EAZA Amphibian Taxon Advisory Group

Best Practice Guidelines

(striped) fire salamander, *Salamandra salamandra (terrestris)*

First edition, July 2021

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Published: 2021

EAZA Best Practice Guidelines disclaimer

EAZA Preamble

Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the “Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria”. These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country. Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

Summary

EAZA Best Practice Guidelines (Striped) fire salamander, *Salamandra salamandra (terrestris)* is the first version of the EAZA Best Practice Guidelines for this species. This guideline has evolved out of the growing concern for extinction of local fire salamander populations due to the introduction of the invasive chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) into Europe. Multiple populations of *Salamandra salamandra terrestris* have collapsed in north-western Europe. Upon the discovery of Bsal, and associated mass mortalities, a captive assurance colony was established in the Netherlands at GAIA Zoo and later also in Rotterdam Zoo. A studbook is managed in ZIMS by GAIA Zoo. In the face of continuous spreading of Bsal into new areas within Belgium and Germany, both countries aim to develop similar ex-situ programs. To ensure collaboration, shared goals and to effectively share knowledge and resources, the multidisciplinary 'Ex-situ Salamandra Group' (ESG) was initiated by scientists, NGOs and zoos from the three bordering Bsal affected countries. Close collaboration and mutual commitment between all partners involved is the strength of this group.

For this ex-situ program, it is necessary to collect available scientific knowledge on genetics, ecology and behaviour, and translate them into practical ways to keep and possibly in a later stage also breed the species. The development of a scientifically based and EAZA (European Association of Zoos and Aquaria) approved husbandry protocol for *S. s. terrestris,* as lies in front of you, is a first product. The complete literature list can be found at the end of this document.

This document consists of two sections:

• Section 1. Biology and field data: this part reflects the taxonomic information and information about the subspecies *Salamandra salamandra terrestris* as this subspecies has been the best studied of all subspecies and it is the focus subspecies for the ESG. It includes data on natural habitat, ecology, behaviour, diet and reproduction;

• Section 2. Zoo management: this part includes suggestions about the enclosures, feeding, social structure, breeding, handling, transportation, veterinary problems of the fire salamander and recommended research to extent and improve this guideline.

These Best Practice Guidelines are for current keepers who wish to expand their knowledge about this species to take care of the animals in the best possible way, but also for future keepers need for basic information. It is recommended to consult the guidelines and to contact the TAG-members for any questions or problems.

Acknowledgements

We would like to thank the colleagues and other experts who have contributed to compose these Best Practice Guidelines by sharing their experiences. All members of the Ex-situ Salamandra Group (ESG) are thanked for their positive comments and support. Their logos are on the front of this document.

Special thanks go to Matt Goetz (Durrell Wildlife Conservation Trust / Jersey Zoo), Gerardo Garcia (Chester Zoo), Olivier Marquis (Paris Zoo, Muséum National d'Histoire Naturelle de Paris), Mark de Boer (Rotterdam Zoo), Benjamin Tapley (Zoological Society London) and Jay Redbond (Chester Zoo) for their extensive review and help. Alex Kloor (the Netherlands), Rodrigo Palacios (Spain), Billy Head (United Kingdom), Philip Gerhardt (Germany) and Caroline van der Ploeg (Boschhoven Clinic for Animals, the Netherlands) are thanked for providing important photo's.

We thank Uwe Seidel (Germany) for constructive discussions regarding this document.

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Citation: Bogaerts, S., S. Lötters, A. Spitzen-van der Sluijs, K. Preiβler, B. Caspers, P. Oswald, C.J. Michaels, T. ter Meulen, T. Reinhardt, A. Martel & F.Pasmans, 2021. EAZA Amphibian Taxon Advisory Group, Best Practice Guidelines (striped) fire salamander, *Salamandra salamandra (terrestris*). - *First edition*. European Association of Zoos and Aquariums, Amsterdam, The Netherlands.

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Striped fire salamander (*Salamandra salamandra terrestris*) coming out of its hide (Frank Pasmans).

Section 1. Biology and field data

1.1 Taxonomy

ORDER: Caudata Fischer von Waldheim, 1813
FAMILY: Salamandridae Goldfuss, 1820
GENUS: *Salamandra* Laurenti, 1768
SPECIES: *Salamandra salamandra* Linnaeus, 1758
COMMON NAMES: Fire salamander (English), Feuersalamander (German), Salamandra común (Spanish), Salamandre tachetée (French), Salamandra pezzata (Italian), Vuursalamander (Dutch)
SUBSPECIES: *Salamandra salamandra terrestris* Lacépède, 1788
COMMON NAMES: Striped fire salamander, banded fire salamander (English), Gebänderter Feuersalamander (German)

Note to the reader: These Best Practice Guidelines focus on the subspecies *Salamandra salamandra terrestris* as this subspecies is subject to the outbreak of the chytrid fungus *Batrachochytrium salamandrivorans* (Bsal). Most available ecological studies also refer to this subspecies. Besides the subspecies *S. s. terrestris*, 12 other subspecies of *S. salamandra* exist, as well as several related species (*S. atra, S. lanzai, S. algira, S. corsica* and *S. infraimmaculata*) (Sparreboom, 2014). Although many aspects of their ecology, morphology, life history and behaviour are quite similar, others may differ from that of the (sub)species discussed here. These in general are influenced by the habitat and climate in which they occur. For instance, the surface activity of many Mediterranean *Salamandra* species and subspecies is often depending dependent on winter rainfall. In summer they are not active at the surface. In nature, high altitude species like *S. lanzai* and *S. atra* have a very short activity season in summer of sometimes only a few months. This means that there are some differences in husbandry practices such as reproduction cycles compared to *S. salamandra*. However, the basic management when it comes to the enclosure, feeding, handling, biosecurity etcetera is, in our experience, the same for all *Salamandra* species. In fact, most of the other species or subspecies mentioned have been kept and bred successfully under similar conditions as described here for *S. s. terrestris*. We used photos of *S. s. terrestris* as much as possible but photos from other species or subspecies were used if none were available.

1.2 Morphology

1.2.1 Description

Salamandra salamandra terrestris is a moderate-sized, stocky subspecies of the fire salamander. The trunk is robust and slightly flattened, and the tail is almost cylindrical in cross section. The skin is smooth and shiny (Sparreboom, 2014). The tail, legs and toes are relatively short. The tail is shorter than the body (Thiesmeier & Grossenbacher, 2004). Head moderately flattened, almost as long as wide. Eyes entirely black, large and protruding, snout rounded and large and conspicuous parotids, with gland openings visible. The parotoid macroglands located behind the eyes are widely elongated. Additional parotoid glands are distributed in two parallel lines along the spine. The striped pattern is typical in most of the distribution area of *S. s. terrestris*, but in Germany it can change to spotted patterns more typical of the subspecies *S. s. salamandra*. The type locality, the place where the first specimens of this subspecies were collected, is Normandy, France. This species exhibits sexual dimorphism; males are slenderer and have longer legs and forearms than females.

Adult males can be recognised by their grossly swollen vent area in breeding season. However, this swollenness fluctuates seasonally and may not always be obvious (Feldmann & Klewen, 1981). Adult females do not show the pronounced swelling of the cloaca region and overall have a plumper build than males. Pregnant females in particular are very plump (see Fig. 1).

Figure 1. Cloaca of *Salamandra s. terrestris* male (left above and below) and female (right above and below) (Frank Pasmans).

1.2.2 Length and weight

Adults may reach a maximum total length of ca. 200 mm (tail included) but are usually smaller; males 14-16 cm and females 15-17 cm in total length (Thiesmeier & Grossenbacher, 2004). Adult males usually weigh less than 20 g and females mostly more than 20 g up to over 50 g if they carry larvae (Thiesmeier, 2004). Klewen (1985) presents a mean weight of 17.2 g for males (n = 400) and a mean weight of 21.9 g for females (n= 200). All animals were measured in summer when none of the females was gravid.

1.2.3 Colouration

The basic colour of the animals is a deep shiny black. Additionally, dorsal colouration exhibits variable yellow colour patterns that usually consist of two continuous or interrupted yellow stripes along the back. Specimens with irregular spots and without stripes are common and also occur within populations of striped specimens (Sparreboom, 2014). The striped pattern is typical for adult salamanders; juvenile and sub adult salamanders often exhibit yellow spots on the dorsum, which eventually darken in the midline to form the striped pattern. (Sparreboom, 2014).

Every individual has a unique colour pattern that can be used for individual identification starting when the animals are about 1 to 1.5-year-old (e.g. Thiesmeier & Günther 1996; Bogaerts, 2002, Speybroeck & Steenhoudt, 2017) (see also paragraph 2.5.1). The border from black to yellow is always discrete and there are usually no black markings within yellow parts (Fig. 2). Exceptions are the yellow portions of the parotoid macro glands behind the eyes that always possess tiny black spots. Ventral colouration is black with an irregular pattern of yellowish markings of variable size and amount; here, black and yellow are less discrete. Commonly, ventral surfaces of hands and feet are entirely black. Dorsal yellow bands typical range from pale to bright lemon yellow. Animals displaying an erythristic (Fig. 3) or other colour pattern have been reported from different areas but are rare (Fig. 4). Hobbyist breeders however have bred a line of amelanistic specimens for over 25 years (Concaro, 2004; Fig. 5). In addition, a breeding line originating from the Solling area, Germany, has created many odd colours in captivity (examples in Seidel et al., 2012).

Salamandra s. terrestris also exhibits sexual dichromatism. Males display more dorsal yellow coloration than females (Preißler et al., 2019). A laboratory study revealed that individuals raised as larvae under good nutritional conditions develop more yellow coloration than individuals raised under limited nutritionally constrained conditions (Caspers et al., 2020). Larvae in the poor-condition treatment group received food twice a week, while larvae in the rich-condition group received food six times a week. The amount of food (*Chironomus* larvae) given to each larva was the same at each feeding. It indicates that yellow colouration may be energetically costly to produce.

Figure 2. Different patterns of *Salamandra s. terrestris* found within one natural population (Sven Gippner).

Figure 3. *Salamandra s. terrestris*, captive bred line of specimen with orange instead of yellow bands (Sergé Bogaerts).

Figure 4. Hypermelanistic (brownish spots and stripes instead of yellow), *Salamandra s. terrestris* male found in Flanders, Belgium (Frank Pasmans).

Figure 5. Amelanistic *Salamandra s. terrestris* bred in captivity in France (Sergé Bogaerts).

Fire salamander larvae are grey-brown in colour mottled with black and metallic dots and the ventral parts are whitish (Sparreboom, 2014). They also have moderately long gills; the tail crest starts in the middle of the body; the tail-tip is blunt. From the beginning, all larvae of *Salamandra* species display distinct yellow patches at the base of their legs that make them distinguishable from other urodelan larvae (Thiesmeier, 1990) (Fig. 6). They develop the black and yellow pattern throughout metamorphosis and the first months after metamorphosis the pattern can still be changing (Fig. 7).

Figure 6. Two larvae of *Salamandra s. terrestris* in a small stream. On the left, a larger (older) one near metamorphosis; on the right, a smaller (younger) one with shorter tail (potentially due to predation/cannibalism?). The characteristic yellow markings at the base of each limb are clearly visible. Ardennes, Belgium. 20th July, 2019 (Sergé Bogaerts).

Figure 7. Three newly metamorphosed *Salamandra s. terrestris* found under one stone showing different stages in development of the typical black and yellow colouration pattern (Sergé Bogaerts).

1.3 Physiology

Salamandra s. terrestris seems to prefer temperatures between 5-20°C. When provided with sufficient humidity, *S. s. terrestris* prefers temperatures between 17-21°C (Beukema et al., 2020). However, body temperatures measured in the field during salamander activity are consistently below 17°C (Beukema et al., 2020). Even during heat waves, fire salamanders are buffered from high temperatures, with body temperatures only exceeding 20°C on very rare occasions (Beukema et al., 2020). *S. s. terrestris* can withstand temperatures a few degrees below zero, as low as -5.2°C for a few hours (Thiesmeier & Grossenbacher, 2004) and survive in temperatures exceeding 25°C. However, exposure to such high temperatures may cause stress and is only advised to use to treat Bsal infections (Blooi et al., 2015) (see paragraph 2.8).

1.4 Longevity

In the wild, specimens can live for 15-16 years (Heine & Thiesmeier, 2010), but probably 20 years and perhaps more (Thiesmeier, 2004). This age has been repeatedly attained in captive specimens, while even much older animals of 43 years (Schmditler & Schmidtler, 1969) and over 50 years have been reported (Böhme, 1979).

1.5 Biogeography, ecology and conservation

1.5.1 Distribution

Salamandra s. terrestris is known from a large geographic range over much of Western Europe (Fig. 8). The distribution ranges from northeastern Spain (Catalonia and eastern Pyrenees), France, Belgium, Luxembourg and the southeasternmost Netherlands into western and northern Germany. The striped fire salamander also occurs in Switzerland north of the Alps. The geographic range of this subspecies partly overlaps with other subspecies and natural hybrids with subspecies of *Salamandra salamandra* are known from Germany (with ssp. *salamandra* see Thiesmeier & Grossenbacher, 2004). And there are probably also hybrid zones in the Maritime Alps (with ssp. *gigliolii*) and in the Pyrenees (with ssp. *fastuosa*) (Thiesmeier & Grossenbacher, 2004). The latest most in-depth assessment of the genus *Salamandra* diversity and evolutionary relationships to date, shows that currently recognised subspecies are paraphyletic, along with over-splitting of the Central and East European populations, probably based on regional variations in phenotypic traits like colour pattern, the evolutionary diversity of *Salamandra salamandra* is highest on the Iberian peninsula (Burgon et al, 2020).

