Methodological challenges in avian nestbox temperature manipulation experiments: a review with recommendations

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Experimental manipulation of nest temperature - particularly in nest boxes, given ease of access and control - has become increasingly used for understanding the ecological impacts of climate change on wild birds. While field experiments are crucial for establishing causal links between temperature variation and biological responses, they present numerous logistical challenges. Moreover, reproducibility between such studies remains a challenge, largely due to heterogeneity among methodologies. Here, we comprehensively review published nestbox temperature manipulation studies, and document widespread variation in heating protocols and temperature measurement approaches. We also present a case study of a pilot temperature manipulation experiment in blue tits (*Cyanistes caeruleus*), documenting the project from planning through field implementation. By outlining methodological constraints and logistical pitfalls, we demonstrate the value of transparently reporting setbacks to guide future research. Furthermore, we highlight how temperature logger placement and calibration can introduce substantial variation in recorded nest temperatures. Specifically, we show that small changes in logger position can shift apparent treatment effects by an order of magnitude, revealing a critical source of methodological inconsistency that is rarely discussed in the literature. Building on key lessons from our field experiment, we outline a practical framework for nestbox temperature manipulation experiments, emphasising pilot testing, adaptive field protocols, welfare safeguards, and standardised temperature measurement. By transparently reporting the logistical barriers encountered in our pilot study, we provide a practical roadmap to improve rigour, comparability, and welfare outcomes in avian climate change research.

Introduction

As global temperatures rise and extreme climatic events become more frequent and intense with ongoing climate change (IPCC, 2023), there is growing urgency to understand how wildlife responds to these altered environmental conditions, especially during their most vulnerable developmental stages. In altricial bird species, nest microclimate is frequently a key determinant of egg development, nestling growth, and fledgling success (Bleu et al., 2019; Corregidor-Castro & Jones, 2021; Larson et al., 2015; Perez et al., 2020). Observational studies relying on ambient temperature data have formed the basis of much of our current understanding of important associations between climate and avian life histories (Satarkar et al., 2026). However, while these correlative approaches are tremendously valuable, they are often limited by their inability to disentangle the causal effects of nest temperatures from confounding environmental variables, particularly at the microhabitat scale most relevant to developing eggs and nestlings.

To overcome these limitations, researchers have increasingly turned to experimental manipulations of nestbox temperatures in the wild. The widespread adoption of nestboxes in ecological research for cavity-nesting species like great tits (*Parus major*), blue tits (*Cyanistes caeruleus*), and tree swallows (*Tachycineta bicolor*) has facilitated such experimental approaches, providing easy access to free-living birds and their microenvironment. By altering thermal conditions during critical phases such as incubation and the nestling period, such experiments potentially allow the examination of causal links between microclimate and the physiology, growth, behaviour and fitness of both offspring and parents.

Despite these advantages, the design and execution of temperature manipulation experiments in natural settings can be complicated by methodological challenges and field conditions. Unpredictable weather, such as cold spells, heatwaves, or heavy rain, can override intended temperature differences between treatments, introducing noise and reducing experimental control. In addition, practical constraints such as difficulty accessing remote field sites, power supply limitations, and the need for frequent nest access without excessive disturbance can limit the duration and intensity of manipulations. Together with welfare considerations for wild animals, these factors can make even the most carefully planned experiments difficult to implement consistently.

An additional challenge is the striking heterogeneity in methods reported across the literature. Studies differ widely in their choice of heating and cooling apparatus, the duration and magnitude of temperature treatments, and the monitoring and reporting of manipulated nest temperatures. For instance, experimental heating of boxes has been achieved using candles (Tov & Wright, 1993), heatpacks and various electronic devices (Figure 1C; see full references in Supplementary Table S1). While these methodological choices are often understandably tailored to the specific goals and practical limitations of each project, such variation can hinder reproducibility and complicate comparisons across studies.

Moreover, the technical details of nest temperature monitoring, which are central to establishing the size of the treatment effect, are described with varying levels of detail in the literature. These details include thermologger placement within the nestbox, calibration procedures, and whether, and how, ambient or microhabitat conditions around the box (for example, nestbox orientation, shading, or canopy cover) are recorded. There is a clear need for greater transparency and standardisation of these methodological aspects to enable more robust experimental designs across different ecological contexts.

