HERPETOLOGICAL JOURNAL, Vol. 12, pp. 163-168 (2002)

INVASIVE POPULATIONS OF *XENOPUS LAEVIS* (DAUDIN) IN CHILE

GABRIEL LOBOS[1] AND G. JOHN MEASEY[2]

[1]*Laboratorio de Hidronomía, Facultad de Ciencias Forestales, Universidad de Chile, Santiago, Chile*

[2]*Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*

Invasive populations of *Xenopus laevis* are known from the UK, USA and Chile, although there is poor documentation of the latter. Currently, four administrative Regions in Chile are reported as having established populations. Fieldwork during the austral winter of 2001 was conducted in order to assess the density and diet of *X. laevis* populations in two localities. At one site, fewer than 30 adults were captured and a population 4 times this size was estimated. At the second site, nearly 2000 adults were trapped and a population of nearly 20 000 was estimated. This yielded density estimates of 0.37 and 0.25 clawed frogs m^{-2} respectively. However, significant bias in the sex ratio of animals caught at each site suggests that the populations may be even larger. Stomach contents of a sub-sample of animals revealed a diet consisting primarily of zoobenthic and zooplanktonic components. Further work is required to assess the extent to which this anuran affects the biodiversity of indigenous aquatic invertebrate, fish and amphibian populations.

Key words: African clawed frogs, exotic species, invasive amphibians, South America

INTRODUCTION

The African clawed frog, *Xenopus laevis* (Daudin), is regarded as a standard for amphibian studies; renowned for its ease of laboratory maintenance and simple stimulation of breeding (Gurdon, 1996). *X. laevis* has a wide natural range, covering most of sub-Saharan Africa with five commonly recognized subspecies: the largest of these, *X. l. laevis*, is commonly encountered in South Africa, Swaziland, Lesotho, Zimbabwe, Botswana and Namibia (Poynton, 1964; Kobel *et al.*, 1996). Within its native range, this subspecies is noted for its rapid invasion of man-made water bodies and anthropogenically disturbed areas (Evans *et al.*, 1997), and in the Cape region it has displaced the naturally occurring acid black-water species *X. gilli*, contributing to the inclusion of the latter in the red data book (Picker & DeVillers, 1989).

There have been a number of publications concerning extralimital populations of *Xenopus laevis* (all of which are of *X. l. laevis* and will be hereafter referred to as *X. laevis*), and these were reviewed by Tinsley & McCoid (1996). Since then, a number of studies have raised concern that the size and impact of *X. laevis* populations are increasing in the UK and USA (Measey, 1998, 2001; Crayon, in press). Indeed, concerns over predation pressure on native aquatic invertebrates and vertebrates by introduced populations of *X. laevis* seem to be well founded (Lafferty & Page, 1997).

In this study, we begin by reviewing the literature concerning introduced populations of *X. laevis* in Chile, to determine the areas affected by invasion. Literature on other invasive amphibians concurs that impact is positively related to density in extralimital habitats (e.g. Kupferberg, 1997; Kraus *et al.*, 1999; Crossland, 2000). Therefore, we have concentrated on population density of *X. laevis*, using two sites in the Metropolitan Region of Chile: Antumapu (studied by Lobos *et al.*, 1999) and Rinconada (a previously undocumented locality for *X. laevis*). Diet analyses were carried out on a sub-sample of animals captured.

Correspondence: G. J. Measey, Institut de Recherche pour le Développement, 32 Avenue Henri Varagnat, 93143 Bondy Cedex, France. *E-mail*: John.Measey@bondy.ird.fr

LITERATURE REVIEW OF *XENOPUS LAEVIS* IN CHILE

Chile is a recognized biodiversity hotspot (Myers *et al.*, 2000), and has 43 known species of anurans, of which 33 are endemic to this narrow strip on the west coast of South America (Formas, 1995). The clawed frog was first mentioned in the Red Data book for Chile by Glade (1983), who called for studies to investigate the effects of this invading anuran. Veloso & Navarro (1988) also mentioned *X. laevis* in their summary of reptiles and amphibians of Chile, giving only general geographic and ecological distributions: the administrative region 'Metropolitan' (Santiago and surrounds), and semi-arid Mediterranean regions (altitude 250-500 m). Jaksic (1998) and Hermosilla (1994) described *X. laevis* from the Metropolitan and VIIIth Regions (Fig. 1), dating the entry of this species into Chile to the 1970s.

