Original Article

Dynamic dichromatism and effects of high temperatures on male coloration plasticity in an ectotherm model

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ABSTRACT

Climate change is increasing the temperatures of wild environments, but the impacts on evolutionary ecological processes, such as sexual selection, are still under-studied. In ectotermic tetrapods, male coloration is a pivotal trait in social contexts. During the reproductive season, males can enhance the dichromatic coloration of certain body regions. However, it is unknown how reproductive dichromatism might interact with the increasing temperatures that cause body temperatures to fall within the upper range of preferred temperatures. We hypothesize that sexual dichromatism could be dynamic, i.e. show changes, being reinforced during the reproductive period of the species. However, high temperatures associated with climate change could disrupt reproductive colour patterns by inducing plastic changes. We test this in the social lizard species *Tropidurus spinulosus* in a laboratory setting by applying three thermal treatments: one reproducing current thermal conditions and two simulating scenarios of climate change. Our results highlight that male coloration is enhanced during the reproductive season in key body regions for social displays. At the preferred temperature of the species, males could darken their ventral coloration; however, no such change was observed at high temperatures. Therefore, increasing temperatures could impact the capacity of males to adjust their coloration plastically during the reproductive season.

Keywords: sexual selection; global warming; coloration; reproductive investment; lizard

INTRODUCTION

Mean surface temperatures of wild environments are increasing as a result of climate change and modifying several biological processes (Hughes 2000, Lee et al. 2023). From an evolutionary ecology standpoint, a major challenge is to forecast the impact of increasing temperatures on the mechanisms of sexual selection (Cornwallis and Uller 2010). Sexual selection is a great force that models the evolution of ornaments, communication, sexual dimorphism, and gonadal investment, among many other traits (Andersson and Simmons 2006). However, many gaps remain in our knowledge of the fundamental principles of sexual selection, among them how environmental modifications affect mechanisms in different stages of the reproductive process (Ingleby et al. 2010). Reproductive success might be influenced by the thermal environment during the precopulatory stage owing to alterations in secondary sexual traits that can impact important dynamics, such as mate choice (Bestion et al. 2015, Holleley et al. 2016,

Rossi *et al.* 2021, 2023). Therefore, given that reproductive traits can express plasticity in relationship to external factors and the action of ecological and evolutionary processes (Cardozo and Chiaraviglio 2011, López Juri *et al.* 2020), the mechanisms of sexual selection might be affected profoundly by increasing temperatures. Furthermore, considering that sexual selection traits are energetically costly, it is possible that high temperatures might aggravate the energetic expenditure specifically in ecto-therms, owing to their strict relationship with the thermal environment (Angilletta 2009).

Among secondary sexual traits, coloration is often a precopulatory trait used by males to deliver information in intraand intersexual contexts mainly in male-biased dichromatic species subject to intense sexual selection (Pérez I de Lanuza *et al.* 2013, 2014). Conspicuousness is a trait of coloration frequently used to determine its importance as a signal (Renoult *et al.* 2017). It is defined as the contrast between the studied character

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and a reference in a colour space (e.g. contrasts between sexes, or changes in coloration in the same individual) (Perez i de Lanuza and Font 2015, Valdez and Benitez-Vieyra 2016). In male lizards, coloration is a character of great functional value because it signals male-male interaction strategies (Baird 2013, Rossi et al. 2022, Naretto and Chiaraviglio 2023). Besides, it is a character of selection in the context of mate choice (Rossi et al. 2023) and it is directly related to reproductive potential because it is correlated with testis size (McDiarmid et al. 2017, López Juri et al. 2020). Moreover, dichromatism, which is the difference in coloration of males and females of the same species (Badyaev and Hill 2003), could be dynamic (i.e. show changes) between the non-breeding and breeding seasons to exacerbate sexual signals (Rossi et al. 2019). It can change within the reproductive season following the social context (López Juri et al. 2020) or show variations in plasticity in relationship to seasonal factors such as temperature (Cadena et al. 2018). For example, bearded dragon coloration varies between cold and warm seasons in body regions important for signalling conspecifics (Cadena et al. 2018). Moreover, during the warm reproductive season, lizard coloration shows greater changes with increasing experimental temperatures (Smith et al. 2016). However, it is unknown how dynamic dichromatism might interact with the increasing temperatures attributable to climate change that cause body temperatures to fall within the upper set of preferred temperatures, close to the maximum temperatures (pejus range) (Telemeco et al. 2019).

