

HUSBANDRY GUIDELINES

Pickersgill's Reed Frog –(Hyperolius pickersgilli)



Figure 1 Female Pickersgill's Reed Frog (Ian du Plessis, 2017)

**CREATED BY:
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**IN ASSOCIATION WITH:
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Disclaimer: This manual assembles basic requirements, best practices, and animal care recommendations based on experience taking care of Pickersgill's Reed frogs over a sustained period to maximize capacity for excellence in animal care and welfare. The manual should be considered a work in progress since practices continue to evolve through advances in scientific knowledge. The use of information within this manual should be in accordance with all local and national laws and regulations concerning the care of animals. The recommendations included are not meant to be exclusive management approaches, diets, medical treatments, or procedures, and may require adaptation to meet the specific needs of individual animals and particular circumstances in each institution. Commercial entities and media identified are not necessarily endorsed by JCPZ.



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Glossary

Activity budget	Time spent by an animal in various activities such as resting, moving, sleeping, eating, hunting, etc.
Adult	A sexually mature frog capable of reproducing
Amplexus	A type of mating behaviour exhibited by some externally fertilizing species in which a male grasps a female with his front legs as part of the mating process, and at the same time or with some time delay, he fertilizes the eggs as they are released from the female's body
Behavioural enrichment	Activities that provide captive animals a wider choice of natural behaviours in which to engage
BMP-S	Biodiversity Management Plan for Species – a document developed to help applicants identify, protect and manage a native species
BPA	Bisphenol A is a chemical found in many hard plastics that we use every day. BPA is an endocrine disruptor and can imitate the body's hormones, thus interfering with the production, secretion, transport, action, function, and elimination of natural hormones. Higher doses have been linked to infertility and other health problems
Buoyancy deficits	Reduction or even loss of floating ability
Chitinophilic	Having an affinity for chitin
Chronic stress	Induced stress that an animal cannot escape from over a long period of time. Over time can lead to deterioration of physical wellbeing
Coelomic effusions	Accumulation of fluids in the main body cavity
Developing tadpoles	A tadpole older than 48 hours up until the appearance of the first limb
Ecchymotic haemorrhage	Subcutaneous discolouration caused by blood seeping into tissues from ruptured blood vessels
Eggs	Frog embryos that are encased in a few layers of gelatinous material
EKZNW	Ezemvelo KwaZulu-Natal Wildlife
Environmental enrichment	An animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being
Erythema	Redness of the skin, caused by dilation of veins
Froglets	A small frog that has recently developed from a metamorph. It has no tail
Granulomatous	Inflammatory response composed of immune cells called macrophages which attempt to wall off substances they cannot eliminate
Gut loading	Giving feeder insects food items that will improve their nutrient content making a more balanced meal for insectivores. E.g., providing crickets with fresh fruit and vegetables increases carotenoid concentration
Histiocyte	Stationary, phagocytic cells of the immune system
Hydrocoelom	Distension of coelom with fluid
Hyper-	Prefix for over. Meaning too much of a substance. E.g., hypervitaminosis
Hypo-	Prefix for under. Meaning too little of a substance. E.g., hypovitaminosis
Insurance population	captive breeding of specific strain/specie with the aim of re-introducing back into the wild
IUCN	International Union for Conservation of Nature
JCPZ	Johannesburg City Parks and Zoo. See Joburg Zoo
JCPZ – ARP	Johannesburg City Parks and Zoo – Amphibian Research Project
Joburg Zoo	Johannesburg City Parks and Zoo
Keratinophilic	Having an affinity for keratin
LLDPE	Linear, low-density polyethylene
Metabolic Bone Disease	Also known as MBD is a disorder of bone strength usually caused by abnormalities of minerals (such as calcium, phosphorus or magnesium) and vitamin D.



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Metamorph	Tadpole that is undergoing metamorphosis into an adult frog. Characterised by the appearance of the limbs and the fully developed lungs. Tail is still present.
Mucopurulent	Containing or composed of pus or mucous
NEMBA	National Environmental Management Biodiversity Act, 2004
Neoplasia	The presence or formation of new abnormal growth of tissue
NRC	National Research Council
Oedema	Accumulation of fluid beneath the skin and in other body cavities
Oncogenic	Causing development of a tumour
Panophthalmitis	Inflammation of all layers of the eye, including the intraocular structures
Petechiation	State in which the skin of an individual is covered in small, blood-filled spots caused by minute haemorrhages
PRF	Pickersgill's Reed Frog
Reverse osmosis	Water purification process where a partially permeable membrane is used to remove ions, unwanted molecules, and larger particles from water. Also referred to as RO water
Short Tongue Syndrome	A disease where the cells within the tongue, are gradually keratinised. This keratinisation makes the tongue lose its elasticity and therefore unable to catch prey
SoP	Standard Operating Procedure
Sporozoite	Motile, spore-like stage in the life cycle of some parasitic sporozoans. Typically the infective stage in the cycle
Sporozootic	Pertaining to sporozoites
Stressor	A cascade of physiological events designed to prepare the body for homeostatic challenge—the so-called “fight or flight” response
Tadpoles	Aquatic, larval stage that has a tail and gills.
Visceral congestion	Impaired outflow from a tissue resulting in accumulation of excessive body fluids
% W/V	Percentage weight by volume
Young adult	Frogs that have recently developed their mature colouration and have started calling
Zoonotic	Disease of animals that is capable of infecting humans



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Introduction

Background

According to the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species (IUCN, 2019), an estimated 32 - 53 % of amphibians are threatened with extinction primarily due to habitat loss, pollution, fires, climate change, disease and over-exploitation ([Table 1Table 1](#)). The IUCN's Technical Guidelines on the Management of *ex-situ* Populations for Conservation (IUCN/SSC, 2014) currently stipulate that all 'Critically Endangered' and 'Extinct in the Wild' taxa should be subject to *ex-situ* management, to ensure recovery of wild populations. Based on the number of species deemed Critically Endangered in [Table 1Table 1](#) it is almost impossible to establish successful *ex-situ* populations for all species. Therefore, the selection of amphibian species for *ex-situ* conservation must be done cognizant of the limited resources and capacity of the available *ex-situ* facilities/institutions around the world.









Legend Key	Red List Category	Number of species	Percentage in category
	Extinct (EX)	38	0.6
	Extinct in the Wild (EW)	1	0.02
	Critically Endangered (CR)	489	7.8
	Endangered (EN)	787	12.6
	Vulnerable (VU)	715	11.4
	Near Threatened (NT)	381	6.1
	Least Concern (LC)	2,316	37.0
	Data Deficient (DD)	1,533	24.5
	TOTAL	6,260	

Table 1 IUCN Red list of threatened amphibian species in 2018

The JCPZ Amphibian Research Project's (JCPZ-ARP) objectives

The Amphibian Research Project (ARP) of the Johannesburg City Parks and Zoo (JCPZ) was initiated in 2006 with the objective of conserving selected South African endangered amphibian species by creating and establishing sustainable insurance populations. The project's other objectives were to compile detailed husbandry manuals and protocols to successfully breed and maintain these endangered species *ex-situ*, for introduction, reintroduction, or reinforcement in the wild. The first Endangered species bred in this project was the Pickersgill's reed frog (*Hyperolius pickersgilli*) (hereafter abbreviated as PRF).

Taxonomy, Genus and Status of Pickersgill's reed frogs

The species was first described by Lynn R.G. Raw in 1982 and is named after the herpetologist Martin Pickersgill, who discovered the species at Mount Edgecombe in 1978. Its habitat area has diminished rapidly over the years because of extensive urban development and wetland drainage. When *Hyperolius pickersgilli*, whose classification is given in [Table 2Table 2](#), was first considered for inclusion in the ARP, its Red List classification was Critically Endangered B2ab(ii,iii) (SA-Frog & IUCN, 2010). The species is now listed as Endangered B1ab(ii,iii)+2ab(ii,iii) ver 3.1 (IUCN & SA-FRoG, 2016) due to:

- It's very small area of occupancy (9 km² as of the 2009 assessment); This was with further monitoring and surveys found to be a grave underestimate of the range of the species.
- The severe fragmentation of its habitat.
- The continuing decline in the area of occupancy, extent and quality of habitat, and number of locations.

Kingdom	Animalia
Phylum	Chordata
Class	Amphibia
Order	Anura
Family	Hyperoliidae
Genus	<i>Hyperolius</i>
Species	<i>pickersgilli</i> (Raw, 1982)
Conservation status	Endangered

*Table 2 Taxonomic classification of the Pickersgill's reed frog
Adapted from IUCN & SA-FRoG (2016)*

A Biodiversity Management Plan (BMP) for the Pickersgill's reed frog was gazetted in 2017 (Tarrant & Armstrong, 2013) as per the provision of the National Environmental Management Biodiversity Act (NEMBA) of 2004. This greatly assisted conservation efforts by enabling the development of national norms and standards for the management and conservation of the Pickersgill's reed frog. The BMP influenced the conservation efforts of JCPZ in creating an insurance population.

Morphology

The PRF is a small reed frog, measuring up to 30 mm in length. Mature males are smaller than mature females (22 mm and 28 mm respectively) (Raw, 1982). The snout is slightly pointed and extends just beyond the nostrils. The rear toes have webbing between them, and all the digits have well-developed sucker discs. The call is a soft, high-pitched, staccato chirp (“prrrreet”).

Aside from the size differences, adult PRFs normally have a clear sexual dimorphism in terms of colouring. Females can typically be recognised by their bright yellowish-green coloured back ([Figure 1](#)~~Figure 1~~, [Figure 4](#)~~Figure 4~~). Males can be recognised by their dark yellow throat, and brown body colour, with a light-coloured lateral line running from the nose over the eye and then along the side to the groin area on each side of the body ([Figure 3](#)~~Figure 3~~). This stripe may be bordered with a thin black line ([Figure 1](#)~~Figure 1~~) (Schwan, Adams, & Dawood, 2016). In both sexes, the inner thighs, toes, and fingers lack pigmentation. Juvenile specimens are brownish, and sexes are difficult to distinguish from each other at this stage. The dorsal skin is granular in texture, and the ventral skin is smooth.



Figure 2 Pickersgill's reed frog's sexual dimorphism. Left side, female, right side, male (photo by Michael Adams)

However, staff from JCPZ and Ezemvelo KZN Wildlife (EKZNW) observed different variations in colouring from the 'norm' of adult frogs at various natural sites in fieldwork excursions carried out from 2017-2019. At some sites males were also green in colour but still had the lateral lines present, whereas females were found to range from a whitish-beige colour to light- or dark green. It has been reported that amphibian specimens change colour in response to age, lighting and radiation levels. Genetically, it has been found that the PRF forms a single genetic population and has relatively good heterozygosity (Kotze, et al., 2019).



Photo: Donovan Marais

Figure 3 Male Pickersgill's reed frog (photo by Donovan Marais 2020)



Photo: Donovan Marais

Figure 4 Female Pickersgill's reed frog (photo by Donovan Marais 2020)

Home range and natural habitat

The PRF is a small frog species endemic to KwaZulu-Natal, South Africa (Variawa, 2015). While the extent of occurrence of the species is 4768 km², the area of occupancy of the PRF is calculated to be only 12 km² which includes a few wetlands which are all located within 15 km of the coast as shown in [Figure 5](#) (IUCN SSC Amphibian Specialist Group & South African Frog Re-assessment Group (SA-FRoG), 2016). The PRFs occur in coastal mosaic bushveld and grassland at low altitudes (up to 380 m above sea level) (Variawa, 2015). Here they use stagnant (low oxygenated) temporary water bodies and are surrounded by dense, sedge-like vegetation (Measey, 2011); (IUCN SSC Amphibian Specialist Group & South African Frog Re-assessment Group (SA-FRoG), 2016). The vegetation understory is composed of shorter flora, like snakeroot (*Persicaria attenuata*) and indigenous forbs which are used by males to call from. Taller broadleaf vegetation like common reed (*Phragmites australis*), sedges like the giant sedge (*Cyperus dives*) and paper reed (*Cyperus papyrus*) and bulrushes (*Typha capensis*) are used to lay eggs on (Variawa, 2015), (Du Plessis, Armstrong, Malepa, Kanengoni, & Price, 2022). During expeditions to collect frogs, JCPZ and EKZNW staff observed that these frogs were almost always found segregated from other species of amphibians, suggestive of avoidance behaviour given that PRF may be prey of larger species.

