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Front Cover: The jararacussu *Bothrops jararacussu* photographed by Marcelo Ribeiro Duarte in Pindamonhangaba State, Sao Paulo, Brazil, see article on p.16.

THE HERPETOLOGICAL BULLETIN

Contents: Issue 161, Autumn 2022

RESEARCH ARTICLES:

- Evaluation of cloacoscopy for sex determination in the Argentine black and white tegu *Salvator merianae*
FILIPPO SPADOLA, MANUEL MORICI, MARCODI GIUSEPPE, EMANUELE LUBIAN, MATTEO OLIVERI, ALENA
BARTOSKOVA & ZDENEK KNOTEK 1
- Great crested newt *Triturus cristatus*, smooth newt *Lissotriton vulgaris*, and other amphibians in an acidified area of
southern Norway surveyed using eDNA and other methods
LEIF ÅGE STRAND, HEINI K. MÅSEIDE, STIAN GAUPELAND, BEATE STRØM JOHANSEN & AUDUN SLETTAN 5

SHORT NOTES:

- Sexually dimorphic growth and maturity in captive mountain chicken frogs *Leptodactylus fallax*
CHRISTOPHER J. MICHAELS, KIMBERLEY C. CARTER, FRANCESCA SERVINI & BENJAMIN TAPLEY 12
- Segmental muscle twitching behaviour in the flanks of lancehead vipers *Bothrops* spp in response to human approach
MARCELO RIBEIRO DUARTE, DANUSA CAMANDUCHY MAIA, SILVIA REGINA TRAVAGLIA-CARDOSO 16
- Variability and hybridisation in the introduced pond slider turtle *Trachemys scripta* in Romania
ALEXANDRU IFTIME & OANA IFTIME 20
- Diet of the recently introduced white-lipped frog *Leptodactylus fragilis* from a suburban ecosystem in western Cuba
L. YUSNAVIEL GARCÍA-PADRÓN, ROBERTO ALONSO BOSCH & OMAR FALERO ÁLVAREZ 24
- The threat of discarded food and drinks containers to monitor lizards
PRZEMYSŁAW ZDUNEK & KRZYSZTOF KOLENDA 28

SHORT COMMUNICATIONS:

- Limb malformations in introduced populations of midwife toad *Alytes obstetricans* in Great Britain
MARK J. GOODMAN, DAVID J. CLEMENS, LLOYD G. ROSE & STEVEN J.R. ALLAIN 31
- Rediscovery of the marbled caecilian *Epicrionops marmoratus* and first record from Colombia
JUAN DAVID FERNÁNDEZ-ROLDÁN, JOHN D. LYNCH & GUIDO FABIAN MEDINA-RANGEL 34
- Effect of hypoxia on the embryonic and larval development of the Nagaland montane torrent toad *Duttaphrynus*
chandai in India
LAL MUANSANGA, AMIT KUMAR BAL, H. MALSAMWKIMA & H.T. LALREMSANGA 37

NATURAL HISTORY NOTES:

- First case of predation of the Tyrrhenian endemic Sardinian tree frog *Hyla sarda* by an alien water frog *Pelophylax* sp. in
northern Sardinia, Italy
MATTEO RICCARDO DI NICOLA & JORDI RIBÓ FERRER 40
- Deadly snack: mortality of a European grass snake *Natrix natrix* while attempting to feed on a three-spined stickleback
Gasterosteus aculeatus
MARÍA BRAEUNER & CHRISTIAN WITT 42
- The first record of sea anemone *Stichodactyla gigantea* consuming a sea snake *Emydocephalus ijimae*
TAKAHIDE SASAI, TAISHI OKAZAWA, MINORI HASEGAWA & KEI YAMAZAKI 44
- Smooth newt *Lissotriton vulgaris* preyed upon by European starling *Sturnus vulgaris*
MARIO I. SHIMBOV & STEVEN J. R. ALLAIN 46

BOOK REVIEW:

- J.G. Schneider's *Historiae Amphibiorum*: Herpetology at the Dawn of the 19th Century
ROGER DOWNIE 47

Evaluation of cloacoscopy for sex determination in the Argentine black and white tegu *Salvator merianae*

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INTRODUCTION

Approximately one fifth of reptilian species is listed as Critically Endangered, and another one in five species is classed as data deficient (Alroy, 2015). Their low dispersal ability, together with their great morphological and physiological specialisation, makes reptiles highly sensitive to anthropogenic habitat degradation and climate change (Kearney et al., 2009; Jenkins et al., 2014). Ex situ conservation and captive breeding programmes have therefore become fundamental instruments in reptile conservation (Witzenberger et al., 2011). Moreover, artificial insemination techniques seem to be close to standardisation (Mattson et al., 2007; Molinia et al., 2010; Oliveri et al., 2017). A basic knowledge of reptile reproductive morphology and anatomy is then necessary in order to standardise methods for artificial insemination and early sex recognition (Oliveri et al., 2016). Advanced endoscopic technique has proven to be the ideal method for this purpose, being safe and relatively non-invasive.

Sex recognition in the lizard family Teiidae is challenging. Jaw-muscle dimension (Naretto et al., 2014) and snout-vent distance have been described, but these methods entail a high risk of misdiagnosis (Kratochvíl et al., 2003). Probing of the hemipenial pouches (Hall, 1978; Funk, 2002) may be used in adult tegu lizards, but not in hatchlings. Hemipenial eversion is another possibility but subsequent infection and necrosis of everted hemipenes after injection of saline solution have been reported and eversion with manual massage is challenging in large lizard species with highly muscular tails. It is also dangerous in lizard taxa that perform caudal autotomy and may cause serious injury if it is not performed gently (Reed et al., 2012). Evaluation of the presence of post-cloacal spurs (three slightly protruding scales) is a suggested sex determination technique in lizards of the family Teiidae (Sprackland, 2009). A post-cloacal spur is present on both sides of the tail base in male Argentine black and white tegu. In females, post-cloacal spurs can be also present, but of a smaller dimension than in the male.

Ultrasound imaging and diagnostic endoscopy have both

proved to be useful tools for sex determination in lizards. In monomorphic lizards, ultrasound has been used to investigate the development of ovarian follicles and for sex determination (Morris et al., 1996; Schumacher et al., 2001; Gartrell et al., 2002; Prades et al., 2013). However, gonadal ultrasound is only useful when applied to adult, sexually mature lizards (Reed et al., 2012; Stetter, 2006). Cloacoscopy has proved a feasible method of sex determination in many reptile species (Schildger et al., 1989; 1999; Kuchling, 2006; Spadola et al., 2009; Divers, 2010; Innis, 2010; Selleri et al., 2013; Martínez-Silvestre et al., 2015; Perpignan et al., 2016; Spadola et al., 2021). However, to date no information on cloacoscopy of tegus has been published.

The aims of the current study were to i) describe the cloaca in adult male and female Argentine black and white tegu (*Salvator merianae*) using cloacoscopy, and ii) validate this technique for sex determination of hatchlings.

MATERIALS & METHODS

The lizards

A total of 25 Argentine black and white tegus (*Salvator merianae*) were included in the study. Lizards were referred to the Veterinary Teaching Hospital (University of Messina) from private breeders and were divided in two groups according to the age: the first group included six adult tegus (1.8–2.2 kg), while the second group included nineteen 15-day old hatchlings. First, the sex of the adults was determined by the presence of post-cloacal spurs (PSSD) then the results were verified using ultrasonography to detect the ovaries in female tegus (Mylab 40Vet + linear transducer 7.5–12 MHz, Esaote, Italy). The adults were submitted to endoscopy and ultrasound for other clinical reasons and the photographs generated were subsequently used as an aid to identify the sex in the hatchlings. Once the sex was unequivocally determined in all 6 adult tegus, then cloacoscopy (CSD) was undertaken with the hatchlings.

Cloacoscopy

The lizards were restrained manually and positioned in

dorsal recumbency on an electric heating pad (30 °C Bosch PFP 1031; Bosch, Germany). For cloacoscopy the following equipment was used - a rigid arthroscope (4 mm diameter, 0°, 8.5 cm length, Olympus medical, Japan) with a working sheath connected via one port to a syringe (60 mL, Pic solutions/Artsana, Italy), camera (Telecam DX-II, Karl Storz, Germany) and a documentation system (TELE PACK, Karl Storz, Germany). The endoscope was gently introduced into the vent of the lizard to visualise the proctodeum, urodeum and coprodeum. To obtain cloacal dilatation and a better visualisation of anatomical structures, there was continuous flushing with sterile saline solution (0.9 % NaCl, S.A.L.F., Italy) combined with 3 ml/L lidocaine chlorohydrate (2 % Lidocaine, Esteve, Italy) from the syringe. After the cloacoscopy, all lizards were kept in the clinic for 24 h to monitor the condition of their health.

One veterinarian (MdG) used PSSD on the 19 hatchling tegus, this was followed by CSD performed with two veterinarians (FS, MM), who were not informed of the results of PSSD. All photographs taken during CSD were then presented to two veterinarians not involved in any of the earlier sex determination work. Since the data were qualitative, numerical statistical testing was not used but instead the reliability of agreement between the results obtained from PSSD, cloacoscopy and final observers was examined with the Fleiss' kappa (K) nominal scale (Fleiss, 1971). The K values range used to measure nominal scale agreement was from 0 to 1.

RESULTS

Details of the cloacoscopy

Using cloacoscopy, the proctodeum of adult tegus was immediately visualised as the endoscope was inserted through the vent. Advancing cranially with the probe, the urodeum (Fig. 1) was seen ventrally. It was present as a small central mucosal fold upon which the urinary papillae could be recognised. In adult female tegus, a mucosal recess was present just beyond the central mucosal fold; this recess was divided in two vaginal pouches by a vertical mucosal slit (Fig. 1A). In adult male tegus the central mucosal fold was not seen (Fig. 1B). In 15-day old hatchling tegus, similar differences between males and females were found (Fig. 2). In the coprodeum the anal sphincter was easily overcome and by passing the endoscope through that the rectal chamber could be seen (Fig. 3). During the whole procedure the lizards did not show any signs of discomfort.

Post-cloacal spur sex determination compared with ultrasonography and cloacoscopy

Sex determination in adult tegus using ultrasonography revealed 3 males and 3 females; by comparison, PSSD suggested the correct sex in five of the six cases (83.3 % accuracy).

In the case of hatchlings, PSSD suggested 7 females and 12 males but CSD revealed 9 females and 10 males. PSSD failed to identify the sex correctly in the case of 4 males and 3 females (Table 1S, see Supplementary Materials), so it was accurate for only 13 of the 19 hatchlings (68.4 % accuracy).

The two blind-trial observers confirmed the results

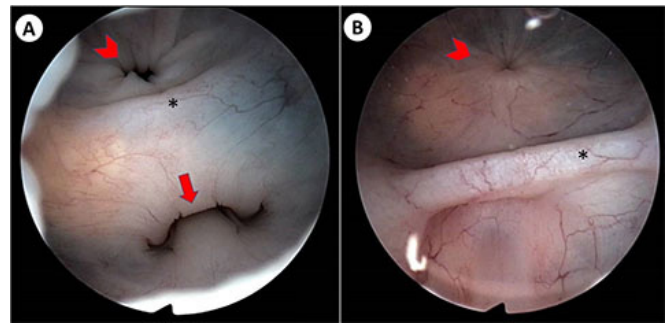


Figure 1. The urodeum of adult *Salvator merianae* visualised by cloacoscopy - **A.** Female, **B.** Male. In the female a mucosal recess (red arrow) is present, and it is divided in the two vaginal pouches by a vertical mucosal slit. In the male the recess was not visualised. An additional horizontal slit (asterisks) divides the urodeum from the coprodeum (arrowheads). When cloacal chambers are not dilated by the saline flush, this horizontal slit covers the 'genital' part of the urodeum, leaving exposed just the central mucosal fold, with the coprodeum connecting directly to the proctodeum.

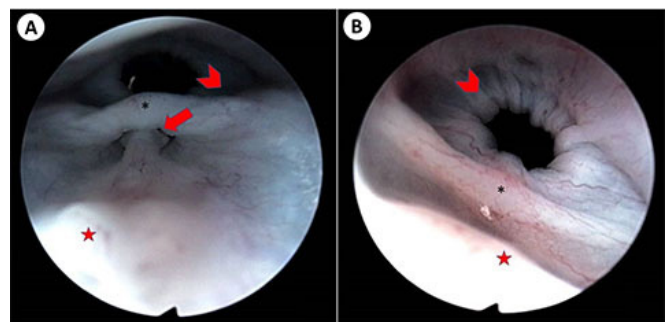


Figure 2. Urodeum of 15-day old hatchling *Salvator merianae* visualised by cloacoscopy - **A.** Female, **B.** Male. Just beyond a mucosal fold (urinary papillae, stars), a mucosal recess (red arrow) and vaginal pouches have been shown even in post-hatchling (15-day old) *S. merianae*. An additional horizontal slit (asterisks) divides the urodeum from the coprodeum (arrowheads). The urodeum differences allow early sex diagnosis.

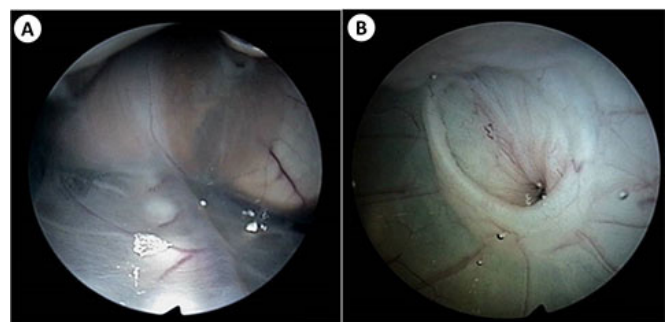


Figure 3. Rectal chamber of adult *Salvator merianae* - **A.** When dilated, the rectal chamber shows the presence of coelomic organs and is highly vascularised, **B.** The rectocolic valve in the rectal chamber

obtained using CSD (Table 1S). There was poor agreement between the PSSD results and those obtained by CSD operators and blind-trial observers ($K = 0.479$). Cloacoscopic sex determination showed instead perfect statistical agreement between cloacoscopy operators and the subsequent blind-trial observers ($K = 1$).

DISCUSSION

In our experiment, PSSD in young tegus was accurate only in 68.4 % of cases, hence it cannot be considered a reliable method of sex determination in *S. merianae* hatchlings. Instead, cloacoscopy proved to give very accurate sex determination, due to the considerable anatomical differences between sexes. Moreover, cloacoscopic sex determination showed a close statistical agreement between cloacoscopy operators and subsequent blind-trial observers. Cloacoscopy is an important tool in the study of cloacal anatomy. Cloacoscopy and cystoscopy have also been proposed as useful methods of sex determination in young chelonians by indirect visualisation of gonads through the urinary bladder wall (Selleri et al., 2013; Martínez-Silvestre et al., 2015); recently cloacoscopy has been used for sex determination in lizards (Morici et al., 2017), and to aid artificial insemination in snakes (Oliveri et al., 2017; 2016). There is great variability in cloacal morphology between reptile species (Morici et al., 2017; Oliveri et al., 2016; Spadola et al., 2009; 2015; 2016), and the physician should achieve a detailed knowledge of these differences before attempting sex determination through cloacoscopy.

Cloacoscopy has proven to be a safe and minimally invasive technique, comparable in invasiveness to a simple cloacal flushing. Our results demonstrate that CSD represents a highly accurate method for sex determination in *S. merianae*. This method can be considered a useful tool in ex-situ conservation programmes of endangered Teiidae. Moreover, it can be easily used as sex determination method by reptile specialists. We strongly encourage the use of CSD in other squamate species in order to establish accurate references.

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Great crested newt *Triturus cristatus*, smooth newt *Lissotriton vulgaris*, and other amphibians in an acidified area of southern Norway surveyed using eDNA and other methods

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ABSTRACT - Acid rain for many decades has led to severe acidification of waters in southern Norway. Acidic water can be fatal to gill-breathing vertebrates (i.e. fish and larval amphibians). Great crested newt *Triturus cristatus* (GCN) - seems to be less tolerant of acidic water than other Norwegian amphibians. Not until 2015 was GCN recorded in Agder, the southernmost county in Norway, when the larvae of this species were found in two ponds. The aim of our investigation, in late spring and summer 2021, was to find out whether GCN was still present in these two ponds and ten others in the same area, which are surrounded by peat bogs and forest. Since this is a marginal and acidic area with probably low numbers of individuals and low detectability, we used three survey methods in combination (funnel traps, nets, and eDNA) and also measured water conductivity and pH. At the same time, the occurrence of other amphibians in the area were investigated; the smooth newt *Lissotriton vulgaris*, the common toad *Bufo bufo*, the common frog *Rana temporaria* and the moor frog *Rana arvalis*. Using traps and nets, GCN was found in four ponds but in only two of these ponds by eDNA. However, GCN eDNA was detected in three other ponds, showing that a combination of methods gave the most complete result. eDNA of the common toad and the common frog were detected in (almost) all samples but there were few records from traps or nets. Smooth newts were detected in almost all ponds by traps, nets and eDNA, while none of the methods detected the moor frog. Especially when a species is rare at a location, eDNA analysis may be the most efficient method of detection. However, only trapping and netting can give information about breeding. Water pH in late spring and early summer varied from 4.7 to 5.6 (median pH 5.1), which makes this area marginal for amphibian reproduction.

INTRODUCTION

Amphibians are in decline globally with populations disappearing from locations where they were once abundant (Halliday, 2008). Some of the factors behind these declines include the destruction of habitats, invasive alien species, diseases, and acid rain (Dolmen, 2018). The great crested newt *Triturus cristatus* (GCN) is found in the northern parts of Europe and is categorised as 'Near Threatened' (NT) in Norway (Artsdatabanken, 2021). It is far less common than the smaller smooth newt *Lissotriton vulgaris* and vulnerable to further reduction in population size (Dolmen, 2008). It is therefore important to map the distribution of this species so that it can be better protected. Surveying of rare species like the GCN with traps and hand nets can be time consuming and challenging, with the risk of not detecting the presence of the species in areas with few individuals. By combining these methods with environmental DNA (eDNA) analysis of water samples, it may be possible to detect species in a shorter time and improve the overall detection rate. eDNA analysis has been used successfully to study the presence and distribution of a wide range of species (for a review see Ruppert et al., 2019), including aquatic species and amphibians (Ficetola et al., 2008; Rees et al., 2014; Ruso

et al., 2019). eDNA normally becomes undetectable in fresh water less than one month after the target species has left the water (Dejean et al., 2011). However, acidic water might accelerate the degradation of eDNA (Seymour et al., 2018).

Many decades of acid rain has led to acidification of water and watercourses, especially in the southernmost parts of Norway. The decline in freshwater fish populations in parts of southern Norway is associated with increasing acidity in rivers and lakes. The chief cause of increased acidity is acid precipitation which is the product of the emission, oxidation and long-distance transport of air pollutants, particularly sulphur dioxide (Leivestad & Muniz, 1976). There have been a number of liming projects to prevent fish deaths in lakes and rivers (Sandøy & Romundstad, 1995). Water with a very low pH is fatal to gill-breathing vertebrates (i.e. fish and larval amphibians), as it causes ion loss over the gills, and exposure to toxic aluminium compounds which can be dissolved from the bedrock. Tolerance for acidic water varies between different species of fish and amphibians, but the problems start at around pH 5.