In ectotherms, high temperature increases metabolic rate (Schulte 2015). This leads to a reduction in energetic reserves, altering the energetic balance of the individual (Angilletta 2009), hence influencing colour production and maintenance (Mészáros et al. 2019). Some studies demonstrate that temperature can cause changes in the extent of male coloration in the short term (Smith et al. 2016). In particular, pigment-based colours could be variable in different thermal conditions owing to their lability and possible targets of trade-offs depending on the energetic balance of the individual (Olsson et al. 2013). Likewise in ultraviolet coloration, the maintenance of the underlying dermal components can be costly and dependent on temperature (Bajer et al. 2012, Olsson et al. 2013). Climate change has increased the frequency of heatwaves, which can be defined as a time span of \geq 5 days during which the average temperature is above the average (or maximum) seasonal temperature (World Meteorological Organization 2016). Therefore, an understanding of how increasingly warm environmental conditions might interact with the expression of coloration, both visible and ultraviolet, is relevant to predict effects on social reproductive interactions.

Tropidurus spinulosus is a medium-sized lizard (female snout-vent length, 97.21 ± 0.94 cm; male snout-vent length, 115.74 ± 0.70 cm) and an interesting ectotherm study model because reproductive individuals of both sexes have specific thermal requirements, in that they select higher body temperatures than non-reproductive individuals and show thermal stability (Juri *et al.* 2018). Furthermore, *T. spinulosus* shows marked sexual size dimorphism (López Juri *et al.* 2020). Males show greater body size, head width and tail perimeter than females (López Juri *et al.* 2020). They also show basal dichromatism in the non-reproductive season (Rossi *et al.* 2019). In particular,

the coloration of the flanks and ventral regions is male biased (Rossi et al. 2019). The reproductive coloration has not been studied. However, dichromatism could be exacerbated during the breeding season owing to its importance for conspecific interaction (Rossi et al. 2022). For example, colourful males usually dominate aggressive contests (Rossi et al. 2022) and are more attractive to females (Rossi et al. 2023). Moreover, the breeding season, which starts in September and lasts until December, coincides with a seasonal increment of environmental temperatures. The population also shows a variable operative sex ratio (OSR), which starts equal in September and shifts to male biased towards November/December (López Juri et al. 2020). Our study population dwells mainly in rocky outcrops that can have arboreal vegetation in the surroundings (Rossi et al. 2023). Lastly, it is one of the southernmost species of the genus (Cruz et al. 1997), and lizards from temperate regions can react rapidly to warmer conditions (Zhang et al. 2010, Tang et al. 2012, Kubisch et al. 2016).

We hypothesize that sexual dichromatism could be dynamic between seasons, being reinforced during the reproductive period of the species. However, high temperatures associated with climate change could disrupt reproductive colour patterns of males by inducing plastic changes within the pejus range. Consequently, our aim was to test whether periods of increasing temperatures that lead to body temperatures within the upper range of preferred temperatures could affect coloration of reproductive males of *T. spinulosus*. We predict that high temperatures will reduce colour conspicuousness and extent of male coloration, taking into account both visible and ultraviolet patterns. Furthermore, in the pejus range of temperature, the colour changes could be associated specifically with those body regions and colour bands that show seasonal plasticity.

MATERIALS AND METHODS

Capture and handling

Reproductive females and males of *T. spinulosus* were captured in the private reserve Los Chorrillos, Tanti, Córdoba province, Argentina (31°23′33″W, 64°35′48″S) during two consecutive breeding seasons (September–December, 2017 and 2018). Temperatures in this mountainous region of Córdoba vary between the non-reproductive and reproductive seasons of the species (autumn mean of 14.80°C and spring mean of 19.63°C, respectively; data provided by the National Weather Service of Argentina).

