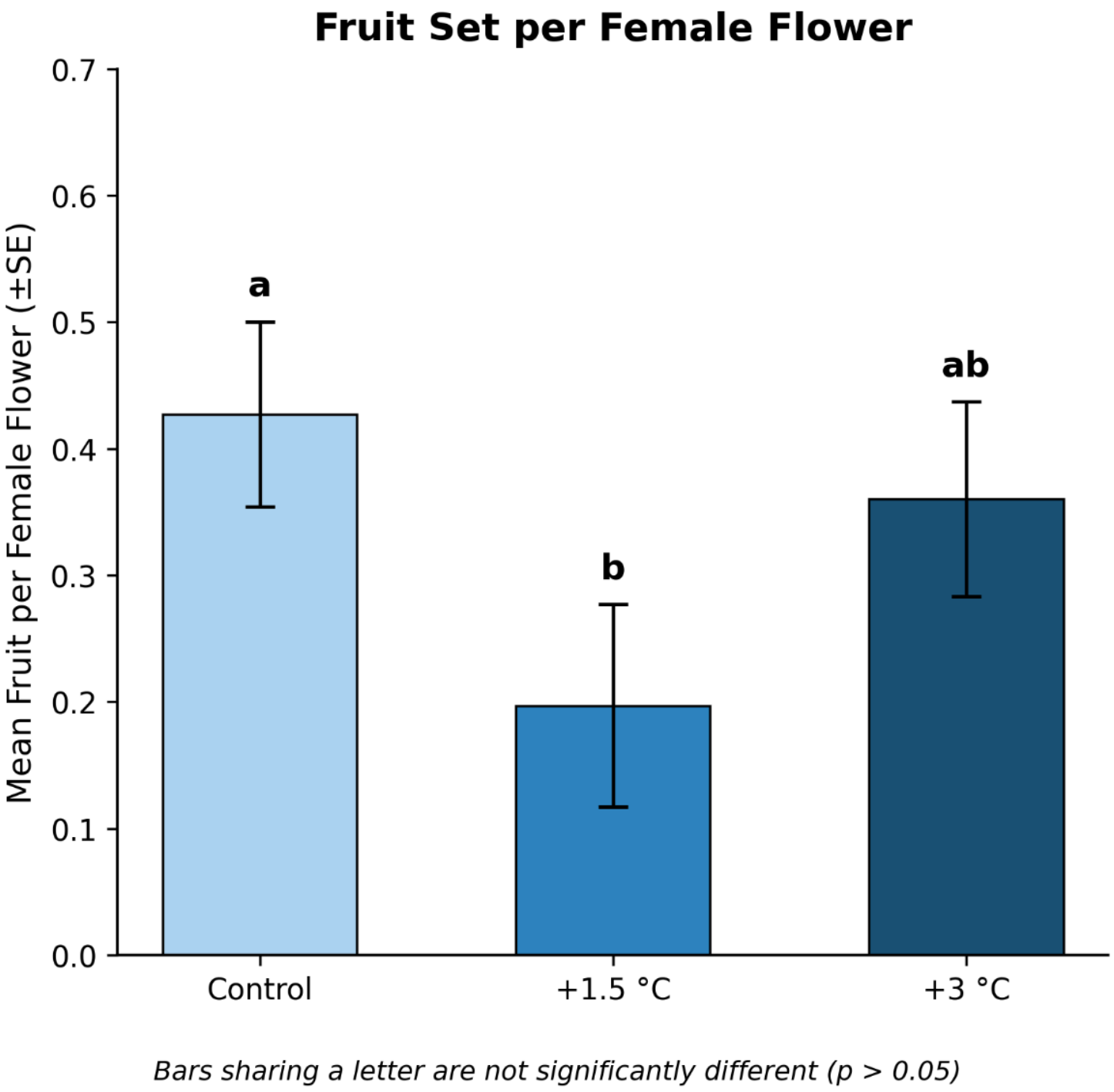


Figure 5

Effects of temperature treatment on *Cucurbita pepo*fruit set per female flower. Fruit set differed among treatments (Kruskal–Wallis); the +1.5°C treatment produced significantly fewer fruits per female flower than control (Holm-corrected Mann–Whitney, p = 0.002), while the +3°C treatment was intermediate and did not differ significantly from either control (p = 0.365) or the +1.5°C treatment after Holm correction (U = 123, p = 0.060). Letters above bars indicate pairwise differences; bars sharing a letter are not significantly different (α = 0.05). Bars represent mean ± SE.

