

FERAL *XENOPUS LAEVIS* IN SOUTH WALES

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Despite its prominence as the "standard laboratory amphibian", the ecology of the African clawed frog, *Xenopus laevis*, has been neglected. Feral populations have been documented in several countries with Mediterranean climates, but established populations are also known from the UK. Long term studies of individually-marked *X. laevis* in South Wales reveal large demographic fluctuations and the ability to migrate overland. Maximum longevity recorded from recapture of marked individuals was 14 years. Diet analysis demonstrates a major reliance on benthic invertebrates and zooplankton components of the pond fauna. Skeletochronological studies of growth rings in bone showed that lines of arrested growth are formed annually and allow calculation of age. Data on population age structure indicate successful recruitment is infrequent, with dominant cohorts originating in perhaps only four summers during the past 20 years.

INTRODUCTION

The African clawed frog, *Xenopus laevis*, has been intensively studied in the areas of developmental, cell and molecular biology (Gurdon, 1996). However, ecological studies of this principally aquatic amphibian remain scarce. Accidental and deliberate introductions of *X. laevis laevis* to alien environments are associated with its use in human pregnancy diagnosis, as a laboratory animal, and in the pet trade in the 1950s and 1960s. A recent review of feral populations (Tinsley & McCoid, 1996) noted that this species has colonized a variety of lentic habitats world-wide, but principally in Mediterranean climates similar to that of its native southern Africa.

The most intensively studied feral populations are those in California (McCoid & Fritts, 1980*a,b*; 1989; 1993; 1995; McCoid, Pregill & Sullivan, 1993; McCoid, 1985) where *X. laevis* has colonized several river drainages (Tinsley & McCoid, 1996). California has a Mediterranean climate (Walter, Harnickell & Muller-Dumbois, 1975), and McCoid & Fritts (1995) found that *X. laevis* grows year-round in optimal conditions, maturing in eight months, and has an extended breeding season.

The climate of the UK appears to be ill-suited to this southern African species. However, feral populations of *X. laevis* in the UK include a number of isolated reports as well as two established populations, on the Isle of Wight (now possibly extinct) and in South Wales (Tinsley & McCoid, 1996; Lever, 1977). This report summarizes a long-term study of the Welsh population of *X. laevis* from 1982 to 1996 and a shorter more intensive study of one site within the area (1994 to 1996). Detailed accounts of these studies are described elsewhere (Measey & Tinsley, in prep.).

MATERIALS AND METHODS

Long-term studies of populations of *X. laevis* were conducted in two adjacent watercourses in South Wales, from 1982 to 1996. The catchment areas of

these watercourses are below 100 m a.s.l. and lie within 8 km of the coast, exposed to westerly winds, which markedly increase the salinity of the water. The catchments are well drained; much of the area is either cultivated or pasture. A 2 km length of the river and associated ponds, confined to a steep sided U-shaped valley (hereafter referred to as the "Valley"), was sampled. A small calcareous stream runs through open pasture and then through a number of man-made terraces (hereafter referred to as the "Stream"). A man-made pond, studied intensively from November 1994 to August 1996, lies 0.5 km south-west and 15 m above the river on the top of a hill (hereafter referred to as the "Pond").

X. laevis were trapped using modified fyke nets baited with liver. Traps were set at dusk and collected at dawn. *X. laevis* caught were sexed and weighed, the snout-vent length (SVL) was recorded, and then they were marked using a dye injector ("Panjet", Wright Health Group Ltd, Dundee) with unique combinations of spots on the ventrum (Wisniewski, Paull, Merry & Slater, 1979). Beginning in 1994, *X. laevis* caught were freeze-branded with three digit numbers (Daugherty, 1976).

Population estimates were calculated using the triple catch method (Begon, 1979) and a zero-truncated geometric model based on numbers of marked animals recaptured from the population (Seber, 1973) at the Pond.

