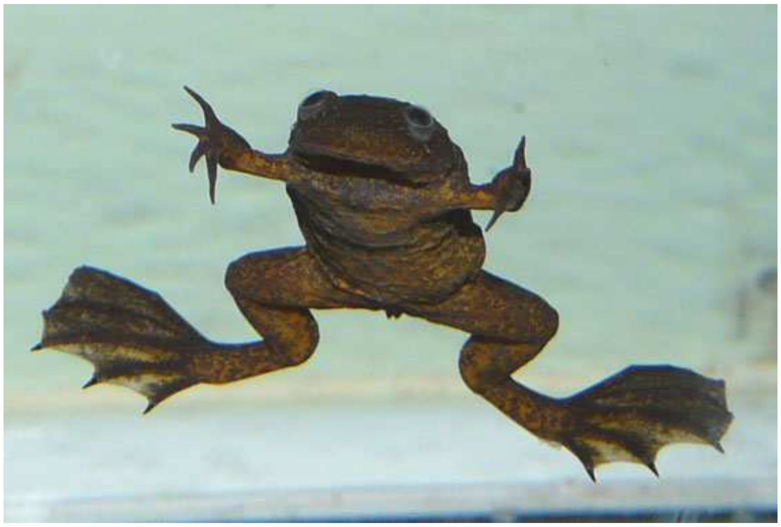
# Species profile: Lake Oku clawed frog (*Xenopus longipes*)



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Cover image of female *Xenopus longipes* by David Blackburn.

Refer to this document as: Browne RK[1*], Blackburn DC[2], Doherty-Bone T[3] 2009. Species profile: Lake Oku clawed frog (*Xenopus longipes*).

**Summary**

The Critically Endangered Lake Oku clawed frog (*Xenopus longipes*) conforms to IUCN Red list, Amphibian Ark guidelines and EDGE assessments as a priority candidate for a conservation breeding program supported by a Taxon Management Plan.

The entire population of this fully aquatic species occurs in Lake Oku at 2,200m on Mount Oku in North West Province, Cameroon. This population is under threat of extinction from the possible introduction of exotic fish, disease, invasive species and habitat modification. In 2008 a population census of *X. longipes* recognised the safety of removing founders to establish an international conservation breeding program.

*Xenopus longipes* is an excellent species to integrate a conservation breeding program with a wide range of research; virtually nothing is known about its biology. Its husbandry requirements, as predicted through the other *Xenopus* species in captivity, and through being a small aquatic species, should enable the keeping of appropriate numbers in simple facilities. In all there are approximately 15 species of *Xenopus* and 2 species of *Silurana* in captivity.

*Xenopus* can be housed adequately in plastic troughs, and typically do not need special temperature regulation. Another exceptional attribute of *X*. *longipes* is that quarantine can be readily accomplished. *Xenopus longipes* consumes artificial compound feed, potentially eliminating the need for live food with associated risks of disease transmission. *Xenopus longipes* will also feed on enriched crickets, which although live feed are less likely than aquatic feed to transmit pathogens. *Xenopus longipes* are totally aquatic eliminating the possibility of the introduction or spread of pathogens through complex substrates such as soil.

To maintain genetic variation, biobanking is a developing theme in amphibian conservation breeding programs. Another exceptional attribute of *X. longipes* is the potential for successful cryopreservation of the sperm, based on application in both *X*. *laevis* and *X*. *tropicalis* for genetic strains. The extension of this technique to *X. longipes* would enable the indefinite maintenance of genetic diversity and enable both greater security and reduction of the necessary captive population. To facilitate the perpetuation of the genetic variation of the Lake Oku clawed frog if possible, an assessment using microsatellite DNA techniques should be used, though this may be complicated due to the polypoidy of this species. .

Antwerp Zoo houses a conservation breeding population of *X*. *longipes* (~50) and is encouraging collaborations with other institution with populations (~90 in total). Other species of *Xenopus* are not of high conservation priority, with the exception of *Xenopus gilli*, of South Africa, threatened by invasion of habitat by and hybridization with *X*. *laevis*.

Conservation programs for *X*. *longipes* offer an exceptional opportunity for sustainable population management integrated with benefits to local communities. The foundations for conservation at Lake Oku are supported by a tradition in the Oku community, with the lake being considered sacred and deserving protection. There is also some protection already offered to the forest surrounding the lake as a government "Plantlife Sanctuary", with the rest of the mountain forest managed by the community. However, ongoing conservation efforts will be needed for guaranteeing continuing political support.

Educational and research and opportunities with *X. longipes* are provided by:

- Unique habitat in Lake Oku with its surrounding forest of high conservation significance.
- Lake Oku being a conservation focus for other species particularly birds.
- Critically Endangered status.
- Good display characteristic of *X*. *longipes* as adults.
- *Xenopus* spp. being a major contributor to biomedical, genetic, and biological research.
- The reciprocal use of biotechnologies for conservation.
- Unique chromosome compliment.
- Surrogate *Xenopus* spp are readily available.
- Parallels to the Endangered, Cape Platanna *X*. *gilli*.

**Introduction and aims of this document**

With the Lake Oku Clawed Frog (*Xenopus longipes*) becoming part of an international Taxon Management Plan (TMP) for zoos and associated institutions, an updated profile on this species is therefore necessary. This document is aimed at anyone who holds or may hold this species in their collections, as well as researchers planning projects on captive animals. Information includes an overview of published work, and findings of recent expeditions so far unpublished. This accompaniment to the TMP is hoped to serve as a detailed introduction to *X. longipes* for anyone who becomes involved in its conservation, or conservation of similar species or habitats.