Nestbox temperature manipulations are typically motivated by the need to approximate future climate scenarios and to isolate the direct effects of temperature on developing offspring and parents. In practice, however, these manipulations simultaneously alter the developmental environment as well as the thermal cues birds respond to. This dual role of temperature, as both constraint and cue, means that common design choices (treatment magnitude, developmental phase, logger placement) can profoundly influence which biological question is actually being tested.

We have three main objectives here:

i. to systematically review published nestbox temperature manipulation studies and identify common methodologies and their caveats;
ii. to provide a detailed account of a small-scale temperature manipulation experiment in blue tits illustrating practical challenges encountered in the field;
iii. to offer practical recommendations grounded in empirical experience, aimed at improving the design, execution, and transparency of future experimental studies.

Ultimately, our aim is to provide a comprehensive resource for researchers designing temperature manipulation experiments in wild bird populations. We discuss whether current temperature manipulation protocols actually deliver the thermal contrasts they are assumed to provide, while offering the conceptual framework and practical guidance necessary for rigorous, transparent, and effective study design and implementation in this rapidly developing field.

Systematic review of nestbox temperature manipulation studies

We conducted a systematic literature search according to the methods described in (Foo et al., 2021). A Web of Science search using "(temperature manipulation OR heat OR temperature elevation OR cool* OR cold* OR microclimate OR therm*) AND (experiment*) AND (avian OR bird* OR 'cavity nest*' OR passerine) AND (nest OR nestbox) NOT insect NOT plant" yielded 306 results on 26 November 2025. After adding citation-based search results (total N=314), and abstract screening, 54 papers were retained, of which 42 were deemed relevant for this paper (see Supplementary information for excluded studies). Three additional studies were identified at a later stage (in March 2026) and have been incorporated into our synthesis and Supplementary Table S1.

Since the early 1990s, 45 studies (see Supplementary Table S1 for full citations*) have employed some form of nestbox warming/cooling, primarily during incubation or nestling-rearing (Figure 1B). These experiments are geographically clustered in a narrow latitudinal band in temperate Europe and North America (87.5% of studies between 35º N to 60º N; Figure 1A); are taxonomically biased, with 65% of all studies focusing on three cavity-nesting passerines: tree swallows, great tits, and blue tits (Figure 1D); and predominantly use wooden (21 studies) or wood-concrete (10 studies) nestboxes, which differ markedly from natural cavities in their baseline microclimatic properties and humidity profiles (Sudyka et al., 2023). While early work used heat-generating light sources under nests (Bryan & Bryant, 1999; Londoño et al., 2008) or electric resistors (Meijer et al., 1999), most modern studies favour inexpensive and convenient air-activated heatpacks, which have been claimed to increase nest temperatures up to 10ºC above control nests (Dawson et al., 2005; O'Brien & Dawson, 2007). However, reported temperature differences relative to controls (ΔT) are highly variable (heating range of 0.6 to 8ºC, cooling range of -0.6 to -8.2 ºC) (Figure 1E); at the same time, the stated locations within the nestbox where temperature was measured is also very variable (Figure 1F).

Supplementary Table S1 synthesises these methodological details across 46 studies (including this study), highlighting the profound variation in temperature treatments, apparatus used and measurement approaches. Cooling experiments are notably less common, comprising only 12 of 46 studies. This likely stems from the practical challenges involved, as cooling apparatus (most commonly icepacks; Figure 1C) typically require more frequent replacement and are harder to standardise. Heightened welfare concerns are also relevant, given that young hatchlings cannot yet regulate their own body temperature and are therefore particularly vulnerable to cold exposure (Satarkar et al., 2026).

