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Front Cover: Male San Salvador rock iguana (*Cyclura rileyi*). A Natural History Note on this species can be found on page 27. Photograph credit: James St. John, Wikimedia Commons.

Interanal seam loss in Asian turtles of the *Cuora flavomarginata* complex (Testudines, Geoemydidae)

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ABSTRACT - The taxonomy of Asian box turtles of the genus *Cuora* is complicated by the description of numerous valid and invalid taxa over the last several decades. However, some characteristics used to differentiate species are questionable. Members of the *C. flavomarginata* complex are defined by some, but not all, taxonomists as having reduced interanal seam lengths relative to other species. We examined the ratio of interanal scute seam length divided by midline anal scute length in *C. flavomarginata* and *C. evelynae*. Hatchlings show a seam that divides 100% of the anal scute along the midline. As individuals increase in carapace length, there is a tendency for the percentage to decrease, especially in females, although there is considerable overlap. We suggest that the decrease in interanal seam length is due to abrasion of the plastron on the substrate as turtles grow larger and older. Differences in habitat substrates across the range of the species may contribute to the wide variation we observed.

INTRODUCTION

Turtles of the Asian genus *Cuora* have a complicated taxonomic history, in large measure due to the description of hybrid forms as distinct taxa (Parham et al., 2000; Parham et al., 2001; Shi et al., 2005; Stuart & Parham, 2007). Twelve species are currently recognized with 17 terminal taxa including all currently recognized species and subspecies (van Dijk et al., 2014). Ten of those terminal taxa were described since 1980, a reflection of the increasing resolution possible through application of relatively new molecular techniques (Honda et al., 2002; Tiedemann et al., 2014). Still, the distinctiveness and natural distribution of some taxa remain unresolved (e.g., Ernst et al., 2011). For example, the yellow-margined box turtle (*C. flavomarginata*) has been a subject of taxonomic disagreement. The *C. flavomarginata* complex, as defined by Ernst et al. (2008) to include only the species *C. flavomarginata* (Gray, 1863) and *Cuora evelynae* Ernst & Lovich, 1990, has not met with universal acceptance. Recent authors recognize the consistent differences between the taxa and the vicariant nature of their allopatry, but suggest that the latter is a subspecies of the former (Honda et al., 2002; Ota et al., 2009).

Turtles of the *C. flavomarginata* complex have been recognized by some taxonomists as having reduced interanal seam lengths (relative to midline anal scute length) in many individuals, but this condition has had variable use as a taxonomic character. For example, it has been mentioned in descriptions or shown in illustrations of *C. flavomarginata* by Boulenger (1889), Stejneger (1907), Mao (1971), Bonin et al. (2006), and Ota et al. (2009); and *C. evelynae* by Ernst & Lovich (1990). In contrast, this condition was not included in descriptions by Gray (1863, 1870), Siebenrock (1909), Hsu (1930), Pope (1935), Pritchard (1967, 1979), Obst (1986),

Ernst & Barbour (1989), or Zhao & Adler (1993). Is the loss of the interanal seam with shell growth a legitimate and unique characteristic of turtles in this complex? Or is it, as has been suggested by some authors, that the ratio of interanal seam length divided by the midline length of anal scutes (hereafter interanal seam percentage or IANSP), decreases as body size increases because of environmental abrasion over the life of an individual? Neither hypothesis has been satisfactorily studied. In this paper we examine variation in IANSP in the *C. flavomarginata* complex. For consistency with our earlier publications, we refer to members of the complex as species, with full recognition that other authors consider them to be subspecies. The distinction between the two approaches has no effect on our analysis: they are sister taxa either way. We hypothesized that larger turtles would have smaller IANSPs and tested the strength of this relationship in our analyses.

METHODS AND MATERIALS

A total of 125 turtles were examined including: 38 *C. evelynae* from the Ryukyu Islands; and 50 Taiwan and 37 southern China *C. flavomarginata* (see Ernst et al., 2008, for straight-line measurements taken on each specimen). To calculate IANSP we divided seam length between the paired anal scutes on the plastron by midline anal scute length. For comparisons of IANSP, adult males, females and juveniles of the two species were analysed separately due to the potential effect of sexual size dimorphism (Gibbons & Lovich, 1990). Sex was determined by examination of secondary sexual characters. Turtles that did not clearly exhibit these characters were considered to be juveniles. Even though the two *Cuora* taxa are closely related (Ernst & Lovich, 1990; Ernst et al., 2008), we tested for differences in IANSP between them before continuing our analyses.

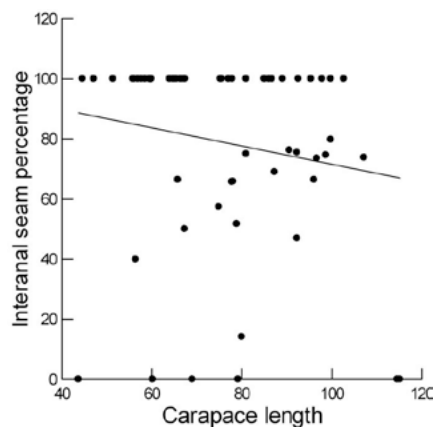


Figure 1. Interanal seam percentage vs. carapace length (mm) of juvenile *C. flavomarginata* and *C. evelynae* combined.

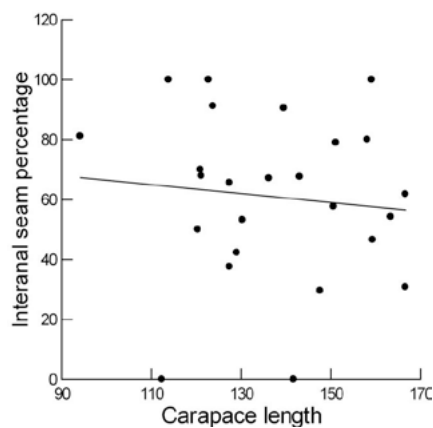


Figure 2. Interanal seam percentage vs. carapace length (mm) of adult male *C. flavomarginata* and *C. evelynae* combined.

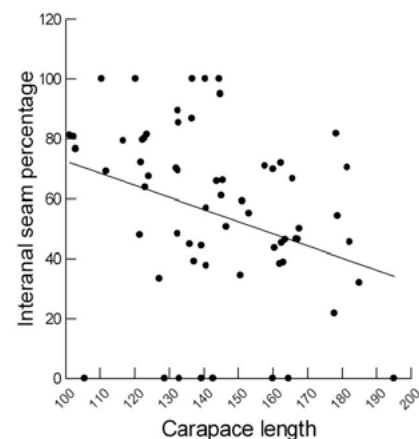


Figure 3. Interanal seam percentage vs. carapace length (mm) of adult female *C. flavomarginata* and *C. evelynae* combined.

Because ratio data do not always satisfy the assumption of normality for parametric tests, we used nonparametric analyses. Spearman correlation coefficients (r_s) were calculated to examine the strength of the relationship between carapace length (CL) and IANSP. Since we hypothesized that the correlations between those two variables would be negative (e.g., as CL increased, IANSP decreased) we evaluated test statistics against one-tailed critical values. Mann-Whitney U Tests and Kruskal-Wallis tests were used to compare median values of IANSP between and among sexes and size classes (e.g., males, females and juveniles), evaluating test statistics against two-tailed critical values. Statistical analyses were conducted using STSTAT 12 (version 12.02.00) at an alpha level of 0.05.

RESULTS

The variable IANSP did not differ between *C. flavomarginata* and *C. evelynae* for either females (Mann-Whitney U = 395.0, $df = 1$, $P = 0.78$), males (Mann-Whitney U = 76.0, $df = 1$, $P = 0.96$), or juveniles (Mann-Whitney U = 195.0, $df = 1$, $P = 0.08$), so the taxa were combined for subsequent analyses, but separated according to sex. Values for IANSP varied greatly among females, males, and juveniles (Table 1) but the median and mean values were higher in juveniles than in adults. Median IANSP values were statistically different among juveniles, males, and females (Kruskal-Wallis Test = 8.67, $df = 2$, $P = 0.01$). When comparing the median IANSP values for juveniles to the median value for males and females combined, the results were again significant (Mann-Whitney U = 1079.0, $df = 1$, $P = 0.004$). However, median IANSP was not significantly different between males and females (Mann-Whitney U = 721.0, $df = 1$, $P = 0.47$). Plotting transformed IANSP against CL of juveniles showed a wide scatter with many values of 100% and a few of 0% (Fig. 1). Spearman rank correlation coefficients between CL and IANSP were all negative, as hypothesized. The coefficient was not quite significant for juveniles ($r_s = -0.26$, $0.10 > P > 0.05$), or males ($r_s = -0.18$, $0.25 > P > 0.05$), the latter as shown in Fig. 2. The coefficient was significant for females ($r_s = -0.36$, $P < 0.0025$)

even though 0-100% values were again recorded across a wide range of CLs (Fig. 3).

| | n | Min | Max | Median | Mean | SD |
|-----------|----|-----|-----|--------|------|------|
| Juveniles | 36 | 0 | 100 | 75.3 | 71.0 | 33.3 |
| Males | 25 | 0 | 100 | 65.6 | 60.9 | 27.7 |
| Females | 64 | 0 | 100 | 58.1 | 54.9 | 29.3 |

Table 1. Summary statistics for interanal seam percentage in *C. flavomarginata* and *C. evelynae*, combined.

DISCUSSION

The majority of hatchlings of both *C. flavomarginata* and *C. evelynae* that we examined did not have a distinct indented seam between their anal scutes. However, the position where such a seam should occur is usually represented by a medial linear raised area. In this study, we considered this elevated area to represent an interanal seam. Thus, all hatchlings begin life with a 100% IANSP. Examination of hatchling/juveniles with longer CLs, however, revealed an IANSP less than 100% in many, producing a scattering effect (Fig. 1). Such a phenomenon is also revealed when IANSP is plotted against CL of both adult males (Fig. 2) and adult females (Fig. 3). Since adult females are slightly larger than males in mean CL (Gibbons & Lovich, 1990; Ota et al., 2009), the closer values for median and mean IANSP of juveniles and males (Table 1) may be due to some large juveniles being misidentified as males and some small males being misidentified as juveniles.

We suggest that these scattered patterns are indications of wear caused over time when the individual turtle crawls in contact with a hard abrasive substrate in its natural habitat. The turtles we examined are from several localities, likely exposing individual specimens to different substrate textures that cause variation in the rate and extent of shell abrasion. The anal scutes in these *Cuora* taxa are the largest or second largest of the six paired plastron scutes (Ernst et al., 1997) potentially exposing them to a disproportionate amount of wear relative to the other scutes on the bottom of the shell. Turtle shells

are well-known to wear smooth with prolonged exposure to abrasive conditions, obfuscating fine annual or semi-annual growth rings on the shell (Germano, 1988; Germano & Bury, 1998) that are often no wider than the contact seam between the anal scutes we examined. Unfortunately, the substrates at the collection sites of the turtles were not recorded, so we have not been able to test this hypothesis. However, if the loss of IANSP with increased CL (i.e., growth) is genetically controlled, the pattern of IANSP should be more predictable than shown in our figures. We conclude that the loss of IANSP in turtles of the *C. flavomarginata* complex is apparently more influenced by environmental microhabitat substrate texture. While the tendency toward a reduced IANSP with size/age does not appear to be genetic and taxonomically useful, many hatchlings/juveniles and some adults retain a 100% IANSP. Adults that retain the full IANSP may live on soft substrates. The presence of the raised linear medial strip between the anal scutes of hatchlings supports this possibility.

We have also recorded loss of IANSP in 92% of the *Cuora galbinifrons* (n=104) we have examined, and only 4% of the *C. amboinensis* (n=671), but not in any other species of *Cuora*. In contrast, we have rarely, if at all, found IANSP loss in the several thousand individuals of emydid species that we have studied during our careers. Consequently, we recommend further study of the possible genetic influence in IANSP in turtles of the genus *Cuora*.

