Wild fire salamanders (*Salamandra salamandra*) prefer natural housing conditions *ex-situ*

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Abstract. The European fire salamander (S*alamandra salamandra*) is threatened by infection with the chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*), which has caused massive population declines in several European countries. One attempt to conserve the genetic diversity of fire salamanders is to keep individuals of affected or vulnerable populations in *ex-situ* assurance populations. However, literature knowledge about appropriate husbandry conditions and behaviour in captivity is inconsistent. In this study, we investigated the preference of wild-caught fire salamanders in a choice test with two different husbandry setups: a natural setting and a minimalistic setting. The former provided parts of the environment, with biotic materials, such as moss, soil and tree bark. The latter is mostly used by specialized breeders, more artificial, but also easier to clean, with e.g. soft paper towels as a substrate. Fire salamanders had the possibility to choose their preferred zone to stay over a period of 60 days. We documented the individual hiding places and activity patterns. Initially, especially female fire salamanders showed a preference for the more natural zone and the majority of fire salamanders preferred the moss for hiding. However, as the experiment progressed, there was a notable increase in the amount of time individuals spent in the other zone, indicating the need for a diverse habitat when keeping fire salamanders in captivity. Our results thus provide valuable information on *ex-situ* husbandry of fire salamanders.

Keywords. Husbandry, *Bsal*, natural housing, functional housing, hidden Markov model, Activity, Preference

Introduction

Amphibians are currently facing great extinction risk, with 40.7 % of all species classified as threatened (HOULAHAN et al. 2000; IUCN SSC AMPHIBIAN SPECIALIST GROUP 2023; LUEDTKE et al. 2023). Among others, Chytridiomycosis is the cause of a severe biodiversity loss, including over 200 anuran species (SCHEELE et al. 2019; FISHER & GARNER 2020). However, not only anurans, but also caudates are threatened by Chytridiomycosis. A pathogen related to the 'common' chytrid fungi is *Batrachochytrium salamandrivorans* MARTEL et al., 2013 (hereafter referred to as "*Bsal*"). This fungus has recently emerged in Europe and causes massive extinction events, especially known in the European fire salamander (*Salamandra salamandra* (LINNAEUS, 1758)); hereafter referred to as "fire salamander" (MARTEL et al. 2013; LÖTTERS et al. 2020).

Fire salamanders predominantly occur in deciduous woodland with streams and ponds to deposit their larvae (THIESMEIER 2004; BOGAERTS et al. 2021). They prefer cool temperatures (3–12 °C) and high humidity (> 75 %) (BOGAERTS et al. 2021). These preferences are also shared by the pathogen *Bsal* (MARTEL et al. 2013), which makes the fire salamander an "ideal" host. Once a population is infected, *Bsal* causes a significant reduction of the population size (LÖTTERS et al. 2020). The current (2023) IUCN species status of the fire salamander is Vulnerable, with a decreasing population trend. Several measures have already been assessed to prevent the introduction and spread of *Bsal*, such as informing the public how to prevent the spread of the pathogen, or to keep the pathogen from spreading via host removal, fencing and disinfection (IUCN SSC AMPHIBIAN SPECIALIST GROUP 2023). Yet, there is still an increasing number of *Bsal* outbreaks in in–situ populations. One potential approach to prevent fire salamanders from local extinction is to collect them from vulnerable habitats and to keep them *ex–situ*. Eventually, individuals could then be released in a less endangered region or to their region of origin, once this habitat is considered safe again (THOMAS et al. 2019).

To ensure welfare of animals in *ex-situ* programmes, science-based evaluations of husbandry regimes are needed. Housing amphibians and reptiles is common and often conducted with large numbers of animals among hobbyists, traders, or zoos. Evidence-based husbandry guidelines for these animals are, however, often not available (LINHOFF 2018). Most housing conditions are based on what keepers observed as the original environment of the animal or what "works well" according to breeders' own experience, whereas measures based on scientific evidence are rare (ARBUCKLE 2013). Fire salamanders have a long history of being kept in captivity, both as pets and as laboratory animals for scientific research (SEIDEL & GERHARDT 2016; BOGAERTS et al. 2021). However, housing conditions (e.g., provision and type of substrate, hiding options or water containers) vary widely. One method is a naturalistic approach, which mimics their natural habitat. Alternatively, a minimalistic approach utilises a very simple setup (explained in more detail below). Both the natural as well as the minimalistic approach meet the minimal requirements of fire salamanders, such as the regulation of body temperature and hydration. In the following, we provide an overview of the two different housing conditions.

The more natural approach is attempting to simulate the natural environment of fire salamanders. Thus, the terraria are equipped with organic materials such as soil, dried (beech) leaves, natural hiding places, and a water container to provide sources of hydration. Some facilities, such as those at Bielefeld University, use moss to ensure suitable humidity inside the terraria, as it can store moisture. However, the moss needs to be moistened regularly, and it is important to check for growing mould. To avoid accumulations of bacteria, faeces must be removed weekly, which could be challenging due to the dark soil and cleaning the boxes may be time-consuming. Furthermore, the moss needs to be replaced from time to time (e.g., bi-annually). GERHARDT & SEIDEL (2019) established another successful and more artificial housing approach for breeding fire salamanders, which, as they reported, resulted in high longevity and high reproductive success of their animals. The salamanders are kept in opaque plastic boxes, which are also used in the food industry and are easy to stack. The setup is more convenient concerning workload for breeders. Dry newspaper is used as a substrate, as it can be easily removed and changed. It is recommended that the newspaper be changed every three to six weeks. Rocks are used as hiding places and the box is closed with a lid, or several boxes are stacked on each other to reduce space. Light and air circulation are ensured with holes on the sides of the boxes. Humidity is provided by a water container and a "wetbox". The wetbox is a small plastic box filled with moss, which can be accessed through a hole in the lid that is small enough to maintain high humidity inside (GERHARDT & SEIDEL 2019). This approach has the advantage that the bacterial and fungal growth is limited. It provides different microhabitats, which are essential for a salamander's survival and welfare. In summary, cleaning the box is quicker and easier than in the natural housing system; however, the substrate needs to be exchanged more often.

Appropriate husbandry conditions are crucial for an animal's welfare, but unfortunately for wild fire salamanders there is no clear evidence of what those animals need or prefer. Keepers agree with basic conditions, i.e. "best practice guidelines", to ensure the animal's welfare. However, virtually nothing is known about the conditions that are preferred by the animals themselves and provide them with the possibility to live under suitable conditions. Due to the increasing necessity of saving fire salamander populations and their genetic diversity, this study investigated their housing preferences in captivity. We conducted a choice test, offering individuals a choice between two distinct habitats. Over 60 days, we observed their space use and activity within the experimental setup. Given the established effectiveness of simultaneous choice tests in assessing animal preferences, we hypothesized that fire salamanders demonstrate a preference for one housing condition over the other. A preference, in turn, will give us insights into which housing conditions may increase animal welfare in captivity.

Materials and methods

Study species

The fire salamander is distributed throughout central, western and southern Europe, predominantly in deciduous, mixed forests with waterbodies for larval development, streams and ponds nearby (THIESMEIER 2004). Adults are primarily terrestrial, but females deposit fully developed larvae in streams or ponds where they remain until metamorphosis. The adults are mostly nocturnal and exhibit a seasonal lifestyle that is highly dependent on weather conditions, including humidity, temperature, and wind (THIESMEIER 2004).

Collection and transport

During the main activity periods of fire salamanders in central Europe (i.e., spring and autumn), we captured 30 adult fire salamanders during rainy nights: in spring (9 April 2022, n=4; 27 April 2022, n=11) and autumn (13 September 2022, n=15), from the Kottenforst near Bonn, Germany. The sex of a fire salamander was determined based on the shape of the cloaca and the body shape (THIESMEIER 2004). Before transportation, the animals were tested in a standardized behavioural trial (i.e., tests for activity, exploration, boldness) in the field for another experiment (MÜHLENHAUPT et al. 2025). Subsequently, the animals were placed in individual transport boxes (35x20x15 cm). The transport boxes were filled with damp, dead leaves, and some dead branches as shelter. To maintain proper humidity, tap water was sprayed inside the boxes after collecting the animals and before transporting them to the Behavioural Ecology Department at Bielefeld University, Germany, the morning after collection.

Upon arrival, the fire salamanders were individually kept in a climatized room, each in a separate husbandry plastic box that represented their “choice arenas” (Fig. 1). To allow them to acclimate to the new environment, they spent the first night in a “neutral zone” without access to the zones of interest (natural and artificial). A mesh separated this area from the others, thereby allowing the animals to visually and olfactorily sense both presented habitat types.

Experimental setup

To test which type of housing condition the wild-caught fire salamanders prefer, we designed a simultaneous choice arena in which each individual could choose between two habitat types. The experiment lasted for 60 days, during which the daily hiding position was monitored. During this time, we video-taped the enclosures for 30 days to record activity and preference of the fire salamanders in 7-day intervals. Due to time constraints, we were only able to do recordings for 30 days. To cover the whole duration of the experiment, we recorded the setups for seven days, followed by seven days of no recording.

The choice arena (60x40x27 cm) consisted of an opaque plastic box, which was separated into three zones (Fig. 1 and Fig. S1 in Supplement A). The natural and artificial zones were evenly divided by a wall to prevent the individual from reaching the other side directly; instead, the individual had to pass a neutral zone to reach the other zone. To prevent the salamanders from escaping, a lid covered the edges of the arena, leaving the centre open. The neutral zone had no specific substrate and no hiding spots, only a dish with tap water and food on occasion (Fig. 1).

One preference zone represented the “natural” type of housing, which consisted of one layer of soil, a mixture of fresh moss and grass and one piece of bark for hiding. The moss on the natural side was moistened every 4th day to keep it fresh and humid. The artificial zone was equipped with six layers of kitchen paper as a substrate, which was lightly moistened with tap water to stick to the ground at the start of the husbandry experiment. The artificial zone also contained a brick and a wetbox as hiding opportunities. As wetbox, we used a plastic box (10x10x6 cm) filled with moss (without soil). A hole allowed the fire salamander to enter and leave the wetbox. The moss inside the wetbox was moistened once a month, as the closed box itself ensured that the moss stayed humid for a long time. For one half of the choice arenas, the natural zone was on the left side and *vice versa* for the other half.

To test for seasonal differences, we conducted the experiment with 15 individuals per season (spring and autumn). The choice arenas were distributed in patches of four arenas inside a climatised room. The room had a regulated temperature of 14 °C/8 °C in a 12:12 day/night cycle. Four cameras (Camera Security DVR System by Elro; DVR74S) with night vision were used to record the location and behaviour of the animals, with one camera recording four arenas simultaneously. We used one arena, without a salamander, as a control to analyse climate conditions (temperature and humidity) of the natural and the artificial zone as well as the wetbox, respectively. This control arena was fully equipped, and dataloggers were attached to the wall in each preference zone and checked regularly during the experimental period. A third datalogger was attached to the lid inside the wetbox.

The recordings started in the morning after acclimation. After removing the mesh, the individuals could move freely to all areas of the arena. Throughout the experiment, the individuals were checked daily at around 10 am without handling them in order to observe changes in the overall health status. During the check-ups, the daily hiding spots were noted. Fresh tap water was provided *ad libitum* in a flat bowl. Food (i.e., pieces of annelids, *Lumbricus terrestris* LINNAEUS, 1758) was provided every 8th day, and during the second half of the experiment, every 16th day as the fire salamanders showed low interest in the food. Leftovers of the annelids were removed the following day. Each zone was checked every day and faeces were removed.

At the end of the experiment, the individuals were tested for *Bsal* before they were returned to the location in the Kottenforst where they had been collected. All *Bsal*-tests were negative.

