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THE SUSTAINABLE UTILISATION OF THE AFRICAN CLAWED FROG, *Xenopus laevis* (DAUDIN)

by

Ché Weldon

In partial fulfillment of the requirements for the degree of

MAGISTER SCIENTIAE IN ZOOLOGY

A thesis submitted in the

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Co-supervisor: Mr. M.T. Seaman

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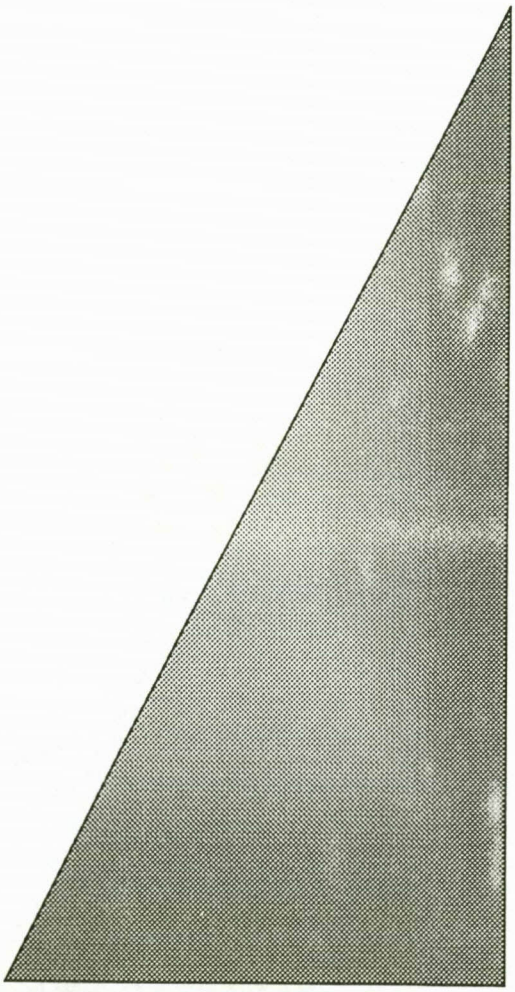
Chapter 1

Introduction and literature overview



INTRODUCTION AND LITERATURE OVERVIEW

> *"Ask anybody what uses a frog can be put to and you'll get replies ranging from a meal for a Frenchman to pets for small boys. Ask a hospital laboratory worker, though and the first thing she'll think of is the frog test for pregnancy."*
>
> Marian Kuhl (1970)

Smith (1838-1949) was the first herpetologist in southern Africa to make a substantial contribution to the taxonomy of amphibians (Adler 1998). Having described more local taxa than any other person, Smith is considered by some as the father of herpetology in southern Africa (Spawls 1991). Then followed the well-known taxonomic work of Boulenger (1910) and later Hewitt & Power (1913) and Hewitt (1926, 1932). It was Poynton (1964a) who set the standard for the classification and distribution of amphibia in southern Africa. He also made numerous contributions to our understanding of zoogeography of Anura in southern Africa (Poynton 1960, 1964b, 1987, 1992). The illustrated field guide of the Anura of South Africa by Passmore and Carruthers (1979, 1995) is most often used today for quick identification of species by both professional and amateur herpetologists. Poynton and Broadley (1985a, 1985b, 1987, 1988, 1991) in their series "Amphibia Zambesiaca" also made a major contribution.

Power (1926) and De Villiers (1929) carried out the first ecological studies of South African amphibia. Important works to follow include those of Rose (1962), Wager (1965) and Stewart (1967). Pioneering work on the ecology of South African Anura by Balinsky (1969) is considered to be the basis of ecological studies of Anura in the sub-continent.

Numerous authors compiled guides to expand our knowledge on the Anura of South Africa. The first to appear were those of localities in the former Transvaal, namely the Kruger National Park (Pienaar, Passmore & Carruthers 1976), the Witwatersrand (Carruthers 1976) and the Suikerbosrand Nature Reserve (Carruthers & Carruthers 1979). This paved the way for many more to follow in short succession. Jacobson (1982)

helped bridge a gap with his ecological study on the reptiles and amphibians in the Nylsvley Nature Reserve. The field guide of Lambiris (1988) contained information about the amphibians of the Natal Drakensberg and Lambiris (1989) of Kwazulu-Natal. Contributions on the frogs of the Orange Free State were the taxonomic and distributional study of De Waal (1980) and a field guide by Du Preez (1996).

One of the widespread anuran species in the sub-continent is the African Clawed Frog, *Xenopus laevis*. It has been known to Science for the past 200 years. Daudin (1803) first described this frog under the name of *Bufo laevis*. During the nineteenth century the attention of various systematists was focused on the animal and practically every investigator renamed it, until at the end of the century most zoologists generally accepted the name *Xenopus laevis*. It is commonly known as the "Platanna"- derived from the old name "Plathander", which in turn refers to its flat hands. It is also known by the colloquial name of "Plattie". The generic name *Xenopus* is derived from the Greek words "xenos", meaning strange or unusual, and "pous", for foot, while the specific name *laevis* means smooth or slippery in Latin (Du Preez 1996).

Xenopus belongs to a unique family of frogs, the Pipidae. Members of the family are strictly aquatic frogs equipped with large, fully webbed feet. This, together with the combination of small dorsally placed eyes and poorly developed or absent eyelids, the absence of a tongue and the presence of a lateral line system, are the most significant morphological characteristics that distinguish the pipids from other anuran families (Duellman & Trueb 1986, Mattison 1992). The geographical range of the family includes tropical South America east of the Andes and adjacent Panama, and sub-Saharan Africa.

The distribution of the *Xenopus laevis* species complex (*X. l. laevis*, *X. l. poweri*, *X. l. petersi*, *X. l. victorianus* and *X. l. sudanensis*) form a south-north succession which generally corresponds with the relatively cooler highlands between the Cape of Good Hope and the plateaux of Cameroon and Nigeria (Tinsley, Loumont & Kobel 1996). This range excludes the Zaire Basin. *X. laevis* does not occur in much of the hotter lowlands of eastern Africa. Within the boundaries of South Africa, *X. l. laevis* is a common species which occurs from the Western Cape Province northwards, excluding the extreme north

of the Northern Cape Province, northern Kwazulu-Natal and eastern Mpumalanga Province. It occupies any permanent body of water such as ponds, dams, streams, rivers and water holes.

Reproductive biology and the effect of physical factors on the breeding habits of *X. laevis* have for many years been the subject of investigations. Leslie (1890) was the first to note the time of breeding of Cape *Xenopus* populations. The earliest successful attempts at breeding *Xenopus* in captivity were by Beddard (1894) at the gardens of the London Zoological Society. Bles (1901, 1906) achieved similar results by imitating natural conditions for breeding (raising the temperature from 15°C to 22°C, simulating rain and introduced algae for tadpole feed). It was evident that if optimal environmental conditions were established, *Xenopus* would occasionally breed in captivity. However, the methods used were neither dependable nor practical. If optimal conditions were not created the female frogs would not ovulate (Shapiro 1936a, b). The gap was bridged by Hogben, Charles and Slome (1931) when they discovered that ovulation in the female *Xenopus* could be induced by pituitary stimulation. The response of the female to the injection of an anterior pituitary hormone suggested that simultaneous injection into the male might induce mating, and the laying of fertile eggs. Various workers used this technique and successful breeding resulted (Andres, Bretscher, Lehmann & Roth 1949, Hobson 1952, Nieuwkoop & Faber 1956).

Xenopus laevis is one of the most intensively used animal species in medical and biological research and in teaching. The first great prominence achieved by *X. laevis* in scientific laboratories was in the early 1930s. Medical history was made by Shapiro and Zwarenstein (1934) when they developed the *Xenopus* test for the diagnosis of early pregnancy in humans. Then, almost overnight, a universal demand developed for the South African *Xenopus*. At first, animal dealers exploited this demand by catching large numbers of *Xenopus* for export. The methods employed in catching were often wasteful as only the usable females were selected from a catch. The balance was simply discarded and had to find their own way to the nearest water (Hey 1986). As a consequence, the supplies from natural sources were rapidly being depleted in the Cape Peninsula and its precincts. Dr. Louis P. Bosman, a leading medical pathologist at the time, realised what

was happening and prevailed upon the Cape Provincial Administration to introduce protective legislation and to sponsor research on the artificial cultivation of *Xenopus* at the Jonkershoek Inland Fish Hatchery near Stellenbosch, South Africa (Hey 1945). In August 1941, the Curator was authorised to propagate *Xenopus* at the Jonkershoek Hatchery for medical and scientific purposes. Concrete tanks were constructed for holding supplies of *Xenopus* that were collected from farm dams, initially in the Stellenbosch vicinity, and later further afield from Paarl, Caledon, Malmesbury and Piketberg. In the same year local deliveries were made and the first shipment was sent to America (Hey 1945).

At the same time research was also in progress at the Hatchery to develop a technique for culturing the frogs on a large scale in captivity. Initial experiments were concerned with induced breeding by using drugs, and were based on the research of Shapiro and Zwarenstein, but these were not successful (Hey 1949). Research was then directed at inducing the frogs to spawn by natural means. It was found in 1940 and again in 1941 that organically matured water seeded with zooplankton (specifically *Daphnia magna*) stimulated the breeding of *Xenopus*. By August 1945 the construction of large ponds were completed and *Xenopus* were cultivated on a large scale (Hey 1949). Sales increased ten fold within four years and for more than thirty years thousands of *Xenopus* were sold annually both locally and abroad. The collection, cultivation and selling of *Xenopus* from Jonkershoek was stopped in 1975 when attention was rather focused on indigenous fish species like the scarce yellowfish, *Barbus capensis*. Sales of *Xenopus* were left to private undertakings (Hey 1976).

According to Elkan (1960) the published literature on amphibian pathology is extremely sparse and the knowledge concerning the amphibian reaction to the factors commonly causing disease is even less incomplete. It has usually been easier and cheaper to obtain new animals than to attempt to investigate or treat disease problems of amphibians (Crawshaw 1992). More often the most important consideration in the combat of disease has been prevention measures rather than post-infection treatment. The main causes of morbidity in amphibians are parasites, tumours, inflammatory conditions and fungal infection (Elkan 1960).

Nearly one half of the cases presented by Elkan (1960) in his summary of pathological case studies in amphibians were caused by invertebrate parasites such as trematodes, cestodes and nematodes. Nace (1968) highlights the fact that when obtained from nature, amphibians are invariably parasitised, while those raised in the laboratory have a lower incidence of infection because of the absence of intermediate hosts. He further states that completely parasite-free colonies have not yet been developed.

Information on diseases of *Xenopus* is scattered through the literature, summarised in reviews (Reichenbach-Klinke & Elkan 1965, Walton 1964, 1966-1967). Tinsley (1996a) reports that although *Xenopus* may carry a range of pathogens, diseases will only develop once a physiological disorder has occurred due to malnutrition, temperature change or other environmental stress. Even in captivity *Xenopus* are extremely hardy animals provided they are kept under healthy conditions and are well fed. When kept under crowded holders as can be experienced under captive or culture conditions however, the mortality can be heavy as diseases soon start to appear that can rapidly assume epidemic proportions (Hey 1949). If not detected soon enough and the animals treated, such an epidemic can cause a drop in condition and eventually result in grave losses.

The most commonly reported disease in frog culture, to which *Xenopus* is equally vulnerable, is the so called "Red-Leg" disease caused by a variety of usually gram negative bacteria such as *Aeromonas, Pseudomonas, Mima, Citrobacter* and *Proteus* (Crawshaw 1992; Pariyanonth & Daorerk 1995).

Besides the risk posed by the parasites to the culture of *Xenopus,* there is a risk associated with the export of wild-caught animals. The animals have carried their natural parasite infections to the export countries. Some of the animals were released in the wild and today feral populations exist in Europe, Chile, Ascension Island and the United States (Loveridge 1959, St. Amant & Hoover 1969, Zacuto 1975, Pefaur 1994, Tinsley & McCoid 1996). Lafferty and Page (1997) found three internal parasites from a feral population at the Santa Carla River in California. A danger exists that the parasites could switch hosts with some of the native frog species occurring within the same region.

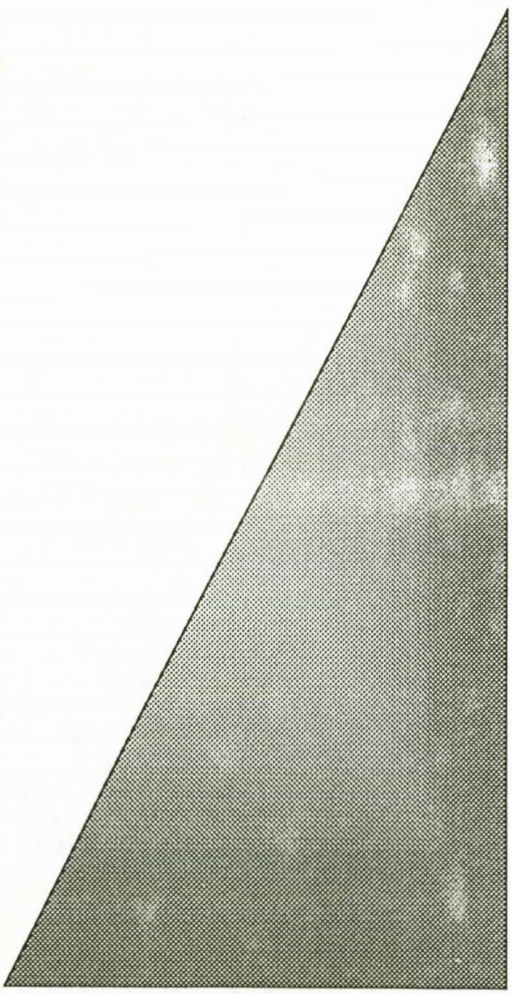
Frogs that eventually reach the end-user have been collected in one of two ways, either they were caught from wild populations in South Africa or they were bred in captivity at various facilities around the world. The main objective was to investigate effects of collecting and breeding procedures on the sustainable utilisation of *Xenopus*. To achieve these objectives the study is divided into four components:

- **Market research**: to establishing needs of the end-users by utilising questionnaires, e-mail and telephone interviews.
- **Field studies**: to determine the factors affecting the developmental biology of *Xenopus laevis*, and size of tadpoles and on community structure of populations.
- **Captive breeding**: to investigate induced-breeding, the effect of water volume on tadpole development, and the effects of growth in different types of enclosures and different feeds on growth of the frogs.
- **Experiments on control of parasites**: to study the effect of host captivity on parasite infection levels and the treatment of parasites with different drugs.

The **Introduction and literature overview** (Chapter 1) is followed by a description of the **General study area, material and methods** (Chapter 2) that contains the sources of material and only those methods that are generally applicable to all of the chapters. Chapter 3 deals with **Market research on the utilisation of *Xenopus***. The next three chapters, **Tadpole development and population dynamics of wild *Xenopus laevis* populations** (Chapter 4), **Aspects of captive breeding and husbandry of *Xenopus laevis*** (Chapter 5) and **Infection levels, parasite survival and control of parasites** (Chapter 6) are each presented in the format: Introduction, Material and methods, Results and Discussion. The combined results and their implications from Chapters 3 to 6 are considered in the **General discussion** (Chapter 7). The thesis is concluded with a **Summary** (Chapter 8). **References** for all chapters follow (Chapter 9) and published papers have been added as **Appendices**.

Chapter 2

Study area, general material and methods



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STUDY AREA, GENERAL MATERIAL AND METHODS

2.1 STUDY AREA

Xenopus laevis were obtained at six earth-walled dams from farms and protected areas surrounding Bloemfontein and neighbouring towns. Names of the dams and experimental fields in which the frogs were used are given in Table 2.1.

Table 2.1 The sources of post-metamorphic frogs indicating the three main experimental groups for which the frogs were used.

DAM	EXPERIMENT		
	Mark and Recapture	Breeding	Parasite
Dam van Trane	*		
Vallei van 7 damme	*		
Rustig	*		
Duraan farm		*	*
De Dam	*	*	*
Nuwe Orde			*

2.1.1 Dam van Trane

Situated at the western outskirts of Bloemfontein 29°05'S, 26°10'E (Fig. 2.1). An example of a medium-sized (3000m^2) semi-permanent dam, which receives its water purely from local runoff and dries for one to two months of the year. Approximately 80% of the dam is invaded by aquatic vegetation. Dam van Trane ("Dam of Tears") is a natural heritage site and access to the perimeter is strictly controlled, which minimises disturbance by the public.

2.1.2 Valley of Seven Dams

One of seven dams is in a protected area, in the northern suburbs of Bloemfontein, 29°04'S, 26°12'E (Fig. 2.1). It is a small (1000m^2) permanent dam with dense reeds in the southern area. The surrounding area has numerous hiking paths, is frequented by the public and as a consequence suffers from vandalism from time to time.

2.1.3 Rustig

A large (50 000m^2) permanent dam on the north-western periphery of Bloemfontein, 29°03'S, 26°11'E (Fig. 2.1). It is deep (exceeding 2m) with brown turbid water. Aquatic vegetation is absent.

2.1.4 Duraan farm

The farm is situated 45km north-east of Bloemfontein, 30°50'S, 29°45'E (Fig. 2.1). The inflow area of the dam forms a deep donga before expanding towards a red-earth dam wall. Aquatic vegetation is restricted to the shallower water near the edges.

2.1.5 De Dam

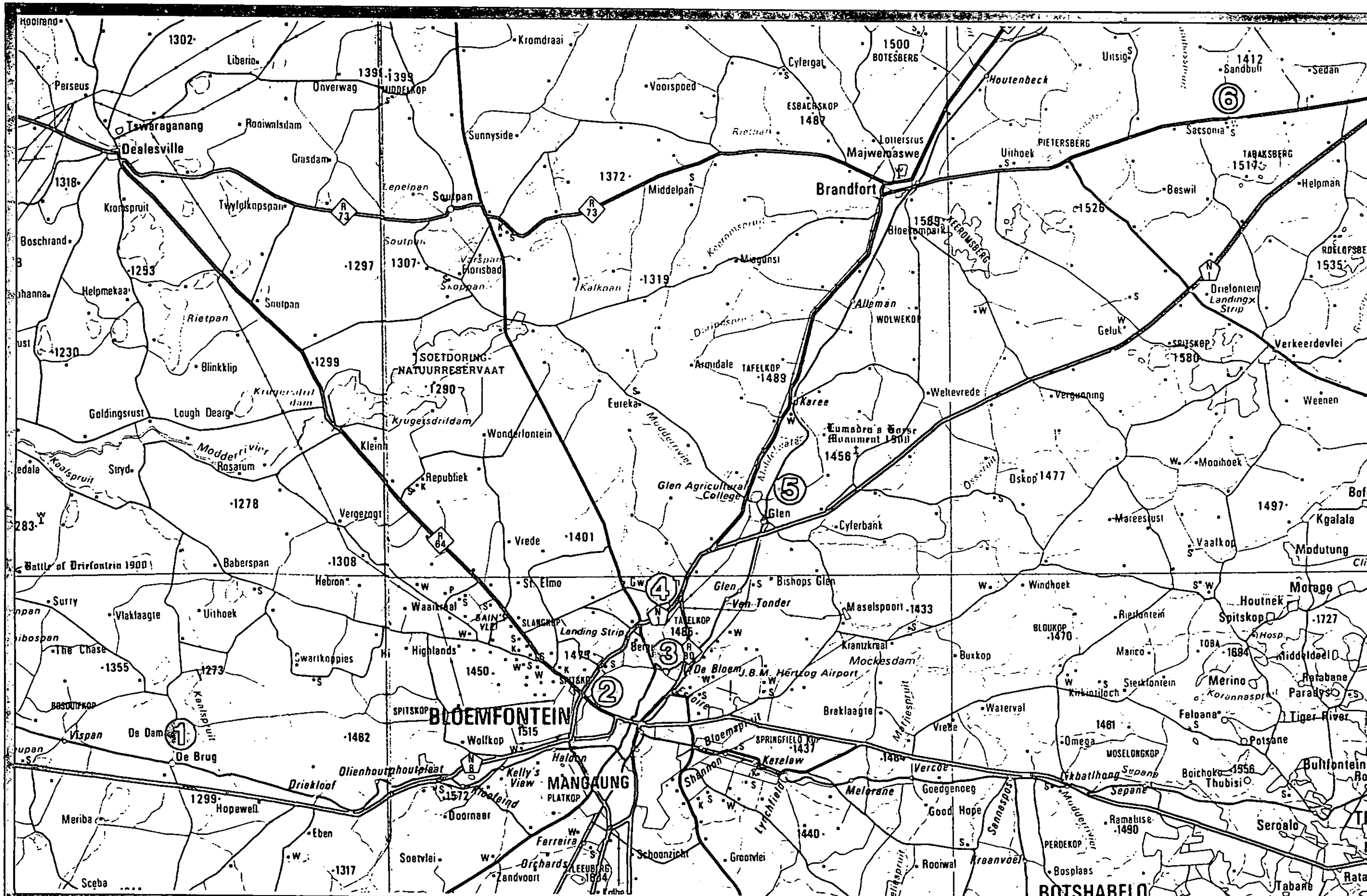
The farm (29°07'S, 25°48'E), is situated near the small settlement of De Brug west of Bloemfontein (Fig. 2.1). The dam has a thick layer of sludge and was the smallest (500m^2). The water is used for irrigation purposes but never dries up as water from a borehole is continuously being pumped into the dam. More or less 50% of the dam is covered by reeds.

2.1.6 Nuwe Orde

The farm is situated on the road between Brandfort and Winburg (Fig. 2.1). Frogs were collected from a large, permanent dam (28°39'S, 26°44'E). The catchment area consists of surrounding pasture. The water colour is brown. No aquatic vegetation was observed.

Figure 2.1

Map showing positions of collecting sites in and around Bloemfontein. Abbreviations: 1, De Dam; 2, Dam van Trane; 3, Valley of Seven Dams; 4, Rustig; 5, Duraan farm; 6, Nuwe Orde.


Hooirand
Perseus
Liberio
Onverwag
MIDDELKOP
Kromdraai
Voorspoed
Cyfergat
1500
BOTESBERG
Houtenbeck
Uitsig
1412
Sandbult
Sedan
ESBACHSKOP
1487
Tswaraganang
Dealesville
Rooiwalsdam
Grasdam
Sunnyside
Majwemasweu
Lottersrus
Brandfort
PIETERSBERG
Uithoek
Sacsonia
TABAKSBERG
1318
Kromspruit
Twyfelkopspan
Lepelpan
Soutpan
1372
Middelpan
Beswil
Helpman
Boschrand
1297
1307
Varspan
Florisbad
Skoppan
Kalkpan
1319
Keeromspruit
Miegunst
1589
Bloemspruit
KEEROMSBERG
c1526
ROELOFSBERG
1535
1253
Helpmekaar
Soutpan
Rietpan
Alleman
WOLWEKOP
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Verkeerdevlei
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Lough Dearg
Krugersdriftdam
Wonderfontein
Eureka
Modderrivier
Karee
Weltevrede
Vergunning
Weenen
Modderrivier
Kleinh
Koalspruit
Styrd
Rosarium
Republiek
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Glen
1456
Oskop
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Mooihoek
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Vergezogt
Vrede
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Cyferbank
Mareesrust
1497
Kgalala
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Modutung
Battle of Driefontein 1900
Baberspan
1308
Hebron
Waaikraal
BAIN'S VLEI
SLANGKOP
St. Elmo
Landing Strip
Glen
Bishops Glen
Ven Tonder
Windhoek
Morago
Houtnek
Surry
Vlaklaagte
Uithoek
Maselspoort
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Marico
TOBA
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Koalspruit
SPITSKOP
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De Bloem
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Estoire
Braklaagte
Steirkfontein
Kirkintilloch
Merino
Korannaspruit
Ratabane
Paradys
Vispan
De Dam
De Brug
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Wolfkop
Bloemspruit
SPRINGFIELD KOP
1437
Marthiespruit
Vrede
Waterval
Omega
1461
Felpana
Tiger River
Potsane
MOSELONGKOP
Olienhoutplaat
Driekloof
Kelly's View
Haldon
MANGAUNG
PLATKOP
1572
Rooifeind
Shannon
Kerelaw
Vercoe
Melorane
Goedgenoeg
Good Hope
Sannaspos
Ikbatlhong
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Schoonzicht
Grootvlei
Lynchfield
1440
Rooiwal
Kraanvoël
PERDEKOP
Ramalise
1490
Bosplaas
BOTSHABELO
Seroalo
Tabane
Ratau

2.2 MATERIAL AND METHODS

2.2.1 Collecting of frogs

The method for collecting post-metamorphic *Xenopus* is based on the aquatic nature and feeding behavior of the frog and therefore allows only *Xenopus* to be caught. Strongly oudoured bait is used to lure frogs into traps as *Xenopus* rely strongly on their olfactory sense for locating food. Two sizes of home-made funnel traps were constructed for the purpose. The smaller sized traps (20*l*), were made from plastic buckets, while 200*l* metal drums were used for the larger ones. Traps were placed near the water's edge with one third protruding above the water level. This allowed for adequate respiration by the trapped frogs. Small traps were baited with marrowbones and left in the water for 48 hours. The number of traps used varied from six to ten according to the size of the dam. A waste product of a crop maroho (*Amaranthus* spp.) used for the brewing of a local beer was used as bait for the large traps. One to three large traps were used at a time and left for two weeks in the dams. On removal of traps, the contents was immediately emptied into a container half-filled with dam water as frogs (especially small ones) easily suffocate from the froth produced by the beating of skin secretion.

2.2.2 Care of experimental frogs

Collected frogs were transported to and sorted at the university. Frogs required for experiments were separated from the rest and placed in metal holding tanks (Fig. 2.2a) in dechlorinated water at a density of one frog per litre. They were fed on chopped beef liver (5mm cubes), as much as they could consume in 20 minutes. Tanks were cleaned and water replaced once a week. Frogs that were not being used were kept in a large concrete dam converted to a semi-natural environment (Fig 2.2b). These frogs were left to fend themselves on natural food.

Figure 2.2

Photographs of facilities where frogs were kept in captivity:

a) Metal holding tanks of experimental frogs.

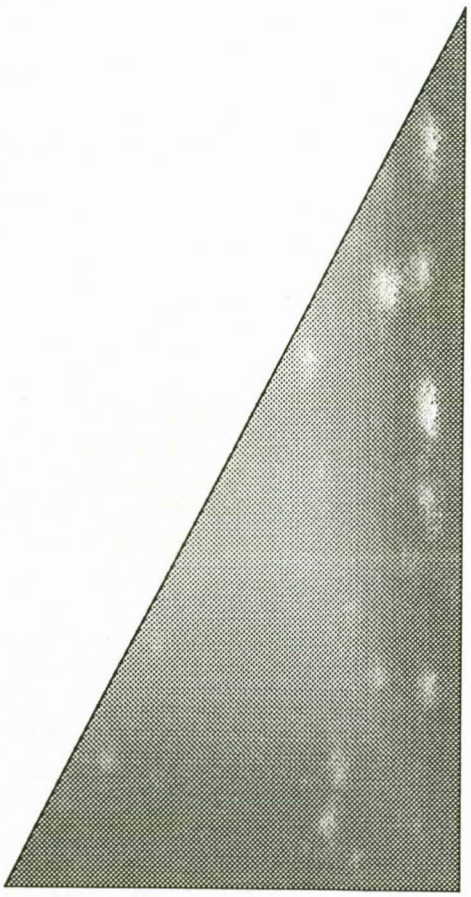
b) Concrete dam in which *Xenopus laevis* stock was kept until required for experimental purposes.


a


b

Chapter 3

Market research on the utilisation of *Xenopus*



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MARKET RESEARCH ON THE UTILISATION OF *Xenopus*

3.1 INTRODUCTION

For years, *Rana* spp., followed shortly by *Xenopus*, have dominated the research involving anurans. A recent survey by Major & Wassersug (1998) indicates that the use of *Xenopus* has now surpassed that of *Rana* as a laboratory animal. The demand for this popular research animal continues to increase.