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Captive husbandry and breeding of file-eared tree frogs, *Polypedates otitophus* (Boulenger, 1893) (Amphibia: Anura: Rhacophoridae)

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ABSTRACT - Six *Polypedates otitophus* were reared from small juveniles to adult breeding size over a period of 18 months. An account of captive husbandry and breeding is provided. Clutch size ranged from 44 – 119 eggs. Eggs hatched after ten days and tadpoles attained total lengths of 85 mm. Metamorphosis took 74 – 84 days at 22 – 26 °C.

INTRODUCTION

The file-eared tree frog (*Polypedates otitophus*) is a large nocturnal rhacophorid frog from in Borneo and Java. (Inger et al., 2004; Inger & Steubing, 2005; Riyanto et al., 2009). Matsui et al., (2014) recently split the species on morphological and genetic grounds, the populations in Sumatra are now assigned to *P. pseudotitophus*. *P. otitophus* is an occupant of primary and disturbed forest and tea plantations from 0 - 1, 500 meters above sea level (Malkmus et al., 2002; Inger & Steubing, 2005). *P. otitophus* feed on invertebrates and have been observed consuming small sympatric *Rhacophorus dulitensis* (Sheridan et al., 2012). *P. otitophus* is a foam nesting species and breeds along or above streams and pools which are usually turbid (Tapley B. pers. obs). Eggs are laid in foam nests which are suspended 30 cm above the surface of the water on low plants (Malkmus et al., 2002). When eggs hatch the tadpoles drop into the water below.

Lifespan and age at sexual maturity in the wild have not been reported. The maximum lifespan in captivity is also unknown. Iskandar (2004) states that *P. otitophus* does not do well in captivity although details of captive management were not reported. Despite being Least Concern this species is poorly known in the wild and in captivity (Inger et al., 2004).

METHODS

Six captive-bred juveniles of this species were obtained in September 2012 from a private breeder in Hungary. Specimens measured 50 mm SVL at the time of acquisition. Two individuals exhibited malformation of the spine and the breeder stated that the frogs had not been exposed to UV-B radiation. The cause of the malformation could have been developmental or due to inappropriate nutrition and/or lighting.

The group was initially housed in a 40 x 40 x 40 cm glass vivarium with a mesh panel in the lid. As this frog is an arboreal rainforest species it is likely to be exposed to UV-B radiation in day time retreat sites. UV-B radiation is important for the synthesis of vitamin D₃ which plays an important role in calcium metabolism, muscle development, organ formation, muscle contraction and immune and nervous system function (Whitaker & Wright, 2001). Captive *P. leucomystax* develop metabolic bone disease when UV-B radiation is not provided, even when prey items were dusted with vitamin D₃ containing supplements (Tapley B, pers. obs.). UV-B provision was therefore considered important for the captive maintenance of *P. otitophus*. This was provided by mounting a UV-B emitting lamp (1150 mm T5-HO UV-B) fluorescent tube (D₃+ 12%UV-B Reptile Lamp, Arcadia Products plc, Redhill, UK) above the mesh of the vivarium top. UV Index (UVI) readings were taken on a monthly basis with a Solarmeter 6.5 UV Index Meter, UVI gradients were measured through the mesh lid and ranged from a UVI of 0 – 4.0 at the level of the frogs' dorsal surfaces. The floor of the vivarium had a built-in aquatic area which measured 40 x 12 cm and was filled with aged tap water to a depth of 3 cm. Compacted organic peat free compost was used as a substrate. A single bird's-nest fern (*Asplenium antiquum*) provided a resting site for the juvenile frogs. Branches were angled from the water into the back top corners of the vivarium and it was on these branches that the highest UVI reading (UVI 4) was measured.

The frogs rapidly outgrew their enclosure. When the largest individual measured 65 mm SVL, all specimens were transferred to a custom-made 100 x 75 x 35 cm breeding vivarium (Custom aquaria, Rushden, UK). The tank was designed to recreate the breeding sites of this frog; a pool with overhanging plants and branches. The base of the vivarium was filled with aged tap water to a depth of 20 cm. *Ficus binnendijkii* and *Dracaena compacta* were planted into two planting compartments. Branches and

fronds of the lady palm (*Rhapis excelsa*) were added for additional resting sites. A mesh panel in the roof of the vivarium allowed for the same lighting array described above. UVI readings were taken on a monthly basis with a Solarmeter 6.5 UV Index Meter and were measured through the mesh lids and ranged from UVI of 0 – 6.0 at the level of the frogs' dorsum. Photo period was 12:12 all year round. In the non-breeding season, full water changes were carried out every week with aged tap water. The water was heated to 27°C with a 300 W aquarium heater (All Pond Solutions Ltd, West Drayton, Middlesex), this was to maintain high ambient humidity and to keep temperatures stable in the enclosure as there was little temperature control in the room in which these frogs were housed. Ambient temperatures within the tank ranged from 23°C and 26°C (night/day summer) to 20°C and 25°C (night/day winter), and temperatures of 28°C were recorded on the branches directly below the UV-B emitting lamp, the highest temperature in the enclosures therefore overlapped with the greatest UV-B radiation which replicates what we would expect to occur in nature. The overlap of the highest temperature and highest UV-B level is important as the formation of D₃ is dependent on the thermal isomerisation of its precursors (Webb, 2005; Tapley et al., 2015).

The enclosure was lightly misted with aged tap water at approximately 21:00 hrs on a daily basis. The enclosure and all furnishings were thoroughly scrubbed with a brush and water on a weekly basis. No chemical cleaners or disinfectants were used.

Juvenile specimens were fed every three to four days on live invertebrates, predominantly crickets (*Gryllus assimilis* and *G. bimaculatus*). Feeder insects were released into the enclosure in the evening corresponding with the frogs' peak activity time. This was considered important as the food insects were consumed when they were still coated in the dietary supplement. Once the frogs attained a SVL of 65 mm they were occasionally offered 4th instar locusts (*Schistocerca gregaria*) and cockroaches (*Dubia blaptica*). Prior to being offered to the frogs, all feeder insects were placed in a polythene bag and dusted with a high-calcium multivitamin and mineral supplement containing vitamin D3 (Nutrobal, Vetark Ltd., Winchester, UK). Approximately 8 prey items were offered to each frog at each feeding event.

RESULTS

The only male began vocalising in mid-February 2014. Female specimens were frequently observed toe tapping in response to the vocalisations of the male. This has been documented in other *Polypedates* species; *P. leucomystax* females toe tap to attract vocalising males, in response to the toe tapping male frogs move closer to, and eventually locate the females (Narins, 1995). Our captive *P. otilophus* spawned from April until November. As the females were not individually identifiable and because we did not observe all egg laying events, inter-clutch intervals



Figure 1. Amplexant *P. otilophus*.

Figure 2. Foam nest deposited on *F. binnendijkii*.

and the number of clutches per female was not recorded. Amplexus is axillary (Fig. 1). Fourteen fertile foam nests were produced by the five females from April 10th - November 4th 2014. Egg laying was observed to take place five times in the early morning at approximately 06:00 hrs. Three of the nests were deposited on *F. binnendijkii* leaves (Fig. 2), and 11 clutches were deposited on the glass sides of the vivarium. On 19th May 2014 the process of nest construction was observed in its entirety, the process took approximately 45 minutes. Once the foam nest had been constructed the female created a chamber in the centre of the nest using circular motions of her hind feet, eggs were then deposited in the cavity in the centre of the nest.

Eggs were left in-situ because we believed that oviposition sites selected by the frogs were likely to be optimal for egg development. Three nests were dissected within 24 hours of laying and contained 42 - 119 eggs. Foam nests were sprayed on a daily basis with aged tap water. A receptacle containing shop bought bottled mineral water (2 cm deep) was secured directly below the foam nest to catch the tadpoles as they hatched. Larvae emerged from the foam nest approximately 10 days after laying and most still had large yolk sacs.

Larvae were kept in the receptacle for 48 - 72 hours until they were free swimming and then transferred to a 30 x 20 x 20 cm (12 litre) tank, without substrate, for rearing. Shop bought bottled mineral water was used for rearing as the local tap water was extremely hard and not deemed appropriate for the species as in nature they occur in microclimates fed by rain water which is normally soft (Poole & Grow, 2008). Oak leaves (*Quercus robur*) were added to acidify the water. Water parameters were as follows: pH: 7.5, KH: 80-120 mg/L, GH: 120 mg/L. Air stream sponge filters were used for mechanical and biological filtration. Twenty percent water changes were carried out on a weekly basis. Filter medium was cleaned and rinsed in the water removed during the water change at each partial water change. Water was heated with a 50 W aquarium heater (All Pond Solutions Ltd, west Drayton, Middlesex). Water temperatures during the larval period ranged from 22°C – 26°C. Larvae were fed ad libitum every day on commercial fish foods, Tetra Pro Colour and

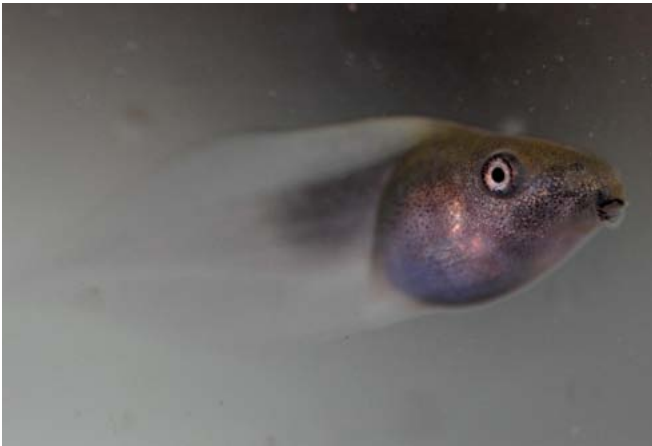


Figure 3. *P. otilophus* larvae prior to the development of the characteristic longitudinal stripes.



Figure 4. Metamorph *P. otilophus* exhibiting characteristic longitudinal stripes.

Tetra Pro Algae (Tetra Werke Melle, Germany) and Hikari algae wafers (Kyorin Food Industries, Ltd. Japan).

When the larvae exceeded 60 mm (Fig. 3) they were deemed too large for the rearing tank and they were transferred to the aquatic area in the adult frogs' enclosure (40 litres), water parameters were the same as above. Two weeks prior to metamorphosis, larvae developed the characteristic longitudinal dark stripes and at this point attained a total length of 85 mm. The first larva metamorphosed 74 days after hatching (Fig. 4), all of the clutch metamorphosed within 10 days.

Metamorphs were housed in the same set up used to house the 50 mm juveniles that were originally acquired. Metamorphs were offered *Drosophila* and 1st instar crickets (*G. assimilis* and *G. bimaculatus*). Prior to offering to the frogs, the feeder insects were placed in a polythene bag and dusted with Nutrobal®. Specimens started feeding 10 days after the tail had fully resorbed. Metamorphs were fed daily until six weeks of age after which, the feed interval was gradually increased to once every three days.

DISCUSSION

Clutch size was smaller in *P. otilophus* than documented in most other *Polypedates* species. Mean clutch size of *P. leucomystax* in Thailand was 476.94 and in Singapore 261.49 (Sheridan, 2008). *P. leucomystax* is likely to be a species complex and variation in the clutch size of *P. leucomystax* could be attributed to undocumented diversity within the species. Clutch size in *P. otilophus* was also smaller than recorded in *P. maculatus*, which ranged from 275 – 719 (Monhanty & Dutta, 1988) and *P. braueri* where clutch size ranged from 400 – 500 (Yang, 1998). *P. otilophus* egg diameter was not measured and it is not possible to state whether or not *P. otilophus* produce fewer but larger eggs than other *Polypedates* species.

Malkmus et al., (2002) report that in the wild, *P. otilophus* larvae attain a total length of 80 mm. Captive reared larvae exceeded this and attained a total length of 85mm, considerably longer than published reports. We believe 85mm to be the greatest total length reported for any *Polypedates* larvae. Larvae of *P. colletti* reach 32.8 mm

total length (Hass & Das, 2008), *P. leucomystax*; 50 mm (Malkmus et al., 2002), *P. macrotis*; 60 mm (Malkmus et al., 2002; Inger and Steubing, 2005) *P. maculatus*; 50 mm (Daniels, 2005) and *P. teraiensis*; 47mm (Tamuly & Dey, 2014). Larvae of some *Polypedates*, however, remain undescribed and may grow larger.

