The Ecology Of Tadpoles
In A Temporary Pond In The Western Cape
With Comparisons To Other Habitats

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ABSTRACT

This work centres on the tadpoles in a temporary pond in the middle of Kenilworth racecourse, Cape Town, South Africa. Trapping was carried out over two wet seasons and five species were found, *Cacosternum platys*, *Microbatrachella capensis, Strongylopus grayii, Tomopterna delalandii* and *Xenopus laevis*. Three of these species were trapped and their position in the pond was related to environmental variables. Water depth, water temperature, oxygen concentration, and substrate type were all found to correlate to the position of the tadpoles in the pond. Over a 24 hour period, sampling every three hours, tadpole species divided the pond spatially and were trapped in different numbers in different. Previous work has shown that some tadpole species can adjust their time to metamorphosis. When *S. grayii* and *C. platys* tadpoles were tested, there was no significant difference between the drying and control water treatments for either species. The food items the tadpoles were eating in the Kenilworth ponds was studied using gut contents analysis. Tadpoles were taken from the pond once a week and their gut contents were compared to the contents of the water column and differences were looked for between the species. These results suggest that the tadpoles do not select for certain food items and do not partition their environment, between species, using food. A similar study was then carried out in a tropical environment in North Luangwa National Park, Zambia. Here, 12 genera were collected and still the tadpoles' guts contained the same quantities of food items as was found in the water column. The final part of this work is concerned with the tadpoles' thermal tolerance. *S. grayii* was selected as a study species and 15 sites were visited across its distribution. These sites included the northern and southern ends of the distribution in the winter rainfall area as well as three sites with a different rainfall regime and six sites that were on the same latitude but at different altitudes. The results showed that the tadpoles of *S. grayii* are probably able to produce heat shock proteins that will last up to two weeks, however, the critical thermal maxima (CTM) of the tadpoles did not show a trend with latitude or altitude. The largest difference was seen between the tadpoles from the summer and winter rainfall regions.

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The summer rainfall tadpoles had a much higher CTM than the tadpoles from the winter rainfall regions before and after acclimation.

This study is a very broad insight into tadpole ecology in the Western Cape. It has highlighted the fact that little work has been carried out here recently and more needs to be done to establish what environmental changes are occurring, and if these will affect the ability of these tadpoles to develop to metamorphosis under these new conditions.

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DECLARATION

I. SHopkins, declare that “The ecology of tadpoles in a temporary pond in the Western Cape with comparisons to other habitats” is my own work and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Amphibians

The class Amphibia is represented in South Africa only by the Anura, although the Gymniophona are present in the tropics to the north. Of the 5283 species of anurans known globally (Frost 2006), 117 occur in South Africa, including Lesotho and Swaziland (Minter et al. 2004). Amphibians generally have a biphasic life cycle. Although exceptions are known, adults lay eggs which develop into free-swimming tadpoles. The tadpole stage allows the exploitation of environments that the adult does not use. Tadpoles are an important part of the pond community; they reduce the standing crop of suspended particles and change the nitrogen composition of the pond. They can even change the whole phytoplankton community of a pond away from filamentous blue-green algae (Seale 1980). They serve as the prey for many animals higher up the food chain such as predatory insects, birds, fish, frogs and salamanders (Alford 1999).

In general, anurans spend the first part of their life as aquatic herbivores and the last part as terrestrial carnivores. The anuran larval stage is dedicated solely to growth whereas the adult stage is concerned with reproduction, dispersal and survival in harsh weather conditions (Wilbur 1980).

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In a typical cycle, eggs are usually laid in water, and each egg is surrounded by a jelly membrane. This jelly is not used for nutrition and possibly has a role in thermoregulation, as it will cool slowly at night and heat slowly during the day (Savage 1938). After a period of growth there is a pulse of hatching as many new tadpoles enter the pond, followed later by a decline in tadpole numbers as predation, desiccation and metamorphosis occur (Wilbur 1980).

1.2 Tadpole Development

The time it takes for a tadpole to reach metamorphosis varies from weeks to years, and depends upon a number of factors. This species-specific variation reflects genetic adjustments to interactions between the relative growth rates and mortality risk. Tadpoles that remain in the pond longer have more chance to feed and grow, while at the same time being more exposed to the risk of predation (Rudolf and Rödel 2006). A tadpole that has more food will grow quicker and metamorphose sooner. Tejedo and Reques (1994) tested this using four treatments and measuring the time to metamorphosis, size at metamorphosis and survival to metamorphosis. The largest metamorphs came from the high food treatments, but the first tadpoles to reach metamorphosis came both from the high food, and the high to low food treatments. Tadpoles must balance the risks associated with remaining longer in the pool, such as desiccation, predation and competition for dwindling food supplies, against the potential benefits obtained from the pond environment.

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Anuran larvae utilise a number of different habitats ranging from damp ground and temporary puddles to permanent lakes and rivers. The tadpole habitat may vary between species and the tadpole is adapted to the conditions in which it lives. Temporary ponds have a high productivity and provide an opportunity for rapid growth although some predators may be present. They are less likely, however, to contain the large predators such as fish. The length of time, after filling, before temporary ponds dry out is variable and uncertain. Permanent water bodies are more predictable but often contain the larger predators such as fish. Skelly (1997) suggested that there is a gradient between permanent and temporary ponds and most species can survive along a small section of that gradient. To look at the differences between frogs that breed in temporary ponds and those that breed in permanent ponds Skelly (1997) studied two species. The first was the spring peeper, *Pseudacris triseriata*, that is known to breed in permanent and temporary ponds, and the second was the chorus frog, *P. crucifer*, which is known exclusively from temporary ponds. The results of these experiments showed that the chorus frogs did better in the temporary ponds and the spring peepers did better in the permanent ponds. He concluded that in a temporary pond there are few predators and little time for development so the tadpole stays active and forages, resulting in rapid growth. In a permanent pond there are more predators and more time so the tadpole can lead a more sedentary life and grow at a slower pace. There is a trade-off between rapid development and the risk of predation because foraging increases predation risk.

1.3 Predation on Tadpoles

Predation is an important determinant of tadpole survival. Predation has been studied in terms of the survival of tadpoles (Petranka et al. 1998), tadpole defences (Kats et al. 1988), including noxiousness (Brodie and Formanowicz 1987) and the changes tadpoles make in behaviour when put under predation pressure (Adams and Claeson 1998, Azevedo-Ramos et al. 1992, Feminella and Hawkins 1994, Laurila et al. 1997, Peacor 2002, Relyea and Yurewicz 2002, Richardson 2001, Thiermann and Wassersug 2000, Van Buskirk 2001). Relyea (2002) reported on the effect of predators regarding the growth, development and morphology of tadpoles while other reports have concentrated solely on the effect of predators upon the morphology of tadpoles (McCollum and Leimberger 1997, Relyea 2001a, Relyea 2002, Relyea and Werner 2000, Van Buskirk and McCollum 1999), and the colour patterns of tadpoles in the presence of predators (Altig and Channing 1993, Caldwell 1982). Predators influence the distribution of tadpoles in a pond (Bradford 1989, Downie et al. 2001, Hero et al. 1998, Ultsch et al. 1999). Many studies have examined the predators in this interaction, reporting on prey preference (Brodie and Formanowicz 1983, Richards and Bull 1990), cues given off by the predator (Kiesecker et al. 1996, Mantifel 1995, Mantifel and Zhushev 1996) and tadpoles themselves being predators (Brendonick et al. 2002, Petranka and Kennedy 1999). Predation does not act upon the tadpole alone; other influences shape the lives of tadpoles in conjunction with it. The tadpoles of *Pseudacris crucifer* and *P. triseriata* have the pressure of predation as well as pond drying. Permanent ponds have a suite of different predators to temporary ponds which causes and increase in the tadpoles body size, abundance and time to metamorphosis (Skelly 1996).

Even though fish are usually not present in temporary water there are still many invertebrates, birds and mammals that can prey on the tadpoles. Some of these predators are known to produce cues that can change the tadpoles' behaviour, morphology or alert the tadpole to the predator's presence. *Rana temporaria* tadpoles faced with a dragonfly larva (*Aeshna juncea*) that had been fed on tadpoles decrease their activity (Laurila et al. 1997) and *Phrynomantis microps* tadpoles swarm in the presence of a predator such as *Anax imperator* or *Hoplobatrachus occipitalis* larvae (Rödel and Linsenmair 1997). McCollum and Leimberger (1997) studied *Hyla chrysoscelis* and the effect dragonfly larvae had on their morphology. Tadpoles raised in the presence of predators had deeper tails and black spots. They concluded that the deeper tail would allow the tadpole to escape the predator and the colour on the tail would direct the predator to the tail and away from the body.

1.4 Competition between Tadpoles

Competition has been studied between tadpoles of the same species (Browne et al. 2003, Gillespie 2002, Griffiths et al. 1993, Scott 1990, Semlitch and Caldwell 1982), different species (Alford 1989, Alford and Wilbur 1985, Anssi 2000, Bardsley and Beebee 2000), and between tadpoles and non-anuran species (Brönmark et al. 1991, Holomuzki and Hemphill 1996, Mokany and Shine 2002a, b, Morin et al. 1988). The effect of competition upon the distribution (Skelly 1995), behaviour and morphology (Relyea 2002) and size at metamorphosis (Travis 1984) as well as the position of the tadpole in the pond (Waringer-Löschenkohl 1988) have been examined.

Competition between tadpoles affects their growth rates, time to metamorphosis (Semlitch and Caldwell 1982) and survival (Scott 1990). The growth rate decreases with increasing competition whereas the time to metamorphosis increases. This was reported in *Scaphiopus holbrookii* tadpoles placed at different densities in containers by Semlitch and Caldwell (1982). *Ambystoma opacum* larvae placed in field enclosures at different densities have different survival rates. Increasing the larval density decreases the survival rate (Scott 1990). Competition can also affect the tadpole's behaviour; *Lithobates sylvatica* tadpoles increase their activity in the presence of competitors (Relyea 2002).

Predation and competition interact. The absence of predators can cause an increase in competition between tadpoles as well as other species (Werner and Anholt 1996). The relationship between the size of the tadpole, predation and competition was studied by Werner and Gilliam (1984). The effect of predation and competition at the tadpole stage may produce differences in adults. This has been examined to see if there is an effect on the adults' fitness (Relyea 2001b, Relyea and Hoverman 2003, Tejedo et al. 2000). Tadpoles of *Lithobates sylvatica* exposed to predators develop into frogs with large limbs and narrow bodies (Relyea 2001b). Tadpoles of *Hyla chrysoscelis* exposed to competition have a reduced rate of survival as post metamorphs and are morphologically different to post metamorphs that have not been exposed (Relyea and Hoverman 2003). Post metamorphs of *Pelophylax lessonae,* and *P. ridibundus* that have been tadpoles in a competitive environment are smaller and jump shorter distances than frogs grown in low density environments (Tejedo et al. 2000).

1.5 Amphibians on the Cape Flats

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The Cape Flats is a low-lying sandy area, where temporary pools form during the winter and spring rains. Biogeographically, the frogs of the Southwestern Cape Assemblage (Alexander et al. 2004) are characterised by extremely high endemism and species richness. Up to 30 species have been recorded per half-degree grid. The following species are known to occur on the Cape Flats, and might be expected within the study site (Taxonomy throughout after Frost et al. 2006).

Bufonidae

Vandijkophrynus angusticeps (Smith 1848).

This species is recorded in the same grid cell as Kenilworth Racecourse (Cunningham 2004a). The adults are obvious during the breeding season in their preferred habitats, such as the sandy flats around Stellenbosch. However, they breed in small shallow pools, and were not found at the study site, which is a deep pool.

Amietophrynus pantherinus (Smith 1828).

This species is known from the southern adjacent grid cell (Minter et al. 2004), and might be expected to have occurred historically in the Kenilworth area. It has a peculiar coastal distribution, however, and does not reach as far inland as Kenilworth Racecourse (De Villiers 2004a).

Amietophrynus rangeri (Hewitt, 1935).

This species has been recorded in the same quarter-degree grid cell as Kenilworth Racecourse (Cunningham 2004b). This species is a summer breeder, and can be heard calling from November in the south-western Cape. The ponds at Kenilworth Racecourse do not persist after December, and are therefore not available for *A. rangeri* to breed in.

Hyperoliidae

Hyperolius horstockii (Schlegel, 1837)

This small reed frog is reported from the same quarter degree grid cell as Kenilworth Racecourse (Braack 2004). It is a summer breeder, with egg clutches recorded in October and November (Braack 2004). It is thus temporally separated from the species able to breed in the winter pools on the Cape Flats. This easily recognised species was not heard or seen at Kenilworth Racecourse during the study.

Hyperolius marmoratus (Rapp, 1842)

This tropical reed frog has recently expanded its range to the Cape Peninsula (Bishop 2004) with many breeding populations known. The range expansion appears to be due mainly to the introduction of garden plants, which serve as an ideal temporary habitat during a road journey (Bishop 2004). This species is, however, a summer breeder, and is not expected to breed in the winter ponds at Kenilworth Racecourse.

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Semnodactylus wealii (Boulenger, 1882)

This species has been recorded in an adjacent quarter-degree grid square, south of Kenilworth Racecourse (Du Preez 2004). In the south-western Cape it has a coastal distribution, and may not extend inland as far as the study site. It breeds in spring, so the winter pools of the Cape Flats may not be available to this frog for breeding. It was not heard or seen at Kenilworth Race Course during the study.

Brevicepitidae

Breviceps gibbosus (Linnaeus, 1758)

This species has been reported from the same quarter degree grid cell as the study site (Harrison and Minter 2004). It is widespread on the Cape Flats. All the members of this family are direct developers, not requiring ponds for tadpoles to develop.

Breviceps rosei (Power, 1926)
Rose's rain frog has been reported from the same grid cell as the Kenilworth Racecourse (Harrison 2004). This species is a direct developer.

Pyxicephalidae

Cacosternum platys (Rose 1950)
This small endemic occurs widely on the Cape Flats, and breeds at Kenilworth Racecourse. The distribution is confused with that of *Cacosternum boettgeri* in the Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland (Scott 2004), as older museum records do not distinguish between the two, although it has a different advertisement call (Channing 2001). This was one of the species included in the present study.

Cacosternum capense (Hewitt, 1925)
This small frog, endemic to the south-western part of South Africa, is known from the same grid cell as Kenilworth Racecourse. It breeds in shallow temporary pools that form in loam or clay soils (De Villiers 2004b). The centre of Kenilworth Racecourse is deep sand, and this species was not found at the study site during the study.

Amietia fuscigula (Duméril and Bibron, 1841)
This large, widespread river frog has been recorded from the grid cell where Kenilworth Racecourse is situated. This distinctive frog was not found at the study pond. It prefers waterways and permanent ponds (Channing 2004a), as its tadpoles may take a year or more to develop.

Strongylopus grayii (Smith, 1849)
This widespread and abundant species is able to flourish in modified habitats, and is a common garden inhabitant. It is present in all grid cells in the south-western Cape (Channing 2004b), and was breeding in the study pond. It was included in the present study.

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Tomopterna delalandii (Tschudi, 1838)

This species is common in the south-western Cape, and is present at Kenilworth Racecourse (Channing 2004c). It was included in the present study.

Microbatrachella capensis (Boulenger, 1910)

This very small frog is both endemic to a small area of the south-western Cape, and categorised as Critically Endangered, due to habitat loss and modification (De Villiers 2004c). It is present at Kenilworth Racecourse, and breeds in the winter pools. This species was included in the present study.

<u>Pipidae</u>

Xenopus gilli (Rose and Hewitt, 1927)

This aquatic frog is endemic to the extreme south-western Cape, where it has been recorded from the same grid cell as Kenilworth Racecourse (De Villiers 2004d). Despite intensive searching, including listening for calls and dip netting for tadpoles, no evidence of this species was found in the study pond at the racecourse. If it was historically present, it may have been replaced by *Xenopus laevis*, known to be highly invasive (Measey 2004). Burger and Cohen (1998) suggest that this species may be present at the racecourse.

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Xenopus laevis (Daudin, 1802)

This common frog is widespread in South Africa (Measey 2004), and is present in the ponds at Kenilworth Racecourse. It was included in the present study.

1.6 This Study

The Cape Flats is a focus for many housing and other developments. These have already converted most of the natural habitat, but small relict patches still exist, such as that in the centre of the Kenilworth Racecourse. The racecourse site was selected to investigate the tadpole community occupying temporary winter pools. Details of the study site are presented in Chapter 3. The main focus of this study was the community of tadpoles that occur in the ephemeral ponds in the centre of Kenilworth Racecourse.

This study looked at some of the South African tadpoles which have had little attention and feature infrequently in the scientific literature. Western Cape tadpole and frog assemblages are not well known. A brief overview of the intended study is outlined below. This study began with determining basic information such as the species of anurans present at the pond, when they are present, and in what numbers they occur.

Practically all the tadpole research reviewed was based on experiments undertaken in laboratory tanks or small outdoor microcosms, which were manipulated according to the aim of the experiment. My study is based on determining the variables in the pond *in-situ*. The *in-situ* technique reduced potential laboratory effects such as feeding, temperature chamber and handling. Temperature and oxygen concentration preferences and a number of other variables (detailed below) were examined.

Tadpoles in temporary pools, when faced with uncertain rainfall, must adopt a strategy of reaching metamorphosis as early as possible, to avoid mortality. Metamorphosis will only happen once a growth threshold has been reached, that depends upon the level of food. This complex relationship has been modelled by Rudolf and Rödel (2006). I examined the ability of two species of tadpoles to reduce the time to metamorphosis in an experiment where the water level in the experimental ponds was decreasing.

Tadpoles feed mainly on algae. There are two conflicting views in the literature: one suggests that tadpoles are indiscriminate feeders (Wassersug 1974), while the other suggests that bottom-dwelling tadpoles will feed on a different set of algae to tadpoles living in the water column. Harrison (1987) examined feeding preferences by looking at gut contents. It is possible that these tadpoles use food to partition the habitat to avoid competition, as has been shown for "*Bufo" bufo* and *Rana temporaria* (Harrison 1987). The diets of many South African tadpoles are not known. This study sheds light on the diets of the three most abundant species of tadpoles in a natural community.

These results were compared to a brief study of the diets of a tropical tadpole community in North Luangwa National Park in Zambia that was occupying temporary ponds.

Southern African tadpoles are found in water at various temperatures, from pools under ice on top of the Drakensberg, to temporary pools in rock tanks in the desert that reach 40°C. As global climate change is a real threat in South Africa the ability of tadpoles to cope with increasing temperatures is of conservation interest. Tadpoles can acclimatise to small increases in water temperature, resulting in changes to their Critical Thermal Maximum (CTM) (Cupp 1980) and upper thermal limits (Brown 1969). *Strongylopus grayii* was selected for critical thermal maxima experiments as the species has a large range which covers both summer and winter rainfall regimes. A widespread species such as this with a common ancestor, but living in different and variable environments, may be able to adapt to increased temperatures. The results of this experiment offer insight into the ability of this species to survive under conditions of increased ambient temperatures.

The critically endangered micro frog *Microbatrachella capensis* occupies relatively few ponds along the coast (De Villiers 2004c). As the habitat becomes more modified there may be fewer ponds for breeding. The breeding success of this species in a pond where there are four other widespread successful species will indicate if it is able to compete in other ponds where a number of species may be breeding.

1.7 Key Research Questions

1. Which frog species use the temporary ponds in the study site for breeding?
2. Which species of tadpoles occur simultaneously in these ponds?
3. How long do cohorts of tadpoles remain in the pond?
4. How many tadpoles make up the community? Does this vary through the season?
5. Which species of tadpoles are most and least abundant in the pond?
6. What are the temperature preferences for the different species of tadpole?
7. What are the oxygen concentration preferences for the different species of tadpole?
8. What are the water clarity preferences for the different species of tadpole?
9. Do tadpole species prefer to be active at a particular depth (or depth range) in the pond?
10. Do tadpole species prefer to be active over particular substrates or near particular kinds of vegetation?
11. How do the tadpoles of *Strongylopus grayii* and *Cacosternum platys* respond when placed in an environment where the water is drying?
12. Do tadpoles in a temperate temporary pond partition the food resources?
13. Do tadpoles in a tropical temporary pond partition the food resources?
14. Does the Critically Endangered micro frog breed successfully in the pond with four other species?
15. Do *Strongylopus grayii* tadpoles from warmer northern inland sites have a higher critical thermal maximum (CTM) than tadpoles from cooler southern sites?
16. Do *S. grayii* tadpoles from higher elevations have lower CTMs than those from low altitude?
17. Do *S. grayii* tadpoles from the summer rainfall region have higher CTMs than tadpoles from the winter rainfall region?

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1.8 Species Descriptions

The following are descriptions of the species investigated at the Kenilworth Racecourse study site. The species examined from the North Luangwa National Park are dealt with in Chapter 7.

Family Pyxicephalidae

Cacosternum platys

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Fig. 1.1. A photograph of a *Cacosternum platys* from the Kenilworth racecourse site.

Common name: Flat dainty frog

Description: This is a small frog (Fig. 1.1), with large females reaching a length of 22 mm (Channing 2001). The body is elongated with a characteristic thin head and neck. The limbs are gracile, and the skin of the back varies from smooth (Rose 1950) to slightly rugose. There is no webbing between the toes. Colour pattern is variable, with the back being banded, striped, or uniform, in grey, brown, green, red, or a combination of these. The belly spots may be grey to black (Rose 1950).

Distribution: The exact range is unknown, but it is widespread in the south-western Cape (Channing 2001).

Habitat: Western Cape, fynbos biome

Breeding Habitat: It breeds in flooded grassland and seepages (Channing 2001), and in temporary pools on the sandy Cape Flats.

Conservation: This species was assessed by the Global Amphibian Assessment. It appears to be widespread, although threatened by development. It should is regarded as of least concern (GAA 2006).

Threats: Housing developments and other habitat loss in the Western Cape.

Eggs: About 20 eggs are deposited in shallow water attached to emergent vegetation.

Tadpole:

Fig. 1.2. A photograph of a *Cacosternum platys* tadpole from Kenilworth racecourse pond.

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The tadpole reaches a length of 25 mm. The shape of the body of the tadpole is oval and the tadpole is green, or grey in colour although this is variable (Fig. 1.2). The eyes are on the side of the head and the mouth is positioned anterioventrally. The vent tube is continuous with the fin margin and the papillae around the mouth have an anterior median gap but no posterior gap. The labial tooth formula is 6(2-6)/3. The dorsal and ventral fin margins are nearly parallel. Development time to metamorphosis is not reported.

Notes: On one occasion six adult *C. platys* were found in a small clump of grass of no more than 300 x 300 mm at Kenilworth racecourse.

Microbatrachella capensis

Fig. 1.3. Photograph of a male *Microbatrachella capensis* calling at Kenilworth racecourse.

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Common name: Micro frog

Description: This is a small frog (Fig. 1.3), with large females reaching a length of 18 mm (De Villiers 2004c). The limbs are small, and the snout is short (Hewitt 1926). The skin of the back has no warts (Rose 1950) although it may be slightly rugose. There is slight webbing between the toes. Colour pattern is variable, with the back being grey, brown, or green. The belly is mottled (Rose 1950).

Distribution: This species is now present at fewer than 12 localities (Baard 1989), within only four quarter-degree grid cells (De Villiers 2004c). The range extends from the Cape Flats southeast 140 km to the Agulhas region The Kenilworth racecourse pond population is the only remaining Cape Flats population (De Villiers 2004c).

Habitat: Low lying coastal areas of the fynbos biome on neutral to acid sand.

Breeding habitat: Semi-permanent water bodies where little or no disturbance has taken place (Baard 1989).

Conservation: This frog species has been placed on the IUCN red data list as Critically Endangered due to habitat destruction, which suggests that it has a high risk of extinction (De Villiers 2004c). The GAA lists this species as Critically Endangered. (GAA 2006). The threats are all associated with human activity: draining and filing-in of breeding sites, eutrophication, invasive alien vegetation, silt build up leading to invasion by reeds and bulrushes (De Villiers 2004c).

Threats: The main threats to this frog are human interference and development of habitat (De Villiers 2004c).

Eggs: Groups of about 20 eggs (De Villiers 2004c) are deposited around vegetation in shallow water.

Tadpole:

Fig. 1.4. A photograph of a *Microbatrachella capensis* tadpole.

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This tadpole (Fig. 1.4) reaches a length of 25 mm. It has a high tail and an oval body. The tadpole colour is quite variable but is mostly a green to grey colour. The eyes are on the side of the head and the mouth is anterioventral. The vent tube is continuous with the lower fin margin. The papilla around the mouth have anterior and posterior gaps. The labial tooth formula is 3(2-3)/3(1). Dorsal and ventral fin margins are nearly parallel. Time to metamorphosis is not reported.

Notes: Little is known about this species' ecological requirements (Baard 1989).

Strongylopus grayii

Fig. 1.5. Two photographs of *S. grayii* showing the variation in colour.

Common name: Clicking stream frog.

Description: This is a small but stocky species. The toes are long and only a small amount of webbing is present (Channing 2001). The adult frog is one of the larger species at the study pond. The male reaches 35 mm and the female grows to 64 mm. Frogs are variable in colour, ranging from green to brown with darker markings, and occasionally they have a vertebral stripe or band (Channing 2001).

Distribution: This species is found from the Western Cape eastwards to Kwa-Zulu Natal and northwards to Limpopo Province (Channing 2004b).

Habitat: The species occurs in the fynbos biome, succulent Karoo, Nama Karoo, savanna, grassland, thicket and forest biomes. Outside of the southwest part of the species' distribution it is restricted to the uplands (Channing 2004b).

Breeding habitat: This frog breeds in a variety of water bodies including small dams, ponds, pools, ditches and shallow seeps. The frog is known to lay eggs out of water (Rose 1950).

Conservation: *Strongylopus grayii* is widespread and not threatened (Channing 2004b).

Threats: This species is not threatened.

Eggs: About 200 eggs are deposited in vegetation above water level (Channing 2001).

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Tadpole:

Fig. 1.6. Photograph of a *Strongylopus grayii* tadpole.

The tadpole (Fig. 1.6) reaches a length of 50 mm. The tail fin is high and the body is oval in shape. The colour is variable but usually brown to grey-green, but will get lighter if put against a lighter background. Some tadpoles caught were cream in colour with small grey spots. The eyes are positioned on the upper side of the head and the vent is on the right side of the fin margin. There is a gap in the anterior papilla but the posterior papilla are continuous. The labial tooth row formula is 4(2-4)/3(1). The jaw sheaths are pigmented to the base. Tadpoles of this species will eat those of *Cacosternum* and even unwell conspecific tadpoles (De Villiers 1929). They are reported to take three months or more to reach metamorphosis (Wager 1965).

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Notes: The male frogs call when the air temperature drops rather than when the rains start. This response means that they are the first species to start calling and one of the first species to have tadpoles in the winter pools.

Tomopterna delalandii

Fig. 1.7. Photograph of an adult *Tomopterna delalandii*.

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Common name: Cape sand frog.

Description: The adult is stocky (Fig. 1.7) and has an enlarged metatarsal tubercle that is used for digging. The toes are slightly webbed (Rose 1950). The female of this species can reach 50 mm in length (Channing 2001). The species is light grey to green in colour with the ventral side white (Rose 1950).

Distribution: This frog ranges from the Cape Peninsula to Port Alfred in the east and to Steinkopf in the north (Channing 2004c).

Habitat: The species is found on flat sandy areas in lowlands and valleys (Channing 2004c).

Breeding habitat: Adults will move to water to breed in the wet season, utilising pans, vleis and dams (Channing 2004c).

Conservation: No conservation action has been recommended (Channing 2004c).

Threats: None.

Eggs: Clutch size is 2500, laid singly or in small groups (Channing 2004c).

Tadpole:

Fig. 1.8. A photograph of a tadpole of *T. delalandii.*

This is a large tadpole that may reach a length of 44 mm. The tail fin is gently rounded dorsally and ventrally. The body is oval in shape and a grey to green colour. The eyes are on the upper side of the head (Fig. 1.8) and the mouth is anterioventral. The vent tube is on the right side of the fin and the papillae around the mouth have an anterior gap. Metamorphosis takes 25-35 days.

Notes: During the breeding period this species was the last to start calling at the study pond.

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Family Pipidae

Xenopus laevis

Fig. 1.9. Photograph of an adult *Xenopus laevis*.

Common names: Platanna, African clawed frog.

Description: This frog (Fig. 1.9) is the most distinct of all of the frogs at the study pond. They have a pointed head, with broadly webbed feet, claws on three toes, and no tongue (Rose 1950). Females may reach 130 mm in length (Channing 2001). In colouration they have a light ventrum and a dark green and brown mottled dorsum.

Distribution: *Xenopus laevis* is present across South Africa except for the extreme arid regions of the Kalahari and the east of the Northern Cape Province (Measey 2004).

Habitat: This frog requires permanent water bodies. It is present in natural water bodies as well as in man-made dams and sewage purification works (Measey 2004).

Breeding habitat: *X. laevis* will breed in most water bodies but do not breed in running water (Measey 2004).

Conservation: *X. laevis* is widespread and not threatened.

Eggs: Clutch size is up to 350, laid in small groups (Wager 1965).

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Tadpole:

Fig. 1.10. Photograph of the tadpole of *X. laevis*.

This is probably the largest tadpole at the pond. They may reach a length of 80 mm (Channing 2001); however, they were never that large at the Kenilworth racecourse site. The tail fin is shallow with a very thin tip. The body is almost triangular (Fig. 1.10) and is transparent with pigmented spots. The eyes are at the side of the head and there are no papilla around the terminal mouth. The tadpoles do not have any labial teeth or jaw sheaths. The tadpoles remain in the water column, suspended with the head down, and the tip of the tail vibrating. Large tadpoles school at the Kenilworth racecourse site. The tadpole can reach metamorphosis within two months (Tinsley et al. 1996)

Notes: The adult frog will either move to another pond or bury into the mud when the pond dries. They eat insects, small fish, and young and larvae of their own species (Leslie 1890). The adults can breed more than once per season (Measey 2004). *Xenopus laevis* is an invasive species in the USA, Chile, Mexico, France, Indonesia and the UK (Measey 2004), and may pose a threat to other species sharing a breeding pond.

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CHAPTER 2: ENTRY SEQUENCE AND TADPOLE NUMBERS IN THE KENILWORTH RACECOURSE POND

2.1 Introduction

This chapter sets out to answer key research questions one to five. It deals with the species assemblage at the Kenilworth pond and the duration of time that each species is present.

2.1.1 Species assemblage

The assemblage of species and at what point they enter the pond can have a marked effect on the success of the tadpoles. Obviously the species of tadpole found in a body of water reflects the adult species assemblage but also a number of other factors such as the tadpoles' need for permanent or temporary water (Skelly 1997) or even a certain water body type (Gascon 1995, Inger et al. 1986, Skelly 2001). If the tadpole takes many years to metamorphose, *Heleophryne purcelli* for example, then a temporary water body will not suffice. The presence of predators or competitors can affect the presence of a tadpole species in the pond. Adults of *Mannophryne trinitatis* select the pools of water they will place their eggs in by making an assessment of the number of predators and competitors in a pond (Downie et al. 2001).

2.1.2 Sequence of tadpole entry

The timing and sequence that tadpoles enter the pond will also ultimately affect their success. New pools have higher algal concentrations (Wassersug 1975) and the type of algae in the pond fluctuates throughout the season (chapter 6). The type of food a tadpole consumes affects its growth (Brown and Rosati 1997, Kupferberg et al. 1994, Scorgie 1980). Therefore, the timing of entry into the pond may be essential to the tadpoles' feeding success.

Abiotic factors change with time. The pond fills in winter when temperatures are low and persists for many months. By the end of the season the temperature has increased (see Fig. 3.27). The timing of the entry of the tadpoles will affect what water temperatures they are exposed to. Oxygen concentrations change from after a pond fills through the season (Fegraus and Marsh 2000) and the amount of oxygen available to a tadpole can have an effect on bobbing rate (Wassersug and Seibert 1975), stamina (Wassersug and Feder 1983) and developmental speed (Nelsen 1947).

The relationships with other anuran species alter through time, with tadpoles competing for food or predating upon one another. The tadpoles of *Anaxyrus americanus* do better in terms of mass and larval period when introduced before *Lithobates sphenocephalus.* The tadpoles of *Hyla chrysoscelis* are affected by the previous presence of *Lithobates spenocephalus* and *Anaxyrus americanus*. The tadpoles have a decreased survival and size at metamorphosis and an increase in the time to metamorphosis when added to ponds that had contained the other two species (Wilber and Alford 1985). Tadpoles of *Rana boylii* increase standing stocks of algae by decreasing the number of mayfly larvae but also decreased food for other primary consumers (Kupferberg 1997). Tadpoles are affected by non-anuran species in much the same way and competition and predation between mosquito and *Pseudepidalea viridis* larvae relies on which species arrived first (Blaustein and Margalit 1996).

2.1.3 Competition

The number of tadpoles in an area is an important component to tadpole growth and development. Tadpoles can compete for space (Alford 1986) as well as food with other anurans (Browne et al. 2003, Griffiths et al. 1993, Scott 1990).

2.2 Methods

2.2.1 Marking techniques

In the present study the total tadpole community size within the pond was estimated. For this to be successful the tadpoles needed to be marked with something that would last the duration of the season. There are many different ways of anaesthetizing and marking tadpoles. Anholt et al. (1998) looked at three anaesthetics. The first two, chlorotone and benzocaine caused mortality whereas tricaine methane sulphonate (MS222) allowed all the tadpoles to recover within 30 minutes. I used MS222 due to its proven safety.

A number of different methods for marking have been proposed. Vital staining is simple to use and allows batches of tadpoles to be marked simultaneously. However, tadpoles of *Hyla gratiosa* stained with Neutral Red grow more slowly than the unstained control tadpoles and the stain only lasts six days (Travis 1981).

Fin clipping and vital stains were rejected as suitable marks by Anholt et al. (1998) because of an increase in predation risk. They recommended visual implant elastomer (VIE) as 136 out of 160 individuals that were marked with VIE retained their tags. In addition there was no significant difference between the predation rate of dragonfly nymphs (*Anax imperator*) on the marked and unmarked tadpoles. Harvey (2003) also favoured VIE for three reasons: the retention of the mark was higher than with organic dyes, marked tadpoles were not more likely to be cannibalised and marking did not affect survivorship. Mark retention with the VIE is high; tests have shown that adult anurans can retain the mark for at least four months (Marold 2001, Nauwelaerts et al. 2000). For this reason I used VIE to mark the tadpoles in this study.

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2.2.2 Determining the presence of tadpoles at the pond

The onset of calling for each species present was noted at the start of each season. The tadpoles were trapped (see methods in chapter 3) during the wet season (June until December) of 2003 and 2004. For each species the date they were first present in the pond was recorded as well as the duration of each species presence. In the second season the tadpole's stage was recorded to allow for an estimation of number of breeding events for each species. Total tadpole numbers cannot be determined throughout the season. It is possible to show relative numbers of tadpoles in each species assuming the tadpole species are equally catch-able.

2.2.3 Mark and recapture

Tadpoles were marked using a visual implant elastomer which did not affect the tadpoles when grown in captivity. This was tested before the study was carried out at the pond. In May 2003 *S. grayii* tadpoles were collected at stage 30 (Gosner 1960) and ten were marked under the skin with a dorsal pink spot about 2 mm long. Another ten tadpoles of the same species and stage were handled but not marked. These 20 tadpoles were kept in the same tank until all four legs had emerged (stage 42), and the time it took to reach this stage was recorded. The results were then compared using a t- test to make sure that the marking process and the mark itself did not adversely affect the tadpoles.

The results showed no difference ($t = -0.11$, $p=0.69$) in the time to metamorphosis of the marked and unmarked group of tadpoles (data shown in Appendix 1). This pilot study showed that the mark itself did not adversely affect the tadpoles. However, in the pond at Kenilworth racecourse the small mark may make the tadpoles more vulnerable to predation. There are few visual predators at the site, however, and the mark was small. The only problem with the marking techniques was that very small tadpoles, stages 25-26, were impossible to mark owing to the size of the needle (28 gauge). This meant that only tadpoles of stages 27 upwards were marked.

In the second season (2004) mark-recapture attempts were made on two occasions. At each, tadpoles were caught by six people using dip nets. Each netting session ended when there were no tadpoles being caught, after approximately 48 person hours. The tadpoles were all marked and released. A recapture session was carried out a week later, using the same collecting method for 5 person hours. Only the second session produced sufficient recaptures that could be used in a population estimate. The data were used in a Petersen Estimate with Bailey's modification to estimate the number of tadpoles present in the pond (Heyer et al. 1994).

The Petersen estimate with Bailey's modification was used to allow for the use of low numbers (Heyer et al. 1994). This method was used because the pond can be

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considered a closed population for the week between marking and recapturing. The other assumptions of the Peterson method are that the marked and unmarked tadpoles have the same chance of being caught. This is highly likely using the dip net method of recapture as the dip net does not select for certain tadpoles. The fact that the animals do not loose their marks was observed in the preliminary study where marked and unmarked *S. grayii* tadpoles were kept in a tank and the tadpoles retained the marks right through metamorphosis. The final assumption is that all marks are reported upon discovery in the second sample (Heyer et al. 1994). This was facilitated by the fluorescing of the marks in blue light when viewed through orange goggles which made the marks incredibly easy to see.

2.3 Results

2.3.1 Adult calling

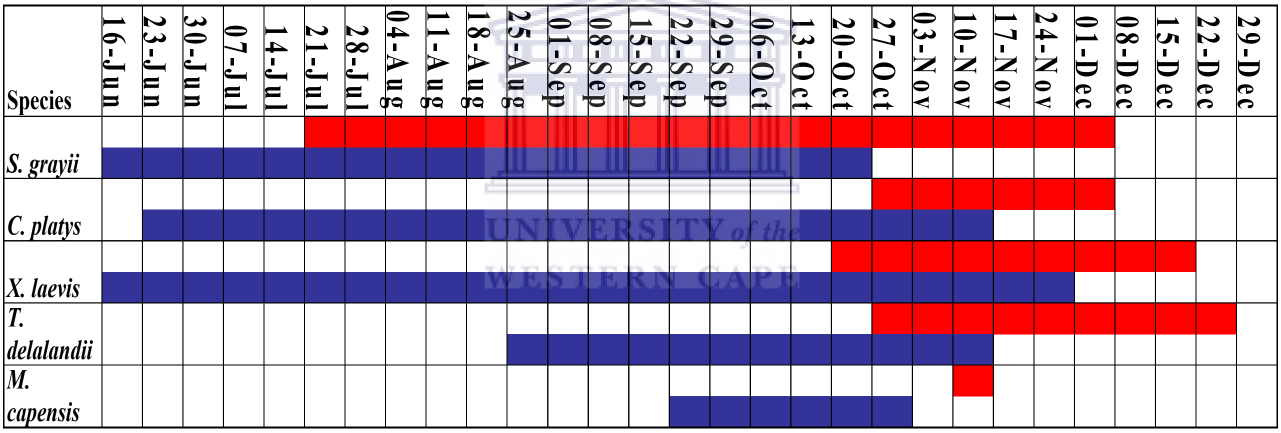
S. grayii adults started calling before the pond contained water, in June of the first season and May in the second. *M. capensis* and *C. platys* only called when water was present in the pond. The number of calling males of *M. capensis* and *C. platys* were similar even though the numbers of tadpoles were not. *T. delalandii* called later on in the season, in October of the first season and August in the second. They were heard in fewer numbers than *S. grayii*, *C. platys* and *M. capensis*.

2.3.2 Tadpole species presence

S. grayii tadpoles were present in the pond as soon as it filled in both seasons. In the first season they were joined by *X. laevis* on the 20th of October. *C. platys* and *T. delalandii* were first seen on the 28th of October and last to appear was *M. capensis* on the 7th of November. In the second season the order in which the tadpoles were trapped was slightly different, with *S. grayii* and *X. laevis* being present from the beginning and then *C. platys* seven days later. *T. delalandii* was not seen until the 25th of August and *M. capensis* on the 23rd of September (Fig. 2.1).

The pond dried at the end of December in 2003, and the last *S. grayii* and *C. platys* were seen on the 1st of December. The last *X. laevis* and *T. delalandii* were seen on the 18th of December. *M. capensis* was only seen for the first and last time on the 7th of November. The second season (2004) was five weeks longer than the first and *S. grayii* were last seen in the pond on the 7th of October, *M. capensis* on the 31st of October and *T. delalandii* and *C. platys* on the 14th of November. *X. laevis* tadpoles were in the pond until the 22nd of November (Fig. 2.1).

The second season started earlier than the first and lasted longer. *S. grayii* was in the pond for 20 weeks in the first season and 19 weeks in the second and *T. delalandii* was recorded in the pond for 9 weeks in the first season and 12 in the second. *C. platys* and *X. laevis* were in the pond for a short time (6 and 9 weeks) in the first season whereas in the second season they were present for longer (21, and 24 weeks). The brief appearance of *M. capensis* tadpoles in the first season (only recorded once in traps) may be an artefact of the low numbers of tadpoles of this species, as in the following year it was recorded in the pond for six weeks (Fig. 2.1).



Species	16-Jun	23-Jun	30-Jun	07-Jul	14-Jul	21-Jul	28-Jul	04-Aug	11-Aug	18-Aug	25-Aug	01-Sep	08-Sep	15-Sep	22-Sep	29-Sep	06-Oct	13-Oct	20-Oct	27-Oct	03-Nov	10-Nov	17-Nov	24-Nov	01-Dec	08-Dec	15-Dec	22-Dec	29-Dec
S. grayii						🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥				
	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦										
C. platys																				🟥	🟥	🟥	🟥	🟥	🟥				
		🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦							
X. laevis																			🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥		
	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦					
T. delalandii																				🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	
											🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦							
M. capensis																						🟥							
															🟦	🟦	🟦	🟦	🟦	🟦									

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Fig. 2.1. Duration of tadpoles trapped in the pond (Red – first season (2003), Blue – second season (2004))

2.3.3 Tadpole species catch rate

Figures 2.2 and 2.3 show the total number of tadpoles caught throughout the season for each species. In the first season (2003) *S. grayii* has a peak in numbers at the start of the season and one in the middle of the season and then the number of *S. grayii* tadpoles trapped declines. *C. platys* has a single peak towards the end of the season and *X. laevis* has a peak in numbers at the same time as the *C. platys*. *T. delalandii* only has a peak in numbers at the very end of the season. No *M. capensis* were caught in traps in this season (Fig. 2.2). In the second season (2004) the tadpoles enter the pond in a different order and their numbers peak at different times. *S. grayii* tadpole numbers peak at the beginning of the season and again in the middle of the season as they did in 2003. *C. platys* numbers peak once in the middle of the season and again toward the end of the season. *X. laevis* numbers peak before *S. grayii* tadpole numbers and are trapped again in small numbers toward the end of the season. *T. delalandii* peak at the same time that the numbers of *S. grayii* peak the second time. *M. capensis* is only present at the end (Fig. 2.3).

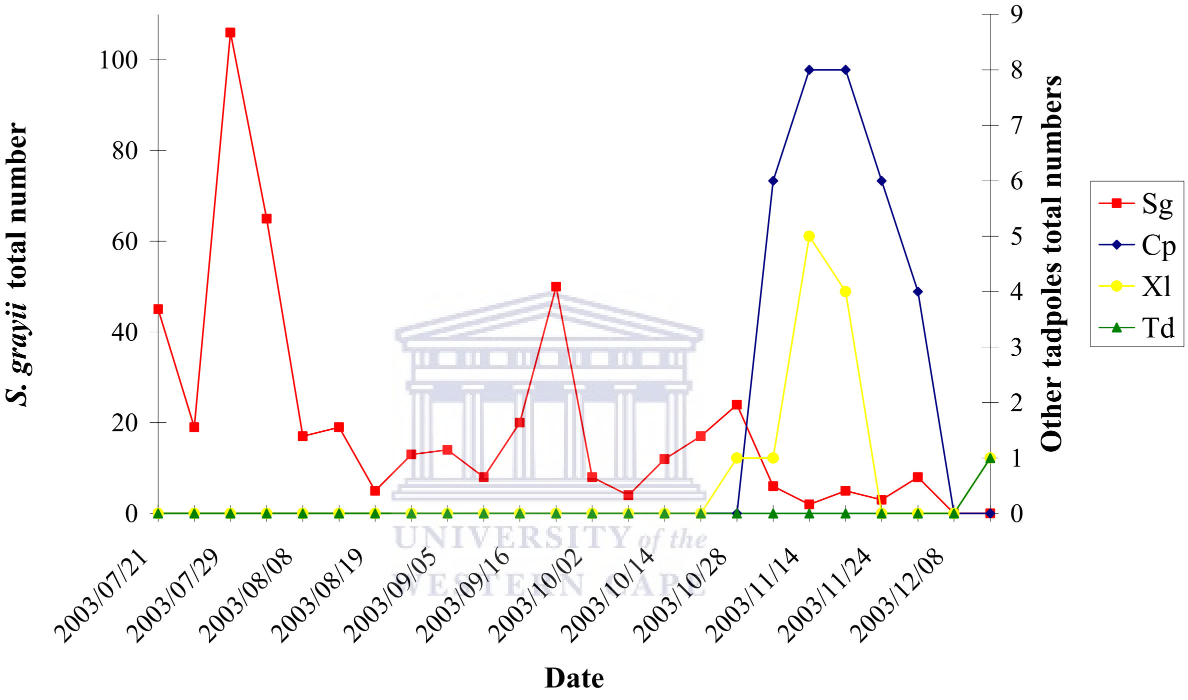

Fig. 2.2. Tadpoles caught in funnel traps 2003. Note that *S. grayii* is plotted on a larger y-axis scale.

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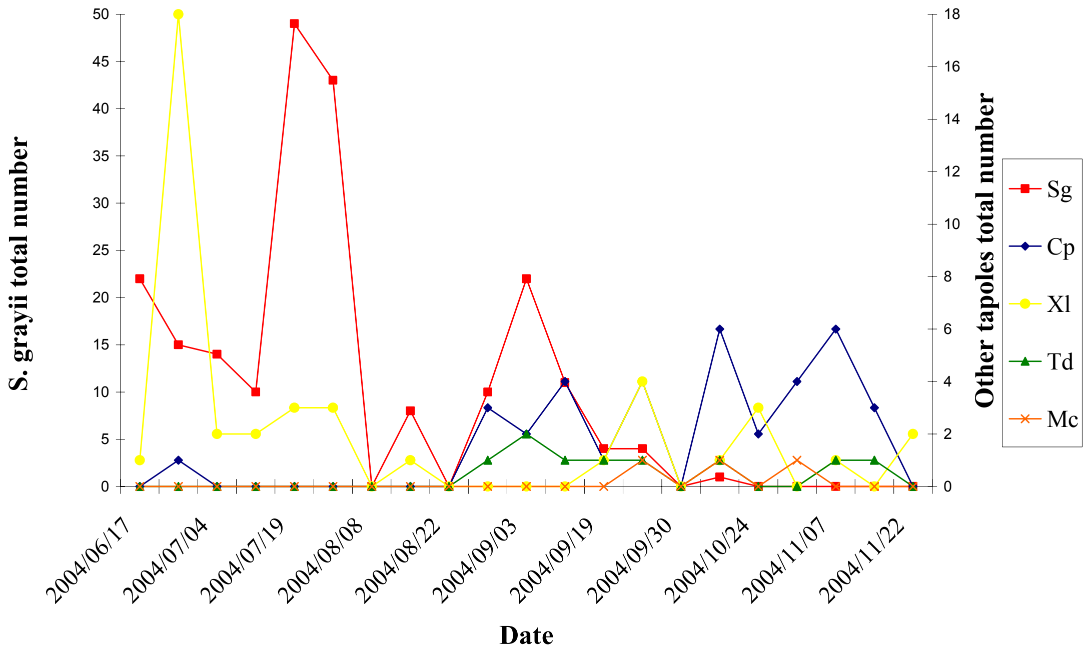

Fig. 2.3. Tadpoles caught in funnel traps during 2004. Note that *S. grayii* is plotted on a larger y-axis scale.

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2.3.4 Tadpole developmental stages in the pond

Figures 2.4-2.6 show the stage, according to Gosner (1960), of the tadpoles that were caught in the traps. *Strongylopus grayii* tadpoles were first caught in mid June and the first stage 42 was captured in mid July (Fig. 2.4). Marked on the graph is an indication of the time to metamorphosis published in the literature and the time it would have taken for the first tadpole caught to reach metamorphosis using the first stage 42 tadpole caught. The first *C. platys* was caught at the end of June in 2004 and the first stage 42 was captured at the beginning of November (Fig. 2.5). The time suggested for *C. boettgeri* to reach metamorphosis is marked on the graph along with the time it would have taken for the tadpole caught in June to reach metamorphosis using the first stage 42 tadpole as a guide. *X. laevis* tadpoles were first captured in the traps in mid June and the first stage 42 tadpole was caught in mid September (Fig. 2.6). The time suggested in the literature for a *X. laevis* tadpole to reach metamorphosis is marked on the graph along with the time between the first tadpole to be caught and the first stage 42 tadpole to be caught.

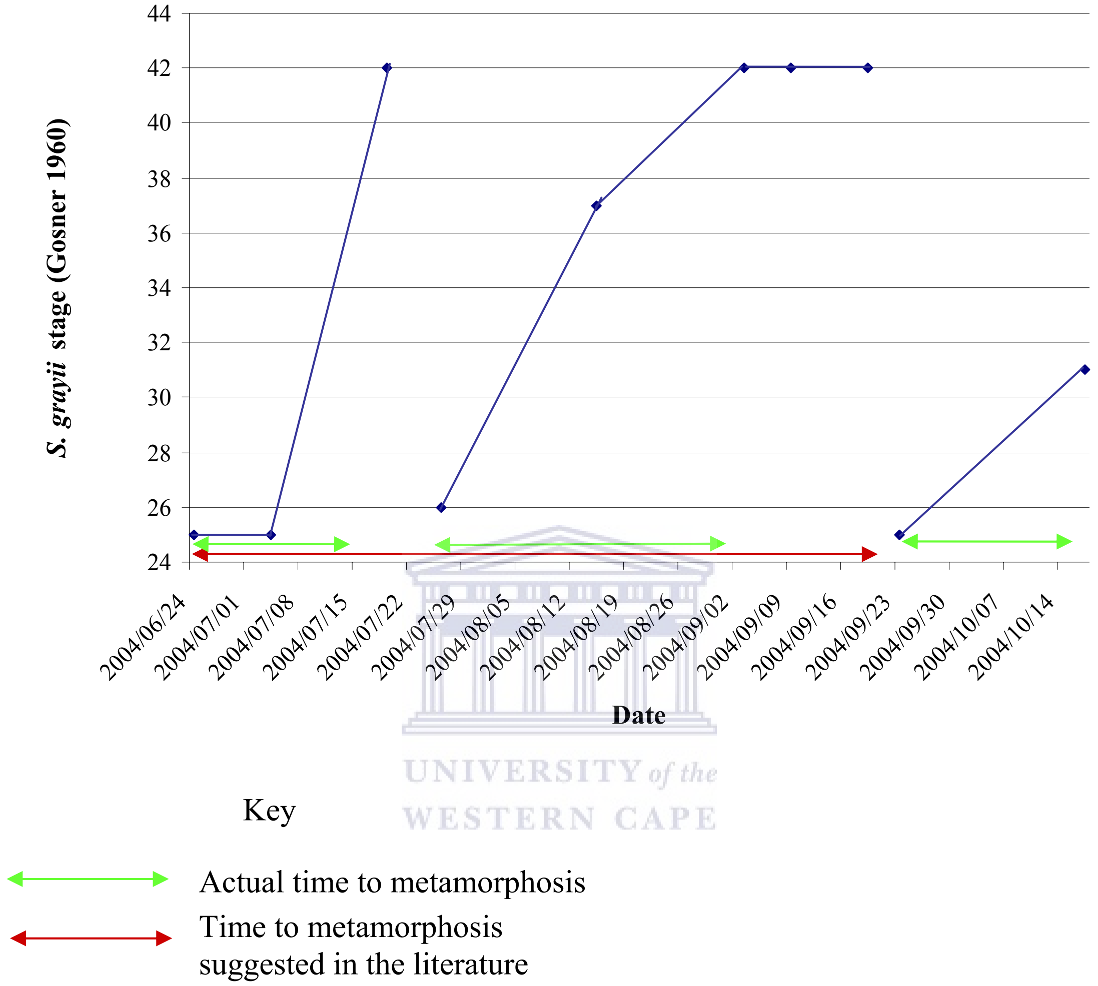

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Fig. 2.4. The stage of *S. grayii* tadpoles caught in traps in 2004.

