Amphibian Taxon Advisory Group Best Practice Guidelines for the Lake Oku clawed frog *Xenopus longipes*

Version 2

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Preamble

Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the "Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria". These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country.

Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

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The information in this Best Practice Guideline has come from a variety of sources including a literature review, the experience of the authors and others in the captive husbandry of *Xenopus longipes* as well as direct observations of the species in the field.

Amphibian husbandry is a rapidly evolving field and there are many aspects that require further research. Breeding triggers for *X. longipes* are still unknown; this area should be a focus of extended research if captive populations are to be viable. The vocalisation of *X. longipes* remains the only one not confirmed and characterised in the whole genus Xenopus (Evans et al., 2015). While hydrophonic recordings have been obtained from Lake Oku which are likely from *Xenopus* (M. Tobias, pers. comm.), confirmation from isolated vocalisations from *X. longipes* (likely in zoos and aquaria) are needed to document and describe vocalisations. This may facilitate monitoring of the species in Lake Oku, including fluctuations in breeding activity.

Captive diets for both larval and post metamorphic amphibians are likely to differ from diets consumed by larval and post metamorphic amphibians in the field. Replicating the wild diet in captivity will likely be precluded by the limited number of invertebrate species that can be reared on scale required for them to form viable live food colonies.

In-situ conservation efforts have been ongoing for over a decade, but should reviewed and reinforced where possible (given the current civil war in progress) considering the current Critically Endangered status of the species. In-situ research has been concluded on the causes of mortality events, though more research is necessary to verify the findings and expand knowledge on the general biology of this species. This will enable provided guidelines useful for its maintenance ex-situ, ideally in Cameroon.

Key husbandry points:
From experience of successful breeding of *X. longipes* in captivity, notable husbandry points include:

1. Replicating the water parameters of Lake Oku is key to rearing the tadpoles of this species.
2. Water quality must be carefully managed when rearing tadpoles, ensuring that the tadpoles obtain enough food whilst also maintaining low levels of nitrogenous waste from animals, which can be extremely labour intensive.
3. Monitoring and management of water quality.

These guidelines have been reviewed and approved by the Amphibian TAG members.

Contents

Section 1. Biology and field data

1.1 Taxonomy and evolutionary history

ORDER: Anura Fischer von Waldheim, 1813
FAMILY: Pipidae, Gray, 1825
GENUS: *Xenopus* Wagler, 1827
SPECIES: *Xenopus longipes*, Loumont and Kobel, 1991
COMMON NAMES: Lake Oku clawed frog

The earliest fossil evidence of the Genus *Xenopus* in sub-Saharan Africa was found in Tanzania and is dated from the Nsungwe Formation (~25 million ya; Oligocene). It resembles diminutive extant species of Xenopus such as *X. longipes* (Blackburn et al., 2019).

The Genus Xenopus, of the subfamily Dactylethrinae of aquatic African clawed frogs, has a complex phylogeny, characterised by a combination of “normal” speciation (with an ancestral species splitting into different descendant species) and “reticulating” speciation via allopolyploidisation (where ancestral species merge into one descendant species), leading to a remarkably high incidence of polyploid species (Evans, 2008). *X. longipes* is a dodecaploid species (12 sets of chromosomes), with a total of 108 chromosomes and a genome thrice as large as that of *X. laevis* (~8 pg/nucleus) despite its smaller egg- and body-size (Miller et al., 2020). Based on molecular and morphological data, karyotypes, and vocalisations, some authors propose three species grouping within the subgenus *Xenopus*: the *amieti*, laevis, and *muelleri* species groups. In consideration of its unfused cloacal lobes, prominent keratinous claw on the prehallux, skin ridge along the first pedal digit from the prehallux and dorsal skin often with small spicules, *X. longipes* is classified in the *amieti* group (Evans et al., 2015). Sequencing of ribosomal DNA (16S) indicates low genetic variation among *X. longipes*, with a maximum divergence of 0.37% between individuals (Quock et al., 2014).

1.2 Morphology

1.3 Weight: Females 1.3-3.7 g; Males 1.1 – 2.3 g (Doherty-Bone, 2009). There is a pronounced difference in body condition between captive and wild animals, captive animals are much heavier than wild animals.

1.3.1 Length: Adult females attain a snout vent length of 32-36 mm, adult males are smaller attaining a snout vent length of 28-31 mm (Loumont and Kobel, 1991). Maximum total length of tadpoles is 98.1 mm (Tapley et al., 2015).

1.3.2 Colouration: The ventrum of adult *X. longipes* are heavily speckled with black spots. The dorsum is dark brown to orange and the ventral surface is grey (Loumont and Kobel, 1991). Tadpoles are largely pale and translucent with widely scattered brown chromatophores. Chromatophores are absent in the area directly below the eye (Tapley et al., 2015).

1.3.3 Description: *X. longipes* is one of the smallest species in the genus *Xenopus* (Fig. 1A & 1B). The body is pear shaped and limbs are relatively long. The digits are long, which gives rise to the scientific name “*longipes*” which means “long foot”. Toes are fully webbed and three digits on the hind feet possess a keratinised claw (Fig 1A). The hind limbs also possess a metatarsal tubercle with keratinised claw. Lower eyelids are small. *Xenopus longipes* has a short subocular tentacle below each eye. *X. longipes* is sexually dimorphic (Fig 2A-C). Male specimens have keratinised nuptial pads on the forearms and female specimens possess cloacal papillae, both of which features become much more obvious during periods of reproductive activity.

Figure 1: Male (A) and female (B) *Xenopus longipes* taken from Michaels et al. (2015).

Figure 2: Keratinised nuptial pads on the forearms of a male (A, C) and cloacal papillae in a female (B) *Xenopus longipes* taken from Michaels et al. (2015).

The body of the tadpole is oval and depressed with dorsolaterally flattened head and body (Fig. 3). The dorsal fin is low and originates at the tail-body junction. The ventral fin is higher than the dorsal fin. The ventral fin originates mid abdomen appears as rounded lobe at the junction between the tail and body. The height of ventral fin diminishes at the point at which the vent tube terminates but gradually increases, reaching its maximum height before tapering off towards the end of the tail. The tail tip terminates in flagellum. The nares are transversely elliptical and parasagittal, situated nearer to the snout than to the eyes. The mouth is terminal and slit-like. During later stages of development, a single, relatively short barbel (Fig. 4), located at each corner of the mouth can be present (Tapley et al., 2015).

Figure 3: *Xenopus longipes* tadpole at Gosner stage 35 (© Benjamin Tapley / ZSL)

Figure 4: *Xenopus longipes* tadpole with barbel (© Benjamin Tapley / ZSL)

1.4 Physiology

Host defense peptides displaying immunomodulatory, chemotactic, and insulin-releasing activities, and cytotoxicity to tumor cells and viruses, have been isolated from skin secretions of many *Xenopus* species but have not been researched in *X. longipes* (Coquet et al., 2016). Aquatic amphibians such as *Xenopus* are known to secrete more ammonia than urea (Cragg et al., 1961).

1.5 Longevity

The longevity of this species in the wild is unknown. Specimens collected from the wild as adults in 2008 are still alive in the captive setting indicating that this species can live at least 13 years; metamorphs take approximately 6 months to reach adult size (Tapley et al., 2015, Michaels et al., 2015).

Field data

1.6 Zoogeography, ecology and conservation

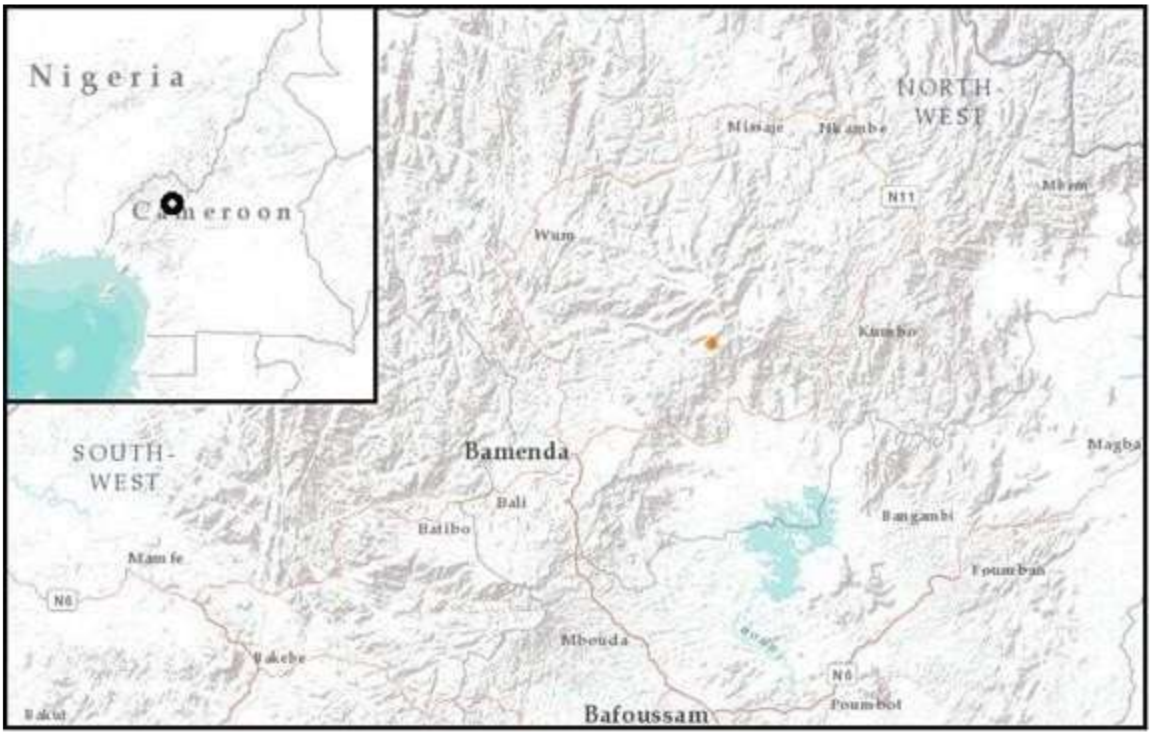

Figure 5: The distribution of *Xenopus longipes*. The orange polygon represents where *X. longipes* are present (IUCN SSC, 2020).