Large scale exploitation started soon after it was discovered that *Xenopus laevis* could be used to diagnose early pregnancy in 1934 (Shapiro & Zwarenstein 1934). Enormous quantities of the species were exported to all areas of the world. Twelve years later the scientific research involving *X. laevis* had become so extensive that Zwarenstein, Shapeika & Shapiro (1946) published a bibliography in which 305 papers were listed. A recent review estimated that there are approximately 4 500 papers on *Xenopus* spp. (Van Dijk[1], personal communication).

Relative ease of maintenance, resistance to disease and a high reproductive output are reasons why *X. laevis* is a popular choice as a laboratory animal. This animal is also extensively used in science. In fact most of our knowledge of human embryology is based on *X. laevis* embryology (Dawson & Bishop 1990). The biggest market for these frogs today is still the various research facilities in genetics, molecular biology, embryology and biochemistry.

X. laevis not only serve as a resource to the science community, but has for decades been sold as bait for angling in South Africa. *X. laevis* is also a pet animal. There is very little published published information dealing directly with *X. laevis* in the pet trade, except for McCoid & Fritts (1980) mention of *Xenopus* as a pet animal in the US. Equally little

known is the use of *X. laevis* as a food source. Hey (1986) reports on *X. laevis* being eaten in South Africa and it is mentioned along with *Pyxicephalis adspersus* and a large ranid, in a list of traditional foods eaten by San in the Southern Kalahari (Steyn 1984).

The aim of this part of the study is to investigate the relevance of *Xenopus* in:

- The international research market. (We have decided to delimit and concentrate on molecular biology and embryology research because the variety of research fields using *Xenopus*, complicates the extent of the requirements; and these are two of the largest disciplines.);
- teaching and research at South African universities;
- the angling industry in the Orange Free State and
- the amphibian pet trade.

3.2 MATERIAL AND METHODS

3.2.1 Questionnaire on the demand of *Xenopus* in molecular and embryological research

An eight-questionnaire (Appendix 3.1) with explanatory letter was electronically sent to 171 persons listed in the "*Xenopus* Molecular Marker Resource White Pages" (URL: http://vize222.zo.utexas.edu/Marker_pages/White_pages.html). A second circular of the same questionnaire was sent to additional addresses received on the return forms.

[1] Prof. D.E. van Dijk, Dept. Zoology, Univ. Stellenbosch, 3 Kleineweide Street, Stellenbosch, South Africa, 7600.

3.2.2 Survey on the use of *Xenopus laevis* in teaching and research at South African universities

Relevant departments forming part of Natural Science and Health Science Faculties of South African universities (Table 3.1) were asked if *Xenopus* formed part of any module that is offered to students in their departments. In addition they were asked if any members of their staff were involved with *X. laevis* research and in what field.

Table 3.1 Table to indicate departments from the Natural Science and Health Science Faculties of South African universities that were contacted with regard to the use of *Xenopus laevis* in teaching and research.

Department	Number of departments
Anatomical Science	1
Biochemistry	6
Health Science	2
Ichthyology and Fishery Science	1
Medical Biochemistry	1
Medical Physiology	2
Neurology	1
Pharmaceutical Chemistry	2
Pharmaceutics	2
Pharmacology	3
Pharmacy	2
Physiology	4
Veterinary Physiology	1
Zoology	13

3.2.3 Inquiry on the use of *X. laevis* in the angling industry

The owners of angling shops in the Free State were interviewed on their involvement with the selling of *X. laevis*. General information was gathered concerning the use of the frogs in the angling industry.

3.2.4 Search on *X. laevis* and the animal pet trade

Internet searches were conducted to determine the popularity of *Xenopus* as a pet frog. Large suppliers of *X. laevis* as well as reptile and amphibian pet stores were consulted on their involvement with the *X. laevis* pet trade.

3.3 RESULTS

3.3.1 Requirements for *Xenopus* by *Xenopus*-related molecular and embryological research.

Thirty-six responses to the survey were received. This accounts for 21% of the field (n=171) to which questionnaires were sent. Thirteen percent of the mail encountered delivery failure while 66% did not reply. Most of the respondents (78%) were associated with academic institutes (universities and colleges). The remainder were from a variety of users such as pharmaceutical and cancer research facilities. The majority of the responses (77.7%) came from the United States, followed by the United Kingdom (8.3%), France (5.5%) and Switzerland and Germany (each 2.8%).

3.1.1.1 Species diversity

All respondents were actively involved in research on *X. laevis*. Most of them (67%) worked exclusively with this species. Seven (19%), also worked with *X. tropicalis*, while five were considering working with this species in the near future. The only other species also used were *X. gilli* and *X. borealis*, each by one laboratory (2.7%). Some respondents mentioned a few species on which work was previously conducted but has since been stopped. These included *X. tropicalis, Rana catesbiana* and *R. pipiens* by one respondent,

X. wittei and *X. vestitus* by another, *X. borealis* by two respondents and *X. muelleri* by one respondent. One worked on albino *Xenopus*, but the species was not mentioned.

3.3.1.2 Type of research

All respondents except three, confirmed their involvement in either molecular or embryological research and often both. Of these, seven (21%) stated that they combined their research with developmental biology. Others (15%) specified their involvement with neurobiology, while still others (12%) specified cell biology. One respondent (3%) combined embryological research with neurological, evolutionary and behavioural (mating systems) research. The remaining three were all involved in ecological research.

3.3.1.3 Suppliers

Frogs were obtained in three ways: ordered from supply companies, bred from in-house colonies and collected from wild or feral populations (Table 3.2). Four of the respondents did not specify who their suppliers are, referring to them only as "commercial suppliers", and were consequently not included in the table. One of the facilities from France import frogs from South Africa but did not specify the supplier and was therefore also excluded from the table. Almost half (47%) of the facilities especially those based in the US, order their frogs from more than one supplier. Facilities with their own in-house colonies (stocked with breeding adults) order from commercial suppliers when necessary.

Table 3.2 Sources of *Xenopus* spp. for the respective facilities in the survey.

Source of supply	Country	Number of facilities*
Nasco	United States	21
Xenopus 1	United States	18
Xenopus Express	United States	6
Pacific Biological Supply Company	United States	4
African Xenopus Facility	South Africa	2
North Carolina Biological Supply Company	United States	1
African Reptile Park	South Africa	1
Xenopus Ltd.	South Africa	1
University of Geneva	Switzerland	1
Private dealer	United Kingdom	1
In-house colonies	United States	10
Collect from feral populations	United Kingdom	1

* The total number of facilities in the table (67), exceed the number of facilities that replied to the questionnaire (36) due to facilities that make use of more than one supplier or other means to obtain frogs.

3.3.1.4 Number of frogs required

The data from Question 4 has been processed to reflect number of frogs required per week for comparative purposes and since most of the facilities operated on a weekly routine. To simplify the data further, the number of frogs required has been categorised into five groups (Table 3.3). The number of frogs needed by the different facilities varied from as few as 24/year to more than a thousand per year. The most frequently required number of frogs is 1-10/week and is required by 48% of facilities. The research of only a single respondent involves the use of tadpoles to the extent of thousands every two days, and was not included in the table.

Table 3.3 The number of frogs required, according to gender, and the number of facilities requiring the frogs.

FROGS / WEEK	FACILITIES REQUIRING			TOTAL
	Male frogs	Female frogs	Gender not specified	
< 1	0	5	6	11
1 - 10	6	4	10	20
10 - 20	2	0	5	7
> 20	0	0	1	1
unspecified	0	0	2	2

3.3.1.5 Importance of gender

The gender of the experimental animals mattered to 25 (69.5%) of respondents, of which 10% need more males and 15% were dependent on equal numbers of males and females. The remaining seventy five percent of the 25 used more females for experimental procedures at any given time. The sex ratio male to female frogs as needed for experimental trials ranged from 1:2 to 1:30.

3.3.1.6 Frog size and age

The age or developmental stage of the frog seems to be more important than size. To the three respondents conducting ecological studies, frog size and age was of secondary importance. One respondent used tadpoles and another, juvenile frogs. All other facilities (86%) require sexually mature individuals. Eleven of the 31 indicated that they order the largest individuals available. A few specified the required age of frogs as: two years and older, older than one year, three to four years and males one to three years and females two to four years.

3.3.1.7 Opinion on a parasite-free frog

Question 7 was unanswered by 11% (=4) of respondents. Seventeen percent replied that they were not interested in the idea of a parasite-free frog, while the remaining 72% agreed that they would prefer a frog without parasites.

3.3.2 Use of *Xenopus laevis* at South African universities

Forty-one departments from 12 universities were contacted and 32 responded. Results from the survey indicate that *X. laevis* was currently being used by nine departments at eight universities (Table 3.4) for a small variety of teaching purposes at both undergraduate and post-graduate level. Correspondents from five departments disclosed that the practice of *Xenopus* dissection during practicals was no longer in use at their respective departments. Only three departments from three universities (Table 3.5) are actively involved in research on *Xenopus* spp.

Table 3.4 Teaching purposes to which *Xenopus laevis* were put at South African universities

University	Department	Purpose of use	Academic year	No frogs /year
Durban	Zoology	Physiology & Anatomy dissection	First	Unknown
Fort Hare	Zoology	Physiology & Anatomy dissection	First	Unknown
Free State	Zoology	Physiology & Anatomy dissection	First	350
		Physiology (thermoregulation)	Third	4 (no killing)
		Parasitology	Honours	5
Port Elizabeth	Pharmacy	Muscle, Neural & Heart Physiology	Undergraduate	40
Potchefstroom	Physiology	Muscle & Neural Physiology	First & Second	Unknown
Pretoria	Zoology	Ecophysiology	Second	30 (no killing)
Western Cape	Physiology	Physiology	Unknown	Very small scale
	Zoology	Physiology & Anatomy dissection	First	Unknown
		Experimental Physiology	Third	Unknown
Witwatersrand	Zoology	Physiology & Anatomy dissection	First	800

Table 3.5 Research involving *Xenopus laevis* at South African universities. Where post-graduate research was involved, the level is given in brackets.

University	Department	Type of research
Free State	Zoology	Ecology and Husbandry (MSc). Parasite-related
Stellenbosch	Zoology	Bioindicator of water pollution
Witwatersrand	Zoology	Bioindicator of wetland quality (PhD). Developmental biology

3.3.3 Relevance of *Xenopus laevis* to the angling industry

The angling shops in the Free State seem to concentrate in three major cities: Bloemfontein (5), Welkom (2) and Bethlehem (1). Seven of the eight shops currently sell *X. laevis*, while the eighth stopped a few years ago. None of the shops kept records of the number of frogs that were sold. The estimate numbers sold differed from one shop to another and ranged between 5 000 and 32 000 per year.

3.3.4 Pet *Xenopus laevis* trade

This pipid makes an ideal pet for those interested in Amphibia (especially in the United States) for many of the same reasons that render it popular to scientists. Most of the large suppliers in the US market *Xenopus* exclusively to research and education institutes. Less than 1%, and usually captive-bred albinos, are sold as pets by the few that do cater for the pet trade.

3.4 DISCUSSION

3.4.1 *Xenopus* in molecular and embryology research

From the current survey it can be gathered that the situation has not changed much in the molecular and embryology fields with regard to the choice of experimental animal. The growing interest in *X. tropicalis* stems from its use in transgenetic experiments. It is likely to become an important genetic model system in the near future.

The array of research interests presented by the survey (Developmental Biology, Neurobiology and Cell Biology) are all facets of embryology and molecular biology and should therefore not be viewed as separate disciplines. The most significant purpose for *Xenopus* in molecular biology is for it to produce positive oocytes. These are used as heterologous expression systems and for studying the molecular aspects of development, especially pattern formation in embryos. According to Hamilton (1976) the study of *Xenopus* development stimulates many inquiries and investigations and can be taken as a model system for development as many of the problems faced throughout the vertebrates are met by solutions found in *Xenopus*.

Much the same range of products is provided by the different suppliers, which would imply that competition exists between suppliers. Any stage of development from fertilised eggs to mature frogs can be purchased. The cost of a sexually mature *X. laevis* female (pigmented) in the United States ranges between $15 and $34. The reason so many facilities make use of more that one supplier can be attributed to the fact that suppliers run out of stock from time to time because of sudden increases in demand. Furthermore, the end users are often dissatisfied with size or overall condition of a shipment and consequently order from someone else. The phenomenon of laboratories having their own breeding colonies is not at all new, it is however becoming more common. Surveys conducted in the United Kingdom indicated that the percentage of facilities carrying out research on *Xenopus* that make use of in-house colonies had risen from 3.2% in 1972 (LAC 1974) to 15% in 1977 (Donnelly 1980). The figure for the current survey, though representative of the molecular research market, is nearly double (27.8%) that of the 1977

UK survey. The direct implication of having in-house breeding colonies is a reduction in orders from commercial suppliers.

Although this survey does not address the total number of *Xenopus* used by all molecular facilities, it does bring into perspective the requirements for male and female frogs. Even though the ratio of frogs needed for experimental trials is 1:30 (male:female), the actual number of males required is equal to and even surpasses that of females. This is because males are often sacrificed and the testes isolated for the purpose of inducing spawning in oocyte-positive females, while the females are reused every 2-6 months for as long as they can produce large clutch sizes. Older females that produce smaller clutch sizes are removed and replaced by young sexually mature individuals.

Some facilities are of opinion that the size of the frog is less important than shape as skinny frogs are poor reproducers, but the general specification for the "ideal" frog is a large, young, sexually mature individual.

Despite the claims of suppliers of parasite free frogs, parasites are sometimes found in these frogs. For some of the facilities it is irrelevant whether their experimental frogs are parasite-free or not as the parasites do not influence the outcome of their research in any way. It can be argued that, since most facilities now are populated by frogs that probably are not parasite free, adding a parasite free animal to these facilities would be defeating the purpose.

3.4.2 Use of *Xenopus laevis* at South African universities

Surprisingly few universities still make use of *Xenopus* for teaching purposes. In some instances the practice has been replaced by computer based simulations, others have left it out of their curriculum completely. The most significant use of *X. laevis* has always been, and remains, as a model system for physiology and anatomy dissection. Universities seem to utilise the minimum number of frogs needed to complete each task and avoid killing where possible. Figures for frogs used at one university shows a gradual annual decrease since 1993 from 1 146 to 841 in 1998. It seems ironic that in the country of origin of this

globally exploited species, only three universities are actively involved in *X. laevis* research. Less encouraging is that only one of the studies is of herpetological interest.

3.4.3 *Xenopus laevis* and the angling industry

X. laevis is a much sought after local live bait for catfish. Only young frogs between the lengths of 40mm and 55mm are used for this purpose. Other fish that are known to take *X. laevis* are yellowfish, bass and trout. Sales continue all year round, but it is especially during the angling season from September till April, that large quantities are sold. Shops that sell to the angling community often cannot keep up with the demand. They are totally dependent on outside sources to replenish their stock and are constantly on the lookout for persons who can deliver. These include a few people who occasionally collect from existing farm dams or farmers themselves who find the frogs by chance when cleaning dams or drinking troughs. Current sales would probably be greatly exceeded if a permanent supplier were available.

3.4.4 The pet-trade

Specialist reptile and amphibian pet shops provide most of the frogs themselves, independently of commercial suppliers. *X. laevis* is often recommended as a "starter pet" for persons interested in keeping a pet frog for the first time. The frog is usually sold as part of a starter kit containing the frog itself, an enclosure, substrate, lighting equipment, cleaning equipment and food supply. Detailed care-sheets with background information on the species, housing conditions and common diseases and the treatment thereof are readily available to the purchaser.

3.4.5 General

No other animal has served scientific and medical research quite like *Xenopus* has. The network of suppliers has established a firm infrastructure and breeding programmes to provide for the needs of the relevant end-users. Wild frogs are continually collected and exported from South Africa to seed captive breeding colonies and provide genetic variation within the colonies of various suppliers. Effective exploitation and utilisation will ensure that this frog will remain a viable economic resource.

Appendix 3.1 Questionnaire sent electronically to members of an Internet Newsgroup and other research facilities to determine the requirements for *Xenopus* in molecular and embryological research.

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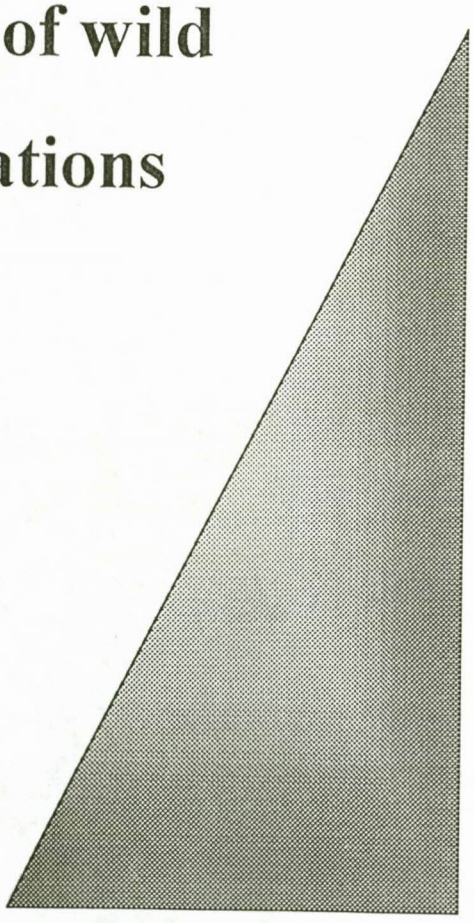
1. State the type of research with *Xenopus* you are involved in.
2. Do you work with any species of *Xenopus* other than *X. laevis*?
3. Where do you obtain the specimens for your current research?
4. How many, and how frequently are frogs required?
5. Does the sex matter? If so, what is the ratio of males to females?
6. Does age or size matter? If so, how?
7. Would you be interested in a parasite-free frog?
8. Who else do you know who is involved in research on *Xenopus*?

Chapter 4

Tadpole development and population dynamics of wild *Xenopus laevis* populations



CONTENTS

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TADPOLE DEVELOPMENT AND POPULATION DYNAMICS OF WILD *Xenopus laevis* POPULATIONS

4.1 INTRODUCTION

The first full account of the development of *Xenopus laevis* was contained in a short paper by Beddard (1894). A 36-page description on the life-history of *X. laevis* by Bles (1906) included for the first time the description and sketching of the development of the embryo.

Early attempts to divide the developing embryo and tadpole of *X. laevis* into identifiable stages were made by Peter (1931) followed by Weisz (1945). It was however the extensive and well defined Normal Table of *X. laevis* by Nieuwkoop & Faber (1956, 1967) that became the standard reference for the developmental stages of this species. In an effort to simplify the staging of anuran embryos and larvae Gosner (1960) proposed a generalised table that consisted of 46 stages.

The Normal Table has been used extensively as a reference for communicating certain phenomena in embryology. Even though the Normal Table can be used for ecological applications, information is lacking on larval development as related to ecological function.

The current harvesting of *Xenopus* from natural sources in South Africa is a practice over which no control is executed for the larger part of the country. The danger of over-exploitation has lead to the apparent depletion of previously rich sources of the frog in the Free State. To allow controlled harvesting of these frogs requires knowledge of the population structure in the wild condition.

The aquatic nature of *Xenopus* offers the ideal opportunity to study the structure of a population. Collecting of frogs with baited traps has been used with great success (Schramm 1986). It is an easy technique that can trap large quantities at a time to ensure adequate material. Furthermore fresh material is always readily available, as frogs can be collected all year round.

The focus of this chapter is on two aspects of the ecology of *X. laevis*:

- Distribution, seasonality and development of tadpoles in the Free State.
- Population sizes and structure of wild populations.

4.2 MATERIAL AND METHODS

4.2.1 Tadpoles

4.2.1.1 Material examined

X. laevis tadpoles examined were part of the collection of the Southern African Frog Atlas Project (SAFAP), collected in a joint effort by L.H. du Preez (SAFAP regional organiser for the Free State), students (including the present author of this thesis) and volunteers during the years 1995 to 1998. Material is housed in the SAFAP collection at the Department of Zoology and Entomology at the University of the Free State, Bloemfontein, South Africa.

4.2.1.2 Collecting and fixing method

The aim was to collect from at least one water body in every quarter degree grid cell (QDGC) within the Free State province. A wide range of water bodies from temporary to permanent, both natural and man made were visited. A collecting net with aluminium frame (handle length, 1m) and nylon net (diameter, 400mm; mesh size, 1mm) was used to scoop up the tadpoles from the water. Tadpoles were immediately fixed in 10% neutral buffered formalin. A field number was allocated to each sample and the date of collection, type of water body, weather data for the 24 to 48 hours prior to collection, grid cell and co-ordinates, were recorded. In addition, a label bearing the field number and collecting data was put into the bottles containing the fixed specimens.

4.2.1.3 Identifications, staging and measuring

As the collection method used does not select for any specific species, the tadpoles of *X. laevis* had to be separated from the rest for each sample. The criteria given in the diagnostic keys of Van Dijk (1966) and Du Preez (1996) for *X. laevis* were used to

distinguish the species. The "Gosner Stage" (Gosner 1960) was used as reference to stage the tadpoles with the aid of a dissection microscope and total length was measured, using vernier callipers.

4.2.2 Mark and recapture

The methodology is based on the principle that by repeatedly catching and marking individuals from a specific locality, the population size can be estimated through integration of data. Estimates of the Dam van Trane population size were determined using a modified version of the Jolly-Seber Stochastic Method (Donnely & Guyer 1994). First the number of marked individuals at risk on day i (M_i) was estimated using the equation:

$$M_i = m_i + \frac{z_i . r_i}{y_i}$$

where: r_i = the number of marked animals released on day i

m_i = the number of marked animals caught on day i

y_i = the number of animals marked and released on day i and caught after day i

z_i = the number of animals marked before day i that are not caught on day i but are caught after day i

Population size (N_i) was estimated as follows:

$$N_i = \frac{M_i (n_i + 1)}{(m_i + 1)}$$

where: n_i = the number of animals caught on day i

The estimations of survival rate (ϕ_i) and gains (g_i) are given by the equations:

$$\phi_i = \frac{M_{i+1}}{(M_i - m_i + r_i)} \qquad \text{and} \qquad g_i = N_{i+1} - \phi_i N_i$$

Standard error for estimate population size was calculated as follows:

$$SE_{Ni} = \{N_i(N_i - n_i)[\frac{M_i - m_i + r_i}{M_i}\frac{1}{(y_i - r_i)}\frac{1}{+ m_i - n_i}]\}^{1/2}$$

Too few marked frogs were recaptured for the data to be subjected to the Jolly-Seber Method for the estimation of population size. Instead the Chapman's Modification of the Petersen Estimate (Donnely & Guyer 1994) was used. It was a more appropriate method to use for the particular data set from Valley of Seven Dams as it corrects for low number of recaptures. Population size was estimated as follows:

$$N_c = \frac{(r + 1)(n + 1)}{(m + 1)} - 1$$

where: r = number of animals caught, marked, and released on day 1

n = total number of animals caught on day 2

m = total number of marked animals caught on day 2

Standard error for N_c was calculated by using the formula:

$$SE_{Nc} = [\frac{(r + 1)(n + 1)(r - m)(n - m)}{(m + 1)^2(m + 2)}]^{1/2}$$

In addition to estimate population size, information concerning community structure (sex ratio and body measurements) was gathered from the mark and recapture study.

4.2.2.1 Sampling regime

Frogs were caught, using the technique described in Chapter 2 from the following sites.

- Dam van Trane (Fig. 4.1a): Frogs were caught once a month from February to November 1997 using ten small traps.
- De Dam (Fig. 4.1b): Frogs were caught once a week during the month of November 1998 using one large trap.

- Valley of Seven Dams (Fig. 4.1c): Frogs were caught once a week from March to June 1999 using six small traps.
- Rustig (Fig. 4.1d): Three large traps were placed once a week from mid August to mid September 1999.

4.2.2.2 Freeze-branding

A set of 10 (numbers 0-9) brand-irons made from bronze wire was used for numbering the frogs. The brand-irons were placed in liquid nitrogen until it stopped bubbling. The numbers (15mm high) were branded onto the ventral surface posterior to the sternum by pressing the brand-irons down for four seconds (Fig. 4.2). Young frogs are difficult to handle and were temporarily anaesthetised before branding by immersion in benzocaine solution (ethyl 4-aminobenzoate) for five minutes and revived by rinsing with dechlorinated tap water.

Frogs from the Dam van Trane were numbered, starting with 1 for the first frog and continuing numerically with each successive frog, while with the Valley of Seven Dams and De Dam, all frogs caught with the first catch received the number 1 and all those caught with the second catch, the number 2 etc.

4.2.2.3 Measuring and weighing

The distance from the tip of the snout to the vent (snout-to-vent-length or SVL) was measured using vernier callipers. Body weight was measured using an electronic scale (to 0.001g accuracy). Only frogs from the Valley of Seven Dams were measured and weighed.

4.2.2.4 Sexing

Females were identified by distinct swollen labial folds that protruded past the vent, compared to the more reduce cloaca of the males. Males were also identified by their palms that blacken during the mating season. The use of morphological characteristics to distinguish sexes is very difficult for individuals smaller than 30mm and was therefore not attempted.