As Supplementary Table S1 reveals, air-activated heatpacks dominate heating protocols (Figure 1C), yet there is striking reported variation in how effectively they can elevate temperatures. Nestbox properties, such as construction and material, likely contribute to this variation. Compared to natural cavities, artificial nestboxes are thermally less stable across breeding stages, showing higher maximum temperatures, larger daily amplitudes, and poorer insulation from peak ambient temperatures (Sudyka et al., 2023). Many temperature manipulation studies further modify nestbox design and material with insulation such as polystyrene, which helps mimic natural cavity profiles more

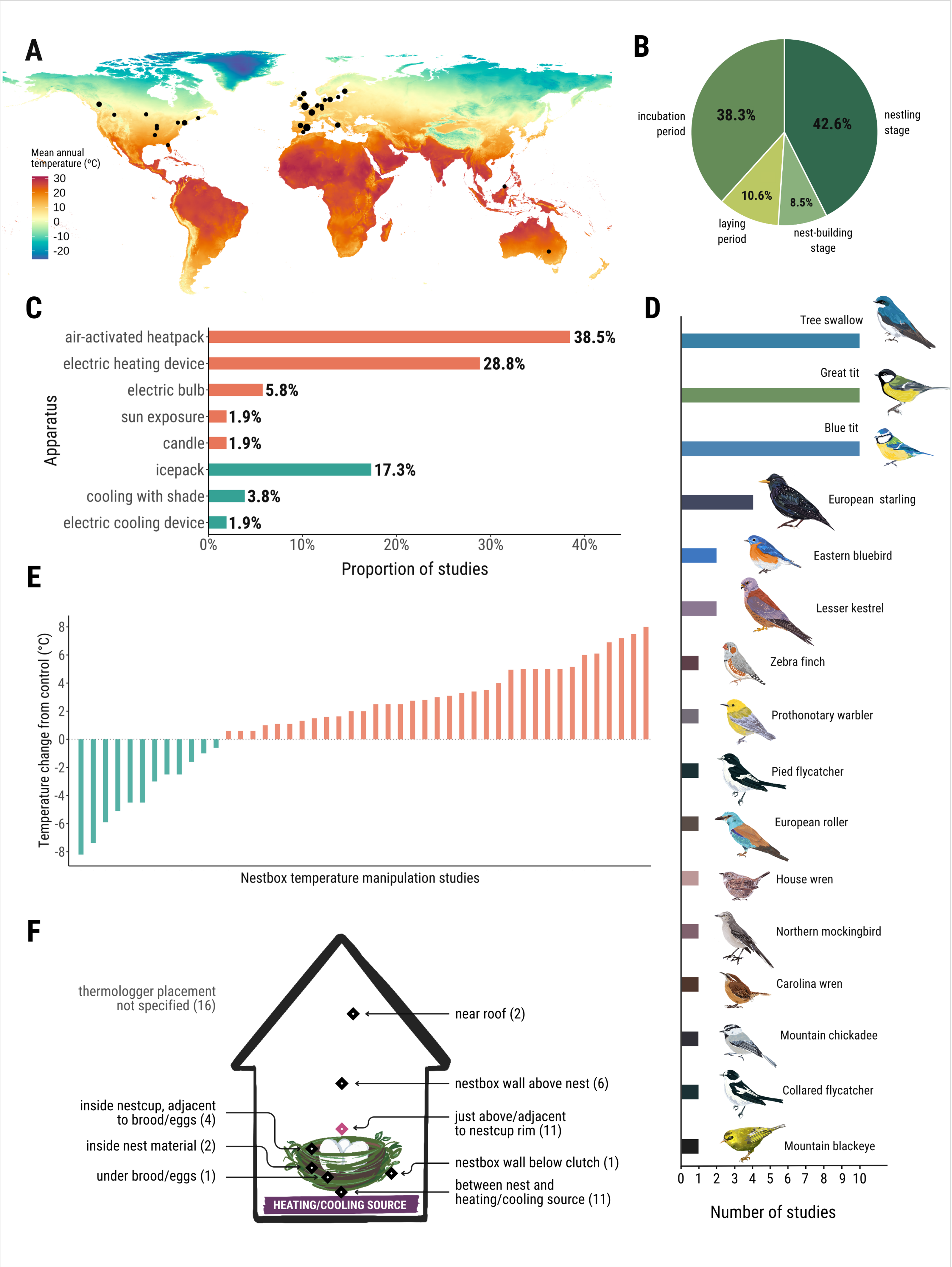

Figure 1. (A) Global distribution of the 46 nestbox warming/cooling studies (including our pilot experiment), showing strong clustering in temperate Europe and North America. Colours indicate mean annual temperature (°C) and the size of each point represents the number of studies per location; (B) Proportion of studies conducted at different reproductive stages, from nest-building through incubation to the nestling period; (C) Proportion of studies using different types of experimental apparatus for warming (red) and cooling (blue);