In Norway, the smooth newt is found breeding in water as acidic as pH 4.6 (Strand, 2002), and larval GCN have been found at pH 4.9 (Dolmen, 1980; Strand, 2011). However, while the smooth newt is commonly found in waters within

the range of pH 5.0–5.4, GCN is more sensitive to acidic water and is only rarely found breeding in water at lower than pH 5.5. The common frog *Rana temporaria* and the moor frog *Rana arvalis* are less sensitive to acidic water and are often found breeding at pH 4.5–5.0. The common toad *Bufo bufo* seems to avoid breeding below pH 5 (Strand, 2002; 2010). In contrast to the other species, prior to 2015 GCN had never been documented in Agder, the southernmost county of Norway, but it is possible that the species had a larger and undetected distribution before the period of acid precipitation.

To our surprise, during a mapping project in Agder in 2015, GCN was found breeding in two forest ponds in Gjerstad municipality in the northern part of Agder (Strand & Stornes, 2015) (Fig. 1). This finding led to new investigations during the following two years, expanding the survey area to include eight municipalities southwest of Gjerstad. However, GCN was not encountered in the expanded area. The new surveys covered 124 bog and forest ponds and in 47 of them aquatic vertebrates (i.e. the smooth newt, the common frog, the common toad and fish) were found. The ponds with aquatic vertebrates had a median pH of 5.7, slightly higher than that for the other ponds (pH 5.0); a statistically significant difference ($p < 0.001$ Mann-Whitney U-test) (calculated from data in Strand & Stornes, 2016; Strand, 2017).

The aim of the present study was to see whether GCN was still present in the two original ponds six years later, and whether the species was found in other ponds within the same area. We also aimed to compare the efficiency of funnel traps and nets with analysis of eDNA for detection of all amphibians in the area, which include the smooth newt, the common toad, the common frog, and possibly the moor frog. In addition, the potential presence of the chytrid fungus *Batrachochytrium dendrobatidis* (BD) that is a global threat to amphibians was also examined in the water samples with the use of eDNA analysis. Finally, we set out to investigate to what extent the acidic water in the area limits the reproduction of amphibians.

MATERIALS & METHODS

Area description and selection of ponds

This study, undertaken in summer 2021, included twelve ponds (Fig. 1), of which ponds #1–#10 were previously investigated in the 2015 survey (Strand & Stornes, 2015). The pond ‘Stemtjern’ is divided into two by a peat bog and is, as in the 2015 survey, treated as two separate ponds (#8 Stemtjern East and #9 Stemtjern West). These ponds lie within an area of 5 km² in a landscape dominated by forested hills with peat bogs in the lower parts; details of the location, area and altitude of these ponds are presented in Table 1S (see Supplementary Material). In Gjerstad (Agder county), the marine level (i.e. the highest sea level after the Ice Age) is 90–110 m above today’s sea level. The soil below this level is characterised by clay, with sediments of sandy soil where rivers flow out during the ice melt. The altitude of the ponds investigated in this study are at 144–236 m (Table 1S), where the soil is dominated by less fertile moraine (Thorsnæs & Lauritzen, 2021).

Field methods

We visited the area on four occasions during late spring and summer 2021 (29 & 30 May, 25 June, 18 July, and 11 August). During the mating season in the spring, the newts are very active and are easily caught with traps. In May, we used collapsible traps, with funnel-shaped entrances at each end, to catch adult newts. The traps, measuring 25 cm in diameter and 60 cm in length, originally designed for removing minnows *Phoxinus phoxinus* from lakes, are used for monitoring GCN in Norway (Dervo et al., 2019). Ten traps were set in each pond and taken up after 24 h, after which the species and numbers of amphibian captured were recorded.

For the detection of amphibian larvae and adults, at all visits we made 10 or more horizontal hauls along the pondbank with long-handled hand nets. The nets were fine-meshed and suitable for catching aquatic animals down to the size of planktonic crustaceans. The mesh frame was approximately 25 cm by 25 cm.

In 2021, water samples were collected from the ten ponds that had been investigated in 2015. Specific conductivity (total ionic score) was measured with a ‘Delta Scientific model 1014’ conductivity meter and read as $\mu\text{S}/\text{cm}$ at 25 °C (K25). The conductivity measures the ionic concentration (hardness) of the water and indicates its buffer capacity (acid-binding capacity). The acidity (pH) was measured electrically with a ‘Polymetron 55N’ pH meter, with an electrode suitable for low-ionic water.

Collection of samples for eDNA analysis and qPCR

Samples for eDNA analysis were collected in May and July 2021. One litre of water was sampled from each pond by collecting sub samples of 100 ml at locations evenly distributed around the pond and combined in a sterile bottle. Samples were then transported to the laboratory and filtered through a cellulose nitrate filter (0.45 μm pore size). DNA was extracted and purified from the filters by using DNeasy® Blood & Tissue Kit (Qiagen) following the manufacturer’s protocol.

Traps and/or nets were used in 11 of the 12 ponds while eDNA samples were taken from all ponds, which means that one pond was only investigated with respect to detecting the amphibians and *B. dendrobatidis* by using eDNA. Also, one of the eDNA samples was a combination of three adjacent ponds (the Rossmyr ponds, ponds #5–#7).

TaqMan qPCR assays with species specific primers and probes (Table 2S, in Supplementary Material) were used for detection of the various species in the isolated eDNA. The 20 μl PCR-mix consisted of 1 x TaqMan Environmental Master Mix (Applied Biosystems), 0.9 μM of each PCR primer, 0.55 μM probe and 3 μl of template eDNA. The qPCR was conducted on a StepOnePlus Real time PCR system (Applied Biosystem) with temperature profile of 50 °C for 2 min and 95 °C for 10 min, followed by 60 cycles of 96 °C for 15 s, 60 °C for 60 s, with fluorescence detection after each cycle. We analysed every sample in triplicate. Genomic DNA from the species and dd H₂O was added as a template in positive and negative controls respectively.

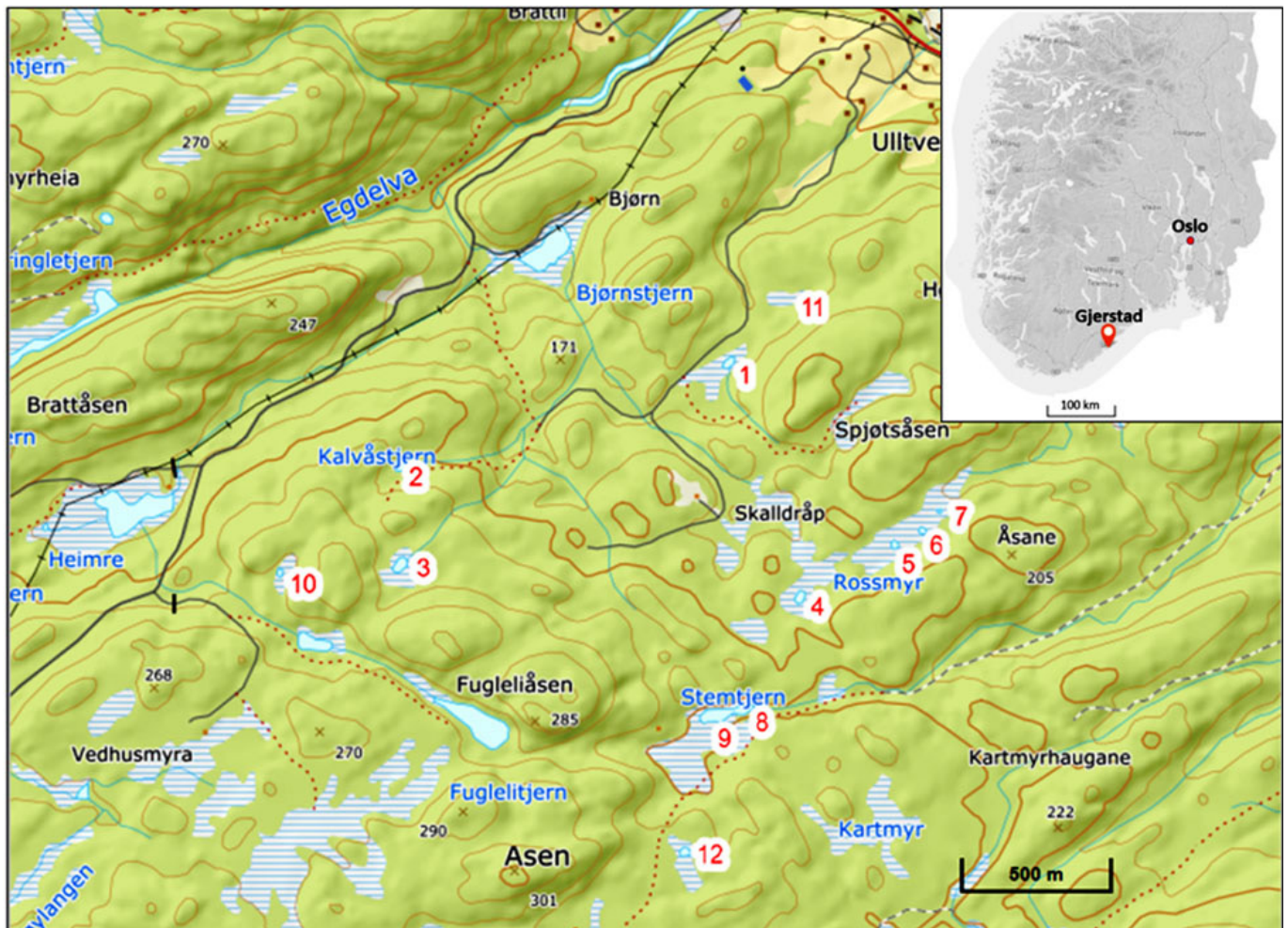


Figure 1. Map of the study area. The numbers indicate the locations of ponds.

RESULTS

Newts

In May 2021, adult GCN and smooth newts were retrieved from the two ponds (#2 & #3) in which they had been detected in 2015 using hand nets (Table 1). Traps were set in six of the other ponds from 2015, and the smooth newt was caught in all of these ponds, in addition, one female GCN was found in pond #10. In June and July 2021, using hand nets, we were able to detect larvae of the smooth newt in only two of the six ponds in which adults had been trapped earlier. Larval GCN were found in one pond investigated that had given a negative result in 2015 (#9), but we were not able to detect larvae of this species in the pond where we had trapped the female. The traps also caught the adult smooth newt in one of the new ponds (#11). In August, larvae of the smooth newt were found in another two of the six ponds where traps were used, which means that reproduction was detected in four of the six ponds where adults were found in May.

eDNA from GCN was detected in half of the 10 eDNA-samples (Table 2), of which three samples came from ponds with negative results using traps and/or nets. One of these samples was a mixture of water samples from the three Rossmyr ponds (#5–#7). The result was negative for one

pond where larvae were found both in June and July (#9). eDNA from smooth newts was detected in all but one of the samples (pond #4).

Anurans and chytrid fungus *Batrachochytrium dendrobatidis*

In May 2021, newly hatched tadpoles of the common toad were found in one pond (#1), visually and by net. This species was not found in any of the other ponds in this survey. The common frog was observed in four ponds, of which tadpoles were caught by nets in ponds #6 and #7. In August, adult frogs were observed visually in ponds #4 and #10, jumping into the water as we approached. The moor frog was not found at any location.

eDNA of the common toad was detected in all samples while eDNA from the common frog was detected in seven (Table 1). Pond #8 (Stemtjern East) was not analysed for the eDNA of the common frog. No eDNA of the moor frog or *B. dendrobatidis* was detected in the surveyed ponds.

Measurements of specific conductivity and pH

The conductivity ranged was 14–19 $\mu\text{S}/\text{cm}$ and showed some increase during the investigation period (Table 1). The largest increases were seen in the three Rossmyr ponds (#5–#7). The pH values were correspondingly low, and in May were pH 4.7–5.3. There was a generally small increase – 0.2

Table 1. Counts of amphibians, using traps and nets, and water chemistry from ponds in Norway, from the first investigation in 2015 and the follow-up investigations in 2021

Pond No.	28 June 2015			29–30 May 2021				25 June 2021			18 July 2021			11 August 2021		
	Net	pH	k25	Net	Traps	pH	k25	Net	pH	k25	Net	pH	k25	Net	pH	k25
1	Lv3, [Lv]1, Bb1	5.5	17	Bb	[Lv]55♂, 22♀	5.2	16	-	-	-	Lv1	5.7	17	Lv40, [Lv]1♀	5.6	17
2	Lv6, Tc4	6	19	[Tc],[Lv]	-	5.5	18	-	-	-	-	-	-	-	-	-
3	Lv21, Tc2	5.9	16	[Tc],[Lv]	-	5.3	16	-	-	-	-	-	-	-	-	-
4	Neg.	4.8	17	[Lv]1♀	[Lv]1♂	4.7	19	Neg.	4.9	18	Neg.	5	17	[Rt]1	4.9	18
5	[Lv]1	5	16	[Lv]	[Lv]14♂, 7♀	5	14	-	-	-	Neg.	5.5	19	Lv1	5	24
6	Lv2	5	17	[Lv], Rt	[Lv]40♂, 14♀	5	16	Neg.	5	21	Neg.	5.2	21	Lv1	4.9	26
7	Neg.	4.9	19	[Lv], Rt	[Lv]14♂, 5♀	5	16	-	-	-	Neg.	5.2	19	Neg.	4.8	26
8	Lv9, [Lv]1	5.6	16	-	-	-	-	Lv10	5.6	18	Lv1	6	17	Lv4	5.3	20
9	Lv4	5.6	19	-	-	-	-	Tc2, Lv25, [Lv]1♀	5.6	19	Tc1	5.8	18	Lv6, [Lv]1♂, 1♀	5.4	18
10	Neg.	5.2	15	Neg.	[Tc]1♀, [Lv]15♂, 14♀	5	16	Neg.	5.4	15	Lv1	5.3	16	[Rt]1	5.3	16
11	-	-	-	-	[Lv]2♂	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: Lv - *Lissotriton vulgaris*; Tc - *Triturus cristatus*; Bb - *Bufo bufo*; Rt - *Rana temporaria*. Abbreviations in brackets refer to adults. Numbers refer to the number of individuals. K25 - conductivity (µS/cm). Neg. = investigated with no finding, - = not investigated/no water sample taken

to 0.5 pH units - from May to July, and a small decrease was seen thereafter.

DISCUSSION

Methodology agreement and discrepancy

Using funnel traps and nets, the smooth newt was found in 11 of the 12 ponds included in our study, while GCN was observed in four ponds (#2, #3, #9 and #10). eDNA from GCN was only detected in two (#2 and #3) of those four ponds. On the other hand, its eDNA was detected in samples from two additional ponds (#1 and #8), as well as in a combined sample comprising three discrete ponds (#5, #6 and #7), i.e. from ponds where the species was not detected by the two other methods. The detection of GCN by eDNA analysis in ponds where it was not detected by other methods demonstrate the sensitivity of eDNA analysis. Three replicates of all eDNA analyses were used in this study in order to avoid false negatives where species detection probability is low, e.g. because of few individuals in the population. Suggestions of how many replicates to use in such studies varies depending on environmental factors and population size. Ficetola et al. (2015) suggest using up to eight replicates, eventually combined with several eDNA extractions, to avoid false negatives where detection probability is low. In our study,

all eDNA analysis replicates were found positive for GCN in ponds where this species was detected by traps or net, while fewer than the three replicates were positive where other methods of detection failed. One could speculate that including a higher number of replicate analyses might have led to the detection of GCN in some of the ponds that were eDNA negative in this study. However, a higher probability of false positives should be taken into consideration in such an analysis scheme. Negative result for traps and nets may be due to the fact that the animals were scarce and so had a low probability of being caught, stayed too far from the shore, or in too deep water so that they were out of reach of traps and nets. However, the negative test with eDNA for the two ponds where the species was detected by traps and nets give rise to speculation: in one of these ponds (#10), only one adult (female) was found in May, but reproduction was not detected. As this pond is situated close to the two ponds where GCN was found in 2015, maybe only a few stray animals were present, releasing too little eDNA into the water for detection. A low abundance and a more restricted spatial movement of the newts during the spawning period could be the reason why we did not detect GCN eDNA in this pond. As shown by Buxton et al. (2017) various environmental factors, population size and breeding and larval development, affect the amount of

Table 2. Quantitative PCR (qPCR) tests for various amphibian species and the chytrid fungus *Batrachochytrium dendrobatidis* from 12 ponds in Norway. Each sample was tested three times, where one or more positive replicates were considered a positive eDNA detection. Each + or – represents one replicate. The positive samples had a Ct-value (cycle threshold) between 29 and 40. ‘+’ eDNA detected (positive sample); ‘-’ eDNA not detected (negative sample)

Pond no. & location	<i>L. vulgaris</i>	<i>T. cristatus</i>	<i>R. temporaria</i>	<i>R. arvalis</i>	<i>B. bufo</i>	BD
1. Spjøstjenn	+++	--+	+-	---	+++	---
2. Kalvåstjenn	+++	+++	+-	---	+-	---
3. Grønbergtjenn	+++	+++	---	---	+++	---
4. Igletjenn	---	---	+-	---	+++	---
5., 6., & 7. Rossmyra combined	+++	+-	+-	---	+++	---
8. Stemtjern East	+++	+-	Not measured		+-	---
9. Stemtjern West	+++	---	+-	---	+-	---
10. Little Grønbergtjenn	+++	---	+-	---	+++	---
11. Torgrymsmyr	+++	---	---	---	+++	---
12. Bjørnåsen	+++	---	+++	---	--	---

eDNA released into the water. In the other ponds that were negative for GCN eDNA (Stemtjern West, #9), GCN larvae were found on two occasions but only at the same place just 15 meters upstream from its adjacent pond (Stemtjern East, #8), which was eDNA-positive for GCN. We speculate that perhaps the eDNA had drifted the few meters from the western to the eastern pond.

The smooth newt was found in all 11 ponds investigated with traps and/or nets. eDNA was found in all samples except in the one taken from the most acidic pond in this investigation (which is probably not suitable for reproduction, see below), showing a high degree of agreement between the survey methods.

At their breeding sites, tadpoles of toads and frogs are normally present in greater numbers than larvae of the newts, and their presence is easily detected using traps and nets during spring and early summer. Despite this, only three anuran breeding sites were found. The low recording rate with funnel traps and nets is in sharp contrast to the detection of common toad eDNA which was found in all the ponds investigated and common frog eDNA that was present in seven of the nine samples taken. A potential explanation for recording eDNA from these species in ponds where no larvae was collected, might be that adult common toads and common frogs moved around in the area, on land as well as in the ponds, and although not successful in breeding left cells that were detected by eDNA-analysis.

Several studies have shown that eDNA analysis is more sensitive than other survey methods when it comes to detecting aquatic species (Sard et al., 2019; Hallam et al., 2021). This might partly be an explanation for some of our findings where eDNA recorded the presence of a given species while the species was not detected with traps or nets. Especially when the given species is rare at a location, eDNA analysis might be the most efficient method. Biggs et al. (2015) demonstrated that GCN was significantly more efficiently recorded by eDNA analysis compared to standard

methods as bottle traps, torch counts and egg searches.

The moor frog was not found in this study by either traps, nets or eDNA, giving good agreement between these methods.