Figure 4.1

Photographs of the four dams at which mark and recapture studies were performed on wild *Xenopus laevis* populations for the estimation of population size.

a) Dam van Trane

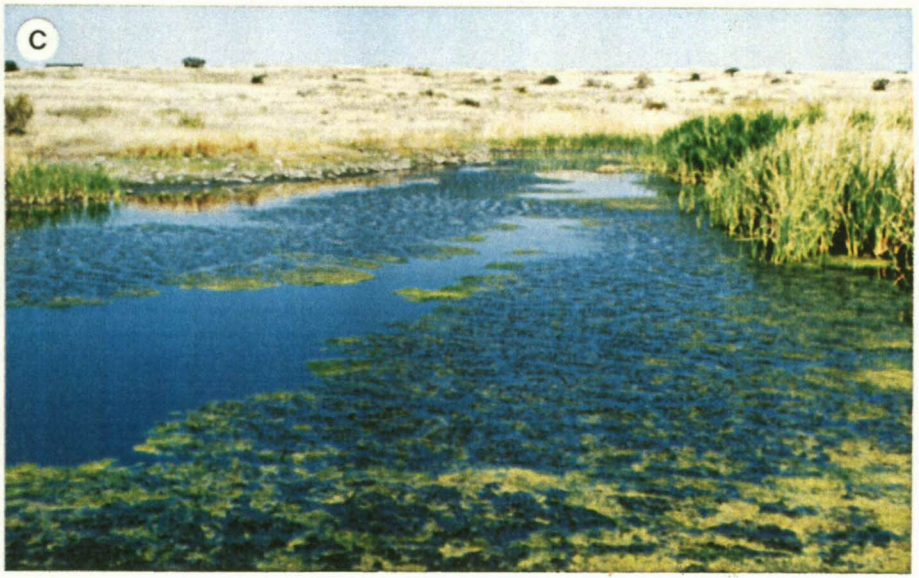
b) De Dam

c) Valley of Seven Dams

d) Rustig


a


b


c

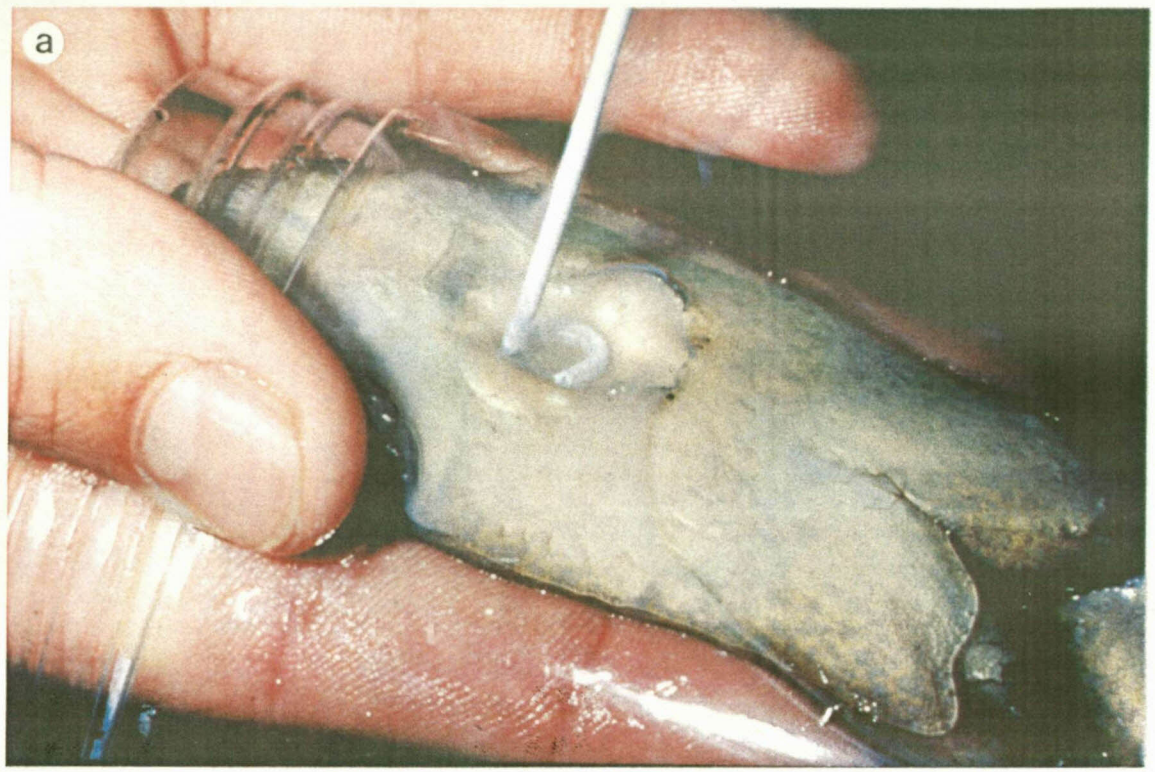

d

Figure 4.2

Photographs showing freeze branding of *Xenopus laevis* for the purpose of mark and recapture studies.

a) Photograph to show how the number is branded onto the ventral surface of the frog, using branding irons (15mm high) cooled in liquid nitrogen.

b) Photograph showing the branded number on a recaptured frog, allowing easy identification of the individual.


a


b
293

4.3 RESULTS

4.3.1 *Xenopus laevis* tadpoles

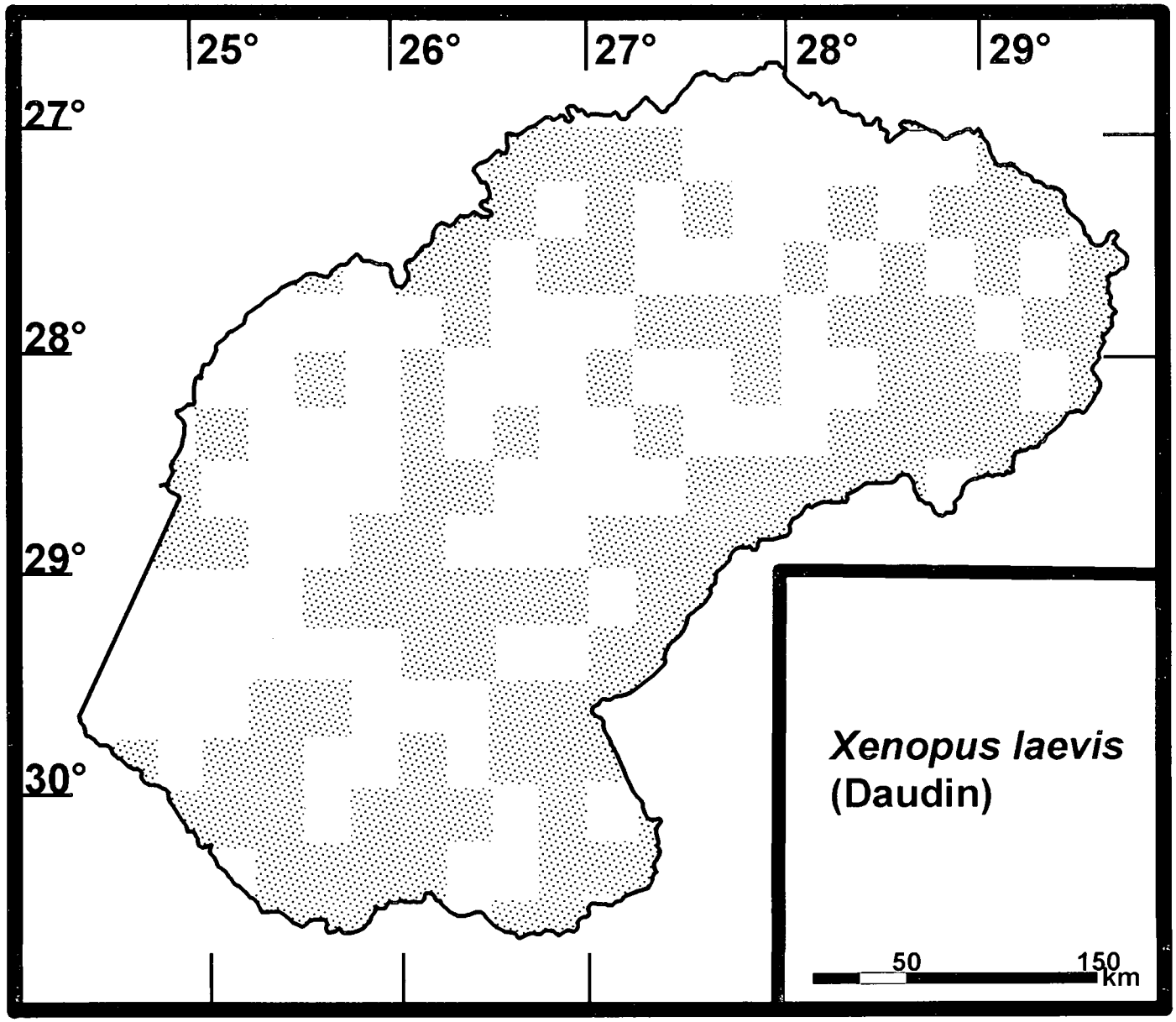
A total of 105 samples, consisting of 711 *X. laevis* specimens were examined. Of the collections 24% contained only a single specimen. However the maximum number of specimens for a sample was 38, and the mean 6.8. Of all samples 47% contained tadpoles of more than one developmental stage (mean, 2.0). The maximum number of stages for a sample was seven and ranged from stage 28 to 43 (15 stages). The biggest range however, 16 (stages 26 to 43) was from a sample containing tadpoles of six different stages.

4.3.1.1 Distribution in the Free State

Newly collected tadpoles as well as fixed specimens from the SAFAP collection are representative of 110 QDGCs within the Free State (Fig. 4.3). This implies that the presence of *X. laevis* can be accounted for in 52% of grid cells. The grid cells concerned are scattered over the entire province, but seem to be concentrated towards the east. Frogs were found in almost any type of water body that included mostly roadside pools, earth-walled dams, and drinking troughs, but also vleis, streams and rivers.

Figure 4.3

Map of the Free State indicating the quarter-degree grid cells from which *Xenopus laevis* tadpoles were recorded.


25°
26°
27°
28°
29°
27°
28°
29°
30°
Xenopus laevis
(Daudin)
50
150
km

4.3.1.2 Development

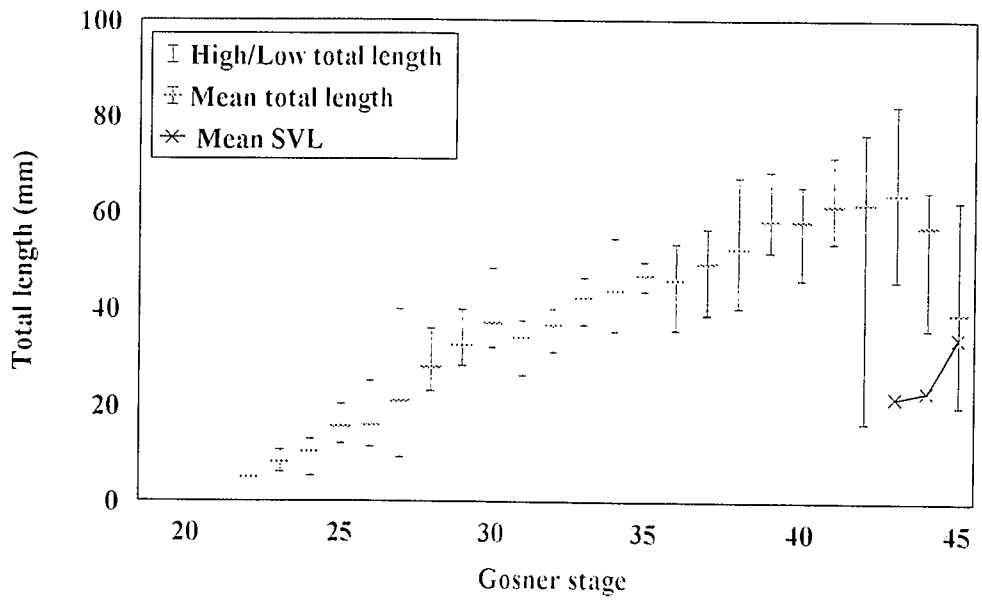
Examples of every developmental stage of the free-swimming tadpole until the end of metamorphoses (stage 22 to 45) were present in the material examined. Stages before stage 22 are generally less than 5mm in length and not yet free-swimming and therefore more difficult to collect.

A stage-length graph was compiled from the collective data of all samples over the four years (Fig. 4.4). Development is accompanied by a steady increase in body length except for stages 30 to 33 up until stage 43. From stage 43 till the end of metamorphosis total body length decreases to a length equal to stage 33 tadpoles. SVL can be measured for the first time in stage 43 tadpoles. Even though total body length decreases during the resorption of the tail, the body from the snout to the vent keeps on growing in length.

The size ranges for each developmental stage were often great but maximum size was often exhibited by a single specimen that was considerably larger than most other specimens of the same developmental stage. The largest specimen was an 82.3mm tadpole in stage 43 of development.

Figure 4.4

Gosner stage-length graph of *Xenopus laevis* tadpoles (stages 22 to 45) obtained from measurements of wild collections. Mean total length is used for each Gosner stage and mean snout-to-vent-length (SVL) for three stages in which it was possible to measure. Variation in total length for specimens of the same developmental stage is also given.


High/Low total length
Mean total length
Mean SVL
Total length (mm)
100
80
60
40
20
0
20
25
30
35
40
45
Gosner stage

4.3.1.3 Seasonal occurrence

Each year a different number of *X. laevis* samples were collected, 33 in 1995, 34 in 1996, 12 in 1997 and 23 in 1998. Only a few of the samples were collected from the same localities. The majority of samples however, were collected from different localities. The first tadpoles to be collected in the new breeding season were stage 24 tadpoles collected on 23 September 1995. The eggs were laid on 19 September as Gosner stage 24 tadpoles resemble *X. laevis* tadpoles at Nieuwkoop and Faber stage 45, which are approximately four days old. Collections continued for eight months spanning from September to mid-April (Table 4.1). For most months tadpoles from an early developmental stage through to late development are represented.

Table 4.1 The number of samples and range of Gosner stages of *Xenopus laevis* tadpoles collected during 1995-1998.

Month	No of samples	Range of stages
January	28	23-44
February	26	28-43
March	33	24-46
April	13	37-46
May	0	–
June	0	–
July	0	–
August	0	–
September	1	24
October	17	22-37
November	72	23-44
December	16	26-45

4.3.2 Population structure of post-metamorphic frogs

4.3.2.1 Population size

Attempts to collect *X. laevis* at the Rustig dam failed repeatedly. Even after four attempted trappings, implementing different combinations of bait and trapping time, no frogs were caught at this locality. The dam does however have a teeming catfish population. The fish were introduced more than two decades ago and have continued breeding ever since. The farmer allows the angling of catfish at the premises from time to time in an ostensible attempt at controlling the population.

During the third week of trapping at De Dam the experiment had a serious attack. A frog collector had cunningly emptied the traps, removed the frogs and then replaced the traps. The result was that only 11 frogs were retrieved for capture 3. This is considerably fewer than the 163 caught the previous week (Table 4.2). The following week traps similar to the ones used for the study were found at the same site, hidden amongst the reeds. Only a single frog could be caught with the experimental traps. The mark and recapture studies performed at Valley of Seven Dams and Dam van Trane did not have the same negative effect on the number of frogs caught after repeated captures. The prevailing circumstances forced the termination of the experiment at De Dam.

Table 4.2 Capture data of the different samples for *Xenopus laevis* at De Dam.

Capture	No. of individuals caught	No. of recaptures
1	245	–
2	163	67
3	11	4
4	1	1

Trapping at Valley of Seven Dams also had its share of setbacks. On two occasions vandals had interfered with the experiment and did extensive damage to traps. Not only were traps lost, but once close to 20 frogs became trapped and drowned because the traps

had been capsized. The calculated values for the estimates of population size from the Chapman's Modification of the Petersen Estimate were plotted on a graph (Fig.4.5a). A fair amount of variability exists for the estimates of population size. This can be as a result of not enough repetitions. The standard error for the population size estimates generally decreases from capture 4 till the last capture (SE_{Nc7} = 39). The population size is therefore estimated between 1 200 and 1 400 with reasonable reliability.

Captures retrieved for Dam van Trane were sufficient for the Jolly-Seber Method to be used. Values for the estimations of population size, survival rate and gain are listed in Table 4.3. The values for estimated population size for captures 5 to 6 stabilise near 1 200 and the estimated standard error decreases to 379 for capture 7 (Fig. 4.5b). Amongst the frogs captured during captures 8 and especially 9, were large numbers of newly metamorphosed frogs. The recruitment of these frogs into the population caused the values for estimated population size to escalate by almost 400 and standard error by 300. The estimated population size of 1 200 is more reliable than 1 481 estimated for capture 9.

Table 4.3 Estimates of survival (ϕ_i), population gains (g_i) and population size (N_i) for Dam van Trane, calculated using the Jolly-Seber Method.

Capture (i)	ESTIMATE		
	ϕ_i	g_i	N_i
1	–	–	–
2	1.445	516	110
3	0.692	-83	675
4	2.195	291	384
5	0.614	476	1 134
6	1.058	-89	1 172
7	1.093	430	1 151
8	1.268	-146	1 432
9	–	–	1 481

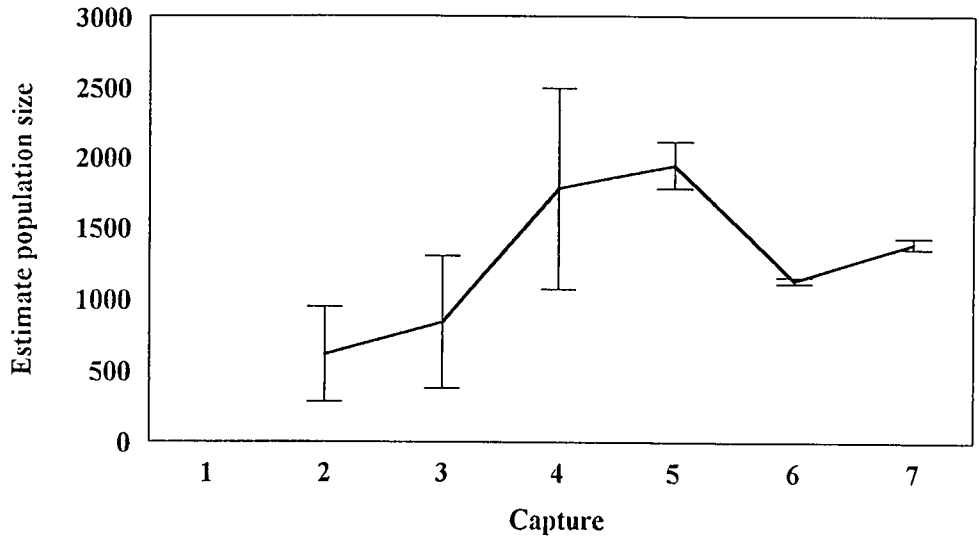
Figure 4.5

Estimates of population size, using repetitive estimation and mark and recapture methodology and indicating reliability of estimates as standard error for:

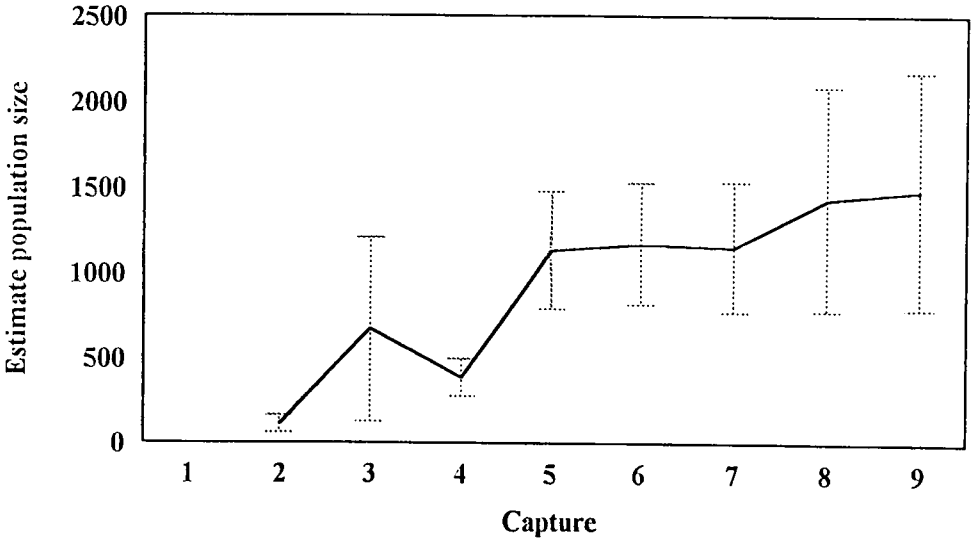
a) Valley of Seven Dams, calculated by using the Chapman's Modification of the Petersen Estimate;

b) Dam van Trane, calculated by using the Jolly-Seber Statistical Method.

a)


3000
2500
2000
1500
1000
500
0
Estimate population size
1
2
3
4
5
6
7
Capture

b)


2500
2000
1500
1000
500
0
Estimate population size
1
2
3
4
5
6
7
8
9
Capture

4.3.2.2 Sex ratio

In all three cases in which frogs were sexed, females were dominant in terms of numbers. Each population however had its own unique gender ratio. The ratio male to female were as follows:

De Dam - 3 : 4 (n = 420)

Valley of Seven Dams - 3 : 5 (n = 301)

Dam van Trane - 1 : 3 (n = 880)

4.3.2.3 Measurements of males and females

The length-mass graph (Fig. 4.6) revealed that the difference between the length-mass relation in male and female *X. laevis* is not significant. Rather the difference lies in the size reached by mature frogs as females can grow to more than twice the mass of males and reach lengths of up to 110mm opposed to the 75mm reached by males.

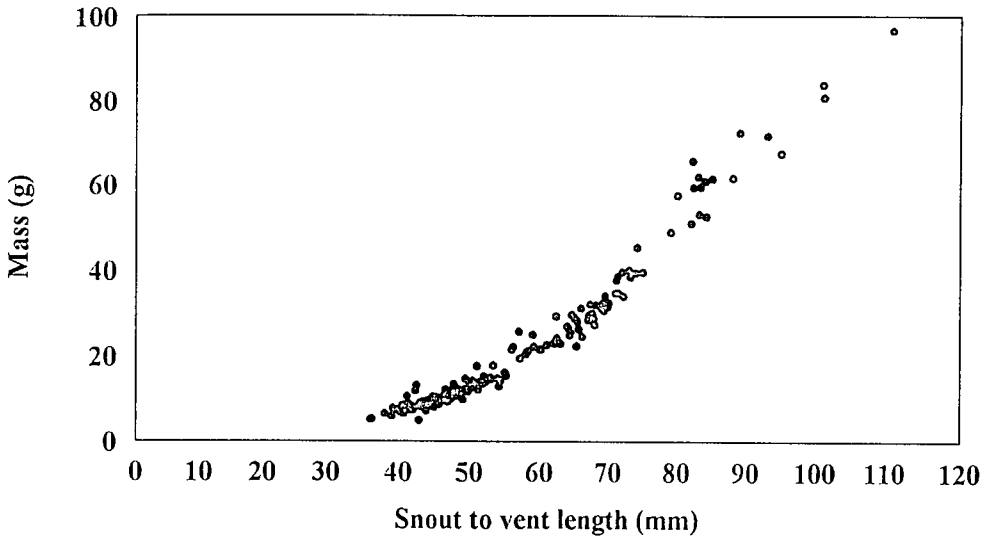
Figure 4.6

Length-mass graphs of *Xenopus laevis* from the Valley of Seven Dams:

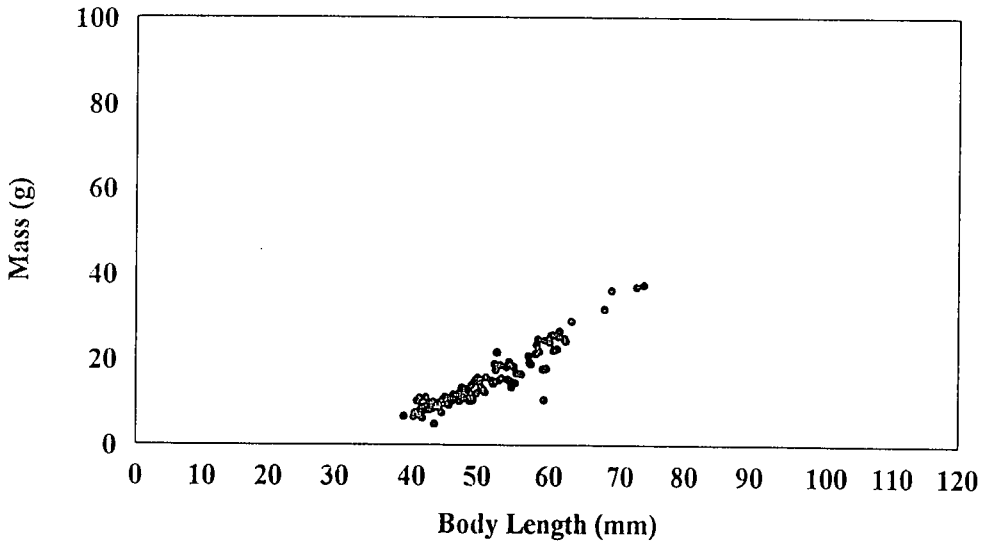
a) Female frogs

b) Male frogs

a)


Mass (g)
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100 110 120
Snout to vent length (mm)

b)


Mass (g)
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100 110 120
Body Length (mm)

4.4 DISCUSSION

4.4.1 Collection methodology and distribution

Traditionally, knowledge of the ranges of animals has been based on observation and collection of adult specimens. Anurans provide a challenge in this regard. As they are conspicuous yet vocal animals, they are more often heard than seen. The advertisement calls of all species differ significantly from one another and are highly reliable characters upon which to base the diagnosis of species (Passmore & Carruthers 1995). For this reason recordings of frog calls provide an accurate record of the presence of a species at a particular locality. The recording of *Xenopus* calls is somewhat more difficult as one needs a hydrophone since calls are emitted under water. Channing & Van Dijk (1995) report that tadpoles remain the obvious collectors' choice for distribution and ecological studies, as they are readily obtainable during the breeding season, independent of weather. The presence of tadpoles at a particular site provides an easier yet effective alternative for mapping the range. The survey becomes even more thorough when the two techniques are used in unison. During daylight hours tadpoles can be collected and recordings of frog calls made at night. Tadpole collection has the added benefit of the gathering of information on the breeding season and on tadpole development.

The distribution of collection sites confirms that the range of *X. laevis* extends throughout the province as documented by du Preez (1996). The availability of water bodies is an important factor in the distribution of this species as it lives in almost any water body. The lower density of collection sites in the drier west corresponds to the smaller number of water bodies, which in turn is a result of lower rainfall. An area of medium density collection sites forms a transitional barrier between the western and eastern extremes that corresponds with the 500mm-rainfall belt given by de Waal (1978). Therefore rainfall plays an important role in the density of this species within its geographical range.

4.4.2 Breeding season and tadpole development

The month of September usually marks the start of the summer rainfall season. The presence of all the free-swimming stages of tadpoles in each of the following six months implies that breeding activity continues throughout this period and some larvae continuously undergo metamorphosis. The last tadpoles to complete metamorphosis do so in April. The breeding season there fore comes to an end some time in March and lasts for six months. Similar findings of the duration of the breeding season on the former Transvaal Highveld, of 6½ months, were made by Balinsky (1969). *X. laevis* is however a winter breeder where it occurs in the winter rainfall region (Shapiro 1936b, Berk 1938). The net result is that tadpoles are present all year round in different parts of the country at different times of the year (see also Channing 1998).

At stage 46 (Gosner's Stage) metamorphosis is essentially complete and the young frogs resemble adults. Event though stage 46 frogs were collected, they were excluded from the results as they varied greatly in size from the reproductively mature frogs. The stages (1 through 21) which are absent from collections, together with stages 22 through 25, for which there were only a few samples (1, 3, 10 and 4 respectively) contain the embryonic or pre-feeding series. Stages preceding 25 are not yet free swimming and become an easy meal for predators including adult frogs. Measey (1998a) found that cannibalism amongst *X. laevis* adults was mostly directed towards eggs but states that the number of tadpoles found in the diet under-represented the extent to which predation occurred.

The dip in body length from stage 30 to 33 might be as a result of poor representation of specimens as only 19 collectively were available for these four stages. Resorption of the tail occurs from stage 40 until completely absent (stage 46). However, total body length only starts decreasing from stage 43, thus the rate of growth in the body without the tail is surpassed by the rate of resorption of the tail during this stage.

The great variation in body size of tadpoles of the same developmental stage (Fig. 4.4) even within a sample implies that there is a large variation in condition between tadpoles of similar stages. It could be possible that tadpoles of different developmental stages from the same sample are of the same age and that the rate of development differs.

1500 073 52

It is important to realise that the information on the tadpoles is a generalised picture from many different localities, but within the same rainfall region. Breeding season might not be as long and variation in tadpole body length as great within a single locality. The size-staging graph that was obtained by plotting body length against developmental stages permits the use of absolute size as a key trait to compare the development with that of *X. laevis* larvae from different rainfall regions.

4.4.3 Dynamics of population size

A study to estimate population density in frogs in West Africa showed that *Euphlyctis occipitalis* reached a biomass of 30.5 to 44.4 kg/ha, at a density of 270 to 1 000 individuals/ha. *Xenopus muelleri*, was calculated to have between 400 and 1550 individuals/ha (Micha 1975). According to Channing *et al* (1995) no empirical density data is available on any frog population in South Africa.

