Universidade de Évora

Avaliação da população de uma nova espécie exótica em Portugal: *Xenopus laevis* (Amphibia: Pipidae)

Patrícia Cátia Isidoro de Amaral

Dissertação para obtenção do grau de

Mestre em Biologia da Conservação

Orientadores:

Prof. Rui Rebelo (FCUL)

Prof. Paulo Sá Sousa (Univ. Évora)

Outubro 2008

Esta dissertação não inclui as críticas e sugestões feitas pelo júri

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Resumo

As introduções de rã africana *Xenopus laevis* têm ocorrido à escala mundial na sequência do seu uso como animal de laboratório e de estimação. Na África do Sul onde é nativa, esta rã tem uma distribuição muito ampla compreendendo todos os tipos de habitats. *Xenopus laevis* é ecologicamente distinta de outras espécies de anfíbios, apresentando características ecológicas e fisiológicas que asseguram o seu sucesso em habitats exóticos e que conferem a esta espécie um elevado carácter invasor. Embora o seu carácter invasor *X. laevis* foi introduzido por toda a Europa, desde a Alemanha a Inglaterra e nos E.U.A. (Califórnia), e Chile. Para alguns destes países esta espécie é considerada como uma praga, com graves impactos quer a nível ecológico como económico. Apesar de ser uma das espécies de laboratório mais estudadas, com investigações centradas sobretudo no seu desenvolvimento e biologia molecular, a informação sobre a sua ecologia é escassa. Recentemente descoberta numa ribeira a sudoeste de Lisboa (Portugal) e sem informação sobre os possíveis impactos a nível do ecossistema nativo, tornou-se importante a realização de um estudo que paralelamente avalia-se a sua distribuição e possíveis impactos na fauna nativa. Assim, para uma melhor compreensão da distribuição desta espécie e dos seus potenciais impactos, por um período de um ano (2006/2007) realizaram-se amostragens periódicas ao longo da ribeira da Lage (Oeiras), caracterizou-se cada ponto de amostragem em termos de vegetação e solo e analisou-se a dieta de 70 indivíduos capturados. Segundo os resultados obtidos, esta espécie encontra-se

actualmente restrita ao troço regularizado da ribeira e a sua ausência de locais mais a montante está aparentemente relacionada com o crescente carácter rochoso do substrato da ribeira. Constatou-se, por outro lado, que se trata de uma população reprodutora e que adultos e girinos coexistem com espécies nativas de anfíbios e peixes. Relativamente à dieta, trata-se de uma espécie extremamente generalista. No entanto, a sua dieta consistiu predominantemente de uma componente bentónica, maioritariamente composta por gastrópodes da família *Physidae*, uma componente nectónica e numa pequena componente terrestre. Verificou-se ainda que esta espécie preda vertebrados nativos incluindo anfíbios e peixes. A nível de biomassa, registaram-se diferenças significativas quando foi analisado o peso de cada grupo de espécies-presa, quando considerado o local e a época de captura.

Evaluation of a new exotic population in Portugal: *Xenopus laevis* (Amphibia: Pipidae)

Abstract

Xenopus laevis has been introduced worldwide as a consequence of its use in laboratories and in the pet market. In its native South Africa, this species is largely spread for all types of habitats. This species is highly distinctive ecologically from other anuran amphibians presenting ecological and physiological characteristics that allow its success in non-native habitats, enabling a highly invasive potential to this species. Although it's invasive character *X. laevis* was introduced over all Europe from Germany to U.K., and also in the USA (California) and Chile. In some of these countries this species is considered as pest with severe ecological and economic consequences. *X. laevis* is one of the most intensively studied of laboratory animals, with investigations centered upon developmental and molecular biology, but ecological information is scarce. Recently, a population of *X. laevis* has been discovered in a stream southwest of Lisbon, Portugal and without no knowledge on the impacts that this species would have in the native ecosystem, it was urgent to study the distribution and the possible impacts that it would have in native fauna. Therefore, for one-year period we conducted periodical sampling across the Lage stream (Oeiras), we characterized the soil and vegetation for each sampling point and we analyzed the stomach contents of the 70 individuals captured. Regarding our results we demonstrated that the presence of *X. levis* is now restricted to the regularized area of the Lage stream and that its

absence from upstream sampling points is probably related with the increasing rocky character of the substrate. On the other hand, the results emphasize that this is a reproductive population with tadpoles and adults co-inhabiting with native amphibians and fishes. The analysis of *Xenopus* diet suggests that this is a generalist species. However, there is a large component of benthonic fauna, especially snails (*Physidae*) and some nekton. Although being less represented, terrestrial prey items had a significant importance in the biomass of the diet of *X. laevis*. This species also preys native vertebrates as amphibians and fishes. Finally, there were significant differences in prey biomass in different places and sampling seasons.

INTRODUÇÃO GERAL

A introdução de espécies exóticas é considerada uma das acções antropogénicas com maiores consequências nos ecossistemas (Mills *et al.*, 1994). Espécies invasoras ou exóticas são todas aquelas que foram introduzidas de forma acidental ou intencional por humanos (Allen & Humble, 2002), ou conseguiram expandir a sua área de distribuição na sequência de perturbações antropogénicas, para regiões geográficas onde historicamente não estavam presentes (Sax, 2001). É reconhecido que o número destas introduções tem aumentado desde o começo das viagens intercontinentais, e consequentemente milhares de espécies exóticas têm sido introduzidas mundialmente (Jaksic *et al.*, 2002). Esta introdução de espécies exóticas pode ter sérios impactos na estrutura e funcionamento dos ecossistemas e ainda ter profundas implicações a nível económico (Liebhold *et al.*, 1995; Mack *et al.*, 2000), podendo rapidamente superar a perda de habitat como a principal causa de desintegração ecológica a nível mundial (Vitousek *et al.*, 1997; Chapin *et al.*, 2000). Geralmente as ameaças à biodiversidade descritas para Portugal são as mesmas que ameaçam os ecossistemas nativos noutros países Europeus: a perda e destruição de habitat, contaminação e introdução de espécies exóticas (Ferreira & Crespo, 2003). No entanto, as introduções bióticas variam de acordo com as condições ambientais e regiões biogeográficas e espera-se que tenham maior impacto em biomas mediterrânicos, como os encontrados no nosso país (Sala *et al.*, 2000).

Os ecólogos há muito tentam explicar a distribuição das espécies através de mecanismos bióticos como a selecção de habitat, competição e predação versus as características abióticas do meio (Dodson, 1970). Destes mecanismos, as interacções bióticas, em particular, são relativamente mais importantes para os ecossistemas aquáticos do que terrestres (Lodge *et al.*, 1998). Os ecossistemas aquáticos são áreas naturais muito importantes à escala global, sendo habitat primário ou secundário de um grande número de espécies aquáticas e terrestres. A sua presença determina ainda a biodiversidade local de uma região. Nestes ecossistemas a distribuição de cada espécie pode ser mais ou menos heterogénea, apresentando uma estrutura em mosaico que é geralmente resultado da sucessão de regiões com características ambientais diferentes (Romero & Real, 1996; van Buskirk, 2005). Esta heterogeneidade está directamente relacionada com a capacidade de cada espécie em colonizar um novo local e pode ainda ser importante para limitar a sua distribuição e/ou expansão (Shurin & Havel, 2002). Os ecossistemas dulçaquícolas sofrem impactos substanciais, sobretudo porque o Homem vive desproporcionadamente perto das linhas de água e modifica extensivamente as zonas ripícolas. Como consequência, estas modificações da paisagem conduzem a muitas mudanças nas linhas de água, incluindo o aumento da entrada de nutrientes, sedimentos e contaminantes (Sala *et al.*, 2000).

Hoje em dia, a ameaça mais importante para a biodiversidade e funcionamento de ecossistemas dulçaquícolas é, em muitos países do mundo, a introdução de espécies exóticas (Lodge *et al.*, 2000; Sala *et al.*, 2000). Os efeitos nocivos destas introduções são há muito reconhecidos pela comunidade de ictiólogos em virtude da existência de um registo impressionante de invasões de peixes em águas interiores que contribuíram para a perda de muitas espécies nativas por todo o mundo (Leonardos *et al.*, 2008) e tiveram consequências negativas na qualidade ambiental (Zambrano *et al.*, 2001). Actualmente na América do Norte, as espécies exóticas e outras mudanças globais põem em risco uma proporção muito maior da biodiversidade em ecossistemas dulçaquícolas do que terrestres (Lodge *et al.*, 2000). Por exemplo, a colonização e expansão do bivalve *Dreissena polymorpha* no Lago Michigan teve como consequência uma das maiores alterações ecológicas a grande escala nos tempos recentes (Fleischer *et al.*, 2001). Mais recentemente, foi descoberto que o bivalve dulçaquícola do mesmo género *Dreissena rostriformis bugensis* se expandiu do Este para o Oeste da Europa através de um corredor de bacias hidrográficas interligadas por canais construídos pelo homem (Molloy *et al.*, 2007). Também a introdução do lagostim vermelho, *P. clarkii*, na Europa (Lodge *et al.*, 2000), é um exemplo de uma introdução com consequências negativas para as populações nativas e pouco ou nenhum valor económico (Mills *et al.*, 1994).

Apesar do declínio mundial nas populações de anfíbios que tem sido descrito durante a última década, alguns anfíbios podem também possuir características invasoras e provocar impactos consideráveis nas áreas onde são introduzidos. Na Austrália, o sapo *Bufo marinus* modificou fortemente a diversidade e abundância de invertebrados nas áreas de clima tropical, e pode ainda ter modificado os processos ecológicos dentro destes complexos ecossistemas (Greenlees *et al.*, 2006; Semeniuk *et al.*, 2007). O pequeno sapo *Eleutherodactylus coqui* é também reconhecido como um invasor problemático no Havai; o número e tamanho das suas populações continuam a aumentar, sendo considerado uma ameaça económica e ecológica (Kraus & Campbell, 2002; Beard & Pitt, 2005). Por sua vez, a rã africana *Xenopus laevis* tem sido introduzida a nível mundial, como por exemplo na Califórnia (McCoid & Fritts, 1980), Reino Unido (Measey & Tinsley, 1998) e Chile (Lobos, 2004), existindo actualmente populações selvagens deste anfíbio numa grande variedade de latitudes e ambientes (Solís, 2004). Recentemente, esta espécie foi também descoberta na ribeira da Lage, a oeste de Lisboa, Portugal. As introduções acidentais e deliberadas de *X. laevis* em novos ambientes estão associadas com o seu uso em laboratório para diagnósticos de gravidez, como animal de laboratório e na sua comercialização como animal de estimação (Measey & Tinsley, 1998). Esta espécie em particular apresenta importantes características biológicas e fisiológicas que permitem a sua sobrevivência em todos os tipos de ambientes: sobrevive quase sem água, pode movimentar-se por terra, tem uma dieta generalista (que inclui canibalismo da sua própria descendência), sobrevive sem alimento por longos períodos de tempo e suporta uma grande amplitude térmica. O seu desenvolvimento está completo em apenas 8 meses (Cattan, 2004) e sob condições ambientais favoráveis e temperaturas óptimas pode reproduzir-se durante o ano inteiro (Solís, 2004; Measey & Tinsley, 1998). Porém um dos aspectos mais importantes a considerar na introdução de uma espécies exótica, como o *X. laevis*, será o tipo de relações ecológicas que esta possa estabelecer com as espécies nativas (Solís, 2004). Apesar de, não existirem dados em relação aos possíveis efeitos da introdução de *X. laevis* na fauna nativa, os biólogos suspeitam de um impacto nocivo nas espécies de anfíbios (Lobos & Jaksic, 2004; Measey & Royero, 2005). Sabe-se que, o peixe ameaçado *Eycyclogobius newberryi* é uma presa comum para *X. laevis* no estuário do rio Santa Clara, Califórnia (Lafferty & Page, 1997), e no Reino Unido e Chile *X. laevis* provavelmente consome ovos e larvas de anfíbios locais, assim como provoca alterações em cascata ao longo das teias alimentares, através do consumo de macroinvertebrados bentónicos (Measey, 1998; Lobos & Measey, 2002).