Use of eDNA is a sensitive method for detecting animals in nature when they are otherwise hard to observe. This was the case in our study where we detected eDNA of the common frog and the common toad when hardly observing them in the wild. Nevertheless, the use of eDNA has its uncertainties. The risk of false positive eDNA detection due to contamination or unspecific amplification is a concern worth acknowledging. Negative controls and positive controls were included in all our qPCR runs (except for positive control for BD), and the species specificity of the qPCR assays has been validated in other studies and found to be good. It is also possible that the eDNA in some of our samples could have derived from dead amphibians or animal faeces containing amphibian-DNA.

The eDNA analysis used here does not contribute to any quantitative measurements, nor does it give any information concerning the life stage of the individuals. Thus, ideally to maximise the amount of information collected, eDNA analysis should be used in combination with methods such as trapping and netting.

Amphibians and water acidity

The conductivity measurements showed very low values (and most often below 20 µS/cm), which is normal for waters influenced by peat bog. The acid-binding capacity is very poor and explains the very low pH values measured in this survey. All ponds are situated above the marine level and were influenced by peat bogs. The peat (*Sphagnum* spp.) acts like an ion exchanger, by removing Ca⁺⁺ from the water in exchange for H⁺, which reacts with water to form acid substances. In 2015, smooth newts were found in 8 of the 10 ponds included in our updated study, while the investigation in 2021 found individuals of the newt in

all these ponds. Reproduction (evidenced by the presence of larvae) was confirmed in eight of the ponds, and the two ponds apparently without larvae were among the most acidic: Pond #4, where water was at pH 4.7–5.0, and pond #7 where it was pH 5.0–5.2. The water in these ponds was probably too acidic for larvae development. GCN was detected at two new locations, one of which yielded an adult using traps. Despite great effort, larvae were not found at this locality, and we assume that the water was too acidic for the species to reproduce. In the other pond, larval GCN were found at pH 5.6–5.8, which is quite similar to the acidity of two ponds in which the species was found in 2015. Those observations are in line with previous findings (Strand, 2002; 2010).

The number of larvae detected roughly depends on water acidity: in the ponds with pH above 5.5, several larvae of the smooth newt were found, while at lower pH, only one was detected. Another factor which might play a role is the temperature of the surface water. We think that the somewhat meagre larvae detection in June and July might be due to high temperature of surface water, which can drive the larvae to deeper, colder and more oxygen-rich water. In August, after heavy rainfalls, the surface water had cooled, and larvae of the smooth newt were found in two more ponds.

A study by Seymour et al. (2018) found that water as acid as pH 5.3 accelerated the decay of lotic multispecies eDNA. The acidic waters in our study area do not seem to have degraded the eDNA to undetectable levels, as we detected amphibian eDNA in water at pH 4.7–5.0.

In conclusion, reproduction of the newts in Gjerstad is highly restricted by acidic water. GCN was still present in the two original ponds and was found in two additional ponds using traps and nets. However, GCN eDNA was detected in two samples from localities for which the results were negative with traps and nets, and vice versa; was not detected in samples from two ponds where GCN was actually caught by traps and nets. This disagreement between the methods is in contrast to the high agreement between the methods for the smooth newt (which was detected in nearly all ponds) and the moor frog (which was not detected in our study). For the common frog and the common toad, which were found in only a few ponds using traps and nets, and whose eDNA were detected in (almost) all samples, the sensitivity of the eDNA-method makes it better suited to detect a species if only few individuals are present. Since eDNA analyses does not give us information about life stages, sex ratios, body conditions etc., it should ideally be combined with trapping and netting for best results.

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Sexually dimorphic growth and maturity in captive mountain chicken frogs *Leptodactylus fallax*

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ABSTRACT - Sexual dimorphism is commonly encountered in amphibians. Its presence and its ontogeny in a given species has implications for ecology, conservation and captive husbandry. We monitored changes in length and mass of captive mountain chicken frogs *Leptodactylus fallax*. Initially, sexes were no different in snout-vent length or mass, but by about 17 months after metamorphosis females became significantly larger and heavier than males. Diverging growth trajectories between male and female frogs appeared to coincide with the development of secondary sexual characters in males, indicating sexual maturity, while female frogs did not produce nests until the following breeding season, suggesting later reproductive maturity. The absence of similar dimorphism in other large leptodactylids suggests that its presence in *L. fallax* may be linked to its unique and energy-intensive reproductive strategy, which involves extended maternal provisioning of their young.

INTRODUCTION

Sexual dimorphism in size is common among anuran amphibians and often reflects asymmetric investment in reproduction (see review in Wells, 2007). Such dimorphism may result from unequal growth rates between juvenile anurans, and/or from unequal durations of rapid growth before asymptotic growth rates are achieved (Miaud et al., 1999; Gramapurohit et al., 2005; Zhang & Lu, 2013; Otero et al., 2017). Sexual size dimorphism is likely driven by differing reproductive selective pressures linked to relative investment in gametes and competition for access to mates (Zhang & Lu, 2013). Asymmetric growth rates between males and females could inform population management decisions made by captive husbandry practitioners working with anurans in captive conditions by providing clues as to the sex of animals.

Mountain chicken frogs *Leptodactylus fallax* are Critically Endangered and dependent, in part, on captive populations for survival (IUCN SSC ASG, 2017). The species breeds seasonally with female frogs investing heavily in energetically costly maternal care (Gibson & Buley, 2004). Species biology and captive management requirements, both important in conservation strategies, have been heavily informed by data collection from captive populations (e.g. Gibson & Buley, 2004; Tapley et al., 2015; Jayson et al., 2018a&b; Michaels et al., 2021). Much of this information would be difficult or impossible to collect from wild animals due to the small or extinct status of populations, inaccessibility of some remaining populations, and the secretive nature of the species.

We monitored growth in mass and length of captive bred *L. fallax* in a conservation breeding facility over 397 days and used these data to investigate patterns of growth between metamorphosis and sexual maturity.

METHODS

Study animals and husbandry

The study used 12 full sibling, juvenile *L. fallax* captive bred at ZSL London Zoo as part of the Mountain Chicken Recovery Programme, hatched 12 May 2016 and metamorphosed 15–22 July 2016. The frogs were the offspring of a three-year-old captive bred female and a four-year-old captive bred male and were the same animals used as part of a study on dietary calcium provision (Michaels et al., 2021). Animals were exposed to a diurnal thermal gradient of 23–34 °C, a nocturnal gradient of 21–23 °C, an Ultraviolet Index (UVI) of 0–3 correlated with heat and photoperiod of 12:12; full details of husbandry can be found in Michaels et al. (2021). Individuals were identified through Visible Implant Elastomertagging (Nauwelaerts et al., 2000) and photographic IDs. Animals were fed a varied diet of invertebrates following Jayson et al. (2018a). Feeds were supplemented with a 1:1 by weight mix of Vetark Nutrobal® (VETARK Professional, Winchester, UK) and powdered calcium carbonate (product code P0302, Cambridge Commodities, Cambridge, UK), which was dusted onto prey items (Michaels et al., 2014) in the regimens described by Michaels et al. (2021). Frogs consistently held an acceptable body condition of between three and four on the body condition scoring scale developed by Jayson et al. (2018b).

Data collection

As part of routine health checks on all juvenile individuals of this species, frogs were captured by hand at approximately three months of age and weighed using digital balances (Salter Housewares, UK) approximately every 14 days between 4 November 2016 (day 0) and day 168, then a final measurement taken on day 397 following a hiatus in measurements caused by insufficient staffing resources to collect data frequently.

Snout-vent lengths (SVLs) were measured using digital callipers (Transcat, USA) to the nearest millimetre on days 0, 88, 168 and 397. This resulted in four SVL measurements and thirteen mass measurements. Sexual maturity in males was determined based on the development of nuptial spurs (keratinised or otherwise) (Jameson et al., 2019) and the date at which keratinised spurs were first detected was noted to indicate sexual maturity. Females were regarded as being at sexual maturity once they had produced a foam nest. The majority of frogs were exported to other collections at the end of the study, and before first reproduction in females, but for animals that remained in the ZSL collection, the date of first nest production was also recorded.

Statistical analysis

We did not use the von Bertalanffy growth function to model growth as frogs in our data set had not reached asymptotic growth and it was impossible to collect more data (frogs were exported to other collections) (Allen, 1969; Miaud et al., 1999). Instead, we used the *lme4* package in RStudio to build generalised linear mixed models (Bates et al., 2015), fitting the model Response Variable (SVL or Mass) = time + sex + time * sex, fitting frog individual as a random factor to control for repeated measures. We confirmed that model assumptions were met through visual inspection of residuals via the *ggResidpanel* function in R (Goode & Rey, 2019). The *anova()* function of the *stats* package (R CoreTeam, 2021) was then used to test the main effects and interactions sequentially. Data were tested for normality using a Shapiro Wilks test and one-way ANOVAs (*stats* package; R Core Team, 2021) were then used to compare measures between sexes at the start of the study and at the penultimate and final measurements.

RESULTS

Sex ratio, nuptial spur development and nesting

Of the twelve study frogs, seven were sexed as female and five as male. Keratinised nuptial spurs were first observed in male frogs between days 141 and 155, c. 8 months after metamorphosis. The first nests were produced by animals remaining ($n = 4$) in the collection from day 483, c. 20 months after metamorphosis.

Mass data

There was no difference in mass between sexes at the start of the study ($F_{1,16} = 0.32$, $p=0.6$) (Fig. 1A). There was no significant effect of sex as a main effect ($F_{1,66.6} = 2.08$, $p=0.15$). There was a significant effect of time ($F_{1,142} = 529.60$, $p<0.0001$) and sex * time ($F_{1,142} = 64.5$, $p<0.0001$). Model parameters are given in Table S1 (see Supplementary Material). Masses diverged between the penultimate and final measurements (Fig. 1A). Final measurement data were normally distributed (Shapiro-Wilk test, $p=0.469$) and, at the final measurement, there was significant difference between sexes ($F_{1,16} = 53.564$, $p<0.001$). Sexual dimorphism index (SDI; Lovich, J.E. & Gibbons, 1992) at this point was 1.38; mean (\pm SD) mass was 382 ± 24.1 g for females and 276.6 ± 25.4 g for males.

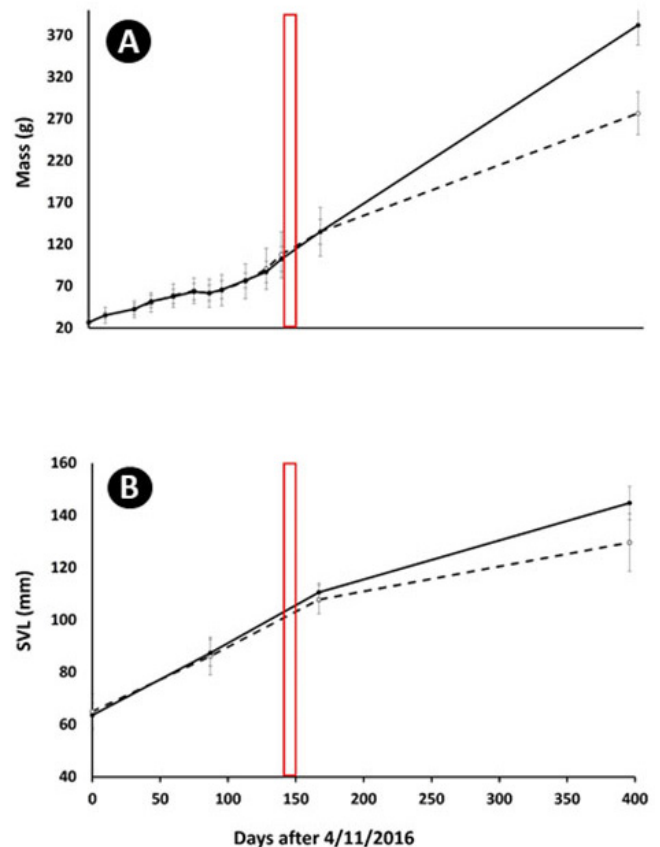


Figure 1. Growth of juvenile mountain chicken frogs in captivity- **A.** Changes in mass, **B.** Changes in snout-to-vent-length. The graphs show male (broken line; $n=5$) and female (solid line; $n=7$) frogs. The red box indicates the period over which keratinised nuptial spurs were first observed in males.

SVL data

There was no difference between sexes in SVL at the start of the study ($F_{1,16} = 0.29$, $p=0.6$) (Fig. 1B). There was no significant effect of sex as a main effect ($F_{1,24.6} = 0.7$, $p=0.41$). There was a significant effect of time ($F_{1,34} = 529.6$, $p<0.0001$) and sex * time ($F_{1,34} = 7.45$, $p=0.01$). Model parameters are given in Table S1. SVL diverged between sexes at between the penultimate and final measurements (Fig. 1B); final measurement data were normally distributed (Shapiro-Wilk test, $p=0.416$) and, at the final measurement, there was significant difference between sexes ($F_{1,16} = 9.119$, $p=0.013$). SDI at this point was 1.11; mean (\pm SD) SVL was 144 ± 6.4 mm for females and 129 ± 11.0 mm for males.

DISCUSSION

Our analyses demonstrated that although sexes were initially no different in SVL or mass, growth trajectories varied such that females became significantly larger and heavier than males by the final measurement at approximately 17 months after metamorphosis. Our models were a good fit with high R^2 values and relatively small confidence intervals around estimates; conditional:marginal R^2 ratios were close to 1:1

for both models and random effect standard deviation was low, suggesting the effect of individual separate from sex was small. Sexual dimorphism in either measure of growth was not apparent at 8 months or before, but was present at 17 months when the next measurement was taken. Unfortunately, no data were available during this period to detect at exactly which point growth trajectories became significantly different. However, keratinised nuptial spurs, a characteristic of sexual maturity, were developed in males at 8–8.5 months post metamorphosis. This coincided slightly before the start of the data gap and it is most likely that growth rates became sexually dimorphic shortly after this point. Our data regarding male sexual maturity at c. 8 months for males and female first reproduction at c. 20 months is congruent with anecdotal evidence from other captive collections (Jameson et al., 2019), but is the first record of this information in the scientific literature. This represents rapid onset of sexual maturity given the size of the species and aligns with the suggestion that the species is short lived but with rapid growth to maturity in the wild (seven years maximum longevity, Guarino et al., 2014) as well as in captivity (longevity 6–12 years, Jameson et al., 2019). These findings are congruent with those of other studies on leptodactylid frogs. Both sexes of *Leptodactylus labyrinthicus* are considered sexually mature at 8 months of age (Silva et al., 2005). Sexual maturity is attained within the first year in *Leptodactylus latrans* with more males attaining sexual maturity within the first year than females (López et al., 2015).

Sexual size dimorphism is likely due to different investment in reproduction (Zhang & Lu, 2013). Typically, large bodied *Leptodactylus* species do not exhibit sexual size dimorphism, or if they do then females are smaller than the males (Camurugi et al., 2017). The SDI in the study frogs was substantial, with females being on average 10 % larger than males and 40 % heavier. There was, however, overlap between sexes such that some males were larger than some females. In *L. fallax*, although males compete to gain access to females and to hold suitable nesting sites (King et al., 2005), females must produce large clutches of eggs and feeder eggs (10,000–25,000 eggs per clutch) and then guard and feed young which results in their own reduced food intake (Gibson & Buley, 2004; Jameson et al., 2019). Larger body size is likely important in meeting the energetic demands of this reproductive mode.

Sexual dimorphism in size has been detected in numerous anuran taxa; larger adult females is a common pattern (Zhang & Lu, 2013). Although in many cases this is due to more rapid growth rates alone (e.g. Ma & Lu 2009; Sarasola-Puente et al., 2011; Otero et al., 2017), it is often driven by delayed sexual maturity and therefore delayed asymptotic growth in females, (Gramapurohit et al., 2005), or due to a combination of growth rates and delayed maturation (Miaud et al., 1999). In a study looking across anuran lineages, Zhang & Lu (2013) found that the typical trend is for lower growth rates but longer longevity in females, leading to eventually larger size in females. In the frogs in this study, diverging growth trajectories appear to coincide with the development of secondary sexual characters in males, indicating sexual maturity, while female frogs did not produce nests until the following breeding season, suggesting later reproductive maturity. Female frogs may have become

sexually mature at the same point, but waited until the next environmentally-triggered breeding season to produce clutches. The development of keratinised spurs, however, coincided with the very beginning of the breeding period that year, and yet females did not produce spawn until the following season, a year later. This suggests that females were not sexually mature at the same time as males and reached this point later, following the first breeding season of male siblings. This coincidence suggests that earlier sexual maturity in male *L. fallax* may at least partially explain the patterns we detected. We did not see evidence for relatively slow female growth rates, so these results only partly align with the wider anuran trends reported by Zhang & Lu (2013). However, this may be explained by the relatively short lifespan and large size of this species (Guarino et al., 2014). Selective pressure for females to adopt a strategy of slower growth protracted over a longer period may not be viable, leading instead to the development of larger female size under accelerated growth at sexual maturity in order to achieve the same fecundity benefit (Zhang et al., 2013).

Unfortunately, management of the wider captive population of this species required animals from this study to be moved to other holders and we were unable to continue following this cohort's growth in order to establish the point of asymptotic growth in these animals. Further research in the captive population should seek to track animal growth over longer periods to better understand this aspect of biology, including data covering a longer period of development after sexual maturity, and potentially from a broader genetic background within the species (all focal animals presented here were full siblings).

Although our data contribute to the wider understanding of the biology of this species, the coincidence of sexual dimorphism in size with the acquisition of binary secondary sex characters, such as spurs, limits its utility in a practical context in terms of sexing animals. However, differing growth rates between sexes from the onset of maturity may suggest differing nutritional requirements between sexes at this point. Given the tendency of this species to develop pathologies linked to calcium metabolism (Tapley et al., 2014; Jayson et al., 2018a; Michaels et al., 2021), it may be particularly important to provide this mineral, and others, with this in mind.

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Segmental muscle twitching behaviour in the flanks of lancehead vipers *Bothrops* spp in response to human approach

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ABSTRACT - *Bothrops* species are known to have a wide range of defensive behaviours against potential threats. Herein we show a frequently observed, but to date undocumented, behaviour in male and female *Bothrops* spp of varying sizes in newly arrived specimens from the wild, short-or long-term captives as well as in captive born individuals. The behaviour consists of irregular, synchronous or asynchronous muscular twitches in segments of the body flanks in otherwise motionless snakes. We named this ‘Segmental Muscle Twitching Behaviour’ (SMTB). We observed fifteen *Bothrops* spp from six species groups for incidence of this behaviour and made videos of snakes during the ‘alertness’ and ‘threatening’ phases of response to human approach. We found the behaviour in only five species, these belong to the monophyletic *Bothrops jararacussu* and *Bothrops atrox* species groups, suggesting a single evolutionary origin of this behaviour. Macroscopically, SMTB varied in the number of segments involved and the twitches had uneven intensities. Although recurrent and often replicable in similar situations during human approach, the behaviour was not displayed consistently. In addition, SMTB can be interrupted, stay inactive for quite some time and then restart. Hypotheses to explain this behaviour are suggested.

INTRODUCTION

The Neotropical pitviper genus *Bothrops* comprises forty six species (Uetz & Hosek, 2021) within six recognised species groups (Werman, 1992; Wüster et al., 2002; Salomão et al., 1997; Alencar et al., 2016). They occur in all the main ecosystems east of the Andes in South America (Hoge & Romano, 1972; Martins et al., 2001; Campbell & Lamar, 2004).

