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Opinion

# Are ectotherm brains vulnerable to global warming?

Iván Beltrán[1,*,@], Suzana Herculano-Houzel,[2,3,4,@] Barry Sinervo,[5,†] and Martin J. Whiting[1,@]

**Elevated temperatures during development affect a wide range of traits in ectotherms. Less well understood is the impact of global warming on brain development, which has only rarely been studied experimentally. Here, we evaluate current progress in the field and search for common response patterns among ectotherm groups. Evidence suggests that temperature may have a positive effect on neuronal activity and growth in developing brains, but only up to a threshold, above which temperature is detrimental to neuron development. These responses appear to be taxon dependent but this assumption may be due to a paucity of data for some taxonomic groups. We provide a framework with which to advance this highly promising field in the future.**

## Highlights

Ectotherm brains are remarkably plastic to changes in developmental temperature; however, their physiological requirements may still make them susceptible to the higher temperatures associated with global warming.

Global warming affects the development of the ectotherm brain because rising temperature stimulate neuronal activity and growth up to a certain threshold, above which it is detrimental to neuron development.

Making predictions about temperature effects on neuronal development and cognition is currently hampered by what might be highly taxon-dependent effects documented in a limited number of species.

We propose that studying the developmental plasticity of the ectotherm brain can help us predict how a wider range of species will respond to global warming.

## Elevated temperatures affect ectotherm development

While elevated temperatures have been shown to affect a wide range of phenotypic traits [1,2] (Box 1), the impact of global warming on brain development has only recently been addressed. The brain was once thought to be a static organ due to its crucial role in controlling important physiological and behavioral processes [3]. However, we now know that brain development is a plastic, self-organizing process affected by a wide range of external factors including stress, diet, and importantly, temperature [4]. Studying the effects of temperature on the brain can help us understand, and possibly predict, changes in reproductive **phenology** (see Glossary) and behavior that have already been observed in many animals likely due to anthropogenic climate warming [5,6]. Although warming has not been uniform across the planet, the average temperature in terrestrial and aquatic environments has increased at an average rate of approximately 0.18°C per decade since 1980 [7]. Moreover, the relative area of land on the planet where animals experience greater average temperatures is expected to increase significantly in the future. For example, areas that experience average temperatures of 29°C are expected to increase from a current (2019) 0.8% to an approximately 19% in 2070 [7,8]. However, the existing literature on temperature-induced **developmental plasticity** in the brain of **ectothermic animals** has not been systematically assessed, preventing us from better understanding effects of rising temperatures on brain morphology and function.

Here, we review the effect of elevated temperature on brains of developing ectotherms; discuss implications of global warming for ectotherm brains as it relates to development and maintenance; and propose a framework for future research in the field.

## Common effects of elevated developmental temperatures on ectotherm brains

We conducted a literature search of studies that performed incubation of embryos and reported changes in the brain extending after birth (Figure 1; see supplemental information online). Overall, it is difficult to make generic statements on the responses to high temperature on the developing ectotherm brain because the reported effects depend on which baseline or control temperatures are tested and thus, how response curves are constructed. Therefore, ecological consequences

[1]Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia
[2]Department of Psychology, Vanderbilt University, Nashville, TN, USA
[3]Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA
[4]Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN, USA
[5]Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, CA, USA
[†]Deceased

*Correspondence:
ivan.beltran@mq.edu.au (I. Beltrán).
[@]Twitter: @IvanBeltran31 (I. Beltrán), @suzanahh (S. Herculano-Houzel), and @lizard_lab (M.J. Whiting).

### Box 1. Why are developing ectotherms particularly vulnerable to global warming?

In ectothermic animals, body temperature is regulated by exchanging heat with their surroundings. Therefore, rapid changes in environmental temperature can alter their behavioral and physiological performance making them especially vulnerable to climatic warming [67,68]. Most adult stages of ectotherms have different physiological and behavioral strategies to maintain optimal temperatures for body functioning, which can potentially buffer them from future elevated temperatures [55]. By contrast, the early thermal environment that developing embryos experience depends on the location and depth of the nest, in the case of oviparous species, or the body temperature of the female in viviparous and mouthbrooding species. Thus, the embryo has little potential to significantly modify its body temperature to cope with environmental challenges and therefore, it is a life stage particularly vulnerable to environmental stress [69,70]. Assessing the thermal sensitivity of embryos to higher incubation temperatures provides crucial information on the species' responses and vulnerability to global warming [69,71].

