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The large invasive population of *Xenopus laevis* in Sicily, Italy

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Abstract. The worldwide spread of invasive species is considered to be one of the main causes of global amphibian declines and the loss of biodiversity in general. The African Clawed Frog, *Xenopus laevis*, shows a strong ability to establish populations and invade various geographic regions. In 2004 *X. laevis* was found in Sicily for the first time. The Sicilian population is probably the largest in Europe with a range of about 225 km^2 in an area characterized by numerous agricultural ponds. This high density of ponds has potentially facilitated the dispersal of *X. laevis*. The frogs can move far from rivers or watercourses by utilizing the ponds as suitable "islands". The analysis of their diet shows that the aquatic larvae of nektonic insects comprise the major portion in terms of mass while small planktonic crustaceans are the most numerous component. There is a significant difference between the diet of adults and juveniles.

Keywords: Amphibians, diet, distribution, invasive species, Sicily, stomach flushing, *Xenopus laevis*.

Introduction

It is well-known that the introduction of non-native anurans in natural ecosystems can have deleterious effects on native species. One of the most notorious examples is the frog, *Rana catesbeiana*, a native of Eastern USA but naturalized across North America (Pearl et al., 2004), Mexico, the Caribbean, Hawaii, Japan (Toshiaki, 2004) and Europe, including Italy (Lanza, 1962; Ficetola et al., 2007). *R. catesbeiana* can cause the decline of autochthonous amphibian populations through competition for resources or through direct predation (Kats and Ferrer, 2003). Other invasive species, such as *Bufo marinus*, can be a cause of mortality to predators due to the presence of deadly toxins (Phillips et al., 2004) or non-native anurans can spread diseases like *Batrachochytrium dendrobatidis* as in the case of *R. catesbeiana* and *Xenopus laevis* (Weldon et al., 2004; Garner et al., 2006).

Because of the high demand for *X. laevis* as a laboratory model, it is one of the most widespread amphibians in captivity (Gurdon, 1996). This use has contributed to the establishment of several allochthonous populations around the world. These have been particularly successful in areas with a Mediterranean climate. *X. laevis* is a pipid frog native to sub-Saharan Africa. The species shows many adaptations to aquatic life, including the retention of the lateral line system after metamorphosis, aquatic chemoreceptors (Elepfandt, 1996a, 1996b; Elepfandt et al., 2000) and a body structure particularly adapted for swimming (Videler and Jorna, 1985). In its natural habitat *X. laevis* lives in stagnant or still waters in ponds or sluggish streams, but may also inhabit fast-flowing water (Tinsley et al., 1996). Non-native populations of *X. laevis* are known in Arizona and California (Crayon, 2005), Ascension Island (Tinsley and McCoid, 1996), Chile (Lobos and Measey, 2002), France (Fouquet, 2001) and Wales (Measey and Tinsley, 1998). Recently *X. laevis* has been found in an area of north-western Sicily (Lillo et al., 2005).

Since the discovery of the Italian population of *X. laevis*, the need for an in-depth investigation on the possible consequences of this introduction has become evident. Although the ecological relationship between this pipid frog and the native amphibians is not clear, its biological features cause marked concern since *X. laevis* has biological features very similar to predatory fish, and predatory fish can, at least,

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strongly affect amphibian populations (Hecnar and M'Closkey, 1997). Therefore the large area occupied by *X. laevis* in Sicily and its potential to disperse further are a cause of great concern.

The aims of this study are: to assess the effective size of the Sicilian range of distribution of this species; to investigate potential dispersal patterns with regard to landscape characteristics; and to analyze its diet. This information is particularly important in order to begin to understand the impact of *X. laevis* on the local amphibian communities and to plan adequate counter-measures against this biological invasion.