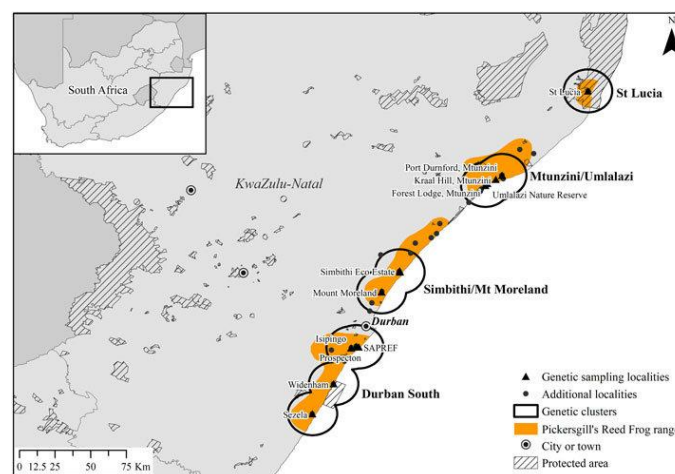


Figure 5 Pickersgill's Reed Frog distribution in KwaZulu-Natal, South Africa (Kotze et al., 2019)

Chapter 1.

Designing of an *ex-situ* Pickersgill's reed frog breeding program

Rationale

The BMP for Pickersgill's reed frog states that one of the goals to conserve the species is to:

"Identify and conduct relevant research to provide information relevant to conservation management requirements, both *in-situ* and *ex-situ*, implement population monitoring protocols, determine relocation and rehabilitation requirements, and ensure that these data are fed back into and inform the overall conservation process".

This is to be done through Action 4.1.2 which aims to:

"Undertake research on the habitat requirements, breeding biology and general husbandry of *H. pickersgilli*, both *in-situ* and *ex-situ*".

JCPZ undertook to set up an *ex-situ* breeding project. To have a successful amphibian breeding project, it was imperative to meet the correct husbandry requirements of the species. Regarding the PRF, the husbandry guidelines had to be established first and followed throughout the project and needed to be in line with the gazetted Biodiversity Management Plan (BMP). An operational plan divided into 9 steps was formulated and followed to determine and establish the husbandry requirements of the PRF as shown in [Figure 6](#). A non-endangered species, the marbled reed frog (*Hyperolius marmoratus*), was identified as a surrogate to assist with the designing of operating systems and protocols which were subsequently modified and used for the PRF. Based on the success of this approach at JCPZ, it is recommended that an operational plan like this be adhered to, to ensure successful breeding and release of PRF and other amphibians.

Operational Project Plan

Step 1: Identification and desktop study of threatened and surrogate species

- Identify surrogate species (in this case *Hyperolius marmoratus*), which mimics target species (PRF), to design husbandry manuals, and to draft Standard Operating Procedures (SoPs)
- Perform desktop study on their natural distribution, life-history, nutritional requirements, breeding behaviour, interactions, social dynamics, seasonal adaptations, feeding/food sources, veterinary needs, water quality and general habitat
- Develop husbandry protocols and SoPs guided by the desktop findings.
- Design and set up enclosures.
- Review agreements and Memorandum of Understanding (MoU) as well as apply for ethical approval and necessary permits.

Step 2: Inception of breeding of surrogate species

- Implement surrogate species breeding project.
- Review protocols regularly.
- Improve the guidelines of husbandry methods.
- Evaluate the *ex-situ* environment, housing and feeding system for suitability and sustainability over at least a 2-year period.

Step 3: Evaluation of surrogate species' breeding and husbandry protocols

- Complete evaluation of steps 1 and 2.
- Update husbandry protocols based on practical findings.
- Prepare for introduction of Endangered species (in this case PRF).
- Perform additional research and literature review in preparation for species-specific husbandry for the PRF.

Step 4: Inception of breeding of Endangered species based on surrogate protocols

- Start breeding Endangered species (PRF) based on husbandry protocols developed from surrogate species.
- Record all information on responses of target species.

Step 5: Evaluation of Endangered species' breeding and husbandry protocols

- Improve the guidelines of husbandry methods according to the requirements of the PRF.
- Review regularly the husbandry protocols for suitability for PRF.
- Continue breeding of PRF to produce a sustainable insurance population to F1 and F2 generation.

Step 6: Preparation for release of Endangered species

- Identify suitable release sites.
- Prepare administrative requirements for release and post-release monitoring (e.g., permits, transport).
- Prepare specimens for release based on protocols (e.g., tagging of specimens, desensitization).

Step 7: Release and post release monitoring

- Release specimens into approved site.
- Monitor specimens post-release.

Step 8: Write up of findings and publications

- Prepare publications – husbandry manuals and journal publications.

Step 9: Protracted maintenance of insurance population

- Evaluate protocols continually.
- Monitor frogs post release.
- Maintain insurance population at the Zoo facility.

The SoPs need to be in line with the husbandry methods to ensure that the quality of life for this species is the same or improved in comparison to their natural habitat. This includes the study of any possible risks e.g., health concerns/ zoonotic infections and outbreaks, stress or environmental changes. Veterinary and health check protocols should be implemented into a preventative and treatment protocol.

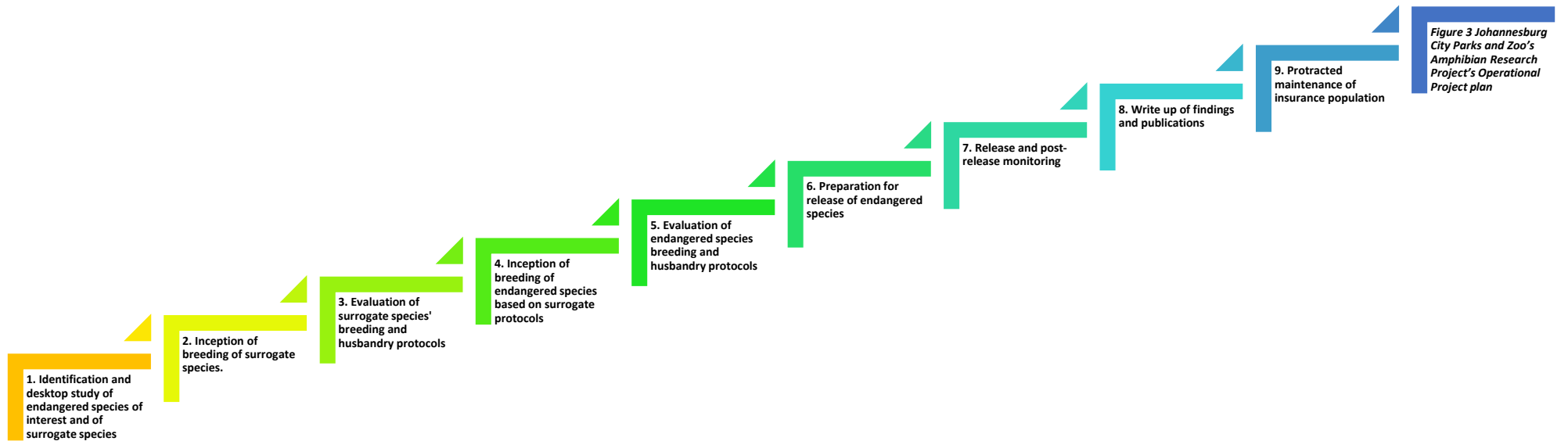


Figure 3 Johannesburg City Parks and Zoo's Amphibian Research Project's Operational Project plan

Figure 6 Johannesburg City Parks and Zoo's Amphibian Research Project's Operational Project plan

Chapter 2.

Environmental requirements

Introduction

The PRF is a habitat specialist, occurring in coastal wetlands, and is considered an “indicator species” in that its presence, absence, or relative well-being reflects the overall health of coastal wetlands. It is sensitive to different environmental stress factors including poor hygiene, too high or too low temperatures, poor water quality or insufficient humidity. While seasonal fluctuations do occur in nature and are important for triggering rest periods and stimulating natural behaviour/breeding, they should not exceed tolerable levels. In captive breeding, the number of life stages that the PRF goes through necessitate that a unique and specific environment needs be provided for each life stage at the correct interval (Du Plessis, Armstrong, Malepa, Kanengoni, & Price, 2022). Proper hygiene protocols should be followed for all enclosures, and before handling any specimens.

Hygiene

Both personal and environmental hygiene should be practiced. The first step is to make sure the PRFs are kept in a room that can be locked to limit access, and only authorised personnel should be allowed into the room. Fomite transmission can be minimised by using a footbath placed at the doorways when entering/leaving the area where the PRFs are kept. The footbath should contain a disinfectant medium and absorbent material to hold the disinfectant and prevent evaporation (e.g., sponge, cloth). An ideal disinfectant should be non-toxic, non-corrosive and have high efficacy against gram positive and negative bacteria, fungi and viruses. There are none that match these criteria, but the closest is the F10© disinfectant and treatment range which are based on a combination of a quaternary ammonium compound, benzalkonium chloride, and a biguanide compound, polyhexamethylene biguanide (PHMB). The antimicrobial mechanism of action of the F10© products is derived not only from that of each of the individual compounds, but also the novel synergistic action of the components. At JCPZ, F10© is used at a dilution of 1:250 (Du Plessis, personal comm.). The footbath should always be used when entering/leaving the room, even if the person has only briefly left the room.

Personal hygiene is important to make sure no pathogens are spread through human activity (Taylor, 2001). Hand washing must be done before opening an enclosure and/or handling of the amphibians. Hands should be washed with the recommended disinfectant and water, after which the hands must be thoroughly rinsed with reverse osmosis (RO) water. Due to the number of different systems used in the project and husbandry activities, it is important to ensure that all parts of a system are kept clean and free of contamination and infectious agents. As the frogs develop through their various stages, they need to be transferred to different enclosures designed to cater to their life-stage needs (Du Plessis, Armstrong, Malepa, Kanengoni, & Price, 2022). Other activities undertaken include daily husbandry, transfers, and introduction of new specimens.

Powder-free latex gloves should be used always and wetted with Reverse Osmosis treated water (RO water) before working with the specimens or enclosures. Gloves should be changed between enclosures to prevent any cross contamination. If a specimen jumps out of an enclosure and is recaptured, the gloves should be changed before work is continued. Once work is completed gloves should be disposed in a bin and sent for incineration.

Temperature

Amphibians are ectothermic, meaning that they derive heat from their environment. Temperature is therefore one of the most important management factors. If temperature exceeds or drops below tolerance levels, it will have numerous negative effects, such as delayed breeding, delayed development, reduced life expectancy, mortalities, etc.

The KwaZulu-Natal coastal climate is subtropical, often hot and humid, especially in the far north. Coastal temperatures range from the low 20°Cs to high 30°Cs in the summer months and from the low teens to mid-20°Cs in winter, with rainfall of up to 1200 mm falling mostly in summer (Palmer et al., 2011). High temperatures (above 33 °C) present a potential risk by creating a suitable environment for bacterial and fungal growth that can be fatal to the collection. While minimal research has been done regarding the temperature range the PRF can endure before it becomes a stressor, the ideal daily summer temperatures at the JCPZ facility was found to be between 24 and 28 °C. Frogs experiencing temperatures below 23 °C and above 28 °C did not breed. The room temperatures (ambient temperatures) should be from 23 to 27 °C during the breeding season and 16 - 20 °C in the winter or non-breeding season.

Humidity

Another critical aspect of amphibian husbandry is humidity. Insufficient humidity can negatively impact on PRF by drying out the skin too much, which interferes with oxygen transfer, heat dissipation, water balance and disease resistance(REF). Average humidity in Durban, being a coastal city, has been measured from 72 to 80 % (World Weather & Climate Information, 2019); and maintaining these values is therefore recommended for PRFs in captivity. It is important to mimic the natural conditions to promote the breeding potential and maintain the sustainability of the insurance population. Each enclosure that houses specimens should have proper ventilation but must be escape-proof.

If the room temperature is well maintained and the enclosures are sprayed twice daily, the humidity should be constant at a required 75 %. It must be kept in mind that if the humidity fluctuates too much or is not maintained it can become a stress factor as well.

The ambient temperature will affect the humidity inside the enclosure due to changes in evaporation. Some of the older air-conditioning units will dry the air and thus the humidity will drop to unsuitable levels. Heating equipment such as heater-fans and other heaters would cause the evaporation level to increase to such an extent that the air will be dry in a short while as hot air moves upwards, removing moisture in the air with it.

Water quality

The preferred water is RO-treated water or alternatively aged water. The RO water is produced by filtering tap water through a RO machine to remove all minerals and possible contaminants maybe mention the brand manufacturer and country of origin here. As an additional safety precaution, the JCPZ also ages RO water. This is done by collecting the water in a 200-litre food-grade, LLDPE, UV-resistant & BPA-free plastic drum, and leaving it for 3 days. This aging process ensures that there are no residual chlorine particles (0.00 ppm) remaining in the water. Finally, all water should still be tested before use to ensure it is within the required parameters as stated in [Table 3](#). All new water must be tested before use as the slightest contamination can cause mortalities, especially in tadpoles. It is not only the water quality but also the water temperature that needs to be controlled and monitored. The containers holding the new fresh water should not be enclosed as it needs to be kept at room temperature to assist with the acclimatizing of specimens when doing water-changes.