Figure 1. (D) Taxonomic distribution of studies across 16 cavity-nesting bird species, with 30 out of 46 studies focusing on tree swallows (*Tachycineta bicolor)*, great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*); (E) Reported temperature differences between experimental and control nestboxes (Δ°C) for heating (red) and cooling (blue) treatments, illustrating the wide range of achieved temperature manipulations; (F) Reported positions within the nestbox where temperature was measured (with number of studies in parentheses), highlighting heterogeneity in measurement location among studies (All positions are shown in black, except the pink one which shows the position where temperature was measured in our pilot study); *Bird illustrations in (D) by Devi Satarkar.*

closely but can shift internal temperatures by several degrees (Larson et al., 2018). Therefore, in practice, apparently identical heating protocols may generate substantially different realised temperature changes depending on baseline nestbox construction and experimental design modifications, representing an important but frequently unacknowledged source of methodological heterogeneity.

Moreover, there is also considerable variation in temperature measurement practices, which are likely to make formal comparisons of temperature differences difficult. In particular, the placement of temperature loggers (most often discrete coin-shaped *iButtons*) varies widely, meaning that studies may record fundamentally different thermal microenvironments within the same nestbox. Loggers have been positioned directly above the heat source beneath the nestcup (Albert et al., 2023; Dawson et al., 2005; Rodríguez & Barba, 2016), adjacent to nests near the eggs/nestlings (Ardia et al., 2010; Woodruff et al., 2025), or mounted above the nest on the nestbox walls at varying heights (Arct et al., 2022; Duncan et al., 2024) (Figure 1F). Such heterogeneity in logger placement generates substantial variation in recorded temperatures that is independent of treatment effects, thereby undermining not only interpretation of results but also cross-study comparability. Recording intervals also range from 1-60 mins, and information on logger calibration and ambient temperature measurements is often missing. In our review and recommendations, we primarily consider designs where heating or cooling elements are placed beneath the nestcup, as in our own pilot experiment, but note that a few studies apply heat from the roof or walls of the nestbox; full details of heating/cooling apparatus placements are summarised in Supplementary Table S1.

These technical details are largely overlooked, but are a likely source of heterogeneity across studies, which complicates cross-species comparisons and meta-analyses. For instance, both positive effects of increased nest temperatures on survival (Dawson et al., 2005) and negative effects on growth rates (Corregidor-Castro & Jones, 2021; Woodruff et al., 2025) have been reported, which may also stem from variation in heating protocols rather than biology alone. Furthermore, this methodological heterogeneity presents a confusing array of options for researchers planning new experiments or attempting replication, without clear guidance on trade-offs or 'best' approaches for specific aims. Next, we explore some of these methodological challenges in the context of a small-scale study of nest-box inhabiting blue tits.

Experimental heating in blue tit nestboxes: a case study of practical challenges and logistical pitfalls

Decades of detailed life-history data on great and blue tits from Wytham Woods, UK, combined with historic climate records, reveal that extreme climatic events influence growth and survival in a developmental stage-specific manner. In particular, while hatchlings are sensitive to temperature fluctuations in the first week of their development, increased temperatures appear to benefit older nestlings by indirectly affecting their food source or parental provisioning (Satarkar et al., 2026). We repeated these analyses using blue tit data from the same population (smaller dataset spanning fewer years), which yielded similar stage-specific patterns (Satarkar, D., 2025, *unpublished*). To causally test these patterns, we designed a nestbox temperature manipulation experiment targeting blue tits, which are more amenable to experimental manipulation than great tits at this long-term study site. This was intended as a pilot experiment, in order to establish baselines and ethical guidelines. However, the pilot experiment faced substantial field challenges that necessitated protocol adaptations, which we document here to illustrate methodological realities of field experiments of this kind.

Insights from pre-experimental pilot tests

Prior to the field experiment, we conducted calibration tests using unoccupied woodcrete nestboxes (Schwegler) to finalise the experimental layout and validate our target temperature manipulations (ΔT). These pre-experimental tests using three heatpacks in unoccupied nestboxes achieved reliable nest temperature elevations of ~2.3°C across multiple trials (n=6). While these were not designed as formal replicates and varied in setup, the broadly consistent direction of elevations suggested ~2.3°C as a plausible value, which we set as our target effect size. Apart from being invaluable for protocol refinement, insights from these tests also helped explain the marked variation in internal nest temperatures during field implementation.

