


Ex situ Management of Amphibians

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ASSAM STATE ZOO CUM BOTANICAL GARDEN

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1. Indian baloon frog *Uperodon globulosum*: Navendu Page
2. Spade footed burrowing frog *Sphaerotheca brevicepts*: Navendu Page
3. Venated cliding frog *Rhacophorus pseudomalabaricus*: S. U. Saravanakumar
4. Himalayan Newt *Tylotoriton verrucosus*: Karthikeyan Vasudevan

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Precautionary Note:

Amphibian husbandry is a rapidly developing field and it has become apparent that amphibians have complex and varied husbandry requirements and are not necessarily easy to maintain and breed. Many species have not been maintained in captivity, therefore developing appropriate and optimal husbandry and breeding techniques is crucial to the success of ex situ programmes. Tropical species in particular have proven difficult to establish in captivity, due to high levels of endemism combined with a tendency towards high levels of environmental specificity and limited husbandry experience or field data. Herein we present a number of species specific husbandry guidelines. Whilst some of these guidelines are based on direct experience either with the specific species or a closely related species, others (E.g. Clinotarsus, Indirana, Ramanella and Uperodon) have been compiled based on how we would go about maintaining a species in captivity based on field experience and literature reviews.

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Foreword

Lee Durrell
Honorary Director, Durrell Wildlife Conservation Trust

The global amphibian crisis – the unprecedentedly sudden and rapid decline of a whole class of vertebrate worldwide – is now generally acknowledged, as is the key role that ex situ conservation programmes will play in addressing it.

Of all species of globally threatened amphibians, however, zoos hold only 6.2%, far short of the number for which *ex situ* management is considered necessary. Over the last 20 years zoos have increased their efforts to aid amphibian conservation, but more attention must now be turned to species which have been named as high priorities for coordinated conservation breeding programmes.[1]

With a rich amphibian fauna to which newly discovered species are frequently added, India also harbours several internationally recognised hotspots for amphibian diversity. Its zoos have a vital role to play in safeguarding this important aspect of its natural heritage, as well as setting an example for the rest of the world's zoo community to follow.

The initiatives taken since 2008 by the Central Zoo Authority to promote the role of zoos in the conservation of Indian amphibians culminated in a workshop late in 2013 in Guwahati. There was excellent representation from Indian zoos, and invited experts came to discuss with their colleagues the way forward for amphibian conservation in India.

Quite rightly, the workshop focussed on first things first – the prioritisation of species in need of *ex situ* conservation and the development of expertise and capacity in husbandry and breeding by using model species.

This book gleans the knowledge, experience and skills of many people who have managed a wide range of amphibian taxa in captivity. These can be applied to model or 'practice' species in order to hone the competences needed for coordinated conservation breeding programmes for targeted threatened species. Illustrative of the pragmatic approach is the naming of 23 zoological institutions in India and the assignment of 'practice' species to them.

Let us hope that what is learned through 'practice' is put to the test sooner rather than later by the participating zoos, not only for the sake of India's threatened amphibians, but also to enrich amphibian conservation planning at a global level.

[1]Dawson, J., Patel, F., Griffiths, R.A. and Young, R.P. 2015. Assessing the global zoo response to the amphibian crisis through 20 year trends in captive collections. *Conservation Biology* DOI: 10.1111/cobi.12563

Dated : 5 OCTOBER 2015

Preface

Smithsonian Conservation Biology Institute

This booklet, Ex-situ Management of Amphibians is needed now, more than ever to keep populations afloat and save species from extinction. Amphibians are declining rapidly in many parts of the world, in some cases, before even being officially known to science. The amphibian crisis has led to the creation of a Global Amphibian Ark organization, coordinated by the World Association of Zoos and Aquariums (WAZA), the Conservation Breeding Specialist Group (CBSG) of the IUCN's Species Survival Commission, and the Amphibian Specialist Group (ASG). The goal has been to mobilize a truly global network of actors, including building serious capacity to establish genetically viable ex situ populations of the most endangered amphibians. These interventions are benefiting amphibians by buying us time to figure out how to mitigate the threats causing the precipitous declines in nature.

One interesting component to ex situ management and breeding of amphibians is that so much information has remained 'stuck' in people's heads rather than being written down. There also are huge gaps in knowledge. Amphibians have evolved a wide range of life-history traits that help them survive and thrive in habitats ranging from dry deserts to tropical forests to rugged mountaintops. This diversity means that keeping them successfully in captivity often can be considered an art form, practiced best by dedicated students of natural history and zoology. Many discoveries await the careful, attentive observer who cares for these species, documenting (and writing down) their success and failures through trial and error. This strategy of adaptive management accompanied by a scientific approach to answer key questions in nutrition, reproduction and animal welfare (among other disciplines) is desperately needed now. Done well, we can improve our knowledge of amphibian husbandry to a point where we have a decent chance of establishing healthy ex situ assurance colonies that, as possible, can be used later for reintroductions to the wild.

But first things first. In reality, we know little about the basic natural history of many of India's 384 amphibian species, and even less about how to breed them. But the guidelines provided here provide a welcome and critical first step, especially as the information shared is based on real life experiences by experts with vast, practical experiences on a diversity of taxa. We hope others will be spurred on to embark on ark projects of their own, remembering to study not only endangered species, but also the most common. After all, the study of non-threatened species (as surrogates) often provides us the best strategies for saving the rarest. Regardless, we predict that the observations and recommendations provided in this booklet will inspire others to increase both knowledge and ex situ conservation breeding of this incredibly diverse class of frogs and caecilians.

Brian Gratwicke
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Center for Species Survival
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Dated: 22nd September, 2015

Contents

1. Introduction

Worldwide, amphibian populations are declining[1], and India has about 390 species and more than 70% of them are endemic to the region. It also harbours more threatened species of amphibian than any other country in the Indo Malayan realm[2] The Western Ghats and Eastern Himalaya are recognised biodiversity hotspots, with high levels of amphibian endemism[3,4,7]. The amphibians of the Western Ghats are enigmatic and new species are being described with astonishing frequency, it is estimated that just 50% of amphibians in the South Asian region have been described[5] with new amphibian genera being described as recently as 2013[6].

Figure 1 Bronze frog *(Hylarana temporalis) (Brij Kishor Gupta)*

Indian Zoos could play a pivotal role in the conservation management of the countries threatened amphibians. In addition zoos are ideally placed to educate the visiting the public about amphibians and the threats that they face. Currently amphibians are underrepresented in Indian Zoos and only one species *(i.e.* Salamander *Tylototriton verrucosus)* maintained by one institution, Padmaja NaiduHimalayan Zoological Park in Darjeeling. The Central Zoo Authority recognises the need to increase capacity in amphibian zoos.

The Central Zoo Authority (CZA) has made several attempts to strengthen conservation breeding programme for amphibians, by providing training to zoo professionals and promoting ex-situ conservation efforts in different Indian Zoos. In February 2008, in the International Conference organized by CZA on India's Conservation Initiative the conservation breeding programme of Himalayan Salamander was reviewed. In January 2009, workshop on ex-situ conservation for amphibians was organized by CZA in Mysore where preliminary attempts were made to prioritize amphibians for conservation breeding in Indian zoos. In December 2013, the CZA organised a workshop "Building National Capacity for ex-situ Amphibians Management and Conservation" in Guwahati, where a list target and practice species of amphibians were identified. During this workshop the Central Zoo Authority with the assistance of the Durrell

Wildlife Conservation Trust and the Zoological Society of London has strengthened national capacity in amphibian management. More than 80 delegates from all over India representing nearly 40 institutions participated in these workshops. The participants were exposed to the specific requirements of amphibians in the design and management of ex-situ facilities. Participants developed hands-on skills in enclosure design, the management of water flow and quality, temperature and light within the captive facility and the principles of amphibian nutrition, reproduction, biosecurity and conservation education. The potential contribution that ex situ amphibian management could provide to in situ amphibian conservation was also covered.

Figure 2 Narrow mouthed frog *(Microhyla rubra)* *(Brij Kishor Gupta)*

Figure 3 Wrinkled frog *(Nyctibatrchus species) (Brij Kishor Gupta)*

Over the years CZA has paved way for prioritisation of species and preparation of a plan for coordinated conservation breeding for Indian amphibians. With ecology and biology of many amphibians in India remains unknown, it is potentially difficult to keep, establish and breed Indian amphibians. We strongly urge young biologists and zoo professionals to gather information through targeted studies on species in the field before embarking on captive breeding programmes. A step in that direction would be to indentify a model amphibian species for the zoo to practice husbandry techniques with, nevertheless a vital first step for promoting amphibian conservation breeding in Indian zoos. The present guidelines on the ex situ management of amphibians are part of output of the workshop on "Building National Capacity for ex-situ Amphibians Management and Conservation" held at the Assam State Zoo, Guwhati, Assam, India during December, 2013.

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2. Ex-situ Management of Terrestrial toads Bufo species

Figure 1. *Duttaphrynus melanostictus* (Benjamin Tapley).

Introduction

Description

Terrestrial toads are stocky bodied, short limbed and possess parotid glands behind each eye (Fig.1) D. *melanostictus* is polymorphic, colouration and patterning are highly variable, even amongst wild specimens collected in the same areas. This species can be characterised by the black tipped bony warts distributed all over its body and hardened ridges on its head (Daniels, 2005) Females of D. *melanostictus* may grow to 150 mm snout vent length (SVL) and males up to 54 mm SVL (Li Fan et al, 2013).

Distribution

Duttaphrynus melanostictus is a widely distributed species, occurring from Pakistan to southern China and south throughout southern and south east Asia and parts of Indonesia. It can be found from sea level up to 1, 800 m asl (IUCN et al., 2006).

Conservation Status and Threats

Duttaphrynus melanostictus is listed as Least Concern by the IUCN Red List due to its extensive distribution and its tolerance of a wide range of habitat types(IUCN et al., 2006).. It should be noted, however, D. *melanostictus* represents a complex of cryptic species, and taxonomic revision of the complex is required (IUCN et al., 2006).

Habitat and Ecology

Duttaphrynus melanostictus are very adaptable and occurs in a wide variety of habitats, including beach vegetation, various human habitats, natural edge habitats, and closed subtropical or tropical forest (IUCN et al., 2006).

Sexing Individuals

Duttaphrynus melanostictus are sexually dimorphic with females being more than 50% larger than males. Reproductively active males have nuptial pads on the inside of the 1st and 2nd fingers (Fig. 2). Daniels, 2005 reports that in some parts of their range males turn yellow during the breeding season (Fig. 3).

Figure 2. Top. Typical black nuptial pads seen on the first and secnd finger of terrestrial toads (Benjamin Tapley). Figure 3. Male *D. melanostictus* exhibiting yellow breeding colours (Benjamin Tapley)

Reproduction and Larval Development

In drier parts of its range, *D. melanostictus*, breeds during the monsoons (Amphibiaweb). In wetter areas of its range (Including north east India the species reportedly breeds year round (Church, 1960; Ahmed, 2009).

Males congregate around slow moving or still water bodies where they vocalise (Daniels, 2005).

Amplexus, Egg Laying and Larval development

The breeding biology of *D. melanostictus* is well documented. Amplexus (mating position) is axillary in this species. Once in amplexus the female deposits up to 4,000 eggs (Li Fan et al, 2013) which hatch after 48hours (Daniels, 2005). In Chennai metamorphoses takes approximately 45 days (Daniels, 2005).

Longevity and Age at Sexual Maturity

Lifespan in the wild is unknown. In captivity *D.melanostictus* have been recorded to live for over ten years (Csurhes, 2010). Sexual maturity is at least partially governed by size, they are reported to reach sexual maturity when they are 23.0 g (Csurhes, 2010).

Captive Management

Introduction

Duttaphrynus melanostictus is commonly kept by zoos and private individuals, and have been bred on occasion in captivity by private breeders.

Identifying Individuals

Adult specimens of both species are not individually identifiable as specimens do not possess characteristic markings. Adults could be marked with passive integrated transponders (PIT tags). Pit tags should be inserted subcutaneously on the dorsum. Juveniles could be marked with different coloured visible implant elastomer (VIE) but this may not be necessary for rearing purposes

Housing adults

Duttaphrynus melanostictus require relatively large enclosures a floor space of 600 x 100 mm would be the minimum enclosure size for 5 individuals (Fig.4). Height is not important as these toads are poor climbers but the height of the enclosure should allow them to hop freely without coming into contact with the lid, an enclosure height of 500 mm would be sufficient. A wire mesh lid should be provided. A 250 x 150 x 100 mm water dish should be provided and should be deep enough for the toads to immerse their drink patches.

Cover objects are essential when keeping terrestrial toads. 150 mm lengths of PVC pipe or hollowed bamboo make appropriate cover objects, these need to be able to accommodate the toads so the size of the tube will depend on the size of the toad. Live plants can be added to the enclosure as they create humid pockets which the animals may use, the plants also provide visual barriers for the toads.

Lids should be constructed of mesh as glass will block UVB.

Substrate must be provided for this species as they like to dig. The provision of substrate will prevent the enclosure from drying out. Be sure to incorporate a drain in the tank construction. A layer of gravel covered with a permeable matting should be laid underneath a substrate of soil mixed with sand (ratio 2:1). The depth of substrate will depend on enclosure size and the size of the occupants, but must be at least 100 mm deep. The larger the enclosure, the deeper the substrate should be as the substrate will dry out quickly in large enclosures. Substrate does not need to be compacted for this species. Ensure that substrates are not contaminated with fertilisers or other substances that may be toxic to amphibians.

Ensure the substrate has a humidity gradient by pouting some water (or spraying with water) more at the end of the enclosure with no basking light.

Figure 4. Set up suitable for housing and breeding *D. melanostictus* (Zoological Society of London)

Temperature, Humidity and Lighting

Husbandry should be informed by data collected from the field. Basking spot temperatures, UV exposure and gradients, temperature and humidity regimes should mimic the environmental conditions at the collection site. Note that environmental data should be collected at different times throughout the day and year. Suitable ambient room temperatures should vary between 23 and 27˚C (night/day summer) and 20 and 25˚C (night/day winter).

A UV gradient should be provided in conjunction with a basking spot. Arcadia D3+ reptile lamp T5 12% UVB are ideal and a maximum UVI of 3 should be provided for the frogs at their basking spots. Remember that mesh will filter out some of the UVB so UV readings must be taken from below the mesh with a solarmeter 6.5.

Basking spots can be provided with regular incandescent lamps. The basking lamp should be positioned at one end of the enclosure and the temperature on the substrate below the basking spot should range between 30-35°C and not exceed 35°C. Ensure that all lighting is inaccessible to the frogs as they will be burnt easily if they come into contact with lamps.

Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, pH and phosphates. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. A plastic water dish should be provided and should measure 200 x 150 mm. Water depth should be 80 mm, and toads should be able to immerse their drink patch.

An emergent rock should be placed in the water to allow the frogs easy access out of the water.

The enclosure should be lightly misted with aged tap water daily.

Routine Husbandry

All animals should be visually inspected daily. Water dishes must be cleaned out and refilled daily as amphibians often excrete waste products in water. Water quality cannot be judged by eye. Faeces and uneaten food must be removed daily. The enclosure and all furniture must be thoroughly scrubbed (with a brush and hot water, no chemical cleaners or disinfectants) twice a week. Temperatures of the enclosure should be checked at different points of the enclosure regularly to ensure that the correct thermoregulatory gradients are maintained. UV readings must be taken every three months to check that there is a sufficient UV gradient and that the UV emitting lights are still emitting UV.

The substrate must be changed as soon as it looks dirty or if it becomes saturated or unable to retain humidity.

Diet in captivity

Most toads tend to favour small food items and *D. melanostictus* have been observed feeding on ants and winged termites in India (Daniels, 2005). Great care should be taken when feeding captive amphibians ants as ants can be aggressive and may bite and sting the toads, this is problematic because the toads cannot escape the ants if they choose to. Daniels, 2005 reports that D. melanostictus avoid armoured prey items such as beetles and crickets with hard exoskeletons. Some of the commercially available crickets are fairly soft bodied and may be appropriate. Food should be offered after it is dusted with an appropriate dietary supplement, well fed and an appropriate size, no greater in size than the width of the frogs head. Duttaphrynus melanostictus are nocturnal and should be fed in the evening.

Adults should be fed every three to six days (depending on season and condition), juveniles up to six weeks of age should be fed daily.

Reproduction in Captivity

Breeding Enclosure

Increasing the humidity can trigger breeding in *D.melanostictus*. These toads should be cycled in a rain chamber with a sprinkler system for breeding (Fig. 5).

A rain chamber with a floor space of 600 x 1000 mm would be suitable as a minimum size for 2 males and 3 females. It is important that the sex ratio in the breeding enclosure is not biased towards males as toads often form mating balls which may result in the drowning of the females (Mathew & Sen, 2010,). No substrate is required in the rain chamber. Most toads are poor swimmers so it is vital that there are some rocks or logs in the water that the toads can easily leave the water should they choose to do so. A pump powered spray system or fountain would be ideal for breeding this species but a mature biological filter should be incorporated into the rain chamber to maintain water quality. Ideally a sump could be used

Figure 5. A rain chamber suitable for breeding *D. melanostictus*. (Benjamin Tapley)

(Appendix 1). The, lighting and temperature parameters should be maintained as above. Visual barriers such as palm fronds or plastic plants could be used to provide cover for the toads in their breeding enclosure if they fail to spawn after a couple of weeks.

The toads should be fed at least every other day whilst they are in the rain chamber but ensure that any dead insects are removed daily as they will pollute the water.

In closed systems regular water tests should be carried out (Temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration process.

Breeding Seasonality

There is no published report on the

captive breeding of D. melanostictus.

Provision of Breeding Sites

A depth gradient should be provided for the toads so that they can choose their egg laying site, this can be provided by angling slates or paving slabs into the water (Fig.6).

Figure 6. A basic rain chamber used to successfully breed the Vietnamese bony headed toad *(Ingerophrynus galeatus)* in captivity. Note the sloped tile which provided the toads with a depth gradient in the rain chamber (Benjamin Tapley).

Egg Care, Tadpole Husbandry & Development

Most toads lay a paired string of eggs. It is important that the eggs (Fig. 7) are transferred to a mature aquatic system soon after laying so that they are not damaged or exposed to the air by the toads moving around in the rain chamber. You may not wish to keep all the eggs as rearing up to 4,000 tadpoles will be a huge task.

Figure 7. Paired egg string typical of toads.