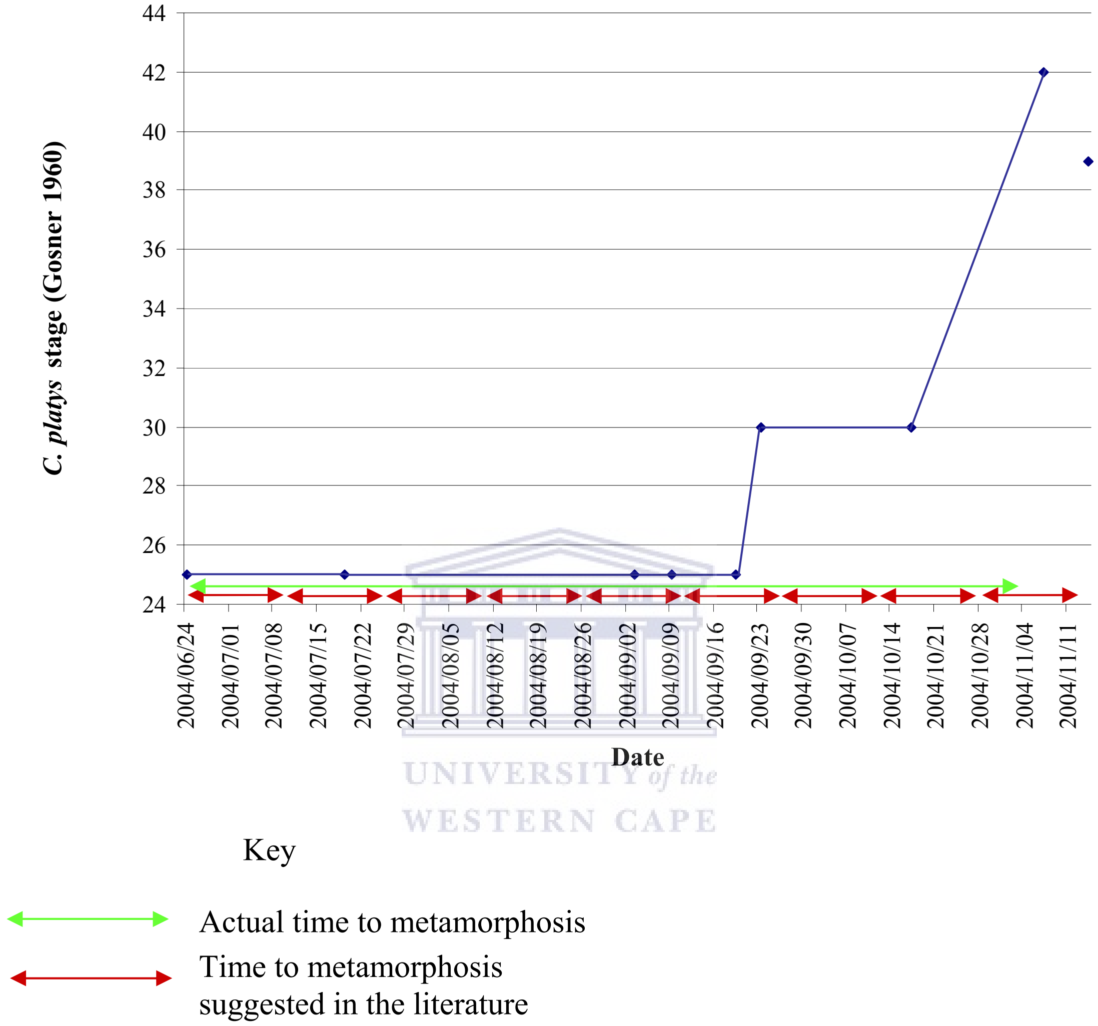

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Fig. 2.5. The stage of *C. platys* tadpoles caught in traps in 2004

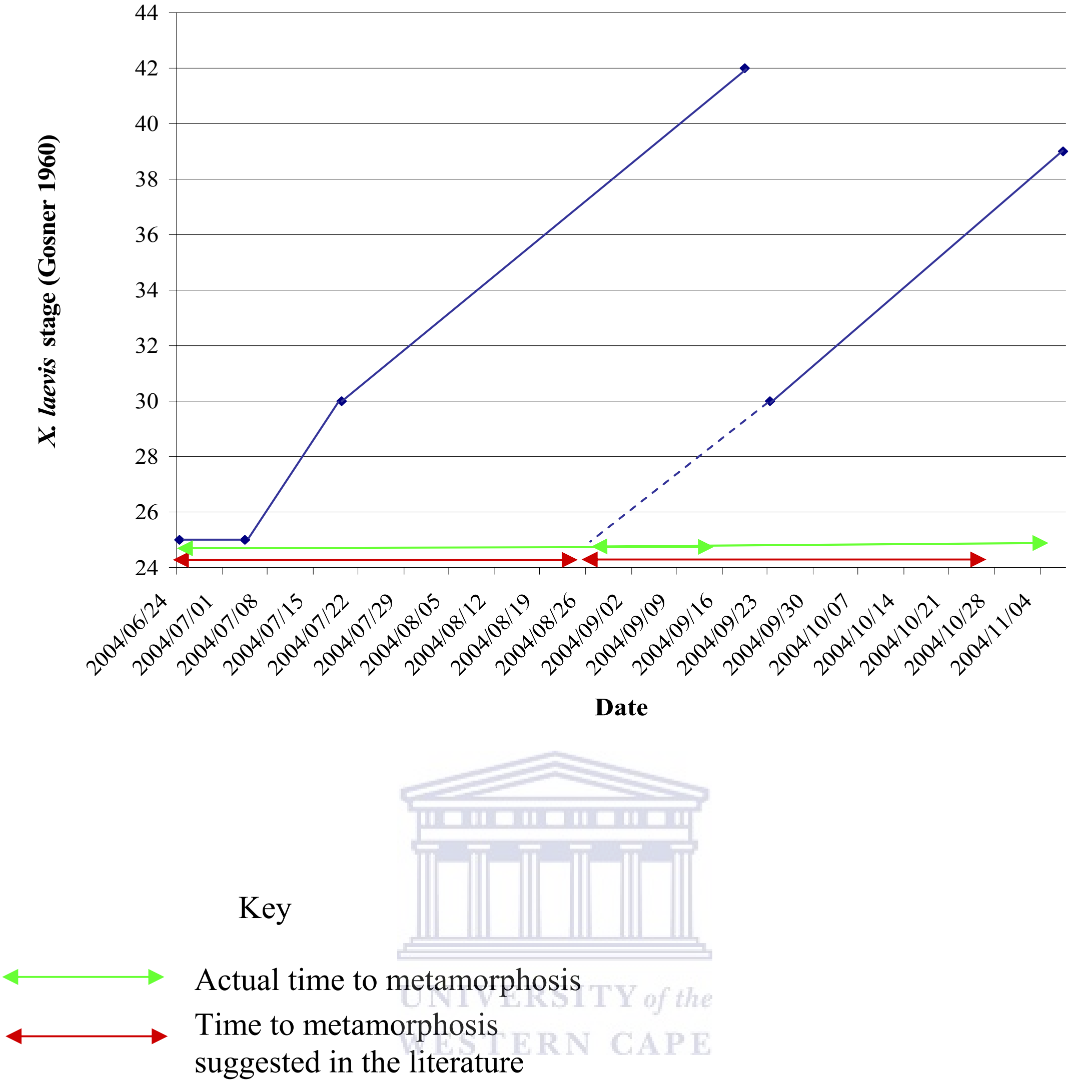

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Fig. 2.6. The stage of *X. laevis* tadpoles caught in traps in 2004

2.3.5 Mark and recapture

In the middle of August 2004 there were 392 tadpoles in the pond and the standard error of this estimate was 5.98 (Table 2.1). These figures were estimates of total tadpole numbers in the pond for a single sampling session not taking into account species or age and are not representative of total seasonal numbers

Table 2.1. The number of tadpoles of all species marked and recaptured over one season from the Kenilworth racecourse pond in 2004.

Date	Caught	Marked and released	Captured 1 week later	Number marked in sample
13.7.04	174	174	67	1
15.8.04	24	24	97	5

2.4 Discussion

2.4.1 The pond tadpole community

The tadpoles at Kenilworth racecourse partition their habitat temporally. In the first season *S. grayii* were seen in the pond on the 21st of July and the next species, *X. laevis,* did not arrive until the 14th of October. This is probably advantageous for *S. grayii* as the pond may contain a large amount of algae that the tadpoles can utilise. Wassersug (1975) suggested that frogs breed after heavy rains because inorganic nutrients are washed into basins and cause an increase in primary productivity. *S. grayii* could be using this increase to their advantage.

In an experiment by Wilbur and Alford (1985) the impact *Anaxyrus americanus* and *Lithobates sphenocephalus* made on the trophic structure of experimental ponds affected *Hyla chrysoscelis* tadpoles that were introduced later. It was concluded that *A. americanus* and *L. sphenocephalus* were in competition with *H. chrysoscelis* as well as causing nutrient loss in the pond and changing the algal structure. The nature of the competition between the species in the pond is considered in Chapter 6.

In the two seasons studied here, *S. grayii* and *T. delalandii* were present in the pond for roughly the same amount of time whether the rains arrived early or late, whereas *C. platys, X. laevis* and *M. capensis* were present for a shorter time as they entered the pond later. Figures 2.2 and 2.3 showed more peaks in *C. platys* and *X. laevis* numbers

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in the second season which could suggest that the adults breed on more nights when the wet season is longer.

The last tadpole was seen in the pond two weeks before the pond had dried in the first season and six weeks before in the second. This indicates that the tadpoles are selecting for the optimum conditions for their growth and are able to leave the pond long before it dries.

Assuming that all of the tadpoles are equally catchable then Fig. 2.2 and 2.3 show a peak of *S. grayii* tadpoles at the start of each season and as their numbers decline there is a peak of *C. platys* tadpoles. These two tadpoles are morphologically quite similar (Chapter 1) and this temporal separation could be a competition avoidance strategy.

The developmental stage graphs (Figs. 2.4-2.6) show the stage of tadpoles caught at the pond in the traps. Firstly this shows that tadpoles under stage 25 were never caught and this is probably because these tadpoles are not strong swimmers. Secondly these graphs show that for *S. grayii* and *X. laevis* there is more than one spawning time as there are stage 25 tadpoles in the middle of the season. The graph of the *X. laevis* stages fits with the peaks seen in the catch rate graph of the same year; however, the other two species do not.

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2.4.2 How many tadpoles occupy the pond?

The visual implant elastomer was tested on captive tadpoles in a preliminary study before it was used at the Kenilworth racecourse pond. The tadpoles raised with marks metamorphosed at the same time and survived as well as the tadpoles with no mark ($t = -0.11$, $p=0.69$). The mark and recapture method has been tested by a number of authors on a number of species and is generally considered a good way of estimating population size (Anholt et al. 1998, Harvey 2003). Jung et al. (2002) and Schmidt (2004) both used mark and recapture in their studies whilst testing other methods. Jung et al. (2002) were trying to estimate the population numbers of *Scaphiopus couchii* and *Hyla arenicolor*. Many techniques were tested and mark and recapture was most effective. Their results show that the larger the pond the higher the tadpole numbers.

The mark and recapture study gave an estimate of 392 tadpoles in August of 2004. The clutch size of a single *S. grayii* pair is 250 to 350 eggs, while *X. laevis* lays around 1,000 eggs per clutch (Channing 2001).There were more frogs calling at any one time than one or two males. However, in one of the dip-netting sessions on the 13th of July 2004 a team of six people spent 8 hours at the pond and caught tadpoles until they could find no more. The total number of tadpoles caught was only 170 and although it is highly likely that every tadpole was not taken from the pond it does show that the estimate of 392 tadpoles in August may be accurate. Schmidt (2004) tested the effectiveness of count data opposed to mark and recapture data; mark and recapture data were preferable. Actual figures calculated for a pond in Plumas National Forest, USA, found that the tadpoles of *Pseudacris regilla* occur in large numbers. The estimate for this pond is that 162,138 tadpoles occur in a pond that is 1,329 m^2 (Baldino et al. 2006). The discrepancy between these numbers and the Kenilworth racecourse site numbers could be due to the tadpoles of *Pseudacris regilla* at this site having a limited amount of time when there is water present and possibly high predation rates causing the adults to flood the environment with young. The frogs at Kenilworth racecourse breed across many nights and perhaps do not have the urgency seen in the pond in Plumas National Forest.

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A mark and recapture study in the Kenilworth racecourse pond environment is difficult because of the high turnover of tadpoles, and the size of the pond. It is important to note that the figure of 392 does not represent the number of tadpoles in the pond for the season rather it is an estimate for a single sampling session only**.**

2.4.3 Projections of tadpole numbers

The mark and recapture estimation of 392 tadpoles is marked on the trapping graph for 2004 (Fig. 2.7). The mark and recapture data were collected when there were few tadpoles being trapped in the funnel traps. There had been a peak of *S. grayii* tadpole numbers two weeks prior to this sampling session and numbers of *S. grayii* and *X. laevis* were rising again. Figure 2.4 suggests that the mark and recapture data were taken when at least the second group of *S. grayii* tadpoles had hatched into the pond. The *C. platys* adults had not yet started to lay large numbers of eggs, although a few tadpoles were present, and *X. laevis* tadpoles were probably from the first adult laying as Fig. 2.6 suggests that the next suite of tadpoles did not enter the pond until the end of August. If there were around 392 tadpoles present in the pond in mid-August then it is predicted that the number of tadpoles present in the pond would get much larger than this, perhaps up to 2500 in July 2004, and 1600 in September 2004. There are successive peaks of tadpole numbers through the season. The first wave of tadpole hatching will encompass the 2500 estimate and the 392 estimate as they are both within the first three months of the pond filling. The tadpole numbers never reach 2500 after the first three months; the highest number of tadpoles trapped in any one week after this is around 8, in mid-October. This is equivalent to the number of tadpoles trapped in the week the mark and recapture was carried out which would give an estimate of total tadpole number in that week of around 400 tadpoles (Fig. 2.7). With estimations of 2500 tadpoles for the first three months of the season and estimations of 400 tadpoles for the second part of the season then an estimate of total number of tadpoles in the pond for the season would be around 2900 tadpoles at least.

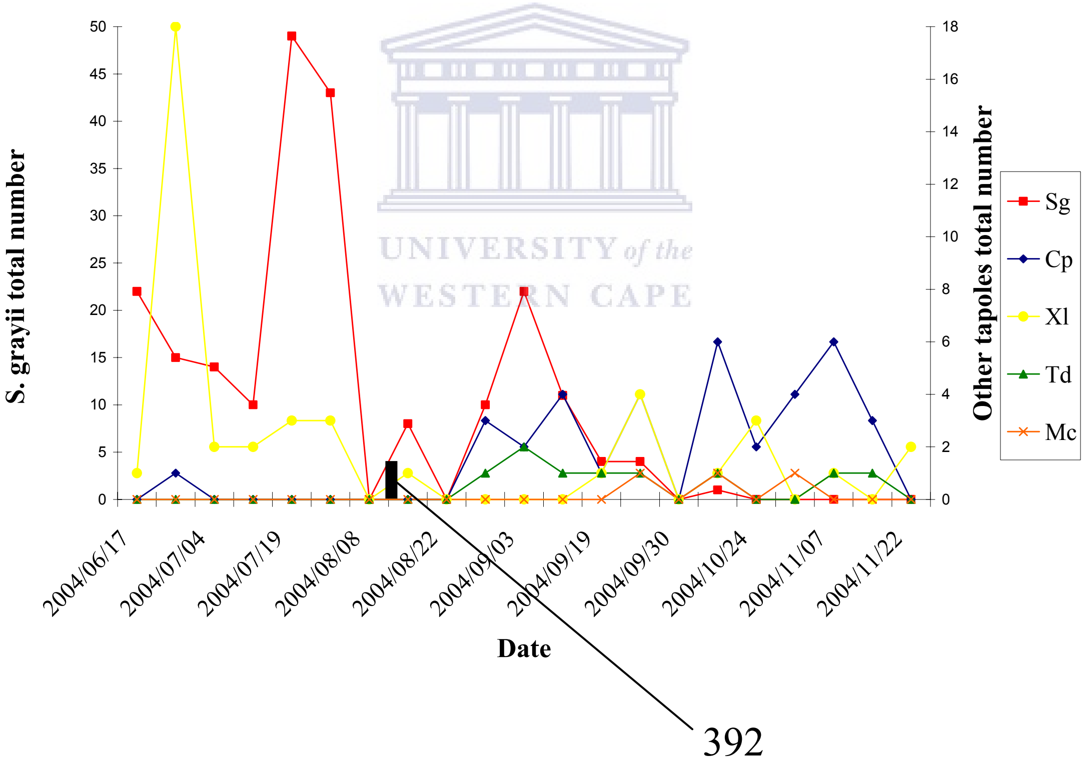

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Fig. 2.7. The number of tadpoles caught in traps throughout 2004. The mark and recapture result is indicated in mid August.

CHAPTER 3: THE TADPOLE ENVIRONMENT

3.1 Introduction

Tadpoles in an ephemeral pond may be distributed randomly throughout the available water volume, or may select to be in some parts of the habitat rather than others. Shallow water may be warmer or perhaps offer more food in the form of algae. Deeper water may offer greater protection from predators. Abiotic factors such as water clarity or oxygen tension may directly or indirectly affect the position of a tadpole in a three-dimensional habitat.

This chapter examines variation in temperature, oxygen concentration, clarity, water depth and substrate in a temporary pond. All of these factors have been shown experimentally to influence tadpoles. The aim here was to investigate these factors in a natural habitat.

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The tadpole stage in the anuran life cycle is devoted to growth (Wilbur 1980). By the time the water has dried up in a temporary pond the tadpole must have metamorphosed. The tadpole, therefore, should select the optimum environment for growth, which may be affected by many different factors (Wilbur 1987). Both biotic and abiotic factors have been examined with reference to tadpoles (Alford 1989, 1999, Merilä et al. 2000, Morrison and Hero 2003, Ultsch et al. 1999, Wassersug and Seibert 1975).

The best understood abiotic factors include water temperature (Merilä et al. 2000, Morrison and Hero 2003) oxygen concentration (Wong and Booth 1994, Wassersug and Seibert 1975) and the depth of the water (Ultsch et al. 1999). Aspects such as whether the water is shaded and the type of substrate the tadpoles prefer are less well understood.

Higher temperatures increase growth rate and decrease the time to metamorphosis (Merilä et al. 2000, Morrison and Hero 2003). Merilä et al. (2000) placed *Rana* *temporaria* at two different temperatures, 14 and 22 ˚C. The tadpoles at the lower temperature took longer to metamorphose than the tadpoles at the higher temperature.

The behaviour of tadpoles can change in anoxic conditions. The number of times a *Limnodynastes peroni* tadpole has to swim to the surface (bobbing rate) increases in anoxic conditions after a certain threshold (Wong and Booth 1994). Wassersug and Seibert (1975) tested the response of *Lithobates pipiens, Pseudacris triseriata, Anaxyrus woodhousii, Spea bombifrons* and *Ambystoma tigrinum* to different levels of dissolved oxygen. The tadpoles' bobbing rate differed between species as different species show different tolerances for anoxic conditions. This partly explains the tadpoles' distribution in the pond as well as habitat partitioning between different species. *Lithobates catesbeianus* tadpoles occur in deeper areas of the pond below the critical oxygen concentration and must come to the surface to breathe. However, they move into shallower water in the winter where the water temperature is higher (Ultsch et al. 1999).

Altig and Brodie (1972) tested the torrent dwelling tadpoles of *Ascaphus truei* for substrate preference. The tadpoles prefer rocks to sand, gravel or pebbles, reflecting their normal habitat. Even visual cues may be important for habitat selection. Wiens (1970, 1972) placed tadpoles of *Rana aurora* and *R. cascadae* on different patterned backgrounds. The tadpoles of *R. aurora* raised on striped backgrounds showed a preference for striped backgrounds whereas the tadpoles of *R. cascadae* raised on square backgrounds preferred square backgrounds. The preference was formed 14-17 days after hatching and is thought to be involved in the tadpoles' selection of an optimum environment in which to grow. On the other hand, Lithobates *pipiens* tadpoles do not have a background colour choice as all specimens prefer white backgrounds (Dunlap and Satterfield 1985).

The experiments summarised above were carried out in the laboratory or in mesocosms such as cattle drinking troughs. The parameters of temperate ponds in the Cape fynbos region, and the spatial distribution of tadpoles in such ponds, have not previously been studied.

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3.1.1 Tadpole trapping

There are many different ways of sampling tadpoles. Each technique has merits and weaknesses depending upon the habitat that is sampled and the data being collected. Seining works well in shallow ponds and habitats with little vegetation. Dip netting works best if there is a large amount of vegetation and enclosure sampling works best if the substrate is uniform and the water is shallow. Rödel (1998) used a metal quadrat 50 cm x 50 cm, which was thrown into the water, and all the tadpoles that were trapped inside were caught. This gave information about the species of tadpole that were present and also the numbers of each species. Trapping is used when the water is deep or the habitat is complex. The benefit of this technique is that the traps can be placed in different parts of the habitat and at different depths and the capture rate for the different traps can be compared. All these techniques assume that all tadpole species are equally catchable (Shaffer et al. 1994).

3.2 Methods

3.2.1 Study site

The study site is located in Cape Town in the Western Cape province of South Africa (33º 59’ 55” S, 18º 29’ 00” E) (Fig. 3.1). A large temporary pond in the centre of Kenilworth racecourse, in the suburb of Kenilworth, was selected for this study (Figs. 3.2, 3.3). This area has a Mediterranean climate with winter rainfall. The average temperature was 18 ºC for the three years (2003-2005) of the study (Fig. 3.4). The average rainfall per month was 35 mm in 2003, 54 mm in 2004 and 51 mm in 2005 (Fig. 3.5). The soil of the racecourse rests on a granite island and consists of a layer of loamy granitic substrate with quatzitic sand on top (Turner 2006). The vegetation is a relic patch of natural Cape Flats acid sand plain fynbos consisting of acidophilic plants such as ericas and proteas (McDowell 1989). Much of the sand plains fynbos in the Western Cape has now been destroyed by housing developments and only 0.013% of this fynbos type remains (McDowell 1989). This unique 45 Ha patch of vegetation has been protected since the racecourse was built in 1882 (Rosenthal 1977). There are at least 275 indigenous plant species present at the site, 17 of which are red data plant species (Turner 2006). The complete list of vegetation at the site can be found in McDowell and Brown (1991) with revisions in Turner (2006). Invasive plant species

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have been managed and kept to a minimum (McDowell and Brown 1991), however, there are at least 61 alien plant species present on the site (Turner 2006). The racecourses owners require the race goers to be able to see right around the track and this means that the vegetation such as trees (which are mostly invasive) are removed (McDowell and Brown 1991).

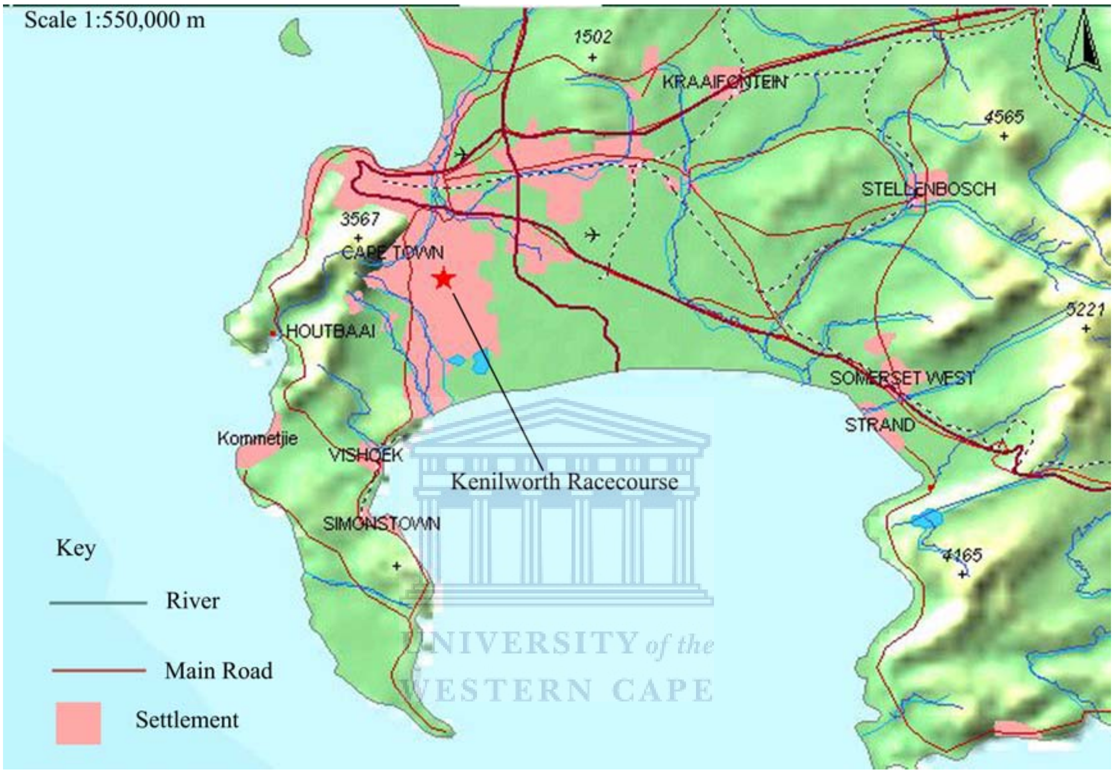

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Fig. 3.1. A map of the peninsula with Cape Town and Kenilworth (33º 59’ 55” S, 18º 29’ 00”) marked. Map taken from Biodiversity GIS SANBI (2006).

Fig. 3.2. A photograph of Kenilworth racecourse from the air, July 2005.

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Fig. 3.3. Aerial photograph of the study pond, July 2005.

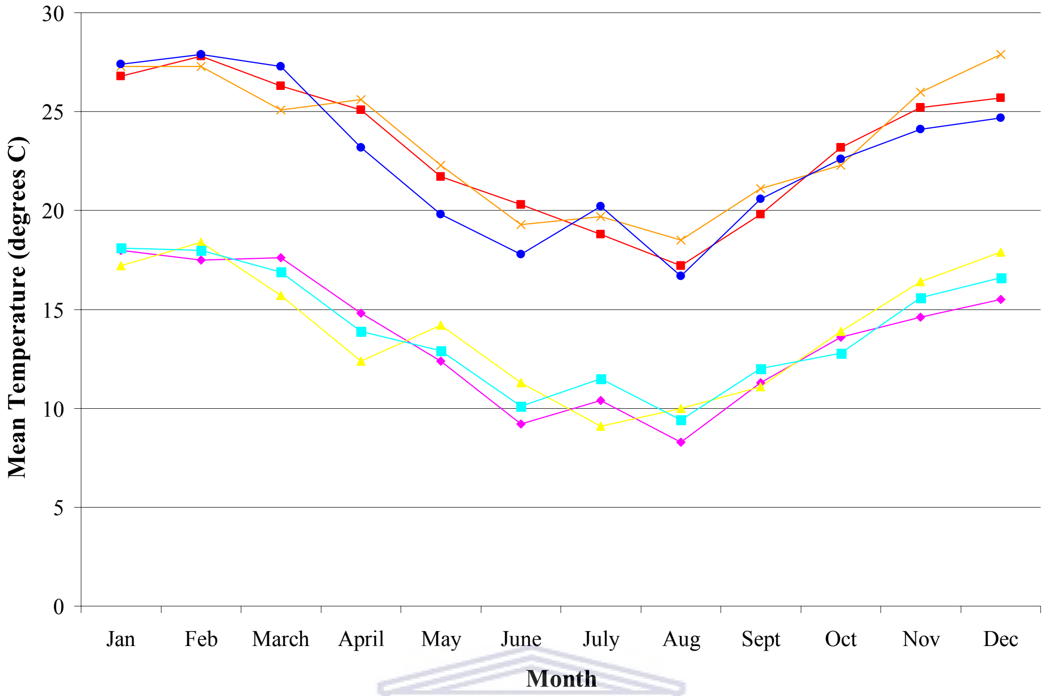

Fig. 3.4. Temperature data for Kenilworth racecourse during the study period. 2003 mean minimum – pink, 2003 mean maximum - red, 2004 mean minimum - yellow, 2004 mean maximum – orange, 2005 mean minimum – light blue, 2005 mean maximum – dark blue (Data from the South African Weather Service).

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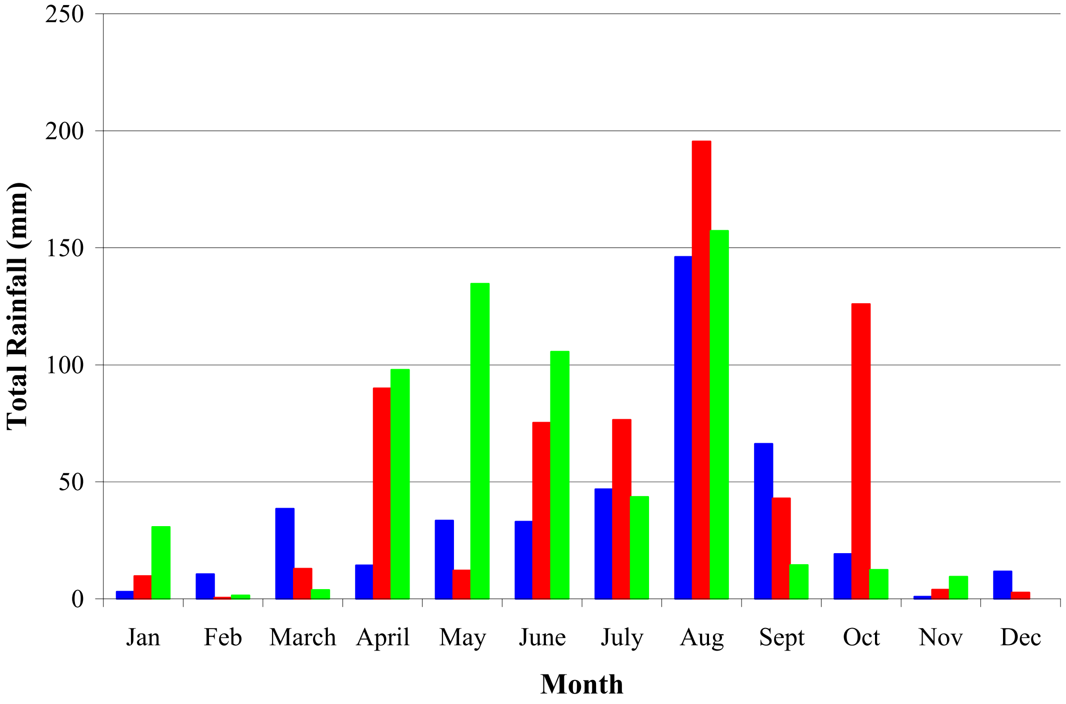

Fig. 3.5. Rainfall data for Kenilworth racecourse during the study period. 2003 monthly total – blue, 2004 monthly total – red, 2005 monthly total – green (Data from the South African Weather Service).

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On the site there are some temporary ponds that are full from about June until December each year. The particular pond selected was 500 m in circumference at its largest and over 1 m deep when it was full with water (Fig. 3.3). Burger and Cohen (1998) suggested that the particular pond selected for the study was one of the most important frog habitats on the site.

Although 16 anuran species may be present in or near the area (Chapter 1) only five species of anurans use the pond to breed. One of these, *Microbatrachella capensis*, is listed by the IUCN as critically endangered (De Villiers. 2004c).

There are a number of possible tadpole predators present in the pond. Adult *Xenopus laevis* are known to eat tadpoles (Channing 2001). There are also avian predators such as the grey heron (*Ardea cinerea*) and a number of insect predators like odonate larvae, back swimmers in the family Notonectidae, and common water scorpions of the genus *Laccotrephes*. Reptiles such as the herold snake (*Crotaphpeltis hotamboeia*) and marsh terrapins (*Pelomedusa subrufa*) are present on the racecourse.

McDowell and Brown (1991) gave the fynbos a very high conservation priority and further stated that the “natural remnant (of fynbos) comprised by far the best example of Sand Plain Fynbos remaining in either the Cape Peninsula or Cape Flats”. They went on to state that the seasonal vleis in the centre of the course were the highest quality in the south-western Cape. This is an ideal site to look at an ephemeral pond tadpole community.

This site was selected because of the presence of the micro frog, the rarity of the vegetation type represented, and the protection that is offered the habitat by the racecourse. The security system made it feasible to leave traps out without any interference.

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3.2.2 Tadpole trapping

The traps were made out of 2 l plastic juice bottles. Each bottle is rectangular in section, measuring 160 mm high with a cross-section of 95 mm. The mouth of the bottle has a diameter of 30 mm. The end of the bottle was cut off and inverted to make a funnel trap (Fig. 3.6).

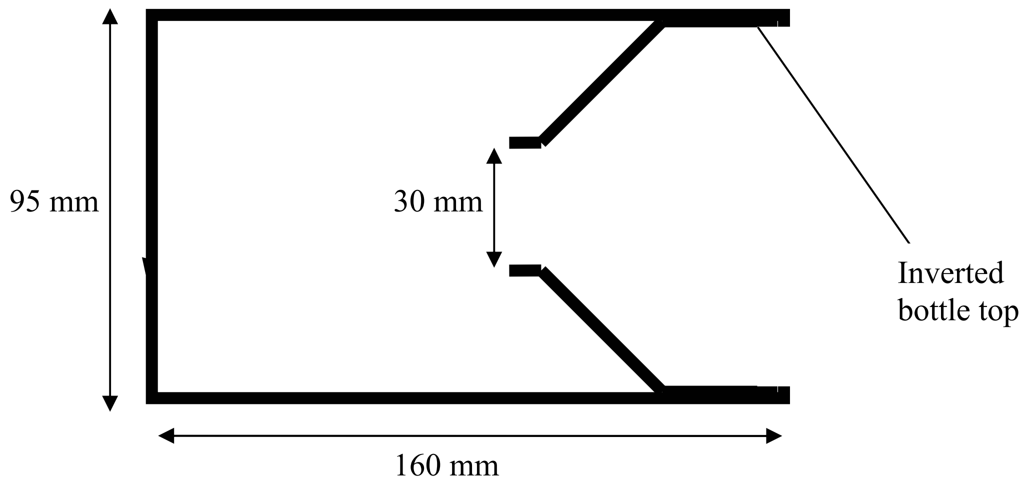

Fig. 3.6. A diagram of a funnel trap that was used for trapping tadpoles.

Bottle traps were placed, unbaited, horizontally in the pond in a random stratified design, which took into account the depth and circumference of the pond using the methods of Shaffer et al. (1994). Random numbers were used to determine the starting point of each transect. Trapping was carried out one night per week through the season.

Transects ran at a right angle to the shore and were placed randomly. Each transect was 5 metres wide, divided into 1 m lateral blocks, and ran from the shore out to a water depth of 1m. Water column depth was divided into five categories: 0-200 mm, 210-400 mm, 410-600 mm, 610-800 mm, and 810-1000 mm. Each of these represents one possible trap point. Trap points were placed at each depth category, in one of the 5 lateral blocks. An example is shown in Table 3.1

Table 3.1. A representation of the trap placement in a typical trapping transect

Lateral blocks	Depth 0-200mm	Depth 210-400 mm	Depth 410- 600 mm	Depth 610- 800 mm	Depth 810- 1000 mm
1	X				X
2					
3			X		
4				X	
5		X			

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At each trap point, three traps were set out; one at the surface, one on the bottom, and one in the mid-water. In the example above, transect 1 would have traps placed in lateral blocks 1, 3, 4 and 5. There would be 5 traps at the surface, 5 on the bottom and 5 in the midwater.

The result of this design is that traps are placed randomly; traps are placed at depths in proportion to the area of the pond at each depth category (Table 3.2); and different substrates were sampled in proportion to the area covered by that substrate. In total over the two seasons 1738 traps were placed in the pond over 45 trapping nights. The number of traps placed in the pond varied due to the pond's circumference but an average of 39 traps was placed in the pond per trapping night. A diagrammatic representation of a typical trap placement in the pond when it is 1 m deep can be seen in Fig. 3.7.

Table 3.2. The number and depth of traps placed in the pond over the two seasons.

Trap depth (mm)	Number of traps placed over both seasons
0 – 100	824
110 – 200	290
210 – 300	217
310 – 400	171
410 – 500	107
510 – 600	72
610 – 700	39
710 – 800	23
810 – 900	6
910 – 1000	3

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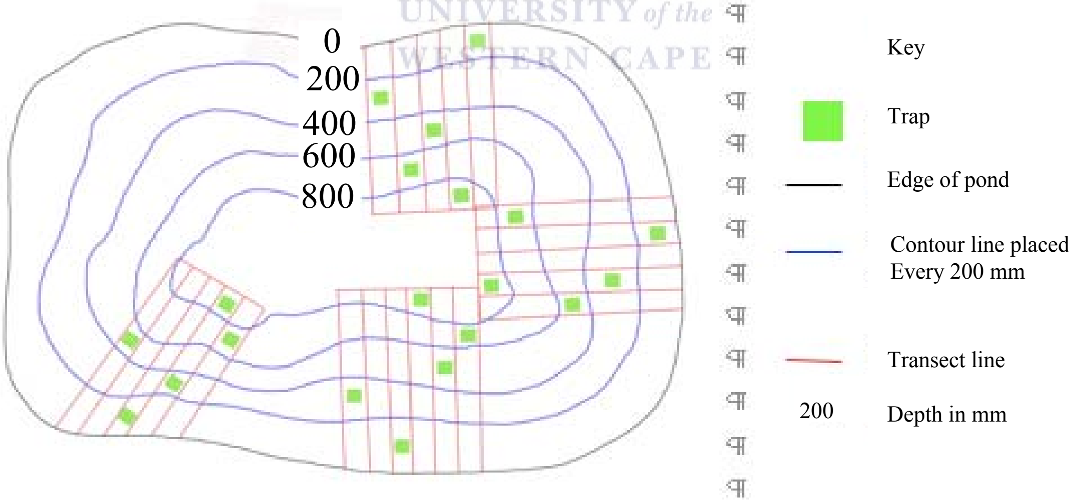

Fig. 3.7. Diagrammatic representation of trap placement, showing how the traps were stratified to water column depth.

A number of observations were taken to describe the habitat around each trap. These included: water temperature and oxygen concentration taken at the trap, clarity of the water around the trap, depth of trap, depth of the water column, and the substrate under the trap. Water column depth was measured from the surface to the substrate. Trap depth was measured from the surface of the water to the entrance of the trap. Water temperature and oxygen concentration were taken at the entrance to the funnel trap using a YSI 55 dissolved oxygen meter. Water clarity was measured using a measuring stick with a 2 mm thick white line drawn around the end. When the white line could no longer be seen the depth of the line was recorded.

The study was initiated in May 2003 and carried out over two seasons. The pond is rain-filled and trapping began as soon as there was water in the pond. Trapping was carried out once weekly and traps were set out in the evening and collected the following morning. Tadpoles were counted, identified (Channing 2001) and staged according to Gosner (1960).

Trapping ceased at the end of the tadpole season, which was defined when the traps were placed in the pond for three successive weeks without catching anything.

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3.2.3 Data analysis

Kendall's Tau was used to test for correlation between the following variables. Trap depth was tested against temperature and oxygen concentration measurements taken at the same position as the trap. Water temperature was also tested against oxygen concentration.

To test if the tadpoles were distributed evenly throughout the pond a Spearman Rank correlation was carried out comparing tadpole numbers against the environmental variables. Temperature data were transformed, because there are only certain temperatures available at any one time in the pond. For example, a tadpole may always be choosing the highest temperature available to it; however, this may be 10 °C on a July evening and 20 °C on an October evening. To solve this problem all the available temperatures for each session were ranked from 1 to 10 where 10 represents the highest temperature available and 1 the lowest. This was done for every trapping night and then these ranks were used in the Spearman Rank correlation. Actual temperatures and ranks can be found in Appendix 2.

The trap results vs. substrates were compared using a chi^2 test as the data were categorical.

For the two least abundant species (*Tomopterna delalandii* (n=10) and *Microbatrachella capensis* (n=3)) no statistical analysis could be performed as the numbers of captures were too low.

3.2.4 Duration of study

In 2003 the pond filled on the 21st of July and was dry 24 weeks later by the 30th of December. In 2004 the pond filled on the 17th of June and was empty 30 weeks later by the 2nd of January 2005.

3.3 Results

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3.3.1 Species present in the pond

The pond at Kenilworth racecourse is used by five tadpole species; *Strongylopus grayii, Cacosternum platys, Xenopus laevis, Microbatrachella capensis* and *Tomopterna delalandii.*

3.3.2 Pond temperatures

Water depth, not unexpectedly, was significantly correlated with water temperature ($\tau = 0.193$, $p < 0.05$) (Fig. 3.8).

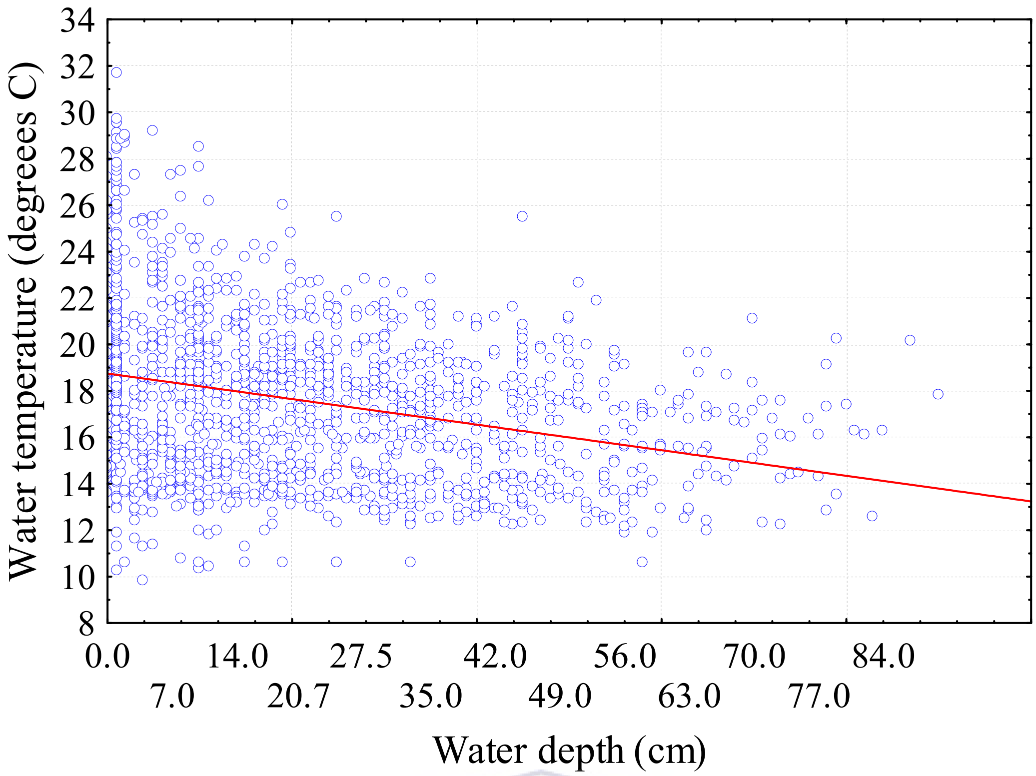

Fig. 3.8. The correlation ($\tau = -0.193$, $p<0.05$) between water depth and water temperature. The red line shows best fit.

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Figures 3.9 and 3.10 show the temperature of the water throughout the second season (2004) (only the second season is shown for simplicity). Fig. 3.9 shows the surface temperature of the pond water. The temperature of the water starts to increase mid-September and the range of the water temperatures increases toward the end of the season. The cooler water at the bottom of the pond shows an increase at about the same time in the season in mid-September (Fig. 3.10).

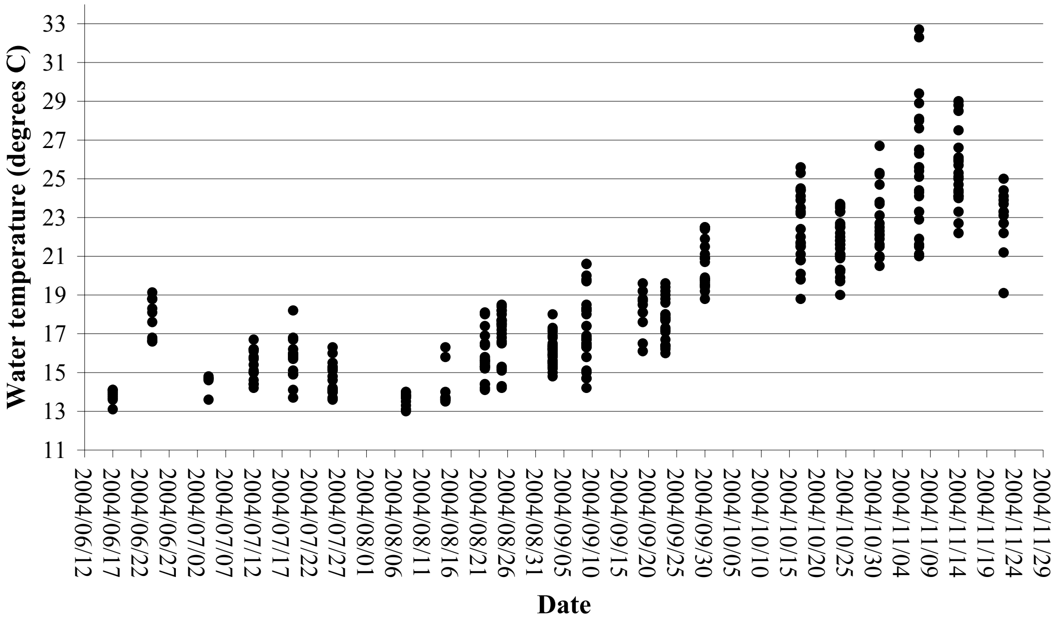

Fig. 3.9. Surface temperature at various parts of the pond in the second season (2004).

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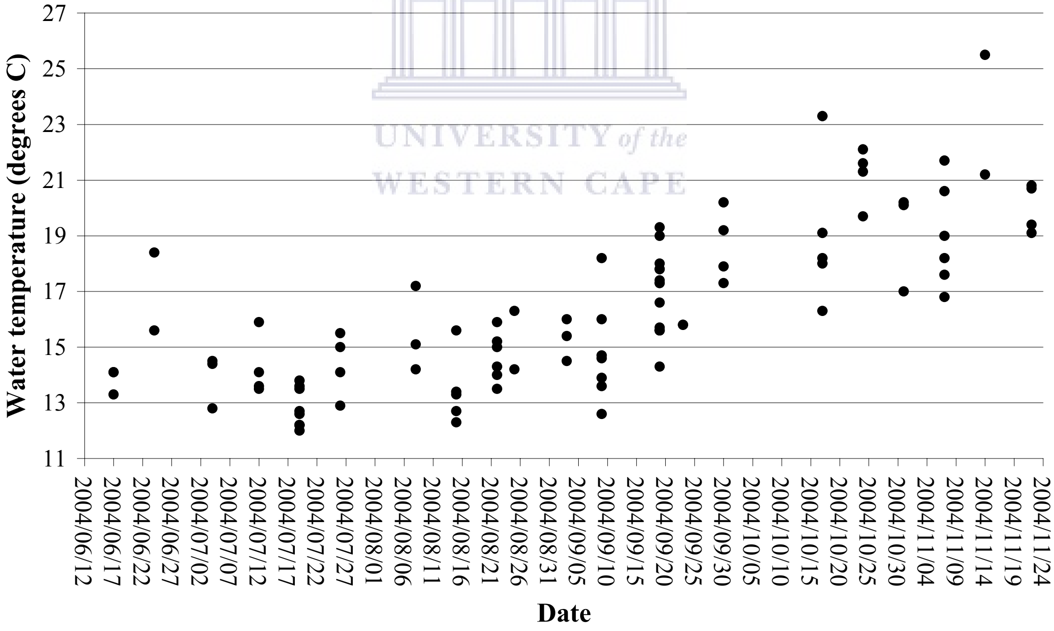

Fig. 3.10. Bottom temperature of the water in the pond throughout the second season (2004).

3.3.3 Oxygen concentration in the pond

The surface water is well oxygenated, with a consistent drop in oxygen concentration in deeper water. Water depth was significantly correlated with oxygen concentration ($\tau = 0.227$, $p<0.05$) (Fig. 3.11). Figs. 3.12 and 3.13 show the oxygen concentration of the surface and bottom water throughout a single season (2004). Only oxygen measurements taken over grass were graphed to control for different oxygen concentrations over different substrates. The graphs show no clear pattern of oxygen concentration changing throughout the season. The surface and the bottom water oxygen concentrations were very variable.

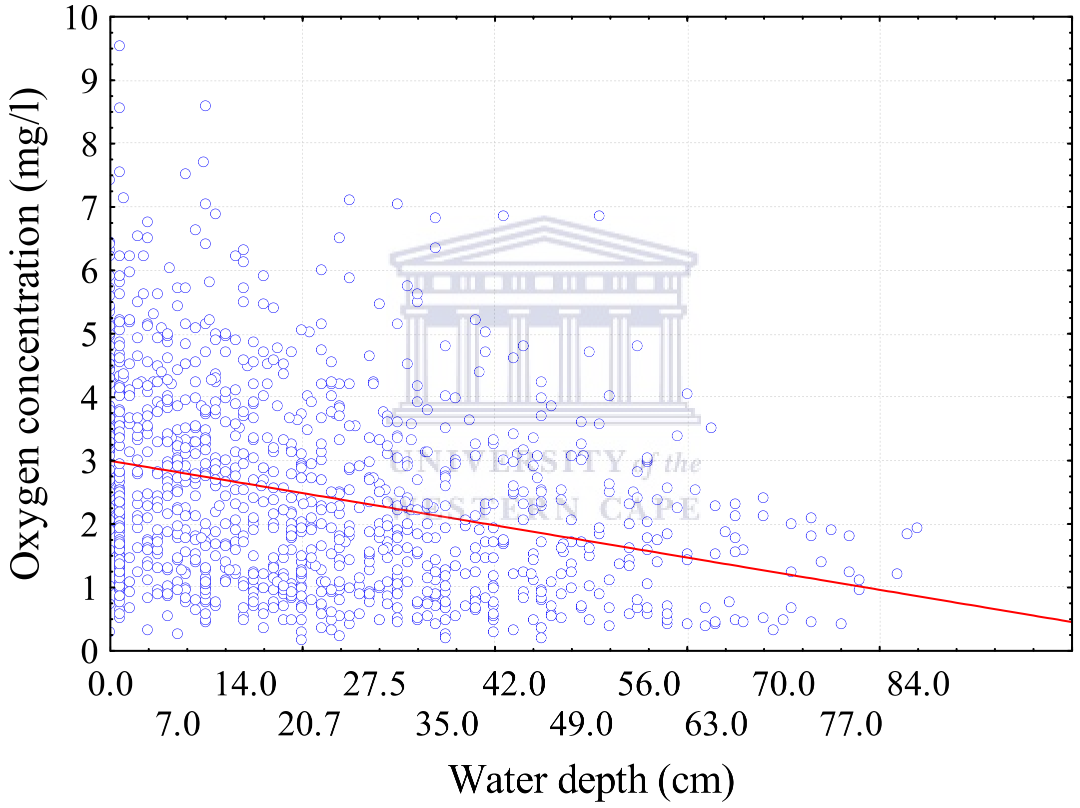

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Fig. 3.11. The relationship ($\tau = -0.227$, $p<0.05$) between water depth and oxygen concentration. Deeper water has a lower oxygen concentration. Red line shows the line of best fit.

