# Citizen science data supports sexual dichromatism but rejects thermal melanism in the European fire salamander (*Salamandra salamandra*)

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# Abstract

## Background

Conspicuous color patterns are traditionally believed to advertise the toxicity of prey to potential predators. However, many aposematic species show drastic variation in coloration, indicating the possibility of other functions of coloration. To study these other functions, we can investigate the influence of inherent (e.g., sex) and external factors (e.g., climate) on color variation. We used the aposematic European fire salamander (*Salamandra salamandra*) to study drivers of variation in the yellow proportion of their dorsal black and yellow coloration based on citizen science data available in a new online database.

## Results

Our results suggest a widespread sexual dichromatism in fire salamanders in Germany with males displaying a larger proportion of yellow than females. This dichromatism persisted even after correcting for a sex-difference in body shape that accounts for some of the variation in the proportion of yellow. Among six investigated putative drivers of this color variation, the proportion of the aposematic yellow coloration increased with latitude and longitude but showed no association with other environmental variables such as temperature or the productivity of the habitat.

## Conclusions

Integrating citizen science data in this study enabled a comparison of fire salamander populations across large parts of their distribution and conclusively supports sexual dichromatism. Given the lack of assocations with temperature-related variables, thermal melanism appears to play no role in fire salamander coloration. Future studies should further investigate potential mechanisms of mate choice in fire salamanders and other selective factors on coloration such as differential predation pressure between both sexes or the role in crypsis. Lastly, multi-national studies supported by citizen science data should fully unravel the extent of color variation in this species.

Keywords:

Aposematism, Amphibians, Coloration, Image analysis

## Background

Color is among the most diverse traits in the animal kingdom. Different abiotic and biotic factors affect the coloration of an animal, both, via evolutionary changes and over the lifespan of an individual [1–4]. For centuries, coloration has attracted ecologists and evolutionary biologists aiming to study the drivers of color differences [1, 2]. However, since studying environmental drivers of animal coloration in the wild would involve sampling across large spatial scales in order to capture gradual changes of environmental variables, studies using a larger distribution area and larger gradients of environmental factors are scarce. Harnessing the data available in social media or on citizen science platforms can drastically increase our sampling effort and thereby, improve the generalizability of the conclusions from our studies [5–7].

One important selective agent of coloration is predation [8]. Both predator and prey can avoid being seen through background matching or disruptive coloration [9]. However, in other cases, animals feature highly conspicuous colors and patterns. If this form of coloration is paired with an underlying defense such as toxicity or spines, this antipredator strategy is termed “aposematism” [10, 11] and can be very effective at preventing predator attacks once the association between the conspicuous coloration and an underlying defense has been established in the predator [12]. In addition to avoiding predation, animal coloration can play an important role in thermoregulation. By increasing the density of melanin, the skin of an animal becomes darker, increasing its ability to absorb radiation [13]. This thermoregulative strategy termed “thermal melanism” can be adaptive for ectotherms inhabiting cooler habitats by aiding in body temperature regulation [14]. Indeed, a cline in melanism along gradients of temperature or it’s proxies for (e.g., geographic latitude or elevation) has already been reported in several taxa [7, 15–17].

In addition to natural selection, sexual selection can also play an important role in the evolution of animal color [18–20]. For example, a costly coloration can be selected through mate choice [18, 20, 21]. Costly coloration involves energetically costly pigments or highly conspicuous color patterns that increase the likelihood of detection by predators, thus constituting an honest signal of mate quality [20]. Furthermore, if coloration is heritable, mating with a partner that displays costly and therefore attractive coloration can result in the production of attractive offspring. Therefore, highly contrasting and elaborate color patterns can evolve in one or both sexes, depending on the mating system (i.e., if only one of the sexes (e.g., females) is choosy, contrasting and elaborate color patterns should evolve in the opposite sex (e.g., males); [22]). In aposematic species, in which coloration is traditionally believed to be linked to predator avoidance only (see above), it has been hypothesized that sexual selection via mate choice could play an important role in maintaining or even enhancing aposematic coloration [4, 23]. Once the link between conspicuousness and prey unprofitability has been established in the predator, the strength of selection by the predator on coloration could weaken. Consequently, sexual selection could take over the role of the main selective agent and induce a sexual dichromatism in the intensity or pattern of the aposematic coloration. Indeed, evidence for the role of sexual selection in shaping aposematic coloration is accumulating [4, 22–25]. In aposematic amphibians in particular, it has been hypothesized that conspicuousness can act as a quality signal and can thus be selected by the choosy sex [26–30].

The coloration of an animal can also change within the lifespan of an individual through phenotypic plasticity, either during development [3, 28] or, in some species, within seconds or minutes as a fast response to environmental stimuli [31]. Common drivers of color plasticity are changes in the environment, such as seasonal changes that challenge individuals to exhibit drastic changes in color to match their phenotype to the environment [32–34]. In times of rapid environmental changes caused by human activity, the extent to which plasticity in coloration can enable organisms to adapt to changes in the environment and whether plasticity can buffer a phenotype-environment-mismatch are open questions [35–37]. For example, given the importance of color for thermoregulation (see above), the question of whether animal coloration changes with increasing temperature due to plastic changes or adaptive evolution is now motivating studies across many different taxa and geographical areas [38, 39].

The European fire salamander (*Salamandra salamandra*) represents an excellent study organism for drivers of color variation, as postmetamorphic individuals have a conspicuous yellow-on-black coloration that is easily quantifiable due to the strong contrast between the two colors [40]. While the coloration of fire salamanders is considered aposematic given the potent toxic substances produced in specialized glands on the skin [41, 42], surprisingly high inter- and intrapopulation variation exists in the dorsal proportion of yellow in these salamanders [29, 30, 43–47]. There is strong evidence for the heritability of color-associated traits [28, 48], and yellow coloration is energetically costly for an individual [41, 47]; however, the proportion of yellow color does not correlate with the potency of the toxins produced by an individual [28, 29, 45, 49]. Therefore, further studies concerning the emergence and maintenance of color variation in fire salamanders are needed to unravel the causes of the remarkable variation in this species.

Previous studies have demonstrated that conditions during the larval period strongly influence postmetamorphic coloration [28, 41, 47]. For example, [41] reported that fire salamanders raised under poor nutritional conditions as larvae had lower proportions of yellow on their back after metamorphosis compared to individuals raised under rich nutritional conditions as larvae, indicating that yellow coloration is energetically costly to produce. Another interesting hypothesis, referred to as “thermal melanism”, has been postulated, which suggests that the proportion of yellow color could also be mediated by environmental temperatures and, accordingly, by elevation above sea level or distance from the equator (i.e., geographic latitude), resulting in more black and less yellow color in cooler environments [43, 49, 50]. However, to our knowledge, no study has found empirical evidence supporting the existence of “thermal melanism” in fire salamanders [45, 47]. Instead, [47] reported an association between habitat productivity quantified as the normalized difference vegetation index (NDVI; an indicator of photosynthetic activity and green biomass) and the hue in the yellow color of fire salamanders but not the proportion of dorsal yellow color. The authors argued that this relationship could indicate that greater resource availability could facilitate the production of the costly pigments (pteridines and carotenoids stored mainly in xanthophores; [45]) responsible for the hue of the yellow color of the skin of a fire salamander.

Two studies of fire salamanders in six populations in Slovakia and three populations in Germany reported a sexual dichromatism with males having a greater dorsal proportion of yellow than females [29, 30], but no such pattern was found in 25 Italian populations [47] (see also [44, 45], who did not find sex-specific differences in the dorsal color patterns of Polish and Spanish fire salamanders). However, to date, no study has considered the confounding effect of body shape, which could also drive sexual differences in the dorsal yellow proportion of fire salamanders. Fire salamanders in Central Europe are usually mostly black on their lateral sides [42, 43], and an individual with a wider body shape (e.g., due to pregnancy) will therefore have a larger proportion of black in dorsal view, as the abdomen is more stretched out and a larger proportion of the sides is visible (Fig. 1).

In this preregistered study (link to preregistration: https://osf.io/92x8w), we used images of fire salamanders from the recently developed web-based software Amphibian and Reptile Wildbook (ARW; [51]), which applies AI-based capture-recapture analysis via pattern recognition. In the ARW, scientists and citizen scientists can upload photos of fire salamanders for population analyses (e.g., [52, 53]). Using images of fire salamanders uploaded to the ARW, we studied variation in the fire salamander dorsal black-to-yellow ratio (B/Y ratio; [28]; low values of the B/Y ratio denote large proportions of yellow) on a large regional scale across wide swaths of the natural distribution of fire salamanders in Germany (Fig. 2). Using 30 pictures (depicting 15 males and 15 females) per location at 20 different locations, we tested for sex-specific differences in the B/Y ratio on the entire body viewed from above as well as on the head. We used coloration on the head specifically, as we expect that entire-body coloration and head coloration will be strongly correlated but that head coloration is not influenced by sex- and season-specific differences in body shape that could confound our results regarding a potential sexual dichromatism (see above and Fig. 1). Furthermore, we tested for correlations between the B/Y ratio and between-site variation in six environmental variables that have been shown to drive animal coloration in previous studies (e.g., [7, 15, 17, 47]). These variables included elevation, average temperature within the period of peak adult fire salamander activity, temperature change over the last 50 years, NDVI, and geographic latitude and longitude. We studied both average temperature and temperature change, as the former represents the current thermic conditions under which the individual developed and thus would affect coloration via developmental plasticity [3], whereas the latter represents how the thermic conditions changed in recent times and thus could represent an important selective agent leading to adaptive evolution [36, 39].

We expected 1) male fire salamanders to show lower B/Y ratios (i.e., more yellow coloration) both on their entire back (body B/Y ratio) and on their head (head B/Y ratio) than females. We also expected 2) no sex-difference in the relationship between body B/Y ratio and head B/Y ratio, indicating the absence of a body shape effect on these ratios. Based on the results from testing hypothesis 1) and 2), we planned to select either the body B/Y ratio or the head B/Y ratio to study the relationship between fire salamander coloration and environmental location-specific factors.

## Methods

All methods were carried out in accordance with our preregistration (link to preregistration: https://osf.io/92x8w), complemented with additional statistical tests as justified in the Statistical analysis section for further details.

### Sampling and processing of images

Using the *Mapped Results* function in the ARW, we mapped fire salamander encounters color coded according to sex. Next, we identified locations with enough fire salamander encounters (i.e., a minimum of 15 males and 15 females). There had to be a distance of at least 5 km between locations [47] to ensure no regular gene flow between populations [54, 55]. If a suitable fire salamander population within a location was identified, we requested for permission to include the fire salamander images provided by the ARW-users in our analysis and active participation in this study. If the users accepted, for each population, we randomly selected and downloaded 15 images of male and female fire salamanders, respectively, assuring that each fulfilled minimum quality criteria [40]. In brief, acceptable images had a sufficient resolution to delineate color patterns and body parts, showed the entire dorsal side of the individual, and were taken as top-view with an approximately 90° angle to the fire salamander. Images failing at least one of these criteria were discarded, and replaced by another randomly selected image from the map. Applying this process, 598 images from 20 locations (15 images per sex per location; for one location, we were only able to obtain 13 images of females; Table 1, Fig. 2) were downloaded. All photos included in this study were taken between 2017 and 2023 and depicted adult fire salamanders (i.e., snout-to-tail-length > 13 cm, as determined by the person taking the photo).