Importantly, most ectotherms have remarkable environmentally induced developmental plasticity [2,66]; as several aspects of their adult phenotype self-organize according to environmental conditions, organisms have a chance to cope with environmental heterogeneity [55,67]. Incubation temperature strongly influences the development of ectotherms with important consequences for hatchling fitness [48]. For instance, elevated temperatures accelerate embryonic development [72], reduce body size [16] and have diverse effects on the physiological and behavioral performance of many ectotherms (reviewed in reptiles [48], fish [73], insects [74], and amphibians [75]).

Importantly, the effects of temperature on living systems are discontinuous. In nonliving systems, Arrhenius kinetics describe how chemical reactions accelerate progressively at increasing temperatures; in contrast, in living systems, the metabolic processes that lead, for instance, to growth, strongly decelerate once temperatures exceed 25°C [9]. Such discontinuity has been attributed to temperature-dependent effects on proteostasis, the processes of folding, chaperoning, degradation and maintenance of protein function, whereby enzymatic rates increase until 24°C, but between 24 and 28°C start to suffer the consequences of increasing rates of protein unfolding, which places a burden on chaperone capacity [9,76]. Temperatures above 28°C rapidly fill that capacity and further increases the burden on proteostasis and diverts chaperone function to a point where protein synthesis is affected, and cell growth rates slow down [9]. An important implication of studying temperature-induced developmental plasticity is the identification of the determinants of common and species-specific impacts of global warming on ectotherms.

will crucially depend on how close a species already is to a threshold temperature above which temperature is unfavorable to brain development. Moreover, the relationship between neuron development and behavior is complex, and mere increases or decreases in certain processes resulting from high temperatures will not necessarily increase an animal's fitness.

### Cell damage and impaired neuronal development

The mechanisms behind heat-induced brain damage seem to be shared across animals and are likely related to disruption of protein synthesis and folding [9], increased anaerobic metabolism and oxidative stress, and loss of cytoplasmic organelles [10]. Accordingly, under temperature elevation, **heat-shock proteins** show increased expression to maintain protein function [11]. Elevated temperatures significantly downregulate genes that control normal brain development [11,12] and reduce the differentiation and survival of motor neurons [13,14], that also have cascading impacts on ectotherms muscular development [13,15]. This result explains why most ectotherms incubated at higher than usual temperatures are smaller and have reduced locomotor performance [16]. The reduced survival of developing neurons may also explain why elevated temperatures reduce the size of the brain in lizards [17] and the **mushroom bodies** (MB) in insect brains [18–20].

### Increased neuronal activity

Warm temperatures appear to have an excitatory effect on the developing nervous system of ectotherms, increasing the activity of neurons in insects (28–35 vs 23°C) [21,22], fishes (30–32 vs 26–28°C) [10,23] and amphibians (19 vs 8°C) [24]. Warmer temperatures increase cholinergic activity in sharks (30 vs 26°C) [10], frogs (19 vs 8°C) [24], and moths (28 vs 23°C) [22]. Similarly, high developmental temperatures stimulate release of excitatory neurotransmitters in frogs (37 vs 24°C) [25], fishes (29 vs 17°C) [26], and moths (35 vs 23°C) [21]. Importantly, the effect

### Glossary

**Aromatase:** an enzyme that catalyzes the conversion of testosterone to estradiol, which then binds to nuclear and membrane-bound estrogen receptors in various target tissues to exert genomic and non-genomic effects.

**Developmental plasticity:** the process by which a genotype produces distinct phenotypes depending on the environmental conditions under which development takes place.