Figure 6 (left): Lake Oku (© Thomas Doherty-Bone). Figure 7 (right): *Xenopus longipes* on endemic sponge in lake Oku (© Brian Freiermuth).

1.6.1 Distribution: *Xenopus longipes* is endemic to Lake Oku in Northwest region, Cameroon (Fig. 5 & 6). Lake Oku is a crater lake situated below the second highest peak in Cameroon, Mount Oku, 2,219 m a.s.l. (IUCN SSC, 2020). Lake Oku has an area of 243 ha.

1.6.2 Habitat

Parameter	Mean Value	±	Unit
Water temperature	17.27	4.17	Celsius
pH	7.58	0.24	-
Total Dissolved Solids	8.72	2.27	Ppm

Table 1: Water temperature, pH and TDS measured at the Lake Oku shoreline (Michaels et al., 2015; modified from Doherty-Bone et al., 2008).

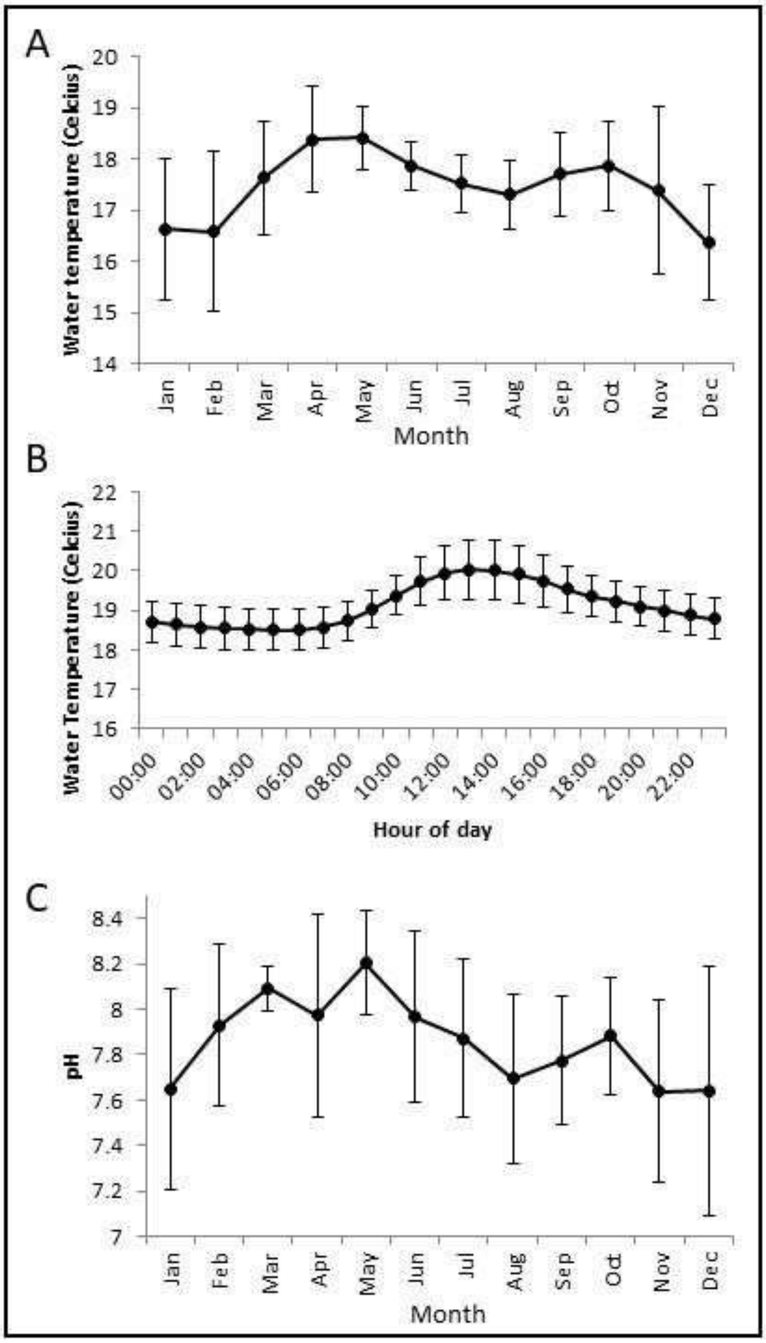

Figure 8: Mean monthly water temperatures recorded from the shoreline of Lake Oku based on 10 years of monitoring. (©Thomas Doherty-Bone).

Lake Oku is a mesotrophic lake (clear water) with a mean depth of 32 m and a maximum depth of 52 m (Kling, 1988). The lake is surrounded by montane rainforest (IUCN SSC Amphibian Specialist Group, 2020.). Large colonies of aquatic plants occur in the lake and include *Ceratophyllum* sp., *Myriophyllum* sp. and the rare *Isoetes biafrana* that is found in only one other lake in Bioko (Cheek et al., 2000; Doherty-Bone, 2014) as well as an undescribed sponge (Fig. 7), which is possibly endemic to Lake Oku (Doherty-Bone, 2014). Temperature data is available from the lake shore and ranges between 17-22.73^{o}C (Fig. 8A-B, T. Doherty-Bone unpubl. data). Data loggers have found water temperature varies throughout the day, peaking to 20.03±0.76^{o}C at 1300hrs and troughing to 18.49±0.51^{o}C at 0500hrs (Fig. 8C). pH at the lake shore ranges from 6.67 to 8.62 (Fig. 8; T. Doherty-Bone, unpubl. data). The water of lake contains very little in the way of total dissolved solids (TDS) (Table 1) and dissolved nutrients (ammonia, nitrate, phosphate) and gases (oxygen, carbon dioxide) have been found to not to significantly vary by season (T. Doherty-Bone, unpubl. data). Frogs are frequently encountered around the lake shore, but have been found to be evenly distributed across the lake at all depths (Doherty-Bone, 2009; T. Doherty-Bone, unpubl. data). It is not known if frogs utilise the lake in its entirety. Lake Oku is an important cultural landmark for surrounding communities, especially (but not exclusively) the Oku people, who view the lake as the resting place of 'Mawes', their deity (Koloss, 2000; T. Doherty-Bone, unpubl. data).

1.6.3 Population: The population of *X. longipes* was monitored (by Oscar Nyinchia & Thomas Doherty-Bone) from 2006-2018, with monthly monitoring from 2008 (surveys halted due to civil war in 2018). The unpublished findings indicate a stable population with no absolute fluctuation by season. It is listed as decreasing by the IUCN SSC Amphibian Specialist Group (2020).

1.6.4 Conservation status: The species is listed as Critically Endangered by the IUCN because its extent of occurrence and area of occupancy is 2km^2, because all individuals are in a single threat-defined location, and because there is a projected decline in the number of mature individuals in view of the compounded effects of disease, of high likelihood of a fish introduction into Lake Oku and of habitat degradation causing subsequent decline in the quality of the water in the lake (IUCN SSC Amphibian Specialist Group, 2020). Collection of amphibians for the commercial pet trade also apparently occurs from Cameroon (Herrel and van der Meijden, 2014), though not yet found to occur at Oku.

As of October 2021, the threatened status of this species is reflected in its ranking as the number 246 global priority for amphibian conservation on the basis of threat and evolutionary history by the Zoological Society of London's Evolutionarily Distinct and Globally Endangered (EDGE) programme (Isaac et al., 2012). It has been found that local authorities at Oku have been overruled by the national government to undertake harmful developments at the lake (in this case forest clearance for tourism and other infrastructure), highlighting that other harmful developments like fish introduction could still happen despite local awareness.

The lake is situated just outside the boundary of a proposed Faunal Reserve on Mount Oku. Lake Oku and its surrounding forest are protected by the national government-sanctioned Kilum-Ijim Plantlife Sanctuary, restricting access to the lake, even though enforcement is difficult and even controversial. BirdLife International conducted a conservation project on Mount Oku involving community management of mountain forest with the local villages for several years but the project ended in the mid-2000s (IUCN SSC Amphibian Specialist Group, 2020). A Conservation Action Plan for Lake Oku was published in 2014, and prepared in collaboration with local, regional, national and international representatives in Oku (Doherty-Bone, 2014). This was scheduled for review in 2018 (5 years since the stakeholder workshop), but the Anglophone Crisis civil war prevented this from happening. The impact of this conflict on this lake and surrounding forest is uncertain.

Figure 9: Dead *Xenopus longipes* in Lake Oku (© Thomas Doherty-Bone).

Between 2006 and 2017, reoccurring enigmatic *X. longipes* morbidities and mortalities were observed in Lake Oku (Fig. 9) (Blackburn et al., 2010; Doherty-Bone et al., 2013; T. Doherty-Bone, unpubl. data). Diseased animals were lethargic, and some presented with obvious limb or skin lesions, characterised by skin ulceration and limb tissue necrosis. The mysterious disease that has affected this species has been monitored since its discovery in 2006, but its causative agent and long-term consequences are still not understood other than its apparent stability amid a numerically abundant frog population (Doherty-Bone et al., 2013; T. Doherty-Bone, unpubl. data). Only one in 48 dead animals tested was positive for a ranavirus, making it unlikely to be the cause of mortality (Doherty-Bone, 2013). While the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), which causes the disease chytridiomycosis (known to be one of the drivers of global amphibian population declines), is present in the region, with some concern it may be a factor of widespread frog declines (Hirschfeld et al., 2016). It has rarely been found to infect *X. longipes* (first recorded in Hirschfeld et al 2016), with none of the morbid specimens testing positive, and is apparently not affecting this species and is not the culprit of the observed die-offs (Blackburn et al 2010; Doherty-Bone et al., 2013). Bd has been shown to infect other species of *Xenopus* without known detrimental impacts at the population level (Tinsley et al., 2015; Ramsey et al., 2010; Rosemblum et al., 2009; Zimkus et al., 2020).