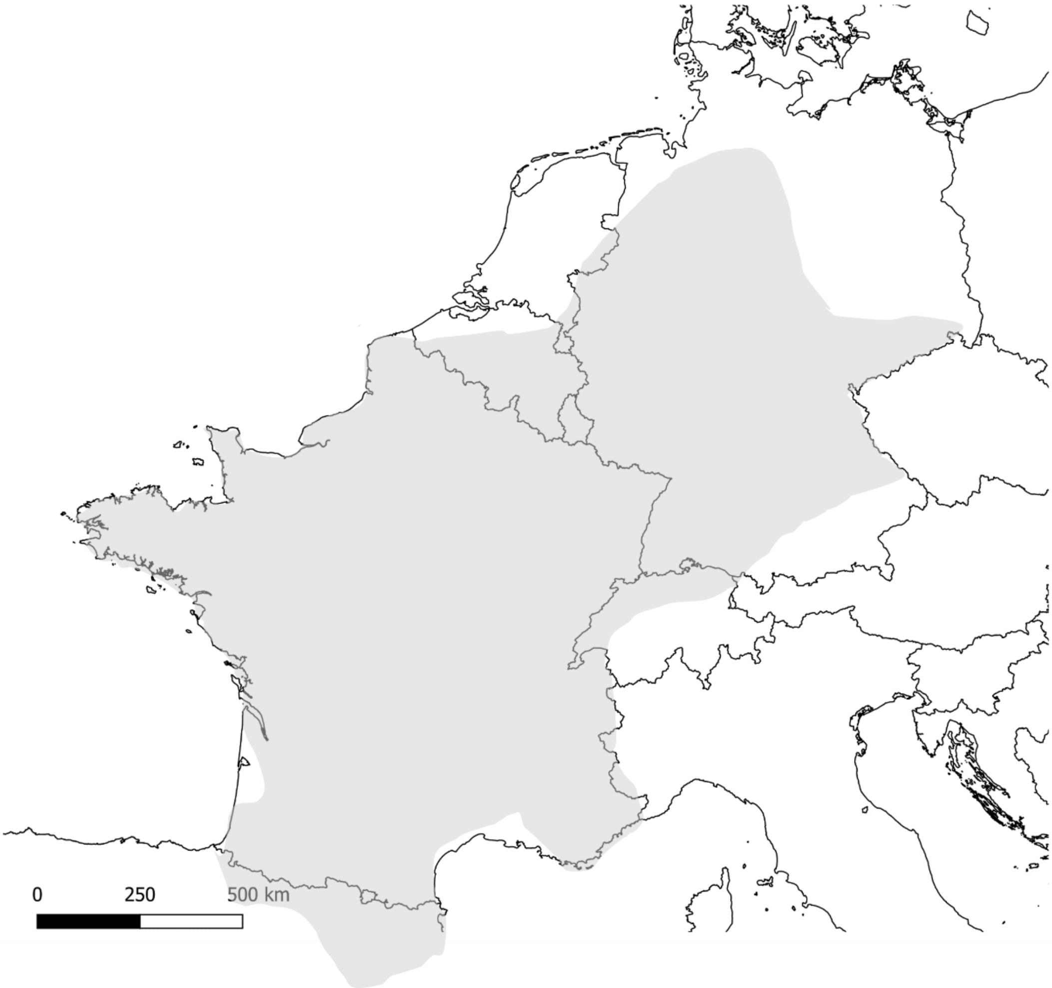

Figure 8. Potential range map of the subspecies *Salamandra s. terrestris,* including the possible hybrid zones with *S. s. fastuosa* in the Pyrenees, *S. s. gigliolii* in the Martime Alps and *S. s. salamandra* in Germany (map by Kathleen Preiβler).

1.5.2 Habitat

Salamandra s. terrestris typically inhabits moist mixed deciduous forests; with predominantly Eurasian beech (*Fagus sylvatica*). In many parts of the range, larvipositioning occurs in clean fish-free headwaters (see Fig. 9). Yet, this subspecies also uses standing waters, particularly in lowlands (but also in for instance Kottenforst, Germany, see Steinfartz et al. 2007). A typical form of standing water that can be used are water filled tracks on forest roads (Fig. 10). Local ecological differences in terms of larvipositioning sites are thus large throughout the range. Fire salamanders can also be found in anthropogenic habitats including parks or cemeteries, in which they larviposit in wells, troughs and all kinds of man-made water bodies (Thiesmeier & Grossenbacher, 2004).

Dense leaf litter layers and mossy areas are used for foraging, but adult salamanders also use roads and pathways due to improved sightlines (males in search of females) and hunting. Shelter is necessary for diurnal hiding, survival of dry periods (e.g. in summer) and overwintering. Apart from rodent burrows, these can also be rotting wood and rock crevices. Populations on limestone-rich soil, often prefer slopes with rocky cave systems or scree material. Occasionally, the salamanders hide some meters below the surface in natural rock-soil corridors to escape the dry or the cold. Also, anthropogenic structures such as house-basements, bunkers and old adits are used, sometimes even throughout the year (where breeding can take place too). The elevation ranges are reported to be 1,000-1,310 m asl in Germany and Switzerland, respectively (Thiesmeier & Grossenbacher, 2004).

Figure 9. Typical habitat of *Salamandra s. terrestris* with breeding stream in a beech dominated forest near Viroinval, Belgium (Sergé Bogaerts).

Figure 10. Temporary breeding pond in mixed forest for *Salamandra s. terrestris* near Stadtkyll, Germany (Sergé Bogaerts).

1.5.3 Population and activity range

Population densities in West and Central Europe have been estimated (with capture-recapture methods) at around 80 salamanders per hectare (Klewen, 1985) to 196 (Seifert, 1991). In very optimal conditions, local densities can exceed 400 animals per hectare (Seifert, 1991).

Despite being considered sedentary animals, active salamanders may move between 35 and 375 m per night during surface activity. Total migratory distances of up to 980 m have been recorded (references in Thiesmeier, 2004). Denoël (1996) indicates home ranges of 5-255 m^2 in Belgium. Hendrix et al. (2017) show that home-ranges of pond-adapted fire salamanders are generally larger than those of stream-adapted salamanders and that pond-adapted adults moved long distances up to 1.9 km.

1.5.4 Conservation status

The IUCN Red List of Threatened Species does not assess subspecies and the species *S. salamandra* is considered 'Least Concern' [LC] Temple & Cox, 2009). However, this assessment is considered out-of-date as it is more than 10 years old. This is sadly the same case for the many regional or national Red lists. Furthermore, the species has not been assessed since the description of Bsal and the associated catastrophic population declines.

The legal status at national levels is differentiated, but in almost every country throughout its range *S. salamandra* is protected by general or special conservation acts and laws, even though it is rarely listed in a high threat category in the national red lists or similar compendia. At the international level, *S. salamandra* is included on Appendix III of the Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats, 1979), which awards the species general protection throughout all European countries.

In case extractions of adult salamanders or larvae from the wild are planned, these extractions must always be in accordance with the national (and in many cases subnational) laws and involvement of the local wildlife management and conservation authorities.

In recent years, the subspecies *Salamandra s. terrestris* has undergone catastrophic population declines at the regional scale due to salamander chytridiomycosis. Salamander chytridiomycosis is a lethal skin disease caused by an invasive fungal skin pathogen Bsal (Fig. 11). This fungus has been introduced from Asia into Western Europe, most likely via the trade with pet amphibians. Bsal is lethal to individuals in the genus *Salamandra* (as well as for some other urodelan species) and is currently known to occur in wild populations in the Netherlands, Belgium, Spain and in Germany (Spitzen - van der Sluijs et al., 2016; Stegen et al., 2017; Martel et al., 2020; Vences & Lötters, 2020).

Salamander chytridiomycosis is an emerging infectious disease (EID). The disease is so far known from ca. 50 populations of *S. s. terrestris,* but also in a few populations of other salamandrid species (e.g. *Triturus marmoratus, Triturus cristatus, Ichtyosaura alpestris, Lissotriton vulgaris*). Monitoring data, as far as available, indicate that most of these populations were free from Bsal up until recently. Although at most outbreak sites, fire salamanders are still found in low numbers, it is expected that pathogen invasion may lead to total extirpation of fire salamander populations in the long-term. The fungus may persist in the system in alternative hosts (such as anurans, newts or perhaps invertebrates) and reservoirs (including soil and water). The spread of Bsal and the associated patterns of local extinction risk are the reason for proposing ex-situ conservation measures (Martel et al., 2013, 2014; Spitzen-van der Sluijs et al., 2016; Stegen et al., 2017; Canessa et al., 2018; Dalbeck et al., 2018). On the website http://www.bsaleurope.com/, the most recent knowledge of Bsal research, distribution and detections and outbreaks is presented.

Figure 11. *Salamandra. s. terrestris* with Bsal infection. Note the rests of shed skin on the dorsal and lateral side and the typical black edged holes on the yellow parts of the head created by the fungus (Frank Pasmans).

The combination of an iconic and charismatic species like the fire salamander and the extirpation of populations in Western Europe gives institutions opportunities to develop education programs with other partners in the field. Both GaiaZOO and Rotterdam Zoo have display panels and these provide information to the public about the current situation and their role and cooperation with the non-governmental organisation, Reptile, Amphibian, Fish Conservation Netherlands (RAVON) in conservation of the species in the Netherlands (see paragraph 2.1.9).

1.6 Diet and feeding behaviour

1.6.1 Food preference

Like other salamanders, *Salamandra s. terrestris* feed on a wide array of arthropods, worms, insects and molluscs (slugs and snails). Prey items can be relatively large, as long as they can be swallowed. Occasionally even other amphibians are also preyed on if small enough, including conspecific juveniles (Pasmans et al., 2014; Seidel & Gerhardt, 2016).

1.6.2 Feeding

The Striped fire salamander is an opportunistic hunter performing ‘sit-and-wait’ predator behaviour. Orientation towards prey is visual, so that prey movement is the stimulus that triggers feeding attempts. The olfactory sense might also play a role, but this is little explored (see Thiesmeier, 2004). Research has shown that juveniles in the first months learn a preference for either slow or fast moving prey items (Luthardt-Laimer & Roth, 1983).

1.6.3 Adult fire salamander nutrition in nature

Several studies on the diet of wild fire salamanders identified prey items that were collected by either stomach flushing or that were removed from the stomach of dissected salamanders (for overviews see Thiesmeier, 2004; Thiesmeier & Grossenbacher, 2004). DNA-metabarcoding was recently applied to fire salamander faeces to estimate prey diversity (Smith et al., in prep.). The findings of all these studies are broadly congruent. Fire salamanders are opportunistic feeders that consume a high diversity of invertebrate taxa. However, Laking et al. (in prep.) recently demonstrated pronounced inter individual differences in prey preference when the salamanders were given a choice. All salamanders consumed woodlice at a low frequency in an experimental setup, some animals clearly preferred gastropods while others preferred annelid worms or mealworms. The implications for the animal's fitness are not clear but may point to different nutritional requirements (or this may be simply a matter of taste, habit or learned behaviour). Besides the consumption of insects, arachnids and worms (Annelida), gastropods and millipedes and/or centipedes are highly prevalent in wild salamanders' diets. Snails, millipedes and possibly centipedes may provide the most significant natural source of calcium, which is typically very low in most insects (Finke, 2002). The natural prey items are typically high in protein, probably with highly variable proportions of fat (depending on invertebrate species and life stage) and low in primary carbohydrates (<10% DM, Finke 2015, Oonincx & Dierenfeld, 2011), which is in line with suggestions that propose lipids and proteins as main drivers of amphibians' energy metabolism (Brenes Soto & Dierenfeld, 2014).

1.6.4 Nutrition of wild salamander larvae

An excellent overview of *S. salamandra* larval diet can be found in Thiesmeier & Grossenbacher (2004). Similarly, to adult fire salamanders, the diet of larvae roughly reflects availability of prey. Diet depends on the water body (lentic or lotic), implying substantial differences in available prey items between habitats (Bletz et al., 2016). Fire salamander larvae detect prey exclusively by movement but can ingest prey items up to about their own body size.

Aquatic insect larvae (e.g. Chironomidae, Plecoptera) and crustaceans (Amphipoda, Copepoda, Cladocera, Ostracoda), Gastropoda and Annelida but also amphibian larvae (including conspecifics) and terrestrial arthropods (fallen on the water surface) may be consumed (Reinhardt et al., 2015). However, observations under natural conditions have shown that a diet of exclusively plankton crustaceans (Copepoda and Cladocera) was not sufficient to sustain a significant growth to metamorphosis but instead triggers diverging feeding strategies and larvae became cannibalistic or ingested terrestrial prey items to reach metamorphosis (Reinhardt et al., 2017).

1.7 Reproduction

1.7.1 Age of sexual maturity

The age at which fire salamanders are sexually mature varies between the sexes. Males can start breeding at 3-4 years of age, females are mostly 5-6 years old when they first mate (Seifert, 1991; Thiesmeier, 2004).

1.7.2 Seasonality/reproductive cycle

Mating can take place from spring into autumn and may throughout the distribution range. Klewen (1985) found that 40% of observed matings took place in July. Larvae are often deposited in spring (March to May), but in some lowland populations in Germany, Belgium and France larval deposition can also occur in autumn or winter (Denöel, 1996; Thiesmeier, 2004; Fig. 12). Furthermore, there is evidence for different genetic and ecological adaptations within populations for either pond- or stream breeding in females in the Kottenforst, Germany (Caspers et al., 2015). Females may also show preference for males from the same breeding-habitat type (Caspers et al., 2009). Pond-breeding females may have larger home ranges, show longer dispersal distances as well as longer larval deposition periods compared to stream-breeding females (Hendrix et al., 2017).

Males may mate with several females, and vice versa. Females may mate with up to four males to increase reproductive success (Caspers et al., 2014b). In some populations 'rival combat' by males is observed, where the same males can be found in the same location each year defending their territory against other males (for details and review see Gerhardt, 2017).

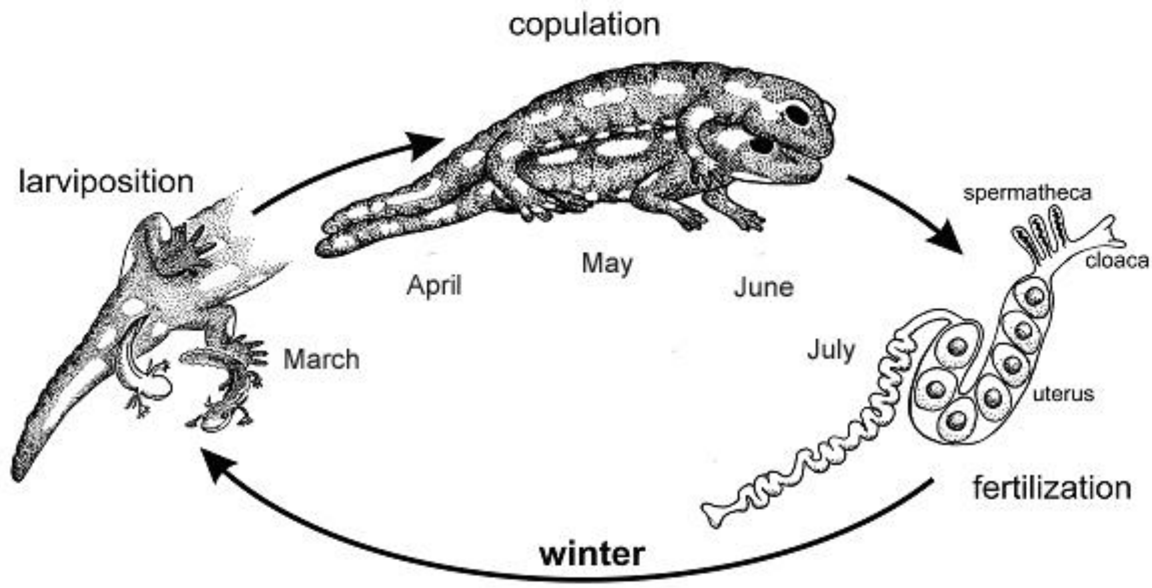

Figure 12. Annual life cycle of *Salamandra s. terrestirs* females (Ulrich Pörschmann).

1.7.3 Sperm storage

Females can store sperm in a specific sperm storage organ- the spermatheca. The sperm of different males is mixed and not layered (Caspers et al., 2014b). Sperm can be stored for several years (two years under laboratory conditions; Caspers, pers. observations), but sperm storage over long periods is probably rare under natural conditions (Steinfartz et al., 2006).

Figure 13. Historic drawing by Mauro Rusconi (1854) showing the different stages in larval development.