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**Associated Amphibian Ark documents**

*Xenopus longipes* Husbandry Guidelines
*Xenopus longipes* Taxon Management Plan

**Status**

Red List Category: Critically Endangered (CR)
Red List Criteria: B1ab(v)+2ab(v)

Date of Assessment: 15 May 2004. Red List Category Rationale: Listed as Critically Endangered because its Extent of Occurrence is less than 100km$^2$ and its Area of Occupancy is less than 10km$^2$, all individuals are in a single location, and there are current and potential catastrophic threats to the continued survival of this species (Tinsley and Measey 2004).

CITES Status (Convention on International Trade in Endangered Species): Not listed.

*Xenopus longipes* is ranked as 34 on the Zoological Society of London, EDGE (Evolutionarily Distinct and Globally Endangered) Programme due to its conservation status and unique genetic characteristics.

Amphibian Ark prioritisation – not prioritised.

In the highly diverse region of Cameroon seven other amphibian species are critically endangered out of a total of 196 species. Of the other 17 species of *Xenopus*, the Cape Platanna *X. gilli*, an endemic of the south-western tip of South Africa is endangered. Regionally, Cameroon ranks 17 in the top 20 for countries for the highest biodiversity of amphibians and within Africa has the highest number of threatened species (IUCN *et al.* 2006) and the highest number of amphibian species per unit area.

**Description**

*Xenopus longipes* was described by Loumont & Kobel (1991). Uniquely among vertebrates (except *X. ruwenzoriensis* and two undescribed species), *X. longipes* is a dodecaploid species probably formed by both hybridization and polyploidization (Loumont and Kobel 1991; Evans et al., 2004). In general the biology of *Xenopus* sp. is very different from that of other frogs and toads (Tinsley et al., 1996). For instance, they have a lateral line system normally found in fish.

*Xenopus longipes* is a small species, with a total length of 28–31 mm in males and 32–36 mm in females. The body is pear-shaped and ventrally flattened, possibly enabling foraging on the substrate. The head is short and broad with a rounded snout. The eyes are relatively large and protrude upwards giving floating frogs panoramic vision of the water surface. A small lower eyelid covers the lower third of the eye, barely reaching the pupil. The dorsal surface of *X. longipes* is golden-brown in colour and is heavily speckled with melanocytes and patterned with irregularly shaped large blotches. Their ventral surface is also covered in melanocytes, with the throat and thighs being yellow-orange. The skin is not entirely smooth, but is covered in tiny keratinous spinules. The back legs are strong and the feet are large and possess large, thin, webbed toes, with prominent keratinised claws.

Tadpole are not known from *X. longipes* but tadpoles of other *Xenopus* are dark in colour and lack keratinous beaks and posses long barbels either side of their mouths, causing them to have a slight resemblance to tiny catfish (Tinsley et al., 1996).

X-rays show males of *X. longipes* having larger and more ossified postero-medial processes of the hyoid plate (D.C. Blackburn, unpublished data). It may be possible to determine sex in live frogs by gently feeling for these large bony processes. When in breeding condition males may be distinguished by nuptial pads, enlarged first digits on the front limbs, and a negligible labial cloaca. Recent fieldwork has found these traits useful in distinguishing males from females. These traits are however difficult to recognise from photographs or after preservation (T. Doherty-Bone, unpublished data).

**Taxonomy and phylogeny**

*Xenopus* are moderately sized aquatic frogs in the family Pipidae, residing mostly in still water bodies including permanent lakes. Pipids have a wedge-shaped, dorsoventrally flattened body, small, upward-gazing eyes, no visible eardrums, unique vocalizing apparatus requiring no inflatable sacs, and a very slippery skin. There are eighteen species of *Xenopus*, all of which are restricted to sub-Saharan Africa. "*Xenopus*" is Latin for "strange foot," because of their enormous webbed, five-toed, three-clawed rear feet. Pipids include the Surinam Toad (Genus *Pipa*) of South America and the diminutive West African *Hymenochirus* and *Pseudohymenochirus*.

Amphibians first evolved around the Carboniferous Period ~ 350 Mya in Panagaea before its breakup into Gondwana and other nascent continents. The Anura emerged around the early Triassic Period ~ 260 Mya, with modern day archaic lineages of the Archaeobatrachia emerging in the late Triassic/early Jurassic, the Neobatrachia diverging in the Cretacious, possibly in response to the emergence of flowering plants (Roelants *et al*., 2007). Putative fossil *Xenopus* in Brazil and support a Gondwanan origin of the Pipidae. Fossil *Xenopus* are found in Africa from the late Cretaceous (100my BP) to the early Pleistocene (10my BP). Molecular clock analysis of phylogeny has suggested that the Pipidae first emerged as far back at the Jurassic Period (200 my BP) (Roelants *et al*., 2007). The occurrence of Pipid fossils in Yemen that may be those of *Xenopus* confirm the existence of the taxon in the late Oligocene (~25 MYa) and reinforces the view that the families distribution was once more widespread geographically than at present (Henrici & Báez, 2001).

Studies of mtDNA infer that lineages of extant clawed frogs originated after the breakup of Gondwana (63.7 Mya) and occurred 50 – 80 Mya. *Silurana* and two major lineages of *Xenopus* now have overlapping distributions in sub-Saharan Africa, and clawed frogs probably originated in central and/or eastern equatorial Africa. Most or all extant species originated before the Pleistocene and polyploidization occurred at least six times in clawed frogs (Evans et al. 2004).