Video analysis

The videos were imported into the software BORIS (Behavioural Observation Research Interactive Software, version 9.3.2) (FRIARD & GAMBA 2016) for video scoring. Before starting the observations, an ethogram was created and coded for the software (see Table S1 in Supplement B). The video IDs only consisted of the date and channel ID (i.e., channel 1 = camera 1) without any information on individual identity to prevent subjective observations. The identity of the arena was represented by the given location (up-left, up-right, down-left and down-right) and later assigned to the respective animal ID in Excel.

Behavioural sampling started the moment the mesh was removed. The first choice of the zone of interest (natural or artificial) was noted. The recording of the inactivity and activity data concluded when the individual disappeared in the first hiding spot and started again once the individual reappeared. Activity was defined as the movement of the body centre, while inactivity represented no movement of the body centre. The movement of the animals was recorded for the entire arena, including the neutral zone. To ensure having the data for the complete surface activity (i.e., the time one individual spent outside their hiding area) available, another code for “out of sight” was added when the individual was outside their hiding spots but not visible due to dead angles by the cameras. Activity data was also divided into day (6 am to 6 pm, when the light was on) and night activity (6 pm to 6 am, when the light was off) and it was noted in which zone (natural, neutral and artificial) the individual was at a given time interval. To visualise different individual activity patterns throughout the 30 days of recording, we used 30-min intervals over the day to create actograms with Excel (Version 2410). Actograms are used to visualise data periodically throughout a day to investigate rhythmical activity patterns of animals (OIKE et al. 2019).

Statistical analysis

The statistical analyses were performed using R (R-version 4.4.1; R CORE TEAM 2023). Prior to testing for differences in temperature and humidity in the respective zones within the arenas (natural, artificial and wetbox), the data were checked for normality using the Shapiro-Wilk-Test and visual plots (Histogram and Q-Q-Plot) (see Fig. S2 in Supplement A and Table S2 in Supplement B). To test for differences in temperature and humidity conditions within the zones, we conducted a Kruskal-Wallis-test and Dunn’s test (R-package: FSA; OGLE et al. 2023) with Bonferroni adjustment to correct for multiple comparisons for the *post-hoc* analysis.

As one indicator of preference, we compared the first choice after habituation using the exact binomial test with the given probability of 0.5 for each habitat. To investigate the preference for a specific zone, we compared the daily hiding spots of all individuals and the surface activity (including activity, inactivity and out of sight) for all three zones.

To analyse the surface activity, we used the percentage of being outside (surper), calculated as the surface activity (sur) divided by the daily recording time (rec) and multiplied by 100, since the daily recording time differed between days due to the recording system (i.e., when the recording for the first day started around 10 am and ended at midnight). The surface activity and recording were both measured in seconds. To investigate differences in surface activity, we fitted a linear mixed effect model with the R package lme4 (BATES et al. 2015) and initially included the fixed effect of habitat type (i.e., preference zone) on the dependent variable surface activity. As a random intercept, the individual’s identity was added due to multiple measures and to control for individual-specific variation. The model was fitted to the data using a step-wise step-up procedure, evaluating the model using the Akaike Information Criterion (AIC) for model comparison (AKAIKE 2011). Further fixed effects were included, such as season, sex, day and their interactions with the variable of interest (habitat type), when they improved the fit until the best minimal adequate model was found. The final model was compared by using the ANOVA to a reduced model to explore the impact of single fixed effects on the data. As the variable sex enhanced the model fit, but no sex could be identified for three salamanders, these individuals were excluded from this dataset, resulting in a total of n = 27. When the effects of the final model were significantly different compared to the reduced one, we applied the pairwise Wilcoxon rank-sum (WRS) *post-hoc* test with the Bonferroni correction to investigate differences within the group. To investigate the contrasts among predictor interactions, we used the lsmeans-package (LENTH 2016) with a Bonferroni adjustment to control for multiple comparisons. Finally, we used the daily hiding spots as an indicator of a fire salamander's preferences by calculating the proportion of how often an individual was found under either the moss or bark, the brick or inside the wetbox during the day. We fitted a linear mixed effect model as described above, however, this time with the proportion value as the dependent variable and the hiding place, sex, and season as fixed effects of interest and the individual identity as the random intercept.

To investigate differences in activity in relation to individual and season-specific differences, we fitted a hidden Markov model (HMM) with the individuals' activity in 30-min intervals using the R package hmmTMB (MICHELOT 2025). The model comprises an observed process corresponding to the data provided and an underlying serially-dependent, unobserved ('hidden') sequence (the state process) to infer individual behavioural processes (ZUCCHINI et al. 2017), such as foraging or movement (VAN BEEST et al. 2019; GOWAN et al. 2021; NAGEL et al. 2021; SCHWARZ et al. 2021), but also processes on population and community level, such as abundance or species co-existence, respectively (MCCLINTOCK et al. 2020). Here, we fitted a two-state HMM with a zero-adjusted gamma distribution to the activity data of the fire salamanders. The estimated state-dependent distributions indicate an inactive state (state 1, when the individual is immobile) and an active state (state 2) (see Fig. S3 in Supplement A and Table S3 and S4 in Supplement B). We used the decoded states of the fitted HMM to examine the overall daily activity patterns of the animals for both seasons by determining the percentage of time spent in state 2 as well as individual differences in activity over the duration of the experiment.

Results

Climate conditions

Climate analysis revealed a difference in temperature and humidity between the zones of the arena (Kruskal-Wallis-test: Temperature: H(2) = 27.86, $p < 0.001$; Humidity: H(2) = 151.48, $p < 0.001$). For temperature, there was a difference of approximately 2.8 % between the natural and artificial zones (natural: 13.17 ± 0.56 °C; artificial: 13.55 ± 0.43 °C; Dunn's Test: z = -4.38, $p_{adj} < 0.001$) and 3.1 % between the wetbox and the artificial zone (wetbox: 13.13 ± 0.44 °C, Dunn's Test: z = -4.72, $p_{adj} < 0.001$). No difference was found in temperature comparing the natural zone and the wetbox (Dunn's Test: z = 0.42, $p_{adj} = 1$).

The wetbox had the highest humidity with the mean value reaching nearly 100% (99.18 ± 0.39%), being significantly different from the natural zone (90.11 ± 5.87%; Dunn's test: z = 7.00, $p_{adj} < 0.001$) and the artificial zone (83.37 ± 4.34%; Dunn's Test: z = 12.28, $p_{adj} < 0.001$). The natural and artificial zones differed on average by 7.8% (moss: 90.11 ± 5.87%; tissue: 83.36 ± 4.34%, Dunn's Test: z = -5.38, $p_{adj} < 0.001$). For further descriptive data on humidity and temperature differences, see Table S5 in Supplement B.

Preference: First choice

During the habituation period, six animals were able to climb over the fence and hid on one side and thus were removed from the first choice analysis (resulting in n = 24). Fire salamanders chose the natural zone significantly more often than the artificial zone (exact binominal test: $p < 0.05$) with 18 individuals entering the natural zone first and six individuals entering the artificial zone first.

Preference: Daily hiding

Fire salamanders hid significantly more often in the natural zone compared to the other zones (0.91 ± 0.19, LMM: t-value = 22.71, $p < 0.001$, Fig. 2, Table 1a). We found fire salamanders in three of the various hiding opportunities, i.e. underneath the moss, underneath the brick, and in the wetbox. Fire salamanders were found significantly more often underneath the moss than in the wetbox or under the brick (WRS: $p_{adj} < 0.001$; < 0.001). All individuals were found at least once hidden underneath the moss in the natural zone, while only three individuals were found at least once under the brick. Individuals showed a tendency to hide more often inside the wetbox compared to under the brick (0.08 ± 0.19, LMM: t-value = 1.97, p = 0.052, Table 1a); WRS: $p_{adj} < 0.05$).

Including sex or season did not improve the model fit. An interaction of sex and season did not influence the proportion of daily hiding places of fire salamanders and was thus omitted from further analysis. For further descriptive data on the daily hiding spot preferences, see Table S6 in Supplement B.

Surface activity

There was a significant effect of habitat type on the surface activity of female fire salamanders. Once outside, females spent on average more time in the natural zone (natural: 7.56 ± 10.08%, LMM: t-value = 2.8, p = 0.005), compared to the respective other options (Fig. 3, Table 1b). Fire salamanders, regardless of sex, did not differ in their use of the artificial and neutral zones (artificial: 2.93 ± 4.87%; neutral: 2.89 ± 5.32%, WRS: $p_{adj} = 0.67$). There was a tendency that fire salamanders spent more time outside their hiding areas as the experiment progressed (LMM: t-value = 1.755, p = 0.08, Fig. 3, Table 1b). We found a significant interaction effect of sex and zone, with females spending on average more time on the natural zone compared to the other zones (LMM: t-value = -3.845, p < 0.001). Males, however, did not show any differences among zones (Fig. 3, Table 1b). While there was no general seasonal effect on the surface activity, fire salamanders from the autumn group spent more time on the natural zone (LMM: t-value = 4.071, p-value < 0.001) compared to all other zones. No differences were observed between the zones during the spring period. For further descriptive data on surface activity separated by season and sex, see Table S7 in Supplement B.

Activity analysis

Overall, the activity pattern depicted by the actograms revealed that wild fire salamanders in captivity remained mostly nocturnal; however, there were some exceptions and high individual variation in activity patterns (see Figure 4 for two examples, the remaining data can be found in Supplement A). In the provided example (Fig. 4), the autumn individual was very active during the night over the whole course of the experiment, while the spring individual showed regular daytime activity at the end of the experiment.

For the HMM analysis regarding activity, state 1 captures *inactive* behaviour, in which the individual has a probability of zero activity of 99.8% (mean activity = 0.73 ± 0.56 minutes if moving), and state 2 captures *active* behaviour, with a probability of zero activity of 5.1% (mean activity = 4.75 ± 3.78 minutes if moving) within the 30-minutes' intervals. The transition probabilities show that individuals stay in state 2 from one 30-min interval to the next with a probability of 82.4%, corresponding to an expected duration of 2.8 hours before switching to state 1; however, once in state 1, they can stay inactive for several hours or even days, depending on the individual (see Fig. S3 supplement A and Table S4 supplement B).

Individuals were more active during autumn compared to spring, as indicated by our HMM approach. We also investigated the circadian rhythm (Fig. 5) for spring and autumn, respectively, and found a similar pattern for each season, again with a lower activity for the spring group. In general, fire salamanders showed an increase in activity shortly after onset of darkness (6 pm–7 pm), followed by a lower activity for 3 hours (7 pm–10 pm). After approximately 10 pm, the fire salamanders showed increased movement activity before reaching a peak of activity at 3 am in the morning (Fig. 5). The activity then decreased until daytime (6 am), and as seen in the actograms, activity during the diurnal period was much lower than during the nocturnal period, which is why these observations were mostly attributed to the inactive state by the HMM approach (Fig. 5).