**Ectothermic animals:** animals that derive the heat they require for body functioning from external sources, such as sunlight or a heated surface. This group includes invertebrates, fishes, amphibians and nonavian reptiles.

**Heat-shock proteins:** proteins that are produced in response to exposure to stressful or abnormal conditions (commonly elevated temperatures), to facilitate cells processes including protein synthesis and refolding, and prevent protein aggregation and denaturation.

**Microglomeruli:** spheroidal structures with high density of synapses located in the mushroom bodies of insects.

**Mushroom bodies:** higher integration centers in the insect brain that process olfactory and visual information. The mushroom bodies are composed of thousands of neurons (Kenyon cells), whose dendrites form a calyx-shaped structure and whose axons form a stalk or peduncle-shaped structure.

**Myelin:** a lipid-rich specialized membrane that surrounds nerve cell axons insulating them to increase the rate at which electrical impulses (i.e., action potentials) pass along neurons.

**Phenology:** the study of the timing of recurring biological events (e.g., reproductive events), and the biotic and abiotic forces responsible for their timing.

**Preoptic area:** part of the anterior region of the hypothalamus. It is recognized as a pivotal region in the central regulation of body temperature in mammals, and is crucial for the control of some male sexual behavior in mice and reptiles.

**Ventromedial nucleus:** refers to a collection of cells located ventrally in the intermediate hypothalamic region. The VMH is important in the regulation of female sexual behavior, feeding, energy balance, and cardiovascular function.