Oviposition in *P. leucomystax* was not dependent on recent rainfall (Sheridan, 2008) and, in captivity, *P. otilophus* were bred without the use of a rain chamber. Reproductive activity did not appear to be temperature dependent as they continued to produce fertile eggs when the temperature of the enclosures had dropped from 23°C and 26°C (night/day) to 20°C and 25°C (night/day) in November 2014.

To our knowledge this is the first documented breeding of this species in the UK. Wild *P. otilophus* are still collected from the wild for the international pet trade (Tapley B, pers. obs.). The global trade in amphibians and the lack of associated biosecurity may facilitate the spread of amphibian pathogens (Garner et al., 2009; Martel et al., 2014). The methods outlined here could be adopted in order to establish this and other *Polypedates* species in captivity which may reduce the number of *Polypedates* of wild caught origin being traded.

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Predation of newts (Salamandridae, Pleurodelinae) by Eurasian otters *Lutra lutra* (Linnaeus)

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ABSTRACT - The remains of newts (Pleurodelinae) were recorded in Eurasian otter (*Lutra lutra*) spraints collected from two river systems on the Gower peninsula, Wales, U.K., between 2005 and 2007. Spraints were collected during detailed field surveys undertaken every two weeks as part of a two-year otter study. Palmate newt remains, mainly vertebrae and maxillae, were identified in 9.27% (43/464) of the spraints analysed by comparison with a reference collection of bones. This study demonstrates for the first time the extent to which otters may consume newts in the UK. Newt remains were recorded in spraints collected during all but two months (February and March) with the largest proportion of occurrences falling between April and July. Conservative estimates of the number of individuals consumed ranged between one and 31 newts per spraint. Newts represent a profitable prey for otters and, as with other amphibians, may represent a seasonally important dietary component with the newts becoming particularly vulnerable during reproductive accumulations. Sporadic but occasionally intense predation of newts by otters may affect both the breeding success and the dynamics of local newt populations. The presence of otters should be taken into consideration when planning conservation and translocation programs for newts. A photographic guide and morphological descriptions of amphibian remains is provided to aid future identification of amphibians in the diets of mammalian predators.

INTRODUCTION

Research into the predation of amphibians has predominantly focused on the impact of alien species of fish, amphibian, and crayfish (Kats et al. 2003); however, amphibians are also preyed upon by a number of mammalian carnivores (Sidorovich & Pikulik 1997, McDonald 2002, Azevedo et al. 2006). The significance this holds for the dynamics and life history strategies of mammal and amphibian populations is poorly understood, representing a gap in our knowledge of the role of amphibians in trophic web dynamics. Investigating predation of amphibians by mammalian carnivores is limited by the difficulty in identifying amphibian remains to family or species level through faecal hard part analysis, which is the most frequently used dietary analysis technique.

Although the Eurasian otter (*Lutra lutra*) - hereafter referred to as the otter - is primarily piscivorous, this species actively exploits non-fish prey, particularly (and unsurprisingly) those species associated with aquatic environments (Carss 1995). As with many terrestrial mammalian carnivores, otter dietary studies usually rely upon the identification of prey remains in faeces or stomachs (Parry, 2010). Amphibian remains are frequently recorded in otter diet (e.g. Weber 1990, Brezeziński et al. 1993, Parry et al. 2011) and in some localities they form an important component of otter diet, particularly during late winter when the availability of fish prey is reduced (Carss 1995) or during spring when anurans aggregate in high densities to spawn (Clavero et al. 2005).

Unlike fish prey, which is usually assigned to family, genus, or even species level, amphibian remains are rarely described in detail. Predation of newts has been recorded in the American river otter *Lontra canadensis* (Toweill 1974), but confirmed predation of newts has only been recorded in a handful of published otter dietary studies (Britton et al. 2006, Pedroso and Santos-Reis 2006, Parry et al. 2011). Furthermore, to our knowledge, newt predation has not been recorded in published dietary studies of other mustelid species that can forage in freshwater environments, such as polecat (*Mustela putorius*), European mink (*M. lutreola*), and American mink (*Neovison vison*).

The scarcity of reports of newts in mustelid diet, in particular that of the otter, seems unusual considering that they occupy sympatric or parapatric habitat to anurans, which frequently occur in mustelid diet (Lode 1997, Sidorovich et al. 2007, Parry 2010). As with many amphibian species, newts aggregate for breeding (Hedlund and Robertson 1989), although these are less dense and subject to greater temporal variation than frogs and toads (Díaz-Paniagua 1992). Despite this, newts are a potential prey resource for mustelids such as the otter and that their availability will increase during the breeding season.

The infrequency of records of newt predation in the literature may be due to difficulties in identifying amphibian prey remains to family and genus level. Only two European studies have recorded the low occurrence of newts forming part of the amphibian diet of the Eurasian otter (Polednik et al. 2007, Cogalniceanu et al. 2010). In this study we report

the occurrence of palmate newts in otter diet on the Gower Peninsula, Wales, United Kingdom, a landscape that contains common frog *Rana temporaria*, common toad *Bufo bufo*, palmate newt *Lissotriton helveticus*, smooth or common newt *Lissotriton vulgaris*, and great crested newt *Triturus cristatus* (Russell, 2002). We also provide a photographic guide and morphological descriptions to aid future identification of amphibians in diets of mammalian predators.

METHODOLOGY

Dietary analysis

Otter spraints were collected at fortnightly intervals between December 2005 and January 2007 under licence (Countryside Council for Wales licence number OTH: SA: 178:2007) from rocks, trees, and boulders in the riparian corridors of the River Clyne (51°35'57"N, 3°59'48"W) and Burry Pill (51°37'21"N, 4°14'31"W) as part of a long-term study of otters on the Gower Peninsula in South Wales. Spraints were stored at -17°C until dietary analysis was undertaken. Individual spraints were soaked for 24 hours in a saturated solution of biological detergent before being rinsed through a 0.5mm sieve and left to air dry. Hard remains were identified using keys (Conroy et al. 2005) and a reference collection of vertebrae collected by the authors including samples from common toad, common frog, palmate newt, smooth or common newt, great crested newt, and slow worm (*Anguis fragilis*). Diet was described as Relative Frequency of Occurrence (RFO %)

The minimum number of individual newts was determined by counting maxillae using the assumption that two maxillae in one spraint sample represented a minimum of one newt. This is a conservative approach as the occurrence of two maxillae in one spraint could reflect the consumption of one or two newts.

Production of images

The images were produced using two programs: CombineZP (www.hadleyweb.pwp.blueyonder.co.uk/CZP/Installation.htm) and Image Composite Editor or ICE (www.research.microsoft.com/en-us/um/redmond/groups/ivm/ICE/). All photographs were taken with a webcam fitted with an adaptor slotting into the microscope (Wild M3Z) eyepiece.

RESULTS

Occurrence of newts in spraints

Vertebrae and maxillae of the palmate newt *L. helveticus* were recorded in 16.90% (36/213) of spraints collected from the River Clyne and 1.23% (11/898) of spraints collected from the Burry Pill (Fig. 1). There was a large difference in the occurrence of palmate newt remains recovered from spraints between the two riparian corridors of the permanent and ephemeral ponds of the rivers, with the vast majority being recorded from the river Clyne. Newt remains were recorded throughout the year with clear peaks in April and June in the river Clyne. The median number of maxillae recovered in spraints containing newts was one. By pairing maxillae, and after accounting for single occurrences in spraints collected on the same day, it was ascertained that a minimum of 95 individual

newts had been consumed by otters between December 2005 and April 2007. The maximum number of maxillae recorded from a single spraint was 62, representing a minimum of 31 individual newts. The majority of occurrences were between March and July, with 32 occurrences representing a minimum of 82 individual newts recorded in spraints during this period (Table 1). In spraints collected between August and November eleven occurrences were recorded, representing a minimum of eight individual newts. Palmate newt remains in four spraints representing a minimum of five individuals were recorded between December and Feb over the period 2005-2007.

| Month | Burry Pill | | | River Clyne | | |
|-----------|------------|-------|------------|-------------|-------|------------|
| | FO % | RFO % | N spraints | FO % | RFO % | N spraints |
| January | 2.53 | 2.90 | 79 | 3.20 | 0.00 | 23 |
| February | 0.00 | 0.00 | 122 | 0.00 | 0.00 | 12 |
| March | 0.00 | 0.00 | 111 | 0.00 | 7.89 | 22 |
| April | 0.00 | 0.00 | 200 | 0.00 | 28.33 | 52 |
| May | 0.00 | 0.00 | 51 | 0.00 | 2.94 | 10 |
| June | 0.00 | 0.00 | 47 | 0.00 | 8.33 | 20 |
| July | 2.88 | 1.72 | 104 | 2.77 | 3.03 | 10 |
| August | 6.06 | 1.19 | 33 | 18.36 | 0.00 | 8 |
| September | 0.00 | 0.00 | 41 | 0.00 | 17.14 | 29 |
| October | 3.13 | 1.81 | 32 | 9.78 | 0.00 | 7 |
| November | 3.57 | 1.69 | 56 | 6.38 | 0.00 | 3 |
| December | 4.55 | 2.70 | 22 | 20.68 | 2.77 | 17 |

Table 1. Frequency (FO) and relative frequency (RFO) percentages of occurrence of palmate newts in otter spraints fortnightly over a two-year period 2005 - 2007.

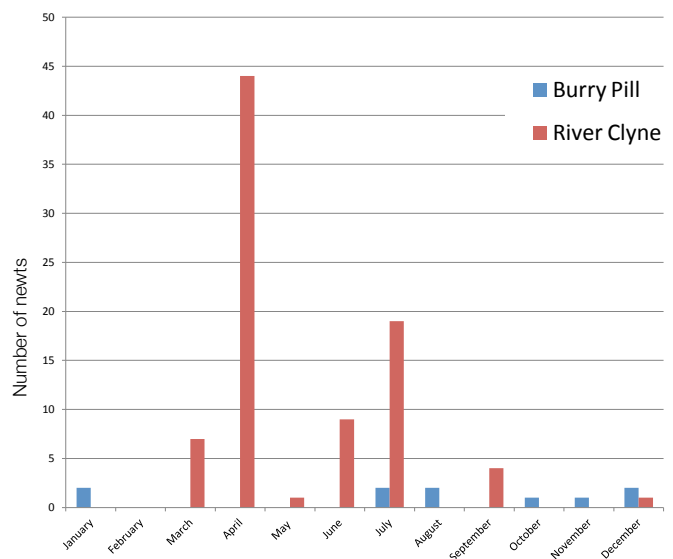


Figure 1. The minimum number of newts predated per month in each of the two river systems sampled.

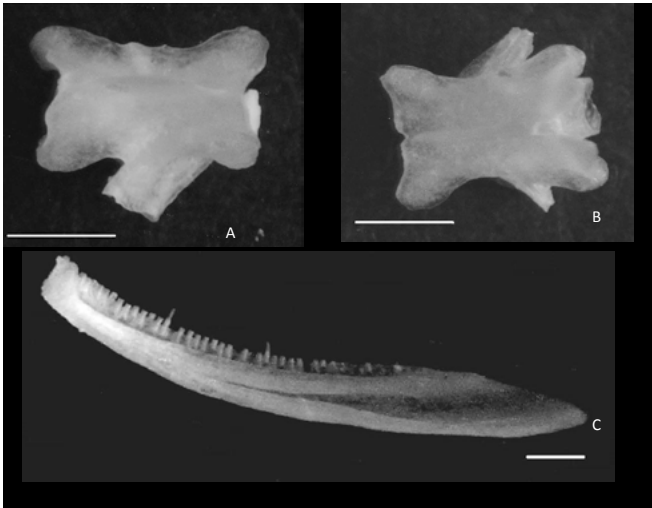


Figure 2 Palmate newt *L. helveticus* - scale bars 1 mm.

