ASPECTS OF THE ECOLOGY AND BREEDING BIOLOGY OF THE AFRICAN BULLFROG, *PYXICEPHALUS ADSPERSUS*

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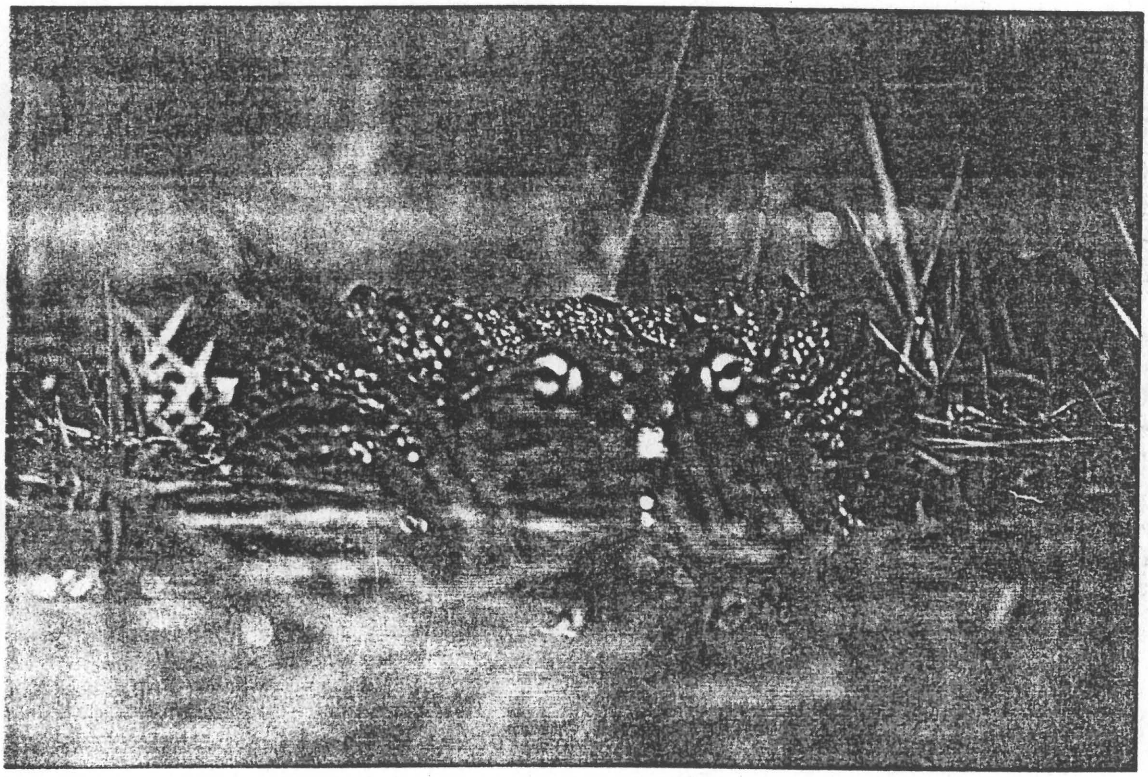

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JOHANNESBURG

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Dedicated to Albert and my family and friends.

ABSTRACT

The African Bullfrog, ***Pyxicephalus adspersus***, is highly sexually dimorphic with adult males exceeding 1kg in body mass and females rarely exceeding 200g. Bullfrogs are extreme explosive breeders completing mate acquisition, amplexus, and oviposition within 48 hours. Territorial males then remain with their tadpoles until metamorphosis 32-37 days later.

I observed mating behaviour and parental care in two marked populations of bullfrogs over three breeding events during the 1992-93 and 1993-94 wet seasons (October-January). Adult males exhibit three distinct size-related mating tactics namely territorial, non-territorial and satellite behaviour.

Here I consider variation in mating success between these three categories of males. Territorial frogs were the largest, most aggressive males and showed intolerance to any intruding conspecific males. Non-territorial frogs gathered in large groups in certain areas of the pan and compete to mate with females that did not mate with territorial males. Competitive interactions between these males were characterised by numerous attempts at amplexus displacement. Satellite frogs were the smallest adult males who adopted concealed non-calling, non-aggressive positions close to territorial males. Satellites attempted to intercept females attracted to territory residents.

Only territorial males remain behind and defend their tadpoles against predators. The presence of territorial males has a positive effect on tadpole survival. Territorial males also construct channels between their tadpole 'nurseries' and the main body of water. These channels function as a means of tadpole movement and also a mechanism whereby males regulate water depth in the nursery to provide optimal conditions for larval development.

Bullfrog offspring are adapted for survival in their unpredictable ephemeral breeding habitats by having two different tadpole morphs. A large, rapidly developing carnivorous tadpole which completes its larval development in 19-21 days and a normal developing, omnivorous tadpole which complete metamorphosis after 31-35 days. Tadpoles are also able to survive extreme temperatures of up to 39°C in their shallow temporary ponds.

Tadpoles of ***Pyxicephalus adspersus*** preferentially associate with kin in the laboratory and in the field form sibling schools in the territories and mixed sibling schools in the non-territorial breeding areas.

PREFACE

Anurans provide a model system for the study of mate acquisition and mating success. In spite of increased interests in this field, many aspects of their mating behaviour and seasonal variation in mating success have yet to be investigated through detailed field studies.

Pyxicephalus adspersus is an interesting species which has received little previous attention. Due to various behavioural traits associated with mate acquisition and parental care, it provided a wide scope for both observational and experimental work.

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ABBREVIATIONS USED IN THE TEXT

ANOVA	Analysis of Variance
C	degrees Centigrade
cm	centimetre
df	degrees of freedom
E	East
fig.	Figure
hr	hour
km	kilometre
Lat.	latitude
Long.	longitude
m	metre
min	minute
mm	millimetre
N	North
n	sample size
ns	non-significant (p>0.05)
p	probability
RFA	right forearm
S	South
sd	tandard deviation
se	standard error
SPL	sound pressure level
x	mean

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THE ADULT POPULATION

CHAPTER ONE

GENERAL INTRODUCTION

1.1 PREAMBLE

1.1.1 ANURAN REPRODUCTIVE STRATEGIES

A reproductive strategy may be viewed as the combination of physiological, morphological, and behavioural attributes that act in concert to produce the optimal number of offspring under certain environmental conditions (Duellman and Trueb 1986). The great diversity in patterns and modes of reproduction in amphibians is associated with differences in fecundity, duration of larval development, reproductive effort, and age at first reproduction. In this interface between developmental and population biology, amphibians are especially noteworthy because of their reproductive diversity and, in many species, complex life cycles (Duellman and Trueb 1986).

Wells (1977a) broadly classified anuran mating systems into two basic types on the basis of the duration of the breeding period, namely, prolonged breeders and explosive breeders. Although it is convenient to distinguish these categories, they actually represent two ends of a continuum from single-night breeding in some species to year-round breeding in others. Prolonged breeding covers breeding periods of more than a month, whereas explosive breeding refers to breeding periods of a few days to a few weeks.

This distinction provided an evolutionary framework for understanding the relationship between social behaviour and ecology in anurans. The length of the breeding season determines the density of males at the breeding site and hence the type and intensity of male-male interactions (Wells 1977a). It also determines the temporal availability of females at the breeding site. Consequently, the length of the breeding season influences the selective pressures which shape the mating system.

The selective pressures favouring explosive breeding in some species and prolonged breeding in others are not altogether clear. In many species, the length of the breeding period is determined by the equability of the climate and the seasonal availability of suitable breeding sites. However, climatic factors only provide a framework of constraints for the evolution of reproductive strategies (Wells 1977a). Ecological factors such as larval competition and predator abundance, and demographic factors such as age specific mortality of adults, age at first reproduction, clutch size and frequency of reproduction by individual females must also be considered (Tinkle, Wilbur & Tilley 1970; Andrews & Rand 1974).

Explosive breeding is usually associated with aggregations of males around suitable oviposition sites and is characteristic of many species that breed in temporary rain pools and other ephemeral habitats (Wells 1977a). These aggregations of males lead to high density populations and often leads to active searching for females in or around the breeding area. Active searching is usually accompanied by intense competition amongst males for possession of females (Tejedo 1993). Complex social organisation is uncommon and active searching behaviour is a response to fact observation assumption that male reproductive success is determined by the rate at which they encounter other individuals (Wells 1977a). The advantage to be gained by encountering an incoming female probably outweighs the disadvantage of repeatedly clasping other males, and thus a calling strategy or the defence of a territory would not be viable since females would be intercepted by searching males before they reached the calling individual (Wells 1977a).

Males in low-density aggregations often space themselves around the periphery of the breeding site and call from stationary positions. Consequently, active searching may be rare or absent in low-density aggregations. Females can approach individual males at their calling sites and select mates. This strategy would reduce physical male-male competition for females and may be a more efficient way for males to obtain mates at low densities. Males may also reduce their vulnerability to predation by calling from concealed locations in vegetation instead of searching in exposed areas (Wells 1977a).

Prolonged breeding is probably more common. Here males are sedentary and acoustic communication allows the identification of the callers' sex and their reproductive state, it facilitates the location of mates, and it permits the establishment and maintenance of individual distance or territory by males (Arak 1983a; Telford 1982; Wells 1977a).

Males vocalise in choruses that probably advertise the location of breeding sites to females and males in surrounding areas (Wells 1977a). The more intensified sound source of a chorus may facilitate easy localisation of breeding areas (Wells 1977a). Within the chorus, male vocalisations play a role in the attraction of females (for a review see Wells 1977,a,b) and in the maintenance of a minimum distance between callers which allows for effective signalling and may enhance a male's chance of securing a mate (Arak 1983b, Telford 1985). Since the advertisement call functions in mate recognition, it is necessarily species-specific and relatively invariable in the features required for recognition (Backwell 1991). Females are presumably attracted preferentially to conspecific calls which are most audible and locatable (Arak 1983a). A male's relative mating success may increase if, for example, his call is louder than surrounding calls and hence heard by more potential mates (Backwell 1991).

The advertisement call is also perceived by conspecific males and may play an important role in the organisation of the chorus (see Whitney & Krebs 1975) and allows for discrimination of signals against background noise (Arak 1983a). Male-male vocal interactions resulting in spatial separation of calling males have been extensively researched in prolonged-breeding anurans (Arak 1983b, Gerhardt 1991). Intermale spacing may improve an individual male's attractiveness by maximising his locatability and/or the distinctiveness of his call (Telford 1985).

1.1.2 BODY SIZE AND MATING SUCCESS

Darwin (1871) proposed two forms of sexual selection; intrasexual in which members of one sex, usually males, compete in displays or fights for mating opportunities, and intersexual selection in which one sex, usually females, chooses a mate on the basis of phenotypic characteristics or territorial resources. During the past two decades significant advances have occurred both in sexual selection theory (e.g. Trivers 1972; Emlen & Oring 1977) and in empirical studies (e.g. Andersson 1982; Clutton-Brock 1991).

The tremendous diversity of reproductive patterns exhibited by anurans renders them excellent subjects for investigating the evolution of mating behaviour (De Orense and Tejedo-Madueno 1990.) The most commonly measured male attribute is body size, because it is usually relevant to mating success. Only a few studies have considered other male traits, especially behavioural ones, in relation to mating success (e.g. Kagarise Sherman 1980; Sullivan 1983, 1987; Ryan 1985).

The relative importance of male-male competition and mate choice by females in producing non-random mating is often inferred by observing size-dependent mating success or size assortative mating, respectively. Although size-dependent mating success and assortive mating are possible outcomes of sexual selection, they do not specify which component of sexual selection is operating (Arak 1983a). Size dependent mating success can result from female preferences for larger males and/or a greater ability of larger males to control resources important to female success (or females themselves) (Howard 1988 a; b). Assortative mating can result from females selecting males of a particular size relative to their own size or competition among males to mate with females of particular sizes (Howard 1988 a; b).

In size-dependent mating, male body size is correlated with mating success. Size-dependent mating in explosive breeding anurans most often results from male-male competition and active searching for females; females often have little opportunity to exercise mate choice (Arak 1983a). Female arrival is asynchronous in prolonged breeders. Males are more likely to influence female choice by vocalising and by defending resources critical for females, such as oviposition sites (Howard 1978; Wells 1977a,b). Females may choose larger males directly on the basis of body size based on size-correlated characteristics, such as call parameters or territory quality (e.g. Robertson 1986; Ryan 1983).

In size-assortative mating, the body sizes of individuals in amplectant pairs are correlated. Size-assortative mating may result from either male-male competition or direct female choice, or both. Intuitively, this pattern seems reasonable for both sexes, because fertilisation success should be maximised when the cloacae are closely apposed (Crump and Townsend 1990). However, although a variety of anurans have been examined for size-assortative mating, it has been found in only a few species (e.g., Davies and Halliday 1977; Halliday 1983; Lee and Crump 1981). In contrast to earlier perceptions, however, an increasing number of recent studies have failed to document non-random mating patterns (Gerhardt *et al.* 1987; Sullivan 1982,1983) or have found intraspecific variability in mating pattern between breeding seasons and/or populations (Elmberg 1987; Woodward 1982a).

1.2 STUDY SPECIES

The African Bullfrog, ***Pyxicephalus adspersus***, is the largest species of anuran amphibian in South Africa with males reaching up to 230 mm in length and 1kg or more in body mass (Passmore and Carruthers 1979). Females are much smaller in size and seldom exceed 120 mm in length and weigh less than 200g (Fig.1.1).

Pyxicephalus adspersus are widely distributed in Central and northern Botswana, mainly along the central watershed in Zimbabwe, across the highveld of the Transvaal and the Orange Free State and into the northern and eastern Cape Province, throughout Namibia, except for its northern and southern limits (Parry 1982). Adult bullfrogs usually appear with the first rains in October or November, but mating only commences after the first heavy downpour (Grobler 1972, Balinsky 1969). They breed in temporary ponds and vleis (grassy areas that fill up after heavy summer rains), and then disperse, presumably to spend the remainder of the year buried and enclosed in a cocoon (Fig.1.2; Parry and Caville 1978). Bullfrogs form cocoon-like structures composed of single or multiple layers of skleratinized stratum corneum which substantially reduce evaporative water loss (Loveridge and Withers 1981). The adaptive advantage of dormancy and cocoon formation, with concomitant reduction in metabolic rate and evaporative water loss, is that the bullfrog can survive the 7-8 month dry season by utilising stored food and water reserves (Loveridge and Withers1981).

1.3 SCOPE AND OBJECTIVES

This thesis is essentially concerned with aspects of the life history and breeding biology of the African Bullfrog (***Pyxicephalus adspersus*)**. The breeding biology of male bullfrogs includes various interesting mate acquisition tactics and forms of parental care that ensure larval survival. The major questions addressed in this work are:

1) What are the major mate acquisition tactics employed by adult male bullfrogs?
2) Is there variation in male mating success, does it vary among the different tactics employed by adult males and between breeding seasons at the two populations?
3) Do tadpoles preferentially associate with kin and what mechanism enables them to do so?
4) How important is male parental care for offspring survivorship?

Figure 1.1 Photograph showing the extreme sexual dimorphism between male and female *Pyxicephalus adspersus.*

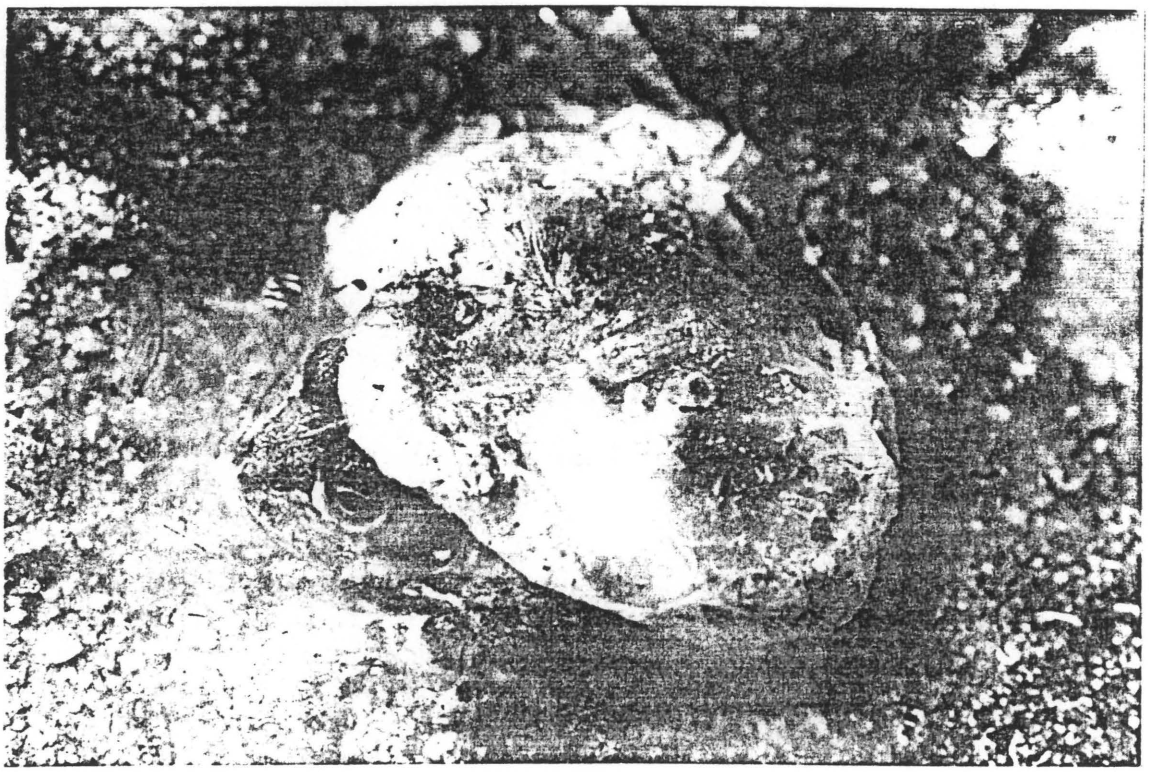


Figure 1.2 Photograph of a bullfrog emerging from cocoon-like structure

1.4 STUDY SITES

Two study sites were chosen in the Pretoria-Witwatersrand area; a temporary pan at the Glen Austin Bird Sanctuary (see Fig.1.3) and a man made dam at Witpoort Bird Sanctuary near Kyalami (Fig.1.4). 13.

1.4.1 GLEN AUSTIN : The Glen Austin pan is situated on remnants of the African erosion surface on the Johannesburg-Pretoria granite inlier above 1500 m (25°58`38"S; 28°10"E). With a diameter of roughly 380 m at the high water line the pan has an open water area of about 11ha. The effective catchment boundary is oval in shape and encloses an area of 65,5 ha. The local drainage pattern within the catchment boundary is centripetal and the knoll is surrounded by a regional centrifugal drainage pattern. The relatively small catchment area of the pan, and the fact that the pan is fed only by rainwater and possibly groundwater, makes the ecology of the pan sensitive to any changes within the catchment area. Roads, boreholes, septic tanks and other human activities have an effect on the behaviour of the pan by reducing and potentially polluting the natural water flow into the pan.

1.4.2 VEGETATION : As part of the highveld grassland biome or Bankenveld (Acocks 1988), the area surrounding the pan consists mainly of dry grassland. The pan is covered mainly in ***Scripus spiciformes*** (sedges). In wet areas the grasses die off to form the organic deposit of the pan floor. Dry areas in the pan are indicated by good ***Scripus spiciformes*** growth. ***Typha capensis*** occurs in the southern half of the pan. Rainfall occurs primarily during the summer months (October-February) with approximately 700-750 mm per annum. During the summer, daytime temperatures are usually above 25°C. At night, temperatures drop into the low 20's. Other frog species that called and bred in the pan were ***Rana angolensis, Tomopterna cryptotis, Schismaderma carens, Bufo rangeri, Bufo gutturalis, Cacosternum boettgeri, Kassina senagalensis*** and ***Xenopus laevis.***

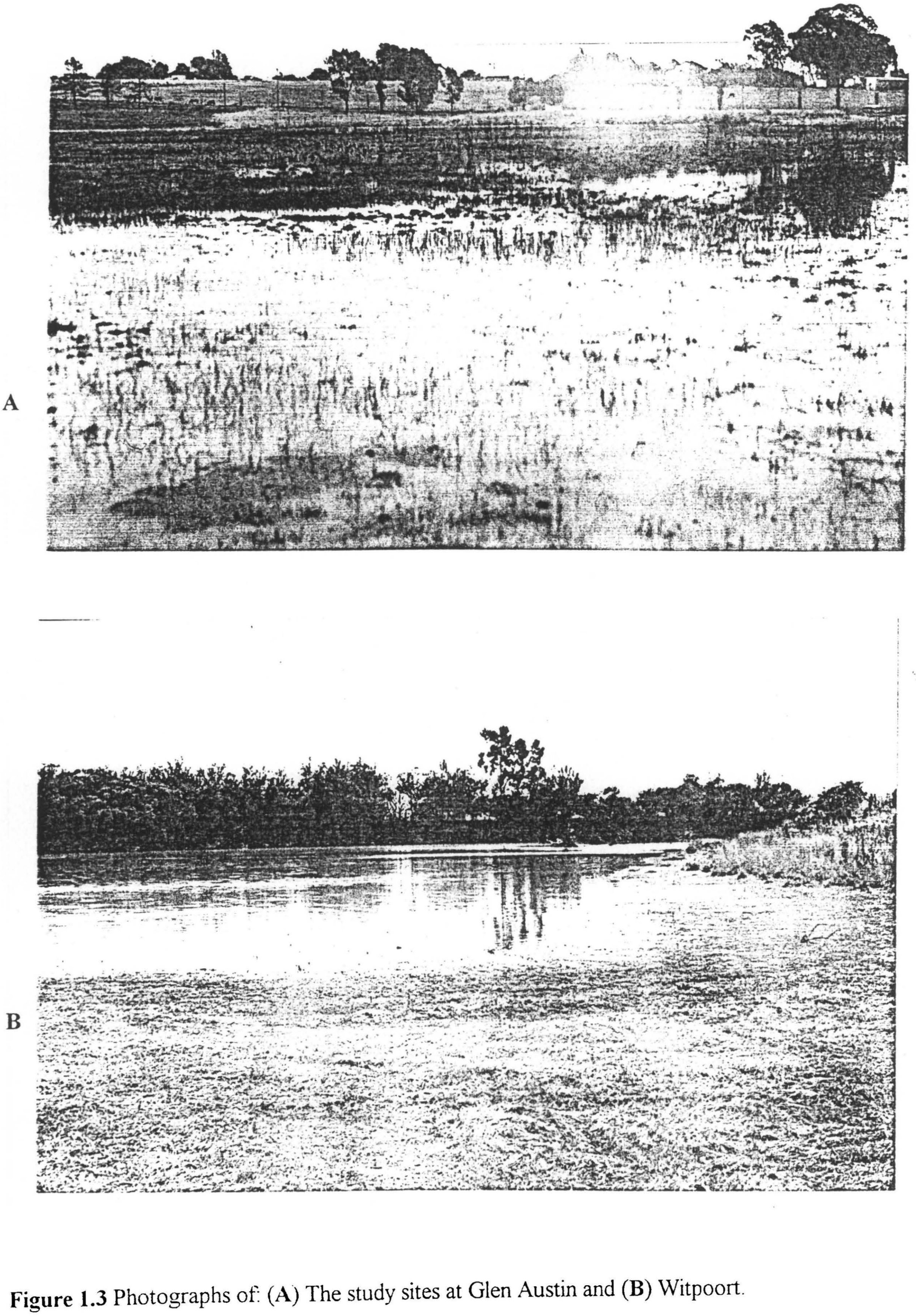


Figure 1.3 Photographs of: (A) The study sites at Glen Austin and (B) Witpoort.

1.4.3 WITPOORT:

The second study site is situated at the Witpoort Bird Sanctuary near Kyalami (25°54`34"S, 28°08'E). The site contains a dam constructed in the 1920's. The dam covers an area of roughly 200 m^2 with a maximum depth of 2m. The dam floods into the surrounding veld during the wet summer months (October-January) providing shallow water (2-4 cm) for the frogs to breed in. A wide variety of grass species occur in the study area, which with various herbaceous plants, has resulted in a stable grassveld. Trees present are largely exotic and provide a variety of habitats for both land and water birds. Rainfall and temperature are similar to Glen Austin. Other frog species breeding at this site were ***Xenopus laevis, Rana angolensis, Schismaderma carens, Cacosternum boettgeri, Bufo rangeri*** and ***Bufo gutturalis.***

1.5 GENERAL METHODS AND MATERIALS

Data presented here result from an intensive observational study conducted during two breeding seasons at Glen Austin and at Witpoort during the following periods, October 1992 to the end of January 1993; October 1993 to the end of January 1994 (Table1.1). Monitoring and collecting individuals in this population during 1992-1994 provided rough estimates of population size and size distribution for each sex. The pan and dam were surveyed for the presence of frogs from the beginning of the first summer rains in October. After the frogs emerged daily observations were made between 0700-1800 hours throughout the breeding season (over 30 days). Typically mating behaviour takes place over a one or two day period. The females and non-territorial males then leave the breeding habitat and only territorial males remain to defend egg clutches until metamorphosis approximately 30 days later.

1.5.1 ADULT POPULATION

Individuals were collected by hand in the bird sanctuaries; along the fence or from the surrounding roads, sexed, measured, marked. Body size (snout-vent lengths: **SVL**) were measured with a plastic ruler to the nearest mm. Right forearm length **(RFA)** was measured to the nearest mm using a dial caliper. Body weight was determined by placing frog in a plastic bag and weighed using a 1000g Pesola spring balanced scale. Frogs were marked for individual identification by heat branding on the dorsum (see Fig.1.4). . Coded brand marks were made using a 1cm branding iron. Branding irons were heated with a gas with a gas flame according to manufactures specifications. Brand marks were treated with an antiseptic cream to prevent infection and frogs were released within 5 minutes of capture. Brand marks had no detrimental or behavioural effect on the frogs as they often resumed calling and fighting shortly after they were marked and returned to the breeding sites. Certain frogs were re-branded depending on the clarity of the brand marks. Branding has been used successfully for other frog species (see Dyson *et al.* 1992, Jennions 1992). Individuals were observed from the shoreline of the pan and dam. Observers minimized movements that would temporarily disrupt breeding activity.

Statistical procedures

The means and distribution of body size measurements of male and female frogs were compared at Glen Austin using analysis of variance (ANOVA) and a two sample T test at Witpoort. An analysis of variance (ANOVA) was used for variation between breeding events and of study site effects. Statistical tests were conducted using Minitab.

Table 1.1 Dates and duration of the breeding events at the two study sites.

BREEDING EVENT	GLEN AUSTIN	WITPOORT
1ST	DECEMBER 23 1992	NOVEMBER 24 1992
2ND	OCTOBER 18-19 1993	OCTOBER 8 1993
3RD	NOVEMBER 24-25 1993	-

Table 1.2 Number of frogs marked during the breeding events

BREEDING EVENT	GLEN AUSTIN	WITPOORT
1ST	251	112
2ND	282	91
3RD	175	-

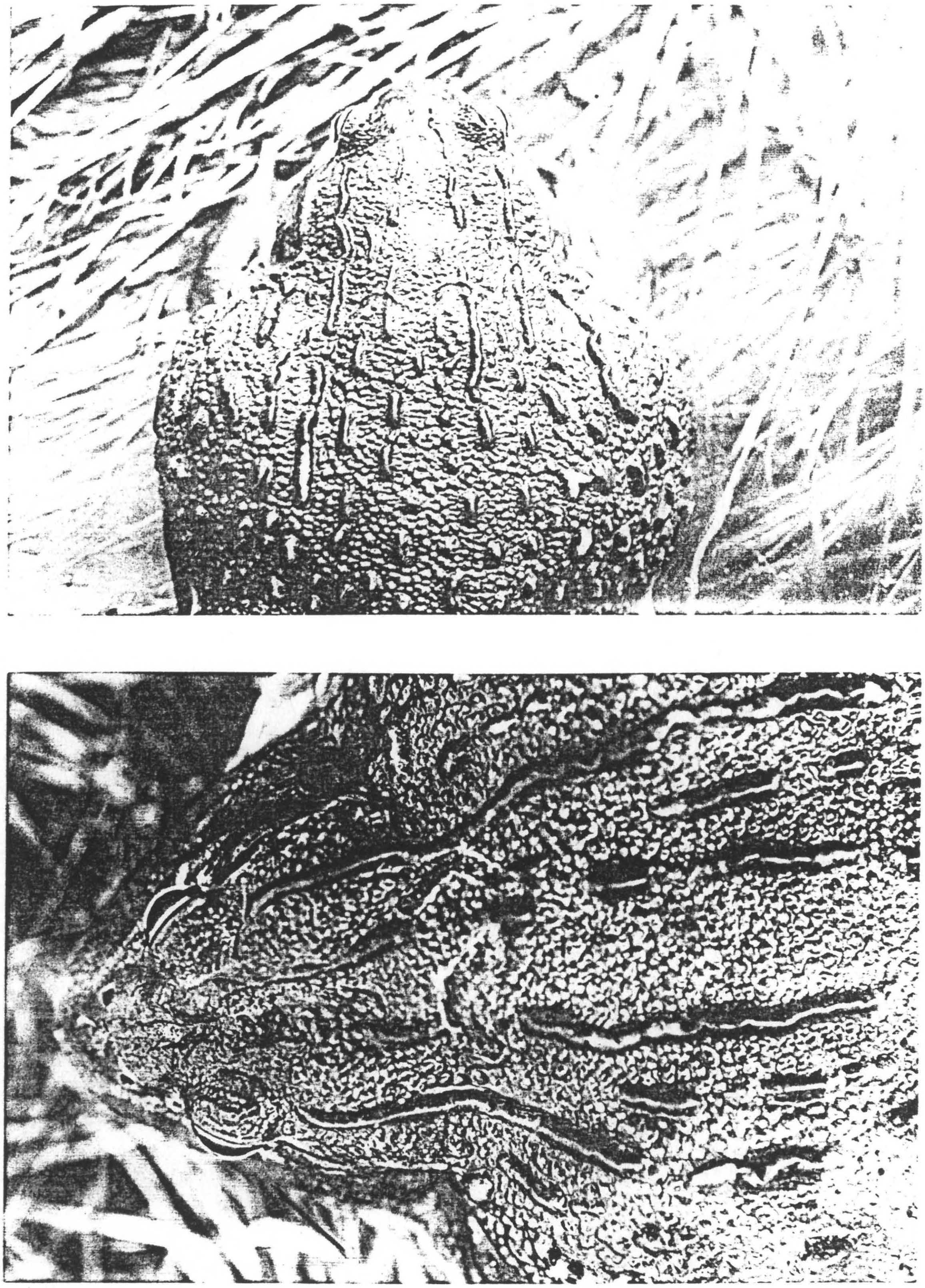


Figure 1.4 Photographs showing brand markings used for individual identification.

General Descriptive Biology of Bullfrog Body Size and Bullfrog Breeding

1.6 EFFECT OF RAINFALL ON BREEDING ACTIVITY:

1.6.1 1992\1993 Breeding Season (1st Breeding Event)

Glen Austin is a temporary rain-filled pan containing water only during the wet summer months between October and February. The pan is a dry grassland area over the winter months and begins filling up after the first heavy summer rains.