Devido às consequências que a introdução de espécies exóticas pode provocar, é necessário compreender algumas das suas características biológicas como a sua distribuição local, abundância, e selecção de habitat, de forma a implementar programas de controlo. O controlo destas espécies, que podem viver numa ampla gama de condições ambientais, pode ser particularmente difícil e é um grande desafio na conservação de espécies ou ecossistemas nativos isolados (Romero-Nájera *et al.*, 2007). Por esta razão, os ecólogos há muito tempo que tentam prever a localização e condições nas quais os organismos exóticos terão mais probabilidade de se tornar invasores (Havel *et al.*, 2005). Contudo, apesar do crescente conhecimento sobre as invasões ecológicas, alguns impactos ainda são pouco compreendidos e muitas vezes a sua valorização económica é baseada em escassa informação (van Wilgen *et al.*, 2006; Measey, 2004).

A presente dissertação reúne dois trabalhos distintos, apresentados sob a forma de artigos científicos. No primeiro artigo tentamos apresentar informação sobre a distribuição local e abundância de *Xenopus laevis* na ribeira da Lage, comparando-as com os seus padrões espacio-temporais já conhecidos para outras áreas. Estimámos a sua distribuição em secções da ribeira com diferente topografia e com diferentes graus de presença humana. A variação temporal na abundância de *X. laevis* foi também considerada. Esta informação irá ajudar a identificar as áreas da Lage onde a espécie é mais abundante e que características físicas estão relacionadas com a sua presença, de forma a propor acções de controlo no sentido da sua captura e possível erradicação.

No segundo artigo, tentámos determinar quais as presas consumidas por *X. laevis* ao longo de um ano na Lage. Foram estimadas a composição e mudanças sazonais da dieta. Esta informação poderá ser uma mais-valia no controlo ou erradicação de *Xenopus*, com o objectivo de promover a conservação da fauna nativa.

ARTIGO I

Local distribution of the introduced african clawed frog *Xenopus laevis* (Daudin, 1802) in Portugal

Abstract

The introduction of some amphibian species has produced alterations of freshwater environments and declines of native species worldwide. The african clawed frog *Xenopus laevis* is one of the most invasive species and for several countries this species is considered a pest producing severe impacts in the entire ecosystem. These species was recently introduced in the southwest Lisbon, Portugal. In the present work we studied the distribution of *X. laevis* in the area where the introduction has occurred: the Lage stream (Oeiras). Our main goal was to determine the area of distribution in this stream, to identify which factors limit clawed frog distribution, which could help to identify the most effective management practices to contain its spread. As a result, *X. laevis* was found in most types of habitats, including man-made pools. Consequently, the soil type of pools was the single predictor variable explaining clawed frog presence in most types of habitats. Our findings can be used to determine which habitats are more likely to be colonized by this species and to develop practical measures to eliminate the few populations existing or to control its spread and minimize its impacts in native species.

Keywords distribution, exotic species, african clawed frog, *Xenopus laevis*, ecological requirements

INTRODUCTION

The human mediated introduction of exotic species is one of the main processes involved in the current biodiversity crisis (Parker *et al.*, 1999; Iriarte *et al.*, 2005), and freshwater ecosystems are particularly vulnerable to this phenomenon (Sala *et al.*, 2000). These introductions, some of them accidental are producing alterations in freshwater environments and are responsible for declines and extinctions of native species worldwide (Lodge *et al.*, 1998, 2000), leading to a growing interest in the study of the processes that enable these species to establish and/or spread into a new area (Shurin & Havel, 2002). In fact, the spread of an introduced species is dependent on its dispersal characteristics (Sakai *et al.*, 2001). Particularly, for invasive species living in linear or discrete habitats, such as freshwater habitats, geographical features which hamper or facilitate spread, such as elevation or the distance to other suitable habitats, may play an important role in its distribution. On the other hand, local characteristics such as habitat size or vegetation structure may also influence its ability to establish in an area. In addition, the quantification of species-environment relationships, may therefore allow a predictive geographical modeling with practical applications (Cruz & Rebelo, 2007). Identifying important environmental gradients and determining the mechanisms that limit species distributions along such gradients have been an effective approach to understanding patterns in community composition. Such studies can also identify attributes of species that contribute to their success in different environments (Wellborn *et al.*, 1996). Hydrographic basins have been proposed as suitable geographical units for biogeographical analysis of fish and amphibians (Real *et al.*, 1993), because they have objective and natural boundaries and some biogeographical processes occur within the limits of the river basins (Romero & Real, 1996). In these systems, water body hydroperiod is a critical gradient along which communities are organized and virtually all major animal taxa inhabiting freshwater ecosystems exhibit restricted distributions along this gradient (Werner & Glennemeier, 1999).

African clawed frog, *Xenopus laevis* (Daudin, 1802), autochthonous from Africa, has been successfully introduced in many countries. More and more frequently, this species has been showing in the pet trade market and their private owners are suspected of dumping them in the wild once the frogs become too large to hold in small aquaria (Jaksic, 1998; Lobos & Jaksic, 2005). A review of feral populations (Tinsley & McCoid, 1996) noted that this species has colonized a variety of lenthic habitats world-wide, but principally in Mediterranean climates, similar to the native home of *X. laevis* in the South African Cape, such as California (McCoid & Fritts, 1995), UK (Measey, 1998a,b; 2001) and Chile (Lobos & Measey, 2002; Lobos & Jaksic, 2005). Within its native range, this species is noted for its rapid invasion of man-made water bodies and anthropogenically disturbed areas (Evans *et al.*, 1997). Lobos & Measey (2002) and Lobos & Jaksic (2005) also suggest that in its native South Africa, these species is thought to use irrigation channels to move into previously unoccupied areas, explaining its rapid spread in Chile. Consequently, if *X.laevis* relies on watercourses and irrigation channels to colonize new ponds, then a clear distribution pattern following such watercourses would be expected. However, if significant numbers of individuals habitually cross the land, a more even invasive pattern could be envisaged (Fouquet & Measey, 2006).

Xenopus laevis is highly distinctive ecologically in comparison with other anuran amphibians. It can survive prolonged periods without water and can migrate overland (Tinsley & McCoid, 1996), in optimal conditions it can grow and breed year-round (McCoid & Fritts, 1995), under food shortage it can feed on its filter-feeding tadpoles (Tinsley & McCoid, 1996; Measey, 1998a; 2002) exploiting a nutrient resource which could not be utilized directly and experimental data demonstrate a tolerance of salinity which is exceptional among amphibians (Tinsley *et al.*, 1996). These characteristics have become important advantages for the survival of populations introduced into new environments. Feral populations were first recorded outside Africa in the 1960s and a long series of new reports continues to the present (Tinsley & McCoid, 1996).

In contrast to the detailed laboratory studies based on other aspects of *Xenopus* biology, there have been few field investigations and most ecological information is based on inference (Tinsley *et al.*, 1996). We studied the distribution of *Xenopus laevis* in the Ribeira da Lage stream (W Portugal), an area recently colonized by this species, with the purpose of determining the relative importance for its spread and establishment of both geographical features and local habitat characteristics. This may help to estimate its potential distribution area and to identify the most effective management practices to contain its spread. This area contains many artificial barriers, like high waterfalls and a coarse substrate. As a result, we expected that clawed frog dispersion would be easier in more homogenous habitat than in areas with artificial waterfalls and in rocky soil, and therefore to find differences in clawed frog distribution between naturalized and non-naturalized areas. In addition, this study was designed on the assumptions that: (1) the population was restricted to the Lage stream, (2) sampling effort was sufficient to establish *Xenopus* presence or absence and (3) all individuals have the equal probability of being sighted. Thus, our main goals were to: (1) determine every site with *Xenopus*, (2) assess its distribution along the stream, (3) localize the areas of reproduction, (4) assess if there is a correlation between the presence of *Xenopus* and the substrate of the site where it occurs, and (5) assess if the vegetation structure affects the presence or reproduction of *X. laevis*.

MATERIALS AND METHODS

Study Area – This study was prompted by the observation of the occurrence of *Xenopus laevis* in two sites of the Lage stream. We conducted our survey on that stream, which is localized in Oeiras, about 20 km W of Lisbon, and runs from the Sintra municipality to the sea coast for about 16km. The study area covers approximately 11 km of this stream, from Cacém to the sea (Figure 1). The stream is almost entirely regularized, with both banks constituted by cement walls, and runs through a varied range of land-use landscapes, including shrublands, farmed areas and city gardens. The Lage basin is more intensively farmed towards its northern limits where its margins are more naturalized; in its southern limit, where the margins consist of cement walls, city parks are more frequent. Stream flow in this disturbed basin is intermediate, with its stronger flow in the Winter. In Summer, water remains in a few pools that are formed along the stream. Some indigenous plant and fish species also occur in Lage stream in this portion of the Oeiras basin. The area also has marked heterogeneity in lithology, edaphology and vegetation. The stream is annually cleared of vegetation by the municipality services and is thus subject to regular disturbance.

Study Sites – We surveyed 16 sites regularly distributed in the Lage stream (Figure 1), all registered in a GPS (eTrex, Garmin). Survey sites included eight regularized stream impoundments and eight naturalized pools along the stream. Using 2004 ortophotomaps and local visits we characterized the habitats bordering each survey site. We recorded the stream width and its flow. We characterized pools by its depth and length and by the soil present in both margins and stream bed. Hydroperiod was designated as either permanent (kept water for all year), intermediate (dried during

Figure 1 Map of the study areas with their location in the Iberian Peninsula, showing the municipality crossed by the Lage stream. Dots represent the stream sampling pools. Balck symbols represent *Xenopus* presence whereas white symbols represent its absence.

some moths of the year), or temporary (dried frequently). We classified overhead canopy cover according to the type of vegetation present (herbs, bushes or trees). To identify the factors responsible for *Xenopus* distribution, each sampling point was characterized using the variables provided in Table 1. Two habitat features which may be related to african clawed frog distribution were studied: soil composition and vegetation cover of the pools.

Table 1 Variables used to characterize each pond.

Variables	Units
Maximum depth	cm
Size	m
Flow velocity	m/s
Substrate variables	
Bedrock	% cover
Rock (>30cm)	% cover
Gravel (7.5-30cm)	% cover
Coarse substrates (2-75mm)	%cover
Sand (0.5-2mm)	%cover
Silt (<0.5mm)	%cover
Leaf litter	% cover
Vegetation variables	
Margins	
Arboreal vegetation	%cover
Arbustive vegetation	%cover
Herbs	%cover
Bare ground	%cover
Water body	
Floating vegetation	%cover
Submersed vegetation	%cover
Emergent vegetation	%cover

We estimated vegetation cover and substrate composition in the shore (margin transects) or inside the water body by identifying the substrate and vegetation in each of 5 (largest pools) or 2 (smallest pools) randomly selected locations spaced out at least 50 cm from each other. The characterization of soil and vegetation present in each location was based on point sampling and performed using a 1meter metal comb with ten teeth spaced by 10 cm each. The soil and vegetation present in each site of contact of the comb's teeth with the ground were registered and each vegetation or soil types were expressed as percentages.