Materials and methods

An investigation area to determine the Sicilian range size and distribution of *X. laevis* was identified from the sites where the presence of this species was already known (Lillo et al., 2005). This area extends for about 300 km^2 around the first site where *X. laevis* was reported. It is mainly agricultural land cultivated with vineyards, olive groves and cornfields. The area is situated in the catchment basins of the Belice Destro and Jato Rivers where there is a large reservoir (Poma Lake) and hundreds of agricultural ponds with surface areas ranging between 100 and 2000 m^2. There are no agricultural channels or temporary flooded fields in the area and therefore *X. laevis* does not have any obvious dispersal corridors except along the rivers.

An agricultural pond near Poma Lake (37°58′55″N-13°07′21″E) was selected for the diet study. It is located at 240 m a.s.l. with maximum dimensions of 20 × 35 m and a depth of about 3 m. The pond is partially encircled by a thin cane-brake and the aquatic vegetation is dominated by Characean algae and *Potamogeton pusillus.* We detected *Rana* synklepton *hispanica* and *X. laevis* in this pond whereas in the neighbouring ponds we also detected *Discoglossus pictus*, *Hyla intermedia*, *Bufo bufo* and *Bufo siculus* (see Stöck et al., 2008).

The study area was sampled from March to July 2005 to verify the presence of *X. laevis*. The search was carried out by visual observation with the aid of dipnets (McCoid et al., 1993), *X. laevis* was considered to be present if adults, tadpoles or spawn was found. The ponds were located with 1 : 10 000 maps (*Cartografia Tecnica Regionale* of Regione Siciliana), aerial and satellite photos. The ponds that were sampled, all located in private land, were selected on the basis of their accessibility, taking care to cover the widest possible area starting from the areas where the presence of *X. laevis* was known. To estimate the size of the distribution area we used the Minimum Convex Polygon (MCP) method, given the fact that the investigated area is homogeneous for physical and biotic characteristics. For the diet study four bi-weekly samplings were carried out in the sample pond between 4 March and 21 April 2006. A plastic dredge measuring 100 × 50 × 100 cm attached to a short rope was quickly dragged through the pond to catch *X. laevis*. This method was preferred to the use of funnel-traps in order to avoid the influence that these could have on diet composition. The use of funnel-traps can induce unnatural feeding behaviour in *X. laevis*, due to the concentration of frogs and prey attracted by the bait used (see Tinsley et al., 1996; Crayon, 2005). Nevertheless dragging must be performed with caution as this method can damage underwater vegetation and, possibly, the spawn of autochthonous amphibians.

The snout-vent length (SVL) of captured specimens was measured with a digital calliper (to the nearest 0.01 mm). To avoid bias due to the conformation and functionality of the pelvis of *X. laevis* (see Videler and Jorna, 1985) the measurements were taken while keeping the frogs with their femurs arranged perpendicularly to the sagittal plane and gently pressing the frogs in a dorsoventral direction. The adults of *X. laevis* were sexed through the secondary sexual characteristics: a protruding cloaca in females and the presence of nuptial pads on the forearms of males. We considered as juveniles any specimens with a SVL less than the minimum length observed in males with nuptial pads, considering that, in general. both sexes reach sexual maturity at the same size (McCoid and Fritts, 1989). The stomach contents were obtained by the stomach-flushing method (Measey, 1998; Solè et al., 2005) using a 50 ml syringe with a 2 mm diameter catheter and suitably filtered water from the pond. The stomach contents were separated and immediately preserved in 90% ethanol and subsequently analyzed in the laboratory with the aid of a steremicroscope. The prey mass was estimated as the mean of the humid weight in alcohol of a known number of specimens of potential prey homogeneous for species, weighed using an electronic precision balance. The weighed invertebrates were captured in the pond using a hand-net with a mesh of 500 μm fixed to a collection jar.

The identified prey was separated into five major types (see Measey, 1998; Lobos and Measey, 2002): Plankton, Benthos, Necton, Terrestrial and *Xenopus*. For the analysis of the diet we calculated: i) the percentage of *X. laevis* eaten items, ii) the percentage of prey occurrence in stomach, iii) the percentage of prey biomass, iv) the mean of items of each taxon ingested from all specimens of *X. laevis* and v) only from specimens eating a specific prey type.