National notification of the first feral populations from Pudahuel, Metropolitan Region (Fig. 1) was made in 1986, and hope was expressed that animals would not move south (Hermosilla, 1994). However, it is thought that *X. laevis* were sent to the Department of Biology, University of Concepción (VIIIth Region) for experimentation and breeding, and subsequently escaped (Hermosilla, 1994). Formas (1995) and Hermosilla (1994) both raised concern about the possible impact of *X. laevis* on native herpetofauna.

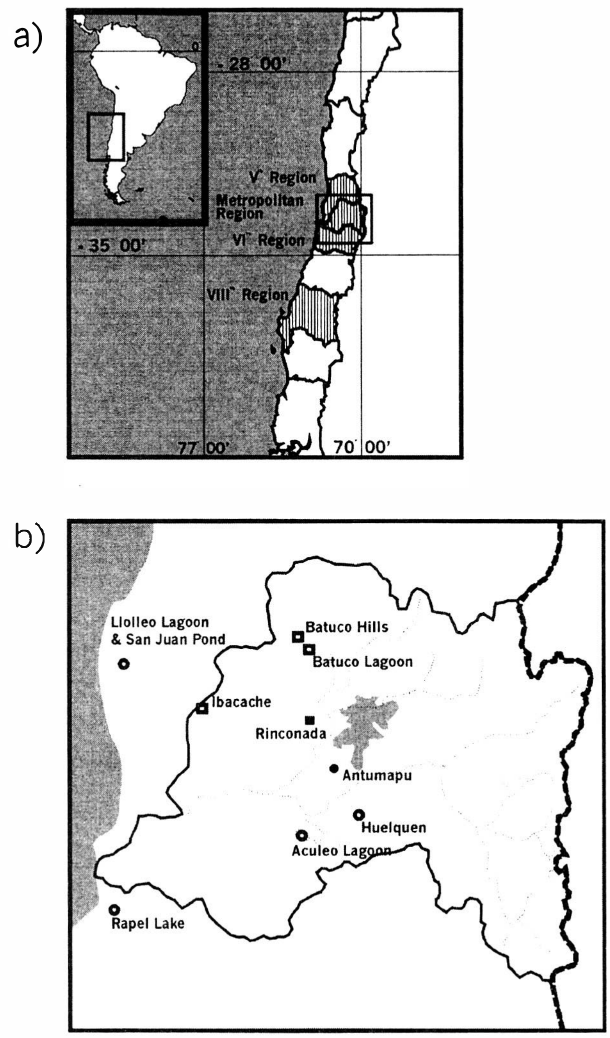

FIG. 1. The reported distribution of *Xenopus laevis* populations in Chile. (a) Vertically shaded areas show political regions of Chile reported to hold invasive populations of *X. laevis* (data from Lobos *et al.*, 1999; Hermosilla, 1994; and this study). (b) Enlargement of (a) showing sites of capture of *X. laevis* in this study (solid symbols), from Lobos *et al.* (1999, square symbols), and recently discovered localities (round symbols). Dotted lines represent major rivers, and the heavy dashed line an international boundary.

In the only published study of the ecology of *X. laevis* in Chile, Lobos *et al.* (1999) analysed the stomach contents of four populations of *X. laevis* in the Metropolitan Region of Chile, including two pristine habitats (Batuco Hills and Ibacache, see Fig. 1), between November of 1997 and February of 1998.

MATERIALS AND METHODS

STUDY SITES

The first locality, Antumapu – in the Pintana (33° 37'S, 70° 39' W), is an artificial pond on the Antumapu campus of the University of Chile. Stone walls form the bank to a depth of 1 m, with a total area of 400 m^2 (40 × 10 m). One third of the pond is overgrown with reeds (*Scirpus californicus*); the rest of the pond has open water with dense mats of algae and submerged alien pondweed, *Elodea canadensis*. The pond is fed and drained by an irrigation channel that links with a number of other dams and ponds.

The second locality, Rinconada Dam (33° 29'S, 70° 54' W), is a large rain-fed dam with a southern wall of about 15 m and a flooded area of approximately 79 000 m^2. The dam feeds a large agricultural research station of the University of Chile at Rinconada. Emergent aquatic vegetation is poor (a few stands of *Cyperus* sp.), but submerged vegetation includes *Eragrostis* sp. and *Ludwigia peploides* at the dam wall. The water, during this study period, was highly eutrophicated with blooms of a blue-green alga (*Microcystis* sp.).