1.7.4 Life cycle from birth to metamorphosis

Female fire salamanders do not lay eggs but release relatively advanced larvae directly into streams and ponds. These larvae leave the egg membrane during the birth process, a reproductive trait called larviposition (Greven 1998). The larvae already have four fully developed legs and are deposited at lengths of 25-35 mm (Krause et al., 2011). It may take a female 1-18 days to deposit all the larvae and she may distribute larvae over different water bodies. The tendency for this bet hedging strategy has been shown to differ between pond and stream breeding fire salamanders in the Kottenforst (Caspers et al., 2015). Stream-breeding females typically use a lower number of deposition events than pond-breeding females. In stream breeding females the larvipositioning phases are commonly associated with events of medium to heavy rain, and flash floods are the most common cause of mortality in stream fire salamander larvae (Reinhardt et al 2018).

Depending on water temperature, water quality, food availability and (probably) predator presence, larvae metamorphose at a total length of 42-65 mm (Thiesmeier, 2004) and under laboratory conditions at an age of 61-110 days (Krause et al., 2011). Sometimes *Salamandra s. terrestris* larvae can grow to up to 2 g fresh body mass before metamorphosis, however the minimum threshold at which a successful metamorphosis can be triggered by clues such as food scarcity, high water temperatures and low water levels is at 0.7 g fresh larval body mass (Reinhardt et al., 2013).

Size/Body mass and colouration at metamorphosis is strongly dependent on the nutritional conditions during larval development (Caspers et al. 2020). In the wild metamorphosis can take place from April till late August but some larvae, particularly those developing in standing and perennial waterbodies, may overwinter and metamorphose in spring of the following year (Reinhardt, pers. observation).

1.7.5 Clutch size

The number of larvae depends on the size and age of the female. Klewen (1985) describes an average of 32 larvae/female, but others report 33 or 22.6 larvae/female (Thiesmeier, 1990; Kopp & Baur, 2000). In Nord-Rhein Westphalen (Germany), litter size ranged between 8-71 larvae (Thiesmeier, 2004).

1.8 Behaviour

1.8.1 Activity

Salamandra s. terrestris is primarily nocturnal. Diurnal activity is only occasionally observed. In general, the activity period is determined by air temperature (above 3°C, ideally between 8-12°C), rainfall and air humidity (75-80%, ideally above 96%). In mild winters and lowland regions, salamanders can be found active throughout the winter. This also applies to animals hiding in adits (Bellenoue et al., 2006; Leeb, 2013).

In regions where winters are harsh, there is a stricter regime. However, activity is very much dependent on daily weather conditions where rain (high humidity) is necessary for activity above ground. Leeb (2013) found salamanders active throughout winter going in and out of brumation. Nothing is known about diurnal activity when the salamanders are in their shelters underground (Thiemeier & Grossenbacher, 2004). In addition to brumation, many *S.s. terrestris* populations are not surface active during the warm summer months (from June to September). This period of inactivity is followed by another peak of activity in autumn until the first frost (Thiesmeier, 2004).

1.8.2 Locomotion

Terrestrial *Salamandra s. terrestris* are slow ground walkers. They can climb, e.g. along rocky cliffs, but do not have an arboreal lifestyle. Adults and metamorphs can swim short distances but can drift or drown easily. Usually water is visited only by females for the release of larvae (Thiesmeier & Grossenbacher, 2004).
Fire salamander larvae swim in a matter similar to the adults using their tails with limbs pulled towards the body when fast movements are needed. General larvae move by slowly walking along the substrate.

1.8.3 Predation

Larvae are known to be predated by insect larvae (like Odonata), crustaceans, fish (especially introduced salmonids), birds and conspecifics (Caspers & Oswald, pers. observation), but even by other amphibians like the Montseny brook newt, *Calotriton arnoldi* (Gerardo Garcia, pers. comment). Fire salamanders produce a skin poison consisting of steroidal alkaloids and multiple other compounds like biogenic amines, peptides and sterols (Lüddecke et al., 2018). The production of alkaloids starts during metamorphosis, although poison concentrations are much lower in larvae and juveniles (Sanchez et al. 2018). Though it is assumed that predation risk is highest shortly after metamorphosis, with carabid beetles and birds but also rodents being the main predators (Caspers et al., 2020), little is known about predation on juveniles and adults. Despite anecdotal records of adults that were eaten by rats, badgers, wild boars and grass snakes, fire salamanders appear relatively safe from regular predation due to their toxicity (Thiesmeier & Grossenbacher, 2004).

1.8.4 Sexual behaviour

According to Sparreboom (2014), a sexually active male stands in an alert posture, with forelimbs extended and head raised. He rapidly pursues a moving female. When the male reaches the female, he nudges her, sometimes going partly astride her dorsum in the process. In some cases, the male may clasp the female's dorsum with his forelimbs while nudging her. Such dorsal clasping is relatively rare in *S. salamandra*. More often the male simply nudges the female and then attempts to clasp her from the ventral side by pushing himself under her body and hooking his forelimbs around hers. Once the male has engaged the female in ventral amplexus, he begins making undulating and shifting movements with his body and tail, a behaviour pattern termed body-shifting (Fig. 14). The male slightly raises his head, laterally swings his head back and forth, rubbing the upper side of his head on the female's chin. The onset of spermatophore deposition is marked by the cessation of body-shifting. The female increasingly presses her vent against the male's tail base, so that the distal part of her tail arches slightly upwards. Sperm transfer is accomplished during ventral clasping. The male deposits one spermatophore. He lifts his vent off the spermatophore and moves his sacrum and tail to the side and so out from under the female. Her vent drops partway towards the spermatophore. The male then draws his sacrum and tail forward under the female's femur without causing her to move her leg. The effect is that the female may thus settle upon the spermatophore, inserting the entire structure in her cloaca. Sometimes the female drops down beside the spermatophore and may lift up and settle upon it, apparently orienting tactually to it (Arnold, 1987).

Figure 14. Amplexus of *Salamandra s. terrestris*, Hesssen, Germany, October 2018. Male below carrying the female before depositing the spermatophore (Philip Gerhardt).

Section 2. MANAGEMENT IN ZOOS AND AQUARIA

2.1 Enclosure

In principal, three types of set-ups can be used successfully to keep, raise and breed fire salamanders. Each of these systems (outdoor enclosures, mainly biotic indoor enclosures and mainly abiotic indoor enclosures) will be briefly discussed and their respective advantages and disadvantages pointed out, including possibilities for public display.

2.1.1 Space requirements and density

Adults The "Minimum Requirements for the Keeping of Amphibians" ("Mindestanforderungen für die Haltung von Amphibien", developed and published by the Swiss chapter of the German herpetological society DGHT Landesgruppe Schweiz, year unknown) recommends housing a single salamander in an enclosure not less than 4 x 2 x total length of the salamander, with an additional 2 x 1 x total length for every additional animal. With a maximum total length of 20 cm for an adult fire salamander, that would result in a minimal enclosure surface of 80 x 40 cm^2 for one animal and 100 x 40 cm^2 for an adult pair. However, these are not strict rules and structuring within the surface can create more space (see Schorn & Kwet, 2010; Seidel & Gerhardt, 2016). The height of the enclosure is less important for these ground dwelling salamanders but some vertical structuring of the terrarium content (for example piles of bark) increases the usable surface area significantly. Since males can exhibit aggressive and territorial behaviour, enclosures should house a single only. Females can be kept in groups without apparent negative effects. If fire salamanders are kept in self-sustaining systems (for example outdoor enclosures in a natural forest habitat where no food is added), an enclosure of 2.4 x 1.2 m^2 proved sufficient to keep one adult male salamander in good health and condition for at least one and a half years, with increased body mass (Laking et al., 2021). Whether this is sufficient for reproducing females remains to be determined.

Larvae Larvae can be kept successfully through metamorphosis at densities up to 1 larva per half litre of water (see for rearing larvae paragraph 2.4.2). The determining factors for densities here are water quality and feeding regime. For water quality, pH should preferably be between 6 – 7 (Thiesmeier, 2004), water hardness may vary between 8-18°dH (German hardness) or 15-32 fH (French hardness), softer water being more prone to pH fluctuations. The maximum levels are for ammonia: 0.02 mg/l, nitrite: 0.1 mg/l and nitrate: 50 mg/l but for ammonia and nitrite, an absence should be the goal (Pasmans et al., 2014). Larvae should be fed ad libitum to avoid cannibalism (see paragraph 2.2.2). Dead prey items that may quickly affect water quality should be removed as soon as possible (Pasmans et al., 2014).

Juveniles Space and density requirements are similar to those mentioned for adults. Juveniles can be kept in groups of similar sized individuals, up to an age of 3 years when they can become sexually mature, after which point territoriality and other aggressive behaviour usually leads to the decline of less dominant animals (Pasmans et al., 2014; Seidel & Gerhardt, 2016).

2.1.2. Outdoor enclosures

Large (± 1 m^2) outdoor enclosures can successfully house fire salamanders with minimal investments in animal maintenance. Such enclosures should prevent animals from escaping, should prevent predators and wild amphibians from entering, and should provide sufficient refuges from heat, cold, flooding and drought. Fire salamanders climb well and the enclosure walls should be smooth and topped with at least 10 cm wide margins. The wall should be inserted in the soil at least 50 cm and should preferably rest on metal wire fence that covers the entire bottom of the enclosure and prevents rodents and others mammals, such as moles and shrews, from digging into the enclosure (which provides escape routes for the salamanders) and which may threaten animals directly by predation.

Location is important and should neither allow fast desiccation of the enclosure nor flooding. A shaded area or artificial shading is recommended. Vertical structuring can be provided by adding a pile of roof tiles, bark and or branches to a depth of minimum 50 cm but preferably of 1 meter. A large part of the surface can be covered with beech leaves to maintain moisture and temperature of the underground retreats. A shallow and easily accessible water container should always be present. Preferably, the enclosure should be fenced off against predators (birds, mustelids, rats *inter alia*) using a wire fence cover, for instance 0.4 x 0.4 cm is used in Gaia ZOO. The latter should not contain Zinc since this may intoxicate the animals and should be rust-proof if metal. Plants can be added to further stabilise the enclosure's climate. If made sufficiently large and constructed well, such systems can be self-sustaining to some degree, without the need of adding food, although population size will still require management (see example Fig. 15).

Figure 15. Natural furnished outdoor enclosure in the shade and public display at GaiaZOO, Kerkrade, the Netherlands. Surface 2 x 2 meter, with 0,8 m height in the back to 0,6 m in the front (Kathleen Preißler).

Advantages:

1. If constructed in the natural range and microclimate, optimal temperature, lighting and moisture regime, closely resembling the situation in nature. Animals are exposed to an environment, similar to the natural one and live natural lives.
2. Requires very little maintenance.
3. Can be furnished to obtain aesthetically pleasing and educational exhibits.

Disadvantages:

1. Higher cost for construction.
2. Biosafety more difficult to guarantee and never 100% waterproof. In practice, there are no indications that salamanders have been infected inside such enclosures with ranaviruses or chytrid fungi from the outside (without the aid of a vector amphibian) but enclosures must be constructed such, that no wild amphibians can enter. Also, the natural enrichment should be biosecure.
3. Possibly the biggest disadvantage is the lack of easy assessment of the inhabitants. Especially during summer and cold periods during winter, fire salamanders show little to no surface activity and months may pass without being able to observe the animals. Second, monitoring the animals should be done at night, which may pose logistic problems. Artificial rainfall can induce activity.
4. Vulnerable to natural disasters (for example trees falling, extreme weather events).

Due to the fact an escape is possible, outdoor enclosures are only recommended within the range of the subspecies.

2.1.3. Mainly biotic indoor enclosures

Such enclosures are the typical terraria, with an organic soil (see Fig. 16 & 17). The terrarium can be constructed from any non-toxic material (glass, plastic). Transparent walls allow easy inspection of the terrarium. Salamanders are escape artists; even plump adults can easily escape in the corners of glass terraria. The terrarium should preferably be placed in a room that mimics natural, optimal conditions of temperature and humidity, following the animals' natural cycle (see paragraph 1.7.3). Although a variety of substrates can be used, clay, loess or sandy clay has several advantages: it keeps moisture well, facilitating the creation of a moisture gradient, faeces are easily visible to be removed, and this substrate requires only occasional replacement. Other organic soils (e.g. forest soil, humus) may be more prone to need rapid changes (e.g. fungal growth). Adding hiding places, a water container and preferably dried (beech) leaves and some extra structure (e.g. branches) results in a suitable environment for fire (and many other) salamanders. Providing some form of soil ventilation (for example by lining the enclosure with a filter mat used for garden ponds) seems beneficial and reduces the risk of build-up of excessive moisture and anaerobic environments (see Pasmans et al., 2014; Seidel & Gerhardt, 2016). Although it is shown that substrates differ in bacterial communities (Michaels et al., 2020) there is no evidence yet that this has implications for fire salamanders, as the microbiome of the skin of fire salamanders has only very few bacteria under natural conditions (Bletz et al., 2017).

Advantages:

1. Requires manageable amount of maintenance, also for large collections.
2. Less prone to quick environmental deteriorations compared to the abiotic systems.
3. May be furnished to create aesthetic enclosures.
4. Facilitates the easy monitoring of the inhabitants.
5. Relatively cheap construction (depending on the enclosure).
6. Stricter biosecurity can be achieved (yet more laborious to dispose waste).
7. Escapes and predation can be easily avoided.
8. Not vulnerable to natural disasters (although air condition may be needed during summer heatwaves).

Disadvantages:

1. Ensuring optimal climate not always easy to achieve.
2. Less exposure of the inhabitants to natural situations (for example natural microbiomes, a large variety of prey items).
3. Requires more intensive maintenance than outdoor natural enclosures.

4. Although less vulnerable to rapid changes, organic soils may quickly shift and become toxic to inhabitants (for example high nitrite concentrations may accumulate). Quick intervention is necessary to avoid mass mortality in these cases; scraping off the top layer and replacing it every 4 months is advised (see 2.1.9).
5. Food uptake is more difficult to monitor compared to the abiotic systems.
6. Prey items have more opportunities to escape consumption (which may result in the loss of dietary supplements, insect mortality and extensive moulding).

Figure 16. Basic biotic indoor enclosures in GaiaZOO. In 2017(above) in a cool basement and in 2019 (below) in a climate-controlled isolated chamber (Sergé Bogaerts).

Figure 17. Basic biotic indoor enclosures in a special transformed and climate-controlled shipping container, Rotterdam Zoo (2020) (Sergé Bogaerts).

2.1.4. Mainly abiotic indoor enclosures

Such enclosures are often used for raising juvenile salamander or quarantine but are equally successful to achieve reproduction. Such containers are mostly some kind of plastic box (which allows easy handling and cleaning), lined with light moist tissue, provided with hiding places and a water container and put in a climate-controlled room. They require consistent maintenance since toxic metabolites such as ammonium quickly accumulate in the substrate and water. Ventilation is provided by covering part of the enclosure with wire mesh or by drilling holes in the sides or lid of the container (Fig. 18).