The Pond was visited at two-weekly intervals. Traps were set for two consecutive nights and physical parameters (water temperature, pH, conductivity and oxygen concentration) were measured. Water samples were taken for chlorophyll *a* analysis and algal composition. Replicate samples of benthic invertebrates were collected with 0.00125 m² cores and zooplankton with a 250 µm mesh net. Samples were preserved in 10% formalin and were subsequently identified, weighed, and sorted. Stomach contents were removed from 20 *X. laevis* (or as many as numbers captured permitted) per visit by stomach-flushing (Legler & Sullivan, 1979)

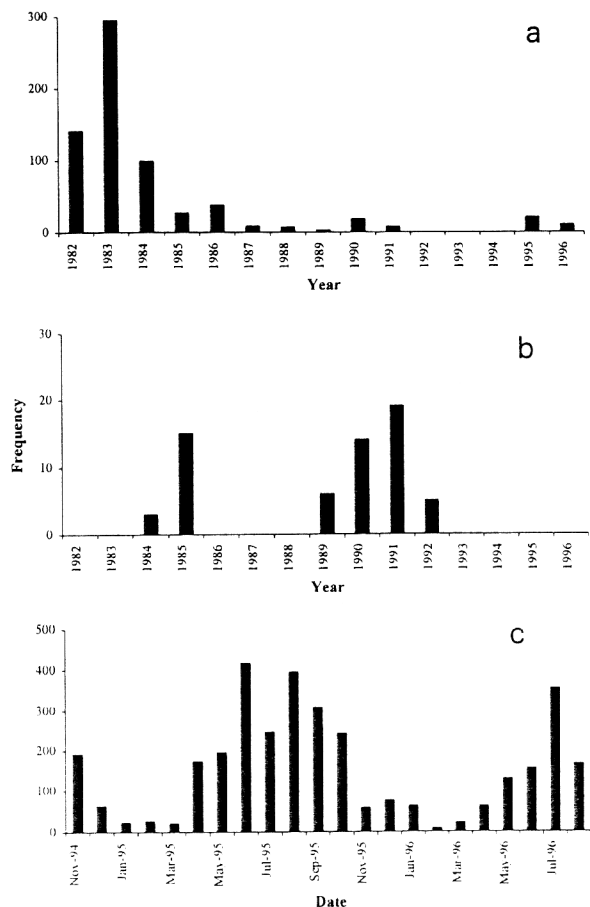


FIG. 1. Frequency distributions of numbers of *X. laevis* captured in (a) the Valley. (b) the Stream and (c) the Pond.

within four hours of capture, and preserved immediately in 10% formalin. A measure of relative abundance of prey in the diet compared to relative abundance in the pond was made using Vanderploeg & Scavia's (1979) relativized electivity index (E^*) for zooplankton and benthic invertebrates.

For age analysis, standard skeletochronological techniques (see Castanet & Smirina, 1990) were employed, based on the second phalange of the fifth toe of 60 *X. laevis* from the Pond in November 1994 and a further 60 in June 1996. Histological preparations were also made from 16 femurs of *X. laevis* captured in the Valley from 1982 to 1990 and seven femurs from the Stream from 1988 to 1993. Toes were processed from 10 *X. laevis* caught in the Valley in 1995.

RESULTS

XENOPUS LAEVIS CAPTURES

A total of 264 *X. laevis* was caught in 677 captures in the Valley from February 1982 to August 1996 (Fig. 1a). In the Stream, there were 62 captures of 38 individually-marked animals from October 1983 to December 1994 (Fig. 1b). Movements between capture sites were recorded for 21% of animals within the Valley, with distances of 0.2-2 km, and 36% of animals from the Stream, with distances less than 100 m. No *X. laevis* were found to move between catchments, and for