Environmental variables were measured at a depth of 0.15 m at 09.00 hr on the second visit to each locality. Water temperature was measured (to the nearest 0.5° C) with an alcohol thermometer, conductivity with a conductivity meter (model 72, ESD inc. Delaware, USA), dissolved O_2 by the Winkler method, and pH with a digital pH meter (EC310, HACH Colorado USA).

DENSITY ESTIMATES

Adults clawed frogs were captured using simple funnel traps (buckets with tight fitting lids, modified by lateral insertion of open cones) baited with liver. Five traps were set at dusk and collected at dawn at approximately two week intervals during March, April and May, 2001. All *X. laevis* caught were sexed using external morphological features (protruding labial lobes in females and formation of nuptial pads on the forearms of males). Animals were then freeze-branded with an individual three digit number (Daugherty, 1976) before being released at the site of capture (see Measey & Tinsley, 1998).

We assumed that this short winter period was not sufficient for significant changes to violate a closed population model (i.e. maturing sub-adults, deaths, im- and emigration), and thus we estimate population size using the Schnabel method, with 95% confidence limits calculated from a Poisson approximation to the Binomial distribution (Krebs, 1999). Density was calculated from the area of each site and its respective estimated population size.

DIET

Here we re-analyse stomach contents of 23 *X. laevis* collected from Antumapu (30 January 1998, Lobos *et al.*, 1999) by placing prey into groups by habitat and consequently by predation mechanism (Measey, 1998). In addition, stomach contents were taken from 21 *X. laevis* collected from Rinconada (10 March, 2001). In both cases, stomachs were removed from fixed animals and the contents enumerated and identified (see Lobos *et al.*, 1999).

RESULTS

A total of 27 adult *Xenopus laevis* was captured in funnel traps from the pond at Antumapu, with six individuals being recaptured during this study. Many *X. laevis* larvae at various developmental stages were observed swimming and breathing at the water surface on each visit. The water temperature was noted as 23° C, pH was 8.6, dissolved O_2 4.1 mgl^{-1} and conductivity 617 μS. At Rinconada Dam, 1913 individual *X. laevis* were caught on all trapping occasions, of which 37 were recaptured. Numbers of *X. laevis* caught in the traps on 4 April far exceeded our expectations (after the low numbers caught at Antumapu) with three of the five traps having so many animals that all air in the trap was excluded, and consequently 323 animals drowned. These animals were returned to the laboratory and fixed. On subsequent trapping occasions at Rinconada, traps were checked on an hourly basis throughout the night, avoiding any further fatalities. No *X. laevis* larvae were observed at Rinconada. The water temperature was noted as 11° C, pH 8.8, dissolved O_2 3.6 mgl^{-1} and conductivity 129 μS.

DENSITY ESTIMATES

Proportions of males and females caught were significantly different, suggesting trapping bias. To account for this, we calculated population estimates for both sexes, and then recalculated population estimates using female mark-recapture data only (numbers shown in parentheses below). Table 1 shows the numbers of captured and recaptured *X. laevis* on each sampling occasion. Using the Schnabel method, the population estimate at Antumapu was 43 (95% confidence limits of 20.1 to 98.4) individuals (39.2 females, with 95% confidence limits of 18.3 to 89.9). Thus, density is calculated as 0.11 (females 0.10) clawed frogs m^{-2}. Observed movement of individuals (Lobos & Garín, 2002) leads us to believe that the open model of population estimate (150 individuals) would more accurately reflect the size of the population. However, low numbers of recaptures prevent a meaningful analysis using an open model. At Rinconada, the estimate of the population of *X. laevis*, using the Schnabel method, was estimated as 19 824 (95% confidence limits of 14 649.9 to 27 583.2) individuals (13 797 females, with 95% confidence limits of 10 200.8 to 20 059.6). Hence, for Rinconada, density can be calculated 0.25 (females 0.17) clawed frogs m^{-2}.

TABLE 1. Capture recapture data for adult *Xenopus laevis* (numbers of males in parentheses) caught at an artificial pond on the Antumapu in the Pintana, Metropolitan Region, Chile; and Rinconada Dam, Metropolitan Region, Chile.