Both Dam van Trane and Valley of Seven Dams are situated in semi-protected areas, which means that frogs have less chance of being endangered by human exploitation. Regulation of population size relies almost completely on the working of natural and environmental factors. Despite the Dam van Trane having more than twice the surface area of the dam at Valley of Seven Dams their estimated population sizes were similar. It can therefore be said that even though the size of a habitat influences the size of a population, it is not an exact determinant of population size.

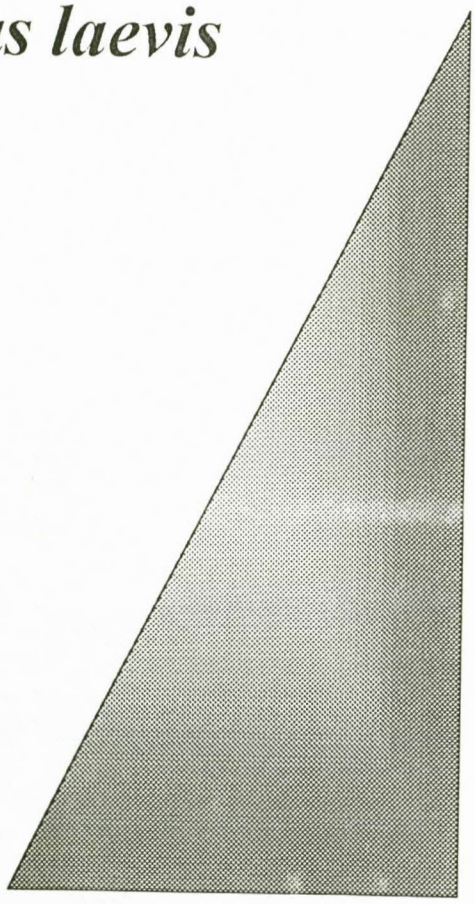
A possible limiting factor for population size at Dam van Trane might be due to its semi-permanant nature. Resulting from the drying of the dam while the breeding season is in progress, many larvae would be lost due to desiccation of the habitat. The breeding season would be reduced and consequently natality would become lower. Furthermore, it would be during this period of fall in water level that predation by wetland birds would be at its highest (personal observation). Numerous predatory birds including reed cormorant (*Phalacrocorax africanus*), grey heron (*Ardea cinerea*), sacred ibis (*Threskiorinis aethiopicus*) and little egret (*Egretta garzetta*) were observed at the Dam van Trane during the drying period taking huge quantities of *X. laevis*, thus fulfilling an important

role in stabilising the population size. Frogs are also known to form part of the diet of catfish (Skelton 1993). It seems that when present, catfish are major predators of *Xenopus*, as can be seen in the absence of the frogs at the Rustig dam. Furthermore none of the localities where *X. laevis* did occur support catfish populations. Another fish known to prey on *X. laevis* is the largemouth black bass (*Micropterus salmoides*) which has successfully been used as a biological control measure against *X. laevis* (Prinsloo, Schoonbee & Nxiweni 1981).

The exploitation of a *Xenopus* population by humans is a potential threat to their survival. As seen at De Dam, continued harvesting a small population over a short period could seriously deplete a population. Repeated trapping was successfully used to control *X. laevis* numbers at a fish culture farm in the Transkei (Schramm 1986). In the same article the author documents that frogs recolonised freshly cleaned ponds within two weeks and that frogs most likely moved in overland. The phenomenon of overland migration has been observed a number of times (Hewitt & Power 1913, Kalk 1960). Therefore recolinisation can only occur if a neighbouring population is within migratory distance of the locality. The exact distance the frogs can travel overland remains to be investigated.

Chapter 5

Aspects of captive breeding and husbandry of *Xenopus laevis*



CONTENTS

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ASPECTS OF CAPTIVE BREEDING AND HUSBANDRY OF *Xenopus laevis*

5.1 INTRODUCTION

The status of commercial frog culture is according to Bardach, Ryther & McLarney (1972) considered to be nebulous. The largest and most widely used attempts at frog culture in the United States are those of the leopard frog *Rana pipiens* and bullfrog *Rana catesbiana* (Gibbs, Nace & Emmons 1971). Frog farming in Thailand has been established for 25 years and is mostly concerned with two local species *Rana tigrina* and *Rana rugulosa* and the imported American bullfrog *R. catesbiana* (Pariyanonth & Daorerk 1995).

The foundation for attempting captive breeding of *Xenopus laevis* was laid by Bles (1906) and was based on imitating the prevailing natural conditions preceding and during the breeding season in South Africa. A number of authors have studied the effect of a range of conditions on the breeding success of *X. laevis* in an effort to attain the optimum breeding procedure that would induce natural spawning. Bles allowed the frogs to hibernate during winter at 15°C. During spring the aquarium temperature was raised to 22°C and twice-daily cooled water was sprinkled to simulate rain. Van der Plank (1935) found that frogs spawn any time the pH is lowered below seven, provided that frogs are healthy. In another experiment by Van der Plank (1939) it was concluded that ultraviolet light is necessary to induce spawning. Experimental evidence by Berk (1938) proved that two of the factors incorporated by Bles (1906), namely temperature and rainfall, determine the activity of the anterior pituitary which in turn controls the sexual cycle of *X. laevis*. Savage (1965) reported that natural spawning is induced by the presence of various unialgal cultures in the aquarium and extended his conclusion in a paper in 1971 having isolated the active materials in algae believed to be responsible for initiating spawning. Du Plessis (1966) came to the conclusion that it could be the fertiliser itself

rather than an algal metabolite that serves as the primary stimulus for spawning as frogs are attracted by fertilised water and spawn before an algal bloom develops.

The only comprehensive report on the large-scale culture of *X. laevis* whereby spawning occurred naturally, is by Hey (1949) from the Jonkershoek Inland Fish Hatchery, Stellenbosch. Water was treated with an inorganic substance that allowed an algal bloom to develop. Successful breeding followed after seeding of the water with zooplankton.

Parallel to the studies on natural stimulus for spawning the technique for inducing spawning by hormone use was developed. Hogben, Charles and Slome (1931) were the first to demonstrate that a gonadotrophic hormone causes ovulation in *X. laevis* females. Shapiro (1936a) obtained fertile *X. laevis* eggs by injecting the female frog with a human pregnancy urine extract while Shapiro (1936b) reported the successful induction of spawning using an extract from the anterior pituitary of a mature *X. laevis*. The popularity of *X. laevis* grew amongst the research community for the study of earlier synthetic processes of oogenesis, and with it the need to maintain an in-house colony.

In teaching as well *X. laevis* became a popular model for demonstrating to pupils the reproductive process and development in Amphibia. These two events resulted in the induction of breeding becoming a popular subject on which to write. Various authors gave accounts of what they believed to be the best procedure for the injection of hormone, care and handling of eggs, tadpoles and young frogs (Cameron 1947, Henriques 1964, Ruddock & Ruffle 1972, Thompson & Franks 1978).

The objective of this chapter is to investigate conditions for raising *X. laevis* tadpoles that included water volume and type of enclosure. Tadpoles were taken through metamorphosis and feeding trials were conducted on the young frogs.

5.2 MATERIAL AND METHODS

5.2.1 Induced spawning

Chorionic gonadotrophin (Pregnyl) was injected into the dorsal lymph sac according to the method described by Van Wyk and Du Preez (1984). Males were injected for three consecutive days, while females were injected on days two and three only (Table 5.1).

Table 5.1 Amounts of chorionic gonadotrophin injected in *Xenopus laevis* to induce spawning (Van Wyk & Du Preez 1984).

DAY	DOSE FOR	
	Male	Female
1	250 I.U. (0.16 ml of a 500 I.U. ampoule)	–
2	250 I.U. (0.16 ml of a 500 I.U. ampoule)	50 I.U. (0.03 ml of a 500 I.U. ampoule)
3	250 I.U. (0.16 ml of a 500 I.U. ampoule)	500 I.U. (0.33 ml of a 500 I.U. ampoule)

Males and females were kept separately until after injection on day 3 when they were placed together in a breeding tank. The tank was fitted with a raised mesh floor to protect the eggs, and placed in a dark breeding room at 30°C.

5.2.2 Treatment of eggs and young hatchlings

After spawning, frogs were removed from the tank and the water with eggs oxygenated. Eggs either adhere to the false floor or the bottom of the tank and start hatching after three days. At this stage the false floor and unfertilised eggs were removed from the tank. The young tadpoles adhered to the sides of the tank until day 5 when they started swimming and feeding.

5.2.3 Experiment 1: Effect of water volume on tadpole development

One hundred four-day old tadpoles were counted and removed from the breeding tank and divided into six separate holding tanks at different densities (Table 5.2). Small tadpoles were transferred with a glass pipette and larger tadpoles with a small aquarium net. Only dechlorinated tap water was used and aerated throughout the course of the experiment. Tanks were cleaned and water replaced twice weekly.

Table 5.2 Tadpole densities in the development study.

Water volume (*l*)	No of tadpoles	Tadpole density
1	32	32//
1	16	16//
2	16	8//
3	12	4//
6	12	2//
12	12	1//

Tadpoles were fed daily on powdered alfalfa just enough not to foul the water. Ten grams alfalfa powder was added to 500ml water, shaken well and left to stand for five minutes to allow larger particles to settle. The top 300ml was drawn off for feeding and the residue discarded.

Development was monitored every week. Ten tadpoles from each tank were placed in a Petri-dish on top of a calibrated graph paper (1mm units). The length from the head to the tip of the tail was read from the paper. Following the measurement tadpoles were staged using the Gosner Stage (Gosner 1960) and a dissection microscope.

5.2.4 Experiment 2: Influence of rearing conditions on tadpole development

Two vinyl swimming pools (2.20m diameter x 0.40m) and two square concrete troughs (3.96 x 0.80 x 0.30m) were used in this experiment (Fig. 5.1). The concrete troughs are sections of a larger system of six concreted troughs (20.00 x 0.80 x 0.30m). Partitions were made from 8mm iron welding rod bent for the frame and covered with 80% shade netting. Enclosures were filled with tap water to the 0.25m level. Each enclosure therefore had a volume of 950*l*. The pools and troughs were covered with hail netting to keep predators out. Water was catalysed with sheep manure and allowed to mature for two weeks.

Both Pools 1 and 2 and Trough 1 were stocked with 950, 4-day old tadpoles while Trough 2 received 1 900 tadpoles. Approximately 2 000 tadpoles were released in another part of the open trough system (approximately 7 800*l*) as a control. The diet of tadpoles from Pool 2 was supplemented with powdered "Sinking Frog Food" (Disa Exporters) prepared the same way as powdered alfalfa. Tadpoles from the remaining three enclosures received no additional food. Enclosures were never cleaned in order to reduce disturbances to a minimum. Loss of water due to evaporation was replaced on a weekly basis.

Ten tadpoles from each enclosure, sampled at random, were staged once a week using the same method as in Experiment 1. Water temperature of the pools and troughs was taken at 12:00 every time tadpoles were staged.

Figure 5.1

Photographs showing types of enclosures in which tadpoles were raised in development studies.

a) Vinyl swimming pools (2.20m diameter x 0.40m)

b) Concrete trough (3.96 x 0.80 x 0.30m)


a


b

5.2.5 Experiment 3: Feeding trials on sub-adult frogs

Feeding trials were conducted in five separate concrete ponds using five different kinds of food (Fig. 5.2a). Each pond was filled to a depth of 0.25m (2.53m^3) with tap water. Thirty, one-week post-metamorphic frogs, obtained from the open trough system mentioned in Experiment 2 were placed in each dam. Dams were covered with plastic hail netting. Evaporated water was regularly replaced and ponds were cleaned once a month.

The five food types tested on the young frogs were: chopped beef liver, pet's mince (i.e. minced meat sold for consumption by pets), commercial puppy pellets, frog pellets and insects. Insects were lured to a 100W light bulb mounted above the dam (Fig. 5.2b). A Perspex plate was fitted around the bulb and a hole cut into the hail netting in the area directly beneath the light. The light was connected to a time switch that was set to go on from 20:00 till 5:00. Frogs from the other four dams were fed twice a week with their respective foods. Portions were equivalent to 5% of the frogs' body weight.

Once a month ten frogs from each dam were collected at random and the following measurements taken: snout-to-vent-length (SVL) and head-width using vernier callipers, body weight using a SAUTER RC 2013 electronic balance (0.001 accuracy).

Figure 5.2

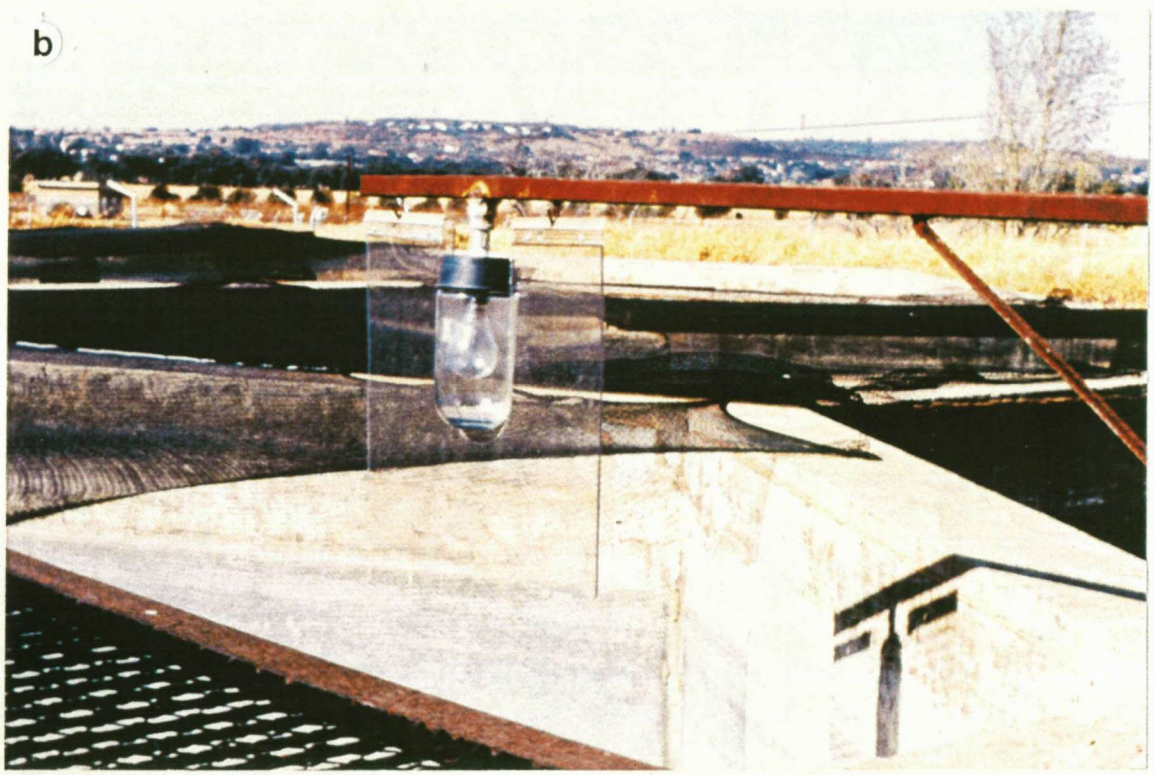
Photographs showing:

a) Concrete dams in which feeding trials were conducted.

b) Light with Perspex plate mounted above one of the ponds to lure and kill insects.

a

b



5.3 RESULTS

5.3.1 Effect of water volume on tadpole development

Tadpoles were kept under experimental conditions and development monitored for 20 weeks. By this stage some of the tadpoles from two density tanks, 1 tadpole// and 2 tadpoles// had completed metamorphosis. Tadpoles from the six populations developed at different rates (Table 5.3). The majority of tadpoles from the same population were at the same developmental stage at any given time and the standard deviations of the Gosner stages were generally small.

Tadpoles at the lowest density (1 tadpole//) developed the fastest. Tadpoles from the high-density tanks, 4, 8, 16 and 32 tadpoles// stopped developing before metamorphosis was completed. Retardation of development sets in from stage 29 through 35 in tadpoles at densities higher than 2 tadpoles// (Fig. 5.3a). Tadpoles from the 4// batch reached stage 35 after 12 weeks and remained at this stage up until week 20. Similarly tadpoles from the 8, 16 and 32 tadpoles// tanks developed up to stage 34 after 11 weeks, 33 after 8 weeks and 31 after 16 weeks respectively when development was ceased.

A Gosner stage-total length graph (Fig. 5.3b) was compiled using the final size (Table 5.4) reached by tadpoles at any particular stage as tadpoles often remained at a developmental stage for more than one week during which time an increase in total length was witnessed. There is almost no difference between the sizes of tadpoles at the same developmental stage from different densities except for stages at which retardation occurred. Individuals from the 4 tadpoles// tank increased in size while at their terminal stage for 7 weeks before it too was arrested. Eight, 16 and 32// tadpoles increased in size at their last developmental stage for respectively 8, 10 and 3 weeks before growth was arrested. This meant that tadpoles at their final stage reached greater lengths than tadpoles of a lower density at the same stage. Tadpoles from the 1// tank first started completing metamorphosis at the start of week 17 and during week 18 the last tadpoles completed metamorphosis. The mean snout-to-vent length of the young frogs was 26.7mm. By week 20 all the tadpoles from the 2 tadpole// tank had completed metamorphosis to produce slightly smaller post-metamorphic frogs of 24.8mm mean snout-vent-length.

Table 5.3 Mean values and standard deviations in paraphysis of the Gosner stages of tadpoles raised at different densities.

Tadpole density	Week										
	0	1	2	3	4	5	6	7	8	9	10
1/l	23.1 (0.1)	27.2 (0.4)	29.2 (0.6)	32.0 (0.0)	34.0 (0.0)	35.0 (0.0)	36.0 (0.0)	36.4 (0.6)	36.4 (0.8)	37.3 (2.1)	37.8 (1.1)
2/l	23.0 (0.0)	23.7 (0.4)	27.2 (0.7)	29.8 (0.8)	33.0 (0.0)	33.9 (1.1)	34.8 (0.8)	35.1 (1.2)	35.4 (1.2)	35.4 (1.3)	36.0 (1.8)
4/l	23.2 (0.2)	24.1 (0.3)	27.5 (0.9)	29.1 (0.5)	31.9 (0.6)	32.6 (1.1)	34.5 (0.6)	34.4 (0.8)	34.5 (1.1)	34.4 (0.9)	34.6 (4.0)
8/l	23.0 (0.0)	23.9 (0.8)	28.9 (3.8)	28.9 (0.7)	30.9 (1.7)	31.9 (1.7)	32.6 (2.1)	33.0 (2.3)	33.1 (2.1)	33.0 (2.1)	33.4 (0.2)
16/l	23.1 (0.1)	23.3 (0.4)	26.6 (0.4)	28.3 (0.6)	29.8 (0.0)	31.1 (0.8)	32.6 (0.4)	32.3 (1.1)	33.6 (1.1)	33.3 (2.1)	33.2 (0.8)
32/l	23.0 (0.0)	23.1 (0.3)	25.5 (0.6)	27.1 (0.8)	28.3 (0.9)	28.9 (1.9)	29.1 (1.9)	28.9 (4.9)	29.4 (0.4)	30.4 (1.8)	29.6 (1.2)

Tadpole density	Week									
	11	12	13	14	15	16	17	18	19	20
1/l	38.0 (0.0)	40.2 (0.0)	40.4 (0.9)	41.9 (2.5)	42.7 (3.0)	43.6 (3.5)	45.1 (3.5)	45.2 (3.5)	45.4 (3.4)	45.6 (3.5)
2/l	36.3 (1.5)	36.9 (1.0)	37.2 (1.3)	37.8 (1.6)	39.5 (2.8)	40.1 (2.8)	42.2 (3.3)	43.6 (4.8)	45.3 (2.6)	45.4 (3.3)
4/l	34.4 (1.1)	35.5 (1.2)	35.3 (0.8)	35.3 (1.2)	35.3 (1.2)	35.5 (1.2)	35.5 (1.3)	35.6 (1.2)	35.6 (0.7)	35.6 (1.1)
8/l	34.2 (1.7)	34.6 (1.7)	34.6 (1.5)	34.7 (2.3)	34.7 (2.1)	34.8 (0.9)	34.7 (1.4)	34.8 (0.9)	34.7 (1.1)	34.8 (1.2)
16/l	33.4 (1.7)	33.3 (1.4)	33.2 (1.6)	33.3 (1.9)	33.4 (1.6)	33.4 (1.3)	33.4 (1.5)	33.4 (1.3)	33.5 (1.0)	33.4 (1.1)
32/l	30.9 (6.8)	29.8 (1.2)	30.5 (1.9)	30.0 (2.0)	30.9 (2.2)	31.0 (1.5)	31.2 (1.5)	31.4 (1.5)	31.2 (1.7)	31.3 (1.2)

Table 5.4 Mean values and standard deviations in paraphysis of the total lengths of tadpoles raised at different densities.

Tadpole density	Week										
	0	1	2	3	4	5	6	7	8	9	10
1/l	11.2 (0.2)	19.6 (0.6)	23.1 (0.3)	28.8 (0.4)	32.7 (0.8)	36.7 (1.1)	39.1 (3.5)	40.3 (2.2)	40.2 (4.3)	42.2 (2.6)	43.8 (2.3)
2/l	11.2 (0.1)	12.9 (0.3)	16.4 (1.2)	22.8 (1.5)	25.4 (1.7)	32.1 (3.2)	34.2 (3.1)	36.0 (3.6)	37.1 (3.8)	39.2 (1.3)	40.0 (0.7)
4/l	11.2 (0.4)	13.3 (0.4)	16.4 (0.7)	21.7 (1.6)	26.1 (2.7)	30.5 (3.4)	33.4 (3.4)	33.1 (1.8)	34.2 (4.5)	35.2 (4.7)	35.2 (2.1)
8/l	11.2 (0.2)	12.7 (1.1)	17.5 (1.4)	21.6 (2.1)	24.0 (2.4)	28.9 (0.1)	27.2 (3.2)	29.2 (4.9)	30.6 (3.0)	28.6 (1.6)	30.2 (2.3)
16/l	11.2 (0.3)	12.4 (0.9)	16.4 (0.9)	20.6 (1.4)	23.6 (1.6)	27.7 (4.1)	27.5 (1.7)	29.3 (1.9)	30.6 (2.4)	32.0 (3.0)	32.1 (1.8)
32/l	11.2 (0.1)	12.4 (0.8)	13.9 (1.4)	17.7 (1.8)	20.3 (1.4)	26.4 (4.7)	22.7 (2.4)	23.0 (1.6)	23.5 (0.8)	25.7 (2.6)	25.0 (1.3)

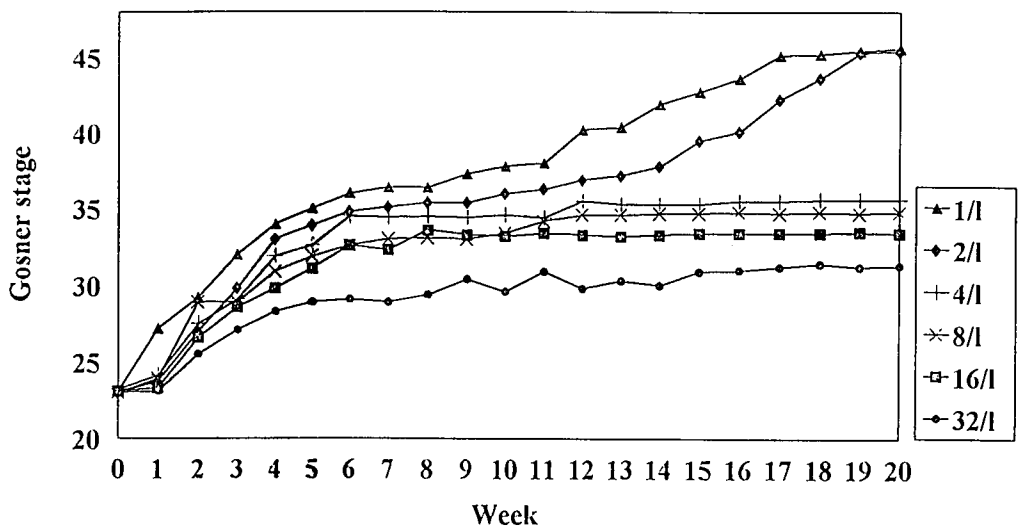
Tadpole density	Week									
	11	12	13	14	15	16	17	18	19	20
1/l	44.2 (1.9)	44.6 (1.6)	45.9 (1.8)	46.7 (3.2)	47.2 (3.3)	43.0 (3.4)	31.4 (3.0)	26.7 (2.4)	27.3 (4.2)	28.0 (2.8)
2/l	41.5 (2.4)	42.1 (3.2)	42.4 (3.3)	43.2 (4.3)	43.4 (3.0)	44.0 (4.6)	45.8 (3.2)	45.0 (2.8)	44.6 (3.2)	41.8 (4.5)
4/l	35.4 (4.4)	37.8 (4.6)	38.7 (4.4)	38.8 (4.8)	39.2 (5.0)	39.0 (4.9)	40.0 (5.3)	41.1 (2.7)	41.0 (3.1)	41.1 (4.0)
8/l	32.3 (3.3)	33.3 (1.0)	34.0 (1.3)	34.3 (2.0)	35.0 (1.4)	34.6 (4.3)	34.4 (4.8)	35.0 (3.7)	34.9 (1.1)	35.0 (3.7)
16/l	32.3 (3.3)	31.7 (3.3)	27.0 (3.5)	31.6 (3.8)	32.1 (3.7)	32.6 (3.7)	32.5 (3.0)	32.5 (3.7)	32.6 (3.3)	32.8 (2.2)
32/l	24.8 (2.8)	25.1 (2.2)	27.0 (5.4)	28.5 (2.3)	29.2 (2.0)	29.2 (1.1)	30.8 (2.0)	31.2 (1.7)	31.2 (1.5)	31.1 (1.5)

Figure 5.3

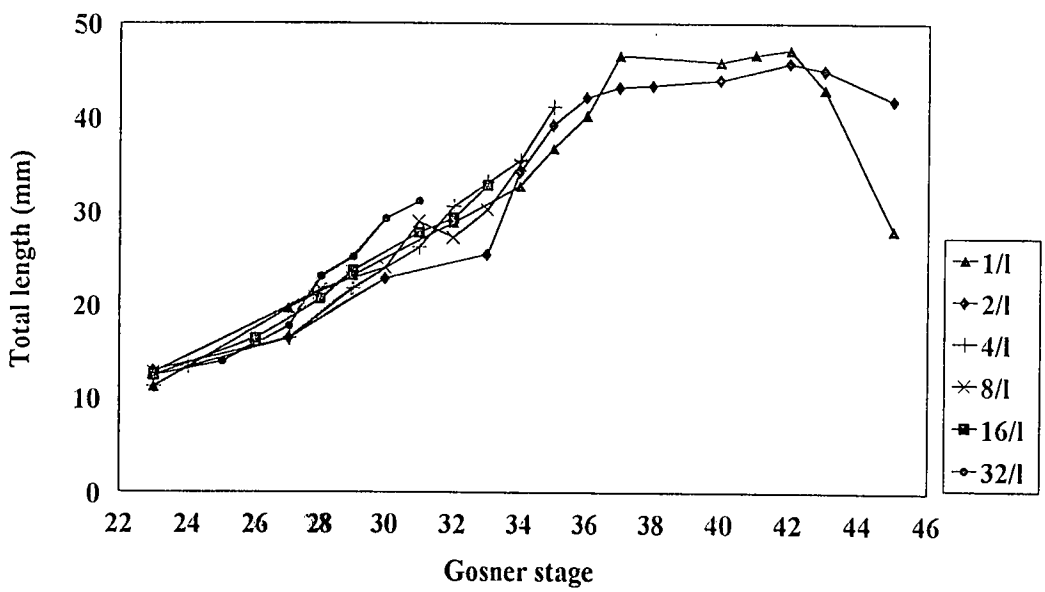
a) Line graph of the mean values of the Gosner stages of tadpoles raised at six densities indicating developmental rates.

b) Stage-length graph indicating the relation of mean total length of tadpoles raised at the six densities, using the final size reached at every stage.

a)


Gosner stage
45
40
35
30
25
20
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Week
1/l
2/l
4/l
8/l
16/l
32/l

b)


Total length (mm)
50
40
30
20
10
0
22 24 26 28 30 32 34 36 38 40 42 44 46
Gosner stage
1/l
2/l
4/l
8/l
16/l
32/l

5.3.2 Influence of rearing conditions on tadpole development

Tadpoles from all the enclosures successfully made it through metamorphosis. The last group (Trough 2) completed metamorphosis after 16 weeks (Table 5.5). Developmental rate up until the first time tadpoles completed metamorphosis differs for each enclosure. Tadpoles from the open trough system developed the most rapidly and were the first to complete metamorphosis, which occurred after 11 weeks.