The size of the tank is not important, the stocking density is. Tanks should be stocked with no more than 5 tadpoles per litre. Filtration is vital (see appendix one to inform your choice of filter). Aged tap water can be used to rear tadpoles. Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, hardness, pH and phosphates. Water parameters should be informed by field data but remember that not all breeding sites may have optimal water quality, especially in urbanised environments where these toads can often be found. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. Check the water in closed systems and carry out regular water tests (temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration and tadpoles. Ideally a sump should be used to increase the volume of water of your system and to house all the life support systems (filters etc). If using an internal canister filter ensure that it is not too strong as the tadpoles could be sucked into the inflow of the filter and may have difficulty feeding. Direct the

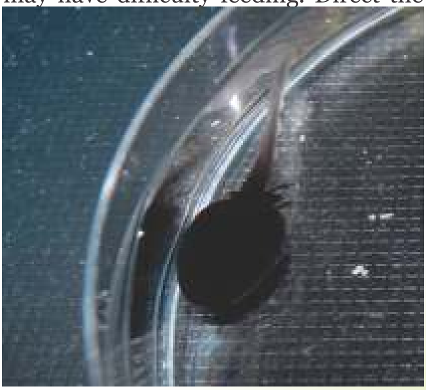

Figure 8. A typical toad tadpole is black in colour

outflow to the surface of the water for oxygenation.

Toad tadpoles are typically black in colour. When they first hatch they will not move and it is perfectly normal for them to sometimes rest on their side. If you look on the underside of tadpoles when they first hatch you should be able to see the yolk sac. The tadpole will not start swimming or feeding until this ac has been absorbed. Once the newly hatched tadpoles have dropped off the egg strings it is important to remove the remaining egg jelly as this will decay and pollute the water. Substrate is not necessary but tadpoles like to hide. Dried Indian almond leaves (*Terminalia catappa*) can be added, they also soften the water which would be beneficial in hard water areas. Partial (10-20 %) water changes should be carried out regularly, the frequency of water changes must be governed by frequent water testing.

Tadpoles should be fed daily, once they start free swimming. They should be fed on a variety of food. At Durrell the tadpoles of the Vietnamese bony headed toad were successfully reared on a powdered tadpole food (components: ground tropical fish flake, grass pellet, trout pellets, tubifex, river shrimp, spirulina algae and cuttlefish bone). Food should be available to the tadpoles at all times but it is important that large amounts of uneaten food do not pollute the aquarium. Tadpole feeding regimes must be modified, as the tadpoles grow they will eat more.

Once the front limbs appear and the tail starts to reduce it is important that the metamorphosing toadlets 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 high.

Figure 9. Enclosure for rearing juvenile terrestrial toads (Benjamin Tapley).

Rearing Metamorphs

Metamorphs can be housed in small plastic containers. A 350 x 200 x 300 mm enclosure is suitable for up to 12 individuals, these groups should be divided amongst larger enclosures as individuals grow. It is important that rearing tanks are relatively small so that high ambient humidity can me maintained and that the juvenile toads can find food easily. Covering the lid with a fine breathable cloth or fleece will prevent the food escaping and ensure that humidity remains high. The cloth will block dome of the UV emitted from the lights so you must experiment and ensure that the UV light and temperature gradients are still suitable. Compacted damp soil mixed with sand would make a good substrate, and this must be replaced as necessary. It is important that the substrate is compacted when housing metamorphs as the soil particles easily stick to the skin of the toadlets. A shallow water dish must be provided (water depth 10 mm). It is essential that the water dish is easy for the metamorphs to climb out of (a rock or small branch in the water dish facilitates access). When transferring toadlets from the tadpole tank make sure that they are placed in the water dish and not on the substrate as they may not be quite ready to leave the water, they will choose when they are ready. Cover objects are extremely important microhabitats for small toads. Bark hides and leaf litter should be added to the enclosure. The furnishings in th enclosure should not be too complex or it will be difficult for the young toads to fid food. The metamorphs can be raised at the same room temperature as the adults (23 - 27˚C), and must be misted with room temperature tap water twice daily. It is vital that metamorphs and juveniles are provided with UV at this critical growing stage (Arcadia D3+ reptile lamp T5 12% UVB), this must be given in a gradient with a maximum UVI of 2 (Fig. 9).

Do not provide metamorphs with a 35°C t basking spot as this will dry them out rapidly and will likely kill them. A lower temperature basking spot can be provided with incandescent lamps positioned at one end of the enclosure. The temperature on the branching below the basking spot should not exceed 28°C. Small tanks will rapidly overheat so ensure that there is still a cool area in the enclosure. Metamorphs should be fed daily until about six weeks of age with pin head crickets and (occasionally) Drosophila. All food items should be dusted with appropriate dietary supplements. After 8 weeks the feeding interval can be gradually increased to once every 3 days.

Health

Problems Encountered in Captivity

Toads are often sit and wait predators, their calorific intake often exceed their need and obesity is common in captive toads.

Record keeping

Things to include.

Individual identification, weight, feeding records, temperature records, UV records, water quality test results, health records, behavioural or reproductive observations.

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3. Ex-Situ Management of Microhylid frogs Kaloula, Microhyla, Ramanella & Uperodon species

Figure 1. Top *Ramanella variegata* (Sandeep Varma).
Figure 2. Middle top *Kaloula taprobanica* (Sandeep Varma)
Figure 3. Middle bottom *Uperodon systoma* (Sandeep Varma)
Figure 4. Bottom *Microhyla rubra* (Sandeep Varma)

Introduction

Description

Indian Microhylid frogs (Fig.1) are characterised by the small to medium size, small heads, invisible ear (Typmanum) ad squat body (Daniels, 2005).

Distribution

Ramanella variegata, Kaloula taprobanica, Uperodon ayatoma, Microhyla ornata and Microhyla rubra are distributed over much of peninsular India. *Kaloula pulchra* occurs in Southeast Asia, Southern China and North East India (IUCN et al., 2006).

Conservation Status and Threats

All of the above species are listed as Least Concern (IUCN et al., 2006).

Habitat and Ecology

Ramanella variegata (Fig 1). can be found in various types of forest, grassland and pastures as well as urbanised areas. It is found in dry soil areas with ground cover. They are often found in association with termite mounds in India (IUCN et al., 2006). They are explosive breeder in temporary pools and breeding starts with the onset of the monsoon rains (Daniels, 2005).

Kaloula pulchra are commensal with humans and can rapidly colonise urban habitats. It is adaptable and was presumably originally a wetland /riverbank/forest edge. It can still be found in dry and evergreen forests. It is a nocturnal and fossorial species and breeds in small pools (IUCN et al., 2006). Kaloula are able to climb well when looking for shelter (Daniels, 2005).

Kaloula taprobanica (Fig.2). is a fossorial, nocturnal species which can be found in dry forests, coconut and rubber plantations, wetlands and areas close to human habitations. It is most often found under leaf-litter, in loose soil, and under logs and other ground cover. Breeding and larval development take place in pools (IUCN et al., 2006).In Chennai this species breeds from October-November (Daniels, 2005)

Uperodon (Fig. 3). are completely fossorial that burrow in loose, moist soil up to a depth of 2 meters (Daniels, 2005). They have been observed in dry forest areas, plains, home gardens and agricultural areas. Adults surface during the summer monsoons. Breeding occurs during the monsoon rains. Males call from the banks of torrents or paddy fields (IUCN et al., 2006). In Pakistan, termites are reportedly the main food of this species.

Microhyla ornata and *M. rubra* (Fig. 4). inhabit dry forest, shrub land, grassland, agricultural land and urbanised areas. They can found in loose soil, amongst leaf-litter, and under logs and other ground cover. Breeding takes place in still waters and paddy fields. (IUCN et al., 2006). They are nocturnal but can also be active during the day in the breeding season. Microhyla breed during the onset of the monsoon rains (Daniels, 2005)

Sexing Individuals

Microhylid frogs can be difficult to sex, females tend to be larger than the males.

Amplexus, Egg Laying and Larval development

Ramanella variegata is an explosive breeder in temporary pools. The tadpoles are bottom dwellers and metamorphosis is thought to occur in less than a month (Daniels, 2005).

Kaloula pulchra breed in pools (IUCN et al., 2006). Larval development take place in still pools (Fig 5 & 6).

Kaloula taprobanica breed in pools (IUCN et al., 2006). Tadpoles metamorphose 30-45 days after the eggs are laid (Daniels, 2005).

Uperodon globulosus lay egg masses which float on the water surface (IUCN et al., 2006). Whilst mating the pair glue themselves together with a sticky secretion, eggs measure 1-1.5mm in diameter when they are laid (Daniels, 2005).

Uperodon systoma lay egg masses which float on the water surface at the onset of the monsoon rains (IUCN et al., 2006).

Whilst mating *M. ornata* glue themselves together with a sticky secretion, up to 1,300 eggs are laid by each female (Fig. 8), the eggs are 0.4mm when laid and take 48 hours to hatch. The tadpoles are plankton feeders and metamorphose in 2-3 weeks (Fig. 7).

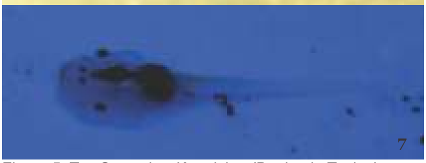

Figure 5. Top Spawning *K. pulchra* (Benjamin Tapley)
Figure 6. Middle. *K. pulchra* tadpole (Benjamin Tapley)
Figure 7. *Microhyla* tadpole (Sandeep Varma)

In India the reproductive biology of *Microhyla rubra* has not been studied in great detail but in Sri Lanka egg clutches consisted of 400 eggs and tadpoles took 77 days to metamorphose at 22-24°C (Bowatte & Meegaskumbura, 2011).

Longevity and Age at Sexual Maturity
Lifespan in the wild is unknown.

Captive Management

Introduction
Kaloula pulchra are established in captivity but are infrequently bred. This species was bred at Durrell but many of the eggs were infertile and the 12 individuals that metamorphosed died less than two weeks after hatching despite feeding and growing. Daniels (2005) reports that an attempt to maintain U. systoma in captivity failed.

Figure 8. Developing Microhylid *eggs* (Benjamin Tapley)

Identifying Individuals
Adult specimens of all species have distinctive markings on the dorsal surface, photographic identification would be appropriate for adults of this species.

Housing adults
A floor space of 600 x 600 mm would be the minimum enclosure size for 5 individuals of *Kaloula* and *Uperodon* species. 8 *Microhyla* or *Ramanella* could be maintained in this size enclosure. Height is not important as these frogs rarely climb. The height of the enclosure should allow them to hop freely without coming into contact with the lid, an enclosure height of 700 mm would be sufficient. A wire mesh lid should be provided. A 200 x 150 x 100 mm water dish should be provided and should be deep enough for the frogs to immerse their drink patches.

Cover objects are essential when keeping terrestrial frogs. 150 mm lengths of PVC pipe or hollowed bamboo make appropriate cover objects, these need to be able to accommodate the frogs so the size of the tube will depend on the size of the frog. Live plants can be added to the enclosure as they create humid pockets which the animals may use, the plants also provide visual barriers for the frogs.

Lids should be constructed of mesh as glass will block UVB.

Substrate must be provided for this species as they all dig. The provision of substrate will prevent the enclosure from drying out. Be sure to incorporate a drain in the tank construction. A layer of gravel covered with a permeable matting should be laid underneath a substrate of soil mixed with sand (ratio 2:1). The depth of substrate will depend on enclosure size but must be at least 150 mm deep for *Kaloula*, *Ramanella* and *Microhyla* and at least 300 mm for *Uperodon*. The larger the enclosure, the deeper the substrate should be as substrate will dry out quickly in large enclosures. Substrate does not need to be compacted for this species. Ensure that substrates are not contaminated with fertilisers or other substances that may be toxic to amphibians.

Ensure the substrate has a humidity gradient by pouting some water (or spraying with water) more at the end of the enclosure with no basking light. These are litter frogs and are well camouflaged, a layer of leaf litter should be provided for these frogs as they may experience some level of stress without it.

Temperature, Humidity and Lighting
Husbandry should be informed by data collected from the field. Basking spot

temperatures, UV exposure and gradients, temperature and humidity regimes should mimic the environmental conditions at the collection site. Note that environmental data should be collected at different times throughout the day and year. Suitable ambient room temperatures should vary between 23 and 27˚C (night/day summer) and 20 and 25˚C (night/day winter).

A UV gradient should be provided in conjunction with a basking spot. Arcadia D3+ reptile lamp T5 12% UVB are ideal and a maximum UVI of 3 should be provided for the frogs at their basking spots. Remember that mesh will filter out some of the UVB so UV readings must be taken from below the mesh with a solarmeter 6.5.

Basking spots can be provided with regular incandescent lamps. The basking lamp should be positioned at one end of the enclosure and the temperature on the substrate below the basking spot should range between 30-32°C and not exceed 32°C. Ensure that all lighting is inaccessible to the frogs as they will be burnt easily if they come into contact with lamps.

Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, pH and phosphates. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. A plastic water dish should be provided and should measure 200 x 150 mm. Water depth should be 80 mm, and frogs should be able to immerse their drink patch. An emergent rock should be placed in the water to allow the frogs easy access out of the water. The enclosure should be lightly misted with aged tap water daily.

Routine Husbandry

All animals should be visually inspected daily. Water dishes must be cleaned out and refilled daily as amphibians often excrete waste products in water. Water quality cannot be judged by eye. Faeces and uneaten food must be removed daily. The enclosure and all furniture must be thoroughly scrubbed (with a brush and hot water, no chemical cleaners or disinfectants) twice a week. Temperatures of the enclosure should be checked at different points of the enclosure regularly to ensure that the correct thermoregulatory gradients are maintained. UV readings must be taken every three months to check that there is a sufficient UV gradient and that the UV emitting lights are still emitting UV.

The substrate must be changed as soon as it looks dirty or if it becomes saturated or unable to retain humidity.

Diet in captivity

Uperodon are reportedly termite specialists (Daniels, 2005; IUCN et al., 2006). Kaloula will readily accept small live insects in captivity. A study on the diet of wild Microhyla heymonsi in Indonesia showed that more than 50% of the diet is made up of ants but they consume a wide variety of small invertebrates (Erftemeijer & Boeadie,1991). Great care should be taken when feeding captive amphibians ants as ants can be aggressive and may bite and sting the toads, this is problematic because the toads cannot escape the ants if they choose to. The feeding habits of Ramanella are unknown, like other similar sized frogs they probably feed on invertebrates. Food should be offered after it is dusted with an appropriate dietary supplement, well fed and an appropriate size. Microhylids have small mouths so the food should be no greater in size than the distance between the yes. of the frogs head. Microhylid frogs are usually nocturnal and should be fed in the evening.

Adults should be fed every three to six days (depending on season and condition), juveniles up to six weeks of age should be fed daily.

Reproduction in Captivity

Breeding Enclosure

Increasing the humidity may trigger breeding in this species. These frogs should be cycled in a rain chamber with a sprinkler system for breeding (Fig. 9).

A rain chamber with a floor space of 600 x 1000 mm would be suitable as a minimum size for 2 males and 3 females (Kaloula and Uperodon) or 4 males and 4 females (Microhyla and Ramanella). It is important that the sex ratio in the breeding enclosure is not biased towards males this may be stressful for the females). No substrate is required in the rain chamber at first, but a 1cm deep

Figure 9. A rain chamber suitable for breeding C. curtipes (Benjamin Tapley)

lining of nontoxic clay could be trialled if the frogs have not spawned after one month. Based on their morphology most microhylids are poor swimmers so it is vital that there are some rocks or logs in the water that the frogs can easily leave the water should they choose to do so. A pump powered spray system or fountain would be ideal for breeding this species but a mature biological filter should be incorporated into the rain chamber to maintain water quality. Ideally a sump could be used (Appendix 1). Water depth is extremely important when breeding microhylids. A gradient should always be provided. During breeding Kaloula and Uperodon will duck under the surface during egg laying and the water depth needs to be deep enough to allow this to happen. The water depth should be 150 – 200 mm at one end of the rain chamber. The, lighting and temperature parameters should be maintained as above. Visual barriers such as palm fronds or plastic plants could be used to provide cover for the frogs in their breeding enclosure if they fail to spawn after a couple of weeks.

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 as they will pollute the water.

Never try and separate Microhylids once they begin mating as many species glue themselves together during the process, pulling them part could damage their skin.

In closed systems regular water tests should be carried out (Temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration process.

Breeding Seasonality

Breeding aggregations of these species can be found at the start of the monsoon.

Provision of Breeding Sites

A depth gradient should be provided for the frogs so that they can choose their egg laying site, this can be provided by angling slates or paving slabs into the water

Egg Care, Tadpole Husbandry & Development

When eggs are laid it is best to leave them in the rain chamber and remove the frogs, many microhylid eggs float on the surface of the water and moving them may make them sink, it is unknown what impact tis would have on egg development. You may not wish to keep all the eggs if the clutch size is large as rearing large numbers of tadpoles and providing food for all the metamoprhs will be a huge task.

The size of the tank is not important, the stocking density is. Tanks should be stocked with no more than 5 tadpoles per litre.. Filtration is vital (see appendix one to inform your choice of filter). Aged tap water can be used to rear tadpoles. Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, hardness, pH and phosphates. Water parameters should be informed by field data but remember that not all breeding sites may have optimal water quality. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. Check the water in closed systems and carry out regular water tests (temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration and tadpoles. Ideally a sump should be used to increase the volume of water of your system and to house all the life support systems (filters etc). If using an internal canister filter ensure that it is not too strong as the tadpoles could be sucked into the inflow of the filter and may have difficulty feeding. Direct the outflow to the surface of the water for oxygenation.

When the eggs first hatch the tadpoles will not move and it is perfectly normal for them to sometimes rest on their side. If you look on the underside of tadpoles when they first hatch you should be able to see the yolk sac. The tadpole will not start swimming or feeding until this ac has been absorbed. Once the newly hatched tadpoles have dropped off the egg mass it is important to remove the remaining egg jelly as this will decay and pollute the water. Substrate is not necessary but tadpoles like to hide. Dried Indian almond leaves (Terminalia catappa) can be added, they also soften the water which would be beneficial in hard water areas. Partial (10-20 %) water changes should be carried out regularly, the frequency of water changes must be governed by frequent water testing.

Tadpoles should be fed daily, once they start free swimming. They should be fed on a variety of food. At Durrell Kaloula tadpoles were reared to metamorphoses on a powdered tadpole food (components : ground tropical fish flake, grass pellet, trout pellets, tubifex, river shrimp, spirulina algae and cuttlefish bone). Food should be available to the tadpoles at all times but it is important that large amounts of uneaten food do not pollute the aquarium. Tadpole feeding regimes must be modified, as the tadpoles grow they will eat more.

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. Emerging rocks or aquatic plants are suitable. Once they emerge they are prone to drying out and easily die if the humidity is not high.