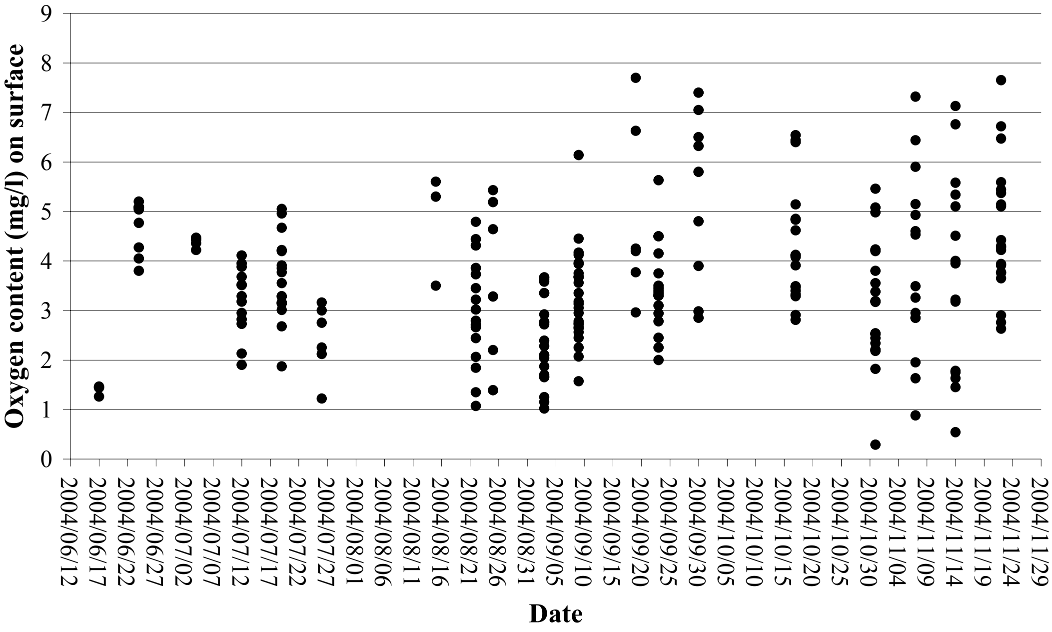

Fig. 3.12. Oxygen concentration of the surface water of the pond over one season (2004). Only readings taken over grass were plotted.

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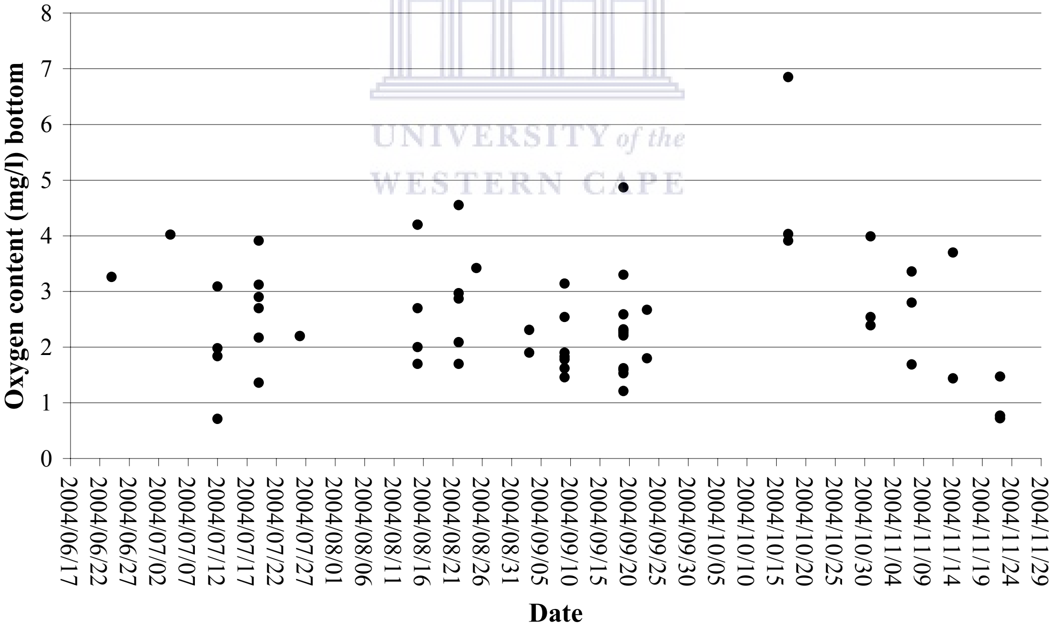

Fig. 3.13. Oxygen concentration of the water at the bottom of the pond over one season (2004). Only readings taken over grass were plotted.

3.3.4 Relationship between temperature and oxygen concentration

Water temperature was not significantly related to oxygen concentration ($\tau = 0.006$, $p>0.05$) (Fig. 3.14). When the data are tested week by week the results are varied; for example at the start of the season on the 24.6.04 the water temperature was not related to the oxygen concentration of the water ($\tau=0.09$, $p>0.05$) but on August the 15th of the same year the oxygen concentration was significantly related ($\tau=0.452$, $p<0.05$).

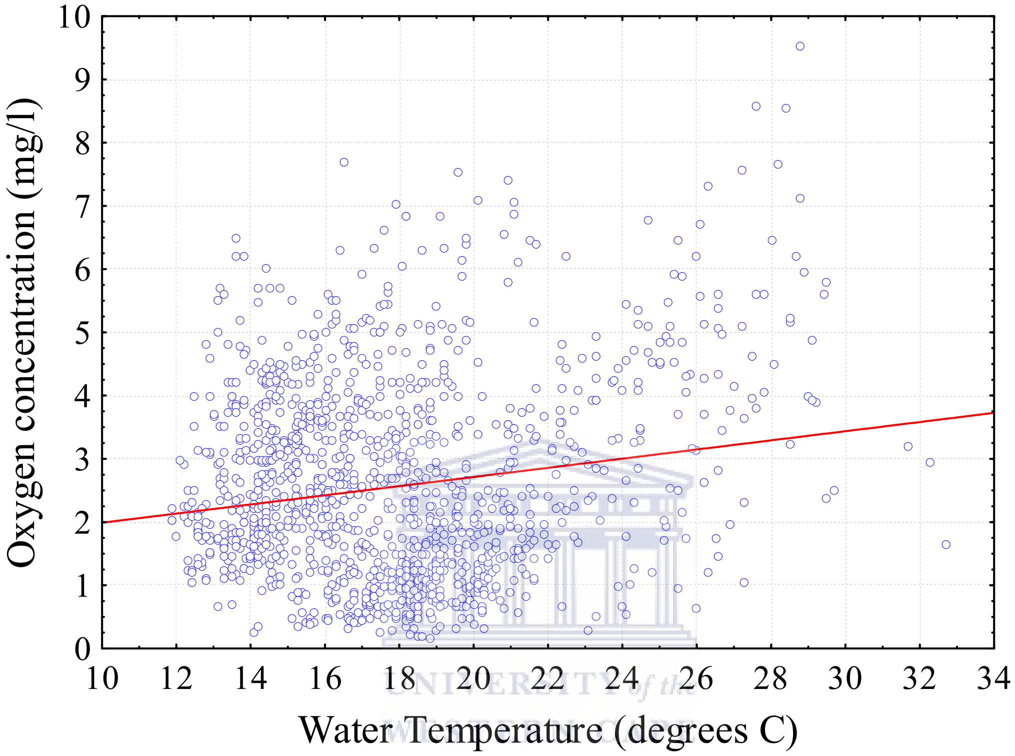

Fig. 3.14. The relationship between the water temperature and oxygen concentration. The correlation between water temperature and oxygen concentration is not significant when all of the data are analysed together ($\tau = 0.006$, $p>0.05$). Red line shows the line of best fit.

3.3.5 Pond depth through the season

In 2003 the pond filled rapidly at the end of July and the beginning of August. On the 2nd of October 2003 the pond depth was 1.03 m which was the deepest measurement taken in that season. By the end of October the water depth started to decrease and the last water depth measurement taken at the pond on 18th December was 520 mm. In 2004 the pond filled to 450 mm after a period of rain in June and steadily increased in depth until the end of July when it reached 1.05 m. The depth of the pond started to decrease at the beginning of September and the last depth measurement was 720 mm taken on the 22nd of November 2004 (Fig. 3.15).

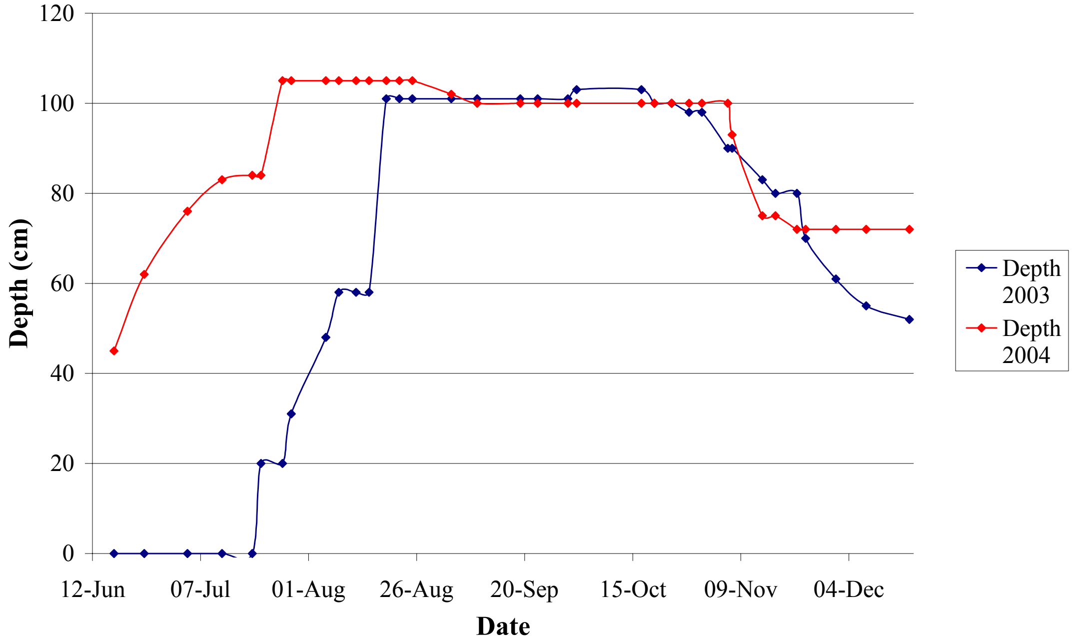

Fig. 3.15. The depth of the study pond for the 2003 and 2004 seasons.

For all trapping results see appendix 3-7

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3.3.6 *Strongylopus grayii* tadpoles

S. grayii tadpoles were the most commonly trapped species. They are not evenly distributed about the pond. There was a significant correlation with the numbers of tadpoles trapped per hour and the position of the trap in the water column ($R = -0.67$, $p = 0.00$). The tadpoles are trapped more often in shallow water columns (Fig. 3.16), and in the traps placed near the water surface ($R = -0.62$, $p = 0.00$) (Fig. 3.17). Table 3.3 shows the mean number of tadpoles caught at each trap position. This shows the position of the tadpoles in relation to the vertical and horizontal strata of the pond. The tadpoles are caught in greater numbers in the 0 -200 mm deep water and in 800 – 1000 mm deep water and at trap depths of 610 – 700 mm. This last result does not conform to the pattern shown in the rest of the diagram. The tadpoles were in low numbers and not present in traps placed at 710 – 1000 mm and in 600 – 1000 mm deep water. In general tadpoles were found at the top of the water column, at the edge of the pond and along the bottom of the pond until the 510 – 600 mm water depth.

S. grayii tadpoles were trapped in higher numbers in the warmer parts of the ponds (R = 0.83, p = 0.00) (Fig. 3.18). More tadpoles were trapped in water that had a low oxygen concentration (R = -0.62, p = 0.04) (Fig. 3.19) but the clarity of the water was not correlated to the number of tadpoles that were present (R = -0.02, p = 0.92). More tadpoles were found in traps placed in the leafy plants and reeds than over the mud or grass (χ^2=25.42; p<0.05; Fig. 3.20).

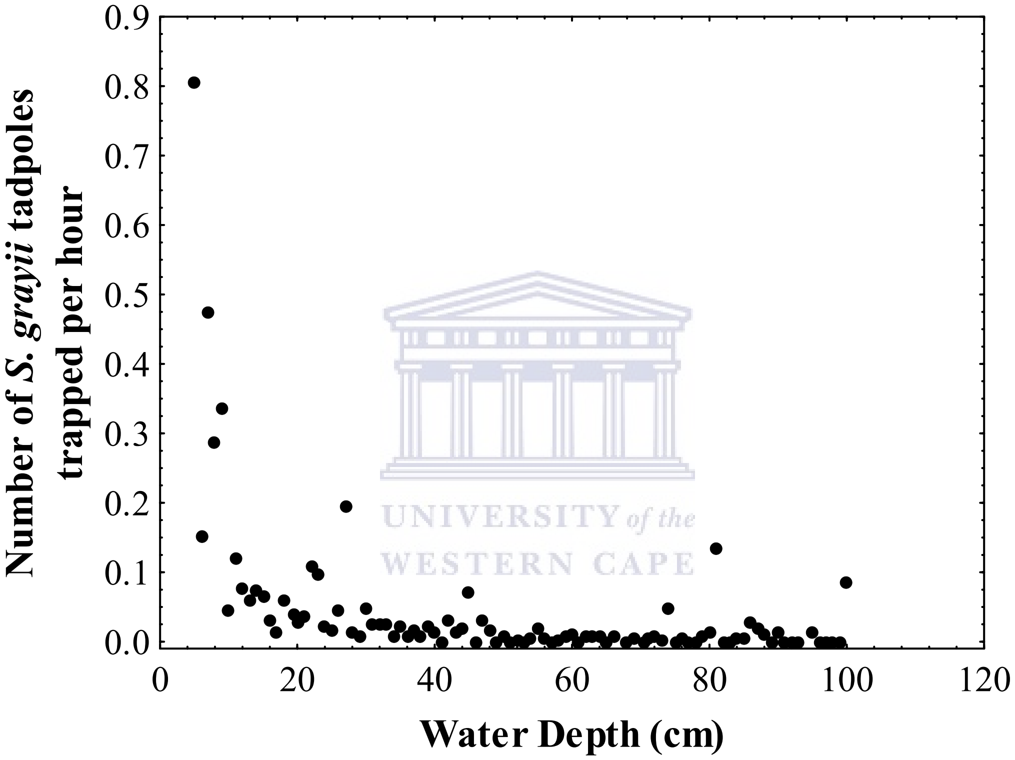

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Fig. 3.16. The number of *S. grayii* tadpoles trapped per hour in relation to the depth of the water column in which the trap was placed.

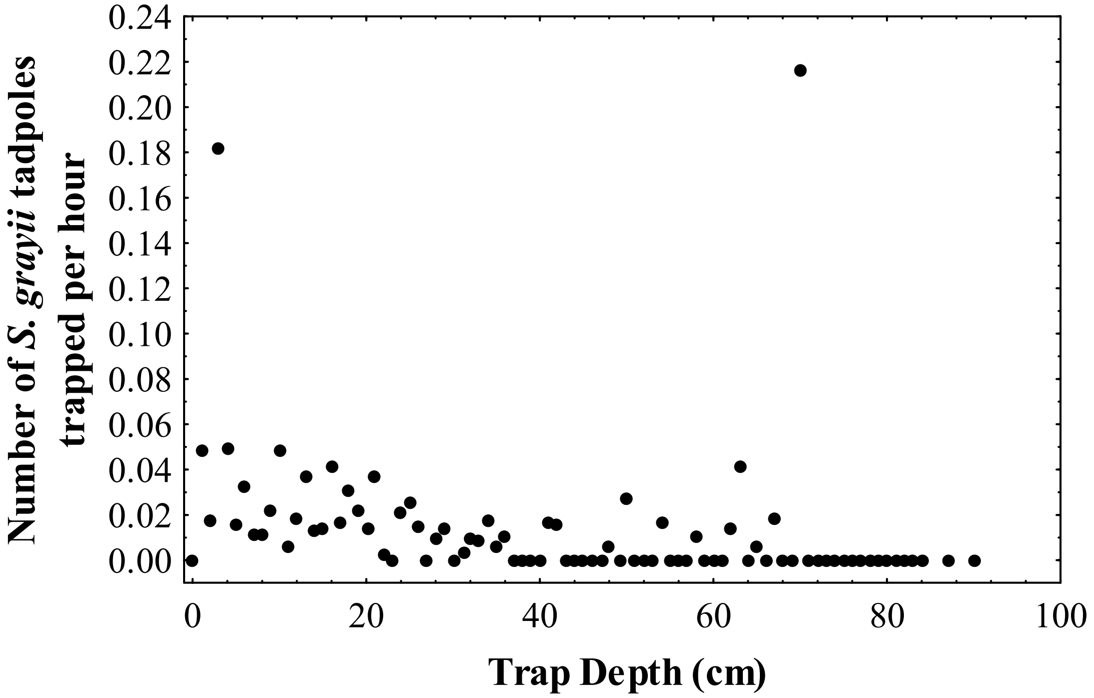

Fig. 3.17. The number of *S. grayii* tadpoles trapped per hour in relation to the trap depth.

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Table 3.3. The positions of the traps in the pond (taken in mm) and the mean number of *S. grayii* tadpoles caught in each trap position for both seasons.

Depth (mm)					
0 -100	0.068	0.025	0.008	0.012	0.010
110 - 200	0.093	0.026	0.009	0.007	0.008
210 - 300		0.021	0.012	0.003	0.011
310 - 400		0.011	0.009	0.001	0.008
410 - 500			0.010	0.004	0.004
510 - 600			0.000	0.007	0.000
610 - 700				0.010	0.062
710 - 800				0.000	0.000
810 - 900					0.000
910 - 1000					0.000

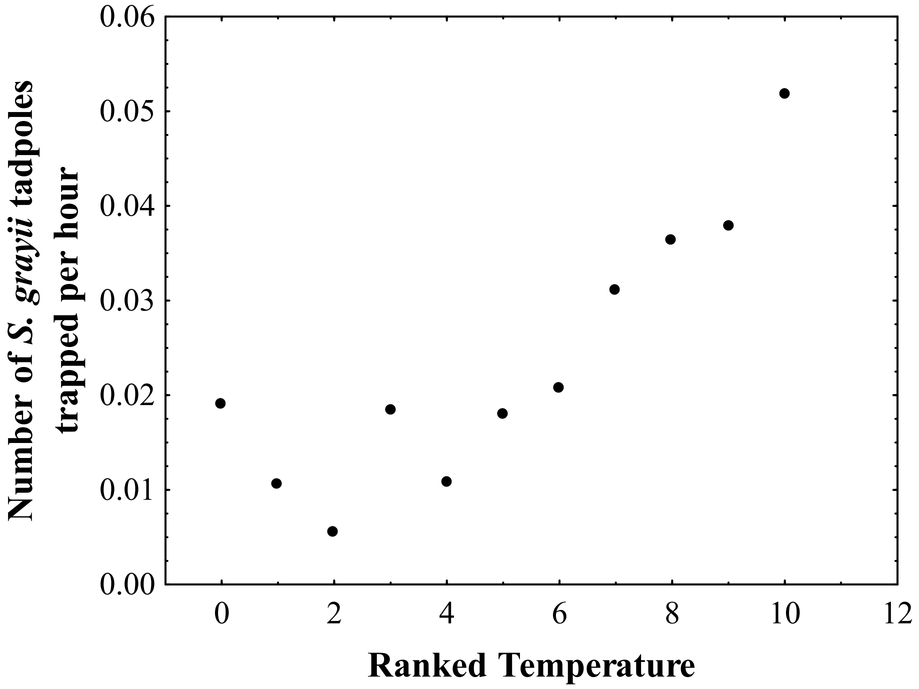

Fig. 3.18. The number of *S. grayii* tadpoles trapped per hour vs. the different temperatures available to them. Temperatures were ranked from 1 to 10, see methods section for procedure. Tadpoles were found more frequently in the warmest water available to them (real temperatures given in Appendix 2).

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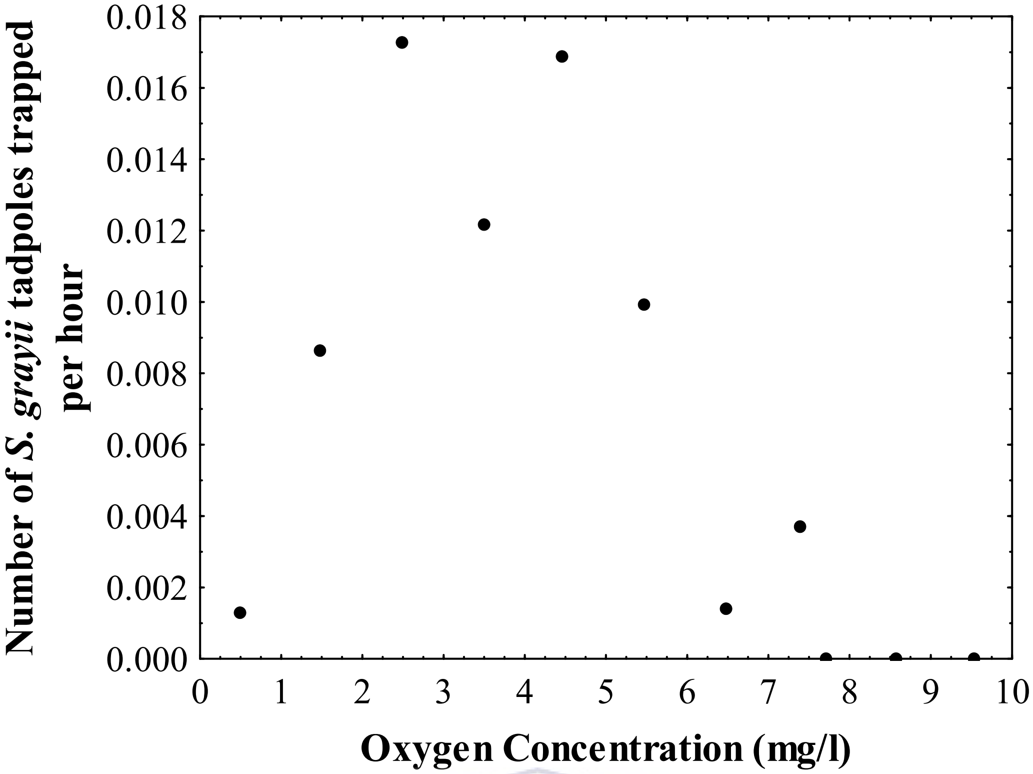

Fig. 3.19. The number of *S. grayii* tadpoles trapped per hour at different oxygen concentrations.

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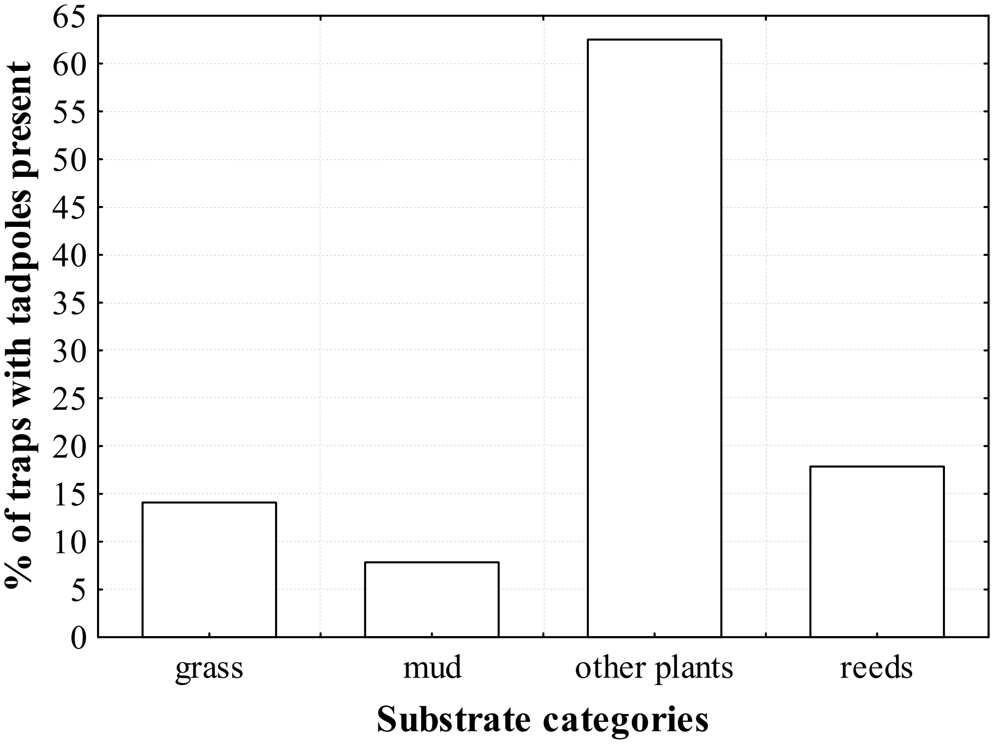

Fig. 3.20. The percentage of traps with *S. grayii* tadpoles present over the different substrates.

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3.3.7 *Cacosternum platys* tadpoles

C. platys tadpoles were trapped in the shallower water column (R = -0.40, p = 0.00) (Fig. 3.21) and in traps that were closer to the surface (R = -0.67, p = 0.00) (Fig. 3.22). The tadpoles were caught in low numbers along the bottom of the pond. Table 3.4 shows the positions of the traps in the pond and the mean numbers of tadpoles that were caught in the traps. This shows that the tadpoles were caught in high numbers in traps placed at 0 – 100 mm and in the 0 – 600 mm water column depth as well as in traps placed 110 – 300 mm deep and in water 800 – 1000 mm deep. The tadpoles were caught in low numbers along the bottom of the pond. They were caught in traps in the warmer parts of the pond (R = 0.83, p = 0.00) (Fig. 3.23) and in water that had a lower oxygen concentration (R = -0.77, p = 0.01) (Fig. 3.24). Tadpole catches increased in murky water (R = -0.72, p = 0.00) (Fig. 3.25), however, the number of tadpoles trapped over different substrates was not significantly different.

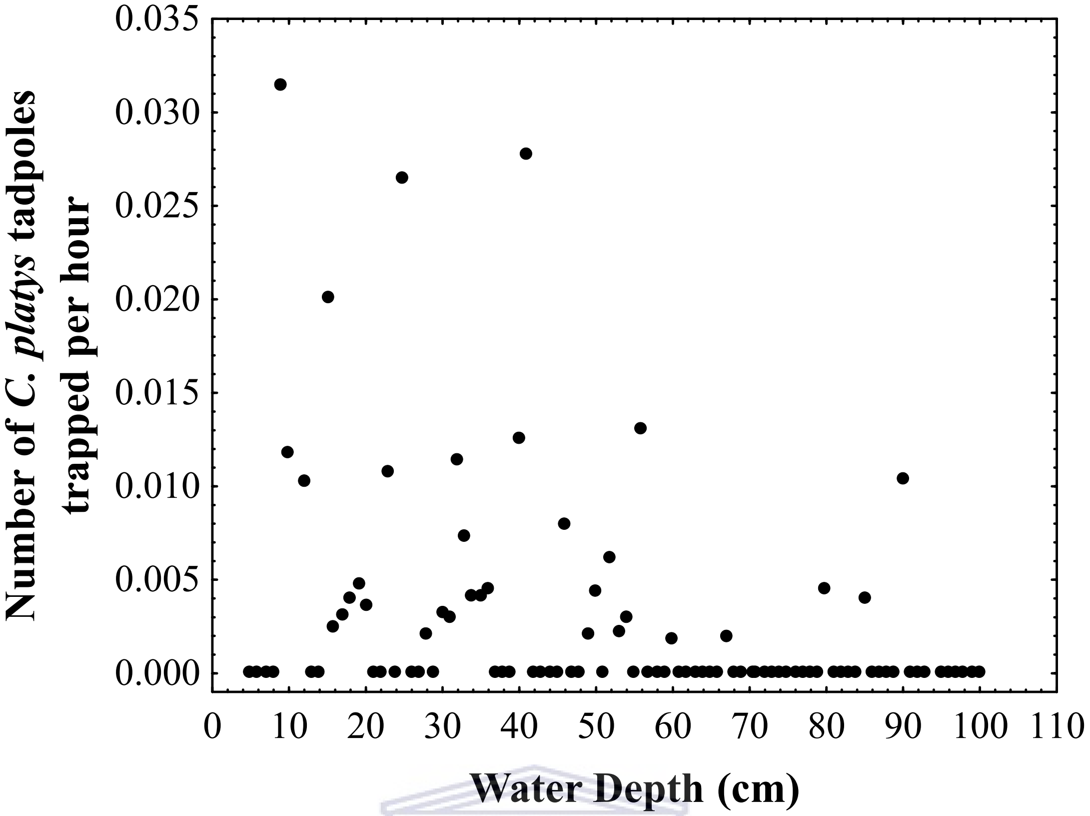

Fig. 3.21. The number of *C. platys* tadpoles trapped per hour in different water column depths

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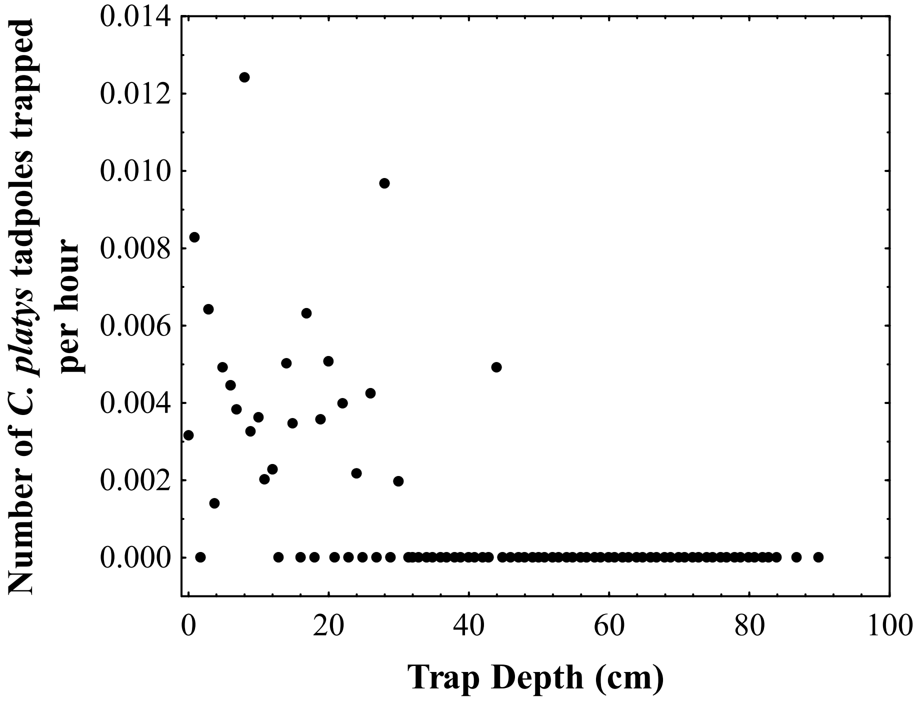

Fig. 3.22. The number of *C. platys* tadpoles caught per hour at different trap depths.

Table 3.4. The position that *C. platys* tadpoles were caught in the pond over the two seasons looking at the water column depth as well as the depth that the trap was placed at (taken in mm). The mean number of tadpoles at each trap position is given in the centre of the cell

Depth mm					
0 -100	0.001	0.002	0.007	0.004	0.000
110 - 200	0.000	0.000	0.006	0.008	0.000
210 - 300		0.000	0.002	0.001	0.000
310 - 400		0.000	0.003	0.003	0.000
410 - 500			0.000	0.000	0.000
510 - 600			0.000	0.003	0.000
610 - 700				0.000	0.002
710 - 800				0.000	0.000
810 - 900					0.000
910 - 1000					0.000

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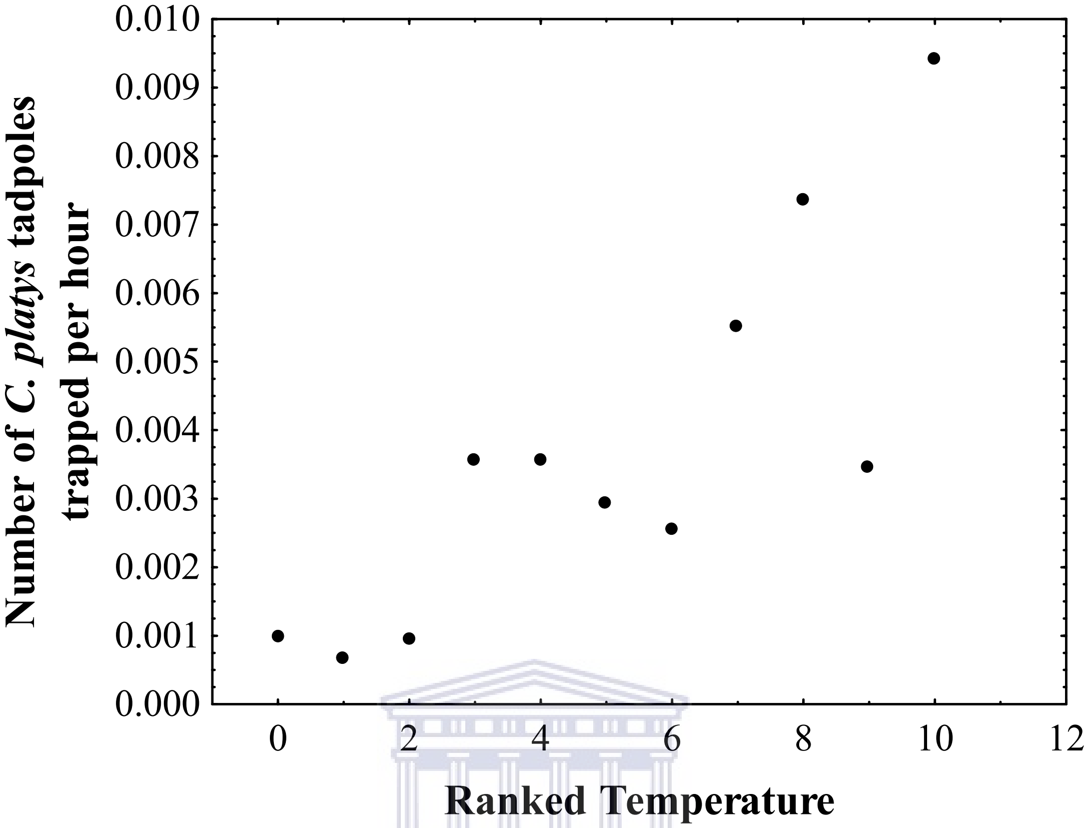

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Fig. 3.23. The number of *C. platys* tadpoles trapped per hour vs. available temperatures (See appendix 2 for actual temperatures).

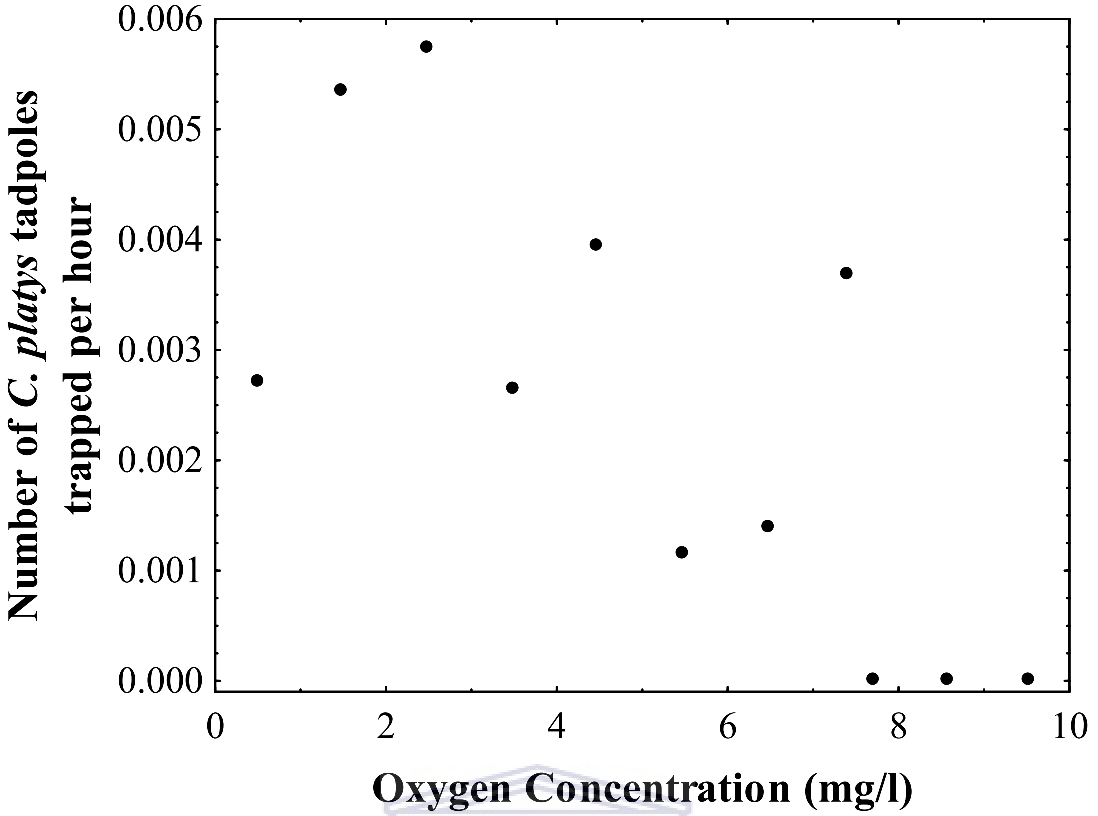

Fig. 3.24. The number of *C. platys* tadpoles caught per hour vs. oxygen concentration.

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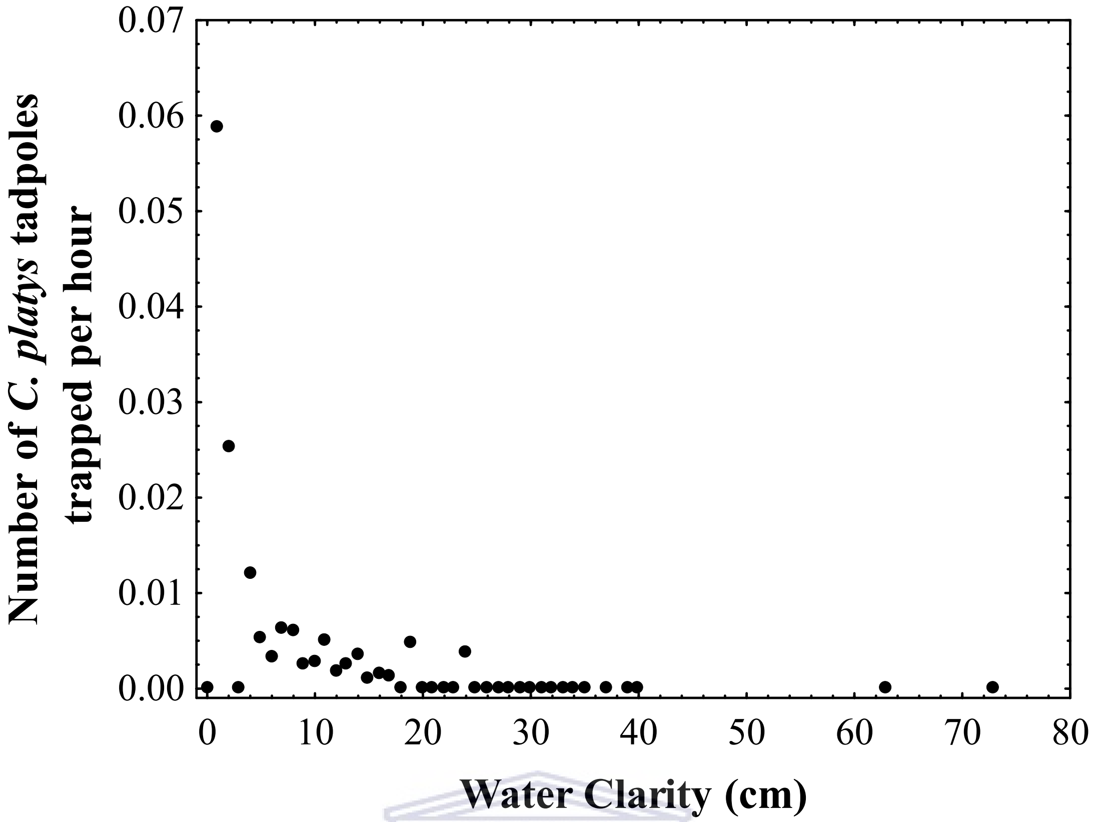

Fig. 3.25. The number of *C. platys* tadpoles caught per hour vs. water clarity.

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3.3.8 *Xenopus laevis* tadpoles

X. laevis tadpoles were not evenly distributed about the pond. There was no correlation between the water column depth and catch rate (R = -0.01, p = 0.92), however, tadpoles were caught more often in traps placed closer to the surface of the water (R = -0.44, p = 0.00) (Fig. 3.26). Table 3.5 shows the position of the traps that caught the *X. laevis* tadpoles and the mean number of tadpoles caught in each trap. The tadpoles are trapped most often in traps placed at 0 – 200 mm depth and in water 400 – 800 mm deep. Tadpoles were not trapped in traps placed at 800 – 1000 mm deep or along the bottom of the pond. The temperature of the water was not correlated to the catch rate (R = -0.36, p = 0.27) neither was the oxygen concentration of the water (R = -0.41, p = 0.21). The water clarity showed no correlation to the catch rate (R = -0.28, p = 0.07) and the Chi ^{2} test showed that the tadpoles were not caught in different numbers over different substrates.

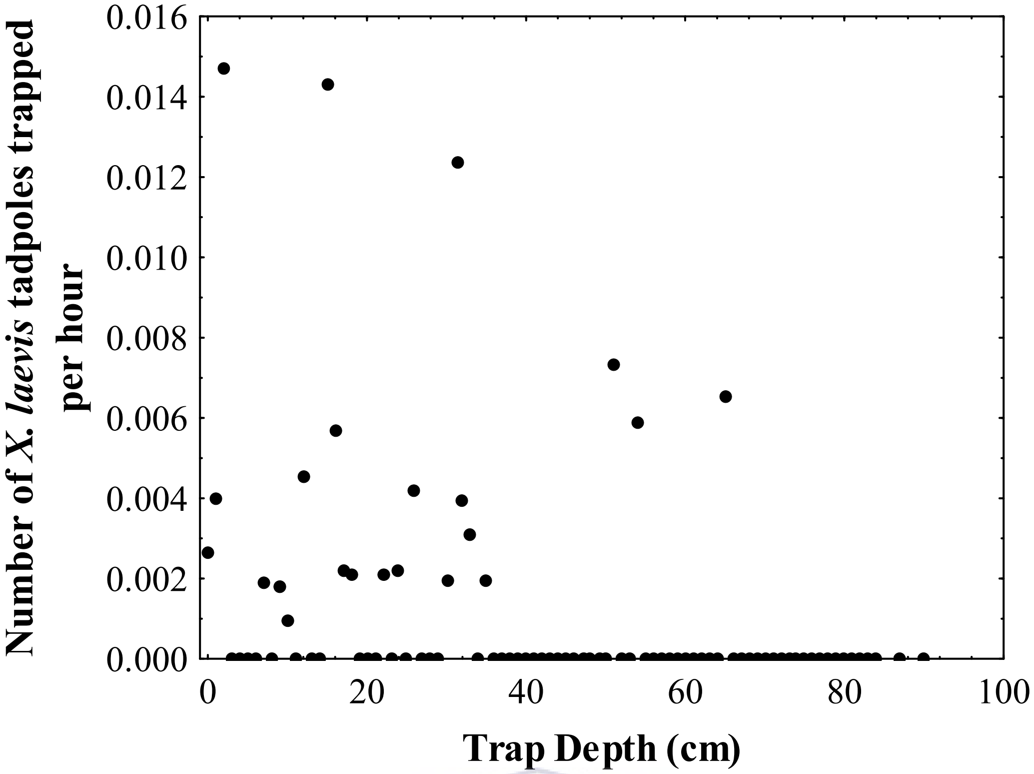

Fig. 3.26. The number of *X. laevis* tadpoles caught per hour at different trap depths.

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Table 3.5. The position of the traps in the water column (taken in cm) and how many tadpoles of *X. laevis* were caught. The amount given in the centre of each cell is the mean number of tadpoles caught in both seasons

Depth (mm)					
0 -100	0.005	0.007	0.005	0.000	0.000
110 - 200	0.000	0.003	0.002	0.004	0.008
210 - 300		0.000	0.003	0.000	0.010
310 - 400		0.000	0.000	0.000	0.000
410 - 500			0.000	0.001	0.000
51 0- 600			0.000	0.000	0.000
610 - 700				0.000	0.000
710 - 800				0.000	0.000
810 - 900					0.000
910 - 1000					0.000

3.3.9 *Tomopterna delalandii* and *Microbatrachella capensis* tadpoles

These tadpoles were trapped in low numbers. It is possible that they are trap avoiding or they may just be in relatively low numbers compared to the other tadpoles in the pond. Over the two seasons ten *T. delalandii* tadpoles were trapped, one in 2003 and nine in 2004. The single tadpole in the first season was trapped in December but during the second season the tadpoles were trapped from August through to November. These tadpoles were trapped over a wide range of trap depths (10 – 660 mm) in a wide range of water column depths (160 – 730 mm). The water temperature where they were found in ranged from 16.2 ºC to 27.5 ºC and the oxygen concentration of the water ranged from 1.02 – 4.62 mg/l. All *T. delalandii* tadpoles were caught over grass and mud.

In the first season a single *M. capensis* was found at the start of November. It was not trapped in one of the funnel traps but collected in a dip net for photography. This meant that no data were collected about the habitat in which it was caught. In the second season three *M. capensis* tadpoles were trapped in the funnel traps at the end of September and during October. Two of these tadpoles were caught in traps placed on the surface of the water and the other was in a trap placed at a depth of 270 mm. The water column depth where these tadpoles were caught ranged from 150 to 560 mm and the water temperature from 18.6 ºC to 24.4 ºC. The oxygen concentration ranged from 3.3 to 4.08 mg/l and the water clarity ranged from 50 mm to 80 mm. All *M. capensis* tadpoles were caught over grass.

3.3.10 Tadpole trapping vs. water temperature

Tadpoles of *S. grayii* and *C. platys* select for the warmest water available. The average temperature of the pond increases throughout the season, however, tadpoles are present in the pond in higher numbers at the start of the season when the water is at its coolest. Figure 3.27 shows the relationship of tadpole numbers vs. water temperature.

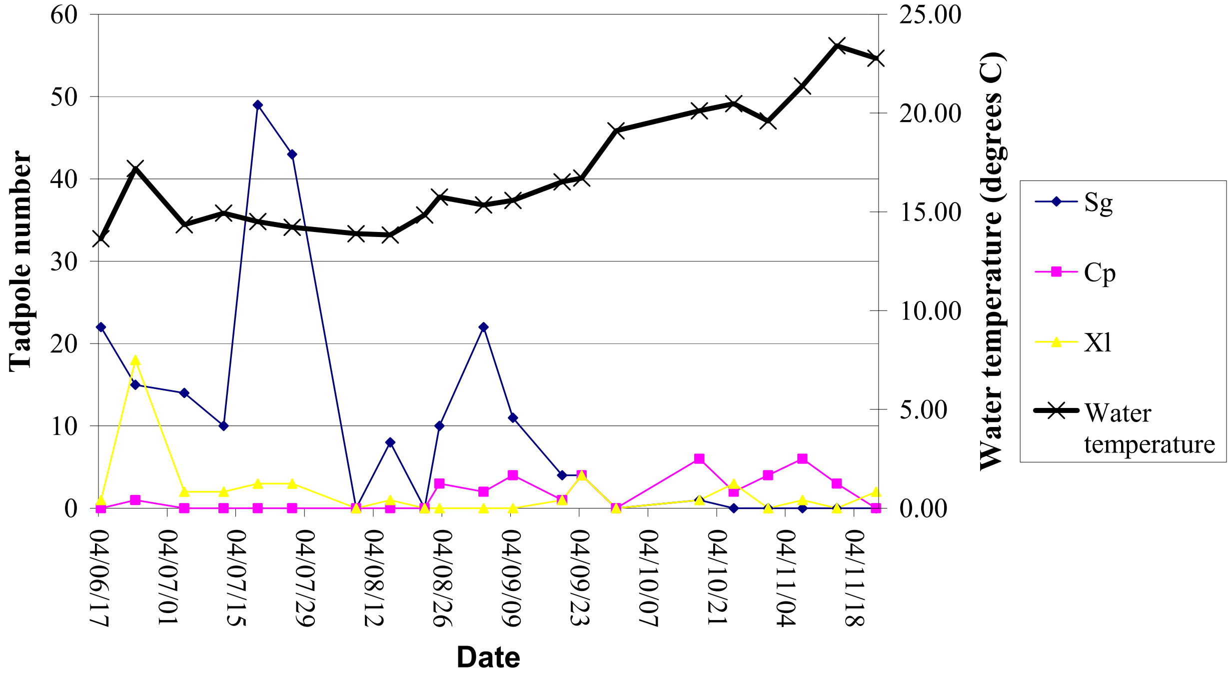

Fig. 3.27. The numbers of tadpoles of the three common species trapped in the pond throughout the 2004 season plotted against water temperature.

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3.3.11 Tadpole trapping vs. oxygen concentration

Figure 3.28 shows the tadpole numbers caught, against oxygen concentration in 2004. There are two peaks in oxygen concentration; one at the end of June and one in mid August, followed by peaks of *S. grayii* about two weeks afterwards in mid July and a smaller one at the end of August. Rainfall events on the 13th of June and the 9th and 15th of August would result in increased oxygenation of the water, and could initiate breeding, resulting in tadpole peaks two or three weeks later.