Lizards were transported to the laboratory and housed individually in plastic containers measuring 30 cm \times 25 cm \times 20 cm under regulated light (9–17 h, Zoomed UVB 5.0 UV tubes) during the experimentation. Lizards were fed one larva of *Zophoba morio* and one *Tenebrio molitor* per day, and water was provided *ad libitum*; vitamins (TetraReptical) were administered two times per week diluted in 10 ml of water (in a ratio of one part vitamins to three parts water (Martin and Lopez 2006). To test the effect of temperature on male coloration, the temperature within the containers was controlled following the treatments detailed in the 'Experimental design' section below.

Ultrasound assessment of the reproductive structures

To study the dynamics of dichromatism, it was necessary to characterize the colour of both males and females in 'reproductive condition' and compare it with basal dichromatism of the sexes (Rossi *et al.* 2019). Therefore, we determined the reproductive condition of captured males and females by evaluating their reproductive structures using ultrasound scanning (Portable Sonosite 180 Plus). Reproductive males had enlarged testes (width range, 5–10 mm) and a hyperechogenic aspect; reproductive females presented with advanced follicles that ranged from 8 to 14 mm in diameter, with a markedly hyperechogenic aspect (López Juri *et al.* 2018).

Processing of reproductive spectra

To characterize dichromatism during the breeding season, we compared the coloration of 10 body regions between reproductive males (N = 27) and females (N = 23). In a previous study on basal dichromatism in the non-reproductive season, we identified discrete colour bands with uniform spectral characteristics within the body regions of males and females (Rossi *et al.* 2019). Therefore, in each body region, we took three standardized measurements of reflectance with a spectrophotometer (Ocean Optics USB 4000) for each colour band by looking for patches of ≥ 2 mm size to avoid chimeric spectra (Badiane *et al.* 2017).

Processing of spectra and quantum catch calculations followed the procedure of Rossi et al. 2019. Briefly, spectra were imported and smoothed in R. To quantify the chroma of the spectra, we calculated the relative contribution of different bands of the spectral range to the total brightness (variable S1, function summary.rspec, pavo package; Maia et al. 2019). The colour bands were as follows: S1U (ultraviolet), minimal wavelength to 400 nm; S1V (violet), minimal wavelength to 415 nm; S1B (blue), 400-510 nm; S1G (green), 510-605 nm; S1Y (yellow), 550-625 nm; and S1R (red), 605 nm to maximal wavelength (Maia et al. 2019). Tropidurus spinulosus colour bands have the following spectral signatures: yellow, green, and blue show a similar peak at 540 nm and a high saturation of S1G, but the yellow band shows a higher saturation of S1Y and S1R and the blue band of S1B; orange and red colour bands show a peak reflectance at 625 nm, but the orange band shows a higher saturation of S1Y and S1R, whereas the red coloration shows higher saturation of the shortwavelengths colours S1U, S1V, and S1B. To characterize reproductive dichromatism, a lizard visual model was built using the data of Norops sagrei (Fleishman et al. 2016), the phylogenetically closest species to T. spinulsosus with available visual model data and that lives in habitats similar to our study species (Loew et al. 2002). Quantum caches were calculated for each spectrum, and patches were then contrasted using a receptor-noise model (Vorobyev and Osorio 1998) to obtain chromatic (dS) and achromatic distances (dL) in just noticeable difference (JND) units between females and males.

