Ex-situ Management of





Amphibians In Indian zoos

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Melanobatrachus indicus, also known as the Malabar black narrow-mouthed frog. It is endemic to wet evergreen forests of southern Western Ghats in Kerala and Tamil Nadu states of India.

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PREFACE

Amphibians are integral to critical ecosystems and are central to many cultural stories and beliefs, including the more modern popculture. They are known to be indicator species for fragile ecosystems. The complexities of amphibian decline in relation to climate change and the rapidly dispersing fungal disease, chytridiomycosis ("chytrid") have been at the forefront of conservation planning consultations.

Globally, ex-situ programs for amphibians with interdisciplinary approaches play a significant role in the development of conservation action plans. The fact that the rate of decline of several species can leave behind the most rigorous in-situ conservation efforts, captive assurance populations have been recognized as the beacon for survival for many amphibian species. Thus, making zoos an integral part of a global response to the amphibian crisis.

This document on ex-situ management of amphibians in Indian zoos is a step to support the Indian zoo community in their efforts to house amphibians and streamline their husbandry protocols based on their local conditions.

I hope this document will be useful to the zoos in their endaevour to house amphibian species and advance captive care standards and contribute positively to ex-situ species conservation efforts.



Dr Sanjay Shukla, IFS Member Secretary, Central Zoo Authority

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1. Introduction and need

In the face of overwhelming threats that amphibians face, the urgency of bringing populations at risk of extinction into the fold of conservation breeding is an important step. With more than 8,425 amphibian species globally, there is m6uch to be learned about their natural history and captive husbandry requirements. In the face of overwhelming threats that amphibians face, the urgency of bringing populations at risk of extinction into the fold of captive conservation is an important step forward. This has been repeatedly recognised as an important activity in global Amphibian Conservation Action Plans (ACAP) since 2005 (Mendelson et al, 2005). Globally, the zoological community continues to employ resources and expand capacity in managing amphibian species collections. This also serves as a tool to educate the public and gain their support for amphibian conservation.

Captive populations contribute to 1) research on amphibian biology and disease, 2) development of improved husbandry guidelines, 3) training and capacity building in range countries, and 4) mitigation of threats in the wild. Captive populations would serve this purpose only if they are implemented as part of a plan that includes Conservation Needs Assessment (CNA) and prioritization of efforts, research on amphibian biology and disease, development of improved husbandry, training and building capacity in range countries, and mitigation of threats in the wild. As the program develops, captive amphibian populations can facilitate many goals that are listed in6 the ACAP, Decision Support Systems (DSS) for rapid response to die-offs, support studies on the biology of amphibians, diseases impacts and their mitigation. This has been recognized as an important activity in global Amphibian Conservation Action Plans (ACAP) since 2005 (Mendelson et al, 2005).

2. Global Trends in Amphibian collections in zoos

The Global Amphibian Assessment (GAA), conducted the first comprehensive assessment of the conservation status of all known amphibian species for the IUCN Red List in 2001. The Alliance for Zero Extinction (AZE) identified hundreds of sites worldwide that held entire, global populations of Critically Endangered or Endangered species, and marked them for protection. However, site conservation alone was not sufficient, and the Amphibian Conservation Action Plan (ACAP) came into effect to identify issues and action to address the crisis, including ex-situ conservation measures. A number of organizations, namely WAZA's Amphibian Ark, the Amphibian Red List Authority (ARLA), the IUCN Species Survival Commission (SSC), Conservation Planning Specialist Group (CPSG), and the Amphibian Specialist Group (ASG) stepped in to act as a global network of breeding programs linked to conservation and research.

Several institutions have made efforts to focus attention to exsitu conservation of amphibians. However, amphibians are often small, shy and inconspicuous; by virtue of which they do not attract crowds and often miss out on resources and funding opportunities. Thus, despite being among the most threatened taxa, they are grossly under-represented in captive collections. On the basis a study conducted in 2017, only 7.1% of all the recognised species were housed in zoological collections. Of these, 76% were anurans, 22% caudates and 2% caecilians. Only about a quarter of the species represented among all collections are Globally Threatened Species (GTS), and a large chunk of them are distributed among very few institutions abroad. The remaining three-quarters are non-GTS species and have no direct ex-situ conservation need, since their populations are stable in the wild.

However, it is possible that they could act as flagship species, to raise awareness for the highly threatened taxa. The limitations to ex-situ management of amphibian species extends even further. Large populations of a threatened species are often housed within a single institution, or at specialized breeding facilities within the country of origin. This puts them at a risk in the event of disease or natural disaster. Further, meeting husbandry requirements can be difficult, given the lacunae in ecological data and the specialist nature of most amphibians. Breeding them is difficult, and very few zoos have reported success rates so far.

A closer mutually beneficial cooperation for ex-situ conservation action between 'traditional' commercial zoos and 'nontraditional' research institutes or specialist groups is recommended by Amphibian Ark. Although the generalised impression is that zoos and aquariums have, with a few exceptions, failed to establish and maintain breeding populations of threatened amphibians, it is important to note that the underlying analysis so far has been a broad and global approach to investigate the overall response to the amphibian crisis. It does not take away from the fact that some zoological institutions have gained a lot of experience in terms of research, husbandry, ecology and in-situ and ex-situ conservation, as well as in the education of visitors and decision makers.

3. Scope of this manual

Captive Management

The manual attempts to support zoos in India to develop sustainable ex-situ facilities for amphibians. Frogs, salamanders and caecilians are three distinct groups of amphibians. They can be maintained in captivity with relatively less cost. For successful management of amphibian populations and based on their complex life history, four types of enclosures should be designed for each species.

- (i) Adult
- (ii) Breeding
- (iii) Hatching and larvae/tadpole rearing
- *(iv)* Metamorph rearing.

Given the diversity of amphibians, reproductive modes and specialized requirements, in this manual, we present ex-situ management practices for the three groups of amphibians using a focal species for frog- *Minervarya agricola*; and salamander - *Tylototriton himalayanus*. For frogs and salamanders, the husbandry protocols have been used and further standardized based on the guidelines by Gupta et al. (2015).

For caecilians we provide a broad framework of ex-situ management options. As the husbandry practices for caecilians are not fully developed, we do not recommend captive management of caecilians in zoos in India at present.

Captive management of Minervarya frogs

1. Introduction

The Genus Minervarya has 31 known species of frogs and they are found throughout India. They are small frogs that grow up to 3 cm in length. They have unique combination of characters among Ranoid frogs in possessing a forked omosternum (the anterior element of the sternum which projects forward from between the clavicles), a rectal gland in most of them, rudimentary webbing, dorsal skin showing longitudinal folds, "Fejervaryan" line on side of belly and upper lip with a white horizontal line (Dubois et al. 2001). For morphological character description, refer to Annexure1. The species resemble each other and close examination of them can provide an unambiguous identity. For the purpose of this manual, we have provided a description of live frogs of Minervarya agricola, a widespread species found in central and south India. It is a small sized frog with snout to vent length not exceeding 3 cm. Snout is pointed and the nostrils are rounded. The snout is longer than wide and the space between the eyes on the head is convex. The eyes have an oval pupil. Tympanum or the external ear is indistinct and the fold of skin from the eve to shoulder is also indistinct. A small pineal opening is present between the anterior borders of upper evelids. 'Vomerine teeth' are small bony projections at the roof of the oral cavity. Vomerine teeth are absent, tongue is heart shaped without any projections on the surface, and the jaws don't have any toothlike projections. Skin on the head, back and flanks smooth, towards the cloaca there are some glandular warts. Some

longitudinal warts are present on the dorsal skin surface. Symmetrical, faint and a thin transparent line runs along the flanks referred to as 'Fejervaryan line', in the background of an opaque pale white belly. Small rounded projections referred to as 'rictal glands' are present at the junction of the jaws and below the tympanum. They are sometimes single or few in a cluster of varying sizes. Rictal gland is present in some species and absent in some others in the genus. Other large glands are absent in the frogs. Ventral parts of head, body and throat are creamy white with grey spots. Webbing on the feet is dark grey. Males have secondary sexual characters. They develop 'nuptial spines', a patch of very small whitish or transparent spines on the dorsal surface of the first finger. Vocal sacs that are strongly pigmented and they develop in mature males. They are in the form of a pouch near the neck region. Dorsal skin colour varies, and the most common background colour is dark brown with olive green blotches. Sometimes the frogs have a prominent dorsal vertebral line, which can be brightly coloured. Dorsal surface of limbs is patterned with dark blotches. The fingers and toes are pointed. There are tubercles on the base of the feet on the digits. The webbing on the feet can differ in species. The front feet have hardened skin as they serve to take the force of landing during their leap. The hind feet are folded when they are sitting and the underside of the thigh has a sensitive skin that has several blood vessels. This makes it look red in appearance.

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Minervarya agricola showing variation in dorsal coloration and tympanum shape (Photo: Krishna Komunduri)

2. Founder population

When populations are sourced, they should be collected from geographically representative populations to bring a reasonable level of genetic diversity in the founder population. Since Minervarya frogs are highly cryptic, collection of geographically distant populations needs extra care. During this process, local adaptations should be taken into consideration, and populations that are in very different climatic zones should be avoided. Taxonomically identified population of 20 adult females and 10 adult males could be considered as a founder population for the species. All frogs should be tagged using Passive Implant Transponder (PIT) tags (see Annexure 2). Oral swabs can be collected as genetic samples to test the genetic variability in the population.

3. Housing adults

3.1 Space requirement

Floor space of 100 cm x 30 cm x 40 cm would be the minimum enclosure size wherein 6-8 (1:2 sex ratio) individuals of M. agricola could be maintained. The enclosure can be made with appropriately sized mesh on the hood/lid to allow for natural light and may have material like UV transmitting acrylic on the sides to retain heat and humidity. Glass blocks UV and therefore, it is not suitable. A covered drain hole with a fine mesh should be provisioned to drain water out of the enclosure. The outlet must be covered with a cap The height of the enclosure will allow for the use of vertical space with enrichment material. Clean unchlorinated ground water should be provided. Water should be deep enough for the frogs to immerse their drink patches and hop freely without coming into contact with the lid. For *M. agricola*, the enclosure can be raised on one end and 2 cm depth of water can be maintained at the deep end of the enclosure. It should be ensured that roughly half of the enclosure has standing water always.

3.2 Hides

Hides are essential as some frogs show elevated levels of stress, if they are constantly exposed. Live plants can be added to the enclosure as they create humid pockets and also provide visual barriers for the frogs. Enclosure architecture and enrichment material should not have sharp projections or be transparent. A few shards of clay pottery of differing sizes or a small potted plant could also be placed inside an exhibit. While choosing covers and enrichment, consider the following points:

- a) visual barriers and hides,
- b) use of three-dimensional space,
- c) texture of substrates,
- d) ease of maintaining hygiene

3.3 Substrates:

The benefit of most naturalistic substrates is that they provide burrowing species places to hide. The pH of the substrate should be considered, for example some mosses are rather acidic which may irritate some species' skin.