The comparison between the percentages was carried out through the χ^2 test. The comparison between the means was carried out through the t-test.

Results

A total of 631 ponds were counted in the investigation area. Of these 96 were inspected and *X. laevis* was found to be present in 52 ponds (54.2%), and absent in the remaining 44

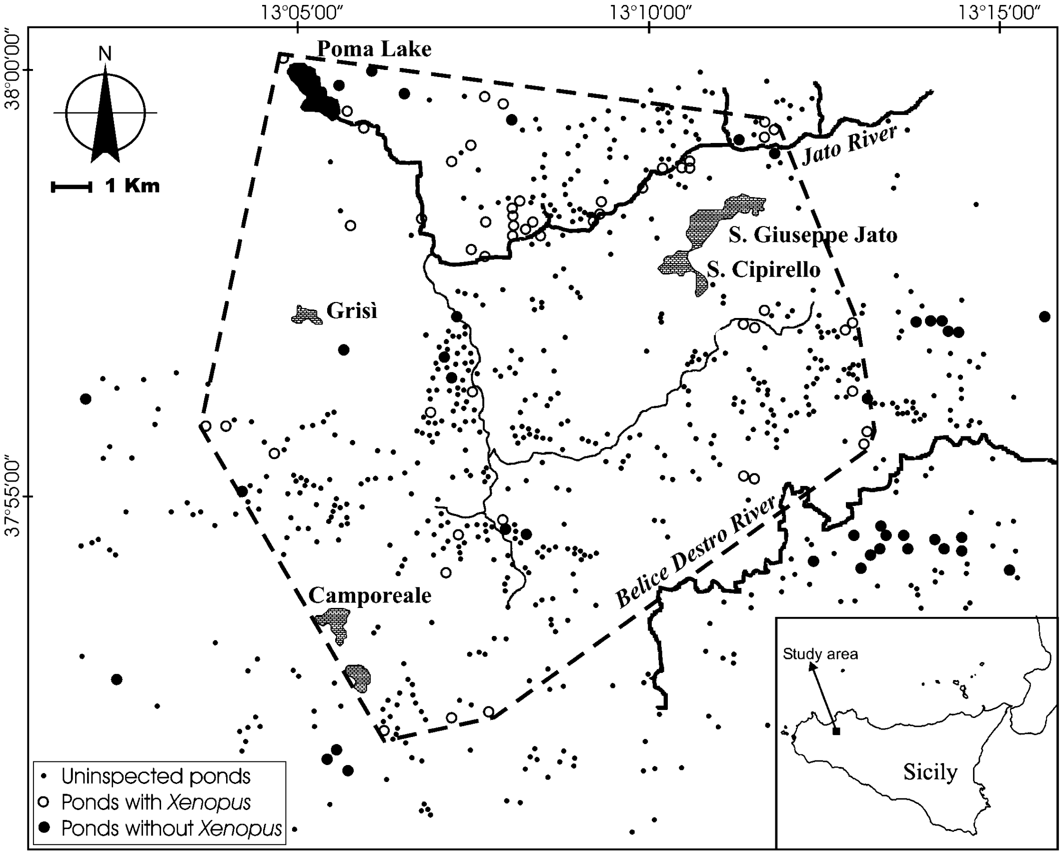

Figure 1. The distribution of *Xenopus laevis* in Sicily. The dotted line represents the species' range estimated with Minimum Convex Polygon (MCP).

(45.8%). The present range of distribution falls in the catchment basins of the Belice Destro and Jato Rivers with an extension of about 225 km^2 from the Jato River valley (North bound) to Camporeale (South bound) (fig. 1). It seems likely that the species has not (yet) colonized the ponds to the south of the Belice Destro River. We found *X. laevis* in ponds quite far from flowing water (maximum distance = 7.2 km). The most isolated pond containing *X. laevis* is about 480 m away from the nearest neighbouring pond.

