DOI: 10.21005/AAPZ2023.66.2.9

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2023, 367(66)2, 90–99

Received 19 Sep 2022
Revised 22 Mar 2023
Accepted 11 Apr 2023

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THE PROPOSAL FOR MONITORING OF *XENOPUS TROPICALIS* (GRAY, 1864) IN WEST AFRICA

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Abstract. The main aim of the study was to present the suggested methods of monitoring *Xenopus tropicalis*, belonging to the subgenus *Silurana* of the Pipidae family. This species is a model animal, in laboratories it is kept in different conditions than the conditions of the natural environment, which proves its adaptation to a new habitat. In addition, an amphibian inhabiting West Africa has been found in the USA in the wild. This may indicate a potential threat as an invasive species. Active monitoring of this species can provide information about the spread and adaptation to new habitats. So far, no method for monitoring *X. tropicalis* has been proposed. The proposed method of assessing the condition of the species is based on field studies – the presence and abundance of individuals, while the assessment of the habitat includes many factors such as: the size and depth of the reservoir, its shading and overgrowth of the water surface, the presence of other animals (fish, other species of amphibians, reptiles) and nutritional base (presence of aquatic and terrestrial invertebrates). The proposed monitoring methods can also be used for other species of the Pipidae family that lead a typically aquatic lifestyle.

Key words: *Xenopus tropicalis*, *Silurana* sp., Pipidae, monitoring methods.

INTRODUCTION

Description of the species

Xenopus tropicalis (Gray, 1864) belongs to the subgenus of *Silurana*, genus *Xenopus*, the Pipidae family – tongueless frogs, to which it belongs, among others known and widespread species *Xenopus laevis* which is a model animal in the field of, inter alia, genetics and embryology, maintained in laboratories around the world (Harland and Grainger 2011). After many years of research on this species, due to its large size and longtime of sexual maturation (Cannatella and Trueb 1988), it is slowly being replaced by a smaller representative of its kind – *Xenopus tropicalis* (Halliday 2016). Both species mentioned above have their genome and embryology known (Grainger 2012).

X. tropicalis, together with species from the subgenus *Silurana*, is a small representative of the above-mentioned family of amphibians. It reaches an average body length of 51–59 mm, and males are smaller and less bulky than females. This amphibian has a streamlined and flattened body, an olive to dark brown color on the dorsal side, and there may be lighter spots

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between the eyes or on the back. The color of the ventral side ranges from white to dark gray (Fig. 1). Small black dots cover the whole body to varying degrees. Coloration may vary depending on where the specimens come from. They have pigment-free warts on the dorsal side (Kunz 2001) and side-line organs that resemble sutures. They are especially numerous around the eyes and the lateral and abdominal sides of the body. The head is triangular, rounded, with small eyes. The forelegs end with long fingers. Hind legs are massive, muscular, and the toes are held together by webbing. *Xenopus* amphibians have 3 claws on the inner toes of each foot. A characteristic feature of the *X. tropicalis* is the presence of a 4th claw on the metatarsus. The clawless front and back fingers end in a bulb-like tissue (Evans et al. 2015).

Fig. 1. *Xenopus tropicalis* (photo J. Stojak, 2020)

In addition to the difference in body structure and size, a feature indicating sexual dimorphism is a protruding cloaca in females, while males have mating cushions on their forearms, most often dyed dark, which appear after reaching sexual maturity and, unlike many species of amphibians, are visible throughout the entire life, and not only in the mating season.

In terms of structure, *X. tropicalis* is confusingly like other species belonging to the *Silurana* subgenus: *X. calcaratus*, *X. epitropicalis* and *X. mellotropicalis*. There are several ways to distinguish the species:

– number of sideline organs (Table 1),
– body size (Table 2),
– male mating sounds,
– genetic tests (Table 3).