Rearing Metamorphs

Figure 10. Enclosure for rearing juvenile Micohylids (Benjamin Tapley).

Metamorphs can be housed in small plastic containers. A 350 x 200 x 300 mm enclosure is suitable for up to 12 individuals (Fig. 10), these groups should be divided amongst larger enclosures as individuals grow. It is important that rearing tanks are relatively small so that high ambient humidity can me maintained and that the juvenile frogs can find food easily. Covering the lid with a fine breathable cloth or fleece will prevent the food escaping and ensure that humidity remains high. The cloth will block dome of the UV emitted from the lights so you must experiment and ensure that the UV light and temperature gradients are still suitable. Compacted damp soil mixed with sand would make a good substrate, and this must be replaced as necessary. It is important that the substrate is compacted when housing metamorphs as the soil particles easily stick to the skin of the froglets. A shallow water dish must be provided (water depth 10 mm). It is essential that the water dish is easy for the metamorphs to climb out of (a rock or small branch in the water dish facilitates access). When transferring froglets from the tadpole tank make sure that they are placed in the water dish and not on the substrate as they may not be quite ready to leave the water, they will choose when they are ready. Cover objects are extremely important microhabitats for small toads. Bark hides and leaf litter should be added to the enclosure. The furnishings in the enclosure should not be too complex or it will be difficult for the young frogs to find food. The metamorphs can be raised at the same room temperature as the adults (23 - 27˚C), and must be misted with room temperature tap water twice daily. It is vital that metamorphs and juveniles are provided with UV at this critical growing stage (Arcadia D3+ reptile lamp T5 12% UVB), this must be given in a gradient with a maximum UVI of 2 (Fig. 9).

Do not provide metamorphs with a 32°C t basking spot as this will dry them out rapidly and will likely kill them. A lower temperature basking spot can be provided with incandescent lamps positioned at one end of the enclosure. The temperature on the branching below the basking spot should not exceed 28°C. Small tanks will rapidly overheat so ensure that there is still a cool area in the enclosure. Metamorphs should be fed daily until about six weeks of age with pin head crickets and (occasionally) Drosophila. All food items should be dusted with appropriate dietary supplements. After 8 weeks the feeding interval can be gradually increased to once every 3 days.

Health

Problems Encountered in Captivity

Obesity.

Record keeping

Things to include.

Individual identification, weight, feeding records, temperature records, UV records, water quality test results, health records, behavioural or reproductive observations.

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4. Ex-situ Management of Leaping frogs Indirana species

Figure 1. Indirana leptodactyla (Benjamin Tapley).

Introduction

Description

Frogs of the genus Indirana (Family Ranixalidae) can be distinguished from most other sympatric frogs by the following characteristics small in size (less than 80 mm snout vent length (SVL), brown in colouration, prominent tympanum (ear), fingers and toes terminating in discs, round pupil (Daniels, 2005).

Distribution

Indirana are endemic to the Western Ghats Mountains of south-western India (Daniels, 2005) and the genus has an altitudinal range from 0 - 2,000 masl (Nair et al., 2012).

Conservation Status and Threats

Several species re known only from their type localities (IUCN et al., 2006). The conservation status of Indirana is summarised in table 1.

Species	IUCN status	Distribution range (km^2)
Indirana beddomii	Least concern	1250
Indirana brachytarsus	Endangered	550
Indirana semipalmata	Least concern	700
Indirana leithii	Vulnerable	500
Indirana leptodactyla	Endangered	600
Indirana diplosticta	Endangered	350
*Indirana gundia**	Critically endangered	50
Indirana phrynoderma	Critically endangered	100
*Inditana longicrus**	Data deficient	50
*Indirana tenuilingua**	Data deficient	50

*Known from only one locality.

Table 1. Conservation status of Indirana (Nair et al., 2012)

Habitat and Ecology

Indirana frogs are ground dwelling and live on the forest floor of evergreen and semi deciduous forests, usually in association with streams with wet rock faces (IUCN et al., 2006; Tapley et al., 2011; Nair et al., 2012). *Indirana* breed during the monsoon rains (Daniels, 2005). Vocalisations consist of short calls, males of some species call simultaneously (Kadadevaru et al, 2000; Kuramoto & Dubois, 2009) suggesting males may form loose leks (Nair et al., 2012)

Sexing Individuals

Females are usually larger than the males. Relative tympanum size is greater in male frogs (Nair et al., 2012). Male Indirana in breeding condition have nuptial pads on the first finger (Daniels, 2005; Gopalan, et al., 2012; Nair et al., 2012).

Figure 2. Eggs of I. *semipalmata* (Benjamin Tapley)

Egg Laying and Larval development

Indirana lay their eggs in humid areas in clear poos at the base of rocks or in rock crevices (Daniels, 2005). *Indirana bedomii* is reported to lay eggs in shallow pools (20 mm in depth) away from streams (Veeranagoudar et al., 2009). Indirana leptodactyla is reported to lay up to 30 eggs per clutch in the grassy margins of ponds (Rao, 1920). Tapley, et al., reported that I. semipalmata lay clumps of up to 343 eggs on sheltered wet rock faces and even on tree bark (Fig 2.).

Indirana tadpoles (Fig. 3). are extremely specialised, they have long tails with reduced fins which suit their specialised locomotion on usually vertical wet rock surfaces and even on wet tree bark away from any permanent streams (Veeranagoudar et al., 2009; Tapley et al, 2011). Tadpoles are exposed to visually orientated predators in such environments and rely on camouflage, early hind limb development and muscular tails which allow them to make quick jumps when disturbed (Annandle, 1918, Kuramoto & Joshy, 2002; Veeranagoudar et al., 2009; Tapley et al, 2011). The incredible jumping ability of *Indirana* tadpoles allow them to escape microhabitats which may dry out rapidly.

Figure 3. Typical *Indirana* tadpole (Benjamin Tapley)

Longevity and Age at Sexual Maturity

Lifespan in the wild is unknown.

Captive Management

Introduction

Indirana is not currently kept in captivity.

Identifying Individuals

Not all *Indirana* possess patterns suitable for photographic identification. Adults of the larger species could be marked Adults could be marked with passive integrated transponders (PIT tags). Pit tags should be inserted subcutaneously on the dorsum. Juveniles could be marked with different coloured visible implant elastomer (VIE) but this may not be necessary for rearing purposes.

Housing adults

Indirana require specialist housing due to their unique microhabitats. An enclosure measuring 600 x 6000 x 600 mm would be the minimum enclosure size for 8 individuals (Fig.4). A wire mesh lid should be provided. Water should be provided in two forms, a pool and a wet rock surface.

Substrate must be provided for this species as it is unknown whether or not they like to dig. The provision of substrate will prevent the enclosure from drying out. Be sure to incorporate a drain in the tank construction. A deep layer of gravel (200 mm) covered with a permeable matting should be laid underneath a substrate of soil mixed with sand (ratio 2:1). The depth of the sands / soil substrate should be at approximately 50 mm deep. Ensure that substrates are not contaminated with fertilisers or other substances that may be toxic to amphibians.

Ensure the substrate has a humidity gradient by pouting some water (or spraying with water) more at the end of the enclosure with no basking light. These frogs and are well camouflaged, a layer of leaf litter should be provided for these frogs as they may experience some level of stress without it.

Cover objects are essential when keeping terrestrial frogs. Plants can be added to the enclosure as they create humid pockets which the animals may use, the plants also provide visual barriers for the frogs. Small lengths of PVC pipe or hollow bamboo may also be beneficial. Lids should be constructed of mesh as glass will block UVB.

Figure 4. Set up suitable for housing and breeding Indirana (Brian's Tropicals)

The water pool van be created by by providing a large indentation in the gravel (Fig. 4). The sides of the pool can be maintained by growing live plants on the substrate, or using pebbles or bricks to create the edge of the pool. Filtration must be incorporated into this design to maintain water quality. In closed systems regular water tests should be carried out (Temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration process. A wet rock face can be created by securely angling a slate or other flat rock surface against one side of the enclosure with the bottom of the slate being firmly positioned in the pool of water (Fig. 4). A submersible pump with adjustable flow can be buried in the gravel. The so that water trickles down the rock face. It is important flow is adjustable, a powerful stream of water will prevent tadpoles from feeding and may wash them off the rock surface.

Temperature, Humidity and Lighting

Husbandry should be informed by data collected from the field. Basking spot temperatures, UV exposure and gradients, temperature and humidity regimes should mimic the environmental conditions at the collection site. Note that environmental data should be collected at different times throughout the day and year. Suitable ambient room temperatures should vary between 23 and 27°C (night/day summer) and 20 and 25°C (night/day winter).

A UV gradient should be provided in conjunction with a basking spot. Arcadia D3+ reptile lamp T5 12% UVB are ideal and a maximum UVI of 3 should be provided for the frogs at their basking spots. Remember that mesh will filter out some of the UVB so UV readings must be taken from below the mesh with a solarmeter 6.5.

Basking spots can be provided with regular incandescent lamps. The basking lamp should be positioned at one end of the enclosure and the temperature on the substrate below the basking spot should range between 30-32°C and not exceed 32°C. Ensure that all lighting is inaccessible to the frogs as they will be burnt easily if they come into contact with lamps. These frogs can often be found on dappled forest floor during the day (Pers. obs) and so the provision of UV lighting is essential.

Lighting is also important for the growth of alga on the flat rock surface (Important tadpole food).

Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, pH and phosphates. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature.

The enclosure should be lightly misted with aged tap water daily.

Routine Husbandry
All animals should be visually inspected daily. Dead food items and faecel material must be removed from the water daily as amphibians often excrete waste products in water. Water quality cannot be judged by eye. Faeces and uneaten food must be removed from the enclosure daily. The enclosure and all furniture must be thoroughly scrubbed (with a brush and hot water, no chemical cleaners or disinfectants) twice a week but do not scrub the wet rock surface as algae will grow on it and this is the most important food for the tadpoles. Temperatures of the enclosure should be checked at different points of the enclosure regularly to ensure that the correct thermoregulatory gradients are maintained. UV readings must be taken every three months to check that there is a sufficient UV gradient and that the UV emitting lights are still emitting UV.

The substrate must be changed as soon as it looks dirty or if it becomes saturated or unable to retain humidity.

Diet in captivity
The feeding habits of these frogs are largely unknown, I. *semipalmapata* have been observed feeding on termites (pers obs). Like other similar sized frogs they probably feed on invertebrates. Food should be offered after it is dusted with an appropriate dietary supplement, well fed and an appropriate size, no greater in size than the width of the frogs head. *Indirana* are nocturnal and should be fed in the evening.

Adults should be fed every three to six days (depending on season and condition), juveniles up to six weeks of age should be fed daily.

Reproduction in Captivity

Breeding Enclosure
Increasing the humidity may trigger breeding in this species. These frogs should be cycled in their regular enclosure for breeding (Fig. 4).

Breeding Seasonality
There is no published report on the captive breeding of *Indirana*. In the wild, breeding aggregations of this species can be found at the start of the monsoon.

Provision of Breeding Sites
A depth gradient should be provided for the frogs so that they can choose their egg laying site, this can be provided by angling slates or paving slabs into the water (Fig.4).

Egg Care, Tadpole Husbandry & Development
Leave the eggs in the same enclosure as the parents, I. *semiplamata* often sit beside their egg clutches and the skin of the adult frogs could have anti fungal properties which prevent the eggs getting mouldy. Some frogs are also known to eat frog eggs, egg guarding by adult individuals could prevent this from occurring. Eggs are sometimes laid in quite exposed places and they could easily dry out. A fogger should be placed in aquarium to maintain high ambient humidity. The fogger can be connected to a timer, and turned on for two minutes every hour. You may need to adjust this if the enclosure becomes too wet or eggs still appear to dry out.

When the eggs first hatch the tadpoles will not move and it is perfectly normal for them to sometimes rest on their side. If you look on the underside of tadpoles when they first hatch you should be able to see the yolk sac. The tadpole will not start feeding until this ac has been absorbed.

Tadpoles feed on wet rock surfaces and maintain the tadpoles is probably the most challenging aspect of *Indirana* husbandry. The rock surface may become depleted of alga very quickly. It is therefore important that several spare rock surfaces are prepared ahead of planned breeding trials. These can be set up in an adjoin well lit aquarium with a similar water fall like system. As a large resort suitable rocks could be removed from the field. Great care should be taken when working with enclosures with tadpoles as you don't want the tadpoles to jump off the rock as they may not be able to find the rock and food source easily once they leave it. Any movement incursions into the tank should involve slow movements and the reaction of the tadpoles to the keeper closely observed. Do not remove the existing rock if more food needs to be added, simply place the new rocks on or beside the existing rock surface, making sure that they too are covered by a trickle of water.

Rearing Metamorphs

Once the tadpoles metamorphose (tail totally absorbed) they can be housed in small plastic containers. A 350 x 200 x 300 mm enclosure is suitable for up to 12 individuals (Fig. 9), these groups should be divided amongst larger enclosures as individuals grow. It is important that rearing tanks are relatively small so that high ambient humidity can me maintained and that the juvenile frogs can find food easily. Covering the lid with a fine breathable cloth or fleece will prevent the food escaping and ensure that humidity remains high. The cloth will block dome of the UV emitted from the lights so you must experiment and ensure that the UV light and temperature gradients are still suitable. Compacted damp soil mixed with sand would make a good substrate, and this must be replaced as necessary. It is important that the substrate is compacted when housing metamorphs as the soil particles easily stick to the skin of the froglets. A shallow water dish must be provided (water depth 10 mm). It is essential that the water dish is easy for the metamorphs to climb out of (a rock or small branch in the water dish facilitates access). When transferring froglets from the tadpole tank make sure that they are placed in the water dish and not on the substrate as they may not be quite ready to leave the water, they will choose when they are ready. Cover objects are extremely important microhabitats for small frogs. Bark hides and leaf litter should be added to the enclosure. The furnishings in the enclosure should not be too complex or it will be difficult for the young frogs to find food. The metamorphs can be raised at the same room temperature as the adults (23 - 27°C), and must be misted with room temperature tap water twice daily. It is vital that metamorphs and juveniles are provided with UV at this critical growing stage (Arcadia D3+ reptile lamp T5 12% UVB), this must be given in a gradient with a maximum UVI of 2 (Fig. 9).

Do not provide metamorphs with a 35°C basking spot as this will dry them out rapidly and will likely kill them. A lower temperature basking spot can be provided with incandescent lamps positioned at one end of the enclosure. The temperature on the branching below the basking spot should not exceed 28°C. Small tanks will rapidly overheat so ensure that there is still a cool area in the enclosure Metamorphs should be fed daily until about six weeks of age with springtails, pin head crickets and (occasionally) Drosophila. All food items should be dusted with appropriate dietary supplements. After 8 weeks the feeding interval can be gradually increased to once every 3 days. Providing small enough prey items for Indirana could be very difficult as the froglets are tiny when they metamorphose. It is important that adequate stocks of live food are available well in advance of breeding.

Health

Indirana leithi in Maharashtra have tested positive for *Batrachochytrium* *dendrobaitidis*, the fungus which causes the disease chytridiomycosis (Dahanuker et al., 2013)

Problems Encountered in Captivity

Non applicable.

Record keeping

Things to include.

Individual identification, weight, feeding records, temperature records, UV records, water quality test results, health records, behavioural or reproductive observations.

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5. Ex-situ Management of Bicoloured frog *Clinotarsus Curtipes*

Figure 1. *Clinotarsus curtipes* (Benjamin Tapley).

Introduction

Description

Bicoloured frogs are stocky bodied and thin limbed ranid frogs (Fig.1) *C. curtipes* is polymorphic, colouration is highly variable, even amongst wild specimens collected in the same areas. Female The tips of the fingers and toes terminate in small discs, the toeas are fully webbed (Daniels, 205). *Clinotarsus curtipes* may grow to 75 mm snout vent length (SVL).

Distribution

Clinotarsus curtipes are endemic to the Western Ghats Mountains of south-western India (Daniels, 2005) from 500 - 2,000 mask (IUCN et al., 2006).

Conservation Status and Threats

Clinotarsus curtipes is listed as Near Threatened by the IUCN Red List as its extent of occurrence is approximately 20,000 km2, and the extent and quality of its habitat are probably declining, thus making the species close to qualifying for Vulnerable. (IUCN et al., 2006). Tadpoles of this species are collected for human consumption (Stuart et al., 2008)

Habitat and Ecology

Clinotarsus curtipes is a terrestrial leaf-litter frog, found in evergreen to semi-evergreen moist forest, and into dry deciduous forest. It is also found in lightly degraded areas (IUCN et al., 2006). The abundance of *C. curtipes* is related to large water bodies and canopy-covered habitats (Krishna & Krishna, 2005).

Sexing Individuals

Clinotarsus curtipes are sexually dimorphic with females much larger than males (Fig. 2).

Figure 2. Amplectant C. curtipes, note the small size of the male (Benjamin Tapley)

Reproduction and Larval Development

These frogs breed in deep, perennial water bodies such as along slow-flowing streams and in water tanks with canopy cover and dams (Hiragond et al., 2001, Krishna and Krishna, 20051,2) Males congregate around slow moving or still water bodies where they vocalise (Daniels, 2005). Large numbers of frogs congregate around breeding sites after dusk and emit their advertisement calls from 1820hours until 0400hrs. Female *C.curtipes* have been documented vocalising from the same location at a breeding site over multiple nights (Krishna and Krishna, 2005[1]). Females who were still vocalising late in the breeding season and were holding territories were observed fighting and chasing off intruding females who arrived late at the breeding site and such fights were not recorded among females who arrived early in the breeding season in the presence of many males (Krishna and Krishna, 2005[1]). Males are territorial and have been fighting during the breeding season (Tapley & Purushotham, 2011). Both males and females are known to vocalise at the breeding sites (Krishna & Krishna, 2005 1).

Amplexus, Egg Laying and Larval development

Amplexus (mating position) is axillary in this species. (Daniels, 2005).It is thought to be egg laying, the reproductive biology of this species is not well studied. The tadpoles of *C.curtipes* are thought to feed on alga and are amongst the largest tadpoles in the Western Ghats and can attain a total length of 9 cm (Fig 3.). Tadpoles have three glands on the dorsal surface which excrete a white fluid when handled, it is not known whether or not this fluid is toxic (Fig 4.). These tadpoles form large shoals and kin recognition is known in this species, each shoal being composed of siblings (Gururaja KV pers.com).