Gerhardt & Seidel (2019) show that this system can even function with dry newspaper for the permanent keeping of adult salamanders. Several containers can be stacked, strongly reducing space requirements. This way of keeping causes strong debates among hobbyists, but so far there is no evidence of associated welfare issues (Figure 19).

We, for the time being, recommend this type of enclosure mainly for quarantine situations or for raising juveniles.

Figure 18. Simple furnished abiotic enclosure for raising just metamorphosed juveniles (lid has been taken off and is not on the picture, see figure 35 for a similar enclosure with lid) (Sergé Bogaerts).

Figure 19. Permanent system designed by Uwe Seidel. Transformed plastic boxes used in the food industry (Gerhardt & Seidel, 2019).

2.1.5 Thermal requirements

Temperature cycles should follow the natural seasons within the subspecies' range, with low temperatures (2-6°C) during the winter months up to maximum 20°C in summer (more southern subspecies are tolerant of slightly higher temperatures of up to 23-24 °C in the day during summer). In outdoor enclosures within the natural range of *Salamandra s. terrestris*, optimal temperature regimes can be easily achieved by providing proper vertical structuring of the substrate. Providing access to subterranean retreats at minimum 50 cm below the soil surface in an enclosure placed in the shade provides a temperature gradient, offering the salamanders a variety of thermal and humidity opportunities.

When housed indoors, an unheated basement can provide appropriate temperature and humidity conditions that seem to match fire salamanders' preferences year-round. Such rooms typically are thermally fairly stable and temperatures rarely exceed 20°C in summer, while frost is highly unlikely. Less ideal but sufficient in case outdoor or subterranean housing is not possible, are artificially climate-controlled rooms (air conditioning). Since this lowers relative air humidity, the use of humidifiers and air conditioner systems that blend circulated and external air is encouraged. The use of cooling is highly recommended for any condition where temperatures exceed 25°C for several days (see paragraph 1.3). Thermal gradients are not used in fire salamander enclosures (see Seidel & Gerhardt, 2016). Rotterdam Zoo is currently experimenting with thermal gradients, so far it is unclear if they could be beneficial.

Brumation in fire salamanders should be considered rather a period during which surface activity is restricted due to prohibitively low surface temperatures, than a period of complete inactivity (as is the case in many reptiles). Fire salamanders may emerge during mild winter nights. Fire salamanders remain active at temperatures above 5°C and continue feeding. For brumation, animals can be left in outdoor enclosures provided sufficient frost-free opportunities are available (minimum depth of 50 cm below the surface, flooding must not be possible). When kept indoors and temperatures do not drop below 10°C, artificial brumation in brumation boxes in a regular fridge provides an alternative (Fig. 20). Since a gradual reduction of temperature reflects the natural situation, this could be advocated in captivity. However, no obvious negative effects have been seen when fire salamanders were moved from 10-15 °C to brumation boxes at 6°C (bearing in mind these temperatures only result in reduced metabolic activity) (Pasmans, Bogaerts, pers. experiences). Brumation boxes can be small (minimum 300 cm² for an adult fire salamander), should contain a hiding place and access to moisture (preferably including a water bowl with clean water but excessive moisture should be avoided (a constantly wet environment has been suggested to increase health problems) and can be kept between 4-6°C. Substrate can consist of a variety of materials ranging from tissue to more natural substrates (forest soil, clay) but must be kept clean (assuring low concentrations of potential toxins such as nitrogen metabolites) at all times. Sufficient ventilation should allow gas exchange. Preferably, each brumation box contains a single animal. Under these conditions, the animals can be kept between November and March. Regular checks (once weekly) of the container and the animal is recommended and substrate and water must be exchanged when necessary (when the water is dirty and substrate depending on the type of substrate see paragraph 2.1.3). Winter temperatures are one of the areas where different forms / subspecies of *S. salamandra* vary most, and the specific parameters at the locality of origin should be taken into account before brumation is undertaken.

Figure 20. Brumation of fire salamanders in individual boxes in a controlled refrigerator in GaiaZOO (Sergé Bogaerts).

2.1.6 Lighting

Lighting cycles should follow natural light cycles in the range of *Salamandra s. terrestris*, meaning few hours of light during winter (8 hours) and up to 16 hours in summer. During brumation at low temperatures (e.g. in fridges), lighting does not seem to be necessary, which is in line with underground conditions in nature. Since fire salamanders (including their larvae) are mostly nocturnal, lighting intensity and composition (for example presence of UV radiation) could be of less importance. Indeed, fire salamanders post metamorphosis are only rarely encountered during daylight hours, typically becoming active (often several hours) after dusk and exposure to any significant amounts of UVA or UVB radiation in nature is highly unlikely. Fire salamanders have been kept for decades in indoor enclosures without additional UVA or UVB radiation with no obvious effects on animal welfare, health or reproduction; subclinical effects cannot be ruled out, of course. However, there must be a source of vitamin D_3 if they are not exposed to UVB radiation. Outdoor enclosures are preferably shaded to avoid excessive sunlight from entering, heating and drying the enclosure (north to north east orientation are ideal). LED lights provide a cheap, long lasting and relatively little heat emitting light source for indoor enclosures and light cycles can be easily adjusted using timers (see also Seidel & Gerhardt, 2016).

2.1.7 Humidity and ventilation

While fire salamanders are typically active on the surface during periods with high humidity, they should be offered a moisture gradient in their enclosure. Continuous exposure to a wet environment has proven to have negative effects on the animals' health. The build-up of stagnant water in soil should be avoided to avoid anaerobic microbial processes that result in the accumulation of toxic metabolites. In outdoor enclosures, a moisture gradient is most easily provided by vertical structuring, with deeper layers typically having a higher relative humidity. In indoor enclosures, humidity gradients can also be easily provided, yet can be maintained both horizontally (for example, spraying 1/3 of the terrarium surface regularly) and vertically (for example, providing stacked pieces of bark). Dry places and retreats should be present in any salamander terrarium. Ventilation is important, making sure enough air refreshment can occur.

A water container is the easiest way to avoid dehydration and should always be present. This can be small (minimal the size that the largest animal can sit entirely in the container) or larger, to taste. In captivity, salamanders are often seen bathing and water depth should allow the animal to submerge itself (approximately 3-5 cm). Important here are 1) easy access to and from the water container (salamanders can drown) and 2) optimal water quality at all times (we advise quality of drinking water for human consumption, pH around 7, absence of any significant levels of nitrite or ammonium, nitrate levels maximum 50 mg / L, moderate to high hardness preferred given increased capacity of buffering pH). Salamanders tend to defecate in their water containers, prey items can drown and these will all result in rapid build-up of for example ammonia or nitrite. Water in such containers must be replaced at least twice a week (or if it is dirty at a daily check-up immediately) and water containers should be scrubbed and disinfected at the same time to prevent bacterial or algal build-up. Using two water containers for each tank is an easy way, while placing the new, clean the old and leave it to dry for the next time.

For pregnant females, even these smaller water containers suffice for larval deposition. Of course, larvae should be transferred to aquaria the same day when detected. In exceptional cases, a water bowl may not be advisable (e.g. animals during recovery from anaesthesia, sick animals that have a higher risk of drowning, animals undergoing certain treatments, during transportation etc.). For such cases, a pad of very wet paper towel on a plastic tray can be provided so animals can dehydrate without the risk of drowning.

Spraying salamander containers with water promotes activity and helps maintaining moisture levels. Again, excessive spraying may result in build-up of moisture, which should be avoided. The number of spraying events should be dictated by the humidity of the enclosure, the season, the temperature and the size of the enclosure. There should be a good balance between spraying and letting the enclosure dry (similar to the natural raining events under natural conditions). Spraying once a week is usually sufficient (but in air conditioned rooms spraying may need to be more frequent). Outdoor enclosures exposed to rain generally need no additional humidification (provided these always should contain an easily accessible water container). In the above mentioned enclosures used in the study of Laking et al. (2021), besides a small water dish, no extra water was added to these enclosures, apparently without any negative effect on the animals, even during the very dry summer of 2018 and relatively limited access to underground retreats.

In dry abiotic enclosures, spraying should be avoided as it results in rapid moulding of the paper substrate. Animals maintained in these setups display normal behavioural patterns and reproduction without ever being sprayed (Michaels, Seidel, pers. comm.).

2.1.8 Enrichment

Every salamander terrarium or aquarium should contain a sufficient number of hiding places, at least one hide for every animal present. Ideally if moisture gradients are being created, there should be one hiding place for each animal in each extreme of the various microhabitats. Preferably, hiding places should cover a substrate with different moisture levels, so the animals can choose their spot. For ease of cleaning (preferably non-chemical disinfection but rather thermal disinfection), ceramic (unglazed) hiding places are often used. An easy way to make low cost, eagerly accepted hiding places, is by cutting off part of a clay dish for flowerpots, to create an entrance. Several of these dishes can be stacked to provide a moisture gradient. Other materials include (cork) bark or brick stones with holes. Although the latter provide excellent hiding places, they have two disadvantages: they prevent easy access of the keeper to the animals and cannot be easily cleaned. Faeces can accumulate in these bricks and start to mould. Such refugia may be cleaned by immersion in very hot water and scrubbing with a toothbrush.

Fallen (not green) beech or oak leaves (both decompose slowly and provide tannins) collected from forest soils can be added to any container to provide extra decoration. These increase the surface area of the enclosure and help maintaining a moisture gradient. Addition of further structures (for example branches or bark) may enrich a salamander cage further and help the animal in visually assessing its enclosure.

Plants like ferns and moss can also be used for decoration (see Schorn & Kwet, 2010; Seidel & Gerhardt, 2016), however they are not relevant for the salamanders.

2.1.9 Public display

Public display is not an easy task for an amphibian that is mainly night-active and prefers rather low temperatures. For a public display an imitation of the natural habitat is a logical thought, however this will make the salamanders invisible during normal visiting hours (Fig 21). GaiaZOO and Rotterdam Zoo both show the animals in a similar basic biotic enclosure as the animals that are kept behind the scenes. Salamanders are visible, but not active (Fig. 22). The use of reverse lightning may be suitable for exhibiting this species. Fire salamanders seem to possess trichromatic vision (Przyrembel et al., 1995) so the use of red lightning is not recommended, as they will still perceive it.

Figure 21. Natural planted terrarium where salamanders during the day are not visible (Frank Pasmans).

Figure 22. Information panel and terraria in Rotterdam Zoo, explaining threats and the cooperation with the NGO RAVON (Reptile, Amphibian, Fish Conservation Netherlands) (Sergé Bogaerts).

2.1.10 Maintenance, cleaning and disinfection

The intensity of cleaning and disinfection heavily depends on the enclosure set-up. Self-sustaining systems like large outdoor enclosures do not usually require any maintenance (apart from keeping vegetation within limits and ensuring a minimum water level and quality in the water container), provided that they have appropriately low stocking densities. In "classical" terraria containing organic soil, hiding places and a water container, cleaning consists of replacing the water in the water container at least twice weekly and removing dead prey items and faeces. Organic soils that are well aerated do not need very regular replacement, once every 4 months has been shown to suffice for terraria that contain densities of salamanders as advised under paragraph 2.1.1., but must be more frequently e.g. when the substrate smells, becomes overly saturated etc. Using test strips, the nitrite concentration of soil may be assessed and, as with the water container, soil must be replaced in case significant levels of nitrite are detected.

Soils should be replaced immediately if animals exhibit any skin anomalies (often a darkening and roughening of the skin, often first seen on the belly). As an indication, the level of ammonia, nitrite and nitrate can be estimated in the soil by using the same test strips that are used by aquarists to assess water quality. Skin discolorations are not solely caused by soil contamination however, and disease and stress processes must be investigated at the same time as substrate replacement in these circumstances.

The higher the density and the more systems shift to abiotic, "clean" enclosures (especially lined with moist tissue/paper), the more vulnerable these are too rapid deterioration of substrate and water quality. The latter require very consistent maintenance, with at least twice a week changes of tissue and the water container.

During regular maintenance of a colony of healthy fire salamanders, there is very little need of actual disinfection of materials; cleaning (for example of the water container) suffices. Disinfection becomes necessary when enclosure contents would be disposed in natural systems (which is highly undesirable). Heat treatment (oven) can be used to kill ranavirus and chytrid fungi (heating for one hour at 70°C or above (preferably, but depending on the heat tolerance of the materials to be disinfected), making sure that this temperature is effectively reached in the core of the material that needs disinfection). Chlorine (hypochlorite, 4%) kills most viruses, fungi and bacteria, is cheap but has pronounced ecotoxicity. Potassium peroxymonosulphate (Virkon) and quaternary ammonium compounds provide less toxic alternatives. For all chemical disinfectants, the general principle is that the presence of organic material reduces efficacy. Thorough cleaning before disinfection is necessary. For in depth information, see Pessier & Mendelson III (2017).

2.2 Feeding

The following proposal for dietary composition of fire salamanders are inferred from available knowledge of natural diets (see paragraph 1.6.3), known composition of commercially available feeder invertebrates and known or expected nutritional disorders. Future studies on optimal diets for captive salamanders will undoubtedly add new and relevant insights that should result in maximal fitness and optimal development, growth and reproduction. Simple questions like how many prey items are fed each week per animal are not easy to answer.

2.2.1 Diet for captive terrestrial fire salamanders

Optimal nutrition for captive amphibians is poorly understood, and available information is typically generalised across the entire Class, rather than being species specific (Ferrie et al., 2014). Ideally, nutritional analysis of wild diets and the assessment of captive diets against this (see Jayson et al., 2018 for an example of this process) should be conducted. Without this knowledge, captive diets for amphibians including fire salamanders must be designed based on the wild diets of the animals in question, considering basic nutritional requirements for terrestrial vertebrates, and in response to specific nutritional disorders encountered in captive populations. The wild diet of fire salamanders is discussed in paragraph 1.6, and shows that these amphibians are generalists, feeding on most invertebrates of appropriate size that they encounter. The nutritional profile of this diet, however, is unknown. Ferrie et al. (2014) provide estimated nutritional requirements for most macro- and micro-nutrients for amphibians based on those empirically developed for poultry, cats, dogs and fish and in the absence of specific data, these may use for reference when designing captive diets for fire salamanders. Being typically dependent on live prey as post-metamorphic animals, standardised prepared diets are not generally available for these animals (but see below), and so the nutritional constituents of available food items are also poorly known and highly variable.

Nutrition can have key effects on the clinical health and welfare of fire salamanders. Currently, there are few diagnostic tools to quantitatively assess the nutritional health of fire salamanders beyond clinical pathologies, however, and some pathologies in captive animals may result from unidentified nutritional problems. Ideally, the body condition and blood biochemistry of captive fire salamanders should be similar to those of healthy, wild animals (but no reference values are available and further research into normal wild blood values is required). Variations can be expected for seasons and life stage. Information regarding blood biochemistry, hematology and scaled mass indices (*sensu* MacCracken & Stebbings, 2012) from healthy, wild fire salamanders, or a body condition scoring system (Jayson et al. (2018b), could serve as reference values. Radiography can be used to assess skeletal health, especially checking mineralisation of bones via radiopacity and conformation. As well as clinical health, nutrition may have impacts on conservation success; for example, *Salamandra* larvae fed with poor diets as larvae metamorphose not only smaller, but also with a reduced amount of yellow colouration, which leaves them more vulnerable to predation (Caspers et al., 2020).