Survey Methods – We completed surveys over a one-year period in 2006/2007. Each site was visited bi-monthly from October 2006 to September 2007. In each site we determined the presence of egg masses, tadpoles and adults. Egg masses were prospected in transects, counting the number of egg masses in each site and the number of eggs present in each one. The sampling of tadpoles in each site was performed with use of a dipnet; we conducted three dipnet sweeps on both margins and on the stream bed. Adults were captured with an electrofishing set SAMUS-752GN (PDC), with a frequency of 30 Hz. Electrofishing (performed in 2-3 15' periods, separated by 10') started in July, when the water level was low enough to allow access to the deep reaches of the stream. Effort was made in each visit to determine if there were egg masses, tadpoles or juveniles that could confirm the presence of a reproducing population.

Adult *Xenopus* were removed and conserved in the cold, later they were transferred to alcohol (75%).

Data Analysis – We first determined if pools with and without X. laevis did not differ in maximum widths/areas and in maximum depths using t-tests. We then performed one logistic regression to identify the factors correlated with Xenopus distribution at Lage stream. We followed the procedure of building multivariate logistic regressions suggested by Hosmer & Lemeshow (2000). First, we conducted a univariate logistic regression for each predictor variable (all variables in Table 2), and selected the independent variables with P-values < 2 test.χ0.25 in the Wald. For those variables that were not selected, we computed several transformations: creation of class variables by partitioning continuous variables into two groups of similar sample size, or by partitioning percentage variables in two groups – presence or absence; see Table 2). If the P-values of the Wald test were significant for any of the new variables, it was considered evidence of non-linearity and the new variable was included in the model and used in subsequent analysis. Finally, the selected variables were included in a backward, iterative, stepwise regression. The choice of the final model was based on the maximum likelihood ratio test (G test) (Hosmer & Lemeshow, 2000).

Distribution maps were constructed using the program ArcGis vs 9.1 using digitalized topographical maps and SPSS version 11.5 for Windows was used for all statistical analyses.

Table 2 Variable transformations for the logistic regression.

Variables	Class transformations
Substrate variables	
Bottom	
Rock bottom (Bedrock + Rock)	Presence (0/1)
Gravel	Presence (0/1)
Coarse substrates	Presence (0/1)
Sand	Presence (0/1)
Sand bottom (Leaf litter + Silt)	Presence (0/1)
Margin	
Rock margin (Bedrock + Rock + Rock wall)	Presence (0/1)
Gravel	Presence (0/1)
Coarse substrates	Presence (0/1)
Sand	Presence (0/1)
Sand margin (Leaf litter + Silt)	Presence (0/1)
Vegetation variables	
Margins	
Land vegetation (Arboreal + Arbustive + Herbs)	Presence (0/1)
Bare ground	Presence (0/1)
Water body	
Submersed vegetation	Presence (0/1)
Emergent vegetation	Presence (0/1)
Bottom vegetation (Leaf litter + Bare ground)	Presence (0/1)

RESULTS

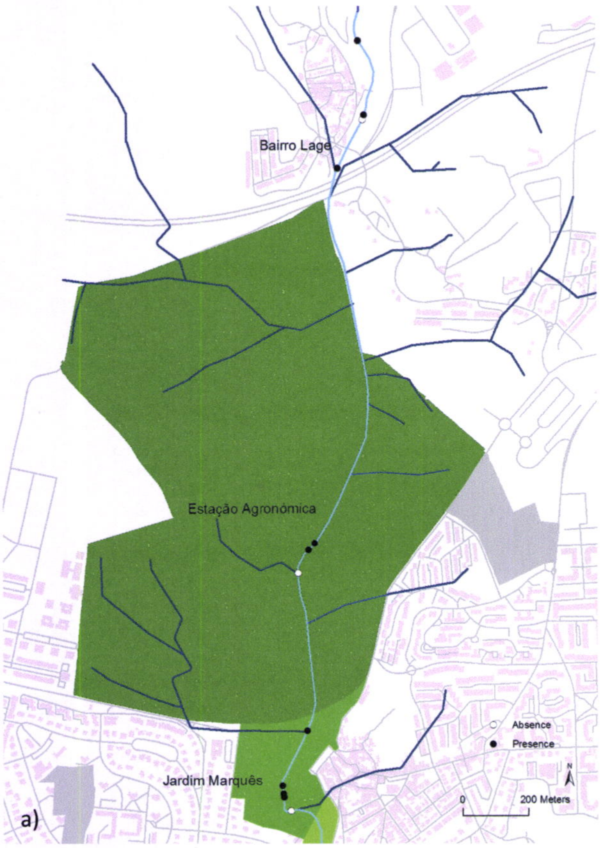
In the Lage stream, 8 of the 16 sampling points had an established african clawed frog population. Tadpoles of *Xenopus laevis* were present in at least 9 sampling points, but in one of these we didn't see or capture adult frogs. Five of the 16 sampling points had no african clawed frog and were excluded from the sampling effort.

We identified egg strings, tadpoles and adults of *X. laevis* at three main areas along Lage stream: Jardim do Marquês, Estação Agronómica and Bairro da Lage (Figure 2). Adults were first found in March 2007 and egg strings and tadpoles were first found in May.

The distribution of this species is up to now restricted to the regularized section of the stream but very close to its upper limit, at least for adults. Neither eggs nor adults were found in the naturalized section/points of Lage.

Across all 16 pools, soil composition varied significantly. There was a dominance of sandy soil in Jardim do Marquês, Estação Agronómica e Bairro da Lage, where *Xenopus* is present, while the remaining pools have mostly a rocky composition. The vegetation structure of the pools also varied: in the water body there is submersed and emergent vegetation, while in the margins it is common the presence of herbs. Vegetation size in the margins becomes higher from downstream to upstream.

In table 3 we show the single best univarite models selected in the logistic regression, only one variable was selected and the percentage of variance in the distribution data explain was significant ($P > 0.069$; Table 3) for all models obtain. These variables will be submitted to a backward, iterative, stepwise regression to obtain a more robust model equation to explain our data.



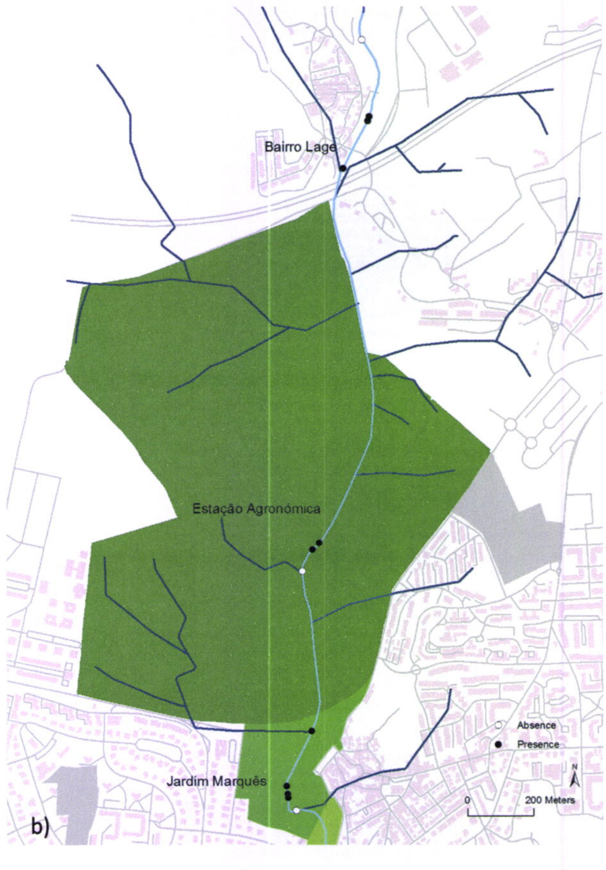


Figure 2 Map of the sampling area with *Xenopus laevis* distribution in three main areas at Lage stream: a) adult *X. laevis* distribution and b) *X. laevis* tadpole distribution. Black circles represent the species presence and the white circles represent its absence.

Table 3 Coefficients (*ß*) and *P*-values obtained via the Wald χ^2 test for the variables obtained in the univariate logistic regressions for each soil and vegetation type and for all sampling pools. Nagelkerke r^2 test values obtained in each model and the significance are also presented.

Variables in the equation	*ß*	S.E.	Wald	*P*
Rock bottom (Bedrock + Rock)	-0.144	0.079	3.296	0.069
Rock margin (Bedrock + Rock + Rock wall)	-0.051	0.042	1.514	0.218
Sand (in margins)	-0.190	0.134	2.011	0.156
Bare ground (in margins)	-0.065	0.055	1.417	0.234

Single best multivariate model of *X. laevis* distribution are presented in Table 4. In the model, only two variables were selected and the percentage of variance explained by the model was not high (Nagelkerke r^2 tests > 0.548; Table 4), however it explained more than half of that variation. A rock bottom and a sandy margin were the only two predictor variables explaining *X. laevis* distribution in the stream. These variables negatively affected the probability of *X. laevis* presence in all pools, but with different coefficients (*ß*) (Table 3). This coefficient was higher (meaning a higher probability of no-colonization of pools) for pools with sandy margins. For pools with a rock bottom, this coefficient was lower but still has high negative effects for the presence of *Xenopus* (Table 4).

Table 4 Coefficients (*ß*) and *P*-values obtained via the backward stepwise test for the variables obtained in the multivariate logistic regressions for each soil and vegetation type and for all sampling pools. Nagelkerke r^2 test values obtained in each model and the significance are also presented.

Variables in the equation	*ß*	S.E.	*P*	Nr^2
Rock bottom	-0.154	0.092	0.095	
Sand (in margins)	-0.209	0.143	0.143	0.548
Constant	3.192	1.533	0.037	

Discussion

This represents the first report of distribution data for a new introduction in Portugal, *Xenopus laevis* (african clawed frog). It is thought that this introduction has possibly taken place around 1978 near the laboratories of Instituto Gulbenkian de Ciência (IGC), located in one of the margins of Lage stream.

Previous work on the reasons for distributional differences between amphibian species has emphasized the role of hydroperiod through limitation of larval period or through indirect effects on the community of competitors or predators (Werner & Glennemeier, 1999). However, with the development of human society, human activities have become one of the main reasons for limiting species distributions, both direct and indirectly. In Oeiras municipality, the process of urbanization has since a long time occupied terrestrial habitats, and roads have lead to loss and habitats fragmentation. As a result, Lage stream crosses one of the most densely populated areas of Oeiras municipality. Beyond that, the stream is regularized almost in its entire length and most of the pools are artificial. However, as this species has a life cycle essentially aquatic (Gurdon, 1996), human pressure does not seem to have a negative effect in its distribution. Indeed, throughout sub-Saharan Africa, *Xenopus* is favored by human activities: the needs for domestic and agricultural water supplies coincide with the requirements of *Xenopus*, and the toads readily colonize newly constructed dams and wells (Tinsley *et al.*, 1996). On the contrary, some species may like live with human because the increasing of farmland or ponds (Sun, 2007).