Evolutionary biologists consider the Pipidae comprise of two sub-families: the Pipinae (*Pipa*, *Hymenochirus* and *Pseudohymenochirus*) and the Xenopodinae (*Xenopus*, *Silurana*). *Xenopus* chromosome numbers are multiples of 18 and *Silurana* multiples of 20; The *Silurana* currently includes two species: *tropicalis* and *epitropicalis*. However, developmental biologists generally ignore this taxonomy and instead recognize *Silurana* as *Xenopus*. The species in the genus *Xenopus* have chromosome numbers that vary from tetraploid (36 chromosomes; many species) to dodecaploid (*X. ruwenzoriensis*, *longipes*); the chromosomal echelons of the genus are believed due to hybridization-induced endoreduplication (multiplication of chromosome numbers).

Allopolyploidization has occurred on multiple occasions in *Xenopus* giving rise to tetraploid, octoploid, and dodecaploid species. The four subgroups of the *Xenopus* group include the *X*. *fraseri* group; the *X*. *laevis* group; the *X*. *muelleri* group; and the *X*. *longipes* group; however, this classification was devised before molecular phylogenetic analyses and these divisions are now not entirely mutually exclusive. *Xenopus longipes* is dodecaploid, which means it has 12 sets of chromosomes that have resulted from both hybridisation and chromosome doubling. The Lake Oku clawed frog has dodecaploid compliment of 108 chromosomes in each of its cells.

Tinsley and Jackson (1998) showed that susceptibility to *Protopolystoma* (Monogenea, Polystomatidae) in *Xenopus* may be interpreted as indicating a powerful influence of host polyploidisation and hybridisation on susceptibility to infection and parasite speciation. The unusual chromosomal compliment of *X*. *longipes* could therefore be associated with resistance to parasites.

**Geographic range, distribution and habitat**

Biogeographic Realm: Afrotropical. *Xenopus longipes* is endemic to Lake Oku on Mount Oku, Cameroon Highlands, North West Province, Cameroon. It possibly could occur elsewhere in the Cameroon highlands, but there are few lakes with ecological characteristics similar to Lake Oku (GAA; Figure 1); preliminary investigation at other isolate crater lakes have so far not resulted in the discovery of additional undescribed *Xenopus* species (D.C. Blackburn, unpublished data). Lake Oku lies on the watershed between the Sanaga and the Bénoué river systems (EDGE 2008).

Lake Oku lies close to the summit of Mount Oku (3,011 m) in what is though to be a crater, and is roughly circular, 5 km in perimeter, and 50 meters in maximum depth (Kling, 1988; T.Doherty-Bone, unpublished data). Lake Oku is situated in the middle of the Cameroon Volcanic Line, a series of volcanic massifs 2000–3000 m high that receives abundant rainfall. The higher parts of these massifs are covered by montane forests isolated by grassland and savanna-type vegetation (Figure 1).

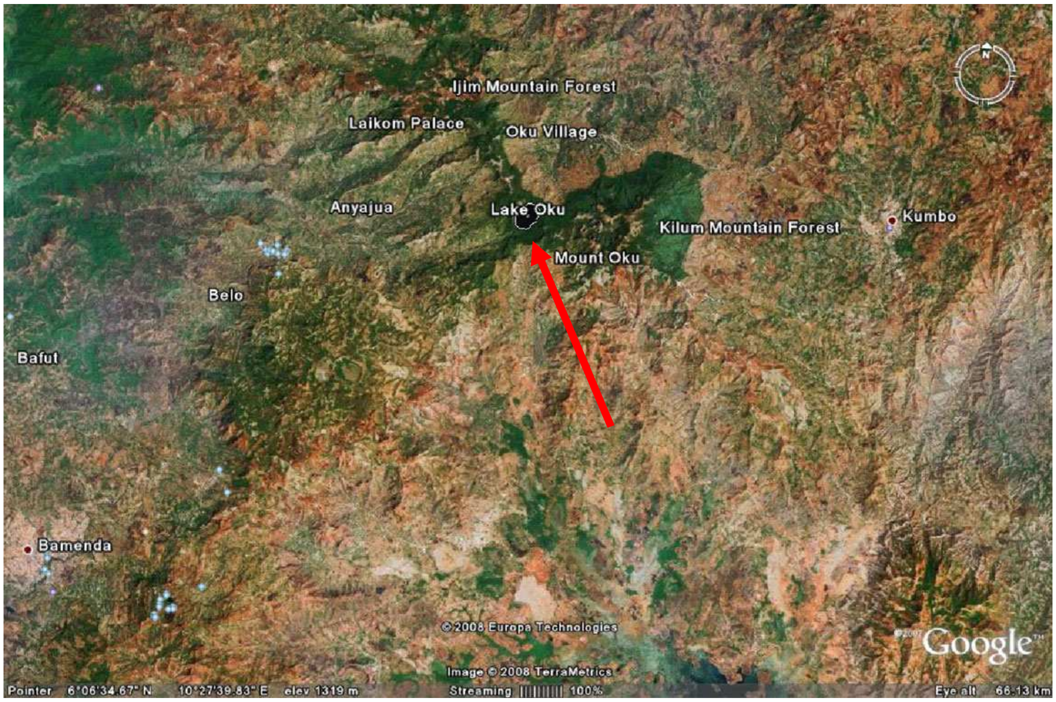



Figure 1. Lake Oku, North-West Province, Cameroon, Latitude/Longitude**:** 6 12'02.5"N, 10 27'35.4"E.