**Table 1** Overview of the study populations with the corresponding number of photos of females ($n_{♀}$) and males ($n_{♂}$) included in this study as well as the mean value of the body black-to-yellow ratio ($\bar{x}_{body\ BY\ ratio}$) and the head black-to-yellow ratio ($\bar{x}_{head\ BY\ ratio}$) by population (differences between the sexes in the ratios were independent of population; see Statistical analysis and Results). GPS coordinates, elevation (in m above sea level), average temperature (in °C), temperature change (in °C) and the Normalized Differential Vegetation Index (NDVI) for each location are given as well. See Environmental data collection in the Methods section for further information on how these data were obtained.

| **Population** | ***n*♀** | ***n*♂** | $\bar{x}_{body\ BY\ ratio}$ | $\bar{x}_{head\ BY\ ratio}$ | **Latitude** | **Longitude** | **Elevation** | **Aeverage Temperature** | **Temperature Change** | **NDVI** |
|---|---|---|---|---|---|---|---|---|---|---|
| Altenau | 15 | 15 | 2.18 | 1.25 | 51.806 | 10.438 | 446.1 | 11.004 | 1.161 | 0.710 |
| Bad_Harzburg | 15 | 15 | 1.52 | 0.79 | 51.868 | 10.569 | 349.5 | 11.989 | 1.385 | 0.710 |
| BGBI | 15 | 15 | 1.64 | 0.95 | 52.014 | 8.510 | 173.9 | 13.122 | 0.986 | 0.635 |
| EBWOB | 15 | 15 | 1.45 | 0.73 | 52.380 | 10.819 | 116 | 13.311 | 1.299 | 0.633 |
| Ehrenberg | 15 | 15 | 1.04 | 0.62 | 51.039 | 13.016 | 225.3 | 13.092 | 1.311 | 0.598 |
| Floeha | 15 | 15 | 1.83 | 1.05 | 50.863 | 13.081 | 323.7 | 12.841 | 1.387 | 0.629 |
| FRD | 15 | 15 | 1.42 | 0.85 | 51.918 | 8.876 | 203.1 | 13.011 | 0.999 | 0.634 |
| Gartensalamander | 13 | 15 | 1.34 | 0.63 | 50.173 | 7.621 | 384 | 13.289 | 1.160 | 0.695 |
| Heidelberg | 15 | 15 | 2.12 | 1.20 | 49.362 | 8.705 | 259 | 14.546 | 1.302 | 0.644 |
| Ilsenburg | 15 | 15 | 1.29 | 0.72 | 51.851 | 10.669 | 306.4 | 11.128 | 1.234 | 0.679 |
| Kaesenbachtal | 15 | 15 | 2.32 | 1.44 | 48.534 | 9.041 | 408.5 | 13.154 | 1.171 | 0.684 |
| KF | 15 | 15 | 3.33 | 1.25 | 50.667 | 7.083 | 174.8 | 13.979 | 1.176 | 0.594 |
| KO-Bienhorntal | 15 | 15 | 2.00 | 1.14 | 50.344 | 7.610 | 129.3 | 13.813 | 1.090 | 0.642 |
| Kruppwald | 15 | 15 | 2.52 | 1.30 | 51.403 | 6.995 | 99.5 | 14.033 | 0.993 | 0.591 |
| Muelheimer_Wald_Rottbachtal | 15 | 15 | 1.93 | 1.07 | 51.380 | 6.842 | 64.6 | 13.337 | 0.885 | 0.584 |
| Ratinger_Wald | 15 | 15 | 1.97 | 1.04 | 51.312 | 6.866 | 75.6 | 14.110 | 0.927 | 0.613 |
| Remscheid_Eschbachtal | 15 | 15 | 2.19 | 1.31 | 51.152 | 7.170 | 157.4 | 13.004 | 1.301 | 0.727 |
| Wasserburgertal | 15 | 15 | 1.77 | 1.29 | 47.895 | 8.852 | 639.9 | 12.245 | 1.207 | 0.698 |
| Wernigerode | 15 | 15 | 1.19 | 0.51 | 51.821 | 10.742 | 259.9 | 12.609 | 1.320 | 0.679 |
| Wuppertal_Kothener_Wald | 15 | 15 | 2.05 | 0.86 | 51.255 | 7.190 | 259.7 | 13.053 | 1.237 | 0.687 |

Following the instructions provided by [40], the fire salamander body, excluding the legs, was cropped out and saved as a new image. Additionally, the head was cropped out and saved as a separate image to compare overall body coloration with head coloration (Fig. 3). The B/Y ratio was then automatically quantified via the Python script provided in [40]. All image processing and analyses were conducted by two researchers (RH and MS) who were blinded to the sex and location of the fire salamander in each picture.

## Environmental data collection

For each population, a centroid was determined as the approximate center of the fire salamander encounters. Using the GPS coordinates of the centroid for each population, the elevation above sea level (in m) was obtained via the free web tool “Elevation Finder” (https://www.freemaptools.com/elevation-finder.htm). The average temperature (in °C) for each location was calculated on the basis of the centroids via a fine-scale (0.25° × 0.25°) temperature dataset of daily temperatures dating back to 1950 [56]. We only considered temperatures during the period of fire salamander activity between the beginning of March and the end of October, as fire salamanders in Germany hibernate during the winter in underground shelters that are likely to constitute constant-temperature environments [42]. Furthermore, the average temperature was calculated from 2000 to 2015, as this timeframe likely covers the time the individuals were born and matured (an average age of 7 to 9 years was reported for fire salamanders in different countries in Europe; however, a maximum age of 25 years has also been reported in the literature [42, 57]). Thus, this value represents the average temperature to which the fire salamanders were exposed during ontogeny and adult life. Similarly, the average temperature between 1950 and 1965 was calculated from the same dataset [56] and then subtracted from the average temperature from 2000 to 2015 to approximate how much a specific location changed in average temperature over those 50 years. While all locations experienced an increase in temperature (Table 1), between-site variation in this temperature change could be an important driver of evolutionary change in color through it’s effect on previous generations of fire salamanders. NDVI data were obtained via the fine-scale (0.05° × 0.05°) MODIS vegetation index dataset (https://www.cen.uni-hamburg.de/en/icdc/data/land/modis-vegetationindex.html). The NDVI per location was also averaged for March to October in the period of 2000 to 2015.

## Statistical analysis

All statistical analyses were carried out in R version 4.2.2 [58]. Before the analyses, we checked our data for unexplainable outliers and ensured that the statistical models did not include any strong collinearity between predictor variables following the protocol outlined in [59]. We did not find any unexplainable outliers and proceeded as follows. All figures presented in the results section were created using the R package ggplot2 [60].

We used linear mixed effects models to study variation in body B/Y ratio and head B/Y ratio. All LMMs were fitted via the function *lmer* from the lme4 package [61]. We $\log_{10}$-transformed the body B/Y ratio and head B/Y ratio for all models to ensure that the assumptions of linearity, homoscedasticity and normality of our models were met, as confirmed via the function *check_model* in the performance package [62]. To test the significance of the fixed effects in our models, we used the *summary* function provided by the package lmerTest [63]. To find the most suitable random effect structure for each model, we used the function *ranova* provided by lmerTest [63]. If our initial more complex random effect structure (i.e., a random slope for *sex* and random intercept for *population*) was not a significantly better fit to the data (based on a *p*-value > 0.1) than a simpler random effect structure (i.e., just a random intercept), we chose the simpler random effect structure for the final model. For each model, we present the initial and the final random effect structure below but in the results section we present only the results from the final model. To determine the role of sex-specific differences in body shape on the black-and-yellow coloration of fire salamanders, we used the body B/Y ratio and the head B/Y ratio in separate LMMs as dependent variables. As a fixed effect, the models included *sex* (categorical: female or male). As random effects in both models, we included a random intercept of *population* to reflect the paired nature of samples from the same population and a random slope for *sex* to control for random variation in sexual color differences between populations [64]. For the model on body B/Y ratio, the random intercept and slope structure provided a marginally better fit ($AIC_{\text{random intercept and slope model}} = -93.219$) than the random intercept model ($AIC_{\text{random intercept model}} = -91.922$; $p = 0.071$, so we kept the former but we removed the random slope for the model on head B/Y ratio ($AIC_{random\ intercept\ and\ slope\ model}$ = 45.511; $AIC_{random\ intercept\ model}$ = 42.512; $p$ = 0.606). We also studied the correlation between the body B/Y ratio and head B/Y ratio (both $log_{10}$-transformed) to investigate an effect of body shape (see above) by using Pearson's correlation test in the package stats [58]. Finally, we studied sex-differences in the relationships between the body B/Y ratio and head B/Y ratio by using a LMM. The LMM included the $log_{10}$-transformed head B/Y ratio as dependent variable, as well as the $log_{10}$-transformed body B/Y ratio, sex and their interaction as fixed effects. The $log_{10}$-body B/Y ratio was centered around it's mean to improve the interpretability of the intercept [65]. Any sex-differences in slopes would indicate an effect of sex-specific differences in body shape on the body B/Y ratio and a significant intercept would additionally indicate differences in the mean head B/Y ratio compared to the mean body B/Y ratio. Initially, this model included a random intercept for population,  a random slope of sex by population, as well as a random slope of the body B/Y ratio by population to control for population-specific differences in the head B/Y ratio as well as population specific differences in the sexual dichromatism of the head B/Y ratio and population specific differences in the relationship of the body B/Y ratio with head B/Y ratio. The model including a random intercept of population and a random slope of the body B/Y ratio for each population as well as the initial model provided the best fit (all $p < 0.004$) but the two models were not significantly better when compared with each other ($AIC_{initial\ model}$ = -591.19; $AIC_{random\ intercept\ of\ population\ and\ random\ slope\ of\ body\ B/Y\ ratio\ model}$ = -592.20; $p$ = 0.322). Therefore, we picked the simpler model (i.e., the model including a random intercept of population and a random slope of the body B/Y ratio) for the final analysis.

Using the model described above, we found evidence for sex-specific differences in the relationship between the head B/Y ratio and the body B/Y ratio that are likely a consequence of sex-specific differences in body shape (for more details see the results). Therefore, in accordance with our preregistration, we used the head B/Y ratio to study the role of environmental variables on fire salamander black-to-yellow ratio. We present corresponding results from the models using the body B/Y ratio in the supplementary information. We used separate models for each environmental variable (i.e., elevation, average temperature, temperature change, NDVI, latitude, longitude) with the mean-centered (see above) environmental variable, sex and their interaction as fixed effects. We also divided elevation by 100 in order to have all environmental variables on similar scales. As random effects, we included a random intercept for population as well as a random slope of sex by population. Following the same step-wise reduction of the random effect structure as described above, we removed the random slope in every model as it did not improve the model slope (all $p > 0.477$). Our preregistration did not include geographic latitude and longitude as potential drivers of fire salamander coloration. However, based on earlier reviewer suggestions, we included these variables and therefore, we explicitly explore spatial variation in coloration. Since all the environmental variables showed weak to moderate pairwise correlations (tested using Spearman's correlation tests in *ggpairs* in the GGally package [66]; all $\rho < 0.690$; see Supplementary Fig. 1), we also used one model that included all centered environmental variables (elevation divided by 100) as well as sex and the interactions of each environmental variable with sex to study the effect of each variable on the head B/Y ratio while controlling for the effect of each other environmental variable [67]. As random effects, this model included a random intercept for population and a random slope of population by sex. The random slope was subsequently removed as it did not improve the model fit ($AIC_{\text{random intercept and slope model}} = 58.791$; $AIC_{\text{random intercept model}} = 55.345$; $p = 0.758$). We used the function *emtrends* provided by the package emmeans [68] to determine if the slope for males is significantly different from 0. As the study populations are not evenly distributed across Germany (Fig. 2), we tested for residual spatial autocorrelation of our models using Moran's *I* provided by the function *Moran.I* in the ape package [69] but found no evidence for residual spatial autocorrelation (all $p > 0.670$).

# Results

## Sexual dichromatism

Male fire salamanders had both, a higher proportion of yellow on their entire body as well as on their head only (i.e., lower body B/Y ratio and head B/Y ratio) than females (Fig. 4; Table 2). In both sexes combined, the body B/Y ratio and the head B/Y ratio were strongly positively correlated (*Corr* = 0.792; $p < 0.001$). However, the slopes differed between the sexes, with males showing a greater increase than females (Fig. 5; Table 3; see Supplementary Fig. 2 for regression plots by sex and population). Overall, fire salamanders of both sexes had a higher proportion of yellow on their heads than on their entire body (i.e., lower head B/Y ratio than body B/Y ratio; Table 1; Table 3, see Supplementary Fig. 2 for population differences). For further analyses, we continue with the head B/Y ratio.