Thermal images of the heated boxes (Figure 2A) revealed a non-uniform distribution of heat within the nestbox. Importantly, iButtons placed at different heights within the nest (Figure 2B) recorded marked differences in temperatures, with temperature elevated up to 12.4°C immediately above the heatpacks (below the nest material) (Figure 2D), but only ~2.3°C at the nest rim where nestlings would be located (Figure 2C). This gradient highlights the strong insulating effect of nesting material, which has important implications for how nest temperatures are measured and reported, but also has reassuring animal welfare benefits by shielding young nestlings from excessive heat at the source. Nest height is therefore another critical factor that should ideally be standardised in nestbox temperature manipulation experiments. Furthermore, these findings illuminate the variable temperature measurement approaches detailed in Supplementary Table S1, where thermologger placements range from below nests to wall-mounted positions several centimetres above the nest (Figure 1F). Incidentally, higher temperature changes are achieved when loggers are reported to be closer to the heat source. This variation likely contributes to the wide range of reported temperature differences across studies, even when similar heating apparatus are used.

Methods

We conducted the heating experiment in 14 (7 heated, 7 control) nestboxes occupied by blue tits in Wytham Woods, Oxfordshire in spring 2025 (Supplementary Figure S1). The nestbox breeding population of blue tits in this mixed-deciduous woodland has been monitored using standardised procedures since the early 1960s (Perrins, 1965). We paired boxes by hatch date, and used a coin toss to randomly assign each box within a pair to either the heated or control treatment.

Our original protocol targeted dual developmental stages: hatchling (0-7 days post hatching (dph)) and nestling (8-15 dph), with 4 heated + 4 control nests per stage (total N=16). However, high early-stage mortality led us to abandon the hatchling stage on welfare grounds, expanding instead to 14 nests (7 heated, 7 control) for the nestling stage only. A cooling treatment was also initially planned but abandoned prior to field implementation, owing to welfare concerns about exposing young hatchlings to reduced temperatures.

The final experimental protocol consisted of placing two heatpacks (*UniHeat 72h*) below the nestcup when nestlings were 9 days old. The treatment lasted 5 days, with heatpacks replaced every 2 days. Before placing the heatpacks, all nestlings were transferred to a cloth bird bag and the nestcup was carefully lifted to place the 2 packs. Nestlings were then returned to the nest, and five individuals per brood were ringed. These marked nestlings were weighed every 2 days until day 15 post hatching, which is when nestlings are ringed in this population. To measure in-nest temperatures as experienced by nestlings and parents, we secured iButton thermologgers (*Thermochron DS1922L*) with duct tape to the nestbox wall at the edge of the nest cup, approximately at the height where nestlings were (shown as a pink dot in Figure 1F and Figure 2B), and recorded temperature every 30 minutes.

Results

Across the 7 heated boxes, mean in-nest temperatures were elevated by ~0.6°C relative to paired controls, substantially lower than the ~2.3°C increase achieved in pre-experimental calibrations using three heatpacks. Linear mixed models showed no significant difference in growth rates between treatments after heating began ($p > 0.8$), but control chicks were consistently heavier than heated chicks by approximately 0.6g from day 8 onward ($p < 0.01$ at all ages tested). However, sample sizes were too small for robust statistical inference. For the originally planned hatchling stage (0-7 dph), we observed mortality in 2/3 heated boxes vs 1/3 control boxes, prompting discontinuation of this stage.

As biological outcomes are not the focus of this methodological review, detailed temperature time series, growth curves, and model details are provided in Supplementary Figures S2–S4; the following section instead details methodological deviations and key lessons learned.

Methodological and logistical barriers: lessons from the case study

During field implementation, we had to deviate substantially from our planned protocol in response to unforeseen constraints, highlighting logistical challenges that could not be fully anticipated at the design stage. For instance, our pre-experimental tests demonstrated that deploying three heatpacks could reliably raise nest temperatures by ~2.3°C on average. However, structural limitations of the nestbox restricted us to two packs, in order to allow parents sufficient space to provision the developing nestlings. This reduced the temperature difference to ~0.6°C on average.