- Sphagnum moss: High-quality sphagnum moss is soft, provides more burrowing/hiding opportunities (refugia), and has antimicrobial properties that can be a good choice for some quarantine applications. Used moss can also be sterilized and re-purposed for other applications.
- 2. Astroturf: This plastic grass-like material is inert and easy to disinfect, as long as it is rinsed thoroughly afterwards, and can be cut to fit any size or shaped enclosure. It is mold-resistant and can be maintained in semi-aquatic enclosures.
- 3. Paper towels: Paper towels work well in quarantine situations but may dry out quickly, requiring frequent monitoring to ensure that they are sufficiently moist. The moist environment also provides a substrate perfect for bacterial

growth, but daily changes can keep problems to a minimum; it is recommended to change the paper towels at least every 48 hours, if not sooner. Unbleached paper towels are recommended, as white or bleached paper towels may contain traces of chemicals such as dioxin.

4. Foam rubber: Foam rubber can be purchased in various thicknesses from fabric stores. This material can be cut to any shape and works well for quarantine situations where cleanliness is a priority, as it can be disinfected. It can also be used for isolated specimens that need or require a softer surface for medical purposes (i.e., broken limbs or rostral rubs). There is some concern about the potential release of dioxins with foam and its potential to harbor harmful bacteria.

Organic and natural substrates:

- Coconut (coco) fiber: This substrate has grown in popularity because it is resistant to breakdown, lasts for a year or more, and makes an environmentally friendly alternative to peat moss. Soak the coco fiber brick overnight in water and squeeze out excess moisture prior to placing it at the bottom of the enclosure. If used on top of a layer of gravel, insert a piece of shade cloth or fiberglass screen cut to fit between the gravel and the fiber to prevent the fiber from mixing with the gravel.
- Potting soil: In general, soil is not a good choice for use with amphibians. Potting soil tends to become compacted and become permanently oversaturated with water under terrarium conditions. If there is no other alternative, use only steam-sterilized potting mix without vermiculite, perlite, or

other artificial additives such as fertilizers. Potting soil can harbor and encourage the establishment of nematodes and other parasites, so its use should be limited. However, some fossorial amphibians do best on a soil substrate and using organic soil products and substitutes may be appropriate in these cases.

- 3. Rocks and gravel: Gravel is a useful, inexpensive, and relatively easy to clean substrate. However, it is heavy and can lend an unnatural look to the vivarium. Aggressive feeders may ingest the gravel by accident, as this can cause a digestive track impaction. A generous layer of moss on top of the gravel layer can reduce the risk of accidental gravel ingestion.
- 4. Sand: This is relatively inexpensive and must be well rinsed before use. Select a grain size that is not powdery, but that has some substance to it. Note that sand, if consumed in substantial quantities, can pose an impaction hazard similar to gravel.

Adult frogs when brought from the wild need a period of acclimatization. This may be done in an off-display enclosures of similar size as the display enclosure. During this period sides of the enclosure should be made opaque by covering it with a colored paper on the outside. This allows the frog to gauge its leap and not hit the wall or hood of the enclosure. After a month, the cover sheet can be removed one side at a time and post quarantine and health assessment the collection can be moved to display enclosures.

3.4 Temperature, Lighting and Water Quality

Enclosure climatic conditions can be monitored by fixing a thermohygrometer on the inside of the enclosure and readings should be taken without opening the lid. Ambient room temperatures should vary between 23 °C and 27°C (night/day) during summer and 20 °C and 25°C (night/day) during winter.

The temperature on the substrate below the basking spot should range between 30- $32^{\circ}C$

Frogs will take a few days to a week to habituate to an enclosure. Once they have habituated it is not advisable to keep changing the regimes of light, temperature and humidity. Vitamin D3 plays a critical role in regulating calcium metabolism, as well as important roles in muscle contraction, organ development and the functioning of the immune and nervous systems. In the majority of vertebrates, vitamin D3 is synthesized via exposure to the ultraviolet B radiation (UVB) present in sunlight. The synthesis of vitamin D3 in the skin requires heat, therefore heat must be provided in tandem with UVB radiation, and temperatures matching with the wild. Some amphibian species might also receive vitamin D3 through some diets although the extent of this is largely unknown; some food supplements provide vitamin D3 artificially. Ultraviolet light gradient should be provided in conjunction with a basking spot. A D3+ reptile lamp that provides 12% UVB is ideal and maximum UV should be available to the frogs at their basking spots. If spot lighting is not possible, then the UV light should be kept on for 6 hours during the day. UV readings can be recorded to check the intensity using a solarmeter. Glass prevents available UV, therefore, UV permeable Perspex/Acrylic should be used. All lighting should be kept insulated so that frogs do not come directly in contact with lamps. Ultraviolet light gradient should be provided in conjunction with a basking spot for frogs. It helps amphibians in calcium metabolism and avoids decalcification of bones.

Water quality is an extremely important component of amphibian husbandry. Monitoring water quality is vital to successfully rearing of healthy, captive amphibians. Fluctuating water parameters create stress for the individuals, therefore it is better to maintain constant conditions. Most zoos use municipal water sources which are generally treated with chlorine, or bleach. This is toxic for amphibians, should they come in contact with it. Therefore, it is advisable to allow excess chlorine to evaporate by keeping the water exposed to sunlight for a day. Avoid using water which has previously been in contact with other amphibians as this may risk disease transmission. Reverse osmosis (RO) systems will produce water that is pure, but it is not suitable for amphibian osmoregulation. RO water can be made suitable either by adding salts or a known quantity of tap water. Avoid water that has been stored in metal tanks or exposed to toxic substances that could leach into the water. Water parameters should be tested frequently in newly established systems. (Annexure 4)

A commonly used system for keeping aquatic and semi-aquatic animals is a semi-closed system, in which a combination of filtration and water changes are employed in order to maintain water quality. Small changes in water quality are permissible. In the closed system, frequency of water changes will depend on the stocking density, the temperature of the water and the amount and type of food given. The outflow of the filter should be adjusted to meet the requirements of the amphibian species. Some tadpoles experience stress if water flow is rapid. The mouth parts, their position and the tail shapes are indicative of the niches of the tadpoles. This information can be used to plan the husbandry and enrichment for tadpoles.

Mechanical, chemical and biological filtration systems are available. They have their advantages and disadvantages. The choice of the system will determine how the husbandry practices will be standardized.

Water should be tested regularly for chlorine, ammonia, nitrite, nitrate, pH and phosphates. Ensure the temperature of the water is at equilibrium with the room temperature. Measured quantity of water should be used to provision the enclosure. The enclosure should be lightly misted with aged tap water (ie dechlorinated water) daily. The substrate and sides of the enclosure can be misted twice a day.

Chilling systems can be installed to cool water temperature in enclosures. Circulating cool water in the enclosure can be a way to keep enclosure temperature matching with their natural environment. Alternatively, air-conditioners can be installed and temperature maintained at 26-28°C.



Figure: Schematic layout of off-exhibit enclosure for *Minervaya* frogs on the bench with natural water treatment tank below

4. Handling and Captive Husbandry

At the time of handling and restraint, safety of personnel and the well-being of the amphibian must be taken into consideration (Wright, 2001). Effort should be made to minimize stress. The skin of many species of anurans and salamanders produces toxic and inflammatory secretions, some others secrete mucilaginous secretions that might cause skin irritation. 1. Handling frogs without clean hands (with clean water) can be dangerous as their skin in permeable to the chemicals and secretions. Wash hands or wear non-powdered vinyl gloves.

2. Avoiding direct contact all-together is preferable by using a small aquarium net or disposable utensils.

3. In case handling is needed, Wet the palms of hand and place thumb on the back of the frog, just below its head. Scoop-up the frog with fingers around the torso. The frog or toad should be laying on the fingers while your thumb is holding the frog gently in place.

All animals should be visually inspected once a day atleast. Water dishes must be cleaned out and refilled daily as frogs excrete in the water. Feces and uneaten food must be removed daily. The enclosure and all covers must be thoroughly scrubbed with a brush and hot water, and not chemical cleaners, such as bleach, twice a week. Temperature and humidity of the enclosure should be checked at different points to ensure that the correct thermoregulatory gradients are maintained. UV light readings must be taken every three months to check that there is a sufficient UV gradient and that the UV lights are functioning.

Alternatively in the case of fixed display enclosures, there could be a provision for a skylight and mesh covering along the width of the enclosure. The positioning of the enclosure with respect to sunlight should be an important consideration.

In the case of off-exhibit movable units, they can be sunned for a period of 30 minutes every day with a mesh cover. Exposure to diffuse sunlight should be taken up with the highest level of caution, as direct sunlight will dry up frogs and will change the temperature and humidity conditions rapidly inside small enclosures. It is not recommended unless there is careful monitoring of the enclosures.

In winter, amphibians hibernate and during peak summer they aestivate. While it is risky to create conditions of aestivation, hibernation in amphibians can be promoted. It is naturally triggered when the substrate is cold and dry. Hibernation spots can be loose stones, logs, loose soil. Once frogs hibernate they will not feed and they should not be disturbed. They will emerge out of hibernation when the conditions are made favorable. This can be done by simulating natural conditions that trigger their emergence.

5 Nutrition and Diet in captivity

Frogs eat live insects usually. Some frogs can be trained to feed from the tip of a glass rod or blunt forceps. Food should be offered after it is dusted with an appropriate dietary vitamin supplement. For details of the nutritional requirement of amphibians refer to Ferrie et al. (2014). Majority of the nutritional needs of amphibians are met if the diet provided to them is appropriate. For this, insects (grasshoppers) can be reared with clean and nutritious food. Since frogs get their nutrition through the grasshoppers, care should be taken to feed the grasshoppers with a variety and rich vegetable diet. For details on rearing of insects see Annexure. Frogs eat live insects usually. Some frogs can be trained to feed from the tip of a glass rod or blunt forceps. Majority of the nutritional needs of amphibians are met if the diet provided to them is appropriate. For this, insects like grasshoppers and crickets can be reared clean and fed with nutritious food. Since frogs get their nutrition through their feed, care should be taken to feed the insects with a variable and nutrient-rich vegetable diet. For details on rearing insects see Annexure 3. For details of the nutritional requirement of amphibians refer to Ferrie et al. (2014).

