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The roles of acclimation and behavior in buffering climate change impacts along elevational gradients

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Abstract

1. The vulnerability of species to climate change is jointly influenced by geographic phenotypic variation, acclimation, and behavioral thermoregulation. The importance of interactions between these factors, however, remains poorly understood.

2. We demonstrate how advances in mechanistic niche modelling can be used to integrate and assess the influence of these sources of uncertainty in forecasts of climate change impacts.

3. We explored geographic variation in thermal tolerance (i.e. maximum and minimum thermal limits) and its potential for acclimation in juvenile European common frogs (*Rana temporaria*) along elevational gradients. Further, we employed a mechanistic niche model (NicheMapR) to assess the relative contributions of phenotypic variation, acclimation and thermoregulation in determining the impacts of climate change on thermal safety margins and activity windows.

4. Our analyses revealed that high elevation populations had slightly wider tolerance ranges driven by increases in heat tolerance but lower potential for acclimation. Plausibly, wider thermal fluctuations at high elevations favor more tolerant but less plastic phenotypes, thus reducing the risk of encountering stressful temperatures during unpredictable extreme events. Biophysical models of thermal exposure indicated that observed phenotypic and plastic differences provide limited protection from changing climates. Indeed, the risk of reaching body temperatures beyond the species' thermal tolerance range was similar across elevations. In contrast, the ability to seek cooler retreat sites through behavioral adjustments played an essential role in buffering populations from thermal extremes predicted under climate change. Predicted climate change also altered current activity windows, but high-elevation

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populations were predicted to remain more temporally constrained than lowland populations.

5. Our results demonstrate that elevational variation in thermal tolerances and acclimation capacity might be insufficient to buffer temperate amphibians from predicted climate change; instead, behavioral thermoregulation may be the only effective mechanism to avoid thermal stress under future climates.

Key-words: acclimation; activity restrictions; behavioral thermoregulation; Bogert effect; global warming; mechanistic niche modelling; NicheMapR; thermal-safety margins

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Introduction

Anthropogenic climate change is a major threat to global biodiversity, stimulating numerous attempts to predict the vulnerability of populations, species and ecosystems (Thomas *et al.* 2004; Williams, Jackson & Kutzbacht 2007; McCain & Colwell 2011; Buckley & Kingsolver 2012; Orizaola & Laurila 2016). Projected rates of climate change may hinder species' abilities to adapt to novel conditions or to track their climatic requirements through dispersal (Araújo, Thuiller & Pearson 2006; Quintero & Wiens 2013). Thus, intraspecific phenotypic variation, acclimation, and behavioral thermoregulation are critical for species' persistence in a warming climate (Kearney, Shine & Porter 2009; Richter-Boix *et al.* 2015).

Macrophysiological studies on the vulnerability of ectotherms to climate change have flourished in recent years, largely due to an increasing availability of physiological data and fine-resolution climate layers. Many of these studies have suggested that species occupying warmer and more stable environments will be disproportionately vulnerable to warming (i.e. species occupying lower elevations and latitudes; Deutsch *et al.* 2008; Tewksbury, Huey & Deutsch 2008; Duarte *et al.* 2012; but see Overgaard *et al.* 2011; Overgaard, Kearney & Hoffmann 2014). A number of recent investigations have challenged this view, however, demonstrating that thermal safety margins (i.e. the difference between experienced maximum temperatures and heat tolerance, *sensu* Sunday *et al.* 2014) and acclimation potential of species from high elevations and latitudes may not be as high as previously thought (Gerick *et al.* 2014; Sunday *et al.*

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2014; Gunderson & Stillman 2015; Gunderson, Dillon & Stillman 2017). In addition, intraspecific variation in environmental tolerances, acclimation capacity, and the potential for behavioral thermoregulation to buffer species from thermal extremes have largely been overlooked in forecasts of species' responses to climate change (Valladares *et al.* 2014; Buckley, Ehrenberger & Angilletta 2015).

Environmentally-driven phenotypic and genetic variation among populations is widespread (Conover & Schultz 1995; Linhart & Grant 1996). Yet, compromises between local adaptation, acclimation and behavioral thermoregulation are emerging as key determinants of vulnerability to climate change. Behavioral thermoregulation may weaken selection on thermal tolerance and the potential for thermal acclimation – a process known as the 'Bogert effect' (Bogert 1949; Huey & Kingsolver 1993; Huey, Hertz & Sinervo 2003; Muñoz & Losos 2018). Further, local adaptation to warmer environments may reduce acclimation potential (Stillman 2003; but see Calosi, Bilton & Spicer 2008). The trade-off between high tolerance and reduced potential for acclimation of thermal performance may, in turn, increase the vulnerability of populations to environmental change (Buckley *et al.* 2015; Gunderson & Stillman 2015). Thus, we need to develop a deeper understanding of geographic variation in thermal tolerances, acclimation potential and behavioral thermoregulation, as well as an increased appreciation of their potential interconnections.

In ectotherms, daily and seasonal thermal fluctuations exert strong selective pressures on thermal traits (Richter-Boix *et al.* 2015; Gutiérrez-Pesquera *et al.* 2016). This could partly explain the observed wider thermal tolerance breadths (i.e. the difference between maximum and minimum critical thermal limits) towards higher elevations and latitudes (Addo-Bediako, Chown & Gaston 2000; Gutiérrez-Pesquera *et al.* 2016; but see Brattstrom 1968). Shorter growing season lengths at high elevations and latitudes also restrict the time available for growth and development (Dahl *et al.* 2012). Consequently, individuals may need to expose themselves to large daily thermal fluctuations to exploit transient food resources (Nicieza & Metcalfe 1997). In doing so, however, organismal body temperatures could approach critical thermal limits. Accurate prediction of these risks for terrestrial species requires an understanding of the processes of heat exchange (i.e. convection, radiation, conduction, evaporation and metabolism; Heath 1964; Tracy 1976; Kearney & Porter 2009) and behavioral

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thermoregulation; air temperatures alone may be uninformative of these events. Moreover, the choice of appropriate spatial and temporal scales is crucial to capture effective thermal environments and extreme temperatures that, however short, may be more important than the daily average in constraining a species' or a population's long-term persistence (Kearney, Matzelle & Helmuth 2012; Gerick *et al.* 2014).

Here, we experimentally study geographic variation in thermal tolerance limits and the effects of thermal acclimation on those limits in a temperate amphibian species. Amphibians are one of the most vulnerable groups to global change, including climate change. Our study models were juvenile European common frogs (*Rana temporaria*) from populations distributed along elevation gradients. We chose this life-cycle stage because it has been understudied despite being one of the weakest links in terms of climate change impacts. Newly metamorphosed individuals must rapidly gain structural size and reserves prior to the onset of winter, but high summer temperatures can impose severe temporal restrictions since they are predominantly diurnal (Vences *et al.* 2000), and are thus more likely to encounter highly stressful temperatures than are the nocturnal adult or aquatic larval life stages. Moreover, the thermal tolerance of newly metamorphosed amphibians is typically lower than that of larval or adult stages, very likely due to the energetic costs of metamorphosis (Cupp 1980; Lambrinos & Kleier 2003; Navas *et al.* 2007; Enriquez-Urzelai *et al.* 2019). Thus, a trade-off can arise between overwintering survival (enhanced by growth and reserve accumulation) and summer survival (subjected to physiological constraints, i.e., thermal tolerance and evaporative water loss). Therefore, global warming will likely increase the risk of thermal stress for juvenile amphibians.

To realistically capture the potential thermal extremes experienced by juvenile amphibians, we apply a thermodynamically-grounded mechanistic niche model (NicheMapR v1.3; Porter *et al.* 1973; Kearney & Porter 2017; Kearney *et al.* 2018; Kearney & Porter 2020) in combination with daily gridded weather data for Europe (Haylock *et al.* 2008; Brinckmann, Krähenmann & Bissolli 2016). NicheMapR integrates a microclimate model of the conditions above- and below-ground for a certain level of shade, with an animal biophysical model that solves coupled heat- and mass-balance equations to predict constraints on body temperatures given an individual's behavior, morphology, and available microclimates. Specifically, we address the following questions: 1) Do juvenile *R. temporaria* differ in thermal tolerance and acclimation potential along elevational gradients? 2) How does geographic variation in phenotype, acclimation potential, and behavioral thermoregulation influence the species' vulnerability to climate change? 3) How will exposure to thermal stress and resulting activity restrictions vary with climate change at different elevations?

Materials and Methods

STUDY SYSTEM

Rana temporaria is widespread through most of Europe and consequently encounters markedly different thermal environments across its geographic range. The Iberian Peninsula, as one of the main glacial refugia (Recuero & García-Paris 2011; Veith *et al.* 2012; Vences *et al.* 2013), harbors multiple lineages diverged during climatic oscillations (Veith *et al.* 2012; Dufresnes *et al.* 2020). During 2015 (August-October) and 2016 (June-August) we sampled juvenile individuals (~2 weeks after metamorphosis) from two replicate elevational gradients corresponding to two different lineages of *R. temporaria* (from central and eastern Cantabrian Mountains, hereafter '*central*' and '*eastern*'; Choda 2014). We sampled between 65 and 95 individuals from a total of seven populations (Table 1).