ANTUMAPU			
Date:	31.03.01	12.04.01	26.04.01
Day	1	13	27
Captured	6 (0)	22 (2)	5 (0)
1st marked		3 (0)	2 (0)
2nd marked			1 (0)
Released	6 (0)	22 (2)	5 (0)
RINCONADA			
Date:	04.04.01	20.04.01	08.05.01
Day	1	17	34
Captured	467 (131)	810 (178)	673 (86)
1st marked		8 (2)	5 (0)
2nd marked			24 (0)
Released	144 (38)	810 (178)	673 (86)

DIET

Table 2 shows the stomach contents of *Xenopus laevis* in Antumapu, consisting mainly of benthic elements (75%) – snails (*Physa* sp. 64.17%) and chironomid larvae (10.44%), both of which are tolerant of poorly oxygenated water. Zooplankton consisted solely of ostracods, which made up 17.91% of the total numbers of prey consumed.

Stomach contents of the 21 Rinconada *X. laevis* show the major component of the diet was zooplankton (95.24%), consisting of cladocerans (60.67%) and ostracods (35.57%). Other items were from benthic (3.07%), nektonic (0.27%) and terrestrial (1.41%) elements (Table 2).

DISCUSSION

The central area of Chile is one of five regions of the world to have a Mediterranean climate, along with the South African Cape and California (di Castri, 1991). In California, McCoid & Fritts (1995) reported the year-round growth of *Xenopus laevis* in what they described as 'optimal conditions', with an extended breeding season, and maturation in as little as eight months. This is considered a prime reason for the frog's rapid establishment and continuing expansion of range there (McCoid & Fritts, 1995). Here we discuss our results and the possible impacts of this invasive anuran in the Mediterranean region of Chile.

Density estimates recorded here show great variability and dependence on various food sources. It is unlikely that the population of *X. laevis* at Rinconada Dam remained closed *sensu stricto* over the trapping period; for example, many potential predators were observed at the dam (e.g. *Nycticorax nicticorax*, night heron; *Casmerodius alba*, great egret). However, we consider that any resulting fluctuation would not significantly change a population of this size, and hence we consider that the closed population estimate (19 824 individuals) is acceptable at this locality. The large number of animals found at Rinconada Dam is consistent with findings at Edwards Airforce Base, California (J. Crayon personal communication), but an order of magnitude greater than those estimated in South Wales (Measey & Tinsley, 1998). Rinconada Dam acts as a reservoir for surrounding farmland, which includes vinyards.

Sex ratios at both localities were found to be highly skewed in favour of females, which may be more attracted to the baited traps used in this study. Biased sex ratios have been previously observed in California and

TABLE 2. Analysis of prey items recovered from stomachs of 24 *Xenopus laevis* caught on 30 January 1998 at a pond on the Antumapu in the Pintana, Metropolitan Region, Chile; and 21 *Xenopus laevis* caught on 10 March 2001 at Rinconada Dam, Metropolitan Region, Chile.

ANTUMAPU

Prey items	Total	% *X. laevis* eating (n=24)	Items ingested mean	Items ingested range	% composition
Zoobenthos					**74.61**
Diptera					
Chironomidae (larvae)	67	12	5.58	1-12	10.44
Mollusca					
Physa sp.	412	20	20.60	3-33	64.17
Zooplankton					**17.91**
Crustacea					
Ostracoda	115	21	5.48	2 – 12	17.91
Nekton					**5.45**
Coleoptera					
Hydrophilidae	7	6	1.17	1 – 2	1.09
Dytiscidae	5	2	2.50	2 – 3	0.78
Odonata					
Libellulidae (larvae)	1	1	1.00		0.16
Coenagrionidae (larvae)	16	9	1.78	1 – 3	2.49
Aeshnidae (larvae)	4	2	2.00		0.62
Hemiptera					
Corixidae	1	1	1.00		0.16
Notonecta sp.	1	1	1.00		0.16
Terrestrial					**2.02**
Coenagrionidae	1	1	1.00		0.16
Lepidoptera					
Noctuidae	6	6	1.00		0.93
Dermaptera					
Forficula sp.	1	1	1.00		0.16
Coleoptera					
Buprestidae	1	1	1.00		0.16
Hymenoptera					
Formicidae	2	2	1.00		0.31
Arachnida					
Araneae	1	4.17	1.00		0.16
Crustacea					
Isopoda	1	4.17	1.00		0.16

attributed to the trapping method, as use of seine nets did not reveal skewed ratios (J. Crayon personal communication). If both populations are considered to have equal proportions of males and females, then estimates made using only females could be doubled for total population estimates (i.e. including uncaught males). This would suggest even larger populations of *X. laevis* both at Antumapu (499) and Rinconada (39 647), as well as a doubling of density and impact.