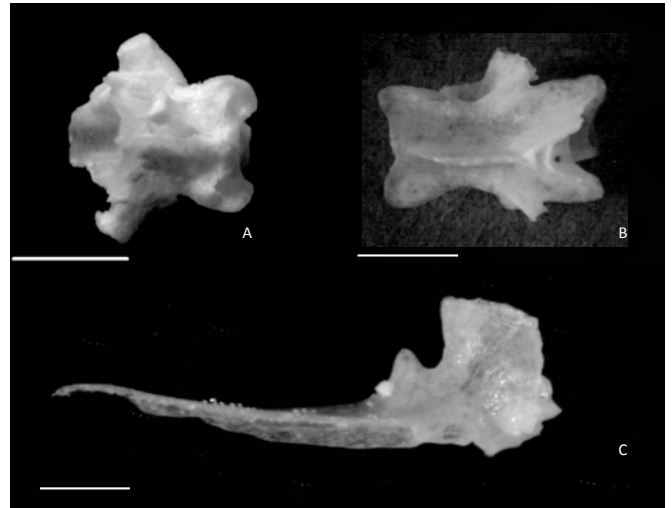


Figure 3 Smooth or common newt *L. vulgaris* - scale bars 2 mm.

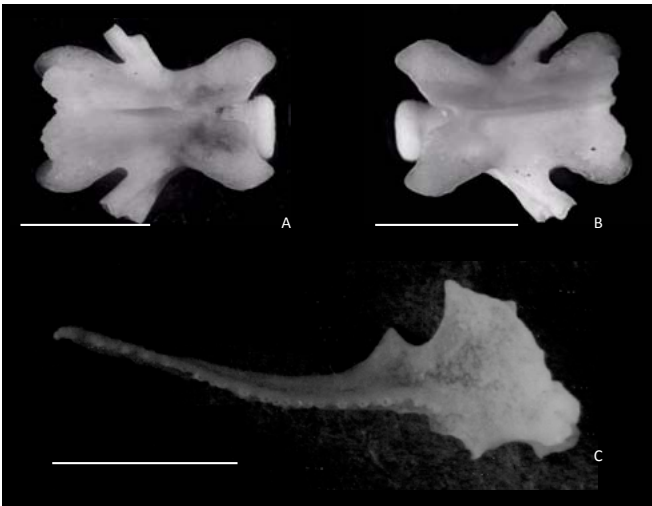


Figure 4 Great crested newt *T. cristatus* - scale bars 2 mm.



Figure 5 Frog *R. temporaria* - scale bars 2 mm.



Figure 6 Toad *B. bufo* - scale bars 2 mm.

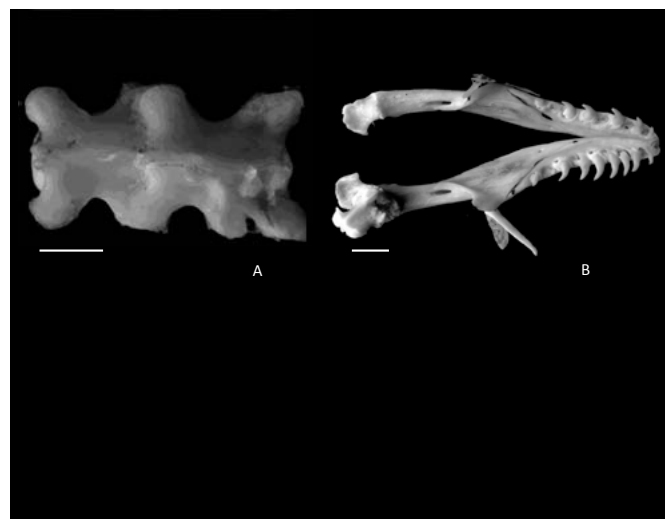


Figure 7 Slow worm *Anguis fragilis* - scale bars 2 mm.

Figures 2 - 7. Amphibian and reptilian remains used for identification in spraints. A and B mid-trunk vertebrae, C maxilla except Fig. 6 B maxilla.

Identifying newt remains

Newt vertebrae (Figs. 2a, b – 4a, b) are easily distinguishable from anuran vertebra (Figs. 5a, b and 6a, b) but resemble those of the slow worm (Fig. 7a). The ventral surfaces of newt vertebrae have distinct transverse processes, which form between two and four tubes, often associated with the transverse foramen. In contrast, slow worm vertebrae have short transverse processes. The centrum of a newt vertebra is convex both anteriorly and posteriorly, i.e. is amphicoelous. Newt maxillae (Figs. 2c – 4c) are very different from anuran maxillae (Figs. 5c and 6c), which are also very different from each other; the slow worm maxilla (Fig. 7b) clearly differs from all of these.

DISCUSSION

It is clear that otters have the ability to actively exploit non-fish prey, particularly those associated with wetland areas (Kruuk 2006). Otters are explorative foragers turning over rocks and entering aquatic vegetation to flush out prey (Kruuk 2006), so newts are likely to be encountered during normal foraging patterns. In the UK, adult newts reach 9 - 15 cm (Chinery 1995), bringing them into the typical prey size range for otters (Kruuk and Moorhouse 1990). Additionally, newts are likely to be relatively easy to catch compared with many fish species, therefore representing an efficient energy gain for relatively little cost. This leads to the question why newt remains have not been recorded more frequently in otter spraints, particularly when other amphibian species, notably the anurans, are recorded relatively frequently (Weber 1990, Clavero et al. 2005). It was suggested by Chanin (1985) that either newts are not being predated or that they are being missed through standard spraint analysis. The evidence presented here shows definitively that newts are actively predated by otters, which suggests that where newts are consumed, they are being overlooked, possibly due to the lack of identification aids.

The greatest number newts inferred in the spraints was detected between March and July which, based on observations made at breeding sites on Gower (Russell 2002), appears to coincide with spawning accumulations, although it is acknowledged that there can be some variation in the timing of newt breeding depending upon environmental conditions (Díaz-Paniagua 1992). The data presented in this paper confirm previous observations that spawning amphibians are extremely vulnerable to mammalian predation (Clavero et al. 2005). Otter predation on newts was reduced during winter months when torpid newts would have been in terrestrial and fresh water refuges that would limit access by otters. Nevertheless over-wintering amphibians at low population densities still appear to be vulnerable to occasional otter predation, and suggests that otters are more adept at foraging in the varied terrestrial environments associated with aquatic systems than has been previously thought. The marked difference in newt predation between the two river systems is likely due to idiosyncratic differences in lotic habitats adjacent to the main river channels, with River Clyne being associated with a larger number of ponds than the Burry Pill.

The lack of newt records in previous studies investigating

otter diet could be in part due to newts being generally less available to otters than other amphibians groups. Breeding newts are more discrete than the highly vocal anuran species, which should reduce their vulnerability to predation. When foraging on land, otters rely primarily on olfaction and audition to locate their prey (Chanin 1985, Dunstone and Gorman 1998), so the lack of vocalisation in newts may provide a degree of protection. However, tactile and visual cues are the primary senses used by otters foraging in water, a medium where audition and olfaction are of limited use (Chanin 1985, Dunstone and Gorman 1998). In particular, otters utilise their highly sensitive vibrissae to detect underwater prey movement (Dunstone 1998, Lanszki et al. 2006), undertaking explorative foraging in submerged and emergent vegetation (Kruuk 2006). Consequentially, newts in breeding ponds are likely to be as vulnerable to predation as other amphibians. All newt species exhibit aquatic lekking (Malmgren and Tholleson 1999) and males undertake courtship displays involving considerable amounts of movement, flashes of colour, and the production of tail vibrations (Halliday 1975, Beebee 1980). Such behaviours will make them very conspicuous to a foraging otter. Moreover, otters are able to learn foraging techniques maternally (Watt 1993), so it is not unfeasible that the location of a seasonally rich amphibian patch could be passed down the matriline.

Although there is some difference in body lengths between newt species (Harrison et al. 1984, Malmgren and Tholleson 1999), this can be subject to geographical variation (Harrison et al. 1984, Arntzen and Wallis 1999). Additionally newts show tail regeneration (Harrison et al. 1984) and there is a degree of overlap in total body length between species due to sexual dimorphism (Harrison et al. 1984, Malmgren and Tholleson 1999). Fundamentally, it is not possible to estimate body length based on vertebrae remains in spraints as it cannot be assumed that all vertebrae from an individual newt are recovered. Neither is it possible to determine accurately the number of newts predated from vertebrae only. Studies carried out on newts have identified few differences in head morphology between sex and between species (Malmgren and Tholleson 1999); thus, it is unlikely that maxillae alone can be used for species identification. In newts, body size and age are not strongly correlated (Halliday and Verrell 1988) so estimations of age cannot be obtained from hard remains in spraints (Steward 1969, Hagstrom 1977). Otters are likely to select for the largest individuals as they will be the most conspicuous, representing the best predation profit, and a large body size is associated with reduced swimming and running speed in newts (Gvozdik and Van Damme 2006).

The effect of otter predation on newt population dynamics may currently be underestimated. Newt populations undergo considerable long-term fluctuations, and intensive predation can be a causative factor in long-term population declines (Beebee and Griffiths 2005). Although newts were a relatively infrequent food item, spraints collected during one fortnightly survey in April 2006 contained a minimum of 44 individuals, demonstrating that otters can locally consume newts in surprisingly large numbers. It is unknown if this had a significant impact on the newt population as no previous

data for abundance within the study area was available. Otters also predate species such as invasive crayfish, which not only consume adult newts but also the larval stages and eggs (Alford and Richards 1999) and this otter feeding behaviour could provide some benefit to newt populations. Localised predation by otters and other mammals, which forage in habitats associated with aquatic systems, such as mink (*Mustela vison*) and polecat (*Mustela putorius*), may influence the demographic and breeding success of newt populations (Dunstone 1993, Lode 1997). Worldwide amphibian populations are under threat with up to one third of all species undergoing severe declines (Wake 1991, Stuart et al. 2004); however, otter populations in the UK are recovering their former range (Mason and Macdonald 2003, Jones and Jones 2004) and this may impact on recovering amphibian populations. Increased predation pressure on newt spawning aggregations could be highly detrimental to local newt populations.

Non-fish prey is frequently a seasonally important component of otter diet, particularly when fish are less available (Carss 1995, Jurajda et al. 1996), so the continued decline of amphibian populations is also a concern for otter conservation as there are no obvious groups to substitute for the decline in amphibian prey. How otters will respond to the reduced availability of amphibians remains to be seen. It is likely that they will switch to alternative prey, particularly those which are abundant at times of year when amphibians traditionally form an important dietary component. There may be an increase in the consumption of semi-terrestrial and terrestrial prey groups leading to the possibility of increased intra-guild competition within the Mustelidae. Low prey diversity has also been linked to increased predation on commercial fish stocks (Jacobsen 2005) and a reduction in the availability of non-fish prey, such as amphibians, may be accompanied with increased raiding of commercial fishponds that remain well stocked at times of the year when wild fish are less available.

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Trialling amphibian ladders within roadside gullypots in Angus, Scotland: 2014 impact study

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ABSTRACT - Gullypots (roadside drains) have long been a problem for amphibians and other animals as a source of entrapment and certain death. Where the problem prevails close to migration routes for amphibians, the threat to local populations is significant and can devastate numbers each breeding season. Combined with existing known threats such as road death, road salting, agrichemicals, pollution and habitat damage, the dangers of gullypot entrapment can further imperil local populations to an unsustainable level. In the first UK trial of its kind, this study aimed to mitigate the gullypot problem with the installation of a simple ladder device which can be tailored and retrofitted in all circumstances. The authors adapted a design, trialled it in a known problem location and recorded findings. This study aimed to determine if the ladders can be ascended by UK widespread species, demonstrate the effectiveness of the amphibian ladders and quantify the extent of the problem using a robust dataset.