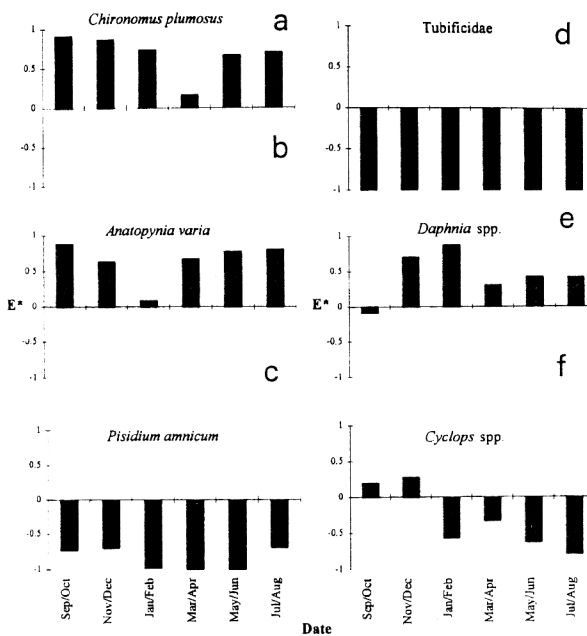


FIG. 2. Vanderploeg & Scavia's (1979) electivity index (E^*) for numbers of zoobenthic and zooplanktonic invertebrates recorded in the stomach contents of *X. laevis* from the Pond. Each bar represents the average of two months of two-weekly samples.

this reason the sites were treated as separate populations. In 46 visits to the Pond, 3358 captures were made of 913 individually-marked *X. laevis* (Fig. 1c). Two animals marked in the Pond were subsequently found in other localities within the Valley 0.75 and 1.5 km away (direct distances). Time elapsed between recaptures at different locations was generally greater than one month, but one female was recorded as travelling 0.2 km (direct distance) in less than 48 hrs.

Throughout the entire period, 47% of *X. laevis* trapped in the Valley were caught only once, whilst others were caught up to 12 times including two individuals whose captures spanned all 14 years of the study. Individual panjet marks made on these animals in 1982 were readily identifiable in 1996. All numbers freeze-branded onto animals in 1994 were still easily distinguished at the end of the study in August 1996.

POPULATION ESTIMATES

Population estimates of *X. laevis* in the Valley, by the triple catch method on contiguous trapping data, revealed a rapid decline from 408 in 1982 (upper and lower confidence limits not calculable) to 64 (+152, -45) in 1984, and a very small population in 1995 (16±2). The population in the Stream was estimated by the triple catch method as 15 (±1) in 1990. From November 1993 to December 1994, despite intensive trapping, no *X. laevis* were caught in the Stream. The population at the Pond was found to be a good fit to a zero-truncated geometric model of numbers recaptured ($\chi^2 < 0.001$, $df = 22$) to predict numbers left uncaught, and together with those caught gave a total population of 1276 *X. laevis*.

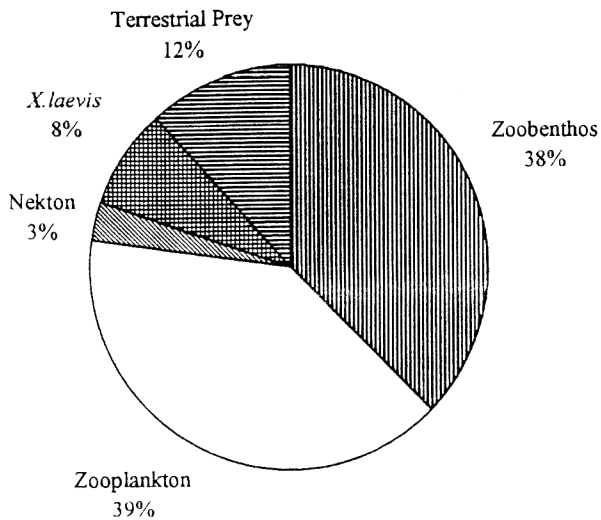


FIG. 3. Proportions of dietary components in the stomach contents of *X. laevis* from the Pond averaged over 12 months.

