Sex-specific divergence in microhabitat use and activity patterns in fire salamanders: temperature matters

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Abstract

Human-driven climate change and habitat degradation cause alterations in the spatial and temporal availability of natural resources, forcing wildlife to adjust their interactions with the environment in order to survive. To identify changes and promote effective conservation measures within the limited suitable habitats available, it is crucial to better understand habitat use. Activity patterns influence the exposure to both risks and opportunities, whereas microhabitat selection reflects fine-scale decisions that can buffer individuals against environmental stressors and facilitate access to essential resources. Amphibians are especially vulnerable to climate change and habitat degradation, making them suitable models for studying the impact of these stressors on different aspects of behavior. To investigate activity patterns and microhabitat selection under nearly undisturbed conditions, we conducted standardized transect walks in a minimally

disturbed habitat of the fire salamander (*Salamandra salamandra*), an emblematic amphibian of the Vienna Woods, between mid-November 2023 and mid-November 2025. Based on data from 316 unique adult individuals, our results reveal sex differences in activity depending on the temperature of the surrounding microhabitat, with females preferring higher temperatures than males. The observed sex ratio differed significantly between seasons, but males were more active than females throughout all seasons. Individuals were disproportionately associated with dead wood and trees compared to their availability, suggesting a preference for these microhabitat structures. In line with the results for the microhabitat use, the spatial activity was unevenly distributed throughout the whole study site, with higher densities where dead wood accumulated and lower densities around open areas. Insights into habitat use can help predict a species' ability to cope with novel and rapidly changing conditions and facilitate the development of effective conservation strategies.

Keywords: Caudata, thermal preference, dead wood, seasonality

Introduction

Temperate forests have been exposed to anthropogenic pressures for about 7000 years (Gilliam, 2016). Anthropogenic land use change is the biggest driver of biodiversity loss (Jaureguiberry et al., 2022), with habitat degradation and fragmentation causing alterations in the availability of habitat structures, such as decreases in dead wood (Debeljak, 2006). These habitat structures can affect the local climate, creating a mosaic of microclimates within a forest (Milling et al., 2018). Forest edges, often created by habitat fragmentation, are more exposed to weather extremes such as droughts (Sun et al., 2025), which are expected to intensify due to climate change (IPCC, 2023). In the light of ongoing changes in climate and land use, further intensification of climatic stressors can be expected. For wildlife, an immediate mitigation strategy to climatic stressors could be shifts in behavior, such as alterations in microhabitat selection (Beever et al., 2017). To identify changes in behavior, a better understanding of the interaction between organisms and their (micro)habitat is essential (Endler, 2025). Activity patterns and microhabitat selection influence the exposure to both risks and opportunities (Railsback et al., 2005), and reflect fine-scale decisions that can buffer individuals against environmental stressors or facilitate the access to essential resources (Scheffers et al., 2014).

Due to their dependence on the surrounding temperature to regulate their own body temperature and metabolism (Kern et al., 2015), the activity level of ectotherms, such as amphibians, is affected by their habitat's microclimate. This susceptibility makes amphibians a suitable model for studying organism-microhabitat interactions and how these may inform policies focused on the preservation and improvement of habitats in a

time of constant change. Fire salamanders, for example, have a highly permeable skin, through which they exchange water and gases with the environment, making them strongly dependent on humidity (Wells, 2007), and particularly vulnerable to both climate change and habitat degradation (IUCN SSC, 2024; Luedtke et al., 2023; Womack et al., 2022). As a result, they endure unfavorable conditions by seeking shelter in more suitable microhabitats (e.g., frost-proof shelters during hibernation; Thiesmeier & Günther, 1996; Leeb, 2013). Fire salamanders are commonly assumed to be found in association with dead wood, which is also known to provide valuable habitats to birds, bats, insects, and lichens, among others (Winter & Möller, 2008). However, quantitative evidence of the importance of dead wood for the occurrence and/or abundance of fire salamanders is, to the best of our knowledge, still lacking.

Here, we use a standardized sampling approach to study activity patterns and microhabitat preferences in wild fire salamanders over a two-year period in a minimally disturbed site. Because previous research generally suggests higher activity in males (Schulte et al., 2007; Leeb, 2013; Kiss et al., 2021; Zhao et al., 2023), we hypothesize that (1) males are active across a broader range of temperatures than females. Further, we expect (2) the observed sex ratio to differ between seasons and (3) to find activity hotspots unevenly distributed across the sampling transects. As commonly assumed (e.g., Thiesmeier & Günther, 1996), we also expect our study to (4) provide quantitative evidence that fire salamanders are more likely to be found close to dead wood accumulations than to any other structure available in their habitat.

Methods

To investigate activity patterns and microhabitat selection under undisturbed conditions, we performed standardized transect walks in a minimally disturbed fire salamander habitat in Vienna between mid-November 2023 and mid-November 2025. While our data could not be recorded blindly, randomized sampling along the transects and standardized measurements were implemented.

Study species

The fire salamander (*Salamandra salamandra*) is widely distributed across its geographic range, which spans from Portugal to Bulgaria (IUCN SSC, 2023). It inhabits moist mixed deciduous forests, often dominated by Eurasian beech (*Fagus sylvatica*) (Alcobendas et al., 1996; Manenti et al., 2009), and abundant mosses and leaf litter, which are considered essential habitat structures because they are used for foraging invertebrates (Bogaerts et al., 2021). Generally, mating can take place throughout the year under suitable conditions (Thiesmeier, 2004). However, for populations around Vienna, we have observed two peaks, in spring and autumn, with the main mating season taking place from September to October. This is followed by a period of overwintering, where shelters, such as micromammal caves, hideouts in deadwood, and under stones, are necessary due to a decrease in temperatures and humidity below activity levels. Fire salamanders are mainly ovoviviparous/larviparous (Buckley et al., 2007), with fertilized eggs developing in the uterus of the females. In spring, usually between March and May, gravid females deposit their larvae mainly in clean, fish-free streams or shallow ponds (Steinfartz et al., 2007). While fire salamanders are frequently considered nocturnal or crepuscular

(Klewen, 1985; Thiesmeier & Günther, 1996; Schlüpmann, 2008a), diurnal surface activity can be locally observed (Leeb, 2013; Szydlik, 2025; Velo-Antón & Cordero-Rivera, 2017). Subterranean habitats are considered essential during multiple phases of the life cycle (Balogová et al., 2017), but they go occasionally over ground for mating or foraging. In general, their activity is influenced by different weather conditions such as air temperature, precipitation, and air humidity (Bogaerts et al., 2021). The highest surface activities are observed during spring or autumn nights (Thiesmeier, 2004) with temperatures between 7 and 11°C and relative air humidity above 85% (Schlüpmann, 2008b). Throughout this study, we refer to “activity” as that occurring above the ground.