**Table 2** Summary of the linear mixed effects models used to investigate differences in the body B/Y ratio and the head B/Y ratio of female and male fire salamanders across 20 locations in Germany. The model output is on the $\log_{10}$ scale, as the dependent variables were $\log_{10}$-transformed prior to analysis. The model coefficient estimates ($\beta$) are presented with their standard errors (*SE*) for the fixed effects. Test statistics ($t$) and $p$ values are given, and all significant effects ($p < 0.05$) are presented in bold. Variance estimates ($\sigma^2$) for the random effect and residual variance are given. For categorical predictors, reference levels are presented in parentheses after the model parameter name.

| Model | Model Parameter | Model Output | | | |
|---|---|---|---|---|---|
| Body B/Y ratio | *Fixed Effects* | $\beta$ | *SE* | $t$ | $p$ |
| | **Intercept (Female)** | **0.26** | **0.03** | **9.73** | **< 0.01** |
| | **Sex (Male)** | **-0.12** | **0.02** | **-5.46** | **< 0.01** |
| | *Random Effects* | $\sigma^2$ | | | |
| | Intercept (Population) | 0.01 | | | |
| | Sex (Male) | < 0.01 | | | |
| | Residual | 0.04 | | | |
| Head B/Y ratio | *Fixed Effects* | $\beta$ | *SE* | $t$ | $p$ |

| | | | | |
|---|---|---|---|---|
| Intercept (Female) | -0.03 | 0.03 | -0.73 | 0.47 |
| **Sex (Male)** | **-0.11** | **0.02** | **-5.43** | **< 0.01** |
| *Random Effects* | $\sigma^2$ | | | |
| Intercept (Population) | 0.02 | | | |
| Residual | 0.06 | | | |

**Table 3** Summary of the linear mixed effects model investigating differences in the relationship of the head B/Y ratio with the body B/Y ratio by sex. The model output is on the log$_{10}$ scale, as the dependent variable was log$_{10}$-transformed prior to analysis. The model coefficient estimates ($\beta$) are presented with their standard errors (*SE*) for the fixed effects. Please note that log$_{10}$ body B/Y ratio was centered around the mean ($\bar{x} = 0.199$). Test statistics (*t*) and *p* values are given, and all significant effects ($p < 0.05$) are presented in bold. Variance estimates ($\sigma^2$) for random effects and residual variance are given. For categorical predictors, reference levels are presented in parentheses after the model parameter name.

| **Model Parameter** | **Model Output** | | | |
|---|---|---|---|---|
| *Fixed Effects* | $\beta$ | *SE* | *t* | *p* |
| **Intercept (Female)** | **-0.07** | **0.01** | **-5.11** | **< 0.01** |
| **Log$_{10}$ body B/Y ratio** | **0.83** | **0.05** | **16.95** | **< 0.01** |
| Sex (Male) | 0.01 | 0.01 | 0.84 | 0.40 |
| **Log$_{10}$ body B/Y ratio : Sex (Male)** | **0.16** | **0.05** | **3.13** | **< 0.01** |
| *Random Effects* | $\sigma^2$ | | | |
| Intercept (Population) | < 0.01 | | | |
| Log$_{10}$ body B/Y ratio | 0.02 | | | |
| Residual | 0.02 | | | |

## Investigating environmental correlates with color variation

We found a negative correlation of the head B/Y ratio with latitude for both sexes (i.e., in the north, fire salamanders are more yellow on their heads; Table 4; Fig. 6A) and a negative correlation of the head B/Y ratio of males with longitude (i.e., in the east, males are more yellow on their heads; Table 4B; Fig. 6B) while this association was non-significant in females (p = 0.078; Table 4A). None of the other environmental variables showed a significant relationship with the head B/Y ratio in single predictor analyses (all $p > 0.139$; Table 4; Supplementary Fig. 3). All these relationships vanished in the model including all environmental variables simultaneously (all $p > 0.200$; Table 5A) with the exception of a just non-significant negative correlation of the males' head B//Y ratio with longitude (i.e., in the east, males have more yellow on their heads). While the males' head B/Y ratio relationship with temperature change was significantly different to the relationship of the females' head B/Y ratio with temperature change (Table 5A), the slopes of both, the males' and females' head B/Y ratio with temperature change were not significantly different from zero (Table 5B). In line with the previous analysis, all models investigating the relationships of the environmental variables with the head B/Y ratio indicated that males have a lower head B/Y ratio (i.e., more yellow on their heads) than females (all $p < 0.001$; Table 4; Table 5A; Fig. 6).

**Table 4A** Summary of the linear mixed effects models used to investigate the relationship between each environmental variable with head B/Y ratio. The model output is on the $\log_{10}$ scale, as head B/Y ratio was $\log_{10}$-transformed prior to analysis. Please note that elevation was divided by 100 in order to have it on a similar scale to the other variables. All environmental variables were centered around their means in order to facilitate better model interpretation ($\bar{x}_{\text{Elevation}} = 2.524$; $\bar{x}_{Average\ Temperature} = 13.030$; $\bar{x}_{Temperature\ Change} = 1.177$; $\bar{x}_{NDVI} = 0.653$; $\bar{x}_{Latitude} = 50.950$; $\bar{x}_{Longitude} = 9.040$). The model coefficient estimates ($\beta$) are presented with their standard errors (*SE*) for the fixed effects. Test statistics ($t$) and $p$ values are given, and all significant effects ($p < 0.05$) are presented in bold. Variance estimates ($\sigma^2$) for random effects and residual variances are given. For categorical predictors, reference levels are presented in parentheses after the model parameter name. **B** Estimated slopes for males for each environmental variable.

**A) Model Summaries**

| **Environmental Variable** | **Model Parameter** | **Model Output** | | | |
|---|---|---|---|---|---|
| | *Fixed Effects* | $\beta$ | *SE* | $t$ | $p$ |
| Elevation | Intercept (Female) | -0.03 | 0.04 | -0.71 | 0.48 |
| | **Sex (Male)** | **-0.11** | **0.02** | **-5.44** | **< 0.01** |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
|  | Elevation | 0.02 | 0.03 | 0.68 | 0.50 |
|  | Sex (Male) : Elevation | -0.02 | 0.01 | -1.63 | 0.11 |
|  | *Random Effects* | $\sigma^2$ |  |  |  |
|  | Intercept (Population) | 0.02 |  |  |  |
|  | Residual | 0.06 |  |  |  |
| Average Temperature | *Fixed Effects* | *β* | *SE* | *t* | *p* |
|  | Intercept (Female) | -0.03 | 0.03 | -0.74 | 0.47 |
|  | **Sex (Male)** | **-0.11** | **0.02** | **-5.44** | **< 0.01** |
|  | Average Temperature | 0.02 | 0.04 | 0.64 | 0.53 |
|  | Sex (Male) : Average Temperature | 0.03 | 0.02 | 1.61 | 0.11 |
|  | *Random Effects* | $\sigma^2$ |  |  |  |
|  | Intercept (Population) | 0.02 |  |  |  |
|  | Residual | 0.06 |  |  |  |
| Temperature Change | *Fixed Effects* | *β* | *SE* | *t* | *p* |
|  | Intercept (Female) | -0.03 | 0.03 | -0.75 | 0.46 |
|  | **Sex (Male)** | **-0.11** | **0.02** | **-5.42** | **< 0.01** |
|  | Temperature Change | -0.34 | 0.23 | -1.47 | 0.16 |
|  | Sex (Male) : Temperature Change | 0.07 | 0.13 | 0.50 | 0.62 |
|  | *Random Effects* | $\sigma^2$ |  |  |  |
|  | Intercept (Population) | 0.02 |  |  |  |
|  | Residual | 0.06 |  |  |  |
| NDVI | *Fixed Effects* | *β* | *SE* | *t* | *p* |
|  | Intercept (Female) | -0.03 | 0.04 | -0.72 | 0.48 |
|  | **Sex (Male)** | **-0.11** | **0.02** | **-5.43** | **< 0.01** |
|  | NDVI | 0.17 | 0.82 | 0.21 | 0.84 |
|  | Sex (Male) : NDVI | -0.61 | 0.45 | -1.37 | 0.17 |
|  | *Random Effects* | $\sigma^2$ |  |  |  |
|  | Intercept (Population) | 0.02 |  |  |  |
|  | Residual | 0.06 |  |  |  |
| Latitude | *Fixed Effects* | *β* | *SE* | *t* | *p* |
|  | Intercept (Female) | -0.03 | 0.03 | -0.82 | 0.42 |
|  | **Sex (Male)** | **-0.11** | **0.02** | **-5.43** | **< 0.01** |
|  | **Latitude** | **-0.06** | **0.03** | **-2.28** | **0.03** |
|  | Sex (Male) : Latitude | -0.00 | 0.02 | -0.20 | 0.84 |
|  | *Random Effects* | $\sigma^2$ |  |  |  |
|  | Intercept (Population) | 0.02 |  |  |  |
|  | Residual | 0.06 |  |  |  |

| | | | | | |
|---|---|---|---|---|---|
| Longitude | *Fixed Effects* | *β* | *SE* | *t* | *p* |
| | Intercept (Female) | -0.03 | 0.03 | -0.80 | 0.43 |
| | **Sex (Male)** | **-0.11** | **0.02** | **-5.43** | **< 0.01** |
| | Longitude | -0.03 | 0.02 | -1.85 | 0.08 |
| | Sex (Male) : Longitude | -0.01 | 0.01 | -0.75 | 0.45 |
| | *Random Effects* | *σ²* | | | |
| | Intercept (Population) | 0.02 | | | |
| | Residual | 0.06 | | | |

**B) Estimation of Slopes for Males**

| **Environmental Variable** | **Output** | | | |
|---|---|---|---|---|
| | *β* | *SE* | *t* | *p* |
| Elevation | -0.01 | 0.03 | -0.20 | 0.85 |
| Average Temperature | 0.06 | 0.04 | 1.54 | 0.14 |
| Temperature Change | -0.28 | 0.23 | -1.18 | 0.25 |
| NDVI | -0.44 | 0.82 | -0.54 | 0.60 |
| **Latitude** | **-0.07** | **0.03** | **-2.41** | **0.02** |
| **Longitude** | **-0.04** | **0.02** | **-2.30** | **0.03** |

**Table 5A** Summary of the linear mixed effects model investigating the relationship of head B/Y ratio with each environmental variable simultaneously. The model output is on the $\log_{10}$ scale, as head B/Y ratio was $\log_{10}$-transformed prior to analysis. The model coefficient estimates (*β*) are presented with their standard errors (*SE*) for the fixed effects. Please note that elevation was divided by 100 and that all environmental variables were centered around their means in order to facilitate better model interpretation (for means of each variable, see Table 4). Test statistics (*t*) and *p* values are given, and all significant effects ($p < 0.05$) are presented in bold. Variance estimates ($\sigma^2$) for the random effect and the residual variance are given. For categorical predictors, reference levels are presented in parentheses after the model parameter name. **B** Estimated slopes for males for each environmental variable.

**A) Model Summary**

| **Model Parameter** | **Model Output** | | | |
|---|---|---|---|---|
| *Fixed Effects* | *β* | *SE* | *t* | *p* |
| Intercept (Female) | -0.03 | 0.03 | -0.81 | 0.43 |

| | | | | |
|---|---|---|---|---|
| **Sex (Male)** | **-0.11** | **0.02** | **-5.45** | **< 0.01** |
| Elevation | -0.02 | 0.06 | -0.35 | 0.73 |
| Average Temperature | -0.03 | 0.09 | -0.32 | 0.75 |
| Temperature Change | -0.40 | 0.42 | -0.97 | 0.35 |
| NDVI | 0.59 | 1.57 | 0.38 | 0.71 |
| Latitude | -0.08 | 0.06 | -1.34 | 0.201 |
| Longitude | 0.00 | 0.04 | -0.14 | 0.89 |
| Sex (Male) : Elevation | -0.05 | 0.04 | -1.35 | 0.18 |
| Sex (Male) : Average Temperature | -0.08 | 0.05 | -1.48 | 0.14 |
| **Sex (Male) : Temperature Change** | **0.60** | **0.25** | **2.38** | **0.02** |
| Sex (Male) : NDVI | 1.53 | 0.96 | -1.60 | 0.11 |
| Sex (Male) : Latitude | -0.05 | 0.04 | -1.25 | 0.21 |
| Sex (Male) : Longitude | -0.03 | 0.02 | -1.60 | 0.11 |
| *Random Effects* | $\sigma^2$ | | | |
| Intercept (Population) | 0.02 | | | |
| Residual | 0.06 | | | |

**B) Estimation of Slopes for Males**

| **Environmental Variable** | **Output** | | | |
|---|---|---|---|---|
| | *β* | *SE* | *t* | *p* |
| Elevation | -0.08 | 0.06 | -1.18 | 0.25 |
| Average Temperature | -0.11 | 0.09 | -1.23 | 0.24 |
| Temperature Change | 0.20 | 0.42 | 0.49 | 0.63 |
| NDVI | -0.94 | 1.57 | -0.60 | 0.56 |
| Latitude | -0.13 | 0.06 | -2.11 | 0.05 |
| Longitude | -0.04 | 0.04 | -1.12 | 0.28 |

## Discussion

### Sexual dichromatism in fire salamanders

Using a semi-automated analysis of photos of fire salamanders across Germany taken by scientists and citizen scientists, we show that males have a higher proportion of yellow on their entire dorsal surface as well as on their head only compared to females. While body B/Y ratio and head B/Y ratio were strongly positively correlated, this relationship was more positive (i.e., a steeper slope) in males than in females. This indicates that a female with a high body B/Y ratio (i.e., low proportion of yellow on her body) has a lower head B/Y ratio (i.e., higher proportion of yellow on her head) than a male fire salamander with an equal body B/Y ratio (Fig. 5). Most fire salamanders in Germany have only very little yellow coloration on their sides, and females usually have a wider body shape than males (due to pregnancy; [42, 43]). Therefore, the more stretched out bodies of females make the sides appear more visible from above, reducing the proportion of yellow when viewed from above. This sexual dimorphism in body shape likely influenced the sexual dichromatism in body coloration observed in this study and in previous studies [29, 30]. The head coloration is less affected by changes in body shape due to pregnancy or feeding status and therefore, comparing the head coloration between sexes is less problematic. Since the yellow proportion on the head is also higher in males than in females, however, the sexual dimorphism in body shape can only partly explain the observed sexual dichromatism. Nevertheless, we advise caution for researchers aiming to study sex-specific differences in dorsal coloration of fire salamanders in the future as differences in body shape will likely need to be accounted for when comparing females with males.