A line graph of the Gosner stages against time (Fig. 5.4) illustrates that the only real difference between the populations was the time it took to complete metamorphosis. Temperature in the pools was approximately 2°C higher than in the troughs and ranged between 22.5 and 29.0°C measured at 12:00. Tadpoles from Trough 1 took one week longer (15 weeks) to complete metamorphosis than those from Pool 1 even though both batches were raised at a density of 1 tadpole/*l*. Metamorphosis was reached fastest for the tadpoles from Pool 2 (13 weeks) which received additional food. Tadpoles from Trough 2 raised at a density of 2 tadpoles/*l* developed the slowest, taking 16 weeks to complete metamorphosis.

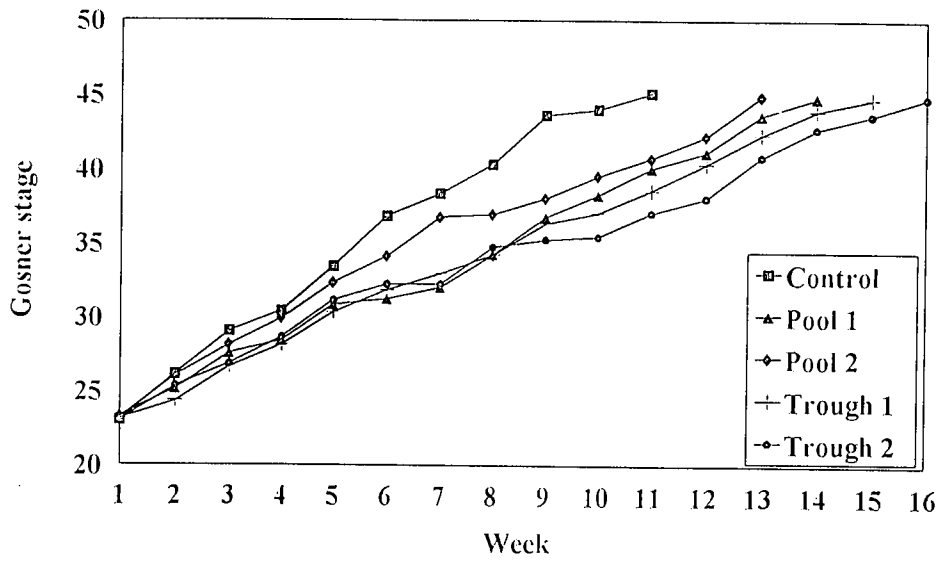
Table 5.5 Mean values and standard deviations in paraphysis of the Gosner stages of tadpoles reared under different holdin conditions. The point when 50% of a sample contained tadpoles that completed metamorphosis is indicated with an asterix each population.

Enclosure	Week							
	1	2	3	4	5	6	7	8
Control	23.0 (0.0)	26.1 (1.1)	29.0 (0.4)	30.4 (0.5)	33.4 (0.3)	36.8 (1.4)	38.3 (2.3)	40.3 (1.1)
Pool 1	23.3 (0.3)	25.1 (0.4)	27.5 (1.3)	28.4 (1.3)	30.8 (0.4)	31.2 (0.7)	32.0 (1.7)	34.2 (0.9)
Pool 2	23.2 (0.2)	26.0 (0.8)	28.1 (0.6)	29.9 (0.8)	32.3 (0.1)	34.1 (2.1)	36.7 (0.4)	36.9 (1.7)
Trough 1	23.1 (0.1)	24.3 (0.3)	26.6 (0.7)	28.1 (0.6)	30.3 (0.3)	31.8 (1.3)	32.9 (1.1)	34.2 (1.5)
Trough 2	23.0 (0.0)	25.3 (0.2)	26.8 (0.2)	28.6 (0.4)	31.1 (1.2)	32.2 (0.9)	33.2 (0.8)	34.7 (1.0)

Enclosure	Week							
	9	10	11	12	13	14	15	16
Control	43.6 (1.3)	44.0 (1.6)	*45.1 (0.9)	45.2 (1.1)	45.3 (1.3)	45.5 (0.7)	45.8 (0.9)	45.8 (1.4)
Pool 1	36.7 (2.4)	38.2 (1.2)	40.0 (1.0)	41.1 (1.1)	43.6 (1.4)	*44.8 (0.8)	44.9 (1.5)	45.3 (1.6)
Pool 2	38.0 (0.8)	39.5 (0.8)	40.7 (2.1)	42.4 (1.4)	*44.9 (0.7)	45.0 (0.6)	45.1 (0.9)	45.2 (1.1)
Trough 1	36.3 (0.4)	37.0 (2.0)	38.5 (0.8)	40.3 (2.2)	42.3 (2.0)	43.9 (0.7)	*45.7 (1.1)	44.9 (1.8)
Trough 2	35.2 (1.6)	35.4 (1.3)	37.0 (0.4)	38.0 (0.9)	40.8 (0.6)	42.7 (1.2)	43.1 (0.5)	*34.8 (1.2)

Figure 5.4

Line graph showing development of tadpoles raised in different enclosure types and under different conditions. Each line terminates when 50% of tadpoles completed metamorphosis.


Gosner stage
50
45
40
35
30
25
20
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Week
Control
Pool 1
Pool 2
Trough 1
Trough 2

5.3.3 Feeding of sub-adult frogs

The mean values and standard deviations of the SVL and body weight of the frogs after five months of feeding are found in Table 5.6 and Table 5.7. Growth curves obtained from SVL and body weight follow a similar pattern (Fig. 5.5). Frogs that received only insects from the light trap showed very poor growth with the first real marked increase in body weight occurring after four months. Apart from the poor value of insects as fed in this study, no difference was apparent in the value of other foods. Apparently better results were obtained with frog pellets over liver followed by pet mince, dog food and lastly insects.

Table 5.6 Mean values and standard deviations in paraphysis of snout-vent-lengths of *Xenopus laevis* fed on different foods.

Food type	Week											
	0		1		2		3		4		5	
Insects	27.7	(2.1)	29.3	(3.2)	31.3	(2.5)	32.0	(1.5)	33.3	(2.5)	33.5	(1.3)
Dog food	29.8	(1.0)	30.0	(2.1)	31.1	(1.7)	33.4	(2.4)	34.5	(1.6)	34.9	(2.6)
Pet's mince	29.2	(0.9)	31.7	(2.5)	34.9	(1.6)	35.1	(2.3)	36.3	(2.4)	37.4	(2.3)
Liver	29.1	(1.8)	29.2	(1.6)	32.9	(1.4)	34.2	(3.4)	36.0	(3.1)	38.1	(1.4)
Frog pellets	29.5	(1.1)	31.3	(2.4)	33.3	(0.8)	35.6	(1.3)	37.6	(1.8)	39.8	(1.7)

Table 5.7 Mean values and standard deviation in paraphysis of the body weight of *Xenopus laevis* fed on different foods.

Food type	Week											
	0		1		2		3		4		5	
Insects	2.19	(0.2)	3.21	(0.6)	4.29	(0.4)	4.41	(0.6)	5.12	(0.3)	5.36	(0.6)
Dog food	2.56	(0.8)	3.38	(1.0)	4.60	(0.1)	5.12	(0.5)	5.72	(0.4)	6.43	(0.1)
Pet's mince	2.54	(0.3)	4.00	(1.1)	5.29	(0.3)	6.58	(0.9)	7.05	(1.0)	7.77	(0.5)
Liver	2.56	(0.7)	4.16	(0.5)	5.40	(0.7)	6.60	(0.3)	7.53	(0.8)	8.81	(0.9)
Frog pellets	2.19	(0.4)	3428	(0.9)	5.61	(0.7)	7.13	(0.7)	8.81	(1.2)	9.72	(0.2)

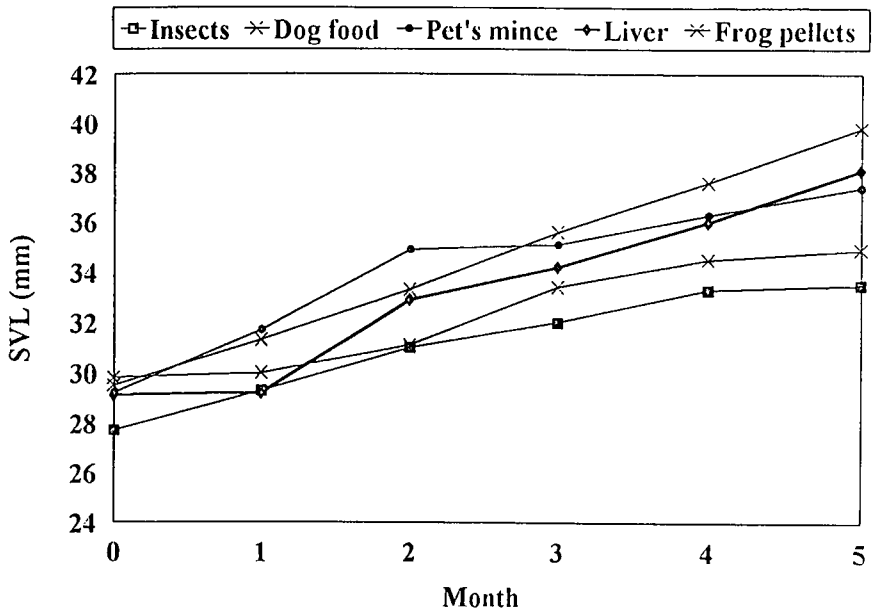
Figure 5.5

Growth rates of post-metamorphic frogs fed on insects, dog food, pet's mince, liver and frog pellets presented by:

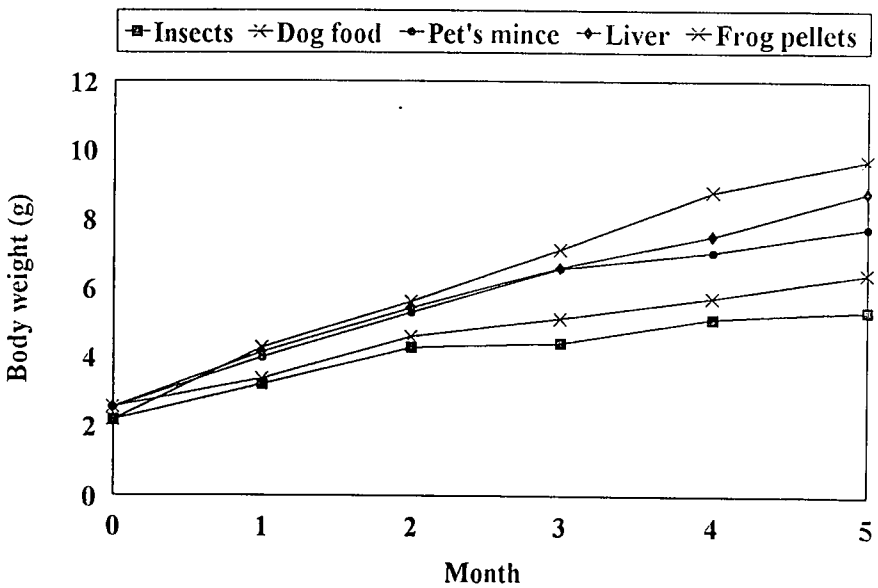
a) Snout-to-vent-length (SVL)

b) Body weight

a)


Insects
Dog food
Pet's mince
Liver
Frog pellets
SVL (mm)
42
40
38
36
34
32
30
28
26
24
0
1
2
3
4
5
Month

b)


Insects
Dog food
Pet's mince
Liver
Frog pellets
Body weight (g)
12
10
8
6
4
2
0
0
1
2
3
4
5
Month

5.4 DISCUSSION

5.4.1 The effect of population density on tadpole development

It has been established for a number of *Rana* species that high density or the crowding of tadpoles leads to the retardation in growth of these tadpoles (Richards 1958, 1962, Rose & Rose 1961, Akin 1966, John & Fenster 1975). The same phenomenon of the inhibition of growth has been found to occur in *X. laevis* (Hurwitz 1979). The present study supports this phenomenon.

Development of tadpoles is progressively slower at increasingly higher densities. As tadpoles failed to develop passed stage 35 at a density of 4 tadpoles// (Table 5.3), this has to be below the maximum acceptable density for raising *X. laevis* tadpoles. Though it is possible to raise tadpoles through metamorphoses at a density of 2 tadpoles// it is more time effective to maintain them at a lower density as a lag in developmental stages develops which becomes more significant at higher densities and as development progresses.

The retardation of development is only followed by the retardation of growth in body length after a few weeks have passed. During this time tadpoles grow to a length greater than the maximum length reached by tadpoles that advance beyond that particular developmental stage. Up until stage 35 crowding does not affect the size of the tadpole from the 2 tadpole// population. From this stage on tadpoles from the 1 tadpole// population reach larger sizes than those do from the 2 tadpole// population for each Gosner stage. Sizes of metamorphosing tadpoles are important, as it determines the size of newly post-metamorphosed frogs.

It seems that while growth and development proceed at the same time, the latter may also be dependent on the former as Adolph (1931) found that *Rana* tadpoles had to attain a minimum size before undergoing metamorphosis. Experimental evidence by Hurwitz (1979) indicated that *X. laevis* tadpoles are able to retard the development of other *X. laevis* tadpoles that are at an earlier stage of development.

Development continues normally until stage 29 is reached when retardation starts to occur. At Nieuwkoop and Faber stage 50 (which corresponds with Gosner stage 28) the tectal and tegmental parts of the brain start functioning separately (Nieuwkoop & Faber 1967). The tectum becomes responsible for the co-ordination, vision and perception of the tadpole. Hurwitz (1979) argues that the tadpole may then become visually aware of its neighbours, which may be a cause of the retardation of growth. Colloid formation also begins in the thyroid follicles at Nieuwkoop and Faber stage 50 (Nieuwkoop & Faber 1967). Secretion of thyroid hormones has to be controlled at low levels to ensure the release of the growth promoting substance, somatotrophin (STH) as an increase in thyroid hormones slow down or even cease growth (Steinmetz 1952, 1954).

5.4.2 The influence of rearing condition on rate of tadpole development

The crowding effect on tadpole development becomes evident once again in tadpoles from the concrete troughs. Conditions in the troughs were exactly the same except Trough 2 was stocked with twice as many tadpoles. As a probable result a lag of one week appeared in the development of the tadpoles in Trough 2.

Tadpoles of *X. laevis* can be taken through metamorphosis one week earlier if raised in a vinyl enclosure rather than one of concrete as tadpoles from Pool 1 took 14 weeks and those from Trough 1, 15 weeks to complete metamorphosis. It can therefore be deduced that a vinyl enclosure favours the development of tadpoles as opposed to an enclosure of concrete. It is possible that the slightly higher temperature attained in the pools favoured the more rapid development of the tadpoles.

Temperature is one of the factors that influence the rate of development of eggs and tadpoles. The most rapid development from egg laying to metamorphosis of the large frogs found in the former Transvaal Highveld, namely 31-33 days, is achieved by the Giant Bullfrog *Pyxicephalus adspersus* (Balinsky 1969). Many of the breeding habits of *P. adspersus* are directed at utilising high temperatures, such as spawning at the height of summer and spawning in shallow water which becomes very warm (Balinsky 1969). It can therefore be said that together with intrinsic high growth rate, high temperatures, within an optimal range, aid the rapid development of the tadpoles. The water temperature of the

enclosures measured at midday of 22.5-29.0°C fall mostly within the range of temperature found by Balinsky (1969) to result in good development of *X. laevis* embryos, namely 25.5-30.0°C. It must however be realised that the temperature of small bodies of water varies considerably during day and night and that the maximum temperature is often prevalent for a few hours at the most.

The dominant aquatic flora in the concrete troughs were periphytic algae, while planktonic algae dominated in the vinyl pools. Since *X. laevis* tadpoles are obligate suspensions feeders, less food is available to them in the troughs. Periphytic algae do not seem to be able to grow on the plastic surface allowing phytoplankton to bloom. The higher suspended food content in the plastic pools might also be responsible for the more rapid growth of tadpoles in this enclosure.

Even though it is possible to rear tadpoles from the early embryo through metamorphoses in a naturally "organically matured" enclosure, development is more rapid if the tadpole diet is supplemented with some external food source. This can be seen in tadpoles from Pool 2, which metamorphosed one week earlier than those from Pool 1 after receiving additional powdered frog food. Adolf (1931) and Richards (1958) explained that the amount of food available to tadpoles was a causative factor of the retardation of growth. Hurwitz (1979) claims that while food may be a factor in preventing the growth and development of tadpoles below a certain threshold of feeding, a distinctly separate factor appears to be responsible for the inhibition of growth and development in the crowded situation.

5.4.3 Feeding of sub-adult frogs

Choosing the correct food on which to raise *X. laevis* is vital for obtaining the highest biomass over the shortest time. Traditionally beef heart or liver was used as the standard diet for *Xenopus* held in captivity. Liver is however expensive and often difficult to attain especially if a large colony of a thousand frogs or more is maintained. Dog pellets, pet's mince and the use of insects offer much cheaper alternatives, but may compromise rapid growth of frogs. However, there was no apparent difference in the value of the prepared foods.

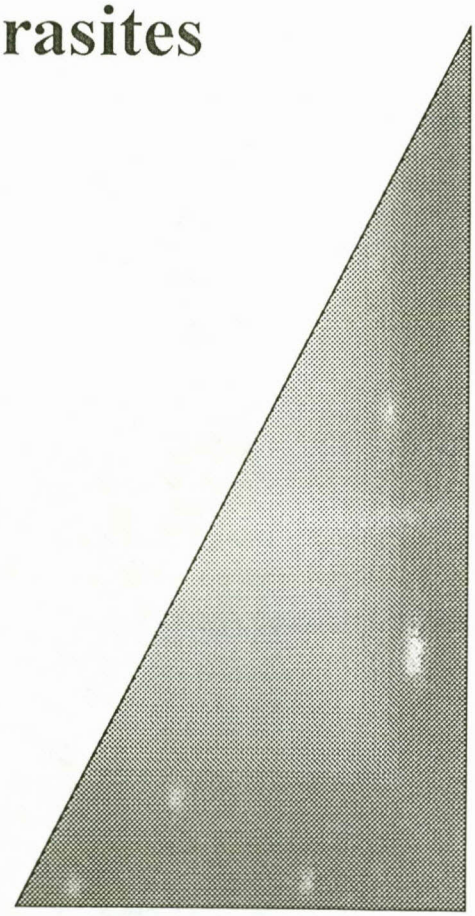
Making use of flying insects should not be considered as an option for raising *X. laevis* for very poor growth resulted from it. Elepfandt (1996) explains that *X. laevis* may detect prey, including insects, by waves caused in the surface. Another mechanism of terrestrial prey capture is described by Measey (1998b) whereby *X. laevis* visually detects its prey and captures it outside and just above the water surface. Either the light trap did not work as efficiently or the frogs did not often take insects that had fallen in the water. Another possible explanation for the poor growth might be because of the time of the year the experiment was conducted. The last four months extended into autumn when generally fewer insects are expected. Even if this were the reason, feeding by insect light trap remains a poor method as one can not have good growth when insects are abundant, followed by poor growth when insect numbers decline during the colder months.

Despite the dog pellets having a high nutritional value it too resulted in unsatisfactory growth. The pellets do not have a strong odour and since the main method of food detection in *Xenopus* is through olfaction, pellets often disintegrated before being detected by the frogs. After liver, frogs attained a slightly higher biomass from pet's mince. Pet's mince was easily detected and ingested by the frogs.

A survey conducted by Major & Wassersug (1998) on the maintenance of *Xenopus* at research facilities around the world indicated that 74% of facilities used some type of dry commercial fish or frog food as the only food source, 17% used organ meat in the form of beef liver or heart and the remainder of the facilities (9%) varied the diet by giving the animals both dry commercial food and organ meat. Because no significant difference in the current study was attained between feeding with pet's mince, liver and frog pellets, any one of these three foods can be used for the rearing of *X. laevis*.

Chapter 6

Infection levels, parasite survival and elimination of parasites



CONTENTS

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INFECTION LEVELS, PARASITE SURVIVAL AND ELIMINATION OF PARASITES

6.1 INTRODUCTION

Despite reports of amphibian disease going back to the first decade of this century, knowledge of amphibian pathology and medicine has not kept pace with that of other animal groups. The existence of this situation, Crawshaw (1992) attributes to the fact that it has usually been easier and cheaper to obtain new animals than investigating or treating medical problems.

The most commonly reported disease in frog culture is Red Leg, of which *Aeromonas hydrophila* is a major but not an exclusive pathogen. The disease has been studied in great detail and preventative measures as well as treatment have been suggested by many that encountered the pathogen (Koivastik 1950, Nace 1968 and Ruddock & Ruffle 1972).

Amphibians, particularly wild-caught ones are host to a bewildering array of metazoan and protozoan parasites, but for the most part, clinical disease attributable to parasitism appears rare (Crawshaw 1992). Collectively the species of *Xenopus* carry a parasite assemblage of over 25 genera representing seven invertebrate groups (Tinsley 1996a). Various aspects of the parasites of *Xenopus laevis* including diversity, life cycle, evolution and disease have been well-studied (Southwell & Kirshner 1937, Elkan & Murray 1952, Pritchard 1964, Thurston 1970, Macnae, Rock & Makowski 1973, Tinsley & Whitear 1980, Tinsley 1996a & 1996b).

Disease and parasites become a problem in frog breeding when they are confined in high concentration, under-fed and are kept in unhygienic conditions (Hey 1949, Negroni 1996). If present in high numbers, parasites could lead to drop in condition and seriously reduce growth rates of the animals.

Given the possible threat parasites pose to frog culture it was firstly decided to monitor parasite infection in *X. laevis* while the host was kept in captivity for an extended period. Secondly, the elimination of parasites of *X. laevis* was attempted through treatment of the host with different drugs.

6.2 MATERIAL AND METHODS

6.2.1 Testing the effect of captivity on parasite infection

Frogs collected at the Duraan farm and De Dam (Chapter 2, pp. 11) were subjected to captive conditions to determine whether parasite infection levels were influenced by prolonged host captivity. Directly after collection 170 frogs were placed in a metal holding tank in 170*l* dechlorinated tap water. Ten frogs were immediately dissected to establish the natural parasite diversity and infection levels. At the end of each month 10 more frogs were removed and dissected to monitor parasite infection. The tank was cleaned at the same time and filled with 10*l* less water than for the previous month. This procedure was repeated for 11 months.

6.2.1.1 Dissection procedure

Frogs were anaesthetised in benzocaine solution for 10 to 15 minutes prior to dissection. First the external surface of the frogs was scanned for any parasites. Next the body cavity was exposed, and together with the surface of the organs, examined for parasites. The following organs were dissected from the animals and placed in a petri dish containing amphibian saline for further dissection: gall bladder, heart with surrounding pericardium, urinary bladder and alimentary canal consisting of the oesophagus, stomach, duodenum and rectum. Lastly the buccal cavity, bile ducts, nostrils and Eustachian ducts were examined. All dissections were carried out with the aid of a ZEISS dissection microscope.

6.2.2 Testing the effect of drugs on parasite infection

Frogs from the Duraan and Nuwe Orde farms were used to find a treatment for the elimination of the parasites of *X. laevis*. Ten frogs were initially dissected as a control group using the same procedure as described above.

6.2.2.1 Treatment of frogs

Three commercial brands of anthelminthics for sheep remedy, Ivomec (product of Logos Agvet), Levisol (product of Milborrow) and Cestocur (product of Bayer) were individually tested for their effectiveness in treating the parasites. A combination of Ivomec and Cestocur was also tested.

Drugs were administered using three different techniques:

- **Direct application to the stomach** - a rubber canula was inserted through the mouth and oesophagus to the stomach and the drug injected.
- **Subcutaneous injection into dorsal lymph sac** - the needle was inserted subcutaneously on the thigh of the frog 10mm from the lateral line, gently pushed under the skin and through the membrane of the dorsal lymph sac where the drug was injected.
- **Dipping** - the frog was exposed to the drug solution for 24 hours.

Combinations of the drugs, doses and techniques were administered to the frogs (Table 6.1.). Each treatment was first administered to a single frog, which was observed for a further 24 hours, to test whether it was safe to use on the host. If lethal to the host, the dose was reduced and if apparently safe, the treatment was administered to 10 frogs simultaneously. A dilution series was prepared for each drug and tested on the frogs to determine the optimum doses. After treatment, frogs were kept for a week to allow the drugs to work and fed every second day to aid in the elimination of dead parasites from the alimentary canal and urinary bladder. The frogs were then sacrificed and a full parasite count made.

Table 6.1 The drugs and techniques used for attempting the killing of parasites of *Xenopus laevis*.

DRUG	ACTIVE AGENT	TECHNIQUE		
		Stomach	Inject	Dip
Ivomec	Ivermectin		*	*
Levisol	Levamisole hydrochloride			*
Cestocur	Praziquantel	*	*	*
Cestocur-Ivomec	Praziquantel & Ivermectin			*

6.2.2.2 Statistical analysis

Due to the non-parametric nature of the data on parasite infection, due to the small number of test animals, the Mann-Whitney U-test was used to determine the significance of differences in parasite intensity levels after drug treatment with parasite intensity levels in the control group (representing parasite intensity before treatment). The test compares the medians of two ranked samples. The test was carried out on two sets of data:

1. Combined parasite intensity levels of all the species in the control group with those of the experimental group for each of the seven treatments.
2. Parasite intensity levels of each individual species in the control and experimental groups were compared for each individual treatment.

6.2.2.3 Calculating the cost of treatment

The costs involved in the treatment of the parasites of *X. laevis* were calculated for the recommended doses of the successful treatments.

6.3 RESULTS

6.3.1 Parasite survival in captivity

The control group was dissected in March 1998 shortly after capture. Six parasites from five invertebrate groups were found to infect the frogs from the De Dam and Duraan farms (Table 6.2). Prevalence ranged between 20 and 90% for the different parasites and mean intensity was generally low (never more than 3.7) except for *Tylodelphys xenopodis* (22.3).

Table 6.2 Parasites found in the *Xenopus laevis* control group (10 individuals) from the De Dam and Duraan farms. Infection data is given for each species.

Parasite	Infection site	Prevalence (%)	Mean intensity
NEMATODA			
Camallanus kaapstaadi	Oesophagus	50	1.6
PLATHYHELMINTHES			
Cestoda			
Cephalochlamys namaquensis	Duodenum	90	3.7
Valipora campylancristrota	Bile ducts	30	2.7
Monogenea			
Protopolystoma xenopodis	Urinary bladder	70	1.3
Digenea			
Tylodelphys xenopodis	Pericardium	70	22.3
ACARI			
Xenopacarus africanus	Nostrils & Eustachian ducts	20	1.5

All six species of parasites were still prevalent at the end of the 11 months of host captivity. Prevalence and mean intensity for each species show a different pattern throughout the captive period (Fig. 6.1).