Study site

This study was conducted at the “Wilhelminenberg”, a region in the north-western part of Vienna, Austria (WGS84, 48.221°N, 16.280°E, 361 m.a.s.l). The study site is located in the central-eastern part of the geographic range of the fire salamander (*Salamandra salamandra salamandra*) in Europe, close to the distribution gap starting at the Viennese basin. The study site itself is located close to an urban settlement. However, due to restricted access to the site (the University of Veterinary Medicine Vienna is granted the use of the property by the city of Vienna), minimal anthropogenic disturbance influences the fire salamanders within the area. The forest is dominated by beech trees (*Fagus sylvatica*) in different age classes with almost no undergrowth. The whole area provides a high amount of dead wood in different stages of decomposition, which can serve as microrefugia during unfavorable weather conditions (Pabijan et al., 2023). Due to the

absence of conventional forestry practices at the study site, such as logging and the removal of dead trees, for at least 30 years, a constant accumulation of dead wood has taken place. A breeding stream (Anderbach) crosses the area in the northern part of the study site (Figure 1).

Data collection along transects

Field sampling was conducted between mid-November 2023 and mid-November 2025. Four representative plots were established at a distance of about 180 m from each other, evenly distributed throughout the study site (Figure 1). For orientation, the transects were marked with barrier tape around trees. The data collection was conducted by monitoring along the adjacent pair of line transects within each plot. Two line transects, belonging to an adjacent pair, were 10 meters apart from each other and 5 meters away from the border of the plot. Only individuals within the plots were assessed for the study. The length of the plots ranged between 160 m and 180 m, while the width was always 20 m, resulting in an area of approximately 3.600 m^2 covered during each sampling occasion. The sampling order of the plots and transects, as well as the direction of sampling (uphill vs. downhill), was chosen randomly on each sampling occasion.

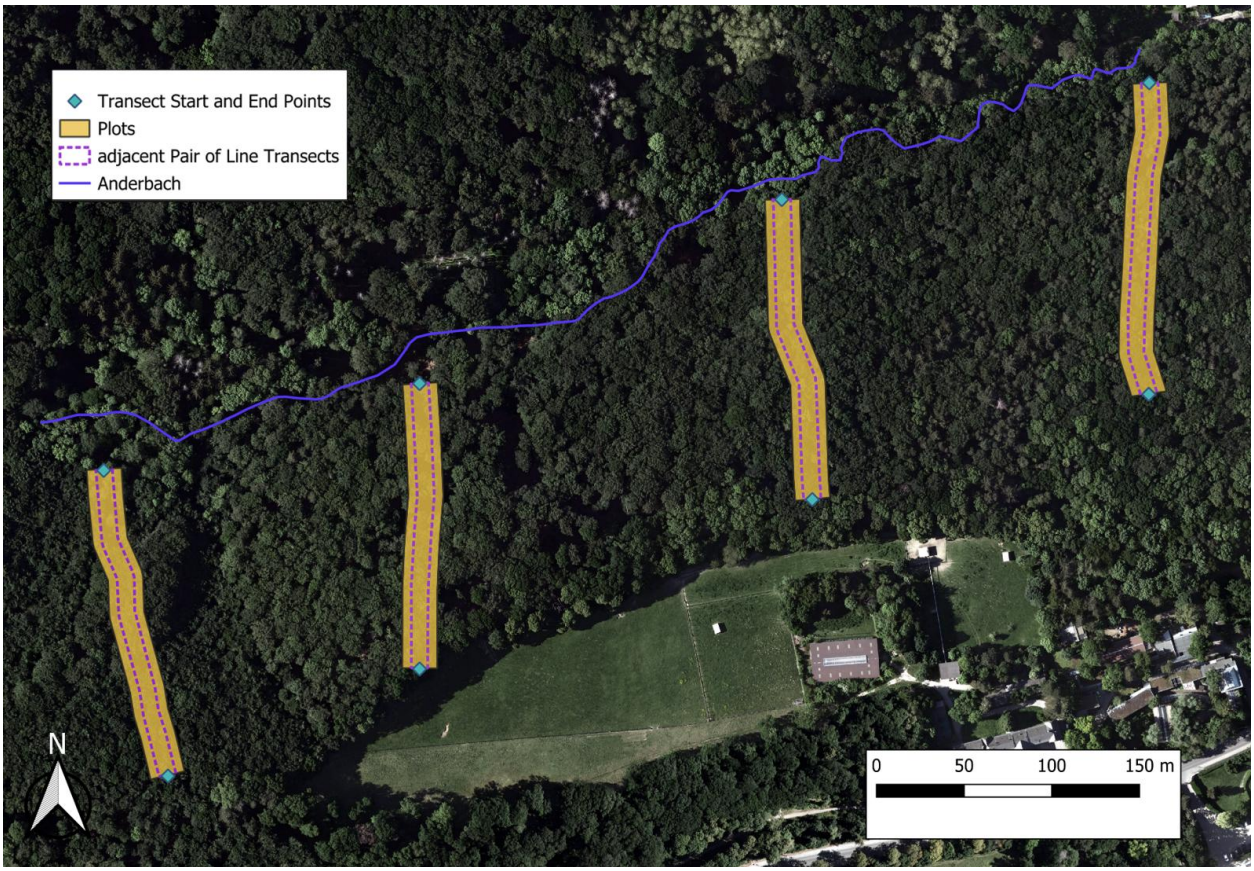


Figure 1. Location of the plots within the study area. Background image: Geoland Basemap Orthofoto (URL: https://www.basemap.at/wmts/1.0.0/WMTSCapabilities.xml)