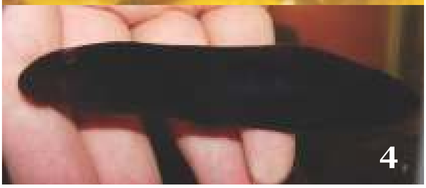

Figure 3. Top. A shoal of C. curtipes larva, note the orange glands (Chetana Purushotham)
Figure 4. Maximum tadpole size of C. curtipes (Benjamin Tapley)

Longevity and Age at Sexual Maturity

Lifespan in the wild is unknown.

Figure 5. The mottled underside of C. curtipes (Benjamin Tapley).

Captive Management

Introduction

Clinotarsus curtipes is not currently kept in captivity.

Identifying Individuals

Adult specimens of both species have distinctive markings on the ventral surface (Fig. 5), photographic identification would be appropriate for adults of this species.

Housing adults

Clinotarsus curtipes require relatively large enclosures, a floor space of 600 x 1000 mm would be the minimum enclosure size for 5 individuals (Fig.6). Height is not important as these frogs rarely climb. The height of the enclosure should allow them to hop freely without coming into contact with the lid, an enclosure height of 500 mm would be sufficient. A wire mesh lid should be provided. A 250 x 150 x 100 mm water dish should be provided and should be deep enough for the frogs to immerse their drink patches.

Cover objects are essential when keeping terrestrial frogs. 150 mm lengths of PVC pipe or hollowed bamboo make appropriate cover objects, these need to be able to accommodate the frogs so the size of the tube will depend on the size of the frog. Live plants can be added to the enclosure as they create humid pockets which the animals may use, the plants also provide visual barriers for the frogs.

Lids should be constructed of mesh as glass will block UVB.

Substrate must be provided for this species as it is unknown whether or not they like to dig. The provision of substrate will prevent the enclosure from drying out. Be sure to incorporate a drain in the tank construction. A layer of gravel covered with a permeable matting should be laid underneath a substrate of soil mixed with sand (ratio 2:1). The depth of substrate will depend on enclosure size and the size of the occupants, but must be at least 100 mm deep. The larger the enclosure, the deeper the substrate should be as the substrate will dry out quickly in large enclosures. Substrate does not need to be compacted for this species. Ensure that substrates are not contaminated with fertilisers or other substances that may be toxic to amphibians.

Ensure the substrate has a humidity gradient by pouting some water (or spraying with water) more at the end of the enclosure with no basking light. These are litter frogs and are well camouflaged, a layer of leaf litter should be provided for these frogs as they may experience some level of stress without it.

Figure 6. Set up suitable for housing and breeding *C. curtipes* (Zoological Society of London)

Temperature, Humidity and Lighting

Husbandry should be informed by data collected from the field. Basking spot temperatures, UV exposure and gradients, temperature and humidity regimes should mimic the environmental conditions at the collection site. Note that environmental data should be collected at different times throughout the day and year. Suitable ambient room temperatures should vary between 23 and 27˚C (night/day summer) and 20 and 25˚C (night/day winter).

A UV gradient should be provided in conjunction with a basking spot. Arcadia D3+ reptile lamp T5 12% UVB are ideal and a maximum UVI of 3 should be provided for the frogs at their basking spots. Remember that mesh will filter out some of the UVB so UV readings must be taken from below the mesh with a solarmeter 6.5.

Basking spots can be provided with regular incandescent lamps. The basking lamp should be positioned at one end of the enclosure and the temperature on the substrate below the basking spot should range between 30-32°C and not exceed 32°C. Ensure that all lighting is inaccessible to the frogs as they will be burnt easily if they come into contact with lamps.These frogs can often be found on dappled forest floor during the day (Pers. obs) and so the provision of UV lighting is essential.

Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, pH and phosphates. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. A plastic water dish should be provided and should measure 200 x 150 mm. Water depth should be 80 mm, and frogs should be able to immerse their drink patch. An emergent rock should be placed in the water to allow the frogs easy access out of the water.

The enclosure should be lightly misted with aged tap water daily.

Routine Husbandry

All animals should be visually inspected daily. Water dishes must be cleaned out and refilled daily as amphibians often excrete waste products in water. Water quality cannot be judged by eye. Faeces and uneaten food must be removed daily. The enclosure and all furniture must be thoroughly scrubbed (with a brush and hot water, no chemical cleaners or disinfectants) twice a week. Temperatures of the enclosure should be checked at different points of the enclosure regularly to ensure that the correct thermoregulatory gradients are maintained. UV readings must be taken every three months to check that there is a sufficient UV gradient and that the UV emitting lights are still emitting UV.

The substrate must be changed as soon as it looks dirty or if it becomes saturated or unable to retain humidity.

Diet in captivity

The feeding habits of this frog are totally unknown, but like other similar sized frogs they probably feed on invertebrates. Food should be offered after it is dusted with an appropriate dietary supplement, well fed and an appropriate size, no greater in size than the width of the frogs head. *C. clinotarsus* are nocturnal and should be fed in the evening.

Figure 7. A rain chamber suitable for breeding *C. curtipes* (Benjamin Tapley)

Adults should be fed every three to six days (depending on season and condition), juveniles up to six weeks of age should be fed daily.

Reproduction in Captivity

Breeding Enclosure

Increasing the humidity may trigger breeding in this species. These frogs should be cycled in a rain chamber with a sprinkler system for breeding (Fig. 7).

A rain chamber with a floor space of 600 x 1000 mm would be suitable as a minimum size for 2 males and 3 females. It is important that the sex ratio in the breeding enclosure is not biased towards males this may be stressful for the females). No substrate is required in the rain chamber. Based on their morphology *C.curtipes* are probably poor swimmers so it is vital that there are some rocks or logs in the water that the frogs can easily leave the water should they choose to do so. A pump powered spray system or fountain would be ideal for breeding this species but a mature biological filter should be incorporated into the rain chamber to maintain water quality. Ideally a sump could be used (Appendix 1). The, lighting and temperature parameters should be maintained as above. Visual barriers such as palm fronds or plastic plants could be used to provide cover for the frogs in their breeding enclosure if they fail to spawn after a couple of weeks.

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 as they will pollute the water.

In closed systems regular water tests should be carried out (Temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration process.

Breeding Seasonality

There is no published report on the captive breeding of *C. curtipes*. In the wild, breeding aggregations of this species can be found at the start of the monsoon.

Provision of Breeding Sites

A depth gradient should be provided for the frogs so that they can choose their egg laying site, this can be provided by angling slates or paving slabs into the water (Fig.8).

Figure 8. A basic rain chamber used to successfully breed the Vietnamese bony headed frog (Ingerophrynus galeatus) in captivity. Note the sloped tile which provided the toads with a depth gradient in the rain chamber (Benjamin Tapley).

Egg Care, Tadpole Husbandry & Development

A description of the eggs of *C. curtipes* have not been described. When eggs are laid transfer them to a mature aquatic system soon after laying so that they are not damaged or exposed to the air by the frogs moving around in the rain chamber. You may not wish to keep all the eggs if the clutch size is large as rearing large numbers of tadpoles and providing food for all the metamoprhs will be a huge task.

As the tadpoles of this species are large and form large shoals, tank size is important. A tank measuring 1,000 x 500 x 500 mm would be suitable for a maximum 250 tadpoles. Tanks should be stocked with no more than 1 tadpole per litre. Filtration is vital (see appendix one to inform your choice of filter). Aged tap water can be used to rear tadpoles. Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, hardness, pH and phosphates. Water parameters should be informed by field data but remember that not all breeding sites may have optimal water quality. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. Check the water in closed systems and carry out regular water tests (temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration and tadpoles. Ideally a sump should be used to increase the volume of water of your system and to house all the life support systems (filters etc). If using an internal canister filter ensure that it is not too strong as the tadpoles could be sucked into the inflow of the filter and may have difficulty feeding. Direct the outflow to the surface of the water for oxygenation.

When the eggs first hatch the tadpoles will not move and it is perfectly normal for them to sometimes rest on their side. If you look on the underside of tadpoles when they first hatch you should be able to see the yolk sac. The tadpole will not start swimming or feeding until this ac has been absorbed. Once the newly hatched tadpoles have dropped off the egg mass it is important to remove the remaining egg jelly as this will decay and pollute the water. Substrate is not necessary but tadpoles like to hide. Dried Indian almond leaves *(Terminalia catappa)* can be added, they also soften the water which would be beneficial in hard water areas. Partial (10-20 %) water changes should be carried out regularly, the frequency of water changes must be governed by frequent water testing.

Tadpoles should be fed daily, once they start free swimming. They should be fed on a variety of food. At Durrell ranid frog tadpoles were successfully reared on a powdered tadpole food (components: ground tropical fish flake, grass pellet, trout pellets, tubifex, river shrimp, spirulina algae and cuttlefish bone). Food should be available to the tadpoles at all times but it is important that large amounts of uneaten food do not pollute the aquarium. Tadpole feeding regimes must be modified, as the tadpoles grow they will eat more.

Once the front limbs appear and the tail starts to reduce it is important that the metamorphosing frolets are given the opportunity to emerge from the water as

Figure 9. Enclosure for rearing juvenile C. curtipes (Benjamin Tapley).

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 high.

Rearing Metamorphs

Metamorphs can be housed in small plastic containers. A 350 x 200 x 300 mm enclosure is suitable for up to 12 individuals (Fig. 9), these groups should be divided amongst larger enclosures as individuals grow. It is important that rearing tanks are relatively small so that high ambient humidity can me maintained and that the juvenile frogs can find food easily. Covering the lid with a fine breathable cloth or fleece will prevent the food escaping and ensure that humidity remains high. The cloth will block dome of the UV emitted from the lights so you must experiment and ensure that the UV light and temperature gradients are still suitable. Compacted damp soil mixed with sand would make a good substrate, and this must be replaced as necessary. It is important that the substrate is compacted when housing metamorphs as the soil particles easily stick to the skin of the froglets. A shallow water dish must be provided (water depth 10 mm). It is essential that the water dish is easy for the metamorphs to climb out of (a rock or small branch in the water dish facilitates access). When transferring froglets from the tadpole tank make sure that they are placed in the water dish and not on the substrate as they may not be quite ready to leave the water, they will choose when they are ready. Cover objects are extremely important microhabitats for small frogs. Bark hides and leaf litter should be added to the enclosure. The furnishings in the enclosure should not be too complex or it will be difficult for the young frogs to find food. The metamorphs can be raised at the same room temperature as the adults (23 - 27˚C), and must be misted with room temperature tap water twice daily. It is vital that metamorphs and juveniles are provided with UV at this critical growing stage (Arcadia D3+

reptile lamp T5 12% UVB), this must be given in a gradient with a maximum UVI of 2 (Fig. 9).

Do not provide metamorphs with a 35°C t basking spot as this will dry them out rapidly and will likely kill them. A lower temperature basking spot can be provided with incandescent lamps positioned at one end of the enclosure. The temperature on the branching below the basking spot should not exceed 28°C. Small tanks will rapidly overheat so ensure that there is still a cool area in the enclosure. Metamorphs should be fed daily until about six weeks of age with pin head crickets and (occasionally) Drosophila. All food items should be dusted with appropriate dietary supplements. After 8 weeks the feeding interval can be gradually increased to once every 3 days.

Health

Problems Encountered in Captivity

Non applicable.

Record keeping

Things to include.

Individual identification, weight, feeding records, temperature records, UV records, water quality test results, health records, behavioural or reproductive observations.

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6. Ex-situ Managemnt of Common tree frogs Polypedates species

Figure 1. Top *Polypedates leucomystax* (Benjamin Tapley). Bottom *Polypedates maculatus* (Benjamin Tapley)

Introduction

Description

Common tree frogs are slender rhacophorid tree frogs with a pointed snouts and prominent eyes (Fig.1). P. leucomystax is polymorphic, colouration and patterning are highly variable, even amongst wild specimens collected in the same area (Church, 1963). The end of each toe terminates in a large disc, enabling this species to climb well. Females of P. *leucomstax* may grow to 80 mm snout vent length (SVL) and males to 60 mm SVL. Males of *P. maculatus* can measure 34-57mm SVL and females up to 89mm SVL (Amphibia web, Daniels, 2005)

Distribution

Polypedates leucomystax is a widely distributed species, occurring in northeast India, Bangladesh, Nepal, western Yunnan (China) and most of mainland southeast Asia. The species is also found throughout much of Indonesia and the Philippines. It has been introduced to Japan and Papua New Guinea. It can be found from sea level up to 1, 500 m asl (IUCN et al., 2006).

Polypedates maculatus is restricted to south Asia including most of peninsular India, Sri Lanka, Nepal, Bhutan and Bangladesh (IUCN et al., 2006).

Conservation Status and Threats

Both species are listed as Least Concern by the IUCN Red List due to its extensive distribution and its tolerance of a wide range of habitat types(IUCN et al., 2006). It should be noted, however, that *P. leucomystax* represents a complex of cryptic species, and taxonomic revision of the complex is required (IUCN et al., 2006).

Habitat and Ecology

Polypedates are very adaptable and occurs in a wide variety of habitats, including beach vegetation, various human habitats, natural edge habitats, and closed subtropical or tropical forest (IUCN et al., 2006).

Sexing Individuals

Polypedates are sexually dimorphic with females reaching maximum SVLs up to 50% larger than males. Reproductively active males have nuptial pads at the base of the first finger.

Reproduction and Larval Development

In drier parts of its range, *P. leucomystax*, breeds during the wet season. In wetter areas of its range the species reportedly breeds year round. *Polypedates maculatus* breeds during the monsoon rains (Amphibiaweb)
Males congregate around slow moving or still water bodies. They sit in elevated positions and call to females to attract

them. The call is a nasal quack. Females of *P. leucomystax* respond to the males vocalisations by tapping their toes, the males then move closer to the females (Narins, 1995).

Amplexus and Nest Production

The breeding biology of *P. leucomystax* is well documented. Amplexus (mating position) is axillary in these species. Once in amplexus the female deposits up to 500 eggs (Average egg diameter = 1.85 mm) on a surface overhanging water (Duellman & Trueb, 1994). The male fertilises the eggs and both sexes secrete mucus. The male uses his hind legs to whisk this up into a foam. This process can take 12 hours. The resulting ball of foam measures approximately 100mm across (Yorke, 1983). Mass nesting has been observed in the wild, the resulting egg mass measured a metre across (Gaulke & Cadiz, 2002). The surface of the nest hardens after a couple of hours (Staniszewski, 1995). In captivity females can produce more than one nest per breeding season (pers. obs.).

The breeding biology of *P. maculatus* is not been studied in detail but the reproductive strategy is identical to *P. leucomystax*.

Longevity and Age at Sexual Maturity

Lifespan and age at sexual maturity in the wild are unknown. The maximum lifespan in captivity is also unknown; to date captive P. leucomystax have been recorded to live 6 to 8 years. Sexual maturity can be reached at one of age in captivity.

Captive Management

Introduction

P. leucomystax is commonly kept by zoos and private individuals, and has been bred in captivity for decades. *Polypedates maculatus* is not established in captivity.

Identifying Individuals

Adult specimens of both species are not individually identifiable as specimens do not possess characteristic markings. Adults could be marked with passive integrated transponders (PIT tags). Pit tags should be inserted subcutaneously on the dorsum. Juveniles could be marked with different coloured visible implant elastomer (VIE) but this may not be necessary for rearing purposes (Fig. 2).

Figure 2. *P. leucomystax* metamorph marked with VIE (Benjamin Tapley)

Housing adults

At Durrell *P. leucomystax* was housed and bred in two different types of enclosure. The first enclosure was a 500 x 500 x 900 mm glass vivarium with a secure mesh lid. A partition divided the floor of the vivarium into a water area (70 mm deep) and a land area. Coarse gravel was used as the substrate for the land area. This would be the minimum enclosure size for 2 males and 2 females. The second enclosure was a converted plastic storage unit. It measured 600 x 800 x 600 mm, and had a wire mesh lid. A 250 x 150 x 100 mm water dish was provided, this would be suitable for 6 adult individuals. No substrate was provided in this enclosure (Fig. 3).

Branching is essential when keeping arboreal tree frogs. Multiple bamboo lengths (at least 30 mm diameter) are ideal. The ends of the bamboo used for branching should be sealed to prevent live food hiding. 150 mm lengths of PVC pipe or hollowed bamboo (70 mm diameter) can be attached to the bamboo branching to provide hiding places for the frogs. Branches should be positioned at different angles within the enclosure with some under the basking spot and UV light and others facing away, this will allow the frogs to regulate their body temperature, rate of water loss through their permeable skin and UV exposure. Live plants can be added as they create humid pockets which the animals may use as perching sites, the plants also provide visual barriers for the frogs.

Lids should be constructed of mesh as glass will block UVB.

Substrate can be provided for this species although it is not essential. The provision of substrate will prevent the enclosure from drying out. Be sure to incorporate a drain in the tank construction. A layer of gravel covered with a permeable matting should be laid underneath a substrate of soil mixed with sand (ratio 2:1). The depth of substrate will depend on enclosure size. The larger the enclosure, the deeper the substrate should be as the substrate will dry out quickly in large enclosures. Substrate should be compacted to prevent soil sticking to the skin of the frog. Ensure that substrates are not contaminated with fertilisers or other substances that may be toxic to amphibians.

Ensure the substrate has a humidity gradient by pouting some water (or spraying with water) more at the end of the enclosure with no basking light.

Temperature, Humidity and Lighting

Husbandry should be informed by data collected from the field. Basking spot temperatures, UV exposure and gradients, temperature and humidity regimes should mimic the environmental conditions at the collection site. Note that environmental data should be collected at different times throughout the day and year. At Durrell *P. leucomystax* were maintained in a room with ambient temperatures which varied between 23 and 27˚C (night/day summer) and 20 and 25˚C (night/day winter).

A UV gradient should be provided in conjunction with a basking spot. Arcadia D3+ reptile lamp T5 12% UVB are ideal and a maximum UVI of 3 should be provided for the frogs at their basking spots. Remember that mesh will filter out some of the UVB so UV readings must be taken from below the mesh with a solarmeter 6.5.

Basking spots can be provided with regular incandescent lamps. The basking lamp should be positioned at one end of the enclosure and the temperature on the branching below the basking spot should range between 30-35°C and not exceed 35°C. Ensure that all lighting is inaccessible to the frogs as they will be burnt easily if they come into contact with lamps.

Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, pH and phosphates. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. A plastic water dish should be provided and should measure 200 x 150 mm. Water depth should be 80 mm, and frogs should be able to immerse their drink patch. An emergent rock should be placed in the water to allow the frogs easy access out of the water.

The enclosure should be lightly misted with aged tap water daily.