Table 1. Summary of lateral line counts in amphibians of the subgenus *Silurana* (Evans et al. 2015)

Species	N	Eye ridges	Oral lateral lines	Medial lateral lines	Lateral lateral lines	Ventral lateral lines
X. tropicalis	15	10,3 (8–12)	10,7 (9–12)	17,9 (16–21)	19,9 (17–22)	18,3 (16–21)
X. calcaratus	17	10,4 (9–13)	10,3 (9–12)	16,4 (15–19)	18,4 (15–21)	17,4 (14–21)
X. epitropicalis	1	12,0 (12–12)	11,0 (11–11)	21,0 (21–21)	23,0 (23–23)	19,0 (19–19)
X. mellotropicalis	4	11,0 (8-12)	11,3 (10–13)	17,3 (16–19)	19,0 (16–19)	14,3 (10–18)

Table 2. Body sizes by gender of amphibians of the subgenus *Silurana* (Evans et al. 2015)

Species	Female (mm)	Male (mm)
X. tropicalis	51–59	42–44
X. calcaratus	44–59	–
X. epitropicalis	56–68	43–49
X. mellotropicalis	53–58	45–51

Table 3. Ploidy of species from the subgenus *Silurana* (Evans et al. 2015)

Species	Ploidy
X. tropicalis	diploidy
X. calcaratus	tetraploidy
X. epitropicalis	tetraploidy
X. mellotropicalis	tetraploidy

Xenopus tropicalis is the second smallest compared to other species of the subgenus *Silurana*. *X. calcaratus* is characterized by uniform, dark coloration also on the ventral side and *X. mellotropicalis* is also uniformly colored in bright colors.

Species differ in the vocalization they make. *X. tropicalis* has a trill-type call with higher intensity modulation than in other species. *X. mellotropicalis* and *X. calcaratus* has a burst-type call. *X. epitropicalis* has a biphasic call, which is a unique call type in the subgenus, and has longer interpulse intervals (~22 msec) than other species of *Silurana* (~10 msec) (Evans et al. 2015).

Species biology

X. tropicalis lives in the forests of the equatorial zone, in which two seasons follow each other – the dry season (October-March) and the rainy season (April-September), during which they are active and start breeding. The female lays from 1,000 to 6,000 individual eggs, 0.7–0.8 mm in size. The spawn is usually folded near the water surface and sticks to floating plants. Tadpoles hatch after a few days and begin to feed after an average of 4 days. This species is sexually mature at the age of 4–6 months.

Research by Imasuen and Aisien (2016) shows that *Xenopus tropicalis* in the natural environment feeds mainly on aquatic invertebrates and, to a lesser extent, on terrestrial: flies, annelids, bugs, dragonflies, crickets, termites, cockroaches as well as amphibian eggs and tadpoles. As representatives of Pipidae, they have no tongues. They push their prey into the mouth with the front paws, and if the food is too large, they tear it apart with the claws on the feet of the hind legs.

Habitat requirements

X. tropicalis inhabits shallow, warm, free-flowing or standing, overgrown reservoirs in forests of the equatorial zone (Evans et al. 2015). It spends the dry season buried in silt or soil (Kunz

2001 after Arnoult and Lamotte, 1968), and actively hunts and breeds in the rainy season (Kunz 2001). The reservoirs inhabited by *X. tropicalis* are often dark (brown), and the water is very soft (below 1°dH), acidic (Nguetsop et al. 2004) and nutrient-poor (Table 4). There is lush vegetation around the reservoirs such as trees (*Mitragyna stipulosa*, *Pterocarpus* sp.*, Mitragyna ciliata*), shrubs, herbaceous plants and ferns that cast a shadow over the reservoir, which may dry out periodically in the dry season. The water surface is additionally covered by plants such as: *Lemna* sp., *Azolla* sp., *Ceratophyllum demersum* and *Nymphaea lotus*. The reservoir is characterized by a great variety of species. In its vicinity there are such animals as shrimps, leeches, other various amphibians (*Ptychadena* sp., *Hoplobatrachus occipitalis*, *Sclerophrys maculatus*, *Bufo regularis*), fish (Clariidae, Cichlidae), reptiles (e.g. red-headed agama *Agama agama*, *Psammophis cf. phillipsi*, the black-necked cobra *Naja nigricollis*) and small mammals (e.g. *Crocidura* sp. and *Rattus rattus*) (Akani and Luiselli 2001).