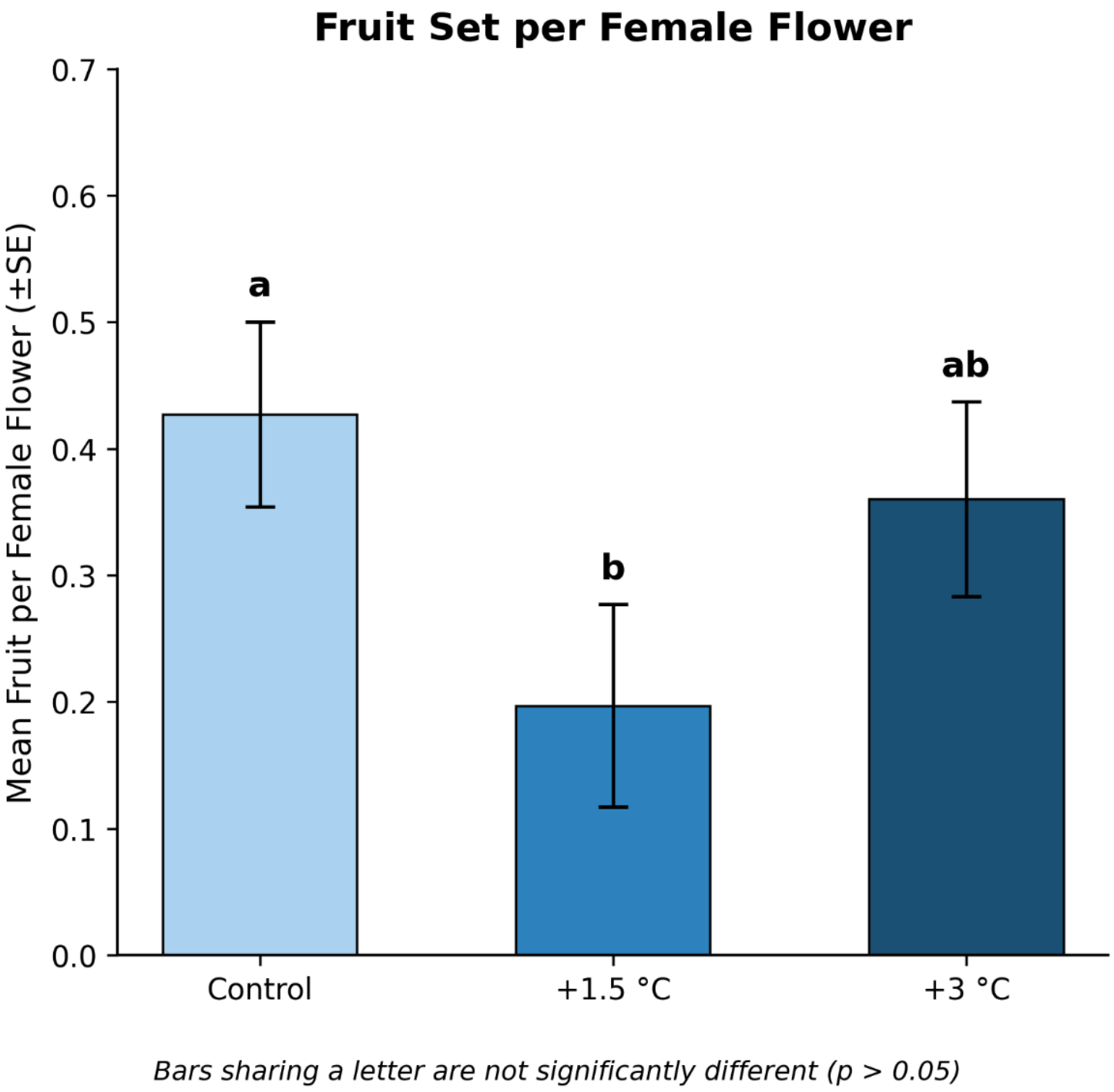


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