Parameter	Measurement
NH ₃ NH ₄	0 mg
NO ₂	0 mg
NO ₃	0 mg
CL	0 ppm
Hardness	Soft
Oxygen (O ₂)	Low
pH	6.5-7
Temperature	19°C

Table 3 Water quality parameters tests done

Chapter 3. Reproduction

Breeding behaviour

In the natural environment, males climb to higher positions on the reeds, where they start calling to attract females. These places are often concealed in dense vegetation (Tarrant J. A., 2017). When a female is attracted, the male will attach himself on the female's back, grasping the female with his front legs as part of the mating process, and at the same time he fertilizes the eggs as they are released from the female's body. This action is called amplexus.

Two other variations of external fertilization were observed in captivity:

- Amplexus is delayed because the female lays eggs in the absence of the male but returns afterwards with the male who mounts on her back and passes sperm on the ova, thus fertilizing them.
- The female lays eggs again without a male's presence. The male comes later and passes sperm over the eggs while the female is absent. It is unclear if it only occurs in an *ex-situ* environment or if this occurs *in-situ* as well.

Life stages and development

Observations *ex-situ* by JCPZ identified seven distinct life stages in the development of the PRF (du Plessis et al 2022). In previous literature, only five stages were recognized. The needs of the life stages necessitated different management approaches and their development timeframes were mapped. These stages are shown in [Figure 7](#) and [Table 4](#).

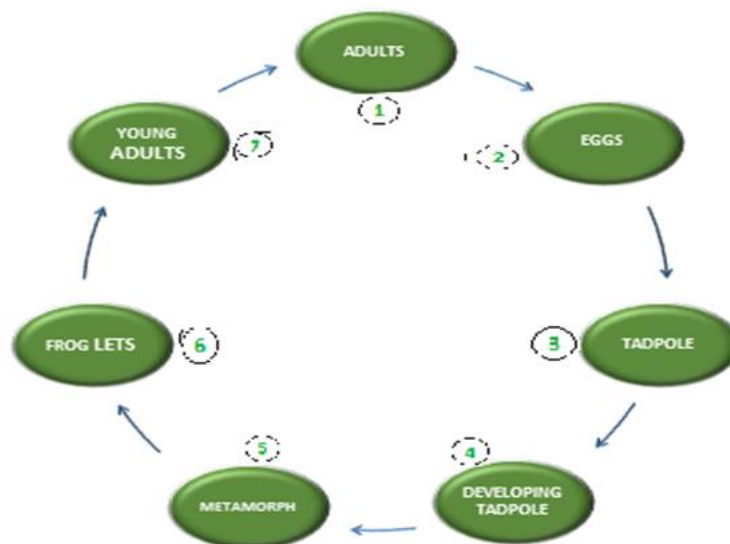


Figure 7 PRF developmental life-stages

LIFE STAGE		DEVELOPMENT INDICATORS AND TIME FRAMES
1.	Eggs	Fertile eggs display a two-tone colour with a whitish top part and a darker bottom part. The fertile eggs will hatch 6 – 8 days after laying.
2.	Tadpoles	Newly hatched tadpoles are black in colour and remain dark for the first 24-48 hours. After 48 hours, they change to a sandy-beige colour. After six weeks, the tip of the tail turns black for a few days, before returning to its normal sandy-beige colour. This is the first indication that limb development has started, and the tadpole can then be classified as a developing tadpole.
3.	Developing tadpoles	From 6-8 weeks, developing tadpoles are characterized by swimming upside down, on the left side and then on the right side in line with lung development and limb development.
4.	Metamorphs	After 8 weeks, in 3 – 4 days, once the fourth limb develops the specimen has lungs and will need to breathe air and is able to climb and move about freely
5.	Frogllets	From 8 weeks to 1 year, newly developed froglets have either lost their tail or have a short tail
6.	Young adults	When aged 1 year to 18 months, the frogs have developed their mature colouration and have started calling.
7.	Adults	Adults are mature and are at least 18-months old. Lifespan in captivity for males is 4-6 years and for females is 10-12 years.

Table 4 Life stage and development of PRFs

Chapter 4.

Enclosure design and husbandry

Introduction

Owing to the number of life stages in the PRF's development ([Figure 7](#)), specific husbandry methods and enclosure systems are needed to provide for each phase (Du Plessis, Armstrong, Malepa, Kanengoni, & Price, 2022). The JCPZ has used several enclosure designs, and records were kept of all activities and descriptions of the husbandry requirements for each stage. (An example of the daily sample record sheets is given in Appendix V). Manual management systems are recommended for PRF rather than automatic systems. Historically, automatic systems were utilised, but these were stopped because of the risk of contamination (6 enclosures were linked via filtration of the water system), high capital expense, and risk of flooding (specimens could drown due to exhaustion from prolonged swimming).

Juveniles to adults – General requirements

Enclosure layout

The PRFs are semi aquatic, and therefore require both dry surfaces and water bodies. The ideal enclosure to house adults and froglets is in an escape-proof enclosure with adequate ventilation ([Figure 8](#)). The recommended enclosure dimensions are:

- Natural Vivarium Small/Wide (l x w x h) - 450mm x 450mm x 450mm
- Natural Vivarium Small/Tall (l x w x h) - 450mm x 450mm x 600mm.

These are specialised glass vivaria with lockable, winged front doors (that can open independently of each other), containing a raised, fixed front window, and a removable, stainless steel full screen mesh at the top (e.g. Exo Terra – Natural Terrarium). Thick, clear plastic sheeting is placed over the light and mesh to prevent excessive loss of humidity and heat from the enclosure. This enclosure is kept angled forward at 30° to allow for a pool of water to be present at the front of the enclosure, and for a dry area at the elevated back portion. Substrate and furniture (e.g., moss, leaves, etc.) are placed at the back of the enclosure. Sphagnum moss is preferred, but substances such as peat, fir bark, and coconut husk fibre were used successfully in the past. The substrate is separated from the water by plastic egg crating. The substrate needs to be changed monthly and should not be allowed to be saturated with water (Edmonds, Breeding Blue-back Reed Frog) (Edmonds, Creating a tropical terrarium, 2008).

Substrate and Furniture

Reed stems, *Strelitzia* spp. leaves, or similar, latex-free leaves are placed vertically in the moss, mimicking the natural environment, providing hiding spaces, and serving as surfaces for frogs to lay their eggs on. Vegetation is used by the PRFs to climb to higher places and therefore high vegetation is recommended inside PRF enclosures. All new enclosure furniture needs to be washed with a scrubbing brush using RO water, and all permanent furniture (e.g., rocks) need to be washed with a disinfectant, rinsed well, left to dry for 24 hours, and rinsed once more before it can be used. The JCPZ makes use of a disinfectant that contains benzalkonium chloride and polyhexamethylene biguanide, diluted in RO water at 1:250. Pebbles should be placed in the area at the front, to serve as resting and hiding spots for the frogs while in the water.

Lighting

A suitable ultraviolet (UV) light source is placed directly over the mesh screen to. This UV light should emit a light like that found in shady environments (e.g., tropical habitats, rainforests). The JCPZ uses 38 cm, 14 W tube lights for each enclosure. The lights have an output of 102 $\mu\text{W}/\text{cm}^2$ UVB (26 IU/Min Vitamin D) at 10 cm and 13 $\mu\text{W}/\text{cm}^2$ UVB (3 IU/Min Vitamin D) at 40 cm. The light output is manually measured monthly using a digital UV meter. The lights are left on 9 hours a day. Again brand manufacturer and country of origin

Water requirements and Management

All water for cleaning and water changes is obtained from a 200-litre drum containing a 2 x 200 W submersible heater set at 26 °C. The water in front of the enclosure is kept 2.5 - 3 cm deep. Water changes are performed every 24 - 48 hours, with 100 % of used water removed via siphoning. The enclosure is misted to ensure that a humidity level of minimum 65 % to maximum of 80 % is maintained. RO water is used for both the water body and for misting.



Figure 8 Vivarium used as a breeding enclosure for adult PRFs

Daily husbandry

Before any work is done, the lights above the exhibits are turned on. During daily husbandry, the enclosure sides, leaves, and furniture are cleaned and wiped down with a paper towel. Care needs to be taken when opening or closing an enclosure that houses specimens as the frogs will jump when disturbed and there is a chance they may escape. Only RO water is used to do water changes and to clean any enclosure furniture as the frogs are very sensitive to any cleaning agents or contaminants. Leaves and sticks are replaced at least once a week, while substrates are replaced once a month. Any leftover food is removed from the enclosure before feeding fresh food. The enclosure is then misted with RO water. Temperature and all activities are recorded.

Eggs

Egg collection and housing

Eggs are kept in 5 litre clear plastic jars, with perforated screw-on lids for adequate aeration ([Figure 9Figure-9D](#)). When a clutch of eggs is found within an enclosure, it is first left in place for 24 hours to allow the protective gel to set before moving it to the plastic jars where they hatch ([Figure 9Figure-9A, B, C](#)). If the eggs have been deposited onto leaves, the entire leaf can be transferred to the plastic jar. The jar is usually placed on its side, with the eggs deposited on a leaf and placed inside the jar. The leaf is then placed at an angle, to allow excess water to flow off. This prevents excessive moisture build up around the eggs, thereby preventing decomposition. If the eggs have been laid on the glass, they are left for 24 hours, after which they are carefully removed from the attachment site. This is accomplished by using a flat, small piece of leaf. The leaf is pressed against the surface below the eggs, then slowly moved up while the eggs are misted with RO water. The water softens the gel surrounding the eggs, allowing them to flow onto the leaf.

Husbandry

The humidity in the plastic jars should be at least 65 %, with the temperature ranging between 22 °C and 25 °C. The humidity is maintained by hand-misting with RO water twice a day. This is repeated daily until hatching. Fertile eggs display a two-tone colour with a whitish top part and a darker bottom part. The fertile eggs hatch from 6 to 8 days after laying (du Plessis et al 2022). Eggs that fail to develop by day 8 should be discarded as they are deemed unfertile or damaged. The disposal method is to place the eggs in boiling water for 2 minutes, where after they can be disposed of in regular waste disposal.

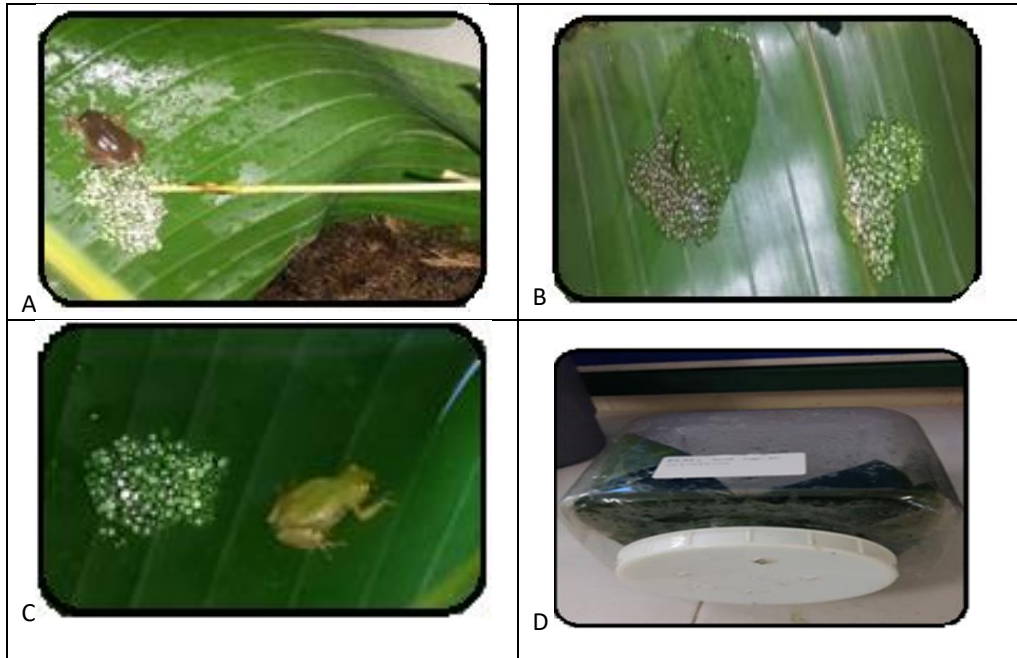


Figure 9 PRF eggs laid on a broad folded leaf (A, B, and C) and a 5-litre plastic jar with a perforated screw-on lid (D)

Tadpoles and developing tadpoles

Housing

Upon hatching, the tadpoles start to move around, and are normally capable of breaking through the protective gel encasing them on their own. The tadpoles then slide down the leaf and drop into the water (Raw, 1982). If hatching is observed, the process can be helped along by spraying the spawn with RO water to flush the hatched tadpoles into the bottom of the jar. Once all the newly hatched tadpoles are in the water the leaf can be removed from the jar. The tadpoles are then collected by sucking them up with a 50 ml catheter tip syringe. The tadpoles are then transferred into a 1-litre clear plastic tub filled with 900 ml RO water at room temperature ([Figure 10](#)~~Figure 10~~). The transfer is done by gently removing the plunger from the syringe and pouring the tadpoles with the water into the tub. It is not necessary to place them with adults, as PRF tadpoles do not receive any parental care and are on their own from the moment of hatching.