Routine Husbandry

All animals should be visually inspected daily. Water dishes must be cleaned out and refilled daily as amphibians often excrete waste products in water. Water quality cannot be judged by eye. Faeces and uneaten food must be removed daily. The enclosure and all furniture must be thoroughly scrubbed (with a brush and hot water, no chemical cleaners or disinfectants) twice a week.

Temperatures of the enclosure should be checked at different points of the enclosure regularly to ensure that the correct thermoregulatory gradients are maintained. UV readings must be taken every three months to check that there is a sufficient UV gradient and that the UV emitting lights are still emitting UV.

The substrate must be changed as soon as it looks dirty or if it becomes saturated or unable to retain humidity.

Figure 3. One set up for housing and breeding P. leucomystax (Benjamin Tapley)

Diet in captivity

At Durrell *P. leucomystax* were fed on live invertebrates, predominantly crickets, cockroaches and locusts. Juvenile animals were fed on live pin head crickets and *Drosophila hydei*. All food items were dusted with Nutrobal® (vitamin and mineral supplement) immediately prior to being fed out. Food should be well fed and an appropriate size, no greater in size than the width of the frogs head. *Polypedates* are nocturnal and should be fed in the evening.

Adults should be fed every three to six days (depending on season and condition), juveniles up to six weeks of age should be fed daily.

Reproduction in Captivity

Breeding Enclosure

Increasing the humidity can trigger breeding in Polypedates. Increasing the humidity can be achieved by increasing the amount that the frogs are sprayed on daily basis. Ideally these frogs should be cycled in a rain chamber with a sprinkler system (Fig. 4).

A rain chamber measuring 500 x 500 x 900 mm would be suitable as a minimum size for 2 males and 2 females. It is important that the sex ratio in the breeding enclosure is not biased towards males as this can be stressful for the females. No substrate is required in the rain chamber A pump powered spray system would be ideal for breeding this species but a mature biological filter should be incorporated into the rain chamber to maintain water quality.

Figure 4. A rain chamber suitable for breeding Polyedates (Andrew Tillson-Willis)

Ideally a sump could be used (Appendix 1). The branching, lighting and temperature parameters should be maintained as above.

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 as they will pollute the water.

In closed systems regular water tests should be carried out (Temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration process.

Breeding Seasonality

In captivity *P. leucomystax* breed during the warmer months. At Durrell this was from April through to October. *Polypedates maculatus* have not been bred in captivity.

Provision of Breeding Sites

Adults need branching over-hanging water on which to make their foam nest. They may also nest in leaves or attach their nests to the side of the enclosure or water dish.

Care of Foam Nest

It may take several weeks in the rain chamber for breeding to occur. Once hardened, the nest can be removed from the enclosure to facilitate collection of tadpoles. Nests can be gently removed from the deposition site and stuck (the underside of the nest remains sticky) to the side of a large plastic container. This container must then raised at a slight angle and the bottom of the container filled with water. This created a deeper area of water (approximately 15 mm) at the end of the container furthest from the nest. It is essential that there is water directly below the nest for tadpoles to drop down into (Fig. 5). The foam nest must be lightly sprayed with tap water daily.

Figure 5. Foam nest of P. leucomystax. (Benjamin Tapley)

Tadpole Husbandry and Development

After hatching, tadpoles can be transferred to a glass tank with a spoon (Fig. 6), they are extremely delicate at this stage so they should be moved with utmost caution. When they first hatch they will not move and it is perfectly normal for them to sometimes rest on their side. If you look on the underside of tadpoles when they first hatch you should be able to see the yolk sac. The tadpole will not start swimming or feeding until this ac has been absorbed. The size of the tank for tadpoles is not important, the stocking density is. Tanks should be stocked with no more than 5 tadpoles per litre. Filtration is vital (see appendix one to inform your choice of filter). Aged tap water can be used to rear tadpoles. Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, hardness, pH and phosphates. Water parameters should be informed by field data but remember that not all breeding sites may have optimal water quality, especially in urbanised environments where these frogs can often be found. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. Check the water in closed systems and carry out regular water tests (temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration and tadpoles. Ideally a sump should be used to increase the volume of water of your system and to house all the life support systems (filters etc). If using an

Figure 6. Polypedats maculatus tadpole (Sandeep Varma)

internal canister filter ensure that it is not too strong as the tadpoles could be sucked into the inflow of the filter and may have difficulty feeding. Direct the outflow to the surface of the water for oxygenation.

Substrate is not necessary but tadpoles like to hide. Dried Indian almond leaves *(Terminalia catappa)* can be added, they also soften the water which would be beneficial in hard water areas. Partial (10-20 %) water changes should be carried out regularly, the frequency of water changes must be governed by frequent water testing. *Polypedates leucomystax* tadpoles metamorphosed after approximately 60 - 90 days at 23 – 26°C.

Tadpoles should be fed daily once they start swimming freely. They should be fed on a variety of food. At Durrell tadpoles were successfully reared on a powdered tadpole food (components: ground tropical fish flake, grass pellet, trout pellets, tubifex, river shrimp, spirulina algae and cuttlefish bone). Food should be available to the tadpoles at all times but it is important that large amounts of uneaten food do not pollute the aquarium. Tadpole feeding regimes must be modified, as the tadpoles grow they will eat more.

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. Emerging rocks or aquatic plants are suitable. Once they emerge they are prone to drying out and easily die if the humidity is not high.

Rearing Metamorphs

Metamorphs can be housed in small plastic containers. A 350 x 200 x 300 mm enclosure is suitable for up to 12 individuals, these groups should be divided amongst larger enclosures as individuals grow. It is important that rearing tanks are relatively small so that high ambient humidity can me maintained and that the juvenile frogs can find food easily. Covering the lid with a fine breathable cloth or fleece will prevent the food escaping and ensure that humidity remains high. The cloth will block dome of the UV emitted from the lights so you must experiment and ensure that the UV light and temperature gradients are still suitable. Damp soil mixed with sand would make a good substrate, and this must be replaced as necessary. A shallow water dish must be provided (water depth 10 mm). It is essential that the water dish is easy for the metamorphs to climb out of (a rock or small branch in the water dish facilitates access). When transferring froglets from the tadpole tank make sure that they are placed in the water dish and not on the substrate as they may not be quite ready to leave the water, they will choose when they are ready. Live plants should be included in the juvenile enclosures. The metamorphs can be raised at the same room temperature as the adults (23 - 27°C), and must be misted with room temperature tap water twice daily. It is vital that metamorphs and juveniles are provided with UV at this critical growing stage (Arcadia D3+

reptile lamp T5 12% UVB), this must be given in a gradient with a maximum UVI of 2. Do not provide metamorphs with a 35°C t basking spot as this will dry them out rapidly and will likely kill them. A lower temperature basking spot can be provided with incandescent lamps positioned at one end of the enclosure. The temperature on the branching below the basking spot should not exceed 28°C. Small tanks will rapidly overheat so ensure that there is still a cool area in the enclosure. Metamorphs should be fed daily until about six weeks of age with pin head crickets and (occasionally) Drosophila. All food items should be dusted with appropriate dietary supplements. After 8 weeks the feeding interval can be gradually increased to once every 3 days.

Health

Problems Encountered in

Captivity

Figure 7. Rostral abrasion in a female P. leucomystax (Benjamin Tapley)

This species appears susceptible to rostral abrasions (Fig. 7). The provision of adequate space is important in minimising / avoiding the occurrence of rostral lesions.

Routine Veterinary Procedures At Durrell Wildlife Conservation Trust

Specimens have been given intra-muscular injections of antibiotics (e.g. Baytril, 10 mg/kg).

All imported specimens were treated against chytridiomycosis by bathing them in Itroconoazole solution (1:99) for five minutes daily for 11 days. There was zero mortality for specimens undergoing this treatment.

Specimens were treated for parasites using Levamisole. Specimens were bathed in baths (60mg/kg) for 30 minutes once a week for three weeks. No mortality was observed for specimens undergoing this treatment.

Record keeping

Things to include.

Individual identification, weight, feeding records, temperature records, UV records, water quality test results, health records, behavioural or reproductive observations.

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7. Ex-situ Management of Gliding frog Rhacophorus species

Figure 1. Top *Rhacophorus malabaricus* (Benjamin Tapley).

Introduction

Description

Gliding frogs are rhacophorid tree frogs with pointed snouts, prominent eyes and extensive webbing between the fingers and toes (Fig.1). These frogs are leaf green in colour. *Rhacophorus malabaricus* is green in colouration with yellow / orange webbing on the feet (Danels, 2005). *Rhacophorus maximus* is the largest tree frog in India and can be distuinguished from other Rhacophorid frogs in the region by thr brown reticulations on the flanks and webbing and a white stripe on the lower jaw (Ahmed et al., 2009). The end of each to in *Rhacophorus* species terminate in a large disc, enabling these frogs to climb well. Females of *R. malabaricus* may grow to 92 mm snout vent length (SVL) (Pers. Obs). *Rhacophorus maximus* may attain a SVL of 93mm ((Ahmed et al., 2009)

Distribution

Rhacophorus malabaricus is restricted to the Western Ghats of India, it has an altitudinal range of 300 – 1,200 masl. (IUCN et al., 2006)

Rhacophorus maximus is known from eastern Nepal, northern Bangladesh, North East India, northern Myanmar, western Thailand and southern China and has been recorded from 500-2,000m asl. (IUCN et al., 2006).

Conservation Status and Threats

Both species are listed as Least Concern by the IUCN Red List due to its extensive distribution and its tolerance of a wide range of habitat types(IUCN et al., 2006)..

Habitat and Ecology

Rahcophorus malabaricus inhabits of tropical moist evergreen forest, deciduous forest, secondary forest and coffee plantations. *Rhacophorus maximus* inhabits lowland to sub montane moist evergreen forest (IUCN et al., 2006).

Sexing Individuals

Rhacophorus are sexually dimorphic, females being much larger than males.

Reproduction and Larval Development

Rhacophorus malabaricus breed after the onset of the monsoon in June and July (Daniels, 2005). Eggs hatch after 4-5 days and larval development is report to take 68 days (Daniels, 2005). *Rhacophorus maximus* breed from February to April in Meghalya (Ahmed et al., 2009).

Males congregate around slow moving or still water bodies. They sit in elevated positions and call to females to attract them.

Amplexus and Nest Production

See fig. 2. The breeding biology of *R. malabaricus* is well documented (Kadadevaru & Kanamadi, 2000). Amplexus (mating position) is axillary in this species. Once in amplexus the female deposits up to 192 eggs on a surface overhanging or next to water (Kadadevaru & Kanamadi, 2000). The male fertilises the eggs (average diameter 2.62 mm) and both sexes secrete mucus. The male uses his hind legs to whisk this up into a foam which is wrapped in leaves by the hind limbs of the frogs (Kadadevaru & Kanamadi, 2000).

Figure 2. Foam nest of *R.. malabaricus* (Benjamin Tapley).

The breeding biology of *R. maximusis* not been studied in the field detail but the reproductive strategy is identical to *R. malabaricus*.

Longevity and Age at Sexual Maturity

Lifespan and age at sexual maturity in the wild are unknown.

Captive Management

Introduction

Rahcophorus maximus is kept by zoos and private individuals, and has been bred in captivity. *Rahcophorus malabaricus* is not established in captivity.

Identifying Individuals

Adult specimens of both species are not individually identifiable as specimens do not possess characteristic markings. Adults could be marked with passive integrated transponders (PIT tags). Pit tags should be inserted subcutaneously on the dorsum. Juveniles could be marked with different coloured visible implant elastomer (VIE) but this may not be necessary for rearing purposes

Housing adults

Minimum enclosure size for 5 individuals (2 males 5 females) 800 x 800 x 1500 mm, a mesh lid must be provided. Due to the jumping nature of these frogs, front opening enclosures are recommended. A 250 x 150 x 100 mm water dish must be provided (Fig 3.).

Branching is essential when keeping arboreal tree frogs. Multiple bamboo lengths (at least 30 mm diameter) are ideal. The ends of the bamboo used for branching should be sealed to prevent live food hiding. 150 mm lengths of PVC pipe or hollowed bamboo (70 mm diameter) can be attached to the bamboo branching to provide hiding places for the frogs. Branches should be positioned at different angles within the enclosure with some under the basking spot and UV light and others facing away, this will allow the frogs to regulate their body temperature, rate of water loss through their permeable skin and UV exposure. Live plants can be added as they create humid pockets which the animals may use as perching sites, the plants also provide visual barriers for the frogs.

Lids should be constructed of mesh as glass will block UVB.

Substrate can be provided for this species although it is not essential. The provision of substrate will prevent the enclosure from drying out. Be sure to incorporate a drain in the tank construction. A layer of gravel covered with a permeable matting should be laid underneath a substrate of soil mixed with sand (ratio 2:1). The depth of substrate will depend on enclosure size. The larger the enclosure, the deeper the substrate should be as the substrate will dry out quickly in large enclosures. Substrate should be compacted to prevent soil sticking to the skin of the frog. Ensure that substrates are not contaminated with fertilisers or other substances that may be toxic to amphibians.

Ensure the substrate has a humidity gradient by pouting some water (or spraying with water) more at the end of the enclosure with no basking light.

Temperature, Humidity and Lighting

Husbandry should be informed by data collected from the field. Basking spot temperatures, UV exposure and gradients, temperature and humidity regimes should mimic the environmental conditions at the collection site. Note that environmental data should be collected at different times throughout the day and year especially for these species which may vary in the preference from site to site due to the range of altitudes in which they are known to occur.

As a guideline for *R. malabaricus* should be housed in a room with ambient temperatures which vary between 23 and 27°C (night/day summer) and 20 and 25°C (night/day winter). *R. maximus* should be kept cooler 18 and 24°C (night/day summer) and 12 and 16°C (night/day winter). A UV gradient should be provided in conjunction with a basking spot. Arcadia D3+ reptile lamp T5 12% UVB are ideal and a maximum UVI of 3 should be provided for the frogs at their basking spots. Remember that mesh will filter out some of the UVB so UV readings must be taken from below the mesh with a solarmeter 6.5. UV is essential for *R. malabaricus* in particular as this species has been observed sitting out during the day in direct sun (Fig 4.).

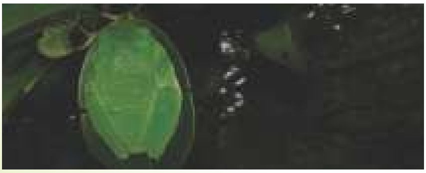

Figure 3. Diurnal resting site of *Rhacphorus malabaricus* (Benjamin Tapley)

Basking spots can be provided with regular incandescent lamps. The basking lamp should be positioned at one end of the enclosure and the temperature on the branching below the basking spot should range between 30-32°C and not exceed 32°C. Ensure that all lighting is inaccessible to the frogs as they will be burnt easily if they come into contact with lamps. During the winter the basking spot for R. maximus should not exceed 25°C.

Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, pH and phosphates. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. A plastic water dish should be provided and should measure 200 x 150 mm. Water depth should be 80 mm, and frogs should be able to immerse their drink patch. An emergent rock should be placed in the water to allow the frogs easy access out of the water.

The enclosure should be lightly misted with aged tap water daily.

Routine Husbandry

All animals should be visually inspected daily. Water dishes must be cleaned out and refilled daily as amphibians often excrete waste products in water. Water quality cannot be judged by eye. Faeces and uneaten food must be removed daily. The enclosure and all furniture must be thoroughly scrubbed (with a brush and hot water, no chemical cleaners or disinfectants) twice a week. Temperatures of the enclosure should be checked at different points of the enclosure regularly to ensure that the correct thermoregulatory gradients are maintained. UV readings must be taken every three months to check that there is a sufficient UV gradient and that the UV emitting lights are still emitting UV.

The substrate must be changed as soon as it looks dirty or if it becomes saturated or unable to retain humidity.

Figure 4. A basic set up for housing Rahcophorus (Benjamin Tapley)

Diet in captivity

Rhacophorus probably feed on large invertebrates and potentially small vertebrates. The feeding habits of *R. malabaricus* and *R. maximus* have not been studied in the field.

Adults should be fed every three to six days (depending on season and condition), juveniles up to six weeks of age should be fed daily.

Food should be offered after it is dusted with an appropriate dietary supplement, well fed and an appropriate size, no greater in size than the width of the frogs head. *Rhacophorus* are nocturnal and should be fed in the evening.

Reproduction in Captivity

Breeding Enclosure

Increasing the humidity can trigger breeding in *Rhacophorus*. These frogs should be cycled for breeding in a rain chamber with a sprinkler system (Fig. 5). A rain chamber measuring 800 x 800 x 1500 mm would be suitable as a minimum size for 2 males and 3 females. It is important that the sex ratio in the breeding enclosure is not biased towards males as this can be stressful for the females. No substrate is required in the rain chamber A pump powered spray system would be ideal for breeding this species but a mature biological filter should be incorporated into the rain chamber to maintain water quality. Ideally a sump could be used (Appendix 1). The branching, lighting and temperature parameters should be maintained as above.

Figure 5. A rain chamber suitable for breeding Rhacophorus (Andrew Tillson-Willis)

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 as they will pollute the water.

In closed systems regular water tests

should be carried out (Temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration process.

Breeding Seasonality

Rhacophorus malabaricus breed at the onset of the Monsson in June and July (Daniels, 2005). In Megahalya *R. maximus* breed from February to April (Ahmed et al., 2009).

Provision of Breeding Sites

Adults need broad laves over-hanging water on which to make their foam nest. They may also nest in leaves or attach their nests to the side of the enclosure or water dish.

Care of Foam Nest

It may take several weeks in the rain chamber for breeding to occur. Once hardened, the nest can be removed from the enclosure to facilitate collection of tadpoles. Nests can be gently removed from the deposition site and stuck (the underside of the nest remains sticky) to the side of a large plastic container. This container must then raised at a slight angle and the bottom of the container filled with water. This created a deeper area of water (approximately 15 mm) at the end of the container furthest from the nest. It is essential that there is water directly below the nest for tadpoles to drop down into (Fig. 6). The foam nest must be lightly sprayed with tap water daily.

Figure 6. Foam nest of *P. leucomystax*. (Benjamin Tapley)

Tadpole Husbandry and Development

After hatching, tadpoles can be transferred to a glass tank with a spoon. Tadpoles are extremely delicate at this stage so they should be moved with utmost caution. When they first hatch they will not move and it is perfectly normal for them to sometimes rest on their side. If you look on the underside of tadpoles when they first hatch you should be able to see the yolk sac. The tadpole will not start swimming or feeding until this ac has been absorbed. The size of the tank for tadpoles is not important, the stocking density is. Tanks should be stocked with no more than 5 tadpoles per litre. Filtration is vital (see appendix one to inform your choice of filter). Aged tap water can be used to rear tadpoles. Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, hardness, pH and phosphates. Water parameters should be informed by field data but remember that not all breeding sites may have optimal water quality, especially in urbanised environments where these frogs can often be found. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. Check the water in closed systems and carry out regular water tests (temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm and nitrate less than 10ppm. No more than 20% of the water should be changed as rapid shifts in water quality will be problematic for the biological filtration and tadpoles. Ideally a sump should be used to increase the volume of water of your system and to house all the life support systems (filters etc). If using an internal canister filter ensure that it is not too strong as the tadpoles could be sucked into the inflow of the filter and may have difficulty feeding. Direct the outflow to the surface of the water for oxygenation.