The expected passive or active antipredator displays described for *Bothrops* spp are easily witnessed during routine interactions with these snakes and include immobility/freezing, fleeing, dorso-ventral body compression/flattening, long duration tongue flicks, tail vibration, body thrash, S-coil, head elevation, strike, and bite (Sazima, 1988; 1992; Greene, 1988; 1997; Araújo & Martins, 2006). Here we document a frequently observed, but to date unreported, behaviour in two species groups *Bothrops* spp., that consists of irregular, synchronous or asynchronous muscular contractions (twitches) in segments of the body flanks in otherwise motionless snakes which we have named ‘Segmental Muscle Twitching Behaviour’ (SMTB).

METHODS

During reception and/or routine maintenance at the snake laboratory or public exhibition cages in the Butantan Institute, São Paulo, Brazil (IBSP), fifteen *Bothrops* spp from six different species groups and four different Brazilian biomes (Amazon, Atlantic forest, Cerrado, and Caatinga)

were investigated. The lineages and species observed were as follows: *Bothrops alternatus* group (including *B. alternatus*, *B. fonsecai*), *Bothrops atrox* group (including *B. atrox*, *B. leucurus*, *B. moojeni*), *Bothrops jararaca* group (including *B. jararaca*, *B. insularis*, *B. sazimai*), *Bothrops jararacussu* group (including *B. jararacussu*, *B. brazili*), *Bothrops neuwiedi* group (including *B. neuwiedi*, *B. erythromelas*, *B. marmoratus*, *B. pauloensis*) and *Bothrops taeniatus* group (only *B. bilineatus*).

The specimens were either donated by the general public, collected by IBSP staff during fieldwork (Project ‘Scales of Biodiversity’ - SB/LCZ or Museu Biológico IBSP - MB) or born in captivity. Their defensive behaviours were videoed during alertness and threatening phases in response to human approach at 09:00 h to 15:00 h with a Nikon D5300 or Sony DSC-HX 300 camera (15 or 29 frames per second). Apart from SMTB, the behavioural responses listed follow the terminology of Greene (1988) and Araújo & Martins (2006).

The snakes were categorised in three groups: newly arrived, captive and captive-born. Details of these snakes are given in Table 1S (see Supplementary Material). The newly arrived specimens were studied immediately from the wild without quarantine, they had not been fed although water was freely available. They were kept individually in plastic cages of different dimensions according to their size (Grego et al., 2021). After their arrival, the subject was gently placed with a snake hook, unrestrained, on the bare ground outside the laboratory, in order to video-record it immediately at a safe distance. Air temperature during video-recording ranged from 22–31 °C. Relative humidity of the air was not measured. The captive group had been collected from

Table 1. Defensive behaviours, including Segmental Muscle Twitching Behaviour (SMTB), and a physiological adjunct exhibited by *Bothrops* spp of the *B. atrox* and *B. jararacussu* groups during human approach

Species	¹ Status	SMTB	Immobility	Head & neck elevation	Head hiding	S-coil	Flattening	Tongue flicking	Tail vibration	Body thrashing	² Hyper-ventilation	Strike
<i>B. atrox</i> ♀	CW	X	-	X	-		X	X	X	X	-	-
<i>B. atrox</i> ♂	CW	X	X	X	-	X	X	X	-	-	-	X
<i>B. atrox</i> ♀	CB	X	X	X	-	X	-	X	-	-	X	-
<i>B. moojeni</i> ♀	NA	X	X	-	-	X	-	X	-	-	-	-
<i>B. moojeni</i> ♀	CW	X	-	-	-		-	-	-	-	-	-
<i>B. moojeni</i> ♀ Juvenile	CW	X	X	-	X		X	-	-	-	X	-
<i>B. moojeni</i> ♂	CB	X	X	X	-	X	-	-	X	-	-	-
<i>B. leucurus</i> ♀	CB	X	X	X	-	X	-	X	-	-	X	-
<i>B. jararacussu</i> ♀	NA	X	X	X	-	X	-	X	-	-	X	-
<i>B. jararacussu</i> ♀	CW	X	X	X	-	X	-	X	X	-	-	-
<i>B. brasili</i> ♂	NA	X	X	X	-	X	-	X	X	-	-	-
<i>B. brasili</i> ♂	NA	X	X	X	-	X	-	-	X	-	X	-

¹CW- captive wild caught, CB- captive born, NA- new arrival²Physiological adjunct**Table 2.** Defensive behaviours observed in *Bothrops* species of four lineages that otherwise have not been observed to display SMTB

Species	¹ Status	Immobility	Head & neck elevation	Head hiding	S-coil	Flattening	Tongue flicking	Tail vibration	Body thrashing	² Hyper-ventilation	Strike
<i>B. alternatus</i> ♂	NA	X	X	-	-	X	X	-	-	X	-
<i>B. alternatus</i> ♂	CW	-	X	-	-	X	X	-	-	-	-
<i>B. fonsecai</i> ♀	CW	-	X	-	-	-	X	-	-	-	-
<i>B. jararaca</i> ♂	CB	X	X	-	-	-	X	-	-	-	-
<i>B. insularis</i> ♀	CB	X	X	-	X	X	X	-	-	-	-
<i>B. sazimai</i> ♂	NA	-	X	-	-	-	X	X	-	X	X
<i>B. sazimai</i> ♂	CW	-	X	-	X	-	X	-	-	-	X
<i>B. neuwiedi</i> ♂	CW	X	X	-	X	X	X	-	-	-	-
<i>B. erythromelas</i> ♀	CW	-	X	-	X	X	X	-	-	-	-
<i>B. erythromelas</i> ♂	CB	-	X	-	X	-	X	-	-	-	-
<i>B. marmoratus</i> ♂	CW	-	X	-	X	X	X	X	-	-	-
<i>B. pauloensis</i> ♀	CB	X	X	-	X	X	X	X	X	-	X
<i>B. bilineatus</i> ♂	CW	-	X	-	X	-	X	X	-	-	-

¹CW- captive wild caught, CB- captive born, NA- new arrival²Physiological adjunct

the wild, subject to double quarantine, and were housed individually in cages made of impermeable transparent plastic material, free from fissures, inert to disinfectants and cleaning chemicals with corrugated cardboard substrate and water freely available (Grego et al., 2021). The only exceptions were a specimen of *B. atrox* and *B. moojeni* that were kept in wooden cages (56 x 37 x 24 cm) with a glass door (Costa et al., 2005). Rooms temperatures were maintained

between 23–26 °C with relative humidity around 60 % monitored with thermo-hygrometers, and a light/dark cycle of 12 h (more details in Grego et al., 2021). They had their behaviour recorded in their individual cage, upon a table, or in a transitory box during sanitising procedures. The captive born group had been kept under the same protocols as the captive group (Grego et al., 2021). Their behaviours were recorded during sanitising procedures. All those snakes in

lineages which showed SMTB had their behaviour recorded between 2005–2019, while those which did not display SMTB were recorded between January–March 2022.

RESULTS & DISCUSSION

Five *Bothrops* spp from the *B. atrox* and *B. jararacussu* species groups showed SMTB during the typical alertness and threatening phases in response to human approach (Table 1; BHS video, 2022a) as well as other defensive behaviours categorised as ‘threatening’ (Greene, 1988; Araújo & Martins, 2006), including hyperventilation (a physiological adjunct) during the alertness phase (Table 1). Macroscopically, SMTB varied in the number of segments involved, and the twitches appear to have uneven intensities. Four other *Bothrops* species groups, represented by ten other species, did not display SMTB but were recorded expressing other defensive behaviours (Table 2; BHS video, 2022b).

When the video footage (shot at 29 frames/sec) is viewed at half speed, the posteroanterior sense of SMTB is revealed and the number of body segments involved. Likewise, better visualised in slow motion video, subtle SMTB has been detected close to the snake’s head, and certainly anterior to the location of the heart (e.g. *B. brazili*, *B. jararacussu*, and *B. leucurus* - BHS video, 2022a). SMTB may or may not occur concomitantly with hyperventilation (*B. jararacussu* and *B. leucurus* - BHS video, 2022a). SMTB cannot be misinterpreted as a byproduct of hyperventilation, a phenomenon witnessed in all six *Bothrops* species groups, as the twitches occur along the flanks only in small segments of otherwise motionless snakes, without volumetric expansion of the snake body as consequence of lung inflation. Although recurrent and often replicable in similar situations during human approach, macroscopically the twitching behaviour is not displayed consistently either in captivity or in the wild. In addition, SMTB can be interrupted, suspended, and then restarted.

Vipers are shy and secretive creatures that rely heavily on crypsis but, when moving, crypsis may be lost so that they may be more likely to bite (Glaudas, 2021). When dealing with species whose behaviours in the field are poorly known, it is impossible to know whether behaviours recorded in the laboratory are typical of those exhibited under natural conditions (Cundall, 1987). Nevertheless, most *Bothrops* spp are endowed with a well-known and predictable defensive repertoire in response to human approach. To date, in the four *Bothrops* lineages in which we did not observe SMTB (i.e. the *B. alternatus*, *B. jararaca*, *B. neuwiedi* and *B. taeniatus* groups) this behaviour has not been observed by other researchers in either wild or captivity (Werman, 1992; Salomão et al., 1997; Alencar et al., 2016; Sazima, 1988; 1991; 1992; Araújo & Martins, 2006). It would therefore appear that this innate behavior is strongly linked to the *B. atrox* and *B. jararacussu* species groups. These two groups are monophyletic which suggests that there is just a single evolutionary origin of this behaviour.

In the case of long-term captive *B. moojeni* and *B. leucurus* kept in our laboratory, SMTB has been displayed routinely in response to human approach, apparently without any detectable degree of accommodation over time. The plethora

of defensive behaviours displayed by pitvipers, particularly in *Bothrops* spp are easily perceived even by laypeople. Perhaps this may be the reason why SMTB has until now not been formally recorded in these two species groups. Because SMTB may be either explicit or subtle, several hypotheses can be suggested as to its purpose: i) as a possible distraction strategy before retaliation, ii) a warning display, iii) propelling blood to the cranial region in order to improve perception of the surroundings, or iv) an action to ensure better anchorage for a strike. In the case of a possible distraction function, it is conceivable that fast-moving, highly reactive predators, such as certain small mammals or birds, could perceive these twitches as strike precursors, and be confused by the large number of ‘false alarms’ generated by this behaviour.

Further research into SMTB could address whether the behaviour is displayed in darkness, the frequency and intensity of twitches under different conditions, and the impact of twitches on blood vessels flux. Furthermore, the distraction hypothesis could be tested in a staged encounter experiment with natural predators.

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Variability and hybridisation in the introduced pond slider turtle *Trachemys scripta* in Romania

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ABSTRACT - A qualitative survey of the chromatic and morphological variability of the pond slider turtle *Trachemys scripta* was undertaken in the parks of ten cities in Romania. Large variability was observed, melanistic individuals and intra-specific hybrids were found, and the characteristics of some specimens were such as to imply possible hybridisation with other species. The large morphological variability of the introduced populations of *T. scripta* indicates multiple geographical origins from the native range and very likely hybridisation. These factors contribute to a great diversity in the introduced populations and may influence their capacity for acclimatisation in the new range.

INTRODUCTION

The pond slider turtle *Trachemys scripta* is a North American species that has been disseminated across the world by the pet trade (Rhodin et al., 2017; Uetz et al., 2022). The constant release of unwanted pet turtles has resulted in innumerable concentrations of such animals, many of which have become established and even started to reproduce in their new environments. This species has elicited concern, being listed as one of the ‘100 of the World’s Worst Invasive Species’ (GISD, 2021) and of the ‘Invasive Alien Species of Union Concern’ (European Commission, 2017) - although “little is known of their impact on indigenous ecosystems” (GISD, 2021). Pond sliders are considered invasive or at least potentially so because “Their omnivorous diet and ability to adapt to various habitats, gives them great potential for impacting indigenous habitats” (GISD, 2021). In Romania, the species has been widely released (see, for the distribution, Sos, 2007; Stănescu et al., 2017; Cioflec, 2017-2021; Iftime & Iftime, 2021 and other sources quoted therein), but reproduction has so far only been documented from urban parks in four cities (Cioflec, 2013; Matei & Tudor, 2014; Iftime & Iftime, 2021). However, successful production of hatchlings does not necessarily reflect a healthily reproducing population, as *T. scripta* has a temperature-dependant sex determination mechanism and outside of a certain temperature bracket single-sex clutches may be produced (Wibbels et al., 1998). Nevertheless, the species is long-lived and so, at least in theory, populations can grow for some time before they become self-limiting - or adapt to find optimal egg-laying conditions. The ecological impact of *T. scripta* is in fact little-known for a species so widely claimed to be invasive (see above, GISD, 2021) and studies claiming negative interaction with native European species, especially *Emys orbicularis*, have been met by results to the contrary (see discussion in Iftime & Iftime, 2021). In this context, the diversity of the introduced stock, reflected in the variability of the introduced population, is of paramount

importance, as it provides the material for adaptation and selection in the introduced population, impacting the probability of establishment. To document this, we observed and photographed the chromatic and morphological variation in as many specimens of *Trachemys* as possible in several parks in the cities of Bucharest, Mogoșoaia, Craiova, Râmnicu-Vâlcea, Constanța, Ploiești, Buzău, Târgoviște, Pitești (over the warm seasons of 2020 and 2021) and the thermal resort of Băile Felix (older data, as of 2014). The identification of morphs and possible hybrids of *T. scripta* was based on the descriptions presented in several publications that are quoted in the Observations and Discussion section below.

OBSERVATIONS & DISCUSSION

We found *Trachemys scripta* to be present in all the parks we visited (see Supplementary Material, Table S1). A number of definite forms were commonly observed: the subspecifically defined *T. s. scripta* (Fig. 1A) and *T. s. elegans* (Fig. 1B); *T. s. troosti* (Fig. 1C) which nowadays is relegated to the status of a natural intergrade between the two above-mentioned valid subspecies (Parham et al., 2020); specimens very similar to the ‘southern intergrades’ between *T. s. scripta* and *T. s. elegans* (Fig. 1D); ‘anthropogenic hybrids’ described by Parham et al., 2020 (Fig. 2A) (see also Cioflec, 2017-2021; Iftime & Iftime, 2021). Melanistic specimens were also found (Figs. 2 B & C), as well as some showing morphological similarities to *Trachemys decussata* (Gray, 1831), a native of Cuba (Uetz et al., 2022) (Iftime & Iftime, pers. obs. - Fig. 2D [compare with *T. decussata angusta* (Barbour & Carr, 1940) as illustrated by Rhodin et al. 2017, p. 64], and Fig. 3A). Some specimens show more or less marked morphological similarity to *Trachemys gaigeae* (Hertweg, 1939), native to USA-Mexico border area (Uetz et al., 2022) (Fig. 3B), and *T. decorata* (Barbour & Carr, 1940), native to Hispaniola (Uetz et al., 2022) (Fig. 3C). A specimen with features of both *T. scripta* and *Pseudemys floridana peninsularis*, possibly an intergeneric hybrid, was

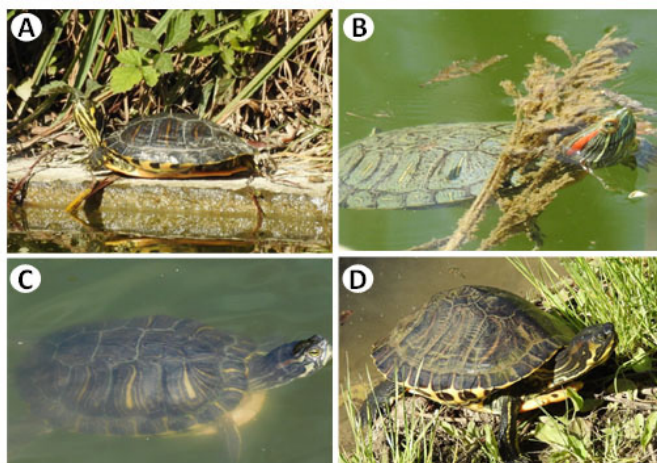


Figure 1. Turtles from various parks in Bucharest, Romania - **A.** Typical *Trachemys scripta scripta*, from lake in Tineretului Park, **B.** Typical *Trachemys scripta elegans*, from lake in Tineretului Park, **C.** '*Trachemys scripta troosti*', from lake in Titan Park, **D.** Probable *Trachemys scripta scripta* - *Trachemys scripta elegans* 'southern intergrade' from lake in State Circus Park

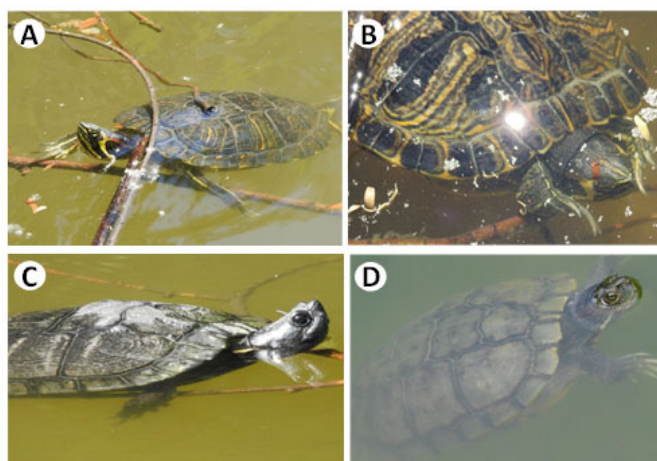


Figure 2. Turtles from the lake in Titan Park, Bucharest, Romania - **A.** Probable *Trachemys scripta scripta* - *Trachemys scripta elegans* anthropogenic hybrid, **B.** *Trachemys scripta* with incipient melanism, **C.** Fully melanistic *Trachemys scripta*, **D.** Probable *Trachemys scripta* - *Trachemys decussata* hybrid

also found (Figs. 4 A & B).

Our observations show a large variability in the introduced *T. scripta* population, including various degrees of melanism. Melanistic individuals are common in *T. scripta*, especially elderly males (McCoy, 1968; Lovich et al., 1990), in both native populations (McCoy, 1968; Lovich et al., 1990; Smith et al., 2016) and introduced ones (Böhm, 2013; Martins et al., 2014; Jablonski et al., 2016). The process of melanisation is hormonally driven (Lovich et al., 1990); both aging (Lovich et al., 1990) and thermal influences (Rowe et al., 2016) appear to be involved. Melanisation can be permanent or transient (Lovich et al., 1990); it goes through various stages with associated colouration patterns (McCoy, 1968; Lovich

et al., 1990; Tucker et al., 1995). Melanisation was linked with behavioural particularities, which may, however, be associated rather with age and/or hormonal changes than with the co-occurring melanisation per se (see discussion in Garstka et al., 1991; Tucker et al., 1995; Stone et al., 2015). In our samples, melanism was seen in adult but not necessarily senescent specimens, mostly in males, but also in a small number of females. Both *T. s. scripta* and *T. s. elegans* appear to undergo melanisation. All stages were seen, from incipient melanisation (Fig. 2B) to all-black individuals (Fig. 2C).

