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

Version 1



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SCIENCES

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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.

Introduction

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 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.

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.

Key husbandry points

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 can be extremely labour intensive.
3. Monitoring and management of water quality.

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

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Section 1. Biology and field data

1.1 Taxonomy

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

1.2 Morphology

1.2.1 Weight: Females 1.3-3.7 g; Males 1.1 – 2.3 g (Doherty-Bone, 2009)

1.2.2 Length: Adults 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).

1.2.3 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).

1.2.4 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. *X. longipes* have 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.

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 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 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 (Tapley et al., 2015).



Figure 1a; Male *X. longipes*. Figure 1b ; Female *X. longipes*.



Figure 2a and 2 C; keratinised nuptial pads on the forearms of a male *X. longipes*. Figure 2 b; Cloacal papillae in female *X. longipes* (Michaels et al., 2015)



Figure 3. *X. longipes* tadpole at Gosner stage 35.



Figure 4. *X. longipes* tadpole with barbel.

1.3 Physiology: Nothing published.

1.4 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 7 years; it is unknown how long frogs take to reach adult size.

Field data

1.5 Zoogeography, ecology and conservation

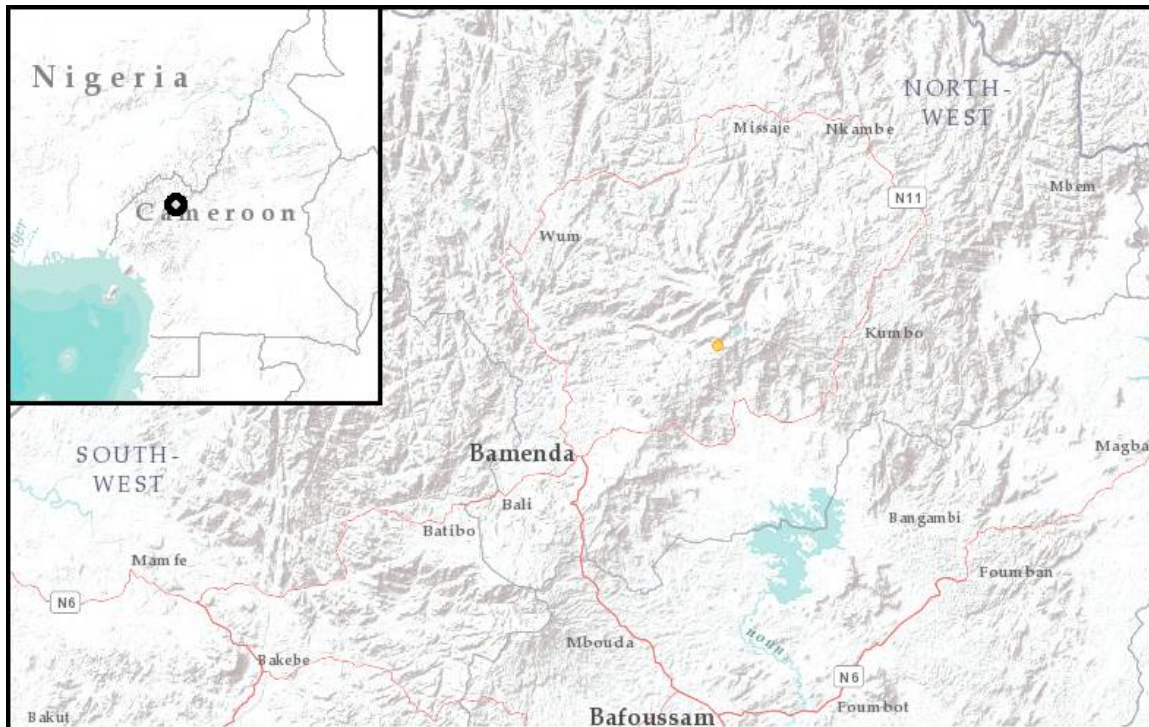


Figure 5: The distribution of *X. longipes*. The orange polygon represents where *X. longipes* are present (IUCN *et al*, 2008).



Figure 6. (left) Lake Oku (Tom Doherty-Bone) Figure 7 (right) *X. longipes* on endemic sponge in lake Oku (Brian Friermuth).

1.5.1 Distribution: *X. longipes* is endemic to Lake Oku in North West 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 *et al*, 2008). Lake Oku has an area of 243 ha.