GEOGRAPHIC VARIATION IN THERMAL TOLERANCE AND PLASTICITY

Upon arrival at the facilities of the Research Unit of Biodiversity (University of Oviedo), juveniles were randomly assigned to one of two acclimation temperature treatments (14 or 24 ºC). These temperatures approximate the average temperatures during the start/end of the activity period (14 ºC) and the hottest period when animals are active (i.e. 24 ºC during July-August; see Fig. S6). We individualized juveniles in plastic containers with access to dechlorinated tap water to prevent dehydration. We placed plastic containers in two different environmentally controlled rooms set at 14 ± 1 and 24 ± 1 ºC, respectively, with a photoperiod of 12L:12D, and let juveniles acclimate for 3-5 days. The acclimation period used here represents enough time to stabilize critical thermal limits after a large change in acclimation temperatures between field and laboratory environments (Brattstrom 1968; Navas *et al.* 2010). We supplied juveniles with small *Acheta domestica* crickets *ad libitum*.

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After the acclimation period, we estimated thermal tolerances (critical thermal maxima and minima, CT_{max} and CT_{min}, respectively). For each population and acclimation temperature, juveniles were assigned to either CT_{max} or CT_{min} experiments. To estimate thermal tolerances, we followed Hutchison's dynamic method (Lutterschmidt & Hutchison 1997). We placed individuals in 100-mL plastic containers with dechlorinated tap water at 20 ºC, and heated or cooled the water at a constant rate of 0.25 ºC per minute using a refrigerated heating bath (HUBER K15-cc-NR; Kältemaschinenbau AG, Germany), for CT_{max} and CT_{min}, respectively. We considered that thermal limits had been reached when individuals remained unresponsive to external stimuli (10 gentle taps with a wooden stick). At that point, we recorded water temperature with a quick-recording thermometer (Miller & Weber; Ridgewood, NY, USA) to the nearest 0.1 ºC. Because of the small size of the individuals, we assumed that body temperatures equalled water temperature (Lutterschmidt & Hutchison 1997; Gutiérrez-Pesquera *et al.* 2016). After CT_{max} and CT_{min} tests, we submerged individuals into cold (14 ºC) or warm water (20 ºC), respectively, to allow recovery. After that, we placed individuals in their respective controlled environment rooms and we verified survival after 24h to ensure that thermal limits were not surpassed. Finally, we weighed each individual to the nearest 0.1 mg.

We used mixed effect ANCOVAs to test for effects of body mass, elevation, lineage, and acclimation temperature treatment. Body mass (*M*) was used as a covariate, and population was treated as a random effect. Elevation (*H* = high and *L* = low, Table 1), lineage ('*central*' and '*eastern*'), acclimation treatment (*14* and *24* ºC), and their interactions were treated as fixed factors. Interactions involving body mass that were nonsignificant (homogeneity of slopes) were removed from models. We visually assessed the normality of residuals using residual distribution and quantile-quantile plots.

BIOPHYSICAL MODELLING

We developed mechanistic niche models using NicheMapR – an R implementation of the biophysical models developed by Porter and colleagues (Porter *et al.* 1973; Kearney & Porter 2017; Kearney & Porter 2020). NicheMapR includes programs that solve heat and mass budgets for both microclimates and animals given terrain and

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weather conditions, and the animal's morphology, behavior, and physiology. In this way it can estimate hourly operative temperatures (T_e) – the steady-state temperatures that the animal could achieve in a given habitat. The microclimate model uses maximum and minimum air temperatures, precipitation, cloud cover, relative humidity, and wind speed values to reconstruct available microclimates. We obtained daily values for these parameters from the ECA&D (Haylock *et al.* 2008) and DecReg/MiKlip (Brinckmann *et al.* 2016) projects for each studied population during 2014 and 2015 (see Supporting Information).

To estimate available microclimates under a climate warming scenario, we examined projections for two time periods (2050 and 2070) using three global circulation models (GCMs: CCSM4, HadGEM2-CC, and GFDL-CM3) and two emission scenarios (*low*, RCP 4.5, and *high*, RCP 8.5). We computed monthly differences between current and projected climates (maximum and minimum temperatures, and precipitation) using WorldClim layers – IPCC5 – at a spatial resolution of 30 arc-seconds (Hijmans *et al.* 2005). We extracted projected monthly anomalies for each population, interpolated those to daily data, and added them to the observed values from ECA&D (see Supporting Information). We used weather data corresponding to the first modelled year (i.e. 2014) as the spin-up period (e.g. for snowpack); we only present results for 2015.

We modelled a medium-sized early juvenile (0.3 g), with its midpoint at 0.5 cm above the ground when active on the surface; a shape equivalent to that of the leopard frog (*Lithobates pipiens*); and assumed that 90% of the skin acted as a free water surface when individuals were active (see Kearney *et al.* 2008 and Fitzpatrick *et al.* 2019 for more information). Since juveniles are predominantly diurnal, we allowed only diurnal activity, bounded within the voluntary thermal maximum (VT_{max}, 18.5 ºC) and voluntary thermal minimum (VT_{min}, 9.5 ºC), selected in a thermal gradient experiment conducted on ~16 juveniles of each of the studied populations (Enriquez-Urzelai *et al.* 2018). When T_e fell outside of these temperatures, we assumed that animals moved underground to the depth with the closest temperature to their preferred temperature (T_{pref}, 13.1 ºC), obtained from the same thermal gradient experiment (Enriquez-Urzelai *et al.* 2018). See Supporting Information for detailed figures showing T_e traces and behaviour for example simulations.

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To quantify exposure to stressful temperatures, we modelled the T_e of non-thermoregulating (remaining above ground) or thermoregulating individuals (able to move underground) in full sun (0% shade) or deep (90%) shade. *Rana temporaria* juveniles remain at the edge of water bodies, where they forage on invertebrates (Vences *et al.* 2000; García-París, Montori & Herrero 2004), and retreat to small cracks and crevices in the soil during the hottest months of the year (U. Enriquez-Urzelai & A. G. Nicieza, personal observation). However, like other similar species (Lamoureux & Madison 1999; Roznik & Johnson 2009; Qi *et al.* 2011), radio-tracked adult *R. temporaria* also use small mammal burrows during summer (U. Enriquez-Urzelai, A. Gandara, A. G. Nicieza, unpublished data). Thus, we allowed thermoregulating juveniles to move up to 30 cm underground to examine any potential change in the effectiveness of behavioural thermoregulation (see Results). Subsequently, we computed the thermal safety margins (TSM: $CT_{max} – T_{e,max}$ and $T_{e,min} – CT_{min}$; *sensu* Sunday *et al.* 2014) of individuals acclimated to 14 and 24 ºC at the studied populations, using population-specific mean thermal limits at each acclimation treatment. Positive TSMs indicate that critical thermal limits exceed experienced thermal extremes, while negative TSMs suggest exposure to temperatures outside the tolerance range.

To explore the impacts of climate change on activity windows for populations at different elevations, we modelled thermoregulating juveniles – using underground retreats up to 30 cm deep – both for current and future climates, and with full sun or deep (90%) shade. We defined activity windows as the number of hours juveniles were predicted to be active during the whole year (*hours year*$^{-1}$). We repeated all simulations with population-specific mean body sizes (see weights in Table 1) but results were almost identical to those obtained employing the overall sample mean size (0.3 g) for all populations. Thus, we only present results for medium-sized juveniles (see Supporting Information for results obtain including population-specific sizes).

Results

GEOGRAPHIC VARIATION IN THERMAL TOLERANCE AND PLASTICITY

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We detected no evidence for heterogeneity of slopes for CT_{max} (no significant interactions between body mass and fixed factors; see Table S1). We found differences in CT_{max} due to mass, acclimation treatment, and elevation. Further, we found differences in acclimation potential due to elevation (significant acclimation treatment × elevation interaction; Table 2). Heavier juveniles showed slightly higher CT_{max} values, and populations from high elevations showed higher CT_{max} but lower acclimation potential than lowland populations (Fig. 1a). Lineage of origin did not affect CT_{max}.

CT_{min} varied with mass and acclimation treatment. We also found differences in acclimation potential of CT_{min} due to mass (mass × acclimation treatment), elevation (elevation × acclimation treatment), and mass within lineage (mass × lineage × acclimation treatment; Table 3). Smaller juveniles showed higher acclimation potential, and this effect was more evident in the '*central*' lineage. Similar to CT_{max}, heavier juveniles showed higher CT_{min} values, and acclimation potential was lower in high-elevation populations compared to lowland conspecifics (elevation × acclimation treatment; Fig. 1b).

EXPOSURE TO THERMAL EXTREMES

Physiological thermoconformity

Niche modelling simulations suggested that, under the current climate and full sun conditions, non-thermoregulating juveniles from both high and low elevations had CT_{max} values that exceeded operative temperatures (i.e., positive TSM), due to effects of evaporative cooling (Fig. 2a). The higher acclimation potential of CT_{max} observed in lowland populations resulted in similar TSM among different elevations when individuals were acclimated to high temperatures (Fig. S10; compare panel a with b). However, acclimation to warm temperatures had minor effects on individuals' TSMs and, for the sake of simplicity, we only present the results of simulations run with thermal limits of individuals acclimated to 14 º C (see Fig. S10 for all parameterizations). Conversely, all high-elevation populations and one low elevation (Huz) population showed negative TSM to cold extremes, for both warm and cold acclimation treatments (Fig. 2b, S10).