Table 1: Statistical results of the mixed linear model analysis (lmer), testing if the zone (natural (N), artificial (A), neutral (NE)), sex (female, male), day or season (spring, autumn) had an effect on the variable a) daily hiding spot (Moss/Bark (M), Wetbox (W), Brick) or b) surface activity. Number of observations and individuals' ID are given for each model, including the conditional and marginal R^2. Coefficient estimates (*β*), confidence intervals (*CI*), the respective standard error (*SE*) and *t*- and *p*-value are provided for fixed effects. For random effects, the residual variance (σ^2) and the variance of the random intercept (τ00) is given. Significant values are highlighted in bold.

a) Daily hiding	N_{obs}=90, N_{ind}=30	$R^2_{conditional}$ = NA $R^2_{marginal}$ = 0.877			
Fixed effects	*β*	*CI*	*SE*	*t-value*	*p-value*
Intercept	<0.01	-0.05 – 0.06	0.03	0.10	0.922
Hiding spot [M]	0.91	0.83 – 0.99	**0.04**	**22.71**	**<0.001**
Hiding spot [W]	0.08	0.00 – 0-16	0.04	1.97	0.052
Random effects					
σ^2	0.02				
τ00 ID	<0.001				
b) Surface activity	N_{obs}=741, N_{ind}=27	$R^2_{conditional}$ = 0.156 $R^2_{marginal}$ = 0.220			
Fixed effects	*β*	*CI*	*SE*	*t-value*	*p-value*
Intercept	1.22	-1.10 – 3.54	1.18	1.03	0.302
Zone [A]	1.02	-1.52 – 3.55	1.29	0.79	0.431
Zone [N]	3.64	1.10 – 6.17	**1.29**	**2.8**	**0.005**
Day	0.03	-0.00 – 0.06	0.016	1.76	0.080
Zone [A]:sex(male)	0.71	-1.62 – 3.04	1.19	0.60	0.548
Zone [N]:sex(male)	-4.56	-6.89 – -2.23	**1.19**	**-3.845**	**<0.001**
Zone [NE]:sex(male)	0.85	-1.48 – 3.18	1.19	0.716	0.474
Zone [A]:season(autumn)	-0.90	-3.38 – 1.58	1.26	-0.71	0.476
Zone [N]:season(autumn)	5.14	2.66 – 7.62	**1.26**	**4.07**	**<0.001**
Zone [NE]:season(autumn)	0.34	-2.14 – 2.82	1.26	0.27	0.786
Random effects					
σ^2	45.97				
τ00 ID	3.75				

Discussion

Letting the fire salamanders choose which habitat they favour gives insights into what the animals prefer and possibly need. Concerning two successful housing types for captive breeding of fire salamanders, with no known or identifiable welfare issues, it is difficult to determine which one is preferred by wild fire salamanders. With our experiment, we aimed to investigate the preference of wild-caught fire salamanders for either natural or artificial housing conditions using a choice experiment.

Preference of conditions

Our results indicated that fire salamanders show a preference for the more natural zone, based on their first choice, daily hiding and surface activity. The natural zone was equipped with soil and moss, which resulted in different climatic conditions compared to the artificial zone. Temperature and humidity are important for salamanders, as they affect body temperature and water balance (CLOUDSLEY-THOMPSON & WARBURG 1997), which in turn can determine their preference. This was, for instance, shown for the terrestrial salamander *Bolitoglossa ramosi* (BRAME & WAKE, 1972; GALINDO et al. 2018). Differences in relative humidity and temperature can also influence the behaviour of other salamanders (SPOTILA 1972) or other ectotherms (e.g., DEZETTER et al. 2023).

Moss in the natural setting provided higher humidity and lower temperatures than in the artificial part, e.g. due to evaporation (CHEN et al. 2019). Choosing the right substrate when keeping fire salamanders is crucial to maintain appropriate humidity levels and should benefit the animal’s welfare. The natural zone and the wetbox had lower temperatures and higher humidity compared to the artificial zone, supporting the assumption that the moss within the arena affected the microclimate and thereby, at least partly influenced the preference of the fire salamanders.

Data from the video analysis revealed that fire salamanders also spent time inside the water dish, which was only present in the neutral zone. Spending time in the water dish might be due to increased humidity needs of the salamanders and may explain the increased time they spent in this habitat as the experiment progressed. One explanation is the need for hydration, as this is an important factor for amphibians. There is evidence that salamanders are also able to draw water from the soil (SPIGHT 1967; SPOTILA 1972). This might explain why the fire salamanders preferred staying within the natural zones and in the wetbox, when the humidity was high enough to reduce water loss, and used the additional water to restore moisture. Another explanation for an increase in the use of the water dish in the course of this experiment might be habituation to the predation-free space. Fire salamanders might have learned that there was no predation risk while exploring the enclosure.

While sex alone was not a predictor of fire salamander surface activity, females spent more time in the natural zone; males showed no preference. Differences in microhabitat preferences between sexes within a species are found all across the animal kingdom (e.g., Birds: ARIDA & BILDSTEIN 1997; Isopods: MERILAITA & JORMALAINEN 1997; Lizards: RECKNAGEL et al. 2023), including amphibians (e.g., BARTELT et al. 2005; ETEROVICK & FERREIRA 2008; VALDEZ et al. 2016; ZHAO et al. 2023). One study found that male fire salamanders preferred deeper leaf litter and stayed close to the larval deposition sites during the breeding season (MANENTI et al. 2017), potentially to increase the chance to find mating partners. However, to our knowledge there is no evidence yet on what females might prefer and what causes these sex-specific differences in their behaviour. As already stated, enough hydration is crucial in the lifecycle of a fire salamander. Especially in spring, females are most likely to be seen near or inside waters for larval deposition (THIESMEIER 2004). Further, it could be hypothesised that females need more hydration due to metabolic requirements for the development of larvae in the uterus. However, whether there are other physiological indications that females need more hydration than males, is not clear. Further data are needed to explore the effects of season and sex on activity in captivity. Other factors could also be responsible for the microhabitat selection, such as the structure or vicinity to the hiding spot.

Fire salamanders hid more often in the natural zone, more precisely underneath the moss and soil layer, compared to under the brick in the artificial zone or the wetbox. Fire salamanders usually hide in dark, cool and damp areas, such as within tree rootstocks, under dead wood or in crevices (THIESMEIER 2004; BÖRDER et al. 2011; SIEGEL 2014). Such hiding spots are usually difficult to access and thus provide protection against predators. The brick in this experiment might have been too small to act as a shelter, which could explain why individuals preferred to stay underneath the moss. Another potential factor causing the avoidance of the brick may be the lower humidity level. Additionally, the wetbox was made of transparent plastic. Although the only way to access the wetbox was through the hole in the lid, the transparency increases light levels inside the hiding spot, which in turn, may not be favoured by fire salamanders. Nevertheless, some individuals demonstrated a tendency to return to the wetbox after having spent a period of time inside, with some returning for several days or even weeks. Whether the light levels affect the choice of a hiding spot of fire salamanders is not clear, but providing a less translucent wetbox might also increase the acceptance of the hiding spot by fire salamanders.

Activity analysis

The hidden Markov model confirmed that fire salamanders, at least those in our study area, are most active at night. They usually appear within the first hours after dusk, which indicates that light is affecting the activity rhythm of fire salamanders (*personal observations*). In the literature, it is stated that individuals usually start to appear at a light intensity of two lux or lower (THIESMEIER 2004). Although the activity differed between the seasons, the overall pattern is similar, with a small peak shortly after darkness and a second, even larger peak after midnight. As fire salamanders are mainly sit-and-wait predators (BOGAERTS et al. 2021), it might be possible that they have been outside during this time but not moving, waiting for possible food to pass by. Thereby, they might have showed no activity after the first peak of activity.

We detected individual differences in activity, with some individuals not being active at all (mainly individuals tested in spring) and others showing high activity levels. The fire salamanders for this study were collected during the activity peaks (spring: mid-April to mid-May and autumn: September to mid-October). However, even during these periods, activity is highly affected by external factors such as temperature, humidity and wind (THIESMEIER 2004) and likely also internal factors such as hunger or the need to find larval deposition sites (although no female deposited larvae during this experiment). We witnessed an increased activity in autumn, which can be explained by the migratory behaviour of fire salamanders heading towards their hibernation sites or their need to find enough food before hibernation. In contrast, activity in spring was comparatively low. In the Kottenforst (Bonn, Germany), the origin of our animals, fire salamanders start leaving their shelters in early February, when the climate is suitable (THIESMEIER 2004). Thus, the spring individuals might have already been at the end of their spring activity peak. These seasonal differences were also mirrored by differences in personality traits of these individuals (see MÜHLENHAUPT et al. 2025).

Reintroduction success of captive animals in the wild

Due to the threat of chytridiomycosis, action is needed to ensure the survival of amphibian populations (Spitzen-van der Sluijs et al. 2016). As it is already clear that *in-situ* conservation is not sufficient to ensure the genetic diversity of fire salamander populations, *ex-situ* programs are the most immediate and viable solution to preserve the fire salamander in Europe (Thomas et al. 2019). However, *ex-situ* conservation should only be practiced when the benefits outweigh the risks (e.g., the impact of removing animals from the wild), the breeding success and the likelihood of the successful release in the wild are ensured, thereby ensuring to keep the genetic diversity (Tapley et al. 2017; Rout et al. 2023). However, according to the IUCN "Guidelines on the Use of *Ex situ* Management for Species Conservation" “time gain” is also an important reason for *ex-situ* insurance populations. The "Establishment of a diverse and sustainable ex situ rescue or insurance population may be critical in preventing species extinction when wild population decline is steep and the chance of sufficiently rapid reduction of primary threats is slim or uncertain or has been inadequately successful to date. Examples include *ex-situ* populations in response to severe disease threat, catastrophic events or continued habitat degradation." (IUCN 2023). This is consistent with the predicament the fire salamander is currently facing.

Animals in captivity can exhibit different behaviours compared to wild ones due to the different environment, selection pressures and the process of domestication (i.e., the process by which animals change for human use, for example as pets or farm animals; Tapley et al. 2015; Rutledge et al. 2023). The environment to which an individual is exposed to, determines what kind of behaviour is displayed (Wilson et al. 2020). The substrate, for example, can encourage additional natural behaviour such as burrowing (Tapley et al. 2014; Zhang et al. 2023). Similar observations were made with the fire salamanders from this experiment, which were mostly hidden between the layers of soil and moss, while there was no option to dig between layers on the artificial side. The freedom to express natural behaviour is one major aspect to consider when keeping animals. This is even more important when there is the intention to reintroduce individuals or their offspring into the wild again (Griffiths & Pavajeau 2008; McPhee & Carlstead 2010).

Animals are able to adjust their behaviour to new conditions, including changes in their environment due to captivity. These domestication processes can also occur in captive-held amphibians (Tapley et al. 2015). For example, captive-bred amphibians show less predator avoidance behaviour compared to the wild conspecifics (KAAIJEVELD-SMIT et al. 2006; LINHOFF 2018). Despite the absence of empirical studies about the differences in behaviour of captive and wild-caught fire salamanders, captive-bred fire salamanders display increased activity levels in the presence of humans (personal observations). This might either be a result of direct interactions with them (e.g., feeding by hand during the day) or simply the absence of predators. In our experiment, we did not observe that individuals left their hiding spots when someone entered the room. Depending on how long fire salamanders must be kept in captivity due to *Bsal*, the housing conditions may encourage or restrict behaviour that does not support survival in the wild. Even a few generations can allow animals to adapt to captivity, which may affect their fitness and therefore have a negative impact on the reintroduction success (SNYDER et al. 1996; ARAKI et al. 2007; CHRISTIE et al. 2012). GRIFFITHS & PAVAJEAU (2008) reviewed different breeding and reintroduction programmes and evaluated the reintroduction success of amphibian species in these programs. By producing multiple generations and developing self-sustaining populations, 13 out of 21 species were ranked as highly successful. Although they have been quite optimistic with their results, TAPLEY et al. (2015) point out that the reviewed programmes only included amphibian populations that suffered from habitat loss in parts of their distribution, and reintroduction may not be as straightforward for species coping with pathogens spreading through their entire habitat. Especially concerning *Bsal*, it is not clear whether individuals of a population can return to their original habitat and how long they need to be kept *ex-situ*, particularly as newts can act as reservoirs of *Bsal* (BENINDE et al. 2021).