Data collection took place at varying times of the day and night during suitable weather conditions, i.e., air temperatures between 3 and 21 °C as well as air humidity above 70 % at least during part of the sampling session (Bogaerts et al., 2021). Weather data were obtained from the ZAMG (Geosphere Austria) using the weather station "Jubiläumswarte", which is located about 1 km west of the study area. Only individuals above the ground were recorded, i.e., checking potential shelters such as micromammal caves, underneath leaf litter, under logs and stones, was entirely avoided to minimize disturbance. The location of each individual was recorded with an Etrex 30 GPS tracker

(accuracy 2 - 4 m). When a fire salamander was handled, a strict hygiene protocol was followed. Individuals were only touched wearing new nitrile gloves, and the handling was kept to a minimum (less than two minutes). Each individual was photographed on graph paper to allow measuring its snout-vent-length (SVL) using the software ImageJ v. 1.54 (Abramoff et al., 2004) and weighed to the nearest 0.1 g using a digital pocket scale (AMIR Digital Scale). The sex of all individuals was determined by the shape of the cloaca, which is more swollen in males than in females (Feldmann & Klewen, 1981). Because reliable sexing is only possible for individuals with a total body length of at least 14 cm (Bogaerts et al., 2021), individuals with a shorter total body length were classified as “juveniles” and were not considered during the study. If uncertain, the sex of the individual was classified as “unknown” (N.A.). All individuals were released at the location where they were captured. Afterwards, the microhabitat temperature was measured at the exact spot where the individual was found using an Infrared thermometer (BOSCH UniversalTemp). The *microhabitat availability* within a 2 m radius of each individual's capture location was characterized by estimating the percentage of available microhabitat types (leaf litter, vegetation, bare soil, dead wood). All kinds of dead leaves and needles on the ground were classified as “leaf litter”, while “vegetation” included different parts of living trees (e.g., trunk, roots), saplings, ferns, grasses and wild garlic, which were close to the ground. Both open soil and stones were aggregated as microhabitat type “bare soil”. The category “dead wood” included standing as well as lying dead wood logs, branches and twigs. This estimation was always done by the same person (KRS). In addition, the exact *(used) microhabitat* on which the individual was first spotted was documented and aggregated in microhabitat types (in brackets). The following

183 microhabitat categories were defined: “on leaf litter” (leaf litter), “on dead wood” (dead
184 wood), “under dead wood” (dead wood), “at dead wood” (dead wood), “on tree (trunk)”
185 (vegetation), “on (tree) root” (vegetation), “at (tree) root” (vegetation), “on bare soil” (soil),
186 “on snow patch” (other).

187 Based on current knowledge (Thiesmeier, 2004) and our own observations on the local
188 populations, we divided the year into five different seasons: hibernation (December 1st to
189 February 14th), larval deposition (February 15th to April 14th), mating season 1 (April
190 15th to June 14th), aestivation (June 15th to August 31st) and mating season 2
191 (September 1st to November 30th).

192

193 *Statistical analysis*

194 All statistical analyses were performed using R (version 4.4.3, R Core Team, 2024) in the
195 RStudio interface (Posit, 2024). Only adults with known sex were included in the analysis.
196 We randomly chose one capture occasion of recaptured individuals to avoid the influence
197 of individuals with multiple captures on our results. To make the code easier to utilize, the
198 “remotes” package (version 2.5.0, Csárdi et al., 2024) was used to install the correct
199 versions of every package, and the relative working directory was set using the “here”
200 package (version 1.0.2, Müller, 2025).
201 We used a Pearson’s Chi-squared test to investigate possible differences in sex ratios
202 between seasons. To test for differences in microhabitat temperatures between the sexes
203 and months, the package “rcompanion” (version 2.5.2, Mangiafico, 2026) was used to
204 first investigate whether the temperature data were normally distributed. Then, we

conducted a two-way ANOVA with type II sum of squares on the linear regression model using the "car" package (version 3.1.3, Fox & Weisberg, 2019) to assess potential differences in the temperature of the *used microhabita*t between sexes and months. Marginal means were calculated with the "emmeans" package (version 2.0.0, Lenth & Piaskowski, 2025). Effect sizes of month and sex on the microhabitat temperature were calculated as partial eta squared using the package "effectsize" (version 1.0.1, Ben-Shachar et al., 2020). For analyzing the microhabitat use, we first performed an MANOVA on the linear model of additive log-ratios to test for potential sex differences between the *available microhabitats* around each individual found. To assess whether individuals were uniformly distributed over different *used microhabitats,* we performed a Pearson's Chi-squared goodness-of-fit test with Monte Carlo simulation and 10,000 replicates, and the standardized residuals were used to investigate the frequency of *used microhabitats*. We then calculated the ratio of *used microhabitats* in relation to their *availability* to assess a possible preference or avoidance for a particular microhabitat. A Pearson's Chi-squared test was performed to test for differences in *used microhabitats* between the sexes. Data curation and manipulation, date adjustment, as well as plotting were performed using the "tidyverse" package (version 2.0.0, Wickham et al., 2019). The heat map was created with QGIS (version 3.42, Münster, QGIS Development Team, 2025). The model validation was done with the "DHARMa" package (version 0.4.7, Hartig, 2024). All the raw data and R-Scripts used for the analysis shown are available on the open-access Zenodo repository (https://doi.org/10.5281/zenodo.22092878).

Results

Across the two-year period, 645 capture events were documented along the transects. Out of these, 430 unique individuals were identified. After removing all juveniles and individuals with unknown sex, 319 data points remained for the analysis. The overall sex ratio was 1:1.9 (110 females, 209 males).

Activity of both sexes throughout the seasons

In line with our hypothesis, we found differences in activity between the sexes across seasons (Pearson's Chi-squared test, $n = 319$, $X^2 = 28.185$, $df = 4$, $p < 0.001$; Fig. 2), such that more males than females were consistently observed. The examination of standardized residuals (mating season 1: F = 4.99, M = -4.99; mating season 2: F = -4.71, M = 4.71) revealed that during mating season 1 (May 15th to June 14th) we observed more females than expected, while in mating season 2 (September 1st to November 11th) the opposite was true.