Water quality and requirements

The PRF tadpoles prefer slow moving or stagnant water with a low oxygen level. The oxygen level of the water is tested with an O₂ drop test, and the desired range is between 0.5 mg/l and 2 mg/l (ideal 1 mg/l). The chlorine, ammonia, and copper content of the water must be 0.00 ppm and pH must be 6.5 – 7.0. All these parameters for the water are based on observations made by the JCPZ team during expeditions.

The water temperature from the 200-litre drum must read 22 °C before being added to water in the plastic tubs housing tadpoles. The temperature in the containers for housing tadpoles ranges from 19 °C to 23 °C. JCPZ observations over a period of 3 years were that at a water temperature of 17 °C – 19°C, the development of tadpoles was stalled, while mortalities were observed at ≤ 16 °C.

Daily husbandry

Compared to all other stages, the tadpole stage is the most vulnerable. Newly hatched tadpoles are black in colour and remain dark for the first 24 - 48 hours. After 48 hours, they change to a sandy-beige colour. After six weeks, the tip of the tail turns black for a few days, before returning to its normal sandy-beige colour. This is the first indication that development has started, and the tadpole is then classified as a developing tadpole (Du Plessis, Armstrong, Malepa, Kanengoni, & Price, 2022) ([Figure 10](#)~~Figure 10~~).



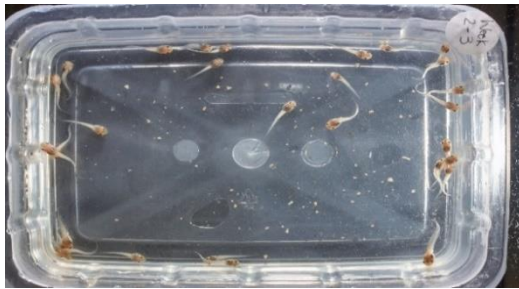







<p>Week 1.</p>		
<p>Week 2 and 3.</p>		
<p>Week 3 and 4.</p>		
<p>Week 5 and 6.</p>		
<p>Week 6 to 8.</p>		

Figure 10 Stages of tadpole development from week 1 to week 8. Photos of tadpoles in a tub (left) and of a tadpole close-up (right)

Tadpoles do not have specific lighting requirements and do well with just ambient light in the room. Water changes are done daily, with 100 % of the water replaced. For tadpoles (≤ 1 week old) and smaller developing tadpoles, contaminated water (faecal matter and old food) is removed via siphoning. Care is taken during this process to prevent any tadpoles from being sucked up, as this may cause mortality. Once the water level gets low (≤ 150 ml), it is topped up to 900 ml. This is done by slowly pouring the water down the side of the container to prevent excess oxygen build-up. Siphoning can then continue until all remaining waste has been removed. Thereafter the water is once again topped up to 900 ml as already described. The larger tadpoles are netted. It should be noted that according to JCPZ observations, tadpoles defecate in response to water changes. This faecal matter is removed before the water is topped up again. The faecal matter initially appears to be in coil spring shape for the first 2 or 3 weeks, after which it assumes a straighter appearance.

The developing tadpoles still have gills and are therefore still dependant on water for respiration as in the tadpole stage. The developing tadpoles are also kept in 1-litre plastic tubs filled with 900 ml of RO water. Tadpoles are kept up to 25 individuals per plastic tub, but the number is reduced to 15 when the tail tip changes colour at 6 weeks. This is done to prevent ammonia spikes in the water, which can be fatal.

Water changes are required daily whereby 90 % - 100 % water change must be done per tub. For water changes, one of two methods can be used:

- The preferred method is to siphon the water out with an air-tube or even a ≥ 20 ml syringe, concentrating on the faecal matter and any leftover food particles. The container where all the siphoned water is decanted is covered with a fine fish net to catch any tadpoles that may accidentally be sucked into the syringe or air-tube. This is a very time-consuming but safe method.
- The second method is to move the tadpoles to a new clean tub. It is done by slowly pouring the water through a fine fish net into a waste container. The net is used to catch all the tadpoles which are then transferred into the newly prepared housing tub. It is of utmost importance to ensure that the parameters of the water in the new container are the same as the previous container. This is a much faster method, but it is much riskier.

Feeding

Tadpoles are fed using a mixture of finely crushed fish flakes and spirulina powder (1-part fish flakes to 2 parts spirulina powder). A pinch of the mixture is added to each tub once a day. Previously JCPZ fed romaine lettuce to the tadpoles in addition to the fish flakes and spirulina. However, this was found to negatively impact tadpole development by delaying hind limb development associated with poor mobility (Becker et al., 2019) and was discontinued.

Changes from tadpole to developing tadpole

During weeks 7 and 8, development takes place very quickly ([Figure 11](#)~~Figure 11~~). The developments are as follows:

- The developing tadpole swims upside down with its mouth parts toward the water surface. The specimens may also remain motionless for extended periods of time. It is postulated that the former is an indication of lung development while the latter is an attempt at energy conservation during development.
- The tadpole then goes through a phase where it swims on its right-hand side with its left-hand side facing up. This may be a sign that the right hind leg is developing, and it has been observed that the right hind leg is the first to be visible.
- Within 1 or 2 days after the development of the right hind leg, the left hind leg develops and is visible. The tadpole swims normally again.
- Within another 1 or 2 days the tadpole starts developing the left front leg and swims on its left side.
- Lastly the right front leg develops within a further 1 or 2 days. After the appearance of the last limb, the tadpole swims normally again.

Due to the rapid development that takes place, it is very important to monitor the specimens closely as some tadpoles may drown if kept in too much water or are not removed in time. Consequently, during weeks 7 and 8 the water volume should be dropped to 150 ml or less. As the dietary needs also change at this point, the water quality needs to be monitored for ammonia spikes. Once the front legs start to develop the water should be decreased once more to about 60 to 80 ml.

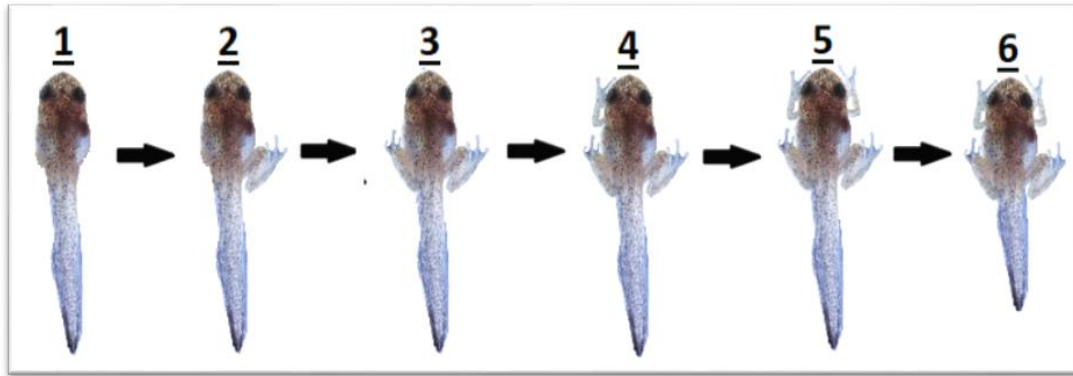


Figure 11 Limb development stages from week 6 to week 8

Metamorphs

Once the fourth limb develops, the specimen has lungs and starts to breathe air directly, and it can climb and move about freely. At this stage it is known as a metamorph, and it is moved to a life stage appropriate enclosure ([Figure 12](#)). This is a 1-litre tub with a perforated lid lined with a wet paper towel. Pebbles are placed inside to allow the metamorphs to climb. The tub is misted so the paper towel remains moist. While metamorphs do not normally feed it would appear, food can be offered on the last day even though the metamorphs may not eat. Once the tail is fully absorbed (after 3 to 4 days) the metamorphs are then transferred to the vivarium enclosure setup for the next stage of their development.

According to JCPZ observations, previously the metamorphs were limited in their jumping ability and could only walk until the tail was fully resorbed. However, the JCPZ found that by changing the diet, the metamorphs were forced to exercise the hind limbs and this assisted muscle development. After the diet change the metamorphs could jump even with the tail present (Becker, Du Plessis, Malepa, & Netsianda, 2019).



Figure 12 Metamorph: in tub (left) and close up (right)

Froglets

Ambient temperatures inside the developing froglets and mature frogs' enclosures are set from 24° to 28°C. Newly developed froglets that would have just lost their tail or only have a short length of tail left to resorb can be housed in similar enclosures as the adults, but instead of tilting the enclosure forward the vivarium is kept horizontal (level). Fine paper-towels are used as substrate for the first 4 weeks. Pebbles are placed inside enclosures on the paper towels to provide the option for the froglets to climb onto the pebbles. No water bowl should be placed inside the enclosure as it poses a drowning risk for the froglets. During this stage the froglets are dependent on oxygen and it's important for them to move around to strengthen their muscles for climbing, walking and jumping.

Within two weeks the newly developed froglets are completely developed and able to move around as the adult specimens but are still quite small compared to the adult. At this stage the specimens can be transferred into a similar enclosure placed horizontally. It should be ensured that all specimens that get transferred are able to walk, climb as well as jump before transferring them (du Plessis et al 2022)



Figure 13 Froglets showing the lack of a tail. Froglets can be brown, yellow, or beige in colour for the first few weeks

Chapter 5.

Nutrition

Nutritional requirements

Nutrient requirements for amphibian species in general and PRFs in particular are mostly unknown (REF, du Plessis et al 2022). In general, when recommending nutrient intakes for species with unknown requirements, the accepted practice is to use published National Research Council (NRC) nutrient requirements of 'related species' (Ferrie, et al., 2014). Nutritional needs of PRFs are strongly related to their veterinary and husbandry needs and vary with their developmental stage. For example, adult PRFs are insectivorous while the tadpoles are herbivorous (feeding on algae). It is therefore very important to provide proper nutrients in an acceptable dietary form to the frogs to ensure they are receiving the best care. Additionally, by providing a variety of insects, the frogs get accustomed to a range of prey items and don't become habituated to one specific food item. This improves their chances of survival when they are eventually returned to the natural habitat as they will take several prey items as or when the opportunity presents itself. Captive amphibians often face deficiencies of calcium, Vitamin A, Vitamin D3 (Donoghue, 1998), and Vitamin B1 (Ferrie, et al., 2014). In addition to these deficiencies, it is also important to pay attention to the Ca:P ratio within the food. [Table 5Table-5](#) provides a summary of the required levels of the most important macro- and micro-nutrients based on the choice of feeder insect.

Energy and lipids

Energy demands of the PRFs vary by stage and is influenced by environmental factors (e.g., higher temperatures lead to increased digestive rate and metabolic rate) (Ferrie, et al., 2014) . Most insect larvae are high in energy and frogs are likely to get adequate energy from an insectivorous diet. The diet must instead be monitored for the diluting effect energy can have on other nutrients.

As there is likely only a minimal amount of carbohydrate in the wild diets, amphibians are likely only receiving a minimal amount of energy from this source (Browne, 2009). They likely receive most of their energy needs from the lipid content of the diet. Excess fat needs to be avoided as fat will reduce voluntary feed intake (VFI), reducing the intake of essential nutrients and leading to deficiencies. Although the NRC (1993) recommendations for fish (as the related species to PRFs) do not provide a dietary lipid level, it is prudent to presume that frogs need a diet with a moderate fat content ($\pm 45\%$) (Hadfield, Clayton, & Barnett, Nutritional Support of Amphibians, 2006). As insects are relatively high in fat (over 19% in the case of an adult cricket), the dietary needs of the PRFs can be supplied by providing feeder insects with proper gut loading.

Protein

According to the NRC, amphibians have a protein requirement of 44 % and high protein levels are associated with fewer developmental abnormalities. Fortunately, most commercially available feeder insects are good sources of protein (Ferrie, et al., 2014).

Calcium and Phosphorus

Calcium is an important macro mineral, playing a role in the function of nerves and muscles, and in bone formation. Phosphorus is also a macro mineral and is important structurally for bones, teeth. It is also a number of important functions within the body which includes forming a part of cell membranes as phospholipids, in energy transfer as ATP, and in phosphorylation of proteins.

Phosphorous levels are usually not an issue in captivity, but feeding levels should be kept in mind as an improper calcium: phosphorous ratio can cause metabolic issues. The ratio of calcium to phosphorus (Ca:P) of 2:1 is necessary to maintain adequate health (Manouria, 2014; Ballard & Cheek, 2017).