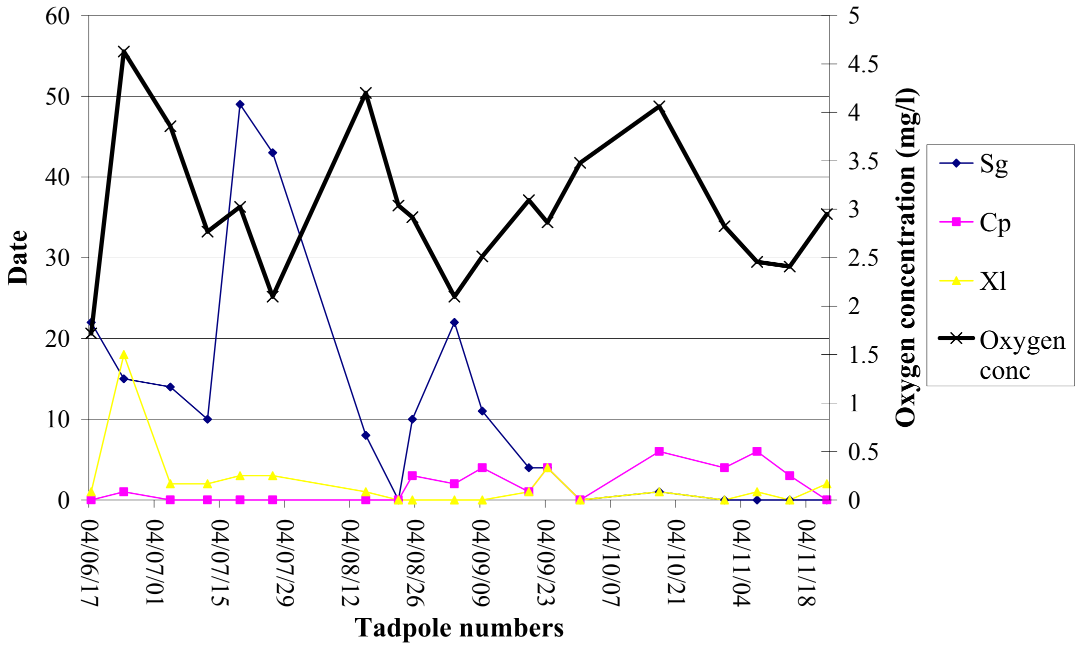

Fig. 3.28. A graph of tadpole numbers and oxygen concentration throughout 2004 showing the three most numerous species that were trapped.

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3.4. Discussion

3.4.1 Species present in the pond

Five species were recorded at the pond in the two study seasons. This is one more than the species collected there by Burger and Cohen (1998) who suggested that 13 species were present at the racecourse and four used this particular pond to breed. Chapter 1 discusses an additional ten species known from the area, but which either breed in summer, are direct developers, prefer different water bodies, or are peripheral to the racecourse.

A study by Parris (2006) suggested that species richness is connected to pond area and pond isolation in urban areas. The maximum number of species at any of the sites in Melbourne was eight and all showed an increasing species richness with increasing pond size. The study in Melbourne took into account the number of roads in a 500 m area around the pond, showing that species numbers decreased with increasing road numbers. The ponds in Kenilworth racecourse can be considered completely isolated due to the urban development, racetrack and an extensive urban network of roads around the site.

In general, tadpoles are not distributed evenly throughout the pond. The abiotic factors recorded here explain some of the distribution. Further studies that separate out the environmental effects upon these tadpoles will be useful. Other factors must be taken into account such as predation, food availability, tadpole density and competition before the distribution of the tadpoles can be fully explained. The purpose of the present study was to examine a tadpole community in nature. Once sufficient data are available to understand the local variables, integrated experimental and field studies will be required to understand the factors driving the system.

3.4.2 Tadpole position

The three tadpole species that were extensively studied occurred in different parts of the water column. For example *Strongylopus grayii* were caught in the surface water of the pond more often in the shallow water column and *C. platys* were caught in high numbers on the surface of the water and not in traps placed along the bottom of the pond. This showed that *C. platys* tadpoles used the body of the water more extensively than *S. grayii*. *Xenopus laevis* was again different, being trapped in only the surface water, never in traps placed on the bottom and sometimes deeper in the water column. The abiotic factors that may have lead to these distributions are discussed later but it is possible that these tadpoles cannot judge temperature, oxygen content or any of the other factor and are perhaps simply staying in the area that they were hatched. The density of the tadpoles of *Spea multiplicatus* remains high at oviposition sites and tadpoles grow larger and survive better in these areas (Pfennig 1990). Pfennig (1990) goes on to suggest that tadpoles may not be able to asses water temperature or oxygen concentration but merely stay in an area that is familiar. This may then give the benefits of increased temperature but means that the tadpole does not have to assess the environment. It is noted in Channing (2001) that *S. grayii* lay their eggs just above the water level in damp places, this would then lead to the tadpoles being hatched into the shallow edge of the pond. Conversely *X. laevis* lays its eggs on submerged vegetation (Channing 2001) which is quite likely to be in the body of the water column as the adult is mostly aquatic. The breeding of *C. platys* is

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unknown but while collecting eggs for a separate study eggs were found attached to submerged vegetation but at the surface of the water column and it the shallow water.

3.4.3 Tadpole trapping and water temperature

S. grayii and *C. platys* distribution was correlated with water temperature. The traps in the higher temperature water collected more tadpoles than the ones in the lower temperature water. Water temperature may be an important factor as the temperature that the tadpole develops at has been shown to affect feeding rate (Warkinten 1992a) swimming performance (Wilson et al. 2000), size (Alvarez and Nicieza 2002, Kaplan and Phillips 2006, Morrison and Hero 2003), time to metamorphosis (Higginbottom 1850, Merilä et al. 2000) and growth rate (Lindgreen and Laurila 2005). Swimming performance plays a role in predator avoidance and size and time to metamorphosis is particularly important to these tadpoles, as they are in a temporary pond (see Dupre and Petranka 1985). There may be additional factors that play a role in swimming performance, size and time to metamorphosis other than temperature, but the importance of temperature can not go unnoticed as its effects have been widely shown (Bradford 1984, Brattstrom 1962, Herreid and Kinney 1967, Merilä et al. 2000 Morrison and Hero 2003, Wilson et al. 2000). The Kenilworth racecourse pond tadpoles need to find the warmest temperatures for optimum growth and time to metamorphosis.

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3.4.4 Tadpole trapping and oxygen concentration

Although water temperature is important in determining the position of these tadpoles, oxygen concentration may also play an important role during tadpole development. *S. grayii* and *C. platys* avoid water with a high oxygen concentration. In tadpoles of *Pelophylax ridibundus* and *P. lessonae* the critical oxygen level was below 3 mg/l (Plénet et al. 2000) and tadpoles of *Euphlyctis cyanophlyctis* have a critical dissolved oxygen level of 5.8 mg/l (Marian et al. 1980). Oxygen levels at the Kenilworth racecourse pond were often below this level. Faster development in increased oxygen concentrations was noted over half a century ago by Nelsen (1947) in three *Lithobates* species, *L. pipiens, L. sylvatica* and *L. palustris.* Tadpoles of *P. ridibunda* and *P. lessonae* are slow to develop under low oxygen levels giving rise to smaller, shorter and thinner tadpoles (Plénet et al. 2000). A low oxygen concentration affects tadpoles in other ways, and tadpoles of *Anaxyrus americanus, Xenopus laevis* and *Lithobates* *berlandieri* all show a decrease in stamina with a decrease in oxygen concentration (Wassersug and Feder 1983). The fact that tadpoles of *S. grayii* and *C. platys* are found in water with low oxygen concentrations suggest that the tadpoles are under some other pressure that would cause them to occur in sub-optimal oxygen conditions. This could be a predator avoidance strategy, as if there are predators in the pond that require high levels of oxygen, then the tadpoles may favour water with a lower oxygen concentration. In a study by Ultsch et al. (1999) on the habitat preference of tadpoles, the tadpoles selected water that was below their critical oxygen tension but was devoid of predators. However, at low concentrations the tadpoles may have to swim to the surface to take in air (Wassersug and Seibert 1975), which could lead to an increased risk of predation if the tadpole must leave its refuge to breathe. Moving to the surface to breathe utilizes energy, which could be saved if the tadpole moved to more oxygen rich water. The tadpoles in the Kenilworth racecourse pond often came to the surface of the water (bobbing) and it is assumed that they were gulping air but this would need further investigation.

3.4.5 Tadpole trapping and water clarity

C. platys tadpoles were trapped more often in water with a low visibility. The reason for this is not clear. Possible explanations for this are that the clarity of the water affects the tadpole's predation risk by visual predators, such as the grey heron (*Ardea cinerea*) that was seen at the pond. As the clarity of the water becomes less the ability of a visual predator to successfully catch a prey item is reduced. Indeed, it has been shown by Spieler (2003) that increased water clarity caused the tadpoles of *Phrynomantis microps* to act as if there were an increased predation risk. In addition, tadpoles not only reduce activity but also seek refuge more often in the presence of predators (Semlitsch and Reyer 1992). Active seeking of a potential refuge may be desirable in the presence of predators, whether hiding is possible in vegetation or through water turbidity.

The amount of UV radiation that reaches the tadpoles in clear water may be higher than in murky water although tadpoles of *Rana cascade* do not avoid increased UV-B radiation, which would suggest that they can not detect it (Belden et al. 2003). Even so, UV radiation reduces the survival of tadpoles when above average levels (Belden et al. 2003, Broomhall et al. 2000, Flamarique et al. 2000). The amount of light that a tadpole is exposed to does not affects its feeding rate and therefore, will not directly affect its growth in that respect (Warkentin 1992a). It is probable that the Kenilworth racecourse tadpoles do not respond to light as the pond is thickly vegetated. The decrease in clarity may be due to an increase in suspended food particles. These factors were not explicitly tested and would require further investigation. Answers to these questions will go a long way to understanding the ecology and behaviour of *C. platys* as, until recently, this species was synonomised with *C. boettgeri* (Scott 2004) and as such, little about them is known.

3.4.6 Tadpole trapping and water column depth

Both *S. grayii* and *C. platys* were trapped in significantly different numbers in different water depths. They were more often in the traps placed in shallow water columns and all three species' catch rate was correlated to trap depth. More tadpoles were caught in traps that were closer to the surface of the water. The trap depth and water column depth may be important due to depth being related to water temperature, predation, UV intensity, food availability or some unknown variable. Water depth was significantly related to water temperature ($\tau = -0.193$, $p<0.05$), with shallow water being warmer.

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3.4.7 Tadpole trapping and substrate

S. grayii were caught in different numbers in traps over different substrates. They were trapped more often in traps over leafy plants and reeds. It is possible that the tadpoles are using the substrate as refuge from predators. Tadpoles will seek out a refuge in the presence of a predator. *Rana temporaria* seeks refuge in the presence of backswimmers, however, the tadpoles only respond with this behaviour to certain predators (Van Buskirk 2001). *Lithobates sylvaticus* and *Hyla versicolor* prefer more complex habitats, remaining in the area of grass and sand rather than selecting just sand, just grass or the bare wire window screening that was used as the un-complex habitat (Formanowicz and Bobka 1989). Perhaps these tadpoles are selecting for a substrate that is familiar to them. This is a mechanism for the tadpole to select an optimum habitat, tadpoles develop this preference between 14 to 17 days after hatching and will probably select for habitats where they hatch (Wiens 1970, 1972).

3.4.8 Success of the critically endangered micro frog

In the first season only one *M. capensis* tadpole was caught. This either suggests that they are very quick to metamorphose, were present but were trap avoiding, or were in such low numbers that they were not caught in the traps. This is worth noting as the Kenilworth racecourse site is one of the few remaining sites with *M. capensis* present and the only remaining site on the Cape Flats, from where it was originally described (Boulenger 1910).

3.4.9 Real ponds are really complex

In short, this study has tried to separate out some of the factors that explain why tadpoles occur in certain microhabitats. The experimental part of the study was carried out to identify factors that influence tadpoles and then the results were compared to more focused literature on the certain aspects of tadpole ecology. The data are by no means complete and other factors, outside the scope of this study, such as predation and competition must be studied before the full picture is seen.

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CHAPTER 4: TADPOLES IN A THREE-DIMENSIONAL HABITAT

4.1 Introduction

According to Pfennig (1990) tadpoles of *Spea multiplicata* will choose their microhabitat with respect to where their mother deposited the eggs. This suggests that the tadpoles do not have to judge temperature or oxygen concentration, or any other abiotic factor as they will already have these conditions selected for them. The tadpoles of *S. multiplicata* have an increased growth and survival rate when reared near the oviposition site.

However, some tadpoles move about the pond throughout the day in relation to the temperature of the water. The tadpoles of *Anaxyrus canorus* crowd in the warmest shallowest parts of the pond during the daytime and move to deeper water during the night where they become inactive (Mullally 1953). *Pseudacris regilla, P. crucifer* and *Anaxyrus boreas* tadpoles all aggregate in specific water temperatures although this was only noted during daylight hours (Brattstrom 1962). In one pond *Rana boylii* tadpoles aggregated in at area with a water temperature of 15 ºC when the rest of the water body was 14 ºC at 9 pm. At 9 am the tadpoles were more dispersed but still in an area of water that was 15.6 ºC where the rest of the water body was cooler. By 10 am the tadpoles had moved to the shallow part of the pond where the temperature was 17 ºC and the rest of the water body was 15 ºC (Brattstrom 1962). The tadpoles of *Ascaphus truei* avoid temperature of over 22 ºC in laboratory experiments and in the field tadpoles avoid areas of water exposed to direct sunlight. Experiments have also shown that these tadpoles' temperature preference do not alter throughout a 24 hour period (De Vlaming and Bury 1970).

The oxygen concentration of a body of water will alter throughout the day. Abbots pool in Somerset, England shows stratification of oxygen concentration throughout the depth of the water with higher levels recorded at the surface. The oxygen concentration changes throughout a 24 hour period with an increase in levels at the end of the day and a decrease during the night. The surface water oxygen concentration starts to rise at the onset of daylight but at 0.25 m depth the increase

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only starts at midday (Happey 1970). Tadpoles of certain species respond to decreases in oxygen by having a reduced stamina (Wassersug and Feder 1983), increasing their bobbing rate (Wassersug and Seibert 1975, Wong and Booth 1994) or by growing and developing slowly (Plénet et al. 2000).

The trapping that was carried out over two years, 2003-2004, gave insight into when the tadpoles entered and left the pond, along with their numbers and distribution within the pond. The results did not show what the tadpoles did within a 24 hour period as the traps were always placed out overnight.

Tadpoles could be changing their position in the pond throughout the 24 hour period to escape predation (Feminella and Hawkins 1994, Van Buskirk 2001) or to obtain food. *Lithobates clamitans* tadpoles move in a cyclic pattern throughout a 24 hour period (Warkinten 1992b). There were no tadpoles in the open water in the morning; tadpoles only appear in the open water during the afternoon and evening and then remain there throughout the night seeking refuge again at dawn. However, there are always more tadpoles present in the vegetation than in the open water at any time of the day (Warkinten 1992b). The tadpoles of *Crinia signifera* and *Litoria ewingi* use the substrate of the pond more in the dark than in the daylight (Peterson et al. 1992). The tadpoles of *Lithobates pipiens* are more active and found more often in the water column when they are in pairs or groups (Golden et al. 2001).

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4.2 Methods

4.2.1 Surveys over 24 hours

On three separate occasions, 16/9/2006, 19/9/2006 and 27/9/2006 the study pond was visited for a period of 24 hours. Four transects were sampled every three hours. Each transect consisted of three water column depths, 20 cm, 50 cm and 100 cm (Fig. 4.1) and six sweep positions (Fig. 4.2) At the 20 cm point a net measuring 20x15 cm was swept through the water three times (position 1). Each sweep was the same length (1 m) and speed (1 sec). Every tadpole caught in the three sweeps was recorded. At the 50 cm point three sweeps of the net were taken through the surface of the water (position 2) and every tadpole caught in the net was recorded. Three sweeps were then taken across the bottom of the pond (position 3) and every tadpole caught was recorded. The last transect point, at 100 cm, had three sampling depths, the surface of the water, (position 4) 50 cm deep (position 5) and the bottom of the water column (position 6). Three sweeps were taken at each depth. Everything that was caught was recorded. The temperature and the oxygen content of the water were recorded at every sampling point in every sampling session using a YSI 55 dissolved oxygen meter. When walking these transects, movement through the pond was slow and shuffling to avoid disturbance and the net handle was extended to 1.5 m so samples could be taken without standing at the sampling point. The sampling procedure was carried out across the same four transects every three hours and then replicated over three sampling periods.

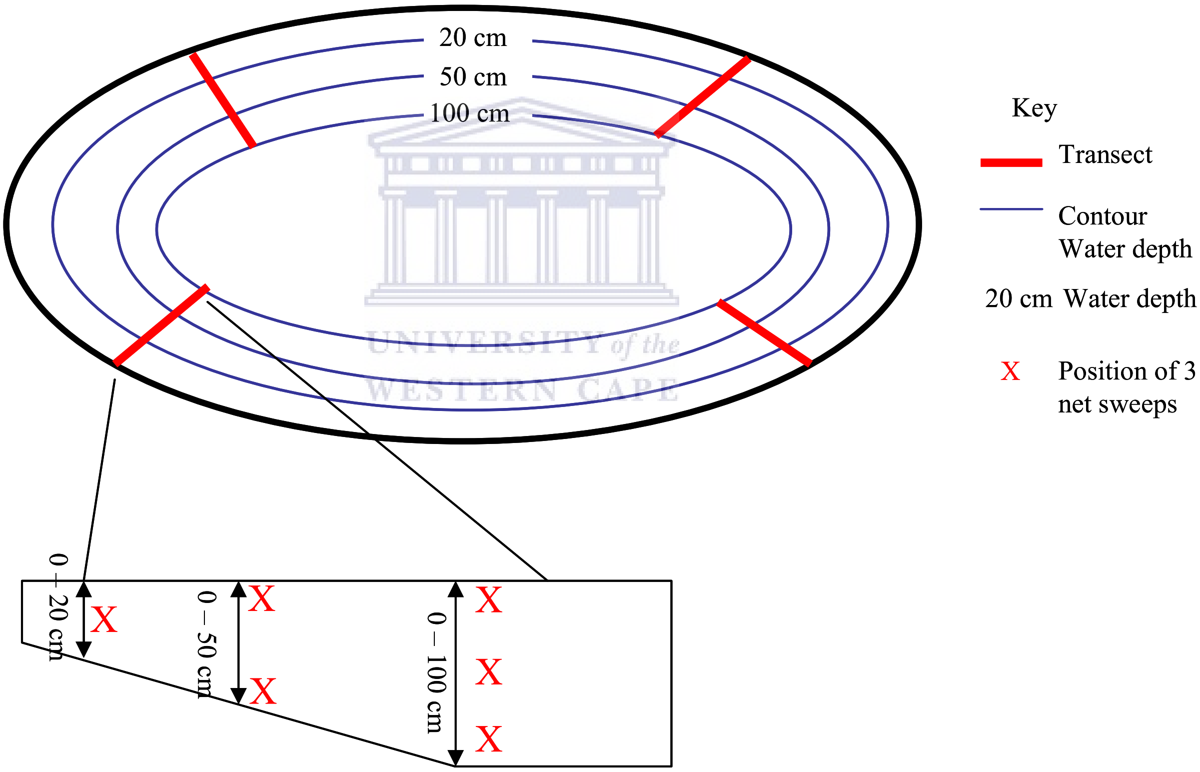

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Fig. 4.1. A diagram showing the position of the transects in the pond with the sampling position for each transect given in the lower diagram.

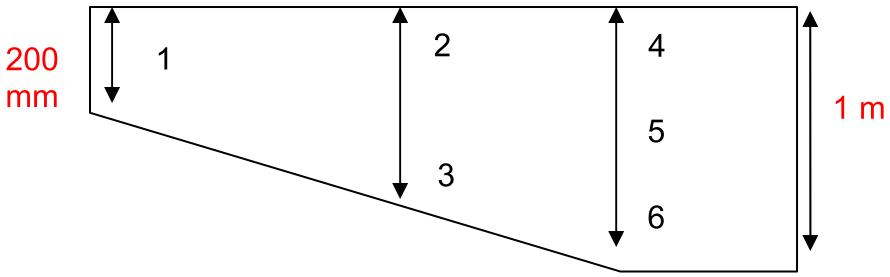

Fig. 4.2. A cross section of the pond with sweep positions used for all transects at all sampling times.

4.2.2 Data analysis

Data were not normally distributed therefore effects of sweep position and time of day on tadpole numbers were analysed using a Kruskal-Wallis ANOVA test. Mann-Whitney U analyses were carried out for the day/ night data. Spearman rank correlations were used to determine any correlation between tadpole numbers and oxygen concentration and tadpole numbers and temperature. All analyses were done separately for each species.

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4.3 Results

4.3.1 Temperature and oxygen over 24 hours

The mean temperature of the pond over the three sampling sessions was plotted against time (Fig. 4.3). The temperature of the water was significantly different over time ($H_{(7,576)}$=168.6975, p=0.000). The significant differences between sampling times are shown in Table 4.1. The oxygen concentration of the water also alters significantly over time ($H_{(7,576)}$=22.522740,=0.0021). A pairwise comparison shows the difference to be between 07h00 and 19h00. Fig. 4.3 shows the oxygen concentration of the water following the pattern of the water temperature with a lag.

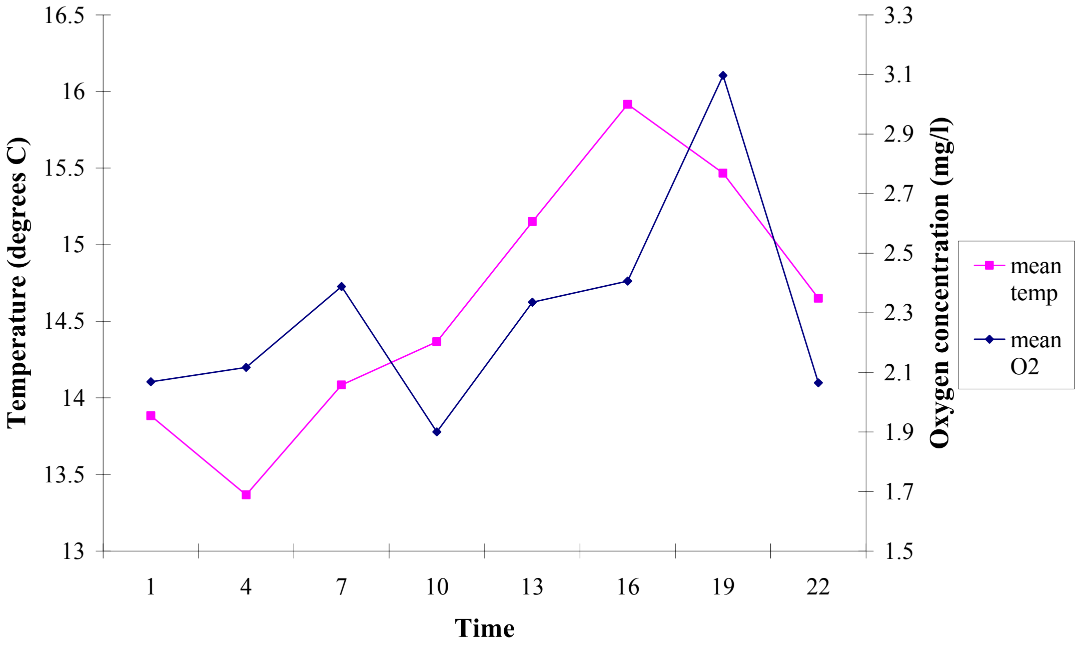

Fig.4.3. Mean temperature and oxygen concentration of the pond water over time.

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Table 4.1. Temperature difference over time (significant values in bold face) (temperatures given in Fig. 4.3).

	01h00	04h00	07h00	10h00	13h00	16h00	19h00
01h00							
04h00	0.090548						
07h00	0.250679	1.000000					
10h00	1.000000	**0.028673**	0.088088				
13h00	**0.000160**	**0.000000**	**0.000000**	**0.000756**			
16h00	**0.000000**	**0.000000**	**0.000000**	**0.000001**	1.000000		
19h00	**0.000003**	**0.000000**	**0.000000**	**0.000020**	1.000000	1.000000	
22h00	0.096197	**0.000000**	**0.000001**	0.271430	1.000000	0.110910	0.493376

4.3.2 Tadpole captures over 24 hours

Over the three 24 hour periods a total of 332 *S. grayii* tadpoles, 537 *C. platys* tadpoles and 204 *X. laevis* tadpoles were caught. Significantly more *S. grayii* tadpoles were found in transect 1 than transect 2 or 3 ($H_{(3, 576)} = 20.37360$, p = 0.0001). Significantly more *C. platys* tadpoles were caught in transect 1 and 2 than in transect 3 ($H_{(3, 576)} =$

25.08579, p = 0.000) and *X. laevis* tadpoles were found in significantly more numbers in transects 3 and 4 than in transect 2 ($H_{(3, 576)}$ = 34.21534,, p = 0.000).

4.3.2.1 *Strongylopus grayii*

Time of day had no significant effect on *S. grayii* numbers ($H_{(7, 576)}$ = 7.110468, p = 0.4175) with similar numbers of tadpoles caught throughout the 24 hour period.

Table 4.2 gives the results of the Kruskal-Wallis ANOVA for *Strongylopus grayii* numbers against sweep position. There was a significant difference in the number of tadpoles caught at each sweep position throughout the 24 hours. However, due to the manner in which the Kruskal-Wallis H statistic is calculated in some cases pairwise comparisons indicate a reduced effect. Consequently, homogenous groups are conservatively determined and may conflict with results obtained across all sweep positions. The high number of zeros in the data influenced the outcomes of the pairwise comparisons, using a median around a zero in some cases, and therefore cannot calculate the significant differences. However, there were significant differences in the pairwise comparisons for 01h00 and 04h00 where a significant difference is shown between groups 1 and 2, 4, 5 and 6 and 19h00 where there was a significant difference between sweep positions 1 and 2, 4 and 5 (Fig. 4.4). Figure 4.4 shows the mean number of tadpoles caught at each sweep position. *S. grayii* always has high numbers of tadpoles in sweep position 1 which is the collecting point next to the shore of the pond in the shallow water. Where significance can be determined, at 01h00, 04h00 and 19h00, tadpoles are in the shallow water at 01h00 and 04h00 and more spread out at 19h00. Interestingly, an *S. grayii* tadpole was caught at least once in every sweep position.

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Table 4.2. The results of the Kruskal-Wallis test for different times of the 24 hour period testing sweep position against *S. grayii* number (data presented in Fig. 4.4).

Time	H	df	p – value
01h00	33.500	5,72	0.0000
04h00	34.799	5,72	0.0000
07h00	34.799	5,72	0.0000
10h00	21.967	5,72	0.0005
13h00	24.342	5,72	0.0002
16h00	19.000	5,72	0.0019
19h00	32.850	5,72	0.0000
22h00	23.066	5,72	0.0003

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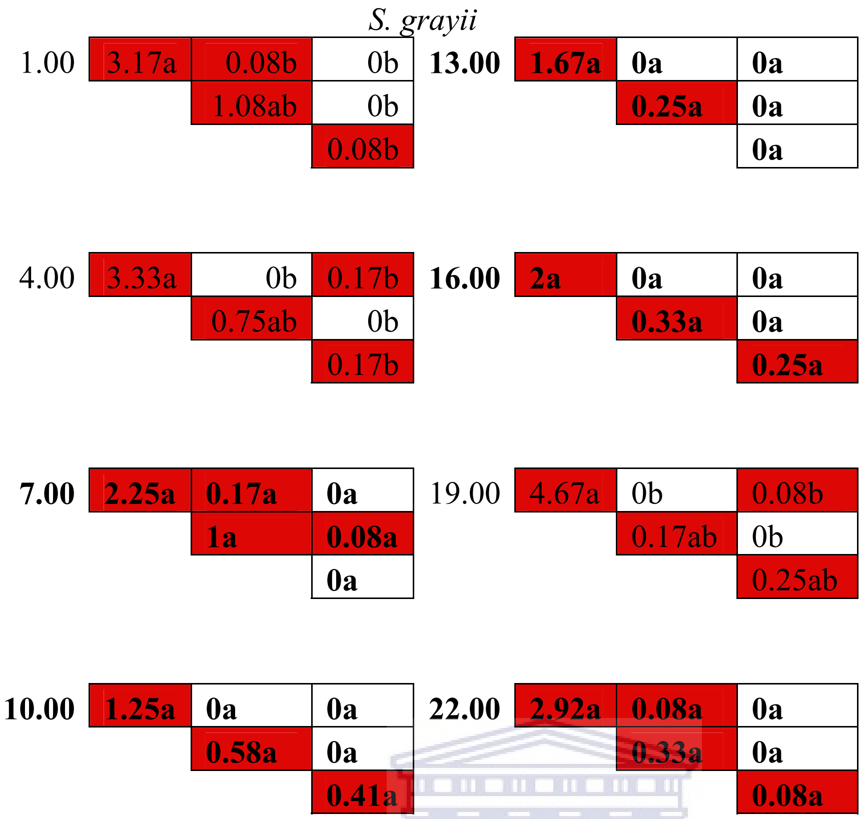

S. grayii

1.00	3.17a	0.08b	0b
		1.08ab	0b
			0.08b

4.00	3.33a	0b	0.17b
		0.75ab	0b
			0.17b

7.00	**2.25a**	**0.17a**	**0a**
		1a	**0.08a**
			0a

10.00	**1.25a**	**0a**	**0a**
		0.58a	**0a**
			0.41a

13.00	**1.67a**	**0a**	**0a**
		0.25a	**0a**
			0a

16.00	**2a**	**0a**	**0a**
		0.33a	**0a**
			0.25a

19.00	4.67a	0b	0.08b
		0.17ab	0b
			0.25ab

22.00	**2.92a**	**0.08a**	**0a**
		0.33a	**0a**
			0.08a

Fig. 4.4. The mean number of *S. grayii* tadpoles caught at each sweep position over time in the three sampling sessions. Letters indicate homogenous groups by *post hoc* pairwise comparison and shading indicates presence of tadpoles. Bold face values indicates significantly different sweep positions (07h00: Kruskal-Wallis $H_{(5, 72)}$ =34.799 p = 0.0000; 10h00: $H_{(5, 72)}$ =21.967 p = 0.0005; 13h00: $H_{(5, 72)}$ =24.342 p = 0.0002; 16h00: $H_{(5, 72)}$ =19.001 p = 0.0019; 22h00: $H_{(5, 72)}$ =23.066 p = 0.0003), however due to the manner in which the Kruskal-Wallis H statistic is calculated in some cases pairwise comparisons indicate a reduced effect. Consequently, in the latter case homogenous groups are conservatively determined and may conflict with results obtained across all sweep positions.

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4.3.2.2 *Cacosternum platys*

The total number of *C. platys* caught at each trapping time differed throughout the day ($H_{(7,576)}$ = 33.56073, p = 0.000). There were significantly more tadpoles caught at 10h00, 13h00 and 16h00 than there were at 01h00 and more tadpoles caught at 13h00 than at 04h00.There is a trend of an increasing number of tadpoles in daylight hours and then a decrease throughout the night (Fig. 4.5).

Cacosternum platys tadpoles were not evenly distributed about the pond throughout the 24 hours. The number of tadpoles caught in each sweep position differed significantly at every sampling time (see Table 4.3 for Kruskal-Wallis results). The higher numbers of *C. platys* caught over the three sampling sessions allowed a more in-depth look as the test was not hampered by too many zeros. The only sampling time where the results showed a significant difference between sweep position but did not indicate homogenous groups was at 01h00 (Fig. 4.6). As explained previously for *S. grayii*, this is due to the manner in which the Kruskal-Wallis H statistic is calculated. At 01h00 more tadpoles were caught in sweep position 1, with fewer being caught in positions 3 and 6. No tadpoles were caught in positions 2, 4 and 5. At 04h00 there was a significant difference in tadpole numbers between positions 1 and 2 with more tadpoles occurring in position 1. At 07h00, 10h00 and 13h00 there was a difference again between positions 1 and 2 but also between positions 1 and 4, 1 and 5, and again between 1 and 6. In each case there were more tadpoles at position 1, which is close to the shore and shallow. At 16h00 there was a difference in tadpole numbers between sweep positions 1 and 3, 4, 5 and 6 with position 1 having the highest tadpole numbers. 19h00 and 22h00 sampling sessions reverted back to the pattern of 07h00 to 13h00 where there were more tadpoles at position 1 than at positions 2, 4, 5 and 6 (Fig. 4.6). At the 04h00 sampling session the tadpoles were more spread across the pond but at all other sampling times tadpoles were caught in select parts of the pond (positions 1 and 3 and once in 2)(Fig. 4.6).

The mean tadpole catch was split into two groups, day and night and the difference in numbers at each sweep position was tested. *C. platys* tadpoles were caught more often during the day at sweep positions one and two (1= z=-2.95, p=0.003; 2= z=-2.78, p=0.005)(Fig. 4.7).

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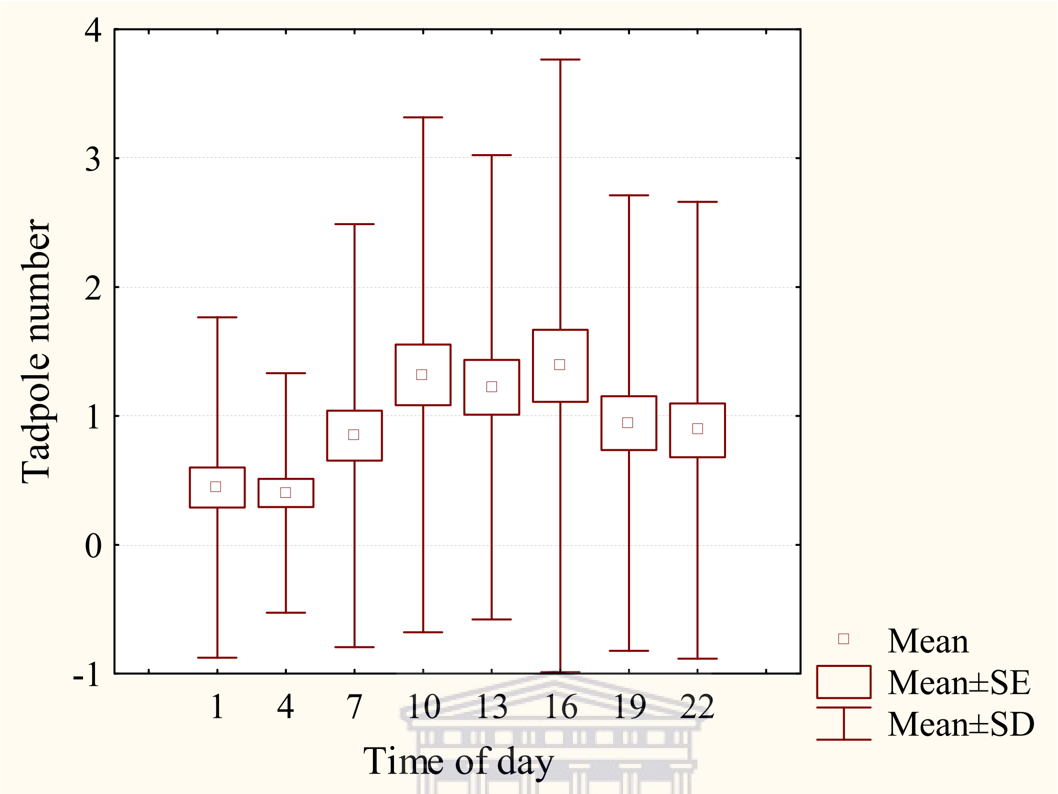

Fig. 4.5. The number of *C. platys* tadpoles caught throughout the 24 hour period, mean number for all three sampling sessions and transect numbers combined.

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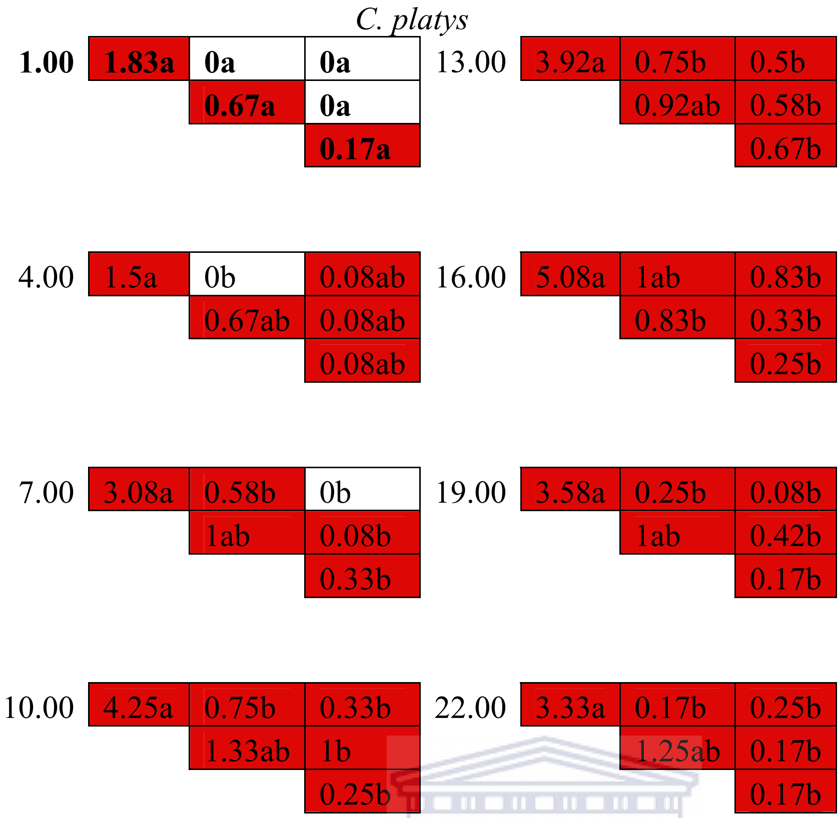
Table 4.3. The results of the Kruskal-Wallis test for different times of the 24 hour period testing sweep position against *C. platys* number (data presented in Fig. 4.6).

Time	H	df	p - value
01h00	29.100	5, 72	0.0000
04h00	28.961	5, 72	00000
07h00	34.533	5, 72	00000
10h00	26.445	5, 72	0.0001
13h00	25.528	5, 72	0.0001
16h00	32.350	5, 72	0.0000
19h00	24.253	5, 72	0.0002
22h00	31.988	5, 72	0.0000



C. platys

1.00	**1.83a**	**0a**	**0a**
		0.67a	**0a**
			0.17a

4.00	1.5a	0b	0.08ab
		0.67ab	0.08ab
			0.08ab

7.00	3.08a	0.58b	0b
		1ab	0.08b
			0.33b

10.00	4.25a	0.75b	0.33b
		1.33ab	1b
			0.25b

13.00	3.92a	0.75b	0.5b
		0.92ab	0.58b
			0.67b

16.00	5.08a	1ab	0.83b
		0.83b	0.33b
			0.25b

19.00	3.58a	0.25b	0.08b
		1ab	0.42b
			0.17b

22.00	3.33a	0.17b	0.25b
		1.25ab	0.17b
			0.17b

Fig. 4.6. The mean number of *C. platys* tadpoles caught at each sweep position over the three sampling sessions. Letters indicate homogenous groups by *post hoc* pairwise comparison and shading indicates presence of tadpoles. Bold face values indicates significantly different sweep positions (01h00: Kruskal-Wallis $H_{(5,\,72)} = 29.100$, p = 0.0000), however due to the manner in which the Kruskal-Wallis H statistic is calculated in some cases pairwise comparisons indicate a reduced effect. Consequently, in the latter case homogenous groups are conservatively determined and may conflict with results obtained across all sweep positions.

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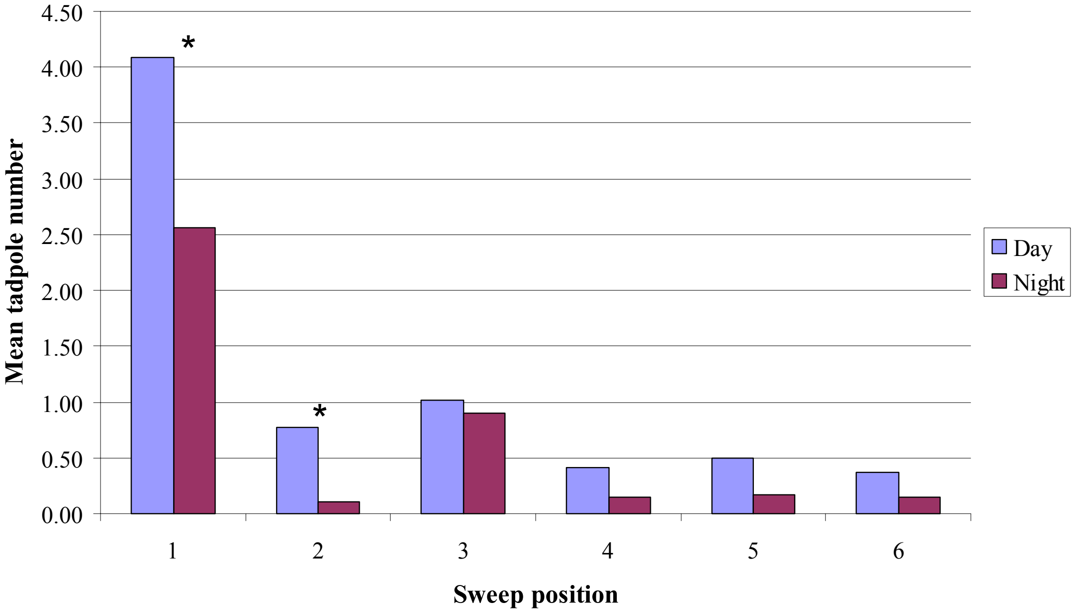

Fig. 4.7. The mean number of *C. platys* tadpoles caught at each sweep position during the day (07h00 – 16h00) and night (19h00 – 04h00). Star indicates significance (p=<0.05)

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4.3.2.3 *Xenopus laevis*

There was no difference between the numbers of *X. laevis* caught at the different times of the day.

Xenopus laevis tadpoles were caught in lower numbers than the other two species. The tadpoles were most often caught in the deeper water and were only once, at 04h00, caught at position 1, which was in the shallow water. The only significant difference was at 19h00 (Table 4.4) where even though the pairwise comparisons showed no homogenous groups (see explanation for same situation in *S. grayii* above) the higher numbers of tadpoles were caught in the 1 m water column (Fig. 4.8). The mean number of *X. laevis* tadpoles trapped during the day and night at each sweep position was tested to ascertain if there was a difference. The tadpoles were caught in higher numbers during the day at sweep positions four and five and higher numbers during the night at positions one, two, three and six. The difference between the numbers during the day and night was significantly different at sweep position three and six (3=z=2.13,p=0.03: 6=z=2.23,p=0.03)(Fig. 4.9).

Table 4.4. The results of the Kruskal-Wallis test for different times of the 24 hour period testing sweep position against *X. laevis* number (data presented in Fig. 4.8).

Time	H	df	p - value
01h00	10.746	5, 72	0.0566
04h00	4.862	5, 72	0.4330
07h00	3.852	5, 72	0.5709
10h00	8.632	5, 72	0.1247
13h00	7.196	5, 72	0.2064
16h00	4.592	5, 72	0.4677
19h00	15.958	5, 72	0.0070
22h00	4.771	5, 72	0.4444

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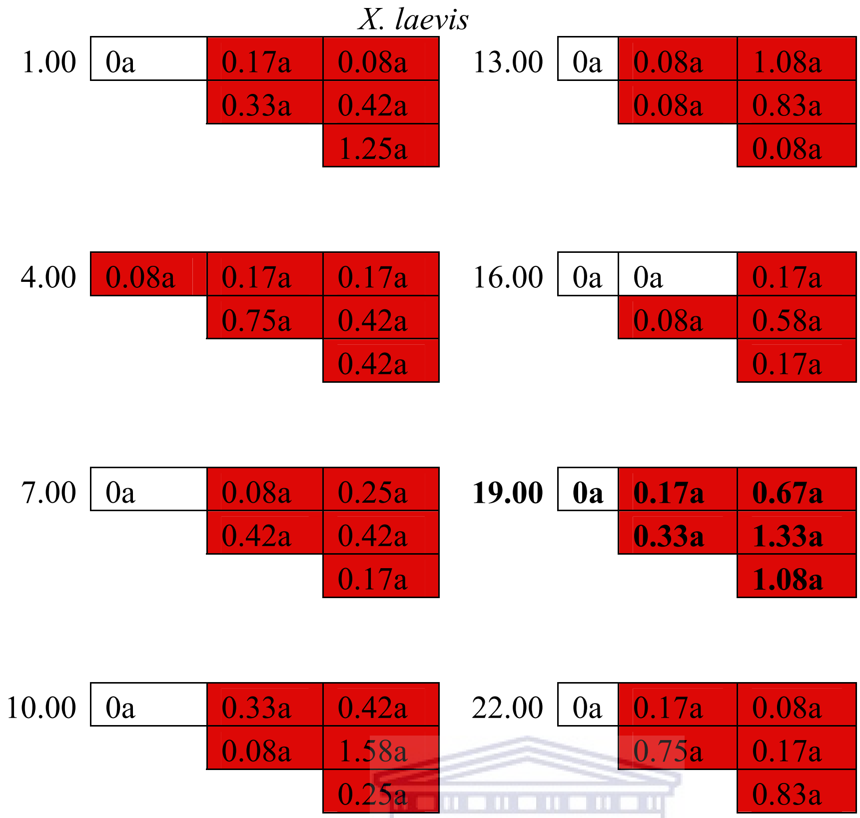

X. laevis

1.00	0a	0.17a	0.08a
		0.33a	0.42a
			1.25a

4.00	0.08a	0.17a	0.17a
		0.75a	0.42a
			0.42a

7.00	0a	0.08a	0.25a
		0.42a	0.42a
			0.17a

10.00	0a	0.33a	0.42a
		0.08a	1.58a
			0.25a

13.00	0a	0.08a	1.08a
		0.08a	0.83a
			0.08a

16.00	0a	0a	0.17a
		0.08a	0.58a
			0.17a

19.00	**0a**	**0.17a**	**0.67a**
		0.33a	**1.33a**
			1.08a

22.00	0a	0.17a	0.08a
		0.75a	0.17a
			0.83a

Fig. 4.8. The mean number of *X. laevis* tadpoles caught at each sweep position over the three sampling sessions. Letters indicate homogenous groups by *post hoc* pairwise comparison and shading indicates presence of tadpoles. Bold face values indicates significantly different sweep positions (19h00: Kruskal-Wallis $H_{(5, 72)} = 15.958$, p = 0.007), however due to the manner in which the Kruskal-Wallis H statistic is calculated in some cases pairwise comparisons indicate a reduced effect. Consequently, in the latter case homogenous groups are conservatively determined and may conflict with results obtained across all sweep positions.

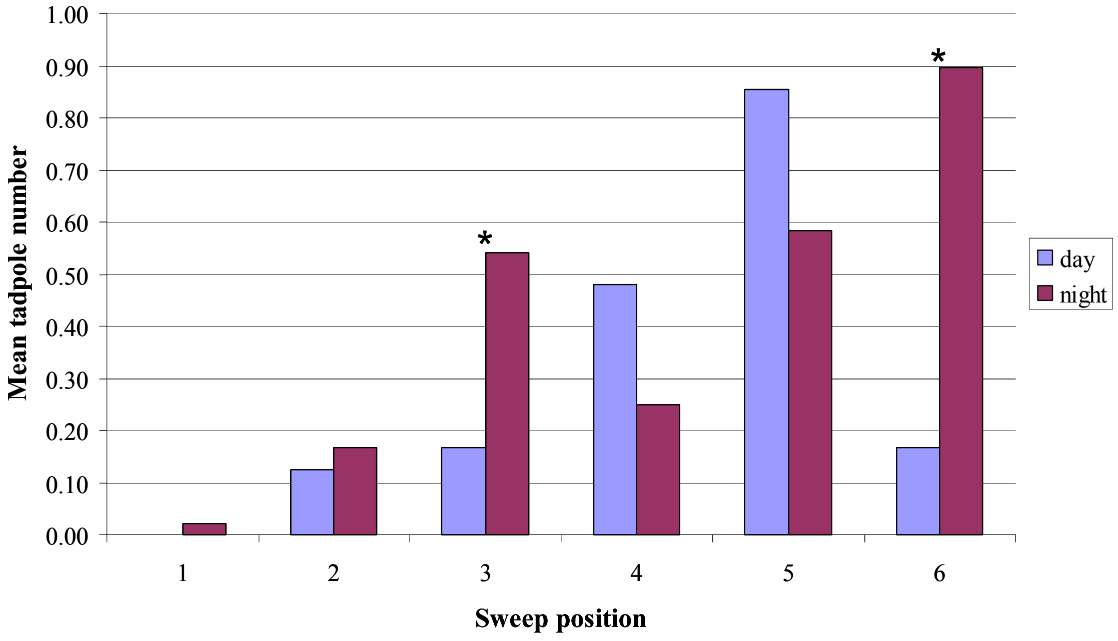

Fig. 4.9. The mean number of *X. laevis* tadpoles caught at each sweep position during the day (07h00 – 16h00) and night (19h00 – 04h00). Star indicates significance ($p=<0.05$)

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4.3.3 Environmental correlations

The correlation with tadpole numbers and water temperature and oxygen varied with species. The number of *S. grayii* tadpoles caught was not correlated to the temperature of the water at the different sweeps, nor was tadpole number correlated with oxygen (Table 4.5). A positive correlation was observed between *C. platys* tadpole numbers and temperature and tadpole numbers and oxygen concentration (Table 4.5; Figs 4.10 - 4.11). Conversely, *Xenopus laevis* tadpoles were negatively correlated to the temperature and the oxygen concentration of the water (Table 4.5; Figs 4.12 - 4.13).

Table 4.5. Spearman rank order correlation. Bold face correlations indicate significance at $p < 0.05$ (Sign indicates direction of correlation).

Tadpole species	Temperature	Oxygen
S. grayii	0.038746	-0.042097
C. platys	**0.273088**	**0.119614**
X. laevis	**-0.130852**	**-0.156380**

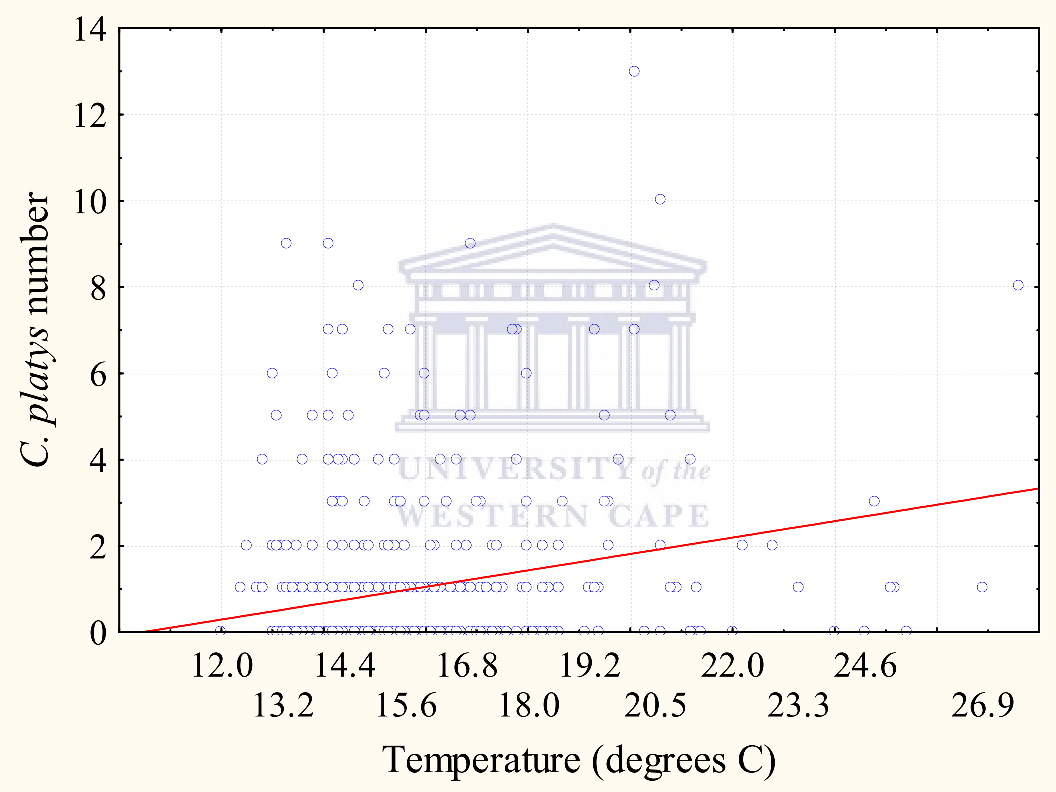

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Fig 4.10. *Cacosternum platys* numbers against water temperature (red line shows the line of best fit).