Our simulations further suggest that, by 2050, evaporative cooling may be insufficient to buffer low elevation populations exposed to full sun from extreme heat.

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By 2070, evaporative cooling may also become insufficient for two high elevation populations (Can and Vid) under full sun conditions; the other two highland populations (Sen and He) will experience T_e close to their CT_{max} (Fig. 2a). Deep shade, by contrast, may protect individuals from overheating under projected climates (Fig. 2c). Regardless of shading level, by 2070, only the lowland population that currently experiences negative TSM to cold extremes (Huz) will be able to tolerate the lowest temperatures during the year, according to the temperature scenarios for 2070 (Fig. 2b, d).

Accounting for behavioral thermoregulation

Under current climatic conditions and both shading levels, retreating to underground retreats as deep as 30 cm allowed juveniles to maintain positive TSM under both hot and cold extremes (Fig. 3a-b); however, by 2050, individuals from both high and low elevations may need to seek deeper retreats to avoid exceeding their CT_{max} when exposed to full sun (Fig. 3a). Even individuals from the population at the highest elevation (He) may need to shelter deeper than 30 cm by 2070 to escape heat stress. Acclimation to warm temperatures had limited impacts on these projections (Fig. S10; compare panel e with f, and m with n). Retreats in deep shade, however, are predicted to remain thermally suitable under projected climates (Fig. 3c).

ACTIVITY WINDOWS

Niche modelling simulations revealed that high elevation populations have reduced opportunities for activity compared to lowland conspecifics under current conditions (Fig. 4). Interestingly, while deep shade (90%) enabled individuals from lowland populations to be active for slightly longer periods, it strongly reduced activity windows in high elevation populations. Shade allowed juveniles from all populations to be active during the hottest hours during summer but prevented high elevation populations from achieving the temperatures required for activity during the coldest seasons.

Most of the studied populations were predicted to show a decline in activity under climate change scenarios when individuals were restricted to full sun. The only exception was the population at the highest elevation (He), for which individuals were predicted to have similar activity windows under current and future climates (Fig. 4a).

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Opportunities for activity of high elevation populations are predicted to decline most in summer and increase in spring, autumn, and winter. Lowland populations will experience reductions in activity during spring and autumn and increases during winter (Fig. 5a, c, e, g). Collectively, reductions in activity hours will exceed increases in full sun conditions under projected future climates (Fig. 4a).

When simulated to experience deep shade, activity windows for high elevation populations under climate change are predicted to decrease in summer but increase in spring and autumn (Fig. 5b, d, f, h). Overall, activity windows in deep shade are predicted to increase under climate change for high-elevation populations but were, nevertheless, predicted to be shorter than when individuals were restricted to full sun. Lowland populations were predicted to experience a marked reduction in activity times during spring, summer, and autumn, and an increase during winter (Fig. 5b, d, f, h). It should be noted, however, that although high elevation populations show an increase in activity times under future climates in deep shade, they will remain more temporally constrained than their lowland counterparts under the same conditions (Fig. 4b).

Discussion

Most species constitute an array of locally adapted and unequally plastic populations (Hereford 2009; Benito Garzón *et al.* 2011; Orizaola & Laurila 2016). Accordingly, thermal traits and their plasticity frequently vary among populations of the same species, typically mirroring changes in the thermal environment (Freidenburg & Skelly 2004; Sinclair, Williams & Terblanche 2012; Sinclair *et al.* 2016). Ultimately, geographic phenotypic variation may result in an uneven sensitivity to thermal extremes across the range of a species and consequently influence forecasts of climate change impacts (Pearson, Lago-Leston & Mota 2009; Matesanz, Gianoli & Valladares 2010; Valladares *et al.* 2014). Behavioral thermoregulation, a mechanism by which animals can buffer themselves against extreme temperatures, will also be decisive for terrestrial ectotherms under changing climates (Kearney *et al.* 2009; Sunday *et al.* 2014). Yet, we are just starting to understand the interplay between environmental tolerances, plasticity, and behavioral thermoregulation, and how this interplay will influence

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climate change impacts (Williams *et al.* 2008; Chevin, Lande & Mace 2010; Huey *et al.* 2012; Jensen *et al.* 2019).

Previous interspecific studies have revealed higher variation in cold tolerance (CT_{min}) compared to heat tolerance (CT_{max}) associated with latitudinal and elevational gradients (Sørensen, Dahlgaard & Loeschcke 2001; Sunday, Bates & Dulvy 2011; von May *et al.* 2017). Recently, von May *et al.* (2017) showed that both CT_{max} and CT_{min} decreased with increasing elevation in tropical anurans (see García-Robledo *et al.* 2016; Pintanel *et al.* 2019 for other examples in ectotherms). At the intraspecific level, patterns of thermal limit variation along environmental gradients seem rather taxa specific. In some ectotherms critical thermal limits decrease with elevation (Miller & Packard 1977) or latitude (Jensen *et al.* 2019); in others thermal limits do not vary along environmental gradients (Gvoždík & Castilla 2001; Buckley *et al.* 2015; Slatyer & Schoville 2016). Our results show that while CT_{max} of juvenile *R. temporaria* increases slightly with elevation, CT_{min} does not, leading to wider thermal breadths in mountain populations. At high elevations, strong radiation along with diurnal behavior can expose temperate montane amphibians to extremely high temperatures (Vences *et al.* 2000). This contrasts with the pattern observed in most tropical high-elevation anurans, which are mostly nocturnal and use shelters during the day to avoid stressfully high temperatures (von May *et al.* 2017; Pintanel *et al.* 2019). Thus, a larger thermal breadth (or a higher CT_{max}) could widen activity windows. Further, the cold tolerance of *R. temporaria* is beyond the freezing point of water, regardless of elevation (Fig. 1b), due to the risk of freezing both at high and low elevations (Muir, Biek & Mable 2014). Previous studies have similarly reported increased thermal tolerance ranges towards higher latitudes and elevations (Gaston & Chown 1999; Araújo *et al.* 2013; Gutiérrez-Pesquera *et al.* 2016). Here, we found that wider thermal tolerance in high elevation populations was driven by small shifts toward higher heat tolerance.

Thermal fluctuations increase towards higher elevation and latitudes and, thus, one might expect concurrent increases in acclimation potential (i.e. plasticity). In a seminal paper, Brattstrom (1968) showed that the potential for thermal acclimation was very limited and remarkably similar between tropical and temperate anurans. Similarly, Gunderson & Stillman's (2015) analysis revealed no change in upper thermal

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tolerance and only a slight increase in CT_{min} plasticity of lizards with latitude. However, the results of other macroecological studies have shown that the acclimation potential in sublethal traits, such as metabolic rates, decreases with increasing latitude (Seebacher, White & Franklin 2015; Gunderson & Stillman 2015). In line with those studies, we found that juvenile *R. temporaria* originating from high elevation populations showed lower acclimation potential, not only in heat, but also in cold tolerance. Although plastic thermal tolerances may be adaptive (Sultan & Spencer 2002), the wider thermal fluctuations and shorter growing season lengths associated with high elevations plausibly favor a variety of physiological adaptations (Brattstrom 1968), including more tolerant and less plastic phenotypes, which avoid paying the costs of plasticity (Dewitt, Sih & Wilson 1998).

Projected rates of climate change exceed the estimated pace of historic niche evolution (Quintero & Wiens 2013). The high similarity in thermal tolerance observed here between phylogenetic lineages of *R. temporaria* in northwestearn Iberia, coupled with only slight differences in heat tolerance between populations from different elevations, suggest that *R. temporaria* may not be capable of adapting to novel conditions through niche evolution (but see von May *et al.* 2017). Moreover, our mechanistic niche models revealed that acclimation provides limited potential to buffer individuals from heat stress under changing climates, in agreement with recent macrophysiological studies (Gunderson & Stillman 2015; Gunderson *et al.* 2017): acclimation to either high or low temperatures had negligible effects on estimates of current and future thermal safety margins, even in the most plastic lowland populations. Indeed, the negligible degree of local adaptation, together with the higher potential for acclimation of lowland populations, led to similar thermal safety margins across elevations, as shown for other ectotherms across latitudes (Overgaard *et al.* 2014). Thus, although we expect biodiversity to shift towards higher elevations (McCain & Colwell 2011), our results show that the risk of overheating will be similar across environmental gradients (Overgaard *et al.* 2014). This could lead to local extinctions of southern populations regardless of elevation in most temperate amphibians. But, the consequences might be particularly acute for species endemic to small regions and mountainous areas (Parmesan 2006; Schwartz et al. 2006), where

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the whole species or evolutionary significant units could go extinct (e.g., Ahmadi et al. 2019).

Taken together, our results suggest that evolutionary potential and acclimation capacity are largely insufficient to buffer juvenile *R. temporaria* from thermal extremes under current and future climates. Instead, our mechanistic niche model revealed that behavioral thermoregulation is a key mechanism to escape extreme cold temperatures, especially for high-elevation populations (Fig. 2-3), as already shown by Ludwig, Sinsch, and Pelster (2013; 2015) for adult *R. temporaria* at high elevations in the Alps. The use of behavioral thermoregulation to avoid extreme temperatures could partly account for the observed low degree of local adaptation, through the 'Bogert effect' (Bogert 1949; Buckley *et al.* 2015; Muñoz & Losos 2018; Farallo, Wier & Miles 2018). In addition, our model suggests that behavioral thermoregulation will be key to compensating the loss of effectiveness of evaporative cooling under future climates.