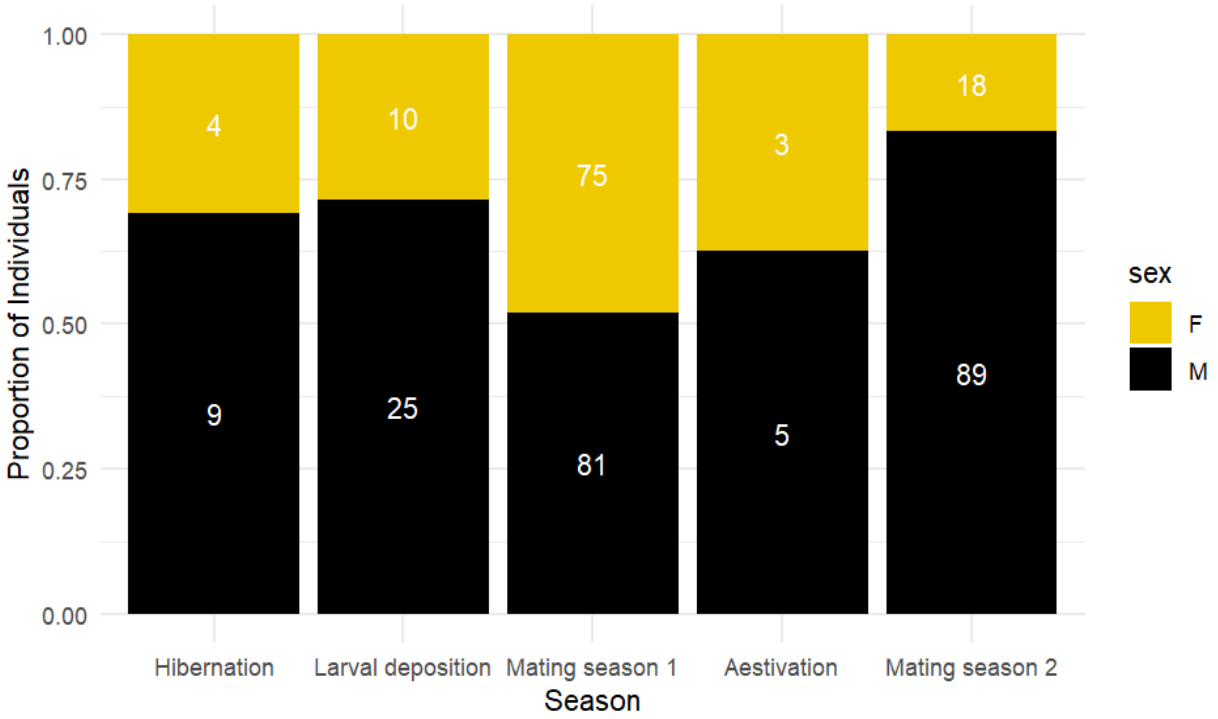


Figure 2. Proportion of female (yellow bars on top) and male (black bars below) individuals during the defined seasons. These self-defined seasons are equivalent to different phases of the adult fire salamander life cycle.

Microhabitat temperature

We found significant sex-dependent differences in the temperature of the microhabitat where individuals were encountered (two-way ANOVA with type II sum of squares, $F(1, 298) = 6.655$, $p < 0.01037$), with females being found in microhabitats with higher temperatures. While the microhabitat temperatures differed across months ($F(11, 298) = 31.402$, p-value < 0.001), our results suggest the general sex dependent pattern to be consistent throughout the year (Supplementary Figures S2 and S3). For males, the peak of density occurred at 7.4 °C, while for females, density peaked at 11.2 °C (Figure 3). The effect size of sex was small (η^2 partial = 0.02), while the effect size of the month of the

year was large (η^2 partial = 0.54). The observed sex dependent differences in activity patterns according to the microhabitat temperature are in line with our hypothesis.