Frogs mainly feed on grasshoppers, small crickets, earthworms, beetles and moths. Feed should be no greater in size than the distance between the eyes. The number of insects provided should be in multiples of two of the frogs housed in the enclosure. The insects should be gently released live into the enclosure. (Culture for Crickets and Drosophila as food given as Annexure 3)

Frogs are usually nocturnal and therefore, they should be fed in the evening. Indoor lighting can be dimmed during the night. Adults frogs should be fed once every three days, juveniles should be fed daily. If healthy populations of grasshoppers are maintained, then instars of different sizes can be fed to frogs of different sizes. Obesity is commonly encountered in the husbandry of frogs. Therefore, a continuous monitoring of body size (snout to vent length) and body weight is important. If there is disproportionate weight gain, then feed should be regulated by isolating the obese frog and altering and monitoring diet.

6. Display and off-exhibit enclosures

These enclosures are of the same dimension as the enclosures used for housing the adults. Typically, 4-6 frogs can be kept in one enclosure. Frogs of different sizes should not be kept in one enclosure. The enclosure base can be kept at 4 feet height from the ground. The glass pane on the display side can be kept at an angle tilting inside and towards the top, so that visitors get a top view. Frogs get used to movement on the display side and stop getting disturbed by their movement Noise and vibrations should be minimized by using thick glass (4 mm).



Figure: Side view off-exhibit enclosure with appropriate dimensions for housing *Minervarya* frogs



Figure: Front view of exhibit enclosure for *Minervarya* frogs with provision for signages and viewer facility

7. Reproduction in Captivity

7.1 Breeding enclosure

This enclosure can be twice the size of the display/off exhibit enclosure. The height should also be increased by 1.5 times. In nature breeding is triggered by humidity. Adult frogs of known sexes can be brought into the breeding enclosure. This enclosure shall be designed to simulate rain. The rain chamber with a floor space of 60cm x 100 cm would be suitable as a minimum size for 3 males and 6 females of *Minervarya*. The male population in each enclosure should be closely watched. They expend energy in breeding and in territorial fights. They

can also disturb and cause stress to females that are laden with eggs. The same individuals can be housed continuously for a period of month with routine spells of rain during the day for 3-4 hours to trigger breeding. No special substrate is required in the rain chamber. If the individuals don't breed for a long time, the substrate can be layered with clay. There should be a sufficient number of perches that are above water so that the frogs can rest and use the water surface periodically. A pump powered spray system or fountain can serve to make rain. The water depth should be 150-200 mm at one end of the rain chamber. Lighting and temperature parameters should be maintained in the same manner as in the display/off exhibit enclosure. Visual barriers are essential to avoid conflicts. Small potted plants can be placed in the center to create visual barriers. Plants or materials used should not have sharp projections or be transparent. The frogs should be fed at least every other day whilst they are in the rain chamber but ensure that any dead insects are removed daily.

Frogs have external fertilization and they spend a considerable amount of time in amplexus outside water and in water. This behavior is an indication of the breeding activity of the pair. Fertilization happens in the water after eggs and sperms are released into the water. Therefore, it is important that the water is not changed routinely. A biological filter would be appropriate to use. The water quality should be monitored regularly in the breeding enclosure (refer to the standards provided in the Annexure).

7.2 Egg Care, Tadpole Husbandry & Development

Eggs are laid in clusters and are referred to as egg mass. The adult frogs should be removed from the rain chamber once eggs are laid. The eggs are usually inside a jelly like mass. Sometimes the egg mass floats and disturbing them leads to detachment of eggs and the eggs sink and might become unviable. Egg masses can be separated by placing them in smaller tubs of water. Usually eggs hatch in 48 hours after laying. All the tadpoles wriggle out of the egg mass and swim freely. Tanks should not be stocked with more than five tadpoles per litre.

Water filtration is vital as there can be accumulation of large amounts of excreta in the tank when tadpoles are reared. Dechlorinated tap water (Tap water left to stand for 24 hours for chlorine to dissipate) can be used to rear tadpoles. Ensure the temperature of the water is at equilibrium with the room temperature.

Not more than 20% of the water should be changed to ensure that there is no change in the water quality. Fish aquarium aerators are useful in making water fit for tadpole rearing. Feed should be made available in small quantities and rationed based on the extent of feed remaining in the tank. Tank water should be cleaned on noticing leftover feed or debris. After hatching the egg mass jelly can be removed as it can change the water quality. Substrate is not necessary but tadpoles like to hide, so some covers can be provided.

Tadpoles are voracious feeders, so constant attention is required for feeding. Tadpoles should be fed daily, or twice daily depending on how much food is left over after feeding. They can be fed on ground tropical fish food, grass pellet, tubifex worms, dried shrimp, spirulina algae, boiled spinach, hardboiled egg yolk and cuttlefish bone. Once the front limbs appear and the tail starts to reduce it is important that the metamorphosing froglets are given the opportunity to emerge from the water as they can easily drown. Rocks or aquatic plants are suitable. Once they emerge, they are prone to drying out and easily die if the humidity is not high.

7.3 Rearing Metamorphs

Metamorphs have hind and forelimbs and have a tail. They can be housed in small plastic containers. A 35 x 20 x 30 cm enclosure is suitable for up to 10 metamorphs of one species. Rearing tanks should be small so that high ambient humidity can be maintained and the juvenile frogs can find food easily. Lid of the enclosure should be made permeable to light/UV with the use of a fine mesh. The enclosures can be placed in dappled sunlight for 30 minutes every day. Plastic container surfaces are suitable for housing without any specific substrate. Water depth of 10 mm should be maintained in the enclosure. Small perches such as rocks and shards of pottery can be put inside from them to climb on. Hides are extremely important microhabitats. The metamorphs can be raised at the same room temperature as the adults, and must be misted with room temperature tap water twice daily. Metamorphs do not require basking spots. Temperature inside any part of the enclosure should not exceed 28°C. Metamorphs readily feed on early instar of grasshoppers and drosophila. Until 6 weeks they can be provided with feed once every 3 days. After 6 weeks the feeding interval can be gradually decreased to feeding once every 2 days. The number of insects provided should be in multiples of two of the number of frogs in the enclosure.

8. Health and quarantine

Ultraviolet light gradient should be provided in conjunction with a basking spot. It helps amphibians in calcium metabolism and avoids decalcification of bones. *Batrachochytrium dendrobatidis* (Bd) will survive in tap water and in deionized water for 3 and 4 weeks, respectively. In pond water, infectivity has been observed for 7 weeks after introduction. The knowledge that water can remain infective for up to 7 weeks is important for the formulation of disease control and quarantine strategies for the management of water that has been in contact with amphibians. To comply with the intentions of OIE listing, amphibians, when moved between countries, should be placed in a different container on arrival; all water, soil, plants, and litter in contact with the amphibian during transport should be adequately disinfected by using techniques capable of killing *B. dendrobatidis*.

9. Disease

Most disease episodes can be avoided if proper hygiene is maintained. Systemic infections are common in amphibians, if sick animals are kept in stressful or crowded environments. Therefore, housing practices have a strong bearing on disease prevalence in amphibians. Post-mortem diagnosis of bacterial and fungal infections could be made by smears of liver and spleen by noticing intracellular bacteria in macrophages and granulocytes (Pessier, 2007). Protozoan parasites also cause disease. Severe Perkinsea infection (SPI) is an emerging disease of frogs responsible for mass mortalities of tadpoles in North America. It is caused by protozoa belonging to the phylum Perkinsozoa that form a distinct group referred to as the Pathogenic Perkinsea Clade that infects frogs. Clinical symptoms include abdominal distension. Post-mortem examinations of internal organs usually show inflamed liver, spleen and kidneys (Isidoro-Ayza et al. 2019). In infected frogs these internal organs might be invaded by Perkinsea-like organisms (Isidoro-Ayza et al. 2019).

Chytridiomycosis is an emerging infectious disease affecting amphibians globally and it is caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). Chytridiomycosis has caused dramatic declines and even extinctions in wild amphibian populations in Europe, Australia, Central and North America (Sreedharan and Vasudevan 2021). However, a lethal outbreak of the infection has so far not been recorded from Asia and therefore, it is referred to as a 'coldspot' of Bd infection (James et al. 2015). Extensive surveys for Bd prevalence in all the biodiversity hotspots in India revealed that they are found in almost all frog populations (Mutnale et al. 2018). The conventional qPCR (Boyle et al. 2004) is not a reliable assay for detection of the pathogen. A new set of primers for qPCR assay has been developed by CCMB to detect for samples from Indian frogs. Chytridiomycosis could be diagnosed by collecting swabs from frogs and the associated data by following the guidelines given in Manual for Biological Sample Collection and Preservation for Genetic, Reproductive and Disease Analyses (Gaur et al., 2017).

Signs of Chytrid

The chytrid fungus *Batrachochytrium dendrobatidis* (Bd) survives in tap water and in deionized water for 3 and 4 weeks, respectively. In lake water, infectivity has been observed for 7 weeks after introduction and this is an important consideration for the formulation of disease control and quarantine strategies for the management of water that has been in contact with amphibians. To comply with the intentions of OIE listing, amphibians, when moved, should be placed in a different container on arrival; all water, soil, plants, and litter in contact with the amphibian during transport should be adequately disinfected by using methods capable of killing *B. dendrobatidis*.

Ranavirus is an infectious disease of amphibians, reptiles, and fish caused by viruses from the genus *Ranavirus* (Chinchar, 2002). There are several different species of ranavirus that cause varying levels of disease in affected animals. It is a DNA virus belonging to the Iridioviridae family. It is detected using qPCR assay. It is clinically manifested in the form of an outbreak resulting in sudden onset of illness and death in large numbers of amphibians over a period of 1-5 days. Clinical signs in amphibians include mild to severe hemorrhage's in the skin,

especially near the base of the hind limbs and the vent opening, lethargy, weak or erratic swimming, buoyancy problems, gasping for air, and mild to severe fluid accumulation under the skin of the abdomen or hind legs. During necropsy fluid accumulation in the body cavity and hemorrhages on the surfaces of the heart, stomach, and liver could be observed.

1	Bacterial	Dermatosepticemia or "red leg syndrome
		Flavobacteriosis
		Mycobacteriosis
		Chlamydiosis
2 My like tha	Mycotic and mycotic- like organisms (other	Zygomycoses
		Chromomycoses
	than chythu)	Ichthyophoniasis
		Saprolegniasis
3 Protozoan p	Protozoan parasites	Amoeba
		Ciliates
		Flagellates
		Sporozoans
4	Metazoan parasites	Myxozoans
		Helminths (Particularly Trematodes And Nematodes)
5	Non-infectious disease	Neoplasia
		Absolute Or Specific Nutritional Deficiencies Or Overloads
		Chemical Toxicities

10. Record Keeping and house keeping

Individual frogs should be tagged using PIT tags (8mm). They should be screened for Bd using swabs once in three months. At this time all frogs should be weighed and measured. While doing so, each frog should be handled by wearing separate non-powdered gloves. The used gloves and plastic ware should be discarded as bio-hazard waste. Use of detergents and bleach in the enclosures and nearby areas should be avoided. Entry of animal keepers should be restricted and strict animal handling and all bio-safety protocols should be followed.