Evaporative cooling is a highly efficient mechanism that allows wet skinned ectotherms, such as amphibians, to dissipate excessive heat (Tracy 1976; Kearney & Porter 2009). Our results show that, due to evaporative cooling, juvenile frogs avoid overheating at all elevations under current climates, even in full sun, as pointed out by Sunday *et al.* (2014). Notwithstanding, by 2050, lowland populations are predicted to be incapable of buffering themselves from extreme heat exclusively through evaporative cooling. Remarkably, by 2070, evaporative cooling may not protect even some high-elevation populations from overheating and others will experience body temperatures close to their maximum heat tolerance (Fig. 2). Thus, although evaporative cooling represents an effective mechanism for amphibians to avoid overheating under the current climate, regardless of elevation, behavioral thermoregulation might become crucial for juveniles to buffer heat stress in the future (Fig. 2-3; Kearney & Porter 2009; Sunday *et al.* 2014; Ruiz-Aravena *et al.* 2014).

Our simulations suggest that juvenile amphibians could potentially avoid high temperatures by seeking deep retreat sites or by restricting their activity to shaded microhabitats. However, the thermal quality of shelters will decrease with climate change. We show, for instance, that typical belowground retreats for *R. temporaria* (30 cm) may become insufficient to buffer rising temperatures at all elevations. Additionally, canopy cover is minimal at the studied sites, especially those at high elevations (Table 1). Thus, at high elevations, current shade levels and currently occupied retreats are unlikely to protect juvenile frogs from extreme temperatures. The availability of suitable microhabitats has been proposed as an important driver of elevational range limits (Wake & Lynch 1976; Jankowski *et al.* 2013) and drives thermal tolerance evolution in tropical mountains (Pintanel *et al.* 2019). Our results similarly suggest that the availability of suitable microhabitats (and microclimates) could be essential for the long-term persistence of *R. temporaria* populations and other temperate amphibians at high elevations. A detailed understanding of on-ground microhabitat structure, such as shade levels and retreat depths, is thus critical to making realistic predictions of species vulnerability to climate change, as well as to informing climate change mitigation strategies. For instance, ensuring thermally suited refugia for juveniles, e.g. around breeding ponds, could minimize the impacts of temperature rises as climate changes, leading to biologically sound, evidence-based mitigation strategies (Cuddington et al., 2013).

In theory, under a warmer climate, populations from colder environments (e.g., the tops of mountains) could benefit from wider activity windows (Levy *et al.* 2016). However, we demonstrate the opposite pattern: warming was predicted to decrease activity windows at all elevations. In general, decreases in activity hours during warmer months exceeded increases during colder months (Fig. 5). Regardless of the available amount of shade, activity windows will remain narrower with increasing elevation. In mountainous areas, weather conditions can change suddenly and unpredictably, and may vary substantially from valley to valley due to the rain shadow effect: while in one valley the weather may be sunny, in the adjacent valley a fog bank or a storm could increase soil and air humidity (Baudier & O’Donnell 2020). This effect could widen the simulated activity periods of some populations but would hardly change the general patterns reported here.

Conclusions

Geographic phenotypic variation, the potential for plastic responses, behavioral thermoregulation, and their interactions may mediate the impacts of climate change on the extinction risk of populations throughout a species’ distribution (Kolbe, Kearney & Shine 2010; Valladares *et al.* 2014; Buckley *et al.* 2015; Gunderson *et al.* 2017). We

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show that mechanistic niche models offer a flexible means with which to integrate and assess the influence of these sources of uncertainty in climate change forecasts (Kearney & Porter 2009; Moran, Hartig & Bell 2016). Applying this modelling framework to juvenile *R. temporaria* suggests that the primary source of forecast uncertainty is the role of behavioral thermoregulation, in particular, the species' ability to seek deeper retreats and shaded microhabitats, or wet microhabitats to rehydrate. In this sense, our results stress the importance of fine-scale patterns of thermal constraints on activity windows (see also Gunderson & Leal 2015). Despite observed phenotypic variation associated with elevational gradients, we found that the risk of reaching detrimental body temperatures under changing climates was predicted to be similar across elevations. Furthermore, although we might expect populations from colder environments to benefit from wider activity windows under climate change (Levy *et al.* 2016), frogs from high elevations were predicted to be more temporally constrained than lowland counterparts under both current and future predicted climates. Hence, climate change vulnerability may be quite similar across elevations, which could have highly detrimental consequences for endemic species and evolutionary significant units inhabiting mountain areas, either as a result of physiological stress or derived from indirect effects (e.g., competition with ecologically similar species).

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Authors' contributions

UEU, MT, AGN, and MRK conceived the idea and designed the study; UEU, MS, ASP, and AGN collected the data; UEU, MRK, RT, and AGN conducted statistical analyses and biophysical models; All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.dz08kprtv (Enriquez-Urzelai et al., 2020).

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Figure 1: **(a)** Heat tolerance – critical thermal maxima or $CT_{\max}$ – and **(b)** cold tolerance – critical thermal minima or $CT_{\min}$ – of *Rana temporaria* juveniles from high and low elevations, acclimated to 14 (*blue*) or 24 ºC (*red*). Boxes represent 95% CIs, bold horizontal lines medians, vertical lines higher and lower values within third and first quantiles, and dots outliers.

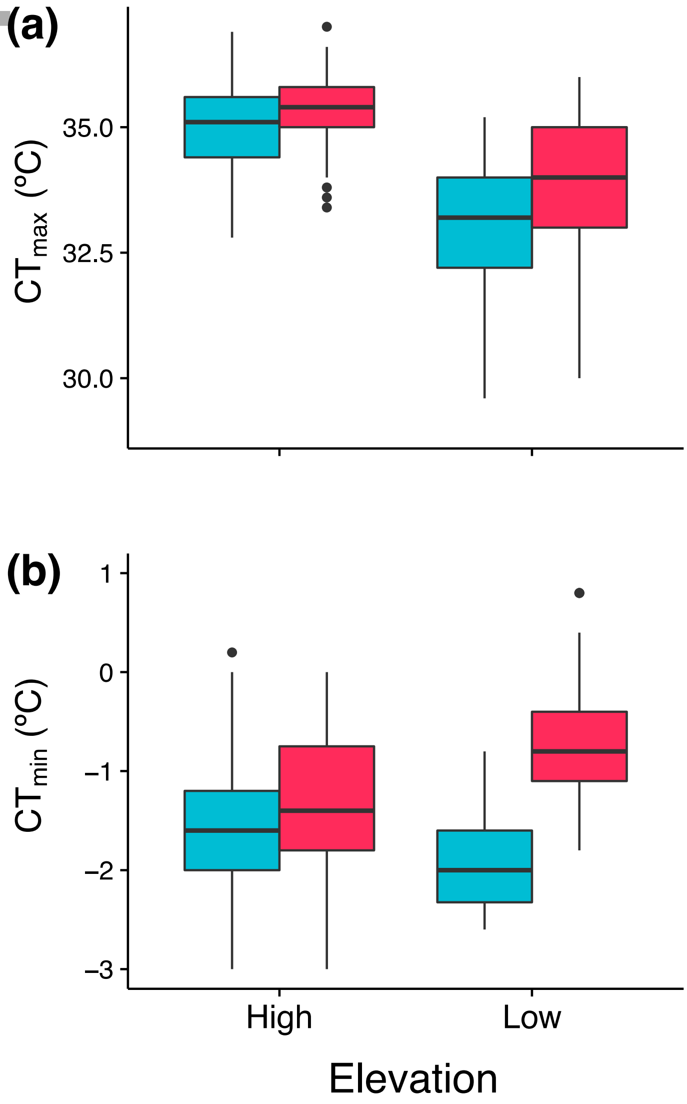

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Figure 2: Thermal safety margins (TSM) of thermoconforming juveniles for different populations ordered by elevation (see acronyms and elevations in Table 1) in (**a-b**) full sun (0% shade) and (**c-d**) deep (90%) shade. Panels (**a**) and (**c**) illustrate TSM to heat extremes, and panels (**b**) and (**d**) TSM to cold extremes. Note that, due to the negligible impact of acclimation, we only present results for individuals acclimated to 14 ºC. See Fig. S10 for all climatic scenarios and acclimation treatments. *Blue dots*: under the current climate; *yellow bars*: under projected climates for 2050; *red bars*: under projected climates for 2070; *orange:* overlap between simulations for 2050 and 2070.