Mimicking the natural environment and thereby maintaining the potential to express natural behaviour reduces the possibility that wild animals might adapt to captivity, and thereby potentially increases the reintroduction success of fire salamanders. However, the costs for maintenance are likely to be significantly higher due to materials and labour and should therefore be outweighed by the benefits. However, as it is currently unknown how long populations and their genetic diversity need to be preserved in the context of the *Bsal*-pandemic, *ex-situ* conservation actions need to be thoroughly planned and housing adjusted, including aspects of cost efficiency.

Conclusion and future research

The spread of Chytridiomycosis is a serious threat to fire salamander populations, and *in-situ* conservation alone will not be able to prevent the loss of fire salamander populations and their genetic diversity. Taking animals into captivity is the most immediate solution, conservationists and scientists can apply (see THOMAS et al. 2019; PENNER et al. 2022). However, ideas about appropriate husbandry of fire salamanders vary among keepers, and certain details need to be evaluated, particularly with the background of a successful reintroduction of individuals to the wild. This includes, among others, maintaining natural behaviour to prevent maladaptive selection of behavioural traits (for example, enhancing activity during daylight due to interactions with caretakers).

To conclude, the results of our study indicate that fire salamanders exhibited a clear preference for natural housing with moss and soil as a substrate. However, they did not demonstrate a clear avoidance of artificial habitats characterized by smooth and relatively dry structures. A heterogeneous habitat, including natural and artificial zones, could be an alternative way of keeping wild fire salamanders to encourage a variety of natural behaviours and provide the necessary microhabitats with sufficient humidity to ensure their wellfare. However, more tests are needed to fully understand which factors lead to the observed preference and how much internal (sex, life stage) and external (climate, light, season, time) factors influence it. As humidity plays an important role, there should be further research on whether the microclimate is responsible for the fire salamanders' habitat selection or the similarity of the environment to the origin itself.

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by the LAVE: Az 81-02.04. 2021.A437. After the experiment, all fire salamanders were released unharmed at the site of capture.

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Figures

Figure 1: Full experimental set-up of the habitat choice arena for fire salamanders. The natural side (top) consists of a layer of soil, moss and a piece of bark and is separated from the artificial side (bottom) by an opaque barrier. The artificial side has six layers of paper towel, a brick, and a wetbox, which is filled with moss. The neutral side connects the natural and artificial sides and provides a dish of water (left).

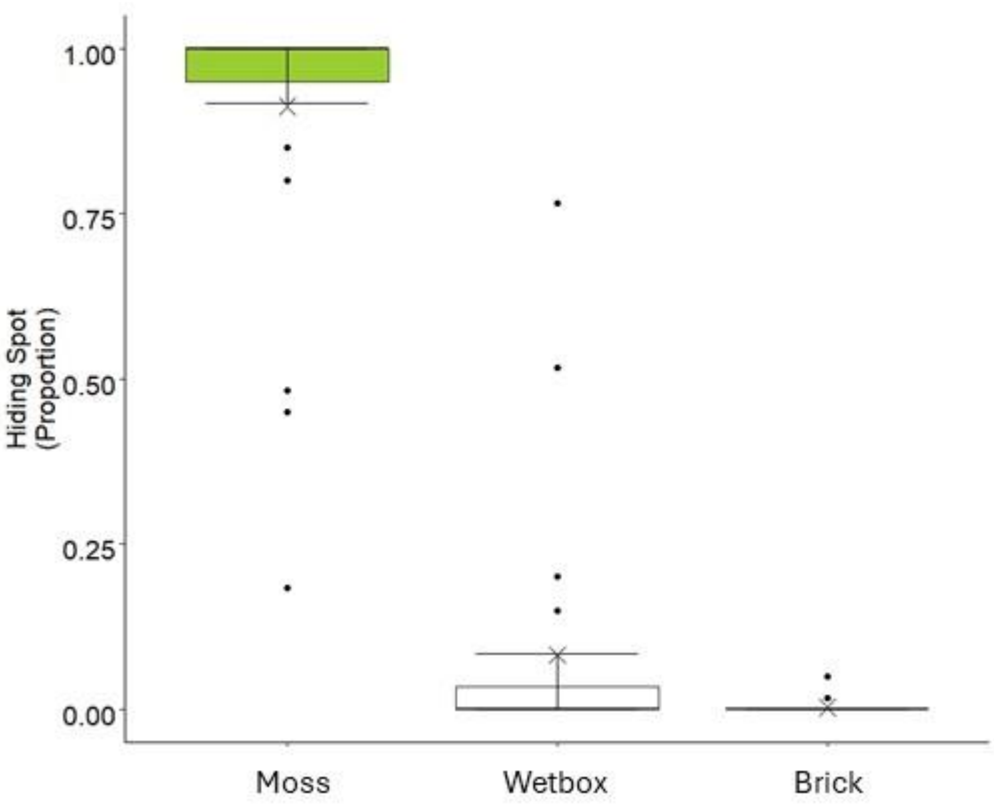


Figure 2: Overall proportion of the daily hiding positions of fire salamanders (compare with Fig. 1), calculated over all 60 days for each individual. The x represents the respective mean values and the thick line the median. The boxes show the quantile range, the whiskers the minimum and maximum and dots are outliers. A linear mixed model and a followed paired Wilcoxon test were used to test differences between each hiding spot.

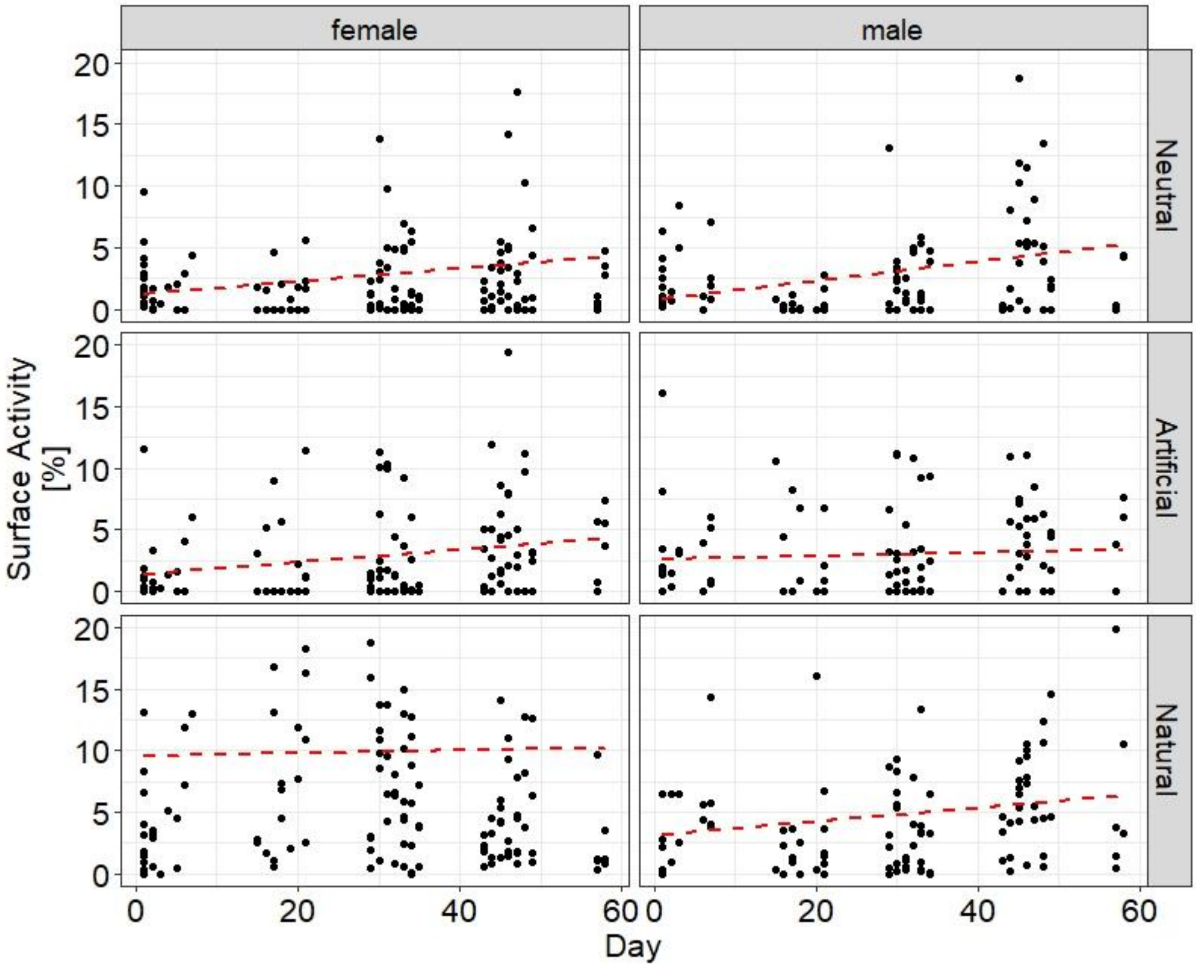
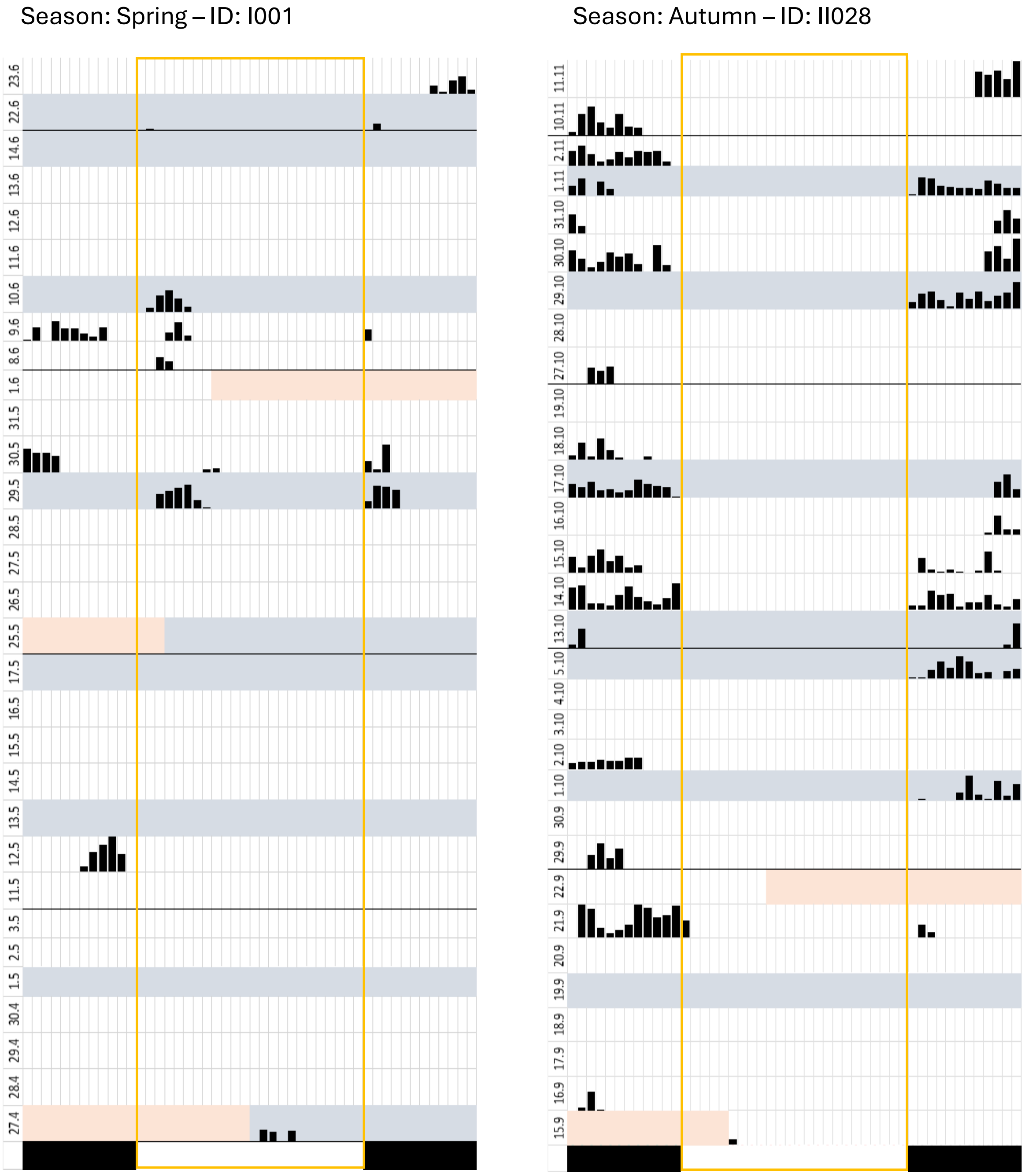


Figure 3: The percentage of surface activity (including “activity”, “inactivity” and “out of sight”) for each individual fire salamander (n = 27) per day, separated by sex and habitat zone over 60 days. The red, dashed line represents the linear trend over the experimental period.