Experimental design: effect of temperature on male coloration

Reproductive individuals of *T. spinulosus* show a set-point range of preferred temperatures (T_{set}) from the minimum 32.5 ($T_{set-min}$) to the maximum 35.7°C ($T_{set-max}$), with a mean preferred temperature (T_{pref}) of 33.6°C (Lopez Juri *et al.* 2018). The pejus range, which includes temperatures above T_{pref} up to temperatures close to the thermal maximum for the species (Telemeco

et al. 2019), ranges from 33.6 to 37.5°C, with the latter being the maximum temperature (T_{max}) recorded in previous studies (Lopez Juri et al. 2018). Therefore, to assess the effect of temperature on male reproductive coloration, we designed three thermal treatments. The first of these was the 'current' thermal treatment, for which the air temperature within each lizard enclosure was set at 28°C, corresponding to the air temperature of their natural habitat during the reproductive season. This leads to a lizard body temperature $(T_{\rm b})$ near to $T_{\rm pref}$ (Rossi *et al.* 2023) and is therefore considered as the control treatment. The other two thermal treatments are related to possible scenarios of surface temperature warming forecast for the end of the century (Allen et al. 2018): an intermediate scenario (RCP4.5), which corresponds to $30^{\circ}C$ (+2°C warming); and an extreme warming scenario (RCP8), which corresponds to 32°C (+4°C warming; Stocker, 2014; Allen *et al.* 2018). Both treatments lead $T_{\rm b}$ within the pejus range but never exceed the species thermal maximum. Individuals were assigned randomly to each thermal treatment. Males were maintained under treatment for 2 weeks. The sample size for each treatment was as follows: N = 17 for the 'current' treatment; N = 23 for RCP4.5; and N = 21 for RCP8.

We organized the containers on three shelves, which corresponded to the three thermal treatments. Shelves were conditioned with dichroic bulbs as the source of warmth, whose thermal output was adjusted through an electrical dimmer. We checked the consistency of the air temperature within the containers in four sample spatial points before the experiments by hanging a thermometer 2 cm above the bottom (air temperature of the corresponding treatment ± 0.6 °C SD). The T_b of specimens during experimentation was also checked daily by taking a single measurement near the cloaca using an infrared thermometer (UNI-T 300s). To evaluate the effect of temperature on male coloration, ultraviolet (UV) and visible pictures were taken before and after temperature treatments (for details see next subsection).

Measurement of changes of male reproductive coloration after temperature treatments

Visible and UV photography

To evaluate changes in male coloration, UV and visible pictures were taken before and after temperature treatments. We placed each individual on a black sheet of cardboard illuminated by an Osram Ultravitalux, a high0pressure arc UV-visible lamp, which emits both UV-A and visible wavelengths, suspended 80 cm above the subject. A Nikon D40 camera, mounted with a 50 mm Nikon lens, was used to take the pictures. Moreover, a wooden slide with a B+W[™] UV-cut filter and a UV/IR filter (Straightedge-U[™]) was fastened to the lens to ensure rapid shifting of the filters, thus avoiding blurred images attributable to movement of the lizards. Standardized ventral, lateral, and dorsal pictures were taken. A black and white standard with 8% and 95% reflectance (assessed with spectrophotometry), respectively, was included in the pictures to account for differences in luminance; a printed standard with relevant colour patches, and whose reflectance was measured through spectrophotometry, was also included in the pictures to allow characterization of visible colour and generate colour spectra with the software COLOURWORKER[™] (Higham *et al.* 2010). For each body region,

we first took a UV picture, then a visible picture using the following settings: depth of field was set at 16 f, ISO 400 and 2 s of shutter speed for UV pictures, whereas for the visible pictures we set f 16, ISO 400 and 1/40 shutter speed.

Colour extent

To quantify the extent of visible coloration, from each set of pictures of an individual we cropped in IMAGEJ (Schneider *et al.* 2012) eight body regions relevant in intraspecific communication according to Rossi *et al.* (2019): dorsum, abdomen, cloaca, chest, throat, flank, head profile, and head.