This work indicates that the distribution of *X. laevis* in Lage stream is basically dependent on site of probable introduction and, weakly, on some habitat characteristics, being the species restricted to the regularized areas of the stream. Indeed, this species, known as a generalist without detectable preferences for habitat type (Tinsley *et al.*, 1996), proved to be able to colonize most water bodies, even the small and shallow ones. It is known that within the geographical range of *Xenopus* there is little evidence of preference for specific biotypes: individuals appear to occupy whatever aquatic habitats are available. However, previous studies state some apparent contradictions: in general *X. laevis* does not often seem to populate large rivers and lakes, and there are indications that it does not tolerate well-established fish communities. On the other hand, several species of *Xenopus* have been reported to occur in large numbers in artificial ponds constructed for fish culture as Tinsley *et al.* (1996) suggest. Lobos & Jaksic (2005) also reported that occasionally the african clawed frog must get in contact with native fishes in Chile. Corroborating these statements, we have captured individuals in large pools were this species coexisted with well-established fish communities.

As expected, there is a high overlap between the distribution of *Xenopus* adults and tadpoles. This did not happen only in two cases at Bairro da Lage: in one sampling pool we captured tadpoles and no adults, and on the other we captured adults and no tadpoles. Although present, tadpoles apparently did not completed metamorphosis successfully, since we did not find any juvenile and for several occasions we observed the sudden disappearance of tadpoles in consecutive sampling days. Many studies had been made concerning the influence of fish predation in amphibian's diversity and distribution (Kats *et al.*, 1988; Zampella & Bunnell, 2000; Pilliod & Peterson, 2001; Bull & Marx, 2002). In all these studies it is suggested that fish have a negative influence, presumably from predation, being the abundance of eggs and larvae of some amphibian species affected by the presence of fish (Bull & Marx, 2002). In resemblance with other studies Pilliod & Peterson (2001) found that the abundance of all life stages of long-toed salamanders and spotted frogs were influenced by fish presence. In fact, he found significantly larger populations of amphibians in fishless than in fish-containing sites. A similar fish influence may be threatening *Xenopus* tadpole survival in Lage stream. Furthermore, studies in Wales demonstrated that *X. laevis* tadpoles did not metamorphose in streams, since the bigger tadpoles were possibly dragged downstream, being reproduction only possible in ponds near streams (Measey, *pers. com.*).

In addition, Hecnar & M'Closkey (1996) suggest that water chemistry appears to have a great effect on amphibians in highly stressed anthropogenic situations. However, the possibility of indirect ecological effects also exists. For example, predators, prey or competitors of amphibians may be affected by water chemistry (Hecnar & M'Closkey, 1996). In fact, there is wide variation in the water chemistry of habitats occupied by *Xenopus* and insufficient information to assess the influence of this on its habitat selection.

With the logistic regression it may be possible to characterize the typical habitats of a species and we can explain the species distribution in terms of probability of occurrence, taking into account both quantitative and qualitative variables (Romero & Real, 1996). Logistic regression can be an appropriate instrument to analyze a broad range of the internal complexity of a distribution area (Romero & Real, 1996). For example, Sjögren Gulve (1994) studied local scale distribution and extension patterns within metapopulations of amphibians and used logistic regression to analyze the differences between pools occupied or unoccupied by *Rana lessonae* in the Swedish coast. Walker (1990) also has used it to generate probability areas of large-scale species distributions and to re-express the distributions of three kangaroo species in Australia in terms of their climatic characteristics. In our case, logistic regression was not a powerful tool; however, it allowed us to forecast the occurrence of *Xenopus laevis* and to identify the environments where the odds of finding this species are high. Nevertheless, we have to pay attention to the fact that this amphibian is cryptic and difficult to census and in these cases non-detections produce biased logistic regression parameter estimates (van Buskirk, 2005; Mazerolle *et al.*, 2005).

In Lage stream, *Xenopus* presence was negatively dependent on substrate characteristics, like "Rock bottom" and "sand in the margins", both selected in the final model. While a negative association with hard substrata is not surprising, given this species preference for burying in the river bed (Tinsley *et al.*, 1996), the negative association with sandy margins is more surprising. However, we found an association between these two variables in the upstream points, located further from the possible introduction site, and corresponding to areas where we did not find adults or tadpoles of *X. laevis*. Thus, pool occupancy may decrease with a rocky stream bottom, which in turn has a positive association with sandy margins.

The recent considerable expansion of the geographical rage of *Xenopus*, which has been a product of man's activities, has actually lead to the reintroduction of *Xenopus* into some areas of former occurrence (Tinsley & McCoid, 1996). Its use as a laboratory animal may be the reason of accidental introduction in Lage stream from the "Instituto Glubenkian de Ciência" (IGC). The species distribution at Lage suggests that *Xenopus* are restricted to the IGC area where the introduction has occurred, and this is the only known introduction for this species in Portugal. Furthermore, introduced *Xenopus* may be very successful in disturbed habitats, especially those created by human activities (artificial water bodies accompanying mining excavations, irrigation schemes, etc.) (Tinsley & McCoid, 1996). As a highly modified stream, Lage has a high number of canals interconnecting with the surrounding streams, but it is also known that *X. laevis* can perform movements by land (Measey & Tinsley, 1998). Fouquet & Measey (2006) conducted the first study that conclusively demonstrated that the main dispersion route of *Xenopus laevis* to new water bodies in western France was by moving over dry land, without reliance on aquatic corridors for dispersal. This is particularly troubling, because it means that their invasive potential is even greater than suspected (Lobos & Jaksic, 2005). It is suggested that if the known release point is taken together with the date of original release, an estimate of terrestrial spread can be made to be approximately 1 km per year. The surroundings of Lage stream at the upstream limits of *X. laevis* distribution are naturalized and less human populated, being a possible corridor for dispersal into new water bodies. In spite of the possible overland dispersion, movements in streams and irrigation channels appear to be much faster (Fouquet & Measey, 2006). The presence of several artificial waterfalls in the Lage stream between Jardim do Marquês and Bairro da Lage does not seem to be an effective barrier to *Xenopus* dispersion.

The absence of major habitat requirements brings question the factors responsible for the geographical distribution of the *Xenopus* (Tinsley *et al.*, 1996). If they did not went upstream, is because it is just a historical introduction and the size structure of frogs suggests that the frogs have not reproduced successfully for the last years. Given the longevity of these animals (15 -27 years in laboratories and exceeding 20 years in the wild) (Tinsley *et al.*, 1996; Tinsley & McCoid, 1996), the population may sustain itself with just a few successful reproduction episodes in a decade.

Acknowledgments

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ARTIGO II

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

Abstract

Aspects of the trophic ecology of *Xenopus laevis,* an amphibian introduced in Eastern Portugal, were analyzed. The current work was carried out in one stream inhabited by the species in Oeiras: Lage stream, and fieldwork was conducted during 2007 in order to assess the diet of *X. laevis* populations in Lage stream. The physic variables from this ecosystem were studied and 70 specimens were captured. *Xenopus laevis* was found in very transformed environments. African clawed frog in Lage stream ate a wide variety and size range of prey. Benthonic and nektonic preys made the greatest contribution to diets. Terrestrial invertebrates were present but made up a small fraction of the diet. In addition, *Xenopus laevis* preyed on native vertebrates which include fishes and amphibians. There were significant differences in prey weight between local and season. These observations demonstrate the high adaptative power of this exotic amphibian, as an important element which facilitates its colonization of the aquatic environments from Portugal.

Keywords amphibian, *Xenopus laevis*, trophic ecology, diet, impact

INTRODUCTION

Human-aided vertebrate introductions have occurred all over the world and, during the last decade invasions have been considered a threat to biodiversity and ecosystem functioning (Jaksic *et al.*, 2002; Fausch *et al.*, 2001, Measey, 1998a). Although accidental and deliberate introductions of amphibians occurs, the anuran *Xenopus laevis* (Daudin, 1802) is associated with its use in human pregnancy diagnosis, as a laboratory animal and in the pet trade (Measey & Tinsley, 1998). As a consequence, today wild populations of this amphibian grow at wide latitudinal ranges and habitats (Solís, 2004). Some are situated in Mediterranean or Mediterranean-like climates, similar to the native home of *X. laevis* in the South African Cape (Measey & Tinsley, 1998; Fouquet & Measey, 2006) such as Chile, California and Arizona, but it also survives in the temperate climates of the United Kingdom and Ascension Island (Tinsley & McCoid, 1996). *Xenopus laevis* is the best known invasive pipid, and its detrimental effects have been documented in these countries (Measey & Royero, 2005).

Within its native range, this species is noted for its rapid invasion of man-made water bodies and anthropogenically disturbed areas (Evans *et al.*, 1997). In fact, in Chile, as in US and Europe reproductive activity has been reported in artificial water bodies, mainly associated to agriculture and frequently euthrophic and polluted. The abiotic and biotic conditions where *X. laevis* has been introduced, and where its populations in most of the cases have grown and expanded, makes evident the peculiar physiological characteristics that allows this species to colonize new areas (Solís, 2004). Therefore, the first steps towards controlling this sort of exotic invasive species are to identify them, determine their geographic origin, their pathways of invasion, their interactions with native species, and the rippling effects they may have on communities and ecosystems (Iriarte *et al.*, 2005). On the other hand, to estimate and compare the impacts of an individual invasive species requires the assessment of three independent factors: the total area occupied, the species abundance, and finally, particular attention needs to be paid to one of the chief attributes of an invasive species - its diet (Parker *et al.*, 1999). Predation in aquatic communities is now widely considered to be of profound importance in structuring prey species diversity, species composition, distribution, feeding and activity levels, and production rates and thus impacts on all aspects of freshwater ecology (Measey, 1998a). In fact, predation by urodeles has been shown to strongly influence the size and distribution of prey in ponds and streams (Dodson, 1970).

Xenopus laevis is one of the most intensively studied laboratory animals, with investigations centered upon developmental, cell and molecular biology (Gurdon, 1996; Measey, 2001). In contrast, ecological studies of this anuran are surprisingly scarce (Measey, 1998a, b). However, concerns over predation pressure on native invertebrates and vertebrates by introduced populations of *X. laevis* seem to be well founded (Lafferty & Page, 1997). These authors found evidences of the presence of the endangered tidewater goby *Eucyclogobius newberrui* in the gut contents of *Xenopus laevis* inhabiting the estuary of the Santa Clara River, California. In both UK and Chile this amphibian probably consumes local amphibian eggs and larvae, as well as causing trophic cascades by consumption of benthic macroinvertebrates (Measey, 1998a; Lobos & Measey, 2002).