Some of the melanisation stages in *T. scripta* are partly convergent with the known pattern of colouration (including melanism) in *T. decussata* (Barbour & Carr, 1940), hence the risk of misidentifying melanistic *T. scripta* as *T. decussata* (Poch et al., 2020). The well-documented hybridisation of *T. scripta* and *T. decussata* (Parham et al., 2013) also confuses the issue. Moreover, diagnostic traits between these and also other *Trachemys* species are often quantitative, difficult to follow, and not always consistent (see, e.g. Barbour & Carr 1940; Seidel, 1988). Many *Trachemys* interspecific hybrids appear to be fertile, and in the complicated context where current species delineation does not follow the objective reproductive incompatibility criterion, the issue of the relationships among putative *Trachemys* species is considered "poorly resolved" (Seidel & Ernst, 2017). In these circumstances, it may be legitimate to advance the hypothesis that some of the morphologically *decussata*-like *T. scripta* specimens observed in Bucharest are of a probable *T. scripta* x *T. decussata* hybrid origin. Indeed, some show a short, rounded snout, relatively large eyes and flat head, relatively flat carapace profile, and traces of radial wrinkling on carapace plates (e.g. Fig. 2D; Fig. 3A). When found individually, and much more so when co-occurring, they are indicative of *T. decussata*, according to some authors (cf. Barbour & Carr, 1940; Seidel, 1988). It is also worth mentioning that a *T. d. angusta* photographed on Grand Cayman Island (Rhodin et al., 2017) may well be a hybrid, showing even less of the typical *T. decussata* traits than the specimen in our Fig. 2D; Grand Cayman is well-known for hybridisation between the two species (Parham et al., 2013, and literature quoted therein). Again, melanistic *decussata*-like *Trachemys* in Bucharest survive the cold season by hibernating, which is not documented for *T. decussata*, and is strongly indicative of the hybrid condition (see also the discussion in Poch et al., 2020).

Other possible hybrids are also present. One specimen found in Pitești (Fig. 3B) suggests, through the cephalic pattern (fragmented right temporal stripe, intense vermiculation pattern on head) a *T. scripta elegans* x *T. gaigeae* hybrid (cf. Stuart & Ward, 2009; Parham et al., 2020). Also, one 'odd' Bucharest specimen suggests, by its morphological features (sharp, conic snout; muted, yellowish temporal stripe; curved but quite short forelimb claws in a male - cf. Barbour & Carr, 1940; Seidel & Inchaustegui Miranda, 1984), a possible introgression from *T. decorata* (Fig. 3C). Another such peculiar specimen (Figs. 4 A & B) may even be an intergeneric hybrid, as it shows a head and carapace pattern reminiscent of both *T. scripta* and *P. (floridana) peninsularis*. *T. scripta* x *Pseudemys* hybridisation has been reliably demonstrated (Soler et al., 2015) and such hybrids are occasionally found in the reptile

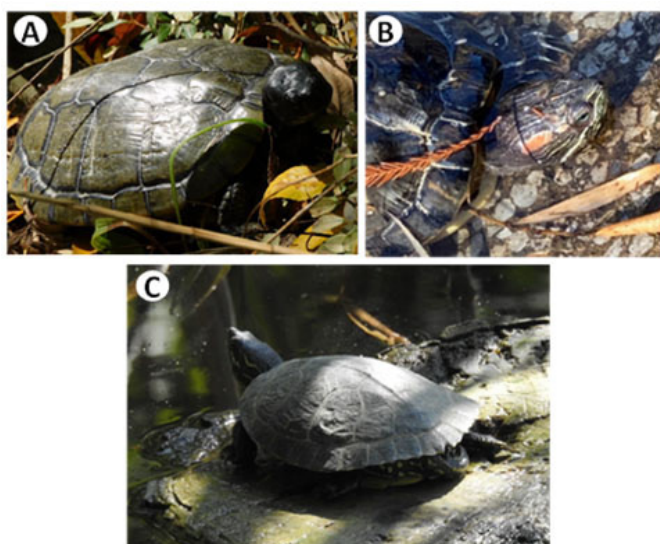


Figure 3. Turtles from various city parks in Romania - **A.** Probable *Trachemys scripta* - *Trachemys decussata* hybrid, Bucharest, from lake in Titan Park, **B.** Probable *Trachemys scripta* - *Trachemys gageae* hybrid, Pitești, from lake in Expo Park, **C.** Possible *Trachemys scripta* - *Trachemys decorata* introgressive hybrid, Bucharest, from lake in State Circus Park

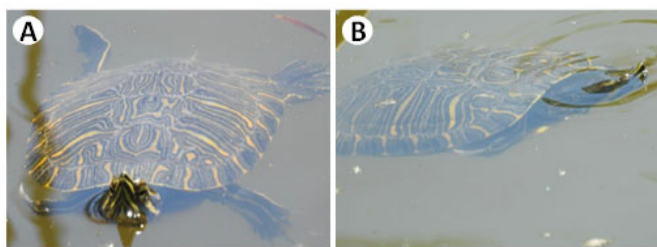


Figure 4. Possible *Trachemys scripta* - *Pseudemys (floridana) peninsularis* hybrid, Bucharest, lake in Titan Park, Romania (**A** & **B** are different views of the same specimen)

trade - see for example *T. scripta* x *P. (floridana) peninsularis* (Underground reptiles, undated) and *T. scripta* x *P. concinna* (The Turtle source, undated). The specimen pictured in the last source is quite similar to Figure 4 in its mix of features, but the 'Romanian' one is somewhat more reminiscent of *P. (floridana) peninsularis*, though we cannot altogether exclude the possibility that it has *P. concinna* ancestry. Such hybrids may have been sold in Romania as well, though not necessarily under the correct label, and therefore could be discarded into urban lakes; similarly, 'cooter/slider' (i.e. *Pseudemys-Trachemys*) hybrids were found in the London area (Langton et al., 2011).

Besides supposed interspecific hybridisation, there is definite evidence of intraspecific hybridisation/intergrading, with numerous specimens displaying features of either 'northern' *T. s. scripta* - *T. s. elegans* intergrades ('*T. s. troosti*'), 'southern' *T. s. scripta* - *T. s. elegans* intergrades, or anthropogenic hybrids between the same subspecies (cf. morphological indications in Parham et al., 2020). Such diversity is indicative of both widespread collection of *T.*

scripta individuals from the native range in the USA and hybridisation in captive-breeding facilities (or even post-release), and suggests great morphological, and presumably genetic, diversity in the introduced population.

To date, the most widespread forms remain the classical subspecies, *T. s. scripta* and *T. s. elegans* (the former now more widespread than the latter, at least in our sampling). It is apparent that the greatest diversity of turtle forms is in Bucharest (Table 1S), where there is considerable variation in diversity between water bodies, followed by Craiova. This probably reflects the intensity of pet trade, which we expect to vary in approximate proportion to city size and general intensity of commerce. The variability in Bucharest would appear to be accounted for by some parks (e.g. Titan Park) being preferred as release sites and perhaps also more favourable to the survival of the new arrivals.

We conclude that introduced populations of *T. scripta* are quite varied in morphology, indicating multiple geographical origins from the native range and very likely hybridisation. These factors contribute to a great diversity in the introduced populations, which may influence their capacity for acclimatisation in the new range. Such diversity may be an asset for a population under selective pressure and the melanism observed in this survey may be a case in point. Conversely, hybridisation with tropical species may be a hindrance in a temperate environment, nevertheless the supposedly hybrid specimens appear to have survived through some winters at least. Further study may establish whether some morphs become better established in the new environment than others and if so then the nature of the selective advantages that have led to this may be revealed.

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Diet of the recently introduced white-lipped frog *Leptodactylus fragilis* from a suburban ecosystem in western Cuba

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ABSTRACT - Diet and trophic interactions are essential for understanding aspects of the natural history of a species and evaluating its role within the ecosystem. A successful coloniser often has the ability to exploit a wide variety of prey during its establishment and may generate negative impacts due to competition with the local fauna. During both the dry and rainy seasons of 2019, in a suburban ecosystem in western Cuba, we analysed the diet of the recently introduced white-lipped frog *Leptodactylus fragilis*. Captured frogs were measured and stomach-flushed and released 24 h after analysis at the site of capture. Ninety-one *L. fragilis* were captured, 73 of them (80.2 %) had prey in the stomach. The most consumed prey were beetles, followed by spiders, and crickets. No difference was observed in prey consumption between seasons. This frog may change its foraging strategy according to the habitat it occupies. In this study, we observed high values of food niche breadth, which may imply a greater competition of *L. fragilis* for food resources with the native syntopic anurans in Cuba. Studies on temporal and spatial dynamics are needed to understand the possible competitive interaction with native amphibians in this area.

INTRODUCTION

A knowledge of diet and trophic interactions is essential for understanding aspects of the natural history, population dynamics, assessment of energy flow, and food webs in ecological communities (Anderson, 1991; Solé & Rödder, 2010). The most obvious potential impact of an invasive amphibian species is competition with native amphibians for food resources and this is likely to be greater when the introduced species occurs at high densities and with no apparent natural predators (Olson, 2011).

In the Caribbean region, 25 amphibian species have been introduced, five of them within the Cuban archipelago (Powell et al., 2011; Borroto-Páez et al., 2015). However, their impacts on the Cuban native fauna has yet to receive an appropriate evaluation. Recently, Rodríguez-Cabrera et al. (2018) reported the presence of an additional introduced amphibian, the white-lipped frog *Leptodactylus fragilis* (Brocchi, 1877) in two localities of western Cuba. These are the first observations of the species outside its native geographic range, which extends from southern-most Texas (USA), through Mexico and Central America, to northern Venezuela, up to the Venezuelan State of Sucre (de Sá et al., 2014). To date there is no evidence of any impacts of *L. fragilis* on native species in Cuba even though Del Castillo et al. (2021) predicted that this species could become successfully established, spreading mainly in open areas. The advertisement call of this species is loud and is audible over a large area, suggesting a potential invasion of the acoustic niche of native amphibian species (Del Castillo et al., 2021).

Leptodactylus fragilis is a generalist predator

(González-Durán et al., 2011), feedings on arthropods (Savage, 2002), especially spiders, beetles, bugs, ants, and cockroaches in the species' natural range (González-Durán et al., 2011; Méndez-Navárez et al., 2014; Arrieta, 2017). In that respect, understanding possible interactions with native amphibian species, and identifying other possible impacts such as resource competition or disruption of trophic webs, is of particular importance for conservation of native fauna. In this contribution, we provide the first analysis of the diet of the recently introduced *L. fragilis* from one of the known localities in Cuba, paying attention to the possible differences between dry and rainy seasons.

MATERIALS & METHODS

Fieldwork

The study was undertaken at Sandino town, Pinar del Río province (22° 4'43" N, 84° 12'10" W, WGS84). We collected frogs at four points along both sides of a 1.5 km stretch of road (from Sandino to Manuel Lazo). The frogs were collected in a suburban environment associated with ditches and ponds in the surroundings of this town (Fig. 1A). To detect the variation in diet with respect to seasons, we sampled in both the dry (April 2018 and February 2019) and rainy (June 2019) season. Frogs were captured by hand at 20:30 h to 22:00 h. Snout-vent length (SVL), the most common descriptor of body size in anurans, and head width (HW), which is related to feeding habits, as it limits maximum prey size (Emerson, 1985), were measured using a caliper to the nearest 0.2 mm. All frogs were stomach flushed (following Mahan & Johnson, 2007) and released at the site of capture 24 h afterwards. The

diet of individuals was determined from placing the stomach contents in vials, fixed with 70 % ethanol, and analysed (later) under a stereomicroscope. Prey items were classified at class or order level, or lower when possible. The investigation was undertaken under research permit P211PR900-003 and no further ethical approval was required for stomach flushing; no frogs died during the procedure.

Data Analysis

We calculated the number of items consumed (N) and percent occurrence of different types of prey ($N_{\%}$); the frequency of occurrence (F , number of stomachs in which a given prey category was found), and its percent ($F_{\%}$) for each prey category. We calculated the trophic niche breadth using Levins' index (B) (Krebs, 1989):

$$B = \frac{1}{\sum_{i=1}^n P_i^2}$$

where P_i = fraction of items in the prey type i . For standardisation of niche breadth (B_A), we use Hurlbert's (1978) proposal: dividing B by the total number of resource states after correcting for a finite number of resources; range = 0 (no diversity, exclusive use of a single prey type, specialist) to 1 (highest diversity, prey items of all categories, generalist).

Mann-Whitney U-Tests were used to compare number and type of prey consumed between seasons. Spearman Correlation tests were calculated for the relationships between SVL/HW, SVL/prey length, and SVL/number of prey per stomach. The Simpson diversity index (D hereafter) (Simpson, 1949) was calculated to compare the variation in diversity of diets among seasons.

RESULTS

We sampled 91 individuals of *L. fragilis* (Fig. 1b), ranging from 31.18 to 43.00 mm SVL (37.04 ± 2.25 ; mean \pm SD), 10.58 to 14.12 mm HW (12.46 ± 0.57). Seventy-three frogs (80.2 %) contained prey in their stomachs, and 19.8 % had empty stomachs. Positive correlation was observed between SVL and HW (Spearman 0.769, $p < 0.001$, $N = 91$). Larger *L. fragilis* consumed larger prey (Spearman 0.370, $p = 0.040$; $N = 58$). There was a negative correlation between SVL and number of prey per stomach (Spearman -0.298, $p = 0.019$, $N = 62$), but there was no significant difference in number of prey consumed between seasons (U-test = 432.0, $Z = -0.690$, $p = 0.490$; $N = 62$). Niche breadth (dry season $B = 8.24$, $B_A = 0.36$; rainy season $B = 8.32$, $B_A = 0.37$) and number of prey items per stomach were similar between seasons.

No significant difference between seasons was observed in type of prey consumed (U-test = 694.500, $Z = -1.041$, $p = 0.298$). In the dry season, 41 (91.1 %) frogs had prey in the stomach, and four had empty stomachs; while in rainy season, 32 frogs (69.6 %) had prey in the stomach and 14 (30.4%) had empty stomachs. We reported 15 prey orders in the stomach of *L. fragilis* and recorded 246 prey items (Table 1S, see Supplementary Materials). The number of prey

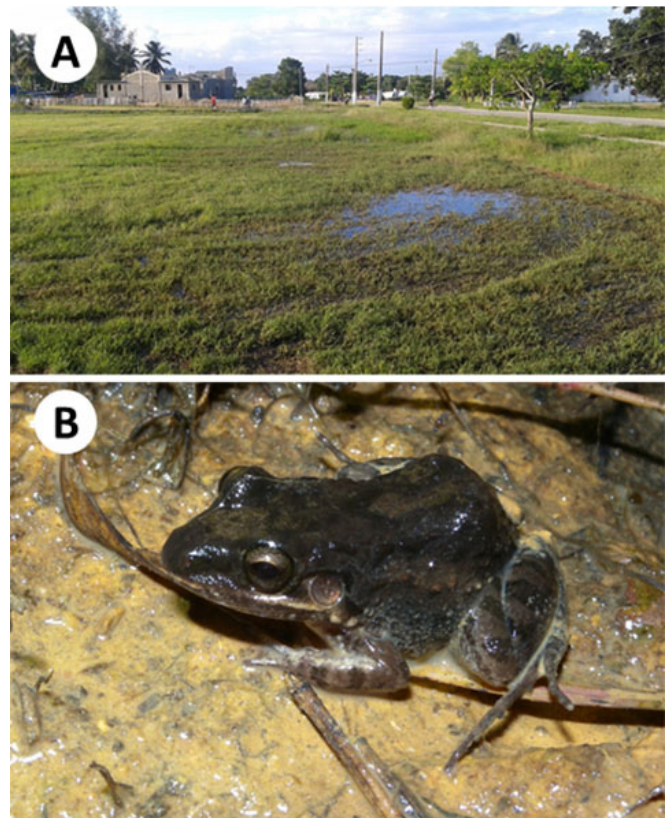


Figure 1. Typical habitat of the study area - **A.** Ditch in suburban environment of Sandino town, **B.** *Leptodactylus fragilis* in the study area

items per stomach in the dry season consumed by *L. fragilis* (mean = 3.3 ± 2.15 , range = 1–10) was similar to that in the rainy season (3.4 ± 3.08 , 1–16). Beetles (Coleoptera) were the most common prey in the stomach samples in this frog, observed in 26 individuals in the dry season ($F_{\%} = 25.2$, $N_{\%} = 22.1$), and in 19 in the rainy season ($F_{\%} = 27.5$, $N_{\%} = 33.0$), and represented by 14 families, followed by spiders (Araneae) ($F_{\%} = 16.9$, $N_{\%} = 16.3$), and crickets (Orthoptera) ($F_{\%} = 6.4$, $N_{\%} = 4.5$) (Table 1S). We identified two gastropod molluscs: the introduced semiaquatic snail *Hemisus brevis* (F. Thiaridae), and the land snail *Zachrysia* sp (F. Camaenidae). Many of the ants (Formicidae) consumed (70.6 %) corresponded to the introduced fire ant *Wasmannia auropunctata*. Other species identifiable to species level in *L. fragilis* stomach samples were the introduced cockroach *Periplaneta americana* and earwig *Carcinophora americana*. Inorganic materials (nylon and a fragment of plastic) were observed in two stomachs. The diversity of prey consumed by this species was only slightly higher during the rainy season ($D = 0.93$) than in the dry season ($D = 0.92$).

DISCUSSION

Prey size is directly correlated with body dimensions in some amphibians (i.e. SVL and HW) (Parmelee, 1999; González-Durán et al., 2011; Lunghi et al., 2018; this study), because a wide head and longer jaws contribute to larger gape (Emerson, 1985). In our study, individuals with larger SVL

tended to consume larger prey, which suggests that body size in *L. fragilis* influence the size of prey consumed (González-Durán et al., 2011; this study).

In our study, the most consumed prey by *L. fragilis* were beetles, observed in the stomach content in both seasons, followed by spiders and crickets. Nonetheless, Toft (1980) defined that wide niches and lower B_A values are distinctive of generalist predators in amphibians. In our study, the higher values of diversity of prey consumed and B_A in *L. fragilis* stomach samples reinforce the generalist/opportunistic predator classification (Taigen et al., 1982; González-Durán et al., 2011). This finding is consistent with previous studies of this species in its native range (González-Durán et al., 2011; Méndez-Narváez et al., 2014; Arrieta, 2017) independent of the habitat that this species temporarily occupies. Some invasive invertebrate species were also observed in the stomach samples of this frog: the semiaquatic snail *Hemisinus brevis* (F. Thiaridae), the cockroach *Periplaneta americana*, and the fire ant *Wasmannia auropunctata*; the latter represents the main content of Formicidae (70.6 %) samples. Other items, such as inorganic material (nylon and a fragment of plastic), were observed in two stomach samples of *L. fragilis*, possibly consumed accidentally while feeding.

Like most leptodactylid frogs, *L. fragilis* is considered a sit-and-wait predator (Taigen et al., 1982; González-Durán et al., 2011). However, in some areas, this species may exhibit a bimodal foraging strategy, depending on which prey are available and abundant in a particular habitat (Méndez-Narváez et al., 2014). *Leptodactylus fragilis* seems to be able to change its foraging strategy according to the habitat it occupies: populations that live around ponds and open areas may exhibit the 'sit-and-wait' strategy (González-Durán et al., 2011, the present study); populations associated with forested areas, and/or livestock, and not necessarily nearby a body of water, may be active foragers (Méndez-Narváez et al., 2014). In addition, *L. fragilis* feeds more in open and certain anthropogenically disturbed areas than in forest-covered areas (Arrieta, 2017).