Our finding of a sexual dichromatism, with males being more yellow than females, is in line with previous studies in Germany [29] and Slovakia [30]; however, fewer populations were tested (three and six populations, respectively). However, two other studies investigating sexual dichromatism in northern Spain and northern Italy, respectively, did not find differences in coloration between males and females [45, 47]. Therefore, our study, which used data from 20 locations spanning the largest area of the natural distribution of fire salamanders so far, provides the first conclusive evidence of widespread sexual dichromatism in this species. One potential cause of this sexual dichromatism is sexual selection driven by mate choice, whereby female fire salamanders prefer to mate with yellower males. Fire salamanders are capable of discriminating yellow from other colors even under low light conditions [70]; therefore, color-based mate choice is theoretically possible in this species. When raised under rich food conditions as larvae, postmetamorphic fire salamanders have a higher proportion of yellow than individuals raised under poor food conditions as larvae, indicating that yellow coloration is likely energetically costly for fire salamanders [41]. However, this difference in yellow proportion disappeared with time when the postmetamorphic fire salamanders were maintained under identical nutritional conditions, which could indicate that the maintenance of the pigments responsible for yellow coloration is also costly [41]. Therefore, the proportion of yellow coloration in fire salamanders could act as a signal of a potential mate's quality, especially when considering that more yellow coloration is more effective at deterring predators [41], although importantly, more yellow coloration does not indicate higher levels of toxicity [28, 29, 45, 49]. In the spadefoot toad *Scaphiopus couchii*, larger males and males with a better body condition have a more dissimilar body coloration to females [71]. Furthermore, clay models that mimic these males in coloration were also preferred by females over clay models of males that resembled females in color and dorsal pattern [71]. Similarly, natterjack toads (*Epidalea calamita*) are sexually dichromatic, and their coloration is correlated with age, body size, hindlimb length, and sprint speed [72]. Although no correlation between color and toxicity has been detected in fire salamanders [28, 29, 45, 49], other factors, such as genetic quality, age or performance capacity, might be identifiable for females via coloration [73]. Male fire salamanders have been reported to prefer sites that are linked to female activity [74] and are frequently observed in an upright position referred to as a "presenter pose" [70, 75]. This pose could provide females with the opportunity to examine a male's coloration and body condition and could therefore play an important role in visual communication during mate choice [75]. Future studies could either directly (e.g., [71]) or indirectly (e.g., [76]) investigate the role of fire salamander coloration in mate choice and whether other traits correlate with a fire salamander's coloration, which would indicate whether color acts as a quality signal. Furthermore, the body parts visible for a female should be further investigated (i.e., "taking the perspective" of a female fire salamander).

Alternatively, this sexual dichromatism could be related to differential selection pressure (e.g., by predators) for both sexes in fire salamanders. As described above, male fire salamanders are often seen in "presenter pose" with their bodies raised and their forelimbs extended, staying motionless for up to several minutes [75]. This behavior could not only be related to intraspecific communication, as the dorsal pattern is more visible for conspecifics when the body of the male is raised, but it could also expose the male to a greater threat of predation (by e.g., *Natrix natrix* snakes and birds of the order Strigiformes; [42, 47]). Furthermore, when tested repeatedly, males were more active than females were (Mühlenhaupt et al., in preparation), which could be another reason males are exposed to a greater threat of predation [77]. Similarly, the development of fire salamander larvae in a female's body might necessitate greater metabolic activity and therefore, the need for higher temperatures, which could be supported by a greater proportion of black in females, i.e., "thermal melanism". For example, [78] reported that males of the ground cricket *Allonemobius socius* had darker cuticles than females did, which could be the result of, e.g., additional sex-specific selection due to temperature-dependent display behavior.

## Differences in the yellow proportion of body and head

An interesting result from our investigations is that fire salamanders have higher proportions of yellow on their head than on their overall dorsal surface. Caspers et al. (2020) used models of fire salamanders with varying proportions of yellow to show that models with higher proportions of yellow received fewer attacks from potential predators [41]. Since the head of a fire salamander is an irreplaceable body part necessary for the survival of an individual, protecting this body part might prove especially important compared with, e.g., the tail (fire salamanders with missing tails are frequently observed (personal observations)). Using coloration to focus or divert predator attacks on specific body parts is a well-known strategy in the animal kingdom [8]. For example, [79] used clay models of lizards with three sections—head, body and tail—that were either black or blue and reported that models with blue sections received the majority of attacks by birds on their blue sections rather than on the black sections. This is especially interesting given that lizards of multiple families have evolved blue tails that can be autotomized [80]. Thereby, attacks are guided toward the body part that can be sacrificed (i.e., is nonvital). In fire salamanders, yellow coloration could act in the opposite way and divert predator attacks from the head. Furthermore, fire salamanders have prominent toxin glands, called parotoid glands, on the sides of their heads that are in almost every case at least partly yellow [42, 43], and the yellow coloration highlights the dark glandular pores.

Advertising these toxin glands might be an effective strategy for avoiding predator attacks. For example, [81] reported that plasticine models of *Epidalea calamita* toads with larger and more conspicuously colored parotoid glands received fewer predator attacks.

The head coloration might also play an especially important role in mate choice of fire salamanders. For example, [82] showed that males of the two extant species of *Salamandrina* have more conspicuous gular coloration. When females are nearby, this coloration is displayed in a stereotyped throat hyperextension while being in a similar "presenter pose" to fire salamanders. Therefore, the head coloration of fire salamanders might play an important role in mate choice. Especially the gular coloration of fire salamanders merits further investigation given that the throat is especially visible during the "presenter pose" (see also [70]).

## Environmental correlates with fire salamander coloration

Several other studies have already attempted to find environmental correlates with the yellow proportion in fire salamander coloration. For example, Burgon et al. (2020) found no relationship between elevation and the frequency of color morphs that vary in yellow proportion and value in *S. s. bernadezi* in northern Spain. Similarly, Barzaghi et al. (2022) found no correlation between the proportion of yellow with NDVI, elevation or predator richness in fire salamanders of northern Italy that likely represent a hybrid of *S. s. salamandra* and *S. s. gigliolii* [43]. In our study, we tested for environmental correlates with yellow proportion in fire salamanders from the widest area of fire salamander distribution yet studied and with individuals belonging to both the *S. s. terrestris* lineage (in western Germany) and the hybrid lineage of *S. s. salamandra* and *S. s. terrestris* (in eastern Germany) [43, 83]. We found evidence for variation in the yellow proportion of fire salamanders along their north-to-south and west-to-east distribution. Given that in our study, fire salamander populations from southwestern Germany to eastern Germany were included, previous studies might have failed to find similar clines due to a smaller sampling area. Interestingly, neither an east-west cline nor a south-north cline can be explained in variation due to underlying environmental factors such as variation in temperature or habitat productivity (i.e., NDVI) as these variables were not significantly associated with the B/Y ratios when tested individually or in models including all environmental variables. One possible driver of this variation could be differences in yellow proportion between genetic lineages across Germany. For example, it is well known that the *S. s. salamandra* lineage and the *S. s. terrestris* lineage and their hybrid lineage differ in dorsal color pattern [43, 44, 83] and it is very likely that the color patterns also affect the yellow proportion (unpublished data). However, these differences between the lineages do not ultimately explain the variation in yellow proportion. A potential explanation for this variation could be differences in the mate choice behavior of females between these lineages as latitude and longitude were more often significantly associated with the B/Y ratios of males than females, especially when accounting for the effects of other environmental variables (i.e., using the models that included all environmental variables). Clearly, further research spanning larger geographic areas and including more lineages of the widely distributed and highly variable European fire salamander is needed. This research could also indicate whether the sexual dichromatism that we have identified for fire salamanders in Germany is also present in other lineages outside of Germany (e.g., in the fire salamander diversity hotspot on the Iberian peninsular [43]). Integrating citizen science approaches and even using data available through apps such as iNaturalist or social media can make such a task feasible [5].

Although the putative role of thermal melanism in fire salamanders has been suggested multiple times [43, 49, 50], neither average temperature nor temperature change showed significant associations with the yellow proportion of the fire salamanders studied here. While comparing the fully black alpine salamander (*Salamandra atra*) with the European fire salamander might lead to this conclusion as alpine salamanders inhabit higher elevated habitats with lower average temperatures [43], the intraspecific variation in yellow proportion of European fire salamanders could not be explained through differences or changes in temperature in this study or former studies [45, 47]. While we were able to cover a span of 3 °C in average temperature (Table 1) and [45] covered a span in elevation of 78 to 1312 m a.s.l., no associations with elevation or average temperature have been found, yet. Therefore, it might be possible that other mechanisms such as behavioral adaptations could play a more important role in thermal regulation of fire salamanders. For example, fire salamanders in Mediterranean areas such as parts of the Iberian peninsular show drastically different activity patterns (i.e., activity during winter) to fire salamanders in temperate climates (i.e., hibernation during winter) [42, 43]. Furthermore, in some regions fire salamanders show diurnal activity whereas in other areas, they are almost exclusively nocturnal [42, 84]. Therefore, future studies could also consider differences in activity patterns across large geographic areas.

We have included temperature change as there was quite some variation in how much the average temperatures have increased in our study locations and as from other studies it is clear that global warming will have a strong impact on the coloration of ectotherms [36, 38, 39, 85]. However, in our study we did not find evidence for an effect of warming on the yellow proportion of fire salamanders. Again, behavioral adaptations such as shifts in phenology (e.g., increased importance of winter and decreased importance of summer for fire salamanders in Germany) might mitigate the changes in temperature as for other sympatric amphibian species shifts in phenology with milder winters have already been shown [86, 87]. An alternative approach to the one we have used here would be to compare the coloration of fire salamanders, preferably from multiple sites, across time (e.g., by using photos and/or specimen from biological collections) to correlate the change in temperature over a span of several decades with the proportion of yellow.

Further alternative explanations of the variation in yellow proportion should also be considered. In other aposematic amphibians such as poison dart frogs (Dendrobatidae), a role of coloration in camouflage has already been shown [88, 89]. Location-dependent differences in background could be associated with differences in coloration of fire salamanders in these locations. While the “greenness” of the habitat (i.e., NDVI) did not show an association with the yellow proportion, other features of the habitat could reduce the detectability of specific color morphs. It can be surprisingly easy to miss a fire salamander when actively searching for these aposematic salamanders (personal observation). A possible role of fire salamander coloration in crypsis has already been proposed [90, 91] but remains to be thoroughly tested.

## Conclusion

This study provides strong evidence for a widespread sexual dichromatism of fire salamanders in Germany through the use of citizen science data. Furthermore, we find evidence for variation in the coloration of the head and the body indicating as of yet underappreciated involvement in different biological functions. Future studies investigating differences in dorsal coloration of fire salamanders should consider differences in body shape between females and males as these will likely affect the dorsal proportion of yellow. The clines in yellow proportion with latitude and longitude that we have found could be the result of differences between lineages of fire salamanders in Germany but the underlying mechanisms are yet to be uncovered. Clearly, more large-scale comparisons, integrating multiple lineages across diverse habitats of fire salamanders, are necessary to better understand the drivers of inter- and intrapopulation as well as intraindividual variation in fire salamander coloration. To facilitate such extensive studies, the implementation of citizen science approaches will be extremely helpful as has been shown for other animals already.