The Cameroon crater lakes on the Cameroon Volcanic Line formed in a series of craters and depressions on formerly active volcanoes that stretch inland approximately easterly from the coast. They include include Barombi Mbo, Bermin, Dissoni/Soden, Benakouma, Kotto, and Mboandong. Most of these lakes are very small, with an area of less than 5 $km^2$. The ancient nature and isolation has led to an extremely high level of endemism in these lakes where over 75 per cent of the fish species and approximately one-third of the aquatic insects are endemic.

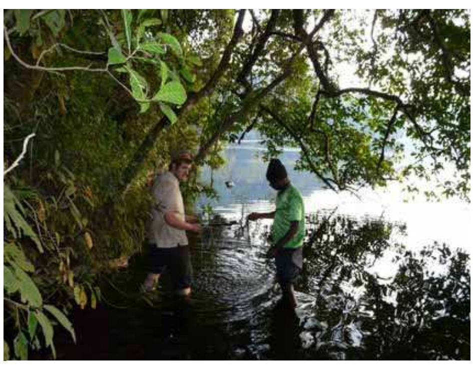
Left: Lake Oku is a small crater lake largely surrounded by forest.

Little is known about the natural history of Lake Oku (Loumont & Kobel, 1991). The shoreline water in Lake Oku approximately varies from 15–19˚C (T. Doherty-Bone, unpublished data). It is approximately neutral in Ph (7), thought to be comparatively low in nitrogen and phosphorus, and has measurable quantities of sulphate (T. Doherty-Bone, unpublished data). The frog is the only known aquatic vertebrate in the lake and consequently is probably a key species in maintaining the aquatic ecology of Lake Oku (Gartshore, 1986; IUCN *et al.*, 2006, T. Doherty-Bone, unpublished data).

With montane forest on its shore, Lake Oku is relatively undisturbed, apparently oligotrophic and has very clear water (Kling, 1988). The forested shores around Lake Oku slope, sometime steeply, into the lake. Around the lake shore there is typically a slight bank. Near the shoreline the lake bottom is covered with fine silt and dead leaves then gradually increases in depth until reaching beds of water weeds at about 2 m depth. These beds are slightly emergent and are observed from the surface as between 5–40 m from shore. The Lake Oku clawed frog is the lake's only *Xenopus* species. The closest other *Xenopus* species, *Xenopus amieti*, is found in streams and pools only hundreds of meters down-hill on the outer slope of the crater.

**Population and demography**

Both the 2006 and 2008 surveys showed *X. longipes* as abundant in Lake Oku. In 2006, over 700 individuals were captured using dip nets along a 16 m stretch of lake shore alone (Doherty-Bone, 2007). Further studies of *X. longipes* population density throughout Lake Oku are needed to estimate the total population. Preliminary mark-recapture results indicate limited migration for some individuals (Doherty-Bone, unpublished data).. The extension of *X*. *longipes* habitat into the lake is unknown. As *X*. *longipes* has only been sampled through active dip-netting and fish traps near the shoreline it is uncertain if these specimens are representative of the overall population. Examination of preserved specimens could reveal the sex ratio and possibly age of the shoreline 'sample population' of *X. longipes*.



Above: Setting traps on the shoreline of Lake Oku.

Above: The trap is set overnight in the shallows.

Left: Several dozen *X. longipes* may be captured overnight in one trap.

**Expeditions and activities:**

Doherty-Bone, 2009, 2008, 2006;
Blackburn et al. 2006, 2004;
Evens et al. 2004;
Loumont & Kobel HR, 1991 (Description); Gartshore, 1986.

**Threats**

*Xenopus longipes* is threatened through the introduction of fish to Lake Oku, through mortality from an unkown disease, and through changes to the aquatic environment of Lake Oku. The nature of the disease causing recently observed mortality has yet to be determined.

"Mass mortalities" of *X. longipes* were witnessed in 2006 (D.C. Blackburn, pers. obs.; Doherty-Bone, 2007). In other parts of the world, mortalities of this nature have often preceded catastrophic declines (Bradford, 1991; Lips 1999). No mass mortality events were observed during surveys in 2004 (B. Evans and D.C. Blackburn, unpublished data). Specimens of *X. longipes* collected in 2006 and 2008 were tested for the presence of the chytrid fungus *Batrachochytrium dendrobatidis* by qPCR, but no specimens (including other sympatric species) were found to be positive for this pathogen (Doherty-Bone et al., 2008; D. Blackburn, unpublished data). Whole-body histology was also performed using whole-body thin section histology on a selection of both healthy and "dying/dead" *Xenopus longipes* collected in 2004 and 2006, but no evidence of the chytrid was found (D. Blackburn and A. Pessier, unpublished data). *Phrynobatrachus* cf. *steindachneri* sympatric to *X. longipes* in 2008 were observed with haemorrhaging thighs (R Browne, pers. obs.). However, this may not be significant as these injuries could be due to handling (D. Blackburn, pers. obs.). As neither qPCR nor histology have demonstrated the presence of the chytrid fungus *Batrachochytrium dendrobatidis* and many specimens have large lesions and tissue necrosis, the possibility remains that the observed mass mortality events are the result of either a short-term temporal change in water chemistry, a pathogen other than chytrid, or a combination of these and other factors. This will be a critically important component for future research. The mass mortality events observed in 2006 remain unexplained.