Following recommendations of the species' 2004 IUCN assessment (Tinsley and Measey, 2004), a captive-breeding programme was attempted by European Zoos in London, Antwerp and Cologne in 2008 and the first successful breeding of this species was achieved by ZSL London Zoo in 2014 (Michaels et al., 2015; Tapley et al., 2015). Only one in the three initial breeding collections currently remains, with a total of 43 captive *X. longipes* currently held by ZSL London Zoo (UK) (Species360, 2022). 12 individuals are maintained at Steinhart Aquarium (USA) (Species360, 2022). These populations are not held in biosecure facilities and are therefore not an appropriate source for any future translocation programme should this be required.

1.7 Diet and feeding behaviour

1.7.1 Food preference: The diet of *X. longipes* in the wild is largely unknown. Stomach contents of other *Xenopus* species include benthic invertebrates and zooplankton which constitute a major portion of their diet (McCoid and Fritz, 1980; Measey, 1998; Lobos and Measey, 2002). Terrestrial invertebrates, aquatic and terrestrial vertebrates have also been reported as food items in other *Xenopus* frogs (Inger and Max, 1961; Lafferty and Page, 1997; Measey, 1998; Bwong and Measey, 2010) although the small size of *X. longipes* may preclude the consumption of terrestrial vertebrates. The only aquatic vertebrates other than *X. longipes* reported to occur in Lake Oku are *Phrynobatrachus njiomock* and *Astylosternus ranoides* (Doherty-Bone, 2009; Doherty-Bone et al, 2013), larvae of these species could potentially be consumed by *X. longipes*. In-situ observations have reported anecdotical cannibalism by frogs of equal size, but this phenomenon was never observed in captivity. This cannibalistic behaviour most likely consists in scavenging on the carcasses of deceased individuals rather than predation, which could enhance the horizontal transmission of pathogens and might explain the strong correlation between abundance and morbidity in the *X. longipes* population (Doherty-Bone et al., 2018).

The feeding ecology of *X. longipes* tadpoles in the wild is unclear – tadpoles when alive are found in the deepest sections of the lake at c. 40-50 m (T. Doherty-Bone, unpubl. data). Tadpoles of congeners are mid water suspension feeders (McDiarmid and Altig, 1999) and this is observed in the morphology and behaviour in captive tadpoles (Tapley et al., 2015).

1.7.2 Feeding behaviour: The feeding behavior of *X. longipes* in the wild is not fully understood, other than casual observations of foraging on small (unidentified) food items and once of multiple animals feeding on one large mantis that had fallen into the lake. “Arthropods and plant material” have been recorded in stomachs of some animals dissected but details were not provided (Blackburn et al., 2010), fragments of an oligochaete worm, chironomid larvae and dasyhelinid larva mixed with sediment and plant material were recorded in the stomachs of preserved specimens (T. Doherty-Bone, unpubl. data) but systematic study of stomach contents have yet to be made.

Ex-situ work demonstrated that chemosensory cues alone could modulate the foraging behaviour of *X. longipes*, with different predation strategies used in response to the scent of benthic (bloodworms) vs. pelagic (glassworms) prey types (Michaels et al., 2018). Indeed, clawed frogs rely on hydromechanical, visual and olfactory cues for prey detection; these latter seem to stimulate complex olfactory pathways, as kairomones released by injured prey were shown to increase the amount of foraging in captive *X. laevis* (South et al., 2020).

Xenopus are tongueless and use their forelimbs to manipulate prey into their toothed jaws (Avila and Frye, 1978), and this has been observed in Lake Oku. *Xenopus* larvae have highly efficient buccal pumping mechanisms (Seale et al., 1982), the floor of the buccopharyngeal cavity is covered with mucus secretory ridges which trap food particles (Wells, 2007).

1.8 Reproduction

1.8.1 Developmental stages to sexual maturity: The reproductive biology of the species in the field is unknown. The reproductive biology of the species has been described from captive specimens (Michaels et al., 2015; Tapley et al., 2015); eggs hatch into tadpoles, which metamorphose into juvenile froglets, which mature into breeding adults.

1.8.2 Age of sexual maturity: Unknown in nature. Captive animals produced eggs at 5-6 months post metamorphosis.

1.8.3 Seasonality of cycling: Unknown in nature.

1.8.4 Clutch size: Unknown in nature, in captivity clutch size ranged from 7-300 eggs (Michaels et al., 2015).

1.9 Behaviour

1.9.1 Activity: In the wild, shallow water activity of post metamorphic *X. longipes* is predominately nocturnal, but during the of the peak wet season they become active during daylight hours (Doherty-Bone et al., 2013; T. Doherty-Bone, pers. obs.). For an overview of diurnal activity budgets in captive *X. longipes* see Dias et al. (2022). *Xenopus* tadpoles are social schooling and active by day and by night (McDiarmid and Altig, 1999). Male *X. longipes* were found more often to dwell in deeper water in Lake Oku amongst aquatic weeds (Doherty-Bone, 2009).

Locomotion: Tadpoles possess barbels, which are very small for the genus, at the corner of the mouth (Fig. 4), these are thought to aid in navigation and mechano-reception (Ovalle 1979; Channing and Howell, 2006). *X. longipes* have reduced barbels relative to congeners. The water in Lake Oku is clear (Kling 1988, T. Doherty-Bone, pers. obs.) and this may explain the shorter barbel length in this species. *Xenopus* tadpoles swim continuously. This swimming is normally downward against their own positive buoyancy and there is little apparent forward movement (Hoff and Wassersug, 1986). The low tail fin of *Xenopus* tadpoles is thought to act as a rudder and reduce yaw making feeding more efficient (Hoff and Wassersug, 1986). *Xenopus* tadpoles are fast swimmers and swimming is most efficient at high speeds, it has been suggested that this efficacy at locomotion at high speed may have evolved in response to predation pressures given the mid water habitats of the tadpoles (Hoff and Wassersug, 1986). Post metamorphic *X. longipes* have fully webbed hind feet and these aid the frog in their locomotion underwater and allow the frogs to give surprising bursts of speed. Typical locomotion in post-metamorphic animals consists of frogs swimming forward using their hind limbs while holding immobile front limbs out in front. Even though several *Xenopus* species have been shown to move overland and to retain traits with terrestrial functions, *X. longipes* stands out within the genus as exhibiting an extreme adaptation to an aquatic lifestyle (Measey, 2016).

1.9.2 Predation: Unknown. Herons can occur at Lake Oku, and Kites have been observed snatching unidentified prey from the lake surface. Frog-eating snakes (*Bothrolycus ater*) have been observed around Lake Oku. Conspecifics act as predators, with post metamorphic frogs consuming eggs, larvae, and other frogs.

1.9.3 Vocalisation: The vocalisation of *X. longipes* has not been described. In captivity audible vocalisations, consisting of metallic clicks typical for *Xenopus* (Tinsley and Kobel, 1996) were heard infrequently and were not closely associated with spawning activity (Michaels et al., 2015). In Lake Oku, recordings with hydrophones have detected vocalisations likely to originate from *X. longipes* (M. Tobias, pers. comm.) but isolation of vocalising individuals is needed to verify these.

1.9.4 Sexual behavior: Axillary amplexus has been observed in situ (Doherty-Bone, 2009) and in captivity (Michaels et al., 2015). The vocalisation of *X. longipes* has not been described.

SECTION 2. Management in zoos and aquaria

2.1. Enclosure

X. longipes may be maintained in aquaria throughout their life (Fig. 10). The enclosure must have a tight-fitting lid or substantial overhang with a gap between the water surface and the lip of the tank to prevent animals escaping.

Figure 10 (left): Breeding for *Xenopus longipes* at ZSL London Zoo (© Benjamin Tapley / ZSL) (Center): Detail Breeding for *Xenopus longipes* at ZSL London Zoo (© Benjamin Tapley / ZSL) and (right): public exhibit at ZSL London Zoo (© Charlotte Ellis / ZSL)

2.1.1 Substrate: For ease of maintenance *X. longipes* can be maintained without substrate. Bare bottom aquaria also eliminate the risk of impaction from eating substrate. Brown and Nixon (2014) looked at resting substrate preferences of *X. laevis* and this species spent more time resting on the bare bottom of glass enclosures than on gravel. Washed aquarium grade silver sand has been used as a substrate for this species with no reported issue. At California Academy of Sciences the exhibit enclosure has a substrate consisting of fabricated naturalistic matting (Universal Rocks, Texas, USA).

2.1.2 Furnishings and maintenance: A recent study by Graves et al. (2023) explored the impact of enclosure background colour on the behaviour of *X. longipes*; behavioural indicators of presumed stress were the lowest in tanks with background colours of green or grey (opposed to black or transparent backgrounds). Aquarium grade plastic pipes and plastic mesh can be used as refugia for the frogs, as well as bog wood, natural stones, unglazed tiles or bricks. Sharp edges and very rough surfaces should be avoided as the frogs have very delicate skins and are prone to sudden erratic bursts of speed that make them vulnerable to injury through collision. Plastic plants can be used for refugia, resting sites but live plants may be preferable; plants such as *Vesicularia dubyana* can be placed in the tank to improve oxygenation of the water, facilitate the removal of nitrogenous waste as well as to provide shelter they can make reproductive management complicated. All eggs, and subsequent early-stage larvae laid in aquaria with live plants at ZSL London Zoo succumbed to fungal growth (Michaels et al., 2015). Spot cleaning should be carried out daily (especially after feeding) using a turkey baster, siphon or net to remove detritus.