Captive fire salamanders may be offered food of several types of origin. In general, relatively soft-bodied terrestrial insects and their larvae, gastropods, annelids and myriapods should be offered. Table I presents the content of the most important nutrients of a selection of commonly available feeder invertebrates. The cited literature usually contains details of other nutrients that are of use for more detailed dietary analyses.

Invertebrates collected from the wild, especially from within the distribution of fire salamanders, may offer the greatest diversity of taxa and nutritional content and so best approximate wild diets, as the majority of invertebrates are not readily cultured or commercially available. The collection of such invertebrates is, however, time consuming and dependent on appropriate weather conditions, which may be problematic especially when maintaining large numbers of salamander. Methods such as pitfall trapping, checking under refugia, digging for earthworms and light trapping may be suitable for harvesting wild invertebrates. Wild collected invertebrates are also inherently risky. Earthworms and gastropods are potential carriers of parasites that may infect fire salamanders, given the widespread role of gastropods and earthworms in parasitic infections. Contamination with pesticides and other agrochemicals may present further threats although, in practice, under north western European circumstances, we have no indications that this actually results in any significant disease. Wild collected invertebrates must also be confidently identified to avoid the introduction of predatory and/or toxic species. The incidental collection of substrate with invertebrates may also allow for the introduction of pathogens or parasites to captive fire salamanders, especially in areas inhabited by wild amphibians.

Commercially produced invertebrates, and especially crickets, are extremely convenient and can be procured in a variety of sizes in order to fit the gape size of the fire salamanders to be fed. Insects from this source are likely to pose less of a risk in terms of chemical and pathogen contamination, although as many commercial producers also deal in live pet amphibians some caution should be exercised. It should be noted that some invertebrates sold by the trade, such as *Lumbricus* earthworms are actually wild collected. Commercial producers are, however, limited to only a handful invertebrate species, mainly restricted to just a few taxonomic groups. Moreover, these invertebrates are exposed to highly limited diets themselves. This means that the nutritional breadth of commercially produced invertebrates is much narrower than wild diets, with particular deficiencies in calcium, vitamins A, D, E and thiamine and omega-3 fatty acids (Finke, 2002; Ferrie et al., 2014; Finke, 2015; Jayson et al., 2018). However, with appropriate supplementation (see below), fire salamanders are routinely reared to adulthood and maintained and bred for decades on commercially available diets, and often on crickets alone.

Invertebrates may be cultured by the institution holding fire salamanders. Such cultures can produce a stable supply of invertebrates, and often a greater diversity of taxa can be targeted than are available commercially; for example woodlice, aphids, and slugs maybe readily cultured. Such cultures often require more maintenance than the fire salamanders themselves, however, may substantially increase resourcing demands, and it can be difficult to produce high enough yields to fully satisfy a large salamander population. Typically, cultures might be maintained as supplements to other primary sources of food.

Although typically dependent on movement to detect prey items, and therefore requiring live invertebrates, fire salamanders can be at least partially adjusted to prepared, non-living diets (see Seidel & Gerhardt, 2016). Moistened pellets and jelly preparations (e.g. Repashy Grub Pie) cut to appropriate size can be offered to fire salamanders using forceps or skewers to create movements needed to trigger a predatory response. Over time, animals may learn to find this food by smell alone and movement is no longer required (Palacios, pers. comm. Fig. 23). This approach to feeding can be time consuming but does allow for a more controlled nutritional input and does not require the maintenance of feeder insects alongside salamanders. The use of vibrating plates as used for snakes has not been tried, but may be of value here, as often lack of movement prevents salamanders from consuming this type of food even when they have located it by scent.

Figure 23. A *Salamandra s. gallaica* feeding on Repashy Grub Pie (Rodrigo Palacios).

Nutritional deficiency in invertebrates may be improved in two main ways; nutritionally enriched diet fed to the invertebrates (Finke, 2003), and application of supplementary powders immediately prior to feeding to salamanders (Michaels et al., 2014). Live feeder insects kept in captivity for any length of time must be maintained properly in order to maximise nutritional content. A nutritionally varied maintenance diet will ensure that alimentary canals are filled with nutritious content, and that the tissues of feeder invertebrates are also of higher nutritional quality (Ferrie et al., 2014). Pellets for laying hens or dog chow, complemented with fresh (pesticide free) carrots and other vegetables, appear to be suitable diets for crickets, tenebrionid beetle larvae and cockroaches in that they contain all required nutrients for other vertebrates (chickens, dogs) and salamanders feeding on insects gut loaded on these ingredient do not typically develop clinical nutritional deficiencies. Locusts, snails and slugs may be offered leafy greens, supplemented for the snails with cuttlefish bone to provide calcium. Cultured earthworms may be kept in an enriched medium, for example stirring in some calcium carbonate, and fed the pellets mentioned above, which must be soaked to soften them in advance (although provision of enriched diets does not necessarily meet with success; Michaels et al. (2021). The maintenance of other potential feeder insects is beyond the scope of these guidelines. As invertebrates will defecate and void nutritious gut contents, they must be eaten within hours of being added to salamander enclosures to retain good nutritional content (Finke, 2003).

Supplementary powders are available from multiple brands but are usually variations on a calcium carbonate base with added vitamins and minerals, especially vitamins D, A, B and E (although some products specifically omit vitamin D – check ingredients before choosing a product). The nutritional impact of these powders on feeder invertebrates has only been quantified for calcium (Michaels et al., 2014). Supplementary powders are a powerful tool in improving nutritional content, but are rapidly shed by feeder insects, which actively clean themselves, and are also lost when salamanders wipe prey items on surfaces during ingestion. The use of smaller prey items reduces the loss of powder during ingestion by salamanders as they are typically swallowed immediately. Dusted invertebrates should be offered in dry conditions where possible, and certainly should not be added to enclosures immediately before spraying, as wet conditions accelerate powder loss. The evidence suggested that little difference exists between brands in retention of powder over time, at least as far as calcium is concerned (Michaels et al., 2014). Wet-skinned invertebrates, such as worms and slugs, are not suitable for dusting as the powder rapidly dehydrates and kills them or is sloughed off with mucus. Earthworms may be dusted as they are ingested by salamanders, but this requires much time and patience to achieve. Supplements should be kept cool, dry and dark; a refrigerator is ideal. Mouldy or heat-exposed products should be disposed of and replaced, even if they appear visually normal.

Several key nutritional disorders have been observed in captive fire salamanders, and diets must be prepared with these especially in mind. Calcium deficiency is probably the most common nutritional deficiency seen in captive fire salamanders, especially juveniles raised in captivity (Fig. 14). Nutritional secondary hyperparathyroidism (NSH), often referred to as metabolic bone disease, describes the outcome of chronic calcium deficiency. Calcium is required for normal neurological and cell membrane function, and if blood calcium levels fall calcium is drawn from the bones, which act as a calcium store, to balance phosphorus levels. Over time, the skeletal system is compromised by calcium extraction or, in juveniles, bones are not properly calcified in the first place. Fractures and deformities of bones caused by trauma or stress applied by muscles leads to skeletally compromised animals as well as complications of such changes, including pain, infections, inability to feed due to jaw malformation, and impaction of the gut due to diverted spinal and pelvic bones. Vertebral fractures may also cause neurological signs. Eventually, neuromuscular signs become evident, including tetany, tremors, gastrointestinal impaction due to loss of peristalsis, and eventually death. Salamanders with calcium deficiency may also develop oedema. Calcium metabolism may be affected by one or both of two factors – calcium availability and calcium absorption. Ensuring a calcium: phosphorus ratio of at least 1:1, but preferably 2 or 3:1 is important in avoiding NSH; wild amphibian diets may exceed a ratio of 8:1 (Jayson et al., 2018). The use of high-calcium invertebrates (e.g. woodlice, snails) and management of invertebrate calcium content through gut loading and dusting (see above) is suitable to avoid calcium deficiency in diets. It must be noted that some invertebrates with high calcium content may be indigestible and the calcium content may not be accessible to fire salamanders, such as black soldier fly larvae which hold much calcium in the cuticle, which is usually passed undigested by amphibians. Calcium absorption occurs passively in the gut at low rates, but active uptake of calcium requires vitamin D_3 and derivatives. This vitamin is sourced from the diet and from exposure to UVB radiation and heat to different degrees in different species of vertebrate (Antwis and Browne, 2009; Baines et al., 2016). It is unclear how important each source of vitamin D_3 is to fire salamanders, or whether passive uptake of calcium is sufficient and in the absence of vitamin D_3. Captive produced invertebrates are low in vitamin D_3, if they contain any at all (Ferrie et al., 2014; Oonincx et al., 2018), but recent evidence shows the UVB irradiation increases this in some species (Oonincx et al., 2018). Although many prey items eaten by fire salamanders are not routinely exposed to high doses of UVB, being as secretive and nocturnal as the salamanders themselves, this is a possible route by which *Salamandra* may derive this nutrient from their invertebrate diet. Dietary supplement powders include vitamin D_3 and this may be used to supplement this vitamin in captivity, bearing in mind that vitamin D_3 toxicosis is also possible in amphibians (Schlumberger and Burk, 1953). Fire salamanders are strictly nocturnal, and spend the day in subterranean retreats, so exposure to UVB radiation is likely minimal; however other amphibians are known to synthesise vitamin D_3 via UVB exposure (Michaels et al., 2015), and even nocturnal reptiles do this, partly due to reflection of UVB radiation into crevices and burrows (Ferguson et al, 2005; Oonincx et al., 2020). As UVB radiation can also be damaging to amphibians (Antwis and Browne, 2009), it is unclear whether it should be provided for captive fire salamanders. This species is typically maintained without UVB provision in captivity without clinical signs of ill health, however. Magnesium is also involved in calcium regulation, through regulation of parathyroidism, and deficiency of this element in the diet may result in perturbation of calcium metabolism (Paunier, 1992). Less is understood about the needs of magnesium, but Ferrie et al. (2014) recommend a diet for captive amphibians comprising 0.04% magnesium.

A second nutritional deficiency that is probably severely underestimated in amphibian husbandry, is hypovitaminosis A (Rodrigues and Pessier, 2014; Clugston and Blaner, 2014; Ferrie et al., 2014). Invertebrates are low in vitamin A or its precursors and, unless supplemented via gut loading and dusting, hypovitaminosis A is likely to occur. Clinical signs are often not obvious but may present as “short tongue syndrome”, or lingual squamous metaplasia, whereby mucous tissue hardens and prevents extension of the tongue for feeding, but can also have more subtle effects on such tissues elsewhere in the body. Vitamin A related skin problems are also frequently observed in captive *S. s. terrestris* (Pasmans and Martel, pers. observ.) and may present as ulcerations (not to be confused with for example a Bsal infection) that resolve after oral supplementation with vitamin A (Fig. 15) as well as poor sloughing and reduced healing abilities (Clugston and Blaner, 2014; Ferrie et al., 2014). Importantly, hypervitaminosis A is also rapidly detrimental to amphibians and over supplementation must be avoided. A concentration of 2,000 – 15,000 IU vit A / Kg is recommended, although this is simply extrapolated from other taxa as data do not exist for vitamin A requirements for any amphibian (Ferrie et al., 2014). Fire salamanders have vivid orange and yellow colouration in their skin, but unlike many other amphibians, this does not fade in captive animals. This suggests that carotenoid pigments do not form part of this colouration; however, carotenoids in the diet may be important to supplement as a precursor to vitamin A, and also due to their other benefits for health (Clugston and Blaner, 2014; Ferrie et al., 2014). The ability of *Salamandra* to convert carotenoids to vitamin A is unknown, however.

Obesity may be an underestimated problem in fire salamanders, potentially negatively affecting life span and reproduction as well as welfare. Obesity is caused by one or both of excessive quantities of food and excessive fat content. Many commercially available invertebrates, especially tenebrionid beetle larvae and waxworms, are very high in fat (Ferrie et al., 2014 and references therein; see Table I), which may contribute to this issue. Although vertebrates are occasionally consumed in nature (see Section 1), these should not form part of the captive diet and especially mammalian or avian vertebrate prey should be avoided entirely. Obese animals may have other nutritional deficits, as a high dietary fat content means that, when adjusted for energy density, nutrient deficiency increases (Finke, 2015). In the absence of a body condition scoring system (similar to the system developed by Jayson et al. (2018b) for mountain chicken frogs) or scaled mass indices based on wild populations (see above), husbandry practitioners should refer to photographs of healthy wild individuals to inform the management of body condition in captive animals. Postmortem examinations of dead animals may also provide insight into levels of obesity in a captive population by directly inspecting fat bodies. It should be borne in mind that body condition does not necessarily reflect body composition, and relative proportions of fat, muscle and retained water may mask or masquerade as obesity. Adjustments to the calorific content of diets should be made slowly to avoid metabolic shock. As well as causing obesity, a high fat diet may disease related to the oxidation of fatty acids due to hypovitaminosis E. While hypovitaminosis E has not been formally described in amphibians, it is as likely to occur as in other vertebrates. Poor body condition may also be encountered in captive salamanders. This is usually linked to inappetence, parasites or insufficient food provision. The latter is easily remedied, but the former two possibilities will require further investigation. For severely malnourished animals, food intake should be increased very slowly to avoid metabolic shock.

Feeder item	**Ca**	**P**	**Vitamin A**	**Thiamine**	**Vitamin E**	**Fat**	**Reference**	**Comments**
Mealworms (*Tenebrio molitor*)	169-184mg/kg	2720-2850MG/KG	< 100 IU / 100g	1.2-2.4 mg/Kg TM	55 IU /Kg TM	31% DM	Barker et al., 1998; Engmann et al., 2013; Ooninckx & Dierenfeld, 2011; Finke, 2002; (Enghoff et	
M*orio* worms (*Zoophobas morio*)	*177mg/kg*	*2370mg/kg*	< 100 IU / 100g	0.6 mg/Kg TM	7.75 IU /Kg TM	*41%DM*		
Wax worms (*Galleria melonella*)	243mg/kg	1950mg/kg	< 100 IU / 100g	13.35 mg/Kg TM	13.35 IU /Kg TM	5*1% DM*		

Juvenile crickets (*Acheta domesicus*)	275mg/kg	2520mg/kg	< 100 IU / 100g	9.65 mg/Kg TM	9.65 IU /Kg TM	10%DM	al., 2014); Wang & Shelomi, 2017;	
Adult crickets (*Acheta domesicus*)	407mg/kg	2950mg/kg	< 100 IU / 100g	19.75 mg/Kg TM	*19.7*5 IU /Kg TM	23%DM	Boykin and Mitchell 2020; Liland et	
Fruit flies *Drosophila spp.*	*0.17%DM*	*1.32%DM*	0-2.2IU/Kg DM	-	*23-*166IU/kg DM	18%DM	al., 2017; Ferrie et al, 2014	
Earthworms *Lumbricus spp.*	444 mg/ kg TM	1590mg/kg DM	328-2400 IU/KG DM	-	*70-229 IU/KG DM*	11-13%DM		Highly variable – some authors find Ca:P rations >1:1
Woodlice *Porcellio* spp.	14% DM,	*1.2%DM*	-	-	-	11.5%DM		
Snail meat (*Achatina*)	586 mg / 100g dry mass (DM)	269 mg / 100g DM	<	-	-	4%DM		
Millipedes (*Tymbodesmus falcatus* and other unnamed spp.)	3.7 - 17.4 g / 100 g DM	-	-	-	-	-		
Black soldier fly larvae (*Hermetia illucens*)	6.6-9.3 g/100g DM	-	*17ug/kg TM*	15.4ppm DM	18.5-*53.3mg/kg DM*	29 g/100g DM		Calcium contained in cuticle, which may not be digested. Vitamin A shown to be increased substantially by gut loading.
Cockroaches (various species)	0.06-0.24%DM	0.37-1.22%DM	0-386IU/Kg DM	2.2ppm DM	17-56 IU/kgDM	14-55%DM		

Table I Content of selected nutritional components of commonly available feeder invertebrates for post-metamorphic fire salamanders compiled from various literature sources. Other invertebrates are available, and these values are only a guide; the exact composition of individual invertebrates can vary considerably based on exact species, diet and other variables. Cells containing '-' indicate no data available.