The use of the dragging method to capture *X. laevis* proved to be very effective. In fact we caught a maximum of 23 specimens with a single dragging. Moreover, the use of dragging in late winter and at the beginning of spring appears to cause significantly less damage since the aquatic vegetation is not fully developed. During the four samplings at the focal pond, 80 specimens were captured, 22 adults (7 males and 15 females) and 58 juveniles. The sex ratio results showed a female bias ($M : F = 1 : 2.1$).

Concerning the diet study, of the 80 specimens captured, 59 (21 adults and 38 juveniles) provided stomach contents. Tables 1 and 2 show the list of the identified taxa with the frequencies for both age classes. The percentage of dietary items shows that planktonic items are the most numerically abundant prey.

As well as skin ingested during the shed, cannibalism has been confirmed by the presence of *X. laevis* eggs and larvae in stomach contents (Tinsley et al., 1996). Eggs and larvae were first found in stomach contents during the third sampling (3 April). This was the first time in

Table 1. Analysis of prey items of 21 adults of *Xenopus laevis* caught between 4 March and 21 April 2006 at a pond near Poma Lake (Province of Palermo, Sicily). * mean of items of each taxon ingested from specimens eating a specific prey type.

Prey categories		Stage	% of *X. laevis* eaten items ($n = 1849$)	% of prey occurrence in stomachs ($n = 21$)	Mean ± SE of ingestion*	Mean ± SE of ingested items per stomach ($n = 21$)	Range	% of total prey biomass (2.421 g)
PLANKTON			**82.5**	**76.2**	**95.3 ± 49.0**	**72.6 ± 38.1**	**1-780**	**3.896**
Crustacea	Calanoidea		10.6	23.8	39.2 ± 21.8	9.3 ± 6.1	2-109	0.211
	Cyclopoidea		15.9	38.1	36.8 ± 21.1	14.0 ± 8.7	2-178	0.317
	Cladocera		55.5	71.4	68.5 ± 32.6	48.9 ± 24.0	1-493	3.138
Insecta	Chaoboridae	larvae	0.4	28.6	1.3 ± 0.2	0.4 ± 0.2	1-2	0.231
BENTHOS			**7.5**	**66.7**	**9.9 ± 2.9**	**6.6 ± 2.2**	**1-40**	**4.065**
Insecta	Chironomidae	larvae	6.8	52.4	11.4 ± 3.1	6.6 ± 2.2	2-36	3.679
		pupae	0.4	38.1	1.0 ± 0.0	0.4 ± 0.1	–	0.324
	Ceratopogonidae	larvae	0.3	14.3	2.0 ± 0.6	0.3 ± 0.2	1-3	0.062
NEKTON			**9.5**	**85.7**	**9.7 ± 1.7**	**8.3 ± 1.6**	**1-27**	**89.387**
Insecta	Ephemeroptera	larvae	5.4	66.7	7.1 ± 1.9	4.8 ± 1.5	1-25	13.900
	Zygoptera	larvae	2.2	71.4	2.7 ± 0.4	2.0 ± 0.4	1-6	38.603
	Anisoptera	larvae	1.2	33.3	3.1 ± 1.1	1.1 ± 0.5	1-9	36.408
	Pleidae *(Plea)*		0.1	4.8	2.0	0.1 ± 0.1	–	0.008
	Leptoceridae	larvae	0.4	23.8	1.4 ± 0.2	0.3 ± 0.1	1-2	0.466
Arachnida	Acarina		0.2	14.3	1.0 ± 0.0	0.1 ± 0.1	–	0.002
TERRESTRIAL			**0.3**	**9.5**	**2.5 ± 1.5**	**0.2 ± 0.2**	**1-4**	**1.701**
Insecta	Curculionidae		0.1	4.8	1.0	0.1 ± 0.1	–	0.071
	Apidae		0.1	4.8	1.0	0.1 ± 0.1	–	1.498
	Thysanoptera		0.1	4.8	1.0	0.1 ± 0.1	–	0.011
	Ephemeroptera	adults	0.1	9.5	1.0 ± 0.0	0.1 ± 0.1	–	0.121
XENOPUS			**0.3**	**4.8**	**5.0**	**0.2 ± 0.2**	**–**	**0.950**
	Eggs		0.3	4.8	5.0	0.2 ± 0.2	–	0.950
OTHER	*Xenopus* exuviae		–	33.3	–	–	–	–
	Vegetal remains		–	23.8	–	–	–	–
	Und. fragments		–	61.9	–	–	–	–
	Und. arthropods		–	38.1	–	–	–	–