Season: Spring – ID: I001

Season: Autumn – ID: II028

Figure 4: Example actogram of two fire salamanders, one for each season (see supplement A for the remaining data). The actogram visualises the rhythmic activity data of individual fire salamanders over the days but only including the days of recording. The y-axis shows the time in days. The lower part of the actogram shows the start of the data recording (starting with day 2) and the upper part shows the last days of recording. Each week is separated by a black line. The day and night rhythm are displayed on the x-axis ranging from: 0 am - 6 am black (night); 6 am – 6 pm white (day) and 6 pm to 0 am

black (night) in 30 minutes intervals. The orange rectangle covers the daytime. The black bars show the activity of the respective individual within 30 minutes. The greater the bar, the higher the activity in proportion to the other days. Grey rows indicate the days the moss habitat was moistened; the melon-coloured cells indicate times at which no recordings have been made.

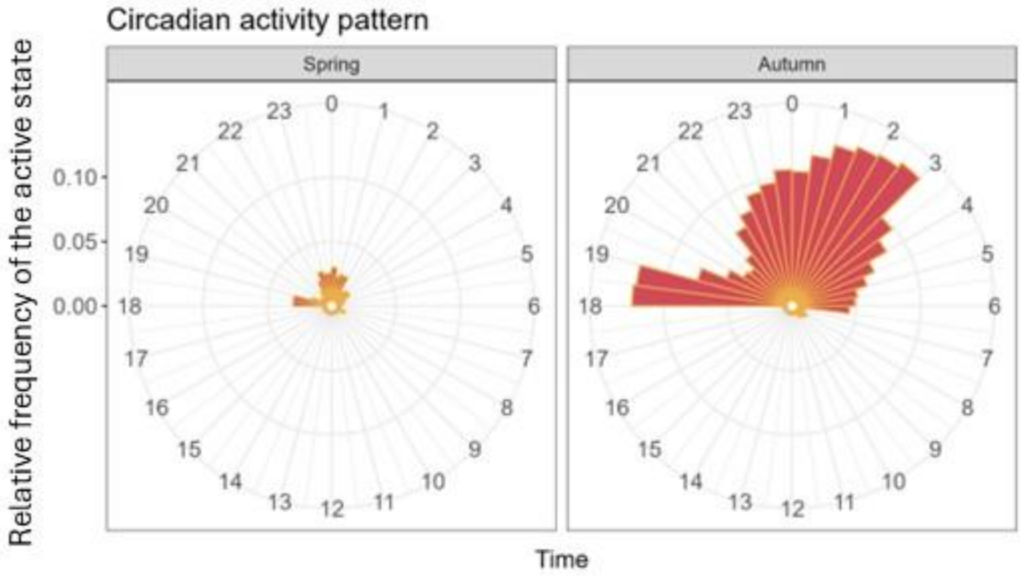


Figure 5: Overall diel pattern of fire salamander activity (state 2) in percentage, averaged over all individuals and the whole recording period, separated into spring and autumn. The centre of the plot shows 0% of activity and the outer circles 5% and 10% of activity, respectively.

Supplementary materials for:

Wild fire salamanders (*Salamandra salamandra*) prefer natural housing conditions *ex-situ*

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Supplementary Materials A: Figures

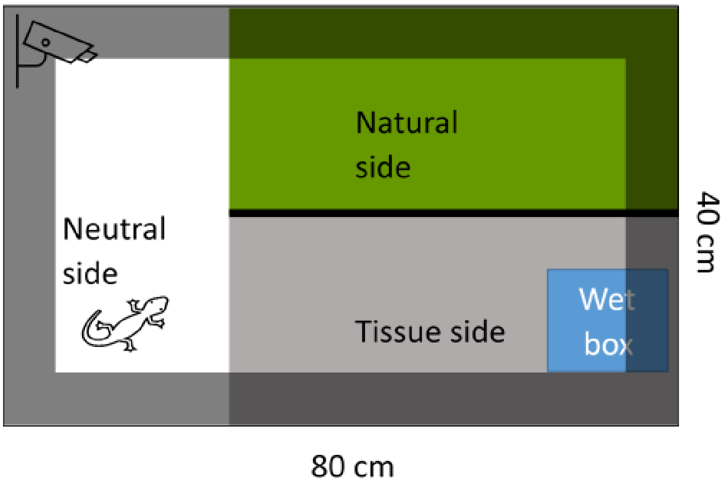


Figure S1: Experimental Setup of one choice arena. The arena is made out of grey, non-transparent plastic.The arena is evenly seperated in the neutral habitat (white), the natural habitat (green) and the tissue habitat (grey). A wall out of plastic seperates the natural side from the tissue side. Inside the tissue side is a wetbox (blue). An open lid (dark grey area) covers the borders of the arena to prevent the fire salamander from escaping. The animals start at the neutral habitat. The whole arena is getting recorded in a 7 day recording, 7 day break manner. After a one night habituation in the neutral zone, the salamander can move in each zone freely.

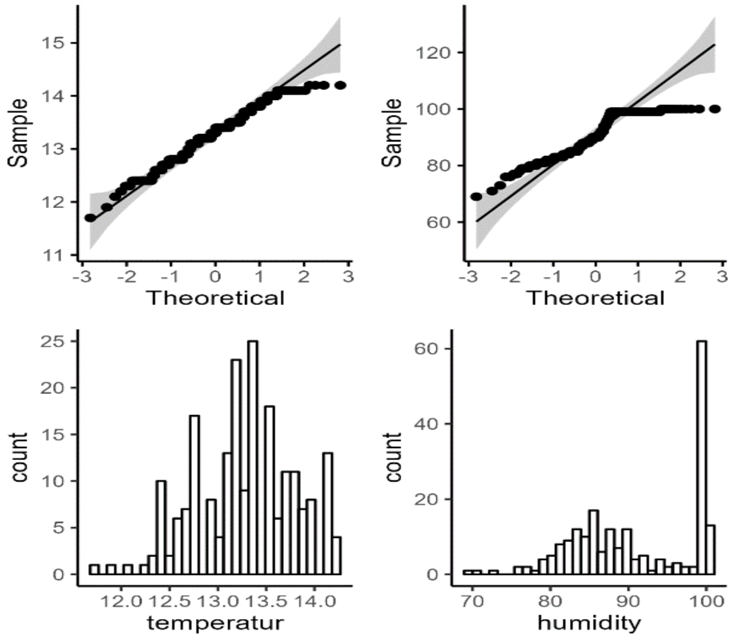


Figure S2: Visualisation of QQ Plots and Histogram for the climate analysis for temperature and humidity. Normality is given, when the black dots are in near proximity to the line for the QQ Plot and follow a Gaussian distribution in the Histogram. Shapiro Wilk test was used as reference and was < 0.05, thus normality was not assumed.

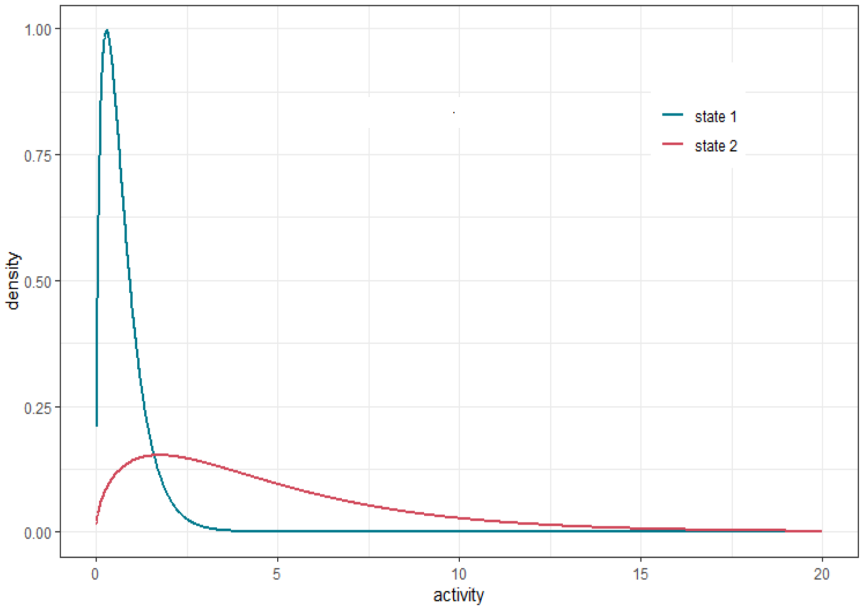
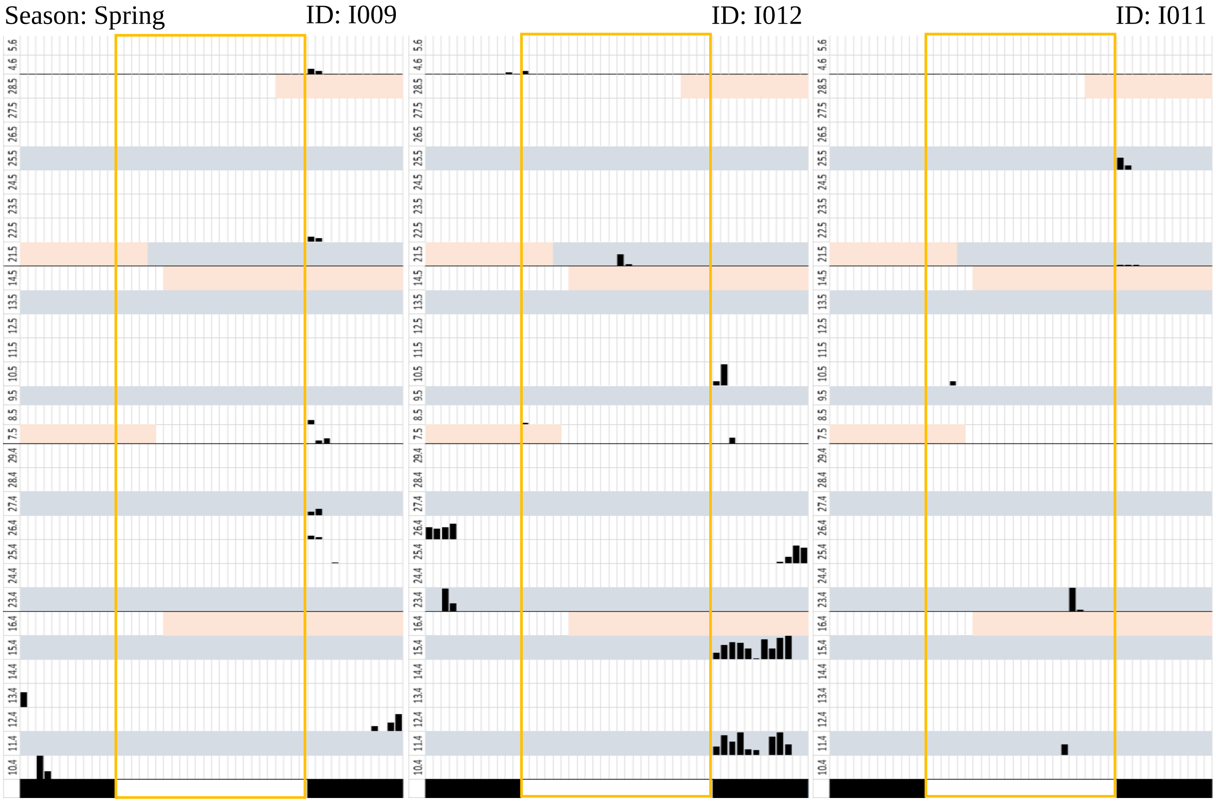


Figure S3: State-dependent gamma distributions of the fire salamanders' activity time within the 30-min intervals. State 1 corresponds to immobile/non-active behaviour (blue line) and state 2 to active behaviour (red line), when fire salamanders were moving. The probability of zero activity for state 1 is 0.998 and for state 2 0.051.