The importance of thiamine (vitamin B_1) deficiencies may equally be underestimated, since nervous disorders in amphibians (*Peltophryne lemur*) and reptiles (*Anolis* sp.) have been associated with hypovitaminosis B_1 (references in Finke, 2015). Commercial feeder insects are low in thiamine and at least some (*Bombyx mori, Anaphe* sp.) contain thiaminases (references in Finke, 2015). The impact on the health of captive fire salamanders is currently unknown.

Providing guidelines for feeding frequency and the number of prey items per feeding session to maintain optimal body condition and nutritional health is difficult. Observations of feeding habits in nature are limited to surface activity but underground foraging of fire salamanders is poorly understood (see 1.6.3). Feeding frequency will be dependent on the age and size of salamanders, nutritional state, temperature, activity levels, and the types and sizes of food items presented. Gerhardt and Seidel (2019) recommend feeding once every 10-14 days for adult fire salamanders and weekly for juveniles. Other experiences (Sergé Bogaerts, Frank Pasmans, Uwe Seidel) suggest that adult salamanders can be fed once per week or even once every two weeks. Michaels and Preziosi (2020) achieved healthy animals and twelve-fold mass increases over 6 months feeding individually housed juveniles every three days on earthworms and black field crickets supplemented with Nutrobal powder at a temperature of 13-17^{o}C. Uneaten food was removed after 24hrs. Ultimately, feeding regimens should be adjusted to achieve and maintain desired body condition, growth rates and overall health. Although juvenile fire salamanders may grow very quickly when offered large amounts of food, this will put additional stress on calcium metabolism and requirements for micronutrients and therefore can lead to increased instances of NSH. As a rule of thumb, all prey items provided must be eaten within 24 hours; if they are not, this may suggest overprovision of food. It is unclear whether providing a larger number of prey items with long (e.g. one week) intervals is better than continuous access to a small number of prey items. Prey items should be offered to salamanders when they are likely to feed immediately; this is usually after dark, especially when precipitation or spraying has occurred. Uneaten prey items should be removed from enclosures after 24 hrs. Persistence of prey animals in salamander enclosures will lead to reduced nutritional quality (see above), increased likelihood of feeder insects (especially crickets) injuring salamanders (Figure 16), and accumulation of dead invertebrates in substrate or water bodies, which compromises hygiene and may promote invasions of mites or flies that can harm salamanders. Constant presence of large numbers of feeder insects may also cause stress to salamanders and depress predatory responses. For post metamorphic salamanders, prey item size can be chosen based on the distance between the eyes of the salamander. Insects and crustaceans should be no longer than the distance between the eyes of the salamander. Earthworms and slugs should be no thicker than the distance between the eyes of the salamander. Snail shells should be easily fitted within the gape of the salamander and can be partially crushed to aid ingestion.

Figure 24. *Salamandra i. infraimmaculata* exhibiting metabolic bone disease (Frank Pasmans). Note the hunched back, particularly obvious near the hindlegs.

Figure 25. X-ray of two *Salamandra algira tingitana*: on the left a captive raised specimen on a diet of crickets and mealworms with little calcium or vitamins added, on the right a salamander that grew up in the wild. Note the difference in calcification of the bones (Caroline van der Ploeg).

Figure 26. *Salamandra s. terrestris* showing skin problems due to vitamin A deficiency (full image above, detail below) (Frank Pasmans).

Figure 27. Captive raised *Salamandra s. gigliolii* showing a large wound caused by non-eaten crickets in the terrarium. The animal died. (Alex Kloor).

A healthy fire salamander will shed its skin a few times in the year. The salamander sheds from front to back in one piece in healthy animals. The skin is mostly eaten afterwards. Watching an animal shed is a rarely seen sight (Fig. 28). If skin if left after a shedding, often on the fingers and feet, it is a sign the animal is not in optimal condition and is best put in quarantine until it eats and sheds normally. The skin that is left can be removed by gently rubbing it off under running water.

Figure 28. A captive bred juvenile *Salamandra algira* during shedding (Frank Pasmans).

2.2.2 Diet for captive larvae

Larvae may be fed a varied diet of small aquatic invertebrates. Although introduction of pathogens is difficult to avoid completely, a diet consisting of zooplankton (Daphnia, Copepoda (but see further), ostracods), *Tubifex* worms, white worms (*Enchytraeus albidus*) and insect larvae (e.g. "blood worm" (Chironomidae), larvae of mosquitoes (*Culex* sp.) results in rapid growth and uneventful metamorphosis. Earthworms of appropriate size are an excellent food item and result in excellent growth – larvae fed earthworms primarily metamorphosed twice the size of those fed *Enchytraeus* as a staple (Michaels, pers. obs.). However, care must be taken not to introduce parasites and predators that may damage or kill larvae (e.g. fish lice, predatory copepods). Bloodworm may be contaminated with heavy metals (Sharifian-Fard et al., 2014) and bloodworm is often of poor quality. However, bloodworm is highly efficient for larval growth (feed conversion ratio of 0.33 as determined on DM in *Pachyhynobius shangchengensis* larvae, Pasmans et al. 2012). When aquatic prey items die, they mould quickly and water quality deteriorates quickly. It is therefore important to only feed healthy, viable prey items. In theory, *Artemia* is a proper food source. However, this is quite labour intensive, needs rinsing before offering and quickly dies in fresh water. Other crustaceans may provide very valuable food items (e.g. *Hyalella azteca*) and can be produced in some quantity. Despite several hobbyists reporting *Tubifex*-associated health problems (feeding of *Tubifex* could result in mass mortality), in our experience (Bogaerts, Pasmans) but also according to Seidel & Gerhardt (2016), feeding these oligochaetes has never resulted in any observed health problem. The condition is that *Tubifex* need proper cleaning and rinsing before providing to the larvae, to remove dead and decaying worms. Finally, captive-bred tadpoles are eagerly eaten by larvae and may constitute an ideal prey item. Frogs can be bred for this purpose in high numbers (e.g. *Discoglossus pictus*, Pasmans et al., 2016). Local legislation should be checked prior to doing this and the amphibian breeding colony that provides the tadpoles must be healthy and free of known pathogens. Although nutritional problems are poorly known in fire salamander larvae, it is likely that similar issues are at play as for animals after metamorphosis, which means that aiming at increasing calcium and vitamin A levels could be equally important. Unfortunately, this is much harder to achieve in aquatic larvae, since gut loading and dusting of prey items is far less achievable. One way to avoid these problems, is to include crustaceans (*Artemia, Gammarus, Hyalella*) in their diets. While these have high calcium levels, it is unclear to which extent they contribute to nutritional vitamin A and precursors for the larvae. Many of these crustaceans are rich in calcium (for example levels for amphipods between 16-25 g /100g DM (Jimenez-Prada et al., 2018) and contain several carotenoids (for example astaxanthin, β, β -carotene-3,4,3'-triol, lutein, zeaxanthin and β-cryptoxanthin, Gaillard et al., 2004). It is currently unclear to which extent salamanders (and their larvae) can convert these carotenoids into active vitamin A. Although some conversion is suggested (Brenes-Soto & Dierenfeld, 2014), dried *Gammarus* based diets are considered the archetypical hypovitaminosis A inducing diets in aquatic turtles. What is far more likely, is contribution of these carotenoids to colour development, since carotenoids are used in chromatophores, where lutein and zeaxanthin could contribute to yellow colouration and β-carotenes, canthaxanthin and astaxanthin to orange colours (Brenes-Soto & Dierenfeld, 2014).

2.3 Social structure

2.3.1 Social interaction

Little is known about the terrestrial phase and social structure of fire salamanders, mainly because of their cryptic, nocturnal lifestyle. Their activity is influenced by temperature, humidity and wind, therefore, sightings and observations of the social behaviour are scarce (Thiesmeier, 2004). Juveniles are rarely observed in the wild, but occasionally have been found in groups in hidden shelters (Bedriaga, 1897; Seifert, 1991). Adult fire salamanders appear to be solitary, but sometimes individuals occupy the same shelter or winter roost (Balogová et al., 2017; Caspers, pers. observations). They show high site fidelity and inhabit a defined home range (Rebelo & Leclair, 2003; Schulte et al., 2007), which might overlap with other individuals or other amphibian species such as newts or toads (Thiesmeier & Günther, 1996). Inter- and intraspecific communication and signalling of amphibians is based on chemical cues (Houck, 2009; Woodly, 2010) and also seems to be involved in mate choice (Rohr & Madison, 2001; Caspers & Steinfartz, 2011). There is no evidence that yellow spots on *Salamandra* have any role in sex communication (Balogová et al., 2015).

Although territoriality and aggression towards conspecifics has been documented in other *Salamandra* species (Bogaerts & Donaire-Barroso, 2005; Gautier & Miaud, 2003; Gautier et al., 2004), it is rarely observed in *Salamandra salamandra* (Kästle, 1986; Gerhardt, 2017) and might be linked to scarcity of resources or reproductive contexts (Thiesmeier, 2004). However, in larvae cannibalism and aggression towards conspecific as well as heterospecific larvae seems to be quite common as a mechanism of competition (Walls & Jaeger, 1987). Thereby, antagonistic behaviour depends on density (Reques & Tejedo, 1996; Manenti et al., 2015) and kinship (Markman et al., 2009; Sadeh, 2012).

Groups of adult fire salamanders in captivity preferably consist of a single male with one or more females. Housing males together could cause stress due to suppression of submissive males. Submissive males may show themselves less, may be less active and as a result, may start to lose condition (Bogaerts, pers. obs.). However, sexually immature small groups (4 -5) of animals can be housed together. Housing small juveniles with larger juveniles or adults is not recommended since cannibalism may occur (Fig. 29). Little is known with regard to mate selection and preferences. Individual preferences may be of minor importance for establishing reproducing, captive populations.

Figure 29. Cannibalism in captive bred juvenile *Salamandra s. terrestris*, leading to the death of the smaller sibling. Picture on page 49 how the salamanders were found and below both after the bigger one spit out the smaller one (Billy Head).

2.3.2 Changing social structure

In general, fire salamanders do not seem to have social structures. However, sociality could be individual specific rather than species specific. In laboratory cases where 2-3 salamanders were kept together some always used the same shelter together, whereas others were always found alone (Caspers, pers. obs.). During the dry summer period, shelters with more than one individual can only occasionally be found and it is more likely to find single individuals in hiding places. Thus, fire salamanders can easily be kept individually, but it is also possible to keep them together in groups when the goal is to simply keep, not to breed them (Caspers, pers. obs.). In these cases, each individual should be provided with at least one shelter each to enable free choice of shelter and the possibility to avoid the other individuals. However, even where outcompetition of submissive males is occurring, all animals may choose a single refugium (Michaels, pers. obs.) and so multiple hides are not an alternative to avoid territorial behaviour (see paragraph 2.3.1) when housing a group of males. Antagonistic interactions or aggressive behaviour are rarely observed (Gerhardt, 2017; Seidel & Gerhardt, 2016). For long term captive assurance colonies, social structure of the group should be a function of the genetic diversity to be maintained. Ideally, animals are kept in pairs.

However, as females mate with more than one male, housing a female with more than one male might be more suited to support natural mating behaviour. Furthermore, as females can store sperm, the exchange of males may not always result in those males being the sires of the clutch.

Known social interactions are limited to mating behaviour and territorial disputes between males. As such, changing the social structure is likely to have implications for animal welfare and health if additional stress can be expected (for example adding a male to an established group that already houses an adult male).

2.3.3 Sharing enclosure with other species

According to the Amphibian Husbandry Resource Guide of the Association of Zoos and Aquariums (Poole & Grow, 2008), multiple species enclosures are suitable for those species that coexist in nature. Fire salamanders co-occur with several other amphibian species and adults hardly show aggression towards conspecifics or heterospecifics (see paragraph 1.9). Since there is competition between amphibian larvae influenced by density and resource availability (Manenti et al., 2015), larvae should be raised in separate enclosures. Housing them with anuran larvae will lead to the anuran larvae ending up as food.

Fire salamanders have been successfully co-housed with several amphibian species, without obvious negative interactions, as long as co-housed species have no predator-prey relationship (e.g. tadpoles and salamander larvae; co-housing any amphibian with another specimen (same species or not) that is of significantly different size is contra indicated). In large enclosures, fire salamanders can be co-housed with for example larger newts or anurans (e.g. *Rana temporaria*) of sufficient size. If signs of stress (e.g., altered behaviour, appetite, posture etc.) or predatory activity are observed, the co-housed species should be removed. Examples of fire salamanders drowned by male *Rana dalmatina* that mistook them for possible conspecific females are known (Grossenbacher, 2006).

2.4 Breeding

2.4.1 Mating

Mating can start in March and last until September, but Klewen (1985) found that 40% of the matings he observed took place in July. Males actively chase females and try to mate. Activity can be induced by spraying water. If selective breeding is the goal, keeping one male and one or two females together is the best option although this might conflict with the male-choice in females (see paragraph 2.3). When females are suspected to be pregnant, they could be kept solitary and wait for them to deposit the larvae in a water bowl. A paint roller tray or another shallow water tray can be used for this. When a young female produces larvae for the first time, unfertilised eggs can also be laid (Fig. 30). Often the first clutch of a female fails (see Bogaerts, 2018; Seidel & Gerhardt, 2016).

2.4.2 Deposit and rearing larvae

Females deposit their larvae from autumn to spring in the available water bowl. The larvae must be reared separately from the parent animals. A simple method to rear a small group of larvae is the use of low plastic storage bins (box, tank) with a lockable lid. Use low trays of 40 x 30 cm surface and a maximum of 10 cm high. A water depth of 5 cm is sufficient. There must be sufficient hiding places (pieces of flower pot shards, oak or beech leaves) and the water must be clean and rich in oxygen. It is sufficient to change the water half twice a week, but this also depends on the feeding schedule. As these larvae occur in nature in rain fed flowing streams but also small standing waters, they do not seem vulnerable to rapid change of natural water quality. One female can take up to 50 days to complete the deposition of all her offspring, which means you can find clutches of larvae spread over time (Caspers et al., 2014a).