Hans Rudolf Kobel (one of the pair of scientists that originally described the Lake Oku clawed frog in 1991) observed that local people actually shun the lake as a sacred place and will not introduce fish into it for this reason. However, the management of this lake is currently a contentious issue in local politics (D. Blackburn, pers. obs.; T. Dohety-Bone, pers. obs.). Cultural change could either negate or reinforce this tradition. With pressures to develop, many elders of the Oku community, as well as the local council have in the past attempted to seek permission to establish a fishery in Lake Oku and continue to seek possibilities to do this (T. Dohety-Bone, pers. obs.).

An insidious threat to the survival of *X. longipes* could be subtle changes to the aquatic ecology of Lake Oku. The aquatic ecology and water chemistry of Lake Oku are of special concern as these have been documented to change quickly in other lakes in the Cameroon Volcanic Line , in the case of catastrophic release of carbon dioxide from Lake Nyos in 1986 (Kling, 1987). Of concern is the paucity of knowledge of the environmental requirements of *X. longipes* and the ecology of Lake Oku. However, even slight increases in nutrients could cause trophic cascades that will affect the overall ecology of the lake. Sources of nutrient imbalance have not been assessed, though may include atmospheric depsotion from industrial pollution, and/or run off from erosion caused by forest loss and degradation.

*Xenopus longipes* could also be threatened by invasion of Lake Oku by other *Xenopus* species. Another *Xenopus spp.* is found in the surrounding region of Lake Oku and within hundreds of meter of the lip of the caldera housing Lake Oku. In SW Africa *Xenopus laevis* has hybridised with the endangered *X. gilli* when the acidity of ponds inhabited by *X. gilli* declined through terrestrial vegetation modification. *Xenopus gilli* lives in extremely acidic ponds that preclude habitation by *X. laevis*. *Xenopus laevis* has invaded southern California and Arizona and legislation prohibiting their possession is in effect in these and numerous other regions. *Xenopus laevis* is also a major in range invader of *X. gilli* ponds in South Africa as the acidity of the ponds becomes less acidic.

## Longevity, fecundity and reproduction

*Longevity*

There is no information on the longevity of *X*. *longipes* except for the small captive populations kept previous to the establishment of the December 2008 conservation breeding populations. A captive populations was established in 2003 and the 4 females were still alive in 2009 (B. Evans pers com). In another facility four females have lived for about 4 years (D. Kelley, pers. com.). Captive *Xenopus laevis* can live 15 years.

Skeletochronology is a method that enables the estimation of age that is suitable even for some tropical amphibians. This method is not always entirely reliable but will provide a first-pass estimate of age. Lines of arrested growth in the long bones are a reflection of variables such as water temperature, seasonal influences of food availability, reproduction can provide valuable information on life history and environmental traits. Skeletochronology has been applied to invasive *Xenopus laevis* populations in the UK (Measey, 2001), and could also be applicable to *X. longipes*.

*Reproduction*

There is little information on the reproduction or fecundity of *X*. *longipes*. Previous to the successful transport of all 88 frogs in December 2009, all males are reputed to have died during transport so there are no previous attempts to breed them in captivity. Fecundity of amphibians can be estimated from dissection of maturing or mature females and, for example, *X. laevis* have tens of thousands of developing eggs, and generally spawns batches of few thousand eggs several times a year.

Tadpoles of known *Xenopus* feed by filter feeding. They adopt a characteristic head-down posture in mid-water and take regular gulps of water. The continuous beating their long filament-like tails create a mouth-ward flow of water, where gills trap fine food particles as small as two microns and transport these to the digestive tract.

Seasonality in other *Xenopus* species appears dependent on rainfall to increase nutrients and plakton levels to feed tadpoles. Seasonality of reproduction of *X*. *longipes* is under current research by Thomas Doherty-Bone.

*Xenopus* possess a bony voice box with two cartilaginous rods that produce a clicking sound that is involved in communication including mating vocalisations.

## Behaviour, feeding and learning

There are two unpublished sources providing information of *X*. *longipes* behaviour; 1) brief above water lakeside observations at Lake Oku, and 2) observations of aquarium populations. These can be considered in relation to generic behaviour of other *Xenopus* species in both nature and captivity. Some species travel overland when forced by swamps drying or during heavy rain, though this has never been recorded for *X. longipes*.

*General:* In both aquariums and in Lake Oku, *X*. *longipes* frequently lie motionless with their limbs and fingers extended. In aquariums at Antwerp Zoo if unfed and undisturbed for some time they sleep in crevices in filter casings. Once disturbed by vibration or through cleaning or water change, they vigorously forage. This behaviour could have resulted from feeding immediately after water change.

*Feeding: Xenopus* feed through generating suction by quickly lowering their hyobranchial apparatus then by lunging forward and grabbing the food with their jaws. Sometimes they shovel food into their mouths with their hands. There is some debate about whether the arm movements are purposeful or partially a by-product of rotation of the pectoral girdle when suction feeding (D. Blackburn pers. com.). They do not have tongues and use their hands to capture and ingest food. Their highly sensitive fingertips, four on each hand, and sophisticated lateral line systems allow them to locate living prey, even when it is concealed in mud and detritus. They have also evolved the ability to locate food by smell and efficiently consume nonliving food items.