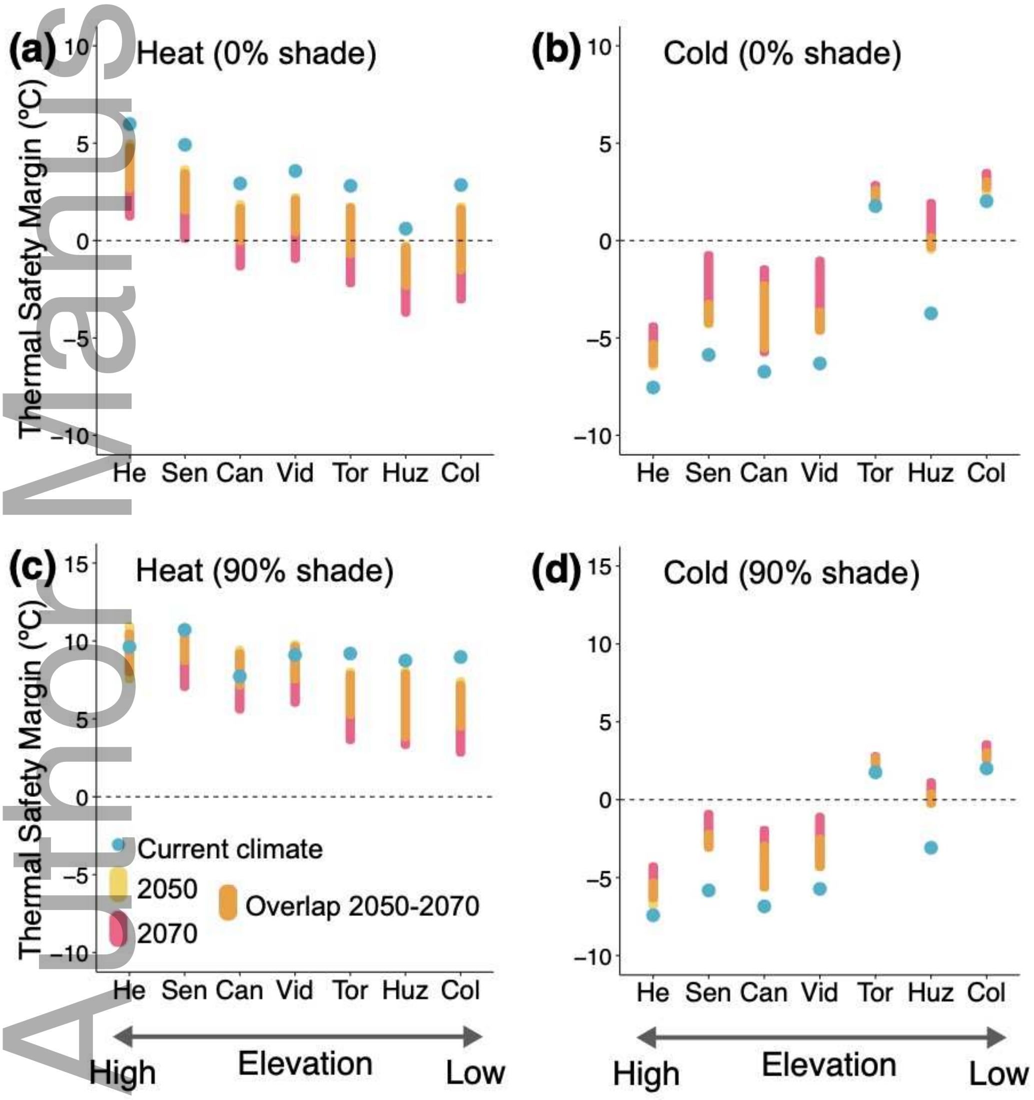

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Figure 3: Thermal safety margins (TSM) of thermoregulating juveniles for different populations ordered by elevation (see acronyms and elevations in Table 1), using retreats of up to 30 cm deep in (**a-b**) full sun (0% shade) and (**c-d**) deep (90%) shade. Panels (**a**) and (**c**) illustrate TSM to heat extremes, and panels (**b**) and (**d**) TSM to cold extremes. Note that, due to the negligible impact of acclimation, we only present results for individuals acclimated to 14 ºC. See Fig. S10 for all climatic scenarios and acclimation treatments. *Blue dots*: under the current climate; *yellow bars*: under projected climates for 2050; *red bars*: under projected climates for 2070; *orange:* overlap between simulations for 2050 and 2070.

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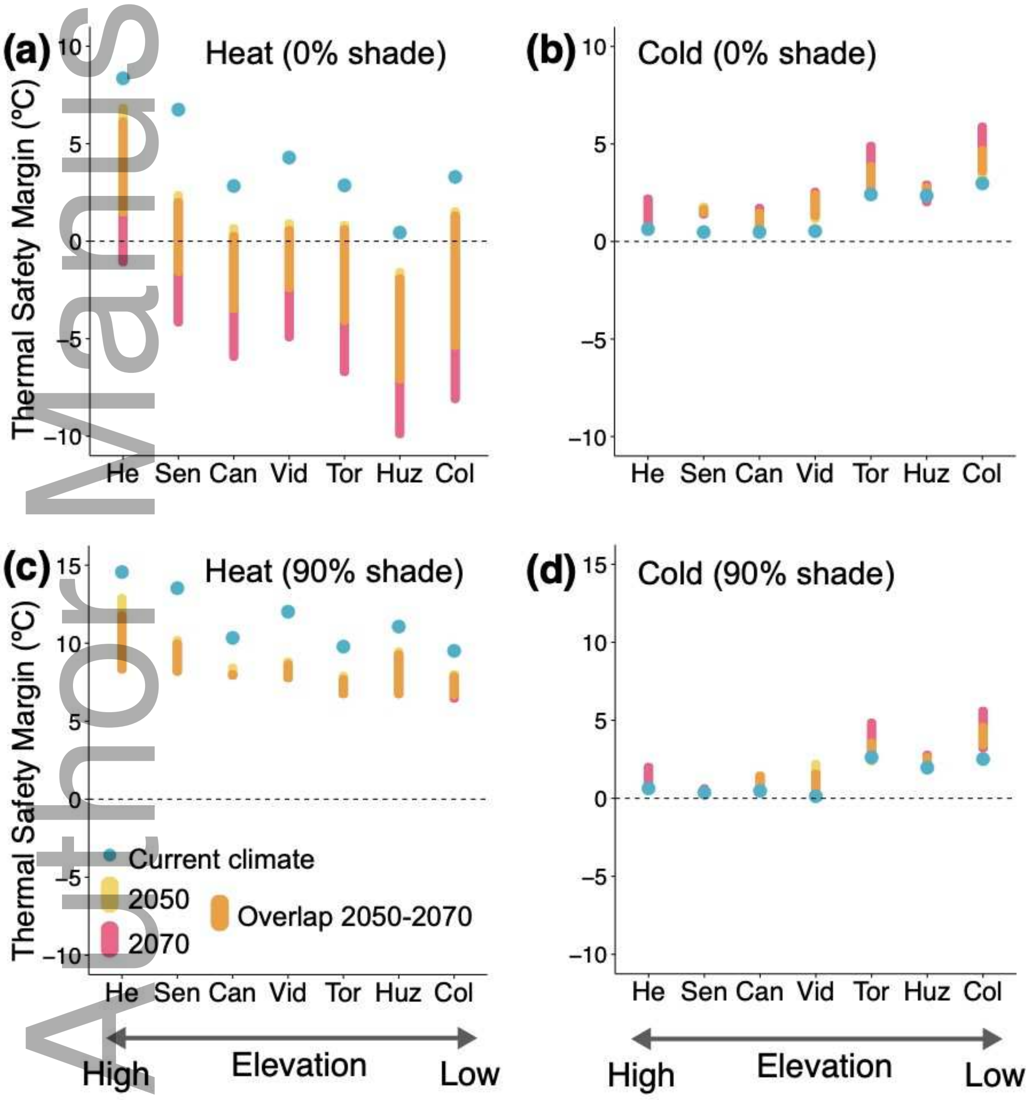

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Figure 4: Activity time in hours for different populations ordered by elevation (see acronyms and elevations in Table 1) under **(a)** full sun (0% shade) and **(b)** deep (90%) shade. *Blue dots*: under the current climate; *yellow bars*: under projected climates for 2050; *red bars*: under projected climates for 2070; *orange:* overlap between simulations for 2050 and 2070.