Bullfrogs remained buried in the ground waiting for the first heavy summer rains to fall. Frogs bury themselves mainly in the soft soil adjacent to the pan as well as on the sides of the roads. Frogs began to emerge at Glen Austin after 55 mm of rain fell on November 9th, 1992. However the majority of frogs emerged only on the 23rd of November, after a 40 mm downpour lasting less than an hour. Frogs emerged between 2200-0200 hours and over 200 frogs were collected, measured and marked. The frogs migrated to the pan from the surrounding areas adjacent to the pan. The females and smaller males hopped towards the pan while the heavier larger males walked towards the pan. Certain frogs migrated over long distances before reaching the pan and began to settle in the temporary rain-filled puddles adjacent to the pan. Extremely hot conditions (32°C) the following day resulted in the temporary ponds drying up. The frogs re-buried themselves during the day and emerged in the evenings between 2000-0200 hours, presumably to feed in and around the pan. The frogs re-emerged on the 22nd of December 1992, after 52 mm of rainfall and remained inactive in the rain-filled puddles during the day only moving towards the pan in the evening and early morning. The second re-emergence culminated in the first breeding event.

The frogs emerged at Witpoort on the 24th of November 1992, after 46 mm of rainfall. The flooded edges of the dam provided a suitable breeding area for bullfrogs. The main breeding area was a section of the dam that had flooded into the surrounding veld, to a depth of 3-5 cm. Breeding activity commenced at around 0900h and lasted throughout the day.

1.6.2 1993\94 Breeding Season (2nd and 3rd Breeding Events)

Frogs emerged at Glen Austin on the 18th of October 1993. Good early rainfall during October (120mm) resulted in the pan being full to capacity for the first time in over 10 years. Mating activity was initiated and lasted for two days. Frogs bred successfully for the second time on the 24th of November, after 132mm of rain had fallen in the preceding month. Frogs bred at Witpoort on the 8th of October 1993, after 63 mm of rainfall. The frogs bred in the flooded veld area of the dam. Further breeding activity was prevented due to continual rainfall (387 mm) during November-December 1993 flooding the veld area to a depth over 10cm which is unsuitable breeding habitat for the bullfrogs.

1.7 SEXUAL SIZE DIMORPHISM

The mean body measurements for adult male and female ***P.adspersus*** for Glen Austin and Witpoort are summarised in Table 2.1 (see Fig.1.5). Male size (SVL) did not differ significantly during the breeding events at Glen Austin and Witpoort. Males from the 3rd breeding event at Glen Austin had longer forearm lengths and weighed more than males from the first two breeding events (**RFA:**F=3.28, $p<0.05$, **Mass:**F=7.62, $p<0.001$). Female body measurements did not differ significantly between breeding seasons at Glen Austin and Witpoort.

BETWEEN POPULATION COMPARISONS: In both populations adult males and females differed significantly in all body measurements (**Glen Austin SVL:**$F_{5,702}=629.0$, $p<0.0001$; **RFA:**$F_{5,702}=329.84$, $p<0.0001$; **Mass:**$F_{5,702}=436.78$, $p<0.0001$;**Witpoort SVL:**$F_{3,199}=441.45$, $p<0.0001$; **RFA**:$F_{3,199}=286.24$, $p<0.0001$ and **Mass:**$F_{3,199}=476.84$, $p<0.0001$). Males at Glen Austin and Witpoort did not differ significantly in body size measurements during the two breeding seasons (**1992, Two sample test: t**=0.21, **p**=0.83, **df**=191 ; **1993, t**=0.78, **p**=0.44, **df**=134). There was no significant differences in right forearm length during the 1992 breeding season (**t**=1.23, **p**=0.22, **df**=177) but there was significant differences in RFA length during the 1993 breeding season (**t**=2.59, **p**<0.01, **df**=172) with males at Witpoort having significantly longer RFA's. Males at Witpoort were significantly heavier than males at Glen Austin during both the 1992 (**t**=4.81, **p**<0.00001, **df**=201) and the 1993 (**t**=4.27, **p**<0.00001, **df**=147) breeding seasons.

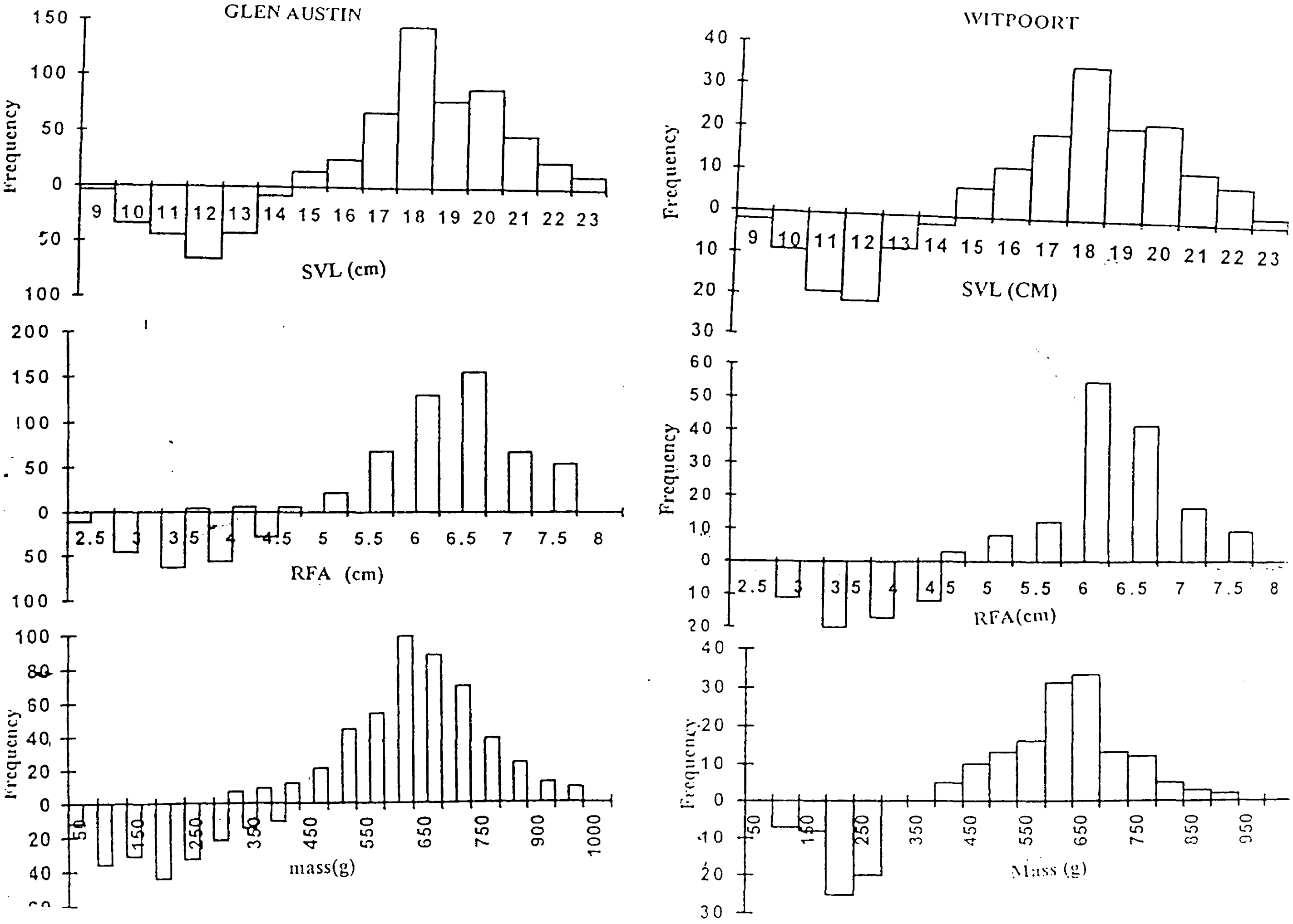


Figure 1.5 Frequency histograms showing distribution of body measurements for adult male and female bullfrogs at Glen Austin and Witpoort during the two breeding seasons.

ORGANISATION OF THIS DISSERTATION :

Chapter one is a general introduction to the study species, study sites and general methods chosen for this project. As bullfrogs are extreme explosive breeders, the effects of rainfall on breeding activity were determined. To show that bullfrogs are highly sexually dimorphic, male and female body sizes are compared. Chapter two examines the social organisation in bullfrogs investigating male-male aggressive interactions, intermale spacing and territoriality. Adult male bullfrogs exhibit three sized related mating strategies namely territorial, non-territorial and satellite behaviour. Territorial males were the largest, most aggressive males in the population, non-territorial males tended to be intermediate in size or large males that could not successfully defend a territory. Satellite males were the smallest adult males adopting a concealed position close to a territorial male. Chapter three discusses the mating success of the various tactics. Chapter four deals with female choice and female fecundity. Territorial males compete for females either directly, by defending the females themselves, or indirectly, by defending resources required by females. The hypothesis of female choice does not adequately explain the results of this study concerning non-territorial males as males initiate and determine the eventual outcome of mating success through amplexus displacement. Chapter five examines the environmental effects on developmental rates and size at metamorphosis of tadpoles. Two distinct phenotypes of tadpoles occur to ensure survival in these ephemeral habitats. Chapter six involves experimental work on environmental effects on tadpole metamorphosis as well as temperature tolerance experiments. Tadpoles are adapted to live in these shallow temporary ponds with extremely high water temperatures. The effects of male parental care on offspring survivorship is also investigated in the field. Chapter seven investigates kin recognition in bullfrog tadpoles, with tadpoles discriminating between siblings and non-siblings in the laboratory.The findings in this study provide a preliminary introduction to the reproductive behaviour of a previously little-studied species. Further studies of *P.adspersus* populations may provide added insight into the reproductive strategies .

CHAPTER TWO

ALTERNATIVE MATING TACTICS

2.1 INTRODUCTION

2.1.1 TERRITORIAL AND AGGRESSIVE BEHAVIOUR

Aggressive behaviour related to site-specific territoriality has been reported in a variety of anuran species. The adaptive significance of territoriality lies in the benefits that accrue to territorial animals by virtue of their access to resources in short supply (Telford 1982; Krebs & Davies 1991). Here it becomes important to distinguish between spacing as a result of site-specific territoriality and spacing which arises from the maintenance of an individual distance (Telford 1982; Krebs & Davies 1991). Site-specific territoriality occurs when intolerance of other individuals centres around particular locations associated with resources (Wells 1977b, Krebs & Davies 1991). Individual distance is a result of intermale spacing without site attachment (Telford 1982; Crump 1988).

Anuran territoriality involves site attachment as well as intolerance of intruders (Stewart & Rand 1991; Wagner 1992). The particular location (territory) is defended with aggressive behaviour and may contain one or more of the following resources required for survival and/or reproduction: oviposition site; courtship area; feeding site; shelter; source of moisture (Wells 1977b; Wagner 1992). In some cases it may give a male direct access to females in an area and enhance his attractiveness to prospective mates in terms of offspring survival and/or increased audibility (Wells 1977b; Howard 1978). Although it is often difficult to distinguish between the maintenance of a territory and that of individual distance, the two types of behaviour may have different functions (Wells 1977b). Individual distances are maintained through hostility without site attachment. Males may occupy different call sites on different nights and there is no defence of a resource which is in short supply (Whitney and Krebs 1975). It may also reduce the number of competitors by preventing nearby males from calling; males would not compete for calling sites but rather for the opportunity to call (Wells 1977b; Haddad

1991). Both territoriality and the maintenance of an individual distance would limit the density of calling males and may force 'excess' males to adopt alternative mating strategies or to establish sub-optimal call sites (Krupa 1989).

Several males may try to mate with a single female, struggling with one another and attempting to dislodge the male most securely fixed to the female back (Wells 1977a).
In the African Bullfrog, ***Pyxicephalus adspersus***, males fight violently among themselves for possession of females and territories (Balinsky & Balinsky 1954).

2.1.2 ALTERNATIVE MATING TACTICS

In anurans, the duration of the breeding season partially determines the intensity of male-male competition and hence the types of alternative mating tactics that are possible. There are clear differences in both the frequency and types of alternative mating tactics exhibited in prolonged and explosive breeders, even when they are different populations of the same species (Tejedo 1988).

The most frequently documented alternative tactics are: ***satellite behaviour***, where non-calling males attempt to intercept females attracted to calling males (Perrill *et al.* 1978); ***amplexus displacement***, where unpaired males attempt to displace amplexing males (Telford & Van Sickle 1989); and ***active searching*** by silent males who clasp any individual they encounter during their searches (Woolbright *et al.* 1990).

Alternative reproductive tactics are usually a consequence of male-male competition for a limited number of females. Competition may limit the number of males that employ the dominant mating tactic, forcing some males to adopt alternative tactics with lower reproductive success (Kodric-Brown 1986). Generally, alternative reproductive tactics yield lower pay-offs than the dominant tactic, although tactics with equal pay-off (Gerhardt et al. 1987) and even higher pay-offs (van den Berghe 1988) have also been reported. Tactics may be reversible or fixed, and either opportunistic, conditional or part of a mixed Evolutionary Stable Strategy/State (Austad 1984). In most anurans, alternative tactics are conditionally employed by smaller males (Howard 1978). Fixed tactics have not been recorded in anurans, but there is often considerable intra-population variation in male size, and tactics are often size-based. Males may be limited to a single tactic for an entire breeding season (Howard 1984). Wilbur *et al.* (1978) argue that females should select the largest available males because large males, on the average, will be older and consequently have demonstrated their ability to survive, and/or large size may reflect rapid growth rates, which may be a demographic advantage.

The aim of this chapter is to examine the social organisation in ***Pyxicephalus adspersus*** with reference to intermale spacing and territoriality.

The major objectives of this chapter are:

1. To determine the patterns of spacing, and/or aggregation of males.

2. To determine whether spacing is a result of territoriality or the maintenance of an individual distance.

3. To document the occurrence of male-male aggressive interactions.

4. To determine the effects of male-male interactions on conspecific males.

5. To document whether amplexus displacement occurs and, if so, whether size influences displacement success.

2.2 METHODS

2.2.1 MALE ARRIVAL PATTERNS : The Glen Austin pan was monitored daily between 0700 and 1800 hours throughout the wet season between October and January 1992-1993 and 1993-1994 (1st,2nd and 3rd breeding events) for the presence of frogs. After the emergence of frogs, individuals were sexed, measured, marked and released, usually within 5 min of capture (see General Methods, Chapter 1). Behavioural data on males were obtained from focal-animal samples. After obtaining multiple focal-animal samples of most males, male movement patterns were determined and the settlement patterns documented. Time of arrival was recorded as well as the location of the territory, territory size, water depth at the territory, water temperature and nearest neighbour distance.

Glen Austin pan was divided into three regions: the southern, western and northern sections (Fig.2.1) based on the settlement patterns of the frogs, the nature of the vegetation, and variation in water depth. Witpoort was similarly monitored daily throughout the wet summer months of 1992 and 1993.

2.2.2 **TERRITORY SIZES:** location and area occupied by territorial males was recorded at the two study sites for the two breeding seasons. A territory is defined as a general area exclusively used and defended by a specific male. The Territories at Glen Austin (Fig.2.1) were situated on the west side of the pan. Territory length and breadth were measured using a flexible 30m tape measure and as the territories were circular/oval in shape; territory area was calculated as πr^2. Territory position and number was marked using bamboo stakes. At Witpoort the territories were situated in the shallow flooded veld area (Fig.2.2) Territories were located along the bank of the dam where water depths did not exceed 10 cm. Territory lengths were measured but territorial borders were not clearly definable. These territories are best described as linear.

2.2.3 NEAREST NEIGHBOUR DISTANCES: Nearest neighbour distances were calculated as the shortest distance from the centre of each territory to the centre of the nearest adjacent territory. Measurements were made using a flexible 30 m tape measure which allowed accuracy to the nearest 1 cm.

2.2.4 MALE-MALE AGGRESSIVE ENCOUNTERS: Observations of male-male aggressive interactions were conducted at both Glen Austin and Witpoort. Fighting was studied by direct observation of aggression towards conspecific males. Aggression was defined as any attempt to displace another male from his territory or place in the aggregation, as well as any attempt to take over a female by dislodging the first paired male. Fights were easily located because attacked males repeatedly utter vocalizations audible over a distance of 50 metres. The durations of fights were recorded using a stopwatch and all participating individuals were monitored. Territorial displacements were recorded with the eventual outcome noted. Aggressive encounters involving non-territorial males were determined as the number of aggressive interactions per male during single hour observations at each aggregation during each breeding event. When amplexus displacement attempts were observed, the participants were identified, and the duration and final outcome of the interaction noted. Aggressive interactions involving satellite males and resident territorial males were also documented. Where possible, individuals involved in aggressive interactions were identified for later body size comparisons as well as comparisons between breeding events.

2.2.5 MALE RESIDENCY: Territories were monitored daily between 0700-1800 hours throughout the breeding season. The presence or absence of the territorial resident male was monitored and duration of residency noted.

2.2.6 MALE BEHAVIOUR: Territorial males were males that actively defended specific sites from intruding conspecific males during the entire, or if displaced, part of the breeding event (Fig.2.3). A male was considered a territorial resident if he was positively identified in a certain territory during the entire breeding event. Territorial residency was also noted and if the territorial resident was displaced the new resident was identified. A male was considered non-territorial if he wandered, or had no definite site attachment during the breeding event and migrated out of the pan after the cessation of breeding activity (Fig.2.4). Satellite males were defined as non-calling, non-aggressive individuals positioned within 2.5 m of a territory holder. Satellite males were easily recognised by the `low' posture they adopt close to resident males.

2.2.7 DESCRIPTION OF DIFFERENT TYPES OF AGGRESSIVE INTERACTIONS

The major behaviour patterns observed were operationally defined as follows.

(1) **Passive**: frogs located within 1.5m of each other ignored or merely orientated towards each other, but did not advance towards each other (**n**=284).

(2) **Chasing**: one frog followed another individual at a speed faster than the normal walking speed (**n**=552).

(3) **Pouncing**: one frog lept towards another and butts the frog away (**n**=564).

(4) **Flinging**: one male grabs another male by the forelimb and flings it backwards, flinging continues until one frog is flung out of the territory (**n**=24).

(5) **Amplexus Displacement**: amplexing pair is attacked by surrounding males which attempt to displace the amplexed male (**n**=404).

(6) **Wrestling and Biting**: Frogs wrestle amongst each other and bite intruders (**n**=212).

(7) **Vocalisation**: relatively intense ‘whoop’ vocalisation given by either male (intruder or resident) during male-male interactions. The call was given before any physical contact occurred, but was also given by the apparent winner of an interaction. The calling

male inflated its body to increase its size; raised itself out of the water with its hindlimbs and walked towards the intruder emitting the call. A shorter encounter call was also documented, a `whoh-whoh' call emitted just before an aggressive encounter.

Statistical procedures

The means and distribution of body size measurements of territorial, non-territorial and satellite males were compared at Glen Austin using analysis of variance (ANOVA). A two sample T test was conducted between territorial and non-territorial males at Witpoort. An analysis of variance (ANOVA) was used for variation between breeding events and of study site effects. A Pearson's correlation coefficient between body size measurements (SVL, mass) and territory area was used at Glen Austin and Witpoort. A two sample T test between males successfully displacing territorial residents were conducted. Statistical tests were conducted using Minitab.

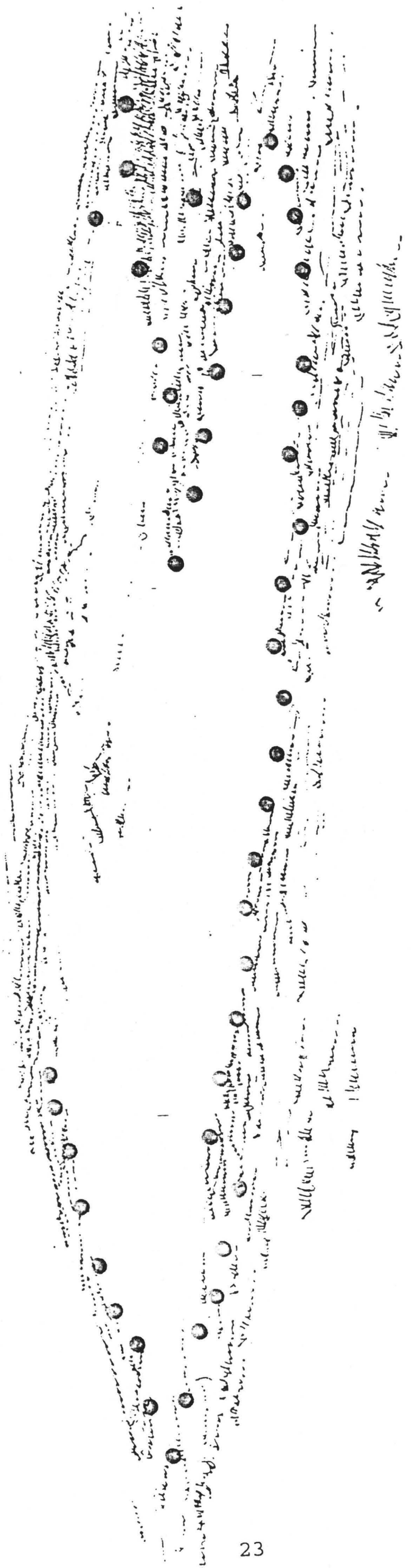


Figure 2.1 Locations of territories at Glen Austin during the two breeding seasons.

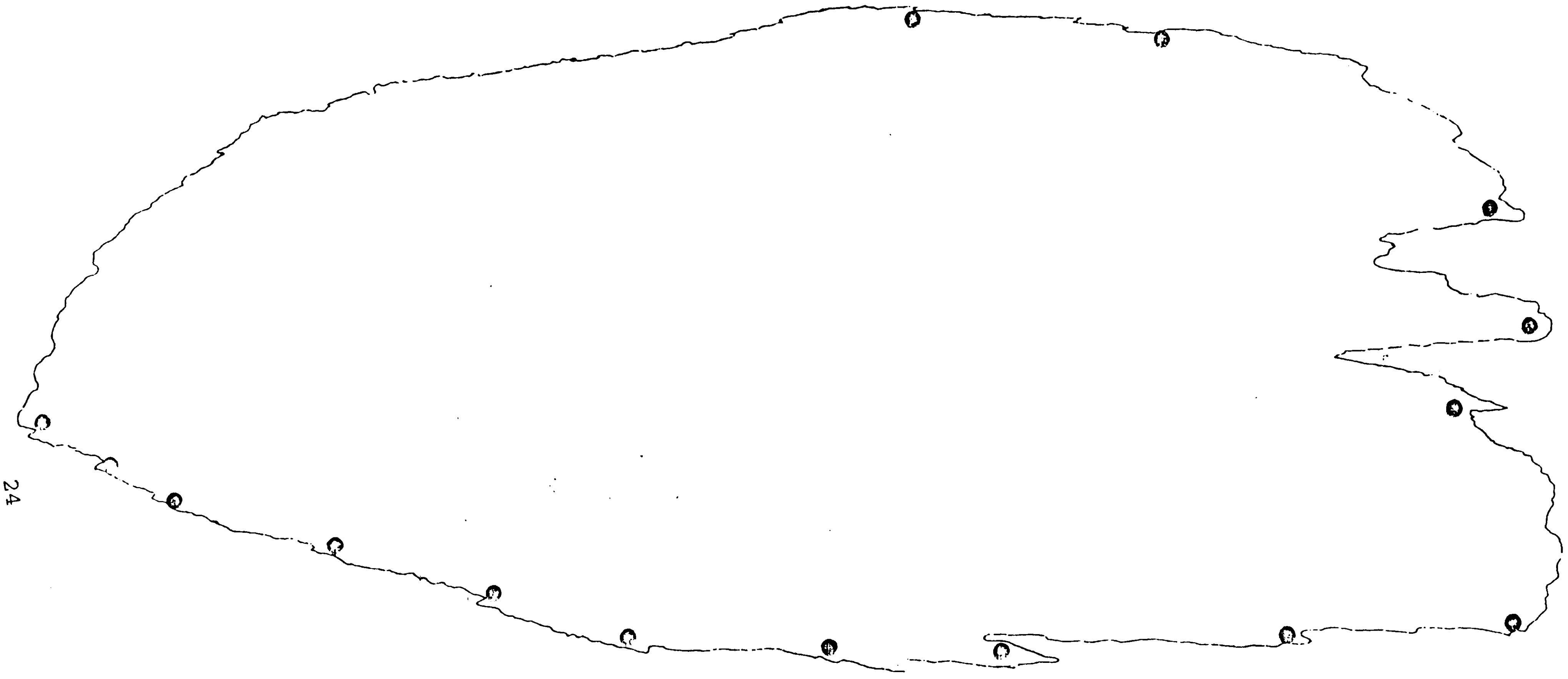


Figure 2.2 Locations of territories at Witpoort during the two breeding seasons.

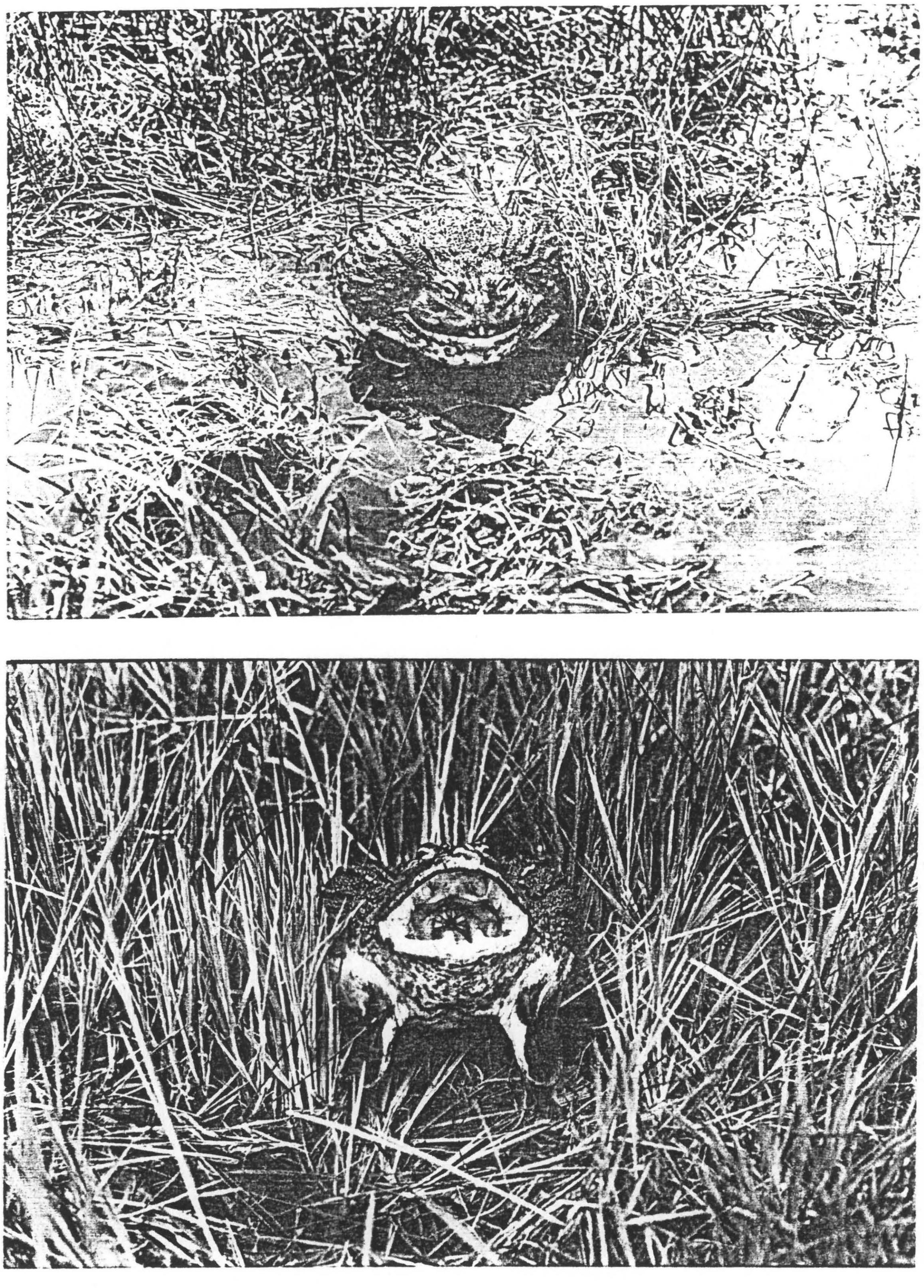


Figure 2.3 Photographs of resident territorial males showing typical aggressive postures

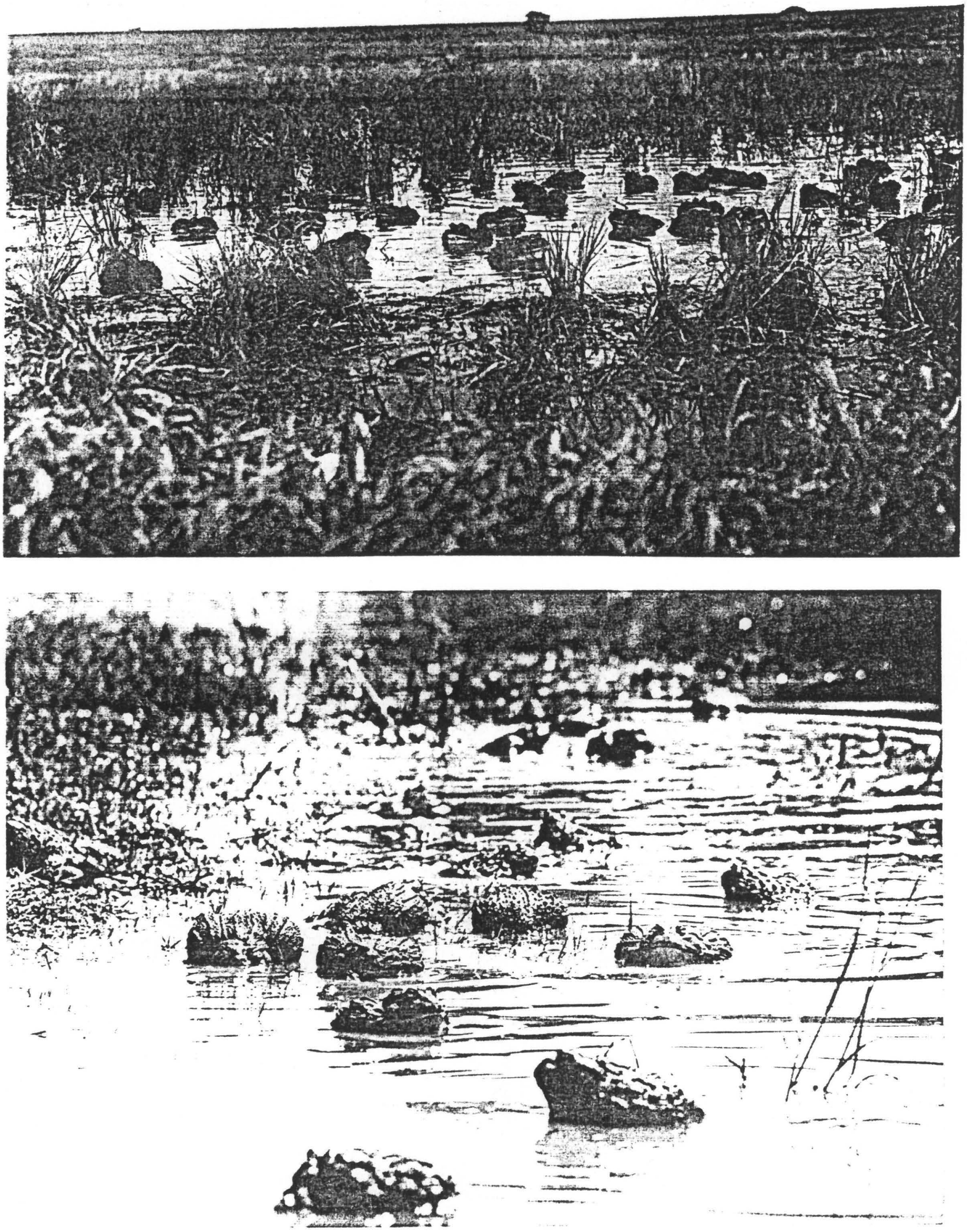


Figure 2.4 Photographs of aggregates of non-territorial males in their breeding arenas.