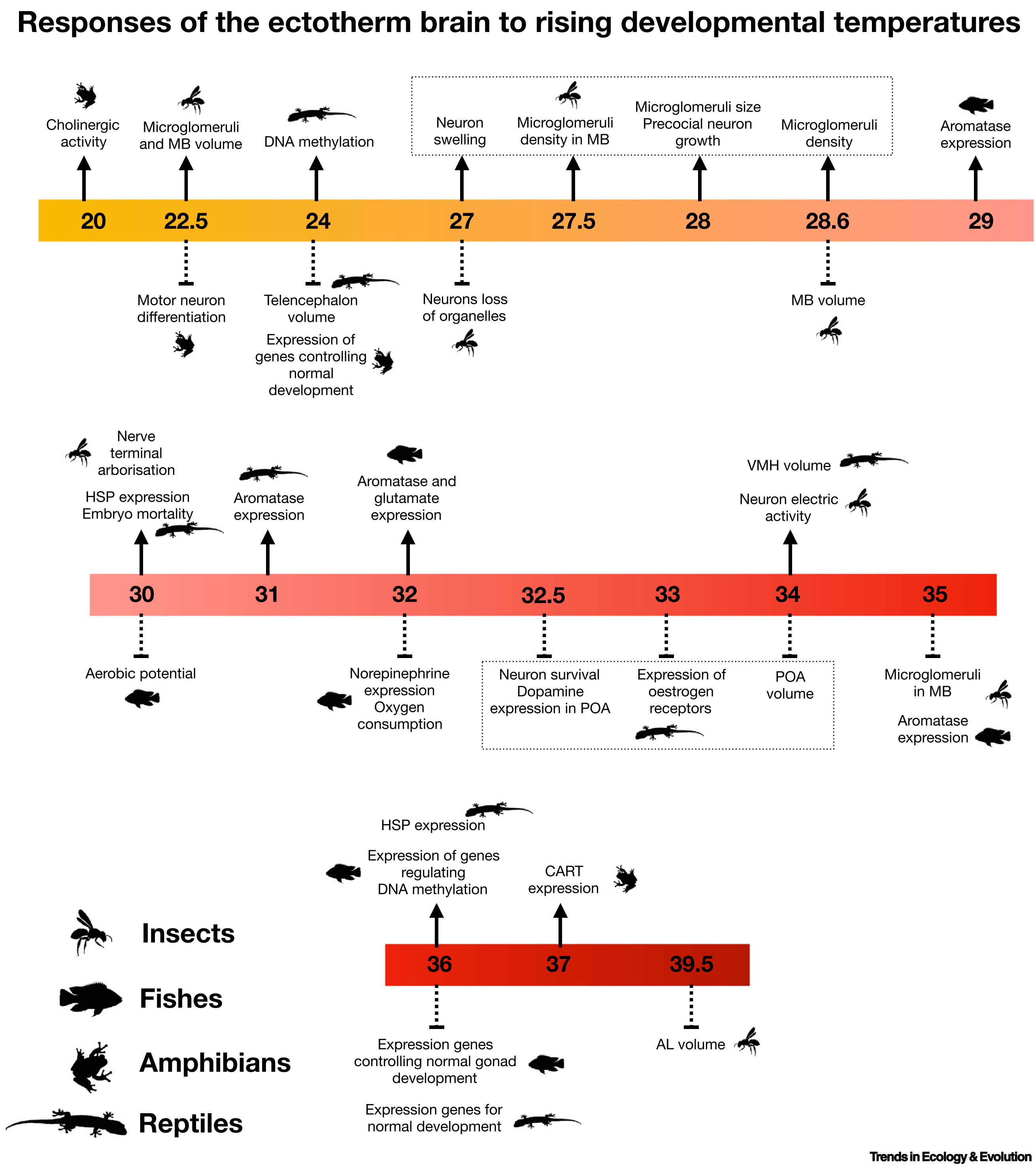


Figure 1. Effect of developmental temperatures on the ectotherm brain. The figure shows commonly reported morphological and physiological changes to the brain for four ectotherms groups, induced by developmental temperatures from 20 to 39.5°C. Arrows pointing upwards indicate processes or functions that increase with increasing temperature, while the broken arrows pointing down indicate process that are down-regulated or reduced. Abbreviations: AL, antennal lobe; CART, cocaine- and amphetamine-regulated transcript neurotransmitter; HSP, heat-shock protein; MB, mushroom body; POA, preoptic area of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus.

of temperature on neurotransmitter levels changes between developmental stages. Therefore, a comprehensive understanding of the consequences of environmental warming on the physiology and behavior of adult ectotherms remains elusive.

### Neuronal growth and proliferation

Warm temperatures promote the formation of terminal dendrites on nerves in flies (30 vs 25°C) [27], increase the number of **microglomeruli** in the MBs of solitary bees (27.5 vs 22.5°C) and ants (28.6 vs 25.4°C) [18,20], and increase neuronal density in the medial cortex of lizards (24 vs 18°C) [17]. In lizards, high temperatures (32.5 vs 26°C) upregulate expression of genes involved in **myelin** production and neurite outgrowth [14]; while temperatures above 34°C reduce the number of microglomeruli in the MBs of honeybees and flies [18,19], and are predicted to reduce the nerve terminal arborization in flies [27]. This evidence suggests that temperatures might stimulate neuronal growth in developing ectotherm brains up to a threshold, above which temperature is detrimental to neuron development. Nonetheless, we must be cautious when drawing conclusions about the fitness benefits that these brain changes can bring to animals.

In vertebrates, brain **aromatase** has been investigated extensively to understand the molecular basis of temperature-dependent sex determination [28]. Aromatase is present in many neural circuits and does not show sexual dimorphism in several fish species [29,30]; therefore, aromatase might do more than regulating reproduction in teleosts. Cells expressing aromatase actively divide and differentiate into neurons in several forebrain regions [31]. Therefore, brain aromatase is associated with the high-proliferative capacity of the teleost brain [26,32]. The expression and/or activity of aromatase in the brain increases with rearing temperature in fishes (29–35°C) [26,33] and reptiles (31–32.5°C) [34,35]. However, it is still unclear how temperature-induced changes in brain aromatase expression are linked to neurogenesis, and if the role of aromatase in neurogenesis is exclusive to teleosts [36].