A shortage of calcium ions in the blood (hypocalcaemia) will cause hormones in the body to mobilise calcium from the bones to maintain nerve impulses and muscle function (Bischof, 2009). Long term shortage of calcium can cause Metabolic Bone Disease (MBD). For adult PRFs, the recommended calcium levels can be achieved by including 0.6 % calcium in the diet ([Table 5Table 5](#)). While juvenile PRFs have a higher calcium requirement than adults (due to their growth needs (Ferrie, et al., 2014); (Manouria, 2014)), the JCPZ has not experienced any MBD in the PRF when using the same supplementation technique as for adults.

Vitamin A (Retinoid)

Vitamin A is an essential micronutrient but is frequently a problem in captive populations fed insect-based diets (Rodriguez & Pessier, 2014). Both hypovitaminosis (under-supply) and hypervitaminosis (over-supply) is possible with Vitamin A; the former is more frequently a problem than the latter.

Hypovitaminosis A is associated with Lingual squamous metaplasia or Short Tongue Syndrome (STS). In STS the cells on the mucous cells of tongue of the amphibian are affected, and become keratinised. This causes the tongue to shorten and become less sticky, making it harder for the amphibians to capture prey. In extreme cases it can lead to starvation (Browne, 2009). A shortage of Vitamin A can be prevented by supplying 0.4 ug Vitamin A/gram bodyweight/week (Hunt Coslik, Ward, & McClements, 2009).

Hyper-vitaminosis A might occur when the concentration of Vitamin A within the food is too high and clinical signs are MBD, anaemia, liver disease, and weight loss (McWilliams, 2008). As hyper-vitaminosis A has only been observed in amphibians which are fed with mammalian liver or immature rodents, it is unlikely to happen in PRFs as all post-tadpole stages of PRF are insectivorous (and insects are normally low in vitamin A). PRFs are therefore unlikely to develop hyper-vitaminosis A when this guideline is followed.

Vitamin D₃ (Cholecalciferol)

Vitamin D, especially vitamin D₃, is another critical vitamin in amphibians. When this vitamin is fed in high quantities, hyper-vitaminosis D develops (McWilliams, 2008), which leads to mineralization of soft tissue like muscles (Hoff, Frye, & Jacobson, 1984). This affects the mobility of the specimen and reduces its ability to catch prey. Hypo-vitaminosis D is caused by a diet insufficient in Vitamin D or by lack of UVB light. Deficiencies of Vitamin D can result in a variety of diseases, including MBD, seizures, oedema, poor growth, reproductive problems, muscle weakness, gut stasis, and hatching failure (McWilliams, 2008). According to the NRC, adult amphibians need 1,111 IU/Kg Vitamin D₃ to fulfil their nutrient requirements. As the PRFs at JCPZ do not show any of the problems mentioned above, the diet is deemed to be sufficient in Vitamin D.

Nutrient	Adult requirement	Nutritional content of commercial feeder insects					
		Adult cricket	Roach	House fly	Mealworm larvae	Super-worm	Soldier fly larvae
Crude protein %	44.4	58.5	47.4	85.8	36.4	32.5	35.1
Crude fat %	-	19.4	25.0	8.3	26.1	29.2	28.1
Calcium %	0.6	0.1	0.1	0.3	0.0	0.0	1.9
Phosphorus %	0.3	0.8	0.4	1.6	0.6	0.4	0.0
Vitamin A IU/kg	2,914	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin B ₁ ppm	12	1.1	2.2	49.2	4.7	1.0	15.4
Vitamin D ₃ IU/kg	1,111	0.0	482	434	0	0	200

*Table 5 Values of important nutrients in feeder insects
Adapted from Ferrie, et al. (2014)*

JCPZ Feeding Programs

Diet and feeding behaviour

During field expeditions, JCPZ and EKZNW staff observed that the potential food for the frogs include mosquitoes, midges, ants, small spiders, small insects, and aphids. Prey is caught opportunistically by ambush methods. The frogs remain stationary on the reeds and capture prey when it comes within striking distance.

Feeding of froglets and adults

The preferred way to feed PRFs is to provide them with a variety of live insects (e.g., crickets, aphids, fruit flies) as shown in [Table 5](#). The feeder insects should be gut loaded for 24 hours to ensure they have a better micronutrient profile. In addition, all feeder insects should be dusted with calcium supplement just prior to feeding to ensure the macro mineral requirements of the PRFs are met. The ingredients in the supplement include calcium carbonate (35 %), ground oyster shell and salt, and the composition is shown in [Table 6](#).

Mineral	Concentration
Calcium %	35.0
Sodium mg/kg	700
Potassium mg/kg	400
Magnesium mg/kg	300
Sulphur mg/kg	400
Iron mg/kg	240
Zinc mg/kg	9
Manganese mg/kg	5
Copper sulphate mg/kg	2

Table 6 Composition of mineral supplement used for Pickersgill's reed frogs

As most amphibians require movement to trigger a feeding response, all prey should be offered live. Frogs will take any prey item they can fit into their mouth, so size appropriate insects should be released into the enclosure to allow the frogs to hunt. The feeding method differs depending on the enclosure setup.

Adult frogs are offered medium sized crickets (9 – 13 mm) or fruit flies. It is important to rather overstock an enclosure than to offer too little food, as food limitation is a stressor. By observing the specimens daily, the body condition can be monitored to establish if enough food items are provided. It is not necessary to place the crickets in a container as the substrate (sphagnum moss) does not accumulate excessive water. The crickets can move freely around until eaten by the frogs.

Juvenile frogs are offered pinhead crickets (crickets of ant size, 1.5 mm) or fruit flies. When feeding the frogs housed in a paper lined vivarium, the crickets should be placed in a shallow container (e.g., lid). Excess water in the paper towel-lined vivaria will cause the crickets to drown, especially pin-head crickets. The frog's feeding response is triggered by movement, and drowned crickets will not be consumed. This leads to wastage and insufficient feeding levels.

When specimens are housed in a 5-litre plastic jar, it is good to add a leaf to the jar before feeding. The pin-head crickets can be placed in the jar, but as the surface of the jar is moist (spraying with RO water) the risk of the crickets drowning is quite high. The leaf therefore provides a surface for the insects to stay on until noticed by the frogs.

Drinking water can be provided in another dish or by the more permanent water body inside the enclosure. As frogs can absorb water through their skin (Hevesy & Krogh, 1935), they should also be able to get into the water. Because of the permeability of the frog's skin, minerals are also absorbed through the skin (MacRobbie & Ussing, 1961).

Life Stage	Food	Quantity of Food	Feeding Intervals
Newly hatched tadpoles	No food required	N/A	Not to be fed within the first 24-hours after hatching
Tadpoles and Developing tadpoles	Powdered spirulina and Sera fish flakes mix	1 x pinch per 20 tadpoles in 900 ml RO water	Daily
Metamorphs	Pinhead crickets	Minimum 5 crickets per specimen	Daily
Frogllets	Pinhead crickets Fruit flies	Minimum 5 crickets per specimen, ad lib	Daily
Young adult and Adults	Pinhead to very small crickets, fruit flies	Minimum 5 crickets per specimen	Daily

Table 7 The feeding program for the various life stages of Pickersgill's reed frogs

Feeding of tadpoles and metamorphs

The first 48-hours after the tadpoles' hatch, they should not get fed as they still have a yolk sac. The specimens are black in colour but change to brown-beige within 48 hours. This is the indication that the specimens are ready to be fed.

For the next 6 – 7 weeks (up until complete tail absorption in the metamorph) these specimens are fed a mixture of finely crushed fish flakes and spirulina powder or algae powder. One pinch of food per day per tub is provided. The JCPZ makes use of fish flakes with the nutrient analyses and ingredients shown in [Table 8](#)

Nutrient	Concentration
Crude protein (Min) %	44.2
Moisture (Max) %	7.1
Crude fat (Min) %	6.9
Crude ash %	12.9
Crude fibre (Max) %	5.1
Vit. A IU/kg	16,800
Vit. B ₁ IU/kg	16
Vit. B ₂ mg/kg	41
Vit. D ₃ IU/kg	820
Vit. E (D, L- α -tocopherol acetate) IU/kg	54
Stabilised Vit. C (L-ascorbyl monophosphate) mg/kg	250
*Ingredients of the fish flakes consist of fish meal, wheat flour, brewer's yeast, Ca-caseinate, gammarus, mannan oligosaccharides (0.4%), cod-liver oil (containing 34% omega fatty acids), spirulina, herbs, alfalfa, stinging nettle, green-lipped mussel meal, parsley, sea algae, paprika, spinach, carrots, Haematococcus algae, garlic.	

*Table 8 Nutrient composition of *fish flakes fed to Pickersgill's reed frogs*

Chapter 6.

Enrichment program

Animal training

Definition

Environmental enrichment is the component of daily animal husbandry focused on how animals interact with their physical and social environment. Behavioural enrichment” – allowing captive animals a wider choice of natural behaviours in which to engage (Young, 2003).

This is a critical component in achieving the highest level of animal husbandry practices particularly in modern zoos.

Objective

The purpose of the PRF behavioural enrichment program is to improve animal welfare by providing opportunities for the animals to express species- typical behaviours and eliminate possible stereotypic behaviour.

Behavioural and environmental enrichment is a vital part of modern captive animal management. By supplying enrichment; animals are provided with mental stimulation, and are encouraged to display natural behaviours, make use of their entire enclosure and make choices about the activities they partake in, thus enabling them to have some control over their own environment. (Young, 2003). The PRF behavioural and environmental enrichment program went a long way to contributing to the specimens’ overall welfare by:

- Increasing positive utilization of space
- Increasing the range of expressed innate behaviour
- Enabling animals to exhibit species-specific behaviours
- Influencing behavioural repertoires and stress levels positively
- Preparing the frogs for release.

Pre- release: Using enrichment as a tool

Although research on environmental enrichment has been more focused on mammalian species; reptiles and amphibians can also benefit when afforded the choice to engage in more innate behaviours. An environmental and behavioural enrichment program used during the *Ex-situ* phase, particularly while preparing the frogs for release, may have aided in the success of the conservation and translocation process of the ARP.

Method

The JCPZ enrichment program is based on a framework developed by Disney’s Animal Kingdoms, Science and Environmental Animal Enrichment Program. The S.P.I.D.E.R. framework allows the zoo to review, refine, and modify this model to fit its own needs (Mellen & MacPhee, 2001).

The acronym S.P.I.D.E.R. stands for; Setting goals, Planning, Implementation, Documentation, Evaluation and Re-adjustments (Mellen & MacPhee, 2001). To evaluate the effectiveness of enrichment activities of the PRF at JCPZ, observations were carried out for; 30 minutes daily, over a period of 18 months; using a random sampling method.

Setting Goals

The goal of behavioural enrichment is to enhance a captive animal's living conditions beyond the primary husbandry requirements needed for survival. There are a number of factors that need to be considered, in detail when setting enrichment goals (Mellen & MacPhee, 2001). These include; the natural history and individual history of the species as well as the exhibit in which the animal is to be housed and which behaviours should be encouraged or discouraged through enrichment activities (Mellen & MacPhee, 2001). There are several categories of enrichment; however, when looking into the requirements of the PRF, the focus was on feeding behaviour, social behaviour, sensory behaviour and enclosure design.

The questions relating to the natural and individual history of the PRF are outlined below:

Natural History

Listed below are the questions we considered for the natural history of the PRF.

- PRF Innate behaviours; communication, feeding, reproduction and migratory behaviour.
- Perception of environment; Habitat and utilization of space.
- Activity Budget; Behavioural Repertoire - time engaged in social behaviour, hunting, feeding, locomotion and resting periods
- Interactive behaviour; Interspecific
- Intraspecific- (other frog species and predators)

Individual History

During the goal setting phase, the PRF's individual history was investigated; the focus was on whether the frogs were wild caught or captive bred. Listed below are the behaviours we considered:

- Problem solving
- Learned behaviour
- Desensitization
- Habituation
- Classical conditioning

Planning

Planning involves the formation of an enrichment program to achieve desired behavioural goals. The enrichment activities should allow animals to have choice and control over their environment.

Plan development decisions included:

Which behaviours to encourage or discourage?

What resources were needed to create the enrichment activities?

Who would be involved?

Would there have been any safety concerns?

Implementation

Implementing an enrichment program is providing for or physically carrying out the enrichment activities.

Implementation required:

Scheduling of enrichment activities

Ensuring the enrichment items were available

Providing activities in order to create novel presentation methods

Varied schedules

Enrichment Activities



Figure 1414 JCPZ Female Pickersgill's Reed Frog

1. Leaf variation (reeds, Cyprus).
2. Rocks – to encourage movement.
3. Feeding variations (crickets/flies).
4. Variety of feeding containers.
5. Alternative or additional substrates.
6. Enclosure furniture rotation.
7. Hiding spots (inside bamboo).
8. Exhibit design.

Documentation

By documenting the enrichment provided JCPZ could evaluate the success or failures of the enrichment activities and make decisions on whether to continue, discard or make adjustments to the activities.