INTRODUCTION

Amphibian population levels continue to be threatened by the fragmentation of their habitats (Beebee & Griffiths, 2000). Development of roads and railways and continual urban growth cut right through the range of habitats the animals require (Beebee, 2013). Declines in frog populations showed significant correlation to the increase in human population density, as well as agricultural intensification and use of pesticides (Beebee, 1973 & Cooke, 1972). It is well known that toads (*Bufo bufo*) travel great distances to return to traditional breeding sites from hibernation and foraging grounds. Individuals continue to move to foraging grounds throughout late spring and summer (May to September) and then return to hibernation habitat in autumn (Buckley & Cole, 2004). Amphibians migrating to breeding or hibernation sites may be forced to cross roads where they are killed in large numbers (Fahrig et al, 1995) or follow kerb lines and crawl over numerous roadside drains (Smith & Sutherland, 2014).

Whilst performing standard drain maintenance during 2008/2009, Tayside Contracts staff observed animals, notably amphibians, trapped within roadside gullypots (Muir, 2011). The predominant species found was common toad (*B. bufo*) but also frogs (*Rana temporaria*) and palmate newts (*Lissotriton helveticus*). Perth and Kinross Countryside Rangers began to survey a variety of locations within the region and discovered that this was not an isolated problem. These initial observations instigated the formation of the Amphibians in Drains Project within Perth and Kinross (Muir, 2011). This three-year project (2010-2012) studied the extent of the problem within the Perth and Kinross area at a variety of sites. From the 1500 gullypots surveyed over this time period, approximately 3000 animals were found trapped. If this number is projected to represent the number of gullypots across the country, the rate of mortality throughout Scotland is significant (Muir, 2013).

The design of the drains allows no escape once the animal has fallen in which results in death by either starvation or drowning (Muir, 2012). Gullypot liners are used throughout the United Kingdom. These are installed below the tarmac to collect debris from the wash off of roads, removing water and preventing debris from clogging the sewage system. As gullypots are fitted with grid covers constructed with widely spaced bars, they allow no protection to amphibians walking over them and result in the animal falling within the gullypot (van Diepenbeek & Creemers, 2012). There has also been speculation that some amphibians may intentionally enter gullypots, mistaking them for ponds.

The gullypot drainage system is in most cases enclosed. In the gullypot wall, there are two ports leading to a chamber. The lower port allows run-off water to pass, the upper port is for maintenance access (rodding point) and is usually fitted with a plastic plug. Amphibians can enter the chamber through the lower port if it is below the water line, or through the upper port if the cover is loose or missing. In both cases, the amphibian is then prevented from travelling further into the system as the chamber itself has sheer sides which cannot be negotiated unless the gullypot and chamber are fully flooded. In this case (during very heavy rain for example) any amphibians entering the chamber may be swept in to the carrier pipe which connects the gullypots. In the urban environment, most gullypot carrier pipes are fed to the sewer system, which is generally toxic and also has no further escape route. In some cases, the carrier pipe will lead to either a chamber or series of chambers, which have upstands of 150mm or to a soak-away system, either a tank or a gravel trap. In these circumstances, there is no further escape route for amphibians and other trapped animals. In rural situations, the carrier pipe may run into roadside ditches.

Because gullypot liners are normally manufactured using plastic, the resulting product is effectively a large diameter

pipe (450mm) and when installed vertically, the sides are either smooth and sheer or corrugated, presenting no opportunities to escape. This results in any trapped animals suffering a lingering death from exhaustion, starvation and/or drowning. During the breeding season, this trapping potentially reduces the adult population of breeding males and females, possibly impacting on the success of local amphibian populations. Installing a ladder provides a means for the amphibians to escape and continue migration (van Diepenbeek & Creemers, 2012).

A variety of measures to reduce amphibian road deaths have been introduced globally (Smith & Sutherland, 2014). In Germany, two sites with significant numbers of migrating amphibians crossing the roads were closed on key migration evenings (Smith & Sutherland, 2014). Wildlife kerbs have been introduced into Perthshire (Muir, 2013). In north Wales, roadside drains in a sensitive Great Crested Newt habitat were moved so there was a 10cm gap between the kerb and the drain. This study showed an 80% decrease in the amount of newts found within the gullypot (Smith & Sutherland, 2014).

A non-governmental organisation, Reptile, Amphibian and Fish Conservation Netherlands (RAVON) conducted lab based studies to determine whether amphibians would use ladder structures to escape from water filled tanks. The trials were successful and Raymond Creemers presented their findings at the 2014 Herp Worker’s Meeting in Bristol, UK. Trevor Rose, one of the present authors and founder of Friends of Angus Herpetofauna, was encouraged by the research performed by RAVON and began planning a trial installation of the ladders within the Angus area. After approaching Angus Council, he was granted permission to install ladders at two sites in Angus which had previously been noted as problem areas for amphibians in drains. 12 drains were identified at Station Road in Carnoustie and 38 drains were identified in Silver Birch Drive, Dundee. The study site, Silver Birch Drive (NO 446 343), is part of a relatively new housing development on the outskirts of Dundee. It is located adjacent to a golf course, which incorporates a variety of ponds that constitute ideal breeding sites for amphibians.

Local residents had previously applied to register the site as a toad migration crossing under the “Toads on Roads” scheme, as a result of observations of the increasing numbers of road-killed amphibians during peak times of migration. Toad warning signs were erected for this purpose. In recent years, anecdotal accounts from a number of residents suggest that the amphibian populations are dwindling. The amphibian ladder study focused on toads and frogs. Palmate newts (*L. helveticus*) were not found during this study but have been recorded in previous years within the study area.

MATERIALS AND METHODS

The study site is approximately 0.7 miles (1.13 kilometres) from drain 1 to drain 38 (Fig. 1). The RAVON ladder design (perforated steel plate) was modified by utilising a length of plain stainless steel which provided a structure for climbing material to be applied. The ladders were made from 125mm wide x 1mm thick stainless steel plate. This was cut to length to suit individual gullypot depth. A foot was formed at the base of each plate by bending a 20mm wide flange at right

angles to the plate. At the top of the ladder, the plate was bent in a semi-ellipsoidal curve. Along both edges of the ladder where it is straight, the edges were flanged to provide stiffness. Enkamat®, the climbing material of choice, was not available in small quantities for the trial therefore jute (hessian) was used as a substitute. It was accepted that this would have a limited service life but it was expected to last for the duration of the trial (approximately six months). The jute was affixed to the steel using push-fit plastic clips.

Prior to the manufacture of the trial ladders, measurements of the gullypots were taken. Each gullypot was measured from the road surface to the full depth of the drain (where possible). In some instances, there was a significant amount of collected substrate, so the measurement was taken to the most firm point which would support the ladder. The height of the collected water was also measured relative to the overall depth, and the gullypot grating was measured for thickness. With these dimensions, the length of steel required could be calculated and the amount of jute determined. Ladders were then custom-built and labelled to suit each gullypot (Fig. 2). Manufacture of each ladder took around 15 minutes.

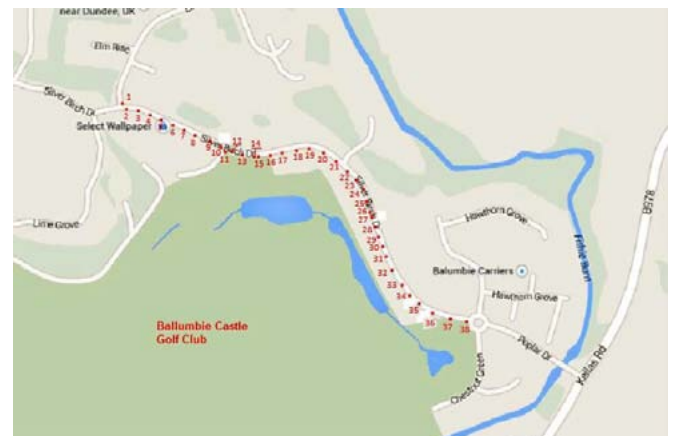


Figure 1. Silver Birch Drive street map showing gullypot locations, sourced from Google Maps (accessed online 29/03/2014) and annotated by McInroy, C.



Figure 2. Selection of trial ladders. Photo credit: Rose, T.

RESULTS

Installation of the ladders took place on the 5th March 2014. The ladder was installed such that the foot was positioned at the perimeter of the gullypot liner, and the top of the ladder then rested against the opposite side underneath the gullypot cover. It was important for the top of the ladder to be level and flush underneath the cover, and where possible, aligned with the gullypot cover grating bars (Fig. 3). This maximises



Figure 3. Ladder installed within a gullypot. Photo credit: Rose, T.

the ease of escape for amphibians. At Silver Birch Drive, the gullypot covers were 50mm thick, which still needs to be negotiated by liberated specimens.

For the trial, two thirds of the drains were fitted with ladders and the remaining third had no ladders, and would be used as comparisons for the study. Un-laddered gullypots were evenly distributed through the site, such that every third gullypot had no ladder fitted. The study took place over three months. Daily visits to the site were undertaken for the first six weeks (05/03/2014 to 16/04/2014). These were usually performed at dusk to witness peak activity. Visits to site continued after 16/04/2014 to monitor the site and continue collecting information on the number of amphibians found within the drains. Seasonal movement had slowed by this point so daily visits were no longer required. The data analysed within this report were from the first six weeks of daily visits beginning from the date of ladder installation.

Each gullypot was visually checked using a powerful lamp to observe any signs of movement. Where large amounts of debris obscured the view, the covers were lifted to ensure no animals were hiding under the leaf litter. Amphibians found in the gullypots without ladders were immediately removed using an amphibian net or gloved hands (since there is no means of escape, it was not necessary to leave them in situ), whereas those in laddered pots were left in situ after they had been identified. Unique markings on each amphibian were used as identification. This method was found to be satisfactory since numbers of individuals in each gullypot at any one time were low, and only required to be monitored for a short period over a few nights. Once noted, individuals in laddered gullypots were then monitored to record whether they later escaped from the drain or remained for a period of time. Where individuals were not attempting an escape after several days, they were removed, recorded, and released in one of the golf course ponds.

Effects of ladders on escape success

In total over the six week period 247 frogs and toads were recorded in gullypots in the study area. One hundred and fifty eight amphibians fell into the 25 laddered gullypots (97 toads and 61 frogs, averaging 6.32 animals found per drain). The 13 gullypots with no ladders entrapped 89 amphibians (73 toads and 16 frogs, averaging 6.85 per drain). Forty-three amphibians were manually rescued from the laddered gullypots (26 toads and 17 frogs), while 115 used the ladders to escape (71 toads and 44 frogs). The number of amphibians that successfully ascended the ladders and escaped (115) represents 72.8% of the total number that fell into laddered gullypots (158). For toads, the percentage rescued was 73.2% (71 of 97, all males), and for frogs, the percentage rescued was 72.1% (44 of 61, including 11 females). There was a substantial variation in numbers of animals entering gullypots, from none at all (10 pots) to 37 in drain number 23. No dead amphibians were found within the drains throughout the study period. The average air temperature from 5/03/2014 to 16/04/2014 when performing surveys was 6.5°C. There was no significant rainfall during the study period and water levels in the gullypots remained unchanged.

Observations

The jute provided a suitable grip for the animals to climb relatively vertical ladders. There was initial concern that the ladders may prove too steep but this was not the case (Fig. 4). *B. bufo* sometimes took a relatively long time to begin ascending the ladders and the duration of the ascent itself could be lengthy. It is not clear to what extent the temperature is affecting this behaviour and the time taken, although logically we would expect slower motion in early spring temperatures of less than 8°C. Most trapped amphibians tended to



Figure 4. Common toad, *B. bufo* on escape ladder. Photo credit: McInroy, C

swim continuously around the perimeter of the gullypot at the water's surface. If the ladder was not within easy reach of the perimeter the animals frequently passed by without attempting to approach it, seemingly oblivious to its presence. Ladder positioning is therefore crucial.

Trapped amphibians were not inclined to attempt escapes when the temperature was very low. This resulted in some individuals spending many days in gullypots if they fell in just prior to a cold spell. Amphibians, especially newts, will use floating items as rafts if they are present. Providing a "raft" in the form a small piece of wood or other material could decrease mortality by offering a refuge if, for example, the temperature drops and until the animal is urged to seek an



Figure 5. Ladder design to be used in continuing 2015 trials, fitted with Enkamat® and raft. Photo credit: Rose, T.

escape. This will be trialed during 2015 (Fig. 5).