Diet determined from stomach contents of animals captured at Antumapu showed a predominance of benthic invertebrates, as seen throughout the natural and non-natural range of *X. laevis* (Tinsley & McCoid, 1996; Measey, 1998). At Rinconada, the stomach contents consisted mostly of zooplankton (cladocerans and ostracods) by number. However, this may give a false impression of contribution of prey to diet, as the mass of individually enumerated zoobenthos (present in all stomach contents) is greater than zooplankton (Table 2b). The large quantities of zooplankton ingested may change with seasonal abundance, as is known in other populations (Measey, 1998). Ostracods have previously been found to pass through the digestive system of clawed frogs unharmed (Measey, 1998).

Predatory impact on benthic species from *X. laevis* may have serious consequences for the biodiversity of Chile's macrobenthic invertebrates in lentic habitats. Absence of vertebrate prey items in these diet analyses

TABLE 2. (continued...)

RINCONADA

Prey items	Total	% *X. laevis* eating (*n*=21)	Items ingested mean	range	% composition
Zoobenthos					**3.07**
Mollusca					
Physa sp.	3	1	3.00		0.09
Diptera					
Chironomidae (larvae)	97	15	6.47	1 – 27	2.98
Zooplankton					**95.24**
Crustacea					
Cladocera	1944	21	92.57	5 – 500	59.67
Ostracoda	1159	21	55.19	8 – 295	35.57
Nekton					**0.27**
Coleoptera					
Dytiscidae	4	3	1.33	1 – 4	0.12
Hemiptera					
Notonecta sp.	5	2	2.50	1 – 4	0.15
Terrestrial					**1.41**
Arachnida					
Acarina	44	12	3.67	1 – 10	1.35
Araneae	2	2	1.00		0.06

cannot be taken to assume that such items are not eaten. Indeed, other alien populations of *X. laevis* have already been shown to prey upon small fish and amphibians (Lafferty & Page, 1997; Crayon, in press). At such high densities, it would seem inevitable that significant impact is made by predation alone; beyond this, exotic aquatic predators to natural water bodies may prompt trophic cascades, altering native species diversity and composition (Measey, 1998). Further secondary impacts may be associated with increased water turbidity and nutrient release, caused by *X. laevis* disturbing the sediment, and a change in population dynamics of native predators.

The Mediterranean region of central Chile has five native anuran species which are listed as vulnerable or endangered (Glade, 1983). Hunting and loss of habitat are presumed to have caused the disappearance of these species from much of their former range. However, like California and the UK, predation by clawed frogs on eggs, larvae and metamorphs of native amphibian species in Chile is an ongoing concern. Studies on the vulnerability of Chilean endemic amphibians, especially the mostly aquatic leptodactylid *Caudiverbera caudiverbera*, are urgently needed to assess the possible effects of the spread of alien invasive species.

Despite a history of 30 years of invasion by *X. laevis* in Chile, to date there have been no comprehensive studies documenting the impacts and spread of this species. Chile has a rapidly expanding viniculture industry that has the potential to take this invader, through extensive irrigation corridors, into new and previously uncolonized areas. Concerns about the potential impacts of *X. laevis* introductions in the US have led to the outlawing of their possession in Arizona, California, Florida, Louisiana, Nevada and Utah (see Crayon, in press). Nevertheless, anecdotal evidence continues to mount concerning the direct predatory effects of this invasive anuran, and indirect effects (such as competition) have yet to be studied. In Chile the potential exists for the high densities of *Xenopus laevis* we demonstrate here to invade a large area of the country, especially when their presence in temperate UK habitats is considered. There is an urgent need for further study of the problems caused by *X. laevis* in Chile and throughout its introduced range.

ACKNOWLEDGEMENTS

We are grateful to Matilde López, Rigoberto Solis, Luz Marina Cairos and Pedro Cattan for time and use of equipment. We also thank Martín Escobar, M. Angélica Vukasovic and Carlos Garín for help during fieldwork. This work was made possible by a Royal Society visiting research fellowship to GJM.

REFERENCES

di Castri, F. (1991). An ecological overview of the five regions of the world with a mediterranean climate. In *Biogeography of Mediterranean Invasions*, 3-16. Groves, R. H. and di Castri, F. (Eds). Cambridge: Cambridge University Press.

Crayon, J. J. (in press). *Xenopus laevis*. In *Status and Conservation of U.S. Amphibians. Volume 2: Species*

Accounts. Lannoo, M. J. (Ed). Berkeley: University of California Press.