### Abbreviations

| | |
|---|---|
| NDVI | Normalized Difference Vegetation Index |
| ARW | Amphibian and Reptile Wildbook |
| B/Y ratio | Black-to-yellow ratio |
| LMM | Linear mixed effects model |

## Supplementary Information

**Supplementary Fig. 1** The correlation of the environmental variables. In the top right hand plots, the Spearman’s correlation test statistics for each combination of environmental variables are shown. “Corr” denotes the correlation coefficient $\rho$. Asterisks represent the $p$-value (* - < 0.05; ** - < 0.01; *** - < 0.001). The diagonal plots show the distribution of the data using density plots. In the bottom left hand plots, you can see the relationship of each combination of the environmental variables as depicted using a regression line. Each dot represents one study location.

**Supplementary Fig. 2** The correlation of the head black-to-yellow-ratio with the body black-to-yellow ratio within each population. The regression lines as well as the dots were colored by sex (red – females, blue – males).

**Supplementary Fig. 3** The relationship of the head black-to-yellow-ratio with **A** elevation, **B** average temperature **C** temperature change, and **D** the normalized difference vegetation index. The regression lines as well as the dots were colored by sex (red – females, blue – males).

**Supplementary Fig. 4** The relationship of the body black-to-yellow-ratio with **A** elevation, **B** average temperature **C** temperature change, **D** the normalized difference vegetation index, **E** latitude, and **F** longitude. The regression lines as well as the dots are colored by sex (red – females, blue – males).

**Supplementary Table 1** **A** Results of the linear mixed effects models investigating the relationship of body B/Y ratio with each environmental variable individually as well as **B** the results from the *emtrends* [68] test of the significance (i.e., difference from 0) of slopes by sex and environmental variable. **C** the results of the linear mixed effects model investigating the relationship of body B/Y ratio with each environmental variable simultaneously as well as **D** the results from the *emtrends* [68] test of the significance (i.e., difference from 0) of slopes for males by each environmental variable. The output is on the $\log_{10}$ scale, as body B/Y ratio was $\log_{10}$-transformed prior to analysis. The model coefficient estimates ($\beta$) are presented with their standard errors (*SE*) for the fixed effects. Please note that elevation was divided by 100 and that all environmental variables were centered around their means in order to facilitate better model interpretation (for means of each variable, see Table 4). Test statistics ($t$) and $p$-values are given, and all significant effects ($p < 0.05$) are presented in bold. Variance estimates ($\sigma^2$) for random effects and residual variances are given for the models in A and B. For categorical predictors, reference levels are presented in parentheses after the model parameter name. If the interactions of Sex (Male) with the environmental variables in the large model presented in B were significant, the slopes for females and males were tested with *emtrends* to investigate if the slopes for each sex were significantly different from 0 and are presented in C.

## Acknowledgements

We are grateful for the helpful comments of two anonymous reviewers of a previous version of this manuscript submitted to Proceedings of the Royal Society B: Biological Sciences that improved the quality of this manuscript. We would also like to thank the participants of the Animal Behaviour Seminar at the Animal Behaviour Research facility of Bielefeld University for fruitful discussions about this project.

## Author contributions

MM, OK, and BAC conceived the idea and designed the study. All authors with the exception of BAC, RH, MS, and OK were involved in sampling. RH and MS conducted the image analysis. MM and NA conducted the statistical analysis. MM and BAC interpreted the results. All authors contributed critically to the drafts and gave final approval for the preprint and the publication.

## Funding

This research was funded by the German Research Foundation (DFG) as part of the CRC TRR 212 (NC³) – project A04.

## Data availability

Data and R code will be made publicly available on Open Science Framework (OSF) upon acceptance of this manuscript for publication.

# Declarations

## Ethics approval and consent to participate

Download of photos from the ARW were approved by the users who uploaded these photos. No ethics approval was necessary for this study as no fire salamanders were touched or elsewise harmed. If animals were touched by researchers, these procedures were approved in alignment with the specific projects.

## Competing interests

The authors declare no competing interests.

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# Figures

**Fig. 1** Representative specimen of female (♀) and male (♂) fire salamanders (*Salamandra salamandra*) with a 2 €- coin as a size reference. Both individuals were photographed in the same night in the Botanical Garden Bielefeld (BGBI), a study location. Females are usually much wider in body shape [42, 43] and, as the lateral side of a fire salamander is usually mostly black, females might have a lower proportion of yellow when viewed from above despite having the same relative amount of pigment cells responsible for the yellow coloration.

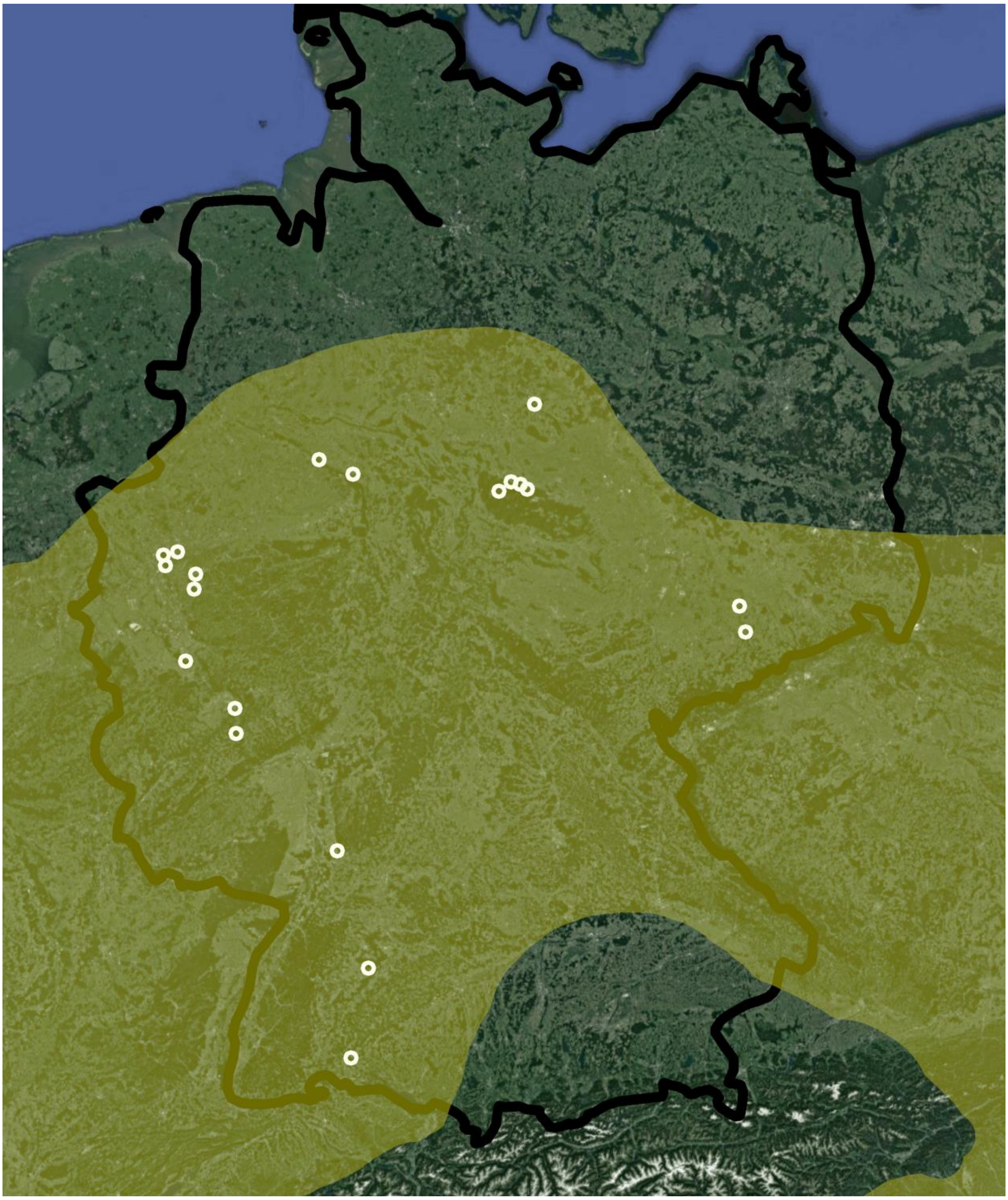

**Fig. 2** Map of Germany with the approximate natural distribution of fire salamanders (*Salamandra salamandra*) (according to [43]) highlighted in yellow. White circles denote the sampling locations.

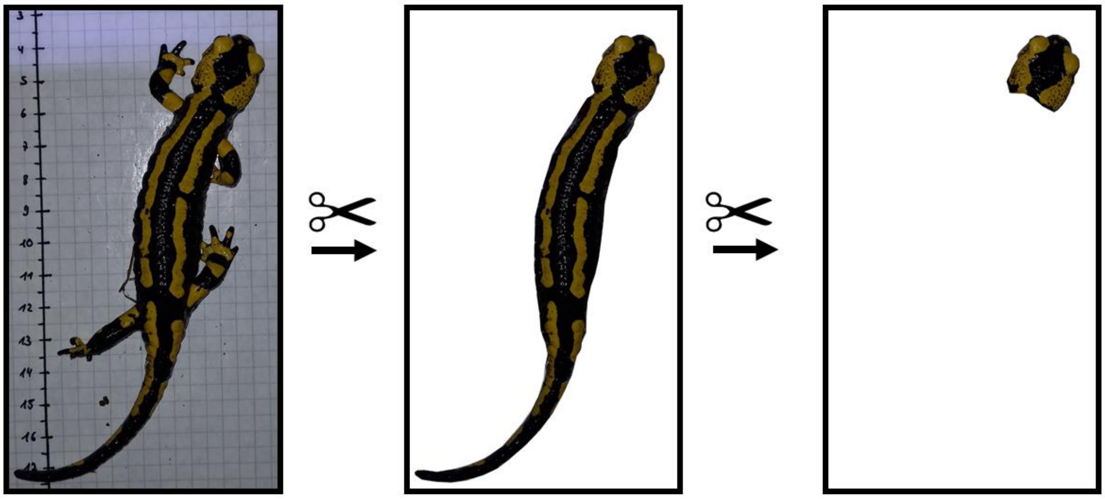

**Fig. 3** Schematic representation of image processing. First, the fire salamander body was cropped as described in [40]. Subsequently, the head of each fire salamander was also cropped out and saved as an additional image to compare overall body coloration with head coloration specifically. Images of the cropped body as well as the head only were then used to automatically quantify the black-to-yellow-ratio (B/Y-ratio) of the fire salamander coloration using the Python script provided in [40]. All image processing and analysis was conducted by two researchers (RH and MS) who were blind to the sex and location of the fire salamander depicted in the image.