2.1.3 Environment: Field data and knowledge of the specific biology of the species should be used to inform captive management (Tapley and Acosta, 2010; Michaels and Preziosi, 2013; Michaels et al., 2014). Replicating conditions from the field has improved captive breeding success of *X. laevis* (Godfrey and Sanders, 2004). A full suite of environmental parameters for Lake Oku are available in Doherty-Bone (2009) and Doherty-Bone et al., (2013), with the 10-year monitoring program of Lake Oku to be submitted for publication soon

2.1.4 Water: *X. longipes* have specific water parameter requirements. The first case of natural spawning in captivity coincided with a shift in the water parameters to better reflect the water parameters in Lake Oku; although there were other husbandry changes such as feeding regime which could have contributed to breeding success (Michaels et al., 2015). Temperature and pH regimes recorded in Lake Oku (T. Doherty- Bone, unpubl. data) should be implemented into the captive management regime of this species. The temperature of Lake Oku increases during the wet season, although temperature changes are not dramatic (Fig. 8). pH also changes with season, pH decreasing in the wet season and increasing in the latter half of the dry season (Fig 8). Total dissolved solids (TDS), conductivity, dissolved solids and gases remain relatively stable throughout the year (T. Doherty- Bone, unpubl. data).

At ZSL London Zoo *X. longipes* are maintained in unmixed and unbuffered reverse osmosis (RO) water. In London, municipal tap water is very hard and alkaline (alkalinity ranges up to 350ppm, pH up to 8.5) and so the RO water still contains some minerals and TDS ranges from 20-37ppm depending on the age and therefore the efficacy of the membranes in the RO system. Temperature can be controlled either by adjusting the ambient temperature or by the addition of a water conditioning system. At ZSL, pH was adjusted by adding small quantities of aged tap water to the system, however it is important to note that the addition of tap water will increase the TDS / mineral content of the water as pH and alkalinity are necessarily linked.

At California Academy of Sciences adult frogs are kept in the aquarium's fresh water cistern water. This is incoming city tap water that is run through a carbon filter to remove chlorine and chloramine. The pH of this water ranges from 7.5 to 8.2 and alkalinity ranges from 0.52 to 1.21 meq/L. Water changes of the frog enclosures are performed based on water quality testing. On average, a 20-30% water change is carried out on a weekly basis to keep ammonia and nitrogenous wastes within specified parameters. Water is changed as values exceed 0.03 mg/L NH_4+, 0.6 mg/L NO_2-, 15 mg/L NO_3-. The temperature is maintained between 18 and 19.5°C.

Adult *X. longipes* do not appear sensitive to the mineral content of the water. Historically, specimens at ZSL London Zoo were maintained in aged tap water which in London had a mineral content of 350ppm. Larvae reared in water where the TDS was above 80ppm died rapidly (Michaels et al., 2015). TDS is a component of water quality which is often overlooked in the husbandry of amphibians. There are other soft water specialists. *Xenopus gilli* from the Cape also appears to be intolerant of hard, alkaline conditions (Rau, 1978) and reproductive success of the hellbender *Cryptobranchus alleganiensis* was improved when TDS levels were decreased as sperm motility improved (Ettling et al., 2013).

As tadpoles are filter feeders and require feeding several times throughout the day, detritus can rapidly build up in tadpole aquaria and this makes nitrogenous waste difficult to manage. In addition, the low mineral content of the water reduces the capacity for biological filtration, which cannot be too powerful in the first place to avoid stripping food from the water column too quickly and to avoid disturbing the tadpoles with a powerful current. At ZSL London Zoo a regime of 10% water changes in the morning followed by 50% water changes in the afternoon, both accompanied by painstaking removal of detritus from the bottom of tanks by siphon and thorough cleaning of air- stream sponge filters in aquarium water, helped to suppress waste.

Regular 10-20% water changes should occur to ensure the maintenance of good water quality in aquaria housing adult animals. At ZSL London Zoo the system housing *X. longipes* has a sump and water changes are carried out by removing and adding water from and to the sump. In addition, accumulated debris and uneaten food are removed from individual enclosures soon after feeding via siphon. Large water changes may dramatically alter water parameters, and this may be highly stressful on the physiology of aquatic animals and should only occur if water parameters deteriorate (particularly if there are spikes in ammonia and nitrite). It is better to have slightly suboptimal parameters than wildly fluctuating parameters. It is not possible to manage what you do not measure. Water should be tested on a regular basis, this will allow the performance of filtration systems to be assessed, can identify one off problems or recurring issues, and assess corrective measures. Important parameters to test on a regular basis include ammonia, nitrite, nitrate, pH and TDS.

2.1.5 Filtration: Filtration requirements differ according to the life stage. Adult animals appear less sensitive to currents and water movement as they are not filter feeders like their tadpoles. For larger aquaria or aquaria with many animals, large external filters are vital. A sump may also be used to increase water volume and filtration capacity. For small aquaria with four or five animals internal box filters or air-stream sponge filters are sufficient. Filter media should be cleaned on a regular basis (depending on the size of the filter and the stocking density), media should be cleaned in tank water rather than tap water as the sudden change in water parameters, as well as chlorine/chloramine in tap water, can be detrimental to the nitrifying bacteria which are vital for biological filtration. There is potential for animals to find their way into the internal mechanisms of filters. For this reason, it is vital that any way into the filter is blocked up with mesh or sealed with silicone. For small juveniles, the use of internal sponge filters or external filters with finely meshed in and out flows may be preferable to internal power filters. The filter should not create anything more than a moderate current and areas protected from any current, which can be created using rocks, tiles etc., are important.

Tadpoles have different filtration requirements. Water currents should be avoided as tadpoles are filter feeders and swim constantly. Tadpoles could become quickly exhausted if they have to swim against a current. Tadpoles could also become starved if filters were to quickly remove suspended food particles from the water column. At ZSL London Zoo air-stream sponge filters with air pressure set to the minimum effective level provided sufficient filtration, with water changes being relied on to maintain optimal water quality.

2.1.6 Dimensions: At ZSL London Zoo 40 x 25 x 25 cm (40 L) aquaria house a maximum of 7 adult individuals. The systems are connected to a single sump, the water volume of the off-show breeding facility at ZSL London Zoo is 945 L including the sump. 40 L enclosures would be appropriate for rearing up to 20 *X. longipes* tadpoles. The exhibit at California Academy of Sciences is a 91 x 91 x 91 cm enclosure, partially filled with water with an emergent layer of live plants. Reported stocking densities range from 1 animal per 13 L to 1 animal per 24 L, floor area as well as water volume should be taken into consideration when stocking enclosures.

2.1.7 Lighting: At ZSL London Zoo *X. longipes* are given a 12: 12 hour photoperiod. Enclosures are lit with Arcadia T5 lamps with 12% UV-B with reflectors (D_3+ 12%UV-B 121 Reptile Lamp, Arcadia Products plc, Redhill, UK), this provides a UVI of 1.0 at the surface of the water. In situ, aquatic frogs are exposed to different levels of UV-B radiation according to the position of the water body, water depth, turbidity of the water and availability of refugia. The UV-B requirements of *X. longipes* in the wild and in captivity are currently unknown. However, an over-exposure of UV-B radiation can be detrimental (Blaustein and Belden, 2003). The developing embryos of *X. longipes* are not pigmented and husbandry practitioners should be cautious about the provision of UV-B during oviposition.

At the California Academy of Sciences the exhibit is illuminated with LED strip lights. Enclosures behind the scenes for adult frogs do not have any additional lighting other than the ambient room lighting. When tadpoles were reared, they were illuminated with LED strip lights.

2.2 Feeding

2.2.1 Basic diet: A variety of food should be offered to compensate for potential dietary deficiencies as knowledge regarding the nutritional requirements of amphibians is lacking (Densmore and Green, 2007). Tadpoles should only be offered food once the yolk sac has been absorbed and they are free swimming. They will not feed until the yolk sac has been absorbed and adding food at this point may only pollute the water. At ZSL London Zoo tadpoles were reared on a suspension of blanched and blended spinach (*Spinacia oleracea*) or nettle (*Urtica dioica*), commercial *Xenopus* tadpole food (Xenopus express Inc, Brooksville, USA), SERA® Micron powdered food (Sera®, Heinsberg, Germany) and *Spirulina* alga, which was strained prior to use to remove larger plant fragments. After several weeks, the diet was changed to only include commercial *Xenopus* tadpole food, Sera® Micron and *Spirulina* to avoid the high oxalate content of spinach (Noonan and Savage, 1999), which may interfere with calcium metabolism (Rosol et al., 1995). All aquaria were maintained at a similar degree of cloudiness with suspended food particles, and additional food was added throughout the day dependent on the rate at which food was consumed in a given aquarium.

At the California Academy of Sciences tadpoles were not reared to metamorphosis but they did grow on a suspension feed of Sera® Micron powdered food Tadpoles were fed with small pipettes. The Sera® Micron suspension was slowly swirled into the water. Tadpoles were fed amounts where they could clear the water within about one hour and were offered food up to three times per day.

At ZSL London Zoo adult frogs are fed once a week on either small red worms (*Eisenia* sp.), bloodworms (Chironomidae), white worms (*Enchytraeus albidus*), *Daphnia* spp., glass worms (*Chaoborus* spp.), micro crickets (*Gryllus bimaculatus* and *G. assimilis*), finely chopped *Lumbricus terrestris* lob worms, pelleted fish foods including Hikari carnivore pellets (Kyorin Food Industries, Ltd, Japan), Arcadia Amphigold and a commercially available *Xenopus* diet (Xenopus express; sinking frog food). Lob worms, red worms and pelleted fish foods form the staple diet at the time of the revision of this document. At the California Academy of Sciences Adult frogs are fed once per week on either live Blackworms (*Lumbriculus variegatus*) or frozen/thawed Bloodworms (Chironomidae). About 2 weeks prior to breeding induction, adult frogs were fed up to four times weekly up through the time of egg laying. It is important that Chironomid worms are well washed for several days before being added to the aquarium as potentially pathogenic organisms, as well as heavy metals and other pollutants, are often abundant in the environments where these worms are collected (Fard et al., 2014). It should be noted that the vast majority of aquatic live-foods commercially available are collected from wild or semi-wild settings and so are unlikely to be less of a disease risk than invertebrates collected by institutional staff themselves.