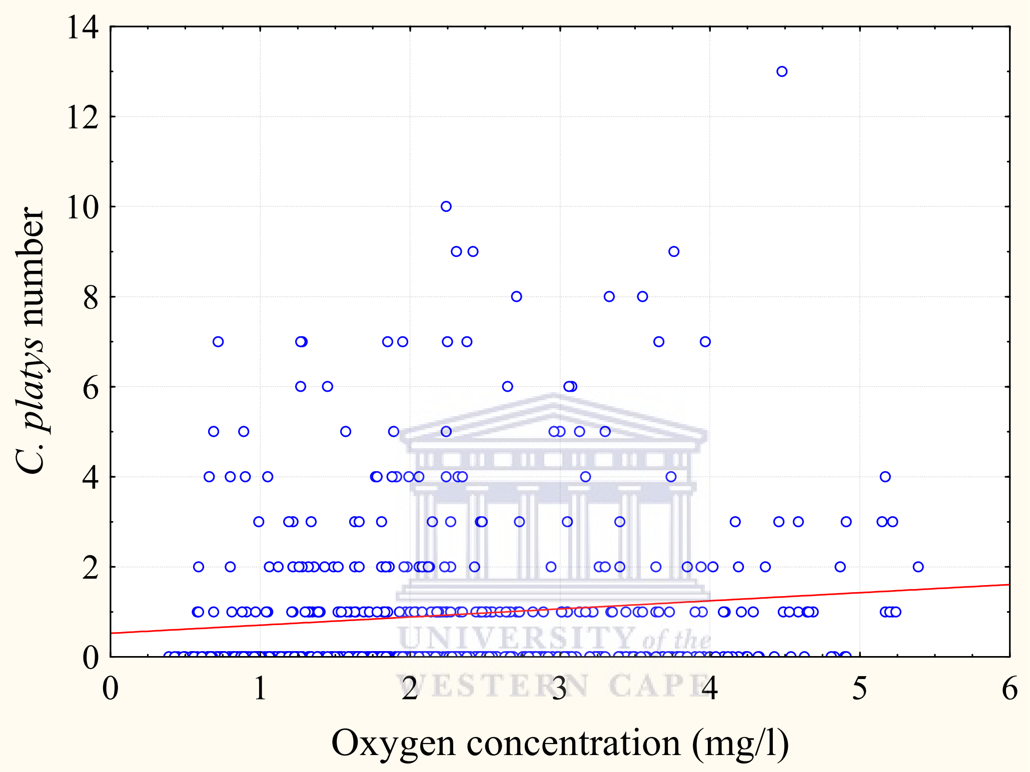

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Fig 4.11. *Cacosternum platys* numbers against oxygen concentration of the water (red line shows line of best fit).

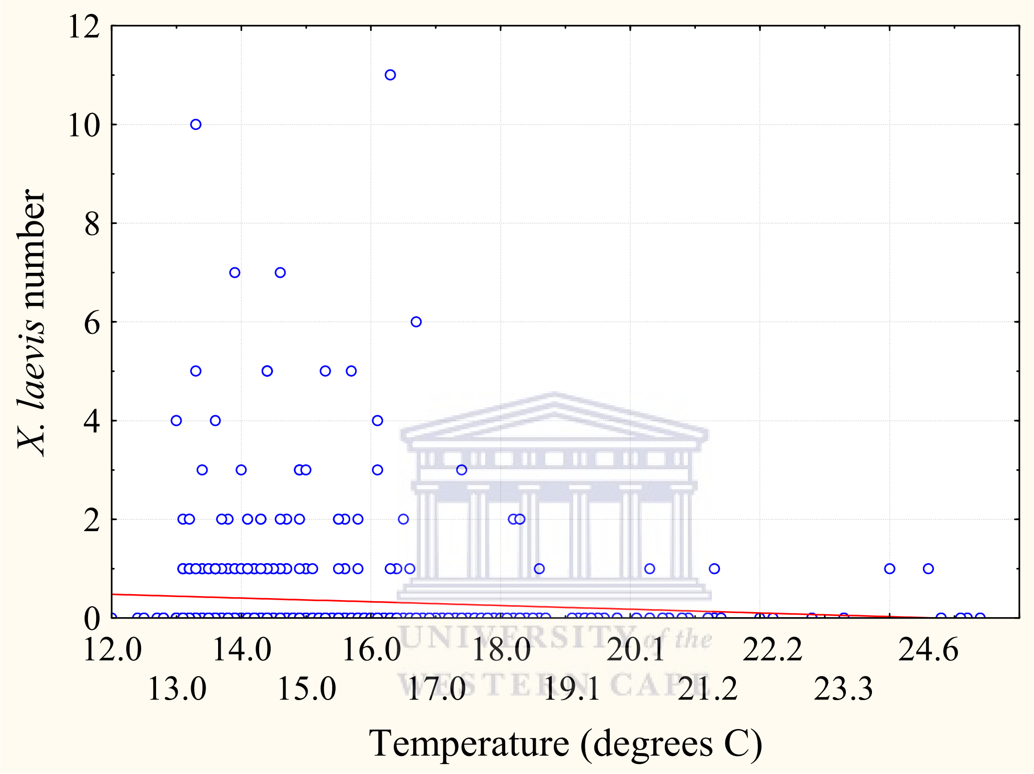

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Fig. 4.12. *Xenopus laevis* numbers against water temperature (red line shows line of best fit)

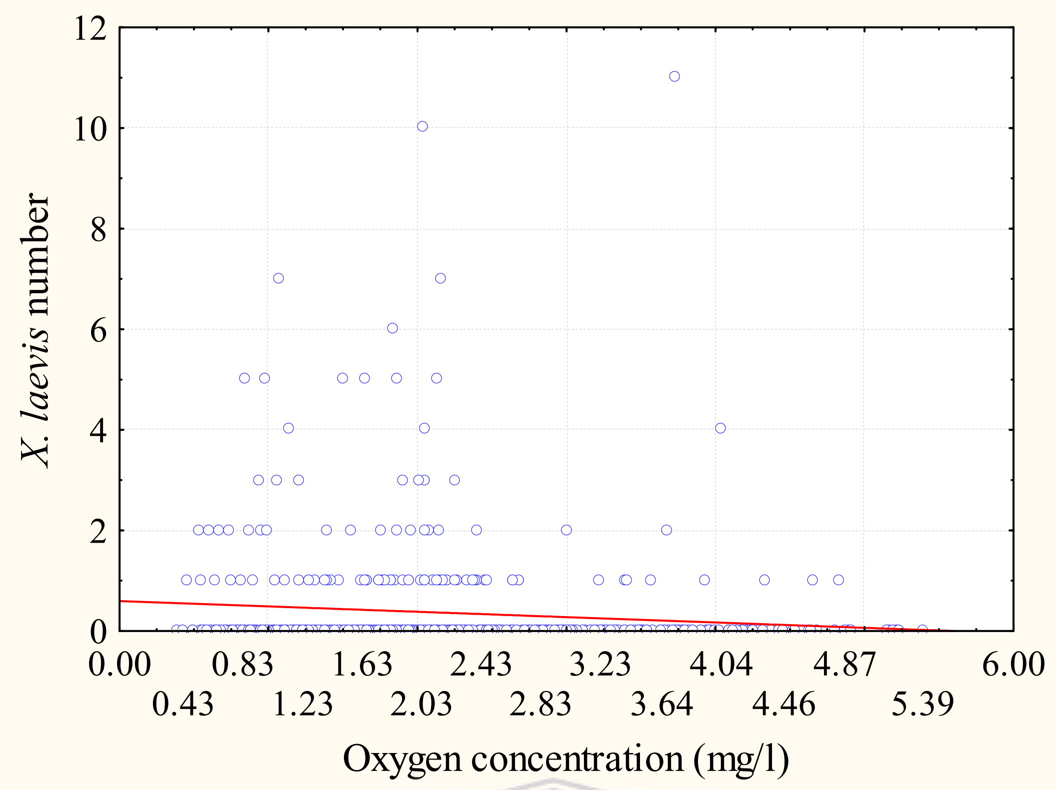

Fig 4.13. *Xenopus laevis* number against oxygen concentration of the water (red line shows line of best fit)

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4.4 Discussion

4.4.1 Tadpole position

This experiment was conducted to study the position of tadpoles throughout a 24 hour period and to relate it to water temperature and oxygen concentration. Previous trapping was carried out over-night, which showed where the tadpoles were generally in the pond during the night. These 24 hour periods were chosen because the three main species, *C. platys*, *S. grayii* and *X. laevis*, were present. At this pond the *S. grayii* and *X. laevis* tadpoles appear before the *C. platys*.

Two of the species, *S. grayii* and *C. platys*, were caught in higher numbers in the shallow water. There were always *S. grayii* caught at sweep position 1 and 3 and *C. platys* were always caught in sweep positions 1, 3 and 6. All of these positions were along the bottom of the pond. The tadpoles of *X. laevis* were not caught in sweep position 1 except for one sampling session at 04h00. There were high numbers of *X. laevis* in the water column (sweep position 4 and 5). The observed difference between the catch of *X. laevis* at the different sweep positions against the catch of the other two tadpole species could be due to the different morphology of the three species. The *X. laevis* tadpoles are more disposed to life in the water column, with a terminal mouth position and transparent body (Van Dijk 1972). Whereas, the tadpoles of *S. grayii* and *C. platys* which are dark in colour with a ventral mouth position, are probably more suited to life on the bottom of the pond. However, Van Dijk (1972) whilst stating that *S. grayii* are bottom dwelling suggests that *C. boettgeri,* which *C. platys* has been synonomised with until recently (Channing 2001) are pelagic due to the lateral position of their eyes and long tail. The difference in body shape between the tadpoles of *X. laevis* and *C. platys* and *S. grayii* could alter the way the tadpoles feed. *Xenopus laevis* has a terminal mouth with no hard mouth parts; it is a filter feeder. The tadpoles of *S. grayii* and *C. platys* have a ventral mouth with keratinised mouth parts which would suggest the ability to remove filamentous algae or projecting material from the substrate (Altig and Johnston 1989, Van Dijk 1972).

Alternatively the difference in tadpole position may be due to the position that the adult frogs lay the eggs. Tadpoles occur in high numbers at oviposition sites. This is possibly an easy way for the tadpole to select the best environment (Pfennig 1990). Adult *S. grayii* lay their eggs above the water line in anticipation of the rain filling the ponds (Channing 2004b). This would result in the tadpoles hatching in the shallow water. The details of breeding of *C. platys* are unknown however, when eggs were collected for another study the eggs were found in small clumps of about 30 eggs attached to vegetation in the shallow water. Adults of *X.* laevis are mainly aquatic and lay their eggs on submerged vegetation possibly giving rise to tadpoles hatching in the centre of the pond (Channing 2001).

The tadpoles of *S. grayii* and *C. platys* stay close to the substrate. The substrate will offer a more complex habitat which in turn will offer protection from predation. The tadpoles of *Lithobates sylvaticus* and *Hyla versicolor* choose a more complex habitat when given the choice of microhabitats of different complexity (Formanowicz and Bobka 1989).The presence of the *X. laevis* in the deeper water could be a predator avoidance strategy in the same way that the other two species may be seeking refuge in the more complex habitats. As has already been discussed, the tadpoles of *X. laevis* are physically different which could alter their predator avoidance strategy. The tadpoles are mostly transparent which may assist in avoidance of visual predators in the open water.

In general the tadpoles of these three species did not show a cyclic movement about the pond as was found in *Lithobates clamitans* (Warkentin 1992b) Tadpoles of *X. laevis* increased in number at sweep position 3 and 6, which is on the bottom of the pond, during the night. The other sweep positions showed differences but none were significant. The trend was that more tadpoles were present during the day at sweep position 4 and 5 and more at night at position 1, 2, 3 and 6. This suggests that the tadpoles of *X. laevis* spend time in the water column in daylight but retreat to the bottom of the pond during the night. This is the opposite of *Lithobates clamitans* which moves from the open water at night to the vegetated water during the day (Warkentin 1992b) and *Anaxyrus americanus* which scatter across the pond during the night but move to the shallow water in daylight (Beiswenger 1977). The tadpoles of *C. platys* were caught in significantly higher numbers in position 1 and 2 during the day. This concurs with the behaviour of *A. americanus* and *Lithobates clamitans* (Beiswenger 1977, Warkentin 1992b). The tadpoles of *Crinia signifera* and *Litoria ewingi* behave in a similar way to *C. platys* and *X. laevis*. The bottom dwelling *C. signifera* is found more often at the edge of the water similar to the *C. platys* tadpoles. *Litoria ewingi* is found more in the deep water nearer the surface like the tadpoles of *X. laevis*. The tadpoles of *L. ewingi* move to the substrate in the darkness just like the *X. laevis* tadpoles seem to do. The position of *C. signifera* and *L. ewingi* is probably a predator avoidance strategy against *Hemicordulia tau* which is more active in the darkness (Peterson et al. 1992).

4.4.2 Environmental correlations

In the previous trapping experiment (Chapter 3) the tadpoles' presence in the shallow water were partially temperature related. During the 24 hours the tadpoles of *C. platys* were found in higher numbers in warmer water. These results concur with Brattstrom (1962) who shows many examples of tadpoles aggregating due to temperature and suggests that tadpoles move to warm water which ultimately increases their metabolism and therefore speeds up their time to metamorphosis. This temperature effect has been shown in *Rana muscosa* (Bradford 1984) and *Anaxyrus canorus* (Mullally 1953). The tadpoles of *R. temporaria* delayed their metamorphosis when placed in 14 ºC water opposed to tadpoles of the same species placed in 22 ºC water (Merilä et al. 2000) and the tadpoles of *R. arvalis* and *R. temporaria* metamorphose later in colder summers (Loman 2002) The tadpoles of *X. laevis* behaved differently. *Xenopus laevis* distribution throughout the pond is negatively correlated to the temperature of the water. This suggests that there is another pressure acting upon these tadpoles.

The tadpoles of *C. platys* were found in significantly higher numbers in water with a high oxygen concentration. This could be because the tadpole can conserve energy by not having to move to the surface for air. In some tadpole species bobbing rates increase with a decrease in oxygen concentration (Wassersug and Seibert 1975) and growth and development slows (Plénet et al. 2000). The tadpoles of *X. laevis* were caught in higher numbers in water with a low oxygen concentration. This difference between the two species has been recorded previously in *Lithobates pipiens* and *Anaxyrus terrestris.* The tadpoles of *A. terrestris* were in water with a higher oxygen concentration than the tadpoles of *L. pipiens*. In this case the development of lungs in the tadpoles occurs at very different stages with *L. pipiens* developing lungs early in development and *A. terrestris* only getting lungs just before metamorphosis. Another ranid that is low oxygen tolerant is *Lithobates catesbeianus*; it has been found in water below its critical oxygen levels. This is possibly a predator avoidance strategy (Ultsch et al 1999).

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CHAPTER 5: THE THREAT OF DESICCATION: DO TADPOLES OF *STRONGYLOPUS GRAYI* AND *CACOSTERNUM PLATYS* METAMORPHOSE EARLIER UNDER DRYING CONDITIONS?

5.1 Introduction

The advantages of temporary ponds to tadpoles include high levels of food (Wassersug 1975, Wilbur 1987, Peterson and Boulton 1999) and fewer predators than in permanent water (Brendonck et al. 2002, Petranka and Kennedy 1999). The temporary nature of the pond is beneficial in these aspects but obliges the tadpoles to metamorphose within a certain time limit. The permanence of a pond can be used to predict which species will be present (Adams 2000, Skelly 1996, 1997, Smith and Van Buskirk 1995, Wilbur 1987). Anurans with long tadpole stages will choose more permanent water whereas tadpoles with a short time to metamorphosis can make use of more temporary ponds. Most species are quite restricted in this respect and must stay within a small section of the gradient from temporary to permanent water (Skelly 1997).

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Tadpoles that are predisposed to use permanent water will forage less and grow slower whereas, tadpoles in temporary ponds forage more and grow faster. This allows the tadpoles in the permanent ponds to reduce predation by moving around less, and the animals in the temporary ponds to grow quickly (Skelly 1997). Anuran species adapted to a temporary tadpole environment may be able to speed up metamorphosis in a drying pond (Laurila and Kujasalo 1999). There is a threshold in the tadpole development that once reached, allows the tadpole to metamorphose depending upon the conditions of its environment (Morey and Reznick 2000). It is not just the temporary nature of the water but how predictable the inundation will be that will determine if the tadpole can speed up development and reach metamorphosis more quickly (Leips et al. 2000, Ryan and Winne 2001). *Crinia signifera* tadpoles respond to declining water levels by accelerating development and decreasing their time to metamorphosis. However, the metamorphs were smaller and survival in the terrestrial stage was lower (Lane and Mahony 2002). The tadpoles of *Rana temporaria* also respond to decreasing water volume by metamorphosing smaller and earlier (Laurila and Kujasalo 1999), but *Hyla cinerea* and *Hyla gratiosa* tadpoles take longer to metamorphose in the drying treatments and metamorphosed at a smaller size than when the water level is kept constant. It is possible that these tadpoles are responding to an increase in density rather than a decrease in water levels (Leips et al. 2000).

In the present study (Chapter 2, Fig. 2.1) the first cohorts of *Strongylopus grayii* tadpoles were present very early in the season for both years. This may offer the advantage of making use of a maximum hydro period, especially in environments where the rainfall is less predictable. This species is widespread, and is known to breed in both permanent and temporary ponds. Do they rely on being in the water as soon as it is present, and is there some developmental flexibility that will enable them to metamorphose and leave a pond early if it starts to dry out? Being in the pond early also means that the species may be able to breed more than once, but this is discussed elsewhere. The question posed is "Can tadpoles of *Strongylopus grayii* reach metamorphosis quicker under conditions of a drying pond?"

On the other hand, tadpoles of *Cacosternum platys* were only found in the pond later in the season. This species is restricted to the South-western Cape, where natural ponds are mostly temporary, filled by the winter and spring rains. The advantages of coming to the pond many weeks after it has filled may include the presence of vegetation for concealment, or perhaps a change in the algal food availability. Whatever the benefits, it remains a risky strategy in terms of pond duration. Although both *Cacosternum platys* and *Strongylopus grayii* were present early in the second season, possibly due to consistent early rains, the ability of a species to survive in a temporary pond implies that in some dry years they will be at risk from the pond drying. *Cacosternum* differs markedly from *Strongylopus* in the time of first pond entry in the first year (2003), (some 14 weeks later), but the same question is therefore even more pertinent: "Can tadpoles of *Cacosternum platys* reach metamorphosis quicker under conditions of a drying pond?"

5.2 Methods

The experimental design was to rear tadpoles from stage 25 to metamorphosis (stage 42), in experimental containers exposed to near natural conditions. In the control group the water level was kept constant, while in the experimental group the water level was decreased by 10% of the original depth per week. Tadpoles of both *S. grayii* and *C. platys* were taken from an adjacent pond for this experiment.

Strongylopus grayii tadpoles at stage 25 were collected from a pond adjacent to the study pond. *Cacosternum platys* tadpoles were reared from eggs produced by two pairs taken from a pond adjacent to the study pond. The eggs were mixed, and the tadpoles were grown to stage 25 (Gosner 1960) in the laboratory at 20°C which took 14 days.

10 experimental containers (22 l plastic basins) were each filled with 20 l of pond water. Small holes were drilled at water level to prevent the water level rising during rain. These were placed at the edge of the study pond. Five tadpoles of *S. grayii* were allocated to each of five containers (25 June 2004), and five tadpoles of *C. platys* likewise on 25 July 2004. The water level was reduced by about 10% per week by removing 2 l of water from each container. After each removal of water, new holes were made to maintain the water at that level in the event of rain. Each container was covered with a layer of shade cloth to prevent predation and disturbance of the water level by larger animals.

10 control containers were set up in the same way, with five tadpoles of *S. grayii* allocated to each of five controls, and five tadpoles of *C. platys* to each of five controls. These were placed at the edge of the study pond. The design of the experiment is shown in Fig. 5.1

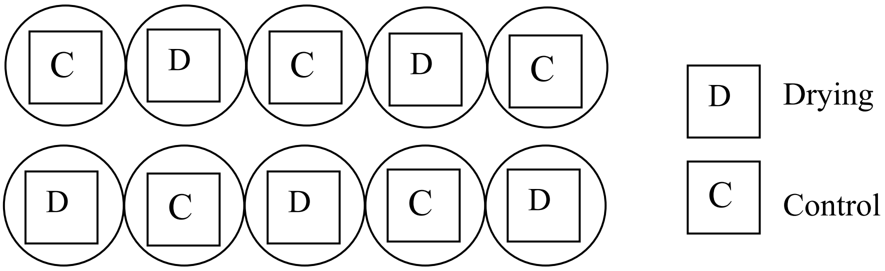

Fig. 5.1. The experimental layout for a single species

The tadpoles in the experimental and control containers were maintained by replacing 1 litre of water with the equivalent of fresh water from the study pond each week. Fish food (Nutrafin) (0.5 g) was added to each container once a week. Mortalities were not replaced and food amount was adjusted accordingly.

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The temperature range during both experiments ranged from 16 to 23°C.

The time taken to reach metamorphosis, defined as stage 42 (Gosner 1960), and survival, was recorded.

5.3 Results

5.3.1 *Strongylopus grayii*

The first *S. grayii* tadpole metamorphosed from the drying treatment 76 days after the start of the experiment. All of the tadpoles had metamorphosed within 104 days for the drying treatment and the control. In the controls 20 of 25 tadpoles survived to stage 42 (Gosner 1960) and in the experimental treatments 16 of 25 survived to metamorphosis. There was no significant difference in time to metamorphosis (ANOVA, F= 0.5, p= 0.5). The results show that although the mean time to metamorphosis is two days shorter in the experimental group, *S. grayii* tadpoles do not speed up their metamorphosis in this drying environment (Fig. 5.2).

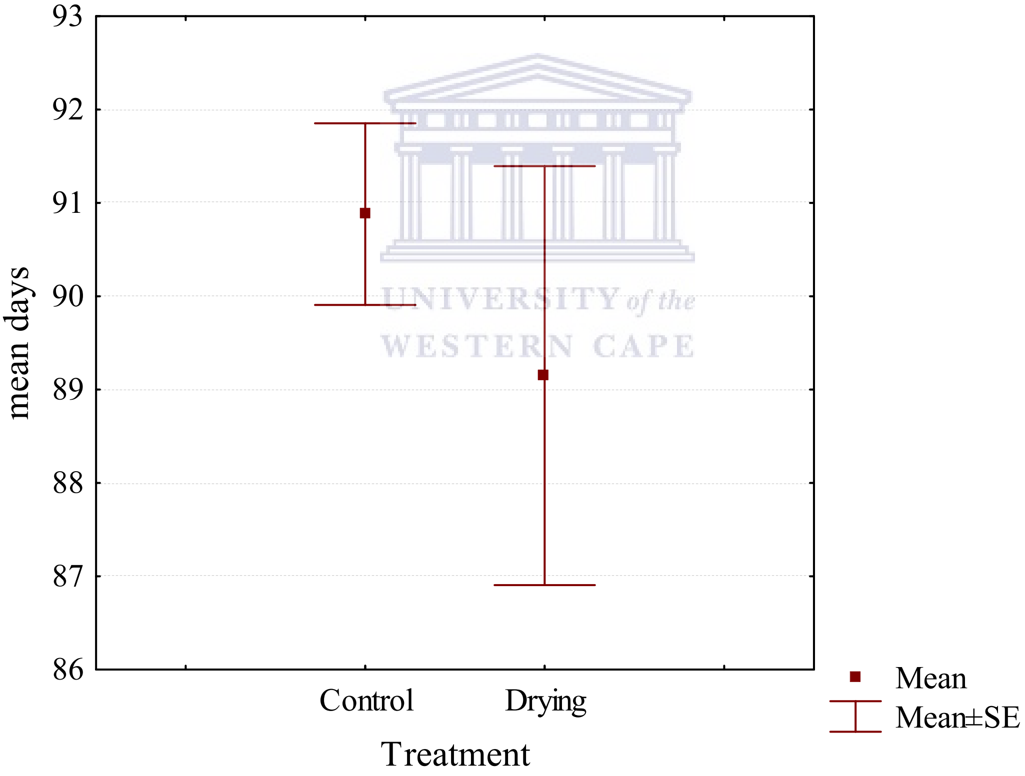

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Fig. 5.2. The mean time to metamorphosis for tadpoles of *S. grayii* in drying conditions and the control

5.3.2 *Cacosternum platys*

The first tadpole metamorphosed from the experimental drying treatment 74 days after the start of the experiment. All of the tadpoles had metamorphosed within 97 days for the experimental and control treatments. In both the controls and the experiments 22 of 25 tadpoles survived. Although the mean time to metamorphosis in the experimental group is three days shorter (Fig. 5.3), the results of the ANOVA showed no significant difference in time to metamorphosis between the two treatments (F= 1.249, p= 0.3).

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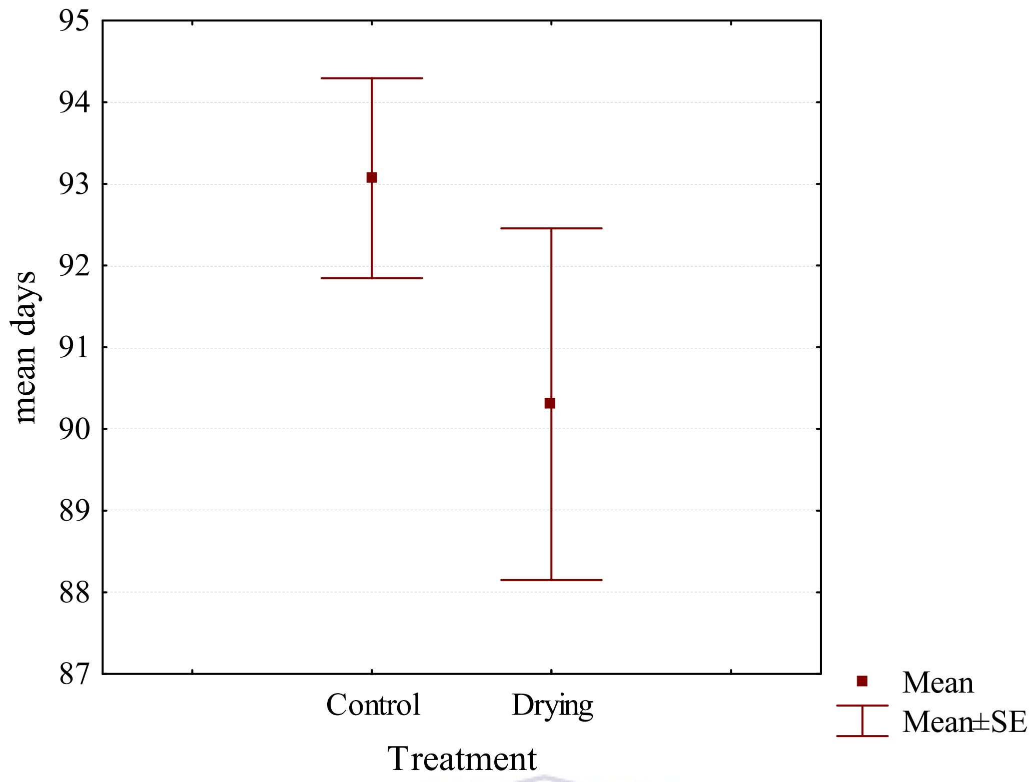

Fig. 5.3. The mean time to metamorphosis for tadpoles of *C. platys* in drying conditions and the control.

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5.4 Discussion

5.4.1 Cues used by tadpoles

Tadpoles may adjust the time to metamorphosis under drying conditions. The cues used might include one or more of the following: water depth, increasing tadpole density (visual or physical encounters increase), water temperature of the drying pond may be less stable, waste product concentrations may increase, or some other chemical cue produced by the tadpoles. This experiment attempts to mimic a real situation, by placing tadpoles in the same environment, but "forcing" the drying by removing water. Rain was allowed to enter the pond as this was part of the natural environment, but the water level was controlled.

5.4.2 Drying has no effect

Neither *S. grayii* nor *C. platys* tadpoles reduced their time to metamorphosis under drying conditions. This has also been reported in *Hyla cinerea* and *Hyla gratiosa* where a similar drying environment did not speed up metamorphosis. Tanks were drained at three different rates and in drying treatments the tadpoles took longer to metamorphose than in the controls (Leips et al. 2000). Some tadpoles, however, do speed up their metamorphosis in reaction to a drying environment. For example, *Lithobates blairi*, *Lithobates shenocephalus*, *Crinia signifera* and *Rana temporaria* all have this ability (Lane and Mahoney 2002, Laurila and Kujasalo 1999, Parris 2000).

It is advantageous for tadpoles to be able to speed up metamorphosis in an unpredictable environment. It would be beneficial to alter the time spent in the water to make use of the time that the water is present and the environment is profitable. Leips et al. (2000) suggests that it is not just the drying that will cause the tadpole to have the ability to speed up its metamorphosis but also the predictability of the inundation. As the Kenilworth racecourse ponds fill every year, as far as I can ascertain, the environment that the tadpoles live in, although temporary, is still predictable.

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The rate of drying in this experiment was based on the middle of three rates used in a similar experiment by Leips et al. (2000). It is possible that under faster rates of drying, the study pond tadpoles might metamorphose sooner. However, that study remains to be undertaken.

The rates of development in laboratory conditions are generally slower than in the field (Channing 2001). This experiment was set up to investigate if two species were capable of reducing the time to metamorphosis under drying conditions.

5.4.3 *Strongylopus grayii* breeding strategy

Strongylopus grayii deposits eggs early in the season, above the existing water level, and often around an empty pond. The eggs continue to develop, but the tadpoles are only released once the water level rises and floods the eggs (Channing 2001). This mechanism permits them to make use of the very first ponds formed. The study pond is present for long enough that multiple clutches can develop. In 2004 at least 3 breeding events took place (Fig. 2.3 and 2.4). The fact that the species breeds more than once indicates that *S. grayii* is well adapted to make use of the temporary ponds in the Western Cape. Wager (1965) reported that KwaZulu-Natal specimens may take “three or four months, occasionally longer”. The present results show that specimens from the south-western Cape have similar developmental times

5.4.4 *Cacosternum platys* breeding strategy

Cacosternum platys deposits small numbers of eggs just below water level attached to vegetation. The eggs developed rapidly in the laboratory to stage 25 tadpoles, taking around 14 days. Early stage 25 tadpoles were trapped in the pond in low numbers from the end of June 2004, but the numbers only increased sufficiently for more developed stages to be trapped from late September 2004 (Chapter 2, Fig. 2.5). The closely related *Cacosternum nanum* from KwaZulu-Natal develops rapidly, taking only 17 days from egg to froglet (Wager 1986). Such rapidly developing tadpoles may be under-sampled when a cohort is present for 17 days or less, although my weekly sampling regime would have at least two opportunities to trap members of each cohort. However, the drying experiment showed that the south-western Cape *Cacosternum platys* tadpoles required a mean of three months from stage 25 to metamorphosis. The time taken for the *Strongylopus* tadpoles to develop was exactly as reported in the literature, suggesting that the developmental time I found for *Cacosternum* is normal.

Two possibilities exist to explain the anomaly. The KwaZulu-Natal species reported may have developed in water considerably warmer that that in the winter ponds in the south-western Cape, suggesting that the Cape species require more time to develop. Alternatively, there may be an error in the literature. *Cacosternum boettgeri* from Pietermaritzburg in KwaZulu-Natal has been reared by D.E. van Dijk (pers. com.) who confirms the short time from egg to metamorph.

Although *Cacosternum* may start to breed early in the season, large numbers of tadpoles are only present much later, when the risk of pond drying has increased. The lower temperatures of the south-western Cape, compared to those in the tropics, would slow development further. Further studies of the breeding strategies of all the species of dainty frogs are required to explain this interesting difference.

5.4.5 Implications for future climate change

Climate change predictions for the Western Cape where the pond is situated suggest that the rainy season will get shorter (Midgley et al. 2005). If pond duration decreases due to climate change, *S. grayii* will still be able to breed once or twice, rather than the three times reported here. *Cacosternum platys*, on the other hand might be unable to produce the surge of tadpoles seen in the present study, and the species would be dependent on the low numbers of tadpoles produced in the early part of the season.

The experiment showed that both *Strongylopus grayii* and *Cacosternum platys* require about three months to reach metamorphosis from stage 25 tadpoles. Neither can speed up development under the experimental drying conditions. Further work on the breeding strategies of other winter rainfall species is required, in order to understand the implications of the predicted reduction in precipitation caused by climate change (Midgley et al. 2005).

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CHAPTER 6: FOOD SELECTION AND COMPETITION IN A TEMPERATE POND

6.1 Introduction

The tadpole stage must exploit the habitat available while it is favourable for growth and then exit as conditions become less beneficial. Tadpoles in temporary ponds must grow quickly to complete metamorphosis before the pond has dried. The length of time it takes for a tadpole to metamorphose is dependent on a number of variables such as predation, competition, food availability and water temperature. The amount of food a tadpole consumes directly affects its growth (Kiffney and Richardson 2001) and the quality of food consumed affects the speed of growth (Brown and Rosati 1997, Kupferberg et al. 1994).

When *Xenopus laevis* tadpoles were fed different types of shop-bought pet food the survival of the tadpoles did not change with the differing treatments but the weight of the tadpole was significantly different depending on what they were fed (Brown and Rosati 1997). The type of food altered the growth of the tadpoles of *Pseudacris regilla* as well as the time it took them to reach metamorphosis and their size at metamorphosis when tadpoles were fed on more natural diets such as filamentous green algae, with a poor quality diet causing the tadpole to metamorphose at a smaller size (Kupferberg et al. 1994).

Tadpoles alter their feeding behaviour in the presence of certain predators and conspecifics. When predators are present tadpoles decrease their activity to avoid detection and in so doing reduce the time they spend feeding (Feminella and Hawkins 1994). However, predation risk increased the growth of *Lithobates catesbeianus;* this is possibly due to the predator excreting nutrients into the water or the fact that tadpoles decrease their activity, which may allow more energy to be used for growth (Peacor 2002). When other tadpoles are present competition can occur which could decrease the amount of food available to the tadpole (Browne et al. 2003, Newman 1994, Semlitch and Caldwell 1982).

Most tadpoles are opportunistic omnivores or detritivores taking food from the water column, substrate and pond sediments, but some tadpoles are oophagous, necrophagous or cannibalistic (Hoff et al. 1999).

The majority of tadpoles have an oral disk with keratinised jaw sheaths, rows of labial teeth and marginal papillae. The tadpole uses the papillae to guide the food to the mouth and then the food is chopped into small pieces using the keratinised jaws. The labial teeth are used for scraping food from surfaces (Hoff et al. 1999). As the tadpole moves water in through the mouth, and out through the spiracle, suitable food particles are trapped on mucus on the internal gill filaments (Hoff et al. 1999). The range of mouthparts is highly diverse in shape, structure and position, as a result of phylogeny and/or adaptation to feeding habits or food types.

Tadpoles may partition the food environment. Bottom dwellers often have a depressed body and feed on detritus whereas the tadpoles in the water column have large dorsal tails and feed on algae or phanerogams (Diaz-Paniagua 1985). For example, the tadpoles of *Rana temporaria* have food from the sediment in their gut contents whereas "*Bufo" bufo* take food associated with the water column (Harrison 1987).

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The ability of south-western Cape tadpoles to partition a habitat and reduce competition by varying their feeding modes was tested in a large temporary pond in Kenilworth racecourse. There are five species of tadpole in the pond at Kenilworth racecourse but owing to the low numbers of two of the species (*Microbatrachella capensis* and *Tomopterna delalandii*) analyses were carried out on the three most abundant species (*Cacosternum platys, Strongylopus grayii* and *Xenopus laevis*). Gut content analyses were carried out along with analyses of potential food items in the water. Four of the tadpoles in the pond (*Strongylopus grayii, Cacosternum platys, Tomopterna delalandii* and *Microbatrachella capensis*) are typical pond tadpoles with keratinised jaws and labial papillae. The *Xenopus laevis* tadpoles have a different mouth shape and position. The mouth is a terminal slit with no keratinised jaw or labial teeth. Tadpoles of different species are often morphologically different and feed on different food items to reduce competition in single water bodies (Harrison 1987, Diaz-Paniagua 1985). Even though the tadpoles at Kenilworth racecourse are similar morphologically, except for *X. laevis*, they may be able to reduce competition by feeding on different items in the water.

The aim of this experiment was to investigate the feeding biology of the three most abundant tadpole species.

6.2 Methods

The pond filled in May 2005 and tadpoles were collected weekly as soon as they were present. Approximately five tadpoles from each species were preserved each week. Two water samples were taken from the pond from the area that the tadpoles were caught weekly; one was preserved with 5% formalin and the other with Lugols solution. Each tadpole gut was dissected out and the contents were stained for 24 hours using Bengal Red and Sienhorst solution, before they were placed on a microscope slide over a grid. The first 100 grid crosses were examined under a microscope (100 x magnification), and the contents were identified. Gut contents were categorised as Single-celled Algae (e.g. *Cryptomonas* spp., *Cyclotella* spp., *Closteriopsis* spp.), Colonial Algae (e.g. *Oocystis* spp., *Sphaerocystis* spp.), Filamentous Algae (e.g. *Anabaena* spp., *Melosira* spp.), Higher Plant Fragments, Animal or Other. Objects such as grit and sand were placed in the category "other". Bacteria were not estimated, although they have been shown to be used as nutrition in some cases (Burke 1933). The water samples were spun in a centrifuge for 5 minutes at 2000 rpm and then placed on a slide and examined in the same way as the gut contents. A Kruskal-Wallis ANOVA was used to test for differences.

6.3 Results

The results are based on 32,800 data points (328 samples each of 100 points). Only three species of tadpoles were present in sufficient numbers for a statistical analysis to be undertaken; *Xenopus laevis*, *Strongylopus grayii* and *Cacosternum platys*. The proportions of different food items, and the significant differences, are presented in Appendix 8.

The first set of tadpoles and water was taken from the pond on the 16th of May 2005. The only tadpoles present were *S. grayii*. *X. laevis* tadpoles were collected from the pond on the 10th of June and *C. platys* tadpoles were only present from the 10th of August. The last tadpoles were sampled in the week of 8th November 2005.

6.3.1 Availability of food in the water column

The availability of food, using the categories above, is presented for the water column in Fig. 6.1

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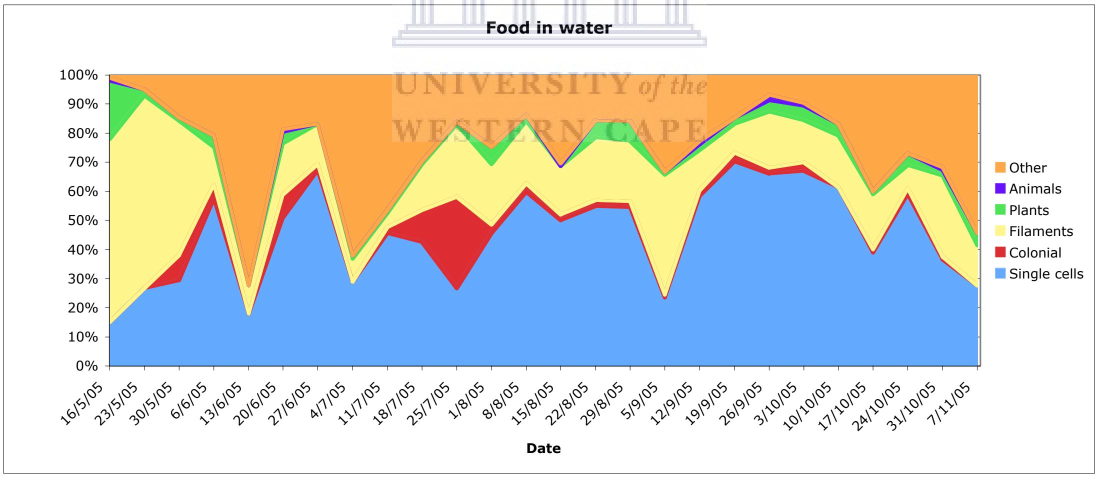

Fig. 6.1. Proportions of food items present in the water column through the 2005 season.

Filamentous algae are dominant in the pond for the first few weeks, before there are many tadpoles. They show a marked decrease after tadpoles enter the pond, and then constitute roughly 15% of all items until the end of the season.

Higher plant fragments are initially also common and are present as a small proportion, around 5%, for the duration of the season.

Single-celled algae are present at the start of the season around 15%, rapidly becoming the dominant food item in the pond, with levels up to 65%.

Colonial algae are less common, only recorded at low levels through the season, around 5%, except for a two-week bloom at the end of July and the first week in August.

Much of the water content appears to be debris and dust, here categorised as “Other”, which fluctuates inversely to the algae as these populations grow rapidly, or are consumed, or die off.

Filamentous and single-celled algae make up the largest proportion of possible tadpole food through the season.

6.3.2 Tadpole diet

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Xenopus laevis

The proportion of items taken by these tadpoles is presented in Fig. 6.2.

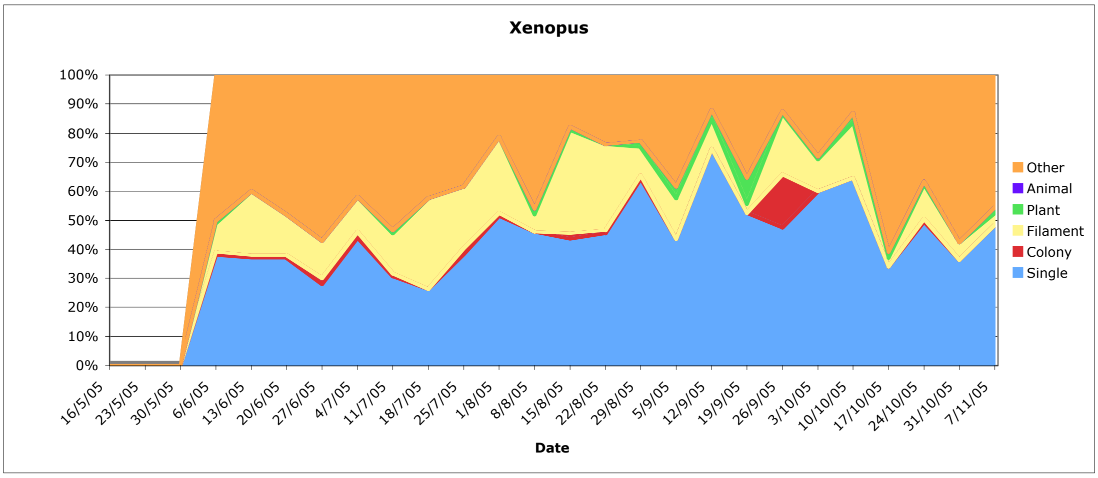

Fig. 6.2. Proportion of items in the guts of 114 tadpoles of *Xenopus laevis*.

Initially, *X. laevis* tadpoles consumed mostly single-celled algae. Throughout the season they continued to feed mainly on single-celled algae, even when these formed a smaller proportion of the available food during the filamentous algal bloom in the first week of September. Filamentous algae were consumed in smaller proportions, but they also made up a smaller proportion of the available items (Fig. 6.1). During the last week of September they consumed relatively more filamentous algae. Animal fragments are only taken in very small numbers. The species was selecting for "other" in the week of 20 June 2005 (p=0.05), but for the rest of the season the proportions of items in the gut are not significantly different from that in the pond water.

<u>*Strongylopus grayii*</u>

The proportions of items in the guts of 99 tadpoles are shown in Fig. 6.3

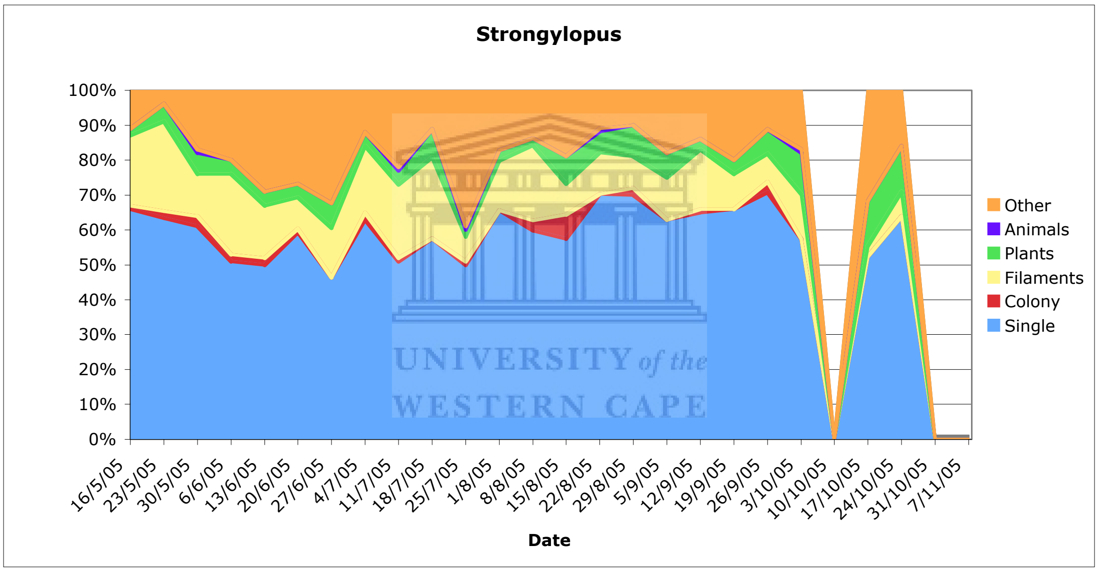

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Fig. 6.3. Proportions of items in the guts of *Strongylopus grayii* tadpoles through the season. No tadpoles were caught in the week of 10 October.

Strongylopus grayii tadpoles took mostly single-celled algae, at levels of up to 70%. Even in the first weeks after the pond filled, when there were large proportions of filamentous algae (around 65%), these tadpoles were eating mostly single-celled algae. They do consume filamentous algae, however, which make up about 15% of the diet throughout the year. This species also took small amounts of colonial algae, which increased for two or three weeks after the bloom of colonial algae in the pond at the end of July. Remarkably, this species also took small but consistent proportions of higher plant remains, even when these were present in relatively small proportions in the water column. Animal fragments were found in very small numbers, similar to the low proportions in which they occur in the pond water.

This species selected for single-celled algae (p=0.026) and "other" (p=0.046) in the middle of June 2005, and for single-celled algae again in the week of 5 September 2005 (p=0.03) (Appendix 8).

Cacosternum platys

The proportions of the gut contents of 70 tadpoles, taken through the season, are presented in Fig. 6.4

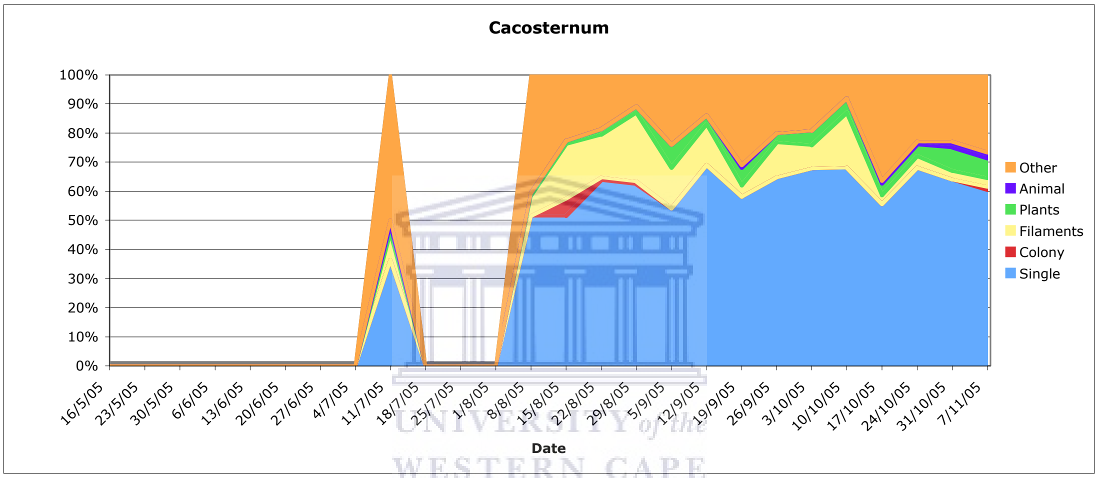

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Fig. 6.4. Analysis of the gut contents of *Cacosternum platys* tadpoles

The first record for this species is the week of 11 July 2005, when the proportions of items in the gut reflect almost exactly those in the pond (Fig. 6.1). The items in the gut for the rest of the season continue this trend. During the last two weeks of the season this species took a larger proportion of animal fragments than previously throughout the season. This species did not show any significant selection of food items from the pond. The contents of their guts did not differ significantly from the contents of the water for the duration of the season (Appendix 8).

6.3.3 Competition between species and food resource partitioning

Xenopus laevis vs. *Strongylopus grayii*

For the most part, these two species do not consume significantly different proportions of food items (12 of the 20 weeks when they are both present in the pond). These two species are therefore competing for the same food resource for much of the season.

These two species can be shown to be partitioning the items in the pond when they are taking significantly different proportions. This happened for only 13 comparisons in 8 of the 20 weeks of the season when both species were present in the pond. These differences relate to the following food items (see Appendix 8):
Single-celled algae (*S. grayii* consuming more than *X. laevis*) – 4 cases
Higher plants (*S. grayii* consuming more than *X. laevis*) – 5 cases
Other (*S. grayii* consuming less than *X. laevis*) – 4 cases

Xenopus laevis vs. *Cacosternum platys*
These two species were collected together for 15 weeks. During this time there were 13 weeks when there was no significant difference in their diet. They are therefore competing for the available food resource.

These two species were taking significantly different proportions of some items in 2 of the 15 weeks that they were in the pond together. These differences were:
Single-celled algae (*C. platys* taking more than *X. laevis*) – 2 cases
Higher plant matter (*C. platys* consuming more than *X. laevis*) 1 case
Other (*C. platys* consuming less than *X. laevis*) – 1 case

Strongylopus grayii vs. *Cacosternum platys*
During the 12 weeks these species co-existed in the pond there was only one significant difference in items consumed.
Plant matter (*C. platys* consumed less than *S. grayii*). These two species thus hardly partition the food resource. They are therefore potential competitors for the food resource.