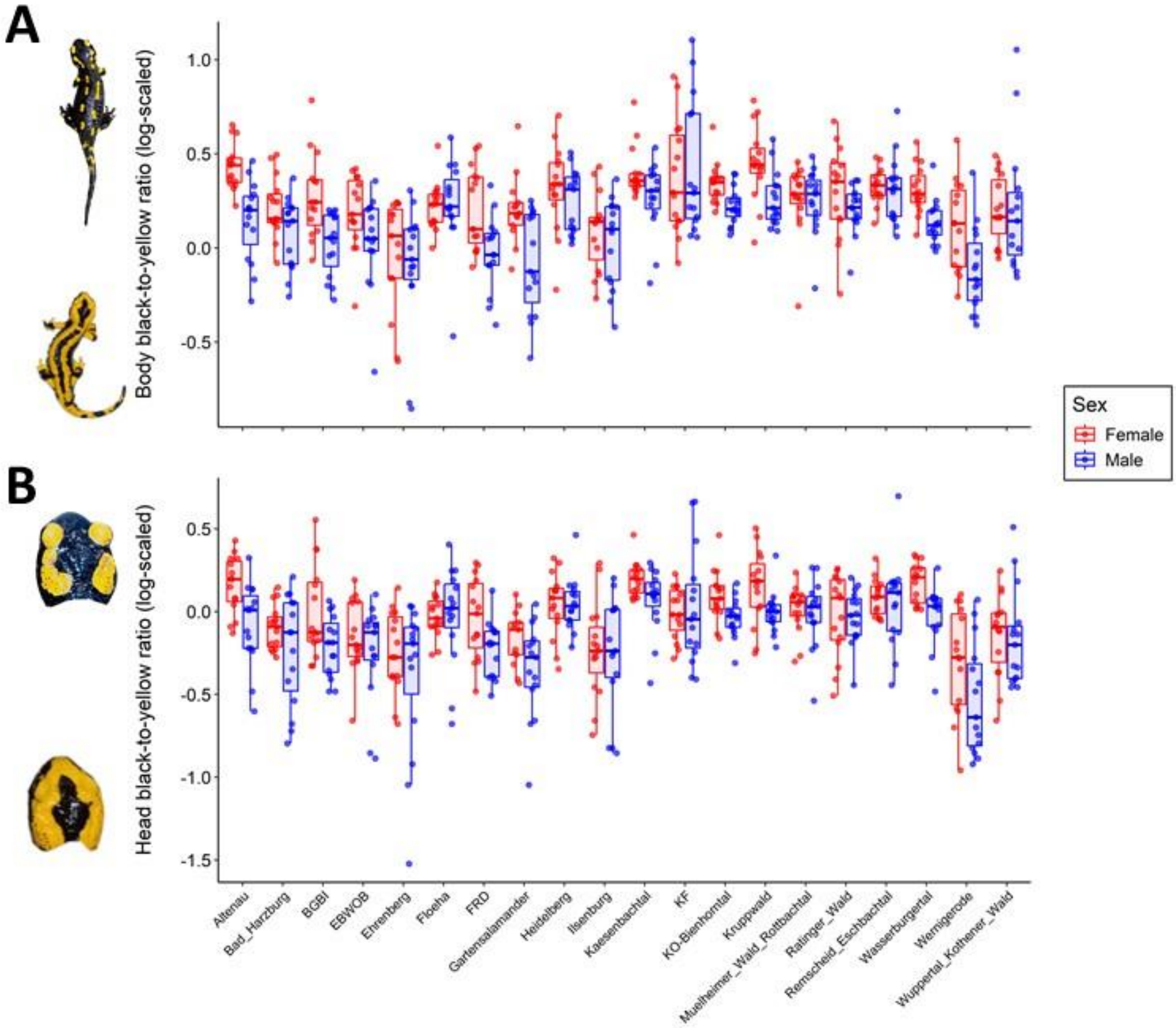

**Fig. 4** Differences in **A** the body black-to-yellow ratio and **B** the head black-to-yellow ratio of female (red) and male (blue) fire salamanders by population. Left to the y-axes, you can see a graphical representation of the coloration of a fire salamander (i.e., very yellow or very black fire salamanders or fire salamander heads, respectively). The legs of the fire salamanders in panel A were not cropped for aesthetical reasons. The boxplots show the median (horizontal bar) and the 2nd and 3rd quartile with the minimum and maximum range outside of the boxes. The dots represent each sample. Please note that the black-to-yellow ratios are on the $\log_{10}$ scale.

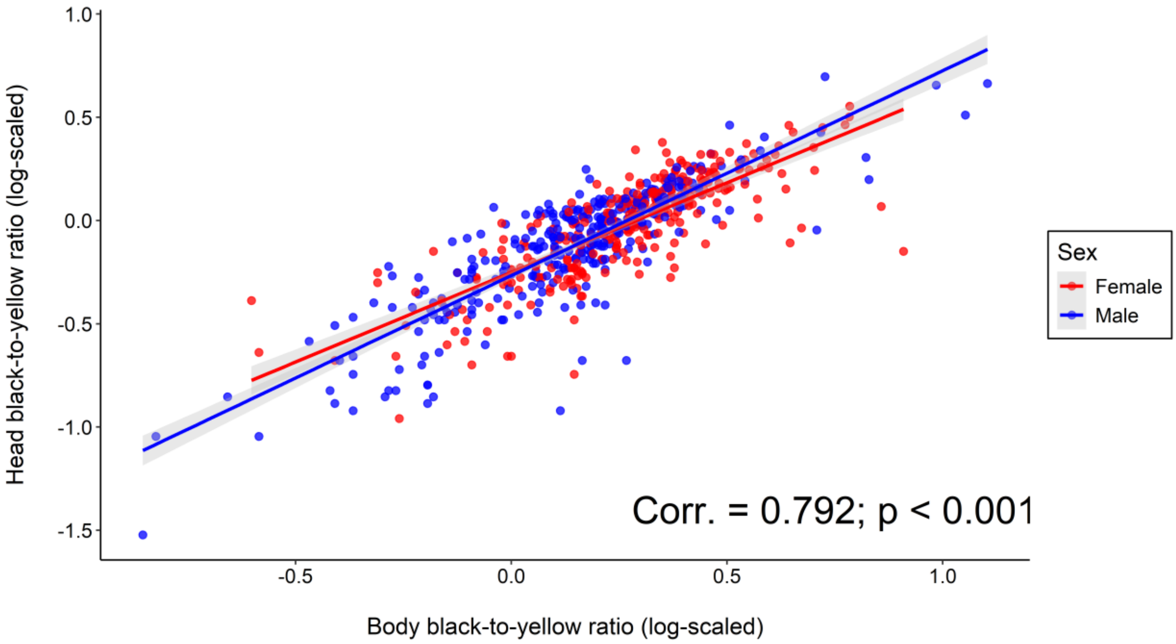

**Fig. 5** The correlation of the head black-to-yellow-ratio with the body black-to-yellow ratio. The regression lines as well as the dots were colored by sex (red – females, blue – males). The correlation test statistics in the bottom right corner represent the results of the Pearson’s correlation test. Please see Supplementary Fig. 2 for a correlation plot by sex for each population.

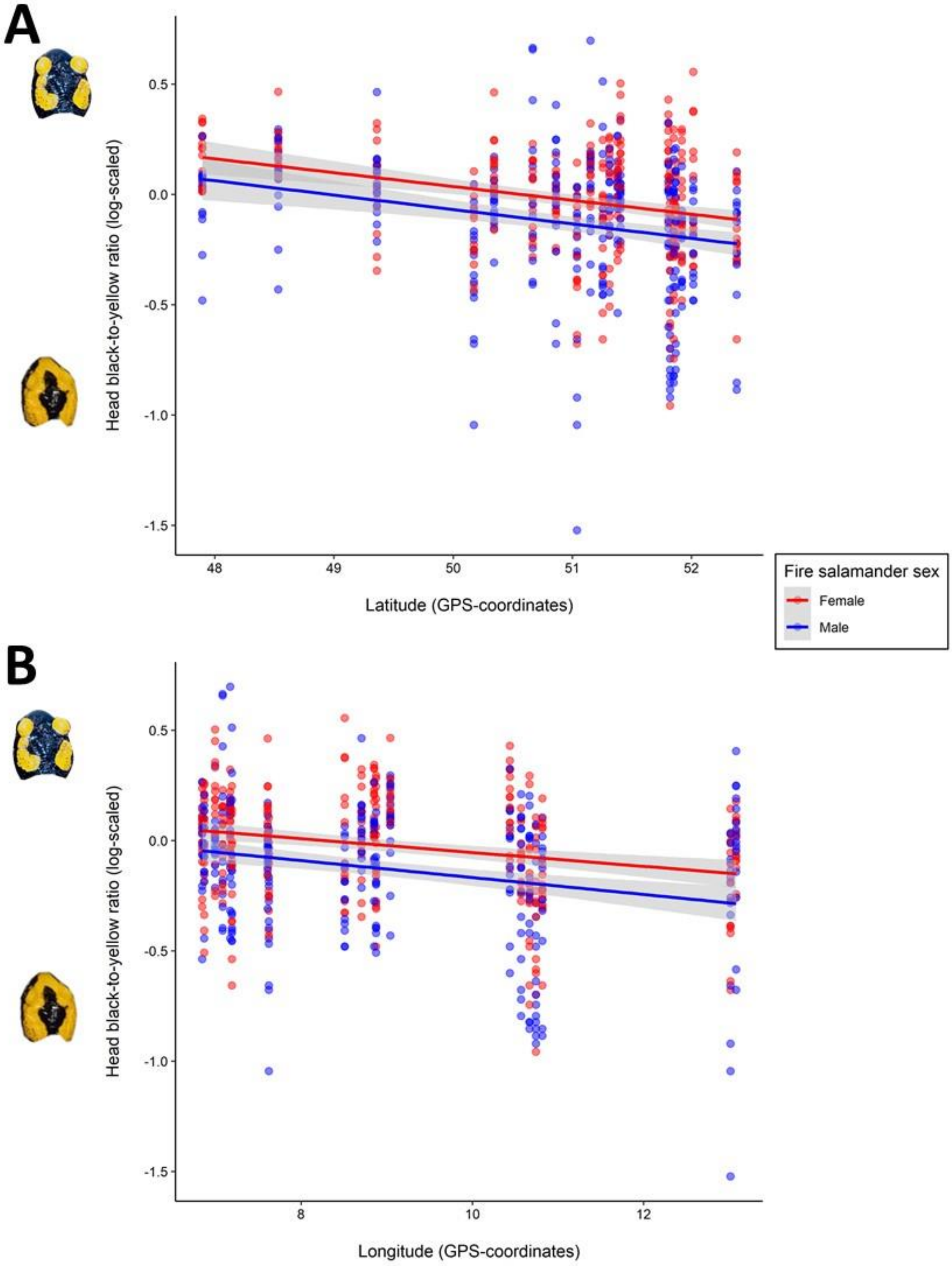

**Fig. 6** The relationship between the head black-to-yellow ratio and **A** latitude and **B** longitude for female (red) and male (blue) fire salamanders. Left to the y-axes, you can see a graphical representation of the coloration of a fire salamander (i.e., very yellow or very black fire salamander

heads). The dots represent each sample. Please note that the black-to-yellow ratios are on the $\log_{10}$ scale and that the relationship between head B/Y ratio and longitude was not statistically significant (Table 4).

## Supplementary Information for the Manuscript titled:

# "Citizen science data supports sexual dichromatism but rejects thermal melanism in the European fire salamander (*Salamandra salamandra*)"