### Region-specific effects of temperature

Developmental temperatures seem to affect some brain regions more than others. In insects, olfactory-innervated brain regions, such as the antennal lobes and the MB calyx, appear to be more sensitive to changes in temperature compared to other regions such as the optic lobes or the central complex [18–20]. In teleost fishes, elevated incubation temperatures (>36°C) have a clear disruptive effect on the hypothalamic–pituitary–gonadal axis [37,38], suggesting that high temperatures have negative impacts on the regulation of fish reproductive physiology and behavior (reviewed for adult stages [39]). In nonavian reptiles, brain regions regulating the expression of sexual behaviors such as the **preoptic area** (POA) and **ventromedial nucleus of the hypothalamus** (VMH) are particularly sensitive to changes in incubation temperature [40,41]. The volume of the POA in the gecko *Eublepharis macularius* increases with elevated incubation temperature from 26 up to 32.5°C, which is correlated with higher aggressiveness [40]. Likewise, the volume of the VMH in female *E. macularius* becomes smaller with incubation temperature up to 32.5°C and is correlated with lower attractiveness [40] – which shows that a similarly increased temperature during development does not have a common effect on different brain regions. Importantly, incubation temperatures above 32.5°C change the correlation between the volume of POA and the VMH, and their behavioral outputs [40].

### Effect of temperature on areas responsible for higher processing

Consistent with elevated neuronal activity and proliferation rates, high temperatures seem to have an excitatory effect on the development of cognitive ability in some ectotherms. The studies performed so far show that honeybees raised at 36°C were better foragers [42] and had better short-term memory compared to bees raised at 32°C [42,43]. Elevated incubation temperatures

(23.6 vs 20.6°C) produce sharks with a better quantity discrimination ability [44]. Skinks incubated at 24°C performed better in spatial and discrimination tasks [45] than those incubated at 18°C; however, high incubation temperatures have negative effects on the learning performance of other lizard species [46,47]. This inconsistency may be related to the fact that the high temperature treatment used in the skinks was relatively cool compared to those used in most studies (27–30°C) investigating the effect of temperature on reptiles [48].

Acute heat stress (39.5°C) during development disrupts associative odor learning in flies, despite having little effect in brain regions that control locomotor performance, memory, and sensory acuity [19]. Nonetheless, seemingly positive effects on cognition can create trade-offs that overall might not benefit an animal. For instance, skinks (22 vs 25°C) and agamids (27 vs 30°C) incubated at high temperatures performed better in spatial learning tasks but had reduced growth rates [49,50]. Similarly, high temperatures (36°C) produced honeybees with better foraging abilities and memory [42,43], but malformations were more common at this temperature [18]. Most studies have explored the short-term effects of elevated temperatures; therefore, it is still unknown if animals in the wild can compensate for the effects of incubation temperature on morphology through increased cognitive ability. Moreover, because high temperatures cause high mortality during development, it is unclear if the results observed so far are due to plastic responses during development or to selective mortality of animals with poor cognitive ability. Further research is needed to test how brain functioning and cognitive ability of ectotherms responds to a wider range of developmental temperatures.

## Predictions for a warmer future

Currently, we have little understanding of what the key variables are that determine how ectotherm brain development responds to temperature. The study of the role of developmental temperatures in ectotherm brains is challenging because of their incredible diversity of ectotherms and the wide range of life histories they exhibit. Also, importantly, we are constrained by a lack of experimental standardization in the field and a dearth of experimental studies that generally lack ecological relevance.

Our literature search suggests that rising temperatures will generally produce animals with a reduced brain size and a reduced number of neurons if temperatures go beyond a certain threshold (e.g., >34°C in insects and >32°C in lizards). However, these responses appear to be highly taxon dependent, which could reflect a specialization to different thermal niches or a lack of experimental data in some groups. Elevated temperatures will likely affect neuronal survival in some brain regions more than others. In insects, regions responsible for processing olfactory cues seem to be particularly vulnerable to increasing temperatures [18]. In fish, reproductive physiology will clearly be affected by rising developmental temperatures [37,51], while elevated incubation temperatures might alter important aspects of reptile social behavior and learning [17,40]. It is important to highlight that these distinct changes observed between ectotherm groups could be an artifact of the species targeted and possibly not due to a group-specific effect of temperature on brain functioning.