Animal collection staff were required to make use of the enrichment scheduling and observation record documents detailing:

- The date enrichment was provided
- The behavioural goal of the enrichment activity
- What enrichment activity was provided
- The behavioural observation notes pertaining to the enrichment activity

Additional tools used:

- Video recordings
- Photographs

Evaluation

Evaluating the results of the documentation to determine the effect of the enrichment activities; by evaluating the success or failure of activities, assisted in making determinations on whether to repeat successful activities and discard or adjust failed activities (Mellen & MacPhee, 2001).

Evaluation of the results needed to happen on a regular basis through:

- Observations
- Meetings
- Individual evaluation of enrichment initiatives from records

Evaluation of Stress

Evaluation of stress on captive bred specimens was an important factor of the pre-release program. Captive bred specimens could have lived in a relatively “stress free” environment than their wild counterparts due to the lack of predators and fluctuations in temperature, water and the amount of available food (Davis & Maers, 2011). We needed to induce stressors in a controlled manner in order to prepare the PRF's for release. A certain amount of stressors induced can be beneficial to captive bred or housed animals as lack of stress could lead to a decrease of innate behaviour. We had to be cautious of the amount of stress induced in order to avoid chronic stress.

Goal Setting	Implementation	Observation	Measures of success & welfare
Natural and Individual behaviour			
Learned behaviour / classical conditioning	Introducing stressors (changes in temperature, changes in food and water quantity and quality)	Changes in movements; Hunting/Eating readily or not	Alertness, Responsiveness Body condition
Innate behaviour	Competition calls from visible males in adjacent enclosures	Communication behaviour	Vocalizations & physical changes (e.g., Pigment changes and postures)
	Offering alternative food items	Feeding behaviour	Normal food consumption
	Breeding stimulation - Spraying enclosure with water and optimal temperatures	Reproduction behaviour	Vocalizations, Amplexus, Egg laying and fertilization
Environmental perception	Providing vertical furniture (reeds, grass, etc)	Migratory behaviour	Vertical migration on reeds
Environmental perception	Enclosure design: size, landscaping, plants, water quantity, hiding spaces, other substrates.	Enclosure utilization	Normal development (juveniles), Normal health and behaviour in adults.
Activity Budget			
Behavioural repertoire	Offering alternative food presentation puzzles	Time hunting and feeding	Body condition
	Changes in furniture, substrate and amount of water (increase or decrease accordingly)	Sitting, hopping, swimming, possible inactivity	Time active and inactive (too much vs. too little)
Intraspecific interactions	Optimal Sex ratios	Mimic natural social structure	Healthy competition
	Housing together or in adjacent enclosures.	Reproduction, Competition, Breeding,	Vocalizations
	Optimal number of specimens being housed together during life stage (juvenile vs adults)	Change in social structure and resultant behaviour during development	Mate selection
		E.g., immature males lower down vs. adult males calling higher up furniture	Breeding success
		Seasonal changes in social structure	
Interspecific interactions	Human interaction during enclosure servicing and feeding	Reaction to humans	Avoidance behaviour
		Fear in juvenile frogs (jumping away) vs. desensitization and habituation in adults	Jumping/hiding/swimming/changing colour Fear response (flight/freeze)

Table 9 Evaluation of enrichment implementation

Re-adjustments

Re-adjustments needed to occur throughout the enrichment framework. Alterations to the enrichment program was required during and after implementation of enrichment activities and, after evaluation of enrichment records had been completed. If the results were poor, identify the cause and revert back to the goal-setting phase.

Conclusion

The behavioural differences between wild caught frogs vs. captive bred frogs were evaluated during the ARP; observations carried out on wild frogs and captive bred frogs that had been released *In-situ*; showed little to no difference in behaviour.

The implementation of enrichment for the PRF played a role in ensuring the survival of captive bred specimens *In-situ*. The enrichment activities were based on the behavioural repertoire of the specie observed in the wild. The enrichment program contributed to the success of the breeding program *Ex-situ* and may have assisted in ensuring the sustainability within the insurance population *In-situ*.

Chapter 7 Veterinary care

Veterinary services

Veterinary services are a vital component of excellent animal care practices and emphasize preventative medicine, a 30-day quarantine period for incoming frogs, and ongoing health assessments (parasitology, chytrid screening, husbandry protocols). The basis of health care for the PRF at JCPZ is a high level of biosecurity and a quarantine-like approach to husbandry to reduce the risk of a disease outbreak. Each PRF life stage is housed differently in isolated vivarium units and monitored daily.

Continued research into amphibian-prone conditions (skin lesions, parasitism, nutritional imbalances, husbandry related conditions) will help determine what is normal and abnormal for the species and help to establish therapeutic regimes. The keeper staff is crucial to and responsible for noticing and alerting curators and veterinarians should any health concerns arise. Animal record keeping is an important element of animal care and ensures that information about individual specimens and their treatment is always available.

Identification methods

As part of the veterinary and population management tools it is important to be able to identify individuals and /or genetic groups. Identification of amphibians comprises either non-invasive or invasive techniques. Non-invasive techniques are most preferable, because the amphibian will not be harmed (Odum & Sonntag, 2010), and stress for the animal is avoided. However, non-invasive identification methods are non-applicable for species like the PRF because they can change colour and they do not appear to have a fixed pattern, which can identify individuals. Another non-invasive method to identify a PRF is to isolate a specimen or a breeding pair. This is applicable if the frogs do not participate in a breeding project. If they do, this method will not be useful either, because swapping the animals to make new breeding pairs will make this method inefficient.

Invasive identification methods are techniques that bring temporary harm to the amphibian and in some instances have caused mortality. Examples of invasive techniques are tagging, ringing or toe clipping. Many of the invasive methods are not applicable to PRFs as these amphibians are simply too small. For instance, a tag with a certain number, or a coloured ring are too big to apply on a PRF. The toe clipping method, however, can be applicable to PRFs (Odum & Sonntag, 2010) , but is not preferred at JCPZ. Another invasive identification technique is to make use of Visible Implant Elastomers (VIE). The elastomers are made of medical grade, silicone-based material constituted immediately before use. The elastomers are injected subcutaneously (S/C) or superficially intra muscularly (I/M). As there are ranges of different colours available, this technique can identify individual frogs satisfactorily. In situations where colours and obvious marks on the animals are undesirable, an invisible elastomer that is only visible under a black light is preferable. Within the JCPZ collection, this is the preferred method.

Quarantine

All incoming specimens undergo at least a 30-day quarantine before they join the collection. This occurs in a separate quarantine facility with the ability to accommodate amphibians. The quarantine is under the charge of a veterinarian and health checks are done, including physical examination, chytrid screening and parasitological screening. Biosecurity is strict and access to the frogs is limited to authorized personnel only. The following are recommendations and suggestions for appropriate quarantine procedures for amphibians:

1. direct and floatation faecal sample
2. swabs for chytrid testing
3. proper records to manage the quarantine period accurately such as
 - i. personnel accessing the quarantine including details such as name, date, time
 - ii. animal identification, arrival dates and quarantine cancellations
 - iii. all mortality, morbidity, disease diagnosis and treatment regimens.

Common diseases and disorders

Keepers and veterinary staff monitor closely for the most common diseases under the following categories (1) nutritional (2) microbiological (3) husbandry related, and (4) parasitic.

Nutritional diseases

Diseases related to nutritional deficiencies or excesses are quite common in captive amphibians. This is because their nutritional requirements are largely unknown, and it is difficult to simulate in captivity what they consume

in the wild (REF). Incidences of neurological and musculo-skeletal abnormalities associated with spindly leg syndrome and paralysis have been attributed to vitamin deficiencies (Wright & Whitaker, 2001); Crawshaw, 2003). The common nutritional conditions have been highlighted in the nutrition section, and the nutrition associated challenges regarding the PRFs have also been reported and how they were dealt with.

Infectious diseases

Bacterial infections in amphibians are generally opportunistic and associated with other problems such as traumatic injury, poor hygiene and following viral infections and mycotic skin infections (Densmore & Green, 2007). One of the most common bacterial diseases affecting captive amphibians is bacterial dermatosepticemia, also commonly known as red leg syndrome. It is a generalized systemic bacterial disease associated with cutaneous erythema, a reddening occurring most often on the ventrum or extremities. Gram-negative bacilli including *Aeromonas hydrophila*, *Citrobacter*, *Proteus*, *Salmonella*, some Gram-positive bacteria e.g., *Streptococcus* and *Staphylococcus* have been linked to this syndrome (Crawshaw 1992; Mauel et al., 2002). The erythema is due to vasodilation, congestion, and petechial, paintbrush, or ecchymotic haemorrhages hence the name red leg syndrome. Other clinical signs associated with red leg syndrome are anorexia, swelling, generalized oedema or oedema localized to extremities or the lymphatic sacs, coelomic effusions, and epidermal erosions, ulcers, sloughing, or necrosis. This disease may also present as sudden death, with few or no overt signs.

Another common disease presenting similar clinical signs to red leg syndrome is flavobacteriosis, or a bacterial disease associated with the genus *Flavobacterium*. Clinical signs and gross findings of flavobacteriosis are nonspecific and include effusions in the lymphatic sacs, hydrocoelom, lingual or corneal oedema, panophthalmitis, petechiation, and visceral congestion (Keller & Shilton, 2002); Olson et al., 1992; (Taylor, 2001). However, it is important to note that causes of effusions into the lymphatic sacs and body cavity of amphibians are many and include ranaviral infections, other systemic bacterial infections, renal disease, lymph- and heart-disease.

Compared with known viruses of other lower vertebrates, there are very few described pathogenic viruses that affect amphibians (Densmore & Green, 2007). Of great concern are Ranaviruses (family Iridoviridae), double-stranded DNA viruses that infect fish, reptiles, and amphibians (Gray & Chinchar 2015) and considered the second most common infectious cause of mortality in amphibians after Chytrid fungus (Chinchar et al., 2002; Price et al., 2014). These viruses have caused amphibian die-offs on five continents; North and South America, Europe, Africa and Asia (Miller et al., 2011; Duffus et al., 2015). Disease presentations vary from sudden death with few or no clinical signs to high percentages of severely affected individuals. Disease is generally systemic and presents acutely with incubation periods ranging from a few days to 2 weeks (Wolf et al., 1969). Clinical signs may include lethargy; anorexia; abnormal body posture, abnormal swimming behaviour, or buoyancy deficits; erythematous skin associated with petechial or paintbrush haemorrhages, particularly around the mouth or base of the hind limbs; raised, vesicular, or erosive skin lesions; and focal to generalized swelling due to effusions in the lymphatic sacs and body cavity (Docherty et al., 2003; Johnson & Wellehan 2005; Wolf et al. 1969). Internally, there may be oedema, enlargement, and haemorrhage or discoloration of numerous internal organs including the spleen, liver, kidney, and gastrointestinal tract.

Currently, the most significant and well-described pathogen of amphibians is Chytrid fungus, (*Batrachochytrium dendrobatidis*, and *B. salamandrivorans*). Chytrids are ubiquitous, keratinophilic or chitinophilic, sporozoic fungi located in moist and aquatic environments and *Batrachochytrium dendrobatidis* research reported its presence in South Africa. Clinical signs include lethargy, dehydration, dysecdysis, hyperaemia of skin, and occasional neurological signs of abnormal posture, loss of righting reflex, and behavioural aberrancies (e.g., absence of fear when approached and captured). Additional gross findings include thickening of the skin (hyperkeratosis) associated with dysecdysis and, when secondary bacterial or other fungal infections are present, ulcers, petechiae, and ecchymoses of the skin and congestion of viscera. Mortality rates may be quite high, clinical signs may be variable or minimal preceding death, and secondary epidermal infections may complicate the diagnosis. At JCPZ regular swabs are taken from the frogs for molecular identification using PCR (although only the test for *B. dendrobatidis* is currently available in South Africa). The tests carried out so far for the *ex-situ* collection have been negative for chytrid.

Another common infectious disease of aquatic lower vertebrates is saprolegniasis, which is caused by water moulds such as *Saprolegnia* spp. and similar agents (e.g., *Achlya* and *Leptolegnia*) (Densmore & Green, 2007). These organisms are ubiquitous in aquatic environments worldwide and they are frequently involved in secondary superficial infections of aquatic anurans. Saprolegniasis may have its greatest impact on amphibian

eggs, resulting in highly variable mortality levels depending on environmental factors and overall condition (or fertility) of the eggs (Blaustein et al. 1994). However, water moulds also commonly invade eggs after death, so it may be difficult to determine whether these organisms represent a causative factor in an egg mortality event (Densmore & Green, 2007). In affected eggs, the capsules may appear to have a thin layer of white fuzz over the surface. Fungal growth is usually white to pale grey but may vary in coloration depending on host, duration of infection, species of mould, and water quality, including suspended particulate matter in the water.

