Diet of invasive clawed frog *Xenopus laevis* at Lage stream (Oeiras, W Portugal)

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We present data on the trophic ecology of *Xenopus laevis*, introduced in two streams at Oeiras, Western Portugal. During 2007, we captured and euthanized 70 individuals during two field seasons (July and September) in one stream. The diet included a wide variety and size range of mainly benthic prey, with water snails (Physidae) being the most important. *Xenopus laevis* also preyed on native fish and amphibians. There were differences in the diet between sexes (males showing higher prey diversity). Our results demonstrate the ability of this exotic amphibian to adapt to a wide range of conditions.

Key words: amphibian, invasive species, Mediterranean climate-type region, trophic ecology

Human-aided vertebrate introductions have occurred all over the world, and are considered a threat to biodiversity and ecosystem functioning (Mooney & Hobbs, 2000; Simberloff & Rejmanek, 2011). Among the introductions of amphibians, those of the anuran *Xenopus laevis* (Daudin, 1802) were associated with its use in human pregnancy diagnosis, as a laboratory animal, and in the pet trade (Weldon et al., 2007). As a consequence, wild populations of *X. laevis* live today at wide latitudinal ranges and in different habitats (Tinsley & McCoid, 1996; Measey & Tinsley, 1998; Solís, 2004; Fouquet & Measey, 2006; Faraone et al., 2008; Rebelo et al., 2010).

The diet of *X. laevis* includes a large taxonomic range of prey, with predominance of aquatic invertebrates, suggesting that this species is a non-selective predator (Measey, 1998a; Lobos et al., 1999). There are concerns over predation pressure on native invertebrates and even vertebrates by introduced populations of *X. laevis.* Lafferty & Page (1997) found the endangered tidewater goby *Eucyclogobius newberrui* in the gut contents of *X. laevis* inhabiting the estuary of the Santa Clara River, California. Crayon (2005) provides a review of documented fish predation by *X. laevis* in California. The aim of this study was to analyze the diet of adult *X. laevis* at Oeiras, Portugal, where the species was discovered in 2007; the introduction is believed to result from an escape of a laboratory stock after a disastrous flood in 1979 (Rebelo et al., 2010).

We conducted our survey on Lage stream, located in Oeiras, about 20 km west of Lisbon. The study area covers approximately the last 11 km of this stream, which flows through shrubland, farmed areas, suburban areas and city gardens. Stream flow is intermediate, with stronger flow during winter. In summer, water only remains in a few pools along the stream. Individual *X. laevis* were captured during July and September 2007 in the three pools where the species was found in 2007 (Rebelo et al., 2010). Frogs were caught using the electrofishing set SAMUS-752GN (PDC), applying a 30 Hz frequency; electrofishing was carried out over two or three 15 minute periods, separated by 10 minutes. The frogs were euthanized by quick freezing at -40 ºC within 2 hours of capture and transported to the laboratory where they were measured (snout-urostyle length (SUL) to the nearest mm). Stomach contents were extracted, weighed to the nearest 0.1g and preserved in 75% alcohol. Sex was determined by direct observation of the gonads. The specimens are deposited at the Museu Nacional de História Natural (MNHN Lisbon; specimen codes MB04-408 to MB04-478).

All prey items were identified whenever possible with the help of a stereomicroscope, after which each prey type was weighed to the nearest 0.01g. Although prey determination was carried out at the lowest taxonomic level possible (species or genus), the results are expressed by grouping prey into higher taxa to facilitate comparisons.

As a measure of trophic niche amplitude we used the standard index of Levins (Krebs, 1999):

$$B_{sta}=B\text{-}1/\,B_{max}\text{-}1$$

where B is the Levins index and B_{max} is the total number of diet elements. The standardized index varies between 0, for specialized diets, and 1, for generalist diets. Diet composition was also expressed as percentage of biomass of each prey type (% B) for all stomachs containing prey items sufficiently intact to allow correct weight estimation.

Our study encompasses 70 *Xenopus laevis* (27 females and 18 males caught in July, and 3 females and 22 males caught in September). All but two stomachs contained at least one identifiable prey item. Many stomachs contained detritus, microalgae and some debris.