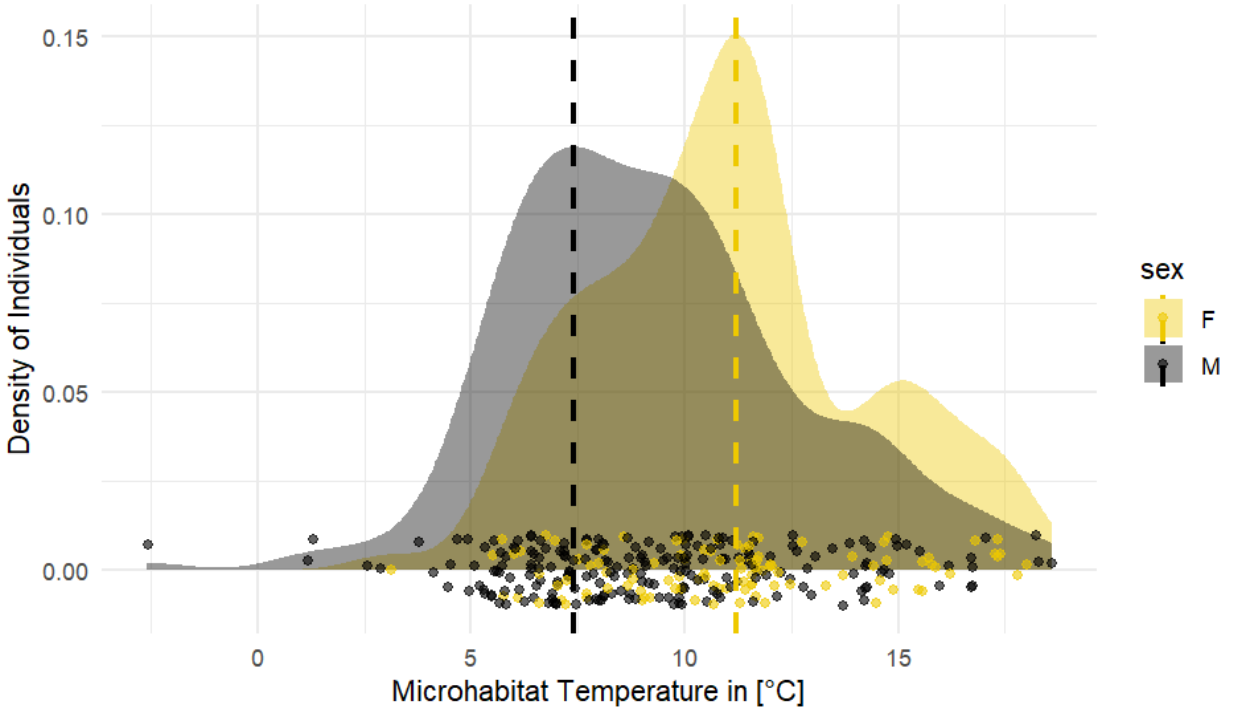
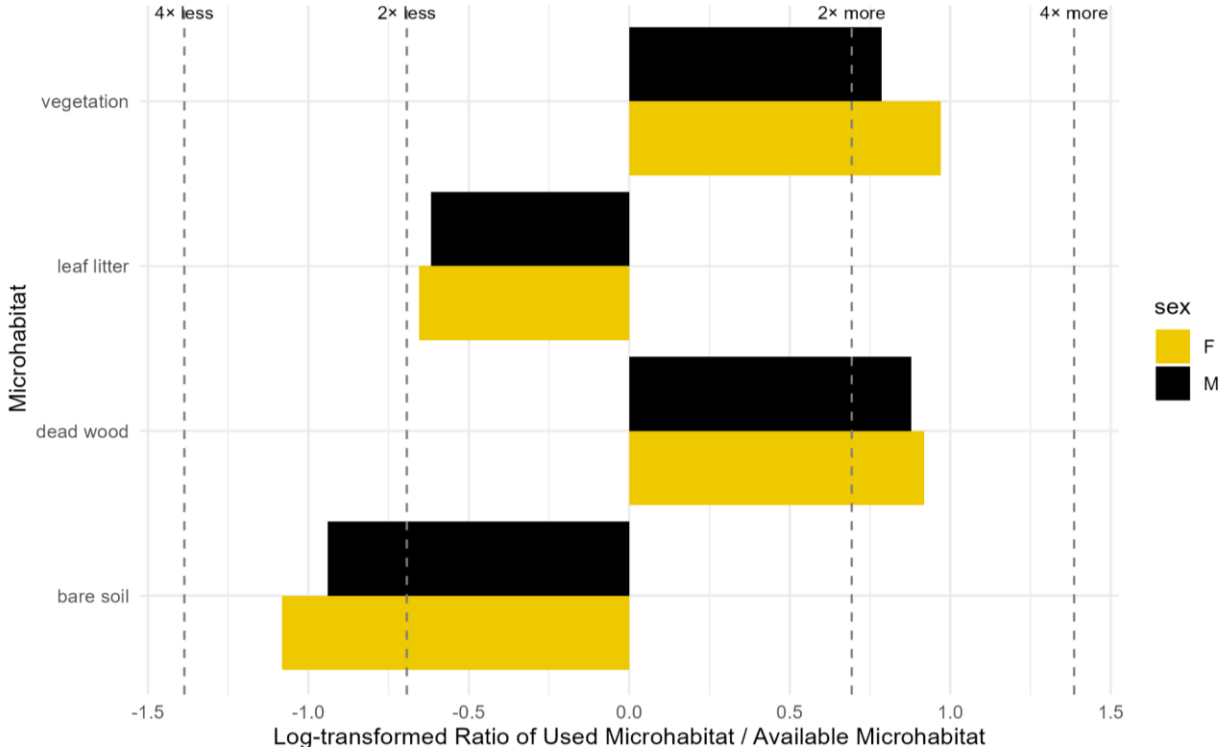


Figure 3. Kernel density plot for the observed activity of individuals at different microhabitat temperatures for female (yellow) and male (black) individuals. The dots along the x-axis represent raw data points and are jittered for better visibility (n = 311, as microhabitat temperature was not documented for eight of the individuals included in the study). The dashed vertical lines depict mean temperature values for females (yellow) and males (black).

Microhabitat selection

First, we performed a MANOVA on the linear model of additive log-ratios to test for potential sex differences between the *available microhabitats*. We did not find any support for sex differences in *microhabitat availability* where individuals were found (MANOVA type II, n = 319, Pillai's trace = 0.015, F(3, 315) = 1.56, p = 0.1984). We found significant

differences in the distribution of individuals across *used microhabitats* (Pearson's Chi-squared goodness-of-fit test with Monte Carlo simulation and 10,000 replicates, n = 319, X^2 = 180.11, df = NA, p < 0.001). The standardized residuals of the counts showed that individuals were found close to dead wood (r = 8.7) and leaf litter (r = 5.2) disproportionately more often than expected by chance. In contrast, fire salamanders were least likely to be encountered on snow (r = -8.8) and bare soil (r = -6.6). The individuals observed on vegetation were within the range expected by chance (r = 1.4). However, when calculating the ratio of how often individuals occurred within each microhabitat class relative to its availability, we found that females were found near dead wood 2.51 times, while males were found 2.41 times more often than could be expected by chance. Near vegetation (living trees), females were observed 2.64 times and males 2.20 times more often than by chance. For bare soil, the ratio was 0.34 for females and 0.39 for males, while for leaf litter it was 0.51 for females and 0.54 for males (Figure 4). The ratio for the microhabitat class "snow" could not be calculated because the amount of snow was not usually assessed (n fire salamanders on snow = 1). Therefore, individuals appear to prefer dead wood and living trees, while avoiding leaf litter and bare soil. It is important to note that, while the *available microhabitat* type "vegetation" included multiple classes (living trees, saplings, ferns, grasses, wild garlic, etc.), fire salamanders were always observed associated with living trees when they were aggregated in the *used microhabitat* type "vegetation". Additionally, no differences in the *used microhabitat* were found between the sexes (Pearson's Chi-squared test with simulated p-value and 10,000 replicates, n = 319, X^2 = 1.59, df = NA, p = 0.8687).



291

292 **Figure 4.** Log-transformed ratio of how often individuals were found associated with different microhabitat

293 categories compared to how much of it was available.

294

295 *Spatial distribution*

296 We observed spatial heterogeneity in activity across the transects (Figure 5). In line with

297 our findings regarding the microhabitat selection, higher densities were observed in areas

298 with larger amounts of dead wood accumulations (Figure 5, panel C, D, F, H, I). A low

299 density was observed at B, marking an open area, as well as in E and G, where more

300 herbaceous vegetation could be found, especially during spring and early summer every

301 year.