In previous studies, we had characterized the dichromatic colours of the species from a model of lizard vision using spectrophotometric data (Rossi et al. 2019). On the flanks, the dichromatism was attributable mainly to male-specific light blue spectra reflected by the outer ventral scales together with medium-wavelength green and long-wavelength yellow spectra. On the belly and throat of males, the dichromatism was attributable mainly to orange spectra. Therefore, in this study, we quantified the extent of these colours. We identified the spectral signatures of each colour from pixels in pictures of 10 individuals, in which the number of pixels sampled was related to the area of the body regions: yellow, 691 pixels; green, 1163 pixels; blue, 1836 pixels; and orange, 1682 pixels. We used these pixels in a supervised classification, namely a cluster analysis, using the 'Random Forest' algorithm of the homonym package in R (Liaw and Wiener 2002). The algorithm partitioned the representative pixels into a training dataset and a test dataset, which was used to validate the classification process. The validation yielded an out-of-bag error of 9%, which is considered low (Millard and Richardson 2015). The algorithm was applied to the cropped images of body regions, whose pixels were thus assigned to a colour band. The extent of each colour band was measured as a percentage of the area of each body region.

Colour conspicuousness

We calculated the mean red-green-blue (RGB) values for each colour band obtained by the supervised classification in each body region. We then generated squares measuring 100 pixels \times 100 pixels with those RGB values. We then imported the RGB squares together with a chromatic standard into COLOURWORKERTM to convert each RGB square into spectral data. Previously, the software was calibrated in a two-step process by: (i) providing it with the spectra for each colour of the chromatic standard; and (ii) training it with spectral data for each colour band adapted from Rossi et al. (2019). Each RGB square was then calibrated using the spectral data of the chromatic standard and converted to a spectrum (Supporting Information, Fig. S1). The resulting spectra were imported into R, where a lizard visual model was applied following the same protocol mentioned above (in the subsection ' Processing of reproductive spectra'). This whole procedure is represented in the panel available as Supporting Information Figure S1. Lastly, colour conspicuousness was assessed by calculating chromatic and achromatic JND contrasts. In particular, we compared spectra of the same colour band, body region, and individual, before and after the thermal treatment.

To study changes in the intensity and extent of UV coloration, we calibrated the camera responses of the UV pictures in IMAGEJ

(Schneider *et al.* 2012) using the black and white standard as reference and fitting an 'exponential with recovery' function to obtain a reflectance value for each pixel (Garcia *et al.* 2013). We then manually selected the body regions under consideration and filtered out black and specular pixels (pixels that reflect near 100% of the illuminating source due to the shiny nature of the surface) by applying a threshold whose lower limit was 5%, which is considered to be the minimum value of true UV reflectance, and an upper limit of 50%, which is the maximal UV reflectance recorded in our species (Garcia *et al.* 2013, Rossi *et al.* 2022). We then calculated the mean pixel reflectance and the total UV extent for each body region.

Statistical analysis

Reproductive dichromatism

To elucidate whether the coloration of T. spinulosus is dichromatic in relationship to the reproductive condition of individuals, we followed the protocol adopted by Rossi et al. (2019). Briefly, we applied a cluster analysis on the dS and dL distances and produced five grouping trees by cutting at different heights in the classification. We set the following three criteria to identify dichromatic clusters that are composed by homogeneous patches relative to visual model stimulation: (i) 90% of the observations within the cluster belonged to a sex; (ii) the total number of patches in the cluster was $\geq 10\%$ of the total patches measured for the body region; and (iii) the candidate cluster was conspicuous and had a IND difference of at least two from all other clusters in the tree. If a cluster that satisfied these requirements was present in at least one of the five trees, we concluded that the body region was dichromatic.

Effect of temperature on male coloration

To evaluate the effect of high temperatures on changes in coloration, for each body region we tested whether the colour bands after the thermal treatment showed changes in conspicuousness using JND values and testing for significance with a Wilcoxon signed-rank test. Our null hypothesis was that JND values were equal between the same colour band before and after the treatment, and the alternative hypothesis was that JND values were greater after the treatment. For the characterization of dichromatism, we used JND = 2, whereas for colour change we also used a threshold of JND = 1 to obtain higher sensitivity, because colour changes in response to temperature increments might be less accented than dichromatic evolutionary patterns. A JND of one theoretically identifies colour patches that are discernible in good illuminant conditions (Siddigi et al. 2004). We tested for relevant effects of temperature on visible colour band extent, UV extent, and UV brightness for each body region through ANOVAs with temperature, colour band, and the interaction between them as fixed factors.