DIET

Densities of common benthic invertebrates in the Pond were seen to fluctuate from August 1995 to August 1996. The major components were tubificid worms, chironomid larvae (*Chironomus* *vr. plumosus* and *Anatopynia varia*), and pea mussels (*Pisidium amnicum*). Electivities for numbers of chironomids (Fig. 2) are generally positive, while those of *P. amnicum* are negative. Tubificids showed total negative electivities as they were completely absent from the stomach contents of *X. laevis*.

The densities and composition of zooplankton were seen to fluctuate from August 1995 to August 1996. The major components were cladocerans (*Daphnia* spp.), whose density peaked in summer, and copepods (*Cyclops* spp.), whose density peaked in autumn. Zooplankton sizes varied during the year, with sizes of both *Daphnia* spp. and *Cyclops* spp. greater in the winter than the summer. Electivities for numbers of zooplankton taxa (Fig. 2e) showed positive selection of *Daphnia* spp. for most of the year, while *Cyclops* spp. showed positive selection mostly in the autumn when density peaked.

Data on stomach contents of *X. laevis* from the Pond were divided into groups by prey habitat and consequently by predation mechanism: zoobenthos, zooplankton, nekton, and terrestrial (Measey & Tinsley, in prep.). The prey category *X. laevis* has been placed in a separate group which comprised larvae and eggs of *X. laevis*. Zoobenthos and zooplankton consistently made up the largest components of stomach contents (Fig. 3), with terrestrial prey items (e.g. beetles, flies and woodlice) peaking in spring and summer. *X. laevis* made an important contribution to stomach contents only during summer 1996 when breeding occurred in the Pond. The contribution of nektonic prey items was consistently low throughout the year. Each prey group showed a large taxonomic range of invertebrate (and vertebrate) prey items, but the majority of prey was made up of very few taxonomic groups.

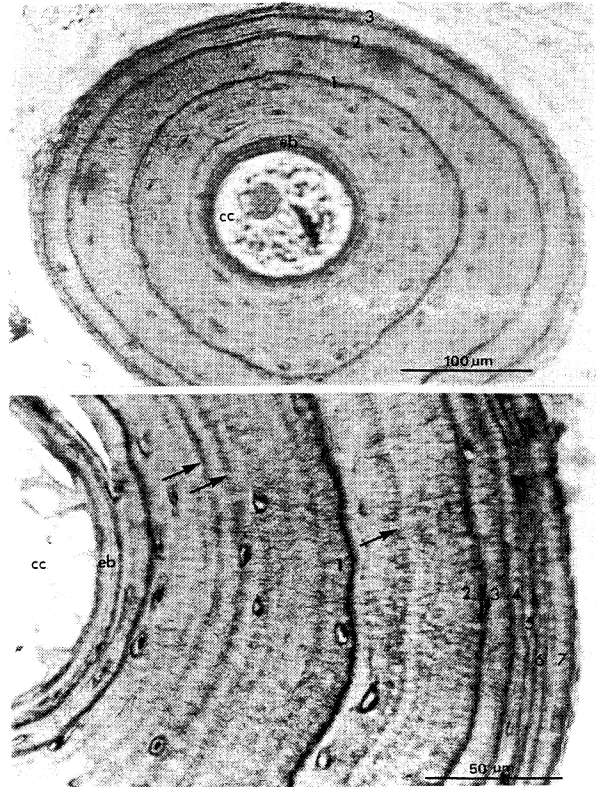


FIG. 4. Transverse sections of phalanges of *X. laevis* from South Wales. (a) a female with SVL 73 mm and 3 LAG from the Pond, and (b) a female with SVL 101 mm and 7 LAG from the Valley. cc central cavity; eb endosteal bone; numbers indicate LAG and arrows false LAG.

MORPHOMETRICS

From first collections at the Pond in November 1994, sex could be determined externally for only 12% of individuals. No internal examinations were made. Mean snout-vent length for juveniles was 51 mm (SE = 0.38, $n = 113$), and for those identified as females 61 mm (SE = 0.30, $n = 13$) and males 62 mm (SE = 0.88, $n = 3$). By July 1996, sex could be determined externally for all individuals, and females were significantly greater than males in both length (mean difference 3.90 mm) and weight (mean difference 2.34 g) (length $F_{1,347} = 79.72$, $P < 0.001$; weight $F_{1,347} = 29.96$, $P < 0.001$).