The 0.6°C difference must be interpreted cautiously, as we observed substantial between-nestbox variation in temperature profiles (Supplementary Figure S3). Some of this between-box variation could likely be attributed to sun exposure and box orientation, which we did not control for but clearly affected nest temperatures. Studies have shown that nestbox structure, orientation, and solar exposure strongly influence internal microclimates (Ardia et al., 2006; Ellis, 2016; Lambrechts et al., 2010; Sudyka et al., 2023), making these critical factors for experimental planning.

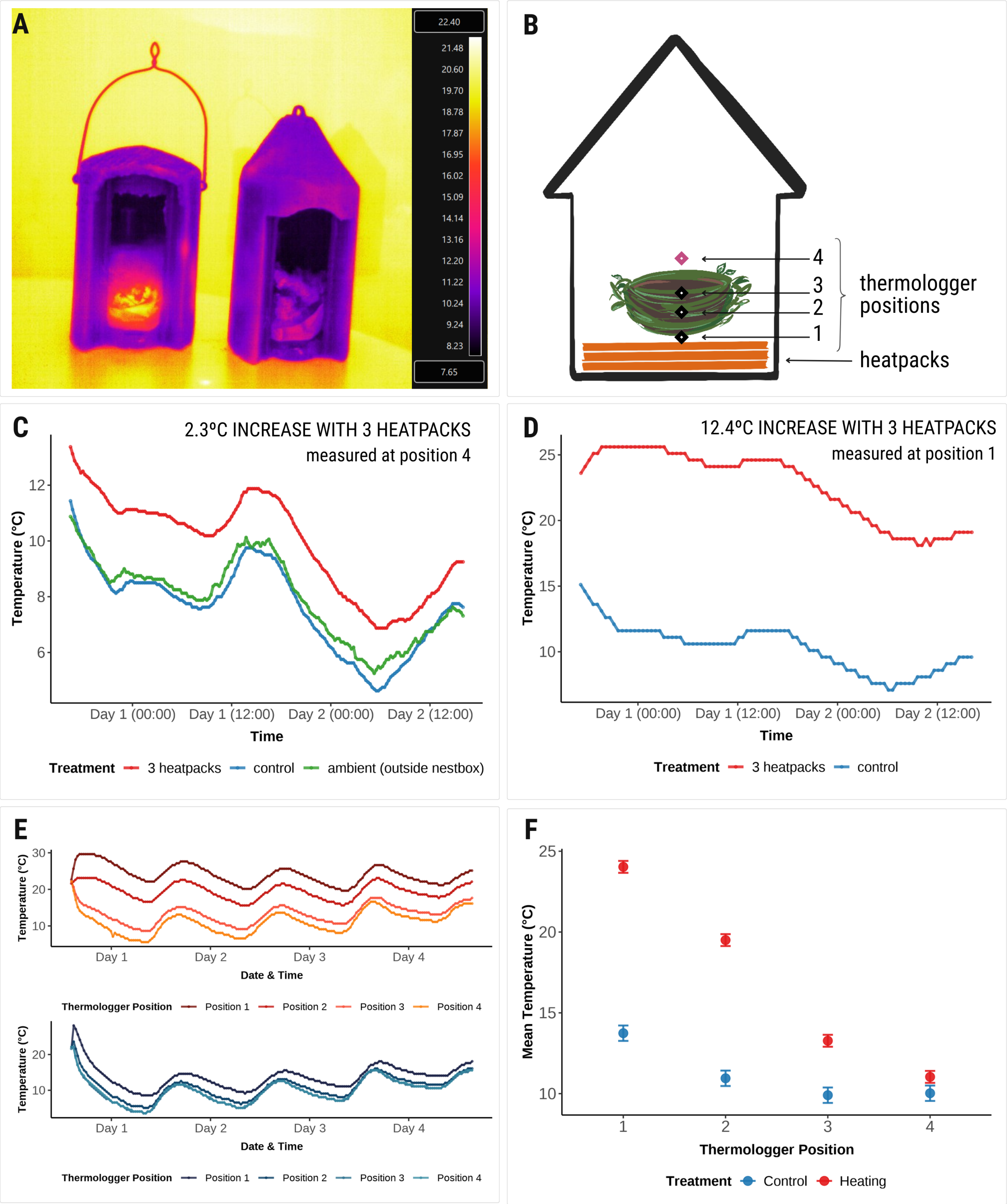

Figure 2. (A) Thermal image of heated and control nestboxes showing non-uniform heat distribution, with hotspots immediately above three heatpacks and cooler regions higher in the nest; (B) Schematic of four *iButton* thermologgers at different positions within the nestbox: position 1 (immediately above heatpacks, below moss), position 2 (inside nest material); position 3 (inside nestcup), position 4 (nest rim, nestling height), along with three heatpacks below the nestcup (position 4 was selected for the pilot experiment: shown in pink); (C) Temperature time series comparing heated (red), control (blue) and ambient temperatures outside nestbox (green), measured at position 4 (Trial 1), showing a ΔT of ~2.3°C on average with three heatpacks; (D) Temperature time series comparing heated (red) vs control (blue) treatments, measured at position 1 (Trial 2), showing a ΔT of ~12.4°C on average with three heatpacks; (E) Time series of temperature profiles across all four positions comparing heated (top panel, red gradient) vs control (bottom panel, blue gradient) nestboxes (Trial 3); (F) Estimated marginal means (±95% CI) from Trial 3, showing mean temperatures by position and treatment; All temperatures measured in °C.

Another significant deviation was the discontinuation of heating during the early developmental stage, when we had intended to heat 3-day-old hatchlings for five consecutive days. However, we observed mortality in early-stage broods, which led us to err on the side of caution. Although the small sample size prevents us from attributing mortality directly to the heating treatment, we prioritised animal welfare and consequently abandoned the original dual-stage design. Furthermore, nestlings younger than seven days lack feathers and cannot thermoregulate independently, making them particularly sensitive to temperature manipulation (Satarkar et al., 2026). Notably, nestling mortality/abandonment rates appear underreported across the nestbox temperature manipulation literature, despite ethical importance and potential impacts on effect sizes.