Actogram: Spring

To reduce repetition, the following paragraph contains a general description of the Actograms on page 42 – 47.

An actogram visualises the rhythmic activity data of individual fire salamanders over the days, but only including the days of recording (10.4. – 5.6.2022, n=4 (ID: I017, I009, I012, I0011); 27.4 – 23.06.2022, n=11) and autumn (15.9– 11.11.2022, n=15). The y-axis shows the time in days. The lower part of the actogram shows the start of the data recording (starting with day 2) and the upper part shows the last days of recording. Each week is separated by a black line. The day and night rhythm is displayed on the x-axis ranging from: 0 am - 6 am black; 6 am – 6 pm white and 6 pm to 0 am black in 30 minute intervals. The orange rectangle covers the daytime. The black bars show the activity of the respective individual within 30 minutes. The greater the bar, the higher the activity in proportion to the other days. Grey rows indicate the days the moss habitat was moistened; the melon-coloured cells indicate time at which no recordings have been made.



Season: Spring

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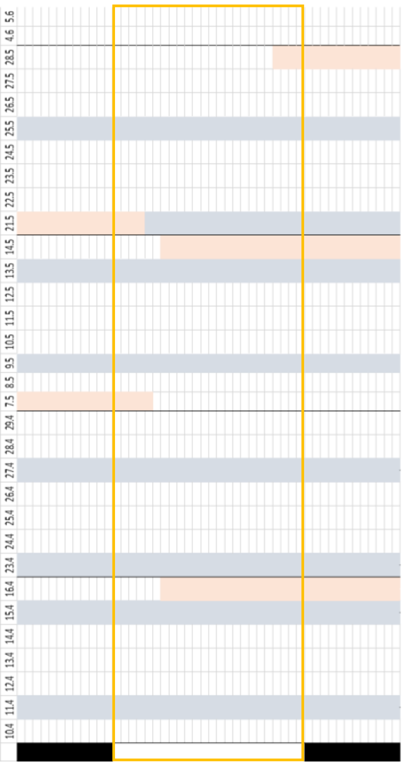
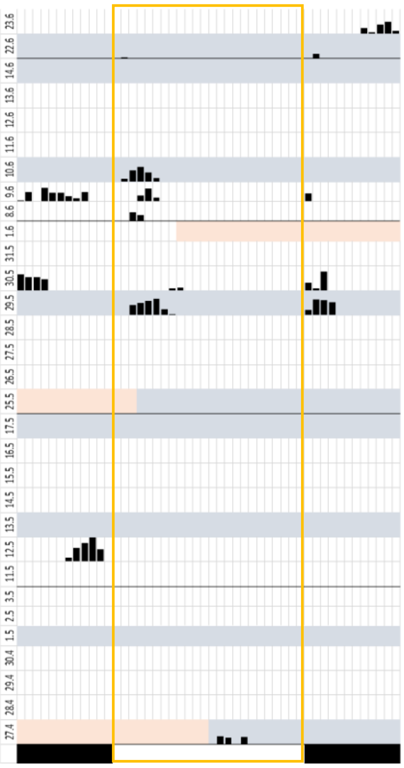
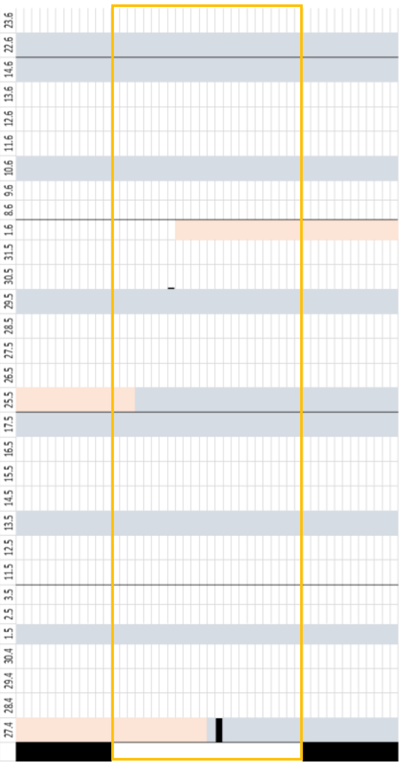


ID: I001

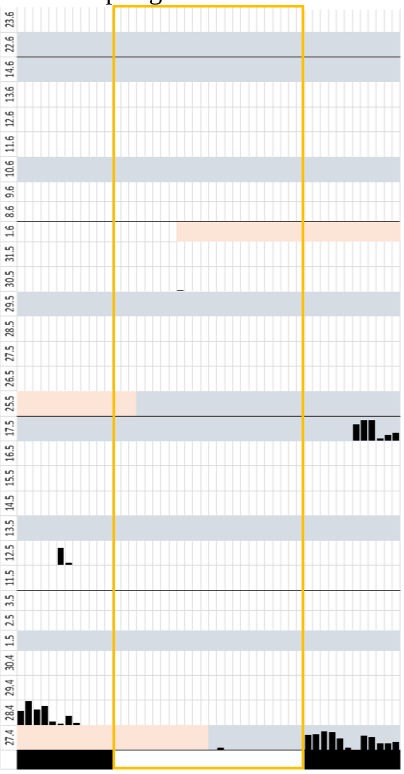


ID: I002



Season: Spring

ID: I004



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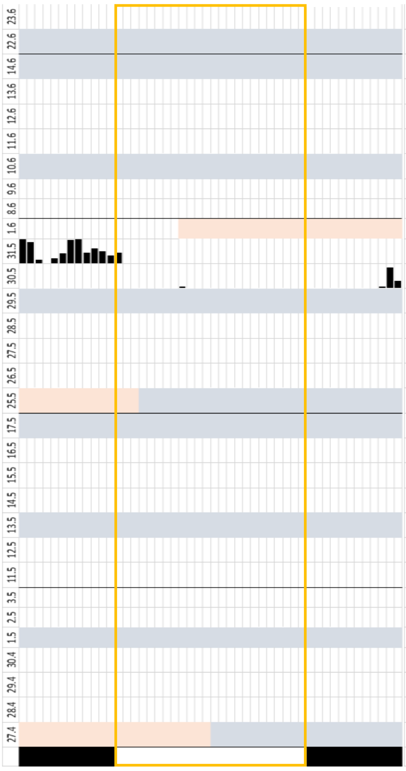
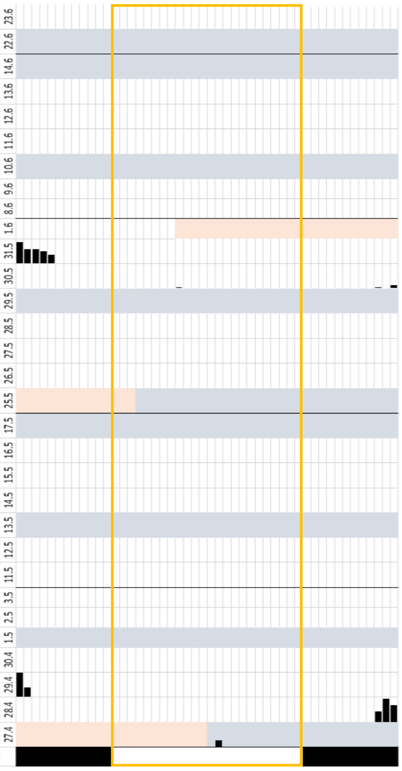
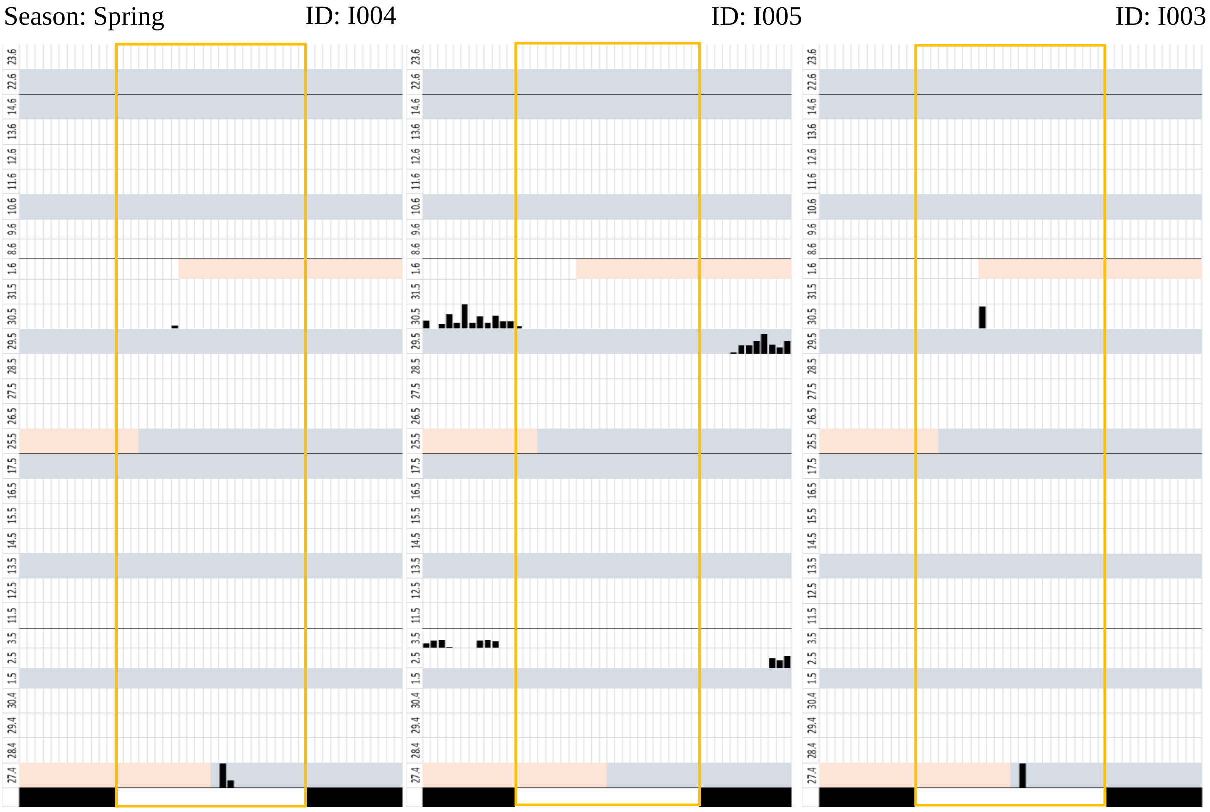
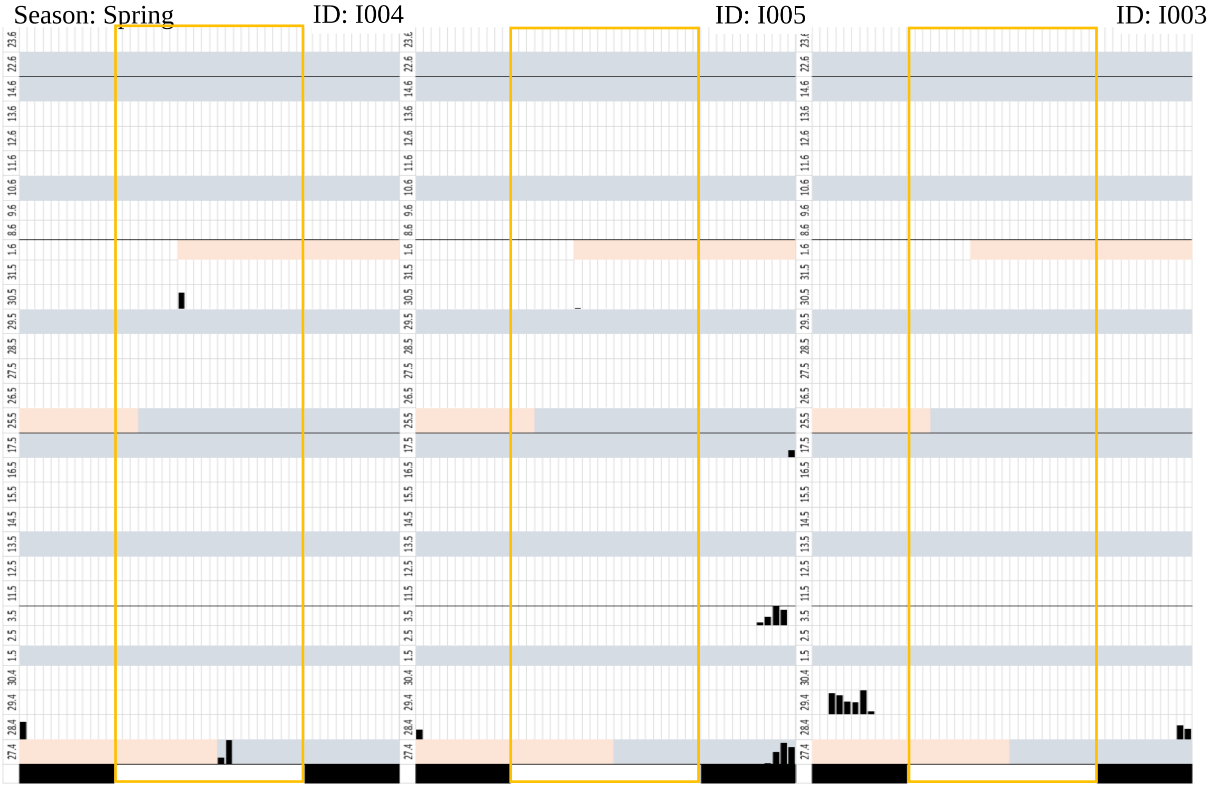