6.4 Discussion

This study has shown some of the complexity of the feeding relationships in a pond, by examining both the availability of potential food items, and the proportions in which these are taken by the three common species of tadpoles. This can be illustrated by the following.

6.4.1 Availability of food in the water column

The factors that are interleaved include the physical conditions as they relate to algal growth, such as the temperature, sunlight pattern, and stability of the pond. Rainfall is a major factor, not just for filling and maintaining the pond, but also as it is associated with blooms of colonial and filamentous algae (Ling 1938, Rao 1955). The peak in rainfall for 2005 occurred in August, when over 150 mm was recorded (Fig. 3.5). But there were large rainfall events throughout the winter. It is not the intention here to ascribe causality, but it is apparent that environmental factors such as temperature, rainfall, and amount of sunlight play a role in the growth and reproduction of these microscopic algae.

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Plant fragments are initially also common, probably as remnants from the previous season.

Before any consumers play a role, there is a vibrant, dynamic pond system with constantly changing proportions of algal populations. The role of tadpoles in consuming algae was not examined experimentally, but the fluctuations in tadpole numbers seen in 2003, and 2004 (Figs. 2.2, 2.3), indicates they may play a role in controlling algal populations, with high numbers of tadpoles potentially able to consume large numbers of algae.

6.4.2 Tadpole diet

The major rainfall events serve to cue the amphibians for breeding, with eggs being laid during and after each event. However, the system is not simple, as *Strongylopus grayii* will deposit eggs when the temperature drops preceding a cold frontal system, long before there is any rain. The eggs develop and tadpoles are released as soon as there is water in the pond (Channing 2001). *Cacosternum platys*, on the other hand, will breed in low numbers initially, waiting for a really large rain before many pairs arrive to breed (Channing 2001). The tadpole consumers thus arrive at the pond at different times, and in various numbers. Each tadpole takes about three months to reach metamorphosis and leave the pond, but during that time many other breeding events will have happened. The pond is occupied by cohorts of tadpoles of various ages, mostly competing for the same food. One view from conventional wisdom is that mid-water filter feeders (e.g. *Xenopus*) take mostly single celled and other algae without any selection, while other tadpoles with hard mouthparts will rasp plant matter to some degree, or feed on debris and small algae found in and on the substrate of a pond. Tadpoles of *S. grayii* possess hardened mouthparts, and would be expected to consume more plant and animal matter than *X. laevis*, but the data show that they actually consume more single-celled algae than *X. laevis*. This latter species can be likened to a vacuum cleaner, as the tadpoles are unable to rasp away material, and must rely on small particles such as algae that are present in the water column.

Much dust and other debris is blown into the pond, which may account for this constituting about 25% of the items in the pond water. As the numbers of algae fluctuate, or the absolute density changes, then the “other” category fluctuates inversely. *Cacosternum platys* is present in large numbers only at the end of the season, when it assumes the feeding role of *Strongylopus* tadpoles, with which it is similar in size and morphology. It is present until the end of the season, some weeks after the last *Strongylopus* has left the pond. By mid-summer the pond is dry.

Competition for food resources occurs when there are sufficient numbers of tadpoles to affect other species using the same food resource. The graphs of relative numbers of tadpoles (Figs. 2.2, 2.3 for 2003, 2004) show that there may be large fluctuations of tadpole numbers, with the possibility of increasing and decreasing competition through the season. Partitioning of the food resource can occur involuntarily if the food is unequally distributed in the pond, and the tadpoles frequent different parts of the pond for reasons not related to feeding. Active partitioning occurs when two species select different proportions of the items present in the pond, when the food is equally distributed. This study shows that on the whole the tadpoles’ guts contain food items in the same proportions as found in the water column. They are acting as indiscriminate suspension feeders as suggested by Wassersug (1974). Diaz-Paniagua

(1985) found that food was not important in niche partitioning between the five species in the Doñana reserve. This would be expected under conditions of high algal populations. The pond at Kenilworth racecourse appears similarly to have a large amount of suitable food for tadpoles.

Further work is required to determine the relative food value of the various components recognised in the pond water. This could be undertaken by breeding pure colonies of algae, and growing tadpoles under these conditions. This is beyond the scope of the present study.

The results of my study contribute to understanding what tadpoles are eating in a natural winter pond, and how common competition is in such a system.

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CHAPTER 7: DO TADPOLES PARTITION FOOD IN A TROPICAL POND? NORTH LUANGWA NATIONAL PARK, ZAMBIA

7.1 Introduction

In northern Zambia there are around 44 frog species (Channing 2001) in two major habitat types, miombo and mopane woodland. The frogs with a tadpole stage make use of the pools and streams that form in the wet season to breed. There is a potential for a large number of different tadpole species to occur in the same water body. Competition for resources such as space or food becomes important when there are many species co-existing in a pond, and these different species may partition their environment to reduce these interactions.

Although tadpoles are often thought of as indiscriminate suspension feeders (Wassersug 1974), *Rana temporaria* and *"Bufo" bufo* had different particles present in their gut contents that could be associated with their position in the pond (Harrison 1987). Bottom dwellers tend to have a depressed tail and eat detritus whereas tadpoles found in the water column had larger dorsal tails and eat algae (Diaz-Paniagua 1985). Diaz-Paniagua (1985) concluded that food is not as important as space in niche partitioning of tadpoles.

Anurans partition their environment to avoid competition in a number of ways. One way is to divide the environment using factors such as water body permanence (Skelly 1996, 1997). One example in the rainforest streams in Borneo sees the 29 species split into four distinct habitat types such as potholes or side pools (Inger et al. 1986). The adults of *Mannophryne trinitatis* from the south of the distribution will select ponds which are tadpole free to deposit eggs; this is presumably to reduce competition (Downie et al. 2001). When tadpoles are placed together they may shift their position to accommodate each other (Waringer-Löschenkohl 1988).

The type of food a tadpole eats affects its mass at metamorphosis and time to metamorphosis even if it does not affect its survival (Kupferberg et al. 1994). These are important factors that will affect the post metamorph's fitness. It would be advantageous for a tadpole to obtain the most food of the best quality available. *X. laevis* and *Lithobates sylvaticus* both move in the water column to a location where the suspended particles are at the greatest concentration (Seale and Wassersug 1979).

7.2 Methods

7.2.1 Study site

The study site was situated on the floodplain of the Luangwa River, Zambia, in the North Luangwa Nature Reserve. The area is centred on 11° 45’ 25” S, 32° 15’ 24” E, at a mean altitude of 790 m. (Fig. 7.1). The area consists of gently undulating flats, on sandy soils with some river clays, with a rocky margin. The vegetation is mopane woodland, with large trees and bushes such as *Marquesia* and *Colophospermum*. Mean monthly temperature in January is between 22.5 and 25.0 °C. Mean annual rainfall in the area is 70 to 80 mm (Ansell 1978), falling mostly in December and January. After rains many small temporary pools form (Fig. 7.2), and after flooding, the Luangwa River leaves behind many small pools.

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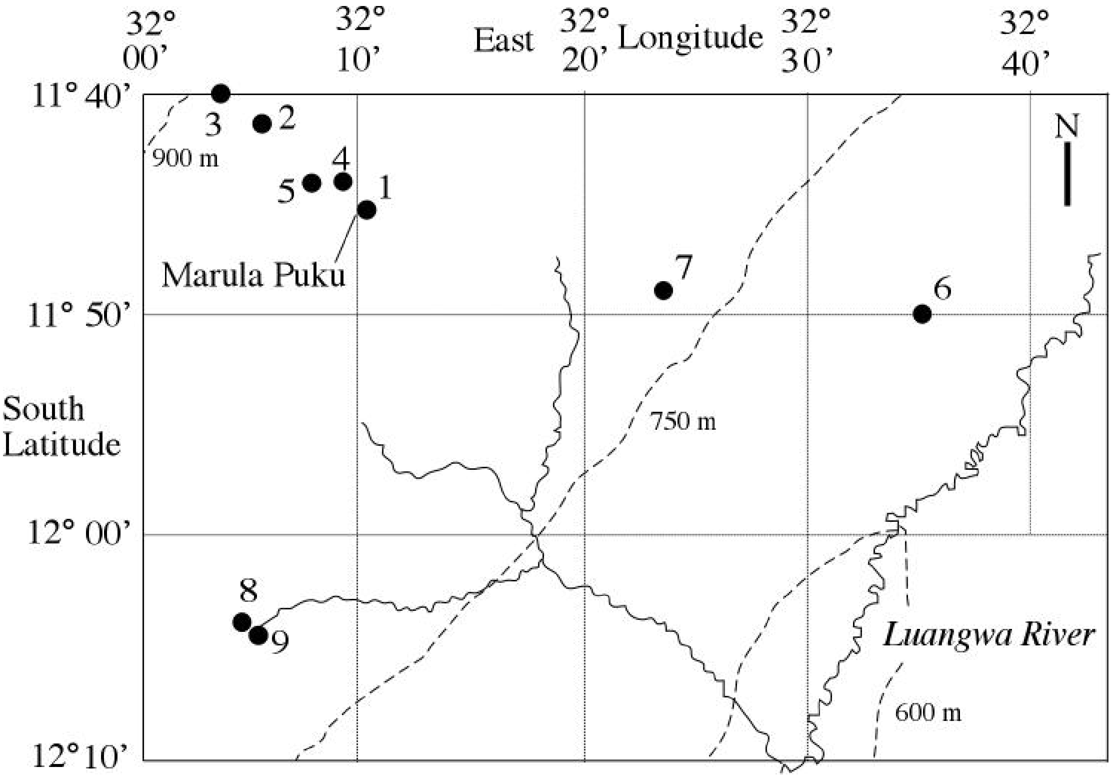

Fig. 7.1. Map of part of the North Luangwa Nature Reserve, showing positions of the nine ponds. Based on satellite imagery (Google Earth 2006).

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Fig. 7.2. Photograph of pond 1 one showing typical vegetation.

Nine small ponds were visited from the 14th to the 17th of January 2005. The position and a brief description of each were noted.

At each pond five tadpoles of each species were collected where possible. Tadpoles were preserved in 10% formalin. Two samples of water were taken from each pond near the tadpoles. One of these was preserved with 10% formalin and the other with Lugols solution.

7.2.2 Tadpole morphology

Each tadpole was measured (body length and whole length), staged, and identified. Tadpole morphology was scored using features that may play a role in determining its position in a pond, and the kind of food it can deal with. The features scored were: Size (as length), tail fin, position of mouth, jaw sheath, and labial tooth rows.

Size may determine how well the tadpole can move through submerged vegetation, and its ability to deal with large plants when feeding.

Tail fin shape is related to the water column; tadpoles occupying deep pools of slow-flowing water have deep tails, while those in shallow water have a generalised tail shape. Tadpoles living in muddy conditions may have reduced tail fins.

Jaw sheaths are hardened with dark keratin; they may be fully or partially keratinised, and can be heavy or lightly built. Modifications of the jaw serrations may be important. Accessory jaw sheaths allow some tadpoles to grind resistant plant material.

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Labial tooth row number is a rough indication of the ability of the larva to scrape food from surfaces. Tadpoles such as *Xenopus* with no hard mouthparts must rely on finding small particles in suspension in the water column. The labial tooth row formula (Altig and McDiarmid 1999) was used: Upper rows are numbered from the lip to the mouth, and lower rows are numbered from mouth posteriorly. Numbers in parentheses indicate rows with medial gaps. A formula of 3 (2-3) / 3 (1) indicates that there are three upper (anterior) rows, with the two rows closest to the mouth (A2, A3) with medial gaps. There are three posterior rows, of which the row closest to the mouth (P1) has a medial gap.

Mouth position is anterior in predatory species, and ventral in bottom dwelling tadpoles.

7.2.3 Tadpole associations

Common species might be expected in all or most of the water bodies, while other species might be quite rare. Do breeding pairs actively select ponds based on the presence or absence of other species breeding there? This question has relevance to tadpole interactions, but is only touched on in the present study. However, pairwise associations were scored for all species collected in the same pond. For each pair of tadpoles an Index of Association was scored, where 0 = no association, and 1 = complete association in all possible ponds. This was calculated as (number of ponds where both species occur / number of ponds occupied by species S_{min}, where S_{min} is the species occupying the smaller number of ponds).

The Expected Association (Proportion of ponds with species Si x proportion of ponds with species Sj) is the probability that two species will co-occur in a pond by chance alone.

A Fisher’s exact test was performed on each tadpole combination for tadpoles present at more than one pond to test these tadpole associations.

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7.2.4 Food items

The tadpole guts were dissected out, and the gut contents were placed in Sienhorst solution and stained using Bengal Red.

The water samples were spun in a centrifuge and the lower phase stained with Bengal Red dye. A sample was placed on a slide under a cover slip and a grid attached to the bottom of the slide. The items present over the first 100 grid crosses were classified and noted, for both the gut contents and water samples. Items were classified into single celled algae, colonial algae, filamentous algae, plant, animal, or other. These are the same categories used in the Kenilworth racecourse pond study in Chapter 6. Differences between tadpole species and between tadpoles and water were tested using a Kruskal-Wallis ANOVA.

7.3 Results

7.3.1 Study ponds

Pond 1 had a diameter of 2.2 m, and a maximum depth of 0.3 m, situated near low-growing shrubs, and surrounded with grass polls, with emergent grass. Five species of tadpoles were collected here: *Afrixalus wittei, Chiromantis xerampelina, Hemisus marmoratus, Leptopelis bocagii,* and *Ptychadena mossambica*.

Pond 2 was in shallow flooded grass, east of the airstrip. Three species of tadpoles were collected: *Hemisus marmoratus, Kassina senegalensis* and *Phrynomantis bifasciatus*. Old nests of *Chiromantis xerampelina* were present overhanging the pond, but no tadpoles were found.

Pond 3 is known as “Elephants’ playground”, and is the most easterly site visited. It is large, with dimensions of 2.5 m x 9.0 m, emptying into a reed-covered swamp. Overhanging trees provided shade. Here tadpoles of the following species were collected: *Amietophrynus maculatus, Chiromantis xerampelina, Hemisus marmoratus, Hyperolius argentovittis, Kassina senegalensis, Leptopelis bocagii,* and *Xenopus muelleri*.

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Pond 4 was a shallow flooded grassy depression, with a diameter of 6 m, and a maximum depth of 0.2 m. The depression was a low point in the surrounding miombo woodland, situated near the airstrip at Marula Puku. Tadpoles of three species were found here: *Hyperolius argentovittis, Kassina senegalensis* and *Phrynomantis bifasciatus*.

Pond 5, east of the airstrip, consisted of a flooded grassy depression, with many water-filled animal tracks. It measured 5 m x 3 m, with a maximum depth of 0.12 m. Four species were present: *Hemisus marmoratus, Kassina senegalensis, Hyperolius argentovittis* and *Chiromantis xerampelina*.

Pond 6 was situated near the Luangwa River. The pond dimensions were 8 m x 2 m, with a maximum depth of 0.3 m. It would have been joined with other nearby ponds after heavy rain. The edges were overgrown with thick grass. Tadpoles of *Hemisus marmoratus, Hyperolius argentovittis* and *Afrixalus wittei* were collected.

Pond 7 was situated on the Luangwa River floodplain. The pond measured 4 m x 3 m, and was 0.4 m deep, with emergent grass. A large bush on one side provided shade. Four tadpole species were collected: *Pyxicephalus adspersus, Pyxicephalus edulis, Hemisus marmoratus* and *Hildebrandtia ornata*.

Pond 8 was a shallow pool in a clearing in miombo woodland. The pond covered 9 m x 6 m, with a maximum depth of 0.3 m. Four species were present: *Ptychadena mossambica, Afrixalus wittei, Hemisus marmoratus* and *Phrynomantis bifasciatus*. Only one tadpole was collected of *P. mossambica.*

Pond 9 was at the edge of a small tributary of the Luangwa River. The substrate was deep mud, and there were trees overhanging the water. Tadpoles of *Hemisus marmoratus, Kassina senegalensis* and *Afrixalus wittei* were present.

The co-ordinates of the ponds, taken with a Garman GPS, are listed in Table 7.1

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Table 7.1 Co-ordinates of the ponds sampled in the North Luangwa Nature Reserve, Zambia.

Pond	South Latitude	East Longitude
1	11° 45’ 39.4”	32° 10’ 22.9”
2	11° 42’ 38.4”	32° 06’ 35.8”
3	11° 40’ 39.1”	32° 04’ 57.2”
4	11° 44’ 28.4”	32° 09’ 20.3”
5	11° 44’ 34.7”	32° 08’ 57.3”
6	11° 50’ 38.3”	32° 35’ 57.8”
7	11° 49’ 33.8”	32° 24’ 49.1”
8	12° 04’ 35.1”	32° 04’ 24.8”
9	12° 04’ 39.1”	32° 04’ 23.8”

The presence of tadpoles in the ponds is summarised in Table 7.2.

Table 7.2 Distribution of tadpole species in the nine ponds sampled

Species	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7	Pond 8	Pond 9	Total
Afrixalus Wittei	X					X		X	X	4
Amietophrynus maculatus			X							1
Chiromantis xerampelina	X		X		X					3
Hemisus marmoratus	X	X	X		X	X	X	X	X	8
Hildebrandtia ornate							X			1
Hyperolius argentovittis			X	X	X	X				4
Kassina senegalensis		X	X	X	X				X	5
Leptopelis bocagii	X		X							2
Phrynomantis bifasciatus		X		X				X		3
Pyxicephalus adspersus							X			1
Pyxicephalus edulis							X			1
Ptychadena mossambica	X									1
Xenopus muelleri			X							1
TOTAL	5	3	7	3	4	3	4	3	3	35

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7.3.2 Tadpole morphology

In total 13 species of anuran tadpoles were present at the nine ponds.

The species of tadpoles present differ in morphology, which may enable them to consume different food, and occupy ponds with different characteristics. The major features of each species are presented in Table 7.3.

Table 7.3 Tadpole morphology summary

Species	Length (mm)	Tail fin	Jaw sheaths	Labial tooth row formula	Mouth position
Afrixalus wittei	16	Medium	Lightly keratinised	0/0	Anterior
Amietophrynus maculatus	17	Narrow	Lightly keratinised	2(2)/3	Anterio-ventral
Chiromantis xerampelina	18	Medium	Lightly keratinised	3(2-3)/3	Anterio-ventral
Hemisus marmoratus	55	Thickened sheath covers front of tail	Lightly keratinised	5(2-5)/4(1)	Anterio-ventral
Hildebrandtia ornate	95	Narrow	Heavy	0/2	Anterior
Hyperolius argentovittis	15	Medium	Lightly keratinised	1/2	Anterio-ventral
Kassina senegalensis	80	Very deep	Heavy	1/2(1)	Anterior
Leptopelis bocagii	17	Medium	Lightly keratinised	4(2-4)/4(1)	Anterio-ventral
Phrynomantis bifasciatus	37	Medium with thin tip	None	None	Anterior
Pyxicephalus adspersus	71	Medium	Lightly keratinised	4(3-4)/3	Anterior
Pyxicephalus edulis	30	Medium	Heavy	2(2)/2	Anterior
Ptychadena mossambica	35	Medium	Medium	2(2)/2	Anterio-ventral
Xenopus muelleri	15	Narrow	None	None	Anterior

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7.3.3 Tadpole associations

Interactions between species of tadpoles are predicated on the tadpoles present in each pond. The thirteen species breeding in the area were found in the following pairwise associations (Table 7.4).

The most common species of tadpole was *Hemisus marmoratus*, which occurred in 8 of the 9 ponds. Some species of tadpoles were only caught in a single pond (*Amietophrynus maculatus, Hildebrandtia ornata, Pyxicephalus adpsersus, P. edulis, Ptychadena mossambica* and *Xenopus muelleri*). *Hildebrandtia ornata,* and both species of *Pyxicephalus* were found together in pond 7.

The expected association and the index of association are presented in Table 7.5.

The Fisher’s exact test showed no significant difference in a tadpole species presence

Table 7.4 Pairwise tadpole associations. Numbers of ponds sharing each association are listed

Afrixalus wittei	*A. w.*											
Bufo maculates	0	*B. m*										
Chiromantis xerampelina	1	1	*C. x*									
Hemisus marmoratus	4	1	3	*H. m*								
Hildebrandtia ornate	0	0	0	1	*H. o*							
Hyperolius argentovittis	1	1	2	3	0	*H. a*						
Kassina senegalensis	1	1	2	4	0	1	*K. s*					
Leptopelis bocagii	1	1	2	2	0	1	1	*L. b*				
Phrynomantis bifasciatus	1	0	0	2	0	1	2	0	*P. b*			
Pyxicephalus adspersus	0	0	0	1	1	0	0	0	0	*P. a*		
Pyxicephalus edulis	0	0	0	1	1	0	0	0	0	1	*P. e*	
Ptychadena mossambica	1	0	1	1	0	0	0	1	0	0	0	*P. m*
Xenopus muelleri	0	1	1	1	0	1	1	1	0	0	0	0

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Table 7.5. Expected Association (above) and Index of Association (below) for pairs of tadpoles.

Afrixalus wittei	*A. w.*											
Bufo Maculates	0.0484 0.00	*B. m*										
Chiromantis xerampelina	0.1452 0.33	0.0363 1.00	*C. x*									
Hemisus marmoratus	0.3872 0.00	0.0979 1.00	0.2937 1.00	*H. m*								
Hildebrandtia ornate	0.0484 0.00	0.0121 0.00	0.0363 0.00	0.0979 1.00	*H. o*							
Hyperolius argentovittis	0.1936 0.25	0.0484 1.00	0.1452 0.67	0.3916 0.75	0.0484 0.00	*H. a*						
Kassina senegalensis	0.2464 0.25	0.0616 1.00	0.1848 0.67	0.4984 0.80	0.0616 0.00	0.0246 0.25	*K. s*					
Leptopelis Bocagii	0.0968 0.50	0.0242 1.00	0.0968 1.00	0.1958 1.00	0.0242 0.00	0.097 0.50	0.1232 0.50	*L. b*				
Phrynomantis bifasciatus	0.1452 0.33	0.0363 0.00	0.1089 0.00	0.2937 0.67	0.0363 0.00	0.145 0.33	0.1848 0.67	0.0726 0.00	*P. b*			
Pyxicephalus adspersus	0.0484 0.00	0.0121 0.00	0.0363 0.00	0.0979 1.00	0.121 1.00	0.0484 0.00	0.0616 0.00	0.0242 0.00	0.0363 0.00	*P. a*		
Pyxicephalus edulis	0.0484 0.00	0.0121 0.00	0.0363 0.00	0.0979 1.00	0.0100 1.00	0.0484 0.00	0.0616 0.00	0.0200 0.00	0.0363 0.00	0.0121 1.00	*P. e*	
Ptychadena mossambica	0.0484 1.00	0.0121 0.00	0.0363 1.00	0.0979 1.00	0.0100 0.00	0.050 0.00	0.0616 0.00	0.0200 1.00	0.0363 0.00	0.0121 0.00	0.0121 0.00	*P. m*
Xenopus Muelleri	0.0484 0.00	0.0121 1.00	0.0363 1.00	0.0979 1.001	0.0100 0.00	0.050 1.00	0.0616 1.00	0.0200 1.00	0.0363 0.00	0.0121 0.00	0.0121 0.00	0.0121 0.00

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7.3.4 Food items

The proportion of items in the water column and the guts of the tadpoles sampled, from each of the nine ponds, are presented in Table 7.6. Significant differences between proportions of food items are indicated in bold. The results are based on 14,000 data points. Out of all of the gut items and different species tested only six times do the contents of one species guts differ significantly from the contents of another. There is only one occasion where the contents of a tadpoles gut differs significantly from the water column and that is in pond eight and *Phrynomantis bifaciatus*

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Table 7.6. The proportion of items in the guts of the tadpoles and in the water column in each of the nine ponds

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Pond	Sample	N	Single celled algae mean	Colonial algae mean	Filamentous algae mean	Plant matter mean	Animal mean	Other mean
Pond 1	Water	1	24	0	26	0	0	50
	Hemisus marmoratus	4	59	0	5	0	0	36
	Leptopelis bocagii	5	**40**	0	11	3	0	46
	Chiromantis xerampelina	5	**65 p=0.02**	0	5	0	0	30
	Afrixalus wittei	1	49	0	5	0	0	46
	Ptychadena mossambica	1	58	0	2	0	0	40
Pond 2	Water	2	29	3	12	0	0	57
	Hemisus marmoratus	3	60	5	11	0	0	24
	Kassina senegalensis	5	50	0	25	2	0	22
	Phrynomantis bifasciatus	5	49	4	22	0	0	24
Pond 3	Water	1	21	0	2	0	0	77
	Hemisus marmoratus	4	47	10	16	0	0	**27**
	Xenopus muelleri	5	23	9	6	0	0	62
	Bufo maculates	4	23	4	4	0	0	**69 p=0.02**
	Chiromantis xerampelina	1	24	0	2	0	0	74
	Hyperolius argentovittis	2	26	2	2	0	0	71
	Kassina senegalensis	1	28	0	1	0	0	71
	Leptopelis bocagii	2	35	8	5	0	0	52
Pond 4	Water	2	48	0	17	0	0	36
	Hyperolius argentovittis	2	42	5	9	0	0	45
	Kassina senegalensis	5	32	3	**58**	1	0	**6**
	Phrynomantis bifasciatus	3	34	3	**8 p=0.04**	0	0	**55 p=0.02**

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Pond 5	Water	1	19	0	3	0	0	78
	Hemisus marmoratus	5	57	1	12	1	0	29
	Kassina senegalensis	2	47	1	37	0	0	16
	Hyperolius argentovittis	2	43	1	12	1	0	40
	Chiromantis xerampelina	1	52	0	8	0	0	40
Pond 6	Water	1	30	0	2	0	0	68
	Hemisus marmoratus	1	36	0	0	1	0	63
	Hyperolius argentovittis	5	40	0	7	0	0	53
	Afrixalus wittei	6	21	0	1	0	0	78
Pond 7	Water	1	27	1	9	1	0	62
	Pyxicephalus adspersus	5	37	1	**8**	0	0	53
	Pyxicephalus edulis	5	35	7	3	0	0	55
	Hemisus marmoratus	5	**33**	1	7	1	0	59
	Hildebrandtia ornate	5	**59 p=0.02**	1	**1 p=0.01**	0	0	39
Pond 8	Water	1	**8**	0	40	1	0	52
	Ptychadena mossambica	1	14	0	27	0	0	59
	Afrixalus wittei	5	31	0	3	0	1	65
	Hemisus marmoratus	5	25	0	27	0	0	48
	Phrynomantis bifasciatus	5	**38 p=0.04**	0	3	0	0	59
Pond 9	Water	2	27	0	4	0	0	70
	Hemisus marmoratus	5	30	0	1	0	0	69
	Kassina senegalensis	1	36	2	2	3	0	57
	Afrixalus wittei	1	36	0	1	0	0	63

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The ponds examined represent a sample of the available water bodies in the area. The nine ponds are similar in their low proportions of higher plant debris and colonial algae, and high proportions of single-celled algae and "other". The ponds differ significantly in the proportion of single celled algae that make up the algal community (χ^2=35.26, p=0.000). Pond four had the greatest proportion of its algal community made up of single celled algae and pond 8 had the lowest. The nine ponds differ in the proportions of filamentous algae, with ponds 1, 4 and 8 having significantly higher proportions of filamentous algae than the other ponds (χ^2=105.93, p=0.000). The portion of other material in the pond water was higher than any other group. There were significantly different proportions in each of the ponds (χ^2=24.85, p=0.002) with particularly high proportions in ponds 3, 5 and 9 (Fig. 7.3).

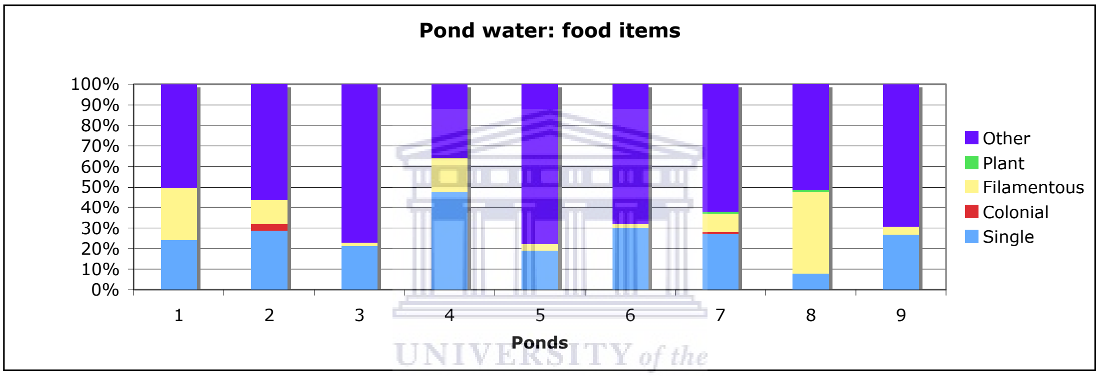

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Fig. 7.3. Food items present in nine ponds sampled on the Luangwa River floodplain and adjacent slopes. Legend: Single = single-celled algae; colonial = colonial algae; filamentous = filamentous algae; plant = higher plant debris.

7.4 Discussion

The visit to North Luangwa National Park was short and only nine ponds could be visited in the amount of time available. There were never less than three genera represented at each site and at pond 3 there were seven different species.

7.4.1 Study ponds

The study ponds represent a sample of the temporary ponds on this part of the floodplain and the lower slopes of the north-western escarpment. The area provides a contrast with the detailed study at Kenilworth racecourse, as there are many more pond breeding species present, and the mean temperature varies from 22 to 25 °C, consistently warmer than the 14 °C during mid-winter in Cape Town. The ponds are turbid but generally well vegetated. The brief visit provided data that serve as counterpoints to the Kenilworth racecourse study.

7.4.2 Tadpole morphology

It is possible to group the tadpole genera caught in Zambia into 7 categories, using their size, mouth position, eye position and jaw type.

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Group 1 includes *Kassina* and *Hildebrandtia* tadpoles. These are both large tadpoles with a terminal mouth. Both tadpoles have eyes on the side of their heads and most significantly, a heavily keratinised jaw suggesting that they could take larger food items or scrape food from surfaces (Channing 2001, Wager 1986). *Hildebrandtia* tadpoles are known to be carnivorous (Rödel 2000)

Group 2 includes *Ptychadena*, *Chiromantis* and *Bufo*. All of these are small to medium in size with a ventral mouth for bottom feeding and eyes on the side of their heads. These tadpoles have a medium keratinised jaw (Wager 1986).

Group 3 includes only *Phrynomantis* tadpoles, as they have a terminal mouth and eyes on the side of their head. These tadpoles filter out particles from the water and have no keratinised mouthparts (Channing 2001).

Group 4 includes *Pyxicephalus* tadpoles. They are large tadpoles with a ventral mouth. This would suggest that these tadpoles scrape food from surfaces. They have eyes on the side of their heads like the previous groups and a medium keratinised jaw (Wager 1986).

Group 5 contains only *Xenopus* tadpoles, with eyes on the top of their heads. These are large tadpoles with a slit of a mouth at the front of the body with no keratinised mouthparts, like *Phrynomantis*. They filter food out of the water column (Wager 1986).

Group 6 includes *Hemisus, Leptopelis* and *Hyperolius* tadpoles. These are all small to medium in size with a ventral mouth, eyes on the side of the head and a lightly keratinised jaw. The mouth position would suggest that the tadpoles take food from a surface but the lightly keratinised jaws imply a certain amount of filter feeding (Wager 1986).

Group 7, the last group contains only *Afrixalus* tadpoles. These are small to medium tadpoles with a mouth at the front end of the body, eyes on the side of the head and lightly keratinised jaws. These filter food from the water column (Wager 1986).

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It would be expected that the differences between gut contents, although few, would be found between species from the different morphological groups. Suggesting either a different feeding mechanism or a different position in the pool of water. Indeed every difference found between gut contents of different tadpole species was between tadpoles of different morphological groups.

Despite the range of morphology in these tadpoles, they show remarkably little food partitioning. Only once, at pond 8, was there a significant difference between the contents of the tadpoles gut contents and the water sample, with *Phrynomantis* selecting single-celled algae above the proportion of these in the water.

The tadpoles of *Rana temporaria* and “*Bufo” bufo* in a single pond contained different algae in their gut contents. *R. temporaria* take food associated with the sediment and *B. bufo* take food associated with the mid-water column (Harrison

1987). The nine ponds examined were very turbid with brown muddy water, which accounts for the high proportion of dust and grit in the guts.

Diaz-Paniagua (1985) suggested that bottom dwelling tadpoles had a depressed body and fed on detritus whereas water column dwellers would have large fins and feed on algae. At the pools in Zambia there were many different types of tadpoles present ranging from *Phrynomantis* and *Xenopus* which have no jaws and *Afrixalus* tadpoles which have very fine delicate jaws to *Kassina* which have thick heavy jaws. The *Hemisus* tadpole is high finned whereas the *Leptopelis* tadpole is slight with thin fins. On the whole these tadpoles did not differ significantly in the proportions of food consumed. I could not recognise any tadpole remains in the guts of the *Hildebrandtia* tadpoles, but they would be expected to digest faster than algal cell walls.

7.4.3 Tadpole associations

The most common species was *Hemisus marmoratus*, which was found in 8 of the 9 ponds. On the other extreme, the heavy-jawed *Hildebrandtia*, known to prey on other tadpoles, was only present in a single pond, along with the widespread *Hemisus marmoratus* (found in 8 of the 9 ponds sampled), *Pyxicephalus adspersus* and *P. edulis*. Both of the *Pyxicephalus* species form schools. It is not possible to determine if the three latter species select the pond because of some preferred quality, such as large volume of water. It is possible that *Hildebrandtia ornata* breeds in ponds in which there are already tadpoles, and the schooling tadpoles of these bullfrogs would be easy to detect. Future research is needed to determine if *Hildebrandtia* spawn in ponds where there are other tadpoles, which might serve as a high-protein food source, or if they select ponds at random.

7.4.4 Food items

All of the tadpole guts collected were full of food.

There is only one significant difference in the proportions of food eaten and the availability of the food in the pond. This difference was between the water column and the tadpoles of *Phrynomantis bifaciatus* in pond 8. There are slightly more differences between the different tadpole species. For example, *Chiromantis* *xerampelina* took significantly more single-celled algae than *Leptopelis bocagii* in pond 1, partly making up the difference by taking less filamentous algae.

Kassina senegalensis tadpoles have heavy keratinised jaw sheaths with lateral accessory plates, enabling them to grind resistant plant material. In pond 4 *Kassina senegalensis* took significantly more filamentous algae than *Phrynomantis bifasciatus*, which has no hard mouthparts and relies entirely on filtering small particles in the water. In pond 7 *Pyxicephalus adspersus* took more filamentous algae than *Hildebrandtia ornata*. However, despite the differences in mouthparts, the tadpoles are largely not partitioning the food, resulting in competition for all food categories.

Tadpoles have little choice in the type of food consumed, as this is determined by extrinsic factors. Apart from competition for food and space, some tadpoles are facultative carnivores, such as *Hildebrandtia ornata* (Rödel 2000). Tadpoles that find themselves in a pond with no potentially carnivorous tadpoles, and as few species as possible that are of similar feeding type (same size and with similar mouthparts), will be at an advantage. The adult frogs determine where to spawn. The selection of breeding pond is beyond the scope of this study, but perhaps pond size and presence of plants play a role.

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Diaz-Paniagua (1985) concluded that food was not a mechanism for niche partitioning in the Donana Reserve tadpoles and it seems to be the same in North Luangwa National Park, Zambia.

7.4.5 Interplay of food and space

The tadpole species, with some few exceptions, do not avoid competition by consuming different proportions of food items. There are many possible pools that the adults can breed in, resulting in a mosaic of different species in pools throughout the floodplain. Although this appears to be almost a random process, not enough is known about adult choice of breeding ponds to evaluate the distribution of tadpoles.

7.4.6 Comparison with the Kenilworth racecourse pond

The five tadpole species in the Kenilworth racecourse pond did not partition the food resource, implying that they were all competing for the algae and other particles. There were some significant differences recorded in the food selection of tadpoles on the Luangwa floodplain. These were rare, however, and depended on the species present in a pond, and their ability to utilize the food. Heavy-jawed species such as *Kassina senegalensis* are able to chew plant stems and deal with filamentous algae, but only when these are present in the pond.

In both the temperate and the tropical environments that were sampled there is little variation in the gut contents of tadpoles of different species from the same water body and little difference between the contents of tadpoles' guts and the contents of the water that they live in. The tadpoles co-exist with others, competing for the same food source.

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CHAPTER 8: THE CRITICAL THERMAL MAXIMA OF *STRONGYLOPUS GRAYII* TADPOLES

8.1 Introduction

8.1.1 Global climate change

Globally, the climate is changing and already these changes have caused distribution shifts in species (Parmesan and Yohe 2003, Root et al. 2003). The most conservative extinction estimates are that 18% of the species in the areas of Central America, Australia, South Africa and Europe will disappear (Thomas et al. 2004).

The earth is warmer now than at any other time in the past 40 million years. There has been a general increase in air temperature of 0.5 °C in the 20th century and minimum and maximum temperatures are increasing all over the globe. The maximum global temperature is increasing at 0.88 °C every 100 years and the minimum global temperature by 1.86 °C every 100 years with the diurnal temperature range generally decreasing by 0.84 °C every 100 years (Easterling et al. 1997).

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In South Africa, the Northern and Western Cape have warmed over the last three decades and the Western Cape will become warmer and drier (Midgley et al. 2005). There will be less winter rainfall and possibly summer rainfall in the east of the Western Cape (Midgley et al. 2005). Since 1967 there has been warming in the minimum temperatures from December to March and then again from July to September and the maximum temperatures have increased in January, May and August (Midgley et al. 2005). Mountainous regions either show little change in rainfall or an increase, but lowlands show a decrease. In general, the predictions for the Western Cape are that it will be warmer with increased summer rain, decreased winter rain and a shortened winter (Midgley et al. 2005). With these rapid changes being brought about species will have to respond in one of three ways, either they will adapt genetically, phenotypically or adjust their physiological state (Helmuth et al. 2005).

8.1.2 Effects of high temperature on ectotherms

Temperature is one of the major environmental factors that affect ectotherms (Angilletta et al. 2002). High temperatures will denature enzymes and perturb ligand binding and may also affect membrane proteins and the cytoskeleton (Bowler 2005). Conversely, individuals must live in a way that keeps their body temperature high enough to carry out metabolic processes. They do this in a number of ways, such as only being present in areas where the temperature is practical or where behavioural thermoregulation is possible (Bradford 1984, Brattstrom 1962, Huey and Kingsolver 1989, Mullally 1953). Temperature affects many different aspects of an ectotherm’s life such as their locomotion (Marvin 2003, Wilson et al. 2000, Wilson and Franklin 2000), heart rate (Chapovetsky and Katz 2003) immune functions, sensory input, foraging ability (Ayers and Shine 1997), courtship (Beebee 1995, Navas and Bevier 2001), feeding and growth (Angilletta et al. 2003, Herried and Kinney 1967, Merilä et al. 2000, Morrison and Hero 2003), metabolic rates (Dutton et al. 1975) as well as their abundance and distribution (Jeffree and Jeffree 1994, Parmesan et al. 1999, Parmesan and Yohe 2003, Pounds et al. 1999, Thomas and Lennon 1999, Walther et al. 2002).

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Anurans, being ectothermic, are probably affected by temperature in all these aspects. Adult frogs can stay below their critical thermal maxima by evaporative cooling (Hoppe 1978) and can stay warm in the cold by basking (Navas 1997). Adult anurans respond to the cold in many ways. In a study by Navas (1997) high elevation frogs that are subjected to frosts and temperature differences of over 40 °C in a 24-hour period were tested. These frogs had a wide performance breadth to cope with the large temperature fluctuations whereas low elevation frogs had a narrow performance breadth. The high altitude frogs had more concentrated haemoglobin and their performance differences were possibly due to increases in mitochondria and changes in the affinity, activity or concentration of enzymes (Navas 1997).

Adult anurans may be able to shift their distributions in line with the changing temperature, or make alterations that will allow them to deal with the changes in temperature and rainfall. However, already the effects of climate change are being seen in amphibian populations. The changes in cloud forest over Monteverde, Costa

Rica are thought to have contributed in the demise of the golden toad (*Ollotis periglenes*) (Pounds et al. 1999). A lift in the cloud forest due to atmospheric warming forced the animals to move into a smaller space and allowed parasites to infect the population (Pounds et al. 1999). An increase in temperature and drought has been correlated with a decline in amphibians in Puerto Rico. Burrowes et al. (2004) studied 11 populations of eight species of *Eleutherodactylus* from 1989 until 2001. Three of the species are now presumed extinct and declines have been correlated with increased periods of drought as well as the pathogen *Batrachochytrium dendrobatidis*. They suggest that the disease is climate linked and when climate change puts the animal under stress and causes the animals to move into a smaller space they will become more susceptible to disease.

8.1.3 Tadpoles

The amphibian larval stage, being mostly confined to water is more vulnerable as it has less opportunity to avoid temperature extremes (Hoppe 1978, Winne and Keck 2005). Even if there is still water present this water may not be at an optimum temperature for these tadpoles or last for the necessary period of time it takes for a tadpole to complete metamorphosis. Many tadpoles rely on shallow temporary water that will be affected if the climate changes; not only in the length of time that the water will be present, or the temperature of the water, but among others, the amount of oxygen that the water contains (Clarke 1993). Climate change may also bring about unforeseen alterations. It may allow modification in vegetation cover (Sturm et al. 2001) and increases in alien vegetation (Bergstrom and Chown 1999, Brown et al. 1997) that could affect the water table that the breeding frogs rely on. Changes in climate may alter the time that amphibians arrive at water bodies to breed. The pond arrival time of *Pelophylax lessonae* and *Triturus vulgaris* is 9 to 10 days earlier with every 1 °C increase in temperature (Beebee 1995). This has been recorded in cases other than anurans in the British Isles. Spring events such as leaf unfolding are getting earlier by about 6 days and autumn events are getting later by roughly 5 days. This has increased the growing season in Europe by nearly 11 days (Menzel and Fabian 1999). In laboratory experiments increasing the temperature can be beneficial to some tadpoles. Tadpoles metamorphose at different rates in different temperatures (Herreid and Kinney 1967, Merilä et al. 2000), developing more slowly and growing to a bigger size in cooler water (Morrison and Hero 2003). Tadpoles select for certain

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temperatures at different stages in development (Dupre and Petranka 1985) and position themselves in optimum temperatures in their natural habitat (Bradford 1984, Brattstrom 1962, Mullally 1953).

8.1.4 Thermal Tolerance

Much is now known about the mechanisms behind thermal tolerance in ectotherms. Phenotypic plasticity makes it possible for individuals to acclimate to gradual changes in temperature. Acclimation is a *within a lifetime adjustment* where constant stress improves the fitness of the animal (Horowitz 2001), and heat shock proteins allow animals to survive sudden increases in temperature for shorter periods of time. The heat shock response is an expression of a set of proteins that will protect the cells from damage. The temperature at which these are switched on is specific to each species and can be altered depending upon stage, growth and acclimation temperature (Barua and Heckathorn 2004, Krebs and Feder 1997). The production of heat shock proteins is expensive to the animal and therefore less is invested in growth or reproduction (Clarke 2003). Phenotypic plasticity is often more important than genetic variability when animals are faced with different temperatures. In a study by Ayrinhac et al. (2004) on cold adaptation in *Drosophila melanogaster;* populations from France and Kenya were tested for cold tolerance after being acclimated at different temperatures and 80% of the variance was due to phenotypic plasticity with only 3.7% due to genetic variation between the populations. However, Hoffmann et al. (2005) reared *D. melanogaster* from eastern Australia under the same conditions and measured their stress resistance (heat being one of the stressors), the populations reacted similarly to the test environments but there were clinal patterns that were independent of the rearing environment.

There are many aspects to the study of the effect of temperature upon ectotherms. Previous studies have looked into animal temperature preference (Bradford 1984, Brattstrom 1962, De Vlaming and Bury 1970, Dupre and Petranka 1985, Noland and Ultsch 1981), the critical thermal maxima (CTM) (Brown 1969, Cupp 1980, Dodd and Cupp 1978, Hoppe 1978, Lagerspetz 2003, Winne and Keck 2005) and minima and their performance at different temperatures (Chapovetsky and Katz 2003, Dutton et al. 1975, John-Alder et al. 1988, Kingsolver and Huey 1998, Navas and Bevier 2001, Van Berkum 1986). The animal’s temperature preference in a controlled environment may not be the same as the temperature the animal experiences in nature as it has other factors acting upon it such as predation pressure and the need to feed (Huey and Kingsolver 1989). The critical thermal maxima is a way of determining thermal requirements and physiology of an organism. There are many different ways of testing for it. Often test animals are heated at a rate that will allow their body temperature to increase at the same rate as the ambient temperature and not so fast that heat shock proteins are produced or so slowly that the animals acclimate (Lutterschmidt and Hutchinson 1997a). In frogs, the animal must have a mass greater than 164.7 g to lag behind a temperature increase of 1 °C a minute (Lutterschmidt and Hutchinson 1997b). An end point is decided upon that is usually loss of righting response, muscular spasms, or heat rigor (Lutterschmidt and Hutchinson 1997b). The CTM of many anuran species has already been determined for adults and larvae although it was suggested that the CTM of adults is just a reflection of the larval stage (Hoppe 1978). In an experiment by Sherman and Levitis (2003) the CTM of different stage tadpoles was tested. The temperature, at which they stopped moving, differed significantly with the more developed tadpoles having a higher maximum temperature. On the other hand the CTM of *Pseudacris triseriata* gets lower with the onset of metamorphosis (Hoppe 1978). In this study animals collected from low altitude (1530 m) sites had a higher CTM than those from high altitude sites (2770 m). As these tadpoles were collected as eggs and reared at the same temperature it can be assumed that this is as a result of genetic variation between the two populations Hoppe (1978). Cupp (1980) found a relationship between geographic range, breeding time and the CTM of five tadpole species. In this experiment the loss of righting reflex as well as the onset of muscular spasms and a lack of movement when prodded were used as the CTM end point. The CTM of the 5 species decreased during metamorphosis and increased again in adults. Noland and Ultsch (1981) examined the roles of temperature and dissolved oxygen on the CTM of *Lithobates pipiens* and *Anaxyrus terrestris*. These tadpoles were placed into stage categories and acclimated at 22, 27 or 32 °C. Increasing the acclimation temperature increased the CTM of the animal. Trade-offs are also a part of temperature tolerance. Frogs adapted to the cold will allocate more resources into muscle structure so that individuals can jump well at low and high temperatures. An example of a frog that does this is *Limnodynastes peronii* (Angilletta et al. 2003).

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A number of anuran species have differing thermal tolerances not just between species but also between stages and populations. *Osteopilus septentrionalis* and *Scaphiopus couchii* were both resistant to high temperatures whereas *Spea hammondii* and *Pseudacris regilla* were not so resistant. This heat resistance is related to previous thermal history and temperature resistance increases with acclimation temperature (Brown 1969).The tadpoles of four different species selected higher temperatures at advanced stages in their development, this selection is to speed up metamorphosis due to drying ponds (Dupre and Petranka 1985). Populations of *Rana temporaria* from North and South Sweden respond differently to variations in temperature. It is possible that these differences are due to genetic divergence (Merillä et al. 2000). Neonates of the diamond back water snake that were born in captivity from 4 different altitudes and differ in their thermal tolerance. These differences are genetic rather than due to acclimating (Winne and Keck 2005).

8.1.5 This study

This study deals with the critical thermal maxima of *Strongylopus grayii*, which is a wide-spread species with populations at high and low altitudes as well as in summer and winter rainfall regions. It must therefore, encounter a wide range of temperatures as it is developing if not on an individual level, at least on a species level.

The aim of this study is three-fold. Firstly, to discern if tadpoles from the warmer northern sites have a higher CTM than the tadpoles from the cooler southern sites as they are exposed to higher temperatures. Secondly, if tadpoles from the low altitude sites will have a higher CTM than the tadpoles from the high altitude sites as they are exposed to higher temperatures. Thirdly, if tadpoles from a summer rainfall region will have a higher CTM than tadpoles from the winter rainfall region of South Africa One of the sites that tadpoles were collected from in the summer rainfall region had a water temperature of 33 °C which is close to the CTM of many of the tadpoles tested here.

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8.2 Methods

Tadpoles of *S. grayii* were collected from 15 sites selected for their different temperature regimes. Three cooler southern sites and three warmer northern sites were visited along with three lower altitude and three higher altitude sites on the same latitudinal line. These were all in the winter rainfall region. After the first heavy rains in the summer rainfall region tadpoles of *S. grayii* were collected from three sites around Graaff-Reinet.

The critical thermal maxima of each tadpole was measured by heating up the water the tadpole was in at 1 °C per minute until the tadpole did not respond to tactile stimulation.

A large pan containing 5 l of water was placed on a gas cooker that was regulated so that the water increased in temperature by 1 °C per minute. Into this pan five wine glasses were placed that each contained 100 ml of water and a single tadpole. The water used was taken from the tadpoles' natural environment. The levels of water in the wine glasses and the pan were approximately equal. The pan water was stirred to avoid hot spots. The tadpole was removed from the warm water after it failed to respond to tactile stimulation with a paint brush, and the temperature was recorded. If the tadpole did not recover after the test, the result was discarded. The CTM of 10 tadpoles was determined in the field at each site and a further ten tadpoles were taken to the laboratory for the following experiment.

Twenty tadpoles (ten tested for their CTM, ten not tested) from each site were taken back to the laboratory and kept at a constant 25 °C (±0.5) for two weeks and the CTM was determined for each tadpole at the end of the acclimation period. This was to test if the CTM was affected by the previous water temperature or was a genetic trait.

The results were tested using a generalised linear model (GLZ) for differences between the sites and species as well as between the field-fresh and acclimated tadpoles.

8.3 Results

8.3.1 Collecting sites

A list of the sites and their locations are given in Table 8.1 and Fig. 8.1.