Xenopus laevis is principally confined to aquatic habitats, although able to move between water bodies (Measey & Tinsley, 1998), and exhibits a variety of feeding mechanisms corresponding to the diverse components of their diet (Measey, 1998a,b). The food of *Xenopus laevis* appears to include a large range of taxonomic preys available in the aquatic environment, with predominance of aquatic invertebrates, suggesting that this species is a non-selective predator. Furthermore, there are reports about the consumption of vertebrates (fish, birds and amphibians) in *X. laevis* stomachs, but they seem to occur just under exceptional circumstances (Tinsley *et al.*, 1996; Solís, 2004). It seems also that the offspring of *Xenopus laevis* may be a significant contribution to the nutrition of the parental population (McCoid & Fritts, 1980); this enables the adult population to exploit a nutrient resource (phyto and zooplankton) which could not be directly available (Tinsley *et al.*, 1996; Measey, 1998a). Cannibalism is an attribute of *Xenopus laevis* which is important for the success of feral (and native) populations (Tinsley & McCoid, 1996), although works of McCoid & Fritts (1980) have evidenced that the finding of *Xenopus* tadpoles in the stomach contents of the adults analyzed in California are of little relevance because of its rare frequency, predation being mostly upon invertebrates.

The aim of this study was to characterize the diet of adult *X. laevis* throughout a year in a stream in Oeiras, Portugal. Dietary composition and seasonal changes in the diet are considered.

MATERIALS AND METHODS

Study Area – This study was prompted by the observation of the occurrence of *X. laevis* in two sites of the Lage stream. We conducted our survey on that stream, which is located in Oeiras coast, about 20 km W of Lisbon, and runs from the Sintra municipality to the sea coast for about 16km. The study area covers approximately 11 km of this stream, from Cacém to the sea (Figure 1). This stream is almost entirely regularized, with both banks constituted by cement walls, and flows through a varied range of land-uses, including shrublands, farmed areas, suburban areas and city gardens. The Lage basin is more intensively farmed towards its northern limit where its margins are more naturalized; in its southern limit, where the margins consist of cement walls, city parks are more frequent. Stream flow in this disturbed basin is intermediate, with its stronger flow in the Winter. In Summer, water remains in a few pools formed along the stream. Some indigenous plant and fish species also occur in Lage stream in this portion of the Oeiras basin. The stream is annually cleared of vegetation by the municipality services and is thus subject to regular disturbance.

Study Sites – We surveyed 16 sites (at least once a month from March to September 2007) regularly distributed along the stream (Figure 1), all registered with a GPS (eTrex of Garmin). Survey sites included eight regularized and eight natural pools. Using 2004 ortophotomaps and local visits we characterized the habitats bordering each survey site. We characterized pools by their depth and length and by the type of soil present in both margins and in the stream bed. Hydroperiod was designated as either permanent (kept water for all year), intermediate (dried during some moths of the year), or temporary (dried frequently). We classified overhead canopy cover according to the type of vegetation present (grass, bushes or trees).

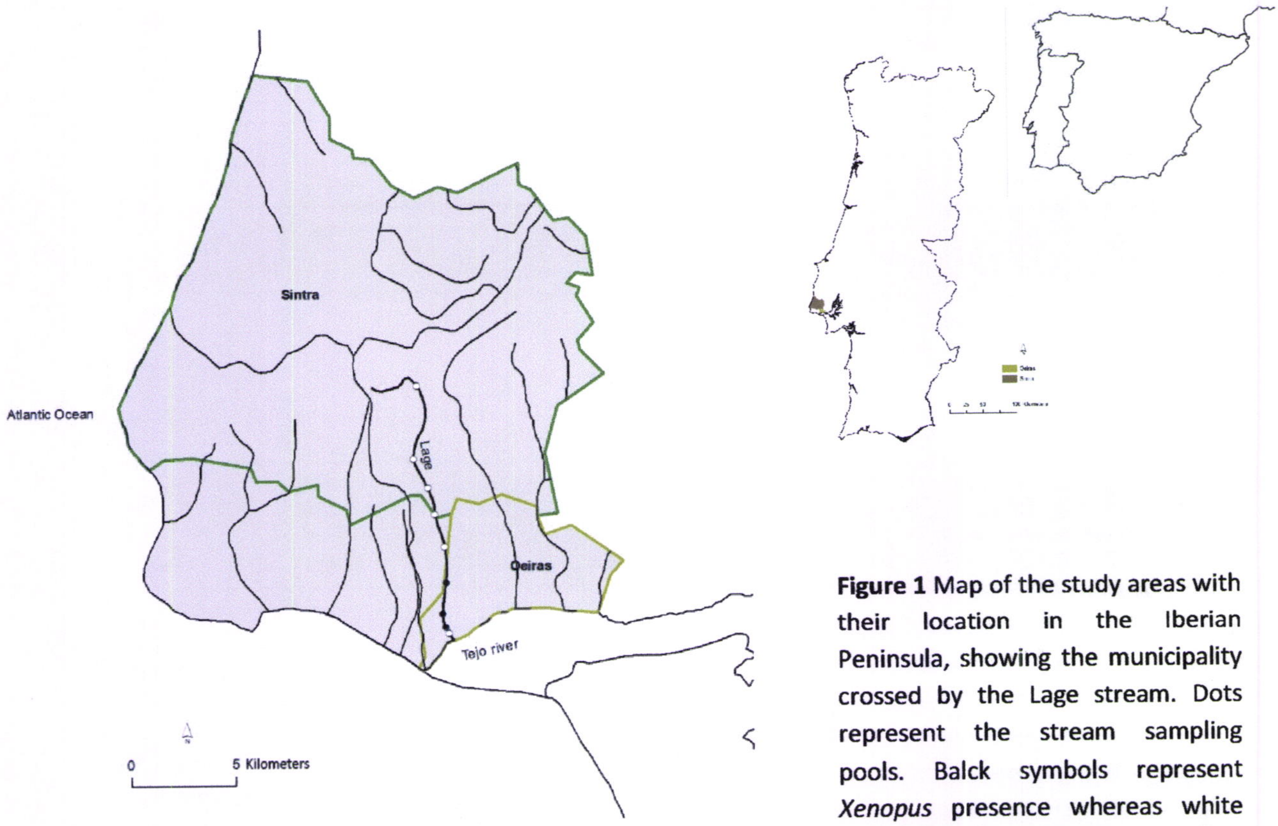

Figure 1 Map of the study areas with their location in the Iberian Peninsula, showing the municipality crossed by the Lage stream. Dots represent the stream sampling pools. Balck symbols represent *Xenopus* presence whereas white symbols represent its absence.

Sampling of *Xenopus laevis* and analysis of stomach contents – From July to September 2007, *X. laevis* were collected by electrofishing. In this period the water level was low enough to allow access to the deep reaches of the stream. The electrofishing set SAMUS-752GN (PDC) was programmed with a frequency of 30 Hz. We performed samplings weekly in 15' periods, separated by 10'. The frogs were euthanized by freezing within 2 hours of capture and later transported to the lab, where they were sexed, weighed, and their snout-urostyle length (SUL) was measured with callipers to the nearest 0.1 mm. At this time all frogs were transferred to alcohol (75%). All frogs were sexed, first based on their secondary sexual characteristics: protruding labial lobes in females and nuptial pads on the forearms of males. Then, frogs were dissected and sex was confirmed by inspection of the gonads. Stomachs were removed from 70 *X. laevis* (all the individuals that were captured). Each stomach was weighted and immediately preserved in alcohol (75%). In the laboratory, the stomach contents were collected and prey items were separated in homogeneous categories; the ones that we couldn't identify were named "unidentified". The items belonging to each prey category were grouped in a Petri dish and the contours of each group were drawn in transparent paper. After this, all prey items were identified with the help of a stereo microscope and all organisms in each taxon were identified to the order, and in some cases to family and species level. The proportion of prey taxa in the diet was estimated by measuring the areas defined by the contour of each prey item with the help of program WinRHIZO Mac Pro V5.0a (Regent Instruments Inc.). We also measured the smallest dimension of the two largest prey items found in each stomach.

Data analysis – To compare prey usage between sexes, and between location and season, graphic representations were made of the relative abundance of prey items in the diet. Comparisons were made between sexes, location and season of capture, taking into account the total amount of prey items in the stomach contents. To estimate the weight of each prey item in each full stomach we used the proportion of the area covered by that item in the Petri dish, and multiplied it by the total weight of the stomach.

A measure of relative diversity of prey in the diet was made using the Shannon-Wiener diversity index:

$$H' = \sum_{i=1}^{S} p_i \ln p_i$$

where **S** is the number of prey item **i** in the diet, **pi** is the relative abundance of prey item **i** in the diet and **ln** is the natural logarithm. The diversity indexes were compared between sexes, and among locations and seasons with a modification of the statistic test of Hutcherson, proposed by Zar (1984).

To examine prey size-selection by *X. laevis*, we calculated the correlation between the maximum average size distribution of preys and the snout-urostyle length of each individual. Preys smaller than 0.01 mm were attributed the value "0.01 mm" to ease the graphic representation, and prey with undetermined size were excluded from this graphic representation.

RESULTS

The results report to the stomach contents of 70 *Xenopus laevis* captured in three main areas of Lage stream: Jardim do Marquês, Estação Agronómica e Bairro da Lage, representing all the captures preformed from July to September 2007 (Table 1).

Table 1 Total *Xenopus* individuals captured in three main areas of Lage stream, representing all the captures preformed from July to September differentiated by sexes.

	July		September		
	Female	Male	Female	Male	Total/Local
Jardim Marquês	21	12	0	4	37
ENA	6	6	0	0	12
Bairro Lage	0	0	3	18	21
Total/Seasson/Sexe	27	18	3	22	

The first place is just about 2 km away from the sea coast, and when the tide is high, there may be some saline intrusion. Beyond Bairro da Lage we didn't see or captured any *X. laevi;* for that reason the sampling effort was focused in these three main locations.

Almost all stomachs had at least one identifiable prey item: 97,1% contained prey and 2,9% (2 individuals) only pink masses of digested content (with no identifiable prey items). Many stomachs contained detritus, some microalgae, and also some wood debris. No other vegetation material was found.

Data on stomach contents were divided up into groups by prey habitat (see Figure 2). Each group shows a large taxonomic range of invertebrate (and vertebrate) prey, but most consumed prey consisted of very few taxonomic groups. Vertebrate prey was found in the stomach of seven (10%) *X. laevis* from the stream. The fish, *Rana perezi* skeleton and egg mass were found to be intact, and one feather (species unidentified) was found in the stomach content of one frog.

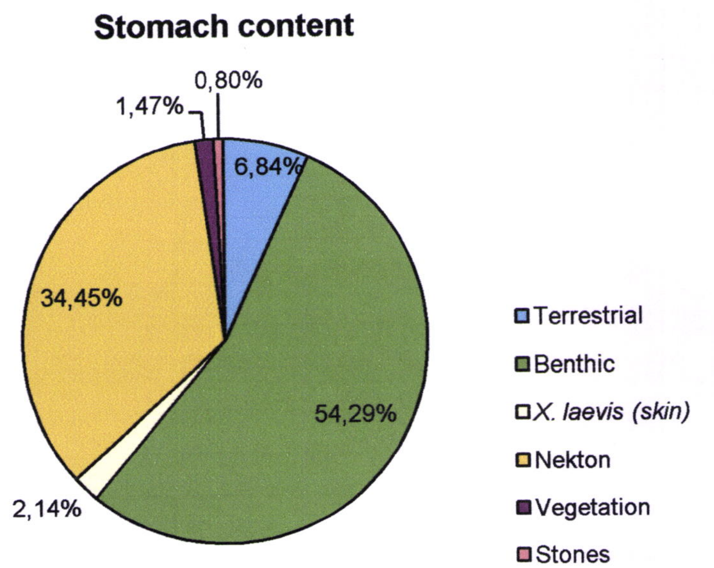

Figure 2 The total contribution of prey types: (a) terrestrial, (b) benthic, (c) *X. laevis* skin, (d) nekton, (e) vegetation, and (f) stone found in the stomach contents of *X. laevis* from Lage stream, Portugal (data collected from July to September 2007).