In Lake Oku *X*. *longipes* can be observed in large numbers at night (and occasionally at daytime) up to the shoreline feeding on the mud substrate. Limited shore side observations in Lake Oku, have shown *X*. *longipes* typically are feeding either close to the bottom, or in the water column, but typically not at the surface. *Xenopus longipes* has been observed as a group feeding nocturnally on a large praying mantis on the surface of Lake Oku (Doherty-Bone pers com). In general other *Xenopus* species are able to tolerate long periods of starvation. It has been observed that some *X*. *longipes* in Lake Oku are quite thin compared to captive specimens under intense feeding regimes (Browne pers obs).

*Sexual behaviour: T*here have been no observations in the field or in captivity of sexual behaviour, of *X*. *longipes* other than occasional amplexus pairs in traps (T. Doherty-Bone, pers. obs.). There are some observations of behaviour of *X*. *laevis* where adults came to the surface after rain although the reason for this is not known. The aquatic habitat of X. *laevis* precludes the holding physical territory and this species has strategies that appear to minimize adult competition. Females of *X*. *laevis* tend to quietly reconnoitre areas above the water's surface for prospective meals, while males often prefer actively searching for food across the bottom.

*Aggression:* In aquariums shoving and biting between *X*. *longipes* have been observed during feeding on the substrate (Browne pers obs).

*Learning: Xenopus laevis* has shown good learning ability for wave and sound stimuli and to some degree for visual and chemical stimuli. They have also shown long term memory and complex learning.

**Defences**

Nothing is known of the defences of the Lake Oku clawed frog. However, *Xenopus laevis* possess chemical defences that give protection against both predators and diseases. These repel many vertebrate predators, especially those found outside of the native range of *Xenopus*. *Xenopus laevis* also generate organic compounds called magainins which have powerful antibiotic, antifungal, antiparasitic, and antiviral actions.

**Senses and vocalisation**

*Eyes*: *Xenopus longipes* rest at the water surface with only their eyes exposed. Their eyes are spherical with binocular control of the area above and on the water surface. The refraction of the eyes is also for vision in air. The eyes dorsal and slightly frontal orientation enables the frog to see the whole hemisphere binocularly. There is no iris contraction enabling scotopic vision in conditions of low light. The eyes of *X*. *laevis* can see red/blue/yellow and green.

There is good vision of form. Visual cues showed that *X*. *laevis* preferred figures with less profile such as a circle over a square or a tripod. A lateral orientation was preferred to a vertical orientation. Larger sizes were preferred to smaller sizes.

*Sub-orbital tentacles*: The suborbital tentacle is a chemosensory structure.

*Lateral line*: Aquatic vertebrates use a mechano-sensory system to decipher currents, obstacles, and active movements of, for instance, predators or prey in the water body. The most primitive of the acoustaco-laeralis organs is the epidermal lateral line organ that is found on aqueous amphibian and primitive fish. This type of organ is activated by water motions that cause a displacement of a free standing, gelatonuus, cupula that protrudes from skin into surrounding water. In *X*. *laevis* this protrudes 0.1 mm above the skin surface.

*Touch:* Specialisation of touch is unknown in *Xenopus*.

*Smell and taste:* For smell *Xenopus* have the principale vomero-nasal cavity found in other frogs, and also a rostral cavity for use in water. These are selected by a dermal fold. Taste buds are located on the palate and the floor of the mouth.

*Captive populations:* There is a 'Rescue population' of 47 *X. longipes* at Antwerp Zoo, and another of 40 for rescue/research at London Zoo (February 2009 census). There are two captive populations of 4 individuals each for research in academic institutions (B. Evans pers com).

*Biomedical utilisation: Xenopus laevis* and *X. tropicalis* are among the most widely utilised vertebrate laboratory model species for genetic, developmental and toxicological studies. *Xenopus laevis* was the first vertebrate to be successfully cloned.

**Conservation Measures**

*In situ components*

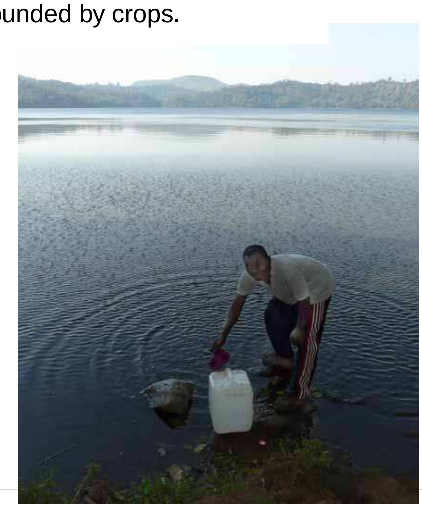
The conservation of *X. longipes* should be integrated into community programs for the sustainable management of ecosystems in the Lake Oku region. These will include generating long term benefits to the local communities from conservation based management of natural resources. Currently direct economic benefit to the Oku community from activities that may impact *X. longipes* include the collection of firewood, harvesting of honey, snaring of wild vertebrates, nearby farming of fish, and provision of services and products for scientific tourism and eco-tourism. In addition members of the community are engaged in scientific activities including monitoring of Lake Oku with support from the Royal Zoological Society of Scotland. Indirect and value stream economic benefits include long term benefit to the Lake Oku community of education and activities derived from the above activities.

Above: The local Oku people have been educated to collect only dead firewood from the forest surrounding Lake Oku. Despite this, there is extreme pressure on the Kilum-Ijim Forest for fuel wood, with the harvest feared to be unsustainable (Birdlife International, 2009; T. Doherty-Bone, per.