Methodological recommendations for nestbox temperature manipulation studies

Published work and our case study demonstrate how methodological choices strongly influence experimental feasibility and outcomes. Building on these insights, we propose a set of practical recommendations for future studies looking to conduct temperature manipulations in nestboxes.

1. To improve reproducibility and comparisons across studies, we propose a **standardised checklist** for reporting methodological details (Table 1)

Component	Details to report
Equipment	type/brand of heating/cooling device, layout, number of devices, replacement frequency
Temperature treatment	planned ΔT, achieved mean ΔT + standard deviation (with n), temperature profiles, duration
Temperature monitoring method	type/brand of thermologger, exact placement (height relative to nestcup/eggs/chicks), logging intervals, calibration details
Multimodal measurements	Measures of ambient temperature, internal nest temperature, nestbox wall temperatures (if measured)
Sample sizes	Planned and actual sample sizes, reasons for reductions (if any)
Developmental stage	Exact age windows (or days during incubation/laying), criteria for selecting developmental stage
Nestbox specifications	Material, size/dimensions, Insulatory material (if used), Nest height (nesting material between heat/cooling source and eggs/nestlings)
Welfare measures	Predefined criteria for terminating experimental treatments, observed mortality/distress (if any), ethical approvals

Table 1. Standardised reporting checklist for nestbox temperature manipulation experiments along with recommended methodological details required for reproducibility and synthesis across studies

2. Thorough **pilot testing** must be conducted prior to full-scale experiments for gaining important insights for experimental design and contingency planning. Indoor and outdoor tests with unoccupied boxes can be used to;
    - characterise temperature gradients within the nestbox,
    - assess how many heating/cooling devices (and their layout) are required for achieving biologically meaningful, but not harmful, temperature differences,
    - identify structural constraints (e.g., sufficient space for adults to provision, insulatory effects of nesting material),
    - quantify between-box variation driven by orientation, elevation, and canopy cover, which can then be either controlled for in design (e.g., stratified allocation) or explicitly modelled.