2.2.2 Method of feeding: Adults frogs should be fed once per week and the amount of food should be adjusted according to body condition. Michaels et al. (2015) speculate that heavy feeding may contribute to spawning activity, and so breeding may be more linked to a threshold in body condition than to external triggers. Tadpoles should be fed 2-4 times a day. The tadpole food should be offered in suspension (the water making the suspension should match the water in the tadpole aquaria to prevent shifts in water parameters). The water in the tank should be cloudy after the food suspension has been added and food should only be offered again when the water is clear. Uneaten / dead prey items should be removed within 24 hours to avoid water pollution.

2.3 Social structure: Specimens can be housed in mixed sex groups, single sex groups or as pairs. Non amplectant animals should be removed from enclosures if amplexus is observed as non-amplectant animals, as well as laying females, may consume eggs (Michaels et al., 2015).

2.3.1 Basic social structure: This area requires more research. Optimal group structures for breeding are not known.

2.3.2 Changing group structure: This area requires more research. Optimal group structures for breeding are not known. *X. laevis* is sensitive to changes in social environment, so adjusting social groups should be done carefully and only when necessary (Holmes et al., 2018).

2.3.3 Sharing enclosure with other species: This should be avoided. *X. longipes* have not been mixed with other species in any captive facility. Mixing *X. longipes* with non-sympatric species has the potential to expose the captive colonies to novel pathogens and this may undermine the research and conservation value of the species in captivity. Moreover, the feeding strategy of *Xenopus* frogs means that tank mates are likely to be seized in predation events, which may lead to the injury of the frog, the attempted prey animal or both.

2.3.4 Population management: The current captive population is limited to just four collections (three institutions; two in Europe and one in the USA) and one private collection Europe. To date the species has only bred naturally in one institution (ZSL London Zoo) where multiple females have spawned on multiple occasions, but larval mortality was high one each of the four attempts that were made to rear them, even under the most successful conditions (thousands of eggs laid, fewer than twenty reared to metamorphosis). Artificial breeding techniques have been successful in achieving spawning at California Academy of Science but all larvae died. The captive population is not formerly managed and not presently viable in the long term.

2.4 Breeding

Figure 11: *Xenopus longipes* spawning in captivity (© Benjamin Tapley / ZSL).

2.4.1 Mating: Amplexus is axillary and in captivity amplectant pairs were only observed during the day.

2.4.2 Egg laying: In captivity spawning behaviour was only observed during the day. The process of oviposition lasted 6.5 hours from initiation to termination of amplexus. Clutch size ranged 7 to 190 per clutch and eggs were deposited singly over all available surfaces in aquaria (Fig. 11). Egg diameter was 1.23 mm one hour after laying, including the jelly. Multiple males were observed attempting to amplex single females. The later males were usually dislodged by vigorous kicking on the part of the first male to have seized the female (Michaels et al., 2015). At ZSL London Zoo the first initial spawning event occurred after increasing the temperature from 17.5 to 19.1°C by adding warm tap water to the system, resulting in a pH shift from 7.5 to 8.09. Subsequently, spawning has been associated by recovery of water temperatures after a sudden drop due to life support failure. However, spontaneous spawning without any clear environmental trigger is also common. These changes in water parameters were undertaken to replicate the seasonal temperature and pH regime in Lake Oku (Fig. 4). The breeding season in the wild has not been documented and there is no evidence that this seasonal change accompanies the initiation of breeding in nature as later spawning events at ZSL London Zoo were not associated with manipulation of water parameters but did follow heavy feeds with earthworms (*Eisenia* sp.).

Figure 12 (left): *Xenopus longipes* hatching, Figure 13 (right): *X. longipes* with eyes and yolk sac Figure 14 (bottom) several newly hatched *X. longipes* (© Benjamin Tapley / ZSL).

2.4.3 Hormone induced reproduction: At California Academy of Sciences all reproduction was hormone induced. Human chorionic gonadotropin (hCG) (Sigma- Aldrich, Inc Saint Louis MO item #C1063- 2,500IU/vial l) after being reconstituted and stored at 4°C (was used to hormonally induce oviposition using the standard breeding protocol for *Xenopus laevis* (Sive et al., 1997) as a reference with dose adjustments after clinical trials and personnel communications with Ben Evans at McMaster Univ Ontario Canada (B. Evans, pers. comm. 2014).

The protocol for mating/inducing ovulation in Lake Oku Clawed Frog *X. longipes* (weight range 3.0-6.0g) was as follows: a "priming dose" of 75IU hCG was given into the dorsal lymph sac of both males and females, followed the next day by an "induction/oviposition dose" of 200IU into the dorsal lymph sac of both males and females. Egg laying occurred 24-48 hours after the second injection.

Attempts to artificially induce breeding in *X. longipes* using human chorionic gonadotropin (hCG) were made three times. Amplexus was observed in all three trials the day after the hCG induction doses were given, and eggs were laid the same day as amplexus was observed. Clutch size from a single female numbered between 300 and 415 eggs; roughly double the clutch size recorded from natural spawning events, though typically approximately 50% of the eggs laid turned out to be infertile.

2.4.4 Hatching: At both ZSL London Zoo and California Academy of Sciences eggs developed and hatched in 2-4 days (Fig. 12). Initially, tadpoles attach to hatch sites via a cement gland (Fig. 13) and eventually developing eyes and pigmentation before becoming free swimming after 2-4 days. Hatch rate was variable (Michaels et al., 2015) between clutches.

<table>
<tr><th>Water TDS (mg/L)</th><th>Refugia (live plants)</th><th>Detritus</th><th>Lighting</th><th>Tannins</th><th>Isolated from adult system?</th><th>Tadpoles survived?</th></tr>
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Table 3: Combinations of conditions used to rear *Xenopus longipes* tadpoles, and the outcome in terms of tadpole survival, after Michaels et al. (2015).

2.4.5 Development and care of young: Tadpole development is temperature dependent (see references within Wells, 2007). At ZSL London Zoo a variety of rearing methods were used in attempts to rear tadpoles (see Table 3). Tadpoles were only reared successfully in water with a very low level of total dissolved solids (TDS; 20mg/L) and in enclosures that lacked live plants and where the accumulation of humic detritus was prevented (Michaels et al., 2015). At ZSL London Zoo, tadpoles only survived in aquaria isolated from the adult system. In one case, water from the adult system spilled over into an isolated aquarium resulting in the loss of nearly all tadpoles housed therein. The reason for this is unknown but it may be the result of toxins from non-aquarium grade pipe work leaching into the system. Mortality of tadpoles remained high until the TDS of the systems fell below 80mg/L. At this point surviving tadpoles began to feed, swim normally and to develop (Michaels et al., 2015). Doherty-Bone et al. (2013) report a TDS of <10mg/L (See Table 1). A TDS of 20mg/L was the lowest possible output from the RO system used at ZSL London Zoo (Pentair PRF, Fileder, Filter Systems Ltd, Maidstone, UK) and this appears to be adequate for larval rearing (Michaels et al., 2015).

At the California Academy of Sciences attempts were made to raise tadpoles in three separate trials which encompassed several methods of managing water quality and feeding frequencies.

Trial 1: Tadpoles kept in slow flow-through mesocosms located within the adult frog enclosure (separated from frogs). Water changes on system were performed based on water quality in order to keep ammonia and nitrogenous waste in specified ranges.
Trial 2: Identical to Trial 1. Feeding quantities differed.
Trial 3: Tadpoles kept in isolated 40 L containers. (28 x 18 x 20 cm) and containers. Some with no filtration. Some with air stream bubble filters.

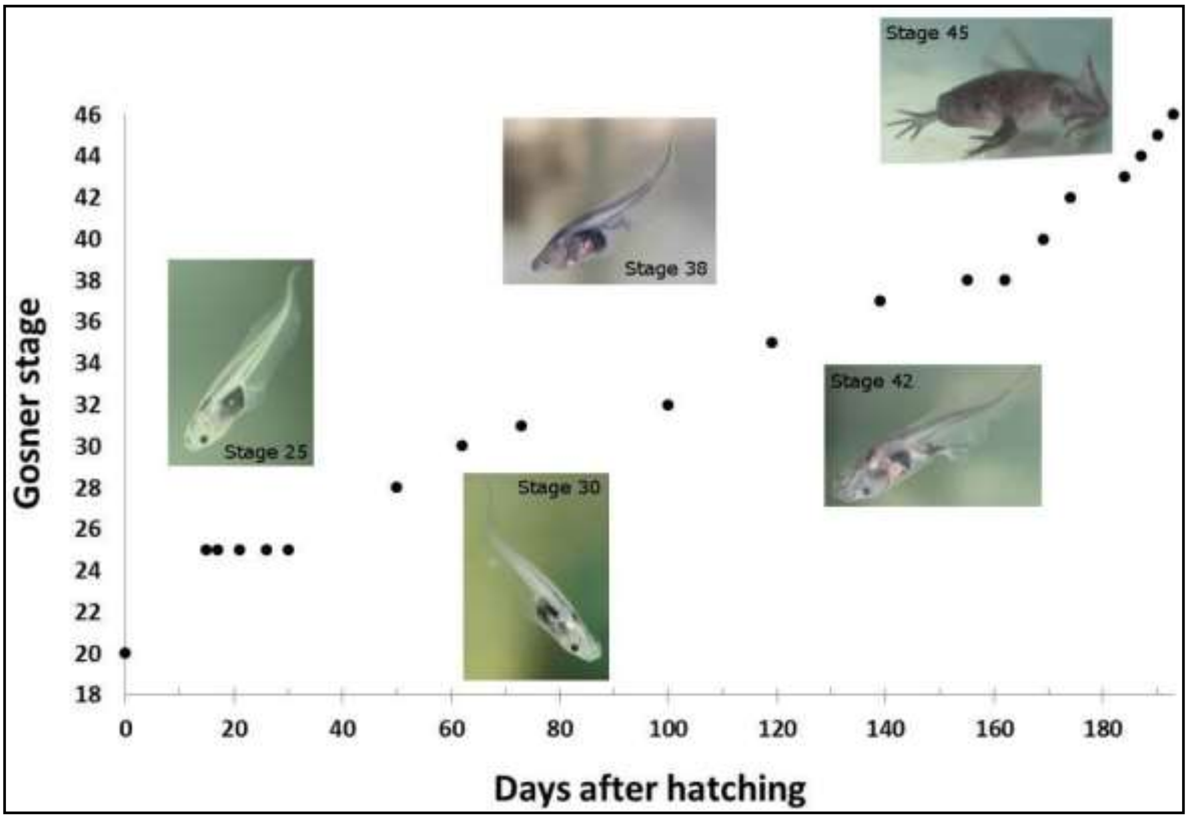
Tadpoles were experimentally raised in three types of water:

1. Fresh water cistern water (linked to adult frog aquarium). pH range of 7.5-8.2
2. Fresh water cistern water treated with the product pH DOWN to reduce the pH to 7.0-7.5
3. Reconstituted deionized water (this water recipe was performed to mimic the *X. tropicalis* and *X. laevis* rearing conditions at UC Berkeley).

None were successful in getting tadpoles past an age of 71 days. The most successful method employed in terms of tadpole age was to house 20 tadpoles in a 40 L aquarium with a slow flowing air-stream bubble filter. The water recipe that proved most successful in terms of tadpole age, was our freshwater cistern water treated with pH DOWN to bring the pH within a range of 7.0-7.5. The TDS was not recorded.

At ZSL London Zoo, tadpole development was slower in *X. longipes* than in congeners. Duration from hatching to metamorphosis lasted 193 - 262 days (Fig. 15; Tapley et al., 2015). In captivity tadpoles

Figure 15: Gosner stage progression of the most rapidly developing *Xenopus longipes* tadpole. Hatching to metamorphosis took 193 days taken from Michaels et al. (2015).



attained a maximum 68.0 mm in total length (Tapley et al., 2015). Tadpoles that were found dead in Lake Oku were considerably bigger with a mean total length of 82.5 mm and a maximum length of 91.8 mm (Tapley et al., 2015). This difference in size could be due to diet, water parameters and temperature regimes differing between the captive environment and Lake Oku. The tadpole of *X.*

longipes is relatively large when compared to the snout vent length of the adult; the maximum length of the tadpole is 91.8 mm, which is 2.5 times longer than the maximum recorded snout to vent length of the adult (Tapley et al., 2015). The relative size of the tadpole when compared to the adult frog is greater in *X. longipes* than in other congeners for which there is a tadpole description. The prolonged larval period maybe linked to a relatively stable habitat of Lake Oku where there is permanent hydroperiod and very low seasonal variation in environmental parameters (Fig. 8). Lower temperatures due to the altitude and no danger of the water body drying out may select for a longer larval phase (Werner, 1986). In *X. gilli*, a species of temperate lowlands in the extreme south of the African Cape, breeds in a habitat where temperatures are comparable with those recorded from Lake Oku. This species also has a relatively long developmental duration of 120 days (Rau, 1978).

2.5 Handling

Figure 16: *Xenopus longipes* marked with visible implant elastomer at California Academy of Sciences (© California Academy of Sciences)

2.5.1 Individual identification and sexing: Adult specimens do not have large distinctive markings and their small size precludes marking with passive integrated transponders. California Academy of Sciences used visible implant elastomer (VIE) injected into the interdigital webbing to individually mark adult *X. longipes* (Fig. 16). VIE marks were found to migrate in *X. longipes* at ZSL London Zoo and this method of individual identification is not currently considered viable in this species (Ævarsson et al., 2022). VI Alpha Tags (Northwest Marine Technology Inc., Washington, USA) were trailed on three adult *X. longipes* in May 2017 but the alphanumeric code was not readable. Frogs were not anesthetised for the VIE and VI Alpha tags procedures (Species360, 2022). Pharmaseq P-chips were trailed in 2018 on the same population but the reader was unable to read the chip once implanted. The reasons that these chips did not read include the P-chip being implanted too deeply; the p-chip flipped over once implanted or that the pigmentation of the frogs blocked the radio frequency transmission from the P-chip to the reader (B. Tapley, pers. obs., 2022). Belly patterns do allow adult photographic identification (Fig. 17) for up to six months but not for the longer term, and not in juveniles (Ævarsson et al., 2022). Tadpoles are extremely delicate and handling them in order to mark them as well as the marking process itself could be detrimental to their health.

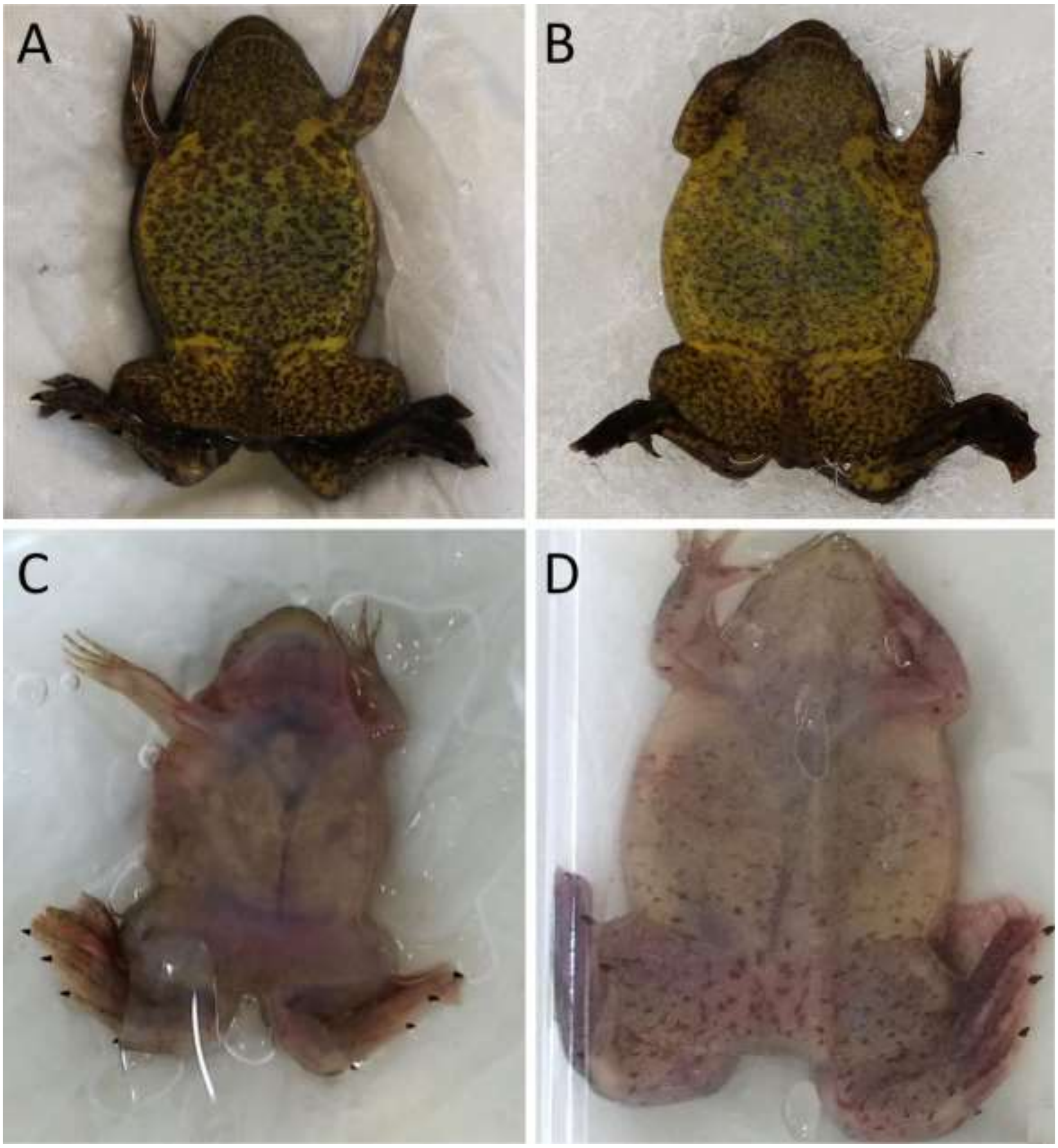


Figure 17: Ventral surface patterns in *Xenopus longipes*. Adult frog over a 24-month period (A & B) and in a juvenile frog over a 6-month period (C & D). Taken from Ævarsson et al. (2022).

2.5.2 General handling: This species should be handled very carefully and only when necessary. Powder free vinyl gloves should be used during handling and servicing as they protect the skin from abrasion, contamination between the species and the handler and the spread of pathogens (CCAC, 2003). Gloves also reduce the transmission of heat from the handler to the specimen. Latex gloves should not be used with *Xenopus* sp. Significant mortality in *X. laevis* tadpoles occurred when exposed to water where latex gloves had been soaked for 24 hours (Sobotka and Rahwan, 1994; Gutleb et al., 2001).

2.5.3 Catching / restraining: The need to restrain individuals in captivity is quite rare. Adult specimens can be captured and handled using a net. Tadpoles are extremely fragile, and nets should not be used to capture them. Instead, tadpoles should be captured by submerging a food grade plastic container into the water beneath the tadpole and lifting the container with the tadpole out of the water. A recent study on captive *X. longipes* developed an ethogram for the species and produced a diurnal activity budget (Dias et al., 2022). This study measured the behavioural impact of a routine health check where frogs were restrained. There were significant effects of the health check on duration of many behaviours (swimming, resting, foraging, feeding, and breathing behaviours) for all frogs. There is a welfare trade-off associated with veterinary monitoring and less invasive monitoring should be undertaken where possible where possible (Dias et al., 2022).