In our study, slightly higher diversity of prey was consumed by *L. fragilis* during rainy season; Arrieta (2017) obtained a similar result. The increase in diversity of prey in the stomach samples of this frog is probably related to prey availability during this season. However, we also detected the highest number of empty stomachs during that period. A plausible explanation could be related to reproductive behaviours. During this season, individuals spend more time in reproductive displays, and have less time to feed, especially at the time of capture (21:00 to 23:00 h). We suggest that this species, even though it is a generalist predator, in the dry season is slightly selective with its prey. In the rainy season, this species spends more time in reproductive activity, and feeds on every prey available in every non-reproductive moment it has (e.g. the 'intermission' of calling activity in males).

Werner et al. (1995) observed that similar body-sized species could overlap in diet, especially if they share the same microhabitat. These authors suggested that the potential strength of competitive interactions among species is likely mitigated by the habitat-related diet differences, and diet overlap declined with increasing body size disparity.

Leptodactylus fragilis has high feeding plasticity, and could become a competitor in newly colonised lands, like Cuba. According to the diversity of prey consumed by *L. fragilis* (Table 1S), the higher value of niche breadth ($B = 8.68$), and its morphological characteristics, we can assume that our studied population is a generalist/opportunistic 'sit-and-wait' predator. The species lives in syntopy with four native amphibian species (*Eleutherodactylus riparius*, *E. goini*, *Osteopilus septentrionalis*, and *Peltophryne empusa*) (Rodríguez-Cabrera et al., 2018). *Eleutherodactylus* frogs, juveniles and sub-adults of *O. septentrionalis* and *P. empusa* are similar in size to *L. fragilis* (Henderson & Powell, 2009; de Sá et al., 2014), and exhibit a similar type of prey consumption to this introduced species (García-Padrón, unpubl. data). Therefore, *L. fragilis* is likely to be in competition with this native species for trophic resources.

Leptodactylus fragilis has certain characteristics that are typical of successful invasive species: it is ecologically tolerant to a broad range of, and rapidly changing conditions; is highly tolerant of human presence (Perry et al., 2008; Powell & Henderson, 2008); is primarily a predator of arthropods (Powell & Henderson, 2008); and is relatively small and capable of rapid reproduction (Kolar & Lodge, 2001). Studies of temporal and spatial dynamics of this food-generalist species are needed to understand its possible competitive interaction with native amphibians in this area.

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The threat of discarded food and drinks containers to monitor lizards

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INTRODUCTION

Monitor lizards (family Varanidae) have a wide distribution in the tropics and sub-tropics including Australasia, south and south-east Asia, Africa, and the Middle East (Koch et al., 2013; Auliya & Koch, 2020). They have an important role in ecosystem as predators, scavengers, and creators of microhabitats for other animals (Doody et al., 2021). Typically, monitors are big lizards and the family includes the largest living lizard, the Komodo dragon *Varanus komodoensis* whose adult males measure up to 3.1 m and weigh up to 100 kg (Jessop et al., 2006). However, there are some small species, e.g. *Varanus sparnus* the adults of which may have a body length of only 23 cm and mass of 17 g (Koch et al., 2013; Auliya & Koch, 2020).

Only 14 of the 68 species listed by IUCN are considered to be of conservation concern although 10 other species are Data Deficient (IUCN, 2021). Currently, the major anthropogenic threats to monitor lizards include the degradation of their natural habitats, uncontrolled tourism, and pet trade (Koch et al., 2013; Zdunek, 2019; Ariefiandy et al., 2021). Locally, road mortality may also limit their populations (Hastings et al., 2019; Ayob et al., 2020). Moreover, monitor meat is traded and consumed as a novelty food, a traditional remedy for a range of ailments (skin diseases, eczema, asthma, blindness), as an aphrodisiac (Uyeda et al., 2014; Nijman, 2015; Zdunek, 2019), and there is still demand for monitor skin from the leather industry (Boscha et al., 2020). Progressive global warming may also cause a decrease in their habitat patch occupancy (Jones et al., 2020).

It is well known that many monitor lizards exploit urban habitats, especially rubbish dumps, which provide food (Kulabtong & Mahaprom, 2015; Karunarathna et al., 2017). Indeed, such littered areas are characterised by higher population abundance compared to undisturbed habitats (Uyeda, 2009; Jessop et al., 2012). Although feeding on organic waste can improve the lizards' body condition and reduce the parasite load (Jessop et al., 2012), availability of such food alters their behaviour and presents them with many threats. Association with rubbish dumps impacts some important aspect of monitor biology including - sex ratio (male-biased; Jessop et al., 2012); movement patterns by reducing species home ranges (Stanner & Mendelssohn,

1987); increased antagonistic interactions and social hierarchy (Uyeda et al., 2015); reduced offspring survival due to cannibalism or predation (Jessop et al., 2012); and reduce reproductive success due to high levels of mate competition (Jessop et al., 2012). Moreover, feeding on anthropogenic food waste can lead to internal injuries and consequently to a slow death (Trembath & Freier, 2005; Gillet & Jackson, 2010). Another, but still understudied threat is getting stuck in food or drinks containers. A monitor lizard may put its head into a container or enter it completely to eat food leftovers or other animals that got stuck therein before. However, getting the head out of the container may be a problem, and such an incident is often fatal. A recent study revealed that beside invertebrates and mammals, reptiles are quite often exposed to such danger. Among 77 reptile-container interactions, 13 (17 %) concerned monitor lizards (Kolenda et al., 2021).

Herein, we highlight the problem of discarded food/drinks containers by presenting new observations of monitor lizards that we obtained from other scientists and naturalists combined with those reported previously (Kolenda et al., 2021). Full details are presented in Supplementary Materials (Table 1S) and include a total of 32 cases of monitors stuck in discarded containers (Fig. 1). Most events occurred in Australia (18, 56 % of all cases). Observations were made on eight monitor species, of which *Varanus acanthurus* was the most common (9 cases, 28 %). None of the species observed were in an IUCN Red List threatened category although *Varanus nebulosus* and *Varanus similis* have not been evaluated. In six containers (19 %), the monitors were found dead. In the remaining 26 (81 %), they were alive at the time of observation, and at least in 22 of these cases they were rescued by an observer or transported to a wildlife rescue. The most common container in which the trapped animals were observed was drinks cans (18, 56 %), followed by food cans (12, 38 %), and a single plastic jar (3 %), and an unrecorded type of container (3 %).

Discarded containers are common litter items in terrestrial environments (Roman et al., 2020). They pose a threat to various groups of animals, including arthropods and small vertebrates as well as large animals such as ungulates, leopards or bears (Kolenda et al., 2021). By themselves mortality due to such containers would not be expected to lead to a population decline, but they constitute one of



Figure 1. Examples of monitor lizards from Australia with their heads stuck in discarded drinks cans - **A–D.** *Varanus acanthurus*, **C.** *Varanus similis*

many factors that synergistically may limit the population size, especially in urban habitats. However, in the case of the Critically Endangered Bermuda rock skink *Plestiodon longirostris*, an endemic lizard of Bermuda, discarded containers are indeed one of the main threats (Davenport et al., 2001). It should be noted that containers such as bottles or cups are also a potential trap for reptiles (for details see Figure 4a in Kolenda et al., 2021), whereas other garbage poses other direct threats to reptiles, e.g. by ingestion or entanglement (Walde et al., 2007; Strine et al., 2014).

Due to the relative rarity of the above-mentioned observations, the real impact of discarded containers on monitor populations remains poorly understood. Taking into consideration reports presented herein, we strongly encourage conservationists to pay attention to this issue. Workshops on conservation of wildlife (such as presented by Bhattacharya et al., 2019) that aim to raise public awareness should mention the harmful effects of litter on animals. Suggestions for limiting impacts should include - to

implement strict regulations regarding littering and waste disposal; to install garbage bins along hiking paths and ensure that they are emptied regularly; and, to engage local communities in rubbish clean-up actions.

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Limb malformations in introduced populations of midwife toad *Alytes obstetricans* in Great Britain

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Amphibians are known to suffer from limb deformities for a number of reasons, including parasitic infection (Johnson et al., 1999), environmental pollution (Taylor et al., 2005), and ultraviolet radiation (Ankley et al., 2002). Deformities may also arise from errors during the regeneration process (Gray & Lethaby, 2010). There have been a few studies investigating the prevalence of malformed limbs in Britain (Ballengee & Sessions, 2009), although there have been several records of deformities for both anurans and urodeles (Jarvis, 2011; Allain et al., 2021; Jordan & Shadbolt, 2021).

The common midwife toad *Alytes obstetricans* is a small, cryptic species found throughout western Europe, although it is an alien species in Britain (Speybroeck et al., 2016). It grows to 5 cm in length and may be found in habitats such as parks, woodlands, gravel pits, and gardens. There are several introduced populations in Britain, one of which in Cambridge has been monitored since 2015 (Allain & Goodman, 2017). Currently, most of the introduced populations are restricted to residential gardens in urban and suburban areas. It is very likely that the first of these *A. obstetricans* populations found its way to Britain in a shipment of nursery plants in the early part of the 20th Century, before being dispersed with human assistance (Beebee & Griffiths, 2000). Currently, the species is monitored to assess distribution, check for disease, and to record any impacts on native fauna.

During surveys since 2015, we have recorded five cases of limb malformation from three separate garden populations of *A. obstetricans* (Table 1), which have been described according to the classification of Meteyer (2000). Photographs of each toad were taken while collecting morphometric data. Individuals were also swabbed for the presence of infectious diseases, such as the amphibian chytrid fungus *Batrachochytrium dendrobatidis*.

Despite having carried out more than 60 surveys across nine different populations, no other instances of limb malformations have been observed. It is likely that individuals exhibiting deformities are uncommon within British populations, with no deformed specimens observed within the Cambridge population since 2017, despite many additional surveys. A considerable number of other amphibians such as common frogs *Rana temporaria*, common toads *Bufo bufo*, and smooth newts *Lissotriton vulgaris* have also been encountered while surveying for *A. obstetricans*. No deformities of any kind have been observed in the native

amphibians present in the same gardens. In Spain, there have been cases of polymelia (Fernández, 2013) and polydactyly (Espasandín, 2017) in *A. obstetricans*.






The locations in which deformities were observed were suburban. This appears to rule out pollution from agricultural run-off as a causative agent, which has been identified as a factor in other studies (Taylor et al., 2005). However, domestic garden pollution due to fertilisers, herbicides and rodenticides cannot be ruled out but this seems an unlikely cause given that other amphibians in the same habitats have been unaffected. Trematode infections in amphibians have previously been proven as the causal factor in some deformities in North America (Johnson et al., 1999), but to date the only such infection recorded in European amphibian populations is from Russia in water frogs of the genus *Pelophylax* (Svinin et al., 2020). Exposure to ultraviolet radiation is unlikely to be the cause of these deformities as *A. obstetricans* eggs are not left in the pond to mature as is the case with other amphibians. Instead, they are carried by the male who seeks shelter underground in warm, damp places. Here, he waits until the tadpoles are mature enough before finding a suitable body of water in which to deposit the eggs (Beebee & Griffiths, 2000). Midwife toads are also largely nocturnal, which when combined with their secretive behaviour, reduces the risk of ultraviolet radiation affecting their developing larvae.

Due to small founder populations and low dispersal rate at each site, it is possible that the *A. obstetricans* populations are significantly inbred. This could be a causal factor in the limb deformities (Williams et al., 2008), but in the absence of genetic testing this remains a tentative suggestion. During future surveys, we will continue to record deformities of both midwife toads and other amphibians to better establishing the frequency of their occurrence.

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Table 1. Limb malformations observed in three British populations of *Alytes obstetricans* in gardens

Date & location	Sex	Weight (g)	SVL (cm)		Notes
28/06/17 Cambridge	F	5.8	3.3		Ectrodactyly - Three digits are present, although digits 1 and 2 are not properly separated and are shorter than normal, and digit 3 is missing completely. Closer inspection of the hand suggested that the metacarpals have not developed correctly
28/06/17 Cambridge	M	3.9	3.4		Ectrodactyly - The tibulare and fibulare have developed correctly but the metatarsals show severe malformation, no phalanges are present
24/08/19 Oundle	M	6.0	3.6		Ectromelia - The tibulare and fibulare of the right hind limb appear smooth and without skin pigmentation, this may be due to the skin being thin and translucent in the area; the pale colour may be the underlying bone
24/08/19 Oundle	M	5.5	3.3		Ectromelia- The tibia-fibula of the right hind limb has the bone terminating further up the leg in the previous case (see above). The tibulare and fibulare are absent, with a kink in the tibia-fibula which may impede mobility
8/05/21 Bedford	-	-	-		Polymely - A second front right limb is present with all digits which also exhibits a 180 ° rotation so the hand faces upwards

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Rediscovery of the marbled caecilian *Epicrionops marmoratus* and first record from Colombia

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A few years ago, during the course of our revision of the Gymnophiona of Colombia, we came across an interesting collection of caecilians from Nariño, Colombia. This collection was housed at Universidad de Nariño and was gifted to Professor Belisario Cepeda-Quilindo by the native inhabitants of Reservas La Planada and Río Ñambí. Unfortunately most specimens lack field notes, collection dates, and specific localities. The specimens were presumably collected sometime during the early 1990s by various biologists visiting these field stations and contain primarily a large series of Gunther's caecilian *Caecilia guntheri*, (seemingly a common species at La Planada) but we detected that a much rarer species was hiding among these; an excellent specimen of *Epicrionops marmoratus* collected by Guillermo Cantillo in Reserva Río Ñambí, Altaquer, Barbacoas, Nariño, Colombia, 27 February 1992.

Epicrionops marmoratus (PSO-CZ 857) was examined under a Zeiss Stemi 2000 stereoscope using entomological pins to facilitate counting the numerous folds found throughout the body. The total fold count was made twice by the senior author in order to avoid miscalculation. A small incision to the commissure of the mouth was made in order to access dentition (i.e. the number of teeth per series), and all teeth were examined directly with the mouth opened. All dental counts were made clockwise from left to right postero-anteriorly; teeth that were not fully exposed outside the gums were not counted. A small, ventral longitudinal incision was made to search for sexual organs. All measurements were taken using a Neiko digital calliper rounded to the nearest 0.1 mm with the exception of total body length, which was determined using a measuring tape (in centimetres) and placed along the body length of the specimen.

We identified this caecilian as *E. marmoratus* because it had the following morphological characteristics, meristics, and measurements. An adult male with a total body length of 290 mm (432 mm in life), body width of 14.4 mm at mid-body point, an attenuation index (i.e. length divided by width) of 20.1 times, and a main brown to reddish body colouration in preservative (70 % ethanol). This individual has a total of 300 folds past the nuchal collars and only the last 26 are caudal folds (Table 1); with seven folds on the second nuchal collar and eight folds interrupted by the vent (Fig. 1). Dentition

and the number of teeth per series differ slightly from those indicated by Taylor (1968) in the original description of *E. marmoratus*, but this is to be expected given that some teeth are missing or concealed by the gums, hence not counted, and the total extent of intraspecific variation is yet to be assessed; premaxillary-maxillaries 12-14, vomeropalatines 13-1-15, dentaries 14-1-14, and inner mandibulars 19-18. Scales begin at the first fold and end at the last fold, where these are subcircular in shape with a straight outline at the margin of inception with the pocket, their overall shape is similar to those indicated in Taylor (1972: 1094, fig. 46). Taking into account that the measurements and meristics of PSO-CZ 857 fall within the range indicated by Taylor (1968: 205) we are confident of our identification.

Table 1. Measurements and meristic data for *Epicrionops marmoratus*

Characters/Specimens	PSO-CZ 857	BMNH 1956.1.15.87	EP 3986
Total length (mm)	290	299	278
Body length (mm)	266.1	278.5	258.8
Tail length (mm)	17.6	20.5	19.2
Head width (mm)	9.2	11	11.2
Body width (mm)	14.4	14	15.2
Tentacle to nostril (mm)	4.1	4.6	4.3
Snout tip to 1st groove (mm)	14.1	12.8	12
Snout tip to 2nd groove (mm)	15.7	15	15
Snout tip to 3rd groove (mm)	17.4	22	21
Total folds (dorsal-lateral count)	300	327	302
Caudal folds	26	25	20
Folds on 2nd collar	7	7	6

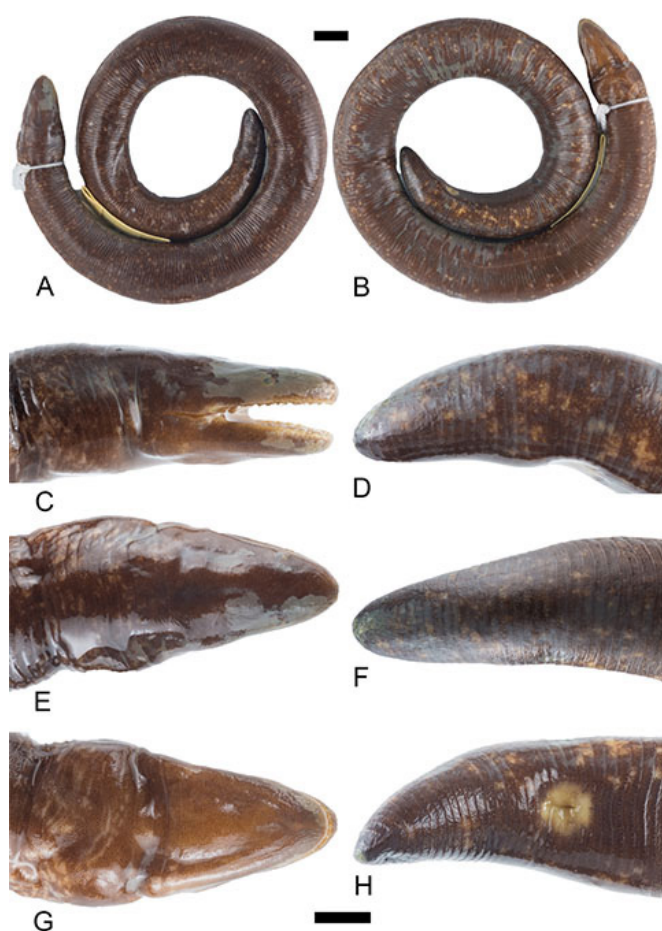


Figure 1. *Epicrionops marmoratus* (PSO-CZ 857) from Reserva Río Ñambí, Barbacoas, Nariño, Colombia - **A.** & **B.** Dorsal and ventral views, **C.**, **E.**, & **G.** Head and nuchal collars in lateral, dorsal, and ventral views, **D.**, **F.** & **H.** Tail in lateral, dorsal, and ventral views. Upper scale equals 9 mm, lower scale equals 4.5 mm.

The main colouration pattern of PSO-CZ 857 seems to vary from the “marbled pattern” indicated in the original description because the ground colour of PSO-CZ 857 is mainly dark brown to reddish brown with light cream or yellow blotches or flecks scattered irregularly along the body. These markings occur more prominently on the flanks and less so on the dorsal and ventral surfaces of the body; in contrast to the cream-white ground colour with lavender spots *sensu* Taylor (1968: 204, 208, figs. 98, 101).