Table 4. Water parameters in the Rumuosi marshes in the Nigerian rainforest of the Niger Delta, habitat of *X. tropicalis* depending on the season (Akani and Luiselli 2001)

Parameter	Wet season	Dry season
Air temperature [°C]	25,6 ± 1,5 (24,0–29,0)	30,8 ± 1,8 (27,6–33,0)
Water temperature [°C]	25,1 ± 0,8 (24,0–26,1)	28,5 ± 1,1 (26,2–30,4)
pH	5,6 ± 0,9 (4,8–6,6)	6,2 ± 1,3 (5,0–6,8)
Electricity [µS]	15,7 ± 5,4 (10,0–23,0)	24,7 ± 6,8 (12,8–30,5)
Salinity [g/l]	0 (0)	0 (0)
Total alkalinity [mg $CaCO_3$/l]	14,8 ± 3,2 (12,0–18,6)	16,2 ± 2,6 (13,1–19,3)

The conditions in which the other species of the subgenus *Silurana* live are similar, except for *X. calcaratus* occurs in a slightly colder place.

Species location in the world

In the natural environment, *X. tropicalis* can be observed in West Africa – from southern Senegal, through southern Gambia, Guinea, Sierra Leone, Liberia, Côte d'Ivoire, Ghana, Thogo, southern Benin, southern Nigeria, to western Cameroon. In the southern part of Cameroon, the distribution area coincides with the occurrence of *X. calcaratus* and *X. epitropicalis.*

Xenopus calcaratus is also found on Bioko Island and *Xenopus epitropicalis* in found in Republic of Equatorial Guinea, Democratic Republic of Congo and Gabon (Burger et al. 2006). The last specie from the subgenus *Silurana*, *Xenopus mellotropicalis*, can be observed in Gabon, Congo and Angola (Ernst et al. 2020).

X. tropicalis is a model animal, kept in laboratories around the world under conditions quite different from those of the natural environment, indicating its ability to adapt to a new environment. In 2021, scientists from the University of Florida confirmed the presence of *X. tropicalis* in the Tampa area, the first report of this species of frog outside its native range in West Africa.

The aim of this study is to propose methods of monitoring the species *Xenopus tropicalis* due to noticeable shortcomings in this field.

CONCEPT OF THE SPECIES MONITORING

When planning the monitoring of the species, it is worth getting acquainted with the literature and reports on the current occurrence (Penner 2011). This will make it possible to determine habitat preferences and narrow down the research area. The most common monitoring methods are visual, acoustic, and traps (Rödel and Ernst 2004).

The concept of monitoring

Xenopus tropicalis, as an unprotected species, is not systematically inventoried in Africa. Reports of its presence in each area come from studies examining the diversity and history of the evolution of *Xenopus* amphibians from West and Central Africa.

Monitoring studies are carried out on common areas and positions for amphibians occurring in each place, on dates consistent with the species' biology (rainy season) and considering individually selected characteristics of the condition of the habitat. *X. tropicalis* is a species with low habitat requirements, therefore the selected habitat characteristics are characterized by a certain universalism – habitats are inhabited by various species of animals.

Due to slight differences in the external morphology of species from the subgenus *Silurana*, the adopted methodology of amphibian monitoring may be problematic, therefore genetic, internal morphology or vocalization analyzes are required to confirm the species.

INDICATORS

The population status is not assessed at site level, as *X. tropicalis* breeds in small reservoirs that dry out periodically. Therefore, only the presence or absence of the species is assessed. However, it is worth entering the numbers of observed specimens as adults (even broken down by sex), juvenile and larvae.

Evaluation of the state of the population

The status of the *X. tropicalis* population is assessed at the biogeographical region level, based on changes in the number of reservoirs inhabited by this species in relation to all monitored reservoirs.