2.3 RESULTS:

2.3.1 FORMATION AND SETTLEMENT OF TERRITORIES

Frogs at Glen Austin settled and established territories in the shallow (2-4.5cm) less densely vegetated western section first, followed by the northern and southern sections. The northern and southern sections are characterised by dense sedge (*Scripus spiciformes)* growth and water depths of between 5-12 cm. The sedges provides a shadow effect due to their dense growth and provides suitable habitat for concealment of satellite males. The western sections are characterised by clumps of sedges surrounded by short grasses. The eastern section of the pan is characterised by denser sedge growth and deeper water (>20cm) making it unfavourable bullfrog breeding habitat.

2.3.2 BODY SIZE MEASUREMENTS

TERRITORIAL MALES

Territorial male body size measurements (**SVL, RFA** and **Mass**) for both Glen Austin and Witpoort during the 1992-93 and 1993-94 breeding season are summarised in Table 2.1. There was no significant differences in all body size measurements of territorial males between breeding events at both Glen Austin and Witpoort.

Between Population Comparison: There were no significant differences in territorial male body size measurements at the two study sites during the three breeding events (**SVL:**$F_{4,131}$=0.30, p=0.875; **RFA:**$F_{4,131}$=2.31, p=0.063 and **Mass:** $F_{4,131}$=2.07, p=0.088).

NON-TERRITORIAL MALES

Non-territorial male body size measurements for Glen Austin and Witpoort are summarised in Table 2.1. Non-territorial males did not differ in all body measurements between breeding seasons at Glen Austin and Witpoort.

Table 2.1 Mean body size measurements (± 1 SD) of territorial, non-territorial, satellite males and females over two seasons, at Glen Austin and Witpoort. Ranges in brackets. Statistical tests (* Anova, F-value; ** T test, t-value)

	Study Site	Breeding Event	(n)	Mean SVL (cm)	Mean RFA (cm)	Mean Mass (g)	Stats. SVL	RFA	MASS	P
Males:	Glen Austin	1st 23 Dec 92	26	20.1 ± 1.3 (18.1-22.5)	6.6 ± 0.6 (5.5-7.5)	617.3 ± 123.5 (450-970)				
	Glen Austin	2nd 18-19 Oct 93	48	20.3 ± 1.5 (16.5-22.7)	6.8 ± 0.4 (5.8-7.6)	687.8 ± 98.3 (480-910)				
Territorial	Glen Austin	3rd 24-25 Nov 93	36	20.4 ± 1.4 (16.5-22.7)	6.8 ± 0.7 (5.8-7.6)	700.6 ± 96.8 (480-970)	2.48 *	1.98 *	2.78 *	>0.05
	Witpoort	1st 24 Nov 92	15	18.8 ± 1.3 (17.5-22.7)	6.6 ± 0.5 (5.7-7.6)	632.0 ± 88.3 (520-900)				
	Witpoort	2nd 8 Oct 93	11	20.2 ± 1.3 (18.7-22.6)	6.9 ± 0.2 (6.7-7.3)	724.5 ± 59.7 (670-870)	0.98 **	1.16 **	1.34 **	>0.05
Non-territorial	Glen Austin	1st 23 Dec 92	146	18.04 ± 1.3 (13.7-21.5)	5.9 ± 0.7 (4.2-7.3)	525.7 ± 86.6 (450-970)				
	Glen Austin	2nd 18-19 Oct 93	134	17.98 ± 1.5 (16.0-21.5)	6.1 ± 0.6 (4.9-7.3)	542.6 ± 88.5 (400-770)				
	Glen Austin	3rd 24-25 Nov 93	96	18.01 ± 1.0 (16.5-22.1)	5.8 ± 0.8 (5.8-7.6)	550.3 ± 83.4 (400-770)	1.86 *	2.14 *	2.31 *	>0.05
	Witpoort	1st 24 Nov 92	61	18.2 ± 1.1 (15.9-22.3)	6.3 ± 0.5 (4.3-7.2)	586.9 ± 70.8 (400-830)				
	Witpoort	2nd 8 Oct 93	56	18.1 ± 1.3 (16.1-20.1)	6.9 ± 0.5 (5.0-7.1)	597.3 ± 80.3 (430-820)	2.06 **	0.52 **	0.32 **	>0.05
Satellite	Glen Austin	1st 23 Dec 92	18	16.0 ± 0.3 (15.2-16.5)	3.9 ± 0.1 (3.4-4.5)	377.2 ± 26.1 (320-430)				
		2nd 18-19 Oct 93	6	16.5 ± 0.2 (16.5-22.7)	5.4 ± 0.4 (4.8-5.7)	460.3 ± 24.5 (430-490)	5.13 **	6.61 **	6.08 **	<0.001
Females:	Glen Austin	1st 23 Dec 92	79	11.5 ± 1.1 (9.5-14.1)	3.7 ± 0.5 (2.5-5.1)	165.3 ± 61.8 (60-400)				
	Glen Austin	2nd 18-19 Oct 93	82	11.8 ± 0.7 (10.1-13.2)	3.8 ± 0.5 (3.0-4.6)	180.1 ± 42.5 (100-310)				
	Glen Austin	3rd 24-25 Nov 93	43	20.4 ± 1.4 (10.0-12.7)	3.6 ± 0.4 (3.0-4.5)	173.5 ± 46.8 (90-290)	0.78 *	1.51 *	1.71 *	>0.05
	Witpoort	1st 24 Nov 92	36	11.8 ± 1.1 (10.2-12.6)	3.9 ± 0.3 (3.2-4.5)	183.4 ± 29.2 (130-260)				
	Witpoort	2nd 8 Oct 93	24	11.95 ± 0.8 (10.9-12.7)	3.9 ± 0.4 (3.1-4.7)	186.1 ± 34.1 (100-240)	1.60 **	0.04 **	0.18 **	>0.05

Between Population Comparison: Body size measurements of non-territorial males did not differ significantly between populations during the breeding events (**SVL:** $F_{4,482}=0.92$, $p=0.454$; **RFA:** $F_{4,482}=1.1$, $p=0.356$) but there was significant differences in body mass ($F_{4,482}=10.88$, $p=0.0001$). Witpoort's non-territorial males were significantly heavier than Glen Austin's non-territorial males during the 1st ($t=4.70$, $p=0.00001$,**df**=104) and the 2nd breeding event ($t=4.64$, $p=0.00001$, **df**=102).

SATELLITE MALES

No satellite males were present at Glen Austin during the 1992 (1st) breeding season. Body measurements of satellite males are summarised in Table 2.1. Satellite males present during the 3rd breeding event were significantly larger in all body measurements (**SVL:**$t=5.13$, $p=0.0015$, **df**=19; **RFA:**$t=6.61$, $p=0.0070$, **df**=14 and **Mass:**$t=6.08$, $p=0.0037$, **df**=16) compared to satellite males present during the 2nd breeding event.

No satellite males were present at Witpoort during the 1992-93 and 1993-94 breeding seasons as the dam edges are sparsely vegetated and satellite males would be clearly visible to territory holders.

2.3.3 COMPARISON BETWEEN TERRITORIAL, NON-TERRITORIAL AND SATELLITE MALES

At Glen Austin there was significant differences in all body measurements (SVL, **RFA** and **Mass**) between territorial, non-territorial and satellite males in all breeding events (**SVL:**$F_{1,171\text{-}2,197}\geq 40.29$, $p=0.0001$; **RFA:**$F_{1,171\text{-}2,197}\geq 28.66$, $p=0.0001$; **MASS:**$F_{1,171\text{-}2,197}\geq 15.2$, $p=0.0001$). Territorial males were significantly the largest, heaviest adult males in the populations. Non-territorial males were average sized males while satellite males were the smallest adult males in the population. However the measurements for frogs at Witpoort were not so conclusive although there was no significant difference in snout-vent length (SVL) between territorial and non-territorial males during the first breeding event($t=3.26$, $p=0.076$), territorial males had larger right forearm lengths (RFA) and increased mass ($t=5.44$, $p=0.023$ and **MASS:** $t=4.28$, $p=0.043$). Territorial males were significantly larger than non-territorial males in all body measurements during the second breeding event at Witpoort (**SVL:** $t=5.50$, $p=0.0001$; **RFA:** $t=9.11$, $p=0.00001$ and **MASS:** $t=6.07$, $p=0.00001$).

2.3.4 TERRITORY SIZES AND NEAREST NEIGHBOUR DISTANCES

Data on territory size and nearest neighbour distances appear in Appendix I. The mean territory sizes and nearest neighbour distances for Glen Austin and Witpoort are summarised in Table 2.3. There was a significant positive relationship between male size and territory area for both populations during the two breeding seasons (**Pearson's Correlation Coefficient, r**=0.536-0.793, **n**=11-48, **p**<0.01, Fig. 2.5) and between body mass and territory area (r=0.263-0.733, n=11-48, p<0.05, Fig. 2.6). There was no correlation between territory area or nearest neighbour distances and frequency of occurrence of aggressive interactions. There was significant differences in territory areas, territory depths and nearest neighbour distances between breeding events at Glen Austin and Witpoort (**Glen Austin Area:** $F_{2,107}$=8.28, p=0.0001; **Depth:** $F_{2,107}$=5.65, p=0.005; **Nearest neighbour distances:** $F_{2,107}$=3.85, p=0.024; **Witpoort Area:** t=1.74, **df**=23, p=0.095; **Depth:** t=2.01, p=0.057, **df**=23 and **Nearest neighbour distances:** t=0.01, p=0.99, **df**=16).

Table 2.2 Mean territory sizes and nearest neighbour distances over two seasons at Glen Austin and Witpoort. Ranges in brackets.

Breeding Event	Number of Males	Territory Area (m^2)	Nearest Neighbour Distance (m)
Glen Austin (1st) Oct 92	26	1.8 (0.95-4.5)	1.32 (0.8-3.7)
(2nd) Nov 93	48	2.1 (1-5.4)	1.39 (1.-5.4)
(3rd) Dec 93	36	1.4 (0.79-2.5)	1.97 (1.-8.7)
Witpoort . (1st) Oct 92	15	1.96 (1.6-2.4)	7.81 (2.1-28)
(2nd) Dec 93	11	2.1 (1.9-2.4)	7.75 (2.1-43.2)

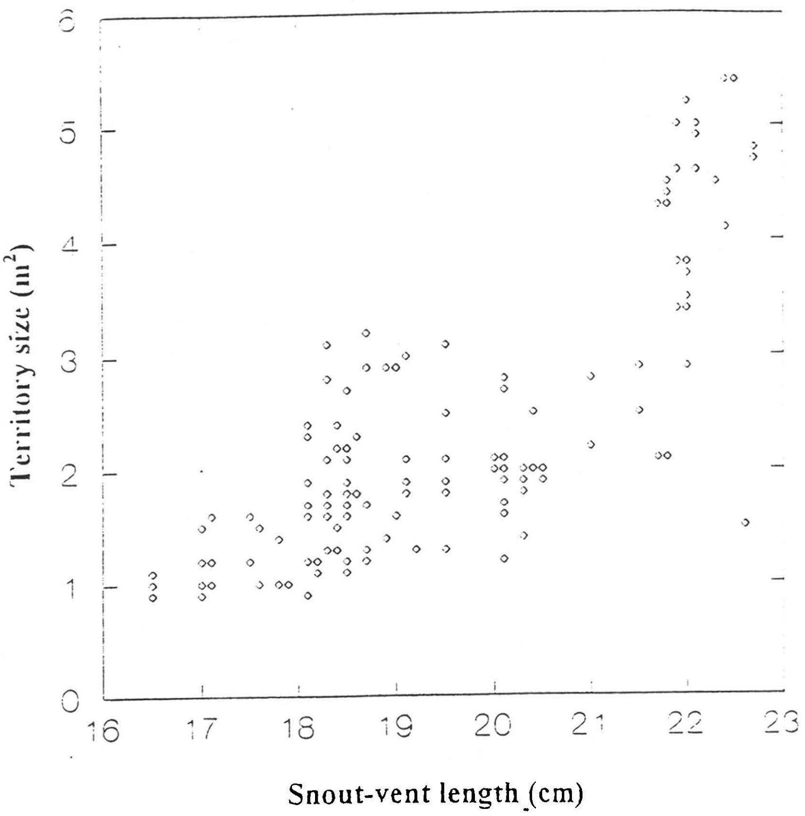


Figure 2.5 The relationship between male snout-vent length and territory size at Glen Austin and Witpoort.

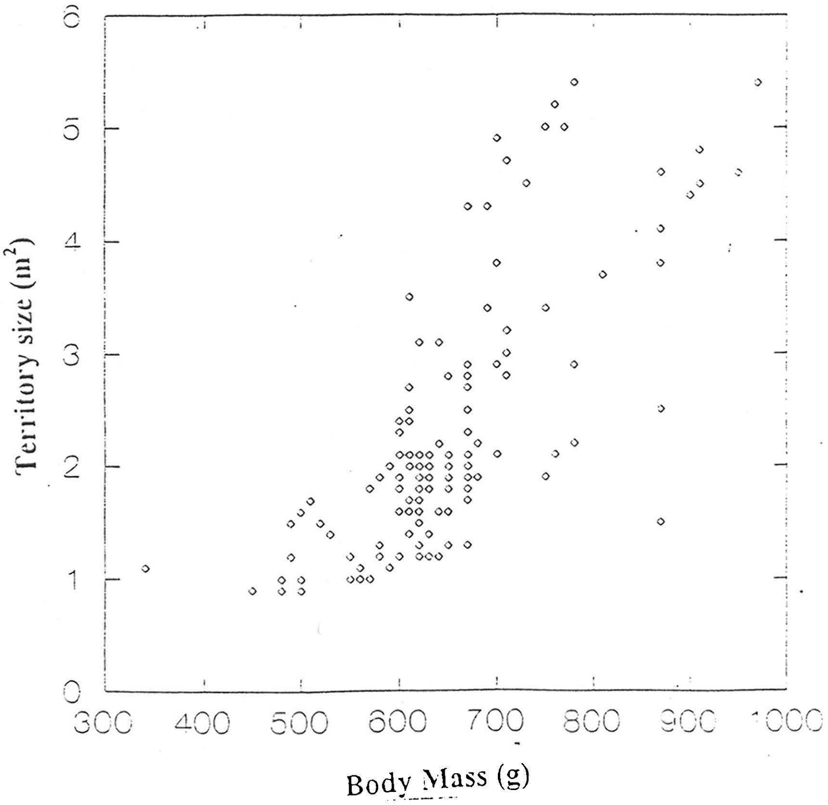


Figure 2.6 The relationship between male body mass and territory size at Glen Austin and Witpoort.

Between Population Comparison: Territories at Witpoort are significantly larger (**$F_{4,131}$**=5.55, **p**=0.0001) and deeper (**$F_{4,131}$**=19.71, **p**=0.0001) than those at Glen Austin. Nearest neighbour distances at Glen Austin are significantly smaller than those at Witpoort (**$F_{4,131}$**=10.23, **p**= 0.0001).

2.3.5 FREQUENCY OF OCCURRENCE OF AGGRESSIVE INTERACTIONS

TERRITORIAL MALES

A male was scored as aggressive if he exhibited chasing, pouncing, wrestling or flinging behaviour. Aggressive males frequently emitted territorial calls and exhibited the aggressive raised posture. Competition for territories was intense. Males that had already established territories engaged in aggressive encounters with migrating intruding frogs. Frogs moved through the territories and settled in areas away from the nearest territorial male. The frequency and duration of territorial male aggressive interactions are given in Table 2.3. Territorial males at Glen Austin (**x**=20.3) were significantly larger than intruding males (**x**=17.8; **t**=8.85, **p**=0.00001, **df**=66) and won 91% of the aggressive interactions. Seventeen territorial displacements were observed, with the original territorial males (**x**=21.2) being displaced by significantly larger males (**x**=18.1; **t**=3.53 , **p**<0.024, **df**=3-9). There was no significant differences in frequency of occurrence of aggressive interactions between breeding events at Glen Austin (**$F_{2,107}$**=0.97, **p**=0.382).

The resident territorial males (**x**=19.5) at Witpoort won 94% of aggressive interactions and were significantly larger than the intruding males (**x**=17.8; **t**=4.61, **p**<0.0006, **df**=19).

Between Population Comparison: Territorial males at Witpoort were involved in significantly more aggressive interactions (**x**=3.4) than territorial males at Glen Austin (**x**=2.0; **t**=3.52, **p**=0.009).

NON-TERRITORIAL MALES: The mean number of non-territorial aggressive interactions at Glen Austin and Witpoort are summarised in Table 2.3.

Between Population Comparison: There was no significant differences in frequency of occurrence of aggressive interactions between non-territorial males at Glen Austin (**x**=3.9) and Witpoort (**x**=3.6, **t**=0.87, **p**=0.76)

Table 2.3 Mean number of aggressive interactions for territorial and non-territorial males, per single hour observations, at Glen Austin and Witpoort during the 1992-93 and 1993-94 breeding seasons. Ranges in brackets.

	TERRITORIAL MALES		**NON-TERRITORIAL**
Breeding Event	**Mean Number of Aggressive Interactions per Male**	**Mean Duration (s)**	**Mean Number of Aggressive Interactions per Male**
Glen Austin 1st Dec 92	2.2 (0-7)	439	3.8 (0-12)
2nd Oct 93	1.8 (0-8)	470	4.2 (0-15)
3rd Nov 93	1.9 (0-8)	458	3.8 (0-11)
Witpoort 1st Nov 92	2.6 (0-9)	414	3.5 (0-15)
2nd Oct 93	4.2 (1-7)	357	3.6 (0-13)

2.3.6 FREQUENCY OF OCCURRENCE OF AGGRESSIVE INTERACTIONS IN TERRITORIAL MALES COMPARED TO NON-TERRITORIAL MALES

Non-territorial males at Glen Austin (x=3.9) have a significantly higher frequency of occurrence of aggressive interactions than territorial males (x=1.96, t=7.68, p=0.0001). However there was no significant differences in frequency of occurrence of aggressive interactions between territorial (x=3.4) and non-territorial males (x=3.6) at Witpoort (t=1.02, p=0.41)

TERRITORIAL MALES

Aggressive interactions include both vocalisations and physical combat and follow a series of stages leading either to escalation or to termination of the encounter (see Fig.2.7). Encounters are always initiated when an intruding male moves into a resident male territory. The resident responds with an inflated raised posture and encounter (territorial) calling which may elicit a reciprocal response from the intruder. The raised inflated posture which accentuates the male's deep yellow throat and bright orange armpits may act as a visual deterrent for the intruding male. The intruder either continues with the aggressive interaction or vacates the territory terminating the aggressive interaction (Fig2.7).

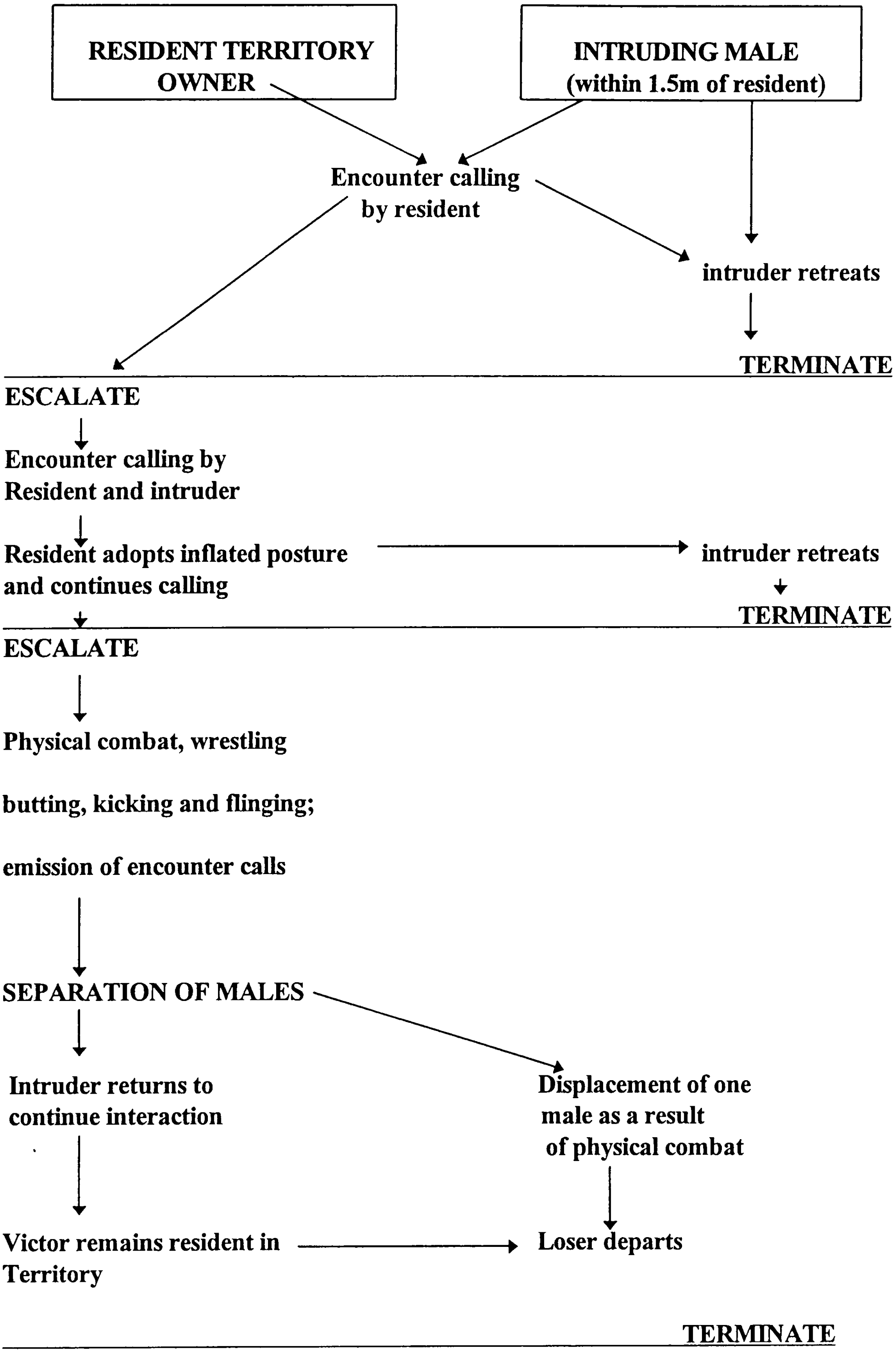
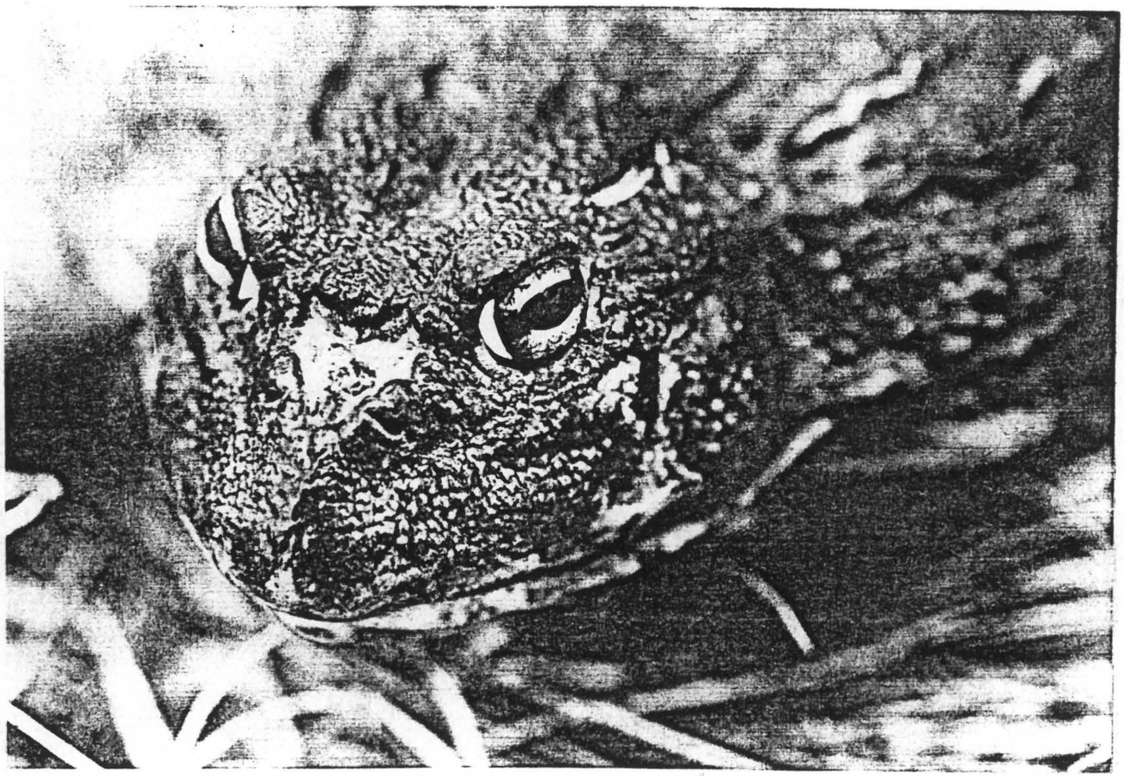


Figure 2.7 Flow diagram of aggressive interactions between territorial ***P.adspersus*** males.



igure 2.8 A photograph showing a territorial male with severe facial scars.

NON-TERRITORIAL MALES

Up to 60 males that were unable to find a suitable territory aggregated in an arena of some $20m^2$ at the periphery of the area occupied by territorial males. Males were individually spaced between 1-5m apart and fought amongst each other for a place in this arena. Fighting was frequent and less intense than territorial encounters. Frogs mainly lunged and butted each other the breeding arena. Males called continuously during aggressive encounters. Due to numerous aggressive interactions between the males in these areas, the surrounding vegetation is flattened completely forming a 'breeding arena'. Non-territorial breeding arenas are up to 20 m^2 in diameter. Non-territorial males adopt a form of 'scramble competition' in acquiring mates. Males rush towards females and attempt to amplex with them. Amplexus displacement attempts are numerous with single males attempting to dislodge the amplexed male.

2.3.8 AMPLEXUS DISPLACEMENT

Successfully displacing males were significantly larger, and heavier than displaced males during all the breeding events at Glen Austin and Witpoort (Table 2.6).

Table 2.6 Observed amplexus displacement attempts at Glen Austin and Witpoort during the 1992/93 and 1993/94 breeding seasons.

Breeding Event	**Amplexus displacemen Attempts (n)**	**Displacing Male SVL (cm)**	**Displaced Male SVL (cm)**	**t**	**p**	**Displacing Male Mass (g)**	**Displaced Male Mass (g)**	**t**	**p**
Glen Austin									
23 Dec 92	50	19.6	17.7	8.38	0.0001	670	550	7.65	0.0001
18-19 Oct 93	140	19.8	16.9	7.37	0.0001	730	540	0.80	0.0001
24-25 Dec 93	78	21.7	17.3	11.1	0.0001	870	650	7.98	0.0001
Witpoort									
24 Nov 92	75	19.9	17.2	9.27	0.0001	753	595	9.01	0.0001
8 Oct 93	71	19.2	17.2	9.99	0.0001	652	493	0.22	0.0001

2.3.19 Change in Mating Tactics over Time

During the first breeding event at Glen Austin there were 26 territorial and 146 non-territorial males present. During the second breeding event; 48 territorial, 134 non-territorial and 18 satellite males were present. Out of the 48 territorial frogs present during the second breeding event; 12 (25%) territorial males returned from the 1st breeding event and 14 (30%) non-territorial males from the previous breeding event became territorial males. Mean increase in body measurements of the returning territorial males was; **SVL=0.5**cm (**range**=0.2-1.2cm); **RFA**=0.5cm (**range**=0.2-0.9cm) and **Mass**=83g (**range**=20-190g). The non-territorial males from the 1st breeding event becoming territorial males in the 2nd breeding event had a mean increase in **SVL** of 1.5 cm (**range**=0- 2.1cm);**RFA**=1.6cm and **Mass**=96g (**range**-70-270 g).

Out of the 134 non-territorial males present during the 2nd breeding event seven (5%) were territorial males during the 1st breeding event and 42 (31%) non-territorial males were recaptured from the 1st breeding event. Mean increase in body measurements were **SVL**=1.7cm (**range**=0-3.7cm); **RFA**=1.2cm (**range**=0-2.1cm) and **Mass**=110g (**range**= -110-320g).

Of the 18 satellite males present during the 2nd breeding event 6 (33%) were non-territorial males during the 1st breeding event. Mean increase in body measurements were **SVL**=1.1cm(**range**=0.4-1.5cm); **RFA**=0.9cm (**range**=0.4-1.7cm) and **Mass**=67g (**range**=-70-210 g).

During the 3rd breeding event 36 territorial males were present; 96 non-territorial and four satellite males were present. Of the 36 territorial males 26 (72 %) returned from the previous breeding event. One non-territorial male returned from the 2nd breeding event and became a territorial male in the 3rd breeding event. Mean increase in territorial body size measurements were **SVL**=0.4cm; **RFA**=0.3cm and **Mass**=30g. Of the 96 non-territorial males 58 (60%) returned from the 2nd breeding event at Glen Austin. No satellite males returned for the 3rd breeding event.

During the first breeding event at Witpoort there were 15 territorial and 61 non-territorial males present. During the second breeding event; 11 territorial and 56 non-territorial males were present. Of the 11 territorial frogs, three (27%) returned while only one (9%) non-territorial male from the previous breeding season became a territorial male. Mean increase in body measurements from the returning territorial males were; **SVL=0.5**cm (**range**=0-1.2 cm); **RFA**=0.2cm (**range**=0.1-0.4cm) and **Mass**=40g (**range**=20-60g).

The non-territorial male from the 1st breeding event becoming territorial male in the 2nd breeding event had a increase in **SVL** of 0.7 cm ;**RFA**=0.3cm and **Mass**=30 g.

Of the 56 non-territorial males present during the 2nd breeding event four (7%) were territorial males during the 1st breeding event and 22 (39%) returned from the 1st breeding event. Mean increase in body measurements were **SVL**=2.9cm (**range**=0-3.4cm); **RFA**=1.2cm (**range**=0-2.4cm) and **Mass**=70g (**range**= -50-220 g).

2.3.20 Male Residency

Territorial Males: Territorial males remained in their territories for an average of 21 days (**range**=0-37) at Glen Austin during the three breeding events. Certain males vacated their territories after oviposition leaving their eggs unattended, others disappeared after the eggs hatched or during larval development. Seven males were found dead or severely injured in their territories defending their offspring from potential predators. Territorial males at Witpoort remained in their territories for an average of 22 days (**range**=0-35 days). There was no significant differences in territorial residency throughout all the breeding events at both Glen Austin and Witpoort ($\mathbf{F_{4,121}}$=0.15, p=0.864)

Non-Territorial and Satellite Males: Non-territorial males vacated the communal breeding area after the cessation of breeding activity. The developing eggs and larvae were left unattended and developed without any form of parental care.

2.4 DISCUSSION

Male anurans rarely employ only one mating strategy, alternative strategies can account for a significant proportion of matings over a single breeding season (Perrill *et al.* 1978). Certain morphological attributes will be beneficial to males that call to attract mates but not necessarily to those actively searching or acting as satellites (Halliday & Verrell 1986). Alternative mating strategies are predicted in those species in which there is variation in male mating success and large differences exist between males with respect to physical attributes and/or experience. In most iteroparous species, the majority of such differences between males are probably explained by variation in age (Howard 1978).

African Bullfrogs, ***Pyxicephalus adspersus*** are extreme explosive breeders completing mate acquisition and oviposition within 48 hours. Despite being explosive breeders they exhibit various mating tactics typical of prolonged breeders. (**a**) Bullfrogs are extremely sexually dimorphic with males being nearly twice the size of females. (**b**) Male and female arrival patterns are asynchronous. (**c**) Males are highly aggressive resulting in frequent aggressive interactions. (**d**) Males exhibit three size related mating strategies namely territorial, non-territorial and satellite behaviour.