We did not see any toads in amplexus using the ladders, nor did we observe any frogs performing an escape. However, on several occasions frogs were witnessed sitting on the tops of ladders but when illuminated by torch they would jump off back into the gullypot. It is possible that as the frogs are more agile and flighty, they are more likely to find the escape route immediately and escape before they can be recorded. RAVON reported that some common frogs and edible frogs (*Pelophylax esculenta*) escaped using ladders with ten minutes

of laboratory trials beginning. Fig. 6 shows a *R. temporaria* using a pry bar as a ladder that was being carried within the collection bucket. For this reason, we consider the overall results of this study and the data collected to be conservative, as it was not practical to attend the site around



Figure 6. Frog using pry bar to escape from the collection bucket. Photo credit: McInroy, C



Figure 7. Leaf litter and debris build-up at the ladder top, which facilitates easier escape through the grating. Photo credit: Rose, T.

the clock (nor all parts of the site simultaneously) so the actual total number of escaping amphibians, especially frogs, cannot be quantified, but is likely to be higher than presented in the recorded data.

Female toads were not recorded escaping from the drains whereas female frogs were. It was unclear if this was due to the climbing material being inadequate to support gravid females or if their ability to ascend was compromised by their condition, or perhaps both. It is possible that the change of material to Enkamat® would improve the escape rates as it provides surer footing. Amplexing pairs had more difficulty in the drains and it is possible that the ladders will be of no benefit to them. This suggests that despite male toads being able to escape there is still the concern that local populations will be compromised due to the continued loss of females. It was often observed that when females were present within the drain, males were more intent on competing for females and coupling with them than attempting an escape via the ladders. However further trials with the Enkamat® product will be required to fully assess suitability for female toads and amplexing pairs.

The small cross-sectional area of the ladders and unobtrusive nature of the construction and installation had no detrimental effect on drainage. During the study, gully cleaning took place, which removed much of the substrate from the gullypots. This resulted in some of the ladders not fitting (no longer reaching the gullypot grating as they were too short) and these were remade accordingly. However, this activity did provide positive feedback from the operators, who reported that the ladders presented no issues for maintenance and refitting after cleaning was a simple operation.

DISCUSSION

Our study showed that ladders reduced entrapment in the gullypots at the study site. Evidently certain drains posed the greatest problems to amphibian migration. Poplar Drive leading onto Silver Birch Drive is the only access into this extensive housing estate. To reduce traffic speed, traffic calming chicanes have been constructed. These provide additional difficulties for amphibian movement. Drains 23 and 36 are within the corners of these chicanes and showed high numbers of trapped amphibians.

The depth of the grating was a cause for concern as this had to be negotiated by escaping amphibians. Male common frogs will usually grow to a body length of approximately 6.5 cm with females slightly larger at up to 8 cm (Beebee & Griffiths, 2000). Female *B. bufo* in the UK can attain 9 cm in length, with males measuring up to 7 cm (Beebee & Griffiths, 2000). These lengths suggest that many toads and frogs will be able to climb out despite the depth of the drain grating. Debris and leaf litter collecting on the landing area of the ladder can also facilitate escape (Fig. 7). The trial revealed a problem with substrate at the bottom of the gullypots requiring some ladders to be remade after gullypot cleaning. It would be beneficial to request the local authority to clean gullypots prior to manufacture of ladders if this study is repeated elsewhere. Once installed in a clean gullypot, substrate build-up causes no further issue as the ladder is self-standing and unaffected by the substrate around it.

CONCLUSIONS

The recorded data and observational evidence suggests it is possible to reduce the number of amphibians trapped during the breeding season within road drains by installing amphibian-friendly ladders. Once hot spots are identified (i.e. gullypots with high catch rates) the appropriate prevention should be introduced. This will minimise amphibian mortality and allow future surveying at new sites. The continued eradication of hot spot traps may help to ensure the future of amphibian populations. The average number of animals found in the drains without ladders was 6.84 compared with 1.72 in laddered drains that were manually released. This shows a significant reduction when ladders were present. Using the combined escape rate of 72.8%, we could expect that 180 of the 247 amphibians recorded may have escaped from the study area (during the period 05/03/2014 to 16/04/2014) if all gullypots had been protected by ladders.

Toad population size for this location has not been determined, so we are unable to assess the effect that ongoing annual mortality due to gullypot entrapment is having. However, we estimate the breeding population to be between 1000 and 2000 individuals. Our assessment of the landscape suggests that the majority of the amphibian population migrates from the north and north east, and must cross Silver Birch Drive to reach breeding pools. The 153 entrapped toads during this 6-week study may represent a significant proportion of the breeding population, and mortality is further exacerbated by road casualties due to traffic (unrecorded data). Anecdotally residents have noticed a decline in toad numbers in recent years and this is also the view of the authors based on numbers recorded between 2009 and 2013. Potentially, this rate of gullypot mortality alone may not be sustainable at this site, for example, simplistically the 153 toads trapped represents 7.65% of a 2000 strong population. Based on this reduction year on year, the population would fall to less than 500 in two decades. Since many more toads would be trapped throughout the year, and other threats including road traffic are affecting them, the population may be in danger of local extinction without mitigation.

The long term aim of this project is to inform local authorities, councils, housing developers, road construction companies etc. on the importance of installing escape methods in drains for amphibians where there are significant populations and / or close proximity of breeding pools. After identifying the numbers of animals trapped within the pits under cattle grids, it is now common practice to provide 'hedgehog ramps' for escape. It is hoped that this study will have similar success. The costs to manufacture the trial ladders amounted to around £10 each, but this could be reduced significantly with mass production. It is essential to try and prevent anthropomorphic activities from influencing the decrease in amphibian populations. The Nature Conservation (Scotland) Act 2004 stipulates that local authorities have the duty to encourage biodiversity conservation within their area (Scottish Government 2004).

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Discoglossus pictus tadpoles: egg cannibalism

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The painted frog *Discoglossus pictus* is a monotypic species distributed in North Africa, Malta and Sicily (Fromhage et al., 2004; Zangari et al., 2006). It is widely distributed in Sicily, where it occupies a broad range of habitats although it is linked mainly to temporary ponds, be they anthropogenic or not (Turrisi, Lo Cascio & Vaccaro, 2007). This species adopts intermediate or opportunistic trophic strategies during the adult stage, feeding mainly on insects and on other terrestrial invertebrates (Licata et al., 2012), while the tadpoles are generalist feeders. Since it is an euriectic species and can occur in several aquatic habitats, painted frogs frequently share their breeding sites with other species of amphibians. In Sicily it coexists with *Pelophylax* sp., *Hyla intermedia*, *Bufo bufo*, *B. siculus* and *B. balearicus*, as well as the invasive *Xenopus laevis* (Lillo et al., 2011).

On 3 April 2009, during nocturnal surveys on a population of *D. pictus*, we observed the predation of *D. pictus* eggs by several tadpoles of the same species (Gosner stage 25; Fig. 1a, b). The observation was made in a breeding site located in a mountain pasture (UTM 33 S 0344451 4217051; alt. 800 m), on the western mountains of Palermo (northern Sicily; Fig. 3). This is a temporary pond usually with a long hydroperiod (> 10 months), measures 60 m in length and up to 150 cm deep. It has plentiful aquatic vegetation and is surrounded by abundant riparian vegetation (Fig. 2). It has already been observed that *D. pictus* tadpoles may feed on other species'

clutches (Escoriza, 2014). In general, cannibalistic oophagy has been observed in several other species of amphibians (Crump, 1983). This is the first time that cannibalistic oophagy has been observed in *D. pictus* tadpoles.

Aquatic organisms living in temporary water bodies are subjected, during the hydroperiod, to intense natural selection and competition, which favour species with successful and efficient strategies. For example, species with synchronised explosive breeding can reduce the risk of cannibalism by limiting the differences in size and development between individuals (Crossland & Shine, 2011). Since *D. pictus* does not have a definite breeding season, spawning events occur at different times, thus creating conditions for cannibalistic oophagy. The potential consequences of this behaviour involve population regulation and may impact on age class structure (Crump, 1983). The benefits of practicing cannibalism are gaining energy and nutrients, as well as reducing the number of possible competitors.



Figure 1. Detail of temporary pond illustrating several *D. pictus* tadpoles eating eggs of its own species (a). Enlarged detail showing a tadpole of *D. pictus* eating eggs of the same species (b).



Figure 2 Photograph of the study area

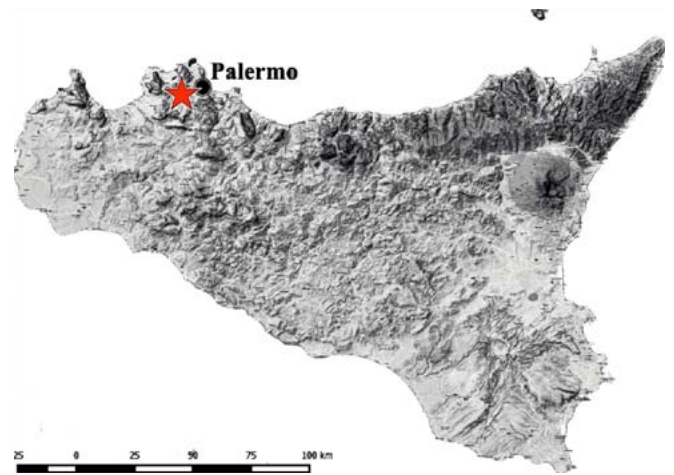


Figure 3 map of Sicily showing the study area (red star).

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Geographical distribution extension and notes on vocalisations of *Ischnocnema penaxavantino* Giaretta, Toffoli & Oliveira, 2007 (Anura: Brachycephalidae)

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INTRODUCTION

The frog genus *Ischnocnema* Reinhardt & Lütken, 1862 comprises 33 species (Frost, 2014), of which 31 are distributed in four species series (*I. guentheri*, *I. lactea*, *I. parva* and *I. verrucosa*). Two further species (*I. manezinho* and *I. sambaqui*) are not allocated in the series (Padial et al., 2014). Currently, there is a gap in knowledge about the vocalisations of frogs in the genus *Ischnocnema*, in which less than 30% have an advertisement call described (Sazima & Cardoso, 1978; Castanho & Haddad, 2000; Giaretta et al., 2007; Oliveira et al., 2008; Conte et al., 2010; Martins & Haddad, 2010; Pombal, 2010; Taucce et al., 2012; Berneck et al., 2013) and only two species have aggressive calls known (Conte et al., 2010; Berneck et al., 2013).

Ischnocnema penaxavantino Giaretta, Toffoli & Oliveira, 2007 is a species from the Cerrado domain and it was previously reported only from the State of Minas Gerais (Giaretta et al., 2007). It is closely related to *I. juipoca* and both may be confounded (Giaretta et al., 2007). *I. juipoca* has been recorded in the Floresta Nacional de Silvânia, State of Goiás, Brazil (Bastos & Pombal, 2001; Bastos et al., 2003; Morais et al., 2012). However, following taxonomy in Giaretta et al. (2007), these specimens may represent *I. penaxavantino*, and not *I. juipoca*. *I. juipoca* and *I. penaxavantino* can be differentiated by size and vocalisation (Giaretta et al., 2007). *I. penaxavantino* is smaller than *I. juipoca*, and its advertisement call is longer, has more pulses, and has higher pulse repetition rate (Giaretta et al., 2007). Herein, we present data about the vocalisations of a specimen collected at the Floresta Nacional de Silvânia, which also allows us to identify specimens in this reserve as *I. penaxavantino*.

METHODS

We studied *I. penaxavantino* (Fig. 1A) at the Floresta Nacional (Flona) de Silvânia (16°39'32" S, 48°36'29" W; about 900 m a.s.l.), Municipality of Silvânia, State of Goiás, Central Brazil. We recorded vocalisations (wav file; 44 kHz; 16 bit) of an *I. penaxavantino* male in April 2009 using a Marantz PMD 660 recorder coupled with a Sennheiser ME66 microphone.