11. Conservation prospects

All species in the genus *Minervarya* are listed as Least Concern in the recent IUCN assessment. With further taxonomic revision there might be more species revealed. However, it is a species that is abundant, and it uses areas like agricultural fields, disturbed habitats and pristine riparian habitats. They perform important ecological function by regulating the insect population. These will thus form good models to develop and streamline the programs in captivity.

Further, development and application of Artificial Reproductive Technologies (ART's) and the incorporation of genome resource banks (GRB's), have been show to serve as powerful tools for amphibian conservation (Tonga et al., 2020). Cryopreservation techniques have been standardized for ~40 Anura species (Browne et al. 2019). ARTs minimises rates of inbreeding and reduction in conservation breeding costs compared with standalone captive management (Howell et al, 2021). Biobanking of conservation dependent species along with captive management could help meeting long term targets of the one plan approach.

Captive management of Himalayan Salamander

1. Introduction

Salamanders around the world are facing decline in populations from fungal diseases, pet trade, habitat loss and climate change. Threats to habitat are particularly dangerous when the animal in question has a narrow distribution range, as any impact could potentially wipe out the entire population. In this scenario, one of the few courses of action is to breed animals in captivity and release them back into suitable habitat or create insurance populations in case populations in natural areas are wiped out due to disease outbreak. Thus, captive breeding of salamanders is an effective mitigation strategy.

Tylototriton himalayanus or the Himalayan salamander is a medium sized with males ranging in size from 87.70 - 63.10 mm (Snout Vent Length-SVL) and females ranging from 97.00–66.00 mm (Snout Vent Length-SVL). Dorsolateral bony ridges present on the head. Head is usually broad and flat, snout is blunt, moderate size eye with granular upper eyelid. The body shows pairs of longitudinal lines of knob-like 16 dorsal warts, with finely granular skin and tail. Tail is compressed laterally, with a well-developed fin fold (Fig. 1). Live newts appear dark to light brown colour on the dorsal region, light brown colour in dorsolateral region, and creamy colouration on the ventral surface. The ventral region of both males and females turns bright orange during the breeding season. In gravid females the protrusion of the cloacal aperture is prominent while the males

of the species lack the cloacal protrusion. The male of this species is reported to produce a feeble 'ptaak' vocalization during the breeding season (Bedi et.al. 2020)



Fig.1 An adult Himalayan Salamander

The distribution of Himalayan salamander is currently known only from the Illam District of Nepal and in Darjeeling District of India (Nag and Vasudevan 2014, Khatiwada 2015). It occurs in an elevation range of 1350-2000 mts above sea level, in streams and ponds that are surrounded by forests, tea plantations and villages (Deuti and Hegde 2007). However, recent surveys have revealed breeding sites between 1000 – 2791 mts above sea level. (Vasudevan et al. 2014). Himalayan salamanders inhabit permanent and ephemeral ponds in the subtropical hill forests. The habitats are characterized as subtropical hill forest and area is dominated by scattered vegetation, for example, *Schima wallichii, Castonopsis indica, Casttonopsis tribuloides, Albizzi sp., Sauraria nepalensis, Rubus ellipticus and Eupatorium adenophorum.* The aquatic vegetation in the pond is characterized by *Acorus calamus, Polygonum* sp. *Nasturtium officinale.* Watercress is a preferred substrate for egg laying (Bedi et al., 2020). Adults are seen in water bodies only during the breeding season that spans from late April to early October. During other months they are terrestrial or semi-fossorial, hide under rocks, logs and leaf and aestivate or hibernate for four to five months (Kuzmin et al. 1994). In Darjeeling hills the salamanders are found at an elevation range between 1350 -2000 mts above sea level. (Deuti and Hegde, 2007)



Habitat of Himalayan salamander in Darjeeling
2. Founder Population

The primary goal of the ex-situ conservation program is to create genetically viable self-sustaining populations (Soule et al., 1986). These populations would ideally resemble the wild population in both genetic diversity and behaviour. Thereby, contributing to increased probability of successful reintroduction (Robert, 2009). For the founder populations - 20 adult females and 10 adult males from different geographic clusters may be considered. For testing the genetic variability in the founder population oral swabs could be taken. For individual identification, individuals should be tagged with PIT tags. The founder population for the captive breeding program of the Himalayan newts in Darjeeling Zoo was brought from Margaret Hope lake in Darjeeling. The program started with 10 individuals in 1990 and in 1998 four more individuals were added to the population from the same lake.

3. Housing Adults

Ten adults of equal sex ratio from the wild or captive population, after genetic and health analysis, can be housed in terrariums measuring 305 cm x 62 cm x75 cm (length x breadth x height) (Fig. 3). About one half of the bottom surface can be filled with water up to a height of 20 cm with an 8 cm gravel layer for higher ground. The newts should be able to fully immerse in water. The other half can be filled with mud, terrestrial plants, and tree barks. Small boulders and barks can be used to create a gradient from the water to the terrestrial section. Some ferns and marsh grasses could be introduced as aquatic vegetation. A

regular tungsten filament bulb can be used for lighting. An incandescent bulb can be kept illuminated at night to attract insects which were readily eaten by the newts. Water filtering and flow arrangement must be installed to always ensure clean water supply to the terrarium and pH of the water was maintained at 8.



Fig.3 Exhibit enclosure of Himalayan newt at PNHZ Park, Darjeeling

3.1 Temperature, Lighting and Water Quality

The temperature of the enclosures should mimic the seasons within the natural habitat of the species; low temperatures (2-6°C) during the winter months up to maximum 25°C in summer. In outdoor enclosures within the natural range of Himalayan salamander, optimal temperature regimes can be easily achieved by providing proper vertical structuring of the

substrate. Providing access to sub-terranean retreats at minimum 50 cm below the soil surface in an enclosure, in the shade provides a temperature gradient, offering the salamanders a variety of thermal and humidity niches. For brumation during the winter months, salamanders can be left in outdoor enclosures with sufficient frost-free microhabitats, for example loose soil with a minimum depth of 50 cm.

Inside the enclosure ultraviolet light gradient should be provided in conjunction with a basking spot. Water should be tested regularly for ammonia, nitrite, nitrate, pH and phosphates. Tap water should be left to stand for 24 hours before it is used so that the chlorine dissipates. Ensure the temperature of the water is at equilibrium with the room temperature. Measured quantity of water should be used to provision the enclosure. The enclosure should be lightly misted with dechlorinated tap water daily. The substrate and sides of the enclosure can be misted twice a day. Sufficient hides and substrate niches need to be provided for avoiding high temperatures and drying.

3.2 Husbandry protocols

All animals should be visually inspected daily. About 20% of the water can be let out on alternate days and filled with fresh water. The water quality should be checked for contamination. Waste matter must be removed daily using a fine net or water filter.

3.3 Diet in captivity

Captive Himalayan salamanders may be given a diverse diet viz Soft-bodied terrestrial insects and their larvae, annelids, Diptera larvae, Coleoptera larvae, Lepidoptera larvae, Odonata larvae, and adult Dytiscid beetles. Occasional supplementation with some invertebrates collected from the wild, especially from within the distribution of Himalayan newts, may offer the greatest balance in nutrition. The collection of such invertebrates is, however, time consuming and dependent on appropriate weather conditions, which may be problematic especially when large numbers of salamanders are maintained.

Wild collected invertebrates are inherently risky. Earthworms and gastropods are potential carriers of parasites and the species of collected invertebrates must be accurately identified to avoid the introduction of predatory or toxic species. Crickets and their larvae are easy to culture for feeding the newts. Other invertebrates may also be cultured by the institution holding salamanders. Such cultures can produce a stable supply of invertebrates, and often a greater diversity of taxa can be targeted.

Although typically dependent on movement to detect prey items, and therefore requiring live invertebrates, Himalayan salamanders can be at least partially habituated to prepared, non-living diets (Seidel and Gerhardt, 2016). Minced meat can be given to the newts 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 the movement trigger is no longer required.

3.4 Display and off-exhibit enclosures

Replication of the natural habitat can be done in display enclosures, however the spotting of the salamaders is not always guaranteed during the visiting hours of the zoo. At PNHZ Park, newts are kept in an outdoor fiberglass biotic terrarium. Individuals may be visible from outside when they are active from May to late October.

A semi-natural pond with enclosed perimeter, surrounded by fine mesh wire to prevent escape and to prevent them from being preyed on by birds, rodents and snakes, can act as a semi-natural breeding enclosure. For easy maintenance and management of the captive population the total area including the pond and its surroundings can be a maximum of 4000 sq feet (20 x 20x 10 feet). The pond should be fitted with a water filtering and replacement system. Temperature and humidity of the enclosure should be natural as the salamanders are being bred in their natural habitat. Temperature, humidity rain gauge recorders can be kept inside the enclosure for keeping records of these abiotic factors. This enclosure could serve to habituation site for salamanders that would be eventually released into the wild.

Small indoor terraria would perform as well as the semi-natural enclosure. These enclosures can be similar to the one described for *Minervarya* frog with elements of stagnant water,

vegetation and misting facilities. These enclosures can be housed with 5-6 salamanders with equal sex ratio. This will allow careful monitoring of their breeding. Once there is egg laying the eggs should be removed and reared in a separate enclosure. This arrangement will allow careful observation and monitoring of breeding activity of salamanders.

4. Reproduction in Captivity

4.1 Breeding Enclosure

Salamanders are known to be active from late April to early October, and during this period, adults emerge from hibernation and move into temporary/permanent ponds for breeding. A separate terrarium measuring at least 300 cm x 60 cm x75 cm (length x breadth x height) could be used as a breeding enclosure. Mating has been observed during daytime and at night. In captivity mating can be induced by spraying water (fig. 4) (Zeigler et al. 2007). The male salamanders are territorial during the breeding season and there is also competition for females. The males attempt to break amplecting pairs by holding the female's hind legs and interfere with the pair formation (Bedi et al. 2020). The number of males in the breeding enclosure should thus be limited to avoid disruption of amplexus.