Howard (1978) reported three similar mating strategies employed by male North American bullfrogs (***Rana catesbeiana***) namely territoriality, male parasitism, and opportunism.

2.4.1 TERRITORIALITY

Territorial behaviour has been recorded in numerous anurans including ***Rana virgatipes*** (Given 1988b); ***Rana clamitans*** (Wells 1978a); ***Rana catesbeiana*** (Howard 1978). Large size may confer an advantage on males in their fights for territories, calling sites and already paired females (Arak 1983b; Robertson 1986). Territorial male bullfrogs, ***P. adspersus*** were the largest adult males at both Glen Austin and Witpoort and show high levels of site fidelity, actively defending their territories against intruding conspecific males.

2.4.2 FREQUENCY OF OCCURRENCE OF AGGRESSIVE INTERACTIONS

TERRITORIAL MALES

Competition for territorial sites was intense. Males that had already established territories engaged in aggressive encounters with migrating intruding frogs. Frogs moved through the territories and settled in areas away from the established territorial males. Territorial males at Witpoort were involved in significantly more aggressive interactions per male than at Glen Austin, because the majority of non-territorial males migrated along the dam edges through the territories to the non-territorial breeding aggregation. In addition, limited suitable breeding habitat resulted in more frequent aggressive interactions. Whereas at Glen Austin, non-territorial males could move through the territories or away from the territorial areas to the breeding aggregation; and there were more suitable breeding areas available with the effect of reducing the number of aggressive interactions

Fighting territorial bullfrog males were significantly larger than intruding males and won betweenn 91-94% of aggressive interactions at Glen Austin and Witpoort during the three breeding events. Twenty two territorial displacements were observed and in each case the displacing male was larger than the original resident male.

The outcome of aggressive interactions in male bullfrogs ***P. adspersus*** seems to be dependent on body size as well as body mass. Larger, heavier males win more aggressive interactions. Residency also plays an important role. When similar-sized males interact, the original resident wins the majority of aggressive interactions. The outcome of aggressive encounters seemed to be independent of body size in ***Atelopus varius*** where in most cases, the original resident was the winner, with the intruder eventually retreating or being driven away from the area (Crump 1988). A resident advantage is nearly universal among territorial animals (Wilson 1975). The reasons for this phenomenon are not well understood beyond the obvious fact that the resident presumably has more reliable information regarding the quality of the territory and thus may invest more energy and effort in a fight (Crump 1988). However, it is unusual for body size to play so unimportant role in determining encounter success for anurans, as large size seems to be important to males of many aggressive species. Often the largest males win encounters as in ***Rana catesbeiana*** (Emlen 1968; Howard 1978), ***Rana clamitans*** (Wells 1977b;

1978a), ***Bufo bufo*** (Davies & Halliday 1977), ***Rana virgatipes*** (Given 1988b). Superior fighting ability of larger males against smaller males has been suggested as a proximate mechanism for non-random mating by size in anurans (Howard & Kluge 1985).

An individual male bullfrog ***P. adspersus*** may defend a specific site for 2-35 days, depending upon his ability to repulse intruders and predators and the presence or absence of tadpoles in his territory. Male parental care may form part of the explanation of the extreme sexual dimorphism of bullfrogs. Males defending breeding sites and offspring need to be larger than females who migrate out of the breeding area after oviposition. Large size may bestow an adaptive advantage in territory defence and predator repulsion.

2.4.3 NON-TERRITORIAL MALES

Non-territorial males are the losers of aggressive contests over territories and appear like territorial males; however, these males do not defend their sites once they have bred. If challenged or defeated by a larger male they vacate their site and find another. Non-territorial males include males that are defeated in territorial encounters, or opportunistic males that are attracted to the aggregation of fighting and displaying males.

2.4.4 COMPARISON OF FREQUENCY OF OCCURRENCE OF AGGRESSIVE INTERACTIONS BETWEEN TERRITORIAL AND NON-TERRITORIAL MALES

Non-territorial males at Glen Austin were involved in significantly more aggressive interactions than territorial males. This was due to the increased density of males in the non-territorial breeding areas, and aggressive interactions of shorter duration. At Witpoort there was no difference in the frequency of occurrence of aggressive interactions between territorial and non-territorial males. This was due to the limited breeding areas resulting in more numerous aggressive interactions between territorial and non-territorial males.

2.4.5 AMPLEXUS DISPLACEMENT

In explosive breeders, the form of male-male competition that favours larger males typically involves contests for females that are already paired (e.g. Davies & Halliday 1978, 1979; Howard & Kluge 1985). Among certain bufonids, non-random mating is accomplished, in part, through amplexus displacement, in which one male dislodges the amplexed male (Lamb 1984). Amplexus displacement is prevalent in bufonid toads but rarely accounts for a significant proportion of mating events (but see Davies & Halliday 1979, Telford & Van Sickle 1989).

Amplexus displacement is prevalent among non-territorial male African Bullfrogs ***P. adspersus***. I observed amplexus displacement attempts during all the breeding events at both Glen Austin and Witpoort. Males readily attempted to displace the initially amplexed male by wrestling, butting and flinging. Amplexed couples were forced into the shallow water (2-4cm) and males would attempt to dislodge the amplexed male. Amplexed females generally played a passive role, assisting neither attacking nor defending males, but would move into deeper water if continually attacked.

Channing *et al.* (1994) recorded only one amplexus displacement in the African Bullfrog (***P. adspersus***). They found that males were extremely aggressive towards other males but appeared to disregard amplexing males. I observed 268 amplexus displacement attempts at Glen Austin during the three breeding events and 146 at Witpoort during the two breeding events. Males readily attempted to dislodge amplexed males with sometimes up to six displacements before first successful oviposition. Although successfully amplexed males had significantly larger forearm lengths than attacking males, forearm lengths are positively correlated with body size and mass. Amplexed males may be relying on body size and mass as well as increased forearm length to remain clasped to females. At a given body size there is no significant differences in forearm length between males and females. Sexual dimorphism in the forelimb has been interpreted, retrospectively, in the context of amplexus displacement (Telford & Van Sickle 1989). Howard & Kluge (1985) and Lee (1986) argued that longer forelimbs confer a selective advantage on males, since males with longer forelimbs may be better able to resist displacement. Lee (1986) tested this idea for several species showing amplexus displacement. He found no evidence of sexual dimorphism in forelimb length and offered several other alternative explanations, but he failed to consider that the prevalence of amplexus displacement may determine the intensity of selection and hence the degree of sexual dimorphism (Telford & Van Sickle 1989). At least for some of Lee's (1986) species, amplexus displacement accounts for a very small proportion of matings and is therefore not a major component of non-random mating (Telford & Van Sickle

1989). Telford & Van Sickle (1989) did not study sexual dimorphism in forelimb length, but found their observed relationship between forelimb length and male mating success at a fixed SVL is inconsistent with the findings of Howard & Kluge (1985) and Lee (1986). It emphasises the fact that single phenotypic characters can neither be used to wholly explain mating success in one species, nor do they necessarily influence mating success similarly in different species (Telford & Van Sickle 1989).

Surrounding males also prevented amplexing couples from depositing partial clutches before butting them out of the breeding arena. The females could prevent oviposition by swimming into deeper water, but this led to some females drowning. Females that had deposited their clutches moved quickly out of the arena or else surrounding males would attempt to amplex them again. Paired females were injured on 22 occasions during attempts at amplexus displacement. Males also mistakenly identified females as small satellite males and some were fatally wounded (**n**=9) in the encounters.

Only in ***Bufo bufo*** (38%) and ***Bufo gutteralis*** (31%) does amplexus displacement account for a high percentage of matings (Davies & Halliday 1979; Telford & Van Sickle 1989). Although amplexus displacement attempts are frequent in ***Bufo americanus***, they resulted in only one displacement out of 57 matings (1.75%) (Howard 1988). Gatz (1981a) reported only one displacement in the 15 pairs (7%) that he recaptured more than once. Similarly, Kagarise Sherman (1980) reported an average of 4.5% displacements per year in ***B. canorus*** and 2.4% in ***B. exul***, and Wells (1979) reported a displacement rate of 3.2% ***B. typhonius***. In contrast, Davies & Halliday (1979) reported a displacement rate of 38.5% for ***Bufo bufo*** and Telford & Van Sickle (1989) reported a displacement rate of 31% in ***Bufo gutteralis***.

Displacement by ***Rana sylvatica*** males is dependent on a number of factors including female body size (Howard & Kluge 1985); when male and female sizes were poorly matched, the males were easily displaced. I did not see this effect directly but the outcome of displacement attempts was determined from the relative sizes of the attacking and defending male.

2.4.6 SATELLITE BEHAVIOUR

Satellite males neither call nor defend an area in the pan, instead they remain in close proximity to territorial males, often less than a metre away and attempt to intercept females attracted to territorial males. The posture of satellite males resembles that of females. Emlen (1968) describes this as a 'low' posture which probably decreases conspicousness, thus reducing aggressive encounters. Territorial males rarely catch satellite males because the characteristic aggressive vocalisation and rather slow approach of the territorial male provides ample opportunity for the satellite to retreat into the grass. Territorial males also do not move far from the territory, thus satellites escape the majority of attempted aggressive encounters.

Male parasitism may best be explained using a model analogous to that proposed by Orians (1969) to describe the evolution of polygyny. As variation in quality of habitat patches increase, young males which can only obtain inferior breeding territories as a result of costly competition with older larger males may better increase their mating success by associating with older territorial males (Howard 1978).

2.4.7 CONCLUSION

Pyxicephalus adspersus exhibit three distinct size related mating strategies namely territorial behaviour using their defended sites as oviposition sites whilst exhibiting site-specific intolerance towards intruding conspecifics; non-territorial behaviour in which dense aggregates of non-territorial males form and amplexus displacement predominates; and satellite behaviour with males adopting a non-calling, non-aggressive posture close to the largest teritorial male.

Territorial males are significantly larger than both non-territorial (opportunistic males) and satellite (parasitic) males. Non-territorial males are significantly larger than satellite males. Thus, territorial males tend to be the largest, most aggressive males in the population, non-territorial males tend to be intermediate in size or large males that cannot successfully defend a territory. Satellite males are the smallest adult males in the population, they are too small to either defend a territory or fight for a place in the non-territorial arena. Satellite males avoid aggressive encounters and decrease the risk of injury and death through their behaviour.

CHAPTER THREE

BEHAVIOURAL AND MORPHOLOGICAL CORRELATES OF VARIATION IN MALE MATING SUCCESS

3.1 INTRODUCTION

3.1.1 ALTERNATIVE MATING STRATEGIES

Anurans are an ideal group for studies of mating behaviour and variation in mating systems because individuals are easily marked, handled and observed (Arak 1983a). Many reviewers also note that external fertilization in frogs allows for accurate quantification of the reproductive success of individuals (e.g. Howard 1988a). External fertilization does not, however, ensure that paternity is known with certainty (Jennions 1992). In studies of animal mating systems alternative mating strategies are often found within either of the sexes (Waltz 1982; Austad 1984; Howard 1984). In anurans two major male mating strategies have been found: **1**) chorusing behaviour, in which males advertise their presence at the breeding pond by intense calling; and **2**) active searching and intense competition for access to females (Hoglund & Robertson 1988). It is argued that the time availability for access to females or the opportunity to control resources necessary for mating determines which strategy is used (Wells 1977; Arak 1983a).

Fighting and searching are commonly found in so called explosive breeders which have a short mating season during which female arrival at the breeding ponds is highly synchronous, in contrast, chorusing is mainly found in prolonged breeders that have a long breeding season during which females arrive asynchronously (Wells 1977).

3.1.2 SEXUAL SELECTION AND SEX RATIOS

Synchronous mating by females in a population has been predicted to affect the form and intensity of male-male competition and female choice in all species (Trivers 1972; Emlen & Oring 1977). Although mating synchrony is a definite factor in mating systems

(Howard 1981; Berven 1981), the effects of male-biased sex ratio must also be considered. Preponderance of males should increase both male-male competition for the relatively scarce females and the likelihood of female choice due to the reduction in time and energy spent locating a large number of males when searching for a suitable mate (Parker 1983).

Emlen & Oring (1977) suggest that the operational sex ratio (OSR), the ratio of the number of adult males to the number of receptive females at any point in time, estimates the intensity of sexual selection. This measure has several potential methodological pitfalls (Kluge 1981) and may be less useful in predicting selection intensity than are measures incorporating the amount of variation in reproductive success (Wade & Arnold 1980; Howard 1983); however, the OSR can serve as a reasonable index of the intensity of male-male competition because it measures the scarcity of females (Howard & Kluge 1985).

In anurans, morphological based deviations from random mating, or at least random pairing, typically take one of two forms: a mating advantage in which larger males have higher reproductive success than smaller males, or assortative mating in which the sizes of mated pairs show a positive covariation. A large-male mating advantage appears to be far more common than assortative mating (Verrell 1983; Lee 1986). Both deviations from random mating are generally interpreted as a consequence of sexual selection. However, the two components of Darwinian sexual selection, choice (often exercised by females) in accepting mates and competition (usually among males) for the possession of mates or resources necessary for reproduction, can produce the same pattern; thus, their relative contributions cannot be assessed solely from the examination of pairing patterns. Moreover, factors other than sexual selection might produce deviations from random pairing (Lee & Salzberg 1989). For example, Lee & Crump (1981) suggested that the apparent assortive mating by size that they documented for ***Tripion petasatus*** might reflect a relationship between size and time of arrival at the breeding site. For explosive breeders in xeric environments where precipitation is highly seasonal, small frogs might rehydrate more quickly than larger ones and arrive at the breeding site earlier. If so, data taken over the span of several hours might show a positive correlation between sizes of frogs in amplexus, even though at any given time, frogs paired at random (Lee & Salzberg 1989).

Several studies have found that large males do not have increased mating success (Fellers 1979). It is questionable to assume that the intensity of selection increases with the duration of the breeding period (Sullivan 1984). For example, primarily as a result of male-male competition, large males have enhanced mating success in anurans with extremely short-duration breeding periods (Berven 1981). Hence, size has been identified as a significant factor influencing male mating success for these species in spite of their short breeding periods (Sullivan 1984).

The calls of male anurans advertise the caller's position to surrounding males as well as to females that may be potential mates (Wells 1977). The calls may simply be a means of attracting females over a long range by signalling the presence of a sexually mature conspecific male (Littlejohn & Harrison 19815; Gerhardt 1987). The call may also provide the female with additional information on the male's suitability as a mate (Gerhardt 1982). There is evidence that females of some species prefer to mate with larger males and that they can determine the size of a male from the dominant frequency of its call (Ryan 1980; Robertson 1986). The sound pressure level (SPL) or amplitude of the calls (Arak 1983b), or temporal parameters of calls such as call rate or pulse repetition rate (Whitney & Krebs 1975) are other parameters of male calls that may be chosen by females. However, there are difficulties associated with assessing size from SPL or the temporal parameters of the calls. Even though SPL may be correlated with body size, it also largely depends on the distance of the listener from the source (inverse square law) and it further suffers from excess attenuation due to vegetation and microclimate and orientation of male (Arak 1983b; Robertson 1984).

To understand the intensity of sexual selection it is not the overall population ratio of males to females that is of importance but rather the OSR. This ratio is strongly affected by the degree of spatial and temporal clumping of the limiting sex. The OSR provides an empirical measure of the degree of monopolizability of mates. The greater the degree of imbalance in the OSR, the greater the expected variance in reproductive success among members of the non-limited sex and the greater the potential for polygamy (Emlen & Oring 1977).

When access to females cannot be controlled through resource defence, male competition may take the form of direct male-male encounters, resulting in differential dominance relationships between the male members of the population (Elmen & Oring 1977). Under what conditions should individual males aggregate and display communally? Such aggregations provide a forum for male-male competition and should increase the variance in reproductive performances among males. The mating success of a high-ranking male would be increased by joining such a group; that of a low-ranking individual might decrease. (Emlen & Oring 1977)

Three mating tactics observed in ***P.adspersus*** will be discussed namely territoriality, non-territorial aggregations and satellite behaviour.

The major aims of this chapter are:

1. What are the major mate acquisition tactics employed by adult male bullfrogs?
2. Do successfully mating adult males differ in size from non-successfull males?
3. Is there seasonal variation in male mating success and does it vary among the various tactics employed by adult males?

3.2 METHODS

3.2.1 BEHAVIOURAL OBSERVATIONS AND MATING SUCCESS

To quantify mating success, the individual identity of males must be known, and ideally the observer should be able to identify the individuals without disturbing them. Monitoring behaviours of known individuals without having to recapture them for identification was necessary for this study. Unique heat brandings were used for individual identification (see General Methods Chapter 1). For both years, the identity of all mating individuals was known.

Observations were conducted throughout the wet season (October-January). Bullfrogs were undisturbed by the presence of the observer. Censuses of numbers of males, females and amplexing pairs were conducted throughout each breeding event. Males were considered to be successful in mating only when egg deposition was observed. Pair formation alone was not considered to be a sufficient measure of mating success because of the chance that a paired male may be displaced from his mate prior to fertilization. Focal-animal observation techniques were used to observe the behaviour of individual frogs. Focal animals were chosen randomly, or if males were already in amplexus observations began immediately. Up to seven field assistants collected simultaneous focal-animal samples. Randomly selected males were observed for between 5-30 min and general aspects of their reproductive behaviour were noted such as whether they were territorial, non-territorial or satellite males. If mating continued for periods longer than 30 minutes observations continued until termination of egg deposition. We timed the mating behaviour exhibited by the focal animal {amplexus, inter-bout duration (period of quiesence) and oviposition) with a stopwatch.

Statistical procedures

Male mating success at Glen Austin and Witpoort as a function of size and mass was determined using a two sample test between successfully mating males and unsuccessful mating males. A correlation between male and female body size and mass in amplectant pairs was conducted.

3.3 RESULTS

3.3.1 MATING BEHAVIOUR

Males clasped females in a typical form of axillary amplexus which lasted from 0.5, to 90.5 min with a mean duration of 15.5 min (**sd**=0.39, **n**=100). Throughout oviposition the amplexed couple remain in shallow water (2-4cm) with the female crawling about rather than swimming. Oviposition commenced between 0800 and 01200 hours and lasted throughout the day, ceasing after 1755h when the frogs dispersed.

Each amplexus involved 1-11 bouts of oviposition, each lasting 7-43s (**mean**=31s, **n**=150). The mean inter-bout duration (period of quiescence) was 54.6s (**sd**=33.4, **n**=100, **range**=10-180s). In most cases, the males, while mounted dorsally on the females, inflate their bodies (Grobler 1972) and see Fig 3.1.

3.3.2 PHENOTYPIC CORRELATES OF MALE REPRODUCTIVE SUCCESS

The operational sex ratios for Glen Austin and Witpoort are given in Table 3.1.

Between 34-71% of males obtained at least one mating at Glen Austin during all breeding events. At Witpoort the proportion of males obtaining at least one mating ranged from between 55-71%. Successfull males were significantly larger and heavier than unsuccessful males at both Glen Austin and Witpoort, (Table 4.2.) There was no significant correlation between either the weights or the SVL's of males and females in mating pairs at Glen Austin and Witpoort (**SVL:** **r**=0.156, **p**≥0.05, **df**=120; **Mass:** **r**=0.119, **p**≥0.05, **df**=125).

3.3.3 MATING FREQUENCIES

3.3.4 Territorial Male Mating Frequencies

The proportion of territorial males that succeeded in obtaining at least one mate ranged from between 87-100% during the breeding events at Glen Austin and Witpoort. Mean male mating success of the territorial males ranged between 0.87-1.31, (Table 3.5). Maximum number of matings obtained by a territorial male was three (**n**=6).

3.3.5 Non-territorial Males

At Glen Austin and Witpoort between 24-64% of non-territorial males succeeded in obtaining at least one mate during the breeding events. The mean mating success of non-territorial males ranged between 0.43-1.03, (Table 3.5). Maximum number of matings achieved by a non-territorial male was five (**n**=10).

3.3.6 SATELLITE MALES

There were no satellite males present at Witpoort or during the first breeding event at Glen Austin. The proportion of satellite males obtaining at least one mate during the 2nd and 3rd breeding event ranged from between 33-100%. Mean satellite male mating success ranged between 0.33-1.0 (Table 3..5). The maximum number of matings achieved by a satellite male was 2 (**n**=1).

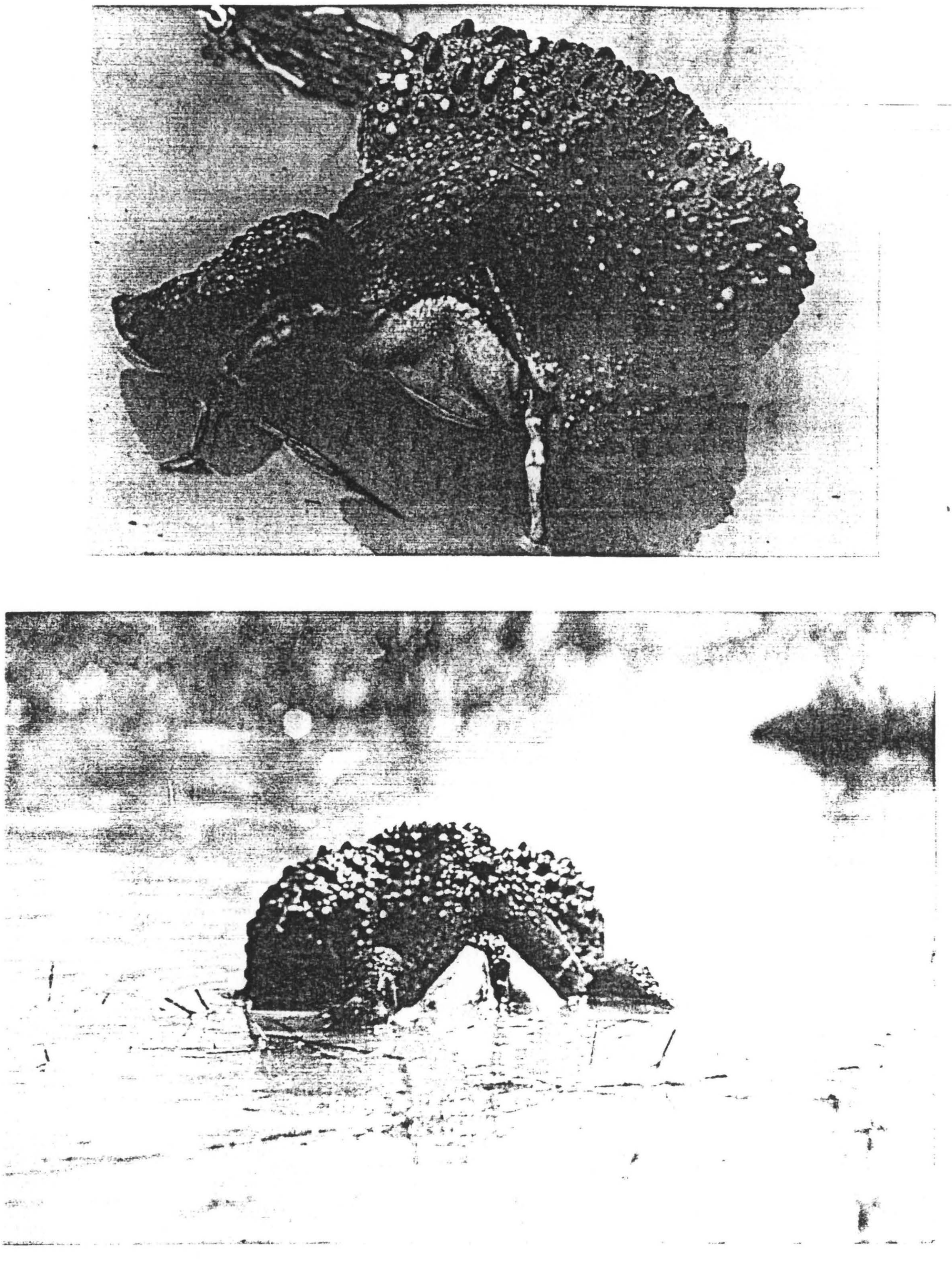


Figure 3.1 Photographs showing amplexus and oviposition in the African Bullfrog *Pyxicephalus adspersus*

Table 3.1 Breeding duration (number of days), and operational sex ratios (OSR) at Glen Austin and Witpoort during two breeding seasons.

Location	Duration (days)	OSR (males per female)
Glen Austin 1992 (Dec)	1	2.2:1
1993 (Oct)	2	2.4:1
1993 (Nov)	2	3.1:1
Witpoort 1992 (Nov)	1	2.1:1
1993 (oct)	1	2.8:1

Table 3.2 Male mating success at Glen Austin and Witpoort as a function of size and weight over two seasons

Year	**Category**	**Mean SVL (cm)**	**n**	**t**	**p**	**Mean Weight (g)**	**t**	**p**
Glen Austin								
1992	Successful	19.6	58			620		
(Dec23)				8.38	0.00001		7.65	0.00001
	Unsuccessful	17.7	114			500		
1993	Successful	19.2	112			613		
(Oct18-19)				10.87	0.00001		10.67	0.00001
	Unsuccessful	17.2	88			479		
1993	Successful	19.1	96			617		
(Nov24-25)				6.90	0.00001		5.78	0.00001
	Unsuccessful	17.7	40			526		
Witpoort								
1992	Successful	18.7	54			614		
(Nov24)				8.38	0.00001		3.66	0.00007
	Unsuccessful	17.7	22			552		
1993	Successful	19.1	37			674		
(Oct8)				7.84	0.00001		7.56	0.00001
	Unsuccessful	17.1	30			550		
Total:	Successful	19.1	357			628		
				8.47	0.00001		7.01	0.00001
	Unsuccessful	17.5	294			550		

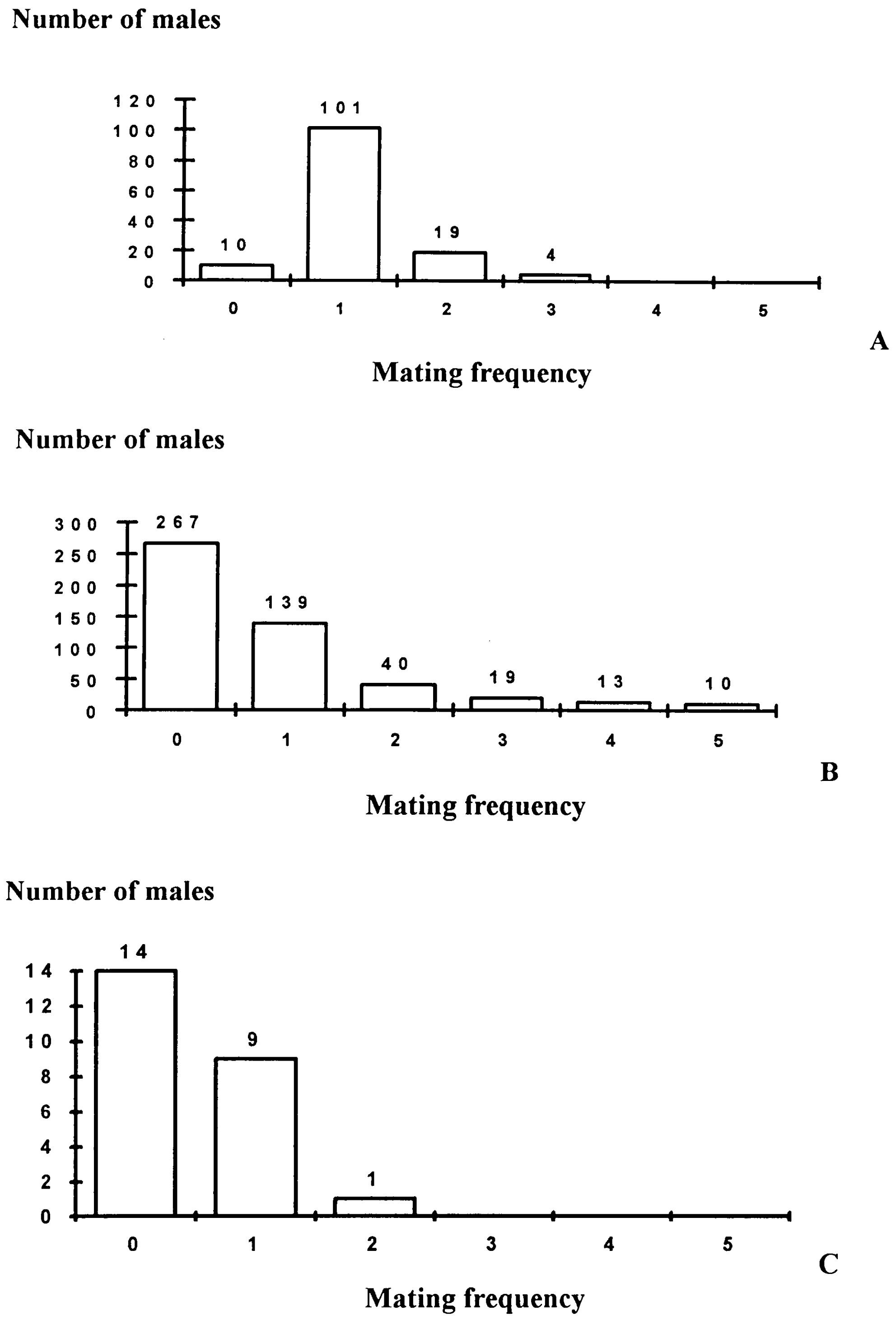


Figure 3.2 The distribution of observed matings of (**A**) territorial; (**B**) non-territorial and (**C**) satellite males at Glen Austin and Witpoort over two breeding seasons.

frequency of mating

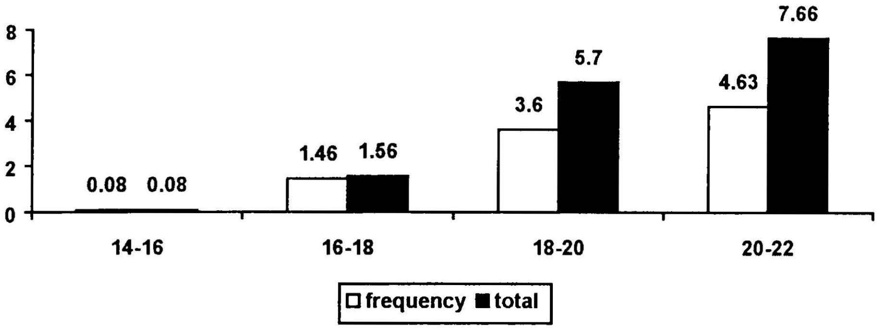


Figure 3.3 The frequency of mating success as a function of male size at Glen Austin and Witpoort during the breeding seasons. Male size classes are on the horizontal axis and mating frequency is on the vertical axis. The hollow bars presents the frequency of successful males in each class, success is defined as obtaining at least one mating. The second measure (solid bars) shows the total number of matings per size class. Both the number of successful males and the proportion of matings per size class increase as a function of males size.

Table 3.3 Mean male mating success of territorial, non-territorial and satellite adult male ***Pyxicephalus adspersus*** over two seasons

Study Sites	**Territorial Males**			**Non-Territorial Males**			**Satellite Males**		
Breeding Event	**No. of males**	**No. of matings**	**Mean Male Mating Success**	**No. of males**	**No. of matings**	**Mean Male Mating Success**	**No. of males**	**No. of matings**	**Mean Male Mating Success**
Glen Austin **1st 23 Dec 92**	26	27	1.04	146	63	0.43	0	0	0
2nd 18-19 Oct 93	48	63	1.31	134	103	0.77	18	6	0.33
3rd 24-25 Nov 93	36	41	1.14	96	88	0.92	4	4	1.0
Witpoort **1st 24 Nov 92**	15	13	0.87	61	63	1.03	0	0	0
2nd 8 Oct 93	11	13	1.11	56	58	1.04	0	0	0
Overall Results	136	157	1.15	493	375	0.76	22	10	0.45

3.4 DISCUSSION

3.4.1 BREEDING BEHAVIOUR AND REPRODUCTIVE BIOLOGY

Breeding in African frogs is strongly dependent on rain, especially in the drier parts of the country where surface water only remains for short durations (Jennions 1992).