Substrate is not necessary but tadpoles like to hide. Dried Indian almond leaves (Terminalia catappa) can be added, they also soften the water which would be beneficial in hard water areas. Partial (10-20 %) water changes should be carried out regularly, the frequency of water changes must be governed by frequent water testing.

Tadpoles should be fed daily once they start swimming freely. They should be fed on a variety of food. At Durrell rhacophorid tadpoles were successfully reared on a powdered tadpole food (components: ground tropical fish flake, grass pellet, trout pellets, tubifex, river shrimp, spirulina algae and cuttlefish bone). Food should be available to the tadpoles at all times but it is important that large amounts of uneaten food do not pollute the aquarium. Tadpole feeding regimes must be modified, as the tadpoles grow they will eat more.

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. Emerging rocks or aquatic plants are suitable. Once they emerge they are prone to drying out and easily die if the humidity is not high.

Rearing Metamorphs

Metamorphs can be housed in small plastic containers. A 350 x 200 x 300 mm enclosure is suitable for up to 12 individuals, these groups should be divided amongst larger enclosures as individuals grow. It is important that rearing tanks are relatively small so that high ambient humidity can me maintained and that the juvenile frogs can find food easily. Covering the lid with a fine breathable cloth or fleece will prevent the food escaping and ensure that humidity remains high. The cloth will block dome of the UV emitted from the lights so you must experiment and ensure that the UV light and temperature gradients are still suitable. Damp soil mixed with sand would make a good substrate, and this must be replaced as necessary. A shallow water dish must be provided (water depth 10 mm). It is essential that the water dish is easy for the metamorphs to climb out of (a rock or small branch in the water dish facilitates access). When transferring froglets from the tadpole tank make sure that they are placed in the water dish and not on the substrate as they may not be quite ready to leave the water, they will choose when they are ready. Live plants should be included in the juvenile enclosures. The metamorphs can be raised at the same room temperature as the adults (23 - 27˚C), and must be misted with room temperature tap water twice daily. It is vital that metamorphs and juveniles are provided with UV at this critical growing stage (Arcadia D3+ reptile lamp T5 12% UVB), this must be given in a gradient with a maximum UVI of 2. Do not provide metamorphs with a 35°C t basking spot as this will dry them out rapidly and will likely kill them. A lower temperature basking spot can be provided with incandescent lamps positioned at one end of the enclosure. The temperature on the branching below the basking spot should not exceed 28°C. Small tanks will rapidly overheat so ensure that there is still a cool area in the enclosure. Metamorphs should be fed daily until about six weeks of age with pin head crickets and (occasionally) Drosophila. All food items should be dusted with appropriate dietary supplements. After 8 weeks the feeding interval can be gradually increased to once every 3 days.

Health

Problems Encountered in Captivity

Being a large gliding frog there is potential for rostral abrasions due to collision with the enclosure sides and or furnishings.

Record keeping

Things to include.
Individual identification, weight, feeding records, temperature records, UV records, water quality test results, health records, behavioural or reproductive observations.

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8. Ex-situ Management of Salamander *Tylototriton verrucosus*

Figure 1. Top *Tylototriton verrocosus* (Shruti Sengupta).

Introduction

Description

It is amedium size salamander with SVL ranging in male 87.70 - 63.10 mm, and 97.00 - 66.00 mm in females, bony ridges are present on head. Head is usually broad and flat, snout is blunt, moderate size eye with granular upper eyelid. The two lines of dorsolateral bony ridges on head are distinct and are widely separated. The flanks have roughly 16 pairs of longitudinal lines of knob-like dorsal warts that are distinct. Skin ventrally and tail finely granular. Tail is compressed laterally, with a well-developed fin fold. It is light brown colour with darker warts in life. This species can produce loud vocalization which has not been recorded so far in other Tylototriton species

Distribution

Anders et al (1998) reported the distribution of the new species in five places in the Illam district Nepal with elevation ranging from 1100 m to 2120m. The distribution of the species is currently known only from the Illam district of Nepal and in Darjeeling district, India (Nag and Vasudevan 2014). The salamanders are more terrestrial in non-breeding seasons (from October to February) and found hiding under the logs, bushes and stones and come to the breeding ponds in early March or April soon after heavy monsoonal showers (also see Schleich and Kästle, 2002).

Conservation Status and Threats

It has not been evaluated so far and information on their population trend assessment is scanty. In India, there is only one study which concludes that despite the high relative abundance of salamander in the area the habitats are disappearing at a rapid rate and recommended raising the IUCN status of the species to "Critically Endangered" because of a reduction in the number of populations higher than 40% in 4 years (Seglie, 2002). According to local people, sharing the same habitat for their day to day activities, the population of T. verrucosus is probably declining, but there is no data that could support this notion at present (Vasudevan et al. 2014).

Habitat and Ecology

The habitats are characterized as the 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*. It occurs in Darjeeling hills from 1350-2000 m asl in streams and ponds that are surrounded by forests, tea plantations and villages (Deuti and Hegde 2007). 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).

Figure 2. Breeding habitat of *T. verrucosus* (Shruti Sengupta).

Sexing Individuals

In body length, tail length and weight females were larger than males in males. There was no distinct colour variation between male and female. The dorsal colouration varied from dark brown to light brown. Tail height is larger in females than in males. The vent length is longer in male compared to female.

Reproduction and Larval Development

Females attain sexual maturity by the age of 3-4 years (Kuzmin et al. 1994). During the breeding season that lasts up to end of monsoon; male salamanders become brighter in colour, while in females the cloaca becomes orange-colored. Clutch sizes vary from 26 to 60 and egg size varies 6 to 10 mm in diameter. The life cycle is completed within one season, except in the case of over wintering larvae (Nag and Vasudevan 2014). The larvae have well developed balancers. The various stages of the life cycle includes egg, early larvae, free swimming larvae, advanced larvae, juvenile newt stage and adult.

Amplexus and Nest Production

Territorial and breeding behaviour include body elevation, lowering of the head, circling, and even biting (Deuti and Hegde, 2007). Courtship and amplexus mostly occurs at night. Amplexus is aquatic and involves the male wrapping the forelimbs around the females from below prior to spermatophore deposition and transfer (see Deuti and Hegde, 2007).

Longevity and Age at Sexual Maturity

Lifespan and age at sexual maturity in the wild are unknown.

Captive Management

Introduction

As a pre-requisite to a successful and long-term conservation breeding program of T. verrucosus, is the design of the existing facility at The Padmaja Naidu Himalayan Zoological Park (PNHZP), Darjeeling. The inputs from field data on microhabitats used by the species in the breeding pools and proximate environmental cue required for initiation of breeding should be taken into consideration in developing the design. Emphasis should be made on housing animals on exhibit and off exhibit in the design. Facilities for water treatment maintenance flow of water and humidity levels in the enclosures need to be incorporated in the design.

Identifying Individuals

Adult specimens of both species are not individually identifiable as specimens do not possess characteristic markings. Adults could be marked with passive integrated transponders (PIT tags). Pit tags should be inserted subcutaneously on the dorsum. Juveniles could be marked with different coloured visible implant elastomer (VIE) but this may not be necessary for rearing purposes

Housing adults

PNHZP has been breeding the salamander since 2000, and in 2008, recommendations were made for a conservation breeding programme with PNHZP as the coordinating zoo and Himalayan Zoological Park, and Sikkim and Manipur Zoo as participating zoos (Vasudevan et al., 2014). In order to make the conservation breeding programmes more efficient and sustainable, a detailed analysis of the requirements for such a facility must be created based on geographical data and environmental indicators. Captive breeding will be carried out based on the strategy described by Ziegler and colleagues (2008) for the closest relative species *T. shanjing*. Chosen individuals from genetic analysis will be housed in terrariums measuring 134 cm x 60 cm x 75 cm (length x breadth x height). About one half of the bottom surface will be filled with water upto a height of 20 cm with an 8 cm gravel layer for higher ground. A sheet of glass will be introduced to divide the terrestrial and aquatic portions. The stratum of the terrestrial section will be formed using moss and tree barks. Small, smooth rocks will be used to create a gradient from the water to shore. Some ferns and marsh grasses will be introduced as aquatic vegetation. Lighting is provided by means of neon tubes and small spotlights. Water and air temperatures will be maintained at 18-26°C and 20-25°C respectively. A water filtering and flow arrangement will be installed to ensure clean water supply to the terrarium at all times and pH of the water will be maintained at 8. The breeding season for the Himalayan newt is in the start of monsoon around late April to early May. Slight reduction in temperature with spraying of water will be used to provide stimulus for promoting mating (Herrmann, 2001; Zeigler et al, 2007).

Figure 3. Breeding on-exhibit enclosure at PNZP, Darjeeling (K. Vasudevan)

Basking spots can be provided with regular incandescent lamps. The basking lamp should be positioned at one end of the enclosure and the temperature on the branching below the basking spot should range between 27-30°C and not exceed 30°C. Ensure that all lighting is inaccessible to the frogs as they will be burnt easily if they come into contact with lamps. During the winter the basking spot for *T. verrucosus* should not exceed 22°C.

Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, pH and phosphates. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. Water depth should be 80-120 mm, and salamanders should be able to immerse completely. An emergent rock, vegetation and small flat rocks should be placed in the water and in the water edge to allow the salamanders easy access out of the water. The enclosure should be lightly misted with aged tap water daily.

Routine Husbandry

All animals should be visually inspected daily. Water dishes must be cleaned out and refilled daily as amphibians often excrete waste products in water. Water quality cannot be judged by eye. Faeces and uneaten food must be removed daily. The enclosure and all furniture must be thoroughly scrubbed (with a brush and hot water, no chemical cleaners or disinfectants) twice a week. Temperatures of the enclosure should be checked at different points of the enclosure regularly to ensure that the correct thermoregulatory gradients are maintained. UV readings must be taken every three months to check that there is a sufficient UV gradient and that the UV emitting lights are still emitting UV. The substrate must be changed as soon as it looks dirty or if it becomes saturated or unable to retain humidity.

Diet in captivity

Adults have broad unspecialized diet and mostly feed on aquatic insects like Dytiscid beetles; Gastropod and Bivalve molluscs (Sphaerium indicum); Decapod crustaceans (land crabs-Potamon potamiscus sikkimense); Lumbricid and Megascolecid earthworms; larvae and pupae of Diptera and Coleoptera and numphs of Odonata, besides tadpoles of common amphibians and eggs from foam-nests of Rhacophorid frogs. Adults sometimes feed on the tadpoles of Bufo verrucosus and Polypedates teraiensis and also feed on snails, slugs, wood-lice and coprophilous insects. The larvae are bottom feeders and mostly feed on bacteria, protozoa, diatoms and zooplanktons. Late stage larvae feed on Chironomid larvae, tubificid worms, soil nematodes and mosquito larvae.

Breeding Seasonality

Salamanders are known to be active from late April to early October, and during this period adults emerge out of hibernation and move into temporary/permanent ponds for breeding.

Provision of Breeding Sites

Adults need leaves of emerging aquatic vegetation and terrestrial grasses and sedges over-hanging water on which to lay their eggs. They may also lay eggs among leaves above water in the pond. The abundance of the vegetation and moisture would avoid exposure and drying up of eggs.

Figure 4. Egg of T. verrucosus in-situ in Darjeeling (Shruti Sengupta)

Husbandry and Development of larva

After hatching, larvae can be transferred to a glass tank with a spoon with utmost caution. When they first hatch they will not move and it is perfectly normal for them to sometimes rest on their side. The larvae will not start swimming or feeding until this ac has been absorbed. The stocking density is important. Tanks should be stocked with no more than 5 larvae per litre. Aged tap water can be used to rear larva. Check the source of your water by testing it regularly for ammonia, nitrite, nitrate, hardness, pH and phosphates. Water parameters should be informed by field data but remember that not all breeding sites may have optimal water quality, especially in urbanised environments where these frogs can often be found. Tap water should be left to stand for 24 hours before it is used in order for the chlorine to dissipate. Ensure the temperature of the water is at equilibrium with the room temperature. Check the water in closed systems and carry out regular water tests (temperature, pH, ammonia, nitrite and nitrate), the results will help you decide the frequency of water changes. pH should not fluctuate, ammonia should measure less than 1 part per million (ppm), nitrite less than 0.1ppm 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. Ideally a sump should be used to increase the volume of water of your system and to house all the life support systems (filters etc). If using an internal canister filter ensure that it is not too strong as the larvae could be sucked into the inflow of the filter and may have difficulty feeding. Direct the outflow to the surface of the water for oxygenation.

Figure 5: Stage 4 and stage 5 larvae of T. verrucosus from Darjeeling.

Substrate is necessary for larva as they like to hide. Dried/autoclaved Indian almond leaves (Terminalia catappa) can be added, they also soften the water which would be beneficial in hard water areas. Partial (10-20 %) water changes should be carried out regularly, the frequency of water changes must be governed by frequent water testing.

Larvae should be fed daily once they start swimming freely. They should be fed on 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 high.

Rearing Metamorphs

Metamorphs can be housed in small plastic containers. A 350 x 200 x 300 mm enclosure is suitable for up to 12 individuals, these groups should be divided amongst larger enclosures as individuals grow. It is important that rearing tanks are relatively small so that high ambient humidity can me maintained and that the juvenile frogs can find food easily. Covering the lid with a fine breathable cloth or fleece will prevent the food escaping and ensure that humidity remains high. The cloth will block dome of the UV emitted from the lights so you must experiment and ensure that the UV light and temperature gradients are still suitable. Damp soil mixed with sand would make a good substrate, and this must be replaced as necessary. A shallow water dish must be provided (water depth 10 mm). It is essential that the water dish is easy for the metamorphs to climb out of. When transferring metamorphs from the larva tank make sure that they are placed in the water dish and not on the substrate as they may not be quite ready to leave the water, they will choose when they are ready. Live plants should be included in the juvenile enclosures. The metamorphs can be raised at the same room temperature as the adults (20 - 25˚C), and must be misted with room temperature tap water twice daily. It is vital that metamorphs and juveniles are provided with UV at this critical growing stage (Arcadia D3+ reptile lamp T5 12% UVB), this must be given in a gradient with a maximum UVI of 2. There is no need for basking spot for metamorphs as this will dry them out rapidly and will likely kill them. A lower temperature basking spot can be provided with incandescent lamps positioned at one end of the enclosure. The temperature on the branching below the basking spot should not exceed 25°C. Small tanks will rapidly overheat so ensure that there is still a cool area in the enclosure. Metamorphs should be fed daily until about six weeks of age with Chironomid larvae, tubificid worms, soil nematodes and mosquito larvae. All food items should be dusted with appropriate dietary supplements. After 8 weeks the feeding interval can be gradually increased to once every 3 days.

Figure 6. Metamorph of T. verrucosus in-situ in Darjeeling (Shruti Sengupta)

Health

Problems Encountered in Captivity

Being a semi aquatic salamander there is potential for limb injuries, skin abrasions due to objects within the enclosure. Periodic scanning of individuals for skin lesions should be taken up as a strain of fungus called Batrachochytrium salamandrivorans (Bs) is known to cause mortality.

Record keeping

Things to include.

Individual identification, weight, feeding records, temperature records, UV records, water quality test results, health records, behavioural or reproductive observations.

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9. Marking of Amphibians

To study large number of amphibians in captivity, 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 method 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.

Figure 1 Passive Integrated Transponder insertion in a frog (B.K. Gupta)

Passive Integrated Transponder Tags (PIT) Advantages:

- Unlimited number of codes
- Painful
- High cost

Figure 2 Reading the Passive Intergrated Transponder Number. (B.K. Gupta)

- Requires handling and tissue penetration
- Limited to use in larger frogs – Animals in lower allowable size class (40-50 mm snout vent length [SVL]) occasionally show Passive Integrated Transponder Tags (PIT)
- PIT tags should only be used for frogs >50mm SVL.

Visible Implant Fluorescent (VIE) Elastomer Tags

VIE tags are silicon-based components - mixed just prior to use A range of colours and injection sites combine to produce a large number of individual codes. This technique has been used on tadpoles, as well as adult frogs with some success.

Pattern Mapping

Advantages

- Low cost - unless software needs to be purchased

Figure 3 Tadpole marked with visble implant elastomer (DWCT)

Figure 4. Marking with Alcian blue dye solution using a "panjet" innoculator (DWCT)

- 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)

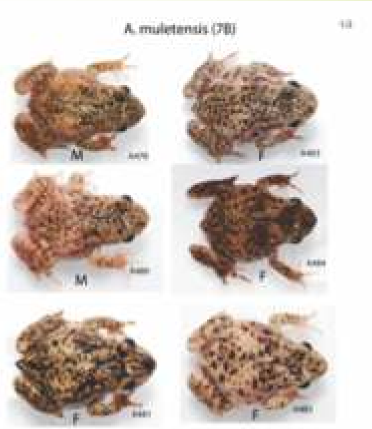

Figure 5. Pattern mapping in Amphibians (DWCT)

- Technology still being developed, i.e. pattern recognition software
- Limited by numbers – cumbersome with a large population
- Time consuming
- Potential temporal shifts in pattern. The application of pattern mapping is limited by the number of species that display unique individual markings.

10. Culturing food for Amphibians

Any captive bird, mammal, reptile or amphibian that would naturally include insects in their diet will benefit from a variety of good quality invertebrate foods.

Attention to this aspect of diet and nutrition can make the difference between captive animals simply surviving in adequate health or thriving and breeding successfully. However providing the food for a captive breeding effort may demand more physical time than the breeding animals themselves; insect culturing is intensive, dynamic and often time consuming.

As per the requirement of animals and suitability of its culutre, we may identify suitable species of invertebrates, with the following objectives:

- Reproducible with good cost/benefit ration
- Gregarious
- Easily identifiable
- Non toxic
- Various sizes for the species they are intended for
- Found naturally in large enough numbers to make gathering founders, a feasible job

Culturing Indian House Crickets:

Indian House Crickets can be cultured easily in zoos. For culturing them new colony may be established by starting with housing of few adult crickets.