For all the ANOVA models, normality was checked by inspecting the normal residual plot and by testing the residuals with a Shapiro–Wilk test. Homoscedasticity was assessed by examining the residual vs. fitted plots.

Ethical statement

This research was approved by the Governmental Environmental Agency and the Ethical Committee of the Instituto de Diversidad

y Ecología Animal CONICET-UNC (protocol numbers: 2/2017 and 12/2019).

RESULTS

Reproductive dichromatism

The reproductive coloration acquired during the reproductive season as perceived by a lizard observer was dichromatic and biased towards males on three body regions: abdomen, flanks, and dorsum. On these body regions, dS contrasts generated male-specific clusters (Table 1).

Furthermore, males showed achromatic dichromatism in the chest and dorsum, where male-specific clusters could be found (Table 1). On the chest, males presented a less luminous coloration than females, with a mix of black and orange scales, whereas on the dorsum the coloration was brighter than that of the females. All the male-specific groups were significant, because JND values were higher than two.

Relative to the non-reproductive season, males showed plasticity in the flanks, because the male-biased cluster intensified during the reproductive season and JND distances were 2.24 points higher than in the non-reproductive season (Table 1; Figs. 1B-E, 2B). In contrast, during the reproductive period, the dorsum of the males showed dichromatism, possibly because males boosted the production of yellow and orange pigmentation (Figs. 1A-D, 2C) and reduced the extent of the green coloration (Fig. 1; N. Rossi, G. Cardozo unpublished data). Despite the absence of dichromatism in the head profile during both the non-reproductive and reproductive seasons, an increase of orange coloration was found in males and of yellow coloration in females (Figs. 1B-E, 2E). The loss of dichromatism in the cloaca was attributable to reproductive males shifting their coloration from yellow to white, with a UV signature similar to that of females (Fig. 2D). On the chest, dichromatism was less intense, possibly owing to a reduction of the orange coloration in males (Fig. 2F). Likewise, in the reproductive season the JND difference between males and females on the abdomen region was lower, because females showed an increase in orange–red coloration. Also on the throat, no dichromatism was found during the reproductive season (Table 1).

Does male reproductive coloration change owing to high temperatures?

The mean T_b of the individuals was higher than the air temperature in each thermal treatment (5.15 ± 0.13°C). Therefore, in the 'current' treatment T_b was near $T_{pref'}$ but in the two highertemperature treatments T_b was in the pejus range (33.6–37.5°C). At a JND threshold of one, in the current thermal treatment male orange visible coloration darkened significantly on the chest and cloaca (sum of positive-signed ranks, V > 140; P < .01; Fig. 3), as did the yellow coloration on the cloaca (V = 86; P = .05). Likewise, the orange band on the chest darkened in the RCP4.5 treatment (V = 185; P < .01). However, at RCP8 no changes in coloration were detected. At a JND threshold of two, no significant changes were shown by the Wilcoxon test. No significant temperature effects were detected for the extent of visible and UV coloration, nor for UV brightness.

DISCUSSION

We observed seasonal changes in coloration of both males and females of *T. spinulosus*. Furthermore, we found that both chromatic and achromatic reproductive dichromatism was male biased. In particular, most males showed more intense coloration than females on the abdomen, chest, flanks, and dorsum. Regarding the effect of increasing temperature on the reproductive coloration of males, in the 'current' treatment and the intermediate warming scenario the coloration of the chest and

Table 1. Body regions that showed dichromatism during the reproductive season. In the column 'Tree clust', we report the first tree in which we found a sex-specific cluster; its absence indicates that no dichromatic clusters were found for the region. Columns 'JND', '%Tot', and '%within' list the three criteria for a cluster to be considered numerically relevant within the region and sex, specifically: 'JND' is the mean difference in just noticeable difference (JND) between the significant cluster and the rest of the clusters of the tree, which should be higher than two to be considered visually discernible; '%tot' represents the percentage of the sampled points included in the significant cluster considering the total for the region of interest, which should be >10%; and '%within' represents the internal composition of the significant cluster and the percentage of points belonging to one of the two sexes, and should be >90%. The column headed '%males' indicates the percentage of males that contributed with their spectra to the significant cluster.