SKELETOCHRONOLOGY

Lines of arrested growth (LAG) were easily discernible in all bones from the Pond population with no false (intra-seasonal) LAG at this site (Fig. 4a). The age structure of the 60 individuals toe clipped in October 1994 is shown in Fig. 5: alongside 28% of individuals estimated to be ages 4-8 years, there is a dominant cohort of animals reflecting metamorphosis in 1993. Of the 60 *X. laevis* toe-clipped in June 1996, 98% had 3 LAG. One individual which was toe clipped on both occasions was found to have increased numbers of LAG from 1 to 3. All bones collected in the Valley contained clear LAG within the periosteal as well as some with false LAG (Fig. 4b). Fig. 6 shows the frequency of the year of metamorphosis (i.e. years preceding LAG₁) to be grouped in the mid and late 1970's, mid 1980's and

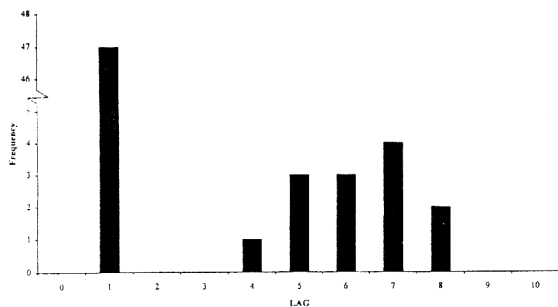


FIG. 5. Frequency distribution of LAG in phalanges of *X. laevis* from the Pond, toe clipped in October 1994.

late 1980's and early 1990's for toes and femurs from the Valley (Fig. 6a). Years of metamorphosis of *X. laevis* from the Stream are shown to be grouped in the late 1980's and early 1990s (Fig. 6b).

DISCUSSION

Population estimates of *X. laevis* in both the Valley and the Stream suggest that numbers have declined sharply from those at the start of the study period. It is thought that contributory factors include predation, particularly the lack of recruitment into the population due to cannibalism (Tinsley, Loumont & Kobel, 1996), and loss of appropriate lentic habitats. Skeletochronological results show conclusively that LAG formation is annual, enabling accurate demographic analysis of the Pond population. This confirms the reproductive potential of *X. laevis*: more than 97% of the population could be traced to metamorphosis during a single summer (1993) (Measey & Tinsley, in prep.).

Documentation of movements of individually-marked animals demonstrates that, although *Xenopus* is principally adapted to aquatic life, travel between habitats involved overland movement. Direct distance between capture sites may not accurately reflect the actual distance and obstacles encountered during these journeys. For the individual found to move 0.2 km in 48 hrs, the route would have included an initial distance overland (150 m), crossing the river and then overland through woodland with dense undergrowth, over a metalled road and into a quarry, where the pond was situated. For the animals marked in the Pond, the most likely route to the habitats in which they were found would be using the river as a corridor. These ponds were both within 20 m of the river, but were not visible from the water surface, and in both cases travel would have involved movement up steep banks and walls. Movement may possibly be stimulated by olfactory cues emanating from the pond, and Savage (1961) has suggested that for *Rana temporaria* the cues may come from phytoplankton. True navigation, homing without landmarks, is known in amphibians (Phillips, Adler & Borland, 1995) and may involve magnetoreception.