Bullfrogs (***P.adspersus***) at Glen Austin and Witpoort emerged and commenced mating after the first heavy summer rains between October and January. Males migrated to the breeding areas forming territories along the edges of the pan or dam, or aggregating in large groups in the non-territorial breeding areas. Grobler (1972) describes how up to 60 males gather in an arena, where dominant individuals occupy the centre. Male-male aggressive interactions occur with males actively defending their territories or positions in the breeding arena from intruding conspecific males. Female arrival is asynchronous, with females arriving throughout the day moving through the territory areas towards the displaying aggregate of non-territorial males. Amplexus occurs in the territories or breeding arena, and is unusual amongst Southern African ranids in that it takes place during daylight. Daytime chorusing could provide information to females and, perhaps incidentally to males, that may be more difficult to obtain at night. Such information is suspected to include variations in water temperature in different areas of the pond: temperature differentials between areas of the pond are maximal during the day due to variations in water depth, vegetation cover, and degree of water circulation (Howard 1978).

Males clasped the females in a typical form of axillary amplexus. Throughout oviposition the amplexed pair remain in shallow water with the female crawling about rather than swimming. Oviposition is carried out in short bursts. The male and female stretch their hindlimbs, lifting their bodies as high up as the length of their legs will allow. The female arches her back downwards with her cloaca about 3-5 cm out of the water. A compact mass of eggs appears and the female ensures maximum fertilization by rubbing her cloacal region on the inner surface of the thighs of the male. The fertilized eggs slide down the posterior surface of the thighs and abdomen of the female and are deposited in the water.

3.4.2 THE OSR

The operational sex ratios (OSR) at Glen Austin was always male-biased ranging from 2.2-2.4:1 over the three breeding events. The male-biased operational sex ratios at Witpoort varied from 2.1:1 to 2.8:1. Other explosive breeders seem to have more varied OSR values than those observed for ***P.adspersus***. ***Bufo pardalis*** varied between 2.4:1 to 6.9:1 (Cherry 1991); ***Rana sylvatica*** the OSR averaged 16:1 (Howard 1985); ***S.multiplicatus*** ranged from 0.13 to 0.44; ***B.cognatus*** 0.03 - 0.19 (Sullivan 1989a). Male-biased OSRs could also result from a sex difference in the time required to attain sexual maturity. It may be that males mature more rapidly than females, a phenomenon that has been documented in a number of temperate anurans (Howard 1981).

3.4.3 LARGE-MALE MATING ADVANTAGE

Large male ***P.adspersus*** have greater mating success than smaller males because of a large male advantage in aggressive interactions. Larger, presumably older males not only physically dominate smaller males but also have increased mating success. The same result was obtained for ***R.catesbeiana*** (Howard 1978).

Amplexus displacement plays a major role in the mating success of non-territorial males. The larger attacking males have significantly higher amplexus displacement rates than smaller males. Larger challenging males always displace smaller amplexed males and amplexus displacement by non-territorial males may best be explained by a form of male-male competition rather than a form of female choice (Chapter 2).

3.4.4 SIZE ASSORTIVE MATING

Size assortative mating based on female choice of a preferred size was not supported by the results of this study. Females of all size categories paired with a wide range of male body sizes. Size assortative mating appears to be rare in anurans, having been recorded primarily in species in which amplexus displacement takes place (***Bufo bufo*****:** see Davies & Halliday 1977; ***Rana sylvatica*****:** see Howard & Kluge 1985; ***Bufo boreas*****:** see Olson *et al.* 1986), but in one species where mate selection is by female choice (***Uperoleia laevigata*****:** see Robertson 1986). In the first cases, assortative mating appears to result from paired males being more difficult to dislodge if males are optimally matched, but in the case of ***Uperoleia laevigata***, females choose males approximately 70% of their bodyweight by assessing the fundamental frequency of the male's call. Again, this can be explained as a genetic benefit, as it is adaptive in ensuring that their cloacas are apposite during amplexus, ensuring fertilization of a high proportion of the female's eggs (Robertson 1990).

3.4.5 TERRITORIALITY

Large territorial males have favourably positioned territories and are able to actively defend them against smaller intruding conspecific males (see Chapter 2). Male mating success may not only depend upon choosing good oviposition sites but also on the ability to defend such sites from contesting males (Howard 1978). Males actively fight for possession of territories before the arrival of females. Territorial male bullfrogs defend resources required by females. Females settle in the male territories or may move to a vacant territory if occupied. Up to three females were found in the territories of certain males. Variation in the ability of males to control high quality territories can influence mating success based on variation in embryo mortality in different egg masses (Howard 1978), although no evidence of this was found in the African Bullfrog.

Territories are not only used for mating and egg deposition but also provide nourishment for the developing larvae. Only territorial males remain after the cessation of breeding activity and defend the developing eggs and larvae from predators and desiccation. The female deposits her eggs in the territory and the male defends the eggs and developing tadpoles from numerous predators including water birds, barbel, carp and terrapins. Territorial males also construct channels from the rapidly drying territories to the deeper main body of water and prevent the desiccation of the eggs and tadpoles (Channing *et al.*1992). Female choice of males may simply be related to territory quality, as opposed to male quality.

Males of two anuran species, ***Rana catesbeiana*** (see Howard 1978) and ***R.clamitans*** (see Wells 1977b,1978), are known to be territorial in the sense of defending resources for their young. In both species mating and spawning take place in the territories, which vary in quality in terms of potential for offspring survival, but for different reasons. In the case of ***R.clamitans*** those territories with more dense mats of vegetation (in which eggs are laid) are favoured, whereas in the case of ***R.catesbeiana***, territories preferred by females have both warmer water (providing a better environment for tadpole development), and have lower spawn predation by leeches, than lower quality ones.

3.4.6 NON-TERRITORIAL BEHAVIOUR

Channing *et al.* (1994) describe the mating pattern of African Bullfrogs (***P.adspersus***) as that of a lek. Males appeared to be maintaining a small area around themselves, defending it against other calling or advancing males, but not usually attacking amplexing pairs. Even males which were extremely aggressive towards other males appeared to disregard amplexing pairs. Sometimes large aggressive males even touched amplexing pairs but ignored them and only one male was recorded dislodging a male from an amplexing pair. Bradbury (1981) has offered a rigorous definition of a lek mating system. It requires that males aggregate and defend display territories solely for mating purposes and that females select their mate from among displaying males. Females must not utilise any resource within the male's display area; this restriction distinguishes lek mating systems from resource defence systems. Any parental care is undertaken only by females, and males defend neither resources nor mates.

Breeding aggregations of non-territorial male bullfrogs are characterised by the lack of a defensible resource and by the extreme synchrony of reproduction. The time available for matings is limited and the females themselves become a rapidly diminishing resource. Sex ratios biased towards males intensify intrasexual competition and further restrict the number of matings most non-territorial males obtain. Female choice in such cases may be impossible, as is the ability of a few males to monopolise several females.

The mating system of explosive breeding frogs is essentially a form of ‘scramble competition’ that often occurs when resources are concentrated in space and time; females in dense aggregations do not approach individual calling males (Wells 1977). The hypothesis of female choice does not adequately explain the results of this study involving non-territorial males. Field observations demonstrated that most pairs formed in this study resulted in males actively grasping females or struggling with other males for control of a single female rather than the females initiating amplexus by approaching a calling male. Any female attempting to move towards a chosen mate was usually intercepted before reaching her mate. Larger non-territorial males were frequently observed to displace smaller males in amplexus and often prevented smaller males from pairing as well as from the fertilization of eggs. Successfully mating males were not necessarily the chosen mates but amplexed males which were able to resist displacement attempts.

3.4.7 SATELLITE BEHAVIOUR

Satellite males adopted a concealed, non-calling 'low' posture in the sedges or grasses adjacent to the resident territorial males and attempted to intercept females. If a satellite male intercepted a female he would amplex with her and begin mating with her almost immediately. Satellites seldom had more than one bout of oviposition with a female before the territorial male displaced him or the satellite fled preventing an aggressive interaction. Although certain satellites mated successfully the eggs were usually deposited on the surrounding vegetation or in extremely shallow water which prevented successful hatching of the clutch. Females also prevented oviposition with satellite males by moving into the male territory, but risked injury from the attacking resident male. Females were also mistakenly identified as satellite males and were severely injured by the aggressive territorial males.

Sexual parasitism in ***Hyla crucifer*** is exhibited far more commonly by small males (Forester & Lykens 1986). Growth in anurans generally is indeterminate so small males are probably young males (Wilbur *et al.* 1978; Ryan 1980, 1983) whose relative reproductive success is untested. An increase in frequency of satellite behaviour with density has been reported for other frog species (Arak 1988b; Krupa 1989: Robertson 1986).

What factors may influence ***P.adspersus*** mating patterns? Female choice based on male vocalizations is unlikely because males only produce mating calls sporadically. Males however exhibit intense intrasexual competition for mates, a characteristic of many explosive breeding anurans (Davies & Halliday 1979; Telford & Van Sickle 1989). Male-male competition, however, may not take the form of direct aggression. For instance, time of arrival at the breeding site may influence male mating success if early arriving males have a better chance of mating. Large male ***B.americanus*** (Gatz 1981b), ***R.sylvatica*** (Howard & Kluge 1985) and ***R.catesbeiana*** (Howard 1978) arrive at the breeding sites earlier than small males. There was no significant variation in size between early arriving male ***P.adspersus*** (07h00-12h00) and later arriving males (12h00-16h00). Males that arrived at the sites later were not smaller than earlier arriving males although the majority of territorial males arrived early.

3.4.8 CONCLUSION

Pyxicephalus adspersus exhibits a short-duration, explosive breeding mating system which is exhibited by anurans that utilise ephemeral sources of water. However there is presently little support for the prediction that such a mating system is necessarily characterised by a synchronously breeding population in which sexual selection is minimal (Sullivan 1989b). The available measures of the intensity of sexual selection, male skewed operational sex ratios, and alternative reproductive behaviours exhibited by explosive breeding male bullfrogs indicate that both male-male competition for mates and to some extent female choice may be occurring in these short duration breeding aggregations.

CHAPTER FOUR

FEMALE CHOICE

4.1 INTRODUCTION

Recent theoretical and empirical studies have focused on the methods of measuring selection (e.g. Arnold 1983; Morris 1989), however it is equally important to be able to detect the mechanisms behind selection (Endler 1986). One reason for identifying the mechanisms of selection, as well as understanding how they interact with one another and other influential factors (e.g. the operational sex ratio), is that this information can aid in predicting the conditions under which selection will be operating (Morris 1989).

Anurans have been studied extensively in relation to sexual selection. Both male-male competition and female choice have been identified as mechanisms that effect selection on male body size in anurans (see Howard and Kluge 1985, Morris & Yoon 1991). However, separating the effects of these two components of sexual selection can be difficult, and therefore their relative importance is often not determined. Using the pattern of mating success (size-dependent mating versus size-assortative mating) to infer which mechanism is operating has been discredited in anurans (Arak 1983a). Instead, intensified field observations of individual males and females prior to mating can be used to indicate the influence these two mechanisms have on mating success (Howard 1988). The behaviour that has been attributed to male-male competition include males seeking out and initiating amplexus with females and males displacing one another during amplexus. Behaviour that suggests female mate choice includes females moving freely through a chorus and initiating amplexus with certain types of males (Morris 1989).

Many sexually dimorphic characters have evolved under the influence of sexual selection, either because they increase an individual's ability to compete for access to members of the opposite sex, or because they increase an individual's probability of obtaining a mate (Darwin 1871). Some of the most elaborate displays in animal communication are those used by males to attract and court females. Male sexual displays may provide information which allows females to identify males as conspecifics or possibly to assess mate quality (Trivers 1972). Darwin (1871) maintained that male sexual displays allowed females to pick more vigorous males and that female choice thus caused the further elaboration of male sexual displays. Vigorous displaying males are preferred by females in a number of organisms, including birds, amphibians, fish and insects (see review in Ryan & Keddy-Hector 1992). The hypothesis that females benefit from selecting males that display vigorously not only requires a relationship between display intensity and phenotypic vigour: males must also exhibit consistent, heritable differences in the intensity of the display (Sullivan & Hinshaw 1992).

Female mate choice has been demonstrated as an important determinant of male reproductive success in several anuran species. In green frogs (***Rana clamitans***), Wells (1977b) found a strong correlation between his measure of the quality of male's territory and male's mating success, and a significant but weaker correlation between male size and mating success. Wells suggested that females preferentially selected males on the basis of territory quality, and that better territories were acquired differentially by larger males. For example, in North American Bullfrogs ***Rana catesbeiana*** males vocalise from their territories and attract females, which choose the larger males on the better territories (Howard 1978). It is not clear if female bullfrogs use traits of the male, the territory, or both when selecting mates.

In summary, the main questions addressed in this chapter are:

(1) Does female mate choice influence male mating success?

(2) Which males do females select as mates?

(3) On what characteristics do females base their choice?

(4) What selective advantages, if any, are obtained by females choosing certain males?

4.2 METHODS

4.2.1 FEMALE MOVEMENT PATTERNS AND FEMALE CHOICE

Marked females were monitored using focal-animal samples from their arrival at the study site to the cessation of the breeding season. Female movement patterns through the pan towards male-occupied territories were also observed. Female settlement patterns amongst territorial males were similarly documented. Quantifying territory quality in the context of resources important for reproduction was beyond the scope of this study. However, certain territorial attributes were determined namely territory size and water depth as well as the sizes of territorial males. Female arrival and settlement patterns were similarly monitored at the breeding arenas of the non-territorial males.

4.2.2 CLUTCH SIZES

To estimate egg production of naturally breeding pairs, I obtained a clutch-female size relationship by collecting 25 amplexed pairs from a pond within 5km of the main study sites. Estimates of clutch size were achieved by allowing pairs of bullfrogs to deposit eggs in a plastic bucket with a lid. Each bucket contained approximately 0.5 litres of water. Pairs usually spawned within a day of confinement. Each egg mass was calculated by placing the egg mass on a tray and by dividing the area of the clutch of eggs, by the mean egg diameter (**n**=250 eggs; 25 from each clutch). Egg diameter was measured with vernier calipers (although this did not provide an accurate measure, it allowed an estimate of ovum diameter). Accurate measures of clutch size produced by naturally breeding territorial individuals were difficult to obtain as the eggs were initially deposited in clumps which rapidly dispersed by the continual patrolling behaviour of the territorial male. Too much disturbance could influence male parental care and cause the male to vacate his territory. Eggs were deposited communally in the non-territorial breeding arenas making the identity of individual egg masses uncertain.

I decided not to calculate clutch size directly by isolating pairs, because removal of pairs could influence subsequent breeding activity. In particular, removal of amplexed males from the population would prohibit them from mating multiply, and eliminate any chance that amplexed males would be dislodged by competing males prior to egg deposition. The latter effect may eliminate a major component of sexual selection (see Howard & Kluge 1985).

Statistical procedures

A two sample T test between female body sizes depositing single clutches and partial clutches as well as of early arriving (0700-1200h) and late arriving females (1200-1800h) was determined for Glen Austin and Witpoort. Correlation coefficients between male's territory size as well as body size measurements (SVL, mass) and the numbers of females encounterd in a male's territory were made for both populations.

4.3 RESULTS

4.3.1 FEMALE ARRIVAL PATTERNS : Females began to arrive after 0700h at Glen Austin and Witpoort migrating through the pan and along the dam shore towards the competing males. Females settled first in the territorial areas eventually moving towards the non-territorial aggregates. Females arriving at the territories would remain concealed in the surrounding border vegetation before moving into the territory. Certain territories contained more than one female with the maximum number of females in a single territory being three (**n**=6). Females arriving at occupied territories would conceal themselves in the surrounding vegetation or remain submerged before entering into the territory. Certain females migrated along the occupied territories searching for vacant territories. Females arriving at the non-territorial breeding arenas would remain hidden in the vegetation or submerged in the deeper water with only their eyes visible. Females would attempt to move towards a certain male but were usually intercepted by surrounding males.

Females arrived throughout the day with the majority arriving between 0700 and 1400 hours. There was no significant variation in body size between early arriving females (0700h-1200h) and late arriving females (1200h-1800h) at Glen Austin and Witpoort during the breeding events (**t** ≥0.68, **p**≥0.121, **df**≥12).

4.3.2 EGG CLUTCH PRODUCTION: Certain females did not deposit an entire clutch with a single male. This happened regularly in the non-territorial breeding areas where amplexus displacements were frequent. At both Glen Austin and Witpoort there was no significant variation in all body measurements between females depositing a single clutch and females depositing partial clutches ($\mathbf{t}\geq 0{,}03$, $\mathbf{p}\geq 0.13$, $\mathbf{df}\geq 12$).

4.3.3 CLUTCH SIZE : Mean egg surface was 5 mm (**range**=3-6 mm) with no significant variation in egg size ($\mathbf{p}>0.05$). The mean clutch size of females mated with territorial males was 2808 (**range**=1000-6000; $\mathbf{n}=36$). There was a significant positive relationship between Territorial male mating frequency and female clutch size ($\mathbf{r}=0.73$-0.87, $\mathbf{n}=11$-45, $\mathbf{p}\leq 0.0001$). The mean clutch size of females mating with non-territorial males was estimated as 1029 (**range**=100-4500; $\mathbf{n}=43$). There was no significant correlation between non-territorial male mating frequency and female clutch size ($\mathbf{r}=0.23$-0.33, $\mathbf{n}=21$-36, $\mathbf{p}\geq 0.05$).

4.3.4 EFFECT OF FEMALE SVL AND MASS ON CLUTCH SIZE : A significant correlation was noted between female size and clutch size ($\mathbf{r}=0.896$, $\mathbf{p}<0.01$) and female mass and clutch size ($\mathbf{r}=0.923$, $\mathbf{p}<0.01$, Fig.4.1).

4.3.5 EFFECT OF MALE SIZE AND TERRITORY AREA ON FEMALE CHOICE : The number of females found in a territory was significantly correlated with male body size in all the breeding events ($\mathbf{r}=0.388$, $\mathbf{p}<0.01$, Fig.4.2). Territory size was also positively correlated with number of females present in the territory ($\mathbf{r}=0.356$, $\mathbf{p}<0.01$, Fig.4.3). There was no significant correlation between territory depth and number of females found in a territory ($\mathbf{r}=0.143$, $\mathbf{p}>0.05$).

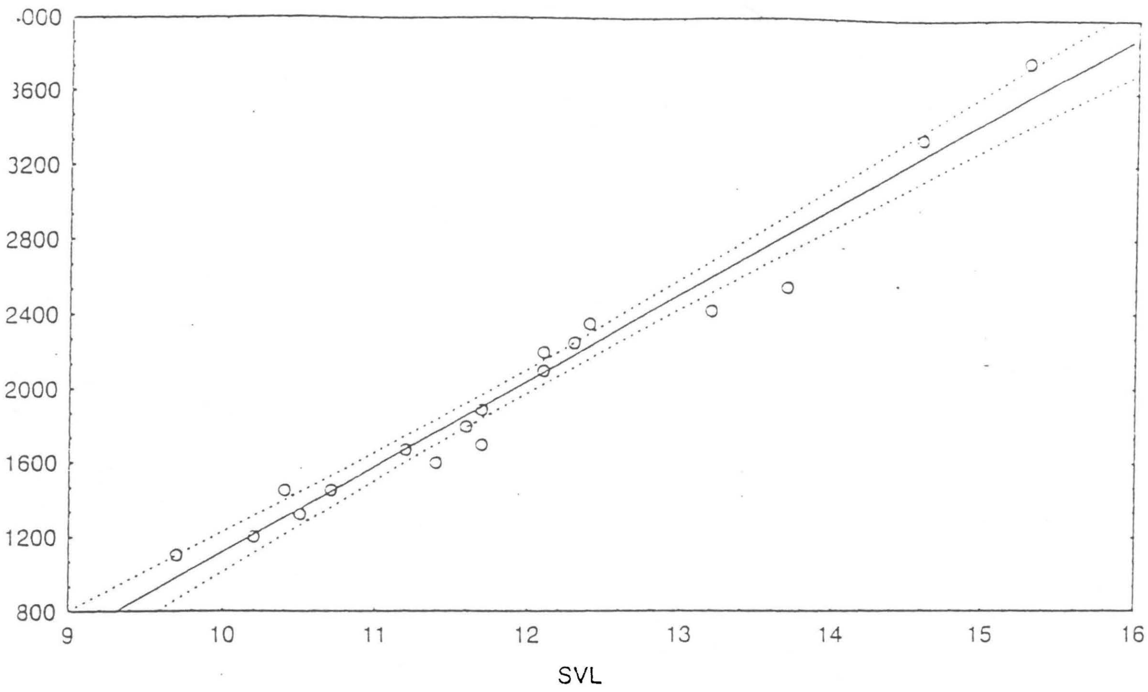


Figure 4.1 The relationship between female snout-vent length (SVL) and clutch size.

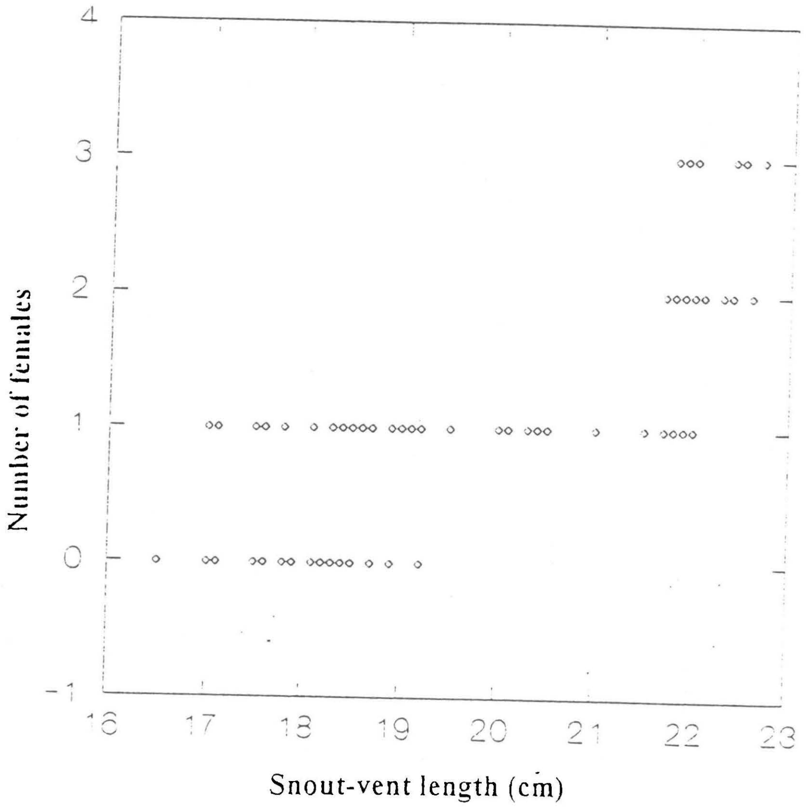


Figure 4.2 The relationship between male body size (SVL) and number of females in a male territory at Glen Austin and Witpoort.

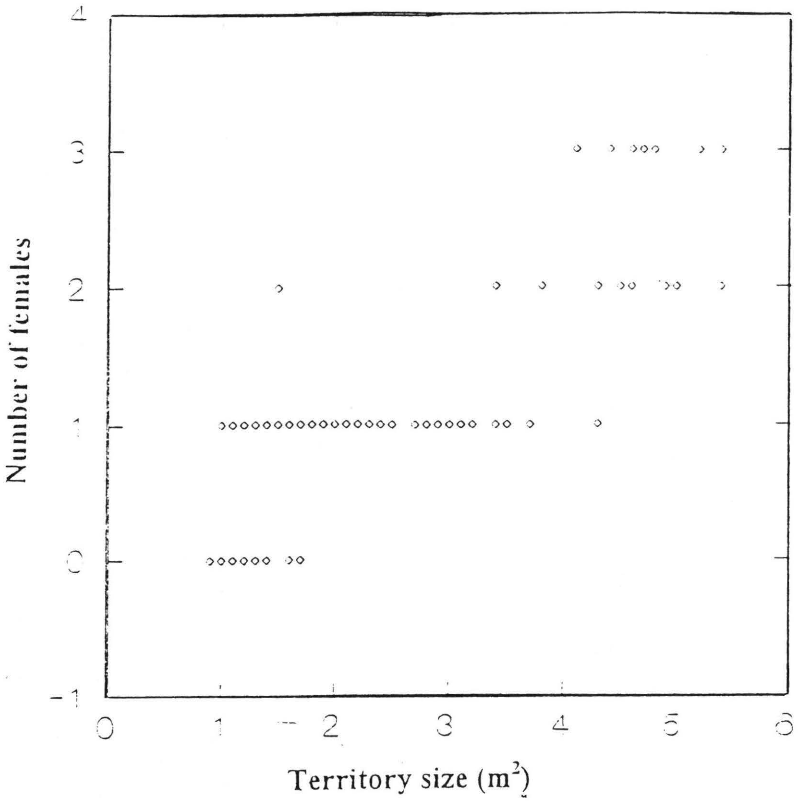


Figure 4.3 The relationship between territory size and number of females at Glen Austin and Witpoort.

4.4 DISCUSSION

Female arrival coincided with an increase in frequency of aggressive interactions between conspecific males. Females arriving in the morning did not differ in size from females arriving in the afternoon. Territorial males are presumably the older, larger males that monopolise matings in their territories and prevent smaller males from attracting females by actively defending these territories from intruding conspecific males. Females may choose large territorial male bullfrogs (see Chapter 3) because they are likely to be older and therefore proven survivors with presumably high quality genotypes. A reliable relationship between body size and age has not been found in anurans and it therefore appears that size only gives a rough indication of age (Robertson 1986).

It is not clear if female bullfrogs are choosing the traits of the male, the territory, or both, when selecting mates. Territory quality may play an important role in female choice. Females must choose a territory to ensure maximum survival of her offspring. These are territories which have sufficient water volume to ensure persistence until the tadpoles hatch and develop, enough vegetative cover to prevent desiccation and heating effects on the developing eggs and favourably positioned to ensure limited predation levels. Male size and mass were positively correlated with the number of females observed in the territories in all the breeding events at Glen Austin and Witpoort. The larger male bullfrogs occupied larger territorial areas; these territories were also favourably positioned. The larger male bullfrogs were successfully able to defend theses larger areas from smaller intruding conspecific males. Water depth in the territories did not seem to be an important factor in female choice. This may be because territory depths were relatively constant throughout the pan on the day the females bred.

Non-territorial frogs occur in aggregations because they are unable to defend a territory successfully from intruding conspecific males. Non-territorial males aggregate in areas where female encounter rate is particularly high (hotspots). Males may gain from `stimulus pooling': by displaying together, they may provide a greater attraction for females and draw in mates from a larger distance (Bradbury & Andersson 1987). Displaying aggregations of non-territorial male bullfrogs may be heard over a great distance. The continual calling and fighting may possibly play an important role in attracting females from the surrounding areas in the pan and veld. This was important at Witpoort as the males had distributed themselves around the entire shoreline of the dam, and thus the females had to migrate over a large distance before reaching the males. Displaying aggregations also have a negative effect as numerous predators such as herons and catfish are attracted to these aggregations, feeding on the smaller females and males.

Amplexus displacement is prevalent in the African Bullfrog. Displacement during the initiation of amplexus was common in the non-territorial aggregations and influenced observed mating patterns by preventing ovipostion. Amplexus displacement prevented any non-territorial males from monopolising a particular female resulting in partial clutches been deposited. A female usually only needs to mate with a single male to fertilise all of her eggs, however she need not copulate with only one male unless mating with more than one incurs some cost to her. A single mating that produces inviable or sterile offspring would claim a large part of a female's effort. Female promiscuity may be a mechanism by which she ensures her clutch is fertilised by more than one male which may incidentally lead to an increased genetic diversity of offspring (Backwell 1991).

Female bullfrogs usually only deposited partial clutches with non-territorial males. Females depositing single clutches were not larger than females depositing partial clutches with numerous males. If a female was releasing eggs when in amplexus with the first male which is displaced and she then mates with a second, she would have, in effect, been promiscuous. However, since this would be due to the male's amplexus displacement rather than the female's choice, this cannot be considered true promiscuity. Satellite males are typically found in a `low' posture adjacent to resident territorial males. Satellite males attempted to intercept females attracted to the large territorial males. Satellites amplexed with the intercepted females and began fertilization immediately. Females mating with satellites only deposited partial clutches, usually in very shallow water (<1cm) or directly onto the grass. No clutches fertilized by satellite males successfully hatched.

CONCLUSION

Territorial males compete for females either directly, by defending the females themselves, or indirectly, by defending resources to which females are attracted. Females visit males solely for mating and males provide parental care. Females may be choosing territorial males for their size, quality of territory, or both. The hypothesis of female choice does not adequately explain the results of this study concerning non-territorial males. Males initiated the majority of pair formations and amplexus displacement plays an important role in the eventual outcome of mating success. Female choice seems to play a minor role in the mating success of non-territorial males. Satellite males are the smallest adult males in the population and cannot successfully defend a territory or displace a male in the non-territorial aggregations. By parasitising territorial males they reduce the risks of severe injury. Females are intercepted and thus have no decisive choice in their mates. Nevertheless females risk severe injury moving into a male's territory whilst in amplexus with a satellite male.

SECTION 2:
THE LARVAL STAGE

CHAPTER FIVE

ENVIRONMENTAL EFFECTS ON OFFSPRING GROWTH AND SURVIVORSHIP

5.1 INTRODUCTION

Amphibians are an excellent group for the comparative study of the evolution of complex life histories as they exhibit a diversity of life histories, reproductive modes, and metamorphic and adult body sizes (Werner 1986). Speculations on the ecology of amphibian life histories have focused on the importance of growth rate in the larval stage (e.g., Wilbur 1980, Pfennig *et al.* 1991). The adaptive significance of the larval stage of anurans is believed to reside in their ability to exploit transient opportunities for rapid growth in temporary ponds (Wilbur 1980; Newman 1987;1988b).

5.1.1 EFFECTS OF EGG SIZE VARIATION AND TIMING OF EGG DEPOSITION ON SURVIVORSHIP

Egg size in anuran amphibians has been viewed as a resource trade-off for females with limited energy for reproduction (Tejedo and Reques 1992). This notion is derived in part from optimality models predicting that for each environment there is an optimal offspring size (Smith and Fretwell 1974; Brockelman 1975). Others have suggested that optimality theory is contradicted by the high degree of intraclutch size variability observed in amphibians (Kaplan 1980; Crump 1981). Egg size variability may be adaptive in unpredictable environments such as temporary ponds where the intensity of competition may vary annually (Tejedo and Reques 1992).

In temporary ponds, the length of the larval period may be an important fitness component because it is inversely related to larval survival (Tejedo and Reques 1992). In some anuran amphibians, individuals that grow relatively fast tend to metamorphose earlier (Wilbur and Collins 1973) and may be less susceptible to size-selective predators

(Brodie and Formanowicz 1987). Further, time to, and size at metamorphosis may have strong ecological implications related to long-term fitness since both can affect juvenile survival, age at first reproduction, or level of adult fecundity (Smith 1987; Semlitsch et al. 1988). The seasonal timing of reproductive events is considered an important aspect of the life history of animals (Alford and Wilbur 1985). Among anuran amphibians there is interspecific variation in the length of the breeding season ranging from a few days to several months (Wells 1977a). Egg and larval survival may influence the period over which adults breed. Three factors can vary with time of egg laying to influence the success of eggs and larvae. First, temperature and duration of water bodies containing eggs and larvae may change with the seasons. Second, the community of predators, competitors and food resources coexisting with tadpoles may also change with the season (Alford 1985 & Wilbur 1989a,b; Morin *et al.* 1990). Third, the quality of eggs laid by females may vary over a season because females breeding at different times differ either in age or in environmental conditions experienced during vitellogenesis (Kaplan 1987), or because evolutionary pressures may have led to females producing eggs of different quality at different variations in the environment experienced by the tadpoles (Crump 1984).