Crossland, M. R. (2000). Direct and indirect effects of the introduced toad *Bufo marinus* (Anura: Bufonidae) on populations of native anuran larvae in Australia. *Ecography* **23**, 283-290.

Daugherty, C. H. (1976). Freeze-branding as a technique for marking anurans. *Copeia* **1976**, 836-838.

Evans, B. J., Morales, J. C., Picker, M. D., Kelly, D. B. & Melnick, D. J. (1997). Comparative molecular phylogeography of two *Xenopus* species, *X. gilli* and *X. laevis*, in the south-western Cape province, South Africa. *Molecular Ecology* **6**, 333-343.

Formas, J. (1995). Anfibios. In *Diversidad Biológica de Chile. Comité Nacional de Diversidad Biológica,* 314-325. Simonetti, M., Arroyo, K., Spotomo, A. and Lozada, E. (Eds). Santiago: CONICYT.

Glade, A. A. (1983). *Libro Rojo de los Vertebrados Terrestres de Chile*. Santiago: Corporación Nacional Forestal.

Gurdon, J. B. (1996). Introductory comments: *Xenopus* as a laboratory animal. In *The Biology of Xenopus*, 3-6. Tinsley, R. C. and Kobel, H. R. (Eds). Oxford: Oxford University Press.

Hermosilla, I. (1994). Un sapo Africano que se queda en Chile. *Comunicación Museo de Historia Natural de Concepción, Chile* **8**, 75-78.

Jaksic, F. (1998). Vertebrate invaders and their ecological impacts in Chile. *Biodiversity and Conservation* **7**, 1427-1445.

Kobel, H. R., Loumont, C. & Tinsley, R. C. (1996). The extant species. In *The Biology of Xenopus*, 9-33. Tinsley, R. C. and Kobel, H. R. (Eds). Oxford: Oxford University Press.

Kraus, F., Campbell, E. W., Allison, A. & Pratt, T. (1999). *Eleutherodactylus* frog introductions to Hawaii. *Herpetological Review* **30**, 21-25.

Krebs, C. J. (1999). *Ecological methodology*. Menlo Park: Benjamin/Cummings.

Kupferberg, S. J. (1997). Bullfrog (*Rana catesbeiana*) invasion of a California river: The role of larval competition. *Ecology* **78**,1736-1751.

Lafferty, K. D. & Page, C. J. (1997). Predation on the endangered tidewater goby, *Eucyclogobius newberryi*, by the introduced African clawed frog, *Xenopus laevis*, with notes on the frog's parasites. *Copeia* **1997**, 589-592.

Lobos, G. & Garín, C. (2002). Behavior, *Xenopus laevis*. *Herpetological Review* **33**, 132.

Lobos, G., Cattan, P. & López, M. (1999). Antecedentes de la ecología trófica del sapo Africano *Xenopus laevis* en la zona central de Chile. *Boletín del Museo Nacional de Historia Natural, Chile* **48**, 7-18.

McCoid, M. J. & Fritts, T. H. (1995). Female reproductive potential and winter growth of African clawed frogs (Pipidae: *Xenopus laevis*) in California. *California Fish and Game* **81**, 39-42.

Measey, G. J. (1998). Diet of feral *Xenopus laevis* in South Wales, UK. *J. Zool., Lond*. **246**, 287-298.

Measey, G. J. (2001). Growth and ageing of *Xenopus laevis* (Daudin) in South Wales, UK. *J. Zool., Lond*. **254**, 547-555.

Measey, G. J. & Tinsley, R. C. (1998). Feral *Xenopus laevis* in South Wales. *Herpetological Journal* **8**, 23-27.

Myers, N., Mittenmeyer, R., Mittenmeyer, C., da Fonseca, G. & Kents, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* **43**, 853-858.

Picker, M. D. & DeVillers, A. L. (1989). The distribution and conservation status of *Xenopus gilli* (Anura: Pipidae). *Biol. Cons*. **49**, 169-183.

Poynton, J. C. (1964). The Amphibia of southern Africa: a faunal study. *Ann. Natal Mus*. **17**, 1-334.

Tinsley, R. C. & McCoid, M. J. (1996). Feral populations of *Xenopus* outside Africa. In *The Biology of Xenopus*, 81-94. Tinsley, R. C. and Kobel, H. R. (Eds). Oxford: Oxford University Press.

Veloso, A. & Navarro, J. (1988). Systematic list and geographic distribution of amphibians and reptiles from Chile. *Mus. reg. Sci. nat. Torino* **6**, 481-540.

Accepted: 27.9.02