The vocalisations were analysed with Cool Edit 96 and Avisoft-SASLab Lite® software. Frequency information was obtained through Fast Fourier Transformation (FFT) (width, 1,024 points). The audiospectrograms and oscillograms were created in overlap (90%) and Window (Flat Top) with the use of package R 3.0.1, Seewave 1.6.4 (Sueur et al., 2008).

We recorded two types (A and B) of vocalisations emitted by *I. penaxavantino* from Flona de Silvânia. Call descriptions followed Gerhardt (1998), Gerhardt & Huber (2002), and Wells (2007). The following acoustic variables were measured: call duration (s), pulse number (pulse/call), pulse duration (ms), dominant frequency (Hz), time interval between calls (s), and call repetition rate (calls/min). We used a Student's t-test to compare the acoustic parameters (call duration, pulse number, pulse duration, and dominant frequency) of the type A and B calls of *I. penaxavantino* from the Flona de Silvânia. We tested for normality within data and homogeneity of variances prior to statistical analysis using Levene's test and, where necessary, calculated t-tests with separate variance estimates (Zar, 1996). We collected the recorded individual (collecting permit number: 15377-4 / Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio) and housed at the Coleção Zoológica da Universidade Federal de Goiás (ZUFG 5267).

RESULTS AND DISCUSSION

The type A call is similar to advertisement calls (Fig. 1B) described by Giaretta et al. (2007). These calls were emitted at irregular intervals that varied from 15.63 to 149.14 s (45.07 ± 47.88 s; N = 8 calls) in which two calls were emitted per minute (N = 1 male). The amplitude of calls increased gradually. The average pulse per call varied from 15 to 18 (17.16 ± 1.33 ; N = 8 calls) with a pulse duration ranging from 0.014 to 0.032 ms (0.0215 ± 0.008 ms; N = 24 pulses). Call duration and dominant frequency varied, respectively, from 0.63 to 0.845 s (0.793 ± 0.068 s; N = 8 calls) and 3385 to 3564 Hz (3455.83 ± 60.35 Hz; N = 8 calls).

We recorded a second type of call (Type B call) emitted for *I. penaxavantino* males. This vocalisation was emitted in response to other males. The type B call (Fig. 1B) is shorter

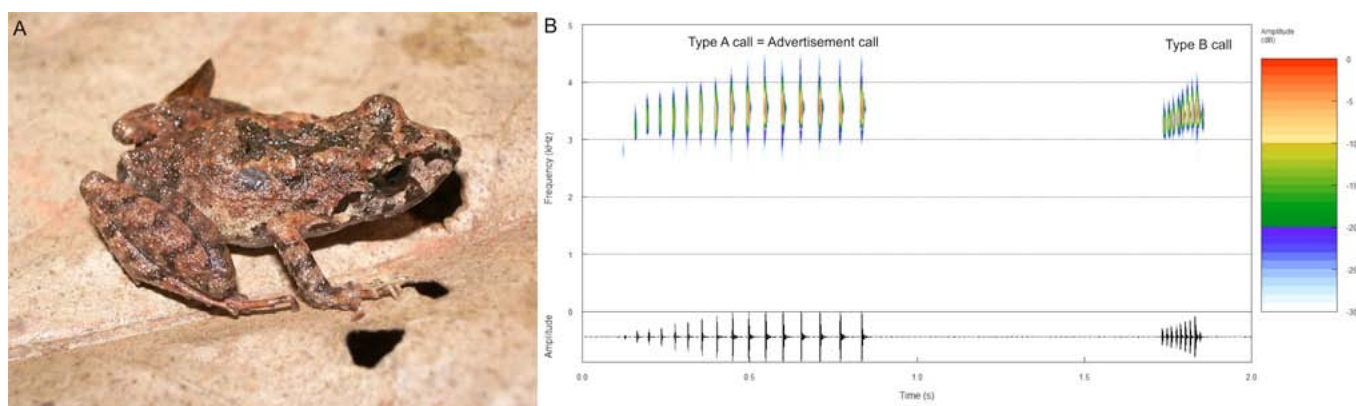


Figure 1. A) *I. penaxavantino* from the Floresta Nacional de Silvânia, Municipality of Silvânia, State of Goiás, Central Brazil. Photograph by Robson Ávila. B) Audiospectrogram (top) and oscillogram (bottom) of the type A (advertisement call) and B calls of *I. penaxavantino* from the Floresta Nacional de Silvânia, Municipality of Silvânia, State of Goiás, Central Brazil. SVL = 15.9 mm; Mass = 0.4 g.

than the type A (advertisement call) with a call duration ranging from 0.108 to 0.147 s (0.127 ± 0.012 s; $N = 8$ calls). This call was also emitted at irregular intervals ranging from 2.013 to 130 s (16.88 ± 42.42 s; $N = 8$ calls). The type B call is composed, on average, of 8.5 ± 0.5 pulses (8–9 pulses; $N = 8$ calls), with a duration ranging from 0.011 to 0.018 ms (0.014 ± 0.002 ms; $N = 32$ pulses). The mean dominant frequency varied from 3246 to 3421 Hz (3359 ± 61.35 Hz; $N = 8$ calls), and the call repetition rate is three calls per minute ($N = 1$ male). We observed that call duration (tsepar. var. = 23.67; $df = 5.25$; $p < 0.001$), pulse number (tsepar. var. = 15.08; $df = 6.22$; $p < 0.001$), and dominant frequency ($t = 2.94$; $df = 12$; $p < 0.05$) are different between the type A and B calls of *I. penaxavantino*. Only the pulse duration (tsepar. var. = 2.15; $df = 5.91$; $p = 0.07$) did not vary between these calls.

The advertisement call of *I. penaxavantino* was described from the type locality, State of Minas Gerais, by Giaretta et al. (2007). The mean values of the acoustic parameters described in this report differ from those presented by Giaretta et al. (2007), but are within the known range for this species. *I. juipoca* was found at the Flona de Silvânia more than 10 years ago (Bastos & Pombal, 2001), but information about vocalisations of individuals in this population was unknown. Giaretta et al. (2007) suggested that such specimens could be assigned as *I. penaxavantino* and also highlighted the importance of requiring bioacoustic analysis.

In this sense, we observed that the dominant frequency and duration of the advertisement call of *I. penaxavantino* from the Silvânia Municipality is similar to that described for *I. juipoca* (0.5 s – Sazima & Cardoso, 1978; 0.7 s – Haddad et al., 1988; 0.51 s, 3300 Hz – Giaretta et al., 2007). However, pulse number seems to be a unique acoustic parameter to strengthen differentiating advertisement calls of *I. juipoca* (10 pulses/call – Sazima & Cardoso, 1978; 9 pulses/call – Haddad et al., 1988; 8 pulses/call – Giaretta et al., 2007) from that within this study (17.16 ± 1.33 pulses/call; range = 15 to 18 pulses/call). Considering these characteristic differences in vocal recordings reinforces our suspicion that specimens of this interesting group found at the Flona de Silvânia are *I. penaxavantino*.

The advertisement calls of some species of the genus *Ischnocnema* have been described in the literature (Castanho

& Haddad, 2000; Giaretta et al., 2007; Oliveira et al., 2008; Conte et al., 2010; Martins & Haddad, 2010; Pombal, 2010; Taucce et al., 2012; Berneck et al., 2013). We observed that the duration of the advertisement call (0.65 to 0.845 s) of *I. penaxavantino* from Flona de Silvânia is lower than that described for *I. hoehnei* (1.3 s – Oliveira et al., 2008), *I. izecksohni* (1.03 to 1.85 s – Taucce et al., 2012), *I. henselii* (20.57 to 26.5 s – Conte et al., 2010), *I. random* (2 to 5 s – Heyer et al., 1990) and *I. guentheri* (9.36 to 13 s – Pombal, 2010). The dominant frequency (3385 to 3564 Hz) of calls in *I. penaxavantino* differed from that described for *I. hoehnei* (2900 Hz – Oliveira et al., 2008), *I. izecksohni* (2250 to 2625 Hz – Taucce et al., 2012), *I. henselii* (2128 to 2501 Hz – Conte et al., 2010), *I. manezinho* (2400 to 2970 Hz – Castanho & Haddad, 2000), *I. sambaqui* (1800 to 2050 Hz – Castanho & Haddad, 2000) and *I. guentheri* (2600 Hz – Pombal, 2010). Herein, we also describe a second type of vocalisation (Type B call) by *I. penaxavantino*. We suggested that this call may have an aggressive function, however further study is necessary to confirm such a hypothesis. In this species, the type B call is shorter than the type A call (advertisement call), with the lowest duration and note number. The aggressive calls of *I. henselii* and *I. nigriventris* have been described in the literature (Conte et al., 2010; Berneck et al., 2013).

In *I. penaxavantino*, the type B call has higher dominant frequency than the aggressive calls of other species of the genus *Ischnocnema* (Conte et al., 2010; Berneck et al., 2013). The call duration (0.108 to 0.147 s), pulse number (8 to 9 pulses/call), and dominant frequency (3246 to 3421 Hz) of the type B call of *I. penaxavantino* differ from those observed for aggressive call of *I. henselii* (0.47 to 0.77 s; 1 to 5 pulses/call; 2214.9 to 3301 Hz, respectively – Conte et al., 2010) and *I. nigriventris* (0.03 to 0.041 s, non-pulsed, 2928 to 3014 Hz, respectively; Berneck et al., 2013).

We conclude that the advertisement call (type A call) of *I. penaxavantino* from the Floresta Nacional de Silvânia is similar to that previously described for this species from the type locality by Giaretta et al. (2007). Furthermore, we observed that pulse number per call is the most important acoustic parameters to distinguish the advertisement call of individuals from Silvânia Municipality referred to *I. juipoca* (Sazima & Cardoso, 1978; Haddad et al., 1988; Giaretta et

al., 2007). Our results allowed us to properly identify species belonging to the *Ischnocema* genus recorded in the Flona de Silvânia, State of Goiás, and also extend, for approximately 260 km, the distribution of *I. penaxavantinho*, which was previously considered restricted to the State of Minas Gerais (Giarretta et al., 2007; Frost, 2014). Finally, this kind of study demonstrates the importance of including vocalizations to differentiate species that are both taxonomically cryptic or similar.

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Epicrates cenchria (Rainbow Boa): Feeding Observation

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The Boid genus *Epicrates* consist of fourteen species belonging to the family Boide, (Wallach, et al., 2014). However, just five species are recognised in the mainland, (Passos and Fernandez, 2008; Rivera, et al., 2011). The Rainbow Boa, *Epicrates cenchria* is a medium-sized boid distributed throughout the entire biogeographic region of the Amazonas, from Colombia, across Venezuela, Guyana, French Guyana, Suriname, Ecuador, Brazil, Peru to Bolivia (Passos and Fernandez, 2008; Rivera, et al., 2011; Wallach, et al., 2014). It is an infrequently encountered terrestrial constrictor that may utilise bushes and low branches when hunting or resting (Savage, 2002). The observations for this species at the Cusco Amazonico show prey including frogs, bird's eggs, bats and rodents as part of their diet, (Duellman, 2005).

Sciurus spadiceus the southern Amazon Red or Black Squirrel, is a large-bodied tree squirrel with an elongated tail, distributed throughout southern Colombia and Venezuela, south through the lowland forest of Peru, Ecuador, western Brazil, and Bolivia, often extending into the foothills of the Andes, (Gwinn, et al., 2012).

On 23 July 2014, between 07:45 – 08:35hrs, we observed an adult *E. cenchria* (ca. 1300mm total length predated on a *S. spadiceus*, (Fig. 1), in the Pacaya Samiria National Reserve at the PV2 (Puesto de Vigilancia 2), (04° 52' 26.0" S, 74° 21' 42.8" O, 103m above the sea level), located in the Loreto

Region on the Ucayama depression, at the confluence of the Marañon and Ucayali rivers, Peru.