Table 8.1. Sites from which tadpoles of *Strongylopus grayii* were collected

Site Number	Group	Date visited	South Latitude	East Longitude	Altitude (m)	Ambient water temperature (°C)
1	South	11/6/05	34° 40’971	19° 33’876	25	8.7
2	South	11/6/05	34° 44’793	19° 41’064	11	10.1
3	South	11/6/05	34° 40’623	19° 54’033	28	13.2
4	Low altitude	9/7/05	32° 35’643	19° 00’557	158	10.3
5	Low altitude	9/7/05	32° 48’513	19° 03’361	225	13.4
6	Low altitude	9/7/05	32° 36’006	19° 00’955	172	14.5
7	High altitude	10/7/05	32° 37’280	19° 07’933	851	10.7
8	High altitude	10/7/05	32° 37’500	19° 06’871	731	15.5
9	High altitude	10/7/05	32° 37’711	19° 09’284	951	12.6
10	North	8/8/05	31° 27’001	19° 09’529	680	15.2
11	North	8/8/05	31° 19’163	19° 07’047	644	10.9
12	North	9/8/05	31° 17’376	19° 03’936	731	10.4
13	Summer	20/11/05	31° 57’832	23° 44’713	1177	16.0
14	Summer	20/11/05	32° 02’796	24° 15’697	1386	21.0
15	Summer	20/11/05	32° 31’352	24° 59’600	759	33.0

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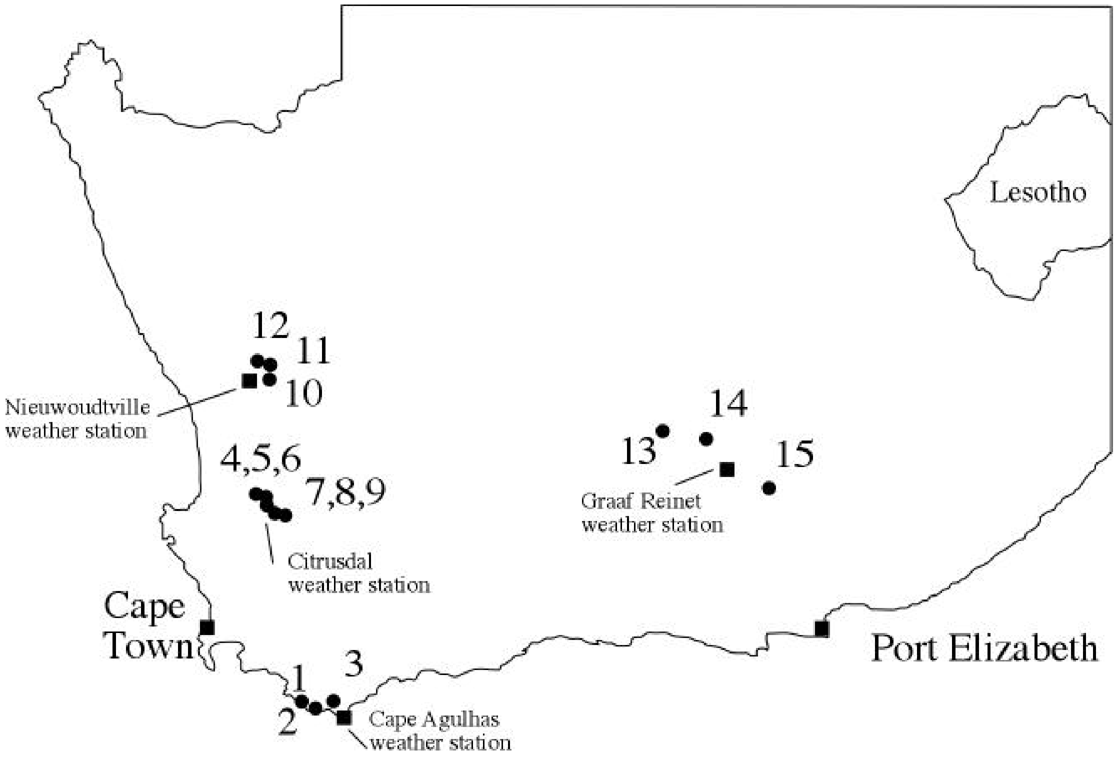

Fig. 8.1. A map of the sites where tadpoles were collected, showing the weather stations. (Data from Garmin Mapsource)

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Figs 8.2-8.5 show weather data applicable to the sites that were visited. Each graph shows the highest and lowest temperature and the rainfall for each month. There are differences between the summer rainfall area temperatures (Graaff-Reinet Fig. 8.5) and the winter rainfall area temperatures (Cape Agulhas Fig. 8.2, Nieuwoudtville Fig. 8.3, Citrusdal Fig. 8.4). Tadpoles were collected in winter for the winter rainfall areas and summer in the summer rainfall areas. The Graaff-Reinet weather station data were used as this is the closest weather station to the sites, although it is lower than the collecting sites and possibly experiences slightly different temperatures.

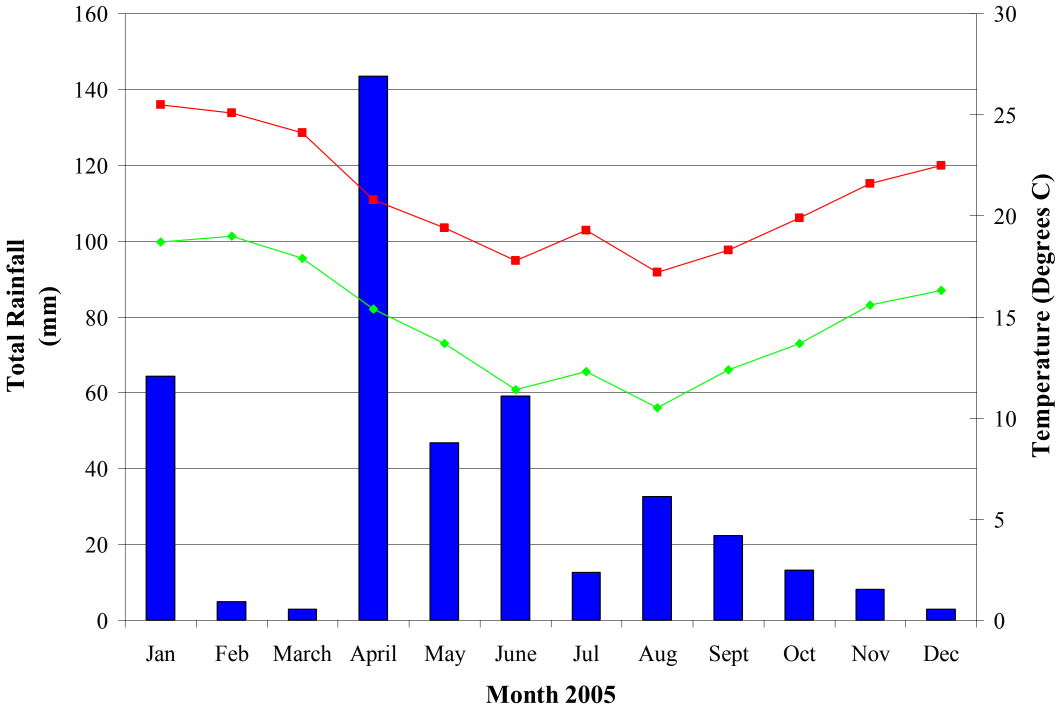

Fig. 8.2. Monthly climate data for Cape Agulhas during 2005. Mean monthly maximum temperature – red. Mean monthly minimum temperature – green. Blue bars show total monthly rainfall. (Data from the South African Weather Service)

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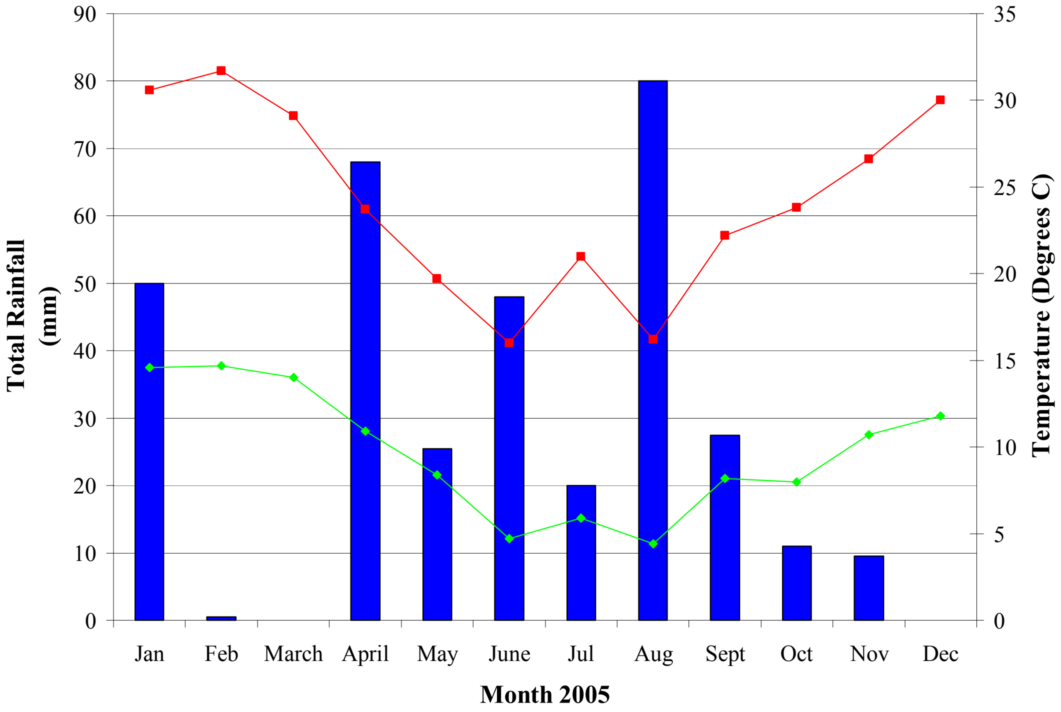

Fig. 8.3. Monthly climate data for Nieuwoudtville during 2005. Mean monthly maximum temperature – red. Mean monthly minimum temperature – green. Blue bars show total monthly rainfall. (Data from the South African Weather Service)

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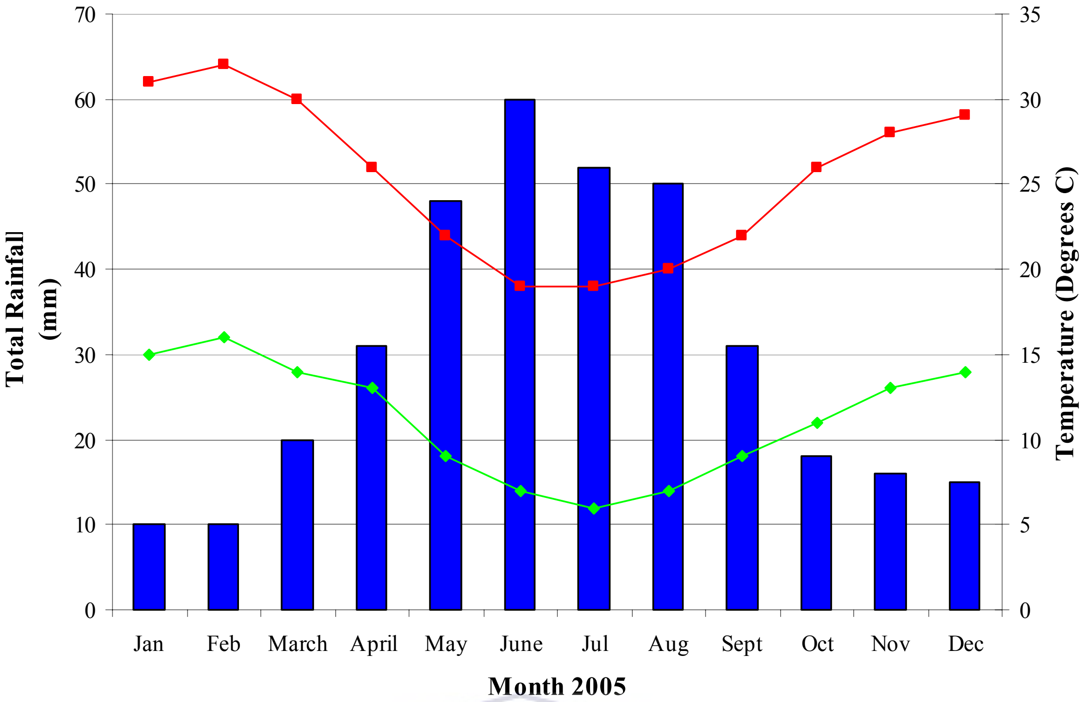

Fig. 8.4. Monthly climate data for Citrusdal during 2005. Mean monthly maximum temperature – red. Mean monthly minimum temperature – green. Blue bars show total monthly rainfall. (Data from the South African Weather Service)

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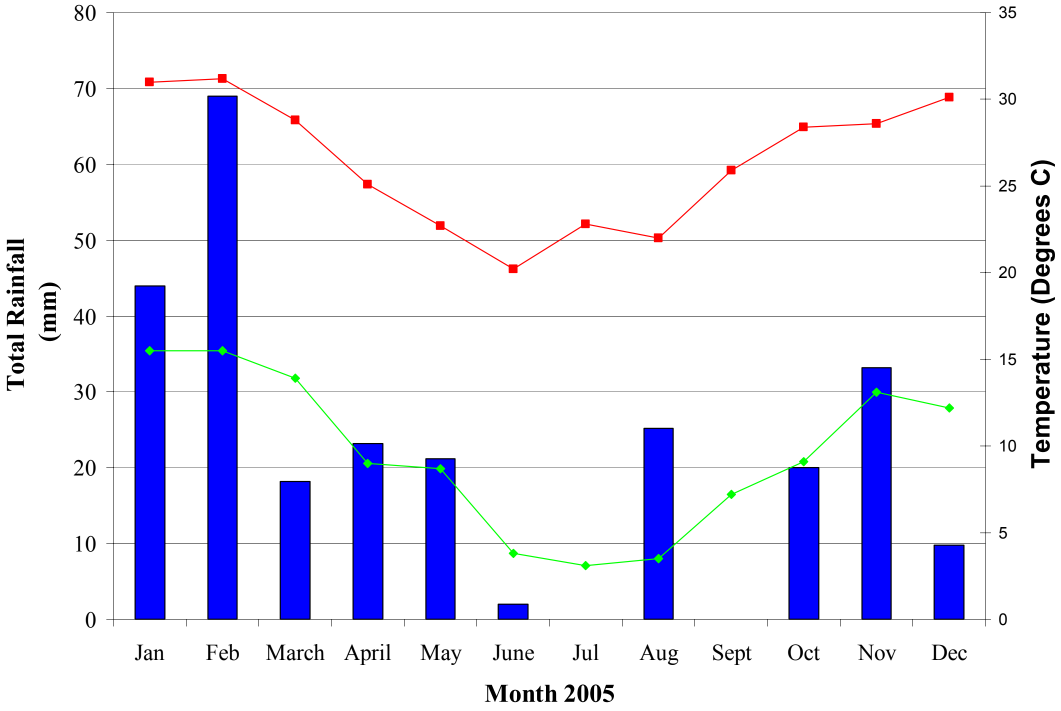

Fig. 8.5. Monthly climate data for Graaff-Reinet during 2005. Mean monthly maximum temperature – red. Mean monthly minimum temperature – green. Blue bars show total monthly rainfall. (Data from the South African Weather Service)

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The results of the field fresh experiments are presented in Table 8.2 for all of the sites.

Table 8.2. Mean field-fresh CTM in °C of tadpoles at each site with number of tadpoles, minimum and maximum temperatures and the range.

Site	n	Mean CTM (ºC)	Min (ºC)	Max (ºC)	Range
1	10	34.60	32.8	35.7	2.9
2	10	34.72	34.1	35.6	1.5
3	10	34.31	32.7	36.4	3.7
4	10	33.78	33.2	34.5	1.3
5	5	34.52	33.2	35.6	2.4
6	10	34.27	32.2	36.6	4.4
7	10	33.70	32.1	34.6	2.5
8	10	33.99	32.4	35.6	3.2
9	10	32.93	32.4	34.0	1.6
10	10	35.59	34.8	35.9	1.1
11	10	34.62	34.4	34.9	0.5
12	10	33.77	32.7	34.6	1.9
13	10	39.52	39.3	39.8	0.5
14	10	38.76	37.5	39.3	1.8
15	10	41.57	40.0	42.9	2.9

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8.3.2 Sites 1-3 (Southern)

At the southern cooler sites (1-3) of the *S. grayii* distribution the mean CTM of field - fresh tadpoles ranged from 34.3 °C to 34.7 °C. When tested with a GLZ the tadpoles from the three sites in the south do not have significantly different CTMs (Wald χ^2 (2) = 1.1 p = 0.6) (Fig. 8.6). When these tadpoles were acclimated at 25 °C the tadpoles that had been previously tested as field-fresh had slightly higher CTMs than the tadpoles that had not been previously tested.

The mean from each of the southern sites when the tadpoles had been acclimated ranged from 35.8 °C to 36.9 °C. The tadpoles that had not previously been tested had CTMs below 36 °C whereas the tadpoles that had already been exposed had CTMs above 36 °C (Fig. 8.6). When this was tested using a GLZ there was a significant difference between previously tested and untested tadpoles (Wald $\chi^2(3) = 14.4$ p = 0.00). The CTMs of the field -fresh and the acclimated tadpoles were significantly different (Wald $\chi^2(6) = 77.2$ p = 0.00) (Fig. 8.6). The field-fresh tadpoles had lower CTMs than the acclimated tadpoles. Acclimating the tadpoles at 25 °C increased the CTM means from ranges of 34.3 °C to 34.7 °C up to 35.8˚C to 36.9˚C.

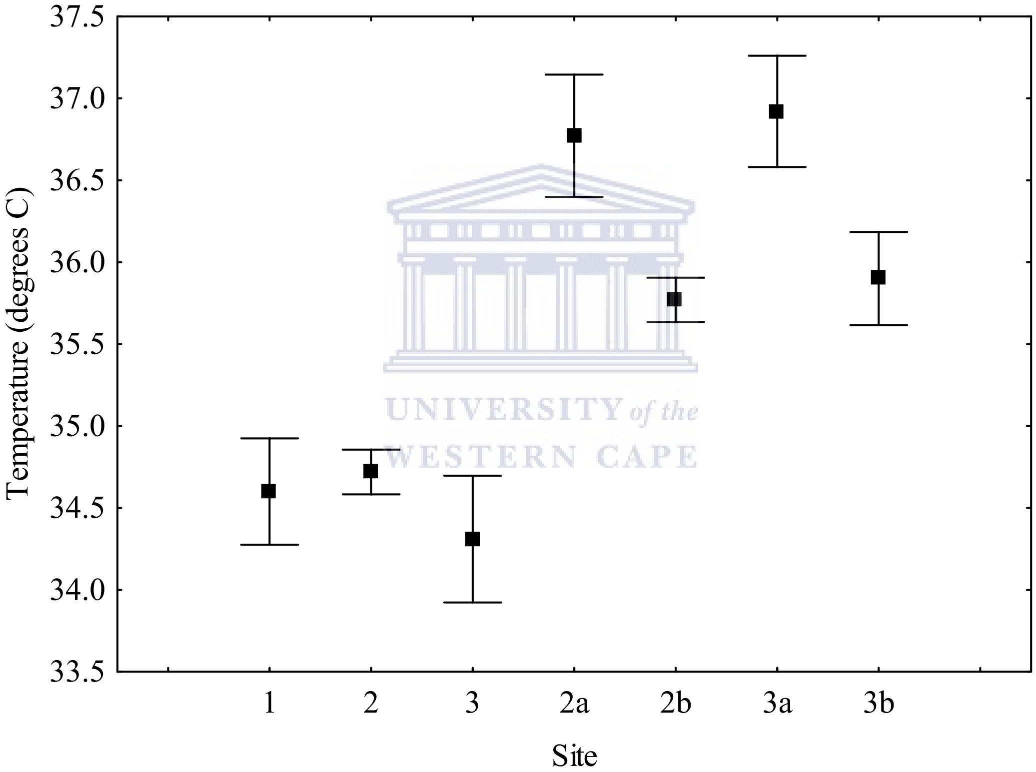

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Fig. 8.6. Mean (±S.E) CTM of *S. grayii* from southern sites showing field-fresh (1, 2, 3) and acclimated results (a, b) (a and b indicate pre-treatment, a - previously tested for CTM, b - naïve to CTM experiment. See Table 8.1 for site details)

8.3.3 Sites 10-12 (Northern)

Strongylopus grayii from the northern sites had CTMs that were significantly different from each other (Wald $\chi^2(2) = 139.6$ $p = 0.0$)(Fig. 8.7) and even when the tadpoles were acclimated the CTM of the different sites were significantly different (Wald $\chi^2(5) = 26.7$ $p = 0.00$) (Fig. 8.7). A multiple comparison test was carried out on the acclimated results. It showed that the previously tested tadpoles of sites 10 and 11; site 10 previously tested tadpoles and site 11 naive tadpoles; and site 10 and 11 naive tadpoles along with site 11 and 12 naive tadpoles were significantly different. These results do not show a difference between individual sites CTMs of naïve and previously tested tadpoles, but do show some variation between the three northern sites. The result from site 12 of the CTM of previously tested tadpoles was discarded because only one tadpole survived. When comparing the field-fresh and acclimated tadpoles from the northern sites there was a significant difference (Wald $\chi^2(8) = 702.1$ $p = 0.00$). The acclimated tadpoles all had a higher CTM (Fig. 8.7).

If the field-fresh results for the northern and southern sites are compared there is a significant difference (Wald $\chi^2(5) = 37.9$ $p = 0.00$). However, when the means and confidence levels are plotted the northern sites show a range which encompasses the southern sites range. The only difference is between site 10 and all of the southern sites (Fig. 8.8). When a comparison is carried out between the acclimated tadpoles there is also a significant difference (Wald $\chi^2(8) = 67.4$ $p = 0.00$). When the confidence intervals are plotted the difference lies only between site 2b, 3b and 10a and 10b (Fig. 8.9).

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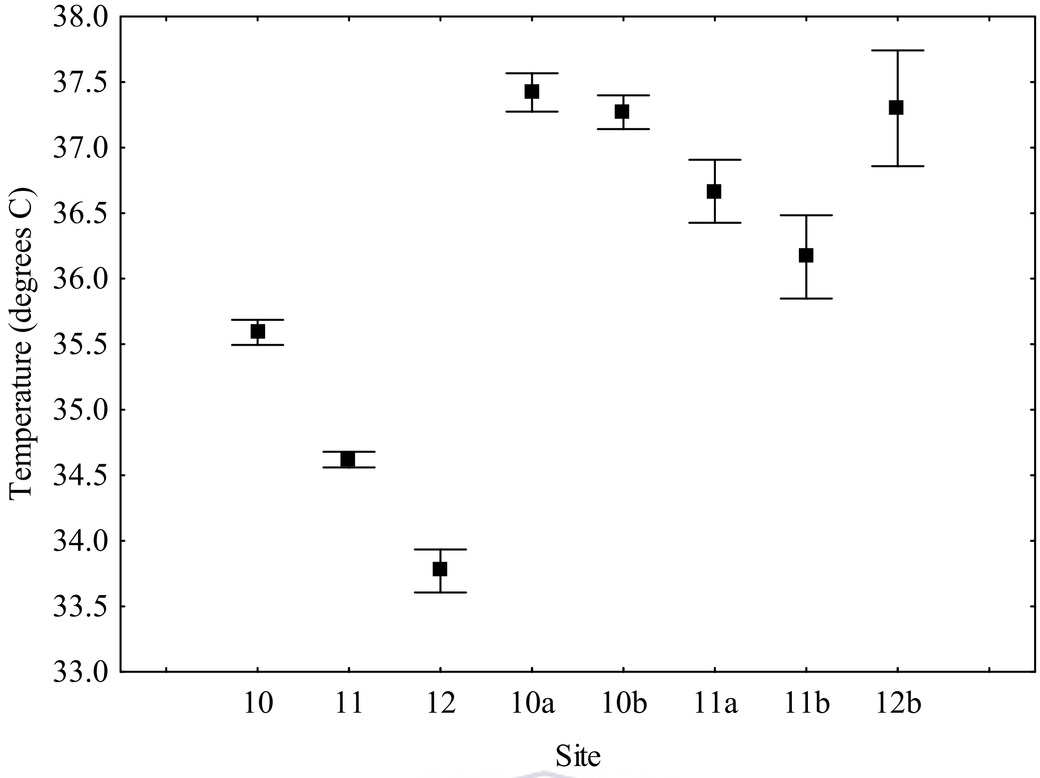

Fig. 8.7. Mean (±S.E) CTM of *S. grayii* from northern sites showing field-fresh and acclimated results (a and b indicate pre-treatment, a - previously tested for CTM, b - naïve to CTM experiment. See Table 8.1 for site details)

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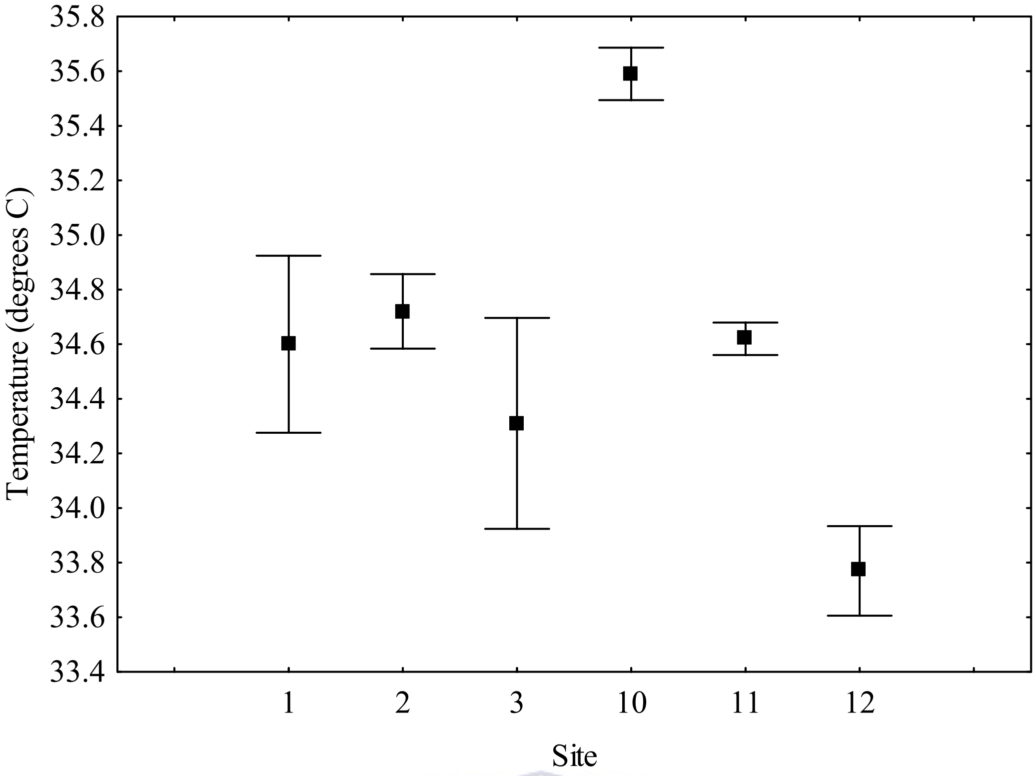

Fig. 8.8. Mean (±S.E) CTM of field-fresh *S. grayii* from southern and northern sites. (See Table 8.1 for site details)

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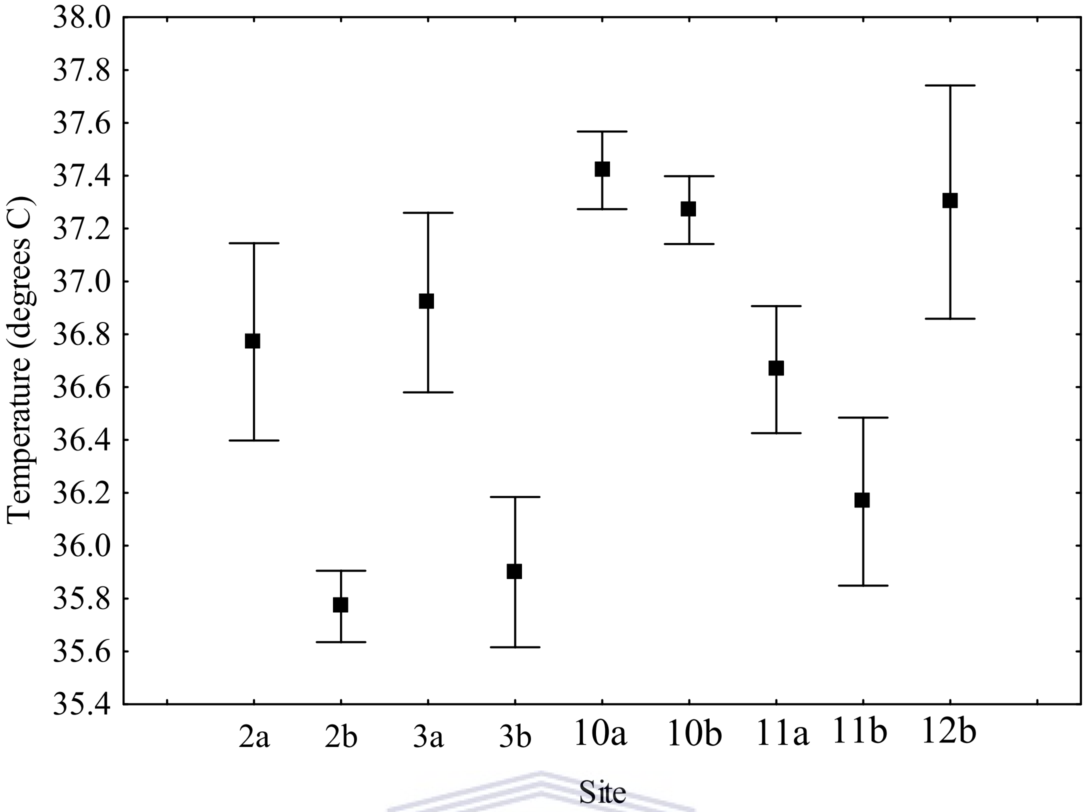

Fig. 8.9. Mean (±S.E) CTM of *S. grayii* after acclimation at 25 °C from southern and northern sites (a and b indicate pre-treatment, a - previously tested for CTM, b - naïve to CTM experiment. See Table 8.1 for site details)

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8.3.4 Higher (sites 7-9) vs. lower (sites 4-6) altitudes

To detect a difference in tadpole CTM vs. altitude, sites were visited that were along the same latitudinal line but at different altitudes, the highest site was 951 m above sea level (site 9) and the lowest was 158 m above sea level (site 4). This gave a difference of 793 m.

The three low altitude sites had a mean range between 33.8 °C and 34.5 °C. There was no difference detected between these three sites (Wald $\chi^2(2) = 2.1$ p = 0.3) (Fig. 8.10). When these tadpoles were acclimated at 25˚C the range of CTM temperatures became 36˚C to 37.4˚C (Fig. 8.10), this is significantly higher than the field-fresh results (Wald $\chi^2(7) = 114.4$ p = 0.00). The significant difference is between the field-fresh and acclimated tadpoles of sites 4 and 6. Site 5 does not show a difference but that site was represented by few tadpoles (Fig. 8.10).

The tadpoles from the high altitude sites had a mean CTM range of 32.9 °C to 34 °C. These were significantly different from one another (Wald $\chi^2(2) = 8.5$ $p = 0.01$) (Fig. 8.11). When these tadpoles were acclimated at 25 °C they showed a difference between the sites (Wald $\chi^2(5) = 51.8$ $p = 0.00$). The mean CTMs ranged from 36.4 °C to 37.5 °C. The tadpoles that had previously been tested from sites 7 and 8 and the naive tadpoles from site 7 all had high CTMs whereas the tadpoles from site 9 and the naïve tadpoles from site 8 had lower CTMs (Fig. 8.11). There was also a significant difference when the field-fresh results were compared to the acclimated results (Wald $\chi^2(8) = 623.0$ $p = 0.00$) (Fig. 8.11).

When a comparison was made between the field-fresh tadpoles from high and low altitude there was a significant difference (Wald $\chi^2(5) = 15.0$ $p = 0.01$) (Fig. 8.12). Once the tadpoles had been acclimated a difference was detected (Wald $\chi^2(9) = 34.7$ $p = 0.01$) (Fig. 8.13). The mean CTMs of the group as a whole ranged from 36.0 °C to 37.5 °C. To establish where the differences lie, as there was a difference between the high altitude sites previously, a multiple comparisons test was carried out. This showed that differences were found between sites with different pre-treatments as well as differences within the high altitude sites. There were no significant differences between the high and low altitude sites.

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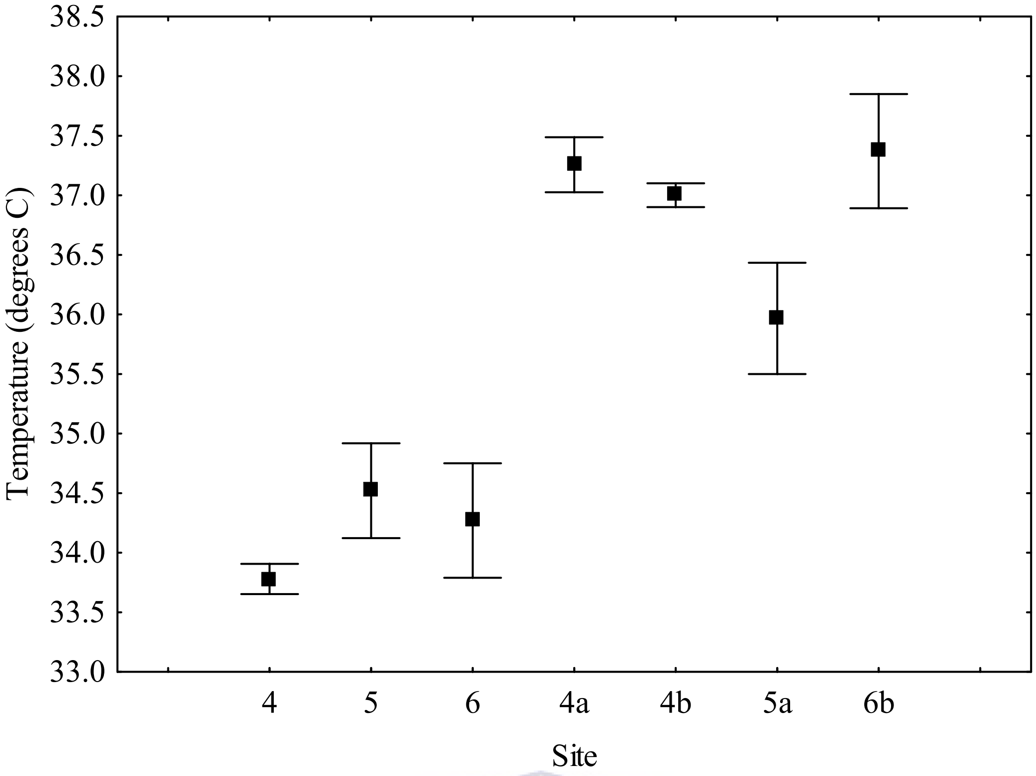

Fig. 8.10. Mean (±S.E) CTM of *S. grayii* from low altitude sites showing field-fresh and acclimated results (a and b indicate pre-treatment, a - previously tested for CTM, b - naïve to CTM experiment. See Table 8.1 for site details)

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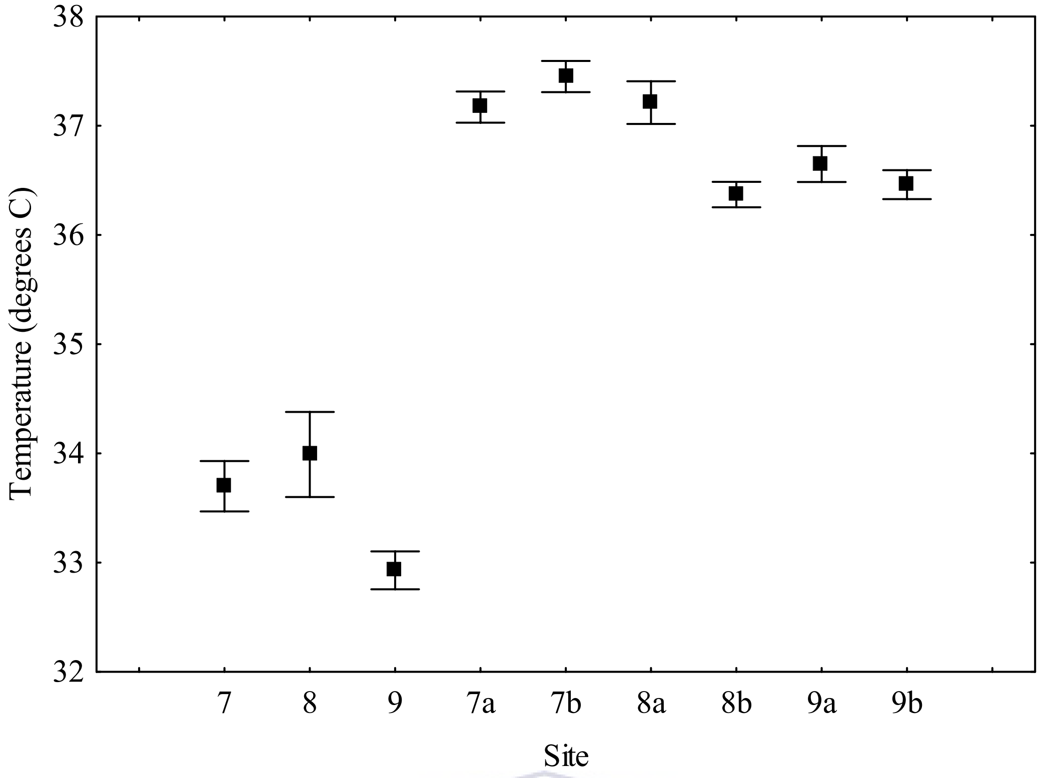

Fig. 8.11. Mean (±S.E) CTM of *S. grayii* from high altitude sites showing field-fresh and acclimated results (a and b indicate pre-treatment, a - previously tested for CTM, b - naïve to CTM experiment. See Table 8.1 for site details)

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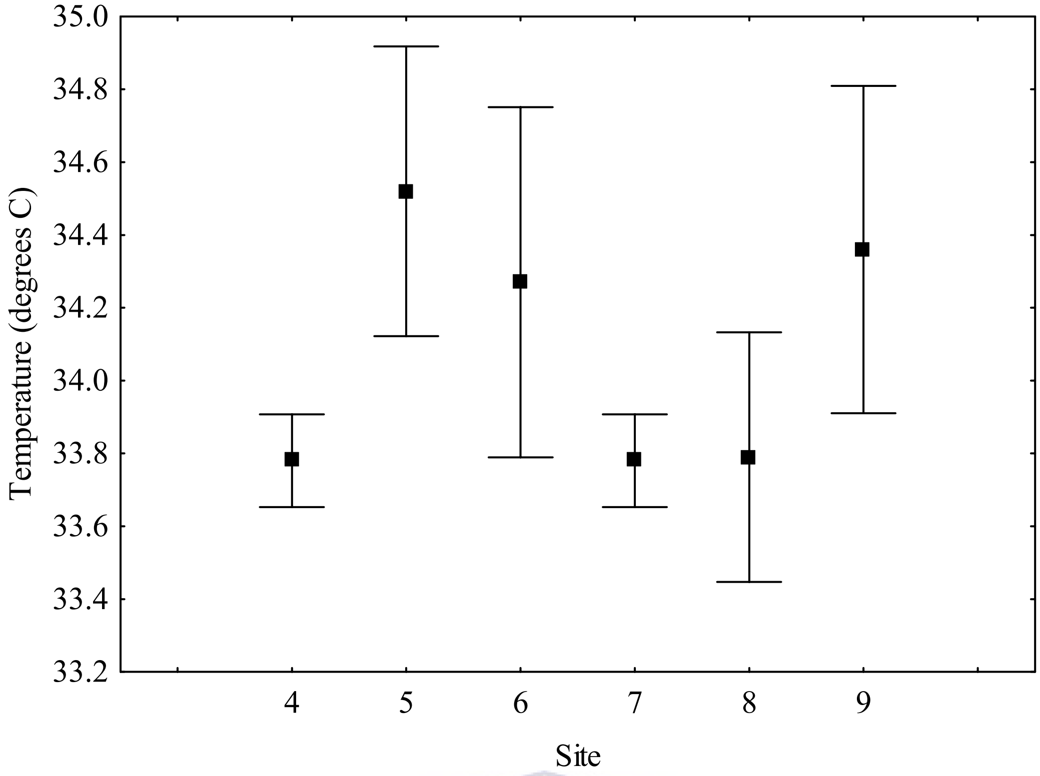

Fig. 8.12. Mean (±S.E) CTM of field-fresh *S. grayii* from low and high altitude sites. (See Table 8.1 for site details)

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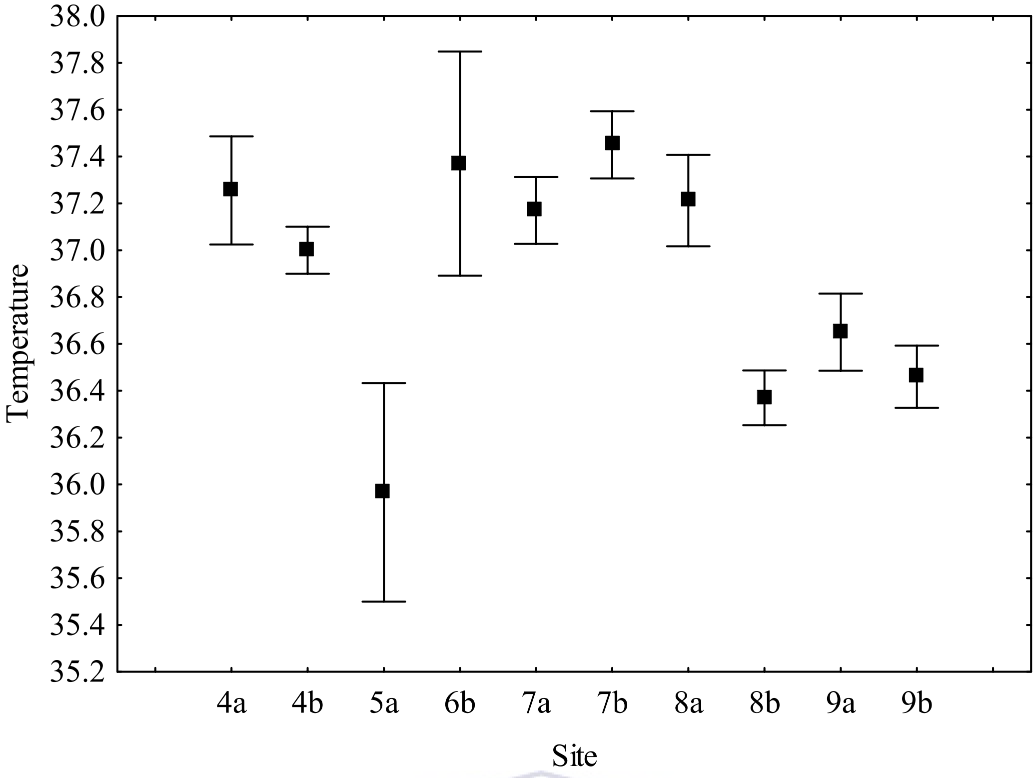

Fig. 8.13. Mean (±S.E) CTM of acclimated *S. grayii* from low and high altitude sites (a and b indicate pre-treatment, a - previously tested for CTM, b - naïve to CTM experiment. See Table 8.1 for site details)

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8.3.5 Summer (sites 13-15) vs. winter regions

The range of *S. grayii* is from the winter rainfall area of the Western Cape through to the summer rainfall areas of the Karoo. The tadpoles of *S. grayii* collected from a summer rainfall area were compared with the tadpoles of the same species from the winter rainfall region. The field-fresh tadpoles showed a significant difference (Wald $\chi^2(2) = 159.2$ $p = 0.00$) between the three sites with the third site having a higher mean CTM (Fig. 8.14). When the tadpoles were acclimated a significant difference was found between the sites (Wald $\chi^2(5) =263.9$ $p = 0.00$). Sites 13 and 14 had a lower mean CTM than site 15. The range of CTM for sites 13 and 14 was small, between 39.7 °C and 39.9 °C. Site 15 was higher at 41.3 °C to 41.4 °C (Fig. 8.14). When comparing the field-fresh summer rainfall tadpoles with the acclimated tadpoles there is a significant difference between the field-fresh and acclimated tadpoles of site 14 with the field-fresh tadpoles having a significantly lower CTM than the acclimated tadpoles (Wald $\chi^2(8) = 446.4$ $p = 0.00$). Sites 13 and 15 showed little difference between the field-fresh and acclimated tadpoles (Fig. 8.14).

When the summer rainfall sites are compared with the winter rainfall sites there is a large difference between the CTMs of the field-fresh tadpoles (Wald $\chi^2(1) =638.4$ $p = 0.00$) (Fig. 8.15). The summer rainfall tadpoles have much higher CTMs than the winter rainfall tadpoles. The mean for the grouped winter rainfall CTM is 34.5 °C and the grouped summer rainfall mean CTM is 40 °C. After acclimation there is still a significant difference between the summer and winter sites (Wald $\chi^2(20) = 1242.7$ $p = 0.00$). The grouped mean CTM for the summer site is 40.4 °C and for the winter sites it is 36.8 °C (Fig. 8.16).

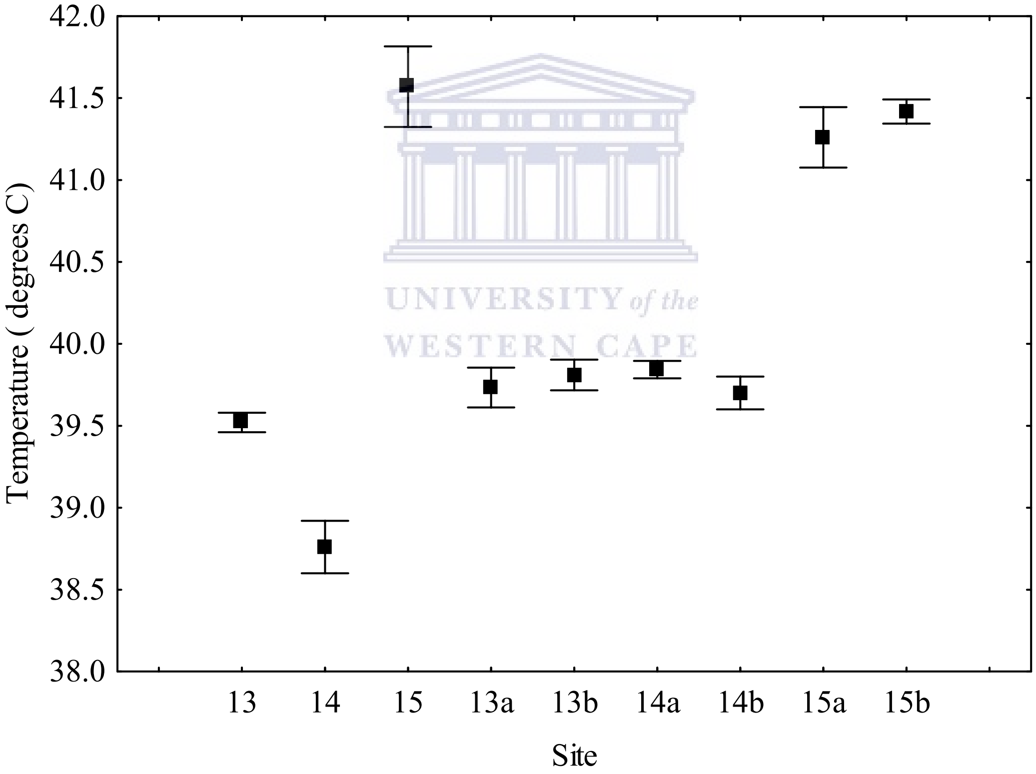

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Fig. 8.14. Mean (±S.E) CTM of *S. grayii* from summer rainfall sites showing field-fresh and acclimated results (a and b indicate pre-treatment, a - previously tested for CTM, b - naïve to CTM experiment. See Table 8.1 for site details)

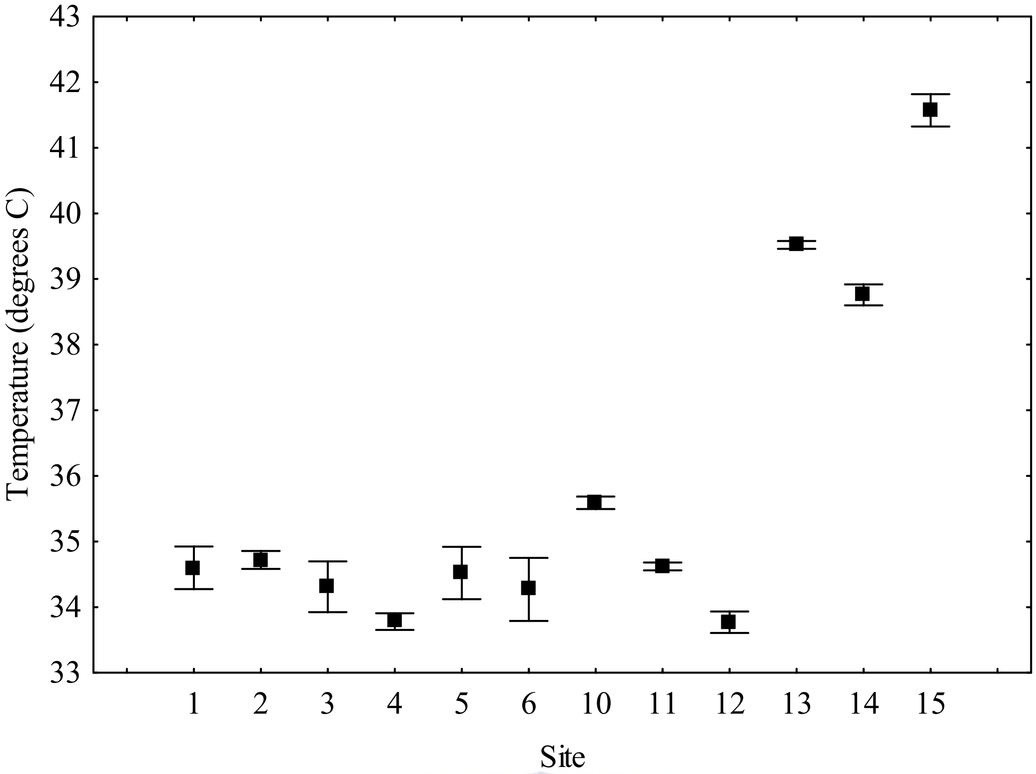

Fig. 8.15. Mean (±S.E) CTM of field-fresh *S. grayii* from summer and winter rainfall sites. (See Table 8.1 for site details)

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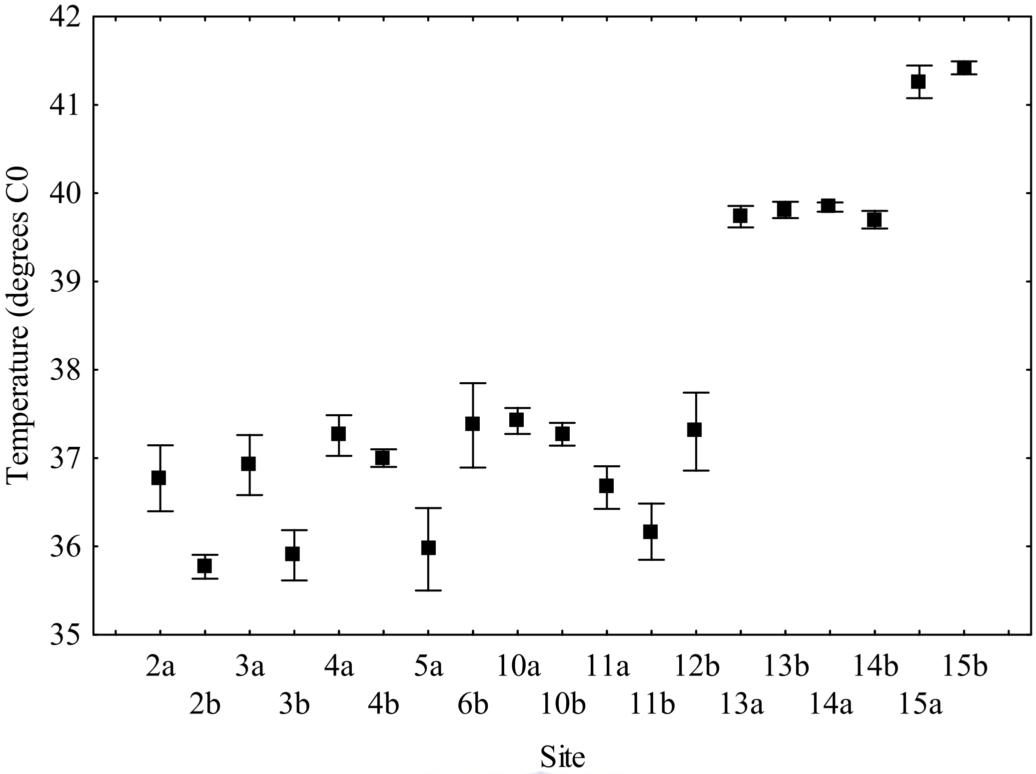

Fig. 8.16. Mean (±S.E) CTM of acclimated *S. grayii* from summer and winter rainfall sites (a and b indicate pre-treatment, a - previously tested for CTM, b - naïve to CTM experiment. See Table 8.1 for site details)

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8.4 Discussion

The aim of this study was to discover the CTM of tadpoles from a widespread species, then acclimate the tadpoles at 25 °C to see if their CTM would alter. The CTM endpoint was chosen to be the loss of response after tactile stimulation, although Lutterschmidt and Hutchinson (1997a, b) suggest that the end point of all CTM experiments should be muscular spasm, as it is a precise measure. However, tadpoles often move in a way that could be mistaken for a muscular spasm. The loss of response endpoint has been used by other authors (Cupp 1980, Sherman and Levitis 2003). The tadpoles were tested as field-fresh and then transported to the laboratory along with a set of tadpoles from the same site that had not been tested. This allowed a control for the effects of previous testing. These results were then compared to tadpoles of the same species in the summer rainfall climatic regime.