Up to 3000 adult field crickets can be comfortably housed in a smooth sided container measuring approximately; 100cm (l) x 80cm (w) x 80cm (d)

Suitable sized nest boxes (20 x 20 x 10cm) must be supplied to enable eggs to be harvested and removed to a separate hatching tub. The nest boxes must be covered with fine metal mesh (0.5cm sq) as spent females will dig up and eat your eggs!

Figure 1 Indian House Crickets (Wikipedia)

The laying medium should be selected for humidity holding and may be compost, topsoil, sand, peat etc

Figure 2 Culture box with used egg trays (DWCT)

Crickets can be fed with fresh fruits (oranges, papaya) and vegetables (cabbage, greens) and a home made cricket cake-diet made of oat powder, skimmed milk powder and sugar.

To ensure that all individuals in the colony have had sufficient opportunity to feed in each 24 hour period, enough food needs to be offered to allow an excess on removal.

The amount of food offered should be

adjusted on a daily basis. Adding large amounts will lead to mould and wastage, as well as wasted time preparing the food, adding the food and removing the food!

Dried foods should comprise the bulk of the diet and do not need to be removed once added. Care must be taken to add just enough to prevent moulding.

Protein content is key, crickets need between 25-30% in order to be robust and productive breeders.

Fresh foods can be scraps, peelings, herbs, greens, forage or whole fruits and veg. Anything non-toxic that will provide moisture without going soggy or mouldy within 24 hours will do.

Basic choices are orange, potato, carrot, sweet potato or apple.

Experimenting for variety is a good idea but avoid soft fruits such as papaya, tomato or plum these will leave your colony with a big hygiene problem.

The most difficult aspect of culturing crickets is maintaining a high level of

hygiene and a standard procedure to run your colony "like clockwork"

Temperature, humidity, density, feeding and hygiene must all be maintained to a high standard.

Culturing Drosophila

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

Figure 3 Drosophila (DWCT)

Cultures of fruit flies are difficult to get consistent and must be carefully managed and adjusted to ensure success. The cultures can smell pretty bad as fermentation of the substrate is a necessary part of the process!

Figure 4 Fruit flies culture containers (DWCT)

The fruit mix is the most nutritious for the flies, providing the best variety of vitamins .

This substrate produces a fast life cycle in the flies, the culture is ready for feeding out 2weeks after it is made, but only normally lasts 7-10 days after the initial peak activity.

Figure 5 Mixture used for culturing the fruit flies (DWCT)

The recipe from Drosophila culture;

- 2 ripe bananas
- 1 ripe papaya
- 2 pears
- 2 apples
- 2 carrots

- multivitamin supplement
- brewer's yeast
- oats

The mix should not be so dry that fly larvae cannot move through it, or so wet that it falls out when you try to harvest flies... this is the hard part!

The mix should be evenly distributed between disposable pint cups, glasses, pots etc.

A piece of cardboard egg tray, wood shaving etc. is used as a platform for the breeding flies themselves.

Each jar should have just 15 *Drosophila* added to begin a new culture

Finally a double layered cloth lid is needed to prevent wild flies breeding with your flightless cultures.

Glass viewing amphibian exhibits with natural vegetation cover.
(Brij Kishor Gupta)

11. Enrichment of Amphibian Exhibits

Today modern zoological facilities strive to provide for the complex needs of animals and aim, as far as possible, to reproduce the natural environment and habitat which should meet the natural needs of the species in their care. However, those who care for wild animals in zoos understand that for many animals, life in a zoo, no matter how well managed, involves compromise. This does not mean that the welfare and care of animals in zoos are necessarily compromised, and does not minimize the ongoing efforts to meet the needs and domains of animal welfare for all species.

The high quality of care provided in a captive environment significantly reduces the time an animal must spend in these pursuits, but does not address the behavioural needs associated with these activities. It has become necessary to provide alternative methods of stimulating natural behaviour of the species housed therein to meet both the mental and physical needs of the captive animal. Enrichment has become essential in zoos to promote species-typical behaviour by providing animals with a naturalistic environment as of similar to wild situations. That environment may include activities that are both challenging and time consuming and may serve several functions, for achieving so, we should know following:

Know your species

Amphibians have complex and varied husbandry requirements and are not always easy to maintain and breed. In captivity keepers should aim to replicate wild micro-environments and conditions as closely as possible and where feasible captive management should be informed by field data as well as knowledge of the biology of the species. Replicating wild conditions may improve captive breeding success.

Figure 1 Terraium type, glass fronted exhibit for ampbhibians with live vegetation. (Brij Kishor Gupta)

Unfortunately, as little is currently known about the micro-climates utilised by most amphibian species in the wild keepers may have to collect these data for themselves or correspond with field biologists in order to obtain these very important data.

Enclosure

Enclosure dimensions will depend on the type of species being kept and typically larger and more active amphibians require larger sized enclosures. Arboreal species should be provided with tall enclosures (E.g. Polypedates spp. Rhacophorus spp. Pedostibes tuberculosus) and terrestrial species (e.g. Duttaphrynus spp. Fejervarya spp. Clinotarsus curtipes) should be provided with a greater amount floor space; enclosure height is less important for these. Note that some species can be incredibly active and jumpy and when they are maintained in small enclosures they may be prone to rostral abrasions.

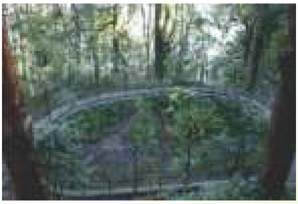

Figure 2 Open, large, naturalistic breeding cum exhibit area for Himalayan salamander at PNHZ Park, Darjeeling (Brij Kishor Gupta)

here are several important factors to consider when selecting an enclosure for amphibians. Enclosures must be escape proof. Due to their permeable skin amphibians may quickly desiccate and die if they escape. The construction material should be easy to clean and preferably be smooth. Wood is not appropriate as it may warp with high humidity. It is not possible to disinfect wood during routine cleaning so this material should be avoided. Many chemicals used to seal wood are toxic to amphibians. Plastic enclosures are a suitable alternative. Any plastic should be food grade as many plastics can leach substances that may be detrimental to amphibian health over time. Plastics may also become brittle when exposed to UVB radiation (an important component of amphibian husbandry). Plastic can be easily cleaned and disinfected. Glass is another alternative; it is readily available, easy to clean and disinfect although glass may break easily. Construction of enclosures using brick and cement should be avoided as these materials may leach minerals into water bodies and may be detrimental to water quality. Amphibians are ectothermic and require a gradient of UVB radiation, temperature, visible light and humidity, therefore amphibian enclosures will need to be lit and appropriate sized meshed apertures need to provide air exchange in the roof or lid of the enclosure. Keeper access is also an important factor to consider for ease of maintenance while minimising chances for escapes while servicing the enclosure. Ideally, drainage holes with taps will need to be provided to facilitate easy cleaning.

Substrate

There are a huge variety of substrates available for amphibians and the type of substrate used will depend on the species being maintained. It is important to consider the depth of the substrate. Fossorial amphibians (e.g. caecilians, *Sphaerotheca* spp.) may require deep substrates for them to burry into. The humidity of the substrate and its potential of water absorption and retention also needs to be considered. As amphibians have permeable skin (and drink through their skin) there should be a moist area of substrate at all times.

Figure 3 Acrylic fronted, terraium type exhibit for amphibians with natural logs and sand as substrate at Coimbatore Zoological Park (Brij Kishor Gupta)

A gradient of humidity in the enclosure should be provided which will allow the amphibian to self-regulate its water balance. For the majority of species water logged substrates are not optimal. Amphibians do need to be given the opportunity to sit in dryer areas within the enclosure and failing to provide some dry areas could result in serious health issues. Substrates should be changed as and when needed. Some amphibians may ingest substrate; this is not always a problem unless the amphibian ingests something it is unable to pass through its gastrointestinal tract (e.g. pebbles / strings of moss), the feeding behaviour of the amphibian should therefore be considered when selecting a substrate. Some substrates can be very powdery and may stick to the skin of an amphibian and interfere with the function of the skin (gaseous exchange etc.). Substrates for surface active amphibians should therefore be compacted to minimise this.

Refugia / furnishings

All amphibians should be provided with refugia. The provision of multiple refugia within an enclosure will make the enclosure more heterogeneous. This is of fundamental importance as amphibians will select sites to regulate their temperature and water balance. Cover objects and visual barriers are also important, amphibians are eaten by a wide variety of other organisms and keeping them in very open enclosures with limited cover can result in severe rostral abrasions, stress and disease. For hides to have the desired effect of providing adequate humidity and to reduce stress, they will need to be individually selected to fit the target species' size and shape so that the animals fit in or under snugly. Arboreal species will require branches as perch / rest sites and some species will be benefit form live plants (e.g. Rhacophorus spp.) which often perch on leaves. Aquatic species (including tadpoles) will also been shown to benefit from refugia. Ensure that any live plants are obtained from sources free from agrochemicals. Also make sure that plants do not have thorns, irritant or toxic sap, hairy leaves/stems or other features that may harm amphibians. Additionally, food insects may feed on plants in terraria before being consumed, so ensure that plant toxins cannot be ingested by amphibians by this route.

Light and temperature

Wild amphibians have evolved in accordance with their environments. They will reconcile requirement and provision by self-regulating their exposure to heat, light and the associated UVB radiation. Optimal UVB, light and temperature levels for a species will be those in their natural habitat, specifically at the microhabitat level for each life-history stage. Where possible the UVB, light and temperature microclimate throughout the year should be established through field studies and used to inform the captive management of the target amphibian species. The UVB and Vitamin D3 requirements of amphibians are largely unknown. It is likely that different species will have different requirements due to their differing life histories and micro-habitats. UVB is important in the synthesis of vitamin D3. Vitamin D3 deficiency is a nutritional problem encountered in captive amphibians. 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 synthesised 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, matching temperatures found in the wild. Some amphibian species might also receive vitamin D3 through some diets although the extend of this is largely unknown; some food supplements can provide vitamin D3 artificially although great care must be taken to achieve the correct dosages and that the supplement is stored correctly and stock is frequently replaced. Dietary supplements are degraded by light, humidity and heat.

Figure 4 Amphibian house at Jersey Zoo, Jersey, Channel Islands, United Kingdom. (Brij Kishor Gupta)

In order to mimic the situation in the wild, heat (infrared radiation), light and UVB should be provided together (i.e. the highest levels of UVB should be associated with the warmest areas of the enclosure, and the lowest levels with the coolest areas). Most importantly, all three must be provided as gradients in the enclosures for appropriate lengths of time each day in order to facilitate self-regulation, with retreats that provide deep shade and no direct UV radiation.

If there are life-history stages that would, in the wild, only be exposed to low-level reflected or diffused UV-B, or none at all (e.g. eggs and tadpoles in tree holes or burrows), then care should be taken to replicate this situation in captivity. The provision of appropriate UV-B radiation to captive amphibians is important for their health and proper development but this field is still in its infancy. We strongly advise caution in its provision as over-exposure can also cause harm. We believe that tolerances to UV-B radiation will be species-specific and related to the historic exposure of animals; individuals that have not been provided with UVB in the past should be slowly exposed to increasing levels until reaching optimal exposure to allow the skin to adapt to radiation.

Amphibians should not be able to come into direct contact with any lamps or heaters as they have extremely sensitive skins and may be easily burnt. All lamps will emit heat but additional heating and cooling may also be necessary. Rooms can be heated and cooled with air conditioning units. Amphibian enclosures should not be placed under such units, or normal fans, as the air movement can cause severe dehydration. Chilling systems can be installed to cool water in enclosures.

Maximum and minimum temperatures in amphibian enclosures in both the cool end and warm end of the enclosures should be recorded on a daily basis. The UV index should also be recorded to ensure that UVB provision is adequate. The UVI index can be measured using a Solarmeter 6.5.

Figure 5 Himalayan salamanders provided with wet mud as substrate at PNHZ Park, Darjeeling. (Brij Kishor Gupta)

Figure 6 Young himalayan salamanders also provided with boulders as substarte and algae as feed at PNHZ Park, Darjeeling. (Brij Kishor Gupta)

Water quality parameter	Recommended levels	Control methods
Water hardness (dissolved Ca and Mg salts)	For soft water amphibians: <75mg litre^{-1} (ppm) of $CaCO_3$. For hard water amphibians: >100mg litre^{-1} of $CaCO_3$.	Soft water: Harden using Ca and Mg salts (only recommended reconstituting RO (reverse osmosis), DI (de-ionized) or distilled water). Hard water: Soften using RO, DI or distilled water.
Dissolved oxygen as O_2 **Gas supersaturation**	>80% saturation Gases maintained at equilibrium with the atmosphere.	Aeration Aerate water until equilibrium with atmosphere is achieved.
Ammonia/ Ammonium-NH_3/NH_4^+	<0.2mg litre^{-1}, N as unionized ammonia	Biological filtration, chemical filtration (with appropriate medium), water changes.
Nitrites NO_2^-	<1.0mg litre^{-1}, ideally 0	Biological filtration, chemical filtration (with appropriate medium), water changes.
Nitrates NO_3^-	<50.0mg litre^{-1}	Removal: photosynthetic action of green plants or water changes.
pH	Generally near neutral, although it is species-dependant. Should avoid pH <6 and >8.	Change water source or add appropriate buffer solution.
Chlorine Cl_2	0	Aerate for 24 hours, or add chemical dechlorinator such as sodium thiosulphate.

Chloramines ($CINH_2$, CIN_2H, CIN_3)	<0.01mg litre^{-1} as CI	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 litre	Carbon filtering and carbonate precipitation (do not use copper piping).
Posphates ($PO^3{}_4^-$)	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.

Water quality parameters: guidelines for keeping amphibians. Adapted from Odum & Zippel (2008).

Water

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, even if these are slightly sub-optimal – of course consistent optimal water quality should always be the goal.

Figure 7 Water quality is extremely important for all amphibians housed at the zoo. (Brij Kishor Gupta)

It is important to know certain parameters of the water source; sources can be tested for dissolved substances, pH and hardness, and treated if necessary to provide the appropriate conditions. Unfortunately the optimal water parameters are unknown for most amphibian species. Most institutions use municipal water sources which are generally treated with chlorine, or chloramines; levels of these chemicals should be tested for and treated accordingly. Other water sources should be treated with caution; avoid using water which has previously been in contact with other amphibians as this may risk disease transmission, or may have been contaminated by dissolved substances. Reverse osmosis (RO) systems will produce water that is too pure for amphibians and using pure RO may cause osmoregulatory imbalances. 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 any other substances that could leach into the water. Low levels of dissolved metals can be toxic to amphibians.

Water parameters should be tested frequently in newly established systems, and tests can become less frequent as the system develops. Testing should be carried out on a regular basis in order to monitor the effectiveness of biological filtration.

The most common system used 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. Water changes should be small and regular (no more than 20% of the original volume of the water body in question). Rapid changes in water parameters can be highly stressful on the physiology of aquatic organisms. The necessary frequency of water changes will depend on the stocking density, the temperature of the water and the amount and type of food given at each feed, as these have an effect on the level of activity within the tank, which in turn affects the amount and character of waste material produced.

Suspended particles can be removed from the water column through mechanical filtration, dissolved harmful substances (such as chlorine) through chemical filtration, and nitrogenous waste material produced by aquatic organisms and the breakdown of organic material can be processed by bacteria living on filter media, through the process of biological filtration. This can be achieved through using separate filters, or single filters with combined functions. The outflow of the filter should be adjusted to meet the requirements of the amphibian. Some tadpoles require extremely fast flowing well oxygenated water whereas this may be detrimental to other tadpoles (e.g. those living in ponds).

Figure 8 Water parameters should be monitored by testing frequently in new established exhibits. (Brij Kishor Gupta)

The size and type of filter used will depend on the size of the enclosure, volume of water and density of individuals being catered for. If larvae are being kept at relatively high densities, large external filters may be necessary to remove waste material; for those kept at lower densities (under 20 individuals per litre), box filters may be adequate. Filters require regular maintenance in order to ensure they continue to function effectively. The frequency with which filters need servicing depends on the stocking density, the amount of food given per individual per feed, as well as the temperature of the water, as this will affect how much waste material is produced.

Mechanical filters remove physical particulates from the system, and these then build up in the filter if they are not cleaned out straight away. Build up should be avoided as it will reduce the capacity of the filter for dealing with further suspended particles, and any organic matter within them may begin to break down and leach harmful substances back into the system.

Chemical filters are designed to remove dissolved substances such as organic compounds from the water as it passes through; activated carbon has been identified as an ideal material for amphibian systems and is commonly used as a chemical filtration medium. It is vital that such filter systems are used in combination with a mechanical filter, as build-up of particulate matter will severely compromise their effectiveness. Chemical filter media will need to be routinely replaced. Be aware that activated carbon will removed medication and tannins from the water column, so may be inappropriate for some systems and under some circumstances.

Biological filtration systems consist of a community of living organisms which act to break down toxic metabolic waste products in the water to less toxic substances; toxic ammonia is broken down to less toxic nitrite which then in turn is converted to less toxic nitrate (which in itself is still less toxic to aquatic organisms). These filters are therefore the most important component in maintaining the health of the aquatic system and the amphibians themselves. Owing to the living component of this filtration system a constant supply and flow of water is required in order to ensure nitrogenous waste is absorbed and that oxygen is available to the bacterial community. Biological filters take time to mature and can be seeded with filter media from established systems, this may risk introducing disease causing agents into new set ups. Alternatively filters can be matured using an ammonium chloride dosing regime. Note that filters are less efficient at processing nitrogenous waste in soft water and at low temperatures.

Consideration must be given to the access points in and out of the water body. Many amphibians are surprisingly poor swimmers and can easily drown.

Plants

Plants are beneficial in amphibian enclosures. Not only do they provide resting sites for amphibians but they also create microhabitats (e.g. humid pockets of air caused by transpiration and also shade from heat and light) as well as oviposition sites for some amphibian species. Plants are particularly important in aquatic exhibits as they will use the nitrogenous compounds for growth, as well as absorbing many other pollutants, and can improve water quality.

Diet

Nutritional problems are a major barrier to successful amphibian husbandry. The nutritional requirements of most amphibians are unknown, and requirements change with life stage for species with tadpoles.

Figure 9 Culturing crickets (Courtesy: DWCT)

Even when the diet is known, it is often impossible to replicate in captivity as captive diets are limited by the commercial availability of food species and the ability to establish breeding colonies of appropriate species, as well as difficulties in providing prey species themselves with suitable diets. Nevertheless, nutrition is a key factor in keeping amphibians healthy and a basis for successful breeding. Great efforts should be made to be able to provide any captive amphibians with as much variety and good nutritional content as possible.