Water moulds may also be primary skin or oral pathogens among larval amphibians. In general, a sustained low-level chronic mortality is the most common presentation, and often an underlying causal factor such as traumatic injury or an infectious agent may also be apparent. Clinical signs of saprolegniasis in larval amphibians include the external appearance of fungal colonies that appear fluffy or cotton-like in texture. Erythematous or ulcerated skin may also be visible. Although infections are generally superficial, affecting the tail, hind limbs, gills, and oral mucous membranes without becoming systemic, lesions can sometimes deeply penetrate and involve underlying tissues.

Husbandry-related diseases

Diversions from ideal environmental conditions may be extremely detrimental to health and may relate directly to development of disease or act as one or more stressors that indirectly predispose animals to diseases. The skin of amphibians is highly permeable and has unique physiologic functions including water and electrolyte exchange and, in some species, respiration. Skin irritation due to water quality problems (e.g., elevated ammonia) is frequently identified in association with epidermal hyperplasia and hyperkeratosis. Concurrent bacterial and fungal infections can be observed but are likely secondary or opportunistic because of a multifocal distribution rather than being present diffusely throughout the lesions (Densmore & Green, 2007). Husbandry-related factors such as water composition, environmental parameters, and nutrition have also been implicated in kidney disease (Ferrie, et al., 2014).

Parasites

Nematodes, or roundworms, are also common helminths that infect amphibians from egg to adult life stages and affect a variety of organs and tissues. Filariid nematodes, which mosquitoes transmit, typically reside only in wild-caught adult amphibians or outdoor amphibian colonies exposed to vectors. Clinical signs usually are vague, but it is occasionally possible to detect those species of filariids whose adults occupy the lymphatic sacs as serpentine nodules or ridges under the skin. Diagnostic methods for filariids include examinations of stained blood smear slides or unstained wet-mount drops of blood for microfilaria. Other helminths reported to infect amphibians and occasionally produce disease include cestodes, acanthocephalans, and hirudineans. Cestodes, or tapeworms, are not commonly isolated from or problematic in amphibian species, but they may produce significant gastrointestinal lesions, gastrointestinal obstruction, and death in heavy infections (Wright K. , 2006). At JCPZ monthly faecal flotation tests for internal parasites are done to check for infestation.

Mortalities and losses

If a specimen is found dead, the carcass is placed in a secure plastic container and taken to the veterinary hospital. A post-mortem is done on the carcass and samples are sent for pathology. The leftover carcasses are incinerated as soon as possible. While no mortalities have been attributed to any of the infectious causes highlighted, tests on two separate clutches of eggs that failed to hatch were positive for *Aeromonas hydrophila*, a gram-negative bacterium and another clutch was positive for the fungus *Glucosporium species*, a soil saprophyte. These seemed to be isolated incidences in that they did not recur, but nevertheless biosecurity processes were reviewed. Some mortalities of tadpoles were linked to low temperatures because of the breakdown in the heating system, which was addressed.

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Annexure I. Excerpt from JCPZ Animal Management Policy

Introduction.

The Animal Management Policy provides Biodiversity Strategy and Action Plan for the Johannesburg City Parks and Zoo, articulates actions through which to implement the vision, strategic objectives and actions necessary for the conservation, protection, use and development of Biodiversity. It also provides an overview of the local, provincial, national and international laws and obligations for biodiversity. Johannesburg City Parks and Zoo is an implementing agent on issues of Biodiversity for City of Johannesburg. This includes but not limited to Protected Areas and Zoo Management. The overall biodiversity vision for the city is to “Conserve and manage biodiversity and the city’s environmental heritage to ensure the delivery of sustainable and equitable ecological goods and services to the citizens of Johannesburg, now and in the future”. The Animal Management Policy (AMP) provides guidelines in terms of principle of Biodiversity Management including but not limited to Acquisition and Disposition of species, Breeding Programmes, Protected Areas Management, Game Ranch Management, Ecological Management and Animal Collection Plans. Should an adequate Animal Management Policy (AMP) not exist, the result would be a lack of proper population management which could result in imbalanced gender ratio, over-extended carrying capacity, intra-species aggression and unnecessary costs relating to resources used.

The prevalence of inbreeding would also be increased should proper animal collection management not be undertaken. An AMP gives an overview of the current state of JCPZ animal collection and the role of each species within it. It is an important source of information to all staff and a key tool that is used to plan the future and progress towards it.

Fundamental to the concept of a collection plan is the notion of species ‘role’. As well as the species role(s), the collection plan also contains some basic information about each species including common and scientific names, geographic range and IUCN Red List threat category and, to manage each species and the collection as a whole, a variety of other operational data is included. Data relevant to managing the species and collection includes the number of each species currently held (males, females and unsexed), the target number for each species, the current and future location in the zoo, and breeding recommendations. Links to husbandry guidelines, diet sheets and relevant in situ field programmes are also provided.

Legislation guiding the policy.

- Biodiversity Act 10 of 2004
- Municipal Finance Management Act, 56 of 2003
- Protected Areas Act, 57 of 2003
- Protection of Animals Amendment Act, No.7 of 1991 (Prevent cruelty to animals)
- Animal Protection Act no 71 of 1962
- Veterinary and Par-veterinary Professions Act, no 19 of 1982
- Animal Diseases Act no 35 of 1984
- Animal Identification Act no 6 of 2002
- Animal Health Act no 7 of 2002
- Societies for the Prevention of Cruelty to Animal Act no 169 of 1993
- Environmental Conservation Act 73 of 1989
- National Forest and Fire Amendment Act of 2001
- Performing Animals Protection Act no 24 of 1935
- Environment Conservation Amendment Act no 50 of 2003
- National Veld and Forest Act no 101 of 1998
- Meat Survey Act no 40 of 2000
- Water Act no 36 of 1998

Acquisition and disposition process.

Acquisition process.

1. Acquisitions must abide with the Supply Chain Management Policy and all relevant laws.
2. All acquisitions should be recorded on the animal record keeping system (ARKS).
3. Donations may only be accepted according to the mission and vision of JCPZ considering the conservation, scientific, educational and exhibit goals.

4. Some animals may be acquired temporarily, such as for holding for governmental agencies, rescued animals or rehabilitation animals etc. These animals should only be accepted if suitable accommodation is available and if they pose no threat to the Zoo's current animal collection.
5. All animals acquired outside the Zoo must undergo a one-month quarantine period during which a health check should be conducted by the Veterinary Department. These animals should be quarantined in an enclosure that is suitable for that species.
6. Animals acquired by birth should be recorded on the ARKS system. Animals with a relatively high neonatal mortality rate such as fish should only be added after 30 days of age.
7. The Zoo must have all the resources necessary to properly care for the animal being acquired.
8. All effort should be made to acquire animals that are part of a managed program such as the African Preservation Program (APP), the European Endangered Species Program (EEP) or the Species Survival Program (SSP) etc.
9. Animals are only to be acquired from sources that operate legally and ethically.
10. Process will include:
 - a. Preparation of memo to deviate from normal acquisition process
 - b. Authorisation and Approval to deviate by the Accounting Officer

Disposition Process.

1. Dispositions must abide with supply chain management policy and all relevant laws.
2. Any animal that is removed from the Zoo's collection may only go to persons able and qualified to care for them properly.
3. Zoo animals may not be disposed of at animal auctions. The process will be monitored through declaration of intent form.
4. Animals from JCPZ protected areas may be sold via different methods which are guided by the laws of South Africa
5. Animals may not be disposed to persons who will sell them at an animal auction. The process will be monitored through declaration of intent form.
6. No animal may be disposed of to an individual or organisation that allows the hunting of animals. The process will be monitored through declaration of intent form.
7. All APP, EEP and SSP animals and animals belonging to a studbook or conservation program can only be removed from the Zoo if instructed so by the Studbook Keeper or Program Co-ordinator.
8. Disposition of domestic animals should be done according to acceptable farm practices and will be subject to all relevant laws and regulations. Standard operation procedures will be developed and guide the process.
9. Live animals may be released into the wild within native ranges if approved by the relevant Provincial and Governmental authorities. Please note that extensive consultation and research is normally required before such releases can take place to ensure the survival of the species.
10. Records of all dispositions (living or dead) should be maintained on the ARKS system in accordance with the:
 - a. WAZA Strategy (The World Zoo and Aquarium Conservation Strategy)
 - b. African Preservation Program (APP)
 - c. The European Endangered Species Program (EEP)
 - d. The Species Survival Programme (SSP)

Disposition of Animals after they have not been sold through direct auction or zoo tender process

- There should be a relationship with other Zoos either National and international which will encourage exchange of animals and direct sale of animals if those animals will add value to the mission of the organisation. The exchange must be of the same value as the animal in a collection
- If animals are not disposed of after tender, there could be loaned out to another credible institution

Policy Statement.

To manage Biodiversity at the Zoo and Conservation Areas in the City of Johannesburg in line with all relevant Environmental Management Acts and other prescripts of Laws.

Policy Outcome.

To have a balanced animal population

- To conserve genetic diversity which is critical to the preservation of biodiversity.

- To ensure that a healthy animal population exists that is managed according to international acceptable husbandry guidelines.
- To establish a well-managed Animal Breeding Programme within the organisation that is formalised, well monitored and updated continuously
- To have Fauna and Flora that is managed in line with relevant legislation.

Policy Objectives.

- To have a balanced animal population in the Zoo and Conservation and Biodiversity areas of the City of Johannesburg by managing the acquisition and disposition of animal collection.
- To ensure that each species has a meaningful role in the collection and to have healthy viable animal populations.
- To implement comprehensive animal breeding programmes in JCPZ that is aligned to the Corporate Strategy.
- To manage genetically healthy animal populations in the Zoo and Conservation and Biodiversity areas of the City of Johannesburg.
- To manage acquisition and disposition of animal populations in line with ecological management plans.

Implementation strategy.

The following criteria will be used when animals are acquired:

1. Conservation - Ark, Rescue, Insurance, Research, Training, Action and *In-situ* Support.
 - a. Ark - Animals Extinct in the Wild (locally, regionally or globally) and which would become completely extinct without *ex-situ* management.
 - b. Rescue - Animals that are facing an extremely high risk of extinction in the wild (locally, regionally or globally) and are being managed in captivity as part of the recommended conservation action.
 - c. Insurance - Animals for which *ex-situ* management may benefit the wild population through breeding as part of the recommended* conservation action.
 - d. Research - Animals undergoing specific research that contributes to the conservation of that taxon or related taxa (this includes clearly defined 'Model' species).
 - e. Training - Animals supporting the training of staff that contributes to the conservation of that taxon or related taxa (this includes clearly defined 'Model' species).
 - f. Action - Animals that encourage understanding of conservation issues and people's individual roles in them, and where possible engage people to take positive action to benefit conservation.
 - g. *In-situ* - Animals held in the collection for which JCPZ supports *in-situ* conservation activity.
2. Research
 - a. Animals undergoing clearly defined pure or applied research that increases knowledge of natural history, population biology, taxonomy, husbandry, disease, health management etc.
3. Learning
 - a. Animals which play a specific role in either formal or informal structured learning programmes or activities carried out by JCPZ. (Education and Marketing Department initiatives)
4. Awareness
 - a. Animals which due to distinctive appearance, behaviour, natural history, biology etc, can be used to inspire the public and develop an awareness of, and empathy for the relationships between species, the environment and people.
5. Temporary welfare/rehabilitation support
 - a. Animals held temporarily by JCPZ in addressing issues of animal welfare, wildlife conflict or confiscations of illegally held animals; normally in support of, and at the request of, conservation agencies / welfare organisations or the community. These taxa are held temporarily only; they are only housed permanently within the collection if another specific role for the animals is identified.
6. Wild animals
 - a. Management of wild animals requires a wildlife census to be completed every second year by 30 September on all bases and units containing wildlife. The census is conducted to determine the number of wildlife species irrespective of whether such species are to be disposed of. In the event of excess wildlife, the following additional information and analysis must be reported:
 - i. The size of the area
 - ii. The number, species and sex removed in the previous disposal action
 - iii. The date and method of wildlife removed in the previous disposal action

- iv. The LSU per species wildlife present
- v. The norm for the grazing capacity of the area (ha/LSU)
- vi. Grazing/browsing ratios

Annexure II. Quarantine recommendations.

Quarantine facility.

A separate quarantine facility, with the ability to accommodate amphibians should exist. An amphibian quarantine should be equipped with isolated vivarium units with heating and ultra-violet lights. Each life stage will be housed/kept differently as per the recommendations for each stage. Amphibians must be maintained at the optimal temperature for the species as determined by the Zoo staff and Veterinarian and agreed upon by the State Veterinarian, in this case optimum ambient temperatures range from 24°C-27°C. If a specific quarantine facility is not present, then newly acquired animals should be isolated from the established collection in such a manner as to prohibit physical contact, to prevent disease transmission, and to avoid aerosol and drainage contamination.