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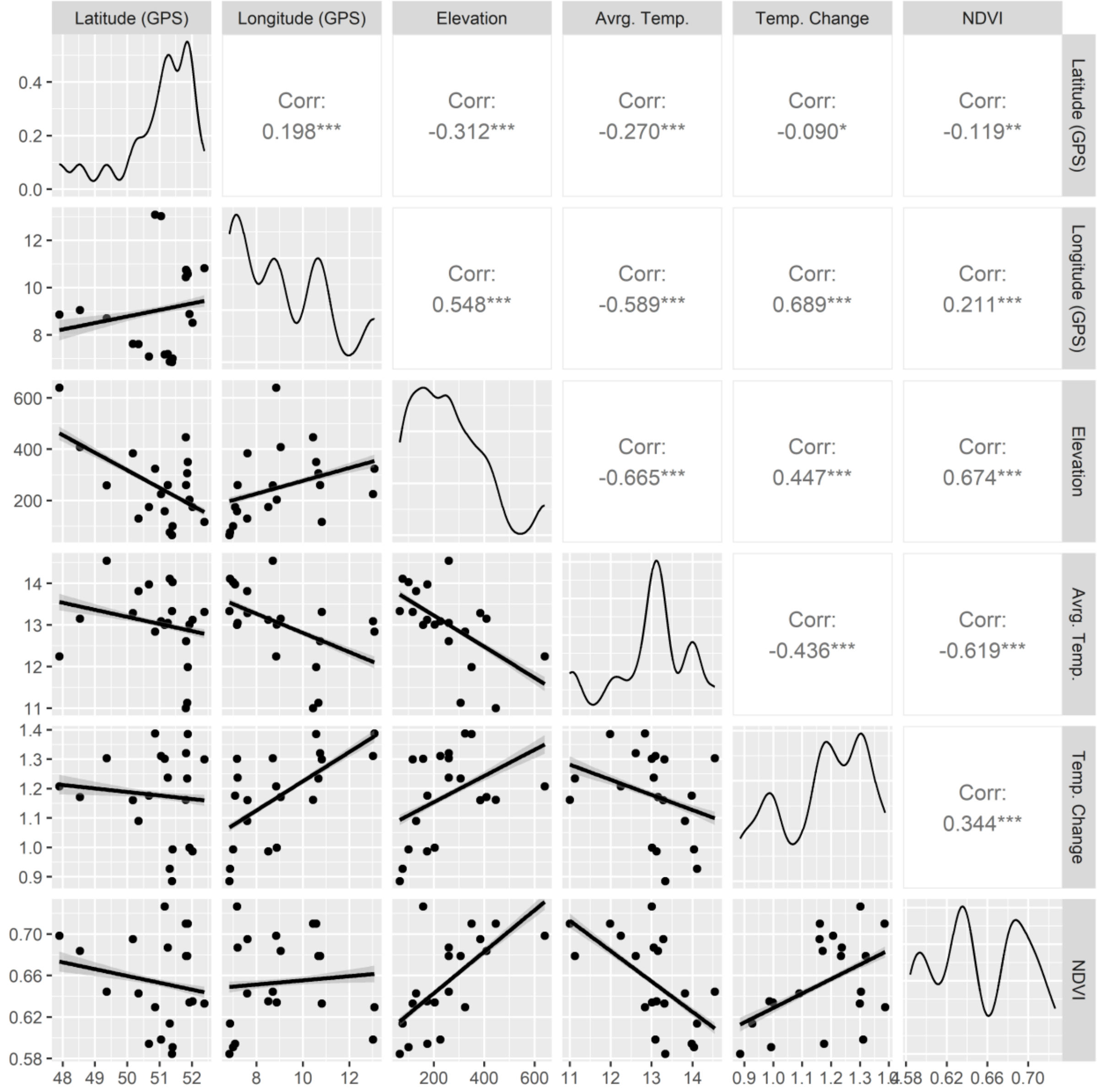
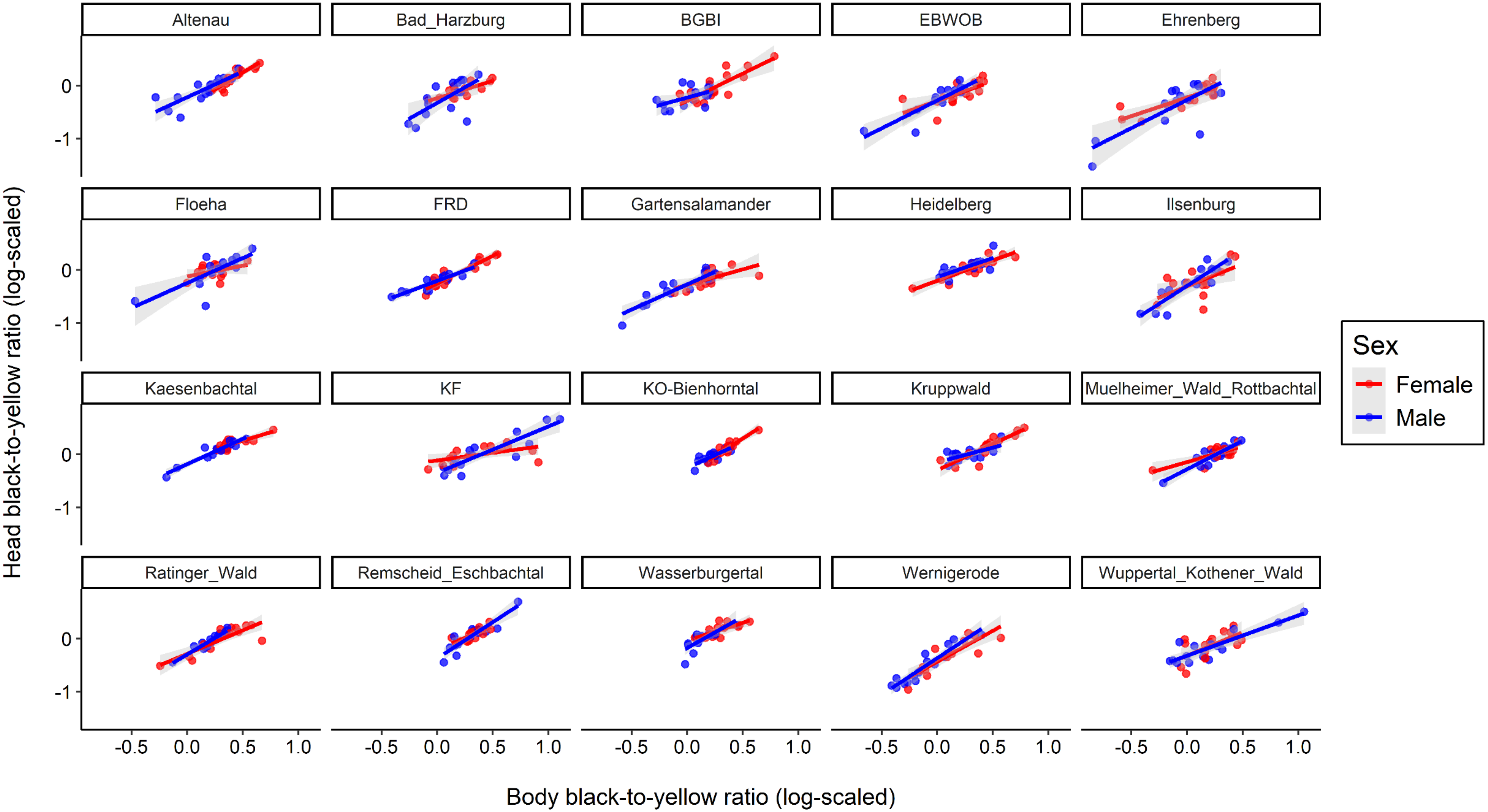
## Summary

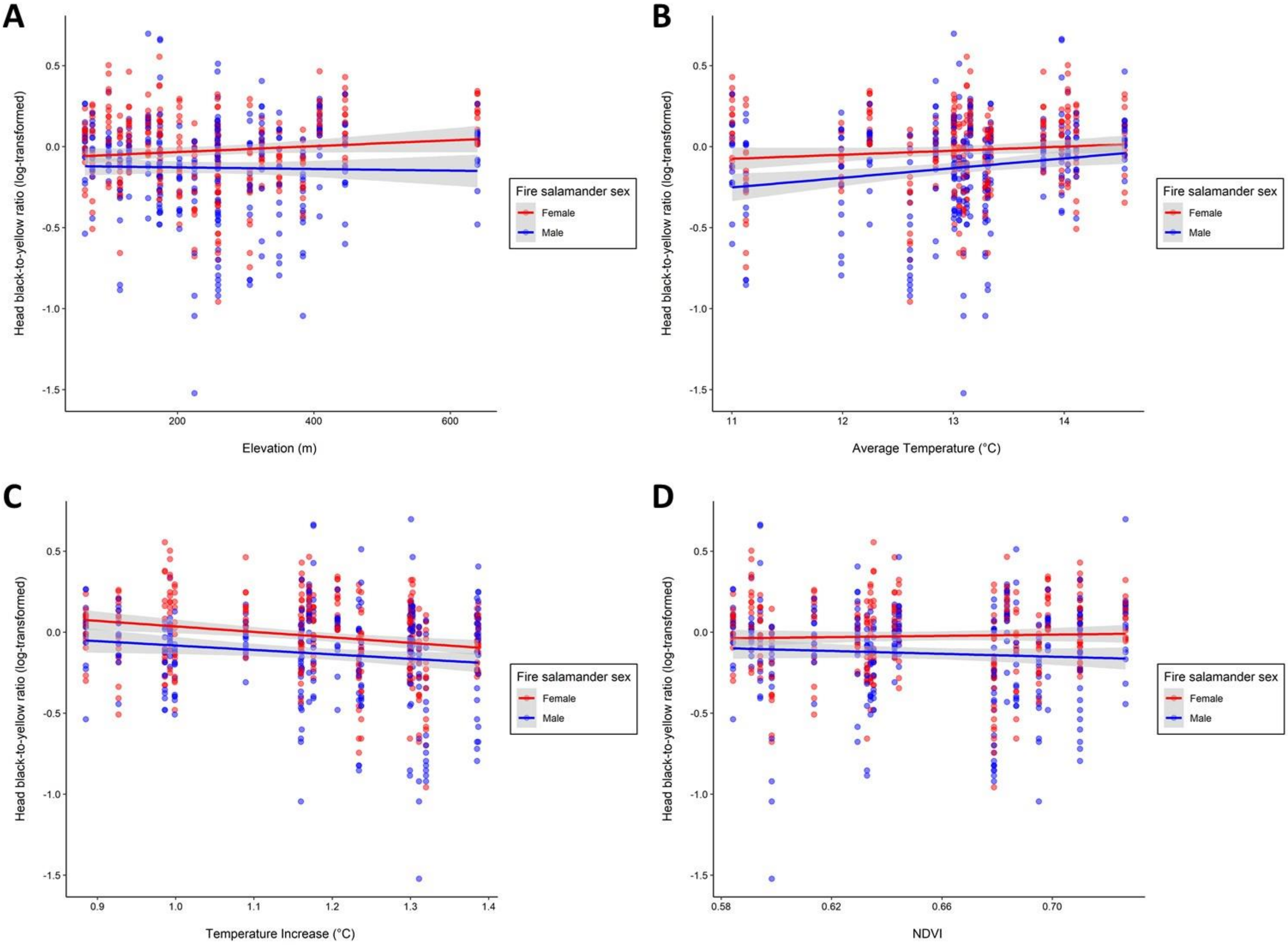
These supplementary information show additional data of the manuscript titled “Citizen science data supports sexual dichromatism but rejects thermal melanism in the European fire salamander (*Salamandra salamandra*)”. The environmental variables used to investigate the influence of environmental factors on the yellow coloration of fire salamanders in this study showed weak to moderate correlations with each other ($\rho < 0.690$; Supplementary Fig 1). Therefore, in addition to models investigating the individual effect of each environmental variable on the black-to-yellow ratio (B/Y ratio) of fire salamanders, we also used a model that integrated all environmental variables and thereby tests the contribution of each environmental variable decomposed of the effect of every other environmental variable [1]. The correlation of head B/Y ratio and body B/Y ratio was sex and population dependent (Table 3). Therefore, we show correlation plots with different colors for each sex and a panel for each population in Supplementary Fig 2. Using body B/Y ratio in separate models including one environmental variable at a time revealed a negative relationship with longitude (i.e., in the east, fire salamanders have more yellow bodies) and a marginally non-significant negative correlation of males’ body B/Y ratio with average temperature but no association with any other environmental variable (all $p > 0.101$; Supplementary Table 1A; Supplementary Table 1B). Using one model including all environmental variables, indicated no effect of any environmental variable on females’ body B/Y ratio, however, this model still indicated that in males, body B/Y ratio declines with longitude (Supplementary Table 1B and C). All models investigating the relationships of the environmental variables with body B/Y ratio indicated that males have a lower body B/Y ratio (i.e., more yellow on their bodies) than females (Supplementary Fig. 4; Supplementary Table 1).



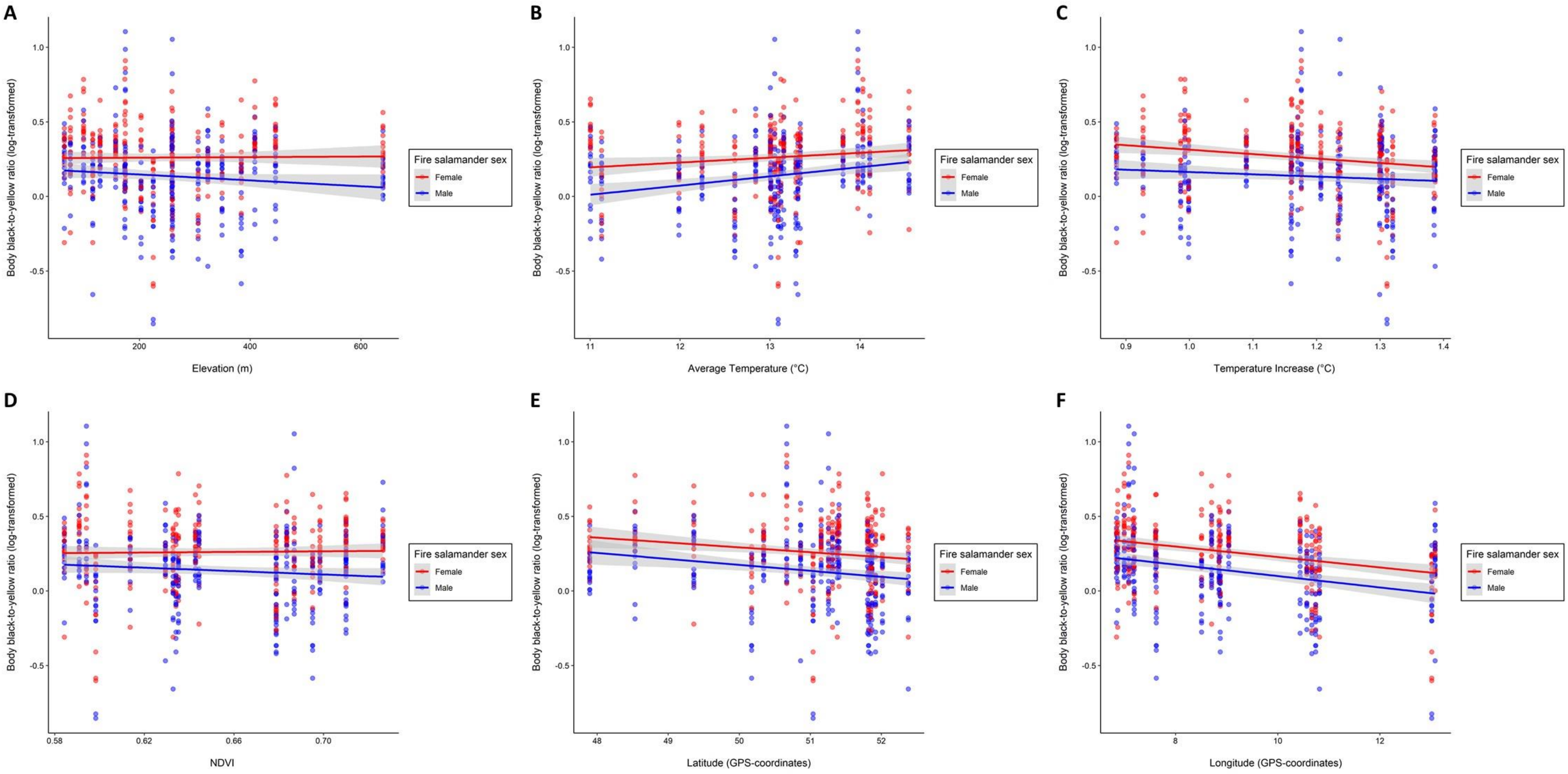
**Supplementary Fig. 1** The correlation of the environmental variables. In the top right hand plots, the Spearman's correlation test statistics for each combination of environmental variables are shown. "Corr" denotes the correlation coefficient *ρ*. Asterisks represent the *p*-value (* - < 0.05; ** - < 0.01; *** - < 0.001). The diagonal plots show the distribution of the data using density plots. In the bottom left hand plots, you can see the relationship of each combination of the environmental variables as depicted using a regression line. Each dot represents one study location.