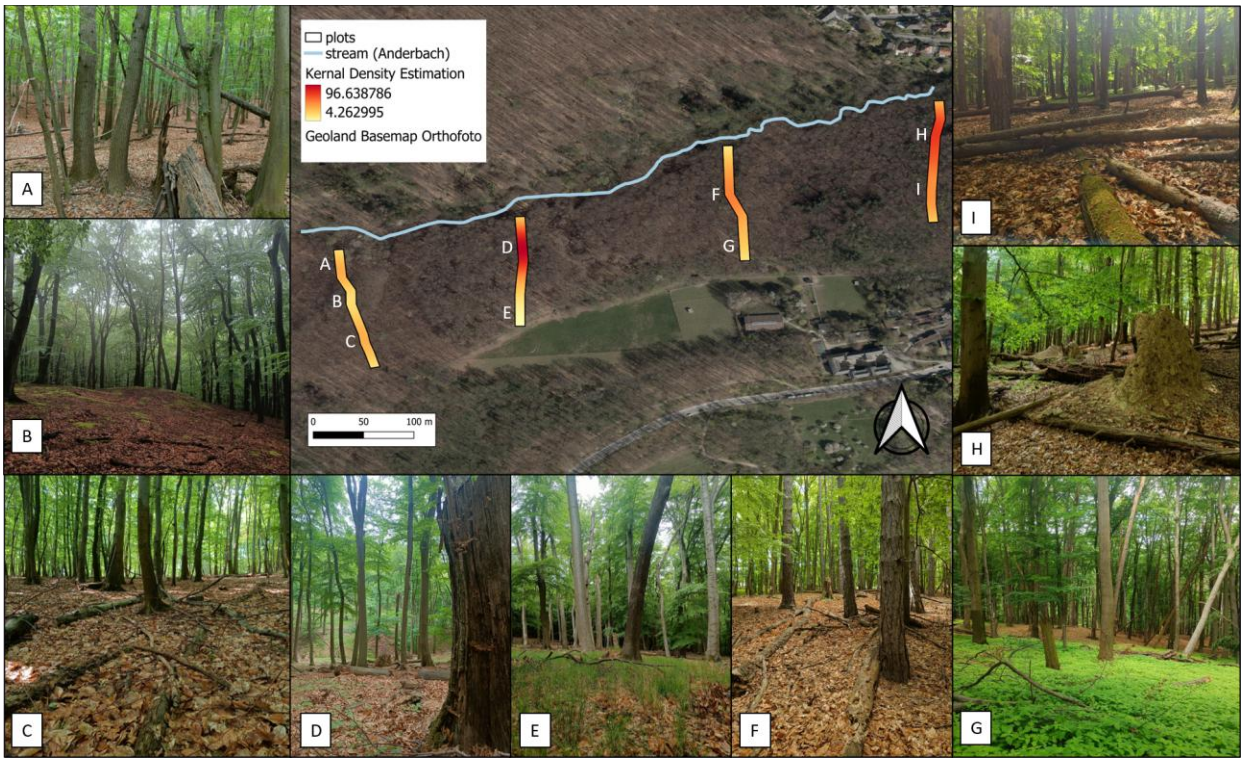


Figure 5. Heatmap visualizing the distribution of all individuals along the transects, including recaptures, juveniles, and those of unknown sex.

Discussion

It has recently been advocated that more research is needed on habitat and microhabitat choice, as not only can they influence multiple aspects of an animal's biology but also have crucial implications for conservation (Endler, 2025). Here, we contribute towards bridging this gap by focusing on an emblematic and widespread European amphibian whose populations are currently experiencing visible declines due, in part, to the destruction and fragmentation of their habitat (IUCN SSC, 2023). Our study demonstrates that fire salamanders prefer habitat structures such as dead wood over open areas. As a consequence, the spatial activity was unevenly distributed throughout the sampling area, with higher densities of individuals where dead wood accumulated and lower densities in

316 open areas. Further, we provide evidence of temperature-dependent differences in
317 activity between the sexes and microhabitat preference. Finally, we found differences in
318 sex ratios between seasons, with more females being observed during mating season 1
319 compared to mating season 2.

320 Regardless of the season, a higher percentage of male fire salamanders was
321 continuously observed. The overall male-biased sex ratio of this study is in line with
322 previous studies (e.g., Golub et al., 2023; Kiss et al., 2021), although female-biased sex
323 ratios (e.g. König, 2021) and even sex ratios (e.g., Rebelo & Leclair, 2003; Najbar et al.,
324 2019; Burgstaller et al., 2021) have also been reported. Differences in sex ratios between
325 seasons, as observed in our study, were also reported by Klewen (1985) and can occur
326 due to differences in reproductive behavior (Ancona et al., 2017). In different amphibian
327 species, larger individuals and those with a higher body condition have been found to
328 reproduce earlier than smaller individuals during the breeding period (Burgstaller et al.,
329 2025; Dittrich et al., 2018). Therefore, the significantly higher percentage of females
330 during mating season 1 compared to mating season 2 could have been explained by a
331 correlation with body size or body condition. However, we found no differences in body
332 weight between females active in mating season 1 and those in mating season 2
333 (Supplementary Figure S1). Crucially, we are unable to rule out the possibility that
334 females included in mating season 1 had also deposited their larvae already. This
335 mismatch could be caused by shifts in weather between the years. Although data
336 collection only took place during similar weather conditions suitable for fire salamanders
337 (air temperatures between 3 and 21 °C and air humidity above 70 % at least during part
338 of the sampling session) based on the information provided by the nearest weather

station, other weather parameters, such as wind and soil moisture, could also influence the activity observed. By directly measuring the microhabitat temperature whenever individuals were found, our study provides thermal information at finer temporal and spatial scales. This differs substantially from previous studies, where environmental temperatures from weather stations have been used to characterize activity patterns in this (e.g., Leeb, 2013) and other ectotherms (e.g., George et al., 2015; Brown & Shine, 2002).