Previous studies documented few prey items: Bernarde and Abe, (2010) studying the feeding habits of snakes in the Spike West in Rondônia, Brazil, registered just unidentified fragments of rodents; Palmuti, et al., (2009) reported the presence of spines of a porcupine in the stomach of one individual of this species, whilst Ramos-Donato, et al., (2012) reported predation of a bat (*Lonchorhina aurita*). Field observations of feeding in *E. cenchria* are limited, but future studies will hopefully increase our knowledge of the dietary habits in nature.

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Figure 1. Adult *E. cenchria* (ca. 1300 mm approximate SVL) predated on a *S. spadiceus* in the Pacaya Samiria National Reserve, Peru. Photograph: D Bowman.



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Crab predation by the San Salvador rock iguana (*Cyclura rileyi*)

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The San Salvador Rock Iguana, *Cyclura rileyi* (Stejneger, 1903) is a species that is endemic to the Bahamas. It is estimated that these iguanas now occupy only 0.2% of their historical range. Currently, populations are confined to small offshore cays and islets (Hudson & Alberts 2004). *C. rileyi* have few natural predators, hence the introduction of dogs, cats, and rats has effectively extirpated them from larger islands and relegated populations to small cays and islets.

The diet of *C. rileyi* is predominantly of plant material, and studies on closely related species of *Cyclura* indicate that their diet is 95% herbivorous (Iverson 1979, Auffenberg 1982, Hines 2011). The two most frequent items found in *C. rileyi* scat were *Borrhichia arborescens* (Tree Seaside Tansy) and *Rhachicallis americana* (Seaside Rock Shrub) (Cyril 2001). There have been few recorded instances of *C. rileyi* exhibiting carnivorous feeding behaviour. Using scat analysis, Buckner and Thomas (2005) identified several putative cases of carnivory including the remains of a dead bird, conspecific hatchlings, legs of a dead land crab, a grasshopper, hermit crab, and various unidentifiable insects. Due to the nature of scat studies, the method of prey capture is unknown and thus, it is unclear if animal remains recovered from scat represent actual predation or scavenging by *C. rileyi*. These findings are also reflected in *C. nubile* in Cuba, where crab was also found in scat samples (Beovides-Casas & Mancina 2006).

Here, we present a novel observation of *C. rileyi* consuming *Grapsus grapsus* (Sally Lightfoot Crab) in the Bahamas. Though the presence of other crustaceans have been detected in scat, there have been no previous observations of the pursuit, capture, and consumption of a live crustacean by *C. rileyi*. Our observations bring to light the need for further studies on the dietary patterns of this critically endangered species. During a daytime hike, on 3 April 2014, we witnessed an adult *C. rileyi* pursue, capture, and consume a single *G. grapsus* (3-4 cm carapace width) on a large (>4m wide) rock in the high intertidal on Manhead Cay (N 24.125503, W -74.449191), off the island of San Salvador, Bahamas. In order to capture the prey, the iguana dashed and grabbed the crab with its mouth, as the crab attempted to evade the iguana by running sideways. After capture, the crab was consumed in less than a minute.

The observation reported here represents the first published record of *C. rileyi* pursuing, capturing, and consuming *G. grapsus*, a common rocky intertidal crab species. With the limited data available we cannot make sweeping predictions, but the observation of *C. rileyi* employing rover-type predation, which requires great agility and practice, alludes to the possibility that carnivory is a larger portion of the diet of *C. rileyi* than currently realised. Predation rather than

consuming plants represents a novel ecological niche for *C. rileyi* and dictates the development of novel adaptations for successful prey capture. Moreover, given the agility and speed of *G. grapsus*, the successful capture of a crab indicates that this iguana likely practiced this hunting behaviour previously and that this behaviour may be prevalent among other individuals.

Active predation could simply be an example of opportunistic carnivory, a tactic not uncommon among other organisms. Clearly, there is much to be learned regarding the use of active predation by *C. rileyi*. Future studies should concentrate on the importance of dietary subsidies for *C. rileyi*, particularly diet items that are marine in origin as these resources may provide a substantial source of energy that is free from the detrimental effects of human activities present in terrestrial habitats.

We thank Fred Diehl for his wisdom and guidance throughout our time in San Salvador. Also, we thank Gerace Research Centre and Duke University Marine Lab for their logistical support. Special thanks to James Nifong, Jennifer Chan, Robb Gaskins, and Chris Dee for their comments and input on the manuscript.

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Hemidactylus flaviviridis (Reptilia: Gekkonidae): Predation on Congeneric *Hemidactylus frenatus* in Dhaka, Bangladesh.

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Nine species of the family Gekkonidae have been reported from Bangladesh, including 6 species of the genus *Hemidactylus* (Chakma, 2009). These geckos are usually arboreal, ground and rock dwellers while some show commensalism, living in close association with humans. Geckos are mainly insectivores with exception of a few herbivores and carnivores while they are preyed upon by a variety of animals (Daniel, 2002). House geckos resemble each other very closely in habits. Among these *H. flaviviridis* is the largest having a total length of about 180 mm, typically living in close association with the human and human-made structures both in urban and rural areas (Chakma, 2009). The main food of *H. flaviviridis* is insects including caterpillars, but sometimes they eat any animal they can overcome, including other geckos and smaller snakes (Bauer 1990). Smaller insects are swallowed whole while larger prey are battered to death, to a manageable softness and eaten; they generally avoid dangerous insects and occasionally show cannibalism (Sahi, 1916; Polis & Myers, 1985). With a total length of 125 mm *H. frenatus* occupies a variety of habitats including trees, stones and human habitations (Chakma, 2009). This species is exclusively nocturnal (Chakma, 2009; Daniel, 2002). Here we report an instance of *H. flaviviridis* preying upon the congeneric *H. frenatus*.

On the 1st September 2014 at 20:35 hrs we observed *H. flaviviridis* attack a *H. frenatus* on a wall at about 9.144 m above the ground at Chunkutia (23.6973873° N, 90.3887799° E) in Keraniganj district, Dhaka, Bangladesh. Both species were identified using the identification key provided by Mahony (2011). The *H. flaviviridis* seized *H. frenatus* at the neck and hit it against the wall three times then remained motionless for about 20 seconds. It gradually moved its jaws around the neck towards the head always maintaining a secure grip on the prey then began to swallow it head first (Fig. 1). At that time the first author took a photograph using flash, which frightened the *H. flaviviridis* causing it to abandon the *H. frenatus* and take refuge in the darkness behind a table. This also frightened the half dead *H. frenatus*, which also ran for shelter behind the table but seized again by the *H. flaviviridis*. The *H. frenatus* attempted to escape by kicking on the predator's head with its hind limb but the *H. flaviviridis* firmed its bite and again remain motionless for about 1 minute and 25s until the *H. frenatus* became weak. The *H. flaviviridis* began to swallow the unfortunate *H. frenatus* head first which took 2 m 53s to consume. After feeding, *H. flaviviridis* moved towards the darker side of wall behind the table.

There are very few records of *H. flaviviridis* feeding on vertebrates and this is the first record of a *H. flaviviridis* feeding on another gecko in Bangladesh. However, several



Figure 1. *H. flaviviridis* preying on *H. frenatus*

authors, for example Sumithran (1982), Dattarti (1984), Kannan & Krishnaraj (1988) and Somaweera (2005) reported *H. leschenaultii* preying on a rat, gecko, skink and a colubrid snake whilst Karunarathna & Amarasinge (2011) recorded *H. hunae* preying upon *Bandicoota bengalensis* (Mammalia: Rodentia).

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Terrapene carolina carolina (Eastern Box Turtle): Railroad nesting

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Terrestrial turtle, *Terrapene carolina carolina* occupies a broad range throughout most of the eastern United States (Ernst & Lovich, 2009). *T. c. carolina* generally seek out dry, open upland habitat with loose, sandy soils in which to construct nests (Hulse et al., 2001; Wilson & Ernst, 2008). *T. c. carolina* populations occurring within Pennsylvania typically nest in early June through July (Hulse et al., 2001). On June 4, 2009, at approximately 16:00 h (United States Eastern Standard Time) the author observed two small groups of female *T. c. carolina* located along a railroad track bordered by woodland habitat near Espy, Columbia County, Pennsylvania USA (41°0'29.2"N, 76°25'24.3"W; WGS84 grid; 150 m elevation). Groups consisted of two and six individuals, respectively. Both *T. c. carolina* groups were watched for approximately five minutes at a distance of three meters. Individuals in both groups were observed exhibiting preliminary nesting behaviour by moving substrate associated with the railroad bed with their hind limbs to excavate a nesting cavity (Hulse et al., 2001). Oviposition was not observed, and it is unknown if nesting was completed or if potential nests were successful.

T. c. carolina have been reported to utilise anthropogenically-impacted habitats such as clearings, fields, and unpaved roadways for nesting (Flitz & Mullen, 2006; Wilson & Ernst, 2008). Additionally, nesting observations associated with railroad beds have been reported in several aquatic and semi-terrestrial turtle species including *Chelydra serpentina*, *Chrysemys picta*, *Clemmys guttata*, *Glyptemys insculpta*, *G. mühlenbergii*, and *Trachemys scripta* (Harding & Bloomer, 1979; Hulse et al., 2001; Ernst & Lovich, 2009). However, observations of *T. c. carolina* nesting in railroad substrate are rare, and this account of *T. c. carolina* nesting groups associated with railroad bedding appears to be unique. The area in which this observation occurred consists primarily of forested and wetland habitat that have been substantially fragmented by roadways and human development. In this location, female *T. c. carolina* may have selected railroad bed substrate to nest when other suitable habitat became unavailable. Railroad beds may be important for *T. c. carolina* populations, as these areas contain open spaces and loose substrate required for *T. c. carolina* nesting (Flitz & Mullen, 2006; Wilson & Ernst, 2008). However, *T. c. carolina* nests that occur in other anthropogenically-influenced habitats (e.g. unpaved roads and clearings) experience high rates of depredation (Flitz & Mullen, 2006).

Railroads in general appear to contribute to mortality or otherwise negatively affect a variety of vertebrate species (Heske, 2015). Negative effects associated with railroad beds have been reported in populations of several turtle species,

including *Clemmys guttata* and *Gopherus agassizii* (Ernst & Lovich, 2009). *T. c. carolina* individuals have a high risk of becoming entrapped between rails on railroad beds and expiring from overheating or other causes (Kornilev et al., 2006), as do other turtle species (Iosif, 2012). Studies are needed to examine *T. c. carolina* nesting frequency and success along railroad beds, particularly within fragmented or otherwise anthropogenically-influenced habitats.

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Lampropeltis getula californiae (California kingsnake): Juvenile aggregation

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At approximately 1515h on 16 November 2014, four juvenile *Lampropeltis getula californiae* were observed aggregated in a cluster on the north side of the lower Pine Ridge Trail in the Ventana Wilderness of the Los Padres National Forest near Big Sur Station, California (within 1 kilometer of 36.247686°N, 121.751531°W, datum: WGS84, elev. = between 400-500 m) (Fig. 1).

The snakes moved slowly over each other while tongue-flicking. The individuals appeared very similar in length (total length 33-38 cm), which is similar to total hatchling length previously reported for this species (Tu et. al., 2002) of 32.6cm mean total length at hatching to 35.9cm mean total length at 25-78 days after hatching. Thus, these snakes were presumably recent hatchlings, though it is impossible to determine if they were clutchmates. Size-assortative aggregation has been reported previously in snakes (Gregory, 2004), though documentation of this behaviour in this species was not found in the literature.

The snakes were found on a steep northwest-facing slope, which had a high degree of shrub and tree cover. The sky was mostly clear. Ambient air temperature was 15-20°C at 1-2m height above ground. The snakes were in a location that likely received direct sunlight for much of the afternoon. Perhaps

the snakes were basking in sunlight, and then aggregated as the sun dropped behind the hills. Such aggregation might very briefly retard heat loss as the sun set, but is unlikely to result in significant heat retention, especially for such small snakes.

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Figure 1: Aggregation of *L. g. californiae* (California kingsnake). Photograph by Michael Powers.

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