Epicrionops marmoratus remains a poorly known species described by Taylor (1968) based on two specimens from western Ecuador, e.g. the holotype (BMNH 1956.1.15.87) from Santo Domingo de los Colorados, and paratype EP 3986, the latter was obtained on the road to Mindo, in Pichincha, Ecuador, at approximately 1400 m a.s.l. while the holotype was obtained at 670 m a.s.l. (Taylor & Peters, 1974). This report raises the total number of caecilian species from Colombia to 35; five of which belong to the genus *Epicrionops*, i.e. *E. bicolor*, *E. columbianus*, *E. marmoratus*, *E. parkeri*, and *E. petersi* (Lynch, 2000; Chaves-Portilla et al., 2021). From a conservation standpoint, the rediscovery of *E. marmoratus*



Figure 2. Geographic distribution of *Epicrionops marmoratus* in western Colombia and Ecuador. Yellow squares represent the previously known records and the red circle represents the new record from Reserva Río Ñambí, Barbacoas, Nariño, Colombia (PSO-CZ 857).

at ‘Reserva Río Ñambí’, corregimiento Altaquer, Barbacoas, Nariño, Colombia (1° 17'16.52" N, 78° 5'38.88" W, 1140 m a.s.l., Fig. 2) not only extends the known distribution of the species 207 km north (in a straight line) but also establishes this natural reserve as the second protected area (after Mindo-Nambillo protected forest in Ecuador) inhabited by this species. Future studies aiming to determine the natural history, behaviour, ecological necessities, and reproductive dynamics of this caecilian could give us a better assessment of its conservation status, which at the moment is classified as Data Deficient according to IUCN.

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Effect of hypoxia on the embryonic and larval development of the Nagaland montane torrent toad *Duttaphrynus chandai* in India

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Oxygen availability is a critical factor in freshwater ecosystems (Maitland, 1978); the concentration of oxygen in water is low relative to air and varies according to biological oxygen demand (BOD), oxygen production by photosynthesis, and water temperature (Dejours, 1981; Ginot & Herve, 1994; Kumamoto, 1975). The oxygen available to amphibian embryos fluctuates widely and is often low enough to affect developmental rate and hatching (Mills & Barnhart, 1999) although relatively few studies have addressed the effects of hypoxia on amphibian embryos (review by Seymour & Bradford, 1995). Kuramoto (1975) suggested that hatched embryos of those frog species that breed in summer or warm waters are smaller and consume less oxygen than those that breed in winter or cool waters. More recently, studies using acute exposures to anoxia (no oxygen) and severe hypoxia (partial lack of oxygen) indicated that, although amphibian embryos are very tolerant of short periods of hypoxia, exposure to acute anoxia inhibits metabolism and can cause death (Weigmann & Altig, 1975; Adolph, 1979; Bradford & Seymour, 1988; Seymour & Roberts, 1991; Seymour et al., 1995). In the case of the salamander *Ambystoma maculatum*, ephemeral ponds with hypoxic conditions had consistent hatching failure and exposure to dissolved oxygen (DO) at < 4.0 mg/L resulted in the death of all embryos before hatching (Sacerdote & King, 2009).

The Nagaland montane torrent toad *Duttaphrynus chandai* is endemic to north-east India described from Nagaland by Das et al. (2013) and was later reported from Mizoram, Bhutan and Manipur (Lalremsanga et al., 2020; Wangyal et al., 2020; Decemson et al., 2021). Herein we report the effects of hypoxia during the embryonic and larval development of *D. chandai*. While studying the breeding and developmental biology of *D. chandai*, we observed breeding sites from three perennial streams surrounding Murlen National Park, Champhai district, Mizoram, namely Tuithing (23° 38'36.39" N, 93° 17'51.70" E; 1597 m a.s.l.), Tuithoh (23° 39'15.17" N, 93° 19'02.58" E; 1129 m a.s.l.) and Kelchi (23° 39'51.55" N, 93° 16'40.19" E; 1122 m a.s.l.) for 22 days from 24 February 2022 to 17 March 2022. However, recordings were taken from only one stream at Kelchi for a period of 10 days where deposition of eggs had taken place in a stagnant pool. Water quality tests for dissolved oxygen (DO) and pH were made daily between 18:00 h – 20:00 h using an oxygen meter (Lutron DO-5510) and pH meter (Hanna HI 991003). Embryos were monitored

in the natural habitat while some larvae along with their natural water were placed in three plastic trays (25 cm × 19 cm × 13 cm) filled with 8 cm depth of water (approx. 4 litres). There were 20 individuals per tray which were observed in the laboratory where the DO level was measured daily. The water temperature fluctuated between 16–18 °C while air temperature was maintained at 16 °C in the laboratory. Water was changed every 24 h with fresh water collected from the microhabitat. The laboratory observations were made under permit no. MZU-IEAC/2018/12 issued by Institutional Animal Ethics Committee, Mizoram University.

The study reveals that *D. chandai* can be found at altitudes greater than 1000 m a.s.l. and breeds in pools with running water (Figs. 1 A & B) at DO concentration of 6.5–7.2 mg/L (mean ± sd, 6.67±0.23, n=10) and pH 6.5–6.9. During the investigation, water temperature in both running and still water of the stream ranged from 11.1–14.3 °C. However, we observed one egg clutch (Fig. 1C) out of more than 15 clutches in an isolated pool with stagnant water (Fig. 1D) having initial DO level of 6.0 mg/L. The DO of the isolated stagnant pool declined from 6.0 mg/L to 2.7 mg/L within 10 days (Fig. 2) while the pH remained relatively stable at 6.6–6.9. We recorded the highest DO level on the fifth day (28 February 2022) (7.2 mg/L) in running waters (Fig. 2) apparently due to a slight rainfall in the area. In the stagnant pool, we observed that embryos at Gosner stage 12 (late gastrula) gradually became pale in colour (Fig. 3B) compared with the normal dark blackish coloration (Fig. 3A); the entire egg clutch died when reaching a DO level of 3 mg/L and below. Within 9–11 days, DO of water in the rearing tray drops to 2.5–3.0 mg/L, larvae at hindlimb bud development (Gosner stage 26) bulged in a star-like shape (Fig. 3D) so that body width was greater by 1.41 mm and tail shorter by 1.76 mm within 2 days compared to normally developing tadpole (Fig. 3C).

This is the first study on the effect of declining DO level on the embryonic development of *Duttaphrynus* toads under natural conditions. It was found that embryos of *D. chandai* are susceptible to minor change in the environmental factors during Gosner stage 10 (dorsal lip) onwards. During the study, the pH of the running water and of the stagnant pool was very similar but the DO levels in the stagnant pool gradually declined from 6 mg/L to 2.7 mg/L within 10 days and the embryos start decaying on the ninth day of monitoring. In addition to this, tadpoles reared in trays tend to show a sign

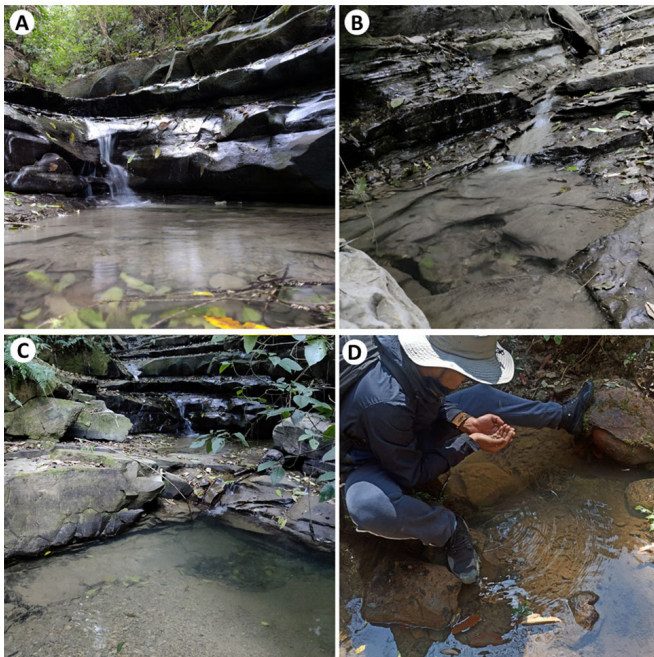


Figure 1. Water bodies in Murlen National Park, Champhai district, Mizoram, India, that support egg clutches and tadpoles of *Duttaphrynus chandai* - **A.** & **B.** Pools with running water, **C.** Egg clutch of *D. chandai* in a stagnant pool (**D.**)

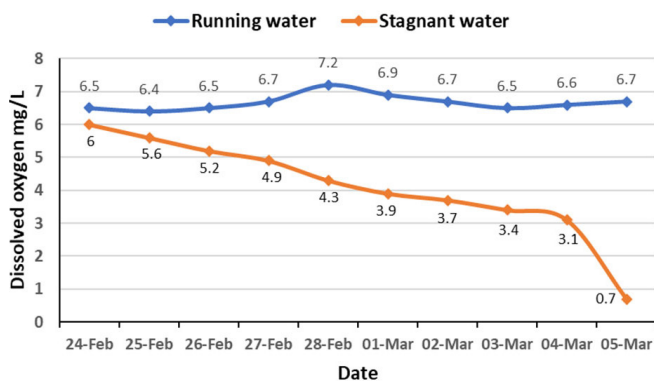


Figure 2. Dissolved oxygen values in running water and a stagnant pool in Murlen National Park, Champhai district, Mizoram

of stress, swimming vigorously, when DO level dropped lower than 3 mg/L, while tadpoles above 3 mg/L remain docile and calm at the bottom of the tray. Kumaroto (1975) showed that DO consumption by embryos of anurans differs species to species and stated that, all else being equal, embryos of species adapted to lower temperatures where higher DO prevails consume more DO as a result of higher metabolic rate. Since our studied species is a winter breeder (February–March), we conclude that these toad embryos and larva need higher concentration of DO for normal development and survival although other toad species such as *D. melanostictus* can thrive in a wider range of DO (4.9–9.6 mg/L) (Shangphliang et al., 2016). This might be one of the reasons why *D. melanostictus* is widely distributed across south and south-east Asia, while the less tolerant sister species, *D. chandai*

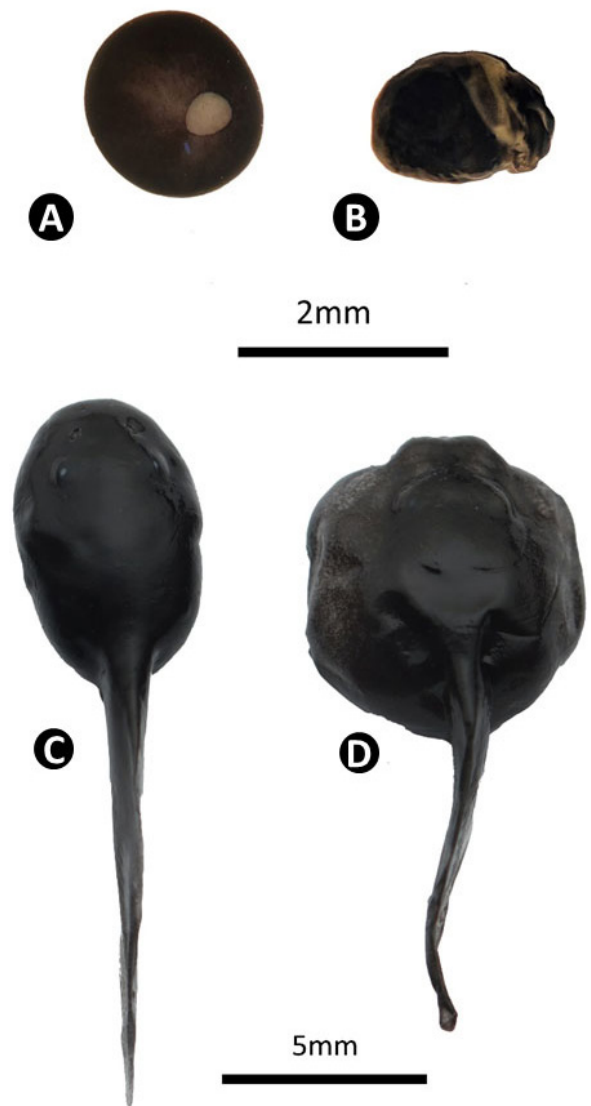


Figure 3. Embryos and tadpoles of *Duttaphrynus chandai* subject to normal and hypoxic conditions - **A.** Normal embryo at Gosner stage 12, **B.** Defected embryo at Gosner stage 12, **C.** Normal larva at Gosner stage 26, and **D.** Abnormal larva with star-like shape body and reduced tail at Gosner stage 26

inhabits limited and selected microhabitats at higher altitude. Should ex situ conservation be required *D. chandai* then it would appear that aeration of eggs and larvae is probably required to rear this species successfully in captivity.

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First case of predation of the Tyrrhenian endemic Sardinian tree frog *Hyla sarda* by an alien water frog *Pelophylax* sp. in northern Sardinia, Italy

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Sardinia is the second largest island in the Mediterranean Sea, located in the centre of its western basin, and represents an important biodiversity hotspot for amphibians (Di Nicola & Mezzadri, 2018; Bellati et al., 2019). The island is currently populated by six species of urodeles and at least six anuran species including two recently introduced *Pelophylax* spp. (Pous et al., 2012; Di Nicola & Mezzadri, 2018; Cossu et al., 2018; Bellati et al., 2019).

Various species of water frog *Pelophylax* spp. are distributed throughout Europe, Asia and North Africa; it is not always possible to base their identification on morphology and bioacoustics, instead molecular analyses are often required (Bellati et al., 2019). In peninsular Italy and Sicily, *Pelophylax lessonae* is present as a native species (with the subspecies *P. l. lessonae* and *P. l. bergeri*); moreover, there is the hybrid taxon *Pelophylax* kl. *esculentus* (see Schultz, 1969 and Bellati et al., 2019 for hybridisation mechanisms) and the alien taxa *Pelophylax* cf. *bedriagae*, *Pelophylax ridibundus* (whose native range may be limited to the extreme north-east of Italy) and *P.r. kurtmuelleri* (Di Nicola et al., 2021; Sindaco & Razzetti, 2021). Alien water frogs are invasive and have been introduced mainly for culinary and scientific reasons (Bruni et al., 2019).

As regards the alien species reported for Sardinia, the low reliability of field identification limits the checklist only to taxa that have been characterised on a molecular basis. So far, the Balkan frogs (*P. r. kurtmuelleri*) and two cryptic taxa of the “*bedriagae*” group (*P. cf. bedriagae*) have been confirmed for the island and their occurrence is related to multiple and independent release events, at least partially due to translocations from central Italy during commercial activities such as plant cultivation, sports fishing and food supply (Bellati et al., 2019). Furthermore, introductions of taxa from the Italian mainland have also been reported in the past (see Corona & Fanzago, 1880; Sochurek, 1955; Tortonese & Lanza, 1968; Lanza, 1983a; Bellati et al., 2019) but the current presence on the island needs to be confirmed.

The water frog species found in Sardinia can exceed 100 mm of snout-urostyle length (Di Nicola et al., 2021). They are active both day and night and their diet consists mainly of invertebrates, but they can prey upon small fish, amphibians (including tadpoles and adults, even of the same species) and also lizards (Nicolau et al., 2014; Plitsi et al., 2016; Bam-e-Zar



Figure 1. Alien water frog preying on a Sardinian tree frog in Lake Baratz, Sardinia

et al., 2019; Katsiyiannis & Tzoras, 2020; Di Nicola et al., 2021).

Here we report a case of predation by an alien water frog of a Sardinian tree frog *Hyla sarda*, an anuran endemic to the Tyrrhenian islands. *Hyla sarda* is a monotypic species found in Sardinia (including some smaller islands), Corsica (including the island of Cavallo) and the Tuscan Archipelago (Elba Island and Capraia). Adults have a snout-urostyle length on average less than 40 mm, with a maximum of 55 mm (Corti, 2007; Di Nicola et al., 2021). It is mainly a nocturnal species and among the Italian tree frogs it seems to be the species most linked to water, found on riparian or emerging vegetation even in the height of summer (Lanza, 1983b; Corti, 2007; Speybroeck et al., 2016). Its predators include snakes, mammals and birds (Corti, 2007; Di Nicola & Mezzadri, 2018) but there are no reports of predation by other amphibians.

On 9 August 2021 at 00:21 h, in Lake Baratz (Province of Sassari, Sardinia; 40° 40'53" N, 8° 13'19" E; 26 m a.s.l., clear weather, Tmin 20 °C, Tmax 35 °C), one of the authors (JRF) observed an adult water frog with a Sardinian tree frog in its mouth (Fig. 1). The tree frog was being swallowed head first with the legs hanging from the mouth. After photographs were taken, the predator moved away keeping the prey firmly in its mouth. The water frog stood on the aquatic vegetation in water less than 5 cm deep, at about 70 cm from

the shore, together with a very large number of congeners, some of which were calling. On the riparian vegetation there were many individuals of *H. sarda* (approximately more than 60 individuals in about a 100 m stretch, none of which were calling) but no individuals were observed on aquatic vegetation together with water frogs. An adult *Bufo viridis balearicus* was also spotted on the shore.

It is known that water-related species such as amphibians are particularly sensitive to changes caused by the presence of non-native fauna (Bucciarelli et al., 2014). In areas where native water frogs exist, the aliens represent a problem due to the ease with which different taxa can hybridise in the wild and due to local competition. Alien species represent a threat also in Sardinia (where there are no indigenous water frogs), since their presence implies competition, predation and the spread of pathogens at the expense of native amphibian fauna (Bellati et al., 2019).

Pelophylax spp. are opportunistic feeders and eat almost any prey they can find, including tree frogs when terrestrial (Katsiyannis & Tzoras, 2020). *Hyla sarda* is categorised as of Least Concern on the IUCN red list and the population in Sardinia is still considered stable (IUCN Italian committee, 2013; IUCN, 2020). However, the recent introduction and expansion of species such as *P. r. kurtmuelleri* and *P. cf. bedriagae* could pose a conservation threat. It is therefore important to carry out further field surveys to assess the extent of the impact of the alien water frogs on native anurans and, if need be, evaluate possible actions to limit the problem.

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Deadly snack: mortality of a European grass snake *Natrix natrix* while attempting to feed on a three-spined stickleback *Gasterosteus aculeatus*

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The European grass snake *Natrix natrix* is widespread throughout Eurasia inhabiting a variety of terrestrial and aquatic habitats. In Germany there are three natricine snake species, *N. natrix*, *Natrix helvetica*, and *Natrix tessellata*, the latter being more aquatic, preferring the benthic regions of lotic systems, and feeding almost exclusively on fish (Hutinec & Mebert, 2011; Weiperth et al., 2014). In contrast, *N. natrix* and the closely related *N. helvetica* mostly feed on amphibians (Reading & Davies 1996; Filippi et al., 1996; Luiselli et al., 2005), even when fish are readily available (Filippi & Luiselli, 2002), and when it comes to aquatic habitats they prefer lentic systems and are mostly observed on the surface (Hutinec & Mebert, 2011). At different life stages and body sizes, grass snake diets shift from feeding on tadpoles, recent metamorphs, and adult toads, frogs, and newts (Reading & Davies, 1996; Filippi et al., 1996; Luiselli et al., 1997; Gregory & Isaac, 2004). Other than amphibians, less frequently they also feed on small mammals, birds, other reptiles, fish, and snails (Gregory & Isaac, 2004; Luiselli et al., 2005; Consul et al., 2009; Hutinec & Mebert, 2011; Šukalo et al., 2014; Lunghi et al., 2018).