We consider that nonaquatic insects might be more vulnerable to global warming because air temperatures are rising at a faster rate than water temperatures [7], and average environmental temperatures are already higher than those expected to minimize the cost of development in non-nesting terrestrial oviparous ectotherms [52]. Nonetheless, the strength of the temperature–size response is greater in aquatic ectotherms, possibly driven by reduced availability of oxygen as water gets warmer [53] and the lower spatial variability in operative thermal conditions that may constrain behavioral responses to changing temperatures relative to terrestrial taxa [54].

Further studies on the general effects of developmental temperatures in the brain will help to assess the vulnerability of ectotherms to global warming.

## Framework for future studies

The lack of experimental standards has complicated the understanding of temperature-induced changes in the developing brain of ectotherms. We provide some basic guidelines to ensure that further research provides comparable findings across taxa and facilitates future metanalyses.

### Diversifying model organisms

Amphibians and nonmodel insects (e.g., beetles and grasshoppers) are under-represented in the field and offer an interesting opportunity for new discoveries. Similarly, researchers can define their model organisms based on their vulnerability to global warming (e.g., tropical species [7,55]). We suggest that wide taxonomic coverage will be more useful for testing for the existence of common patterns for the effect of temperature on the development of the ectotherm brain.

### Determining thermal treatments

It is important to first determine the optimum temperature at which development time is reduced and energy consumption minimized ($T_{opt}$) [52]. Then, researchers should test temperatures below and above the $T_{opt}$, with at least three, but ideally more, different incubation treatments [56–58]. It is only once such curves are systematically obtained for different species that common patterns for the effect of temperature will be found, as well as establishing the degree to which determinants are species specific. This will help us establish a unified theoretical understanding of the effects of global warming on ectotherm brains.

We strongly suggest designing experiments that include ecologically-relevant fluctuating thermal regimes (Box 2). The variance in these fluctuating regimes will depend on the baseline thermal variation that the embryos experience in their nest or microhabitats.

### Applying thermal treatments

Thermal treatments should be applied in the earliest stages of development possible, ideally after fertilization. Although this could be difficult to implement in some species (e.g., mouthbreeders and viviparous species), thermal environments can be modified for reproductive females, allowing also the evaluation of maternal effects [59]. Similarly, thermal treatments should be applied during the entire development; however, in future research, scientists might be interested in determining the sensitivity to temperature of particular developmental stages (S. Radmacher, PhD thesis, University of Regensberg, 2011). Importantly, while testing effects of temperature on brain

### Box 2. Fluctuating temperatures

Some studies have incorporated fluctuating thermal regimes in their incubation treatments, which is a more ecologically realistic approach because it considers natural variation in temperatures during development. In fluctuating thermal regimes, some of the negative impacts of constant high temperature are attenuated. For instance, solitary bees *Osmia bicornis* incubated under fluctuating temperatures (10–25°C) were larger and had larger MBs (with more microglomeruli) compared to bees incubated at a constant temperature of 17.5°C (S. Radmacher, PhD thesis, University of Regensberg, 2011). Similarly, in the ant *Camponotus mus*, fluctuating temperatures (amplitude 3.3°C) that mimic the natural temperature variation in the nest produced pupae with higher numbers of microglomeruli in the MBs compared to pupae reared at smaller or larger thermal amplitudes (0, 1.5, and 9.6°C), or at constant temperatures (25.4 and 35°C) [20]. These studies suggest that fluctuating temperatures can protect insect embryos from unfavorable incubation-induced changes in the brain.

Although few studies have compared the effect of constant and fluctuating temperatures in the development of the brain in vertebrate ectotherms, reptile hatchlings incubated under fluctuating temperatures show more balanced sex ratios, have a larger body size and a higher locomotor performance compared to those incubated at constant temperatures [59,77,78].

development, other variables that influence development such as relative humidity or dissolved oxygen, should be controlled or accounted for [10].