1.5.2 Habitat: Lake Oku is a mesotrophic lake with a mean depth of 32 metres and a maximum depth of 52 metres (Doherty-Bone, 2014). The water in the lake is clear (Kling 1988). The lake is surrounded by montane rainforest (Tinsley and Measey, 2004). 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 17-19 °C (Fig.8). pH at the lake shore ranges from 7.4-8.4. The water of lake contains very little in the way of total dissolved solids (TDS) (Table 1). Frogs are frequently encountered around the lake shore although trapping efforts at the shoreline yielded similar number of adult frogs as trapping efforts at approximately 10 m in depth (Doherty-Bone, 2009). 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).

Parameter	Mean Value	±	Units
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).

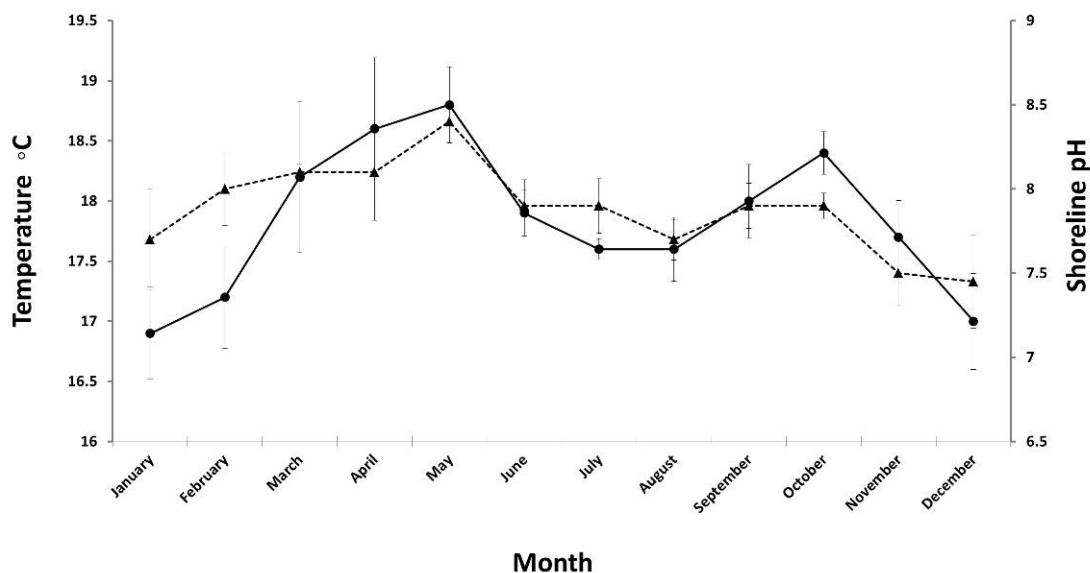


Figure 8. Mean annual water temperatures recorded from the shore line of Lake Oku water temperatures (circles) and pH (triangles) (Michaels *et al*, 2015)

1.5.3 Population: The population of *X. longipes* is currently thought to be stable (Tinsley and Measey, 2004).

1.5.4 Conservation status: The species is listed as Critically Endangered by the IUCN because its Extent of Occurrence is less than 100 km² and its area of occupancy is less than 10km², all individuals are in a single location, and there is a projected decline in the number of mature individuals, due to the high likelihood of a fish introduction into Lake Oku. (Tinsley and Measey, 2004). The threatened status of this species is reflected in its ranking as the number 35 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). Recent observations of the difficulty of the local authorities at Oku to resist developments imposed by the national government (in this case forest clearance for tourism and other infrastructure) have highlighted that harmful developments like fish introduction can still happen despite local awareness.



Figure 9. Dead *X. longipes* in Lake Oku.

Between 2006 and 2010 reoccurring enigmatic *X. longipes* morbidities and mortalities were observed in Lake Oku (Fig. 9), but the impact these mortalities are having on the population of *X. longipes* is unknown (Blackburn et al., 2010; Doherty-Bone et al., 2013). Diseased animals were lethargic and some presented with obvious limb or skin lesions, characterised by skin ulceration and limb tissue necrosis. The causative agent of these morbidities and mortalities remains unknown. No evidence of pathogens was found in tissues examined histologically (Blackburn et al., 2010; Doherty-Bone et al., 2013). Assessing the cause and consequences of these morbidities and mortalities to the

population viability of *X. longipes* is a priority. Recent surveys reveal *Batrachochytrium dendrobatidis* (*Bd*) infection in these frogs (Blackburn, unpublished data). *Bd* is the causative agent of the disease chytridiomycosis which is known to be one of the drivers of global amphibian population declines.