ID: I003



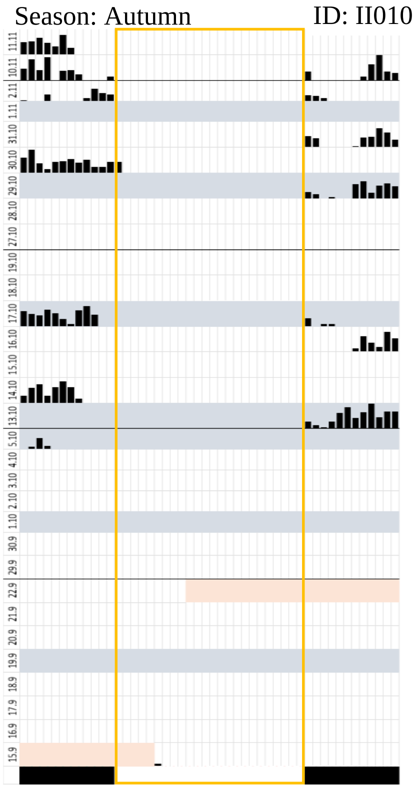


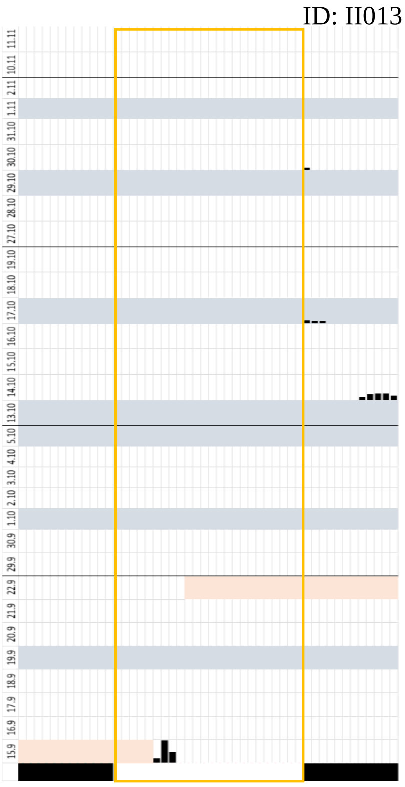
Season: Spring ID: I004 ID: I005 ID: I003

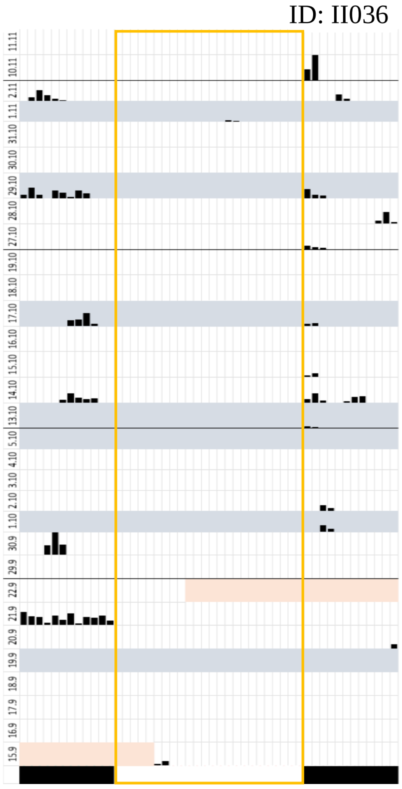


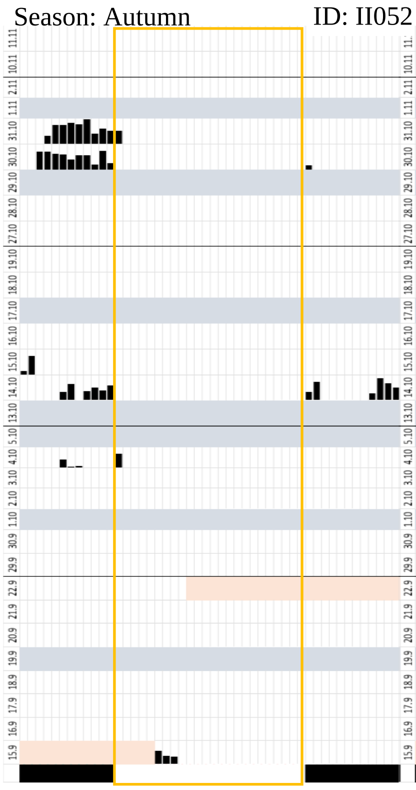
Season: Spring ID: I004 ID: I005 ID: I003