Benthonic fauna were the most frequent component of the diet (54.3%), especially Physidae water snails (41.1%). Nekton macro-invertebrates comprised 34.4% of the total prey consumed. Terrestrial prey (mostly insects) contributed with 4% to the total prey. The small value of the standardized Levins index (B_{sta}=0.1422) indicates a diet based on a few basic prey and on a wide group of occasional prey. The small value is similar to that of demersal fishes (Labropoulou & Eleftheriou, 1997) and tropical hylids with specialized diets (Macale et al., 2008), and is rather uncommon in large amphibians (Vignoli et al., 2009).

Water snails constituted the most important prey biomass, followed by the invasive American crayfish *Procambarus clarkii* (the only Crustacean present at the stream). Vertebrates, including *Rana perezi* skeletons and egg masses were the second most important prey group in terms of biomass (Table 1). Fish remains were in an advanced stage of digestion, and identification was possible for only 3 out of the 11 remains; these were identified as *Cobitis paludica*, a vulnerable Iberian

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Table 1. Analysis of prey items recovered from stomach of 70 *Xenopus laevis* caught at pools on Lage stream, considering the total contribution of prey types: (a) terrestrial invertebrates, (b) vertebrates, (c) benthonic invertebrates, (d) nektonic macro-invertebrates, (e) *X. laevis skin*, (f) vegetation and (g) stones. Estimates are based on the sum of all sampling dates. Identification was conducted to the lowest taxonomic level possible. %*B* – percentage of biomass of each prey type; we only considered the stomachs containing prey items sufficiently intact to allow weight estimation.

Prey	Total	% *X. laevis* eating (*n*=70)	mean number ingested per individual	% composition	%*B* (*n*=27)
a) Terrestrial invertebrates					
Unindentified Diptera	8	3	4	1	0
Muscidae	6	4	2	1	0.4
Coleoptera					
Carabidae	3	4	1	0	0
Hymenoptera					
Formicidae	3	1	3	0	0
Agriotypidae	1	1	1	0	0
Vespidae	1	1	1	0	0
Ichneumonidae	1	1	1	0	0
Hemiptera	1	1	1	0	0
Heteroptera	1	1	1	0	0
Chilopoda	1	1	1	0	0
Unidentified	2	3	1	0	0
Sub total	28	21	17	2	0
b) Vertebrates					
Amphibian skeleton	7	7	1.4	1	-
Amphibian eggs	1	1	1	0	-
Fish skeleton	11	7	2.2	1	-
Unidentified fish eggs	3	3	1.5	0	-
Bird feather (unidentified)	1	1	1	0	-
Sub total	23	19	7	2	15
c) Benthic macro-invertebrates					
Nemathelminthes	79	24	4.35	11	1
Platyhelminthes	5	6	1.25	1	0.4
Annelida					
Achaeta	3	4	1	0	0.4
Molusca					
Physidae	307	51	9.14	41	34
Ancylidae	1	1	1	0	0
Crustacea					
Decapoda	6	7	1.2	1	5
Unidentified	4	6	1	1	0
Sub total	405	99	19	55	40
d) Nekton macro-invertebrates					
Cestoda	4	6	1	1	0
Unidentified Coleoptera	1	1	1	0	3
Dytiscidae	3	4	1	0	0
Unidentified Diptera	1	1	1	0	0
Chironomidae (larvae)	124	31	5.64	17	2
Chironomidae (pupa)	8	4	2.67	1	-
Plecoptera (larvae)	96	51	2.67	13	2
Ephemeroptera	3	4	1	0	0
Unidentified Trichoptera	1	1	1	0	0
Hydroptilidae	1	1	1	0	0
Unidentified Hemiptera	1	1	1	0	0
Gerridae	1	1	1	0	0
Heteroptera	2	3	1	0	0
Unidentified Hexapoda	3	4	1	0	0
Crustacea					
Ostracoda	8	7	1.6	1	0.4
Sub total	257	120	23.58	33	7
e) *X. laevis* skin	16	23	1	2	0
f) Vegetation	11	16	1	2	0
g) Stones	6	4	2	1	0

endemic (Crivelli, 2006) that is abundant at Lage stream. Finally, one single feather (species unidentified) was found in one stomach.