A captive-breeding programme was considered vital in case of a catastrophic collapse of the population due to stochastic factors (Tinsley and Measey, 2004). There are currently 83 captive *X. longipes* held at three institutions: ZSL London Zoo (UK); Cologne Zoo (Germany); and California Academy of Sciences (CAS) (USA) (ISIS, 2015). The populations in Europe are not held in biosecure facilities and are therefore not an appropriate source for any future reintroduction programme should this be required.

1.6 Diet and feeding behaviour

1.6.1 Food preference: The diet of *X. longipes* in the wild is 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 & 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* sp (Doherty-Bone, 2009; Doherty-Bone et al, 2013), larvae of these species could potentially be consumed by *X. longipes*. The feeding ecology of *X. longipes* tadpoles in the wild is unknown. 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).

1.6.2 Feeding: The feeding behavior of *X. longipes* in the wild is unknown, 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 dasyhelminid larva mixed with sediment and plant material were recorded in the stomachs of preserved specimens (Doherty-Bone. Unpublished data) but systematic study of stomach contents have yet to be made. *Xenopus* are tongueless and use their forelimbs to manipulate prey into their toothed jaws (Avila & 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)

1.7 Reproduction

1.7.1 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).

1.7.2 Age of sexual maturity: Unknown.

1.7.3 Seasonality of cycling: Unknown in nature.

1.7.4 Clutch size: Unknown in nature, in captivity clutch size ranged from 7-415 eggs.

1.8 Behaviour

1.8.1 Activity: In nature, post metamorphic *X. longipes* are predominately nocturnal, but during peak wet season become active during daylight hours (Doherty-Bone et al., 2013; T. Doherty-Bone personal observation). *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).

1.8.2 Locomotion: Tadpoles possess barbels 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) 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 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.

1.8.3 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.

1.8.4 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 vocalizations likely to originate from *X. longipes* (Martha Tobias, personal communication) but isolation of vocalizing individuals is needed to verify these.

1.8.5 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. Captive management

2.1 Enclosure



Figure 10. (Left) Enclosures for *X. longipes* at ZSL London Zoo (Michaels et al, 2015) and public exhibit at the California Academy of Sciences

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.

2.1.2 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).

2.1.3 Furnishings and maintenance: 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.

2.1.4 Environment: Field data and knowledge of the specific biology of the species should be used to inform captive management (Tapley & Acosta, 2010; Michaels & 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, Doherty-Bone et al., 2013.

2.1.5 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 (Doherty-Bone, 2009) 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 increasing in the wet season and decreasing as the rains reduce (Fig 8). Total dissolved solids (TDS) remains relatively stable throughout the year (Doherty- Bone, 2009).

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 in order to keep ammonia and nitrogenous wastes within specified parameters. Water is changed as values exceed 0.03 mg/L NH₄⁺, 0.6 mg/L NO₂⁻, 15 mg/L NO₃⁻. 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. *X. 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 removal of detritus from the bottom of tanks by siphon and thorough cleaning of air-stream sponge filters in aquarium water, helped to suppress nitrogenous 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.

2.1.6 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.

2.1.7 Dimensions: At ZSL London Zoo 40 x 25 x 25 cm (40 L) aquaria house a maximum of 7 adult individuals. Water volume approximately 200 L). 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.

2.1.8 Lighting: At ZSL London Zoo *X. longipes* are given A 12: 12 photoperiod. Enclosures are lit Arcadia T5 lamps with 12% UV-B (D3+ 12%UV-B 121 Reptile Lamp, Arcadia Products plc, Redhill, UK) although the current design of the enclosures does not allow for meaningful UV-B exposure. This species has been maintained at ZSL London Zoo for 8 years with no UV-B provision and no clinical symptoms of calcium deficiency. *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), SERA Micron powdered food 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* sp., glass worms (*Chaoborus* sp.), micro crickets (*Gryllus bimaculatus* and *G. assimilis*) and a commercially available *Xenopus* diet (Xenopus express; sinking frog food). 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.

2.2.3 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 amplexant animals should be removed from enclosures if amplexus is observed as non amplexant 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.

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. 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.

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 where multiple females have spawned on four occasions but larval mortality was high. Artificial breeding techniques have been successful 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. *X. longipes* spawning in captivity.

2.4.1 Mating: Amplexus is axillary and in captivity amplexant 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 1.23mm 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. 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) *X. longipes* hatching, Figure 13 (right) *X. longipes* with eyes and yolk sac.