Body region	Tree clust	dS				dL			
		JND	%Tot	%within	%males	JND	%Tot	%within	%males
Abdomen	3	3.28	33	96	82				
Chest						2.01	48	90	93
Cloaca									
Dorsum	2	4.71	17	96	0.68	3.47	60	92	89
Flank	3	7.77	26	98	0.89				
Head									
Head profile									
Leg									
Mouth									
Throat									

Males coloration transition between non reproductive and reproductive season



Figure 1. Transition in male coloration. The top pictures (A-C) portray male dorsal, lateral, and ventral coloration of non-reproductive males during autumn, whereas the bottom pictures (D-F) represent coloration of reproductive males during spring. Notable transitions are the orange patches on the dorsum (A, D); the orange patches on the head and head profile, and the intensification of flank coloration (B, E); and the loss of pigments on the cloaca, the darkening of the chest, and the development of orange pigmentation on the throat (C, F).

cloaca of males was perceived as darker after the treatment. However, contrary to our predictions, extreme environmental temperatures did not cause any significant changes in the perception of visible and UV coloration or its extent.

Reproductive dichromatism that occurred on the flanks and abdomen was attributable mainly to yellow and orange colours, which had already been linked to intraspecific communication (Rossi *et al.* 2019, 2021). The enhancement of males coloration on the flanks corresponds to 10%–15% higher detectability (Fleishman *et al.* 2016). The yellow band of the flanks is correlated with testicular volume and is also an object of selection in mate choice contexts (Rossi *et al.* 2023) and in cryptic female choice (G.Cardozo, N. Rossi, unpublished data). Moreover, the orange–red hues on the abdomen show significant betweenindividual variation and might also signal alternative reproductive and social strategies, because males differing in the intensity of aggressive behaviour also differ in the orange ventral coloration (Rossi *et al.* 2022).

On the dorsal region, males possessed a higher presence of yellow and orange hues in the reproductive season. Although the coloration of this region has often been considered to be ecologically driven, in some lizard species it might contain important cues for social and sexual contexts (Zucker 1994). In *T. spinulosus*, dorsal coloration also contributes to signal dominance in male combat, together with the coloration of the flanks and chest (Rossi *et al.* 2022), and some males raise the head and dorsal crest during combat (Fig. 4). Moreover, *Tropidurus semitaeniatus* exhibits the dorsal region consistently during aggressive encounters between males (Carreira Bruinjé *et al.* 2022). The ventral regions of the cloaca and throat were also dynamic, and during the reproductive season males intensified

the orange coloration on the throat. However, given that females also develop yellow pigmentary coloration, we did not find dichromatism according to the three criteria we adopted. Besides, the coloration of the male cloaca shifts towards white hues. Changes in coloration of the cloaca also occur in T. semitaeniatus with the spermatogenic cycle, in relationship to the presence of mature sperm cells (Ribeiro et al. 2010). On the chest region, males develop predominantly black scales mixed with orange scales during the reproductive season, thus losing chromatic dichromatism while retaining achromatic dichromatism; in other species, the development of black scales and darker coloration is associated with the testosterone cycle (Cox et al. 2008). Regardless of the observed changes, i.e. darkening or fading, it is notable that the colour changes in body regions are associated with behavioural displays. Besides, these changes take place during the reproductive season, suggesting that they could be important signals for the mechanisms of sexual selection.