- ***Camallanus kaapstaadi***: Prevalence was 50% before captivity and was 50% for half of the months in captivity including the last month (February 1999, Fig. 6.1a). Mean intensity was never more than two except for month four and five (July and August 1998) when the averages of worms found were 10 and 11 (January and February 1999).
- ***Cephalochlamys namaquensis***: The only parasite to show a clear reduction in prevalence and mean intensity (Fig. 6.1b). Prevalence was high in the control group at the start of the experiment (90%), but dropped to 40% after four months (July 1999) in captivity. Prevalence then fluctuated between 40 and 60% for the next five months before dropping to 10% in the last month of captivity.
- ***Valipora campylancristrota***: Infection levels for each month showed huge fluctuations from zero infection for month three to a 80% prevalence for month six and a maximum mean intensity of 98 for the last month of captivity (Fig. 6.1c).
- ***Protopolystoma xenopodis***: Prevalence fluctuates between 40 and 90% (Fig.6.1d). Mean intensity was low, not more than three. Dips in infection levels were reached in months one and eight (April and November 1998), while two peaks are reached in months six and ten (September 1998 and January 1999).
- ***Tylodelphys xenopodis***: Prevalence was always high, with a maximum of 100% reported even after six months (Fig. 6.1e). There was no indication of infection levels dropping even after 11 months in captivity (70% prevalence in month nil, March 1998 and in month 11, February 1999). Mean intensity was also very high with a minimum of 20 and a maximum of 100 worms.
- ***Xenopacarus africanus***: The only parasite to have a positive correlation with host captivity time (Fig. 6.1f). Prevalence increases steadily from 20% before captivity to remain at 100% from month seven to 11. Mean intensity follows an apparently related pattern and increases from two to between 30 and 40 for months nine to 11.

Figure 6.1

Infection levels of parasites of *Xenopus laevis* during 11 months of captivity (April 1998 to February 1999). Infection levels for March 1998 represent the control group and indicate infection levels at the start of the captivity period.

a) *Camallanus kaapstaadi*

b) *Cephalochlamys namaquensis*

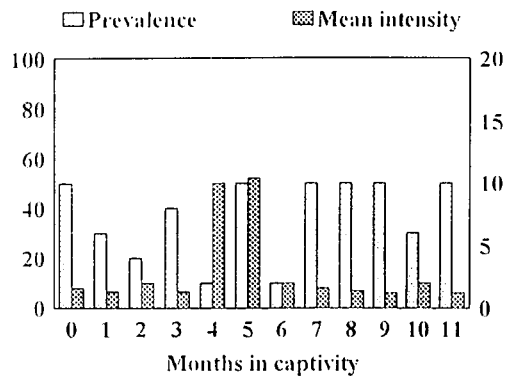
c) *Valipora campylancristrota*

d) *Protopolystoma xenopodis*

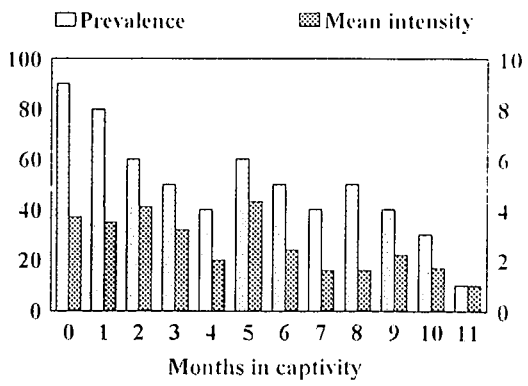
e) *Tylodelphys xenopodis*

f) *Xenopacarus africanus*

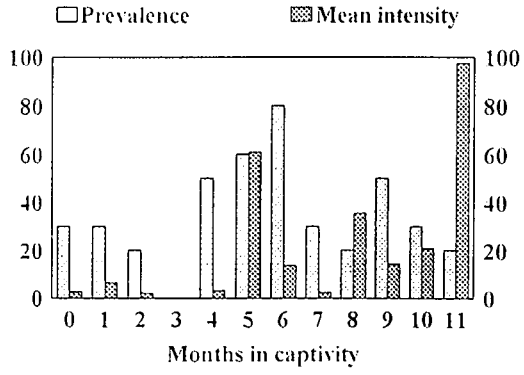
a)


Prevalence
Mean intensity
100
80
60
40
20
0
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11
Months in captivity

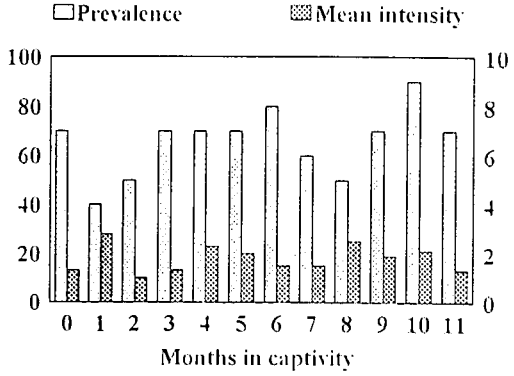
b)


Prevalence
Mean intensity
100
80
60
40
20
0
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11
Months in captivity

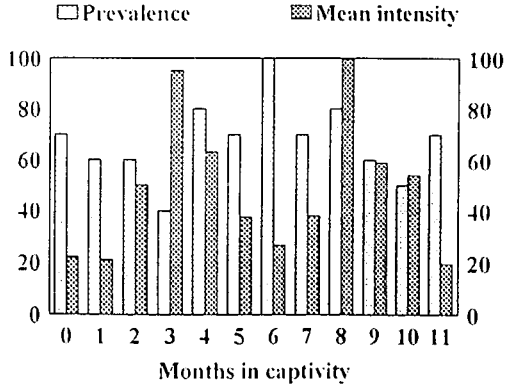
c)


Prevalence
Mean intensity
100
80
60
40
20
0
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11
Months in captivity

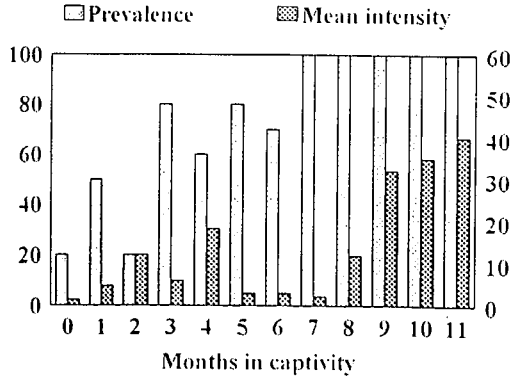
d)


Prevalence
Mean intensity
100
80
60
40
20
0
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11
Months in captivity

e)


Prevalence
Mean intensity
100
80
60
40
20
0
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11
Months in captivity

f)


Prevalence
Mean intensity
100
80
60
40
20
0
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11
Months in captivity

6.3.2 Elimination of parasites

During the current study nine parasites from six parasite groups were found to infect *X. laevis* from the Duraan and Nuwe Orde farms. The prevalence of four of the parasites, *Batrachocamallanus slomei*, *Valipora campylancristrota*, *Gyrdicotylus gallieni* and *Protopolystoma xenopodis* were between 30 and 50% (Table 6.3). Their mean intensities were low, none more than 4.5. The four parasites with high prevalence (70 to 100%), *Camallanus kaapstaadi*, *Cephalochlamys namaquensis*, *Tylodelphys xenopodis* and *Xenopacarus africanus*, varied considerably in mean intensity (2.4 to 27.3). Protozoa were found in the rectum of all the frogs, but no attempt at identification or counting was made. The protozoans were therefore excluded from Table 6.3 and all statistical analysis.

Table 6.3 Diversity and infection levels of parasites found in the control group (ten individuals) of *Xenopus laevis* from the Duraan and Nuwe Orde farms for August 1998.

Parasite	Infection site	Prevalence (%)	Mean intensity
NEMATODA			
Batrachocamallanus slomei	Stomach	30	1.3
Camallanus kaapstaadi	Oesophagus	70	2.4
PLATHYHELMINTHES			
Cestoda			
Cephalochlamys namaquensis	Duodenum	100	3.3
Valipora campylancristrota	Bile ducts	40	4.5
Monogenea			
Gyrdicotylus gallieni	Buccal cavity	40	2.0
Protopolystoma xenopodis	Urinary bladder	50	2.0
Digenea			
Tylodelphys xenopodis	Pericardium	100	27.3
ACARI			
Xenopacarus africanus	Nostrils & Eustachian ducts	80	9.9

6.3.2.1 Comparison of treatments

Direct application of drugs to the stomach was only tried with Cestocur. Each attempt at administering the drug failed as frogs kept regurgitating the liquid immediately after dosage. The only way to keep the drug inside the body was by anaesthetising the frogs before treatment. Both Cestocur and Ivomec were tested by injection into the dorsal lymph sac. The procedure was executed with little effort. Dipping of frogs was carried out in solutions of all three drugs as well as the Cestocur-Ivomec combination.

By comparing the parasite intensity levels of all the parasites for each treatment, or combination drug and technique, one can identify which treatment most significantly reduced parasite intensity levels. All seven treatments showed a significant difference in the combined intensity levels of all the parasites (Table 6.4). Very little difference exists between the smallest p-value namely the combination Cestocur-Ivomec-dip ($p = 0.001$), Cestocur-inject ($p = 0.001$) and Levisol-dip ($p = 0.001$).

Table 6.4 Mann-Whitney U-test comparison of the total parasite intensity levels for each treatment (drug and technique).

Drug	Technique	p
Cestocur	Stomach	0.021
Cestocur	Inject	0.001
Cestocur	Dip	0.003
Ivomec	Inject	0.006
Ivomec	Dip	0.020
Levisol	Dip	0.001
Cestocur-Ivomec	Dip	0.001

6.3.2.2 Effect of each treatment on each parasite

Using the Mann-Whitney U-test to compare parasite intensity levels between control and treated samples for each treatment indicated the effectiveness of each treatment on every parasite species. A significant difference existed in the parasite intensity levels for 11 of the 56 drug-technique-parasite combinations (Table 6.5).

Table 6.5 Mann-Whitney U-test of the effect of each treatment on each individual parasite species, by comparing parasite intensity in control and experimental groups.

Drug	Technique	Parasite	p
Cestocur	Stomach	*B. slomei*	0.257
Cestocur	Stomach	*C. kaapstaadi*	0.571
Cestocur	Stomach	*C. namaquensis*	0.573
Cestocur	Stomach	*I. campylancristrota*	0.879
Cestocur	Stomach	*G. gallieni*	0.545
Cestocur	Stomach	*P. xenopodis*	0.734
Cestocur	Stomach	*T. xenopodis*	0.005
Cestocur	Stomach	*X. africanus*	0.791
Cestocur	Inject	*B. slomei*	0.831
Cestocur	Inject	*C. kaapstaadi*	0.571
Cestocur	Inject	*C. namaquensis*	0.013
Cestocur	Inject	*I. campylancristrota*	0.427
Cestocur	Inject	*G. gallieni*	0.821
Cestocur	Inject	*P. xenopodis*	0.571
Cestocur	Inject	*T. xenopodis*	0.002
Cestocur	Inject	*X. africanus*	0.257
Cestocur	dip	*B. slomei*	0.427
Cestocur	dip	*C. kaapstaadi*	0.545
Cestocur	dip	*C. namaquensis*	0.036
Cestocur	dip	*I. campylancristrota*	0.650
Cestocur	dip	*G. gallieni*	0.821
Cestocur	dip	*P. xenopodis*	0.521
Cestocur	dip	*T. xenopodis*	0.002
Cestocur	dip	*X. africanus*	0.545

Drug	Technique	Parasite	p
Ivomec	Inject	*B. slomei*	0.650
Ivomec	Inject	*C. kaapstaadi*	0.049
Ivomec	Inject	*C. namaquensis*	0.597
Ivomec	Inject	*V. campylancristrota*	0.969
Ivomec	Inject	*G. gallieni*	0.385
Ivomec	Inject	*P. xenopodis*	0.821
Ivomec	Inject	*T. xenopodis*	0.001
Ivomec	Inject	*X. africanus*	0.910
Ivomec	dip	*B. slomei*	0.427
Ivomec	dip	*C. kaapstaadi*	0.131
Ivomec	dip	*C. namaquensis*	0.597
Ivomec	dip	*V. campylancristrota*	0.734
Ivomec	dip	*G. gallieni*	0.212
Ivomec	dip	*P. xenopodis*	0.496
Ivomec	dip	*T. xenopodis*	0.038
Ivomec	dip	*X. africanus*	0.326
Levisol	dip	*B. slomei*	0.650
Levisol	dip	*C. kaapstaadi*	0.096
Levisol	dip	*C. namaquensis*	0.385
Levisol	dip	*V. campylancristrota*	0.597
Levisol	dip	*G. gallieni*	0.131
Levisol	dip	*P. xenopodis*	0.212
Levisol	dip	*T. xenopodis*	0.064
Levisol	dip	*X. africanus*	0.050
Cestocur-Ivomec	dip	*B. slomei*	0.427
Cestocur-Ivomec	dip	*C. kaapstaadi*	0.174
Cestocur-Ivomec	dip	*C. namaquensis*	0.001
Cestocur-Ivomec	dip	*V. campylancristrota*	0.427
Cestocur-Ivomec	dip	*G. gallieni*	0.450
Cestocur-Ivomec	dip	*P. xenopodis*	0.791
Cestocur-Ivomec	dip	*T. xenopodis*	0.001
Cestocur-Ivomec	dip	*X. africanus*	0.257

1. **Cestocur-stomach:** The only parasite for which intensity levels were significantly reduced by Cestocur-stomach treatment, was *T. xenopodis* (p = 0.005, Table 6.5). Prevalence and mean intensity of the experimental group was much the same as the control except for *B. slomei*, which did not occur in the experimental group, and for *T. xenopodis* (Fig. 6.2). Prevalence of the latter was reduced with 50% and mean intensity with more than 80%.

2. **Cestocur-inject:** Intensity levels of two parasites, the cestode *C. namaquensis* (p = 0.013, Table 6.5) and digenean, *T. xenopodis* (p = 0.002) were significantly reduced by Cestocur-inject treatment. Numbers of half of the parasite species was reduced, including one nematode, one cestode, one digenean and one acarid, while mean intensity of six parasites was reduced (Fig. 6.3).

Figure 6.2

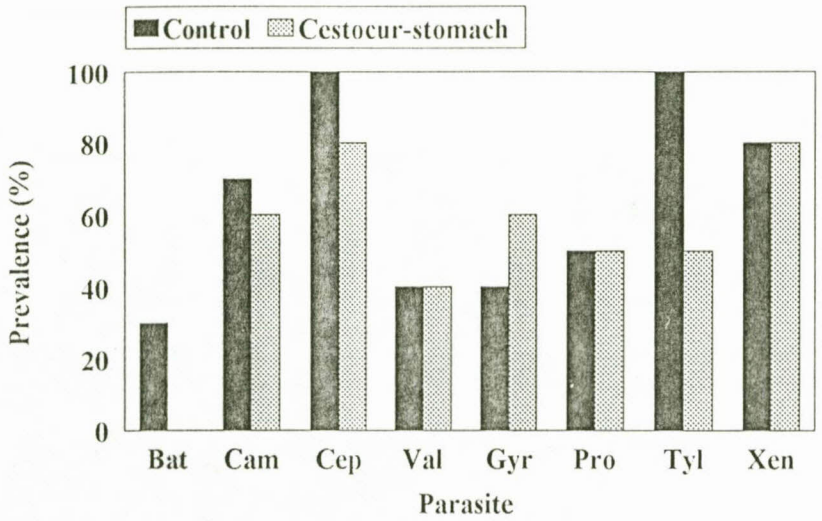
Bar graphs illustrating *Xenopus laevis* parasite infection levels in the control group and frogs treated with the Cestocur-stomach treatment.

Abbreviations of parasites: Bat, *Batrachocamallanus slomei*; Cam, *Camallanus kaapstaadi*; Cep, *Cephalochlamys namaquensis*; Val, *Valipora campylancristrota*; Gyr, *Gyrdicotylus gallieni*; Pro, *Protopolystoma xenopodis*; Tyl, *Tylodelphys xenopodis*; Xen, *Xenopacarus africanus*.

a) Prevalence

b) Mean intensity

a)


Control
Cestocur-stomach
Prevalence (%)
100
80
60
40
20
0
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

b)

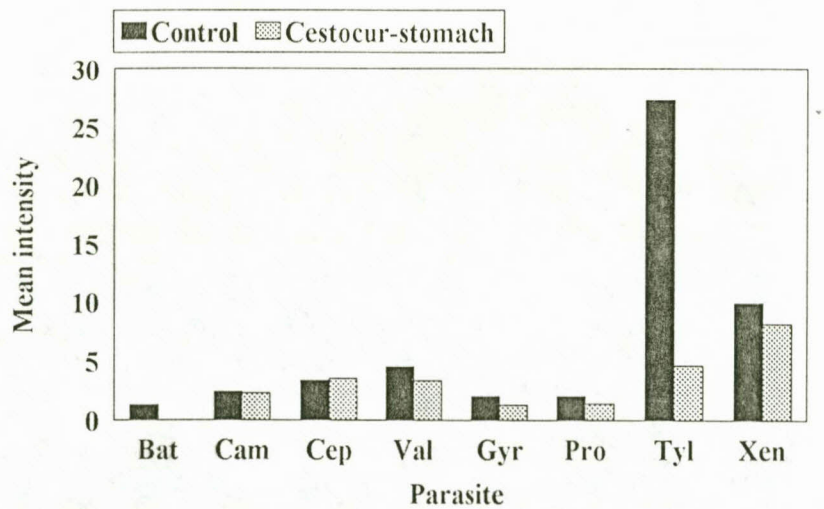

Control
Cestocur-stomach
Mean intensity
30
25
20
15
10
5
0
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

Figure 6.3

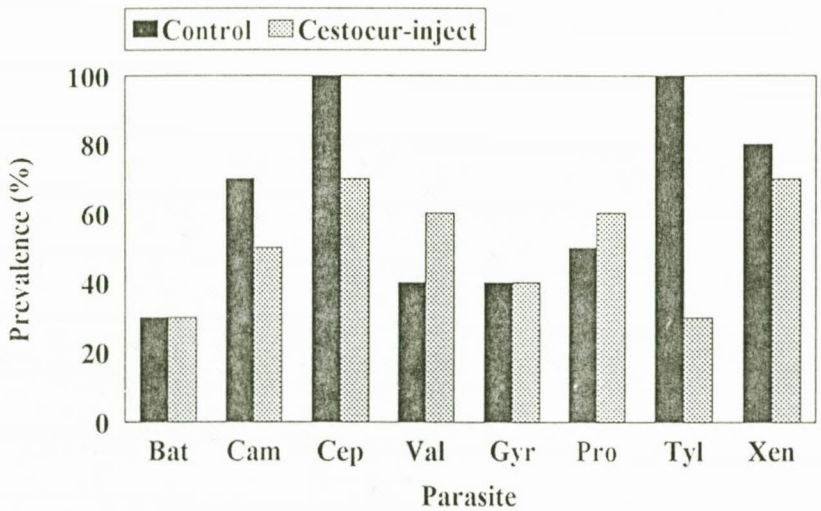
Bar graphs illustrating *Xenopus laevis* parasite infection levels in the control group and frogs treated with the Cestocur-inject treatment.

Abbreviations of parasites: Bat, *Batrachocamallanus slomei*; Cam, *Camallanus kaapstaadi*; Cep, *Cephalochlamys namaquensis*; Val, *Valipora campylancristrota*; Gyr, *Gyrdicotylus gallieni*; Pro, *Protopolystoma xenopodis*; Tyl, *Tylodelphys xenopodis*; Xen, *Xenopacarus africanus*.

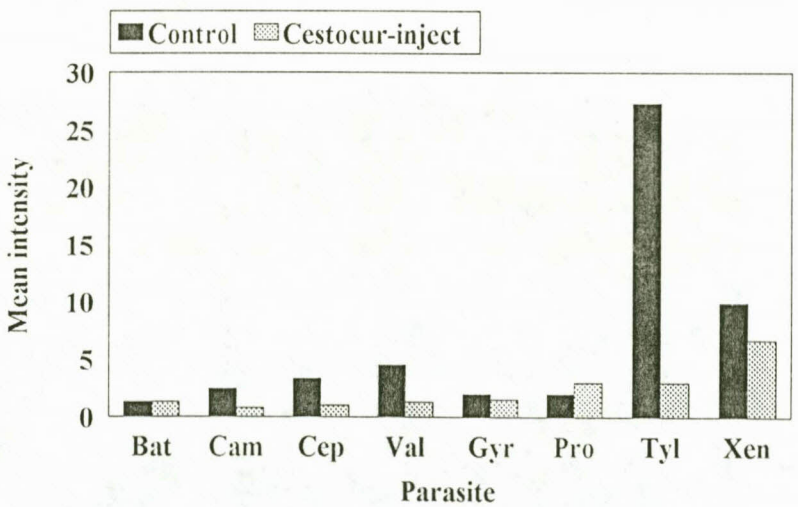
a) Prevalence

b) Mean intensity

a)


Control
Cestocur-inject
100
80
60
40
20
0
Prevalence (%)
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

b)


Control
Cestocur-inject
30
25
20
15
10
5
0
Mean intensity
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

3. **Cestocur-dip:** Again *C. namaquensis* and *T. xenopodis* intensity levels were significantly reduced by treatment with Cestocur-dip ($p = 0.036$, and $p = 0.002$ respectively, Table 6.5). These two significant p-values are accompanied by 60% reduction in prevalence for the two parasites (Fig. 6.4).

4. **Ivomec-inject:** Ivomec- inject was the only treatment to reduce the intensity levels of *C. kaapstaadi* significantly ($p = 0.049$, Table 6.5). Prevalence and mean intensity of both nematodes, *C. kaapstaadi* and *B. slomei*, were however reduced by the treatment (Fig. 6.5). Once again a significant difference existed in the treatment of *T. xenopodis* ($p = 0.001$).

Figure 6.4

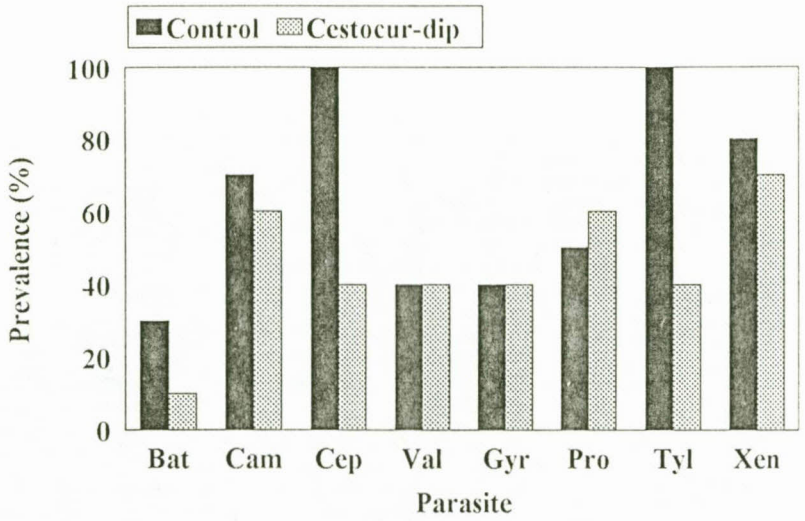
Bar graphs illustrating *Xenopus laevis* parasite infection levels in the control group and frogs treated with the Cestocur-dip treatment.

Abbreviations of parasites: Bat, *Batrachocamallanus slomei*; Cam, *Camallanus kaapstaadi*; Cep, *Cephalochlamys namaquensis*; Val, *Valipora campylancristrota*; Gyr, *Gyrdicotylus gallieni*; Pro, *Protopolystoma xenopodis*; Tyl, *Tylodelphys xenopodis*; Xen, *Xenopacarus africanus*.

a) Prevalence

b) Mean intensity

a)


Control
Cestocur-dip
Prevalence (%)
100
80
60
40
20
0
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

b)

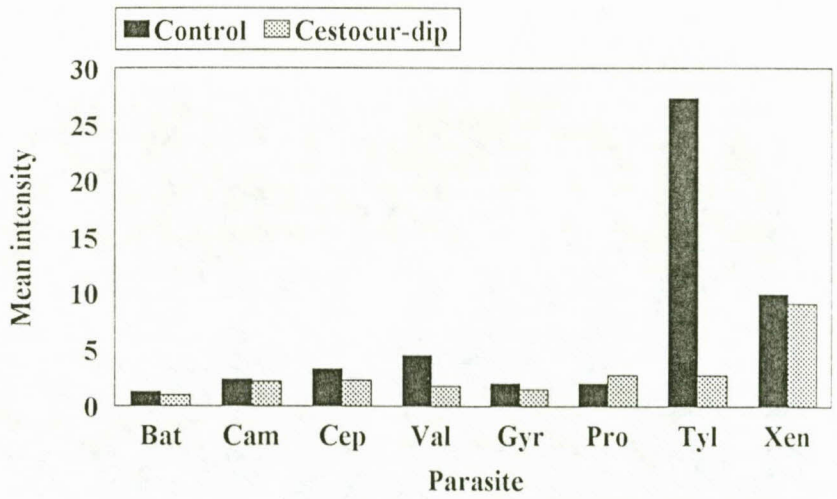

Control
Cestocur-dip
Mean intensity
30
25
20
15
10
5
0
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

Figure 6.5

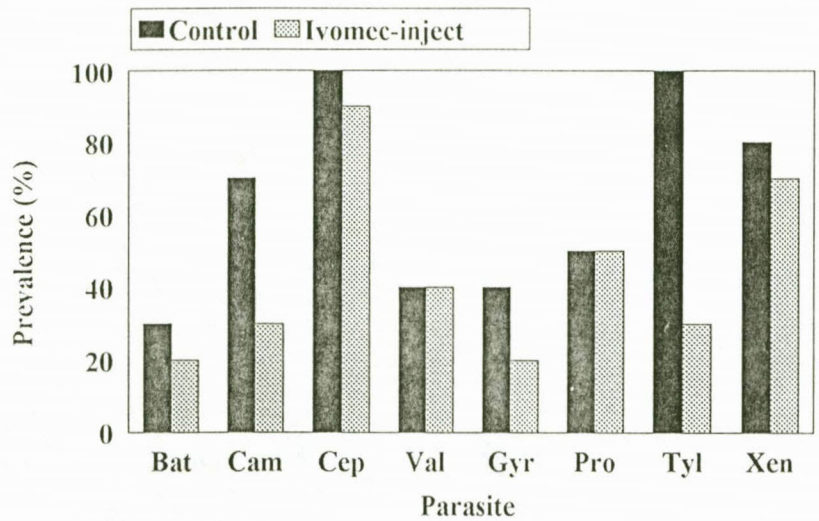
Bar graphs illustrating *Xenopus laevis* parasite infection levels in the control group and frogs treated with the Ivomec-inject treatment.

Abbreviations of parasites: Bat, *Batrachocamallanus slomei*; Cam, *Camallanus kaapstaadi*; Cep, *Cephalochlamys namaquensis*; Val, *Valipora campylancristrota*; Gyr, *Gyrdicotylus gallieni*; Pro, *Protopolystoma xenopodis*; Tyl, *Tylodelphys xenopodis*; Xen, *Xenopacarus africanus*.