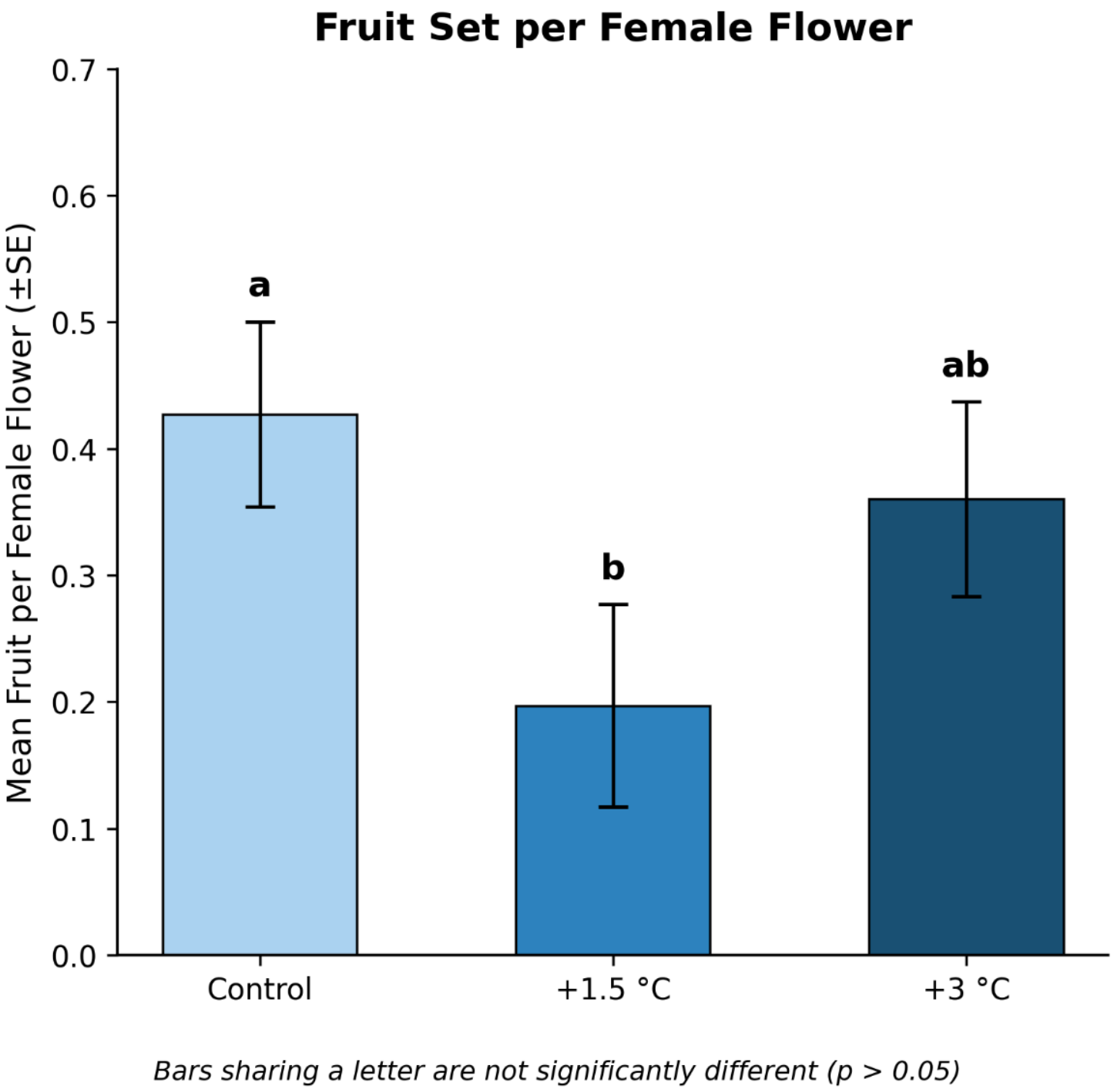


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