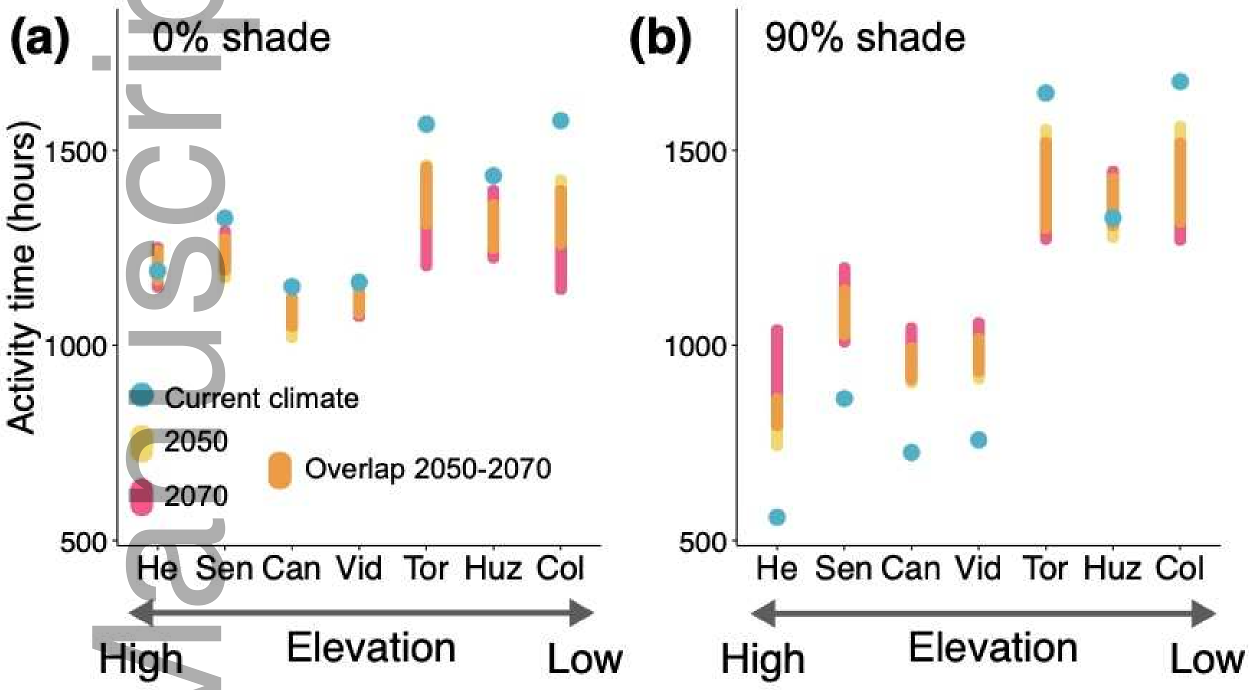

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Figure 5: Monthly differences in activity time relative to current climates under the specified shading levels for **(a-d)** a low emission scenario (i.e. RCP 4.5) and the most benign GCM (CCSM4), and for **(e-h)** a high emission scenario (i.e. RCP 8.5) and the GCM with the highest projected temperature increases (GFDL-CM3). *Blue polygons*: high elevation populations; *Red polygons*: low elevation populations.

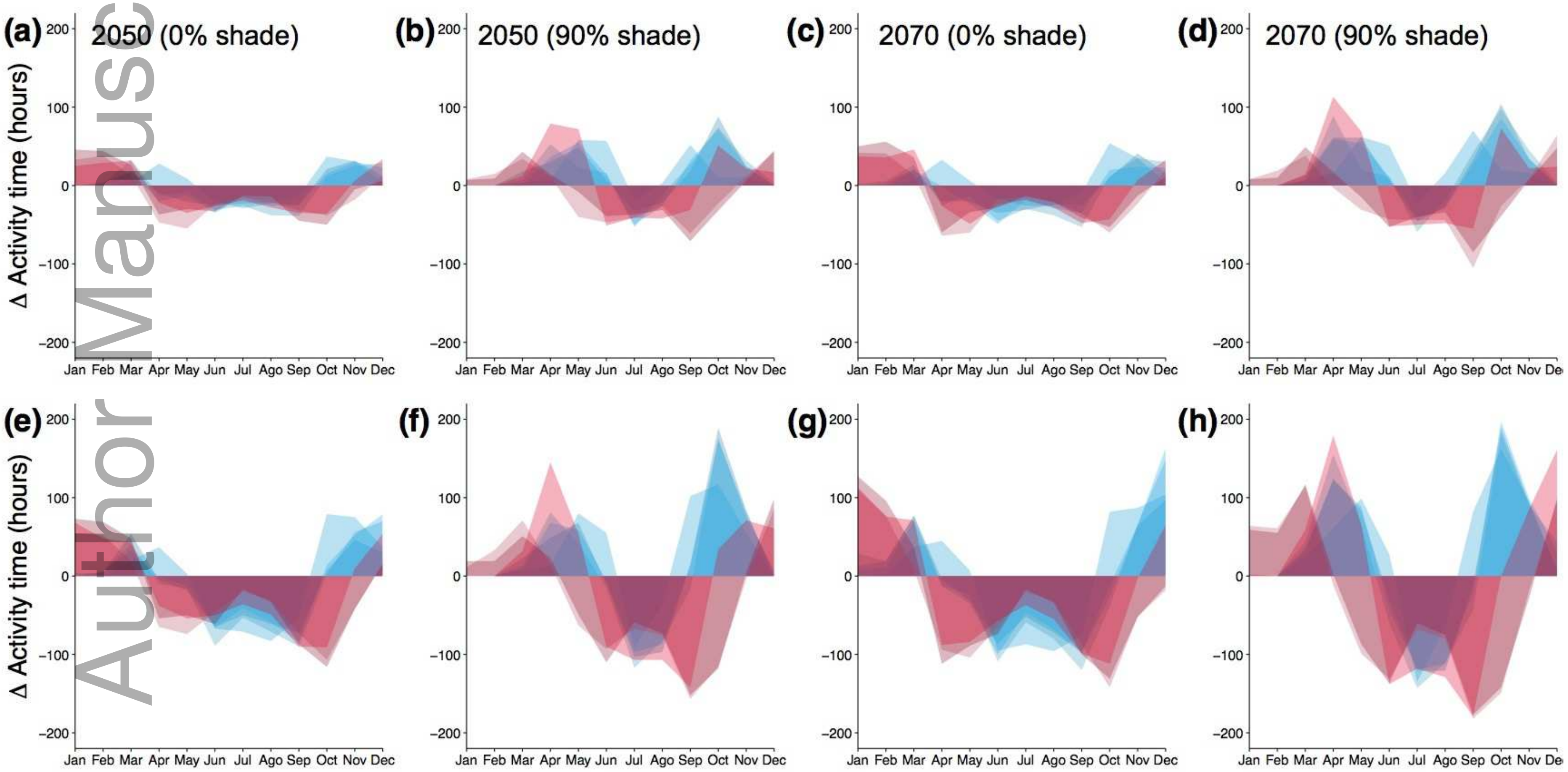

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Table 1: Lineage, sample size (*n*), mean juvenile body size (in g), longitude, latitude, elevation (H = high; L = low), and percentage of canopy cover (i.e. tree and shrub cover; extracted from the Spanish Forest Map at a resolution of 1:50,000; www.mapama.gob.es) for each population.

Population (acronym)	Lineage	*n*	Mean size (in g)	Longitude	Latitude	Elevation	Canopy cover (%)
Candioches (Can)	central	95	0.214	-5.92123	42.99991	1707 (H)	0
Señales (Sen)	central	89	0.309	-5.24043	43.07440	1716 (H)	0
Color (Col)	central	80	0.126	-5.27671	43.29492	377 (L)	85
Tornería (Tor)	central	78	0.156	-4.82462	43.38735	461 (L)	0
Hoyo Empedrado (He)	eastern	80	0.366	-4.75022	43.02275	2076 (H)	0
Vidrieros (Vid)	eastern	77	0.536	-4.60121	42.95523	1438 (H)	0
Huzmeana (Huz)	eastern	65	0.298	-4.23107	43.15771	448 (L)	80

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Table 2: Analysis of covariance for heat tolerance as indexed by the critical thermal maximum (CT_{max}), including mass (covariate; M) and elevation (elev), lineage (lin), acclimation treatment (acc), and their interactions. Population was included as random factor. 'df' stands for degrees of freedom.

	F-value	**df**	***P*-value**
Mass (*M*)	18.62	1, 272	**< 0.0001**
Elevation (*elev*)	13.78	1, 3	**0.034**
Lineage (*lin*)	0.22	1, 3	0.672
Acclimation treatment (*acc*)	37.98	1, 272	**< 0.0001**
elev × *lin*	0.12	1, 3	0.750
elev × *acc*	6.82	1, 272	**0.010**
lin × *acc*	0.66	1, 272	0.418
elev × *lin* × *acc*	0.00	1, 272	0.964

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Table 3: Analysis of covariance for cold tolerance as indexed by the critical thermal minim (CT_{min}), including mass (covariate; M), elevation (elev), lineage (lin), acclimation treatment (acc), and their interactions. Population was included as random factor. 'df' stands for degrees of freedom.

	F-value	**df**	***P*-value**
Mass (*M*)	9.38	1, 261	**0.002**
Elevation (*elev*)	0.00	1, 3	0.982
Lineage (*lin*)	5.13	1, 3	0.109
Acclimation treatment (*acc*)	95.68	1, 261	**< 0.0001**
M × *elev*	0.83	1, 261	0.362
M × *lin*	0.34	1, 261	0.560
elev × *lin*	2.87	1, 3	0.189
M × *acc*	22.88	1, 261	**< 0.0001**
elev × *acc*	26.76	1, 261	**< 0.0001**
lin × *acc*	0.70	1, 261	0.404
M × *elev* × *lin*	0.11	1, 261	0.742
M × *elev* × *acc*	3.05	1, 261	0.082
M × *lin* × *acc*	4.31	1, 261	**0.039**
elev × *lin* × *acc*	0.01	1, 261	0.920
M × *elev* × *lin* × *acc*	0.50	1, 261	0.480