Table 2 shows the stomach contents of *Xenopus laevis*. Benthonic fauna were the largest component of stomach contents (54.29%), especially snails (*Physidae* 41.15%). Nekton consisted solely of macro-invertebrates, which made up 34.45% of the total prey consumed. The terrestrial prey items had a significant importance in the weight of the diet of *X. laevis*, consisting 6,84% of the total numbers of prey consumed (Figure 2). The prey category "*X. laevis*" has been placed in a separate group which comprises shed skin of *X. laevis*.

Table 2 Analysis of prey items recovered from stomach of 70 *Xenopus laevis* caught at pools on the Lage stream in Portugal; considering the total contribution of prey types ((a) terrestrial, (b) benthic, (c) *X. laevis* skin, (d) nekton, (e) vegetation and (f) stones found in the stomach contents. Estimates are based on the sum of all sampling dates. Generally identification was made to the lowest taxonomic level, but due to the state of decomposition of some items, this was not always possible.

Prey	Total	% *X. laevis* eating (n=70)	mean items ingested per eating individual	% composition
a) Terrestrial				**6.84**
Diptera	8	2.86	4	1.07
Muscidae	6	4.29	2	0.80
Coleoptera				
Carabidae	3	4.29	1	0.40
Hymenoptera				
Formicidae	3	1.43	3	0.40
Agriotypidae	1	1.43	1	0.13
Vespidae	1	1.43	1	0.13
Ichneumonidae	1	1.43	1	0.13
Hemiptera	1	1.43	1	0.13
Heteroptera	1	1.43	1	0.13
Chilopoda	1	1.43	1	0.13
Hexapoda				
Unidentified	2	2.86	1	0.27
Amphibian (skeleton)	7	7.14	1.4	0.94
Amphibian (eggs)	1	1.43	1	0.13
Fish (skeleton)	11	7.14	2.2	1.47
Birds				
Unidentified	1	1.43	1	0.13
Eggs				
Unidentified	3	2.86	1.5	0.40
b) Benthic				**54.29**
Nemathelminthes	79	24.29	4.35	10.59
Platyhelminthes	5	5.71	1.25	0.67
Annelida				
Achetes	3	4.29	1	0.40
Mollusca				
Physidae	307	51.43	9.14	41.15
Ancylidae	1	1.43	1	0.13
Crustacea				
Decapoda				
Procambarus clarkii (all body)	2	2.86	1	0.27
Procambarus clarkii (pieces)	4	4.29	1.33	0.54
Unidentified	4	5.71	1	0.54
c) X. laevis				
Skin	16	22.86	1	**2.14**
d) Nekton				**34.45**
Cestoda	5	7.14	1	0.67
Coleopetera	3	4.29	1	0.40
Dytiscidae	1	1.43	1	0.13
Hydrophilidae				
Diptera	124	30	5.90	16.62
Chironomidae (larvae)	8	4.29	2.66	1.07
Chironomidae (ninfa)	96	52.86	2.73	12.87
Plecoptera (larvae)	3	4.29	1	0.40
Ephemeroptera	1	1.43	1	0.13
Tricoptera	1	1.43	1	0.13
Hydroptilidae	1	1.43	1	0.13
Hemiptera	1	1.43	1	0.13
Gerridae	2	2.86	1	0.27
Heteroptera				
Hexapoda	3	4.28	1	0.40
Unidentified				
Crustacea				
Ostracoda	8	7.14	1.6	1.07
e) Vegetation	11	15.71	1	**1.47**
f) Stones	6	4.29	2	**0.80**

There were considerable differences in the total stomach contents among stream areas. Analyzing the total amount of prey consumed by local of capture, in Jardim do Marquês females are mostly consumers of Physidae and Plecoptera; males in turn are big consumers of Chironominae and Nemathelminthe. In Estação Agronómica females' diet consists mainly of Plecoptera, Chironomidae, with a big proportion of unidentified item by its decomposition. Differently, in Bairro da Lage males and females are regularly feeding on Physidae, but males eat more Nemathelminthe than females (Figure 3).

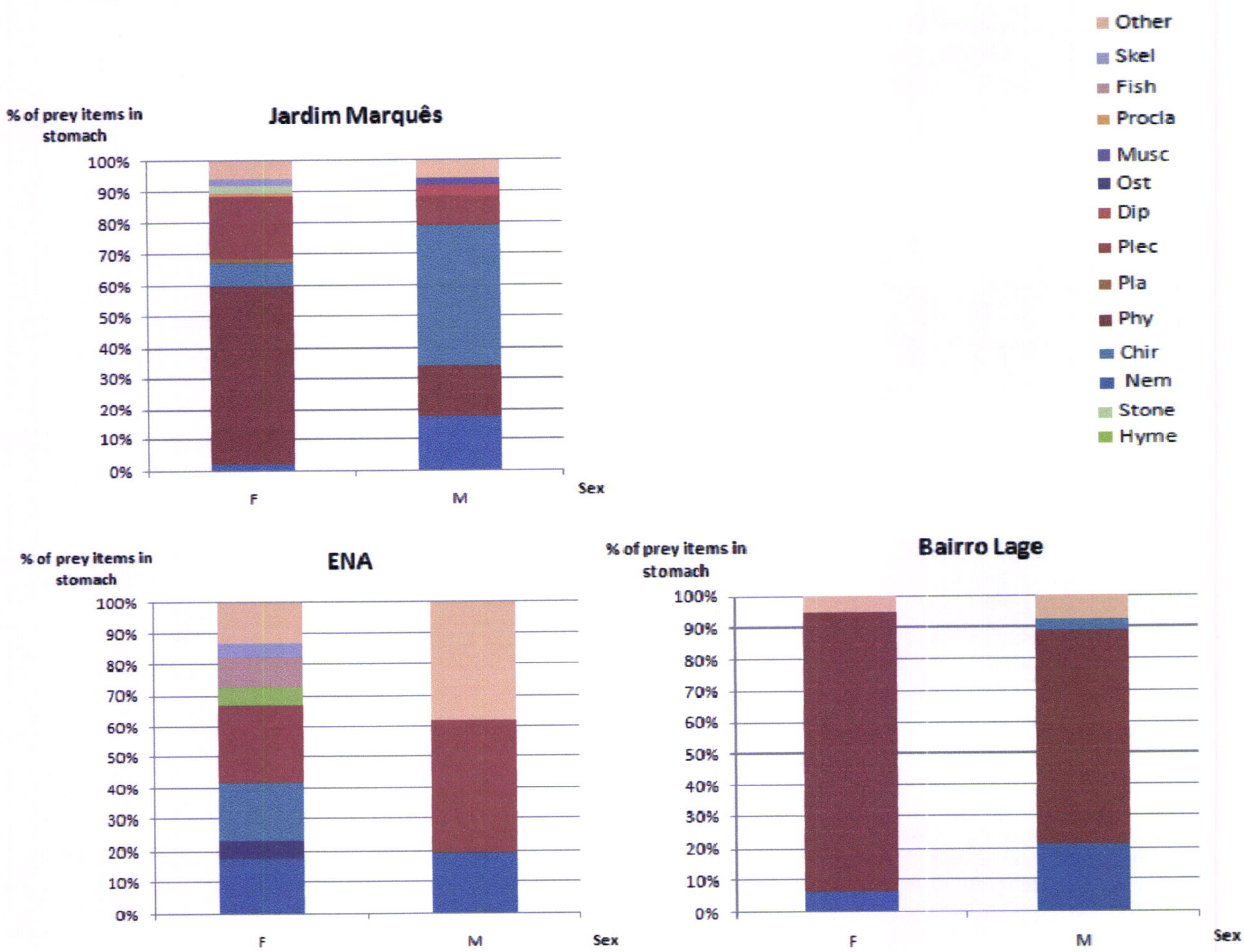

Figure 3 Local diet variation between sexes of *X. laevis* in Lage stream from July to September 2007; **F** represents females and **M** represents male's diet. **Nem** (Nemathelminthe), **Phy** (Physidae), **Plec** (Plecoptera), **Dip** (Diptera), **Fish** (Fish), **Hyme** (Hymenoptera), **Stone** (Stone), **Ost** (Ostracoda), **Musc** (Muscidae), **Skel** (Skeleton), **Chir** (Chironomidae), **Pla** (Platelminthe), **Procla** (*Procambarus clarkii*), **other** (unidentified). "Other" represents all prey items with less than 3% of the stomach content.

The diet of *Xenopus* is distinctly different between sexes and sampling season (Table 1). In general males consume more Chironomidae than females, which in turn feed mostly on Physidae. It's evident that females in July feed on Physidae, Plecoptera and Chironomidae while in September their diet consist mostly of Physidae. Stomach contents of males almost had no variation in prey items; they eat essentially the same amounts of Chironomidae, Physidae and Nemathelminthe in July and September (Figure 4).

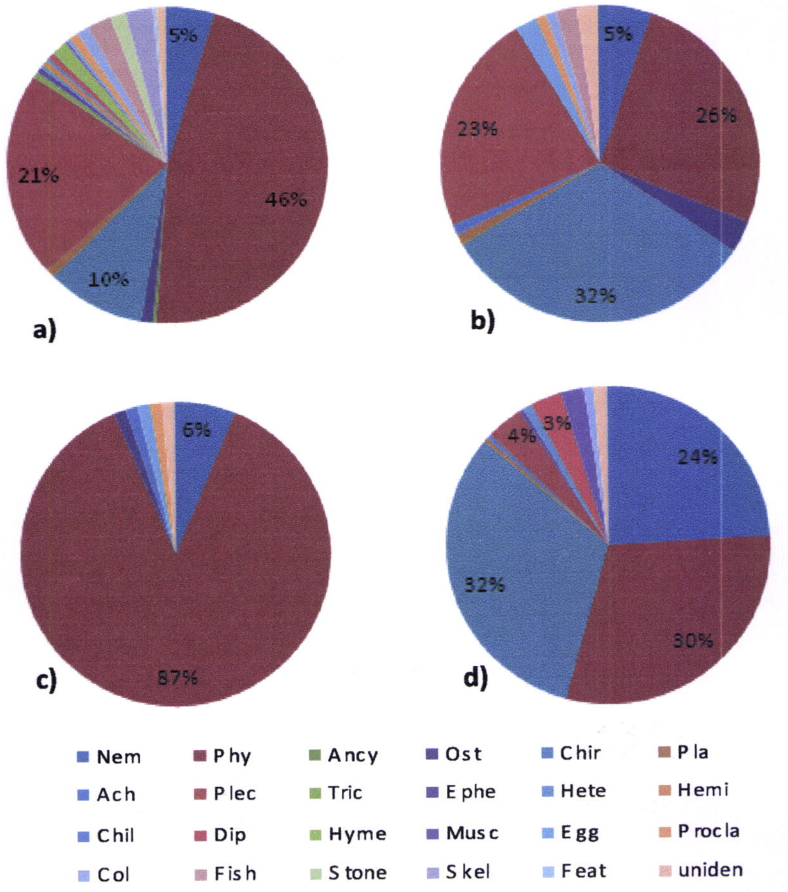

Figure 4 Seasonal diet variation between sexes of *X. laevis* in Lage stream from July to September 2007; a) *Xenopus* females in July, b) *Xenopus* males in July, c) *Xenopus* females in September, and d) *Xenopus* males in September. **Nem** (Nemathelminthe), **Ach** (Achetes), **Chil** (Chilopoda), **Col** (Coleoptera), **Phy** (Physidae), **Plec** (Plecoptera), **Dip** (Diptera), **Fish** (Fish), **Ancy** (Ancylidae), **Tric** (Tricoptera), **Hyme** (Hymenoptera), **Stone** (Stone), **Ost** (Ostracoda), **Ephe** (Ephemeroptera), **Musc** (Muscidae), **Skel** (Skeleton), **Chir** (Chironomidae), **Hete** (Heteroptera), **Egg** (Egg), **Feat** (Feather), **Pla** (Platelminthe), **Hemi** (Hemiptero), **Procla** (*Procambarus clarkii*), **uniden** (unidentified). The prey items with a percentage of occurrence inferior to 3% are not mentioned in the figure.