Fig. 4 Artificial shower system inside the terrarium to provide stimulus for mating

4.2 Egg Care and Larval Husbandry and Development

Females lay eggs on leaves of aquatic plants and terrestrial grasses overhanging the water. The presence of vegetation prevents direct exposure of eggs to sunlight and prevents dessication. Adult females are reported to cannibalise on eggs (Dasgupta 1996) and in captivity, adult newts are reported to cannibalise on their larvae (Kuzmin et al. 1994). Therefore, after hatching, larvae can be transferred to a glass tank with utmost caution. The tank can be 40 x 30 cm surface and a height of 10 cm. Water depth of 5 cm is sufficient. There must be sufficient hiding places (barks, oak or similar leaves) and the water must be clean and rich in oxygen. The larvae do not show any

movement soon after they hatch, they normally rest on their side. The larvae will not start swimming or feeding until the sac has been absorbed. The glass tank should be stocked with a maximum of 5 larvae per litre of water. Dechlorinated water must be used to rear larva. Water should be regularly tested for ammonia, nitrite, nitrate, hardness, pH and phosphates. The results will help in deciding the frequency of water changes. Water parameters should be informed by field data; pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1 ppm and nitrate less than 10 ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration and larva. Larvae should be fed daily once they start swimming freely. They should be fed a variety of food. Once the front limbs appear and the tail starts to reduce it is important that the metamorphosing salamanders are given the opportunity to emerge from the water as they can easily drown. Emerging rocks or aquatic plants are suitable. Once they emerge, they are prone to drying out and easily die if the humidity is not adequate. 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 $(0-5^{\circ}C)$ there is no growth.

Tubifex and *Daphnia*, insect larvae e.g. "blood worm" (Chironomidae), larvae of mosquitoes (*Culex* sp.) can be given as food. Larvae should be kept in aquaria or plastic containers until clear signs of metamorphosis are seen. As soon as the animals enter metamorphosis 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 depth of 3-5 cm and incline the aquarium so part of the surface is dry or place objects (stones, cork bark, etc.) that rise above the water level. This dry part should be covered with hiding places.

5. Health and Quarantine

There is a chance of skin abrasions and limb injuries in captivity which might lead to infection. Apart from this, antiparasitic treatment and regular examination of fresh faeces should be done. Ultraviolet light should be provided as basking spot, if sufficient natural light is not provisioned and temperature gradients must be provisioned.

Batrachochytrium salamandrivorans (Bsal) survives in tap water and in deionized water for 3 to 4 weeks. In pond water, infectivity has been observed for 7 weeks after introduction. Disease control and quarantine strategies for the management of water that has been in contact with amphibians should be employed. To comply with the intentions of OIE listing, amphibians should be placed in a different container on arrival; all water, soil, plants, and litter in contact with the amphibian during transport should be adequately disinfected by using techniques capable of killing *B. salamandrivorans*. Exhibit enclosures should be kept out of bounds for contact from visitors.

5.1 Nutrition

Nutritional deficiency in invertebrates may be improved by: (i) enriching diet fed to the invertebrates cultured (Finke, 2003), (ii) use of supplementary powders just before feed is given to the salamanders (Michaels et al., 2014).

Invertebrates have tissues which are higher in nutritional quality (Ferrie et al., 2014). So far there is no study on nutritional deficiency and its effect on Himalayan salamanders. Such nutritional study would improve our understanding of the diet and the deficiencies in captive populations of the species.

5.2 Disease

Individuals should also be swabbed for detecting *Batrachochytrium salamandrivorans* and *B. dendrobatidis* (Fig. 4). Clinical signs of chytrid infection include anorexia (Fig. 5), skin problems and emaciation. Sick salamanders must be examined by a trained veterinarian and appropriate treatment given. During treatment, manipulation of the salamanders (e.g., injections, force feeding) must be minimised and treatment regimens can focus on topical applications. In anorectic salamanders, unless the animals are emaciated, focus should be on treating the cause of the anorexia, not on force feeding, which causes stress to the animal.



Fig. 4. Swabbing for detection of *B. dendrobatidis*, *B. salamandrivorans*



Fig 5. Emaciated Himalayan salamander with a poor body condition

7 Record Keeping and House Keeping

The salamanders should be tagged with 8 mm PIT tags for individual identification. Records of weight, SVL, health, behaviour and reproductive observations should be maintained. Additionally, records of temperature, humidity, rainfall and water quality tests can also be maintained. Salamanders should be screened for Bd using swabs twice: (i) pre-monsoon season when they start coming out of brumation; (ii) before the onset of brumation in September. The protocol for collection of swabs and the associated data should follow the guidelines given in Manual for Biological Sample Collection and Preservation for Genetic, Reproductive and Disease Analyses (Gaur et al., 2017). The salamanders should be weighed and measured every time they are swabbed. Each newt should be handled by wearing separate non-powdered gloves. The used gloves and plastic ware should be discarded as bio-hazard waste. Avoid using bleach and detergents in newt enclosures and nearby areas. Other non-hazardous sanitization material could be used. Strict bio-security measures should be followed and entry inside the enclosure by the zoo personnel should be restricted.

8. Conservation prospects

Tylototriton himalayanus has not been evaluated by IUCN so far. It is protected under **Schedule II** of Indian Wild life (Protection) Act, 1972. It is a keystone species of the lentic habitats in the Darjeeling hills. Seglie et.al (2003), recommended evaluating Himalayan newt in the IUCN Red List

to elicit the attention of national and International organisations for the conservation of this species. With the description of the species and circumscribing the distribution of the species to eastern parts of Nepal, Darjeeling hills in India, southern parts of Sikkim and Bhutan, the species has a restricted distribution. It is restricted in elevation and thereby, it might be impacted by climate change effects. Pollution and improper land use practices destroy breeding habitats of the Himalayan salamander. Jorephokeri, a small wildlife sanctuary dedicated for the species should be restored to bring back a breeding salamander population in it.

Assisted reproductive technologies (ART) have been employed to reproduce endangered amphibians (Zimkus et al., 2018). Using exogenous hormones about 32 salamander species have been stimulated to mate or induce production of gametes (Browne et al, 2022). In-vitro fertilisation has been successful in five species, and in two species in-vitro fertilisation with frozenthawed spermatozoa has resulted in mature adult individuals. In salamanders it has be demonstrated to increases the success of captive assurance colonies (Marcec, 2016). Recovery of viable frozen thawed sperm and in-vitro fertilization using frozen thawed sperm has been demonstrated in *Ambystoma tigrinum* (Marcec, 2016). These technologies could be harnessed for the conservation breeding of the Himalayan salamander.

Caecilian husbandry practices

1. Captive Management

1.1 Introduction

Gymnophiona is one of the three extant amphibian orders along with Anura (frogs and toads) and Caudata (salamanders and newts) and are commonly known as caecilians, or less frequently as naked-snakes, rubber eels or legless amphibians (Taylor, 1968). Caecilians are worm-like, ringed, limbless and girdle-less amphibians with the majority of adult life cycle inhabiting moist soils in the tropics and subtropics, with a few species being aquatic to semiaquatic (Wilkinson, 2012). Because of their fossorial nature and restricted distribution. caecilians are not only the least specious Amphibia, with 213 species under ten recognized families, but also the least understood vertebrate order (Frost, 2021). Much of the sporadic information available in literature about different aspects of caecilian life cycle, habitat traits, and biology of relevance to their husbandry is based on the information from preserved collections in the museums, opportunistic collections and incidental observations in the field, and under laboratory conditions (Wake and Koo, 2018). More than 20% of caecilians species (41 species) are found in India (Wilkinson et al. 2007; Bhatta et al. 2011; Kamei et al. 2012; Venu et al. 2020; Lalremsanga et al. 2021a, b; Venu et al. 2021a, b). India is inhabited by one endemic family (Chikilidae, four species), three endemic genera: Gegeneophis (12 species); Indotyphlus (two species), Uraeotyphlus (eight species) and the more widely

distributed and Asian endemic genera, *Ichthyophis* (15 species) (Frost, 2021). Western Ghats and parts of Northeast India are inhabited by 40 species, with one species, *Gegeneophis orientalis* being endemic to the Eastern Ghats (Agarwal et al. 2013). The more abundant *Ichthyophis beddomei*, *Uraeotyphlus narayani* and *Gegeneophis ramaswamii* (Venu et al. 2021 submitted) distributed in the Western Ghats, one of the globally recognized hotspots of biodiversity (Myers et al. 2005). Building zoo collection will necessitate collection of individuals from the wild. With ecological considerations in mind and because caecilian husbandry is still poorly understood and a majority of work is still in progress (Wake 1994; O'Reilly 1996), we would urge zoos not to include them in their collection and display until husbandry practices are clearly understood and laid out.



Figure: Habitats of caecilians: (A) Mixed orchards of banana, Arecanut, coffee and paddy fields; (B) Perennial streams associated with mixed orchards.

1.2 Housing adults

Generally, caecilian females are larger and longer than the males of the same age class (Kupfer, 2009). Nevertheless, adult males can be identified by presence of the phallodeum (Venu, pers. observation), and gravid females can be identified by gently palpating the lower side of the abdomen for the strings of eggs. Intra and interspecific competition between caecilians is common both in the wild caught and captive specimens (Seshachar and Ramaswami, 1943;Teodeckiet al. 1998; Measey et al. 2001). Additionally, in view of the intergeneric predation among caecilians (Venu et al. 2016), it is advised to house adult caecilians of the same species with a male and a female together. The terraria can be of dimension 60 cm X 60 cm x 60 cm with a fine soil mixed with cocopeat (1:1) up to 30 cm height. This enclosure can be made of cement or thick glass. The top of the terraria can be fixed tightly with a mesh cover. The entire terraria should be placed on a table with the legs dipped in water to avoid ants.



Figure: Endemic caecilians of Western Ghats: Dorsal and Ventral views of *lchthyophis beddomei* (A, B), *Uraeotyphlus narayani* (C, D) and *Gegeneophis ramaswamii* (E,F)

1.3 Temperature, Humidity and Lighting

Since caecilians live under the soil, it is expected that they prefer temperatures that are relatively equitable and require high moisture. They require a column of soil so that there is a gradient in soil temperature and moisture. Since they are burrowing, the use of light on the surface is an under explored aspect of husbandry.

1.4 Husbandry protocols

The soil in the terraria should not be disturbed. Plastic tubes can be installed at the time of filling the soil to infuse moisture below. Excessive moisture makes the soil compact and interferes with respiration of caecilians. Therefore, the soil should not be made too wet. A peat like composition of soil would be appropriate as it allows sufficient aeration. When soil or any substrate is added care should be taken that it is free of insects that might harm the caecilian. Plant roots might interfere with the movement, therefore, planting inside the terrarium can be avoided. Dead wood can be put in the terraria as furniture and submerged to give caecilians different substrate and increase the complexity of the enclosure. Allowing natural light to fall on the surface is a good practice. If this is provisioned, some covers should be placed on the surface for them to use when they access the surface. Feeding troughs should be cleaned regularly and any remaining food should be removed.