Figure 10 Feed for mansy of the species of amphibians. (Brij Kishor Gupta)

Most post metamorphic amphibians are insectivores and will only feed on live, moving prey items. Invertebrates can be cultured or collected from the field. Food offered to amphibians should be no bigger than the width of the head. All food should be well fed prior to being offered to the amphibian, in addition to being dusted with an appropriate dietary supplement. To encourage natural feeding behaviour amphibians should be fed at times to coincide with their peak activity periods to ensure that they encounter the food and that dietary supplements are still coating the prey item when the food is ingested.

Seasonality and breeding

Many amphibians breed in response to environmental cues, these include, but are not limited to temperature, humidity, precipitation, barometric pressure and changes in water quality. It is important that seasonal changes that reflect the situation in nature are replicated in captivity even if breeding is not the desired outcome as failing to provide animals with the seasonality may cause health issues e.g. females becoming egg bound, obesity etc.

Figure 11 The amphibians should be provided species specific substrate during the period season to meet their biological and behavioural requirement.(Brij Kishor Gupta)

Rain chambers are one of the most common ways to breed amphibians in captivity. These systems recreate rain showers and usually involve a small pump sitting in a few centimetres of water, the pump supplies water to a rain bar. The frequency and duration of the rains can be controlled by using a timer.

Figure 12 Amphibians whose egg laying patter varies, should be provided appropriate artifacts as of to meeting the species requirement (Brij Kishor Gupta)

Routine maintenance

Faecal material and dead feeder insects should be removed from enclosures on a daily basis. Any uneaten food should be removed the day after the animals have been fed as the nutritional value of the food will have deteriorated during the time it is has been in the amphibians' enclosure. Feeding regimes will depend on the species being maintained, the season and life stage of the amphibian. Water dishes should be changed daily and the same attention should be paid to the water source for water dishes as for aquaria. Amphibians often excrete large volumes of urine whilst sitting in water and even if the water appears clean it may not be. Captive amphibians (particularly non-native species) may carry pathogens that are not present in the vicinity of the amphibian holding facility. Waste water from amphibian enclosures should be disinfected following the manufacturer's guidelines. Temperatures should be checked and recorded daily and the function of all aquatic life support systems lights and heaters checked on a daily basis. Special attention should be paid to humidity gradients within the enclosures, if an area has become too dry is can be manually sprayed with water from an appropriate source. Ideally amphibians should be given a visual inspection on a daily basis. Unearthing fossorial species such as caecilians and some frogs may be detrimental to their health and welfare so disturbance should be minimised for these species.

12. Amphibian Diseases

Introduction:

Amphibians are undergoing mass mortality due to various pathogens that include viruses, bacteria, protist metazoans, water molds and fungus. Rana virus disease outbreak in wild as well as captive population has led to mass mortalities in different parts of the world. Bacterium, *Aeromonas hydrophila* is responsible for red legged disease in Rana mucosa. Fungal pathogen *Batrachochytrium dendrobatidis* (Bd) has caused more damage to amphibians than any other known pathogen, and it is responsible for global declines in their population. This is most common disease and it is reported from the Western Ghats in India, therefore, we focus here on Bd fungus.

Batrachochytrium dendrodatidis (Bd) is an aquatic pathogenic fungus that is capable of infecting the frog skin and disrupting osmoregulation resulting in chytridiomycosis. A recently discovered species of fungus, *Batrachochytrium salamandrivorans* (Bs), is also known to cause chytridiomycosis in salamander and newts (Martel et al, 2013). Bd and Bs fungus are closely related and have similar etiology. Bd probably originated from Africa and spread throughout the continent and elsewhere (Weldon et al, 2004). Trading of Xenopus frogs for medical and food purposes resulted in transport of the fungus to different countries. Bd fungus has reportedly caused mass mortality in America, Australia, Africa and Europe; but in Asia the prevalence is low. It is suspected that chytrid infection is emerging in Asia (Swei et al, 2011). So far, only Japan, China, Indonesia, Hong Kong, South Korea and India have reported Chytrid fungus infection in Asia. Nearly 520 frog species have been affected by Bd fungus and population decline has been observed in more than 200 species worldwide (Olson et al., 2013; Fisher et al, 2009).

Bd has two stages, reproductive zoosporangium and motile zoospores. Zoospores which are motile colonize and encyst on the surface of frog skin epidermal cells and penetrate into inner epidermal cell line and develop into thallus. This thallus matures and produces sporangia which release new zoospores. This process causes hyperkeratosis in frogs. The skin becomes thick-walled, which interferes with gaseous exchange, osmoregulation and electrolyte transport which leads to cardiac arrest (Voyles et al, 2009).

Very few treatment options are available for Bd fungus. Previous studies have shown that several symbiotic bacteria have ability to inhibit the growth of Bd fungus. Bacteria can produce anti-fungal compound like Violacein against Bd fungus. Heat treatment is another treatment option which can cure Bs infection. It is therefore necessary for effective surveillance to be able to detect the disease at its early onset in order to successfully manage them in captivity.

Swabbing for Bd/Bs fungus detection: It is is non invasive and sensitive. A fresh pair of plastic or latex hand gloves (NOT powdered gloves, as it will remove moisture from the skin of frogs) should be used for handing each frog. For each frog, take a new sterile cotton swab. A total of 70 swab stroke from the frog's body should be taken as shown in figure 1 (Kriger et al, 2007). For each salamander, take 5 strokes fromeach limbs, 10 strokes from its belly and 5 strokes along the bottom of tail. Used gloves should be disposed using standard protocols, incineration is a preferred method. After sampling, instruments and boots should be washed with alcohol or Sodium hypochlorite. During sampling, swabs can be stored in ice box and later it should be transferred to -20°C.

Clinical symptoms: Common sings of Bd includes discoloration and reddening of frog (fig. 2) skin colour, unusual postures like keeping belly away from ground, hardening of skin, excessively peeling of skinand unusual behaviour like nocturnal frogs becomes active during day time. Bs is known to infect only salamanders, where they show deep ulceration on skin (fig. 3). In some other cases, at sites with low prevalence frogs/ salamander might be found dead. In order to diagnose the disease and testing swabs in a lab is essential.

Diagnosis: It can be done by qPCR which is more sensitive method than normal PCR and histological procedures. qPCR is usually carried out using Bd specific Taqman MGB probe following standard laboratory protocols. Molecular diagnosis for Bd/Bs can be done at Centre for Cellular and Molecular Biology, Hyderabad [see website:http://www.ccmb.res.in/lacones]. As set of 10 swab samples can be sent for analysis. A report on the level of infection in the samples will be conveyed to the concerned persons.

Mass mortality: Andy dead frog should be photographed in different angles and put in separate containers and stored in deep freezer immediately. Necropsy can be performed using standard procedure and tissue samples can be sent to laboratories for forensic analyses. If you encounter a sick frog having symptoms mentioned above keep the frog in captivity and report the symptoms immediately to experts to get their opinion [http://www.amphibians.org/asg/members/].

Supporting data required for swabs: The following information needs to be gathered and recorded for each swab sample for processing the lab.

a) Serial No: Start with Your initials and 0001 (For example, if your name is Robert Frost then your first sample should be labelled RF0001)

b) **Sample ID**: This is a combination of columns A, B and C. First two alphabets of the C and B and the number on A will be the Sample ID

c) **Name of collector**: Please enter your full name

d) **Location**: As accurately as possible. GPS location is preferred.

e) **Altitude**: Elevation in meters if available, if not try to give an estimate. It could be a range.

f) **Max and Min Temp**: The maximum and minimum temperatures on the day of collection in degrees Celsius, if available. If not remember to give a range of temperatures.

g) **Date**: Day, Month and year as per the collection

h) **Habitat**: please use what works but whatever you do make sure that you provide us with a legends and that you remain consistent across all your samples.

(For example, secondary forest or eucalyptus plantation, or riparian zone) Since there are many possibilities, make around 5-6 types and then roughly distribute samples into them.

i) Genus: Give genus of species. Take a photograph of the frog to help in identification.

j) Species: Give species name if sure, if not say ~ and whatever you think the species might be. In such cases try to take good pictures of the individual and send to experts for identifying. Please donot try to guess. This is not a competition.

k) **Snout-Vent length**: Give the value of length from the snout to the cloaca of the frog in mm or cm

l) **Weight**: If possible weigh the frog and present data in grams.

m) **Skin lesions/deformities**: If present, type "yes" and give details, if not just type in "no". See Figure 4.

Fig.1. Pictorial dipiction of frog swabbing and number of strokes as mentioned

Fig.2: Bd infected frog showing reddening of legs

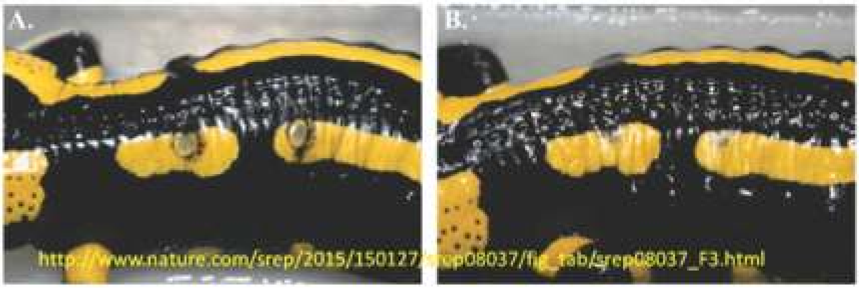

Fig.3: Bs infected salamander (Salamandra salamandra) (A) infected salamander shows patches on skin (B) Uninfected salamander - Adapted from Blooi et al, 2015

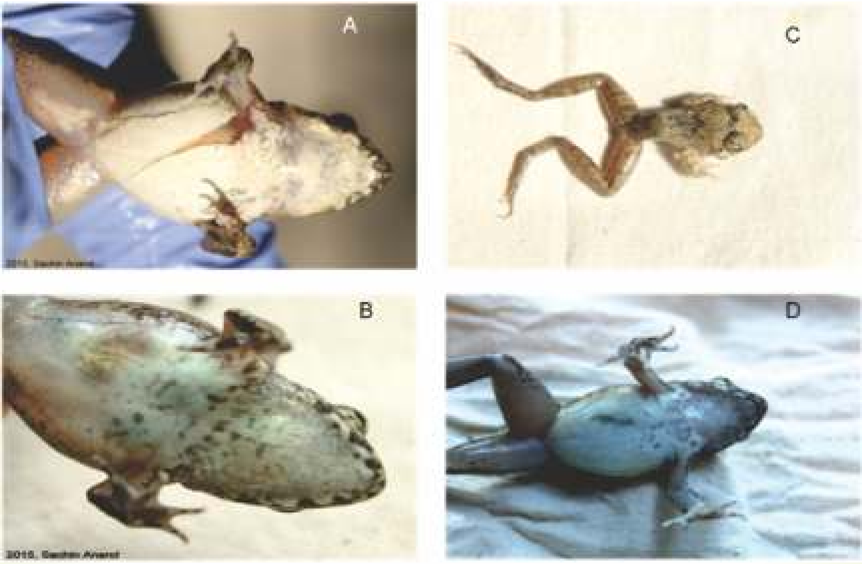

Fig.4: Emaciated or sick frogs A) Fejervarya sp with a red transparent streak on the skin; B) Limnonectes sp with a transparent blackish blotch on the skin; C) Shrunken waist or dehydrated frog (dorsal view); D) Fejervarya sp with grayish blue transparent streak on the skin.

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13. Prioritized Species

List of target species and practice species with the name of zoological parks identified during the workshop for initiating the amphibian housing, exhibit and if required planned conservation breeding in India.

Target Species*

1. *Nasikabatrachus sahyadrensis (WG)*
2. *Melanobatrachus indicus (WG)*
3. *Tylototriton verrucosus (NE)*
4. *Scutiger occidentalis (H)*
5. *Indirana phrynoderma (WG)*
6. *Terorana khare (NE)*
7. *Rhacophorus tuberculatus (NE)*
8. *Ingerana charlesdarwini (AN)*
9. *Bufo hololius (Penninsular)*
10. *Microhyla sholigari (Penninsular)*
11. *Bufoides meghalayanus (NE)*
12. *Rhacopharous pseudomalabaricus (WG)*
13. *Pedostibes kempii (NE)*
14. *Pedostibes tuberculosus (WG)*
15. *Polypedates insularis (AN)*
16. *Nyctibatrachus vasanthi (WG)*
17. *Raorchestes chalazodes (WG)*

* This list will be populated with more species as better assessment of the status of various species is made available.

Zoological Park and their practice species

1. Arignar Anna Zoological Park, Vandalur, Chennai
   a) *Euphylctis cyanophylctis*
   b) *Duttaphrynus melanostictus*
   c) *Ramanella variegata*
   d) *Polypedates maculates*

2. Nandankanan Biological Park, Bhubaneshawar
   a) *Sphaerotheca breviceps*
   b) *Polypedates maculatus*
   c) *Duttaphrynus melanostictus*
   d) *Kalula pulchra*

3. Madras Crocodile Bank Trust, Mamallapuram
   a) *Duttaphrynus melanostictus*
   b) *Sphaerotheca rolandae*
   c) *Polypedates maculatus*
   d) *Ramanella variegata*

4. Chennai Snake Park, Chennai
   a) Uperodon systoma (All India)
   b) *Fejarvarya limnocharis*
   c) *Duttaphrynus melanostictus*

5. Rajiv Gandhi Zoological Park, Pune
   a) *Microhyla ornata*
   b) *Duttaphrynus melanostictus*
   c) *Polypedates maculatus*

6. Padmaja Naidu Himalayan Zoological Park, Darjeeling
   a) *Tylototriton verrucosus*
   b) *Duttaphrynus himlayanus*
   c) *Ichthyophis sikkimensis*

7. Pilikula BiologicalPark, Mangalore
   a) *Hylarana malabarica*
   b) *Indirana brachytarsus*
   c) *Ramanella montana*
   d) *Gegeneophis carnosus*

8. Biological Park, Itanagar
   a) *Polypedates leucomystax*
   b) *Clinotarsus alticola*
   c) *Leptobrachium smithi*
   d) *Rhacophorus maximus*

9. Nehru Zoological Park, Hyderabad
   a) *Duttaphrynus melnostictus*
   b) *Sphaerotheca breviceps*
   c) *Polypedates maculates*

10. Sri Chamarajendra Zoological Gardens, Mysore
   a) *Hylarana malabarica*
   b) *Clinotarsus curtipes*
   c) *Microhyla rubra*
   d) *Ichthyophis beddomi*

11. V.J.B. Udyan Zoo, Byculla Mumbai
   a) *Raorchestes bombayensis*
   b) *Sphaerotheca breviceps*
   c) *Indirana leithii*

12. Bahinabhai Prani Sangrahalaya, Pimpri, Pune
   a) *Rhacophorus malabaricus*
   b) *Raorchestes bombayensis*
   c) *Hylarana malabarica*

13. National Zoological Park, Delhi
   a) *Fejervarya limnocharis*
   b) *Bufo stomaticus*
   c) *Polypedates maculatus*

14. M.C. Zoological Park, Chhatbir, Chandigarh
   a) *Chirixalus dudhwaensis*
   b) *Fejervarya limnocharis*
   c) *Bufo stomaticus*

15. Tata Steel Zoological Park, Jamshedpur
    a) *Bufo stomaticus*
    b) *Uperodon systoma*
    c) *Polypedates maculatus*
    d) *Sphaerotheca dobsoni*

16. Bhagwan Birsa Zoological Park, Ranchi
    a) *Bufo stomaticus*
    b) *Uperodon systoma*
    c) *Polypedates maculatus*
    d) *Sphaerotheca dobsoni*

17. Pt. Govind Ballabh Pant High Altitude Zoo, Nainital
    a) *Duttaphrynus himalayanus*
    b) *Fejervarya limnocharis*
    c) *Paa vicina*

18. Assam State Zoo & Botanical Garden, Guwahati
    a) *Chirixalus simus*
    b) *Kaloula pulchra*
    c) *Fejervarya limnocharis*
    d) *Bufo stomaticus*
    e) *Polypedates leucomystax*

19. Seppai*jala Zoological Park, Sepahijala*
    a) *Kaloula pulchra*
    b) *Fejervarya limnocharis*
    c) *Bufo stomaticus*
    d) *Polypedates leucomystax*

20. Aizwal Zoo, Aizwal
    a) *Kaloula pulchra*
    b) *Fejervarya limnocharis*
    c) *Bufo stomatica*
    d) *Rhacophorus maximus*

21. Manipur Zoological Park, Imphal
    a) *Occidozyga borealis*
    b) *Bufo stomaticus*
    c) *Polypedates leucomystax*

22. Biological Park, Chidiyatapu, Andaman & Nicobar Islands
    a) *Ingerana charlesdarwini*
    b) *Microhyla inornata*

23. Laboratory for Conservation of Endangered Species (LaCONES), Hyderabad
    a) *Duttaphrynus melanostictus*
    b) *Sphaerotheca breviceps*
    c) *Polypedates maculates*

WL- Western Ghats
NE- North East
H- Himalayan
AN – Andaman & Nicobar Islands

Notes

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Message

Securing habitat for threatened species is necessary, but in some instances not sufficient to insure the survival of the species. Pollution, disease, invasive species and other factors within the habitat must be mitigated, and often this cannot happen in time to prevent species extinctions.In such cases the development of captive populations may be the only hope.

With the threat of amphibian extinctions looming large, the ability to maintain and reproduce amphibians in zoos and aquariums is becoming increasingly important. The Amphibian Conservation Action Plan (ACAP) (Gascon et al., 2007), a product of the International Union for the Conservation of Nature Species Survival Commission Amphibian Conservation Summit in 2005, specifically identifies the need for captive programs, "In the face of overwhelming and sometimes urgent threats to many amphibians, such as disease or habitat destruction, the only hope in the short-term for populations and species at immediate risk of extinction is immediate rescue for the establishment and management of captive survival-assurance colonies.".

Drawing on published literature and on the extensive experience of the editors, this volume provides invaluable information relevant to the establishment of those captive colonies called for in the ACAP. While not all of the species for which husbandry protocols are described are threatened at present, many are species for which populations are declining and which may soon be at risk. Additionally, some may serve as models for more endangered species, allowing the development of husbandry skills necessary for working with more threatened taxa.

Assam State Zoo, Guwahati and Central Zoo Authority is to be commended for developing this volume, which will undoubtedly become a much-used reference for all zoos seeking to enhance their ability to care for and conserve amphibians in the South Asia region.

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Amphibian Ark is a partnership among the World Association of Zoos and Aquariums, the IUCN SSC Conservation Breeding Specialist Group, and the IUCN SSC Amphibian Survival Alliance.