Figure 30. From left to right: one unfertile egg, two normal larvae and one malformed larva of *Salamandra algira* (Sergé Bogaerts).

A more robust option is to create a larval nursery. This is a large aquarium with an external filter system and a continuous through-flow of water resulting in a weak current in the aquarium. The larvae are accommodated in plastic boxes of 20 x 20 x 12 cm (maximum 10 larvae per box) that are outfitted with ventilation openings along their sides covered with mosquito mesh. These boxes have floaters attached to ensure floating in the aquarium. The water is channelled back from the filter via a tube whose perforations will supply every single box floating on the surface with a weak jet of fresh water. An aquarium of 100 x 50 x 30 cm will thus offer enough space for raising 80 - 100 larvae to metamorphosis (see Seidel & Gerhardt, 2016; Fig. 31).

An intermediate option is to use filtered aquaria where sibling larvae are kept together, but water quality is maintained by filtration rather than simply water changes.

Figure 31. Larval nursery where groups of larvae are kept in boxes by Uwe Seidel (Sergé Bogaerts).

If food is supplied more frequently, the water must be changed more often due to waste products and uneaten food. Optional, an effervescent stone can provide extra oxygen and light water circulation. Aeration will also prevents a bacteria film on the water surface. Aquatic plants such as water weed (*Elodea* sp.) provide extra oxygen during the day, act as a water quality buffer and also provide shelter, but require lighting to survive (see also Pasmans et al., 2014). Just like the adult animals, the larvae are mainly active at night, but will feed at any time of day or night. The main points for space requirements for larvae are given in paragraph 2.1.1.

The death of a single larvae in a box can quickly result in the death of all the larvae in this box. Therefore, setting up multiple boxes/tanks with groups of up to 10 larvae is advised. The water temperature is preferably kept between 5-15 ˚C. At higher temperatures, accelerated metamorphosis occurs, resulting in smaller and weaker young salamanders. At too low temperatures (0-5 °C) there is no growth.

Cannibalism in fire salamander larvae is very common, especially if the animals are malnourished or there are too few shelters. Legs or tails are often bitten off. These often regenerate but the wounds can be infected by moulds (*Saprolegnia*) (Fig. 32).

Tubifex, *Daphnia* and *Enchytraeus albidus* can be given as food as well as numerous other food organisms (see paragraph 2.2.2). Because frequent feeding is necessary (every other day), it is very important that the water quality remains optimal. For example, dead *Daphnia* can quickly mould. This results in very large amounts of opportunistic pathogens (often *Saprolegnia* sp.) in the water. Water pollution is the most common cause of death for larvae. Rain water (or in some regions tap water as long as it is not heavily chlorinated) is generally sufficient (see for requirements paragraph 2.1.1.). If tap water contains chlorine it should be removed, which can be easily obtained by aerating the water heavily for 24 hours before use. The use of pond water is discouraged, since this may vector pathogens in. Larvae should be kept in aquaria or plastic containers until clear signs of metamorphosis are obvious (meaning: reduction of gills and tailfin, appearance of the bright black and yellow pattern). As soon as the animals enter metamorphosis (which can start after 60 days) easy access to a terrestrial area should be provided to avoid drowning. The easiest way to do so is to lower the water level to a maximum depth of 3-5 cm and cline the aquarium so part of the surface is dry or put in objects (stones, cork bark, etc.) that rise above the water level. This dry part should be covered with hiding places. The salamanders then should have a total length of 5-6 cm and then live on land. As the salamanders will metamorphose over time, parallel set ups will be necessary to remove salamanders that have metamorphosed once a week to a small terrarium.

Figure 32. *Salamandra i. infraimmaculata* larva dying of *Saprolegnia* infection on the head, probably secondary to a bite on the head (note the cotton like structure) (Sergé Bogaerts).

2.4.3 Development and care of metamorphs and juveniles

The young salamanders can be kept individually or in a small group (up to 5) in small plastic terrariums with a surface area of about 20 x 15 cm. It is best to furbish this in a sterile manner for the first three months. In the first few days after metamorphosis, a very damp terrestrial setup until the metamorphed salamanders have lost any gill remnants and sloughed their skins is ideal, as transition to drier conditions immediately can result in osmotic balance problems or drowning if the keeper waits to long to remove animals from an aquarium. This means a solid type of damp kitchen roll (unbleached) as a base that is moistened. Small flowerpot fragments or pieces of bark are placed on top of this. They can be covered with a layer of moss or dry (beech /oak) leaves for extra shelter and to preserve humidity. Bark or flowerpot shards can be stacked to create drier hides higher in the stack, and juveniles readily move between microclimates to osmoregulate. These terrariums are should be cleaned out at least once a week. It is important that the tissue remains moist, but not wet. A small water dish is then not necessary the first three months as it could even lead to drowning of the small salamanders. The enclosure should have a tight closing lid as juvenile fire salamanders are good climbers and can scale the sides and edges of glass and plastic tanks, but excellent ventilation is important too (see Fig. 18 & 36).

The advantage of this system is that the animals and their feed intake can be easily controlled and that a high infection pressure of parasites or extensive fungus formation are less likely to build up. A major disadvantage of this system is that it is labour intensive. The tissue must be replaced at least weekly (preferable a day after feeding) to avoid accumulation of toxic waste materials (in particular ammonia and nitrite) in the tissue paper. In addition, care must be taken to ensure that the terrarium does not dry out. After 2 to 3 months the tissue can be replaced with a layer of clay/loess and with a small shallow water bowl, for example a petri dish (salamanders can still easily drown!). This is then a mini version of the parent terrarium. After 3-5 years the animals can be sexually mature. Males can be identified often after 1,5 to 2 years even before they are able to reproduce.

2.4.4 Malformations

There are various known problems associated with breeding of fire salamanders, of which most malformations are caused by dietary deficiencies, notably metabolic bone disease (see paragraph 2.2.1). Genetic defects that often manifest themselves in colour deviations are common in captivity, such as albinism (see, among others, Concaro, 2004). Albino larvae are much more susceptible to diseases and infections, both as a larvae and as an adult animal (Concaro, 2004).

Animals with colour defects are not the goal in programs that aim to preserve species. For some enthusiasts, deviations like this are popular and can result into a wide variety of strange colour patterns (see Seidel et al., 2012). Sometimes scoliotic or deformed larvae are born. They often die during rearing. Some, however, just grow old, but they mostly do not reproduce. Culling is advised as these are useless for breeding programs.

2.4.5 Population management

For population management, programmes like ZIMS are advised. The advantage of fire salamanders is that after one year (or sooner if photographs are updated frequently) the yellow spots and stripes can be used like a fingerprint so every individual can be recognised by its pattern (see paragraph 2.5.1). For establishing the number of founder animals required to reach a pre-set goal (e.g. preservation of the percentage of genetic diversity over a certain time frame), a generation time of 10 years could be used. Females can store sperm up to two years under lab conditions (see paragraph 1.7.3). Given proper husbandry and nutrition, mortality during raising of juveniles is expected to be very low under correct conditions. Adult mortality is very low, given proper husbandry and nutrition.

2.5 Handling

2.5.1 Individual identification and sexing

Unambiguous identification of fire salamanders can be done either by pattern mapping (non-invasive) or by inserting passive integrated transponder tags (PIT-tags or microchips of 7 mm) in the coelomic cavity (see for instance Ryan et al. 2014, Whitman et al. 2016, Weber et al. 2019). A non-invasive identification is preferred above using PIT-tags.

Pattern mapping can be done using high quality detailed photographs of salamanders against a contrasting background and with indication of size. The major advantages are that this is a non-invasive, cheap and reliable technique. A software tool has recently been developed specifically for *Salamandra s. terrestris* (Speybroeck & Steenhoudt, 2017) and allows individual identification of a large number of animals. One high quality dorsal picture of the entire animal allows its classification using a unique code. Colour patterns change significantly during younger life stages (see example Fig. 33) and a sufficiently stable pattern is assumed to persist in fire salamanders with a total length of 14 cm and more. For smaller animals in captivity, taking pictures with regular, for example 3 month-intervals should allow identification during growth.

Figure 33. Juvenile *Salamandra s. terrestris* of 1.5 years after metamorphosis on a picture when it was a few months old. Notice changes in spots on head, but spots on body, feet and toes help to identify it is the same specimen (Sergé Bogaerts).

Passive integrated transponders (PIT-tags), when applied *secundum artem*, provide an easy, cheap and reliable method to identify salamanders. The major disadvantage is that the animal's snout-vent length needs to be at least 5 cm when using 7 mm transponders. PIT-tags can be inserted subcutaneously or in the coelomic cavity (Ryan et al. 2014, Whitman et al. 2016, Weber et al. 2019). Potential complications are the loss of transponders (which, if performed well, is actually very rare) and its invasive nature. PIT-tags should be inserted by trained persons only (Le Chevalier et al., 2017). The insertion site should be gently wiped clean with a sterile gauze or cotton-tipped applicator. We do not recommend disinfecting the insertion site to avoid possible irritation or even intoxication. After insertion, salamanders can be housed on moist tissue (Fig. 37) for one week in case complications would occur. If inserted properly, side effects are extremely rare (Pasmans, pers. obs.).

For larvae, individual recognition seems possible in a non-invasive way by photographing the pattern of dots on the tail (Eitam & Blaustein, 2002). However, they note this has only been tested in a small group of larvae. Visual Implant Elastomer (VIE) injections could be an alternative method (see Margenau et al., 2018), although it is not suitable for adult fire salamanders as their skin is not translucent. Handling and injecting small larvae is a delicate job; our advice is to start photographing animals from the dorsal side every month once they have metamorphosed, until the pattern becomes stable and then use pattern for individual recognition.

Sexing fire salamanders can be a challenge in immature and sexually inactive animals. Consistent with identification, reliable sexing can be done in animals with a total length of 14 cm and more (see Fig. 4 & 5). Reliably sexing of adult fire salamanders does require expertise. Invasive and non-invasive medical imaging methods work (for example endoscopy, ultrasonography) (Krause et al., 2013). For juveniles and larvae, there is currently no reliable method to identify the animal's sex.

2.5.2 General handling

In general, handling of any amphibian should be kept to the absolute minimum. Fire salamanders are relatively slow animals that can be easily handled when needed. We recommend wearing a novel pair of vinyl or nitrile (non-powdered, no latex) gloves per epidemiological unit (= animals from the same terrarium can be handled using the same pair of gloves). Preferably, the gloves are rinsed with tap water before handling amphibians. Depending on the procedure that needs to be performed, the extent of restraint varies.

Larvae are delicate and should be handled carefully using soft dipnets or using wet tissue. The smaller the animal, the more prone this will be to heat stress, which may result in death of the animal.

2.5.3 Catching and restraining

We recommend handling fire salamanders with the minimal restraint necessary to perform the procedure and to guarantee safety for the animal and the handler. In most cases, this means that fire salamanders can be gently restrained in the palm of a hand. This rarely provokes obvious signs of stress in the animals (secretion of glandular skin content, visible as white droplets: Fig. 34).

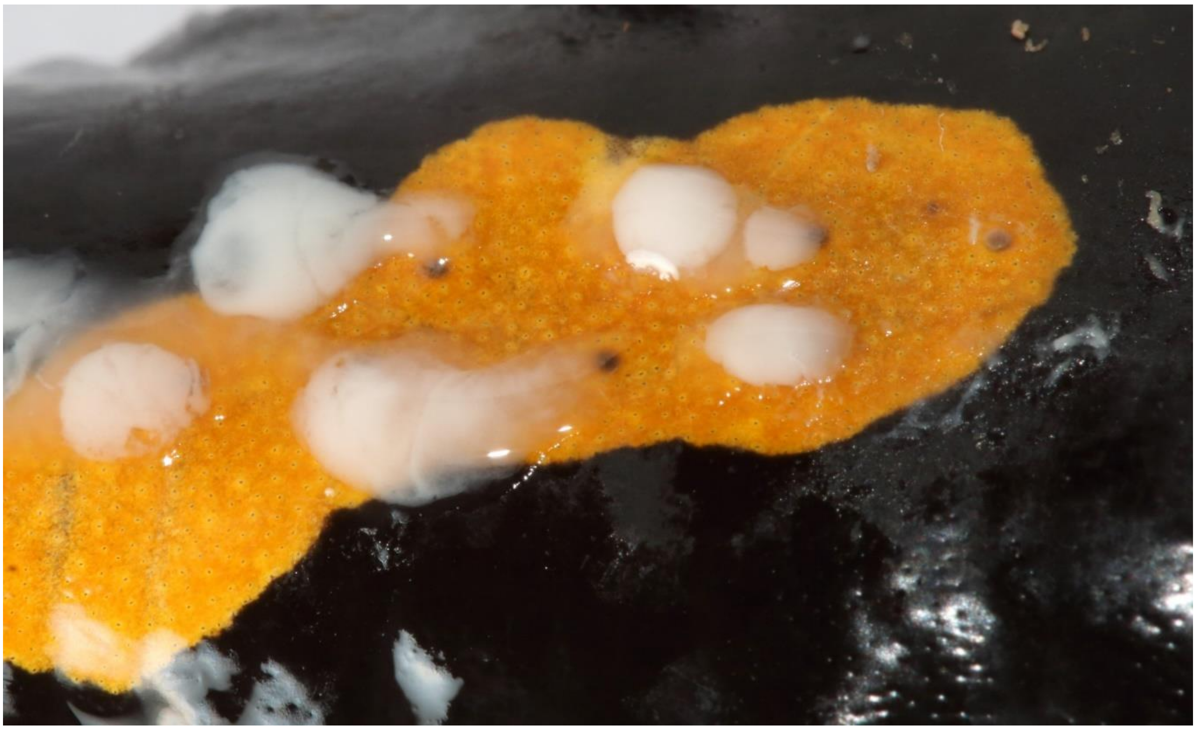

Figure 34. Stress in an adult *Salamandra*: secretion of glandular skin content, visible as white droplets (Frank Pasmans).

Catching terrestrial life stages of fire salamanders can simply be done by lifting the animal from the substrate and loosely restraining it in the palm of one hand. Holding should be done as short as possible, to avoid warming up of the salamanders. Larvae can be caught using soft dipnets. In case animals need more stringent restraining (for example for placing PIT tags, applying medication), the salamander can be held in one hand as shown in Fig. 35). Animals that are often handled are more prone to show signs of stress. Although force-feeding is sometimes advocated in amphibians, these authors rarely (if ever) perform this due to the stress inflicted to the animal.

Figure 35. Correct way of holding an adult fire salamander (Frank Pasmans).