1.4 Diet in captivity

Little is known about the natural dietary requirements of caecilians. Dissection of the gut contents of the preserved caecilians revealed that they are generalist predators and are chiefly detritivores, feeding on ants, termites and earthworms (Measey et al. 2004). Earthworms being their major diet, occasionally they also feed on soil invertebrates such as centipedes, antlions, slugs, thrips and dipteran larvae (Venu et al. 2016).

Health of the captive caecilians could be best maintained by feeding them with locally available earthworm species. Adult earthworms could serve as a better meal for caecilians than the juveniles, as the juveniles are more likely to get cut when the caecilians spin their prey, a general practice by caecilians during feeding (Measey and Herrel, 2006). During the drier seasons of the year, when the earthworms in good numbers are hard to find, chopped pieces of sheep or goat liver would serve as the diet for the captive caecilians. Liver in a coconut shell and placed in the depressions of the soil would make it easy for the caecilians to find its food (Venu, pers. observation).

1.5 Enclosures

Making display enclosures for caecilians is challenging (Fig 3) as they are fossorial. It might be useful to use video footages to show caecilian activity alongside exhibits. Therefore, we restrict our presentation to off-exhibit experimental enclosures. We suggest that zoos should not attempt this unless there is enough expertise in husbandry of caecilians.



Figure: Off-exhibit enclosures for caecilian observation in captivity: Caecilians in an enclosure between two glass plates (A), close-up view of the captive caecilians (B). (Photo by R.K.Browne)

2. Reproduction in Captivity

Reproduction has been achieved in captivity, but it has not been well documented. In order to reproduce caecilians the husbandry practices need to be strengthened.

2.1 Breeding enclosure

The breeding enclosure is the same dimension as the offdisplay terraria. Regular monitoring of the breeding enclosure is essential.

2.2 Egg Care, Larval Husbandry & Development

Majority of the caecilians endemic to India are oviparous in nature and they lay eggs in clutches in underground chambers lying below the topsoil and close to water bodies (Gower et al. 2008; Venu 2008). The mother incubates the eggs by coiling around the clutch, till the larvae are ready to hatch. The growing

larvae are nourished by the stored yolk inside the eggs. Each egg clutch consists of 6-100 eggs with each egg connected by a transparent cord emanating from the jelly coat of the eggs, forming a ball like structure (Seshachar et al. 1982; Bei et al. 2012). The hatched larvae with three pairs of gills enter the surrounding water bodies or water logged soil, remaining there till they attain adulthood. Adult caecilians lead a subterranean mode of life by making burrows in the moist and humus rich soil.



Figure: Egg clutch of ichthyophiid caecilians: Abandoned egg clutch of *lchthyophis beddomei* (A), female *lchthyophis bombayensis* coiling around the egg clutch (B)

3. Health

Caecilian grow rapidly from larvae to adult . Since husbandry protocols are not well established, the best practices for captive management of caecilians is still being worked out.

3.1 Nutrition

Caecilians are carnivorous and they actively feed on live prey such as earthworms or scavenge on carcass at the surface.

3.2 Disease

Caecilians are known to be infected by *Batrchochytrium dendrobatidis* (Gower et al 2013) and the impacts on them are poorly understood. The protocol for screening for infections is similar to other amphibians. The protocol for collection of swabs and the associated data should follow the guidelines given in Manual for Biological Sample Collection and Preservation for Genetic, Reproductive and Disease Analyses (Gaur et al., 2017).

4. Record Keeping

It can be challenging to maintain records for individual caecilians. We recommend that caecilians have natural markings and upon careful examination this can be noted and used for individually identifying them. Weighing, measuring and swabbing them once in 3 months is a good practice. Sexing of caecilians is still being developed, and the method suggested could be improved upon after further standardisation.

5. Conservation prospects

Caecilians show high level of endemism and several species have not been evaluated for their conservation status based on the IUCN criteria. The levels of threat to their population are high and they are impacted by anthropogenic factors. With the unfolding of climate change, these impacts could be further accentuated. Assisted Reproduction Technologies (ART) have only been explored in four Caudata families the Ambystomatidae, Salamandridae, Plethodontidae, and Proteidae (Browne et al., 2022). Eggs of amphibians and caecilians in particular are large and have a large amount of yolk. They cannot be cryopreserved because of the yolk content. Increasing numbers in captivity using ARTs would depend on the development of technologies to recover females from cryopreserved and biobanked cells or tissues (Browne et al., 2022).

Enrichment of Amphibian Exhibits

With amphibian species facing a rising number of threats in the wild, ex situ conservation is critical. It calls for a thorough understanding of captive amphibian husbandry, and is especially important for those amphibian species that have no history in captivity, or are destined for release into the wild through reintroduction programs.

All modern zoo facilities must strive to provide for these complex needs. To serve this purpose, the positive psychological and physiological effects of enrichment have been demonstrated across numerous taxa. Enrichment is primarily of three types - environmental, behavioral and social. Environmental enrichment involves the provision of environmental stimuli necessary for optimal welfare of the animal, behavioral enrichment, the allowance for the animal to express species-specific behaviors, and social enrichment, which caters for social interaction needs by providing access to individuals of the same or different species.

Studies focusing on amphibian enrichment are few. Of these, many have explored the provision of shelter as a form of enrichment. Few have focused on feeding enrichment, and fewer even, on aspects of environmental enrichment. Across all, the study amphibians expressed a clear preference for enriched enclosures over unenriched ones. However, the effects on welfare are yet to be documented and quantified. In order to measure progress, key areas of amphibian biology need to be integrated into enrichment research. What is known of the ecology of the species must be carried over into captive settings, and utilized in a manner that promotes species specific behavior and welfare, while simultaneously simplifying husbandry and care practices.

The following table has been adapted from *Micheals et al.*, "The importance of enrichment for advancing amphibian welfare and conservation goals: A review of a neglected topic" It offers insight and ideas into the measures discussed above.

Key areas of species biology knowledge required for effective enrichment research, potential tools for assessing enrichment needs and effects and areas of amphibian captive husbandry for which enrichment may be important." (Michaels, et al.)

Key areas of amphibian biology, to be integrated into enrichment research		Potential measures of welfare and fitness			Potential areas of captive husbandry for enrichment research focus
Cognition	Learned and hardwired behavioral components	a- individual, phenotypic and genetic variation	Catalog existing issues in captive amphibians and their husbandry	ainst wild populations, where appropriate	Enclosure design • Size • Complexity • Permanent (furniture and decor) • Temporal (novel objects, timed misting) • Refuges • Lighting • Wavelength • Photoperiod • Intensity
	Perception of environment		Behavior and behavioral assays		
Behavior	Natural behavioral repertoires and activity levels of species		Foraging success		Environmental parameters • Gradients • Fluctuation (seasonal and diel)
	Foraging strategies and dietary composition		Growth and development		
	Reproductive behavior • Breeding strategies • Mate choice • Competition for mates' breeding sites		Body condition		Threat stimuli • Predation • Competition • Environmental stressors (e.g., drying ponds)
	Migration and home ranges	iter- and Intr	Hormones • Stress • Reproductive	m parisons ag	Encouraging specific behavioral responses
	Antipredator behavior	I	Micro- and macro-biotas associated with animals • Beneficial communities (mainly skin and gut) • Parasite and pathogen loads	Cc	Nutrition and food presentation • Nutritional content • Temporal variation • Variation in food types (different species of prey animal or algae) • Total abundance
	"Personality" vs behavioral plasticity		Pathologies - Behavioral - Physical (disease, <u>mailormation</u> and pathogen susceptibility)		
Interactions	Intra- and interspecific		Reproductive success		Social enrichment • Presence of conspecifics and non-conspecifics • Stability of social groups • Territory creation and
	As predators, <u>prev</u> and competitors				
Genetics and evolution	Heritability of traits		Survivorship		maintenance • Mate choice • Human habituation
	Detential for colection				

Potential for selection

Education and Outreach

Zoological parks stand at an important intersection of conservation, education, research and awareness. They offer the public a unique opportunity to get a closer look at animals rarely encountered in day-to-day life. Amphibians, due to their small size and general crypsis, often go unseen. These hidden features that make them so fascinating and unique, must be communicated to the public in a way that is immersive, engaging and educational.

Public perception and opinion plays a huge role in decisionmaking processes related to welfare and conservation of species and habitats. Thus, zoo's housing amphibian species have to bear the responsibility of sensitizing people to the natural history, ecology and ethology of the species housed within. It is key to provide the public with all relevant information in a way that encompasses learning opportunities, experiences and activities for all ages and needs.

Amphibians of India

- India boasts of over 440 amphibian species, as per the Checklist of Indian Amphibians, updated and launched on the Zoological Survey of India website on the 15th of May, 2020.
- The species richness extends across Anura (frogs and toads), Caudata (salamanders and newts) and Gymnophiona (caecilians).
- Amphibians are easily stressed animals that are sensitive to external changes. They cannot be physically held or

touched unnecessarily, so all education material needs to be developed in ways that are hands-off and minimally invasive.

Amphibian Alert!

- Amphibian Alert! is a publication of the World Conservation Union (IUCN)/ Species Survival Commission (SSC) -Declining Amphibian Population Taskforce and the American Association of Zoos and Aquariums (AZA) - Amphibian Taxon Advisory Group, in partnership with Seneca Park Zoo Society, Rochester, NY.
- The curriculum is targeted towards children between the ages 7-11, and covers key characteristics of amphibians, threats to existing populations, reasons for decline, and methods to effectively engage with amphibians and nature in the future. The material is extensive, thorough and designed in a way such that it is effective, engaging and interactive.
- The publishers welcome science educators to reproduce any of the lesson plans and field activities, while giving due credit to the originating institution and **Amphibian Alert!**
- The document is available for free at the link below:

http://www.amphibianark.org/pdf/AZAAmphibianAlertCurriculum.pdf

Frog Calls

• An interactive medium -push buttons for sound, tap screensor something similar, to introduce visitors to the wonderful world of frog calls.

• Sounds of common native species should ideally be included.

• Additionally, this segment should introduce types of frog calls - territorial, mating, distress, warning and release calls.

Amphibian Biology

• Frog FAQ's: A quick discussion with visitors or children, that will hold answers to the most commonly asked questions about amphibians. For example:

Why are frogs slimy? What do frogs eat? What is the difference between a frog and a toad? Why do frogs hop? Facts about *Caudata* and *Gymnophiona* species in India (typically not housed in zoos) etc.

Amphibian Behavior

• Screening of videos of various behaviors displayed by amphibians, such as:

Thermoregulation

Breeding behaviours

Reproduction

Parental Care

Egg Laying, etc.

• This session should invite guesses about the behavior, explain the behavior, as well as field questions related to amphibian behavior.

• Additional segments related to etiquettes of behavioral observations, documentation and field notes can be included in the session.