The prevention of the introduction of fish into Lake Oku is of the highest priority. Balancing regional food production in conjunction with conservation should include assuring an adequate supply of protein to reduce the potential for the introduction of fish into Lake Oku. Local education programs should be conducted and signage to present conservation priorities should be established.

Left: A typical farm house on the slopes of Mt Oku built of mud brick and surrounded by crops.

A conservation project has been conducted on Mount Oku for several years by BirdLife International (since 1987). Birdlife International projects build on the convergence of the interests of the conservation community with those of the local population. The projects support communities in the management of forests and the biodiversity of unique and important areas (Birdlife International, 2009).



Right: Collecting water for a camp at Lake Oku. Local conservationists are being trained for ongoing water quality sampling and assessment.

A research project: Conservation Research on Amphibians Unique to Cameroon (CRAUC), by staff of the Natural History Museum, London investigated the status of the critically endangered Lake Oku Clawed Frog and its habitat in Lake Oku (Doherty-Bone, 2008).

Expected outputs include reports on the work to be distributed to local conservation workers; information on endemic species to allow prioritization for conservation; community outreach highlighting the importance of amphibians; an increased capacity for management of species of conservation concern by local conservation managers; formation of long term collaborative links between British and Cameroonian workers.

Baselines need to be evaluated to enable ongoing monitoring of the *X*. *longipes* and the Lake Oku ecosystem. Subsequent work will inform of changes in the conservation status of *X*. *longipes* and the ecology of Lake Oku system. The proposed study by the CRAUC Project (Doherty-Bone 2008) aims to investigate population size of *X*. *longipes*, disease prevalence, demographics and fluctuations, and their environmental correlation. This information will elucidate the immediate level of threat faced by *X*. *longipes* and inform management decisions.

*Ex situ* components

The IUCN Technical Guidelines for the Management of *Ex situ* Populations, recommend that all Critically Endangered species should have an *ex situ* population to guard against extinction. "*A captive-breeding programme should be considered in view of the risk of a catastrophic collapse of the population if a predatory fish species is introduced to the lake.* (GAA 2008)

*Conservation breeding program*

The conservation guidelines of the Amphibian Ark (IUCN/CBSG) for captive breeding programs recommend the preparation of a Taxon Management Plan, a representative founder population, the distribution of the captive population between several institutions, strict quarantine measures, and studies of the *ex situ* population to facilitate both *ex situ* and *in situ* management. In addition the Amphibian Ark requires the preparation of 'Husbandry Guidelines'. Education has a high priority and model programs, like that proposed for the Lake Oku clawed frog, should be used to exemplify the conservation needs of amphibians, the value and contribution of amphibians to humanity, and the role of *ex situ* conservation programs in zoos and the Amphibian Ark.

*Quarantine*: Within Amphibian Ark conservation breeding programs, high quarantine standards are paramount. Particularly, since *X*. *longipes* may have suffered pathogen-related mortalities in the wild the quarantine of their conservation breeding populations should involve sanitary disposal of wastewater (K. Zippel pers com).

*Tagging and record keeping: Xenopus longipes* are about 30mm adult total body length. Consequently they can be tagged with Passive Integrated Transponders, (PIT) or similar tags. This will enable the establishment of a stud book with match DNA samples from the founders. Combined with the proposed DNA study of the variation within the wild population this provides a perfect model basis for studbook records for a captive breeding program.

*Behavioural studies:* There is very little known about the behaviour of *X. longipes.* The role of many behaviours remain unknown including the characteristics and purposes of vocalisations..

*Molecular genetic studies:* Emails were sent to canvas the issue of estimating the genetic variation of *X*. *longipes*. Responses were received from all individuals canvassed (B. Evans, Bob Lacy, T. Doherty-Bone, T. Garner, T. Beebee). The following is a summary of the responses with reference to particularly significant input.

Unique among vertebrates (except *Xenopus ruwenzoriensis*), dodecaploidy makes *X. longipes* of considerable interest for genetic studies. It was probably formed by both hybridization and polyploidisation (Loumont and Kobel 1991; Evans *et al.*, 2004). The polyploidy of *X. longipes* seriously complicates the outcomes of molecular genetic studies, particularly microsatellite studies. Attempts on other polyploid species, such as salmon and some plants, have apparently been reported to be difficult to interperate due to the duplication of genes (Allendorf & Waples, 1996; Frankham *et al.*, 2002). In terms of populations management molecular tools can be used to assess: 1) the effective population size; 2) whether a bottleneck test could be applied to determine whether the effective population is undergoing rapid decline, 3) loss of genetic variation in conservation breeding programs, 4) reproductive strategies.

Molecular genetic tools do not seem to be currently suitable to assess the amount and pattern of diversity in populations of *X. longipes*; polyploidy will make interpretation of the allelic data very difficult. When microsatellites have been applied to polyploid species (the plant, *Epilobrium angustifolium*) exposed to strong population bottlenecks, reduction of genetic diversity was less severe than in diploid sister species (Husband & Schemske, 1997). However, it is still possible to detect a loss of heterozygosity following a population bottleneck (Allendorf & Waples, 1996). Estimating loss of genetic variation in a breeding program uses the current methods of pedigree analysis and management for captive populations (e.g., the PM2000 software). However, presumptions are that the species are sexually reproducing diploids. Researchers have developed some useful tools for monitoring the genetics of species that are capable of selfing, However, good tools for managing species that are polyploid are not currently available. Some of the work that is currently taking place to extend our pedigree analysis methods to uncertain pedigrees (e.g., cases in which there are multiple possible sires) and to group-managed species (e.g., many amphibians!) could possibly be extended conceptually to work for polyploid species. (In a sense, a group of animals maintained without individual animal identification can be considered to be a genetically polyploid super-organism.) A difficulty of developing good pedigree analysis methods, however, will be that there is considerable diversity among polyploid species with respect to how the genes are transmitted (Bob Lacy, pers. comm..).