2.5.4 Transportation

Transportation of fire salamanders should keep the following recommendations into account. Fire salamanders are preferably transported individually. Transportation duration should be kept to the strict minimum. However, salamanders will tolerate longer term transportation provided basic requirements are met. Plastic containers (Fig. 36) with sufficient ventilation, lined with moist tissue and provided with a hiding place (secured so it cannot damage the salamander during transportation) are well suited for transportation. The size of this container should have a surface of at least the total body length of the animal. To avoid trauma during transportation (e.g. by shaking, rough roads etc), the container can be loosely filled with soft material (e.g. Sphagnum moss), which also provides cover. Temperatures in the animal container should not exceed 20°C and should not be lower than 10°C. Adding a datalogger allows tracking environmental temperatures. In case of transportation of several days, regular exchange of the container's contents should be performed to avoid the accumulation of toxic metabolites. Environmental moisture (e.g. wet tissue, moist Sphagnum) suffices to meet the animal's fluid requirements. For transports longer than a day, paper needs to be checked and changed if the animal defecates.

Transportation has been regulated since EU 2018/320 (on 28 February 2018) by the European Commission. An implementing decision on certain animal health protection measures for intra-Union trade in salamanders and the introduction into the Union of such animals in relation to the fungus *Batrachochytrium salamandrivorans* has been issued (EU 2021/361 on 22 February 2021). Check the latest regulations at your local government as this may differ for non-EU countries like the United Kingdom, Switzerland or other non-EU countries.

2.5.5 Safety

Health risks for the person handling the salamander are limited to irritation of mucosa in case of contact with salamander skin secretions. Active spraying of poison from skin glands by fire salamanders may cause severe irritation to e.g. eyes and has been documented (Brodie & Smatresk, 1990), but is very rare and highly unlikely to occur. Fire salamanders will never attempt to bite.

Figure 36. Simple plastic transport enclosure for one fire salamander. A layer of moist tissue on the bottom and moss so the salamander can hide. Note that there are holes in the lid for air exchange (Sergé Bogaerts).

2.6 Biosecurity

Biosecurity should minimise the probability of animal escape and spill over of infectious agents to the environment, while preventing the entry of wild animals and pathogens.

2.6.1 Preventing specific pathogen entry (chytrid and ranavirus)

Establishing specified pathogen-free (i.e. absence of specific pathogens, notably chytrid fungi and ranaviruses) colonies of fire salamanders is crucial to the captive assurance program. In contrast to outdoor enclosures, a closed system (like indoor terraria) provides the opportunity to minimise the exposure to pathogens. A quarantine period of at least eight weeks under veterinary supervision is necessary. During this period, animals should be repeatedly tested for the presence of ranaviruses and chytrid infections. In case of ranavirus infection, animals should not be allowed to the program. Chytrid infections can be successfully treated and when the infection is eradicated, animals can be admitted. Every animal showing signs of clinical disease and every animal mortality should be examined properly by a qualified veterinarian. In case of persisting infections that cannot be treated (for example mycobacteriosis, chlamydiosis), affected animals and contact animals should be excluded from the program. Animals in the program should never be exposed to direct or indirect contact with amphibians of uncertain health status. To avoid entry of pathogens from the environment, enclosures must be protected (e.g. by lids) against wild animals entering the enclosure. Any material used in the animal enclosure should not derive from amphibian habitats without prior disinfection. Feeding wild invertebrates to captive salamanders may be expected to carry some risk of introducing pathogens. However, especially for raising larvae, few options are available and actual introductions are probably very rare. Nevertheless, commercially bred invertebrates should be preferred. Finally, pathogens may be vectored in on fomites (e.g., tubes, buckets, dipnets) or by people (e.g., after handling infected animals). Preferably, a set of materials dedicated to one epidemiological unit should be used. Depending on the setup, the narrowest interpretation of an epidemiological unit is a single enclosure. Handling of amphibians in captivity is preferably done using disposable nitrile or vinyl gloves (one pair of gloves per epidemiological unit) but care must be taken when handling larvae with nitrile, vinyl or latex gloves, which may result in toxicity (e.g. Cashins et al., 2008). Thorough rinsing of gloves is recommended prior to handling amphibians.

2.6.2 Preventing pathogen escape

Even if considered specified pathogen free, every amphibian collection should be considered a potential source of infection for natural populations. Waste should be preferably disposed of using channels that apply thermal inactivation (incinerator). Before disposing waste (including water, substrate) in the environment, this must be decontaminated. Heating (preferably autoclaving) is a proper, environmentally friendly option. Applying chlorine is highly effective but has marked ecotoxicity.

2.7 Veterinary problems: considerations for health and welfare

When conditions for husbandry and nutrition are met, there are few additional issues that would negatively interfere with fire salamanders' health and welfare. The main issues may be the current lack of standards to objectively assess salamander health and welfare, hampering efforts for optimisation. The most common health problems in fire salamander larvae are related to poor water quality, especially elevated ammonium and/or nitrite concentrations. Infections with oomycets such as *Saprolegnia* frequently occur, but are generally considered secondary to for example bite wounds by cage mates. In fire salamanders post metamorphosis, most common health problems are related to inadequate husbandry and nutrition. Healthy fire salamanders withstand temperatures of 25°C without any obvious adverse effects for at least 10 days. However, long term exposure to elevated temperatures appear to increase susceptibility to bacterial diseases such as *Chlamydiales* associated disease (Martel et al., 2012), which typically occur during summer months. Clinical signs are non-specific, with anorexia, abnormal locomotion and skin problems frequently occurring. Persistent exposure to a wet environment without access to drier places supposedly promotes bacterial and fungal infections. Moist substrates and water containers must not contain detectable levels of ammonium or nitrite to avoid intoxication. Such intoxication results in decreased activity and skin anomalies (often a marked darkening of the skin), which is often lethal. As mentioned in the paragraph on nutrition, metabolic bone disease as a consequence of low calcium levels in prey items is frequently observed and can be prevented by supplying feed items with extra calcium. Hypovitaminosis A is also often observed and can be equally prevented by supplying vitamin A through prey items (gut loading and dusting with mineral / vitamin powder). Animals with hypovitaminosis A can be treated by a single injection with vitamin A. Although intestinal parasites frequently occur, they rarely cause health problems but can complicate other, co-occurring diseases. Examination of fresh faeces and antiparasitic treatment of new arrivals during the quarantine period can be recommended (Fig 37). While the chytrid fungus *Batrachochytrium dendrobatidis* infections frequently occur in nature in fire salamanders, they appear to exert few if any negative effects in this species: infections mostly have a low intensity and are transient. Like other amphibians, fire salamanders and their larvae may be infected by ranaviruses, which cannot be successfully treated. Clinical signs include anorexia, skin problems (including ulceration) and haemorrhages.

Sick salamanders must be examined by a trained veterinarian and appropriate treatment given. During treatment, manipulation of the salamanders (e.g., for injections, force feeding) must be reduced to the strict minimum and many treatment regimens can consist of topical application that do not require the insertion of a needle. In anorectic salamanders, unless the animals are emaciated, focus should be on treating the cause of the anorexia, not on force feeding, which causes additional stress to the animal.

Figure 37. Basic quarantine container, with a small water basin, moist tissue and a ceramic hide for individual salamander keeping in a temporary set up (Sergé Bogaerts)

2.8 Specific problems: infections with *Batrachochytrium salamandrivorans*

Infections with *Batrachochytrium salamandrivorans* (Bsal) can cause disease outbreaks in fire salamanders in captivity, with up to 100% mortality. Any fire salamander with skin anomalies (pieces of unshed skin adhering, ulcerations general illness, Fig. 11 & 38) should be examined for the presence of Bsal. Samples can be collected by rubbing the skin of the belly, underside of the tail and one foot 10 times with a cotton tipped swab and sending the swab to a diagnostic laboratory for qPCR analysis. If swabs need to be stored, they can be put in the freezer until further analysis. In case of a dead animal, diagnosis of a Bsal infection consists of the combination of histopathology of the skin and qPCR of a piece of skin or a skin swab. In case Bsal is diagnosed, the following procedure is recommended:

1. delineate the extent of the outbreak and report

All amphibians in the collection (including anurans) must be sampled for the presence of Bsal using cotton tipped swabs and qPCR. Up to four animals can be sampled using the same swab.

In case one animal is found infected, all animals belonging to the same epidemiological unit should be considered infected and separated from other units. One epidemiological unit comprises all animals that may have been exposed by the infected animal (either through direct contact or indirect, for example through the common use of materials (buckets, containers, dipnets etc.) and may thus include several terraria or in some cases even several sites. If animals have recently moved to a different site or institution, report the infection to the receiving site or institution.

Try to trace the source of infection.

An outbreak of Bsal must be reported to the competent authorities (OIE listed disease).

2. isolate and apply strict biosecurity

Infected animals should be housed in a dedicated room where they are treated until the infection is cleared. Preferably, dedicated footwear and clothing for the hospitalisation room should be used. Infected animals should be handled always with vinyl gloves. All materials and content of the terraria (including all waste water) with infected animals and of the hospitalisation terraria should be disinfected prior to disposal (preferably by autoclaving; chemical methods include the use of Virkon S and chlorine (NaOCL 4%). For an overview of disinfectants, see Van Rooij et al. (2017). Every effort should be made to avoid contamination of the natural environment with Bsal.

3. treat all infected and suspect animals

Each animal should be housed individually in a well ventilated container, lined with wet tissue. A hiding place can consist of PVC tube. The container must be put at 25°C for 10 consecutive days (Blooi et al., 25°C). During this period, the temperature (of the container, not of the room!) needs to be monitored and must not drop below 25°C. Tissues must be replaced daily to avoid build-up of toxic waste products. Use a new pair of gloves for each animal. Heavily infected animals in a late stage of infection may still die but many animals will completely recover and skin lesions resolve within a few days.

4. confirm successful treatment

After ending treatment, put the animals at a temperature of approximately 15°C. This will promote the surfacing of infections that have not been eliminated by treatment. Swab the animals at 6 weeks after termination of the treatment. If negative and the animal appears healthy and is eating well, the animal can be considered free of infection. If a positive result is obtained, the complete procedure of treatment and testing must be repeated.

5. defining eradication of the infection

Although the above mentioned treatment is considered efficient, infections with Bsal can remain undetected (Martel et al., 2020). While this is least likely to occur in very susceptible species such as fire salamanders, at least in the Anatolian crested newt (*Triturus anatolicus*), such silent infections have occurred. Flare-ups of such infections can occur. It is therefore recommended to apply strict biosafety measures to an epidemiological unit of salamanders that has been infected in the past (i.e.: disinfecting all materials and contents before disposal, avoid any direct and indirect contact with other units and the natural environment) and remain vigilant in case an animal shows signs of disease. Keep record of the infection history of the collection and report previous infections in case animals are moved to a different institution.

The EAZA Amphibian Taxon Advisory Group announced the launch of the Massive Open Online Course (MOOC) focused on *Batrachochytrium salamandrivorans* (Bsal). An e-course in 4 modules for participants to learn at their own pace about:

- the epidemiology of the fungus: its origin, distribution, risk for biodiversity and mitigation actions
- Veterinary aspects: how to recognize Bsal in the lab and in the field and how to treat and prevent it
- Ex situ conservation: when and how to initiate ex situ conservation, information on husbandry guidelines is provided and on chytrid conservation projects
- Call to Action: How to make conservation management decisions with uncertainties, and which research questions can and should be addressed urgently?

Register here: https://loom.ly/9oSCHBo.

For further questions and/or comments please use the email address: bsal@eaza.net

Figure 38. Close up of typical Bsal lesions, small holes in the skin, with darker edges, here clearly visible on the yellow spots (Frank Pasmans)

2.9 Specific problems: considerations for health and welfare

When conditions for husbandry and nutrition are met, there are no additional issues that would negatively interfere with fire salamanders' health and welfare. The main issues may be the current lack of standards to objectively assess salamander health and welfare, hampering efforts for optimisation.

2.10 Recommended research

Out of the previous section it becomes clear that some questions still need to be answered. They mainly deal with wellbeing, health, fitness and diet. These are the scientific problems to address:

How to assess wellbeing in captive fire salamanders

Husbandry and feeding protocols are currently based on subjective assessment of fire salamanders' health, longevity in captivity and their potential to reproduce. These may suffice for initiation of programmes but the purpose should be to keep salamanders in captivity under, for the animals, optimal conditions. For reference, animals could be considered that stem from large populations, not showing any obvious signs of severe disturbance (e.g. disease, habitat alteration, lack of recruitment etc.). Few proxies for wellbeing in amphibians are known. One possibly usable indicator is the level of corticosteroids. Corticosteroids are associated with chronic stress and are involved in highly diverse processes, including immunity. Establishing a proper matrix for corticosteroid assessment and establishing baseline levels in wild salamanders could yield a more objective method to assess the animals' wellbeing.

How to assess health and fitness in captive fire salamanders

While it is well known that fire salamanders can be kept for well over 20 years in apparently good health, reproducing regularly, it is not known which level of fitness is needed before animals can be released in the wild and how this can be measured (which parameters determine fitness for release in the wild). Currently, about the only measure is a thorough clinical examination of overall health (rendering e.g. metabolic bone disease obvious), combined with calculating the (either or not scaled) body condition index (although reference values are currently lacking). Additional techniques that are routinely used in veterinary medicine could be applied to captive salamanders, provided reference data are available. Notably, proper hematology and blood biochemistry would be a useful instrument to assess overall functioning. Quantifying bone density may be an additional technique to estimate the skeletal calcification. Stamina as often measured in lizards could be an indicator of condition as well, but this has not been investigated for salamanders. Estimating the animals' immune status would be advisable as well but proper and validated methods are currently lacking. Finally, both skin and intestinal microbiomes could be included in an overall fitness assessment. Research should select one or more of these proxies for fitness, taking into account relevance and feasibility. As a reference, optimal fitness could be inferred from data from clinically healthy, wild fire salamanders from healthy populations across life stages and seasons. Captive animals could be followed up based on these baseline data.

Research investigating adaptation to captivity in captive fire salamander populations is also important in order to establish the suitability for translocation. This should look at genetic and phenotypic levels of behaviour, physiology, reproductive investment, phenology, microbiome and other factors.

What is an optimal diet for captive fire salamanders and how to assess?

It is clear from the literature that fire salamanders are rather opportunistic predators, feeding on a large variety of invertebrates, it is not clear to which extent highly prevalent prey items like for instance millipedes contribute to the animals' health (e.g. by the provision of calcium) and whether prey derived substances contribute to the salamander's defence mechanisms, e.g. by incorporation of metabolites in skin secretions (for example against Bsal) as is well known in several anurans. Also, optimal feeding regimes per salamander per week are unknown. Studies on optimal diets for captive salamanders that result in maximal fitness and optimal development, growth and reproduction are necessary. This would be particularly relevant when releasing animals in a natural environment.

How to determine sex in larvae?

There are strong indications that temperature induced sex biases occur in urodeles in captivity, despite the absence of published evidence. To increase efficacy of captive breeding programs, it would be useful to be able to predict the sex ratio of offspring and to determine sex of the larvae at a very early stage.

Cryopreservation

Due to their specialised reproductive phenotype (ovoviviparity), developing techniques for cryopreservation poses additional problems to those in for example frogs (with, mostly, external fertilisation of ova). Developing techniques to collect ova and sperm or fertilised embryos from salamanders, cryopreserve them and successfully thaw them afterwards would open new avenues for ex situ conservation.

Figure 39. *Salamandra salamdra terrestris* in its habitat, near Verviers, Belgium (Sergé Bogaerts)

Section 3: References

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