Female activity peaked at higher temperatures and exhibited an overall higher thermal range compared to male activity. Interestingly, the microhabitat temperatures when activity peaked for males and females are around 7 and 11 °C, respectively, which are the minimum and maximum temperatures at which the highest activity levels in fire salamanders have been previously reported (Schlüpmann 2008b). The observed pattern of highest activity in females at higher microhabitat temperatures than in males was independent of the time of the year. While studying the entrance of a hibernation shelter throughout the winter, Leeb (2013) observed that, on average, female fire salamanders entered the shelter earlier, before temperatures decreased further, and spent more time inside the frost-proof refuge than males. Our results are in line with these findings. Female preference for warmer temperatures has also been shown in mice (Kaikaew et al., 2017) and lizards (Ortega et al., 2016), where this has been interpreted as compensation for a higher energy demand due to an increased heat dissipation rate (Kaikaew et al., 2017), differences in hormone-induced temperature sensitivity generated before maturity (Kaikaew et al., 2017), and a higher physiological performance related to reproduction (Ortega et al., 2016). Gravid female skinks increase their basking behavior in comparison

362 to males and non-gravid females (Schwarzkopf & Shine, 1991), which can lead to an
363 accelerated gestation (Schwarzkopf & Shine, 1991). Across a wide range of taxa,
364 including amphibians, the embryonic developmental time decreases with increasing
365 temperatures within an optimal temperature range for incubation (Gillooly & Dodson,
366 2000). Accordingly, our results suggest that female fire salamanders may choose
367 microhabitats with warmer temperatures to accelerate the embryonic development.
368 However, controlled experiments with knowledge of the reproductive state of the females
369 would be needed to confirm this hypothesis.
370 Insights into microhabitat preferences in fire salamanders are critical for making accurate
371 predictions about how climate change and habitat degradation may further affect this
372 species in general, and each sex separately. When considering the continuous spread of
373 *Batrachochytrium salamandrivorans* (*Bsal*), a chytrid fungus heavily affecting fire
374 salamander populations in Europe (Spitzen-van der Sluijs et al., 2013; Spitzen-van der
375 Sluijs et al., 2016), a better understanding of microhabitat use could help mitigate this
376 threat. As already reported for different amphibians, when infected by a pathogen,
377 individuals can induce a “behavioral fever” by exposing themselves to warmer
378 microhabitats (Giacometti et al., 2026), resulting in an increase in immune performance
379 as well as the inhibition of pathogen replication (Kluger, 2015). Specifically, frogs have
380 been shown to be able to recover from infection of a chytrid fungus by inducing behavioral
381 fever through selecting high-temperature microhabitats (Waddle et al., 2024). A
382 heterogeneous habitat could provide different thermal environments and therefore enable
383 the selection of microhabitats depending on the organism’s specific needs.

Fire salamanders show a preference for certain microhabitat structures (dead wood and trees). These might offer more attractive microhabitats than open areas (bare soil and leaf litter), which appear to be avoided. Consequently, fire salamanders were not evenly distributed over the study area, but rather most abundant around areas with accumulations of dead wood. These findings indicate that habitat structures might provide better conditions for fire salamanders, as they could, for example, ensure lower temperatures during heatwaves (Scheffers et al., 2014). Further, they might provide hiding spots to reduce predation risk (Lombardi & Mali, 2016), more abundant food resources (Lombardi & Mali, 2016), and safe movement pathways (Zollner & Crane, 2003).

Conclusion and future perspectives

Our study provides valuable insights into the habitat selection of fire salamanders, which can improve the formulation or strengthening of effective conservation strategies. Based on our findings, fire salamander conservation initiatives must pay attention to the availability of dead wood and trees, as these provide preferred microhabitat temperatures and a heterogeneous environment that may yield several additional benefits. Therefore, these requirements should be considered when planning or executing local forest management programs, as they are likely to impact other species such as insects and arachnids, which fire salamanders feed on as well. Future studies assessing microhabitat preferences should provide a more detailed classification of microhabitat categories to disentangle, for example, the influence of different types of vegetation. In addition, microhabitat assessments along transects should also be carried out systematically for

random control locations where no individuals are observed (e.g., Mayer et al., 2025) to have a better understanding of microhabitat preference at a broader scale. Extending such studies to multiple sites with differing habitat characteristics would allow their comparison and advance our understanding of what actually drives microhabitat choice at the population and species levels.

Ethical statement

The handling of the animals was in line with the Austrian welfare Act (Tierschutzgesetz) and the permit for handling the fire salamanders was granted by the Magistrat der Stadt Wien, Umweltschutzabteilung (MA 22) to Bibiana Rojas (permit number: MA22-291870/2023) and approved by the Ethics and Animal Welfare Committee of the University of Veterinary Medicine Vienna (ETK-025/02/2026) in accordance with the University's guidelines for Good Scientific Practice.

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Supplementary material

Supplementary materials are published with the article.

Author contribution

Kristin Szydlik: conceptualization (equal), methodology (equal), investigation (lead), data curation (lead), formal analysis (lead), visualization (lead), writing – original draft (lead), writing – review and editing (equal). **Carolin Dittrich:** methodology (supporting), formal analysis (supporting), writing – review and editing (equal), supervision (supporting). **Bibiana Rojas:** conceptualization (equal), methodology (equal), writing – original draft (supporting), writing – review and editing (equal), resources (lead), supervision (lead).

Data and Code availability

The data and the R-script was uploaded to the open access Zenodo repository to make the analysis open and reproducible. It can be found under https://doi.org/10.5281/zenodo.22092878

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Declaration of conflict of interest

None declared.

Declaration of generative AI use

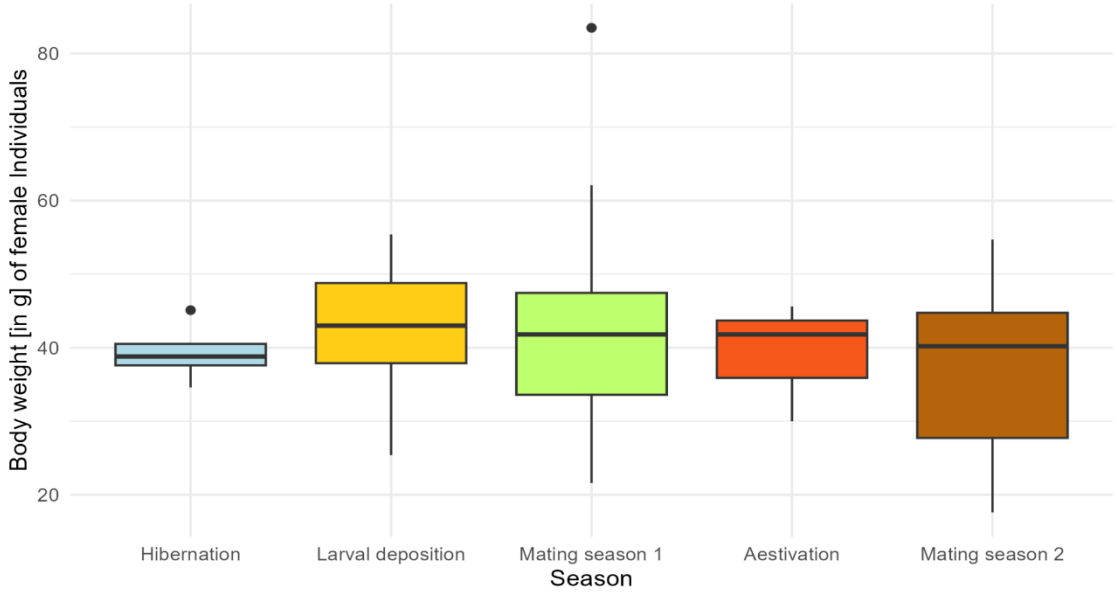
AI was used to clarify error messages obtained in R.