A warning sign prohibiting unauthorized entrance should be prominently displayed at all entrances. Appropriate biosecurity measures must be applied including footbaths at all entry points. Footbaths are to be filled with a disinfectant and changed every 48 hours. Staff that enter/leave the building should move through the footbath. Hands should be washed with a disinfectant (chlorhexidine or similar) and gloves (powder-free latex) should be worn prior to working with animals/enclosures. Once work is completed, gloves should be disposed of in the appropriate quarantine bin (disposed of via incineration) and hands should be washed again before leaving the facility. All waste e.g., used gloves, leftover food, moss etc. should be placed in a disposal bin and incinerated. Provision should be made within the quarantine facility for storing all equipment and food. No tools, equipment, or objects of any kind may be removed from the quarantine facility or transferred from one block to another during the quarantine period.

Quarantine length.

Quarantine for all species should be under the supervision of a veterinarian and consist of a minimum of 30 days (unless otherwise directed by the staff veterinarian). If during the 30-day quarantine period, additional animals of the same order are introduced into a designated quarantine area, the 30-day period must begin over again.

Quarantine personnel.

A keeper should be designated to care only for quarantined animals, or a keeper should attend quarantined animals only after fulfilling responsibilities for resident species. Equipment used to feed and clean animals in quarantine should be used only with these animals. If this is not possible, then equipment must be cleaned with an appropriate disinfectant (as designated by the veterinarian supervising quarantine) before use with post-quarantine animals.

Institutions must take precautions to minimize the risk of exposure of animal care personnel to zoonotic diseases that may be present in newly acquired animals. These precautions should include the use of disinfectant foot baths, wearing of appropriate protective clothing and masks in some cases, and minimizing physical exposure in some species.

Quarantine protocol

During this period, certain prophylactic measures should be instituted. Behavioural notes and feeding records should be kept. An effort to reduce stress during quarantine and an assessment of behaviours indicating that the frogs are adjusting well to their new surroundings is integral to their overall well-being and will serve to judge their future exhibit and program potential. A consistent feeding response and a significant weight gain should be demonstrated prior to exiting quarantine. Individual specimen records will be maintained on all aspects of the animal's health profile. Individual faecal samples or representative samples from large numbers of individuals housed in a limited area should be collected at least twice and examined for gastrointestinal parasites. Treatment should be prescribed by the attending veterinarian. Ideally, release from quarantine should be dependent on obtaining two negative faecal results spaced a minimum of two weeks apart either initially or after parasiticide treatment. In addition, all animals should be evaluated for ectoparasites and treated accordingly. Whenever possible, blood should be collected and sera banked. Either a -70°C frost-free freezer or a -20°C freezer that is not frost-free should be available to save sera. Such sera could provide an important resource for retrospective disease evaluation. The quarantine period also represents an opportunity to, where possible, permanently identify all unmarked animals when restrained. Complete medical records should be maintained and available for all animals during the quarantine period. Animals that die during quarantine should have a necropsy performed under the supervision of a veterinarian and representative tissues submitted for histopathologic examination.

Quarantine procedures.

The following are recommendations and suggestions for appropriate quarantine procedures for Amphibians

4. direct and floatation faecal sample
5. swabs for chytrid testing
6. proper records should be kept to manage the quarantine period accurately including
 - a. personnel accessing the quarantine including details such as name, date, time
 - b. animal identification, arrival dates and quarantine cancellations.
 - c. all mortality, morbidity, disease diagnosis and treatment regimens

Annexure III. Handling and transport.

Handling frogs should be avoided, as frogs are very sensitive animals with delicate skins that serve a very important role in osmosis.

Frogs absorb minerals through their skin which is important for osmosis. However, this ability is very sensitive to influences from outside, like hormones or fluctuation in the pH (MacRobbie & Ussing, 1961). Simply touching a frog might influence the salt level on the skin of the frog, as any sweat on the hands may well disturb osmosis because of the presence of salt. It might even reduce or increase the permeability of the frogs' skin, which disturbs the salt level in the frogs' body (MacRobbie & Ussing, 1961). Furthermore, handling a frog causes a lot of stress, likely due to the temperature fluctuation (DEHP, sd; CCoAC, sd).

When handling a specimen cannot be avoided, the following steps should be followed (DEHP),

- Touch the PRFs with sterile powder-free latex gloves. Change between genetical groups, age groups as well as enclosures.
- Make sure there is minimal handling time with a maximum time of 30 seconds to minimize stress and the risk of infection by form of absorption.
- PRFs should be handled gently, to avoid removal of the protective mucus on the skin, or even cause injuries

When PRFs need to be transported, a health check must be performed prior to travelling. Only when an animal is healthy, and likely to survive the transportation, should it be allowed to depart. Depending on the means of travel a suitable transport method must be used e.g., for international/national flight the standards at outlined by IATA.

For short trips, a disinfected, plastic container (5-litre transparent plastic Jar with a lockable lid) may be used as the transport crate. PRFs must be kept moist during transport, which can be done with wet paper towels or damp moss inside the container. Only RO water must be used. All containers need to be clearly marked as well as the number of animals and origin need to be stated on the container.



Figure 15 Plastic container with label on ready for traveling

For Air Travel, The International Air Transport Organization (IATA) published regulations for Live Animal transportation, in which designs of air transport containers are described (IATA, 2016). Following those regulations will result in suitable air transport containers for the PRF (CCoAC).

For shipping, a double layered container should be used. The inner layer needs to be waterproof, to keep moisture inside. The outer layer should be protecting and insulating the inner container. Holes need to be made in both layers of the container to provide ventilation. The holes should be very small (0,3 – 0,6 cm) and made from the inside, so the sharp edges will be on the outside of the container. The temperature inside the container should be kept between 16°C - 28°C, and sudden fluctuations in temperature should be avoided. PRFs can be shipped to a maximum of 50 individuals per container (CCoAC). Every animal within the shipping container should have 50 ml of personal space, and every animal must be able to be in contact with the bottom with its entire ventral surface. However, providing the shipped PRFs with too much space might cause jump injuries. To prevent this, a cushioning substrate (which may be one of; moistened sponge pieces, dampened sphagnum or dampened sheet moss) and a low container should be used. Do not saturate the substrate with water, this makes the substrate too heavy and might injure the PRF when it shifts (CCoAC). In addition to this paragraph, CITES Transport Guidelines, Section Aph/2 should be taken into consideration (CITES, 1981).

Annexure IV. Hygiene protocol and daily check list.

Hygiene control.

Hands

1. Disinfect hands before touching or handling anything.
2. Put on gloves on entry of any room.
3. Change gloves between species and/or rooms.
4. Change gloves between racks.
5. Discard gloves in the relevant bins (Each room must have their own bin for gloves).

Feet

1. Walk through footbaths on entry and exit of any room.
2. Change the footbath content on a Tuesday and a Friday during the afternoon sessions.
3. Rinse out the substrate in the footbath on replacing the content.

Mops and Brooms

1. Sweep each room after the husbandry protocol is complete.
2. Mop each room with F10 SCXD[®] at 20ml/5L ratio, after the husbandry protocol is complete.
3. Rinse the mops in hot water and leave them upside down to dry before next use.
4. Disinfect the brooms weekly with F10SCXD[®] in a 1:250 ratio and leave them upside down to dry.

Bins

1. Empty all bins and discard all the contents according to the JCPZ waste management policy.
2. Leave the bins in the sun after morning sessions once a week to dry and to be naturally disinfected.

Daily checklist for frogs' husbandry.

Breeding Groups/Original Population & Adults.

07h30

- Switch room and systems lights on
- Perform accountability check on individuals
- Perform systems check
- Take Temperature readings and record in logbook
- Perform Ammonia tests and record in logbook
- Do water change according to the ammonia test results (e.g., ammonia = 0.25 mg/l needs a 25% water change)
- Clean enclosure e.g., remove all faecal matter, spray enclosure with R.O water at room temperature and change substrate is required.
- Check specimens to account for all.
- Feed all specimens with a ratio of 5-8 crickets (pinheads) each dusted with Calcium powders (ensure that crickets are gut-loaded)
- Complete record sheet and daily notes in the book.
- Clean and lock the room

15h30.

- Perform accountability check on individuals
- Perform systems check
- Take Temperature reading
- Do water change according to the mornings ammonia test results.
- Spray enclosure with RO water at room temperature
- Switch room light and systems lights
- Complete record sheet and daily notes in the book.
- Clean and lock the room

Frogllets.

07h30.

- Switch room and systems lights on
- Perform accountability check on individual specimens
- Clean system by replacing the substrate by the developing froglets
- Remove all faecal matter from the enclosures
- Spray enclosure with RO water at room temperature.

- Take Temperature reading and record in logbook under Froglet enclosures section
- Do Ammonia tests and record in logbook under Froglet enclosures section
- Do water change according to the ammonia test results (e.g., ammonia = 0.25 mg/ℓ needs a 25% water change) (Frog let enclosure)
- Feed all specimens with a ratio of 5-8 crickets (pinheads) each dusted with Calcium powders (ensure that crickets are gut-loaded)
- Check and account for all specimens
- Clean and lock the room

15h30.

- Perform accountability check on individuals
- Perform systems check
- Take Temperature reading
- Complete record sheet and daily notes in the book.
- Switch off room light and systems lights
- Clean and lock room.

Tadpoles & developing tadpoles.

07h30.

- Switch room and system lights on
- Perform accountability check on individuals
- Perform systems check
- Take Temperature reading
- Test RO water for Ammonia as well as Calcium before use and record in logbook under Water supply system).
- Do 100% water change by using room temperature RO water only.
- Feed all specimens with Serra Fish-flake & Spirulina mixture. Add blanched Romaine lettuce for the developing tadpoles.
- Clean and lock the room

15h30.

- Perform accountability check on individuals
- Perform systems check
- Take Temperature reading
- Complete record sheet and daily notes in the book.
- Clean room
- Switch room light and systems lights off
- Lock the room

Annexure V. Recording Sheets.

AMPHIBINA RECORD SHEET											MONTH:	YEAR:	
TAXON:				ARKS:	ENCL:			SEX:		:	:		
DATE	TEMP	HUMIDITY	ENCL/CL	W/C	SPRAY	FEACAL	EGGS	FED/(F)	F/TAKEN	SPIDER WEBS	REMARKS	FOOD WASTAGE	SIGN
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
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26													
27													
28													
29													
30													
31													

Daily record sheet for froglets and adults.

RECORD SHEET (TADPOLES)							MONTH:	YEAR:				
TAXON:				ARKS:	TANK:		SEX:	:	:			
DATE	TEMP	NH ₃ NH ₄	NO ₂	NO ₃	O ₂	CL	pH	FED/(F)	W/C	SYSTEM/CH	REMARKS	SIGN
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
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Daily record sheet for tadpoles.

Annexure VI. Release Protocol.

Considerations to be made prior to release.

The release of amphibians can be referred to as Introductions, Re-Introductions or Reinforcements due to environmental influences that directly impacted the population dynamics (genetics, age, gender, etc) or habitat negatively. Once a possible release site is identified the following steps should be followed.

1. Map the geographical region suitable for release
2. Evaluate the environmental conditions (human impact, water quality, etc)
3. Identify the risks (health, predation, pollutions, etc)
4. Assess the diversity of resident fauna and flora
5. Decide on season for release (Preferred time of the year to release specimens is from end September to mid-February)
6. Evaluate rainfall patterns and heat waves
7. Predict potential genetic influences as well as population density
8. Decide on genders (to be released)

Once a suitable release site is identified the following official approvals need to be obtained.

1. All relevant national or/and provincial permits need to be obtained from the relevant conservation bodies
2. Formal letter/s from the landowner/s need to be obtained granting permission for the release and access to the land
3. Availability of *ex-situ* specimens within the required specifications for release

In addition, preparations and conditioning of specimens to be released should be done based on the recorded *in-situ* environment:

1. Food/nutritional changes (crickets that are dusted with CL and gut loaded, fruit flies, etc)
2. Air conditioning (increase or decrease of environmental conditions e.g., temperature, humidity etc.)
3. Light spectrum periods
4. Population density
5. Faecal samples need to be tested weekly to ensure that no endo-parasites are present that cause or present a risk to the specimens or the species within the *in-situ* or *ex-situ* environment as well as cross over introductions.
6. Chytrid fungal testing needs to be done to confirm that all specimens earmarked for release are not infected nor carriers of the fungus.
7. The specimens need to be all marked with elastomers as per marking protocol to facilitate post-release monitoring.
8. Standard husbandry needs to be continued as the daily husbandry protocol.
9. Daily records need to be kept.
10. Transporting of specimens needs to be done according to the transport protocol.

Considerations for release.

A soft release approach is recommended as option one purely based on the lower stress levels compared to a hard release.

The soft release method will include:

1. Holding nets need to be hung up in various sites within the release site.
2. Specimens need to be introduced into the nets during suitable environmental temperatures.
3. These specimens need to be monitored for a few hours while they are acclimatized to the environmental conditions.
4. Small live food items can be introduced into the nets to allow the amphibian specimens to concentrate on the food and so decrease the stress associated with the new environment
5. The specimens can be released out of the nets once they have acclimatized