*Biobanking:* Biobanking is the long term storage of biological samples supported by a data base that records the biotic and environmental history of individual samples. For conservation breeding programs these should include viable sperm, cells and tissues that are representative of the source population.

*Xenopus laevis* and *X. tropicalis* sperm have been successfully cryopreserved. Consequently, it may be expected that the sperm of *X. longipes* can be used to cryopreserve a representative genome of *Xenopus longipes*.

To maintain the integrity of the conservation breeding program for *X. longipes* please do not distribute any individuals without contacting Amphibian Ark Taxon Management Coordinator, Robert Browne, robert.browne@gmail.com). Please also inform Taxon Management Co-ordinator should one be distributing samples to third parties, for the interest of keeping informed of research being conducted, as well as for tutelage.

*Xenopus longipes* should not be released from captive populations due to: 1) an implicit understanding of tutelage with the Lake Oku people and the Cameroonian government, 2) the possibility of pathogen dissemination, 3) the difficulty of restricting harvesting of wild stock if the species is in unrecorded populations.

Caution must be taken with individuals ostensibly claiming to be researchers (please personally contact Dr. Ben Evans phone (office/lab) : 905-525-9140 x 26973/27261).

**Permits**

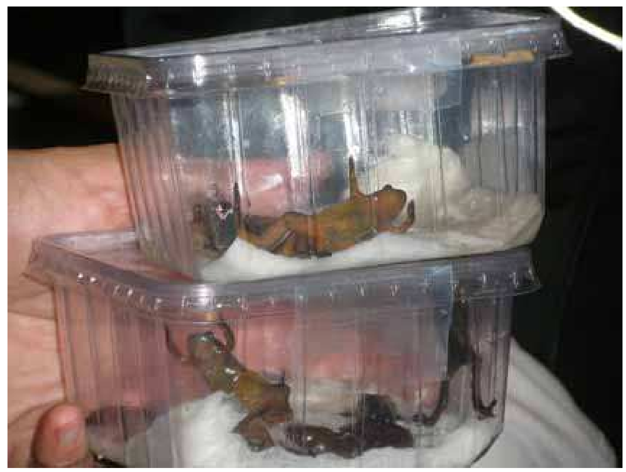
Permits need to be approved from the Cameroon Ministry of Forests and Wildlife. At the local level, the Fon of Oku is also consulted prior to work on and around his community's lake, as well as the relevant community forest managers. (Thomas Dohery-Bone, CRAUC )

**Obtaining rescue population founders and transport**

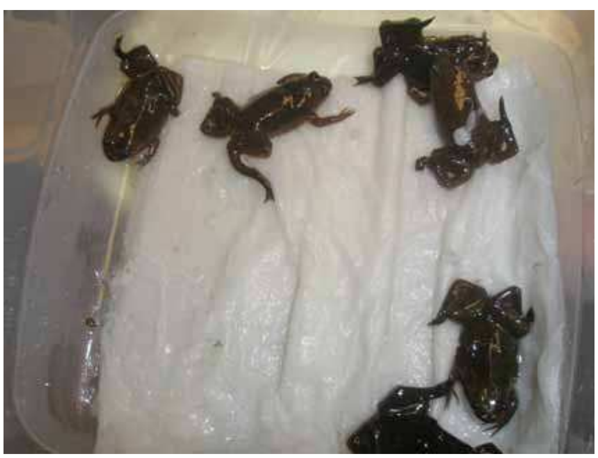
*Xenopus longipes* founders for 'Rescue populations' were transported from Lake Oku to Antwerp Zoo (48) and London Zoo (40) in December 2008. Previous transport of *X*. *longipes* for research populations was reported to have resulted in the mortality of males. Information regarding mortality of males is unclear and whether they died during transport or shortly afterward. Consequently, there was some concern with the December 2008 shipment. At Lake Oku the physiological limitation of *X*. *longipes* to the transport environment were tested by keeping two individuals for two days on a bench in an open shed at Lake Oku in plastic insect containers with wet paper on the bottom. The temperatures ranged from 15–24°C. The morning before leaving, the *X*. *longipes* for the final transport were sampled and placed as 4-6 frogs per plastic insect container. These were placed in a cooler and after reaching Bamenda were cooled by the use of ice, and (when ice not available) frozen fish being placed on top of the boxes inside the cooler. Once arriving in Yaounde the paper was changed daily and moistened with water taken from Lake Oku. The cooler was cooled with ice in plastic bags placed over the plastic insect containers.

*Xenopus longipes* for London Zoo were then transported in plastic insect containers in luggage with ice packs to prevent overheating, and all survived. The *X*. *longipes* for Antwerp Zoo were transported in a cooler with ice and they too all survived. .

Above: On arrival at Antwerp, cooler with 48 *X. longipes* with ice packs and towelling being removed.



Above: On arrival at Antwerp, plastic insect boxes with water saturated paper towel as a substrate.



Left: The *X*. *longipes* were kept at a density of 4-6 per. box.

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