The composition of gut contents was very similar to that found in previous studies of *X. laevis* in an impoundment in Transkei (Schoonbee, Prinsloo & Nxiweni, 1992) and from a stream in California (McCoid & Fritts, 1980a). In common with the present

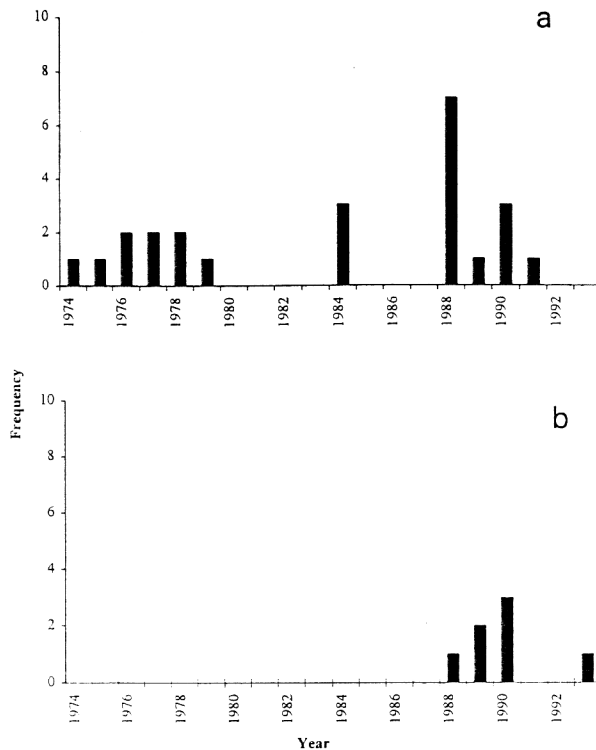


FIG. 6. Frequency distributions of years of metamorphosis (i.e. year preceding LAG) from skeletochronological analysis of femurs and phalanges of *X. laevis* from (a) the Valley and (b) the Stream.

study, tubificids found to be abundant in the environment in Transkei were completely absent from stomach contents. This phenomenon has been observed in fish diets, and is attributed to the unavailability of tubificids, which are found to move deeper into the sediment in the presence of predators (Kornijow, Moss & Measey, in prep.).

In a recent review of diet in *Xenopus*, it was considered unlikely that the large amount of terrestrial prey previously 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 a quarter of the weight of all ingested prey items. Many may have been ingested after falling inadvertently into the pond, where they may have become trapped in the surface tension or fallen to the bottom. However, some may have been caught whilst on land: current laboratory studies have confirmed that *X. laevis* can prey terrestrially on invertebrates (Measey & Tinsley, in press).

The record of two animals recaptured 14 years after first being marked with a panjet represents the longest period reported for *X. laevis*, or any anuran, recaptured from the wild, as well as a record period of panjet mark retention. This also represents a record for the longevity of *X. laevis*, if it is assumed that each animal must have been at least two years old on first capture (Tinsley *et al.*, 1996; Tinsley & McCoid, 1996).

Mark-recapture data for the age of a male which was first caught as an adult in 1982 and toe-clipped in 1995 does not correspond with the skeletochronological re-

sults (11 LAG). The underestimated LAG count was probably due to the difficulty of differentiating LAG at the periphery of periosteal bone in older individuals (Smirina, 1994; Castanet & Smirina, 1990).

Ages of *X. laevis* in both the Valley and the Stream suggest these populations resulted from three groups of years; the mid to late 1970's, mid 1980's and early 1990's (see Fig. 6). With the errors associated with counting outer LAG, it may be that each of these groups of spawning years represents a single successful spawning occasion. This would indicate that there has been only sporadic success in recruitment during the past 20 years, despite more frequent observation of spawning. Reasons for this intermittent recruitment may include cannibalism and dwindling habitats. Cannibalism, of eggs and larvae of *X. laevis*, is well known (Tinsley *et al.*, 1996; Measey & Tinsley, in prep.). Three of the original habitats available to *X. laevis* in the Valley in 1982 are no longer available, forcing adults into other ponds where reproduction is not successful. If there is no recruitment to a population, and predation pressure is persistent, a rapid decline in population numbers would be expected. However, the existence of refugia make the last individuals difficult to eliminate, and as *X. laevis* are explosive breeders, like most other amphibians, there is always a potential for another large expansion in numbers.

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