Due to the lack of data on the monitoring of *X. tropicalis*, the habitat status indicators (Table 5) were determined based on the methodology of valorization of habitats in which the most common specimens are in West Africa (Akani and Luiselli 2001), reflecting the ecological preferences of this species.

Table 5. Indicators of the habitat of *Xenopus tropicalis*

Indicator	Way of evaluation
Geographic region	A – valleys or coastal lowlands, B – upland threshold, C – mountain threshold
The depth of the reservoir (cm)	suitable – 20–30 cm satisfactory – 30–50 cm not suitable – >50 cm
Shading of the reservoir (%)	specifying the degree of shading of the tank in 10% steps: 10–20%… 100%. Most often, shade depends on the nature and height of coastal vegetation.
The degree of overgrowing of the water surface by the vegetation of the reservoir (%)	determination of the degree of overgrowing the water surface in the size ranges: 0–39%; 40–59%; 60–100%
Shore of the reservoir	suitable – flat not suitable – tall
Presence of other animals (fish, other amphibian species, reptiles)	suitable – small population satisfactory – medium population not suitable – large population
The presence of invertebrates	suitable – aquatic invertebrates in predominance over terrestrial invertebrates satisfactory – aquatic and terrestrial invertebrates in the ratio of 1 : 1 not suitable – terrestrial invertebrates overwhelmingly aquatic

The individual components of the aggregate habitat quality index were valorized on a three-point point scale (Table 6).

Table 6. Valorization of *Xenopus tropicalis* population status and habitat indicators

Indicator	Evaluation		
	FV	U1	U2
Geographic region	A	B	C
The depth of the reservoir (cm)	20–30	30–50	>50
Shading of the reservoir (%)	0–60	60–80	>80
The degree of overgrowing of the water surface by the vegetation of the reservoir (%)	65–100%	40–64%	0–39%
Shore of the reservoir	flat	tall	tall
Presence of other animals (fish, other amphibian species, reptiles)	none/small	medium	large
Presence of invertebrates	advantage aquatic	1 : 1	advantage terrestrial

FV – proper condition, U1 – condition unsatisfactory, U2 – bad condition.

Evaluation of the habitat condition

A habitat is in a favorable condition when the appropriate rating in relation to the entire rating is 100–70%, the unsatisfactory rating (U1): 0–30%, while at the same time the bad rating (U2) is not indicated. This means that a habitat that has been assessed as appropriate cannot have a single bad (U2) assessment.

Protection perspectives

When assessing the prospects of *X. tropicalis* behavior at a given site, we consider the condition of the habitat and the current and expected threats. The condition of the population is directly influenced by the condition of the habitat, and in the case of this species: the depth of the reservoir, the shading of the reservoir and the degree of overgrowing of the reservoir's water surface, as well as the height of the reservoir shore. In addition, the indicator of the presence of other animals (competition for food) and the presence of specific groups of invertebrates (type of food) are important.

The evaluation of this parameter is a subjective expert judgment made based on research experience, in addition, it may be supplemented with research in a similar area or scientific literature.

General evaluation

The general assessment consists of the assessment of the condition of the habitat and the assessment of the prospects for the preservation of the species at the site and is determined by the lower of the two features.

MATERIAL AND METHODS

Selection of monitoring areas and their suggested size

In order to delineate the monitoring areas, one can rely on cartographic materials. Habitats where amphibians start breeding are selected – water reservoirs, wetlands, oxbow lakes, periodical puddles. Habitats with at least a dozen small, closely located reservoirs (up to 1 km) should be monitored. The reservoir should not exceed 4,000 m^2, i.e.no fish farming tanks.

Determination of population status indicators

Species presence. The presence of *X. tropicalis* in the test site can be determined by two methods – observation (adults and juveniles) and listening (males during the mating season). The first method, depending on the location, may be imprecise, because the species can be confused with another, related, morphologically similar species. In order to observe the differences (size and number of side lines), it is necessary to catch, examine and photograph individuals.