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 I) after being reconstituted and stored at 4 degrees Celsius (pers. Com. Ben Evans McMaster Univ Ontario Canada, 2014) was used to hormonally induce oviposition using the standard breeding protocol for *Xenopus laevis* (Sive, Grainger & Harland, 1997) as a reference with dose adjustments after clinical trials and personnel communications with Ben Evans (McMaster Univ Ontario Canada, 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 in dorsal lymph sac of both males and females, followed the next day by an “induction/oviposition dose” of 200IU in dorsal lymph sac of both males and females. Egg laying occurred 24-48hrs post 2nd 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.

2.4.4 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 by Fieder) 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 *Xenopus tropicalis* and *Xenopus 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 congeners. Duration from hatching to metamorphosis lasted 193 - 262 days (Fig. 14; Tapley et al., 2015). In captivity tadpoles 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.

Water TDS (mg/L)	Refugia (live plants)	Detritus	Lighting	Tannins	Isolated from adult system?	Tadpoles survived?
20	-	-	-	-	+	+
	-	-	+	-	+	
	-	-	-	-	-	
	-	-	+	-	-	
	+	-	+	-	-	
	+	+	+	-	-	
	-	-	-	+	-	
	-	-	+	+	-	
	+	-	+	+	-	
	+	+	+	+	-	
	+	+	+	-	+	
	+	+	+	+	+	
150	-	-	-	-	-	-
	-	-	+	-	-	
	+	-	+	-	-	
	+	+	+	-	-	
	+	+	+	+	-	
	-	-	-	+	-	
	-	-	-	-	+	
	-	-	+	-	+	
	+	-	+	-	+	
	+	+	+	-	+	
	+	+	+	+	+	
	-	-	-	+	+	

Table 3. Combinations of conditions used to rear *X. longipes* tadpoles, and the outcome in terms of tadpole survival, after (Michaels et al., 2015).

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).

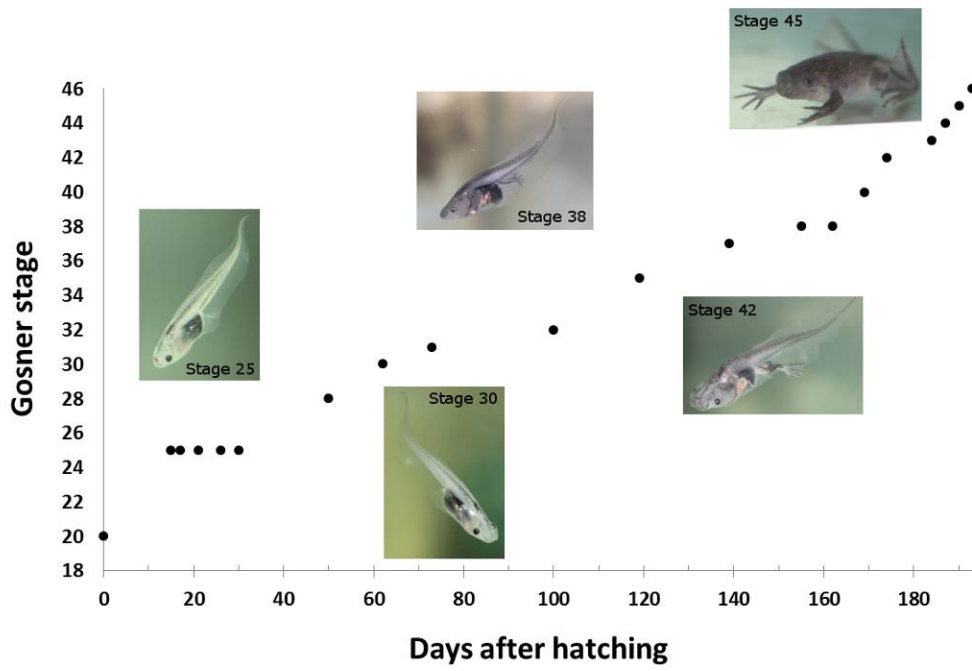


Figure 14: Gosner stage progression of the most rapidly developing *X. longipes* tadpole. Hatching to metamorphosis took 193 days (Michaels et al., 2015)

2.5 Handling



Figure 15: *X. longipes* marked with visible implant elastomer at 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. 15). Frogs were not anesthetized for the procedure. 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.

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.

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 1 Litre water and bathing the frogs for 30 minutes. 2 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 1 litre 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 day 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 future 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.

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