Appendix A: Supplementary Materials

Female's body weight between seasons

To test whether differences in female body weight occur between the seasons, we ran a Kruskal-Wallis test on the non-normally distributed data on body weight (n = 110). The result suggests no differences between the seasons.

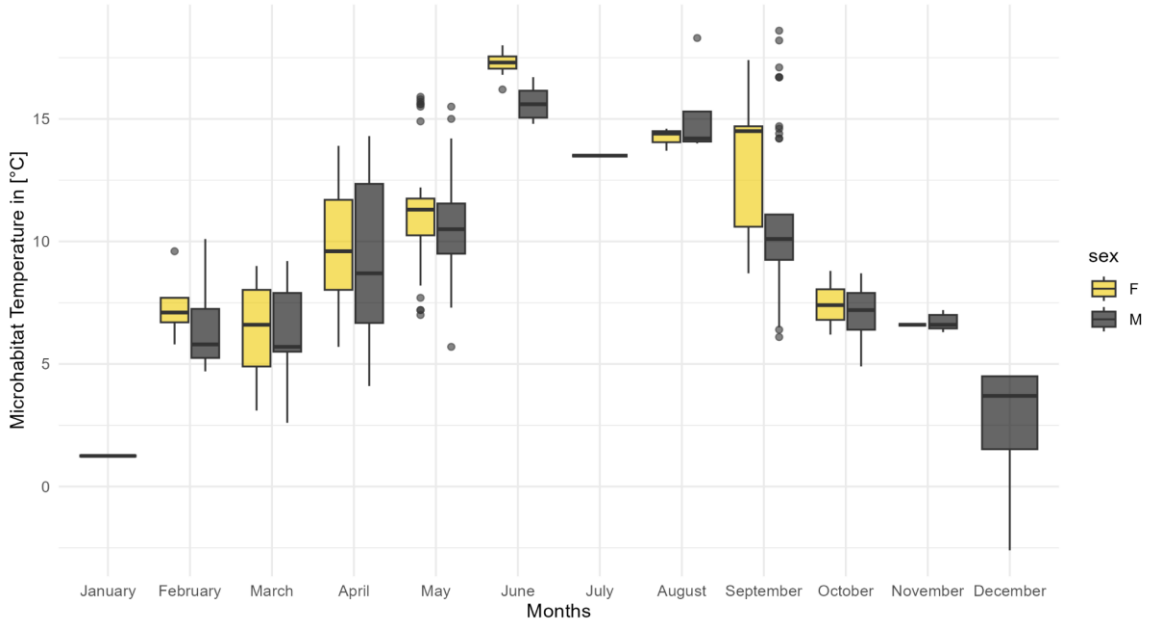
```
        Kruskal-Wallis rank sum test
data: body_mass by season
Kruskal-Wallis chi-squared = 2.5932, df = 4, p-value = 0.628
```



Supplementary Figure S1. Boxplots visualizing female's body weight (n = 110) between seasons.

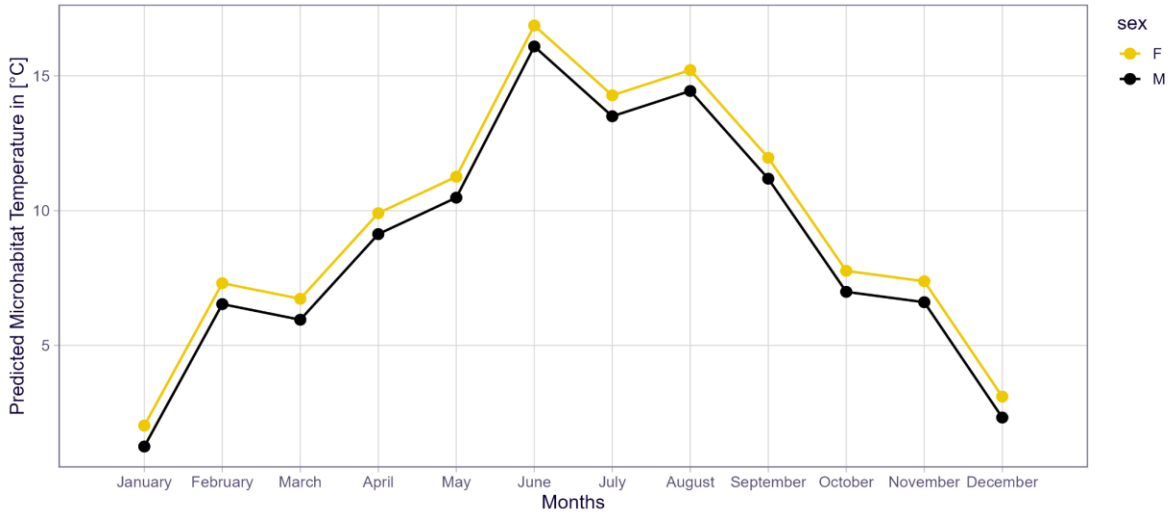
Microhabitat temperature

The microhabitat temperatures for the 311 unique individuals for which microhabitat data were obtained, summarized in boxplots per month and separated by sex. Female fire salamanders were continuously encountered in microhabitats with higher temperatures than males.



Supplementary Figure S2. Boxplots of the microhabitat temperatures in which individuals were found by sex and months.

We built a linear regression model with sex and month as predictors and microhabitat temperature as the response variable, for which we plotted the estimated marginal means to visualize the effect of the predictors. Again, females were found at higher microhabitat temperatures than males throughout the months.



Supplementary Figure S3. Microhabitat temperatures by sex and months predicted by the linear regression model

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