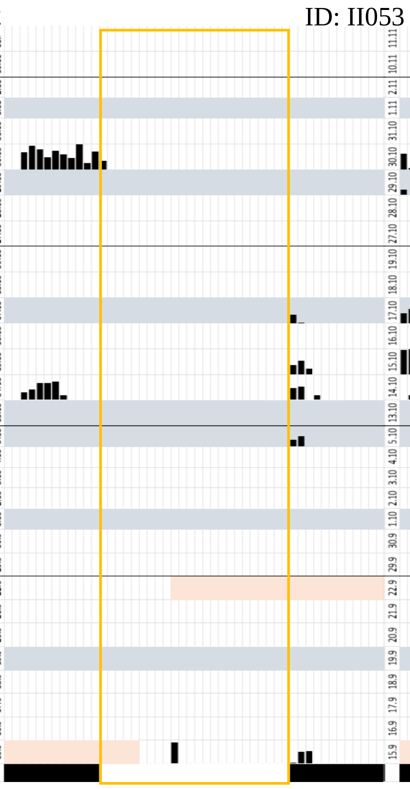
Actogram: Autumn

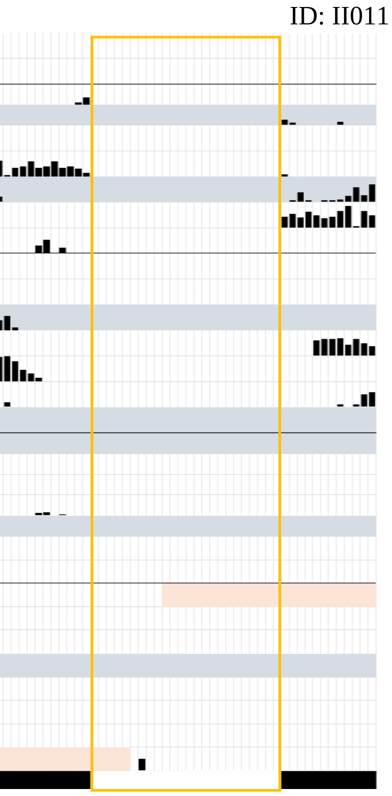


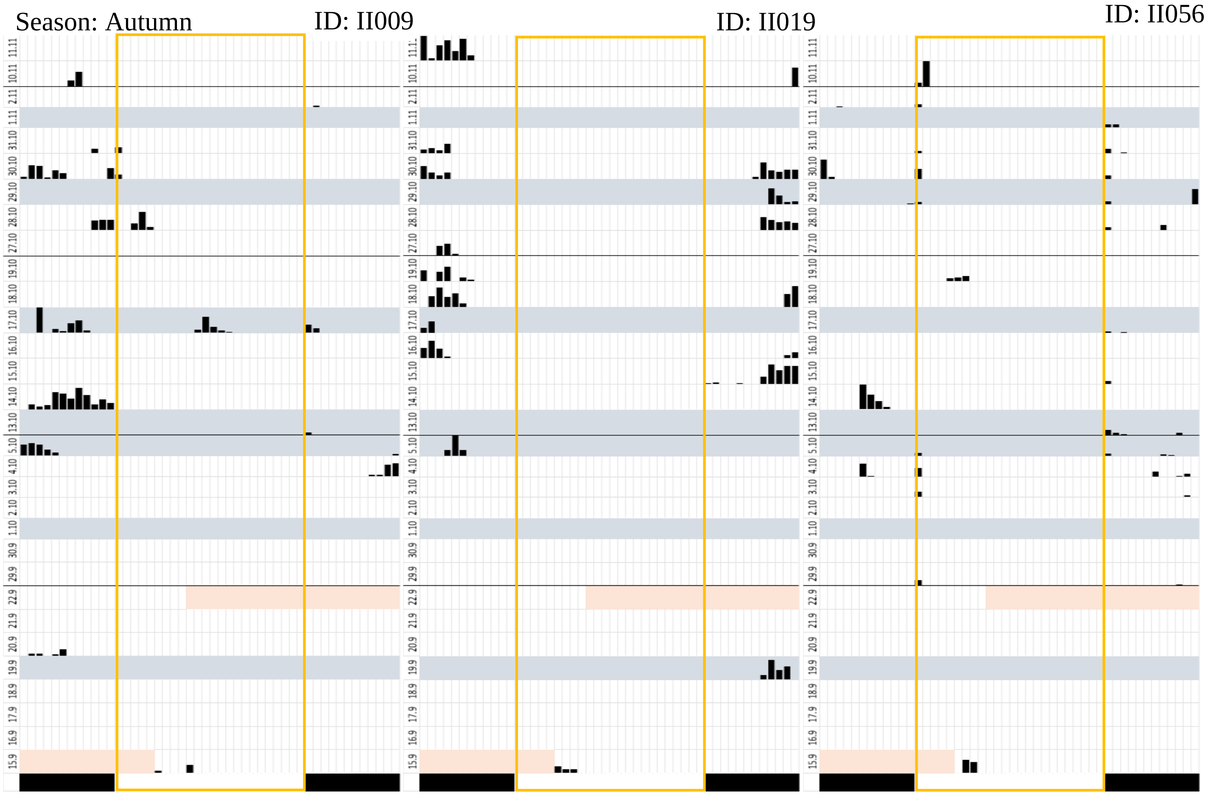








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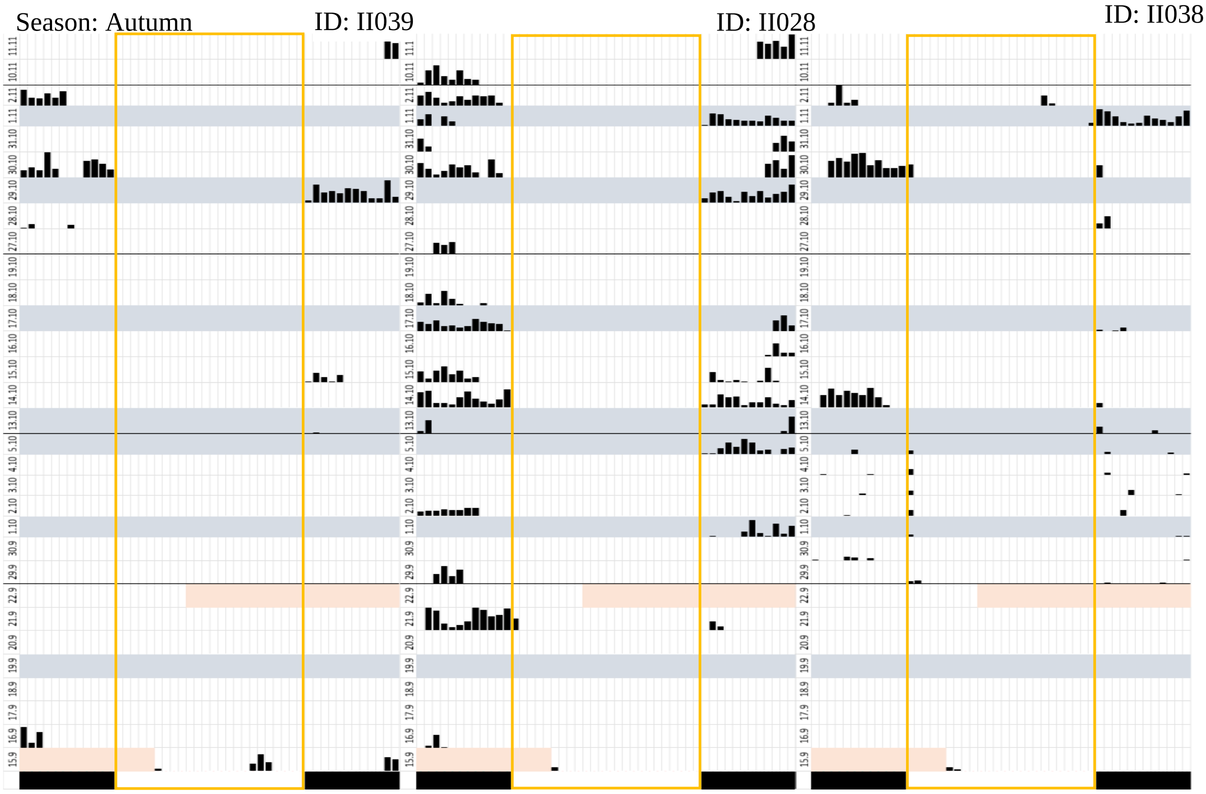
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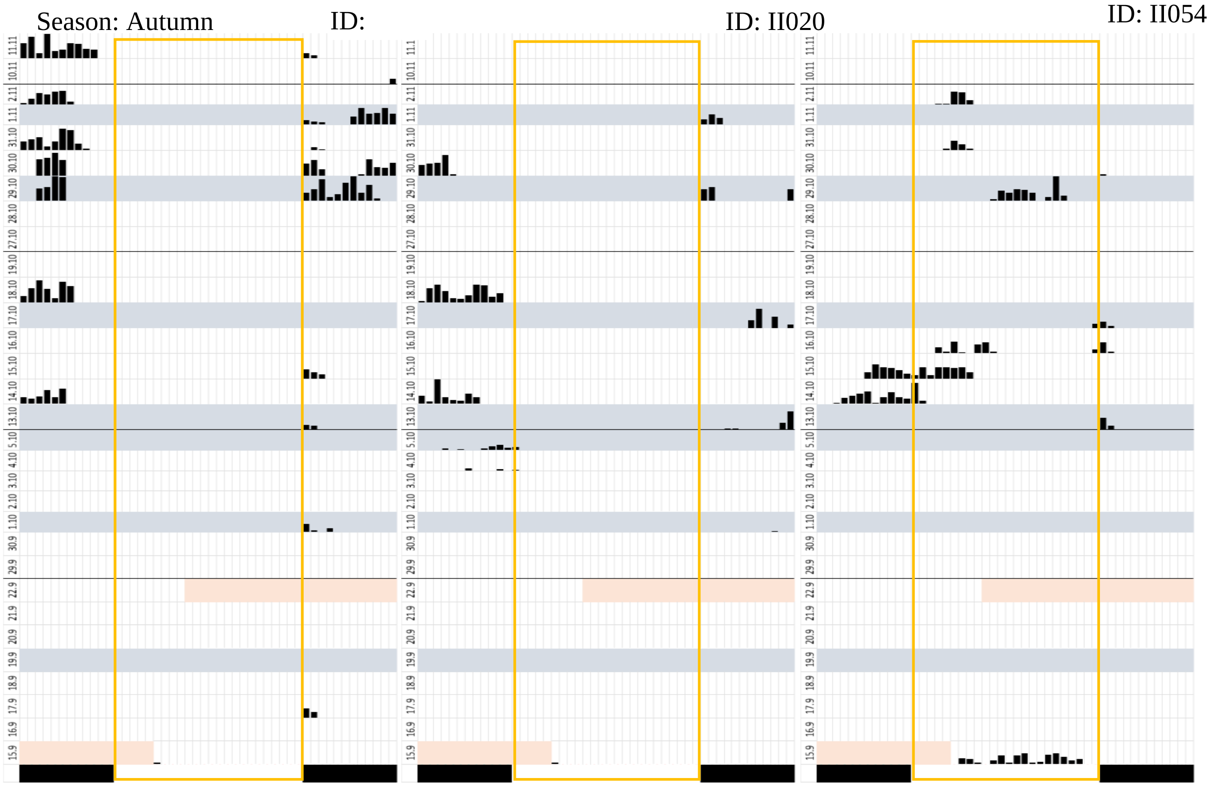
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Supplementary Materials B: Tables

Table S1: Ethogram of all behaviours with description coded for the video analysis in BORIS.

Behaviour type	**Code**	**Description**	
State event	Start of the observation		
State event	Night activity	Focal individual is active during the night	
State event	Night inactivity	Focal individual is inactive during the night	
State event	Night out of sight	Focal individual is outside but not visible during the night	
State event	Day activity	Focal individual is active during the day	
State event	Day inactivity	Focal individual is inactive during the day	
State event	Day Out of sight	Focal individual is outside but not visible during the day	
State event	Neutral zone activity	Focal individual is active in the neutral side	
State event	Neutral zone inactivity	Focal individual is inactive in the neutral side	
State event	Neutral zone out of sight	Focal individual is in the neutral side but not visible.	
State event	Moss side activity	Focal individual is active at the moss side	
State event	Moss inactivity	Focal individual is inactive at the moss side	
State event	Moss out of sight	Focal individual is in the moss side but not visible.	
State event	Tissue zone activity	Focal individual is active at the tissue side	
State event	Tissue inactivity	Focal individual is inactive at the tissue side	
State event	Tissue out of sight	Focal individual is in the tissue side but not visible.	
State event	Water dish	Focal individual spends time inside the water	Not used in analysis
State event	brick	Focal individual spends time on the brick	Not used in analysis
State event	wetbox	Focal individual spends time on the wetbox	Not used in analysis
State event	First choice	Time needed in video 1 after habituation to choose a hiding spot	Not used in analysis

Table S2: Test statistic of the Shapiro – Wilk – Test for the data sets temperature and humidity. If the p-value is < 0.05, the hypothesis that the data is normally distributed, is rejected.

Data	**W**	**p-value**
Temperature	0.9773	0.001922
Humidity	0.889	2.877e-11

Table S3: Estimated state-dependent parameters, i.e. the mean, standard deviation (sd) and the probability of having 0 activity (z), of the zero-adjusted gamma distribution for the activity data. State 1 indicates inactivity and state 2 activity.

	State 1	**State 2**
Activity mean	0.726	4.754
Activity sd	0.557	3.783
Activity z	0.998	0.051

Table S4: Transition probability matrix between state 1, when fire salamanders were inactive, and state 2, when they were actively moving.

	State 1	**State 2**
State 1	0.995	0.005
State 2	0.176	0.824

Table S5: Descriptive data of temperature and humidity for both seasons summarised. Including the number of observations (n), the mean (x̄), the standard deviation (sd) and the median.

Temperature	**n**	x̄	**sd**	**median**
Natural	71	13.17	0.56	13.1
Artificial	71	13.55	0.43	13.5
Wetbox	66	13.13	0.44	13.2

Humidity				
Natural	71	90.11	5.87	90
Artificial	71	83.37	4.34	83
Wetbox	66	99.18	0.39	99

Table S6: Descriptive data of the proportion of the daily hiding for both seasons and all individuals summarised. Including the number of observations (n), the mean (x̄), the standard deviation (sd) and the median.

	n	**x̄**	**sd**	**Median**
Daily Hiding				
natural	30	00.91	0.19	1
wetbox	30	0.08	0.19	0
brick	30	0	0.01	0

Table S7: Descriptive data of the percentage of surface activity for both seasons and sex individually. Including the number of observations (n), the mean (x̄), the standard deviation (sd) and the median.

	n	**x̄**	**sd**	**Median**
Natural				
Spring	70	3.86	7.38	2.15
Autumn	191	8.92	10.6	5.37
Artificial				
Spring	70	3.32	7.41	0.52
Autumn	191	2.78	3.53	1.35
Neutral				
Spring	70	2.47	5.94	0.9
Autumn	191	3.04	5.08	1.26
Sex				
Natural				
female	143	9.9	12.35	4.64
male	104	4.76	5.26	3.48
Artificial				
female	143	2.84	5.75	0.49
male	104	3	3.55	1.72
Neutral				
female	143	2.74	5.91	1.01
male	104	3.03	4.57	1.21