5.1.2 <u>ENVIRONMENTAL CAUSES OF CORRELATIONS BETWEEN AGE AND SIZE AT METAMORPHOSIS</u>

Speculation on the ecology and evolution of anuran life histories has focused on the importance of growth rate in the larval stage (e.g. Wilbur and Collins 1973; Wilbur 1980, 1987). The adaptive significance of the larval stage is believed to reside in the ability to exploit transient opportunities for rapid growth in ephemeral habitats (Newman 1989a,b). This growth opportunity (weighed against the liabilities of predation and desiccation) is considered a major factor contributing to the stability of the complex life history (Wassenburg 1975). Growth rate or body size may be an important component of the fitness of anuran larvae for several reasons. Faster growing larvae may be able to outcompete slower growing larvae through interference or exploitative competition (Wilbur 1980). They may more rapidly attain a size refuge from size-limited predators. They may also have a higher probability of completing development and metamorphosis before temporary ponds dry or before their cumulative probability of encountering a predator becomes too great (Alford 1986). Differences in vulnerability to predation within species of larval anurans may be due to differences in body size (Wilbur 1980). Specifically, larger individuals are thought to be less vulnerable to predation by many predators than are small individuals. A high growth rate would thus convey a lower vulnerability to predation in addition to greater competitive ability and a higher probability of avoiding pond desiccation.

5.1.3 EFFECTS OF DENSITY AND PREDATION ON TADPOLE SURVIVAL IN TEMPORARY PONDS

Causes of tadpole mortality have been extensively studied (Calef 1973; Newman 1987). In temporary ponds, larval mortality is often very high because tadpoles are unable to metamorphose before the ponds dry. Larval survivorship in both temporary and permanent habitats depends on pond conditions. Ecological factors, such as density (intraspecific competition) and food availability, that effect growth rate and length of larval period have been studied in detail under laboratory conditions or in field enclosures (Dash and Hota 1980 and see Wilbur 1980 for a review). These factors are expected to be important because survivorship is thought to be related to size (predation risk) and development time (desiccation risk). While these studies have clearly established that high density has a negative effect on growth and development, the extent to which such effects are responsible for variation in survivorship in natural ponds is not well documented (but see Smith 1983).

Cannibalism (intraspecific predation) is now recognized as a normal rather than an aberrant behaviour, and has been documented in over 1300 species of animals (Crump 1990). Cannibalism frequently occurs under conditions of overcrowding and food limitation (Yorke 1983). Cannibalism obviously benefits the perpetrator by providing a meal, but additional benefits of cannibalism are still a matter of speculation (Crump 1990). Cannibalistic behaviour is, at least in part, genetically controlled in some organisms (e.g., fish, Thibault 1974; flour beetles, Englert & Raibley 1977; rotifers, Gilbert 1980; corn earthworms, Gould *et al.* 1980). Cannibalism should be favoured by natural selection if it increases survivorship and reproductive output (Bobisud 1976).

Organisms such as tadpoles that develop in small, ephemeral aquatic sites and face unpredictable and fluctuating food supplies might resort to and benefit from cannibalism (Crump 1986). Any trait that reduces tadpole developmental time or increases body size at metamorphosis may be advantageous. Faster development should increase the probability of successful metamorphosis. A large body size at transformation should reduce the problems of water balance (because of lower surface area: volume ratio), provide access to a wider range of prey, eliminate some potential predators, and may result in earlier reproductive maturity (Wilbur 1980).

5.1.4 VULNERABILITY OF TADPOLES TO PREDATION: EFFECTS OF BODY SIZE AND DENSITY

Two generalizations follow from the literature on size-specific vulnerability of tadpoles to predation. First, tadpole vulnerability to predation by size-limited invertebrates (e.g., aquatic insects) and certain gape-limited vertebrates (e.g.,fish, tadpoles, and salamanders) decreases with increasing tadpole size until the onset of metamorphosis (Brodie & Formanowicz 1987). The cause for decreasing vulnerability with larger size may be either that larger tadpoles swim more efficiently or that predators are unable to handle larger tadpoles. Second, once a tadpole erupts its front limbs and begins to reabsorb its tail, it once again becomes vulnerable, due to reduced swimming ability. Exceptions to this last generalization are tadpoles such as certain ***Bufo*** and ***Rana*** that develop unpalatability during metamorphic stages (Crump 1984).

Many authors have suggested that differential size-specific predation on tadpoles selects for accelerated development or increased growth rate, large size being an effective anti-predator defence (e.g.Brodie & Formanowicz 1983; Travis 1983). While Crump *et al.* (1991) agree that certain types of predators may act as selective agents for accelerated development in young and in metamorphic tadpoles that only part of the relationship has been investigated to date (Crump *et al.* 1991). Most species of tadpoles are at risk from several different types of predators either simultaneously or at different stages of development (Formanowicz & Brodie 1982). Although many studies have been conducted using small predators such as aquatic insects, fish, tadpoles and salamanders few experimental studies of size-related predation on tadpoles by larger predators such as birds have been done, even though many birds readily eat tadpoles (e.g. Crump *et al.*1991). Presumably the evolution of growth and developmental rates in tadpoles is influenced by, and represents a compromise between, differential predation operating on different stages and sizes of tadpoles by a variety of predators (Crump et al. 1991).

The major questions addressed in this section are:

1.) Is there variation in egg size and the number of days until offspring metamorphosis?

2.) Does the environment affect time to, and size at, metamorphosis in the offspring?

3.) How important is male parental care and channel construction for offspring survivorship?

4.) Are the offspring adapted to tolerate extreme variation in temperatures in their temporary ponds?

5.) Do tadpoles preferentially associate with kin and what mechanism enables them to do so?

5.2 METHODS

African Bullfrogs (*P.adspersus*) usually breed after the first heavy summer rains between October and January. Breeding occurs on the day following a heavy storm. Eggs are either deposited in a male's territory or communally in a non-territorial breeding aggregation. Hatching occurs 48 hrs following oviposition and active schooling and feeding behaviour is first seen on the day following hatching. Observations and monitoring of larval development at Glen Austin and Witpoort occurred daily between 0700 and 1800 hours for the entire duration (31-35 days) for each breeding event. The number of emerging tadpoles and clutch sizes were only estimated due to the large numbers involved. Initial density was estimated visually. Most territories had either very high (>2000 tapoles) or low (<1000 tadpoles) densities. Tadpoles (**n**=120) were randomly selected, every 24hrs, from various territories and measured to the nearest mm until metamorphosis. Because of mortality and predation during the larval period it is desirable to have an estimate of density through larval development. The number of tadpoles are difficult to estimate precisely because the schools of tadpoles are highly mobile and the water in the pan and dam is murky. Tadpole density was therefore not estimated quantitively. By experience and calibrating visual impressions with counts from exhaustive sampling, territories could be placed into high (>2000), medium (1000-2000), or low density categories (<1000). Predation levels of tadpoles were estimated by the density of the tadpole schools and predators were monitored and identified. Duration of tadpole development was noted until completion of metamorphosis. Emerging froglets (**n**=10 from each clutch) were measured to the nearest mm and weighed to the nearest g.

5.3 RESULTS

5.3.1 DEVELOPMENTAL RATES AND SIZE AT METAMORPHOSIS OF TADPOLES: There was little variation in the number of days to, and size of tadpoles at metamorphosis over all the breeding events, so the data were pooled. There were two distinct different-sized tadpole morphs, a large rapidly developing tadpole and a normal developing tadpole. The rapidly developing morph completed metamorphosis between 19-21 days (**x**=20.4, **sd**=0.8, **n**=120 tadpoles,fig.5.3) while normal clutches of tadpoles took between 28-35 days to metamorphose with a mean of 32 days (**sd**=1.3, **n**=120 tadpoles, fig 5.3). The rapidly developing tadpoles were significantly larger than normal developing tadpoles throughout the larval period (**5 days:** **t**=22.44, **p**=0.00001, **df**=12; **10 days:** **t**=32.01, **p**=0.00001, **df**=11; **15 Days:** **t**=38.95, **p**=0.00001, **df**=26). Mean size at metamorphosis of the rapidly developing tadpoles was 31.77 mm (**sd**=1.7, **n**=50, **range**=27-35). Normal tadpoles were significantly larger (**t**=5.31, **p**=0.00001, **df**=36,) with a mean SVL of 33.65 mm (**sd**=0.7, **range**=32-35, **n**=120). There were no significant differences between the number of days to, and size of tadpoles at, metamorphosis at Witpoort during the two breeding events so the data were pooled. There was a significant difference in the number of days until metamorphosis of the rapidly growing tadpoles and the normal developing tadpoles (**t**=8.87, **p**=0.0003, **df**=5). The rapidly developing tadpoles had a mean developmental rate of 23.41 days (**sd**=0.7, **n**=50, **range**=21-25 days) while normal tadpoles took an average of 34.2 days (**sd**=0.9, **n**=100, **range**=33-36 days) until metamorphosis. The mean size of the rapidly developing tadpoles were significantly larger than the normal tadpoles throughout their development (**t**=15.1, **p**=0.0001, **df**=9). Normal developing tadpoles metamorphosed at a significantly larger size than the rapidly developing tadpoles (**t**=4.54, **p**=0.001, **df**=7). Mean size at metamorphosis of the rapidly developing tadpoles was 31.5 mm (**sd**=1.3, **n**=180, **range**=28-33) while that of the normal tadpoles was 32.9 mm (**sd**=0.6, **n**=180, **range**=31.5 -33.2). Tadpoles developed at a quicker rate at Glen Austin during all breeding events compared to Witpoort (**t**>5.60, **p**=0.0001, **df**>16). Glen Austin's newly metamorphosed frogs were significantly larger than those found at Witpoort (**t**>2.18, **p**<0.044, **df**>15).

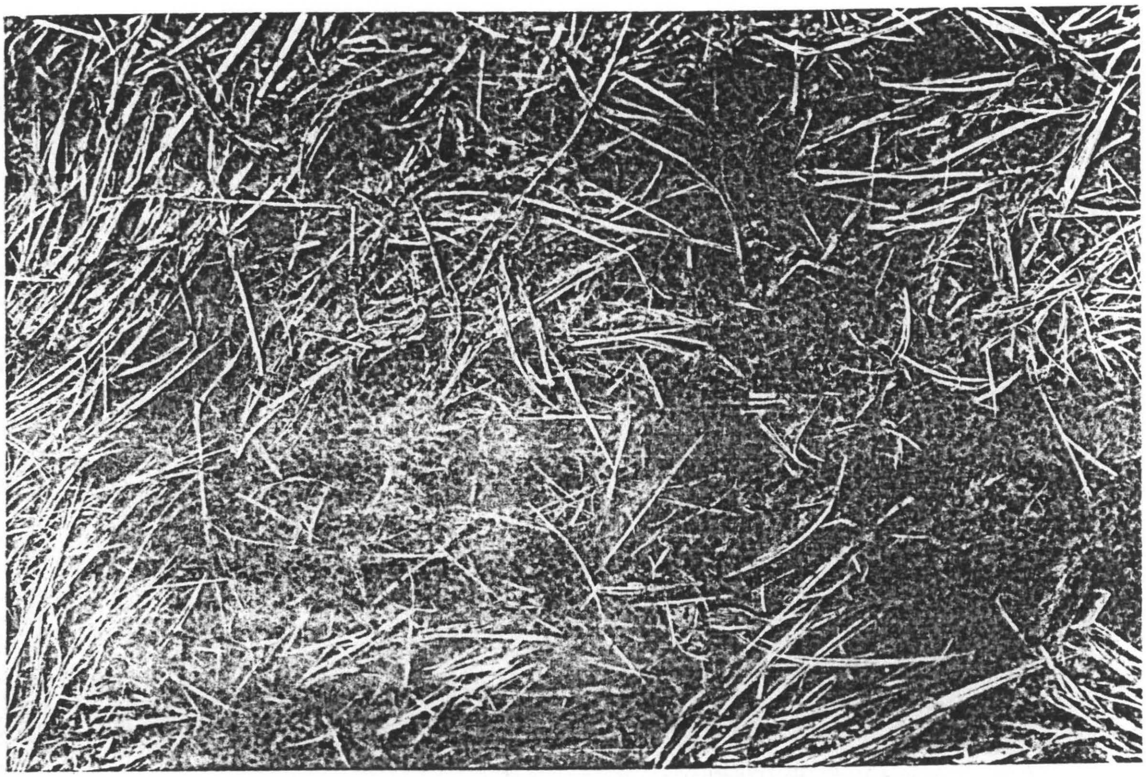


Figure 5.1 A photograph of a typical clutch of African Bullfrog eggs.

Figure 5.2 A photograph of a gregarious school of African Bullfrog tadpoles. Note the two different sized tadpole morphs present in the school.

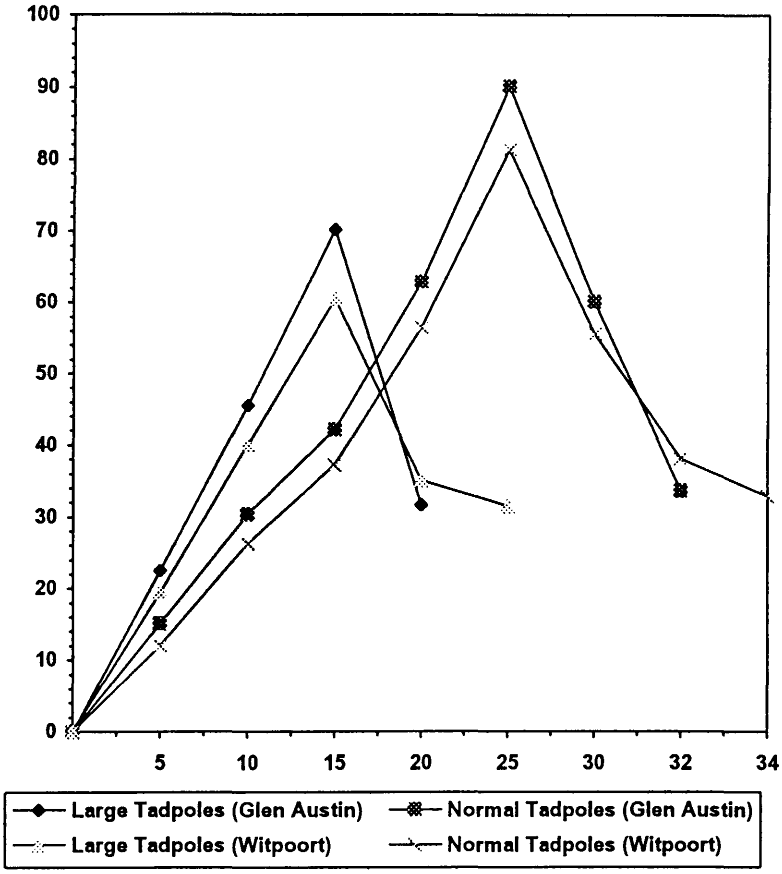


Figure 5.3 Number of days and size at metamorphosis of tadpoles at Glen Austin and Witpoort during the 1992 breeding season.

5.3.3 PREDATION ON LARVAL AND ADULT STAGES

The eggs and larvae of ***Pyxicephalus adspersus*** are heavily preyed upon during all stages of development. Numerous large aquatic invertebrates including predatory, aquatic beetles (Family: Dystiscidae), dragonfly nymphs (***Orthetrum sp.***) and leeches (***Hirrudo sp.***) were observed feeding on eggs and larvae of the bullfrog. At Glen Austin there are no fish predators and survivorship depends critically on the migration, and numbers, of various other predators into the habitat. The dam at Witpoort contains numerous predatory fish species including the sharp toothed catfish (***Clarias gariepinus***) and the carp (***Cyprinus carpio***). Male bullfrogs were observed moving towards a feeding catfish and forcing it away from the breeding area. The carp and the bullfrog breeding activity coincides with the first heavy summer rains and carp were observed devouring whole clutches of eggs and tadpoles. Marsh or helmeted terrapins (***Pelomedusa subrufa***) are common in the pan and dam and have been observed feeding on both the eggs and larvae. A large resident rinkhals (***Hemachatus haemachatus)*** at Glen Austin was often observed feeding on the large unattended tadpoles, floating and feeding from the gregarious school. Platannas (***Xenopus laevis***) are abundant at both Glen Austin and Witpoort and are potential predators. Cannibalism by large tadpoles and juveniles was common in both the field and in the laboratory. Larger tadpoles readily fed on smaller or injured tadpoles. Tadpoles were also observed feeding on the dead body of an adult male bullfrog. Water birds are the major predators of tadpoles at Glen Austin and Witpoort, the main predators being the sacred ibis (Threskiornis aetiopicus), glossy ibis (Plegadis falcinellus), grey heron (***Ardea cinera***), blackheaded heron (***Ardea melanocephala***) and goliath heron (***Ardea goliath***). The herons fed on the developing larvae as well as the small adult females and small male bullfrogs. A strategy adopted by the herons feeding on territorial bullfrog tadpoles was to peck out the eyes of the defending male and feed off his offspring. Emerging juvenile bullfrogs were fed on by numerous avian predators especially the fiscal shrikes (***Lanius collaris***) which hung the bodies on the surrounding barbed wire fences. African marsh harriers (***Circus ranivorus***) and marsh owls (***Asio capensis***) were observed feeding on both juvenile and adult bullfrogs.

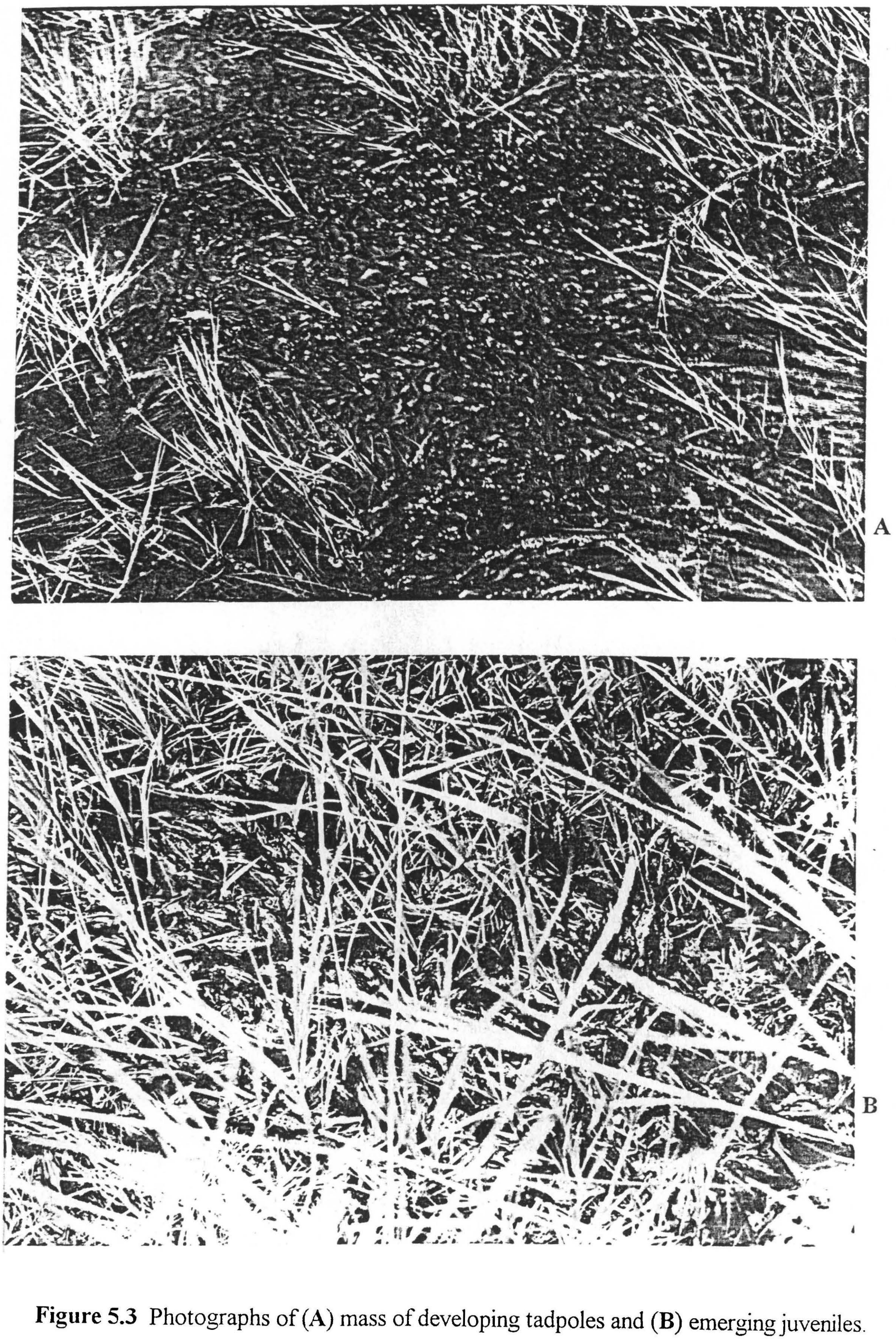


Figure 5.3 Photographs of (A) mass of developing tadpoles and (B) emerging juveniles.

5.4 DISCUSSION

Egg size variability may be adaptive in unpredictable environments such as temporary ponds where the intensity of competition may vary annually (Tejedo and Reques 1992). There was only a small degree of variability in the egg size of the African Bullfrog (***Pyxicephalus adspersus***) although there were two distinct tadpole phenotypes. The abundant food and warm shallow water at the Glen Austin pan lead to the significantly shorter larval period compared to the dam at Witpoort. In temporary ponds, larval mortality is often very high because tadpoles are unable to metamorphose before the ponds dry. Pond desiccation played a major role at Glen Austin's temporary pan while it was not a factor at Witpoort's permanent man-made dam.

Tadpoles of the African Bullfrog (***Pyxicephalus adspersus***) occur as two distinct phenotypes, a large, rapidly developing, carnivorous morph which metamorphoses after between 19-21 days and a normal developing, omnivorous morph which completes metamorphosis after 31-35 days. Large tadpoles made up between 2-5 % of the total clutch of tadpoles. Cannibalism was frequently observed in the field and in the laboratory tanks, with the larger tadpoles feeding on the smaller or injured tadpoles. Having two distinct tadpole phenotypes ensures the survival of a certain percentage of the clutch in these unpredictable ephemeral habitats. A high growth rate would thus convey a lower vulnerability to predation in addition to greater competitive ability and a higher probability of avoiding pond desiccation. Although the large, rapidly developing tadpoles completed their larval development in a significantly shorter period than the normal developing tadpoles they metamorphosed at a significantly smaller size. A large body size at transformation should reduce the problems of water balance (because of lower surface area: volume ratio), provide access to a wider range of prey, eliminate some potential predators, and may result in earlier reproductive maturity (Smith 1987).

Aggregations of fighting, splashing non-territorial males attracted numerous predators. The non-territorial males migrated out of the pan and dam after the cessation of breeding activity leaving their eggs unattended . The majority of eggs associated with non-territorial males at Witpoort were consumed by fish and birds before they hatched and destroyed due to desiccation at Glen Austin. With the synchronous hatching and development of the tadpoles the predators are satiated which ensures maximum survival of offspring. Although predation levels effect the survivorship of larvae, desiccation seems to be the major limiting effect on the successful survivorship of tadpoles.

Anti-predator mechanisms adopted by the gregarious schools of tadpoles included the release of a presumed alarm substance by injured tadpoles, which causes the school to scatter, confusing potential predators and alerting the male. Similar alarm behaviour substances occur in bufonids (Waldman 1982b). Males also 'shepherd' their tadpoles away from areas of heavy predation into deeper waters.

CHAPTER SIX
OFFSPRING DEVELOPMENT AND SURVIVORSHIP

6.1.1 ENVIRONMENTAL EFFECTS ON TIME TO, AND SIZE AT, METAMORPHOSIS IN *PYXICEPHALUS ADSPERSUS*

Traits of amphibians such as growth rate, developmental rate, and size at metamorphosis show considerable intrapopulation variability (Werner 1986). Although these life history traits can be under partial genetic control (Travis 1984), they also respond to environmental factors such as temperature, larval density, water volume, food abundance, and food quality (Wilbur 1980). One trait recently receiving attention is developmental rate of larval amphibians in aquatic sites whose duration is unpredictable (Newman 1988a). Several studies have demonstrated that length of the larval period not only has a genetic component but also varies in response to pond drying (Pentranka and Shi 1987; Newman 1988a, 1988b). Ephemeral rain pools vary unpredictably in duration. Thus, species that breed there are expected to exhibit phenotypic plasticity in developmental rate. This plasticity should be affected by environmental cues (Crump 1989).

I tested two predictions, according to protocols used by Crump (1991), on bullfrogs that breed in temporary rain pools: **1**) tadpoles can accelerate development in a drying aquatic habitat; and **2**) accelerated development in drying ponds reduces body size at metamorphosis.

6.1.2 METHODS

Experiments were conducted using protocols developed by Crump (1989).

To minimize genetic contributions to variance in growth and developmental rates, I used similar-sized tadpoles from one clutch of eggs produced by a marked pair of frogs in the field. Newly hatched tadpoles were placed in a holding tank for the next 5 days and were fed rabbit pellets. I placed ten tadpoles, randomly chosen from the holding tank into each of 30 cylindrical plastic containers (inside diameter 23 cm). Six bowls (60 tadpoles) were assigned to each of five treatments: **(A)** constant 6000 ml water, bowl water depth = 80 mm; **(B)** constant 3000 ml water; bowl water depth = 40 mm; **(C)** constant 1500 ml water (20 mm) **(D)** decreasing amount of water, from 3000 ml to 750 ml (4cm-1cm) and **(E)** constantly increasing water, from 750 ml to 3000 ml (1cm-4cm). For treatment D the amount of water was reduced by 150 ml every third day for the first 12 days; for the next 8 days the amount was reduced by 200 ml daily, and 50 ml was removed on day 21 (= 750 ml water remaining). For treatment E the same amount of water was added instead of removed as in D (750-3000 ml). With this design I could compare developmental and growth rates of tadpoles from a rapidly deteriorating environment to an improving environment as well as a constant high, medium and low level of water. All five of these situations are potentially encountered under natural conditions. The bowls were placed on the laboratory floor in three rows of 10 bowls each. Each row consisted of two bowls from each treatment, in order to spread any extraneous environmental effects (e.g. sunlight or temperature) equally over the five treatments. I changed water in each bowl at the same time daily to minimize the effect of waste accumulation on growth and development. All tadpoles were reared under a 14:10 h light:dark cycle at 22-26°C. Tadpoles in each bowl received 4 rabbit pellets daily. As the front legs of each tadpole erupted, I recorded the date. Within the first hour of forelimb eruption the tadpoles climbed onto the sides of the bowls. At this point each tadpole was removed and put into a covered cup. Once the tadpole had reabsorbed all but 5 mm of tail I determined wet mass (to 1mg) and SVL (to 1 mm). The data was subjected to analysis of variance (water levels as treatments bowls within treatments, and tadpoles within bowls). Pairwise Student t-tests between treatments were calculated.

6.1.3 RESULTS

Pyxicephalus adspersus tadpoles exposed to a rapidly evaporating environment developed significantly faster than siblings exposed to an increasing water level as well as to a constant high, medium and low water level (Fig.6.1)

Tadpoles exposed to a constant high water level treatment required the longest time to reach forelimb emergence (initiation of metamorphosis) (Fig.6.1A). Among-treatment differences were highly significant (**$F_{4,165}$**=496.3, **p**=0.0001). All treatments were significantly different from each other (treatments **A-B, A-C, A-D, A-E, B-C, B-D, B-E,C-D** and **D-E; p**=0.00001). The ANOVA revealed significant variation among bowls within treatments (**$F_{5,54}$**=7.25, **p**=0.009). Bowl effects may have been related to variation in water temperature; tadpoles from bowls in the middle experienced slightly elevated temperatures and, perhaps for this region, developed faster than siblings from bowls exposed to slightly lower temperatures. However, because two replicates of each treatment were assigned to each row, position in the experimental array did not differentially influence developmental rates of tadpoles across the five treatments. Experimental conditions also influenced body size of tadpoles at metamorphosis (Fig.6.1B). An ANOVA revealed significant among-treatment effects (metamorphosis **$F_{4,95}$**=217.2; **p**=0.0001), but no significant within bowl treatment effects. There was significant differences in body mass of the newly metamorphosed tadpoles (**$F_{4,95}$**=289.0, **p**=0.0001, Fig.6.1C)

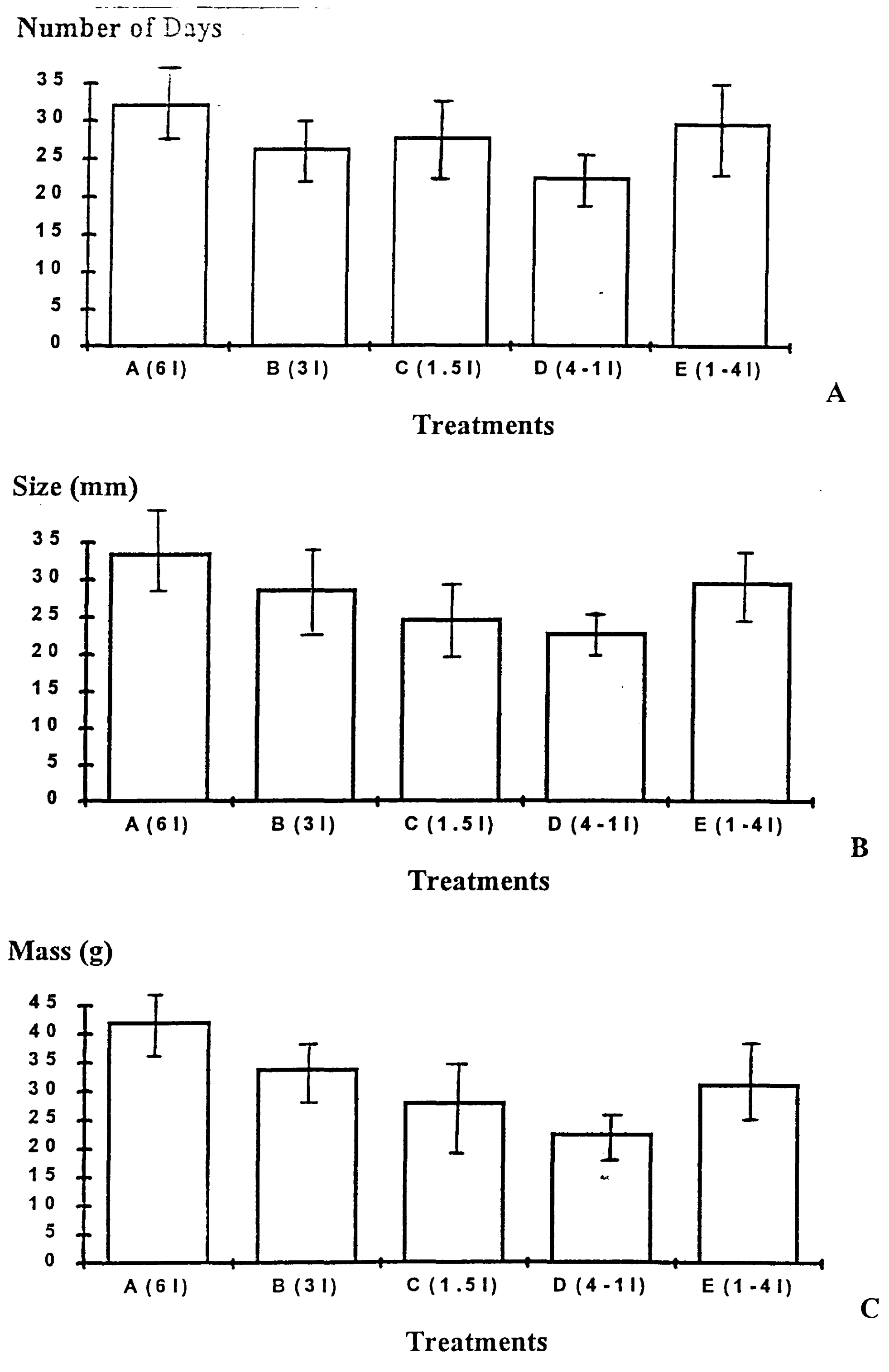


Figure 6.1 Histograms showing the mean (A) developmental time; (B) size and (C) wet mass at metamorphosis for the five experimental treatments.