which the diurnal water temperature went above 20°C, considered to be the optimal temperature stimulus for spawning (see Tinsley et al., 1996). The most important prey category in terms of mass is the nektonic one while zooplankton, even if the most numerically abundant item, represents only a small part of the total ingested mass in the diet of *X. laevis*.

The comparison between the diet of adults and juveniles showed a major tendency for the former to prey on cladocerans and for the latter to practise cannibalism. The differences between the results for the two age classes are highly significant for the percentage of preyed items ($\chi^2_{21} = 76.6$; $P < 0.001$) and very significant for the percentage of prey biomass ($\chi^2_{21} = 40.05$; $P < 0.01$). The t-tests show higher values for adults both for the number of prey ingested ($t_{56} = 2.30$; $P < 0.05$) and for the mean mass of ingested prey ($t_{56} = 2.96$; $P < 0.01$).

Discussion

Although the point of first release for *X. laevis* in Sicily is not known, it is evident that this species has already achieved a remarkable distribution. Currently, the present distribution in Sicily is the largest known in Europe. The French population reached an extent of about 100 km^2 in 2003 (Eggert and Foquet, 2006). *X. laevis* was often considered strongly aquatic because of its distinctive characteristics and it is

Table 2. Analysis of prey items of 38 juveniles of *Xenopus laevis* caught between 4 March and 21 April 2006 at a pond near Poma Lake (Province of Palermo, Sicily). * mean of items of each taxon ingested from specimens eating a specific prey type.

Prey categories		Stage	% of *X. laevis* eaten items (N = 728)	% of prey occurrence in stomachs (N = 38)	Mean ± SE of ingestion*	Mean ± SE of ingested items per stomach (N = 38)	Range	% of total prey biomass (1.768 g)
PLANKTON			**55.4**	**44.7**	**23.7 ± 12.1**	**10.6 ± 5.7**	**1-201**	**1.442**
Crustacea	Calanoidea		3.0	13.2	4.4 ± 1.9	0.6 ± 0.3	1-10	0.030
	Cyclopoidea		3.8	34.2	2.2 ± 0.7	0.7 ± 0.3	1-10	0.038
	Cladocera		48.5	34.2	27.2 ± 14.0	9.3 ± 5.1	1-181	1.373
BENTHOS			**22.4**	**73.7**	**5.8 ± 1.2**	4.3 ± 1.0	**1-25**	**5.001**
Insecta	Chironomidae	larvae	20.6	68.4	5.8 ± 1.1	4.3 ± 1.0	1-20	4.637
		pupae	0.7	10.5	1.3 ± 0.3	0.1 ± 0.1	1-2	0.258
	Ceratopogonidae	larvae	1.1	13.2	1.6 ± 0.4	0.2 ± 0.1	1-3	0.105
NEKTON			**16.3**	**73.7**	**4.3 ± 0.7**	**3.1 ± 0.6**	**1-14**	**75.710**
Insecta	Ephemeroptera	larvae	6.7	47.4	2.7 ± 0.4	1.3 ± 0.3	1-6	8.734
	Zygoptera	larvae	6.7	47.4	2.7 ± 0.8	1.3 ± 0.4	1-14	46.685
	Anisoptera	larvae	1.8	23.7	1.4 ± 0.4	0.3 ± 0.1	1-5	19.697
	Leptoceridae	larvae	1.0	7.9	2.3 ± 0.7	0.2 ± 0.1	1-3	0.593
Arachnida	Acarina		0.1	2.6	1.0	0.0 ± 0.0	–	0.001
TERRESTRIAL			**1.2**	**15.8**	**1.5 ± 0.3**	**0.2 ± 0.1**	**1-3**	**9.496**
Insecta	Curculionidae		0.3	2.6	2.0	0.1 ± 0.1	–	0.181
	Staphylinidae		0.1	2.6	1.0	0.0 ± 0.0	–	0.018
	Formicidae		0.3	5.3	1.0 ± 0.0	0.1 ± 0.0	–	0.096
	Thysanoptera		0.1	2.6	1.0	0.0 ± 0.0	–	0.015
	Ephemeroptera	larvae	0.3	5.3	1.0 ± 0.0	0.0 ± 0.0	–	0.153
Crustacea	Isopoda		0.1	2.6	1.0	0.1 ± 0.0	–	9.034
XENOPUS			**4.7**	**23.7**	**3.8 ± 1.5**	**0.9 ± 0.4**	**1-14**	**8.351**
	Eggs		4.4	23.7	3.6 ± 1.4	0.8 ± 0.4	1-13	7.743
	Larvae		0.3	5.3	1.0 ± 0.0	0.1 ± 0.0	–	0.608
OTHER	*Xenopus* exuviae		–	13.2	–	–	–	–
	Vegetal remains		–	50.0	–	–	–	–
	Und. fragments		–	71.1	–	–	–	–
	Und. arthropods		–	2.6	–	–	–	–