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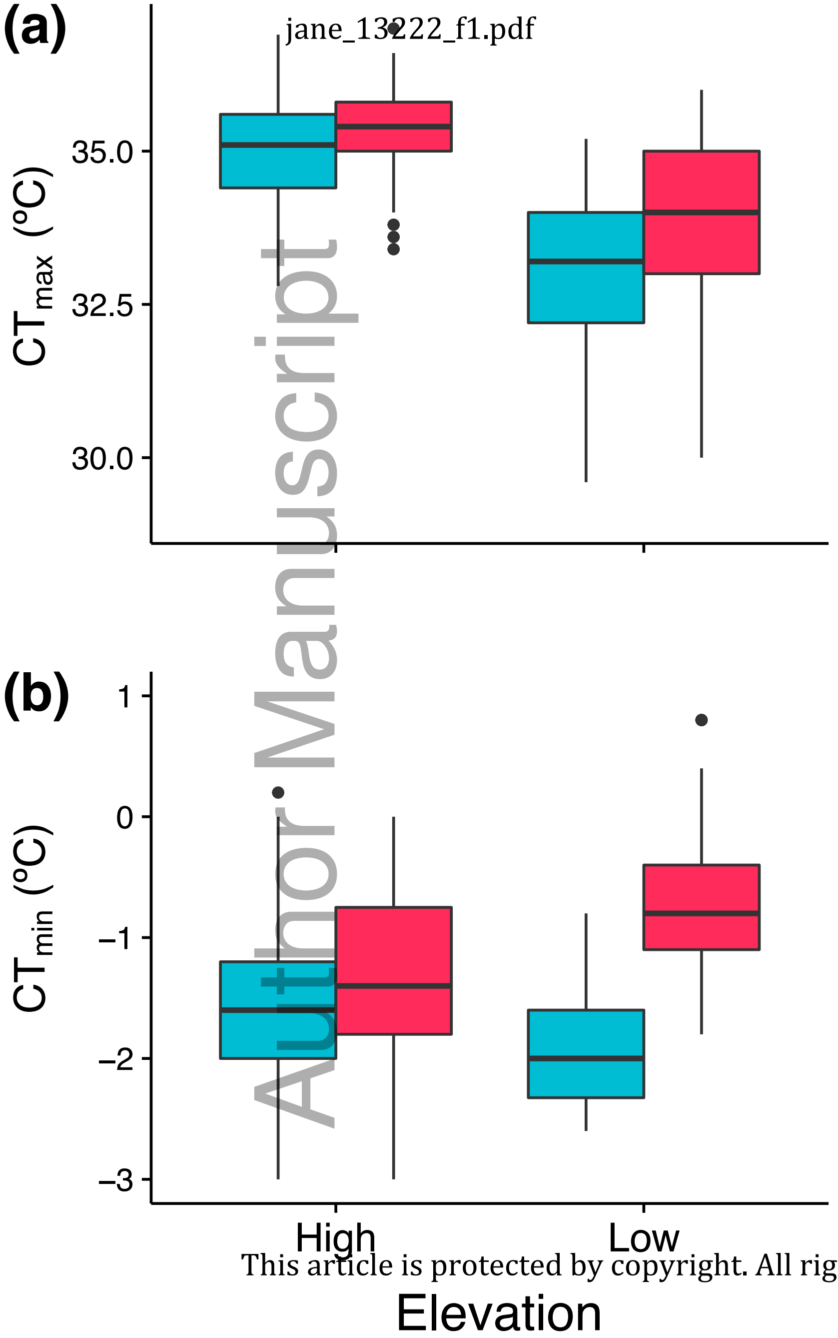

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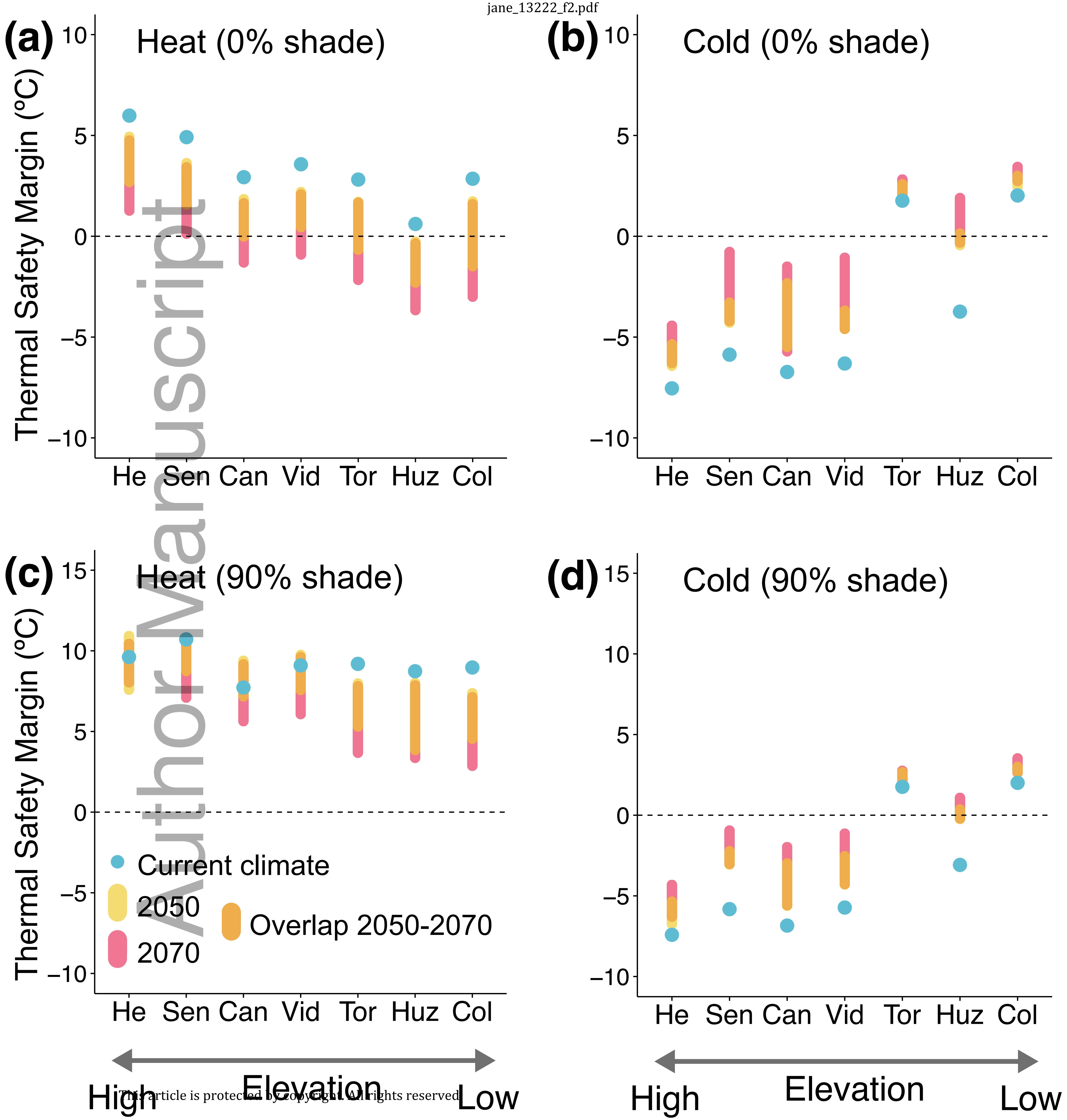

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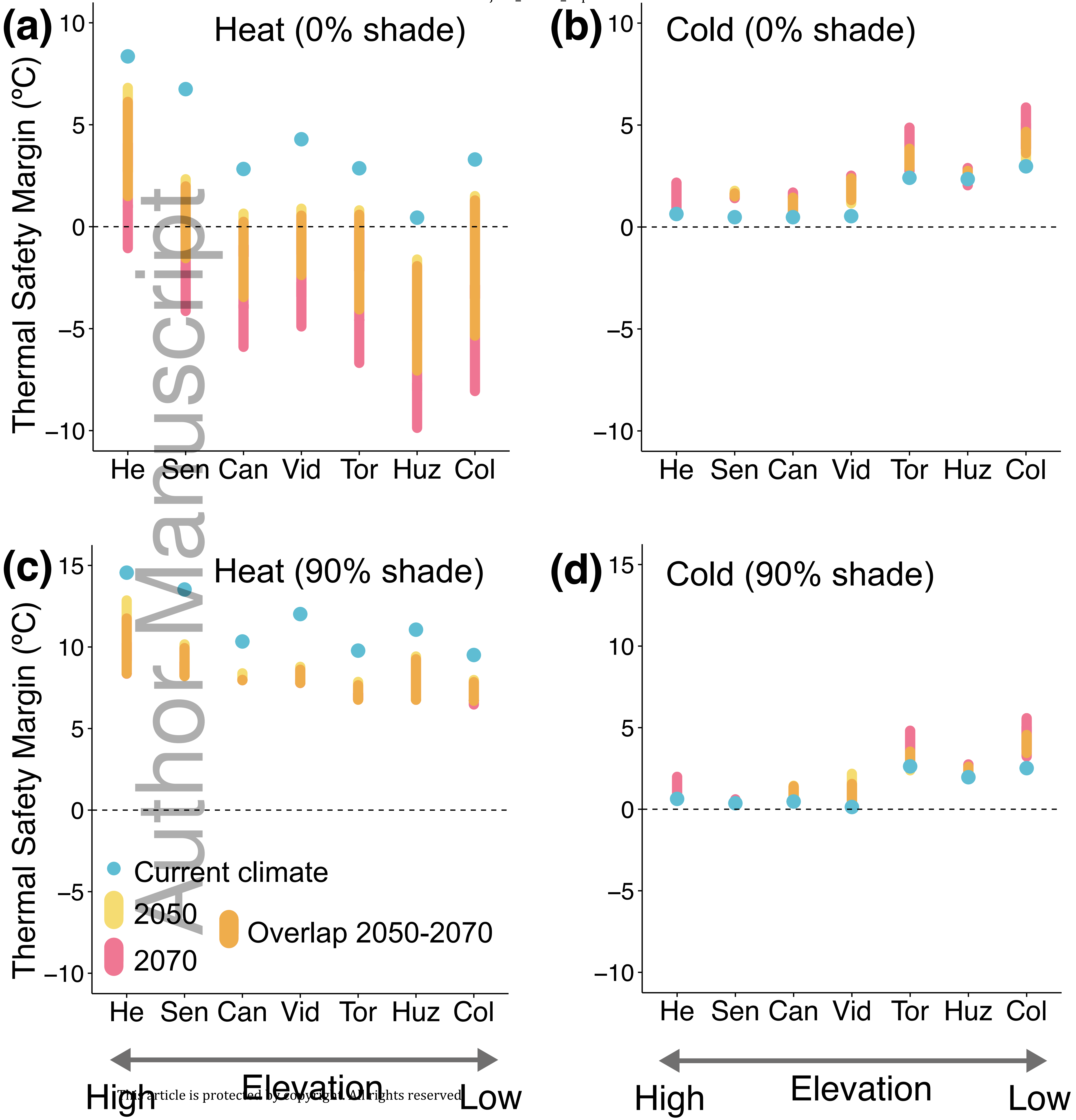