Knowledge of how dynamic dichromatism interacts with increasing temperatures might help us to understand how reproductive strategies could be modified by heatwaves that take place during the reproductive period. For that reason, it is important to consider the effect of the thermal treatments applied on the $T_{\rm b}$ of the individuals. In the 'current' thermal treatment, $T_{\rm b}$ of the lizards was close to $T_{\rm pref}$. In the RCP4.5 treatment, although $T_{\rm b}$ was higher and fell within the pejus range (33.6–37.5°C), $T_{\rm b}$ was below the maximum temperature of the set-point range, $T_{\rm set-max}$, i.e. $T_{\rm b}$ remained within the preferred range. However, in the RCP8 trial $T_{\rm b}$ was above $T_{\rm set-max}$ and close to $T_{\rm max}$.

Contrary to our prediction, the warmest treatment did not cause significant changes to the extent of colour or its stimulation of a lizard visual model. This was true for all body



Figure 2. Changes in the chroma of the spectra of males and females between non-reproductive and reproductive seasons. On the *x*-axis (colour bands) and *y*-axis (proportions), the S1 variable is reported, which represents the relative contribution of different colour bands to the total brightness of the spectra. The boxplot for non-reproductive females is coloured in orange, reproductive females in red, non-reproductive males in light blue, and reproductive males in blue.

regions, including those that showed plasticity between seasons. However, we detected significant changes in the 'current' and RCP4.5 scenarios at a JND threshold of one. Orange bands of the chest in the 'current' and RCP4.5 thermal treatments and the yellow band of the cloaca were perceived as significantly darker after the treatments, which increased the luminosity distance from female patches, whereas in the RCP8 treatment it did not. These *P*-values were not significant when the JND threshold was set at two, possibly meaning that these changes can be detected only when the illumination is ideal (e.g. full sun; Siddiqi *et al.* 2004).

In the 'current' thermal treatment, *T. spinulosus* males might have had suitable and constant thermal conditions. This favoured the maintenance of $T_{\rm b}$ close to the preferred temperatures for longer periods than in the field. Likewise, in the RCP4.5 treatment, $T_{\rm b}$ remained at the bottom of the pejus range. Body

temperature within or near to the preferred thermal range boosts the production of melanin pigments that might contribute to dark coloration (Harmon 2018). Melanin production is context and condition dependent and is energetically expensive in both lizards and birds (McGraw 2008, Bajer et al. 2012). In contrast, the possible thermal stress of the RCP8 treatment might have triggered a higher metabolic rate and an oxidative stress that precluded investment in pigment-based coloration (Cote et al. 2010). Therefore, higher temperature might even hinder the plastic capacity of T. spinulosus to modify its coloration in chromatic body regions used for intraspecific communication. Chest coloration is involved in different behavioural strategies during combat (Rossi et al. 2022). It has been correlated with push-up rate and was displayed mainly by dominant males that relied on behavioural signalling. Furthermore, given that the dark scales develop during the reproductive season, it is possible that they

"Current" thermal treatment Duration: 15 days

Before the thermal treatment

After the thermal treatment

Figure 3. Example of change in male coloration in the control thermal treatment (28°C). Note that the cloaca and chest became darker after the temperature treatment.



Figure 4. Male of *Tropidurus spinulosus* captured in the field after an aggressive encounter. The head and dorsal crest were raised, making the central orange and yellow scales particularly visible.

are under hormonal control and have a role as a secondary sexual character, as in *Sceloporus jarrovii* (Cox *et al.* 2008). Therefore, higher temperatures might restrict the capacity of males to signal their quality honestly by impacting the plastic change of coloration.

In conclusion, increasing surface temperatures could impact precopulatory sexual selection traits. Exposure to high temperatures might impair the plastic capacity of males to invest adequately in precopulatory traits, such as coloration, in those body regions that exhibit dichromatism during the reproductive season. The absence of these plastic changes might modify sexual selection dynamics and might impact directly on the adaptation of the species to climate change. However, future research could determine the true impact of these changing patterns.

SUPPLEMENTARY DATA

Supplementary data is available at *Biological Journal of the Linnean Society* online.

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COMPETING INTEREST

None declared.

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DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

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