Adult and juvenile individuals. We can observe adults and juveniles from April to September, most often in the evening during feeding. Active amphibians will stay near the surface of the water, waiting for the victim to get close enough to be captured. Traps (fish nets) can also be set, but this method requires that you empty them regularly. An alternative is to draw with a herpetological bucket, preferably at the bottom of the reservoir (frightened frogs hide in the silt). Male voices should be recorded and compared with patterns.

Tadpoles. Tadpoles are difficult to identify – tadpoles of different *Xenopus* species do not differ morphologically.

Eggs. *X. tropicalis* eggs are laid individually at the water's surface, most often on floating plants. They are very small and difficult to find and identify.

Determining habitat status indicators

The quality of the habitat should be described based on the indicators as presented in Table 5.

Time and frequency of field research

Monitoring should be carried out during the activity of amphibians – the rainy season (April–September). In extreme months, observations should be extended to wetlands near reservoirs where frogs may wake up/hibernate. The frequency of monitoring works, as in the case of all amphibians – every 3 years.

Research equipment and materials

- Topographic map
- GPS
- Trap – fish net
- Herpetological dipper
- Clothing for work in the field (waders, boots bottoms)
- Flashlight/headlamp
- Gloves
- Camera
- Measure/centimeter
- Species observation cards at the stand, pen

SAMPLE OBSERVATION CARD FILLING

Species card filling at the site	
Species name	*Latin name, author according to the currently binding nomenclature* *Xenopus tropicalis* (Gray, 1864)
Name of the site	*Name of the monitored site* Ekuri, Cameroons
Type of the site	*Enter: research or reference* Research
Protected areas where the position is located	*Nature reserves, national parks, ecological sites, documentation sites, etc.* Parc National de Krup
Geographical coordinates	*Enter the geographic coordinates of the position (GPS)* N 5°45, E 8°85

Altitude above sea level	*Enter the altitude above sea level positions or scope* 800 m
Stand area	Enter the area of the site (m^2) 1000 m^2
Characteristics of the species habitat at the site	*Take into account the general nature of the habitat (reservoir and surroundings); pay special attention to the presence of shallows in the reservoir and the nature of the vegetation in the reservoir and its surroundings* Rainforests, river basins
Information about the species on the site	*Synthetic information about the species occurrence at the site, research to date and other important facts; research results from previous years* The species was found for the first time in 1864, subsequent observations were carried out in the years 1960–1980.
Is monitoring required in subsequent years?	*Enter yes/no, and if "no", justify why it is proposed to resign* Yes
Observer	*Name and surname of the monitoring contractor in the position* Julia Stojak
Observation dates	*Dates of all observations* 20.04.2022; 10.05.2022; 15.05.2022; 30.06.2022; 05.06.2022; 15.06.2022; 30.06.2022

Conservation status of the species at the site

Parameters	Indicators	Indicator value and comment	Evaluation
Population	adult and juvenile individuals	current (3 females, 2 males)	
	tadpoles	current	
	eggs	none	
Habitat	geographic region	B	U1
	the depth of the reservoir	40 cm	U1
	shading of the reservoir	60%	FV
	The degree of overgrowing of the water surface by the vegetation of the reservoir	70%	FV
	shore of the reservoir	flat	FV
	presence of other animals (fish, other amphibian species, reptiles)	small population	FV
	presence of invertebrates	advantage aquatic invertebrates	FV
Conservation prospects		satisfactory prospects due to suitable habitat conditions and low risk of dehydration	FV
Overall evaluation			FV

Additional information card

Other natural values	*Other species of animals and plants observed during the monitoring works which are listed as endangered from various appendices. Mandrillus* leucophaeus
Alien and invasive species	*Observed alien and invasive species.* None
Other comments / other observations	*Any information helpful in the interpretation of the results, e.g. weather conditions, high water level, recent changes in the site (what and when did they occur?), etc.* None
Photographic and cartographic documentation	*Attachments to the database (in electronic version): At least 2 photos per site (species, habitat), boundaries of the research area marked on the appropriate cartographic background*

DISCUSSION

This species is a model animal kept in laboratories around the world in conditions quite different from the natural environment (Table 7), which indicates the ability to adapt to a new environment.