2.5.4 Transportation: Transportation of post metamorphic *X. longipes* should occur in a plastic container packed with wet paper towels or moss in a darkened condition. Sphagnum moss should be avoided as it is very low in pH and may harm animals during transport. Tadpoles should not be transported as they are extremely sensitive. The transportation container should be packed within a Styrofoam cooler to prevent sudden changes in temperature and to provide a buffer against temperature extremes (CCAC, 2003). The transportation container should be kept out of direct sunlight. The packing containers should be placed in a rigid outer shipping container. To prevent jarring during transport, crushed newspaper or foam packing chips can be used to support the packing containers within the outer shipping container. It is advisable to avoid shipping if weather forecasts predict extreme temperatures (CCAC, 2003).

2.5.5 Safety: As with all amphibians, disposable powder free gloves should always be worn to prevent toxins coming in contact with the skin and to avoid the spread of harmful microorganisms such as *Salmonella* being transmitted to the handler.

2.6 Specific problems: Considerations for health and welfare

Water parameters replicating Lake Oku are essential if the species is to be bred in captivity and for successful larval rearing.

Frogs collected from the wild and transported to the California Academy of Sciences tested positive for nematodes and Bd on arrival and were treated for nematodes (ivermectin) and Bd (itraconazole) prior to clearing quarantine.

Nematodes were treated by immersions in ivermectin (1% solution (10mg/ml)). Baths were prepared by adding 10mg (1mL) to 1L water and bathing the frogs for 30 minutes. Two treatments, 14 days apart appeared successful with no ill effect or mortalities noted with the frogs during the two treatments. Treatment appeared successful; post treatment faecal samples were negative for nematodes.

Bd infections were treated by immersions in Itraconazole (10mg/mL); baths were prepared by adding 30mg (3mL) to 1L of water and bathing the frogs for 5 minutes. Post treatment frogs were rinsed before being placed in a clean tank. Treatments were repeated once daily or a period of ten days. No ill effect or mortalities were noted during the 10 days treatment period. Treatment appeared successful, post treatment swabs were negative for Bd.

2.7 Recommended research

UV-B provision is an area that requires further research. Further pathological investigations and further work to determine the biology and ecology of *X. longipes* are required to enable a better understanding of the prospects for this Critically Endangered amphibian. Breeding triggers for *X. longipes* are currently unknown, this area should be a focus of further research if captive populations are to be viable. The vocalisation of *X. longipes* has not yet been described and further attempts to document and describe vocalisation should be made as this may facilitate monitoring of the species in Lake Oku. Tadpoles are challenging to rear and trailing live phytoplankton as a dietary item would merit further research. Captive animals have much higher body condition than wild animals and impacts of this, if any, should be investigated.

Although captive populations of *X. longipes* are not managed under strict enough biosecurity controls to be suitable for reintroduction efforts should they be needed (IUCN SSC, 2014), laboratory techniques for other *Xenopus* exist to generate 'clean' animals (e.g., Kay and Peng, 1992). There is therefore potential to use these techniques to create biosecure cohorts that could safely be used for reintroduction should it be required. Further research in this area is warranted.

Acknowledgements

The captive colony of *X. longipes* was exported under permit from the Cameroon Ministry of Forestry & Wildlife (0928/PRBS/MINFOF/SG/DFAP/SDVEF/ SC and 0193/CO/MINFOF/SG/DFAP/SDVEF/SC), following prior consultation with the Oku community, who also sanctioned access to their lake. We thank Gonwouo Nono LeGrand, Ndifon David, Oscar Nyingchia, Roland Ndifon, Henry Kolem, Matthew LeBreton and Robert Browne for logistical assistance within Cameroon. Field work was supported by the Royal Zoological Society of Scotland, an Erasmus Darwin Barlow grant from the ZSL, a British Ecological Society, European Association of Zoos and Aquaria and Mohammed bin Zayed Conservation Fund. Thanks also to Andrew Cunningham for supporting the initial establishment of this species at ZSL London Zoo and Luke Harding, Zoe Bryant, Iri Gill, Sebastian Grant and George Sunter for the care of the animals at ZSL London Zoo.

References

Ævarsson, U., Graves, A., Carter, K.C., Doherty-Bone, T., Kane, D., Servini, F., Tapley, B., Michaels, C.J. 2022. Individual identification of the Lake Oku clawed frog (*Xenopus longipes*) using a photographic identification technique. Herpetological Conservation and Biology 17: 67-75.

Avila, V.L., Frye P.G. 1978. Feeding behavior of the African clawed frog (*Xenopus laevis* Daudin): (Amphibia, Anura, Pipidae): effect of prey type. Journal of Herpetology 12: 391- 396.

Blackburn, D.C., Evans, B.J., Pessier, A.P., Vredenburg, V.T. 2010. An enigmatic mortality event in the only population of the Critically Endangered Cameroonian frog *Xenopus longipes*. African Journal of Herpetology 59: 1-12.

Blackburn, D.C., Paluh, D.J., Krone, I., Roberts, E.M., Stanley, E.L., Stevens, N.J. 2019. The earliest fossil of the African clawed frog (genus *Xenopus*) from sub-Saharan Africa. Journal of Herpetology 53: 125-130.

Blaustein, A.R., Belden, L.K. 2003. Amphibian defenses against ultraviolet-B radiation. Evolution & development 5.1: 89-97.

Brown, M.J., Nixon, R.M. 2004. Enrichment for a captive environment- the *Xenopus laevis*. Animal Technology and Welfare 3: 87-95.

Bwong, B.A., Measey, G.G. 2010. Diet composition of *Xenopus borealis* in Taita Hills: effects of habitat and predator size. African Journal of Ecology 48: 299-303.

Canadian Council on Animal Care (CCAC), 2003. Species specific recommendations on: Amphibians and Reptiles <www.ccac.ca> Accessed on 20th November 2008.

Channing, A., Howell, K.M. 2006. Amphibians of East Africa. Comstock Pub. Associates/ Cornell University Press, New York, 418pp.

Cheek, M., Onana J., Pollard, B. 2000. The Plants of Mt Oku and the Ijim Ridge, Cameroon. A Conservation Checklist. Royal Botanic Gardens, Kew. 211pp.

Coquet, L., Kolodziejek, J., Jouenne, T., Nowotny, N., King, J. D., Conlon, J. M. 2016. Peptidomic analysis of the extensive array of host-defense peptides in skin secretions of the dodecaploid frog *Xenopus ruwenzoriensis* (Pipidae). Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 19: 18-24.

Cragg, M.M., Balinsky, J.B., Baldwin, E. 1961. A comparative study of nitrogen excretion in some amphibia and reptiles. Comparative Biochemistry and Physiology 3: 227-235.

Densmore, C.L., Green, D.E. 2007. Diseases of amphibians. ILAR Journal 48: 235-254.

Dias, J.E., Ellis, C., Smith, T.E., Hosie, C.A., Tapley, B., Michaels, C. 2022. Baseline behavioural data and behavioural correlates of disturbance for the Lake Oku clawed frog (*Xenopus longipes*). Journal of Zoological and Botanical Gardens 3: 184-197.

Doherty-Bone, T. 2008. Conservation Research for Amphibians Unique to Cameroon (CRAUC) Final Report for work conducted in 2008. 76pp.

Doherty-Bone, T. 2009 Conservation Research for Amphibians Unique to Cameroon (CRAUC) Final Report for work conducted in 2009. 76pp.

Doherty-Bone, T. 2011. Unravelling the mysteries of Lake Oku, where the frog is "Fon" (king). FrogLog 97: 28-30.

Doherty-Bone, T. (Ed) 2014. A Working Conservation Action Plan for Lake Oku. Lake Oku Conservation Action Plan Workshop October 2013, Oku Fon's Palace, Elak-Oku, north West Region, Cameroon. 35pp.

Doherty-Bone, T. M., Gvoždík, V. 2017. The Amphibians of Mount Oku, Cameroon: an updated species inventory and conservation review. ZooKeys 643: 109-139.

Doherty-Bone, T.M., Ndifon, R.K., Nyingchia, O.N., Landrie, F.E., Yonghabi, F.T., Duffus, A.L.J., Price, S., Perkins, M., Bielby, J., Kome, N.B., LeBreton, M., Gonwouo, L.N., Cunningham, A.A. 2013. Morbidity and mortality of the Critically Endangered Lake Oku clawed frog *Xenopus longipes*. Endangered Species Research 21: 115-128.

Doherty-Bone, T. M., Nyingchia, O. N., Tapley, B. 2018. Cannibalism in the Critically Endangered Lake Oku Clawed Frog: a possible cause of morbidities and mortalities? Herpetology Notes 11: 667-669.

Ettling, J.A., Wanner, M.D., Schuette, C.D., Armstrong S.L., Pedigo, A.S., and Briggler, J.T. 2013. Captive reproduction and husbandry of adult Ozark hellbenders, *Cryptobranchus alleganiensis bishopi*. Herpetological Review 44: 605-610.

Evans, B.J. 2008. Genome evolution and speciation genetics of clawed frogs (*Xenopus* and *Silurana*). Frontiers in Bioscience 13: 4687-4706.

Evans, B. J., Carter, T. F., Greenbaum, E., Gvoždík, V., Kelley, D. B., McLaughlin, P. J., Pauwels, O.S.G., Portik, D.M., Stanley, E.L., Tinsley, R.C., Tobias, M., Blackburn, D.C. 2015. Genetics, morphology, advertisement calls, and historical records distinguish six new polyploid species of African clawed frog (*Xenopus*, Pipidae) from West and Central Africa. PLoS One 10: e0142823.