The diet differed between sexes: males consumed more Chironomidae than females, which in turn fed mostly on Physidae. Females fed on Physidae (47% of the diet), Plecoptera (21%) and Chironomidae (10%) in July, while the September sample was too small to yield reliable results. Males consumed the same amounts of the different prey types in both seasons - Chironomidae (26% July, 25% September), Physidae (33% July, 31% September) and Nemathelminthes (23% July, 32% September). The proportion of ingested skin was similar in males (53%) and females (47%). The percentage of stomachs containing sloughed skins was higher in July (63% July, 37% September).

The diet of *X. laevis* appears to include all macroscopic animals available in the aquatic environment (Tinsley et al., 1996), as they are generalist predators that consume a wide variety and size range of invertebrate prey (Measey, 1998a, 1998b). The diet consistently showed a predominance of benthonic invertebrates, both in number and in biomass. These results are similar to those of other studies on *X. laevis*' diet throughout its natural and non-natural range (McCoid & Fritts, 1980; Tinsley & McCoid, 1996; Tinsley et al., 1996; Measey, 1998a; Lobos et al., 1999; Lobos & Jaksic, 2005; reviewed by Crayon, 2005). The main prey items found in this study were similar to what was observed in Chile (Lobos, 2004). The low trophic niche amplitude probably reflects prey availability, which was not evaluated due to methodological constraints. The poor water quality (Câmara Municipal de Oeiras, unpublished data) likely leads to an impoverished prey community, dominated by faunal groups tolerant to non-oxygenated waters, similar to what was observed in Chile (Lobos, 2004).

Measey (1998a) states that the ability of *X. laevis* to catch a range of nektonic prey implies that fish may be consumed. *Eucyclogobius newberrui* was found in the gut contents of *X. laevis* inhabiting an estuary in California (Lafferty & Page, 1997), and there are reports about the occurrence of birds and amphibians in the diet of clawed frogs (Tinsley et al., 1996; Solís, 2004; Crayon, 2005). This conflicts with the conclusions of McCoid & Fritts (1980) that *X. laevis* is a clumsy predator adapted to capturing sessile or slow-moving items. The predation upon vertebrates was confirmed in the present study. Cannibalism in *X. laevis* is well reported in the literature, being an important attribute for the success of invasive and native populations (McCoid & Fritts, 1993). No evidence of cannibalism was found in the present study, but we cannot affirm that it does not exist, as the tadpoles consist almost entirely on easily digestible soft tissues. It is considered unlikely that the large amount of terrestrial prey reported in *X. laevis* stomach contents - up to one quarter of the total prey (Measey & Tinsley, 1998) - originates solely from invertebrates which have fallen or been swept from overhanging vegetation into the water (Tinsley et al., 1996), and *X. laevis* is known to be able to capture prey on land (Measey, 1998b). Evidence for terrestrial foraging was however not found in our study, where only 4% of all ingested prey items were of terrestrial origin. It is not known how often *X. laevis* slough their skin (Measey, 1998a). In this study we found a high frequency of sloughed skins in stomachs in July, which coincides with the onset of mating (Tinsley et al*.,* 1996; Tinsley & McCoid, 1996). No difference was found between males and females, suggesting that the frequency of sloughing is the same for both sexes.

Eradication of invasive populations of *X. laevis* in Mediterranean climates is considered to be difficult, and only a few management or control operations are in place to date (Fouquet & Measey, 2006; Rebelo et al., 2010). Similarly to the studies in California, UK and Chile (Lobos & Measey, 2002), we confirmed the predation by clawed frog on eggs and adults of native amphibians, as well as on native fish at our study site. Furthermore, the Lage and adjacent streams are home to *Iberochondrostoma lusitanicum,* an endemic and Critically Endangered small fish that may be preyed upon by *X. laevis*. While predation upon endemic vertebrates seems infrequent and possibly has a low impact on their populations, there is the possibility of expansion of the clawed frog to nearby protected areas (Rebelo et al., 2010), where the vulnerability of less disturbed ecosystems may be higher, leading to a situation such as found in Chile (Lobos & Jaksic, 2005) or Sicily (Faraone et al., 2008). Our population of *X. laevis* has a relatively small distribution in a contained basin (Rebelo et al., 2010), and may be a threat to native species (this study). That the invasive population should be the subject of a control program (Genovesi & Shine, 2003) was recognized by the Portuguese Governmental Institute for Nature Conservation, and a 5-year eradication program was initiated in 2010.

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