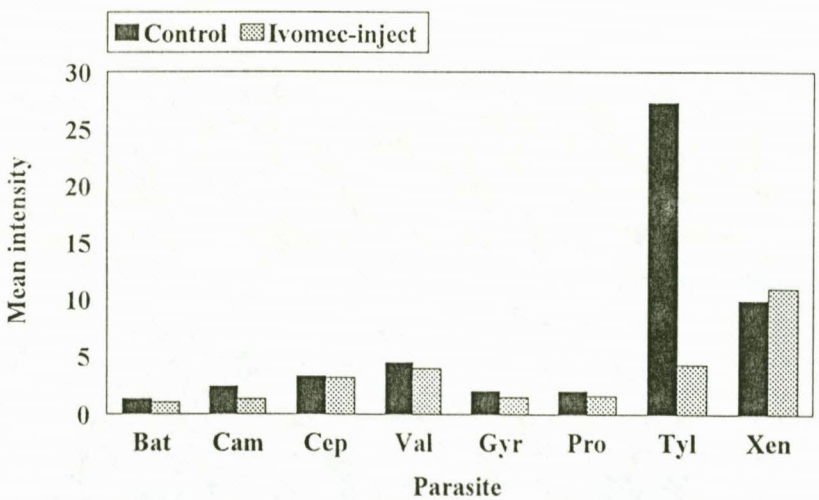
a) Prevalence

b) Mean intensity

a)


Control
Ivomec-inject
100
80
60
40
20
0
Prevalence (%)
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

b)


Control
Ivomec-inject
30
25
20
15
10
5
0
Mean intensity
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

5. **Ivomec-dip:** Just a single parasite, *T. xenopodis* experienced a significant reduction in intensity levels ($p = 0.038$, Table 6.5). The prevalence of seven of the eight parasites experienced a reduction after Ivomec-dip treatment of which only *C. kaapstaadi*, *G. gallieni* and *T. xenopodis* had a 30% or more reduction (Fig. 6.6).

6. **Levisol-dip:** Intensity levels of only a single parasite, the mite, *X. africanus* differed significantly from the control group ($p = 0.050$, Table 6.5). Levisol-dip was also the only treatment to cause a significant reduction in *X. africanus* intensity levels. Levisol-dip was also the only treatment in which a reduction in prevalence of all parasites and in mean intensity of seven parasites occurred (Fig. 6.7).

Figure 6.6

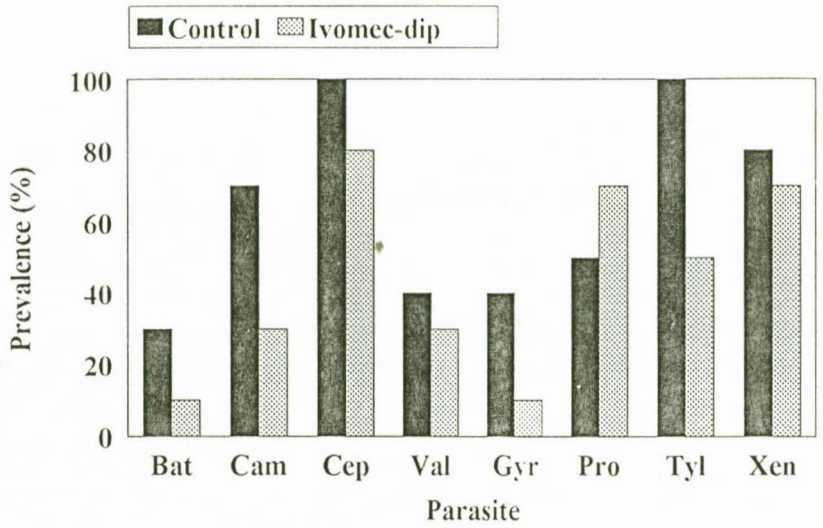
Bar graphs illustrating *Xenopus laevis* parasite infection levels in the control group and frogs treated with the Ivomec-dip treatment.

Abbreviations of parasites: Bat, *Batrachocamallanus slomei*; Cam, *Camallanus kaapstaadi*; Cep, *Cephalochlamys namaquensis*; Val, *Valipora campylancristrota*; Gyr, *Gyrdicotylus gallieni*; Pro, *Protopolystoma xenopodis*; Tyl, *Tylodelphys xenopodis*; Xen, *Xenopacarus africanus*.

a) Prevalence

b) Mean intensity

a)


Control
Ivomec-dip
100
80
60
40
20
0
Prevalence (%)
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

b)

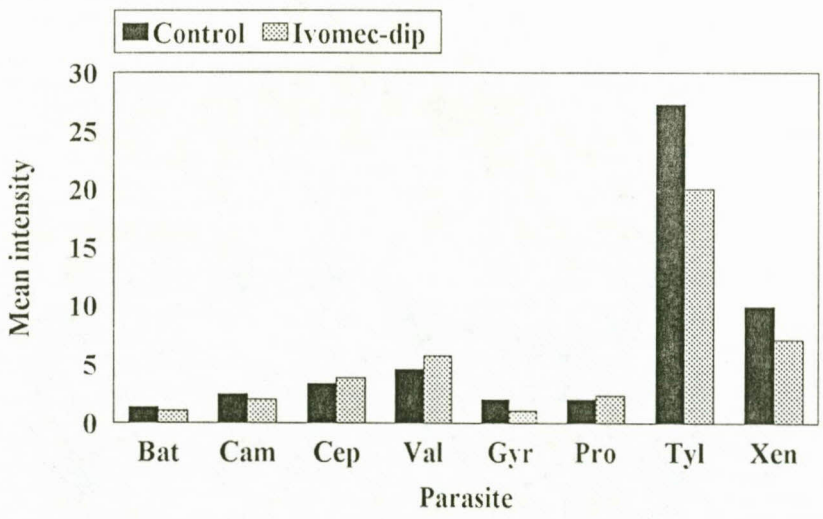

Control
Ivomec-dip
30
25
20
15
10
5
0
Mean intensity
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

Figure 6.7

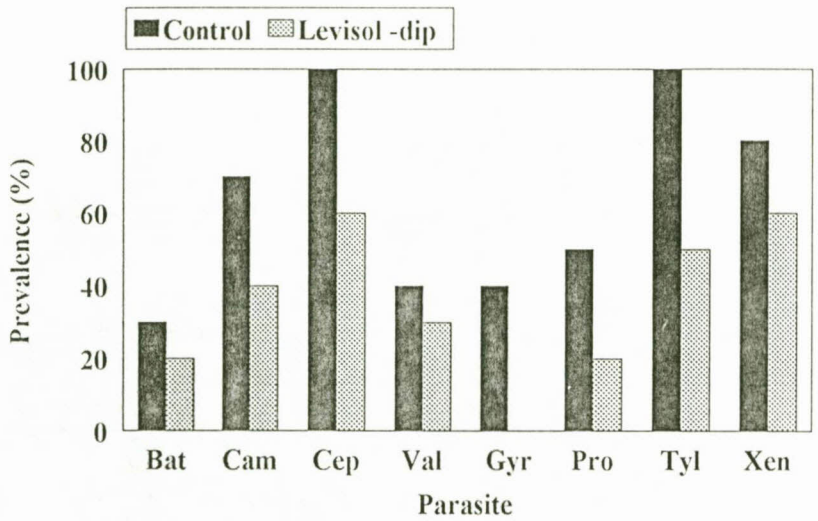
Bar graphs illustrating *Xenopus laevis* parasite infection levels in the control group and frogs treated with the Levisol-dip treatment.

Abbreviations of parasites: Bat, *Batrachocamallanus slomei*; Cam, *Camallanus kaapstaadi*; Cep, *Cephalochlamys namaquensis*; Val, *Valipora campylancristrota*; Gyr, *Gyrdicotylus gallieni*; Pro, *Protopolystoma xenopodis*; Tyl, *Tylodelphys xenopodis*; Xen, *Xenopacarus africanus*.

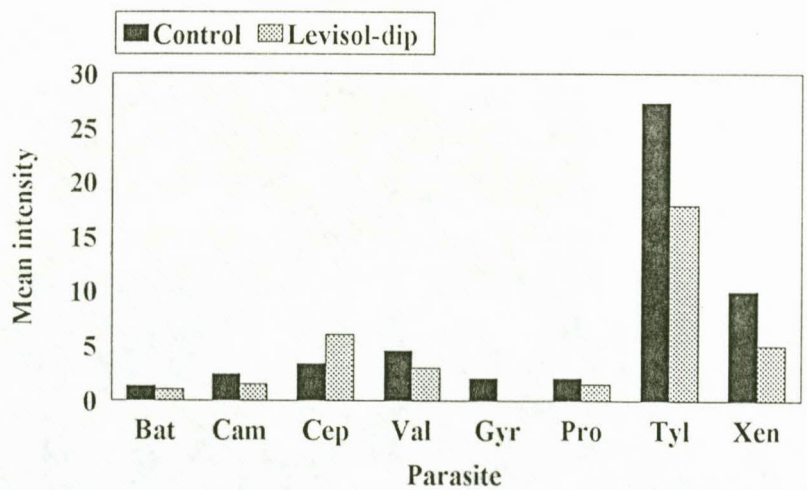
a) Prevalence

b) Mean intensity

a)


Control
Levisol -dip
100
80
60
40
20
0
Prevalence (%)
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

b)


Control
Levisol-dip
30
25
20
15
10
5
0
Mean intensity
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

7. **Cestocur-Ivomec-dip:** Intensity levels of *C. namaquensis* (p = 0.001) and *T. xenopodis* (p = 0.001) were again significantly reduced by treatment with Cestocur-Ivomec-dip (Table 6.5). All eight parasites of the treated sample had a lower prevalence than the control group (Fig. 6.8).

Figure 6.8

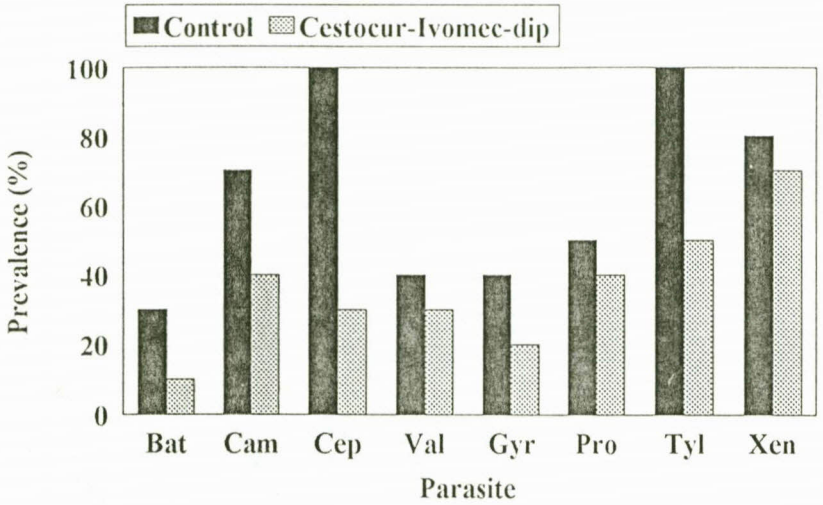
Bar graphs illustrating *Xenopus laevis* parasite infection levels in the control group and frogs treated with the Cestocur-Ivomec-dip treatment.

Abbreviations of parasites: Bat, *Batrachocamallanus slomei*; Cam, *Camallanus kaapstaadi*; Cep, *Cephalochlamys namaquensis*; Val, *Valipora campylancristrota*; Gyr, *Gyrdicotylus gallieni*; Pro, *Protopolystoma xenopodis*; Tyl, *Tylodelphys xenopodis*; Xen, *Xenopacarus africanus*.

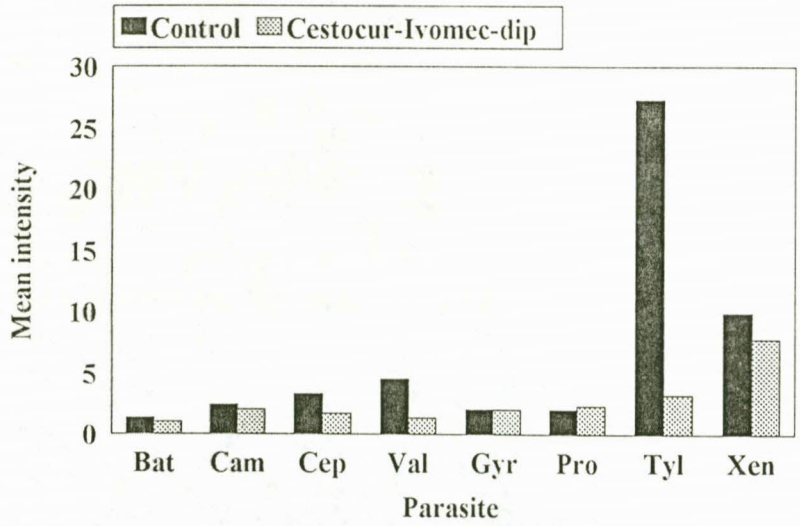
a) Prevalence

b) Mean intensity

a)


Control
Cestocur-Ivomec-dip
Prevalence (%)
100
80
60
40
20
0
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

b)


Control
Cestocur-Ivomec-dip
Mean intensity
30
25
20
15
10
5
0
Bat
Cam
Cep
Val
Gyr
Pro
Tyl
Xen
Parasite

6.3.2.3 Recommended doses and the cost of treatment

The cost involved in eliminating the parasites of *X. laevis* was not calculated for Cestocur-stomach because of the ineffectiveness of this technique. For both Cestocur and Ivomec the dip-technique was cheaper than the inject-technique (Table 6.6). This is because the recommended doses for the dip techniques are smaller than for the inject-techniques. When the same doses of Cestocur and Ivomec, which were safe to use on the host, were combined, the mixture became lethal to the host. However, smaller quantities of the two drugs (0.7mg// Cestocur and 0.012mg// Ivomec) were still as effective when they were used in combination. Because smaller quantities of Cestocur and Ivomec are needed when used in combination, the cost involved (R 0,22/frog) is less than when the two drugs are used separately. This makes the combination Cestocur-Ivomec the most economical choice.

Table 6.6 Comparison of the costs of the drugs calculated for the recommended dosages used in experiments.

Treatment	Recommended dose	Cost/frog (South African rands = 0.16 US dollars)
Cestocur-inject	2mg	R 0.60
Cestocur-dip	1mg//	R 0.30
Ivomec inject	1mg//	R 0.67
Ivomec-dip	0.015mg//	R 0.01
Levisol-dip	12mg//	R 0.60
Cestocur-Ivomec-dip	0.7mg// & 0.012mg//	R 0.22

6.4 DISCUSSION

6.4.1 Parasite survival

The parasites for which infection levels were studied during host captivity belong to one of two groups according to their life cycle patterns. *Tylodelphys xenopodis*, *Valipora campylancristrota*, *Cephalochlamys namaquensis* and *Camallanus kaapstaadi* all have indirect life cycles which consists of two or more hosts. *T. xenopodis* and *V. campylancristrota* have extremely complex life cycles and utilise *X. laevis* as an intermediate host (King & Van As 1997, Jarecka 1970), while *C. namaquensis* and *C. kaapstaadi* occur as adults in the alimentary tract of the frog (Thurston 1967, 1970). *Protopolystoma xenopodis* and *Xenopacarus africanus* belong to the second group, namely a parasite with a direct life cycle for which *X. laevis* serves as only host (Thurston 1964, Fain, Baker & Tinsley 1969).

Reproductive output of *C. namaquensis* and *C. kaapstaadi* would preclude re-infestation of *X. laevis* because the absence of intermediate hosts prevents the life cycle from being completed. Despite the absence of intermediate and final hosts other than *X. laevis*, all the parasites with indirect life cycles survived throughout the captive period. This is indicative of longevity of at least 11 months. The only parasite to experience a severe reduction in infection levels was *C. namaquensis* (Fig. 6.1b). Judged by the rate at which prevalence decreased, the infrapopulation would probably not have survived much longer under the existing conditions. A similar pattern in *C. namaquensis* infection has been observed by Thurston (1970) in both well fed and poorly fed captive frogs.

More longevity records for parasites in captive hosts are available for *T. xenopodis* in excess of 3½ years (Southwell & Kirshner 1937, Tinsley & Sweeting 1974). Tinsley & Sweeting also reported that the prevalence and intensity of metacercaria was not at all effected by prolonged laboratory maintenance. Tinsley & Owen (1975) found longevity in *P. xenopodis* to exceed 1½ years while the record of maximum longevity was found to be about two years (Jackson & Tinsley 1988). As *P. xenopodis* and *X. africanus* were the only two parasites capable of reinfecting *X. laevis*, these were the only parasites for which infection levels might have been influenced by seasonality. The infections of all the other parasites were acquired before capture. According to Tinsley (1972),

temperature is one of the factors that can explain seasonal variation in *P. xenopodis* as it directly influences egg production. The first peak in the infection levels of *P. xenopodis* experienced in winter (months 3 to 5) could be due to relatively high infections in autumn (months 0 to 2) when moderate temperatures were experienced. The crowding of the hosts could further have contributed to a higher transmission than what would otherwise occur in autumn. The same is true for transmission of the parasites for the rest of the captive period. The dip experienced in spring (months 6 to 8) could be as a result of low transmission that occurred in winter. Higher temperatures in spring contributed to the highest infections experienced in the middle of summer (90% prevalence for month 10).

The ability of *X. africanus* to spread to other hosts in captive quarters overshadowed any environmental influence. The rapid spread from 20% to 100% prevalence within seven months was accompanied by an increase in mean intensity. Parasite numbers per individual host were still increasing at the time the last frogs were dissected. The steps in infection levels from 20% to 80% at month three and from 70% to 100% at month seven both saw the appearance of huge numbers of infective larvae. It would appear as if the larvae play an important role in the establishment of the parasite in new hosts.

6.4.2 Pathology

No pathological cases occurred during the study that could be attributed to any of the parasites. Nigrelli & Maravento (1944) attributed the deaths of *X. laevis* to burdens *of T. xenopodis* of up to 150 parasites. The effect of this infection was found to influence cardiac function. The health of the frogs from the current study does not seem to have been influenced by the high parasite burdens of *T. xenopodis* or the occasional high burden of *V. campylancristrota* and the increasing numbers of *X. africanus*. *V. campylancristrota* originally occured in the gall bladder and intestine of fish (Jara & Olech 1964, Jarecka 1970) and is believed to have transferred from introduced fish species, the tench *Tinca tinca* and the carp *Cyprinus carpio* to *X. laevis* (Crous 1999). In fish the parasite is responsible for a number of pathological conditions such as a decline in general health, decrease in growth rate, pathological haemorrhages, necrosis and cellular reactions in the gall bladder and intestines (Jara & Olech 1964, Sapoznikov,

Skvorcova & Laduchen 1974, Körting 1984). None of these conditions have been reported from *X. laevis* due to *V. campylancristrota* infections.

6.4.3 Elimination of parasites

A proportion of wild-caught frogs is always infected with parasites. Some may have potential pathological consequences for the host but by far the most are relatively harmless. *X. laevis* found at the Duraan and Nuwe Orde farms harbour a high diversity of parasites (nine, including protozoans). Should animals begin dying in captivity the tank can be disinfected with quicklime as a first line of defence and the healthy animals replaced (Hey 1949). Use of this procedure alone will always risk the lives of some of the animals. Hence the need for the preventative measure of treating the parasites.

Because none of the parasites found in the current study are responsible for major pathogenic effects very little has been documented on the treatment of these infections. Elkan (1960) noted that *C. namaquensis* were always extruded within 12 hours after the frogs received an injection of urine extracts for the purpose of a pregnancy test. This led him to assume that the Brom-Phenol indicator acts as a vermafuge. Crawshaw (1992) states that for the elimination of nematodes various anthelminthics such as Ivermectin can be used.

None of the drugs tested in the current study had the same effect on all the parasite groups. The only similarity was that all the treatments except Levisol-dip had a significant difference on *T. xenopodis* intensity levels. The effectiveness of each drug is dependent on the parasite group and the method of delivery. Cestocur was most effective against cestodes when applied either using the inject- or dip-technique as it rendered significant p-values ($p \leq 0.05$) for *C. namaquensis* (Table 6.5) and caused a reduction in the mean intensity of both *C. namaquensis* and *V. campylancristrota* (Fig. 6.3 and 6.4). Both Ivomec and Levisol treatment appeared to cause reductions in the prevalence and mean intensity of the nematodes, *B. slomei* and *C. kaapstaadi* (Fig. 6.5, 6.6 and 6.7) although only Ivomec-inject significantly reduced the intensity levels of *C. kaapstaadi* ($p \leq 0.05$). The combination Cestocur-Ivomec had the effects of both of its component

drugs, reducing parasite infection of both cestodes and nematodes (Fig. 6.8). The negligible differences between the p-values of the effective treatments in reducing overall parasite intensity levels, such as Cestocur-Ivomec-dip, Cestocur-inject and Levisol-dip, implies that any one of these three treatments can be used to control overall parasite intensity.

The effectiveness of the drugs on all eight parasites vary according to the techniques that were used. This can be seen in the p-values for the three techniques (stomach, inject and dip) to administer Cestocur and the two techniques (injection and dip) for Ivomec (Table 6.4). This implies that successful control of parasites is as much dependent on the technique of drug application as the choice of drug itself.

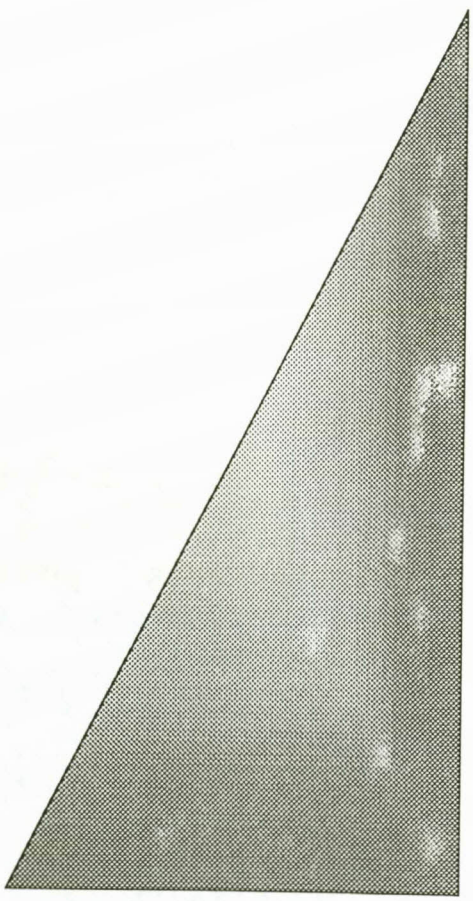
The difficulty of using the stomach-technique discourages its use. The use of the injection-technique is very labour intensive as each frog has to be treated individually and it is a time consuming technique. The dip technique on the other hand allows for many frogs to be treated simultaneously, thus saving both labour and time. Using the dip technique might prove more of a challenge for terrestrial anura, but for the aquatic *Xenopus* it certainly proved to be the superior choice.

Treatment for bacteria and fungus that are the major causes of disease in anurans have been developed and tested. Chloramphenicol, Sulfadiazine, Ringer's solution, Enrofloxacin, Gentamicin and Tetracycline are very effective for the treatement of Red-Leg disease (Koivastik 1950, Nace 1968, Crawshaw 1992, Pariyanonth & Daorerk 1995). Dickenson (1949) used Methylene Blue to treat fungal growths on the body, gastro-intestinal disorders and some other diseases. A parasite known to be responsible for deaths in *X. laevis* is the skin-invading cappillarid, *Cappillaria xenopodus*. This parasite has previously been controlled by injecting Ivermectin into the dorsal lymph sac (Dawson, Schultz & Schroeder 1992).

It can be concluded from this study that the prevalence and mean intensity of all the parasites of *X. laevis* in the present study can significantly be reduced with the treatment of specific drugs and specific delivery techniques. All the effective treatments can be administered at low costs of under R 1.00/frog. The low costs involved and relative ease of administering the drugs makes the treatment of parasites an affordable option for protecting frog populations and preventing the translocation of the parasites abroad.

Chapter 7

General discussion



CONTENTS

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GENERAL DISCUSSION

7.1 THE UTILISATION OF *Xenopus laevis*

During the past century *Xenopus laevis* has been exploitated extensively in South Africa. Early in the twentieth century *X. laevis* was used mainly in research and for teaching in South Africa (Gilchrist & Von Bonde 1919). In the 1930s with the development of the pregnancy assay, which soon led to the export of this species to laboratories around the world. This practice was replaced in the 1960s with the development of a chemical method for determining early pregnancy in women. In South Africa the most frequent use of the animal is as bait by local anglers. Use of *X. laevis* in teaching and research continued and today the biggest applications are in the fields of embryology and biochemistry, while its use in teaching (anatomy and physiology dissection) is declining.

Judging by the outcome of the survey on the demand for *X. laevis* the high and increasing global demand of the animal is not likely to change within the near future. Even though the large supply companies like "Nasco" and "Xenopus One" have breeding programmes in place fit to meet the demands of the scientific community, wild caught frogs from South Africa are required for breeding. In turn increasingly more research facilities are maintaining their own laboratory colonies. Breeding adults are however still ordered from time to time. Advances in maintenance techniques and in enriching the environment for captive laboratory animals and the ease of inducing mating response in *X. laevis* have aided in the culture of this species. Methods dealing with the laboratory housing, health, sanitation, feeding and breeding have already been published (Buttner 1984, Sackin & Sackin 1991, Dawson, Schultz & Schroeder 1992, Kaplan 1993).

Comparing the highly organised and developed *X. laevis* culture in the United States, it is realised that there is room for improvement in South Africa with regards to the selling of *X. laevis* as bait. For eight months of the year during the angling season there is a high demand for the frog, which recently has been in short supply. Customers return home empty handed because bait shops run out of stock. Often weeks pass before shop owners get in fresh supplies of frogs. The need therefore exists for a constant supply of *X. laevis* that can be delivered to the angling shops the angling community.

7.2 THE SUPPLY OF *Xenopus laevis*

The supply of *X. laevis* as bait or for any other market (science, teaching, pet trade etc.) are from collecting from natural sources or from breeding frogs in captivity.

7.2.1 Collecting

There are two ways of harvesting *X. laevis* from wild populations by collecting either the tadpoles or the frogs. The common distribution, ability to migrate overland and adaptability to establish in various water types further enhances the chances of finding wild populations from. If such a population has been located, the effectiveness of using the baited funnel traps will almost guarantee a catch.

Collection of tadpoles is limited to more or less six months of the year depending on the duration of the breeding season. Tadpole collection holds the advantage of non-recurrent visits to *X. laevis* sources as there are no traps involved that need to be re-visited a week or two later to gather the catch. The tadpoles of *X. laevis* congregate in schools in midwater. This facilitates their collection, as it is easier to detect a school of tadpoles than when tadpoles are randomly dispersed. When schools of *X. laevis* are present, many tadpoles can be collected. Tadpoles are however fragile and prone to get hurt especially before metamorphosis starts. Great care therefore has to be taken when handling the tadpoles. Tadpoles can then be reared in captivity until the required sizes are reached.

A consideration when collecting from natural environment is that they are often unreliable and erratic. Temporary water bodies can only be utilised as *X. laevis* sources during the rainy season and for as long as water is present. One also has to be careful not to overexploit the sources. The disappearance of frogs from the Cape flats after the discovery of the "*Xenopus* pregnancy assay" (Hey 1986) should serve as a warning against overexploitation.

7.2.2 Captive breeding

The difficulty of getting *X. laevis* to breed naturally in captivity is overcome by the ability to induce spawning in this species by use of chorionic gonadotrophin to induce mating response. Dawson, Schultz & Schroeder (1992) who had great success with breeding *X. laevis* in the laboratory use each female not more than once a month for breeding, but use males up to three times a month if necessary. Initially reproductively mature frogs need to be obtained from the wild to get the breeding program started.