Fard, M., Pasmans, F., Adriaensen, C., Laing, G.D., Janssens, G.P.J., Martel, A. 2014. Chironomidae bloodworms larvae as aquatic amphibian food. Zoo biology 33: 221-227.

Godfrey, E.W., a Sanders, G.E. 2004. Effect of water hardness on oocyte quality and embryo development in the African clawed frog (*Xenopus laevis*) Comparative Medicine 54: 140-145.

Graves, Dias, J.E., Michaels, C.J. 2022 Effects of Background Color on Stress-Linked Behavior in the Critically Endangered Lake Oku Clawed Frog (*Xenopus longipes*). Journal of Zoological and Botanical Gardens 4: 99-107.

Gutleb, A.C., Bronkhorst, M., Van Den Berg, J.H.J., Murk A.J. 2001. Latex laboratory- gloves: an unexpected pitfall in amphibian toxicity assays with tadpoles. Environmental Toxicology and Pharmacology 10: 119– 121.

Herrel, A., van der Meijden, A. 2014. An analysis of the live reptile and amphibian trade in the USA compared to the global trade in endangered species. The Herpetological Journal 24: 103-110.

Hirschfeld, M., Blackburn, D.C., Doherty-Bone, T.M., Gonwouo, L.N., Ghose, S., Rödel, M.O., 2016. Dramatic declines of montane frogs in a Central African biodiversity hotspot. PLoS One 11: p.e0155129.

Hoff, K.V.S, Wassersug, R.J. 1986. The kinematics of swimming in larvae of the clawed frog, *Xenopus laevis*. Journal of Experimental Biology 122: 1-12.

Holmes, A.M., Emmans, C.J., Coleman, R., Smith, T.E., Hosie, C.A., 2018. Effects of transportation, transport medium and re-housing on *Xenopus laevis* (Daudin). General and comparative endocrinology 266: 21-28.

Inger, R.F., Max, H. 1961. The food of amphibians. Exploration of du Parc National de l'Upemba. Fascicule 64: 1-86.

Isaac, N.J.B., Redding, D.W., Meredith H.M., Safi, K. 2012. Phylogenetically-informed priorities for amphibian conservation. PLoS One 7: e43912

IUCN/SSC, 2014. Guidelines on the Use of Ex Situ Management for Species Conservation. Version 2.0. Gland, Switzerland: IUCN Species Survival Commission.

IUCN SSC Amphibian Specialist Group. 2020. *Xenopus longipes* (amended version of 2017 assessment). The IUCN Red List of Threatened Species 2020: e.T58176A177346697. <www.iucnredlist.org>. Accessed on 06 October 2021.

Kay, B.K., Peng, H.B. 1992. *Xenopus laevis*: practical uses in cell and molecular biology, 36. Academic press. 718pp.

Koloss, H.J. 2000. World-view and Society in Oku (Cameroon). Dietrich Reimerv, Berlin. 484pp.

Lafferty, K.D., Page, C.J. 1997. Predation on the endangered tide water goby, *Eucyclogobius newberryi*, by the introduced African clawed frog, *Xenopus laevis*, with notes on the frog's parasites. Copeia 3: 589-592.

Lobos, G., Measey, G.J. 2002. Invasive population of *Xenopus laevis* (Daudin) in Chile. Journal of Herpetology 12: 163-168.

Loumont, C., Kobel, H.R. 1991. *Xenopus longipes* sp. nov., a new polyploidy pipid from western Cameroon. Revue Suisse de Zoologie 98: 731-738.

McCoid, M.J., Fritz, T.H. 1980. Notes on the diet of a feral population of *X. laevis* (Pipidae) in California. The Southern Naturalist 25, 272-275.

McDiarmid, R. W., Altig, R. 1999. Tadpoles: the biology of anuran larvae. University of Chicago Press. 458pp.

Measey, G.J. 1998. Diet of feral *Xenopus laevis* (Daudin) in South Wales, U.K. Journal of Zoology 246: 287-298.

Measey, J. 2016. Overland movement in African clawed frogs (*Xenopus laevis*): a systematic review. PeerJ 4: e2474.

Michaels, C. J., Das, S., Chang, Y. M., Tapley, B. 2018. Modulation of foraging strategy in response to distinct prey items and their scents in the aquatic frog *Xenopus longipes* (Anura: Pipidae). Herpetological Bulletin 143: 1-6.

Michaels, C.J, Gini. B., Preziosi, R.F. 2014. The importance of natural history and species- specific approaches in amphibian ex-situ conservation. The Herpetological Journal, 24: 135-145.

Michaels, C.J., Tapley, B., Harding, L., Sunter, G., Bryant, Z., Grant, S., Gill, I., Nyingchia, O., Doherty-Bone, T.M. 2015. Breeding and Rearing the Critically Endangered Lake Oku frog (*Xenopus longipes*). Amphibian and Reptile Conservation 9: 100-110.

Miller, K. E., Brownlee, C., Heald, R. 2020. The power of amphibians to elucidate mechanisms of size control and scaling. Experimental cell research 392: 112036.

Noonan, S.C., Savage, G.P. 1999. Oxalate content of foods and its effect on humans. Asia Pacific Journal of Clinical Nutrition 8: 64-74.

Ovalle, W.K. Jr. 1979. Neurite complexes with Merkel cells in larval tentacles of *Xenopus laevis*. Cell and tissue research 204: 233-241.

Pasmans, F., Bogaerts, S., Janssen, H., Sparreboom, M. 2014. Salamanders, keeping and breeding. Nature and Tier: Münster. 247pp.

Quock, R., Blackburn, D. C., Ghose, S. 2014. Conservation Biology of *Xenopus longipes*. AGU Fall Meeting Abstracts. Vol. 2014.

Ramsey, J.P., Reinert, L.K., Harper, L.K., Woodhams, D.C., Rollins-Smith, L.A. 2010. Immune defenses against *Batrachochytrium dendrobatidis*, a fungus linked to global amphibian declines, in the South African clawed frog, *Xenopus laevis*. Infection and immunity 78: 3981-3992.

Rau, R.E. 1978. The development of *Xenopus gilli* Rose and Hewitt (Anura, Pipidae). South African Museum 76: 247-263.

Rauhaus, A., Gawor, A., Perl, R.G.B., Scheld, S., Van Der Straeten, K., Karbe, D., Pham, C.T., Nguyen, T. Q., Ziegler, T. 2012. Larval development, stages and an international comparison of husbandry parameters of the Vietnamese Mossy Frog *Theloderma corticale* (Boulenger, 1903) (Anura: Rhacophoridae). Asian Journal of Conservation Biology 1: 51-56.

Rosenblum, E.B., Poorten, T.J., Settles, M., Murdoch, G.K., Robert, J., Maddox, N., Eisen, M.B. 2009. Genome-wide transcriptional response of *Silurana* (*Xenopus*) *tropicalis* to infection with the deadly chytrid fungus. PloS one 4: e6494.

Rosol, T.J., Chew, D.J., Nagode, L.A., Capen, C.C. 1995. Pathophysiology of calcium metabolism.

Veterinary Clinical Pathology 24: 49-63.

Rosol, T.J., Capen, C.C. 1996. Pathophysiology of calcium, phosphorus, and magnesium metabolism in animals. Veterinary Clinics of North America: Small Animal Practice 26: 1155-1184.

Seale, D.B., Hoff, K., Wassersug, R. 1982. *Xenopus laevis* larvae (Amphibia, Anura) as model suspension feeders. Hydrobiologia 87: 161-169.

Sive, H.L., Grainger, R.M., Harland, R.M. 1997. Early development of *Xenopus laevis*: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sobotka J.M., Rahwan, R.G. 1994. Lethal effect of latex gloves on *Xenopus laevis* tadpoles. Journal of Pharmacological and Toxicological Methods 32: 59.

Species360 2022. Zoological Information Management System (ZIMS) for Husbandry www.zims.Species360.org. Accessed on 29 May 2022.

South, J., Botha, T.L., Wolmarans, N.J., Wepener, V., Weyl, O.L. 2020. Playing with food: Detection of prey injury cues stimulates increased functional foraging traits in *Xenopus laevis*. African Zoology 55: 25-33.

Tapley, B., Acosta, A.R. 2010. Distribution of *Typhlonectes natans* in Colombia, environmental parameters and implications for captive husbandry. Herpetological Bulletin 113: 23-29.

Tapley, B., Michaels, C.J., Doherty-Bone, T.M. 2015. The tadpole of the Lake Oku frog *Xenopus longipes* (Anura: Pipidae) Zootaxa 3981: 597-600.

Tinsley, R.C., Coxhead, P.G., Stott, L.C., Tinsley, M.C., Piccinni, M.Z., Guille, M.J. 2015. Chytrid fungus infections in laboratory and introduced *Xenopus laevis* populations: assessing the risks for UK native amphibians. Biological conservation 184: 380-388.

Tinsley, R., Measey, J. 2004. *Xenopus longipes*. The IUCN Red List of Threatened Species. Version 2015.2. <www.iucnredlist.org>. Accessed on 02 August 2015.

Tinsley, R.C., Kobel, H.R. 1996. The Biology of *Xenopus*. Oxford Scientific Press, Oxford. 464pp.

Wells, K.D. 2010. The ecology and behavior of amphibians. University of Chicago Press. 1148pp.

Werner, E.E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. American Naturalist 128: 319-341.

Zimkus, B.M., Baláž, V., Belasen, A. M., Bell, R. C., Channing, A., Doumbia, J., Fokam, E.B., Gonwouo, L.N., Greenbaum, E., Gvoždík, V., Hirschfeld, M., Jackson, K., James, T.Y., Kusamba, C., Larson, J.G., Mavoungou, L., Rödel, M., Zassi-Boulou, A., Penner, J. 2020. Chytrid pathogen (*Batrachochytrium dendrobatidis*) in African amphibians: A continental analysis of occurrences and modeling of its potential distribution. Herpetologica 76: 201-215.