Table 7. Maintenance conditions of *Xenopus tropicalis* in a laboratory breeding

Parameters	Xenbase – Kristen Kroll	NBRP	CNRS
Temperature of water (°C)	25	24–27	24–26
pH	6,8 (maintained by $NaHCO_3$)	7	no data
Water hardness	hard	HARD	hard
Water conductivity (μS)	1600 (±50)	no data	800–1200
Salinity	RO + synthetic sea salt (Instant Ocean, "Crystal Sea")	dechlorinated tap water + Instant Ocean salt	ELGA microfiltered water with sea salt

It is sensitive only to low temperatures – one of the breeders reports the death of the frogs he breeds when the water temperature drops below 10°C. In Florida, where there is a wild population, high temperatures are observed all year round, which has allowed this species to start breeding.

In Europe, *X. tropicalis* is sold by pet shops both within the country and sent abroad. One of them trades in wild-caught animals, which carries the risk of transmitting parasites found in West Africa. The research by Imasuen and Aisien (2016) shows that X. tropicalis can be a carrier of tapeworms, flukes, nematodes, and spines. In addition, a case of infection with Mycobacterium liflandia has been documented by individuals kept in a research facility that came from a local vendor (Fremont-Rahl et al. 2011). This poses a threat to native animal species through direct infection or by eating a sick individual by another animal.

The risk of developing invasiveness of this species concerns countries where high temperatures persist throughout the year. The threat can be assessed as local because these frogs are not known for moving long distances, as they survive only a few hours outside the water environment.

CONCLUSION

Xenopus tropicalis is a model animal – it served, among others, in the field of embryology and genetics (its genome has been 100% known). It might seem that scientists know all about this, but the biology of the species is not fully understood.

The IUCN lists the species as "Least Concern" because it is widely distributed and is an adaptable species that lives in many habitats, and the population trend appears to be constant.

Monitoring of the species can be carried out using several methods: observation, catching, listening to the sounds of mating season. Regular monitoring of the population in the area of occurrence and in the area located near the area of habitat can show the possible speed of the spread of the species and its ability to adapt to new environmental conditions.

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PROPOZYCJA MONITORINGU *XENOPUS TROPICALIS* (GRAY, 1864) W ZACHODNIEJ AFRYCE

Streszczenie. Głównym celem pracy było przedstawienie sugerowanych metod monitoringu *Xenopus tropicalis*, należącego do podrodzaju *Silurana* z rodziny Pipidae. Gatunek ten jest zwierzęciem modelowym, w laboratoriach jest utrzymywany w warunkach odmiennych od środowiska naturalnego, co świadczy o jego przystosowaniu do nowego siedliska. Ponadto płaz zamieszkujący zachodnią Afrykę został znaleziony w USA w środowisku naturalnym. Świadczy to, że potencjalnie może stanowić zagrożenie jako gatunek inwazyjny. Czynny monitoring tego gatunku może dostarczyć informacji o rozprzestrzenianiu się oraz o przystosowaniu do nowych siedlisk. Do tej pory nie została opracowana metoda monitoringu *X. tropicalis*. Zaproponowana metoda oceny stanu gatunku opiera się na badaniach terenowych – obecności i liczebności osobników, natomiast ocena siedliska zawiera wiele czynników, jak: wielkość i głębokość zbiornika, jego zacienienie i zarośnięcie lustra wody, obecność innych zwierząt (ryby, inne gatunki płazów, gady) oraz baza żywieniowa (obecność bezkręgowców wodnych i lądowych). Zaproponowane metody monitoringu mogą być używane również do innych gatunków z rodziny Pipidae, które prowadzą typowo wodny tryb życia.

Słowa kluczowe: *Xenopus tropicalis, Silurana* sp.*,* Pipidae, metody monitoringu.