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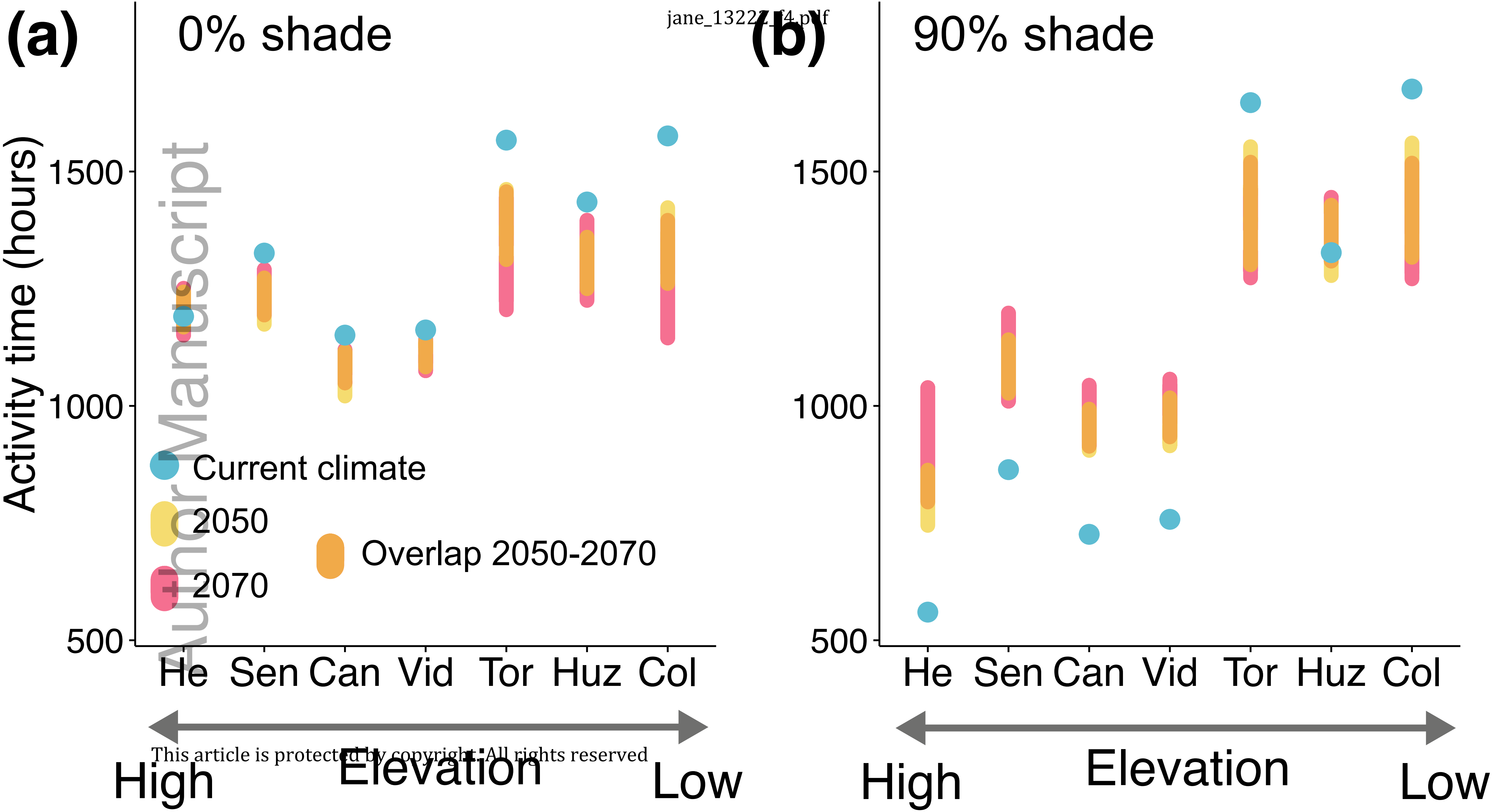

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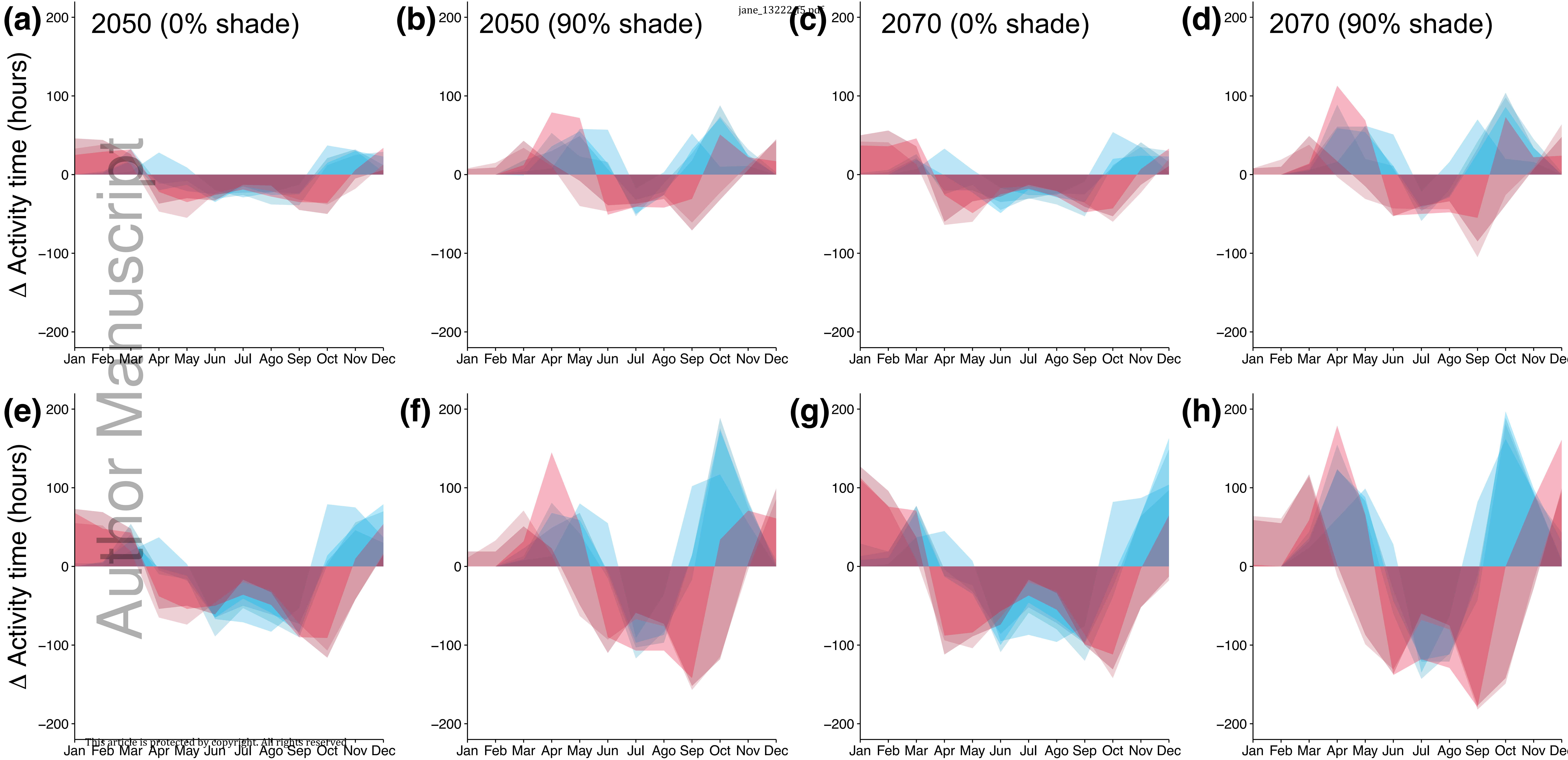

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