3. We recommend focusing temperature manipulations on more **developmentally robust stages**, when thermoregulatory mechanisms are more well-developed. Early-stage hatchlings are likely to be more vulnerable to even modest thermal perturbations, and require especially stringent welfare monitoring. A possible workaround could be to run small trials in live broods to test disturbance levels and welfare responses before scaling up. Cooling experiments warrant particular caution in this regard, given the acute vulnerability of young hatchlings to cold exposure.

4. We suggest **transparent reporting** of deviations from plans and omitted components to provide useful context for interpreting results, and help other researchers avoid repeating the same problems. Even when sample sizes are small, or the results are statistically inconclusive, reporting what did not work contributes valuable methodological knowledge and can help reduce cumulative trial-and-error in the field.

5. Closer **collaboration** across research groups would help build consensus on best practices and

improve comparability. This could include sharing protocols, agreeing on minimum reporting standards, coordinated experiments, setting conventions for temperature measurement and calibration, and common welfare guidelines that are feasible across species and habitats.

Perspectives and future directions

As demonstrated by the extensive methodological variation in the literature and our case study's practical constraints, nestbox temperature manipulation experiments face inherent ecological, logistical and ethical challenges that complicate experimental design, data comparability, and biological inference, despite being a frequently used tool for understanding avian responses to climate change. These limitations can be partially mitigated by rigorous experimental design and well thought-out contingency plans in the face of welfare or logistical concerns.

Despite limited statistical power and minimal temperature elevation achieved, our blue tit case study provides evidence that often overlooked factors like thermologger placement, nest material insulation, nestbox orientation, and welfare-driven protocol changes can strongly influence both temperature profiles and experimental feasibility. Our pilot only achieved a 0.6°C elevation against a 2.3°C target, highlighting how even carefully planned ΔT targets can be difficult to achieve consistently in field conditions. Insights from such statistically inconclusive pilots can help refine expectations and guide design choices in emerging studies of this kind.

While fixed ΔT targets offer experimental simplicity and control, they are typically justified by projected increases in global mean temperatures, which is not entirely representative of real-life conditions experienced by wild birds. Increased temperature variability is another important aspect of climate change, apart from just shifts in average temperatures (Vasseur et al., 2014). Future work could target more ecologically realistic temperature profiles that match observed weather extremes from long-term records. Rather than imposing arbitrary ΔT targets, treatment levels could instead be drawn from historically observed heatwave magnitudes or cold snaps at the study site. Long-term observational datasets can also help identify candidate mechanisms and developmental windows, which smaller, targeted experiments can then be designed to test causally. Integrating long-term observational data with small, carefully designed manipulations may provide a more robust alternative to large, ambitious experiments that are vulnerable to multiple failure modes. This work aims to serve as a comprehensive guide for researchers planning nestbox heating/cooling experiments, and contributes to advancing standardisation and transparent reporting in avian nestbox temperature manipulation research, which is essential if future experiments are to yield comparable, interpretable insights into avian responses to climate change.

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Acknowledgements

We are thankful to the Wytham tit study and the hundreds of fieldworkers who have helped collect long-term data on great tits and blue tits over the years. We owe special thanks to Dr Keith McMahon and Dr Ella Cole, for their valuable insights during the planning and implementation of our pilot experiment, particularly when unexpected challenges during our busy field season required rapid thinking and substantial deviations from our original plans. We also thank Dr Carla Wagener and Dr Thomas Miller for lending us thermal cameras for our pre-experimental pilot tests. Additionally, we are grateful to the AWERB Committee of the Department of Biology for their critical feedback during the planning stages, which substantially improved our experimental design and animal welfare protocols. This work was supported by grants from NERC (NE/X000184/1) and a UKRI Frontiers Award (EP/X024520/1).

Author contributions

All authors contributed to the ideas of this study. Devi Satarkar and David López-Idiáquez conducted the pre-experimental pilot tests and the pilot experiment in Wytham Woods. Devi Satarkar collated primary literature and screened abstracts for the systematic review, with substantial contribution towards summarising methodologies across 46 studies by David López-Idiáquez. Devi Satarkar drafted the manuscript with David López-Idiáquez, Irem Sepil and Ben C. Sheldon providing critical feedback. All authors approved the final manuscript.

Data and code availability

This manuscript is a methodological review and does not report primary datasets. The temperature logger data and R code underlying the pilot experiment figures are available from the corresponding author upon reasonable request. No other data or code are associated with this study.