6.1.4 DISCUSSION

Phenotypic plasticity in growth and development parameters could enhance survival of organisms in highly variable aquatic environments (Caswell 1983; Kaplan & Cooper 1984; Via & Lande 1985). Although within population variability in growth and development parameters has been well documented for numerous species of amphibians, it is not clear how much flexibility can occur in individual larvae (Crump 1989). Models that predict the optimal timing of amphibian metamorphosis (Wilbur and Collins 1973; Werner 1986) assume a compromise between growth and development. As tadpoles near metamorphosis, low food level and drying conditions may trigger accelerated development relative to individuals with abundant food resources in longer lasting sites. In a deteriorating environment, larvae are expected to develop as quickly as possible to escape the site, even though they will be smaller at metamorphosis (Crump 1989).

For those tadpoles in a long-lasting pond, able to remain in water and continue feeding, the larger body size attained at metamorphosis may confer increased fitness. For example, large body size at metamorphosis is correlated with large body size at onset of reproductive maturity in ***Rana sylvatica*** (Berven and Gill 1983) and with earlier reproductive maturity in ***Pseudarcis triseriata*** (Smith 1987). Large body size is advantageous for anurans because larger females generally produce more eggs (Salthe and Duellman 1973) and larger males may be more successful in attaining mates (Howard 1988 and see chapter 3). Other advantages to large size at metamorphosis may include reduced risk of predation, reduced water loss, and increased range of accepted prey items, but these possibilities have not been tested (Crump 1989). Few controlled experiments (Travis 1984; Alford and Harris 1988) have tested the models concerning optimal timing of amphibian metamorphosis. Semlitch (1987) found that most ***Ambystoma talpoideum*** larvae did not initiate metamorphosis and died when the experiment pond dried. Growth was slow, and presumably the larvae failed to reach a threshold body size that would permit initiation of metamorphosis. In a different experiment, Semlitch and Wilbur (1988) found that ***Ambystoma talpoideum*** did respond to pond drying by accelerated development to metamorphosis. Newman (1988a) documented faster development and smaller body size at metamorphosis among

Scaphiopus couchii tadpoles in experimental ponds of short duration, as compared to ponds of longer duration.

My data showed that simulated drying conditions accelerate development of tadpoles of ***Pyxicephalus adspersus***, with the consequence of reduced body size and mass at metamorphosis. Tadpoles in the deteriorating experimental environment (4cm-1cm) developed significantly faster (22 days) but were the smallest at metamorphosis. Tadpoles in a constant 3000 ml (4cm) took an intermediate amount of time (28 days) to develop, but metamorphosed at a significantly larger body size and were followed by the constant 1500 ml (2cm; 30 days) and the rapidly increasing water depth (1-4cm ;31 days). Crump (1989) found that ***Hyla pseudopuma*** tadpoles developed significantly earlier at a small size under simulated experimental drying conditions (25 days). The most unfavourable experimental environment for developmental rate was the constant 6000 ml (8cm) with the tadpoles requiring significantly longer time (34 days) than siblings from the other four treatments, yet metamorphosed at a large size significantly different from the early metamorphosing tadpoles raised in the deteriorating environment. Although the water was changed daily, build-up of toxic waste material may have adversely affected the tadpoles under the crowded conditions of treatment C. Numerous studies have documented the adverse effects of high density on tadpole growth and development (Dash and Hota 1980; Semlitsch and Caldwell 1982). ***Pyxicephalus adspersus*** breed in a variety of habitats, ranging from temporary rain-filled pans to man-made dams that persist for longer periods of time. Tadpoles of this species appear to exhibit phenotypic plasticity in developmental rate in their various habitats. Developmental rate depends on habitat duration as well as numerous environmental factors such as temperature, larval density, food abundance and food quality. My results support the prediction that during unfavourable conditions such as rapidly evaporating ponds, tadpoles should accelerate metamorphosis at the expense of small body size ensuring the survival of certain tadpoles. Under favourable conditions, if the water levels persist, tadpoles should remain in the water, eat, and metamorphose at a larger body size.

6.2.1 TOLERANCE OF HIGH TEMPERATURES IN TADPOLES OF *PYXICEPHALUS ADSPERSUS* IN TEMPORARY PONDS

INTRODUCTION:

The range of temperature over which anurans are active varies, but tropical species are known to be active at higher temperatures than their temperate counterparts (e.g. Brattstrom 1963, 1968; Feder et al. 1982). To some extent terrestrial anurans can control their body temperature below ambient by evaporative water loss (Brattstrom 1970). However, during the larval stage the body temperature may be identical to water temperature, as in aquatic salamanders (Brattstrom 1963, 1970). Tadpoles of some species are reported to select for preferred temperature in a thermal gradient (Wassersug 1974).

Many species of anurans breed in temporary ponds (Wells 1977a; Sullivan 1989). The advantage for these species breeding in such unstable water bodies, is the absence of resident predators and absence or reduction of competitors for the tadpoles, common in permanent bodies of water (Wassersug 1974,1975). Nonetheless, temporary ponds often present the risk of low oxygen tension, overheating or drying up before larval development has been completed. Thus, amphibians breeding in temporary ponds are apparently selected for a shortened larval stage (Abe & Neto 1991).

Pyxicephalus adspersus breed in temporary ponds following the first heavy summer rains (Balinsky & Balinsky 1954). The temporary ponds are often exposed to high temperatures. In this study the upper thermal tolerances were experimentally determined for tadpoles of ***P.adspersus***, based on extreme temperatures recorded in temporary ponds.

6.2.2 METHODS:

The experiments are based on procedures used by Abe and Neto (1991).

Field Data

The water temperatures of temporary ponds were recorded at various sites (**n**=48) and depths with a Sensortek BAT-12 digital thermometer (Accurate to ± 0.1°C). Measurements were made from 0700-1800 hours in the centre of the aggregate of tadpoles to determine the daily temperature variation in the temporary ponds. The temporary ponds were located in a temporary pan situated at the Glen Austin Bird Sanctuary. Body temperatures of the tadpoles in the field were assumed to be the same as those of the surrounding waters (Hutchison 1961; Abe & Neto 1991).

Experimental procedure

Tadpoles of ***P.adspersus*** were collected from the temporary ponds and kept in aquaria at 25 - 28°C for 24 hr prior to use. In each experiment a group of 10 or 15 tadpoles (stages 26-38, Gosner 1960) were removed from the holding aquaria and placed into a 1000 ml beaker filled with 300 ml of aerated water, and placed into a circulating thermostatic bath at 35°C. The temperature of the bath was increased at the rate of 1°C every 5 min, and then kept at a constant temperature of 38, 40, 42 or 44°C for 15, 30 or 60 min. At the end of the experiment the beaker with the tadpoles was removed and placed into a bath at 30°C until temperature equilibrium was reached and then the survivors were counted. All tadpoles were used in only one experiment. The experiments were run between 12h00-16h00 which coincided with the highest temperatures recorded in the field. All tadpoles used in experiments which were returned to the maintenance aquaria were observed for at least three additional days.

6.2.3 RESULTS:

Field measurements

Eggs exposed to temperatures above 38°C did not hatch and were presumably denatured. Tadpoles of ***P.adspersus*** were usually found in water shallower than 10cm. Water temperature ranged from 18 to 39°C (n=150) in the 20 ponds measured. Expressing an average water temperature is meaningless as the temperature of the pond depends on the thermal characteristics of the pond as well as the time of day (Abe & Neto 1991). The thermal characteristics of the ponds depend upon size, depth, bottom type, presence of plants and organic debris (Abe & Neto 1991). There were no large temperature gradients in the ponds with differences of only 4°C recorded between the surface and bottom temperatures. Temperatures seldom exceeded 30°C in the early morning (0700h-1100h) but reached up to 40°C in the hot afternoon (1200h-1500h). The critical temperature range was usually reached between 1300h and 1400h. Temperatures of 40°C rarely lasted longer than 20 min.

High temperature tolerance

The results for high temperature tolerance are summarised in Table 6.1. There was a consistency in the relation between temperature tolerance and developmental stage.

The larger tadpoles (**size** 90-120 mm, Gosner stages 31-38) have a significantly higher tolerance to temperatures above 40°C than the smaller tadpoles (**size** 10-90 mm, Gosner stages 24-30; **t**=7.51, **p**=0.0001, **df**=53). Since no survival was recorded for a 15 min exposure at 44°C, further experiments of 30 and 60 min exposure were not done. Vigorous movements were observed prior to the onset of spasms. Even after reaching an inactive state, some individual recovered upon return to 35°C. No death was recorded in the three days following experiments.

Table 6.1 Tolerance to high temperatures in tadpoles of ***P.adspersus***l (**n**=30 for each temperature). Percentage of tadpoles surviving in brackets.

			Temperature (°C)			
Gosner Stage	**Tadpole Size (mm)**	**Time Of Exposure (min)**	**38**	**40**	**42**	**44**
24-30	10-90	15	30 (100)	30 (100)	12 (40)	n/a
31-38	90-120	15	30 (100)	30 (100)	18 (60)	0 (0)
24-30	10-90	30	30 (100)	30 (100)	3 (10)	n/a
31-38	90-120	30	30 (100)	30 (100)	6 (20)	n/a
24-30	10-90	60	30 (100)	30 (100)	0 (0)	n/a
31-38	90-120	60	30 (100)	30 (100)	3 (10)	n/a

6.2.4 Discussion

Thermal tolerance experiments showed that tadpoles of ***P.adspersus*** can survive the highest temperatures recorded at the temporary ponds and puddles. Even at the highest field recorded temperature of 39°C the tadpoles would be able survive exposure for over an hour. Since the tadpoles were collected from similar conditions and even from the same territory, differences in high temperature tolerance due to acclimation or hardening seems unlikely. Hardening is more rapid in its onset and decay than acclimation (Mannes & Hutchinson 1980). Tolerance experiments run with ***Leptodactylus fuscus*** at 44°C after previous exposure to 42°C 24h earlier did not show any evidence of hardening (Abe & Neto 1991). As shown by Maness and Hutchinson (1980) in two species of amphibians and fish heat hardening is transitory and decreased to the initial level within 24h. In the field hardening may play an important role in heat tolerance with the tadpoles being exposed to gradual increases in water temperature (Abe & Neto 1991). Zweifel (1977) found an increase in temperature tolerance of anuran embryos in relation to stage of development, up to stage 20 in species from various habitats. Abe and Neto (1991) found no difference in temperature tolerance between stages 26-38 which may be an adaptation to breeding in warm water. Embryos of ***Scaphiopus couchii***, an anuran also adapted to breed in warm water, have an early attainment of maximum tolerance and high level tolerance (Zweifell 1977).

Tadpoles of ***P.adspersus*** show an increase in temperature tolerance relative to their developmental stage. The larger tadpoles have a significantly higher tolerance to temperatures above 40°C than the smaller tadpoles. This may be due to hardening or acclimation of tadpoles due to prior exposure to increasing water temperatures. In the field the larger tadpoles are able to lower the water temperature in the temporary pond by the cooling effect due to the circular swimming motion of the school. Temperature measurements taken in the middle of the school are usually between 2-4°C cooler than outside the school. The clockwise swimming motion of the school may also serve to aerate the water in the territory. Field observation showed eggs exposed to temperatures above 38°C did not hatch and were presumably denatured, while tadpoles readily survived temperatures above 38°C. The extreme temperatures recorded in the field were of shorter duration than those measured experimentally, suggesting that tadpoles of ***P.adspersus*** are adapted to live in shallow temporary ponds with extremely high water temperatures.

6.3.1 EFFECTS OF MALE PARENTAL CARE ON TADPOLE SURVIVORSHIP

INTRODUCTION:

Anuran reproductive strategies encompass not only adaptations to ensure fertilisation, but also factors which facilitate the survival of offspring and parental care (Duellman and Trueb, 1986). The evolution of parental care remains one of the few topics in evolutionary biology where unifying principles are lacking (Jennions 1992). Parental care is formally defined as parental behaviour that is likely to increase the fitness of the parent's offspring (Clutton-Brock & Godfray 1991). This definition is, however, sometimes difficult to apply (see Gwynne 1991).

Clutton Brock (1991) in a recent book-length review of parental care focused on five main questions (Jennions 1992). The questions which are best understood are: What are the costs and benefits of parental care? [Answer: the main benefit is increased offspring survival, growth and breeding success, hence increased fitness for parents]; Why does the extent of parental care fluctuate between species [Answer: it varies in relation to the magnitude of the harshness of the environment, and the degree of intra-specific competition]. Less well understood are the questions: What explains male, female and biparental care? Is parental care adjusted in relation to the costs for parents and the benefits for offspring? How is parental care divided between sons and daughters? Parental care has been reported in a number of frogs (Townsend 1986; Summers 1989; Channing *et al.* 1989). Most reports are from terrestrial breeders in the tropics (Wells 1981). Both males and females have been implicated in care. In fact, frogs are unusual in that male parental care is relatively widespread. There are two possible factors that make male parental care more likely to occur in frogs than in other vertebrates. First, the costs of parental care are usually low (Wells 1981). Second, eggs or tadpoles are deposited in the male's territory so that he can provide care for them and continue to attract additional females (but see Townsend 1986). Parental care may take several forms including; guarding eggs or tadpoles against predation (Kluge 1981); preventing desiccation

(Townsend et al. 1984); maintaining access to water (Channing *et al.* 1989); and carrying tadpoles, either to water (Summers 1990) or until development is completed (Ingram et al. 1975).

Channel construction in the African Bullfrog (***Pyxicephalus adspersus***) was previously investigated by Channing *et al.*(1989). They reported that adult male bullfrogs construct channels to allow tadpoles to move from small isolated peripheral ponds to larger more permanent bodies of water (Channing *et al.*1989). I suggest that the channels are not only bridges from one shallow peripheral source of water to the main deeper source of water, but serve to supply cooler water to the rapidly drying breeding ponds containing developing eggs. Channels are constructed in response to deteriorating environmental conditions at the breeding site.

6.3.2 METHODS:

The location and area occupied by territorial males were recorded at the study site. Temperature and water depths of the territories (**n**=48) were monitored hourly and the presence and absence of territorial males and channels were noted throughout the larval development stage. If channels were present they were measured (length and breadth), channel depth was recorded, and time of construction recorded. Observations of the male position as well as tadpole movements after the construction of the channel were documented. Male removal experiments were undertaken with selected males (**n**=10) being removed from their territories to determine the effects of male parental care on offspring survivorship.

6.3.3 RESULTS:

6.3.3.1 Channel Construction (See Appendix II for Data on channel construction)

Bullfrogs emerged after heavy rains and bred in shallow temporary ponds. The breeding ponds were created by the territorial male bullfrogs flattening the surrounding vegetation during courtship with females and aggressive encounters with conspecific males. The female bullfrogs dispersed from the breeding area immediately after spawning. Successive dry days (22-23 December 1992 only 1.2 mm of rainfall; 18-20 October 1993 4.6 mm of rainfall) resulted in the rapid evaporation of water from the breeding ponds.

Channels (**n**=46) began to appear next to the drying breeding ponds which now contained developing eggs. Males vacated five territories which were totally dessicated and the eggs denatured. Channels did not only appear during the dry periods but were also constructed under good rainy conditions (24-26 November 1993; 48 mm of rainfall). Mean channel length was 3.16 m (**range**=0.9-18.7 m, **n**=64) with a mean width of 15.1cm (**range**=13.5-16.7cm, **n**=64) and a mean depth of 4.1 cm (**range**=2.1-4.9 cm, **n**=64). Channel lengths were dependent on the density of the surrounding vegetation as well as the distance from the breeding ponds to the main body of water. Channels were constructed by the adult male bullfrogs, with the majority of channels being constructed during the daytime between 08.00-17.00h (**n**=49) although certain channels were constructed in the evening 18h00-24h00 (**n**=15). Channels were created by the adult males who flattened the surrounding sedges or dug channels through the mud using their hindlimbs. Channel shapes varied from straight to meandering through the surrounding grasses and sedges connecting the breeding ponds to the main body of water. Channels were constructed around obstacles. Temperature differences between the channels and breeding territories varied from 0-3.1°C (**mean**=0.97°C, **sd**=4.2, **n**=142). This was due to the fact that the channels were constructed in the sedges and grasses which had a shadowing effect during the heat of the day (±14.30h) while the breeding ponds had little or no surrounding vegetation as this tended to be flattened during the courtship and breeding process. Not all breeding territories had channels, certain territories were favourably positioned in the sedges, while the resident males of other territories vacated their territories after breeding leaving their eggs unattended. In certain breeding ponds without channels the developing eggs were exposed to temperatures above 38°C (n=14) and no eggs survived. The eggs were either unfertilized or more likely denatured by the extreme high temperature of water in the breeding ponds. Channels also provided fresh water to the rapidly evaporating territories, with an average increase in water depth of 0.545 cm (**range**=0-3.5cm; **sd**=4.4, **n**=64). Although the majority of channels were constructed before the eggs hatched (n=44) some channels were constructed after the tadpoles hatched (n=20). Once the tadpoles hatched they would begin development in the breeding ponds and after between 2-6 days vacate their breeding ponds, moving into the deeper more permanent body of water via the channels. The tadpoles continued their larval development in the main body of water (14 cm deep, surface temperature ± 27° C). Certain tadpoles would move in and out of their breeding ponds during the day, with the resident male either leading or following them along the channel. Other tadpoles only travelled once along the channel as both the channel and the breeding ponds dried up.

6.3.3.2 TADPOLE PREDATION AND SURVIVORSHIP

At Glen Austin during the 1st breeding event, the territorial tadpoles (see Table 6.2) had a significantly higher survival rate compared to the non-territorial tadpoles (**t**=5.71, **p**=0.0001, **df**=13). This was due to the fact that the majority of eggs deposited in the non-territorial areas were either denatured due to extreme temperatures (>40°C) or desiccated. The survivorship of non-territorial and territorial tadpoles was impossible to determine during the 2nd and 3rd breeding events at Glen Austin due to the vast number of juveniles that emerged (over 100 000 during each breeding event). No non-territorial tadpoles survived at Witpoort during the 1st or 2nd breeding events. The communal oviposition sites of the non-territorial males were heavily predated.

The adult male bullfrog plays an important role in limiting predation levels and thus increasing the survivorship of tadpoles. Male removal experiments showed an increase in the level of predation and a decrease in the number of tadpoles surviving to metamorphosis when the resident male is not present (**t**=4.43, **p**=0.0032, **df**=7) and see Fig.6.3. Adult male bullfrogs actively defend their tadpoles and were observed attacking feeding predators. Males remain with their offspring for their entire larval development from between 31-35 days. No adult male was ever observed feeding on tadpoles.

Table 6.2 Offspring survivorship of territorial clutches at Glen Austin and Witpoort.

Breeding Event	Number of Clutches	Clutches Surviving to Metamorphosis	Clutches Dessicated	Clutches Predated
Glen Austin 1st	26	11	6	9
Witpoort 1st	15	9	n/a	6
2nd	11	9	n/a	2

Figure 6.2 Photographs showing channels constructed by males from their territories into the deeper more permanent main body of water.

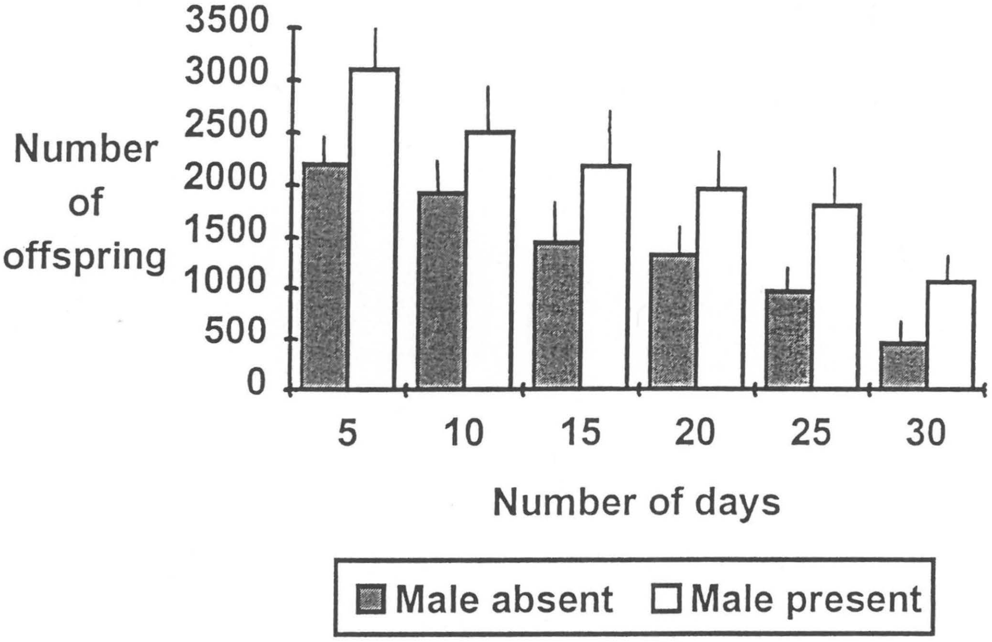


Figure 6.3 Histogram showing the effects of territorial male parental care on offspring survivorship at Glen Austin during the 1992 breeding season.

6.3.4 DISCUSSION:

Adult male bullfrogs attend to egg masses and schools of gregarious tadpoles. This behaviour has been interpreted as parental care (Balinsky & Balinsky 1954) and apparent cannibalism (Carruthers 1983). In addition, males may be responding to environmental cues regarding deterioration in environmental conditions and constructing channels to supply cooler water to the rapidly drying breeding ponds and developing eggs. Males must ensure a constant aquatic environment at least until the tadpoles hatch, enter the channels, and move from the shallow peripheral breeding ponds to the main body of water. My observations support the findings of Channing *et al.*(1989) but also suggest that channel construction is more sophisticated than previously believed, as males seem to anticipate deterioration in environmental conditions as channels were constructed shortly after egg laying as well as after the tadpoles hatched as reported by Channing *et al.* (1989). The presence of the male bullfrog greatly increases the percentage of tadpoles that survive. Not only does channel construction play an integral role in the survivorship of both tadpoles and eggs but male bullfrogs play a vital role in the survivorship off their offspring by actively defending them for the entire duration of their larval development and herding their tadpoles away from heavily predated areas.

CHAPTER SEVEN
KIN RECOGNITION

7.1 INTRODUCTION

7.1.1 KIN RECOGNITION AND KIN ASSOCIATION

It is evident that individuals of numerous species representing various taxonomic groups can discriminate between kin and non-kin (e.g. reviews by Blaustein et al. 1987a,b; Waldman 1988, 1991). The ability of an animal to discriminate between kin and non-kin (often called kin recognition) is an important phenomenon associated with kin selection theory (Hamilton 1964).

Anuran amphibians were among the first vertebrates shown to recognise collateral kin (Waldman 1987). Speculation that the conspicuous coloration and distastefulness of toad larvae (genus ***Bufo***) evolved through kin selection (Wassersug 1973) provided the initial impetus for studies of kin recognition (Waldman and Alder 1979; Waldman 1982). Further work established that kin recognition abilities were not necessarily linked to aposomatic traits. Larvae of numerous species - many palatable and cryptic - were found to recognize and associate preferentially with their siblings and other close kin (see reviews in Waldman 1991; Blaustein and Waldman 1992). Grafen (1990) used data obtained from studies of tadpoles to illustrate how species recognition, in general, may be achieved. He suggested that a species recognition mechanism would work well if, for example, all members of the species had essentially the same smell and some genetic variation in the smell. Then individuals will `acquire a standard (the smell of themselves) which is slightly more like relatives than it is like conspecifics in general. Thus, when an individual uses its species recognition capability to join a tadpole aggregation, it will be biased to join a more related group because such a group is perceived as being closer to the `acquired standard' (i.e. the individual itself) (Blaustein *et al.* 1984). Grafens's point concerning species recognition is well taken. Unfortunately, in many cases, it may be impossible to tell whether kin recognition per se or species recognition is the prime mechanism of kin association (Blaustein *et al.* 1993). However, with regard to tadpoles there is both empirical and inferential support for Grafen's suggestion. For example, O'Hara & Blaustein (1982) suggested that species recognition may be the most parsimonious explanation for kin association in laboratory tests of toad tadpoles. They suggested that toad tadpoles may use the familiar cues emanating from conspecifics to seek optimal habitats or to aggregate with conspecifics to obtain potential benefits from group living (Blaustein 1982 & O'Hara; also see discussion in Blaustein et al.1984).

Experimental results obtained by Pfennig (1989) suggest that kin association in spadefoot toad, ***Scaphiopus multiplicatus***, tadpoles may result from habitat selection due to diet-based environmental cues rather than from social preferences. Tadpoles with similar diets aggregate in the laboratory. In nature, owing to the spatial and temporal proximity of kin within a pond, individuals eating similar foods are likely to be related and therefore may form aggregations (Blaustein *et al.*1993).

One way to resolve the species versus kin recognition problem is to focus on the adaptive value of the recognition phenomenon. Unfortunately, there are significant problems with kin recognition studies in general and with the literature on tadpole kin recognition in particular, because of the lack of supporting data concerning key hypotheses addressing the adaptive value of kin recognition and kin association, and the failure to test hypotheses that are not related to kin selection (see discussion in Blaustein and O'Hara 1987). For example, the most widely cited hypothesis concerning the evolution of kin recognition and kin association in tadpoles suggests that because toad tadpoles are conspicuously black, form large noticeable aggregations and are apparently noxious to their predators, the conspicuousness is aposomatic (Wassersug 1973; Waldman & Alder 1979). Therefore, predators eating a noxious tadpole would avoid other similar individuals nearby. This scenario involves kin selection because the tadpole that is killed by a predator receives some inclusive fitness because its relatives have a reduced risk of predation as a result of its death (Blaustein et al.1991). This is not a satisfactory explanation for the evolution of kin association in frog tadpoles and it does not seem to be parsimonious even for toad tadpoles. For example, all of the frog tadpoles studied by Blaustein and his colleagues (see Blaustein 1988) in the context of kin recognition are palatable to their natural predators, cryptic, and hide under debris when approached. Toad tadpoles generally exist in massive schools, composed of hundreds of kin groups, that the likelihood that kin are associating within the large school is not high. Unless discrete sibling cohorts exist within the massive toad schools, kin selection for the maintenance of unpalatability and conspicuousness in toad tadpoles may not be parsimonious (see O'Hara & Blaustein 1982; Waldman 1982). Many non-kin would also benefit from a predator sampling a distasteful individual and avoiding other members of the group. Even though kin association may be recorded in laboratory experiments, some species lose their preferences after they come into contact with non-siblings during development (e.g.O'Hara & Blaustein 1982). Thus, in nature, maintenance of kin association in tadpoles of species that lose their affiliation for kin after contact with non-kin would be difficult. One alternative hypothesis (among others with experimental support) that is just as plausible as the aposomatic/kin selection hypothesis for the evolution of sociality and dark colouration in toad tadpoles is that masses of dark tadpoles form for purposes of thermoregulation (Guilford 1988).

Although there are good observational data on kin relationships in the field for many vertebrates that suggest that kin associations are based on recognition abilities, tadpoles and ground squirrels have been the only vertebrate subjects that have been studied experimentally in the field. In fact, field experiments of ***Rana cascadae*** tadpoles (O'Hara and Blaustein 1985) reveal much stronger preferences for kin than do laboratory experiments. Therefore, laboratory experiments may be less sensitive assays than field experiments for assessing kin preferences (Blaustein et al.1991).

A general functional model for kin recognition in anurans has proven elusive, but several adaptive scenarios have been proposed. Tadpoles may cooperate with siblings to facilitate growth and development, or they may more effectively deter or respond to predators (e.g. Blaustein 1988; Waldman 1991). Yet tadpoles represent just the first stage in the complex life cycle of anurans, and the ability to discriminate between kin and non-kin might confer selective advantages during later stages. Indeed, experimental evidence that newly metamorphosed frogs associate with their siblings suggests that kin-recognition ability might be retained through metamorphosis, later to be expressed as adults (Blaustein et al. 1984; Cornell *et al.* 1989; but see Waldman 1989).

Natal philopatry can promote interactions among close kin, and may be common among amphibians (reviewed by Waldman and McKinnon, in press).

7.1.2 Intraspecific Associations in Tadpoles and Possible Advantages of Sibling Schools

Living in a group confers a range of advantages on otherwise vulnerable prey. Such advantages include increased probability of predator detection, increased ability to deter or confuse the predator, and dilution of the predator's effect on the group (e.g. Shaw 1978). However these benefits must be weighed against associated costs, such as increased risk of discovery by a predator, and increased competition for resources.

Unpalatability is one strategy that may have evolved to offset the increased risk of discovery by a predator (Griffiths & Denton 1992). Some amphibian tadpoles, for example, posess skin toxins (Formanowicz & Brodie 1982) and show the greatest tendency to aggregate (Wassersug 1973). Moreover, the tadpoles of aggregating species are often darkly coloured and conspicuous, in contrast to the more palatable, cryptically coloured and non-aggregating species (Wassersug 1973,1975). Aggregating tadpoles may run the risk of growth inhibition as a result of crowding (Wilbur 1977). However, Wassersug (1973) suggests that those species that form aggregations show the least susceptibility to crowding effects. Indeed the cumulative stirring of particles from the bottom may actually facilitate feeding in a tadpole aggregation.

7.2 METHODS

7.2.1 Animals and Rearing Conditions

I collected eight clutches of ***P.adspersus*** from Glen Austin bird Sanctuary. Five hundred larvae were reared in aquaria under two basic regimes: **(1)** animals were reared exclusively with siblings in an aerated 20 litre aquarium: **(2)** animals were reared with a mixture of siblings and non-siblings (mixed rearing regime) by placing an equal number of tadpoles from two sibling groups at opposite sides of an aerated 50 litre aquarium divided by 1.5 mm plastic mesh. Partitioning the aquarium allowed the two sibling groups to be reared together and aeration allowed for complete water mixing. There was visual and some tactile contact between groups (see details of this rearing regime in O'Hara & Blaustein 1981).

All eggs were reared under a 14:10 h light:dark cycle at 24 - 26°C. Water was changed in all rearing aquaria every 2-4 days. Tadpoles were fed rabbit pellets and Tetra Min tropical fish food daily.

7.2.2 Apparatus and Standard Testing Procedure

A tank measuring 122x44x30 cm was used to test two tadpoles simultaneously for sibling group preference (figures of the testing apparatus have been previously published; e.g. O'Hara & Blaustein 1981; Blaustein & O'Hara 1986b). A pencil mark was drawn width-wise to delineate the two halves of the tank. To create end compartments for holding stimulus tadpoles, a partition of 1.5 mm plastic mesh was placed 15 cm from each end of the tank. The remaining central portion of the tank was divided longitudinally by an opaque water-tight glass partition allowing tests of two tadpoles simultaneously but independently.