Altenau
Bad_Harzburg
BGBI
EBWOB
Ehrenberg
Floeha
FRD
Gartensalamander
Heidelberg
Ilsenburg
Kaesenbachtal
KF
KO-Bienhorntal
Kruppwald
Muelheimer_Wald_Rottbachtal
Ratinger_Wald
Remscheid_Eschbachtal
Wasserburgertal
Wernigerode
Wuppertal_Kothener_Wald
0
-1
-0.5
0.0
0.5
1.0
Head black-to-yellow ratio (log-scaled)
Body black-to-yellow ratio (log-scaled)
Sex
Female
Male

**Supplementary Fig. 2** The correlation of the head black-to-yellow-ratio with the body black-to-yellow ratio within each population. The regression lines as well as the dots were colored by sex (red – females, blue – males).



**Supplementary Fig. 3** The relationship of the head black-to-yellow-ratio with **A** elevation, **B** average temperature **C** temperature change, and **D** the normalized difference vegetation index. The regression lines as well as the dots were colored by sex (red – females, blue – males).


A
B
C
D
E
F
Body black-to-yellow ratio (log-transformed)
1.0
0.5
0.0
-0.5
Elevation (m)
200
400
600
Average Temperature (°C)
11
12
13
14
Temperature Increase (°C)
0.9
1.0
1.1
1.2
1.3
1.4
NDVI
0.58
0.62
0.66
0.70
Latitude (GPS-coordinates)
48
49
50
51
52
Longitude (GPS-coordinates)
8
10
12
Fire salamander sex
Female
Male

**Supplementary Fig. 4** The relationship of the body black-to-yellow-ratio with **A** elevation, **B** average temperature **C** temperature change, **D** the normalized difference vegetation index, **E** latitude, and **F** longitude. The regression lines as well as the dots are colored by sex (red – females, blue – males).

**Supplementary Table 1 A** Results of the linear mixed effects models investigating the relationship of body B/Y ratio with each environmental variable individually as well as **B** the results from the *emtrends* [2] test of the significance (i.e., difference from 0) of slopes by sex and environmental variable. **C** the results of the linear mixed effects model investigating the relationship of body B/Y ratio with each environmental variable simultaneously as well as **D** the results from the *emtrends* [2] test of the significance (i.e., difference from 0) of slopes for males by each environmental variable. The output is on the $\log_{10}$ scale, as body B/Y ratio was $\log_{10}$-transformed prior to analysis. The model coefficient estimates (*β*) are presented with their standard errors (*SE*) for the fixed effects. Please note that elevation was divided by 100 and that all environmental variables were centered around their means in order to facilitate better model interpretation (for means of each variable, see Table 4). Test statistics (*t*) and *p*-values are given, and all significant effects ($p < 0.05$) are presented in bold. Variance estimates ($\sigma^2$) for random effects and residual variances are given for the models in A and B. For categorical predictors, reference levels are presented in parentheses after the model parameter name. If the interactions of Sex (Male) with the environmental variables in the large model presented in B were significant, the slopes for females and males were tested with *emtrends* to investigate if the slopes for each sex were significantly different from 0 and are presented in C.

| **A) Summaries of the Models Investigating Individual Effects of the Environmental Variables** | | | | | |
|---|---|---|---|---|---|
| **Environmental Variable** | **Model Parameter** | **Model Output** | | | |
| Elevation | *Fixed Effects* | *β* | *SE* | *t* | *p* |
| | **Intercept (Female)** | **0.26** | **0.03** | **8.68** | **< 0.01** |
| | **Sex (Male)** | **-1.12** | **0.02** | **-7.11** | **< 0.01** |
| | Elevation | 0.00 | 0.02 | 0.06 | 0.95 |
| | Sex (Male) : Elevation | -0.02 | 0.01 | -1.70 | 0.09 |

| | | | | | |
|---|---|---|---|---|---|
| | *Random Effects* | $\sigma^2$ | | | |
| | Intercept (Population) | 0.01 | | | |
| | Residual | 0.04 | | | |
| Average Temperature | *Fixed Effects* | $\beta$ | *SE* | *t* | *p* |
| | **Intercept (Female)** | **0.26** | **0.03** | **9.13** | **< 0.01** |
| | **Sex (Male)** | **-0.12** | **0.02** | **-7.11** | **< 0.01** |
| | Average Temperature | 0.03 | 0.03 | 1.01 | 0.33 |
| | Sex (Male) : Average Temperature | 0.03 | 0.02 | 1.51 | 0.13 |
| | *Random Effects* | $\sigma^2$ | | | |
| | Intercept (Population) | 0.01 | | | |
| | Residual | 0.04 | | | |
| Temperature Change | *Fixed Effects* | $\beta$ | *SE* | *t* | *p* |
| | **Intercept (Female)** | **0.26** | **0.03** | **10.22** | **< 0.01** |
| | **Sex (Male)** | **-0.12** | **0.02** | **-5.44** | **< 0.01** |
| | Temperature Change | -0.30 | 0.17 | -1.73 | 0.10 |
| | Sex (Male) : Temperature Change | 0.14 | 0.15 | 0.93 | 0.36 |
| | *Random Effects* | $\sigma^2$ | | | |
| | Intercept (Population) | 0.01 | | | |
| | Sex (Male) | < 0.01 | | | |
| | Residual | 0.04 | | | |
| NDVI | *Fixed Effects* | $\beta$ | *SE* | *t* | *p* |
| | **Intercept (Female)** | **0.26** | **0.03** | **8.67** | **< 0.01** |
| | **Sex (Male)** | **-0.12** | **0.02** | **-7.11** | **< 0.01** |
| | NDVI | 0.08 | 0.70 | 0.12 | 0.90 |
| | Sex (Male) : NDVI | 0.65 | 0.40 | -1.63 | 0.10 |
| | *Random Effects* | $\sigma^2$ | | | |
| | Intercept (Population) | 0.02 | | | |
| | Residual | 0.04 | | | |
| Latitude | *Fixed Effects* | $\beta$ | *SE* | *t* | *p* |
| | **Intercept (Female)** | **0.26** | **0.03** | **9.99** | **< 0.01** |
| | **Sex (Male)** | **-0.12** | **0.02** | **-5.34** | **< 0.01** |
| | Latitude | -0.03 | 0.02 | -1.43 | 0.17 |
| | Sex (Male) : Latitude | -0.01 | 0.02 | -0.38 | 0.71 |
| | *Random Effects* | $\sigma^2$ | | | |
| | Intercept (Population) | 0.01 | | | |
| | Sex (Male) | < 0.01 | | | |
| | Residual | 0.04 | | | |
| Longitude | *Fixed Effects* | $\beta$ | *SE* | *t* | *p* |

| | | | | |
|---|---|---|---|---|
| **Intercept (Female)** | **0.26** | **0.02** | **11.57** | **< 0.01** |
| **Sex (Male)** | **-0.12** | **0.02** | **-5.23** | **< 0.01** |
| **Longitude** | **-0.03** | **0.01** | **-2.97** | **< 0.01** |
| Sex (Male) : Longitude | -0.00 | 0.01 | -0.29 | 0.77 |
| *Random Effects* | $\sigma^2$ | | | |
| Intercept (Population) | 0.01 | | | |
| Sex (Male) | < 0.01 | | | |
| Residual | 0.04 | | | |

**B) Estimation of Slopes for Males for Model presented in A**

| **Environmental Variable** | **Output** | | | |
|---|---|---|---|---|
| | *β* | *SE* | *t* | *p* |
| Elevation | -0.02 | 0.02 | -0.92 | 0.37 |
| Average Temperature | 0.06 | 0.03 | 1.93 | 0.07 |
| Temperature Change | -0.15 | 0.23 | -0.67 | 0.51 |
| NDVI | -0.57 | 0.69 | -0.82 | 0.42 |
| Latitude | -0.04 | 0.03 | -1.42 | 0.17 |
| **Longitude** | **-0.04** | **0.02** | **-2.49** | **0.02** |

**C) Summary of the Model Investigating the Effects of each Environmental Variable Simultaneously**

| | **Model Output** | | | |
|---|---|---|---|---|
| *Fixed Effects* | *β* | *SE* | *t* | *p* |
| **Intercept (Female)** | **0.26** | **0.03** | **9.80** | **< 0.01** |
| **Sex (Male)** | **-0.12** | **0.02** | **-7.21** | **< 0.01** |
| Elevation | 0.02 | 0.05 | -7.21 | 0.76 |
| Average Temperature | 0.01 | 0.07 | 0.13 | 0.90 |
| Temperature Change | -0.12 | 0.35 | -0.34 | 0.74 |
| NDVI | 0.11 | 1.32 | 0.08 | 0.93 |
| Latitude | -0.01 | 0.05 | -0.22 | 0.83 |
| Longitude | -0.03 | 0.03 | -1.00 | 0.33 |
| **Sex (Male) : Elevation** | **-0.08** | **0.04** | **-2.33** | **0.02** |
| **Sex (Male) : Average Temperature** | **-0.14** | **0.05** | **-2.92** | **< 0.01** |
| **Sex (Male) : Temperature Change** | **0.84** | **0.23** | **3.71** | **< 0.01** |
| **Sex (Male) : NDVI** | **-2.21** | **0.85** | **-2.60** | **< 0.01** |
| **Sex (Male) : Latitude** | **-0.08** | **0.03** | **-2.41** | **0.02** |
| **Sex (Male) : Longitude** | **-0.04** | **0.02** | **-2.31** | **0.02** |
| *Random Effects* | $\sigma^2$ | | | |
| Intercept (Population) | 0.01 | | | |
| Residual | 0.04 | | | |

**D) Estimation of Slopes for Males for Model presented in C**

| **Environmental Variable** | **Output** | | | |
|---|---|---|---|---|
| | *β* | *SE* | *t* | *p* |
| Elevation | -0.07 | 0.05 | -1.19 | 0.25 |
| Average Temperature | -0.13 | 0.07 | -1.75 | 0.10 |
| Temperature Change | 0.72 | 0.35 | 2.04 | 0.06 |
| NDVI | -2.10 | 1.32 | -1.59 | 0.13 |
| Latitude | -0.09 | 0.05 | -1.77 | 0.10 |
| **Longitude** | **-0.07** | **0.03** | **-2.48** | **0.02** |

## References

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2. Lenth RV. Emmeans: Estimated Marginal Means, aka Least-Squares Means, 2024. R Package Version 1.10. 5.