When we analyze this results taking into account the weight of each prey category, the prey items with largest biomass and least importance for the total number of prey items are those that have more relevance in weigh analysis. In Jardim do Marquês and Estação Agronómica, prey items "fish" and "*P. clarkii*" are more important than Plecoptera the most eaten prey item by females. There is a large proportion of unidentified prey items that bias these results, with a biomass that varies between 53 and 91% of the total stomach content weight (Figure 5 and 6).

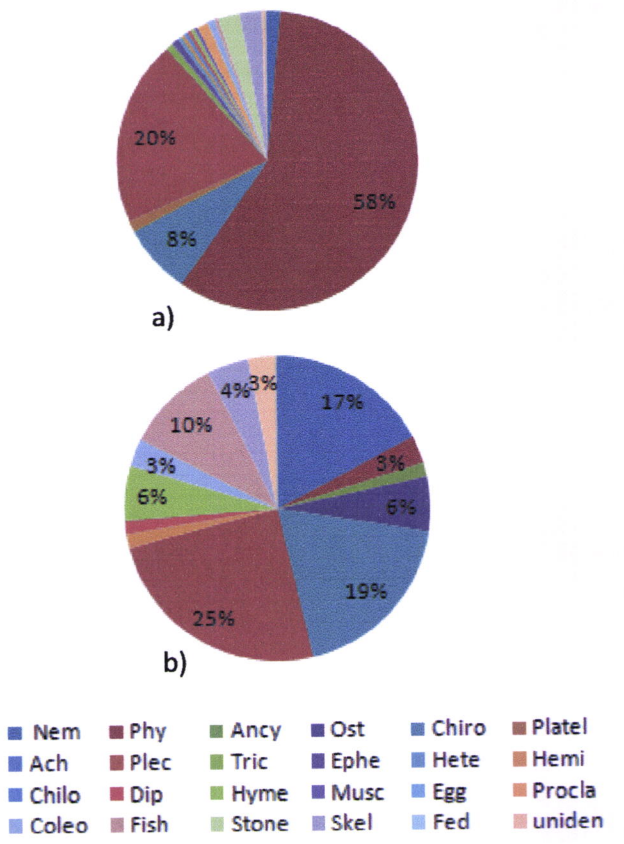

Figure 5 Total number prey item variation in *X. laevis* diet by pool location in Lage stream from July to September 2007; a) Female diet in Jardim Marquês, b) Female diet in ENA. **Nem** (Nemathelminthe), **Ach** (Achetes), **Chil** (Chilopoda), **Col** (Coleoptera), **Phy** (Physidae), **Plec** (Plecoptera), **Dip** (Diptera), **Fish** (Fish), **Ancy** (Ancylidae), **Tric** (Tricoptera), **Hyme** (Hymenoptera), **Stone** (Stone), **Ost** (Ostracoda), **Ephe** (Ephemeroptera), **Musc** (Muscidae), **Skel** (Skeleton), **Chir** (Chironomidae), **Hete** (Heteroptera), **Egg** (Egg), **Feat** (Feather), **Pla** (Platelminthe), **Hemi** (Hemiptero), **Procla** (*Procambarus clarkii*), **uniden** (unidentified). The prey items with a percentage inferior to 3% are not mentioned in the figure.

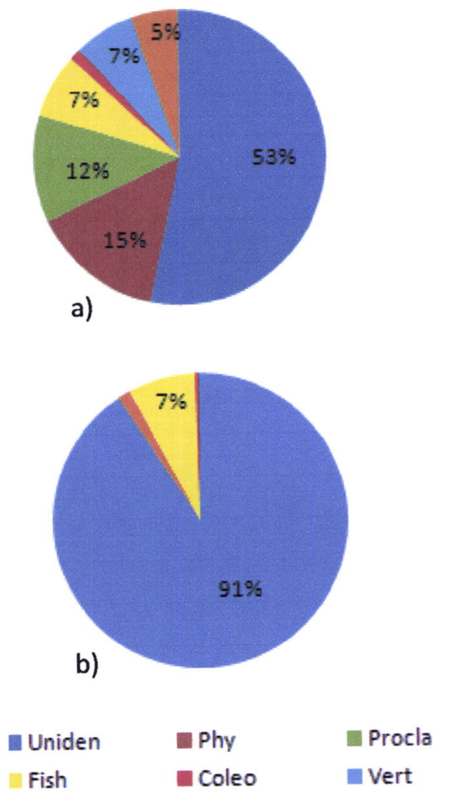

Figure 6 Category prey weigh variation in *X. laevis* diet by pool location in Lage stream from July to September 2007; a) Stomach of female in Jardim Marquês, b) Stomach of female in ENA. **Col** (Coleoptera), **Phy** (Physidae), **Fish** (Fish), **Procla** (*Procambarus clarkii*), **uniden** (unidentified), **Vert** (Vertebrate). The prey items with a percentage inferior to 3% are not mentioned in the figure.

The proportion of ingested skin was similar in males (53,3%) and females (46,7%). However, the percentage of stomachs containing sloughed skins was higher in July 2007.

Considering prey item ingested by *Xenopus laevis* it is clear that there were no significant differences between the sexes (t-test=0.45; df=708,55; n=70), differences were strongly significant only when comparing the diversity indexes for location and season (P<0.001). However, considering the weights of each prey group, samples were found to be significantly different only when comparing the diversity index of Jardim do Marquês and Estação Agronómica (t-test=2.12; df=38,04; n=70; P<0.01) (location) (Table 3). The differences found previous fade away mostly because there is a large proportion of unidentified prey items.

Table 3 t-test results of the diversity index, taking into account the total of prey and the mean weight of each prey item in the stomach content of *X. laevis* in Lage stream; JM represents Jardim do Marquês, ENA represents Estação Agronómica, and Lage represents Bairro da Lage; ns – non significant.

	Number of prey items					Weight of prey items				
	Sex	Location			Season	Sex	Location			Season
		JM/ENA	ENA/Lage	JM/Lage			JM/ENA	ENA/Lage	JM/Lage	
df	708,55	155,34	230,23	264,37	695,27	56,31	38,04	30,42	54,11	59,68
t	0,45	-3,42	9,5	8,24	4,12	1,16	2,12	-0,95	1,38	1,22
P	ns	<0,001	<0,001	<0,001	<0,001	ns	<0,01	ns	ns	ns

In figure 7 we show the minimum average size of the largest prey item for each individual. It is evident that the size of the largest prey items does not necessarily increases with the increase of the SUL. In fact, there's a positive correlation between the minimum average size of prey item and the snout-urostyle length of *Xenopus,* however this correlation was not significant (r=0,14; N=66). The size of some prey items was impossible to measure with electronic calipers, and were considered with a size <0,01mm; likewise we have found a intact skeleton of *Rana perezi* in one stomach content that was impossible to measure; this individual was considered an outlier and not shown in figure 7.

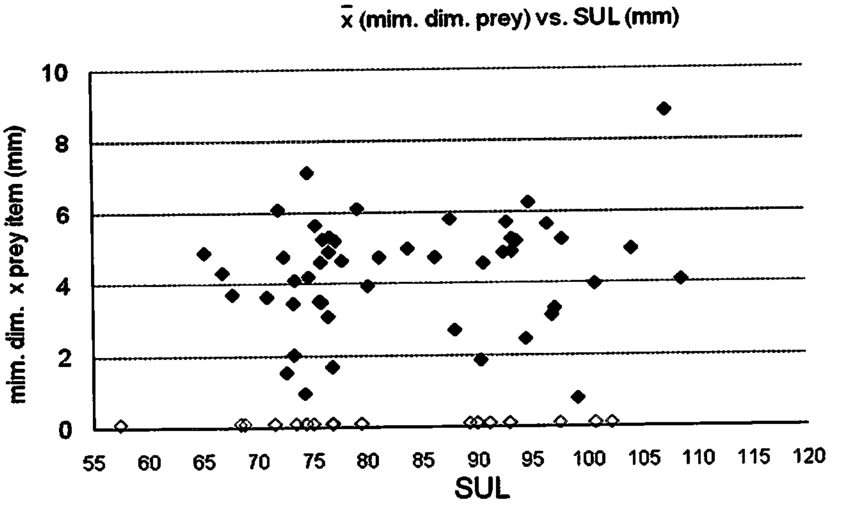

Figure 7 Representation of the maximum size prey item ingested by *X. laevis*, taking in attention the minimum dimension of the prey and the snout-urostyle length measure. We have identified a frog (*Rana* perezi) in the stomach content of one individual that was not represented in the figure; ◆ represents individuals measured, ◊ represents individual too small to be possible to measure.

Discussion

The food of *Xenopus laevis* appears to include everything 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, b). Diet determined from stomach contents of animals captured at Lage consistently showed a predominance of benthic invertebrates, both numerically and by weight. This is consistent with other studies of the stomach contents of *X. laevis* throughout the natural and non-natural range (Tinsley & McCoid, 1996; Measey, 1998a), as in a pond in the South Wales (Measey, 1998a), from a stream in California (McCoid & Fritts, 1980) and in central Chile (Lobos *et al.*, 1999). Regarding our findings, the principal prey in Lage was Physidae, Chironomidae and Plecoptera, but no estimate of the weight of these two last prey items was recorded. This has been observed in the diet of clawed frog population in Laguna de Battuco, Chile (Lobos, 2004), where the principal prey items ingested were Physidae and Chironomidae. This author explains the consumption of this prey by the fact that these two species are very tolerant to non-oxygenated waters, being the most abundant prey in these habitats. In addition, ostracods has previously been found to pass through the digestive system of clawed frogs unharmed, this may suggest that although ostracods may be ingested in large quantities the amount they contribute to the nutrition of individual *X. laevis* may be very low (Measey, 1998a). However, this study conflicts with conclusions of Lobos & Jaksic (2005) that reported for a population at Rinconada de Maipu, based on a sample of 21 stomachs, a numeric dominance of Crustaceans of the diet. Ostracods contributed very little to the diet of *X. laevis* in our study, and their abundance was seasonal.