Defense Mechanisms in Amphibians

A quick module or presentation that covers defensive behaviors in amphibians.

• Frogs generally defend themselves by using calls, color, camouflage or poison; or display behaviors such as urination, playing dead, biting or puffing up their bodies to escape from predators. They also use their well-built anatomy to jump, leap or swim away from external threats.

• As an activity, participants can identify these behaviors across species of frogs and then classify them into Active Defense Mechanisms and Passive Defense Mechanisms.

Captive Care and Husbandry

An event organized by keepers and biologists to demonstrate and speak about aspects of daily care and husbandry, while also touching upon the biology of amphibians.

Talks like these can be part of the daily education and outreach activities of the zoo.

Art & Craft

Creativity can inspire education and action, especially in young children. Following are a few ideas with art and creativity at the center:

• Frog Origami - Fold a little frog into life! Methods for creating origami jumping frogs are widely available online.

• Best out of Waste - Use recycled material like used papers, cardboard cartons, etc. to create an amphibian of choice. Extra points will be given for attention to detail (like the segments of caecilians, number of toes on the frog, etc.).

• Diet Discovery - Create a board with all possible prey items for amphibians. Match the crafted amphibians from above to their preferred prey items.

Acoustics

Amphibians have a keen sense of hearing. High pitch noises can be a trigger for them. Even low frequency noises and vibrations can have consequences for behaviors in them. When amphibian enclosures are planned this aspect should be taken into consideration to provide them with an appropriate acoustic environment.

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ANNEXURE 1

Morphological characters in anurans





Key to taxonomic characters of the "Poster Frog"

1. **Snout tip** can be angular and acute as in the case of the Poster Frog, but it can also be almost absent as in the case of *Nyctibatrachus*.

2. **Cranial ridge** is prominent in Bufonids. This is a keratinized part of the skin and can serve as armor for the animal. They can be faint in young ones because keratin builds up over time.

3. **Canthus rostralis** is the angle from the top of the head to the sides of the head in the region where the pointer is placed. It can be angular or acute or obtuse.

4. **Parietal ridge** is only found in *Duttaphrynus parietalis*. It is a uniquely derived character.

5. **Pupil** is usually elliptical and horizontal but in *Nyctibatrachus* it is diamond shaped. This character was not considered important because much of anuran taxonomy is based on dead specimens and it is difficult to see this in preserved animals.

6. **Paratoid gland** is a glandular projection on the dorsum. It can be oval or diffuse and always in a pair symmetrically arranged on the dorsum. The diffuse paratoid gland has to be carefully observed and it is easier to spot on a live animal than on preserved ones.

7. Keratinized warts are found abundantly on the dorsum of toads. The warts typically have a black tip. The tip can also be conical or rounded or with other interesting shapes.

8. Discontinuous **dorsolateral folds** are usually many running parallel on the dorsum. They start from behind the eye and become diffuse near the vent.

9. Continuous dorsolateral folds run parallel on the dorsum. They may be broken into longer segments. They start from behind the eye and become diffuse near the vent.

10. **Dorsolateral glandular fold**, a pair is found symmetrically arranged on the lateral edge of the dorsum. They can be glandular or non-glandular.

11. **Dorsal skin** surface can be rough, smooth, glandular, warty or wrinkled. It is usually an important taxonomic character.

12. **Transverse bars** on the thigh are found in many ranids. Bars have to be distinguished from blotches which are also found in that region.

13. **Dermal ornamentation** on the anal region is found in the genus *Rhacophorus*. These are fold of skin, usually light colored and prominent.

14. **Tarsal fold** is a fold of skin found at the tibio-tarsal articulation. It is very prominent in live animals. The skin after preservation becomes leathery and can be confusing.

15. **Metatarsal tubercles** are found at the base of the first and the last toe. They are referred to as inner and outer metatarsal tubercles. Their shape and position are important taxonomic characters.

16. **Skin with large granules on the groin** is a common character among Rhacophorids. There is wide variation in the skin of frogs. These granules serve to increase the surface area and help in re-hydration of the animal.

17. **Sub-articular tubercles** are found at the base of the phalange (joints). They are keratinized skin and serve as cushions when they leap and land on hard surfaces.

18. **Vermiculations on the inside of the thigh** is found in many rainds. It does not have a clear taxonomic value, but in the field it helps in identification of some species.

19. **Webbing on feet** varies from species to species. The best way to describe the web on feet is by examining the point of insertion of the inside and outside of the toes. These points can be described as "between the distal and penultimate sub-articular tubercles".

20. **Ventral skin surface with large granules** is found in *Raorchestes* and some *Duttaphrynus*. The shape of the granules can be defined for a species.

21. **Ventral skin surface with fine granules** is also found in *Raorchestes* and some *Duttaphrynus*.

22. **Pointed toe tip** is a derived character from a blunt toe tip. A club shaped toe tip is again different from rounded and it is a derived character.

23. **Nuptial pads** are found in males. They are prominent in live breeding males that need amplexus to fertilize eggs. They are hard structures on the ventral side of the first finger. They are discoloured and sometimes have spines on them.

24. **Palmar tubercles** are again hard pads on the palm and serve to absorb the shock when they land on surfaces. It can also serve to increase the surface area to cling on to surfaces.

25. **Discs with circum-marginal grooves on fingers** are found in both *Raids* and *Rhacophorids.* These are adaptations for arboreal life and life in torrents.

26. **Dermal ornamentation on the arm** is found in the genus Rhacophorus. It is a fold of skin that projects out of the arm. They serve to increase the surface area to enable gliding.

27. **Faint lines called** *Fejervaryan* **lines** are on the flanks of the members of the genus *Fejervarya* and it is an important character for identification of this group of frogs.

28. **Supratympanic fold** is a fold of skin above the tympanum that extends from the posterior corner of the eye to the shoulder. It can be pigmented and sometimes not.

29. **Rictal glands or tubercles** at the base of the tympanum are found in a cluster and sometimes distinctively pigmented. They are also positioned just behind the point where the upper and lower jaw meet. Rictal gland is prominent and elongated. Tubercles are usually small and in a cluster. Their shape and their numbers are taxonomic characters.

30. **Skin surface on the throat region** is usually smooth, but in some frogs it is granular and even warty. Males with external vocal sacs have pigmentation and loose skin here.

31. **Tympanum** is a common feature of frogs, but they are varied in their position, shape and visibility. It can be round, oval or semicircular. It can be hidden, prominent or indistinct.

32. Notch on the tip of the upper jaw is also varied in anurans. They are meant to trap the prey and keep the prey inside the buccal cavity before it is ingested.

33. **Internal nares** are small openings of the respiratory system in the buccal cavity

34. **Vomerine teeth** are special teeth like bony projections from the roof of the buccal cavity. They are in pairs and they are very specific to species. Their arrangement with respect to the nares and the number of teeth and the number of rows of teeth is distinctive.

35. **Shape of the tongue** is also distinctive in Anurans. They can be rounded, oval or bifid.

36. **Papilla on the tongue** is a much debated taxonomic character. Preservation has unknown effects on soft tissue such as the tongue and such characters can get completely masked.

37. Notch on the tip of the lower jaw is usually in the form of an evagination. This can be mirroring the shape on the upper jaw.

38. **Femoral glands on the inside of the thigh** are distinct in *Nyctibatrachus*genus. They are usually colourless or orange in live animals and in preservation they become cream coloured. They are sexually dimorphic and also absent in some species.

Annexure 2

Marking of Amphibians

To study a large number of amphibians in captivity, one needs a reliable technique to identify individuals within populations. Traditionally – toe clipping, freeze and hotwire branding, tattooing & various methods of tagging have been used. These techniques were painful to the animal and had disadvantages too. Thus no longer are recommended.

With the recent developments in technology & looking into the animal welfare aspects, as well as their current acceptability, Passive Integrated Transponder Tags (PIT), permanent methods of marking are being used, however it has several advantages and disadvantages too. These methods have been described in brief:

Passive Integrated Transponder Tags (PIT)

To mark the amphibians with PIT, it is injected into the left caudal body cavity – Subcutaneous – into the dorsal lymphatic sacs of the frogs.

Passive Integrated Transponder Tags (PIT) Advantages:

- Unlimited number of codes
- Painful
- High cost
- Requires handling, tissue penetration and expertise that can be acquired

• 8 mm PIT tags can be used for frogs >30 mm SVL.

Pattern Mapping

Advantages

- · Low cost unless software needs to be purchased
- Allows individual identification of very small frogs, juveniles
- · Non invasive
- · No risk of infection or spread of disease
- · No pain

Disadvantages

- · Useful only in species with unique individual markings
- · Need to handle frogs (as with most techniques)
- Limited by numbers cumbersome with a large population
- Time consuming
- · Potential ontogenic shifts in pattern

Annexure 3

Culturing food for Amphibians

Culturing Indian House Crickets

Rearing crickets and breeding them for feed is a fairly simple process. Crickets require very little room and can be raised either indoors or outdoors, depending on the temperature. They thrive in warm, moist and shady environments.

Development from egg to adult takes about three months. Eggs hatch in about three weeks and crickets are large enough to use in another month.

The most common cricket species used are banded crickets (*Gryllodes sigillatus*), house crickets (*Acheta domesticus*), black field crickets (*Gryllus bimaculatus*) and Jamaican field cricket (*Gryllus assimilis*).

Steps to Start a Cricket Culture

- Ideally, an initial population of about 25-30 healthy, adult crickets is required to start a colony.
- Males and females should be in equal numbers.
 Females are easily distinguishable by the presence of a long egg-laying tube at the end of the abdomen, called the ovipositor.
- Obtain a few tall, smooth edged, 100-liter containers.
 Recommended dimensions are 24 x 12 x 16(h)

inches. Taller boxes prevent crickets from jumping out during routine maintenance.

- Cut out the center of the lid and cover it with a piece of metal window screen fixed into place. This will aid in ventilation of the culture.
- Spread a 1-2 inch layer of vermiculite, perlite or sundried coco peat on the bottom of the container to create a sanitary substrate.

Excess ventilation can cause the substrate and eggs to dry out, rendering the culture ineffective. In such cases, the screen should be partially covered with a fiberglass sheet, as required.

- Fill a 4-6 inch diameter tray with damp, pesticide-free potting soil and place it at one end of the container. This is where the females will lay eggs. Cover the tray with a piece of window screen to keep crickets from burrowing into the soil and eating the eggs. Females will stick their ovipositor through the holes in the screen to lay eggs.
- Maintain the dampness of the egg-laying tray through regular misting. Ensure that the soil is only slightly damp, and not wet or waterlogged.
- Place two small containers, each with a soaked sponge or cotton swabs, on the same side of the tank as the soil. The water level must not exceed ¼-inch. This enables crickets to drink water while preventing drowning, and also maintains humidity.