Prior to each test, the tank was filled to a depth of 6 cm with 34 litres of dechlorinated tap water at room temperature. After 5-10 min, stimulus tadpoles of one sibling group and those from a second sibling group were placed in opposite end compartments and left undisturbed for 15 min. In all experiments 25 tadpoles were placed in each stimulus compartment. One test tadpole was released at the tank centre on each side of the longitudinal partition and allowed to acclimatise for 10 min. The time spent (seconds) in sibling and non-sibling halves (as demarcated by the pencil mark) of the tank was recorded for four 5-min trials at 10-min intervals for all experimental subjects. Similar previous studies of numerous tadpoles using similar techniques indicate that tadpoles typically spend the majority of the time near the stimulus ends of the tank and avoid the central portion (e.g. Blaustein & O'Hara 1983). Observations were taken from behind a plastic blind. Following each test, the tank was drained and thoroughly rinsed. The two tadpoles tested simultaneously were always members of the same sibling group. No test tadpole was tested more than once, and the same stimulus animals were used in no more than five tests. Stimulus groups were alternated from one end to the other between tests. All stimulus tadpoles and test individuals were matched for body size and developmental stage (Gosner 1960).

Tests Conducted

I modified the standard testing regime according to the type of experiment that was conducted. At least 20 different tadpoles were used per replicate in each experiment. The detailed methods for each experiment are outlined below. Testing procedures are identical to those described above for the standard testing procedure

Experiments 1, 2 and 3: Do *P.adspersus* tadpoles discriminate between siblings and non-siblings?

Experiment 1

Tadpoles which were reared with siblings only were tested in early developmental stages.

Experiment 2

Tadpoles reared in mixed regimes of siblings and non-siblings were tested.

Experiment 3

Tadpoles which were reared with siblings only were tested in later developmental stages.

Experiments 4, 5 and 6: do tadpoles prefer to associate with conspecifics?

Relatively little is known about the larval aggregation tendencies of ***P.adspersus*** tadpoles in nature. Therefore, I tested ***P.adspersus*** tadpoles to examine these aggregation tendencies in laboratory conditions. Standard testing techniques were employed (Blaustein & O'Hara 1983).

Experiment 4 and 5

Test animals and stimulus animals were at early stages of development. The stimuli presented to test individuals in experiment 4 were familiar siblings (reared together) and an empty test compartment.

The stimuli presented in experiment 5 were unfamiliar non-siblings (reared apart) and an empty test compartment.

Experiment 6

Test tadpoles and stimulus tadpoles were at late stages of development. The stimuli presented to test animals were familiar and unfamiliar siblings and an empty compartment.

7.3 RESULTS

As in previous studies of kin recognition in anuran larvae conducted in the laboratory (e.g. Blaustein & O'Hara 1987; 1988), test tadpoles swam from end to end within the test tank. Test tadpoles spent relatively more time near the stimulus compartments rather than in the middle of the tank (see Blaustein & O'Hara 1983). Tadpoles constantly swam within the stimulus compartments and generally schooled around the circumference of the stimulus compartments providing continuous visual and chemical stimuli to the test individuals.

Experiments 1,2 and 3: When reared with siblings only, test animals in early developmental stages showed a preference to associate in the portion of the test tank nearest siblings (Table 7.1). The number of tadpoles that spent most of their time in the sibling portion of the test tank was significantly different from random (experiment 1).

Pyxicephalus adspersus tadpoles that were reared with a mixture of siblings and non-siblings did not show a preference for either side of the test tank (experiment 2). The number of animals spending most of their time on the sibling side of the test tank did not differ from random expectation in any replicate or for all replicates combined. Test tadpoles reared in mixed rearing regimes displayed a random association within the test tank. Tadpoles that were reared with siblings, but tested in later developmental stages, showed a strong preference to associate in the sibling portion of the test tank. The number of tadpoles that spent most of their time in the sibling portion of the tank was significantly different from random (experiment 3).

Experiments 4, 5 and 6: *Pyxicephalus adspersus* associate nearest to the stimulus side of the test tank containing conspecifics rather than the side containing no tadpoles (Table 7.2). These results were obtained when tadpoles were in early stages of development (experiments 4 and 5) and in later stages of development (experiment 6). Moreover, these results were obtained with kin and non-kin as conspecific stimuli. This suggests that ***P.adspersus*** tadpoles are positively attracted to conspecifics regardless of whether they are siblings or non-siblings. Therefore, kin recognition in ***P.adspersus*** tadpoles that are in an early and late stage of development is probably based on attraction to siblings rather than aversion to non-siblings.

Table 7.1 Description of standard tests and time distributions of individuals in standard tests.

EXPERIMENT	TEST GROUP	STIMULI	DEVELOPMENTAL STAGE OF TEST TADPOLES & STIMULI	NUMBER SPENDING MAJORIT TIME NEAREST		BINOMIAL P	MEAN TIME SPENT NEAREST SIBLINGS (SE)	t	N	P
				SIBLINGS	NON-SIBLINGS					
EXPERIMENT 1										
REPLICATE 1	A	A(FAMILIAR SIBLINGS)	26-27	1	2	<0.0001	694.6	4.9	20	0.00001
		B(UNFAMILIAR NON-SIBLINGS)					(41.2)			
REPLICATE 2	B	B (FAMILIAR SIBLINGS)	26-27	1	3	=0.001	652.8	2.9	20	0.0068
		A(UNFAMILIAR NON-SIBLINGS)					(47.4)			
REPLICATE 3	C	C (FAMILIAR SIBLINGS)	26-27	1	3	=0.001	668.2	4.0	20	0.0003
		B(UNFAMILIAR NON-SIBLINGS)					(37.2)			
EXPERIMENT 2										
REPLICATE 1	$A_m\psi$	A (UNFAMILIAR SIBLINGS)	26-27	1	8	NS	605.2	0.48	20	NS
		B(UNFAMILIAR NON-SIBLINGS)					(21.3)			
REPLICATE 2	$B_m\psi$	B(UNFAMILIAR SIBLINGS)	26-27		11	NS	547.2	1.3	20	NS
		A(UNFAMILIAR NON-SIBLINGS)					(19.2)			
EXPERIMENT 3										
REPLICATE 1	A	A (FAMILIAR SIBLINGS)	29-30	1	2	<0.001	688.8	7.4	20	0.00001
		B(UNFAMILIAR NON-SIBLINGS)					(39.3)			
REPLICATE 2	B	B (FAMILIAR SIBLINGS)	29-30	1	2	<0.001	690.4	6.4	20	0.00001
		A(UNFAMILIAR NON-SIBLINGS)					(45.2)			
REPLICATE 3	C	C (FAMILIAR NON-SIBLINGS)	29-30	1	3	=0.001	681.5	5.9	20	0.00001
		B(UNFAMILIAR NON-SIBLINGS)					(42.3)			

Table 7.2 Results of conspecific association tests

EXPERIMENT	TEST GROUP	STIMULI	DEVELOPMENTAL STAGE OF TEST TADPOLES & STIMULI	NUMBER SPENDING MAJORIT TIME NEAREST		BINOMIAL P	MEAN TIME SPENT NEAREST SIBLINGS (SE)	t	N	P
				SIBLINGS	NON-SIBLINGS					
EXPERIMENT 4										
REPLICATE 1	D	D (FAMILIAR SIBLINGS)	26-27	18	2	<0.001	800.3	7.8	20	0.00001
		Empty compartment					(36.0)			
REPLICATE 2	E	E (FAMILIAR SIBLINGS)	26-27	17	3	=0.001	707.1	5.71	20	0.00001
		Empty compartment					(27.0)			
EXPERIMENT 5										
REPLICATE 1	F	D (UNFAMILIAR SIBLINGS)	26-27	16	4	=0.0006	663.2	3.56	20	0.0010
		Empty compartment					(25.1)			
REPLICATE 2	G	E (UNFAMILIAR SIBLINGS)	26-27	15	5	=0.021	649.2	2.54	20	0.015
		Empty compartment					(27.2)			
EXPERIMENT 6										
REPLICATE 1	D	D (FAMILIAR SIBLINGS)	30-36	16	4	=0.006	647.5	5.32	20	0.0001
		Empty compartment					(13)			
REPLICATE 2	F	D (UNFAMILIAR SIBLINGS)	30-36	15	5	=0.021	632.4	2.64	20	0.012
		Empty compartment					(17)			

7.5 DISCUSSION

The ontogeny of kin recognition differs among amphibian species (Blaustein & Waldman 1992). In several species, the development of kin recognition is significantly influenced by the rearing regime. African bullfrog, ***Pyxicephalus adspersus***, tadpoles reared with siblings associated with siblings rather than non-siblings in both early and late developmental stages. However, tadpoles reared with both siblings and non-siblings in early development and then exposed to siblings in later development, displayed a random association with respect to siblings and non-siblings. Tadpoles failed to associate preferentially with unfamiliar siblings over familiar non-siblings. These results suggest the existence of a sensitive period early in development during which ***P.adspersus*** tadpoles familiarise themselves with other individuals. Waldman (1981) obtained similar findings in ***Bufo americanus*** tadpoles. Previous tests (Blaustein & O'Hara 1986a) of ***Rana aurora*** tadpoles suggest that each tadpole kin group has a unique chemical signature that is retained when each kin group is reared by itself. However, when tadpoles are reared in mixed sibships, individuals seem to obtain a composite `odour' because test tadpoles taken from mixed rearing tanks failed to discriminate between familiar siblings and familiar non-siblings (Blaustein & O'Hara 1986a). This chemical signal convergence masks the unique signatures of the individual kin groups (see discussion of the `Gestalt Model' by Crozier & Dix 1979 and detailed discussion of the odour transference process in amphibians by Waldman 1985b). Thus in ***Pyxicephalus adspersus*** and other anuran larvae such as ***R.aurora, B.boreas*** and ***B.americanus***, there is a significant `familiarity' component incorporated into the recognition system. Larvae of other anuran amphibian species discriminate between kin and non-kin throughout larval ontogeny whether or not they have been in contact with non-kin during development (see reviews by Blaustein & Waldman 1992; Blaustein et al. 1993).

Sibling association may occur in the early larval stages because tadpoles are using their chemosensory system for aggregation, which is the only intact sensory system in early ages (Blaustein et al. 1993). Tadpoles may form a template for those individuals with genetically based chemical cues (see discussion in O'Hara & Blaustein 1982; Blaustein et al. 1987b; Waldman 1991). If there is a strong genetic component to the recognition signature, then tadpoles will presumably associate most often with kin (see Waldman 1985b). There may be a strong environmental component to the recognition signature. For example, some tadpole species may associate with individuals that have eaten the same types of food and thus their metabolic by-products may permeate the environment with a `familiar' food component that is identified by the tadpoles as they aggregate (Pfenning 1990; Gamboa *et al.* 1991a,b).

Sensory Basis of Recognition

Visual, auditory and chemical cues are used in vertebrate kin recognition (discussed in Blaustein et al. 1987b; Blaustein et al.1993). More than one signal may be involved in the recognition system, a primary signal that enables the animal to orient towards a conspecific group or individual and a secondary signal that may allow the animal to fine tune its primary signal so that it can discriminate between classes of animals such as between kin and non-kin (Blaustein et al. 1987b). For example, in aquatic species, both fish and anuran larvae may use the lateral line to orient towards and swim with conspecifics (Partridge 1982). Laboratory experiments of anuran larvae have shown that visual cues may be used as a primary signal for orientation and schooling (Wassersug & Hessler 1971; Wassersug 1973). However, in fish and amphibian larvae, the more fine-tuned ability to discriminate between parents and offspring and between kin and non-kin appears to be mediated largely by chemical cues (e.g. Blaustein & O'Hara 1982a; Waldman 1985a; Blaustein et al. 1993). These cues may be fixed or variable, depending upon the species (Blaustein et al.1987b).

Tadpoles of ***P.adspersus*** form prolonged aggregations with conspecifics in nature. But, at the oviposition sites of non-territorial males, ***P.adspersus*** tadpoles may mix freely with members of other kinships. As discussed above, contact with non-kin during development causes ***P.adspersus*** tadpoles to lose their preference to associate with kin even in early larval stages. Therefore, it is unlikely that kin association in ***P.adspersus*** tadpoles could be maintained in non-territorial breeding aggregations unless they do not interact with non-kin in nature. Territorial males actively defend their territories from intruding conspecific males and prevent the depositing of unrelated eggs in his territory. Tadpoles hatching in these distinct isolated territories do not come into contact with non-kin and preferentially associate with kin. Territorial males prevent their tadpoles from coming into contact with other kinships by the constant herding away of his tadpoles from other tadpole aggregates.

Possible Advantages of Sibling Aggregations

Previous studies have demonstrated that toad tadpoles are distasteful to a variety of vertebrate predators (Waldman 1982b,1986). Nevertheless, vertebrates do sometimes prey upon tadpoles (e.g. Walters 1975), and although toad tadpoles may have chemical defences against predators, these substances appear not to be acutely toxic to them. After discovering a distasteful individual in an aggregation, a predator may learn that tadpoles are distasteful and then skip over other individuals in the aggregation (e.g. Brodie & Formanowicz 1987).

The expression of aposomatic coloration or conspicuous behaviours may further serve to deter experienced, and thus these traits should also spread among distasteful prey. Although bullfrog, ***P.adspersus***, tadpoles form dense black schools similar to toads they are palatable and are often heavily predated by numerous invertebrate and vertebrate predators. Furthermore, forming dense aggregations does not provide an effective warning signal to potential predators. Schooling with siblings might result in other anti-predator benefits as well. ***Bufo*** tadpoles injured by a predator may warn surrounding individuals of the predator's presence. Tadpoles of many bufonids have a chemical contained in their epidermis that, when released, provokes a fleeing alarm reaction in nearby conspecifics (Waldman 1982b).

Tadpoles of ***P.adspersus*** respond to injured tadpoles with a typical alarm behaviour. When an injured tadpole is placed into the tadpole school they immediately scatter. This `alarm substance' cannot be actively secreted into the water, release is dependent on an individuals' skin being broken, presumably in natural conditions by a predator. As in the apparently analogous alarm behaviour system on many osatriophysean fishes (Pfeiferr 1974,1977), the signalling individual usually does not survive. Unless the behavioural response represents a secondary adaptation to a chemical that serves some other function (William's 1964), alleles for the warning chemical presumably have spread through kin selection (Maynard Smith 1974). Thus release of this pheromone would increase an individual's inclusive fitness only when the kin benefit disproportionately, as when individuals associate in kin groups. Although generally considered to be limited to bufonid species among anuran larvae (Pfeiferr 1977), similar alarm behaviour systems may occur in other species. For example, tadpoles of the eastern spadefoot toad (***Scaphiopus holbrooki***) sometimes respond to crushed conspecifics with typical alarm behaviour (see Waldman 1982b).

Many temperate zone anurans deposit large clutches in ephemeral habitats, and under these conditions individuals should be selected to grow quickly to gain an early size refuge from predators and to metamorphose before the pond dries (Waldamn 1982b). If critical resources are limiting robust individuals might benefit from suppressing the growth of the conspecifics. Although inclusive fitness would presumably be maximized by directing growth inhibition effects towards non-kin rather than toward kin, selection acting on the specificity with which individuals respond to the regulatory substance. Smaller larvae, if their probability of successfully completing metamorphosis is low, might have more to gain indirectly by allowing themselves to be stunted (and perhaps under extreme conditions to be cannibalised by later more vigorous kin) than they could potentially gain by directly competing with these kin (Waldman 1982b).

Tadpoles of the African bullfrog (***P.adspersus***) occur as two distinct phenotypes, a large, rapidly developing, carnivorous morph which metamorphoses after between 19-21 days and a normal developing, omnivorous morph which completes metamorphosis after 31-35 days (see Chapter 5). The large tadpole morphs cannibalise smaller tadpole morphs both in the laboratory and in the field. Pomeroy (1981) noted similar developmental polymorphism in the tadpoles of the spadefoot toad (***Scaphiopus multiplicatus)***. The direct results of the inhibitory effect on growth on smaller siblings by larger tadpoles was not investigated in this study but should provide interesting data in future studies.

CONCLUSION

The larval period represents a short but critical phase in the bullfrog's life history. Bullfrogs deposit large clutches (2000 to 6000 eggs), usually in transient environments, and cohorts suffer enormous mortality, particularly during larval and early post-metamorphic stages. Although tadpoles appear well-adapted for exploiting available food resources, these resources may be limiting, and larval competition consequently may be intense. In addition, predation and desiccation pressures can be substantial. The data presented here show that tadpoles of ***Pyxicephalus adspersus*** preferentially associate with kin in the laboratory and in the field form sibling schools in the territories and mixed sibling schools in the non-territorial breeding areas. Further study is required to delineate the ecological and evolutionary factors that favour kin association, the mechanism by which sibling schools increase larval (and perhaps indirectly, post metamorphic) survivorship, and the possibility that kin recognition abilities are retained after metamorphosis and play some role in adult behaviour.

CHAPTER EIGHT
OVERALL CONCLUSION

The Social Organization of the African Bullfrog *Pyxicephalus adspersus*

Anurans, as a group, have adopted a range of reproductive strategies (contingent on their environmental conditions) which are flexible within species and even within populations (Duellman & Trueb 1986). The studied populations of *P.adspersus*, through sexual reproduction and natural selection, have adapted to specific habitats as a result of the individual's adaptations in phsiology, ecology and behaviour. The reproductive strategies found in these populations are the result of various selection pressures acting on all individuals. Factors such as the temporal pattern of rainfall, the persistence of the ephemeral breeding sites, population density, temperature and variation in predation levels all probably affect the reproductive strategy.

African Bullfrogs, ***Pyxicephalus adspersus*** are extreme explosive breeders completing mate acquisition and oviposition within 48 hours. Despite being explosive breeders they exhibit various mating tactics typical of prolonged breeders. (**a**) Bullfrogs are extremely sexually dimorphic with males being nearly twice the size of females. (**b**) Male and female arrival patterns are asynchronous. (**c**) Males are highly aggressive resulting in frequent aggressive interactions. (**d**) Males exhibit three size related mating strategies namely territorial, non-territorial and satellite behaviour.

Territorial male bullfrogs were the largest adult males and show high levels of site fidelity, actively defending their territories against intruding conspecific males. Territories are not only used for mating and egg deposition but also provide nourishment for the developing larvae. An individual male bullfrog ***P. adspersus*** may defend a specific site for 2-35 days, depending upon his ability to repulse intruders and predators and the presence or absence of tadpoles in his territory. Male parental care may form part of the explanation of the extreme sexual dimorphism of bullfrogs. Males defending breeding sites and offspring need to be larger than the non-territorial and satellite males, and the females who migrate out of the breeding area after oviposition. Territorial males also construct channels from the rapidly drying territories to the deeper main body of water and prevent the desiccation of the eggs and tadpoles .Large size may bestow an adaptive advantage in territory defence, mating success and predator repulsion.

Breeding aggregations of non-territorial male bullfrogs are characterised by the lack of a defensible resource and by the extreme synchrony of reproduction. Non-territorial males are the losers of aggressive contests over territories and appear like territorial males; however, these males do not defend their sites once they have bred. If challenged or defeated by a larger male they vacate their site and find another. Non-territorial males include males that are defeated in territorial encounters, or opportunistic males that are attracted to the aggregation of fighting and displaying males. Amplexus displacement plays a major role in the mating success of non-territorial males. The larger attacking males have significantly higher amplexus displacement rates than smaller males. Larger challenging males always displace smaller amplexed males and amplexus displacement by non-territorial males may best be explained by a form of male-male competition rather than a form of female choice. Although non-territorial males may have increased mating succcess over territorial males, the unattended offspring survival is dependent on favourable environmental conditions and predation levels.

Satellite males are the smallest adult males in the population, they are too small to either defend a territory or fight for a place in the non-territorial arena. Satellite males avoid aggressive encounters and decrease the risk of injury and death through their behaviour. Satellite males adopted a concealed, non-calling 'low' posture in the sedges or grasses adjacent to the resident territorial males and attempted to intercept females. If a satellite male intercepted a female he would amplex with her and begin mating with her almost immediately. Satellites seldom had more than one bout of oviposition with a female before the territorial male displaced him or the satellite fled preventing an aggressive interaction. Although certain satellites mated successfully the eggs were usually deposited on the surrounding vegetation or in extremely shallow water which prevented successful hatching of the clutch. Females also prevented oviposition with satellite males by moving into the male territory, but risked injury from the attacking resident male. Females were also mistakenly identified as satellite males and were severely injured by the aggressive territorial males.

Pyxicephalus adspersus exhibits a short-duration, explosive breeding mating system which is exhibited by anurans that utilise ephemeral sources of water. The available measures of the intensity of sexual selection, male skewed operational sex ratios, and alternative reproductive behaviours exhibited by explosive breeding male bullfrogs indicate that both male-male competition for mates and to some extent female choice may be occurring in these short duration breeding aggregations.

What drives the bullfrog social system? I have shown that intersexual selection is unlikely to play an important role in shaping the breeding behaviour of these animals (Chapter 4) but that intense intrasexual selection takes place with large males becoming territory owners (Chapter 2). Nonterritorial males can, under some conditions, obtain as many matings as territorial males (Chapter 3). However, the survival of a brood within a territory is significantly higher than outside (Chapter 6). I postulate that male territoriality and the accompanying between-male competition is a selective pressure which results in the maximal fitness of males in ephemeral pans. This is because predation, dessication and overheating are major environmental constraints on the fitness of individual bulfrog males. Large males can drive off predators and construct channels for ensuring the survival of their broods (Chapter 6), thus maximising their reproductive fitness.

The findings in this study provide a preliminary introduction to the reproductive behaviour of a previously little-studied species. Further studies of *P.adspersus* populations may provide added insight into the reproductive strategies and their evolutionary consequences. This study has also generated numerous testable hypothesees applicable to other anuran species. Moreover, investigations in the population size/age structure, mating tactics and their flexibility relating to density and other social and ecological pressures will allow a more thorough understanding of the reproductive behaviour and evolution of this anuran.

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APPENDIX I

Territory sizes and nearest neighbour depths at Glen Austin and Witpoort are summarised in Table 8.1.

Male SVL (cm)	**Territory Area (m^2)**	**Territory Depth (cm)**	**Nearest Neighbour Distance (m)**
20.4	3.2	4.5	1.8
18.1	1.4	4.1	2.1
16.5	0.8	4.3	3.2
18.5	1.8	4.3	2.9
18.1	1.6	4.2	2.3
18.3	1.7	4.0	1.2
18.1	1.2	4.8	1.1
18.7	1.5	4.1	1.6
19.2	1.9	3.8	3.2
20.1	2.1	4.1	1.1
22.1	3.5	4.3	1.2
21.7	2.9	4.2	1.5
22.3	4.9	4.0	1.8
20.4	3.6	4.1	2.1
18.1	2.5	4.2	3.2
18.5	1.9	4.5	3.1
17.8	2.1	4.1	1.1
18.1	3.2	4.1	1.2
18.5	4.2	3.8	4.5
18.1	1.8	4.1	1.8
18.3	1.7	4.3	2.1
18.7	2.1	4.2	3.2
19.1	2.4	4.0	2.1
19.2	2.8	4.1	1.2
18.1	1.2	4.2	4.9
17.7	1.7	3.8	2.3
19.2	1.9	4.1	3.2
20.1	2.3	4.1	3.7
22.1	2.9	3.8	1.1
21.9	1.6	4.1	2.1
21.3	1.5	4.3	1.1
20.4	1.5	4.2	4.6
18.1	1.7	4.0	3.2
18.5	1.9	4.1	1.1
17.8	1.5	4.2	1.2
18.1	1.8	4.5	1.5
18.5	1.9	4.2	2.8
18.1	1.3	4.1	1.1
18.3	1.2	3.8	1.2
19.7	1.0	4.0	2.1
20.1	1.5	4.1	2.1
19.2	1.7	4.1	1.1
18.1	1.6	3.5	8.7
18.7	1.9	4.1	1.2
19.2	1.6	4.3	2.1

Male SVL (cm)	**Territory Area (m^2)**	**Territory Depth (cm)**	**Nearest Neighbour Distance (m)**
20.1	1.9	4.2	3.2
22.1	1.4	4.0	2.5
21.7	1.7	4.1	1.8
22.3	4.7	4.2	2.1
21.4	4.2	4.5	3.2
18.1	2.3	4.1	2.1
18.5	2.3	4.1	1.1
18.1	1.2	4.2	1.2
18.1	1.5	4.1	1.5
18.3	1.8	3.8	3.2
18.7	1.4	4.1	2.1
19.1	1.7	3.8	1.2
19.2	1.2	4.1	1.9
18.1	1.1	4.3	2.3
17.7	1.2	4.2	3.2
19.2	1.2	4.0	28
20.1	1.4	4.1	2.1
22.1	1.5	4.2	2.1
21.7	1.4	4.5	1.1
22.3	1.8	4.1	5.6
20.4	1.6	4.1	1.2
18.1	1.7	4.2	1.1
22.5	2.3	4.1	1.2
17.8	1.2	4.3	43.2
18.1	1.8	4.2	1.8
22.7	3.2	4.0	1.1
18.1	1.6	4.1	1.2
18.3	1.8	4.2	2.1
19.7	1.9	4.1	1.2
20.1	2.5	4.2	1.1
19.2	2.8	4.1	1.8
22.3	4.2	4.2	2.4
20.4	2.4	4.1	1.2
18.1	3.1	4.3	1.7
19.5	5.1	4.2	1.4
20.8	3.2	4.1	1.2
17.8	1.2	3.8	2.1
18.1	1.7	4.1	1.2
18.1	1.7	4.1	1.5
18.3	1.7	4.2	1.8
18.1	1.4	3.5	2.1
19.4	1.8	4.2	1.2

		Channel			Territory	Depth (cm)	Territory	Temp. (°C)
T.O.D	Length Date (m)	Width (cm)	Depth (cm)	Temp. (°C)	Before Channel	After Channel	Before Channel	After Channel
11h45	1.4	16.1	4.2	32.1	3.9	4.0	33.4	32.9
12h05	0.9	15.4	4.5	34.2	4.3	4.3	34.9	34.6
13h21	1.7	13.7	4.6	34.7	4.5	4.5	35.8	35.1
13h43	1.2	14.2	4.2	34.1	3.9	4.1	35.8	35.2
13h54	1.4	14.8	3.8	33.8	4.1	4.3	35.7	35.4
14h05	1.5	15.1	4.2	34.5	3.9	4.1	35.2	34.8
14h23	2.1	15.6	4.3	34.7	4.1	4.5	35.3	34.9
07h21	1.3	14.2	3.7	20.4	3.2	3.7	20.4	20.7
07h56	2.1	15.1	3.6	21.3	3.7	3.7	21.3	21.4
08h45	2.3	14.3	3.8	23.2	3.5	3.9	23.5	23.4
10h45	1.1	14.5	4.6	26.3	3.9	4.3	26.9	26.7
12h25	1.2	14.7	4.1	34.2	4.6	4.5	34.6	34.5
13h24	2.4	14.3	4.3	34.7	3.6	4.3	35.6	34.9
14h36	1.3	14.2	3.9	34.9	3.5	3.9	36.5	35.7
15h34	1.7	14.6	4.6	32.1	3.2	4.3	32.1	33.9
16h45	1.9	15.3	4.6	31.2	3.7	4.2	33.7	32.9
17h24	2.6	15.7	4.3	29.6	3.1	3.5	32.1	31.8
?	1.2	14.2	4.9	32.1	?	4.3	?	34.3
?	1.0	16.1	4.1	34.2	?	3.8	?	35.2

APPENDIX II

Data of channels constructed at Glen Austin are summarised in Table 8.2.

Table 8.2 Summarized data on channels constucted at Glen Austin over two breeding seasons.

Date		Channel			Territory	Depth (cm)	Territory	Temp. (°C)
T.O.D	**Length (m)**	**Width (cm)**	**Depth (cm)**	**Temp. (°C)**	**Before Channel**	**After Channel**	**Before Channel**	**After Channel**
10h47	2.9	14.8	4.7	29.7	4.1	4.6	32.7	31.4
11h15	4.5	15.1	4.5	31.7	4.1	4.8	33.8	32.4
11h35	2.1	14.5	4.2	32.3	3.7	4.5	35.2	34.1
12h45	3.2	15.6	4.2	34.2	4.1	4.4	36.1	35.5
13h05	6.7	16.1	4.3	35.1	3.8	4.2	37.8	36.1
13h55	8.5	14.3	4.2	34.7	4.0	4.3	35.2	34.9
14h23	1.4	16.0	4.5	35.7	4.1	4.4	37.1	36.5
15h32	2.1	15.2	4.1	33.1	3.7	4.3	34.1	33.1
16h23	2.9	14.6	4.8	32.1	4.3	4.9	33.2	32.8
?	2.4	15.2	4.1	29.4	?	4.4	?	29.6
?	11.2	16.5	4.3	29.7	?	4.5	?	29.7
?	12.5	15.3	4.2	29.8	?	4.8	?	29.8
?	7.8	16.1	4.5	29.7	?	4.9	?	29.7
11h21	2.1	15.8	4.7	32.1	3.4	4.1	33.1	32.7
11h55	1.7	14.3	4.9	29.4	3.5	4.3	36.1	35.7
12h24	1.1	16.5	4.1	29.7	3.7	4.2	36.7	35.9
13h43	1.9	15.5	3.7	29.8	4.1	4.7	37.8	37.1
14h12	3.4	15.1	5.2	29.7	3.9	4.3	36.8	36.3
15h34	2.3	16.3	4.9	32.1	3.5	4.1	34.1	33.2
15h55	4.2	15.2	3.8	301.	3.9	4.4	33.1	32.1
16h21	1.6	15.9	4.1	29.8	4.2	4.5	32.7	31.9
?	4.6	13.6	3.2	23.5	?	4.6	?	23.5
?	6.8	16.5	2.7	23.5	?	4.3	?	23.5
14h21	4.2	14.6	3.2	34.2	3.7	4.1	35.1	34.8
14h57	2.1	15.6	4.1	35.1	4.1	4.6	35.7	35.4
15h12	1.9	14.5	2.8	33.8	3.9	4.5	34.6	34.1
08h22	1.2	16.7	2.4	25.1	3.7	4.1	25.1	25.3
09h23	2.1	14.5	4.1	25.6	4.1	4.4	26.7	26.1
10h34	2.3	13.5	4.6	29.7	3.8	4.2	31.2	30.8
11h21	2.3	15.5	4.9	32.4	4.0	4.5	34.7	33.5
12h04	4.1	14.6	4.2	34.2	4.2	4.7	35.2	34.7
12h32	1.1	15.1	4.5	34.6	3.2	4.2	36.8	35.7
13h24	2.1	16.1	4.3	33.5	3.9	4.5	35.7	35.2
14h42	2.4	15.6	4.8	35.1	3.9	4.7	36.7	35.9
15h24	4.1	14.7	3.2	32.1	4.1	4.2	33.8	33.2
16h05	1.7	15.2	3.4	29.7	4.2	4.4	30.1	29.8
?	5.6	14.2	4.1	24.1	?	3.6	?	24.3
?	7.4	14.7	2.1	24.1	?	3.9	?	24.3
?	1.1	14.7	4.1	24.2	?	4.6	?	24.2
?	18.7	14.1	2.1	24.1	?	4.3	?	24.4
?	2.3	15.2	3.9	24.3	?	3.8	?	24.6
?	2.1	14.9	4.3	24.5	?	4.1	?	25.1
?	1.8	15.8	3.9	24.7	?	3.9	?	25.3
10h23	2.1	14.7	4.1	28.7	3.7	4.3	28.7	28.7
10h55	1.2	15.1	4.6	29.1	4.1	4.4	29.7	29.5