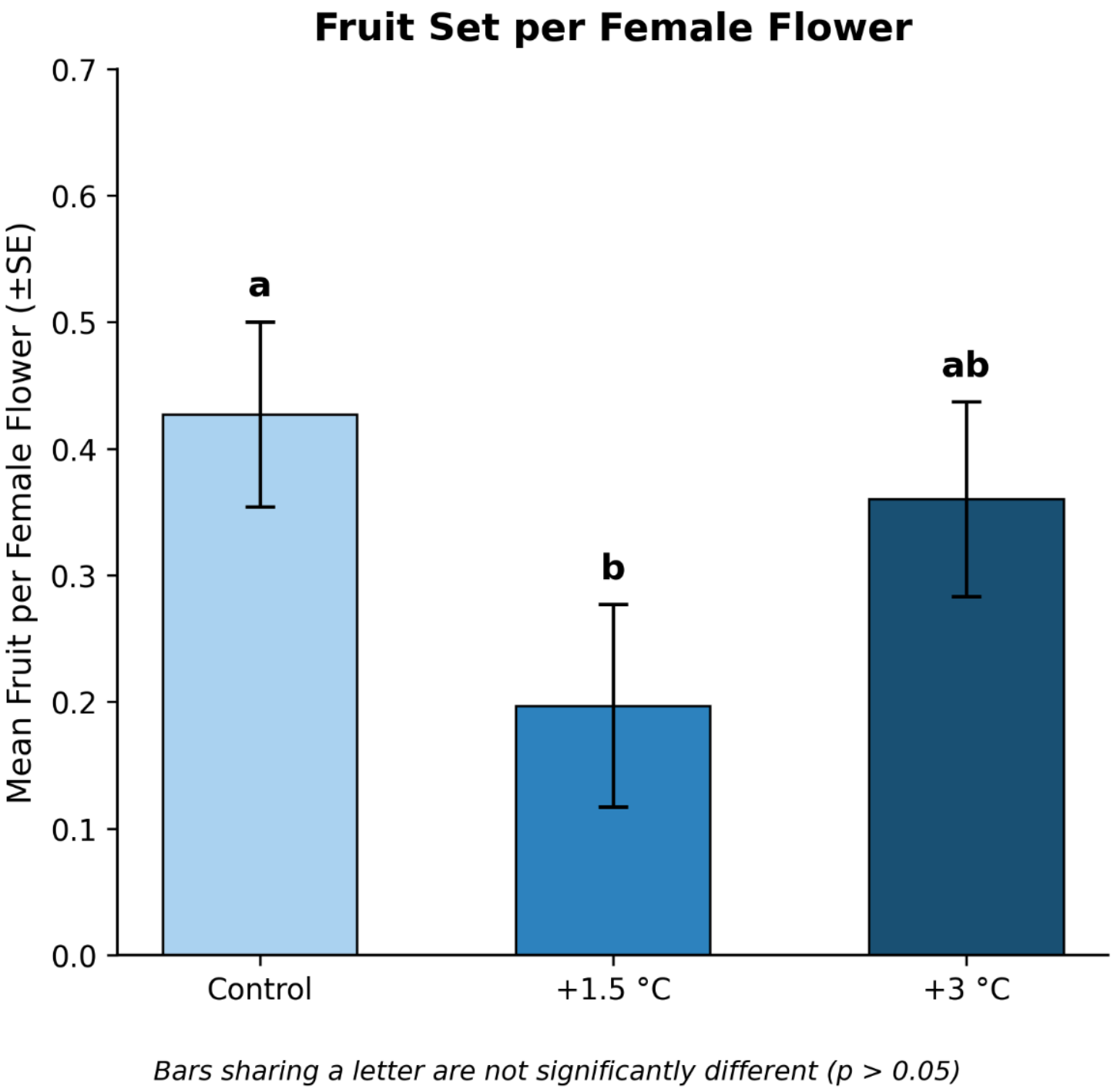


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