Captive breeding programmes of *X. laevis* is advantageous in that it yields larger quantities of frogs than collection from nature. Not only can the number of breeding adults be manipulated, but also survival rates are probably higher than in the wild since tadpoles and frogs can develop and grow in the absence of predators. Tadpoles, sub-adults and adult frogs should be separated to prevent cannibalism. Aquatic invertebrate predators can be removed from enclosures and enclosures covered with plastic hail netting to keep out predatory birds. Ultimately captive breeding programmes of *X. laevis* can produce frogs in the sizes and quantities to suit the needs of research facilities and the angling community alike.

7.3 THE INFLUENCE OF PARASITES ON THE UTILISATION OF *Xenopus laevis*

Wild-caught *X. laevis* have a good chance of being parasitised by a variety of invertebrate parasites. Since disease attributable to parasitism is rare amongst anurans, the general health of the frogs is under no major threat due to parasitism. Furthermore only a few of these parasites have the opportunity for direct transmission once frogs are in captive quarters.

It was shown from the captivity experiment that parasites of the plathyhelminths, nematodes and an acarid have a longevity in captive hosts of at least 11 months. This is supported by Thurston (1970), Tinsley & Sweeting (1974) and Jackson & Tinsley (1988).

For the utilisation of *X. laevis* in research parasites may present an additional variable in controlled physiological studies as documented by Crawshaw (1992) for amphibians in general. As indicated by the survey on the utilisation of *Xenopus*, the majority of foreign research facilities prefer frogs that are not parasitised. Besides obtaining a parasite-free frog to meet the preference of research facilities, it holds merit from an environmental perspective. Reports on feral *X. laevis* populations outside Africa date back 40 years (see Tinsley & McCoid 1996). Lafferty and Page (1997) recently found three internal parasites including *Cephalochlamys* from such a feral population in California. There have been two documented accounts so far where *Cehpalochlamys* was found in non-pipid hosts, by Mettrick (1963) in *Rana angolensis* in Zimbabwe and by Dollfus (1968) in *Dicroglossus occipitalis* in Gabon. The possibility therefore exists that parasites from these feral *X. laevis* populations might be transferred to local anurans.

Captive bred *X. laevis* on the other hand stand less chance of becoming infected with parasites. Treating the frogs with drugs can serve to rid wild-caught frogs of some of the parasites. The choice of drug will allow the target of specific parasites for effective eradication. The relative ease and low costs involved in treating the parasites of *X. laevis* makes it an option well worth considering especially for frogs intended for the export market. It is better to safeguard the frogs against the parasites than to risk the outbreak of diseases or even losses because of infections.

7.4 RECOMMENDATIONS

1. Collection of *X. laevis* from natural sources is important in the supply of this species. Newly caught frogs are required to provide captive breeding colonies with genetic variation to prevent inbreeding from taking place. When collecting *X. laevis* from natural sources it is important to:

- Always release the largest males and females in a catch, because the large frogs produce the most oocytes. In doing so, one will ensure that a portion of the reproductively mature frogs remain with the wild population. The long breeding

season of *X. laevis* ensures that small frogs within the specified sizes to be sold as bait, are available for most of the year from natural sources. When collecting specifically for the angling market sub-adult frogs are required, implying that all large frogs should be released.

- To prevent overexploitation repeated visits to a specific source should be avoided. Sources can be visited on a rotational basis that allow enough time for the populations to recover to their original sizes (i.e. twice a year).

2. Even though it is possible to collect *X. laevis* tadpoles, this method is not recommended. Besides the breeding season lasting 6½ months at the most and the risk of tadpoles injuring, tadpoles still need to be reared in artificial enclosures.

3. If one plans on rearing tadpoles it is better to partake in breeding practices where the frogs are reared from the embryo through market sized frogs. It is vital in the culture of *X. laevis* that:

- Adequate water volume is available to tadpoles (at least 1*l*/tadpole) to prevent crowded conditions that will lead to retardation of growth. Preventing crowding of tadpoles will produce larger tadpoles at metamorphosis, provided they are fed adequately. It is possible that these larger post-metamorphic frogs will reach the required market size in a shorter time because of the head start over the smaller post-metamorphic frogs.
- Enclosures need to be kept in a sanitary state. Cleaning the enclosure once a month should prevent the water from fouling. According to Crawshaw (1992) the provision of a suitable, stable environment is the key for the maintenance of health and to promote longevity of amphibians in captivity.
- The diets of tadpoles and frogs need to be correct. Powdered alfalfa or powdered commercial frog pellets result in satisfactory growth of tadpoles, while commercial frog pellets, chopped liver or pet's mince result in good growth of *X. laevis* in captivity. Feeding frogs twice a week is sufficient to allow normal growth, however more frequent feeding will result in faster growth.

4. After collection, wild-caught frogs should be isolated first and treated for parasites, especially if they are intended for the export market. The supply of parasite-free frogs to angling and bait shops is not important or necessary.

- For the general treatment of parasites, frogs can be exposed to a combination solution of 0.7mg// Cestocur and 0.012mg// Ivomec/frog or 12mg// Levisol for 24 hours or inject frogs with 2mg// Cestocur into the dorsal lymph sac. Treatment should be repeated a week before export.
- 24-hour exposure of frogs to a 1mg// Cestocur solution is recomended for the specific treatment of cestodes.
- For the specific treatment of nematodes, frogs should be injected with 1mg// Ivomec into the dorsal lymph sac.
- As routine procedure captive-bred frogs can be treated on a regular basis for the prevention of, and in the case of accidental acquired parasite infections, the treatment of parasite infections.

7.5 FUTURE RESEARCH

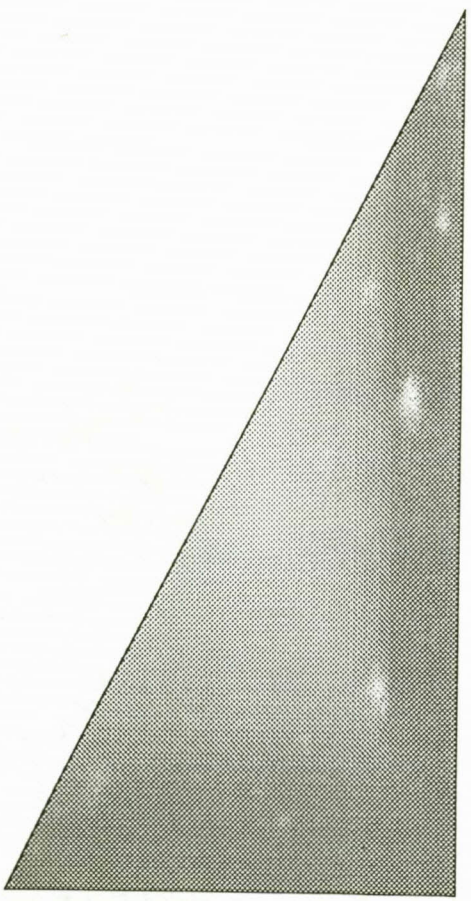
1. More information is needed on the long-term effect collecting from natural sources on the population dynamics of *X. laevis*. The use of mark and recapture techniques can provide an estimate of natural population size. The findings of such studies can aid in the establishment of sensible collecting protocols.

2. Not all the parasites of *X. laevis* have successfully been controlled. Such studies on these parasites could bring about parasite-free frogs. The effects of prolonged use of drugs on parasite resistance to the drugs and on host physiology are still unknown and need to be investigated.

3. Another potential use of *X. laevis* is as a food source for both human and domestic animal consumption. Local communities from a few countries in Africa such as Rwanda, Uganda, Cameroon and Zaire subject *Xenopus* populations to relatively intensive exploitation for human consumption (Tinsley, Loumont & Kobel 1996).

Chapter 8

Summary



SUMMARY

The African Clawed Frog, *Xenopus laevis* has been used extensively in medical and scientific research. For almost a full century this pipid frog has been exported around the world by thousands.

The present study is an attempt to investigate how to manage this important resource of *X. laevis* that will allow this species to remain a commercially viable resource. The approach in this study was as follows:

1. Market research concerning the use of *X. laevis* to various end-users, conducted locally and overseas, indicated that the oocytes of *X. laevis* is used to study the molecular aspects of development. In South Africa *X. laevis* is in high demand as bait for angling, but lacks a regular organised supply. The use of *X. laevis* in teaching at South African universities has declined over the last decade. The sale of *X. laevis* as pets is limited and is mostly confined to the United States.

2. The tadpoles of *X. laevis* collected throughout the Free State province and together with specimens from the Southern African Frog Atlas Project collection were staged to determine their development and measured. A size-stage graph was obtained that can be used to compare the development of *X. laevis* from other rainfall regions or even captive-raised tadpoles to their natural development in the Free State.

3. Mark and recapture studies were performed on wild *X. laevis* populations in and around Bloemfontein to investigate their population dynamics. Factors that determine the size of a population are: size of the habitat and permanency of the water habitat (semi-permanent or permanent). Regulation of population size by predatory birds becomes significant in semi-permanent habitats when water level is low. It can be concluded that uncontrolled harvesting from natural *X. laevis* sources can easily cause a marked reduction in population size.

4. Experiments on tadpoles and sub-adults were conducted to determine the optimum procedures for rearing *X. laevis* in captivity. The volume of water available to tadpoles beyond a density of two tadpoles per litre has an inhibitory effect on development. Factors that further effect tadpole growth rate are: type of enclosure, temperature of environment and tadpole diet. The best growth for sub-adult frogs was obtained by feeding with either Sinking Frog Food (a commercial brand of frog pellets), liver or pet's mince.

5. *X. laevis* were maintained in captivity for 11 months and parasite infections and abundance monitored. The cestode, *Cephalochlamys namaquensis* was the only parasite to experience a reduction in infection levels, while the only parasite to experience an increase in both prevalence and mean intensity was the mite, *Xenopacarus africanus.*

6. Various commercial anthelmintics were tested for the elimination of parasites of *X. laevis*. The delivery technique proved to be as important as the choice of drug itself. The use of drugs to eliminate parasites in *X. laevis* is an effective and affordable method to control parasite infections.

7. It was concluded that to exploit *X. laevis* as a commercial resource on a sustainable basis, efforts should be made to culture the frog following optimum breeding and rearing procedures. The collection method used for frogs from natural sources has to allow for the continued productivity of these populations.

Key words: *Xenopus laevis*, utilisation, tadpole development, population dynamics, collecting method, captive breeding, parasite infection, parasite survival, parasite elimination.

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Geen ander dier het ooit tevore die mediese en wetenskaplike navorsing gedien soos die gewone platanna, *Xenopus laevis* nie. Vir bykans 'n eeu word hierdie padda al uitgevoer na alle dele van die wêreld toe.

Die doel van hierdie studie is om die optimale prosedure vir die benutting van *X. laevis* te ondersoek ten einde te verseker dat die spesie 'n kommersiële hulpbron bly. Die studie is soos volg benader:

1. Marknavorsing oor die benutting van *X. laevis* deur verskeie verbruikers in Suid-Afrika sowel as in die buiteland is gedoen. Die mees veelseggende benutting van *X. laevis* is die gebruik van die oösiete om die molekulêre aspekte van ontwikkeling te bestudeer. Daar is 'n groot aanvraag na *X. laevis* as aas vir die hengelbebdryf in Suid-Afrika, maar 'n georganiseerde verskaffingstelsel ontbreek. Die gebruik van *X. laevis* in onderrig aan Suid-Afrikaanse universiteite het in die afgelope dekade afgeneem. Die verkoop van *X. laevis* as troeteldiere is beperk tot die Verenigde State van Amerika.

2. *X. laevis* paddavisse wat oor die hele Vrystaat versamel is, tesame met eksemplare uit die Suider Afrika Padda Atlas Projek se versameling, is gemeet en die ontwikkelingstadiums bepaal. 'n Grootte-stadiumgrafiek is opgestel wat gebruik kan word om die ontwikkeling van *X. laevis* afkomstig van ander reënvalstreke of selfs van paddavisse wat in aanhouding geteel is, te vergelyk met die natuurlike ontwikkeling van paddavisse in die Vrystaat.

3. Merk-en-hervangsstudies is op natuurlike *X. laevis* populasies van die Bloemfontein area uitgevoer om hul populasiedinamika te bestudeer. Van die faktore wat die grootte van 'n populasie bepaal, is die grootte en standhoudendheid van die habitat. Die regulering van populasiegrootte deur predatoriese voëls is veral wesenlik wanneer die watervlak laag is. Die gevolgtrekking is gemaak dat onbeheerde versameling van *X. laevis* vanuit natuurlike bronne maklik 'n afname in die populasiegrootte kan veroorsaak.

4. Ten einde die optimale prosedure vir die grootmaak van *X. laevis* in aanhouding te bepaal is eksperimente op paddavisse en onvolwasse paddas uitgevoer. Die beskikbare watervolume het 'n inhiberende effek op paddavisontwikkeling indien die digtheid van twee paddavisse per liter oorskry word. Verdere faktore wat die groeitempo van paddavisse beïnvloed, is die tipe beskutting, temperatuur en dieet. Kommersiële padda pille, lewer en troeteldiermaalvleis het die beste groei in onvolwasse paddas gelewer.

5. Parasietgeïnfekteerde *X. laevis* is vir 11 maande aangehou terwyl die infeksievlakke en oorlewing gemonitor is. Al die parasiete het in hul gashere oorleef vir die volle duur van die eksperiment. Die platwurm, *Cephalochlamys namaquensis* is die enigste parasiet wat 'n afname in infeksievlakke getoon het, terwyl die myt, *Xenopacarus africanus* die enigste parasiet is wat toegeneem het in beide persentasievoorkoms en gemiddelde intensiteit.

6. Verskeie kommersiële teenwurmmiddels is getoets vir hul effektiwiteit teen die parasiete van *X. laevis*. Dit wil voorkom of die behandelingstegniek en tipe middel ewe belangrik is. Die gebruik van teenwurmmiddels is 'n redelik effektiewe en bekostigbare metode om parasietinfeksies te behandel.

7. Daar is tot die gevolgtrekking gekom dat daar by die teel en grootmaak van *X. laevis* die optimale prosedure gevolg moet word om te verseker dat hierdie padda 'n volhoubare kommersiële hulpbron bly. Verder moet die versamelingsmetode van die paddas vanuit natuurlike bronne van so aard wees dat dit die voortbestaan van die die betrokke populasies kan verseker.

Chapter 9

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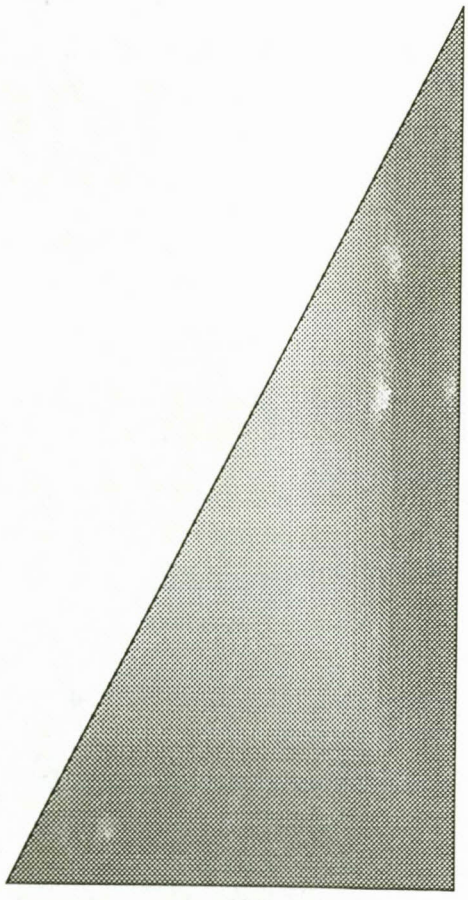

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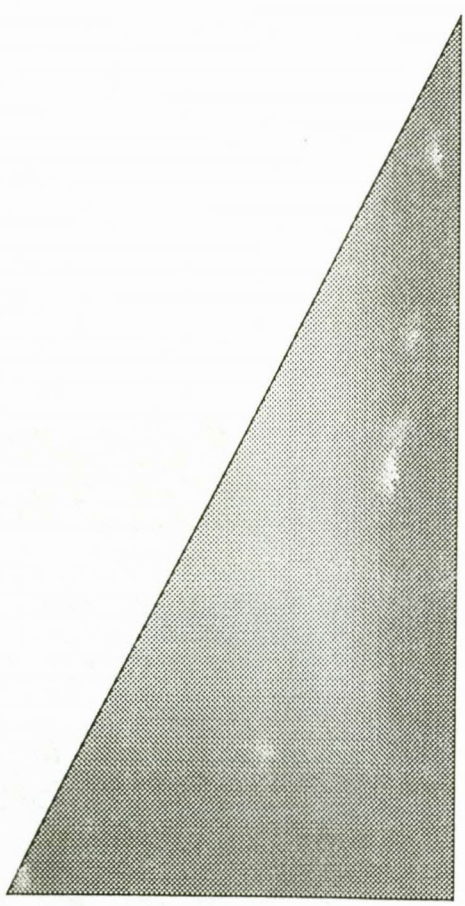
Appendices



Appendix 1

WELDON, C. & DU PREEZ, L.H. 1997. Fixing polystomatid (Monogenea) parasites for scanning electron microscopy. *Mic. Soc. S. Afr. Proc.* 27: 120.

FIXING POLYSTOMATID (MONOGENEA) PARASITES FOR SCANNING ELECTRON MICROSCOPY

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It is a well established fact that fixatives for scanning electronmicroscopical studies do not work equally well for all taxonomic groupings. The correct choice of a fixative that best suits the specimen is vital in obtaining optimal results in any SEM study. Use of an incorrect fixative may cause shrinkage and even structural damage of the specimen. Most authors working with monogenea favoured fixing with glutaraldehyde followed by post-fixation with osmium tetroxide[1]. The aim of the present study was to evaluate different SEM fixatives for polystomatid flatworms.

For the present study *Protopolystoma xenopodis* (Polystomatidae: Monogenea) was selected. *P. xenopodis* is found in the urinary bladder of the anuran genus *Xenopus laevis*.

X. laevis were anaesthetised with MS222 (Sandoz), dissected and the parasites removed. Parasites were transferred to and rinsed in 0.6% saline for 1 h. Parasites were divided in four groups and each group of parasites fixed in a different fixative. Fixatives included: a modified Flemming's solution[2] (1 h at 4°C); 10% neutral buffered formalin (12 h at 4°C), 3% glutaraldehyde (12 h at 4°C) and 3% glutaraldehyde (12 h at 4°C) post-fixed in 1% osmium tetroxide. After fixation, material fixed in Glutaraldehyde was rinsed in phosphate-buffer (15 min at 4°C). Specimens were dehydrated in an alcohol series, critical point dried and mounted with the aid of epoxy resin (Pratley Clear) on pointed brass stubs. Finally the parasites were gold-coated in a Polaron E500 sputter coater and examined in a JOEL 6400 scanning electron microscope.

Parasites fixed in Flemming's solution did not shrink, but extensive damage was caused to the pelicle, with extensive cracks over the entire body of the parasite (Fig. 1). Accordingly Flemming's solution is too severe and not recommendable for polystomatids. Formalin fixed material was reasonably good but some shrinkage did occur with subsequent folding of the integument (Fig. 2). In consequence, the structures are not properly revealed. Fixation in glutaraldehyde gave very satisfactory results with morphology and surface structure being well preserved (Fig. 3). Post-fixation with osmium effected no improvement, although osmium might prove to be useful in badly shrinked material as it does cause swelling of the specimens to a certain extent.

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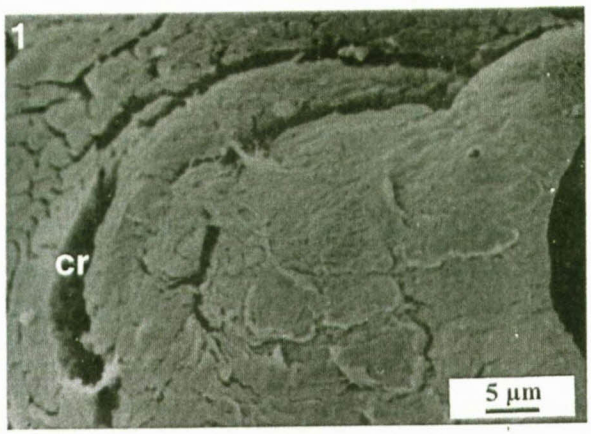

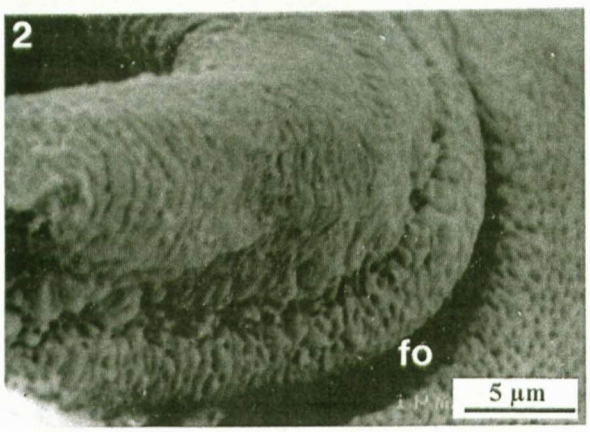

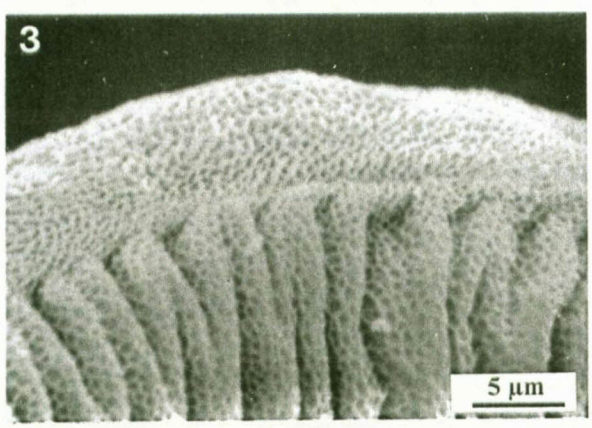

Fig. 1. Parasite fixed in Flemming's solution. Crack (cr).
Fig. 2. Parasite fixed in formalin. Fold (fo).
Fig. 3. Parasite fixed in glutaraldehyde.

Appendix 2

VENTER, J., DU PREEZ, L.H. & WELDON, C. 1999. Ultra structure of ereynetal organ of *Xenopacarus africanus* (Acari, Ereynetidae). *Mic. Soc. S. Afr. Proc.* 29: 77.

ULTRA STRUCTURE OF EREYNETAL ORGAN OF *Xenopacarus africanus* (ACARI, EREYNETIDAE)

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The subfamily Lawrencarinae (Acari: Ereynetidae) includes acarine species known to be parasitic in the nasal cavities of amphibians. *Xenopacarus africanus* is parasitic in the clawed frog *Xenopus laevis* where it is found in the nasal cavities as well as the ducts of eustachius[1]. Two other species of *Xenopacarus* are known, namely *X. kenyensis* from *Xenopus borealis* and *Xenopacarus kivuensis* from *Xenopus* sp. (*fraseri* group)[2]. These different species can be distinguished by various characters, including the structure of the ereynetal organ, a structure unique to the Ereynetidae. The aim of this study was to examine the external morphology of the ereynetal organ of *X. africanus.*

Xenopus laevis infected with *Xenopacarus africanus* were collected at Bloemfontein (South Africa). Hosts were anaesthetised with Benzocaine. Nasal chambers and eustachian passages were opened and mites removed. As mites do not wet easily standard electron microscopy fixatives could not be used and mites were fixed in 70% ethanol. Fixed specimens were dehydrated in an ethanol series and critical point dried. Dried material was mounted on 12,5 mm aluminium stubs with the aid of epoxy resin (Pratley). Specimens were gold coated in a sputter coater (Polaron E5000) and examined in a Jeol Winsem 6400 scanning-electron microscope at 5 KV.

In most genera of the family Ereynetidae the ereynetal organ usually consists of a sac-like structure, sunk deeply into the body of tibia 1 and connected with the dorsal surface by means of a narrow duct[1]. This opening is usually associated with a specialised barbed hair, the poil sensorial satellite. In *Xenopacarus* the ereynetal organ consists of a solenidion with a short barbed hair at its base (Figs. 1&2), which probably represents the poil sensorial satellite[1]. *Xenopacarus kenyensis* and *X. kivuensis* have ereynetal organs in which the solenidion is completely sunk into the integument[2] but in *X. africanus* the solenidion is partly external (Fig. 2). The solenidion has an external length of 4.3 µm and a diameter of 2.0 µm with terminal pole lobed while the barbed hair has a length of 7.0 µm (Fig. 2). The sunken solenidion represents the more advanced form which implies that the partly external solenidion of *X. africanus* resembles the more primitive form[2].

The sunken solenidion resembles Haller's organ found in the family Ixodidae[3]. The function of the ereynetal organ is not yet certain but the fact that the solenidion is partly or completely sunken into the integument indicates that the function might be that of a chemoreceptor. It could be an odour receptor or even have an auditive function[4]. On the other hand the possibility that it could be a mechanoreceptor cannot be excluded. Thus the function of the ereynetal organ needs to be studied in more detail.

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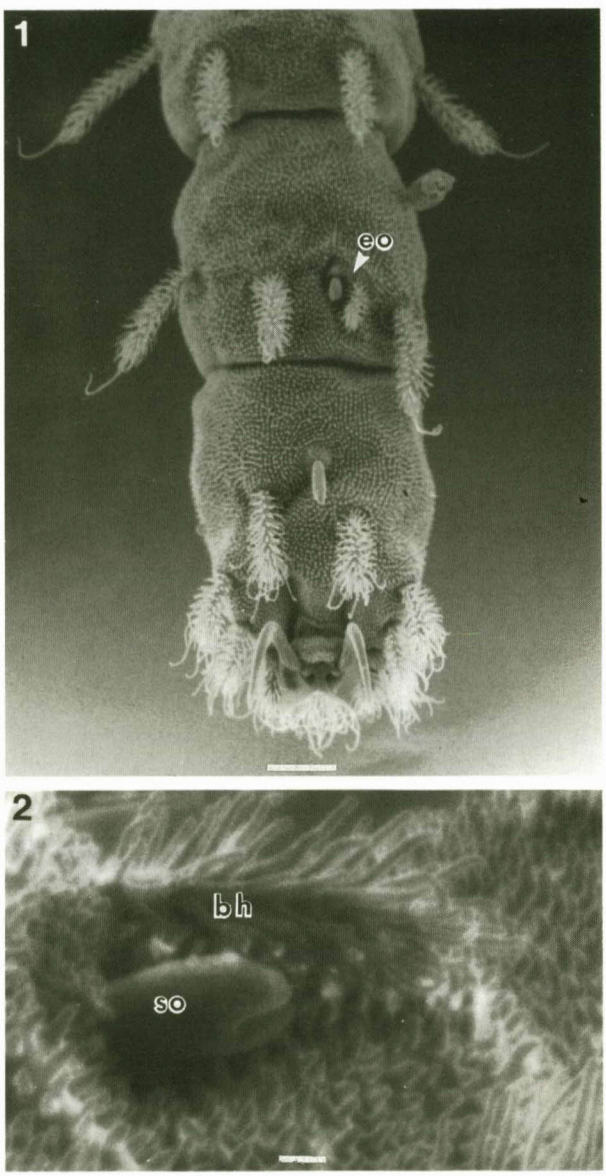

Fig. 1. Tibia 1 of *Xenopacarus africanus* with ereynetal organ (eo).
Fig. 2. Ereynetal organ of *Xenopacarus africanus* with solenidion (so) and short barbed hair (bh).
Scale: 10 µm (1), 1 µm (2).