Reptile prey recognition and predatory behaviour have long been accepted as being innate behaviours, particularly in snakes. Prey is detected mainly by olfaction although visual cues, especially prey movement, are often very important and some prey preference may be influenced by experience (Burghardt et al., 1973; Stimac et al., 1982; Hailey & Davies, 1986). However, all these possibilities seem to be very variable even within the same family, so how much is innate and how much is learned behaviour may depend on the species (Burghardt, 1993). For example, Burghardt (1993) points out that some *Thamnophis* species are more visual and specialist than others of the same genus; Hailey & Davies (1986) point out that *Natrix maura* attacks in response to visual and tactile cues. However, when it comes to *N. natrix*, many of the feeding ecology studies and observations come from terrarium conditions or small studies from a few specific locations within this species' wide distribution (Reading & Davies, 1996; Luiselli et al., 1997; Gregory & Isaac, 2004; Luiselli et al., 2005; Hutinec & Mebert, 2011).

On 20 June 2021 at approximately 12:00 h we made what we believe is the first record of a European grass snake attempting to eat a three-spined stickleback *Gasterosteus*

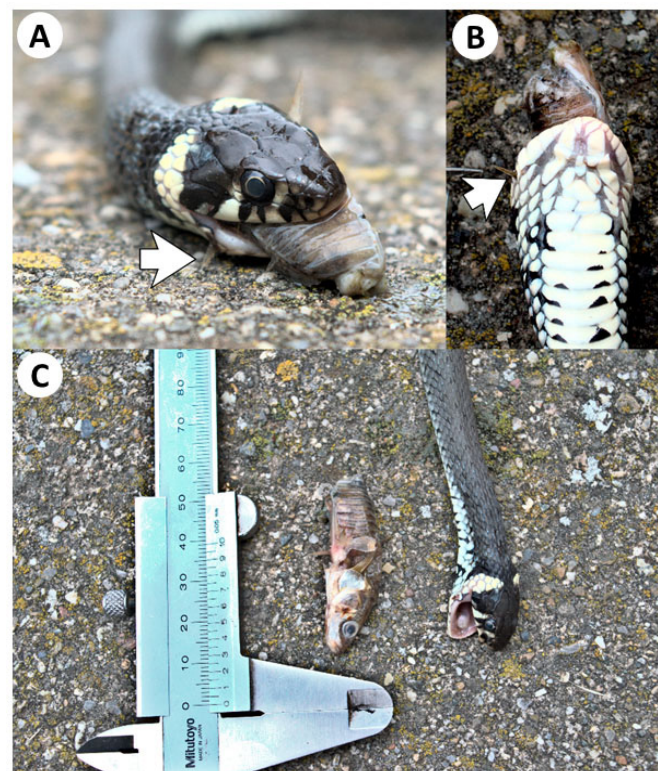


Figure 1. A sub-adult *Natrix natrix* with a *Gasterosteus aculeatus* lodged in its mouth - **A.** & **B.** The first dorsal spine of the stickleback has punctured the infralabial scales of the snake (white arrows), **C.** Both individuals after the authors separated them with a Vernier to give scale; note the stickleback appears as if it had probably died before the snake

aculeatus. A dead subadult *N. natrix* (SVL 46.6 cm, TL 59.1 cm) was found with a three-spined stickleback (TL ~ 4 cm) lodged in the snake's mouth (Fig. 1). The snake was in the shallow waters on the north-eastern shores of Lake Constance, Germany (~399 m a.s.l.; the location is private property). Both creatures were already dead; we cannot estimate for how long, but the stickleback appeared to have been dead longer than the grass snake, most likely the stickleback asphyxiated and died before the snake. Possibly, but we believe unlikely, this might have been a case of

carion feeding. The snake had grasped the stickleback head-first, a behaviour that has been reported on other fish-eating natricines (Hailey & Davies, 1986) and it is also how grass snakes tend to ingest amphibians (Filippi et al., 1996). It is assumed that the stickleback's spines were erected when trying to defend itself and that is when the first dorsal spine punctured the snake's infralabial scales (Figs. 1 A & B) during attempted ingestion. The pelvic spines were locked in place: the left one already inside the snake's mouth and the right one outside, pressing against the corner of the mouth of the snake. All of this caused the fish to get stuck and the snake was not able to manipulate the prey any further nor set it free, leading to their demise. We cannot know whether the snake died close to where it had been hunting or whether it had been washed up from elsewhere.

Throughout the reviewed literature, both Tuniyev et al., (2011) and Weiperth et al., (2014) referred to Khonjakina (1969) (a source we have been unable to verify) as apparently the only other report of a natricine snake (*N. tessellata*) preying on a *Gasterosteus* - but we do not know the outcome from that interaction.

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The first record of sea anemone *Stichodactyla gigantea* consuming a sea snake *Emydocephalus ijimae*

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The main vertebrate predators of sea snakes (Elapidae; Hydrophiinae) are sharks, moray eels, groupers, sea eagles, saltwater crocodiles, leopard seals, and red-banded snakes (Heatwole, 1999; Sasai et al., 2020). There have been a few reports of sea snakes being eaten by invertebrates such as crabs (Voris & Jefferies, 1995), and there are two reports of sea snake interactions with sea anemones. In one report, a live turtle-headed sea snake *Emydocephalus annulatus* was held by a sea anemone (Ineich & Laboute, 2002), and in another report, an olive-headed sea snake *Hydrophis major* exhibited extreme distress immediately after coming into contact with the sea anemone *Entacmaea quadricolor* (Goiran & Shine, 2014). However, in these cases, the sea snakes were alive and were not eaten by the sea anemones. Here, we report the consumption of an Ijima's turtle-headed sea snake *Emydocephalus ijimae*, by the gigantic carpet anemone *Stichodactyla gigantea* and discuss the cause of death of the snake.

Emydocephalus ijimae is a viviparous sea snake species distributed in coral reefs in the region around the Ryukyu Islands in Japan to Taiwan (Ota & Masunaga, 2005). The genus *Emydocephalus* is unusual in being effectively non-venomous, a feature related to its diet which is exclusively the eggs of coral reef fish (Voris, 1966; Heatwole, 1987). *Stichodactyla gigantea* is a common anemone species in the tropical regions of the Indo-Pacific region, where it is found in shallow sandy areas of coral reefs (Hattori & Kobayashi, 2009). This sea anemone is a generalist predator that uses its tentacles to capture prey such as urchins, snails, crabs, shrimps, as well as small fish (Madhu & Madhu, 2017). Further, it is known that they often become host to the clown anemone fish *Amphiprion ocellaris* (Fautin & Allen, 1992).

At 01:09 h on 16 February 2022, we discovered an *S. gigantea* (maximum width of oral disc approximately 330 mm) that was swallowing an *E. ijimae* (snout-vent length, SVL = approximately 300 mm) in the lagoon of the coral reef area of Okinawa Island, Japan (26° 40'16.12" N, 127° 53'15.95" E) (Fig. 1). At this size the sea snake is likely to have been a neonate as *E. ijimae* give birth to their young from November to January which are 280–320 mm in SVL (Masunaga et al., 2003). Initially, the anemone and snake were positioned in the shallows of an area of sand with limestone rock, and the head and tail of the sea snake had been engulfed. By the time of observation, most of the body parts appeared at the

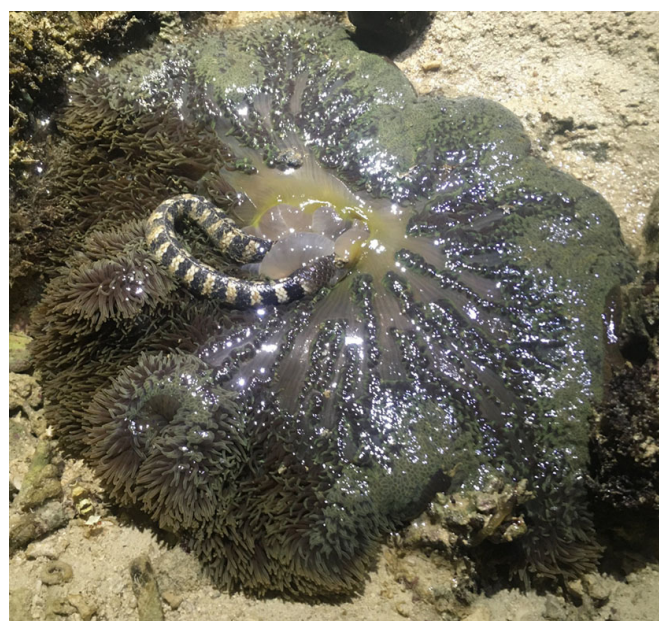


Figure 1. *Stichodactyla gigantea* swallowing an *Emydocephalus ijimae* in the lagoon of a coral reef area

surface of the water because the tide was low. At 01:15 h the observer pulled up the remains of the *E. ijimae*, as the rest of the body was swallowed by then. The upper body, which was approximately 75 mm in length was only skin, and the bone or some internal parts of the body had been digested (Fig. 2). Around the sea anemone there were two small clown anemone fish of approximately 15 mm and 30 mm in length and some squat shrimps *Thor amboinensis*. Their size was measured by photographing them beside a stick of known length that was used later to estimate their length with the Leafareacounter Plus software.

Although *A. ocellaris* spawn close to sea anemones (Ida, 1984) it is unlikely that the sea snake was drawn to the anemone to search for *A. ocellaris* eggs to feed as the clown anemone fish were not of spawning size and, in any case, they usually spawn in summer (Ida, 1984). It would appear that the consumption of sea snakes by sea anemones is rare because ours is only the second recorded case. The current and previous cases (Ineich & Laboute, 2002) of sea snake captured by sea anemones have both involved neonate/



Figure 2. The upper body remains of *Emydocephalus ijimae* with only skin, indicating that some internal parts of the body were digested, a clown anemone fish is visible top centre

juvenile individuals. It has been noted that sea snakes avoid sea anemones (Goiran & Shine, 2014) due to their venom. However, *S. gigantea* is known not to cause a strong stinging sensation, but instead is extremely sticky (Fautin & Allen, 1992). In this case, sea snake species of the genus *Emydocephalus* may be more vulnerable because they are considered to be relatively slow swimmers (Shine et al., 2004; Avolio et al., 2006). Once stuck in the tentacles they run the risk of drowning as sea snakes need to breathe through their lungs even though they can depend on cutaneous respiration to a certain degree (Heatwole & Seymour, 1975). We concluded that the sea snake may have died by drowning, while being unable to escape from the sticky tentacles of the sea anemone, or may already have been dead before it made contact with the anemone.

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Smooth newt *Lissotriton vulgaris* preyed upon by European starling *Sturnus vulgaris*

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The smooth newt *Lissotriton vulgaris* is a common European newt species growing to 11 cm, it is widespread in Great Britain (Speybroeck et al., 2016), and is well adapted to urban environments (Beebee & Griffiths, 2000). Adults breed in ponds in the springtime but both they and the metamorphs can be found in terrestrial habitats throughout the rest of the year. It is here that smooth newts may encounter a broad spectrum of terrestrial predators, including birds.

European starlings are opportunistic feeders and have developed an array of foraging techniques with a particular specialisation that involves 'prying' or 'open-bill probing', which is often used as a feeding technique in searching for invertebrate prey in grassland habitats (Cramp & Perrins, 1994; Feare, 1984). The species' wide geographical distribution, range of habitats, and adaptability of foraging techniques, suggests a diverse diet that includes berries, seeds, and fruits, along with arthropods from the subsoil or soil surface, and even vertebrates. Animal prey is collected mainly during the breeding season and fed to nestlings but is required throughout the year (Feare & McGinnity, 1986).

On 7 May 2021 at 12:30 h during a walk along Jesus Ditch, Cambridge (52° 12'40" N, 0° 07'29" E), a female starling was observed searching for food. At first, it was seen with what was assumed to be an earthworm in its bill but on closer inspection the prey item was seen to be a female smooth newt (*Lissotriton vulgaris*; Fig. 1). The starling spent some time trying to subdue the newt before ingesting it (BHS video, 2022).

European starlings have previously been reported to prey on amphibians, such as newts (Cramp & Perrins, 1994; Feare & Craif, 1999). However, the current observation provides the first definitive evidence of *S. vulgaris* preying upon *L. vulgaris*. There is also a record of the closely related spotless starling *S. unicolor* preying on western spadefoot toad *Pelobates cultripes* in south-western Europe (Martín & López, 1990).

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Figure 1. A female European starling *Sturnus vulgaris* feeding on a female smooth newt *Lissotriton vulgaris* in Cambridge, England

BHS video (2022). Predation of a smooth newt (*Lissotriton vulgaris*) by a starling (*Sturnus vulgaris*). <https://youtu.be/oA7i4zVRukQ>.

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J.G. Schneider's *Historiae Amphibiorum*: Herpetology at the Dawn of the 19th Century

Aaron M. Bauer & Esteban O. Lavilla

Contributions to Herpetology volume 32, Society for the Study of Amphibians and Reptiles, Ithaca, New York. 2022. Ixvii, 777 pp. ISBN 9781946681010 (hardback)



Since the early 1980s, the Society for the Study of Amphibians and Reptiles (SSAR) has been publishing book-length monographs in their Contributions to Herpetology series, under the editorship of Kraig Adler. The series has already included three volumes of *Contributions to the History of Herpetology* (1989–2014). Volume 32 is another historical work, and a very substantial one.

Johann Gottlob Schneider (1750–1822) was a highly regarded German classical scholar, particularly expert in rendering the much copied and error-strewn ancient Greek texts into comprehensible modern Latin. He spent most of his career as Professor of Eloquence at the University of Frankfurt an der Oder, moving later to Breslau (now Wrocław) where he died in post as chief librarian. This may not sound like the potted biography of a herpetologist! However, Schneider wrote on a wide range of subjects (his principal works bibliography runs to 127 items), and natural history, building on the work of the ancients, was one of his main interests from the 1780s until his death.

This was an important period in world history as well as natural history. It included the American and French revolutions and the turmoil of the Napoleonic wars. In natural history, Linnaeus had recently developed his hierarchical binomial naming system for species of animals and plants, his *Systema Naturae* appearing from 1758 to 1767. By the time Schneider became active in the field, Linnaeus' scheme had been widely but not universally adopted, and there was a huge task in progress of devising names and writing descriptions for species, many newly arrived in Europe from wildlife collectors exploring in distant lands. Public museums were rare in this period but many wealthy enthusiasts had established their own 'cabinets' full of exotic specimens.

Schneider was particularly interested in fish, amphibians, reptiles and molluscs, but he did not ignore the warm-blooded groups, birds and mammals. His main contribution on fish was the monumental *Systema Ichthyologiae* (1801)

where Schneider brought to a publishable state the papers of his friend Marcus Bloch who had died in 1799. The book gave names and descriptions, including illustrations, to over 400 species, with about 130 of these names remaining valid to this day. Schneider's *Historiae Amphibiorum*, his main herpetological contribution, appeared as two volumes (in Latin, with minimal illustrations) in 1799 and 1801. So, why has SSAR supported publication of the volume under review?

Bauer and Lavilla contend that, because Schneider wrote in late 18th century academic Latin, and the book has never been translated into English, his contributions have been widely ignored or forgotten. They have not simply provided a readable translation, a significant task in itself, they have also written a helpful biography of Schneider, including a full bibliography. But the most impressive features of their effort are, first, the 1748 fully-referenced footnotes that clarify Schneider's writing and provide information on the current locations of the specimens he described; second, the 275 pages of illustrations, many in colour. Schneider's original included only four plates, black and white illustrations of skeletal parts, but he referred to numerous illustrations already published in other works. Bauer and Lavilla have located all of these and included them in the new volume, meaning that modern readers have an advantage over the originals in being able to link Schneider's text directly to illustrations. They have also compiled three helpful indexes.

A surprise to many may be that the *Historiae Amphibiorum* includes both amphibians and reptiles: the general acceptance of these two taxa as distinct occurred a few years later. Schneider provided accounts of about 70 amphibians and 100 reptiles, with 102 of his descriptions being new to science. He did not include chelonians or geckos, since he had published work on them previously. We currently recognise about 8,000 amphibian species and 12,000 reptiles, so Schneider's compilation indicates what a small proportion of the world's biodiversity was known to late 18th century science.

The book starts with a rather flowery dedication to Sir Joseph Banks, already 20 years into his four decade tenure as President of the Royal Society - and therefore a highly influential figure. Schneider then covers the known amphibians and reptiles grouped under 14 genera (each with a brief diagnosis) and about 170 species. His sources of information included previous accounts: for European

species, these often began with information from the ancients such as Aristotle and Pliny. He then went on to his own observations of museum specimens accessible in Germany: his remarks could be quite critical when he considered a previous account of a specimen to be inadequate or in error. He also had a collection of his own to draw on, and dissected some of these to learn about internal features. Finally, he reported field observations of his own, for example on the breeding behaviour of toads. In total, he named and described 126 species for the first time; 45 of these names remain valid today.

When I began researching the habits of frogs and marine turtles in Trinidad and Tobago, I noticed that several species had first been named by Schneider (1799), so I have been intrigued to read the book where so many names originated. Some of the descriptions are quite brief, especially for non-European species known only from one or a few museum specimens. Others include considerable detail on behaviour as well as gross anatomy. For example, Schneider quotes ancient accounts of the behaviour of toads, said to live only among brambles, sitting in front of beehives with their mouths open, magically attracting bees! I was especially interested in what Schneider had to say about two of the most striking of Trinidad's frogs. The paradoxical frog (*Rana paradoxa* for Schneider, now *Pseudis*) has tadpoles that grow to an exceptional size, then metamorphose to smaller frogs that are almost mature. Schneider had seen specimens of the tadpoles and mentioned "the fabulous information that the common people have about this mythical animal". Sadly, he does not enlighten us further, nor do Bauer and Lavilla in this case. Some had claimed that development was retrograde in this species, from small frog to large tadpole, hence the 'paradox'. Later, Wagler re-named the genus as *Pseudis*, since it represents a pseudo-paradox. Schneider is more helpful on *Rana dorsigera* (now *Pipa pipa*). This species was figured as early as 1719 by Maria Sibylla Merian, described by Schneider as "that glory among women". Merian had spent time in Suriname studying and illustrating a wide range of fauna, especially insects, and she had described parental care in *Pipa*, with eggs incubated on the female's back in individual pockets until metamorphosis is complete.

Schneider gives a detailed account.

Who will find this book interesting? It will attract serious scholars of natural history's origins and progress. My guess is that the book will be of special interest to modern herpetologists who are intrigued by the naming history of the species they work on. I recall my amazement on finding that one of the frogs I worked on in Trinidad had undergone seven name changes. Reading this version of Schneider helps make clear the difficulties the early taxonomists experienced, working with often badly preserved museum specimens whose place of capture was generally poorly documented, and trying to work out if two specimens in different collections belonged to the same or different species. A century after Schneider, the creation of the International Commission on Zoological Nomenclature eventually provided some rules for making decisions on naming disagreements.

Bauer and Lavilla have certainly performed a considerable service to the herpetological community in making Schneider's work accessible and comprehensible to modern readers. Every major natural history library should buy a copy. The book can be ordered from the Society for the Study of Amphibians and Reptiles at a cost of US\$ 85.50 – SSAR members; \$95- non-members; \$120 institutions.

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