The study of Measey (1998a) in South Wales involved *X. laevis* from a pond which would have provided a different availability of potential prey. However, this study and the studies of Measey & Tinsley (1998) and Tinsley *et al.* (1996) suggest a controversy concerning the ability of this species to capture fast motion preys. Measey (1998a) states that the ability of *X. laevis* to catch a range of nektonic prey suggests that if fish are present they may be consumed, and in fact small fish are easily caught by *X. laevis* when they pass close to a waiting individual. In common with the present study, this sit and wait method of capturing nektonic preys may explain their consistently low proportion in the diet. Still, this study conflicts with the conclusions of McCoid & Fritts (1980) that *X. laevis* is a clumsy predator adapted to capturing sessile or slow-moving items.

Xenopus laevis adults and tadpoles co-exist with other native vertebrate species within the same pool, for example the amphibian *Rana perezi* and some native fishes, *Chondrostoma lusitanicum, Cobitis paludica* and *Anguilla anguilla*. Some fish (*Eucyclogobius newberrui*) were found in the gut contents of Californian *X. laevis* inhabiting the estuary of Santa Clara River (Lafferty & Page, 1997), and there are reports about the presence of birds and amphibians in the diet of clawed frog although under exceptional circumstances (Tinsley *et al.*, 1996; Solís, 2004). This phenomenon has been observed in Portuguese *Xenopus* at Lage during this study, where we actually had the opportunity to see an individual actively preying a living eel and we found an amphibian skeleton in the stomach of one individual. The extent of the competitive potential between alien and native frogs cannot be currently appraised, but the predation potential of clawed frogs is troublesome (Lobos & Jaksic, 2005). In this study it was evident that *X. laevis* at Lage preys other vertebrate preys besides their own, including *Rana perezi* adults and this species egg masses. We didn't found other amphibians adults or larvae in the clawed frog stomachs, but it must be recognized that the potential exists. Inhabiting the same polls as their own larvae and with native adult and larvae amphibians, at such high densities, it would seem to be expected that significant impact is made by predation alone. Furthermore, exotic aquatic predators in natural water bodies may prompt trophic cascades, altering native species diversity and composition (Measey, 1998a). Resembling study cases in California, UK and Chile, predation by clawed frog on eggs, larvae and metamorphs of native amphibian species (Lobos & Measey, 2002) in Lage is as well an ongoing concern. Studies on the vulnerability of Portuguese endemic amphibians, especially the most aquatic are needed to assess the possible effects of the spread of alien invasive species as *X. laevis*.

It is likely that the frequent presence of parts of aquatic plants and microalgae in the stomach contents analyzed may be caused by their ingestion together with other more substantial prey, as suggested by Lobos & Jaksic (2005).

Evidence of cannibalism in the diet of *X. laevis* is well reported in the literature, being an attribute which is important for the success of feral and native populations (Tinsley *et al.*, 1996). For example, in California, cannibalism apparently allows colonization of newly created habitats in the absence of an established macro-invertebrate prey fauna (McCoid & Fritts 1980, 1993). No evidence of cannibalism was found in the present study, but we can't affirm that it doesn't exist. Indeed, the tadpole densities in pools fluctuate for the most part of each season; they were very high for few weeks and then declined rapidly. It is probable that the high densities of *X. laevis* adults and the lack of other prey items caused the sudden disappearance of tadpoles (Measey, 1998a).

It is not known how often *X. laevis* slough their skins (Measey, 1998a). In this study, clawed frog showed an increase in the frequency of sloughed skin in stomachs at July 2007, which in literature coincided with the onset of mating (Tinsley *et al.,* 1996;

Tinsley & McCoid, 1996). No significant difference was found between males and females, suggesting that the frequency of sloughing is the same for both sexes.

Although the diet of clawed frogs was dominated numerically by aquatic organisms, terrestrial preys consistently contributed a large proportion to the total mass of prey ingested. In reviews of *Xenopus* diet, it is considered unlikely that the large amount of terrestrial prey reported in stomach contents originates solely from invertebrates which have fallen or been swept from overhanging vegetation into the water (Tinsley *et al.*, 1996). In the present study, items of terrestrial origin make up to 6,84% of the all ingested prey items and 16.32% of the total mass identified. Measey & Tinsley (1998) suggest that many may have been ingested after falling into the pool, where they may have become trapped in the surface tension or fallen to the bottom. However, some may have been caught whilst on land. Additionally, the high proportion of unidentified prey found in *X. laevis diet* may be explained by the fact that the stomach contents may be more or less digested depending if they are feeding during the night or day.

Results in this study clearly demonstrate the non-size selective predation by *X. laevis* on prey item available in pools. Although there is a weak positive correlation between prey size and the snout-urostyle length (SUL), not always a minor individual is associated to a small prey size item. This conflict with studies in South Wales, where *X. laevis* demonstrated a size-selective nature predation, selecting negatively the smallest and the largest size of prey classes (Measey, 1998a). Furthermore, there are seasonal and local effects in prey capture, probably due the impoundments at which pools are daily subjected. Pool size and inhabiting with fish may be another reason for differences in prey capture between locations. However, studies in prey availability and presence of coexisting species in each pool are needed to access the causes of such differences. Native piscine predators of benthic fauna are absent from smaller lentic habitats in the stream. This is partly caused by the extremes of physical conditions which such water bodies undergo, both daily and seasonally. Experimental data for *X. laevis* demonstrate a tolerance to non-oxygenated water bodies and high levels of salinity (Tinsley *et al.*, 1996).

If there is no recruitment to a population, and predation pressure is persistent, a rapid decline in population numbers would be expected (Measey & Tinsley, 1998). Findings that native predators consumed clawed frogs it would be interesting (Lobos & Jaksic, 2005), although it has been hypothesized that the clawed frog is little preyed upon because of its toxic skin (McCoid & Fritts, 1980). But, native predators may not be able to control the invasive frog, simply because it may be already too numerous (Lobos & Jaksic, 2005) or the existence of refugia make the last individuals difficult to eliminate, and as an explosive breeder there is always a potential for another large expansion (Measey & Tinsley, 1998).

Mediterranean habitats have long been recognized as being vulnerable to invasion by many species (Measey, 2001). Eradication if invasive populations of *Xenopus laevis* in such Mediterranean climates is considered to be difficult, although to date no management or control operations are in place (Fouquet & Maesey, 2006). Successful eradication of *Xenopus* populations from an area is unknown, but is still possible and desirable. In south Wales, *X. laevis* is limited by a short growing season and not by the availability of food. This help to explain why this invasive species has not emerged as a threatening invader of South Wales, UK (Maesey, 2001) as in so many other countries.

It is likely that the restructuring of the aquatic macro-invertebrate community by *X. laevis* would not occur in the absence of this invading species, or by any native species (Measey, 1998a). Extensive laboratory and field studies are needed for elucidation on which preys are available in the stream and which native species are more affected by *X. laevis*. Finally, there are two troublesome aspects of the African clawed frog introduction in Portugal. The first one involves its unaided spread upstream using stream canals and refugees to survive to rigorous environmental conditions. Second, the type of interaction that may be occurring between alien and native species needs to be determined. The absence of *Xenopus* predators that naturally control population size can convert *X. laevis* in an emerging invasive species.

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Considerações finais

Os efeitos das espécies exóticas na biodiversidade têm sido descritos como "imensos, insidiosos e geralmente irreversíveis" (IUCN, 2000) e os habitats mediterrânicos são reconhecidos como os mais vulneráveis às invasões por estas (Measey, 2001). Como um país de clima mediterrânico, Portugal é particularmente vulnerável a novas introduções. Sabe-se que nos países com clima semelhante, *X. laevis* tem um carácter já comprovado de praga (Lafferty & Page, 1997).

O presente trabalho é o primeiro estudo realizado que descreve a distribuição e dieta de populações selvagens de *X. laevis* em Portugal. De acordo com os resultados obtidos, ficou demonstrado que a população de *Xenopus* está restrita ao troço regularizado da ribeira da Lage e que as populações existentes estão a reproduzir-se. Também dedicámos particular atenção a um dos principais atributos de uma espécie invasora: o impacto exercido pela predação (Parker *et al.*, 1999), através da análise da sua dieta. A análise da dieta de uma espécie invasora fornece evidências do que está a ser consumido e pode adicionalmente fornecer informação sobre as espécies nativas que mais provavelmente poderão ser afectadas pela sua presença, através de predação ou competição (Park, 2004). Contudo, a análise da dieta, por si só, não pode ser usada para avaliar os impactos do predador sobre as presas. A informação sobre a dieta de uma espécie deve ser usada em combinação com modelos populacionais das suas presas para fornecer uma previsão dos impactos desse nível de predação sobre as mesmas (Park, 2004). Por estas razões, no futuro será importante avaliar a disponibilidade de presas de *X. laevis* na ribeira da Lage. No presente trabalho, este factor não foi analisado, uma vez que não tínhamos indicação *a priori* sobre os tipos de presas consumidos nesta ribeira e a sua realização iria implicar um esforço intensivo para avaliar a abundância de todas as presas disponíveis. Ademais, o tipo de interacção que pode ocorrer entre *X. laevis* e o anfíbio nativo *Rana perezi* necessita ser melhor avaliado, pois a partição de nichos entre estas espécies é ainda desconhecida.

Lafferty & Page (1997) sugerem que um aumento excessivo de caudal pode ajudar *X. laevis* a expandir a sua distribuição para jusante, em ribeiros na Califórnia. Por outro lado, no Chile foi documentada a sua expansão pelas áreas agrícolas do Chile central, usando os canais de irrigação e também através de migrações por terra (Lobos & Jaksic, 2004). À data de conclusão do presente trabalho, uma nova população de *X. laevis* foi descoberta numa ribeira vizinha à da Lage (ribeira dos Ossos) e por este motivo é extremamente importante a monitorização das populações de *Xenopus* na Lage e na ribeira dos Ossos com o objectivo de prevenir mais expansões desta espécie. Seria de interesse prever as possíveis direcções de expansão destas populações para maximizar os esforços de erradicação.

É de opinião geral que as erradicações de populações invasoras de *Xenopus laevis* em climas mediterrânicos são difíceis, apesar de até ao momento não estarem a ser aplicados programas de monitorização ou acções de controlo (Fouquet & Measey, 2006). Em Portugal, a erradicação de *X. laevis* é possível e desejável, enquanto as suas populações se encontram restritas e antes que estas se tornem mais difusas e quaisquer operações de controlo mais dispendiosas. Apesar de erradicações de populações de *X. laevis* numa determinada área sejam desconhecidas, algumas tentativas de controlo têm sido feitas noutros locais. Nestes casos, elevadas concentrações do pesticida Rotenone mostraram-se inúteis na erradicação de *X. laevis* em Los Angeles, porque esta rã pode ter uma elevada proporção de respiração aérea (McCoid & Fritts, 1980). Estudos na Califórnia e no Reino Unido demonstraram que o controlo sobre a comercialização deste anfíbio é crucial para prevenir a sua expansão.

Neste momento, é ainda impossível ter a certeza da data e do local de introdução de *Xenopus* na ribeira da Lage, o que seria apenas possível de avaliar com futuros estudos genéticos focando a idade e origem destas populações.

Finalmente, uma maior atenção aos modelos de distribuição e aos impactos poderá ajudar a orientar os trabalhos futuros. Os ecólogos desde há muito tempo têm prestado muita atenção aos efeitos de espécies exóticas invasoras na biodiversidade nativa. Assim, este estudo poderá ser importante como um aviso que, desejavelmente, conduzirá a um plano de erradicação rápido e eficaz que controlará ou até mesmo erradicará a espécie enquanto está ainda contida numa pequena área.

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