considered quite unfit to terrestrial life (Elepfant et al., 2000).

Therefore the dispersion pattern of this species was often described as strongly related to watercourses and ditches both in the native regions (Measey, 2004) and in the Chilean populations (Lobos and Measey, 2002). Fouquet and Measey (2006) pointed out that in the French population dispersal by terrestrial movement may be prevalent. The French population lives in an oceanic climate region where abundant rainfall may cause temporary flooding useful as dispersal corridors for the frogs (Eggert and Fouquet, 2006). Our data suggests that overland migration may also be prevalent in Mediterranean climate regions such as Sicily, where the land surface is dry or arid for most of the year and where irrigation channels are absent. Many ponds colonized by *X. laevis* are in fact quite far from rivers. It is probable that *X. laevis* could disperse over land during the short periods of rainfall through a step-by-step process influenced by the distribution of ponds. In this "jumping island dispersal" model (Pielou, 1979), ponds could act as "islands" with suitable features for the colonization and the development of reproductive populations. These populations will be new dispersions for the species (Pielou, 1979). The greatest distance between two potentially linked knots within the frogs overland movement seems to be at least 480 m. This is in fact the distance between the most isolated pond containing *X. laevis* and the closest pond to it. In the area colonized by *X. laevis*, pond density attains a value of 13.7/km^2. There is a similar pond density in contiguous areas without *X. laevis*. Thus it is probable that the spread of the species is still ongoing.

Despite *X. laevis*'s sex ratio generally being unbiased both in natural populations (Tobias et al., 1998; Du Preez et al., 2005) and in laboratory experiments (Pickford et al., 2003; Levy et al., 2004; Hayes et al., 2006), Lobos and Measey (2002) found a female biased sex ratio in allochthonous populations in Chile ($M : F = 1 : 2.3$). These authors considered the possibility of a sampling bias due to the catch method (funnel-traps). Nevertheless our different catch method also confirmed a sex ratio biased towards females. There is much evidence of some anthropogenic causes of imbalance in the sex-ratio in *X. laevis*: water in which Bisphenol A (Pickford et al., 2003; Levy et al., 2004), Estradiol (Carr et al., 2003), industrial wastewater (Bögi et al., 2003) and Atrazine is present can cause the feminization of *X. laevis* larvae (Hayes et al., 2006). The focal pond is located in an area strongly affected by agricultural activities, where it is probable that herbicides and fertilizers have accumulated in the water. At the same time it is known that, in amphibians, males and females adopt different behavioural strategies (Marsh, 2001). These differences in behavioural strategies may result in different probabilities of capturing specimens of one sex rather than the other. It would be appropriate to evaluate the effective sex ratio in the Sicilian populations through mark and recapture studies in order to minimize sampling errors. Eventually it may be necessary to investigate if this sex ratio bias is due to to anthropogenic or behavioural causes.