### Evaluating changes to the brain

Future research should run anatomical and physiological studies in parallel when possible. This will help us understand how changes in gene expression, enzymatic activity or neurotransmitter levels correlate with changes in neuron size or density. The use of a specific technique will depend on the resources and interests of the researchers; however, the possibilities are wide and include traditional stereological methods [60], brain imaging [61], isotropic fractionation [62], transcriptomics [63], and immunohistochemistry [26,40].

### Collecting and reporting data

Information that is rarely reported but is crucial for these types of studies includes the threshold temperatures that induces brain malformations or embryo mortality; the exact stage when embryos enter the experimental conditions; a detailed description of the landmarks used to dissect brains into specific regions; and complementary measurements such as behavioral assays or basal metabolic activity. As with any study, we strongly encourage researchers to make data public in online repositories (e.g., GenBank, Dryad, and OSF). This would allow researchers to explore relationships (or non-significant effects) that were not reported but are of interest to other researchers.

## Concluding remarks

Research to date shows that the brains of ectotherms have a remarkably plastic response to changes in developmental temperature. Importantly, the effect of rising temperatures is not a linear, universal response but rather the consequence of physiological changes that depends on the starting temperature [9]. For instance, increasing developmental temperatures up to 30°C enhances cognitive abilities in honeybees, sharks, and some lizards, but higher temperatures disrupt the reproductive physiology and growth of fish and amphibians. Similarly, up to a threshold temperature, higher incubation temperatures promote brain activity and neuronal growth, after which, we observe a reduction in neuron differentiation and survival. Importantly, fluctuating temperatures can protect ectotherm embryos from unfavorably high incubation temperatures. This evidence supports recent claims that given enough habitat diversity to buffer rising temperatures and perhaps a longer time to adapt, many ectotherm species will respond positively to a marginal increase in air temperatures [64] (see Outstanding questions). Other views consider that phenotypic and behavioral plasticity might not protect ectotherms from global warming. Behavioral plasticity can constrain or slow the rate of adaptive evolution by shielding the genotype from the effects of selection (e.g., digging deeper nests) [65]. Similarly, phenotypic plasticity can be maladaptive if it does not match changes to the future environment [66]. Further research is necessary to investigate the fitness value of traits that are affected by elevated temperatures.

We suggest that insect brain development might be more vulnerable to global warming because of the negative effect of elevated temperature on their development costs [18,19]; however, we have a poor understanding of what determines how animals respond to temperature, highlighting the necessity for research in this area.

The study of temperature-induced changes on developing ectotherm brains provides valuable information on how global warming might impact the physiology and behavior of animals and consequently, their ecological responses, and whether they will be sufficient under projected warming scenarios. Moreover, these studies also offer an interesting opportunity to understand the evolution of developmental plasticity across animals.

### Outstanding questions

What are the thermal limits for temperature-induced developmental plasticity of the ectotherm brain? These limits can be taxon-dependent and/or related to constraints in neuron development, brain volume, energetics, etc.

What influence does fluctuating temperature have on brain development in ectotherms? Will (and how does) global warming and other threats to biodiversity such as habitat degradation, affect the natural fluctuation of temperatures experienced by developing ectotherms?

What are the key variables that determine how animals respond to changes in developmental temperature? How similar are optimal temperatures for brain development among ectotherms? Is there a common threshold among ectotherms for the stimulating effects of temperature on the nervous functioning?

Are the temperature-induced changes in the brain during development fixed? Is it possible that the observed negative effects disappear or are ameliorated during the course of the animal's life?

Will these plastic changes in brain development help animals adapt to new environments? Or, will phenotypic plasticity in brain development be maladaptive? For instance, changes to the brain through plasticity may incur trade-offs in the development of other organs. How often do plastic changes in the brain drive, or are favored by, natural selection?

Considering the negative effects of high temperatures on the brain development of ectotherms, how does the endotherm brain manage to function at such high temperatures? What are the mechanisms behind this capability? And are ectotherms capable of evolving similar responses?

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## Declaration of interests

The authors declare no interests.

## Supplemental Information

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