- Place an additional container on the opposite end for feed.
- All containers and/or trays should be dug into the soil, with just the rims visible, so that the crickets can easily crawl into them.
- Use cardboard egg cartons or loose, stacked cardboard sheets to create vertical habitat for the crickets to occupy. These can be about 8-10 inches tall, and must be kept dry to prevent the formation of mould.
- Use a small digital thermometer to keep track of the temperature. Crickets thrive at temperatures between 20-32°C, and are more likely to breed at warmer temperatures, i.e., closer to 29-32°C.

Feed and Care for the Adults

- Crickets can eat a wide variety of foods. While insect feed is commercially available, a diet of fruit and vegetable scraps, grains, poultry feed and a few pellets of dog/cat kibble (dry food) works just as well. Uneaten food must be removed daily to prevent rot and mould.
- Water containers must be checked and filled every day.
- The tray of potting soil for egg laying must be lightly misted when required. Again, care must be taken to ensure that the soil is damp, and not wet or waterlogged.

- The legs of tables holding cricket colonies can be set in small cans with a little oil or water in the bottom. This will keep ants and other climbing insects from reaching the colonies.
- In case of low external temperatures, one might have to employ the use of heat lamps in order to keep the temperature in the ideal range.
- Good sanitation is essential. Cages must be inspected frequently and dead crickets must be removed regularly.

Incubation of Baby Crickets

- When heat and humidity are optimal, the male crickets will begin chirping, a sign that mating has begun.
- After 7-10 days, the container of potting soil should be full of eggs. Eggs resemble miniature grains of rice.
- Place the container (without its screen) in another 100l container and keep it warm and humid. Within a week, baby crickets, called nymphs, will begin to hatch.
- The baby crickets will need the same setup as the adults a vermiculite, perlite or coco peat substrate with containers for food and water (with sponges or cotton swabs) and the same daily care routine.
- Once the new container of baby crickets starts chirping, place a tray of potting soil in it so they can lay eggs and continue the cycle.

Harvest

- Adult crickets reach their full size within two months.
- However, they also can be removed at different stages of growth, as per feeding requirements.
- Crickets can be fed live. The process of chasing and catching live feed is enriching for captive amphibians, and additionally helpful for new individuals that are reluctant to feed from forceps or in the presence of a keeper.
- However, ethically, crickets marked for consumption can be separated into boxes, and placed in a freezer for 6-8 hours, where they go painlessly into a state of hibernation until they eventually die.
- They can then be removed, thawed and fed.

The Cricket Farming Cycle

- After each harvest, discard the substrate and potting soil and disinfect all containers.
- The cycle can then be started again.

For detailed protocol refer to: Semple L., and K. Vasudevan. 2015. Grasshopper protocol: A husbandry protocol for rearing of insect feed for amphibians. CCMB Internship Program Report, 16p. (<u>www.ccmb.res.in</u>)

Culturing Fruit Flies (Drosophilidae)

There are two common species of fruit fly reared for feed : *Drosophila melanogaster* and the slightly larger *Drosophila hydei*. *Drosophila melanogaster* is the best known and most widely available species.

Fruit flies are prolific breeders and cultures do not take up much room. They are ideal food for a number of species of amphibians.

Steps to Start a Fruit Fly Culture

- Obtain multiple discarded/ recycled clear plastic bottles of 500ml capacity. 1I bottles can be cut in half and used similarly.
- Put slices of ripe, unpeeled fruit in the bottle. To start the culture aim for about a 2cm depth of fruit in the jar. A list of fruits and other supplements are listed in the following section.
- Ideally the bottle needs an opening between 2.5cm and 5cm at the top.
- This can be fashioned by using a paper cone in place of a lid. The tip of the cone should be cut out, so that it is just slightly larger than an adult fruit fly. The wider end of the cone sits at the mouth of the bottle. Excess

paper should be folded and tied or taped over the edges. The cone should resemble a funnel of sorts.

• This allows fruit flies to be funneled in, while preventing any escapees.

Feeding

- Suggested list of feed for Drosophila:
 - Papaya
 - o Banana
 - Apple
 - Pear
 - Multivitamin supplements
 - Activated Dry Yeast
 - Oats
- Oats help soak up excess juice and moisture from the fruits. This prevents larvae from drowning.

Pupation Space

- To create a self-sustaining culture of fruit flies, it is important to provide a space for pupation.
- Most often, once big enough, larvae will climb up the side of the bottle and pupate near the lid. A piece of wood resting diagonally within the bottle can provide easy access to the top and more pupation space for larvae.

Breeding Time

- A single *Drosophila* culture lasts for 7-10 days, and then a fresh culture has to be started.
- It is recommended that cultures at different stages are kept running so that there is a constant supply of flies.
- As with any insect, the life cycle of a fruit fly is greatly influenced by temperature. Anything from 10-30°C should give a successful culture of fruit flies. Similar to crickets, breeding success will be greater at higher temperatures.

Annexure 4

Water quality standards

Water quality is an extremely important component of amphibian husbandry. Monitoring water quality is vital to successfully rearing healthy captive amphibians; fluctuating water parameters create stress for the individuals, therefore it is better to maintain constant conditions.Recommended water quality parameters adapted from Odum & Zippel (2011) https:// www.amphibianark.org/wp-content/uploads/2018/08/Water-Quality-Odum-Zippel-2011.pdf

Water quality parameter	Recommended levels	Control methods	
Water hardness (dissolved Ca and Mg salts)	For soft water amphibians: <75mg litre ⁻¹ (ppm) of	Soft water:	
ou unu mg ouno,	CaCO _{3.}	Harden using Ca and Mg salts (only recommended	
		reconstituting RO (reverse	
	For hard water amphibians: >100mg litre ⁻¹ of CaCO ₃	distilled water).	
	······g·····	Hard water:	
		Soften using RO, <u>DI</u> or distilled water.	
Dissolved oxygen as O ₂	>80% saturation	Aeration	
gas supersaturation	Gasses maintained at	Aerate water until	
	equilibrium with the atmosphere	equilibrium with atmosphere is achieved	
Ammonia/ Ammonium-	<0.2mg litre ⁻¹ , N as unionized	Biological filtration, chemical	
NH₃/NH₄⁺	ammonia		
	<1 0mg litro-1 ideally 0	appropriate medium), water changes	
Nitrites NO ₂ -	<1.0mg ime_, ideally 0		
		Biological filtration, chemical filtration (with	

Water quality parameter	Recommended levels	Control methods
Water berdress (disselved	For ooft water emphibione:	Soft water
Ca and Mg salts)	<75mg litro-1 (ppm) of	Hardon using Ca and Ma salts (only
Ca and Mg saits)	CaCO.	recommended reconstituting PO
	0a003.	(reverse osmosis) DI (de-iopized) or
	For hard water emphibiane:	distilled water)
	>100 mg litro-1 of CoCO	Llord water).
		Soften using RO. DI or distilled water
	>80% saturation	Aeration
supersaturation	Gases maintained at	Aerate water until equilibrium with
supersaturation	equilibrium with the	atmosphere is achieved
	atmosphere	
Ammonia/ Ammonium-	<0.2mg litre-1, N as unionized	Biological filtration, chemical filtration
NH₃/NH₄⁺	ammonia	(withappropriate, medium), water changes
Nitrites NO ₂ -	<1.0mg litre-1, ideally 0	Biological filtration, chemical filtration
		(withappropriate, medium), water changes.
Nitrates NO ₃ -	<50.0mg <u>litre-1</u>	Removal: photosynthetic action of green
		plants or water changes.
pH	Generally near neutral,	Change water source or add
	although it is species-	appropriate buffer solution
	dependent. Should avoid pH	
	<6 and >8	
Chlorine Cl ₂	0	Aerate for 24 hours, or add chemical
		dechlorinator such as sodium
		thiosulphate
	40.04 m m litera 1 m o Ol	
Chioramines (CINH ₂ ,	<0.01mg litre-1 as CI	Use chemical treatment specific for
CIN ₂ H, CIN ₃)		Chioramines such as Prime®
		(3240) (120) (200)
		BA 50050, USA). Filters for this
		purpose are available
Copper (Cu)	<0.05mg litre	Carbon filtering and carbonate
		precipitation (do not use copper piping).
		,,
Posphates (PO ³ 4 [·])	Toxicity species-specific;	Lower levels of phosphates using
	EPA recommends limit of	phosphate sponges and filters
	10mg litre-1; 1mg litre-1 is	
	effective for preventing pipe	
	corrosion	

Nitrates NO ₃ ·	<50.0mg <u>litre-1</u>	appropriate medium), water changes. Removal: photosynthetic action of green plants or water changes.
рН	Generally near neutral, although it is <u>species-</u> <u>dependent</u> . Should avoid pH <6 and >8	Change water source or add appropriate buffer solution
Chlorine Cl ₂	0	Aerate for 24 hours,
Chloramines (CINH₂, CIN₂H, CIN₃)	<0.01mg litre ⁻¹ as Cl	Use chemical treatment specific for chloramines such as Prime® (Seachem Laboratories Inc., Madison, GA 30650, USA). Filters for this purpose are available
Copper (Cu)	<0.05mg litte	Carbon filtering and carbonate precipitation (do not use copper piping).
Phosphates (PO₃₊)	Toxicity species-specific; EPA recommends limit of 10mg litre ⁻¹ ; 1mg litre ⁻¹ is effective for preventing pipe corrosion	Lower levels of phosphates using phosphate sponges and filters

Most Zoos use municipal water sources which are treated with chlorine, or bleach. This water is toxic for amphibians. Therefore, sanding the water to allow excess chlorine to evaporate should be practiced. Avoid using water which has previously been in contact with other amphibians as this may risk disease transmission. Reverse osmosis (RO) systems will produce water that is pure, but it is not

suitable for amphibian osmoregulation. RO water can be made suitable either by adding salts or a known quantity of tap water. Avoid water that has been stored in metal tanks or exposed to toxic substances that could leach into the water. Water parameters should be tested frequently in newly established systems.

A commonly used system for keeping aquatic and semi-aquatic animals is a semi-closed system, in which a combination of filtration and water changes are employed in order to maintain water quality. Small changes in water quality are permissible. In the closed system, frequency of water changes will depend on the stocking density, the temperature of the water and the amount and type of food given. The outflow of the filter should be adjusted to meet the requirements of the amphibian species. Some tadpoles experience stress if water flow is rapid. The mouth parts, their position and the tail shapes are indicative of the niches of the tadpoles. This information can be used to plan the husbandry and enrichment for tadpoles.

Mechanical, chemical and biological filtration systems are available. They have their advantages and disadvantages. The choice of the system will determine how the husbandry practices will be standardized.

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Amphibians

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