The diet analysis points out a considerable difference between the mass contribution and the numeric contribution of the prey types. For example, small crustaceans are the most numerous prey items ingested by both adults and juveniles but contribute only 2.8% of the total ingested mass. Odonates and mayflies are fewer in numbers but represent, along with some less common nektonic groups, the most important mass contribution both for adults (89.4%) and juveniles (75.7%). It should be noted that the quite high contribution in mass of terrestrial prey (5.2%) is strongly influenced by a single ingestion of a big isopod. The percentage occurrence of prey in stomach contents shows that nektonic and benthic prey are the most frequently ingested, followed by planktonic prey species. Planktonic prey is frequently ingested but the quantity of ingested items is quite variable, as suggested by the high standard error (SE) values. This variability could be the result of incidental ingestion during feeding influenced by the relative density of the concentrations of small crustaceans present in the water.

As would be predicted by size, the adults ingest a significantly greater mass of prey than juveniles. But it is more difficult to explain the significant difference between the prey types ingested by the two age classes. This difference could possibly be explained by a difference in prey preferences or by a difference in microhabitat use between the two age classes. Our observations tend to support microhabitat differences as an explanatin since, it is easier to catch juvenile specimens of *X. laevis* by dragging the dredge or the dip-net along the pond shore, whereas it is easier to catch adults by dragging in the middle and in the deepest parts of the pond. This spatial segregation could result in the observed prey preferences merely reflecting the differential prey availability in these different microhabitats.

A comparison of the percentages of ingested prey of *X. laevis* (fig. 2) in the populations in Sicily, Wales (Measey, 1998) and the two populations in Chile (Lobos and Jaksic, 2005) shows that zooplankton represents the numerically most abundant prey group only in Sicily and in Wales. The high availability of chironomid larvae in Wales and the frequent ingestion of *Physa sp.* in Chile contribute significantly to the trophic differences beween these two popu-

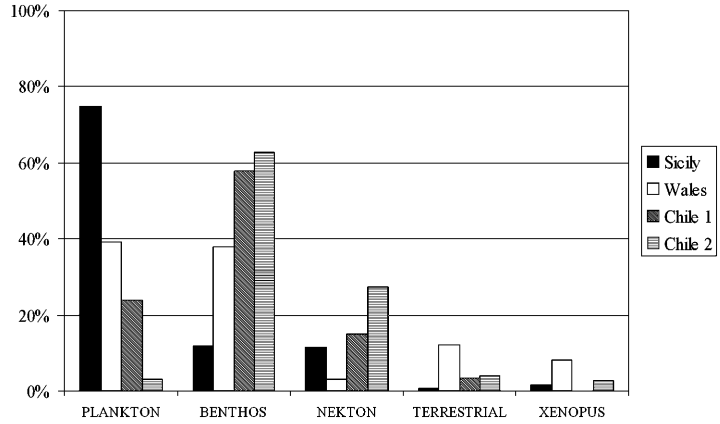

Figure 2. Percentage of numeric prey occurrence in four feral populations of *Xenopus laevis*. See "Discussion" for details.

lations. These results should be considered with caution because the data obtained by Measey (1998) are the result of an annual sampling, whereas the Chilean data (Lobos and Jaksic, 2005) and our data are the results of seasonal samplings.

Although we observed the tendency towards cannibalism of other *X. laevis*, we did not observe any evidence of predation on the eggs or larval stages of autochthonous amphibians. However, we also did not observe any spawn or tadpoles in the pond during the sampling period. Future studies should explain if the presence of *X. laevis* could affect the syntopic populations of autochthonous amphibians. During the sampling period we did, in fact, record the presence of larval and newly-metamorphosed *Discoglossus pictus*, *B. bufo* and *Hyla intermedia* in some neighbouring ponds, some with and others without *X. laevis*.

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