8.4.1 Southern sites

The tadpoles from the southern sites had relatively low CTMs which increased when they were acclimated at 25 °C. After acclimation the naïve tadpoles attained lower CTMs than the previously tested tadpoles. This is probably due to heat shock proteins (HSPs) that would have been produced by the tadpoles when they were tested as field-fresh. If this is the case then it shows that the proteins stay with the tadpole for longer than two weeks. This has been reported previously in *Drosophila melanogaster* which produce HSPs when thermally stressed. Animals will produce these proteins after being introduced to low intensities of heat stress and then will be able to tolerate higher temperatures when tested. HSPs increase in concentration during and after the thermal stress (Krebs and Feder 1997). HSPs protect cells from damage and the temperature at which they are produced are species specific (Barua and Heckathorn 2004).

8.4.2 Northern sites

Northern tadpoles showed a significant difference between the field-fresh CTMs. These tadpoles were all taken from a small area around Nieuwoudtville along the same stretch of river. However, it is possible that each site was occupied by a different population and that the CTM is slow to change. Nieuwoudtville is on an escarpment surrounded by dry Karoo so it is possible that many populations have been forced closer together as the season dried. After acclimation the sites were still significantly different, which shows that the difference in CTMs of the field-fresh tadpoles was not due to different conditions in the environment. The acclimated CTMs were, however, significantly higher than the field-fresh CTMs.

When the northern and southern sites were compared a significant difference was found between site 10 and the southern sites. The difference between the acclimated tadpoles again shows a difference between site 10 and sites 2b and 3b. These tadpoles do not show a trend in CTM with latitude. Other examples where CTM shifts throughout the latitude of the range of the species are well documented such as in the diamond back water snake (Winne and Keck 2005) and the widespread *Drosophila melanogaster* (Ayrinhac et al. 2004, Hoffmann et al. 2005) and larvae of frogs like *Anaxyrus americanus* and *Xenopus laevis* (Sherman and Levitis 2003) although this is not always the case. The trend found by McMahon and Payne (1980) in the fresh water snail, *Physa virgata*, was that the CTM changed in an east-west cline rather than a north-south cline. In Nieuwoudtville the maximum temperature is higher than in Cape Agulhas exposing the tadpoles to higher temperatures; however the minimum temperature in Nieuwoudtville is lower than in Cape Agulhas which may alter the tadpoles CTMs.

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8.4.3 Altitude

The lower altitude sites all had similar CTMs. However, the higher altitude sites differed significantly in their CTMs. When both sets of tadpoles were acclimated the low altitude tadpoles showed no difference between the three sites and naive and previously tested whereas the high altitude tadpoles showed a difference. These differences were between sites 7 and 9 and sites 8 and 9 but sites 7 and 8 were similar. Site 9 was at the highest altitude at 951 m and site 8 was lower at 731 m. The site with the highest CTMs is the lower site (site 8). This could be explained by looking at the distribution of the sites. Both sites 7 and 8 were on the western side of the mountain and site 9 was on the eastern side. The eastern inland slopes are drier and presumably warmer. When just comparing sites 7 and 8 the naive tadpoles, once acclimated, show a significant difference with the lower site having a lower CTM. There was a significant difference between the field-fresh results and the acclimated results for both the low and high altitude sites. When the low and high altitude field-fresh results were compared there was no significant difference between the high and low altitude sites and after a multiple comparisons test the only difference was found between two low altitude sites and sites 5 and 7 (p= 0.01). The differences between the acclimated tadpoles from the high and low altitude sites were not due to altitude and were between sites at the same elevation and often between the different pre-treatments (naive and pre-tested). Many studies have suggested that altitude is a factor that determines the CTM. For example tadpoles of *Pseudacris triseriata* have different CTMs between montane (2770 m) and piedmont (1530 m) sites (Hoppe 1978). Tadpoles from the higher altitude sites have a lower CTM than tadpoles from the low sites. These altitudinal differences are not limited to anurans. Other ectotherms have been recorded to alter their CTM with altitude. Anolis lizards have different CTMs with changing altitude and also alter their sprint speed (Van Berkum 1986). Perhaps the tadpoles tested here did not cover a large enough range of altitude. Future work could test tadpoles from a greater range of altitudes. Tadpoles from populations in the Drakensberg could be tested against tadpoles on the same latitude at sea level.

8.4.4 Summer vs. winter rainfall areas

The tadpoles of the same species in the summer rainfall area must encounter different climatic conditions than the tadpoles in the winter rainfall region. The field-fresh CTMs from these sites showed a difference between the sites. Site 15 was significantly higher than the other two. This was also seen in the acclimated tadpoles and when these were compared the only site that altered the CTM with acclimation was site 14. This is possibly because the tadpoles are already at the acclimation temperature in the field or that they cannot adjust their CTM any further as they are already at its maximum range. The sites that the tadpoles were taken from showed variation in temperature between 16 °C at 8.00 hrs to 33 °C at 15.00 hrs. The highest water temperature recorded at any of the other sites was 15.5 °C. When the summer and winter sites CTMs are compared the summer rainfall sites are significantly higher and even when acclimated the summer rainfall tadpoles still give a CTM that is significantly higher. *Drosophila melanogaster* has a wide distribution. Flies were taken from Prunay, France and Mombassa, Kenya and raised at three different temperatures. 80% of the variation found was due to the temperature they were raised at and is therefore considered phenotypic plasticity and only 3.7% was considered to be a genetic difference (Ayrinhac et al. 2004).

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It would be expected that tadpoles from a species with a large range such as *S. grayii* would have a more variable CTM than tadpoles from a species with a small range such as *S. springbokensis.*

8.4.5 Can these tadpoles respond to global climate change?

The tadpoles of *S. grayii* can increase their CTM when faced with slightly warmer water. However, there will come a point where the tadpoles can no longer adjust to the heat. Certain experiments in this chapter suggested that these tadpoles had heat shock proteins which will protect the animals from sudden increases in temperature.

Global climate change presents more problems than just adjustment to warmer water. The temperature may increase at a rate that the tadpoles cannot keep up with and the amount of standing water present may decrease or not persist for as long. With urbanisation in South Africa these animals may not be able to shift their distribution enough to keep up with the changing temperature.

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CHAPTER 9: GENERAL DISCUSSION

9.1 Mesocosms vs. field studies

There are several advantage and disadvantages to studying an animal in its habitat. Firstly, the study is more realistic and will reduce laboratory effects. Secondly the study is more difficult as controls cannot be provided and the full scope of all of the forces acting upon the animal can never be dissected out and explained.

9.2 Different origins, but similar solutions

Most of the literature I have cited, reports on studies of genera and species not present in South Africa. Despite the separate origin of the African frog species (recently placed in the Afrobatrachia by Frost et al. 2006), the problems faced by tadpoles in temporary ponds in terms of the best time to enter the pond, the preferred oxygen and temperature regimes, the use of microhabitat and the use of food resources are similar worldwide. It is not surprising, therefore, that tadpoles in a pond in the southern tip of Africa, have evolved similar responses to those elsewhere.

Differences might only become apparent when conditions are unfavourable. For example, there were few differences found between the gut contents of *Xenopus laevis* tadpoles, with no hard mouth parts, and the other tadpole species with well developed mouth parts. If there was a decline in the algae in the water column then the tadpoles with well developed hard mouth parts would be more capable of scraping food from grass and restios.

9.3 Answers to research questions

9.3.1 Which frog species use the temporary ponds in the study site for breeding?

Out of the 16 anurans that could possibly be present at the pond only five were seen in the present study: these were; *Cacosternum platys, Microbatrachella capensis, Strongylopus grayii, Tomopterna delalandii* and *Xenopus laevis.*

9.3.2 Which species of tadpoles occur simultaneously in these ponds?

All five tadpole species occur at the pond together at some stage in the ponds existence. Some spend longer in the pond together than others. For example in the second season (2004) *S. grayii, C. platys* and *X. laevis* spent 18 weeks in the pond at the same time but *T. delalandii* only spent five weeks with these species.

9.3.3 How long do cohorts of tadpoles remain in the pond?

This question can only really be answered by following a cohort of each species though to metamorphosis but in this study the stages of tadpoles were recorded. This meant that an estimate of the time between when the tadpoles of a particular species were seen and the first stage 42 tadpole was seen could be deduced. The first stage 42 *S. grayii* was trapped just over 2 weeks after the first tadpoles were caught in the pond. The first tadpoles were present in the pond the day the pond filled. In the literature the time to metamorphosis is considered to be three months (Channing 2001). This phenomenon cannot be explained, as the tadpoles will have had a pond containing large amount of algae and possibly little competition, however, the time is remarkably short. The eggs of *S. grayii* are deposited on a damp substrate and some development occurs whilst the tadpoles are contained within the eggs. It is possible that these more advanced tadpoles were present in a small puddle that was not noticed when the site was checked frequently for the presence of water. The tadpoles may have been developing in a small amount of water which then joined to the pond when the pond filled.

The graph of tadpole stage for *C. platys* suggested that these tadpoles were taking longer than the two weeks stated in the literature to develop (Scott 2004). The time of two weeks was taken from the description of *C. boettgeri* which *C. platys* has been grouped with. Whilst it is possible that stage 42 tadpoles were present in the pond before any were caught in the traps it also highlights the need for a full description of the tadpole of *C. platys*. This tadpole may develop in an entirely different way to the tadpole of *C. boettgeri*.

The tadpoles of *X. laevis* were different from *S. grayii* and *C. platys*. The graph of tadpole stage suggests the time to metamorphosis between the first stage 25 being seen in the pond to the first stage 42 being seen was almost the same as is stated in the literature. The first stage 42 tadpole was caught 11 weeks after the first stage 25 tadpole was caught. In the literature the time to metamorphosis is stated as 2 months (Measey 2004).

9.3.4 How many tadpoles make up the community? Does this vary through the season?

The number of tadpole species present in the pond throughout the season varies as the season progresses. In 2003 *S. grayii* had the pond to itself for 13 weeks before the next species arrived. Then within a week *C. platys, X. laevis* and *T. delalandii* had arrived. *Microbatrachella capensis* was only caught for one week, two weeks later. In the second season (2004), *S. grayii, C. platys* and *X. laevis* all arrived within a week of one another and these three tadpole species were alone in the pond until week ten when *T. delalandii* arrived. The first *M. capensis* was caught four weeks later and for the next five weeks all five of the species were present at the pond. This ended when *S. grayii* was not caught after the 20th of October. By the 17th of November only *T. delalandii* was left in the pond.

9.3.5 Which species of tadpoles are most and least abundant in the pond?

The relative abundance of each species can only be suggested assuming that all of the tadpoles are equally catchable. If this is assumed then the tadpoles of *S. grayii* are by far the most abundant species at the pond for both seasons. In the first season the next most abundant species is *C. platys* and in the second season it is *X. laevis*. In total over the two seasons; 683 *S.* grayii tadpoles, 68 *C. platys tadpoles,* 54 *X. laevis* tadpoles, 10 *T. delalandii* tadpoles and 3 *M. capensis* tadpoles were caught.

9.3.6 What are the temperature preferences for the different species of tadpoles?

The catch rate of the three most abundant species was tested against water temperature in two of the experiments. The first was the trapping carried out over two years and the second was the trapping carried out over the three 24 hour periods. Each species responded differently. The tadpoles of *S. grayii* were trapped more often in warmer water in the two year study but did not show a preference in the 24 hour study. The tadpoles of *C. platys* showed a preference for warmer water in both studies. The tadpoles of *X. laevis* behaved differently preferring cooler water in the 24 hour experiment and showing no significant preference in the two year study.

9.3.7 What are the oxygen concentration preferences for the different species of tadpoles?

As with water temperature, the three most abundant species catch rate was compared to the oxygen concentration of the water they were caught in. The results varied between species and between experiments. The tadpoles of *S. grayii* were caught more often in water with a low oxygen concentration in the two year trapping experiment but showed no preference in the 24 hour experiment. The tadpoles of *C. platys* gave different results in the two experiments being caught more in water with a high oxygen concentration in the 24 hours and low concentration in the two year experiments. The tadpoles of *X. laevis* only showed a significant difference in the 24 hour experiment where they preferred water with a low oxygen concentration.

9.3.8 What are the water clarity preferences for the different species of tadpole?

The only species that showed any significant difference in catch rate in different water clarities was *C. platys*. These tadpoles showed a preference for less clear water. The other two species showed no difference. It is possible that this factor is not important to them or that the clarity measurements were not on a fine enough scale for differences to be detected.

9.3.9 Do tadpole species prefer to be in a particular depth (or depth range) in the pond?

The tadpoles of *S. grayii* and *C. platys* show the same preferences for water depth. In the two year study both species were caught more often in traps placed in the shallow water and near to the surface. In the 24 hour study the preferences were looked at more closely and both species showed a preference for shallow water and the bottom of the shallow water, near the substrate. The tadpoles of *X. laevis* showed a different preference. In the two year experiment the tadpoles were trapped in greater numbers at the top of the water column and in the 24 hour study the preference was for the deeper water but positioned in the mid to top of the water.

9.3.10 Do tadpole species prefer to be active over particular substrates or near particular kinds of vegetation?

The only tadpole species to show a significant preference for a particular substrate was *S. grayii* in the two year study; where tadpoles were caught in higher numbers over leafy plants.

9.3.11. How do the tadpoles of *Strongylopus grayii* and *Cacosternum platys* respond when placed in an environment where the water is drying?

The tadpoles of *S. grayii* and *C. platys* did not respond to a drying environment in this experiment. Perhaps they cannot speed up their time to metamorphosis or the drying conditions provided did not prompt the reaction.

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9.3.12. Do tadpoles in a temperate temporary pond partition the food resources?

There were very few significant difference seen between the tadpoles gut contents and the water column and between the contents of different species guts. This study suggests that tadpoles in this temporary pond are ingesting whatever is in the water column.

9.3.13. Do tadpoles in a tropical temporary pond partition the food resources?

The results for the gut contents analysis in the tropical ponds were similar to the temperate pond. There were few significant differences seen between the content of different species guts and between tadpole guts contents and the water column.

9.3.14. Does the Critically Endangered micro frog breed successfully in the pond with four other species?

The number of calling male micro frogs equals the number of calling *C. platys* but the tadpole numbers do not reflect this. It is possible that the tadpoles of this species are trap shy but the traps were clear plastic and were not baited. This meant that the tadpole would have to be swimming in the area to be trapped. Dip-netting was carried out for other purposes and throughout the whole three years only 28 *M. capensis* tadpoles were ever caught. This suggests that the tadpoles of *M. capensis* and are not doing well in the Kenilworth environment.

9.3.15. Do *Strongylopus grayii* tadpoles from warmer northern inland sites have a higher critical thermal maximum (CTM) than tadpoles from cooler southern sites?

In both the field fresh and acclimated tadpoles there was no pattern in CTM showing a difference between Southern and Northern sites.

9.3.16. Do *S. grayii* tadpoles from higher elevations have lower CTMs than those from low altitude?

The difference in CTMs of tadpoles from different altitudes can only be tested on from sites along the same latitudinal line to negate latitudinal variation. The sites chosen differed in altitude by 793 m. There was no difference detected between the high and low altitude tadpole CTMs.

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9.3.17. Do *S. grayii* tadpoles from the summer rainfall region have higher CTMs than tadpoles from the winter rainfall region?

There is a large difference between the CTM of tadpoles from the summer and winter rainfall regions this difference is seen in the field fresh and acclimated tadpoles.

9.4 The Critically Endangered micro frog

Microbatrachella capensis is the only representative of the genus *Microbatrachella*. The frog occurs in only four sub-populations over an area of less than 10 km^2 (De Villiers 2004c). As has already been mentioned, throughout the three years of fieldwork at Kenilworth racecourse only 28 *M. capensis* tadpoles were found. The Kenilworth racecourse site is one of the very few remaining sites where these animals are present (De Villiers 2004c). The literature states that the animals have very specific habitat requirements and do not do well in disturbed habitat (DeVilliers 2004). The precise reason for this is not known and more investigation needs to be carried out to uncover the reasons, as many other frog species in the area have numerous healthy breeding populations. Once the precise requirements (breeding site and otherwise) for this frog are known then the centre of Kenilworth can be protected from whatever it is that is causing the numbers to decline.

9.5 Implications for global warming

Recently, there has been an increase in the literature focusing on global climate change, and as the animals studied here are so rainfall-dependent then the changing environment will have an effect on them in the future if it has not done so already. As was seen in the trapping work presented here, tadpoles select for temperature. It is not proven why the tadpoles at Kenilworth racecourse selected for higher temperatures but examples from elsewhere have shown that tadpoles metamorphose quicker and alter swimming performance in warmer water (Merilä et al. 2000, Wilson et al. 2000).

The future climatic predictions for Western Cape show that the rainfall will become patchier and less rain may fall in total (Midgley et al. 2005). This will shorten the amount of time that the water is present for the frogs to breed in and the tadpoles to reach metamorphosis. In the drying experiment the tadpoles could not speed up metamorphosis and this may be true for many of the local species. Of more concern would be the many *Vandijkophrynus* and *Amietophrynus* species that breed in very shallow puddles which may not even be present if the rainfall changes. The species that is of most concern in all of this is *Microbatrachella capensis*. This frog species has a small range that occurs on low-lying land that is under threat from development as well as the effects of climate change.

Another aspect to this work was the CTM experiments that showed that tadpoles in the summer rainfall region of their range were experiencing high temperatures and were probably already at their upper limits of temperature tolerance. An increase in temperature in these areas could cause these small pools in the Karoo to become too warm for the frogs to breed in. The *S. grayii* species studied here, being widespread, will most likely not be affected by this as a species but it could cause local population

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extinctions and any species that have small ranges in this area may find it hard to locate a suitable breeding site.

9.6 Tadpole life in a pond

The pond at Kenilworth racecourse fills in about a month once the rains have started. Instantly *S. grayii* tadpoles are present due to the adults laying eggs before the rains, and the tadpoles develop within the egg capsule. This is probably advantageous to the species with less competition and higher algae numbers although the start of the season is the time when the pond water is at its coolest. *Strongylopus grayii* tadpoles are joined by *X. laevis* tadpoles early in the season. As the season progresses, *Cacosternum platys* enters the pond and last to arrive are *M. capensis* and *T. delalandii.* During the season the temperature of the water increases and the oxygen concentration fluctuates. The general trend is that *X. laevis* tadpoles prefer the deeper water but stay at the top of the column in a school. This alters slightly at night when tadpoles swim deeper. Interestingly, the tadpoles of *X. laevis* do not school when the marsh terrapin is present. The other tadpoles much prefer the shallower water and stay near the substrate. They spread out slightly at night. Some of the tadpole species show a preference for high or low temperatures and oxygen concentrations and this may be a way to partition the pond to avoid competition. They do not partition food resources. All of the species took in much the same food types and seldom deviated from the proportions found in the water column. This suggests that the algal composition throughout the water is uniform as tadpoles of *X. laevis* which prefer deeper water have similar algae in their guts to the other species, which seem to prefer the warmer shallower water.

Eggs are laid on more than one occasion probably coinciding with heavy rainfall events. There are influxes of new tadpoles often at the start of the season and the numbers decline as the tadpoles are predated upon or leave the pond after metamorphosis. Towards the end of the season the *T. delalandii* and *M. capensis* arrive. This may be a strategy to avoid the competition when the other species are flooding the pond with tadpoles or a chance for the tadpoles to develop in warmer water. Although the tadpoles arrive late, all are out of the pond before it dries out in mid-summer.

9.7 Conservation of Kenilworth racecourse

Microbatrachella capensis, which is present at the Kenilworth racecourse site, is on the IUCN red data list as critically endangered (De Villiers 2004). As has already been mentioned, even though plenty of adult male frogs call at the site very few *M. capensis* tadpoles were captured and this could be exacerbated by the climate changing or any type of development taking place in the area. These frogs already have a very limited range and losing this piece of land would decrease the amount of suitable land considerably. The populations at this site should be monitored closely, taking into account tadpole numbers and recruitment into the adult stage as well as the number of adults calling. The fire regime that is in place at Kenilworth racecourse needs to be looked at very carefully as burning large pieces of land in a hot fire could decimate the frog populations; not only with mortality due to the fire but also with a lack of food sources for the adult frogs and a change in the chemistry of the water on the land after these burns.

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9.8 Recommendations

A major concern arising from the work carried out for this thesis is that tadpole species are understudied in South Africa. Most of the references presented here were from studies conducted overseas. More work needs to be undertaken to understand anurans in South Africa.

The site at Kenilworth Racecourse needs to be protected and the small micro-frog population needs to be monitored. The reason why they are breeding in such low numbers needs to be investigated. The quality of the water must be checked and any disturbance encountered must be stopped. The nutrients getting to the pond from the area around it should be assessed. Even during the time I was there eutrophication in the form of an increase in the amount of green slime across the top of the pond was noted.

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Appendix 1: The number of days to metamorphosis for *S. grayii* tadpoles marked with VIE.

Marked/ Unmarked	Number of days to stage 42
Marked	60
Marked	60
Marked	61
Marked	61
Marked	61
Marked	61
Marked	61
Marked	62
Marked	65
Marked	65
Unmarked	60
Unmarked	60
Unmarked	60
Unmarked	61
Unmarked	61
Unmarked	61
Unmarked	62
Unmarked	62
Unmarked	65
Unmarked	66

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Appendix 2: The actual water temperatures and the ranks used for analysis.

Date	Real temp	Ranked temp
25-Jul-03	10.3	0.0
25-Jul-03	10.8	2.4
25-Jul-03	11.3	4.8
25-Jul-03	11.3	4.8
25-Jul-03	11.4	5.2
25-Jul-03	12.4	10.0
28-Jul-03	13.4	0.0
28-Jul-03	13.4	0.0
28-Jul-03	13.4	0.0
28-Jul-03	13.4	0.0
28-Jul-03	13.6	5.0
28-Jul-03	13.8	10.0
05-Aug-03	11.6	0.0
05-Aug-03	11.8	0.8
05-Aug-03	12	1.6
05-Aug-03	12	1.6
05-Aug-03	12	1.6
05-Aug-03	12.2	2.4
05-Aug-03	12.3	2.8
05-Aug-03	12.9	5.2
05-Aug-03	13.3	6.8
05-Aug-03	13.4	7.2
05-Aug-03	13.4	7.2
05-Aug-03	13.4	7.2
05-Aug-03	13.5	7.6
05-Aug-03	13.8	8.8
05-Aug-03	14.1	10.0
08-Aug-03	13.1	0.0
08-Aug-03	13.1	0.0
08-Aug-03	13.4	3.3
08-Aug-03	13.4	3.3
08-Aug-03	13.4	3.3
08-Aug-03	13.4	3.3
08-Aug-03	13.4	3.3
08-Aug-03	13.4	3.3
08-Aug-03	13.5	4.4
08-Aug-03	13.5	4.4
08-Aug-03	13.5	4.4
08-Aug-03	13.6	5.6
08-Aug-03	13.7	6.7
08-Aug-03	14	10.0
12-Aug-03	9.8	0.0
12-Aug-03	10.2	5.0
12-Aug-03	10.4	7.5
12-Aug-03	10.6	10.0
12-Aug-03	10.6	10.0
12-Aug-03	10.6	10.0
12-Aug-03	10.6	10.0

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12-Aug-03	10.6	10.0
12-Aug-03	10.6	10.0
12-Aug-03	10.6	10.0
12-Aug-03	10.6	10.0
29-Aug-03	14.5	0.0
29-Aug-03	14.5	0.0
29-Aug-03	14.6	0.7
29-Aug-03	14.8	2.0
29-Aug-03	14.8	2.0
29-Aug-03	15	3.3
29-Aug-03	15	3.3
29-Aug-03	15.2	4.7
29-Aug-03	15.6	7.3
29-Aug-03	15.8	8.7
29-Aug-03	16	10.0
05-Sep-03	12.3	0.0
05-Sep-03	12.9	2.9
05-Sep-03	13	3.3
05-Sep-03	13	3.3
05-Sep-03	13.2	4.3
05-Sep-03	13.3	4.8
05-Sep-03	13.3	4.8
05-Sep-03	13.4	5.2
05-Sep-03	13.5	5.7
05-Sep-03	13.7	6.7
05-Sep-03	13.9	7.6
05-Sep-03	14.2	9.0
05-Sep-03	14.4	10.0
05-Sep-03	14.4	10.0
10-Sep-03	14.1	0.0
10-Sep-03	14.8	4.7
10-Sep-03	15.4	8.7
10-Sep-03	15.4	8.7
10-Sep-03	15.4	8.7
10-Sep-03	15.4	8.7
10-Sep-03	15.5	9.3
10-Sep-03	15.5	9.3
10-Sep-03	15.5	9.3
10-Sep-03	15.5	9.3
10-Sep-03	15.5	9.3
10-Sep-03	15.5	9.3
10-Sep-03	15.5	9.3
10-Sep-03	15.6	10.0
10-Sep-03	15.6	10.0
16-Sep-03	16.3	0.0
16-Sep-03	16.6	0.7
16-Sep-03	19.1	6.1
16-Sep-03	19.3	6.5
16-Sep-03	19.3	6.5
16-Sep-03	19.3	6.5
16-Sep-03	19.4	6.7
16-Sep-03	19.4	6.7

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16-Sep-03	19.4	6.7
16-Sep-03	19.6	7.2
16-Sep-03	19.6	7.2
16-Sep-03	20	8.0
16-Sep-03	20.1	8.3
16-Sep-03	20.2	8.5
16-Sep-03	20.9	10.0
23-Sep-03	17.4	0.0
23-Sep-03	17.7	1.9
23-Sep-03	17.8	2.5
23-Sep-03	18	3.8
23-Sep-03	18	3.8
23-Sep-03	18	3.8
23-Sep-03	18.3	5.6
23-Sep-03	18.4	6.3
23-Sep-03	18.6	7.5
23-Sep-03	18.7	8.1
23-Sep-03	18.8	8.8
23-Sep-03	18.8	8.8
23-Sep-03	19	10.0
23-Sep-03	19	10.0
02-Oct-03	11.3	0.0
02-Oct-03	16.7	8.2
02-Oct-03	17.1	8.8
02-Oct-03	17.3	9.1
02-Oct-03	17.4	9.2
02-Oct-03	17.4	9.2
02-Oct-03	17.4	9.2
02-Oct-03	17.6	9.5
02-Oct-03	17.6	9.5
02-Oct-03	17.7	9.7
02-Oct-03	17.7	9.7
02-Oct-03	17.8	9.8
02-Oct-03	17.9	10.0
07-Oct-03	16.3	0.0
07-Oct-03	16.3	0.0
07-Oct-03	16.3	0.0
07-Oct-03	16.3	0.0
07-Oct-03	16.4	0.9
07-Oct-03	16.5	1.8
07-Oct-03	16.6	2.7
07-Oct-03	16.7	3.6
07-Oct-03	16.8	4.5
07-Oct-03	16.8	4.5
07-Oct-03	17.3	9.1
07-Oct-03	17.4	10.0
07-Oct-03	17.4	10.0
14-Oct-03	19.1	0.0
14-Oct-03	19.1	0.0
14-Oct-03	19.1	0.0
14-Oct-03	19.2	0.2
14-Oct-03	19.2	0.2

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14-Oct-03	19.2	0.2
14-Oct-03	19.4	0.7
14-Oct-03	19.4	0.7
14-Oct-03	19.4	0.7
14-Oct-03	19.5	1.0
14-Oct-03	19.5	1.0
14-Oct-03	19.5	1.0
14-Oct-03	19.6	1.2
14-Oct-03	19.6	1.2
14-Oct-03	19.6	1.2
14-Oct-03	19.6	1.2
14-Oct-03	19.6	1.2
14-Oct-03	19.7	1.5
14-Oct-03	19.8	1.7
14-Oct-03	19.8	1.7
14-Oct-03	20.1	2.4
14-Oct-03	20.1	2.4
14-Oct-03	20.1	2.4
14-Oct-03	20.4	3.2
14-Oct-03	20.4	3.2
14-Oct-03	20.4	3.2
14-Oct-03	20.5	3.4
14-Oct-03	20.8	4.1
14-Oct-03	20.8	4.1
14-Oct-03	20.8	4.1
14-Oct-03	20.9	4.4
14-Oct-03	21.1	4.9
14-Oct-03	21.1	4.9
14-Oct-03	21.1	4.9
14-Oct-03	21.1	4.9
14-Oct-03	21.2	5.1
14-Oct-03	21.2	5.1
14-Oct-03	21.2	5.1
14-Oct-03	21.2	5.1
14-Oct-03	21.2	5.1
14-Oct-03	21.2	5.1
14-Oct-03	21.4	5.6
14-Oct-03	21.4	5.6
14-Oct-03	21.4	5.6
14-Oct-03	21.4	5.6
14-Oct-03	21.4	5.6
14-Oct-03	21.4	5.6
14-Oct-03	21.6	6.1
14-Oct-03	21.6	6.1
14-Oct-03	21.6	6.1
14-Oct-03	21.7	6.3
14-Oct-03	21.7	6.3
14-Oct-03	21.7	6.3
14-Oct-03	21.8	6.6
14-Oct-03	21.8	6.6
14-Oct-03	21.8	6.6
14-Oct-03	21.9	6.8

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14-Oct-03	21.9	6.8
14-Oct-03	21.9	6.8
14-Oct-03	22.6	8.5
14-Oct-03	22.6	8.5
14-Oct-03	22.6	8.5
14-Oct-03	23.2	10.0
20-Oct-03	17.8	0.0
20-Oct-03	17.8	0.0
20-Oct-03	19.8	3.3
20-Oct-03	19.8	3.3
20-Oct-03	19.8	3.3
20-Oct-03	19.9	3.5
20-Oct-03	19.9	3.5
20-Oct-03	19.9	3.5
20-Oct-03	20	3.7
20-Oct-03	20.1	3.8
20-Oct-03	20.1	3.8
20-Oct-03	20.1	3.8
20-Oct-03	22.4	7.7
20-Oct-03	22.4	7.7
20-Oct-03	22.4	7.7
20-Oct-03	22.6	8.0
20-Oct-03	22.6	8.0
20-Oct-03	22.6	8.0
20-Oct-03	22.8	8.3
20-Oct-03	22.8	8.3
20-Oct-03	22.8	8.3
20-Oct-03	23.3	9.2
20-Oct-03	23.6	9.7
20-Oct-03	23.8	10.0
20-Oct-03	23.8	10.0
20-Oct-03	23.8	10.0
28-Oct-03	11.9	0.0
28-Oct-03	15.1	4.0
28-Oct-03	15.3	4.3
28-Oct-03	15.5	4.5
28-Oct-03	15.5	4.5
28-Oct-03	15.6	4.6
28-Oct-03	15.6	4.6
28-Oct-03	15.7	4.8
28-Oct-03	16	5.1
28-Oct-03	16.1	5.3
28-Oct-03	16.1	5.3
28-Oct-03	16.2	5.4
28-Oct-03	16.3	5.5
28-Oct-03	16.3	5.5
28-Oct-03	16.3	5.5
28-Oct-03	16.4	5.6
28-Oct-03	16.4	5.6
28-Oct-03	16.5	5.8
28-Oct-03	16.5	5.8
28-Oct-03	16.6	5.9

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28-Oct-03	16.7	6.0
28-Oct-03	16.7	6.0
28-Oct-03	16.8	6.1
28-Oct-03	16.8	6.1
28-Oct-03	16.9	6.3
28-Oct-03	17	6.4
28-Oct-03	17	6.4
28-Oct-03	17.1	6.5
28-Oct-03	17.1	6.5
28-Oct-03	17.2	6.6
28-Oct-03	17.2	6.6
28-Oct-03	17.3	6.8
28-Oct-03	17.3	6.8
28-Oct-03	17.4	6.9
28-Oct-03	17.4	6.9
28-Oct-03	17.5	7.0
28-Oct-03	17.6	7.1
28-Oct-03	17.7	7.3
28-Oct-03	17.7	7.3
28-Oct-03	17.7	7.3
28-Oct-03	17.9	7.5
28-Oct-03	18	7.6
28-Oct-03	18.2	7.9
28-Oct-03	18.2	7.9
28-Oct-03	18.3	8.0
28-Oct-03	18.3	8.0
28-Oct-03	18.4	8.1
28-Oct-03	18.4	8.1
28-Oct-03	18.4	8.1
28-Oct-03	18.4	8.1
28-Oct-03	18.5	8.3
28-Oct-03	18.5	8.3
28-Oct-03	18.8	8.6
28-Oct-03	19	8.9
28-Oct-03	19	8.9
28-Oct-03	19.2	9.1
28-Oct-03	19.2	9.1
28-Oct-03	19.3	9.3
28-Oct-03	19.4	9.4
28-Oct-03	19.6	9.6
28-Oct-03	19.7	9.8
28-Oct-03	19.7	9.8
28-Oct-03	19.7	9.8
28-Oct-03	19.9	10.0
06-Nov-03	16.7	0.0
06-Nov-03	16.7	0.0
06-Nov-03	16.8	0.2
06-Nov-03	16.8	0.2
06-Nov-03	16.8	0.2
06-Nov-03	16.8	0.2
06-Nov-03	16.8	0.2
06-Nov-03	16.9	0.5

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06-Nov-03	16.9	0.5
06-Nov-03	16.9	0.5
06-Nov-03	17	0.7
06-Nov-03	17.1	0.9
06-Nov-03	17.1	0.9
06-Nov-03	17.1	0.9
06-Nov-03	17.1	0.9
06-Nov-03	17.1	0.9
06-Nov-03	17.2	1.1
06-Nov-03	17.2	1.1
06-Nov-03	17.2	1.1
06-Nov-03	17.2	1.1
06-Nov-03	17.3	1.4
06-Nov-03	17.3	1.4
06-Nov-03	17.3	1.4
06-Nov-03	17.4	1.6
06-Nov-03	17.4	1.6
06-Nov-03	17.4	1.6
06-Nov-03	17.5	1.8
06-Nov-03	17.7	2.3
06-Nov-03	17.7	2.3
06-Nov-03	17.8	2.5
06-Nov-03	17.8	2.5
06-Nov-03	18	3.0
06-Nov-03	18.1	3.2
06-Nov-03	18.1	3.2
06-Nov-03	18.3	3.6
06-Nov-03	18.3	3.6
06-Nov-03	18.3	3.6
06-Nov-03	18.4	3.9
06-Nov-03	18.4	3.9
06-Nov-03	18.4	3.9
06-Nov-03	18.5	4.1
06-Nov-03	18.8	4.8
06-Nov-03	18.8	4.8
06-Nov-03	19	5.2
06-Nov-03	19	5.2
06-Nov-03	19	5.2
06-Nov-03	19.1	5.5
06-Nov-03	19.2	5.7
06-Nov-03	19.4	6.1
06-Nov-03	19.5	6.4
06-Nov-03	19.6	6.6
06-Nov-03	20	7.5
06-Nov-03	20.1	7.7
06-Nov-03	20.1	7.7
06-Nov-03	20.2	8.0
06-Nov-03	20.8	9.3
06-Nov-03	20.9	9.5
06-Nov-03	20.9	9.5
06-Nov-03	21.1	10.0
14-Nov-03	17.5	0.0

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14-Nov-03	17.7	1.3
14-Nov-03	17.8	1.9
14-Nov-03	17.8	1.9
14-Nov-03	17.8	1.9
14-Nov-03	17.9	2.5
14-Nov-03	17.9	2.5
14-Nov-03	17.9	2.5
14-Nov-03	17.9	2.5
14-Nov-03	17.9	2.5
14-Nov-03	17.9	2.5
14-Nov-03	18	3.1
14-Nov-03	18	3.1
14-Nov-03	18	3.1
14-Nov-03	18	3.1
14-Nov-03	18	3.1
14-Nov-03	18.1	3.8
14-Nov-03	18.1	3.8
14-Nov-03	18.1	3.8
14-Nov-03	18.2	4.4
14-Nov-03	18.2	4.4
14-Nov-03	18.2	4.4
14-Nov-03	18.2	4.4
14-Nov-03	18.2	4.4
14-Nov-03	18.2	4.4
14-Nov-03	18.2	4.4
14-Nov-03	18.2	4.4
14-Nov-03	18.3	5.0
14-Nov-03	18.3	5.0
14-Nov-03	18.3	5.0
14-Nov-03	18.3	5.0
14-Nov-03	18.3	5.0
14-Nov-03	18.3	5.0
14-Nov-03	18.3	5.0
14-Nov-03	18.3	5.0
14-Nov-03	18.4	5.6
14-Nov-03	18.4	5.6
14-Nov-03	18.4	5.6
14-Nov-03	18.4	5.6
14-Nov-03	18.4	5.6
14-Nov-03	18.4	5.6
14-Nov-03	18.4	5.6
14-Nov-03	18.5	6.3
14-Nov-03	18.5	6.3
14-Nov-03	18.5	6.3
14-Nov-03	18.5	6.3
14-Nov-03	18.6	6.9
14-Nov-03	18.6	6.9
14-Nov-03	18.7	7.5
14-Nov-03	18.7	7.5
14-Nov-03	18.8	8.1
14-Nov-03	18.9	8.7
14-Nov-03	18.9	8.7

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14-Nov-03	19	9.4
14-Nov-03	19	9.4
14-Nov-03	19.1	10.0
14-Nov-03	19.1	10.0
14-Nov-03	19.1	10.0
17-Nov-03	17.6	0.0
17-Nov-03	18.2	1.7
17-Nov-03	18.3	1.9
17-Nov-03	18.3	1.9
17-Nov-03	18.3	1.9
17-Nov-03	18.3	1.9
17-Nov-03	18.3	1.9
17-Nov-03	18.4	2.2
17-Nov-03	18.4	2.2
17-Nov-03	18.4	2.2
17-Nov-03	18.5	2.5
17-Nov-03	18.5	2.5
17-Nov-03	18.5	2.5
17-Nov-03	18.5	2.5
17-Nov-03	18.6	2.8
17-Nov-03	18.6	2.8
17-Nov-03	18.6	2.8
17-Nov-03	18.7	3.1
17-Nov-03	18.7	3.1
17-Nov-03	18.8	3.3
17-Nov-03	18.8	3.3
17-Nov-03	18.8	3.3
17-Nov-03	18.8	3.3
17-Nov-03	18.9	3.6
17-Nov-03	18.9	3.6
17-Nov-03	18.9	3.6
17-Nov-03	18.9	3.6
17-Nov-03	19	3.9
17-Nov-03	19	3.9
17-Nov-03	19	3.9
17-Nov-03	19.1	4.2
17-Nov-03	19.1	4.2
17-Nov-03	19.2	4.4
17-Nov-03	19.2	4.4
17-Nov-03	19.2	4.4
17-Nov-03	19.2	4.4
17-Nov-03	19.2	4.4
17-Nov-03	19.3	4.7
17-Nov-03	19.5	5.3
17-Nov-03	19.5	5.3
17-Nov-03	19.5	5.3
17-Nov-03	19.6	5.6
17-Nov-03	19.6	5.6
17-Nov-03	19.6	5.6
17-Nov-03	19.7	5.8
17-Nov-03	19.8	6.1
17-Nov-03	20	6.7

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17-Nov-03	20.1	6.9
17-Nov-03	20.1	6.9
17-Nov-03	20.2	7.2
17-Nov-03	20.3	7.5
17-Nov-03	20.4	7.8
17-Nov-03	20.4	7.8
17-Nov-03	20.6	8.3
17-Nov-03	20.7	8.6
17-Nov-03	21.2	10.0
24-Nov-03	18.8	0.0
24-Nov-03	19.6	3.5
24-Nov-03	19.7	3.9
24-Nov-03	19.7	3.9
24-Nov-03	19.7	3.9
24-Nov-03	19.8	4.3
24-Nov-03	19.8	4.3
24-Nov-03	19.9	4.8
24-Nov-03	19.9	4.8
24-Nov-03	19.9	4.8
24-Nov-03	19.9	4.8
24-Nov-03	19.9	4.8
24-Nov-03	19.9	4.8
24-Nov-03	19.9	4.8
24-Nov-03	19.9	4.8
24-Nov-03	20	5.2
24-Nov-03	20	5.2
24-Nov-03	20	5.2
24-Nov-03	20	5.2
24-Nov-03	20.1	5.7
24-Nov-03	20.1	5.7
24-Nov-03	20.1	5.7
24-Nov-03	20.1	5.7
24-Nov-03	20.1	5.7
24-Nov-03	20.1	5.7
24-Nov-03	20.1	5.7
24-Nov-03	20.1	5.7
24-Nov-03	20.2	6.1
24-Nov-03	20.2	6.1
24-Nov-03	20.2	6.1
24-Nov-03	20.2	6.1
24-Nov-03	20.2	6.1
24-Nov-03	20.2	6.1
24-Nov-03	20.2	6.1
24-Nov-03	20.2	6.1
24-Nov-03	20.3	6.5
24-Nov-03	20.3	6.5
24-Nov-03	20.3	6.5
24-Nov-03	20.3	6.5
24-Nov-03	20.3	6.5
24-Nov-03	20.3	6.5
24-Nov-03	20.3	6.5
24-Nov-03	20.4	7.0

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24-Nov-03	20.4	7.0
24-Nov-03	20.4	7.0
24-Nov-03	20.4	7.0
24-Nov-03	20.4	7.0
24-Nov-03	20.4	7.0
24-Nov-03	20.4	7.0
24-Nov-03	20.5	7.4
24-Nov-03	20.5	7.4
24-Nov-03	20.6	7.8
24-Nov-03	20.6	7.8
24-Nov-03	20.6	7.8
24-Nov-03	20.7	8.3
24-Nov-03	20.8	8.7
24-Nov-03	21.1	10.0
01-Dec-03	20.1	0.0
01-Dec-03	20.2	0.1
01-Dec-03	20.4	0.3
01-Dec-03	20.5	0.4
01-Dec-03	21.1	1.0
01-Dec-03	21.2	1.1
01-Dec-03	21.2	1.1
01-Dec-03	21.4	1.4
01-Dec-03	21.4	1.4
01-Dec-03	21.5	1.5
01-Dec-03	21.5	1.5
01-Dec-03	21.6	1.6
01-Dec-03	21.6	1.6
01-Dec-03	22	2.0
01-Dec-03	22.4	2.4
01-Dec-03	22.8	2.8
01-Dec-03	23.4	3.4
01-Dec-03	23.9	4.0
01-Dec-03	24.5	4.6
01-Dec-03	24.8	4.9
01-Dec-03	25.2	5.3
01-Dec-03	25.2	5.3
01-Dec-03	25.5	5.6
01-Dec-03	25.7	5.8
01-Dec-03	25.8	5.9
01-Dec-03	26	6.1
01-Dec-03	26.2	6.4
01-Dec-03	26.3	6.5
01-Dec-03	26.6	6.8
01-Dec-03	26.6	6.8
01-Dec-03	26.7	6.9
01-Dec-03	26.9	7.1
01-Dec-03	27	7.2
01-Dec-03	27.3	7.5
01-Dec-03	27.3	7.5
01-Dec-03	27.8	8.0
01-Dec-03	29.1	9.4
01-Dec-03	29.5	9.8

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01-Dec-03	29.5	9.8
01-Dec-03	29.7	10.0
08-Dec-03	18.1	0.0
08-Dec-03	18.5	1.8
08-Dec-03	18.6	2.3
08-Dec-03	18.6	2.3
08-Dec-03	18.6	2.3
08-Dec-03	18.7	2.7
08-Dec-03	18.7	2.7
08-Dec-03	18.7	2.7
08-Dec-03	18.7	2.7
08-Dec-03	18.8	3.2
08-Dec-03	18.8	3.2
08-Dec-03	18.8	3.2
08-Dec-03	18.9	3.6
08-Dec-03	18.9	3.6
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19	4.1
08-Dec-03	19.1	4.5
08-Dec-03	19.1	4.5
08-Dec-03	19.1	4.5
08-Dec-03	19.1	4.5
08-Dec-03	19.2	5.0
08-Dec-03	19.5	6.4
08-Dec-03	19.7	7.3
08-Dec-03	20.3	10.0
18-Dec-03	20.3	0.0
18-Dec-03	20.7	0.4
18-Dec-03	21.1	0.7
18-Dec-03	21.3	0.9
18-Dec-03	21.6	1.1
18-Dec-03	22.1	1.6
18-Dec-03	22.3	1.8
18-Dec-03	22.4	1.8
18/12/2003	23.2	2.5
18/12/2003	23.3	2.6
18/12/2003	23.7	3.0
18/12/2003	24.3	3.5
18/12/2003	24.5	3.7
18/12/2003	24.7	3.9
18/12/2003	24.8	3.9
18/12/2003	25	4.1

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18/12/2003	25.4	4.5
18/12/2003	25.5	4.6
18/12/2003	26	5.0
18/12/2003	26.6	5.5
18/12/2003	27.2	6.1
18/12/2003	27.5	6.3
18/12/2003	27.6	6.4
18/12/2003	27.6	6.4
18/12/2003	27.8	6.6
18/12/2003	28.4	7.1
18/12/2003	28.5	7.2
18/12/2003	28.5	7.2
18/12/2003	28.7	7.4
18/12/2003	28.8	7.5
18/12/2003	29.2	7.8
18/12/2003	31.7	10.0
17/06/2004	12.7	0.0
17/06/2004	12.9	1.4
17/06/2004	12.9	1.4
17/06/2004	12.9	1.4
17/06/2004	13.1	2.9
17/06/2004	13.2	3.6
17/06/2004	13.3	4.3
17/06/2004	13.3	4.3
17/06/2004	13.6	6.4
17/06/2004	13.7	7.1
17/06/2004	13.7	7.1
17/06/2004	13.8	7.9
17/06/2004	13.8	7.9
17/06/2004	13.9	8.6
17/06/2004	14	9.3
17/06/2004	14	9.3
17/06/2004	14	9.3
17/06/2004	14	9.3
17/06/2004	14.1	10.0
17/06/2004	14.1	10.0
17/06/2004	14.1	10.0
17/06/2004	14.1	10.0
17/06/2004	14.1	10.0
17/06/2004	14.1	10.0
24/06/2004	15.4	0.0
24/06/2004	15.6	0.5
24/06/2004	15.7	0.8
24/06/2004	15.8	1.1
24/06/2004	16.2	2.1
24/06/2004	16.3	2.4
24/06/2004	16.3	2.4
24/06/2004	16.5	2.9
24/06/2004	16.6	3.2
24/06/2004	16.6	3.2
24/06/2004	16.7	3.5
24/06/2004	16.7	3.5

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24/06/2004	16.8	3.8
24/06/2004	17	4.3
24/06/2004	17.1	4.6
24/06/2004	17.2	4.8
24/06/2004	17.5	5.6
24/06/2004	17.5	5.6
24/06/2004	17.6	5.9
24/06/2004	18.1	7.2
24/06/2004	18.3	7.8
24/06/2004	18.4	8.0
24/06/2004	18.8	9.1
24/06/2004	18.8	9.1
24/06/2004	18.8	9.1
24/06/2004	18.8	9.1
24/06/2004	19.13	10.0
04/07/2004	12.8	0.0
04/07/2004	13.3	1.9
04/07/2004	13.4	2.2
04/07/2004	13.5	2.6
04/07/2004	13.6	3.0
04/07/2004	13.6	3.0
04/07/2004	13.6	3.0
04/07/2004	13.7	3.3
04/07/2004	13.8	3.7
04/07/2004	14.1	4.8
04/07/2004	14.2	5.2
04/07/2004	14.2	5.2
04/07/2004	14.3	5.6
04/07/2004	14.3	5.6
04/07/2004	14.4	5.9
04/07/2004	14.4	5.9
04/07/2004	14.4	5.9
04/07/2004	14.4	5.9
04/07/2004	14.4	5.9
04/07/2004	14.4	5.9
04/07/2004	14.5	6.3
04/07/2004	14.5	6.3
04/07/2004	14.6	6.7
04/07/2004	14.6	6.7
04/07/2004	14.7	7.0
04/07/2004	14.7	7.0
04/07/2004	14.7	7.0
04/07/2004	14.7	7.0
04/07/2004	14.8	7.4
04/07/2004	14.8	7.4
04/07/2004	14.9	7.8
04/07/2004	15	8.1
04/07/2004	15.1	8.5
04/07/2004	15.2	8.9
04/07/2004	15.2	8.9
04/07/2004	15.5	10.0
12/07/2004	13.1	0.0

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WESTERN CAPE

12/07/2004	13.3	0.5
12/07/2004	13.3	0.5
12/07/2004	13.4	0.8
12/07/2004	13.5	1.1
12/07/2004	13.5	1.1
12/07/2004	13.5	1.1
12/07/2004	13.6	1.3
12/07/2004	13.6	1.3
12/07/2004	13.8	1.8
12/07/2004	13.9	2.1
12/07/2004	14.1	2.6
12/07/2004	14.1	2.6
12/07/2004	14.2	2.9
12/07/2004	14.2	2.9
12/07/2004	14.4	3.4
12/07/2004	14.4	3.4
12/07/2004	14.4	3.4
12/07/2004	14.5	3.7
12/07/2004	14.5	3.7
12/07/2004	14.6	3.9
12/07/2004	15	5.0
12/07/2004	15	5.0
12/07/2004	15	5.0
12/07/2004	15.1	5.3
12/07/2004	15.1	5.3
12/07/2004	15.3	5.8
12/07/2004	15.3	5.8
12/07/2004	15.4	6.1
12/07/2004	15.4	6.1
12/07/2004	15.4	6.1
12/07/2004	15.4	6.1
12/07/2004	15.5	6.3
12/07/2004	15.6	6.6
12/07/2004	15.7	6.8
12/07/2004	15.7	6.8
12/07/2004	15.8	7.1
12/07/2004	15.9	7.4
12/07/2004	16	7.6
12/07/2004	16.1	7.9
12/07/2004	16.2	8.2
12/07/2004	16.2	8.2
12/07/2004	16.5	8.9
12/07/2004	16.7	9.5
12/07/2004	16.8	9.7
12/07/2004	16.9	10.0
12/07/2004	16.9	10.0
19/07/2004	11.9	0.0
19/07/2004	11.9	0.0
19/07/2004	12	0.2
19/07/2004	12.2	0.5
19/07/2004	12.2	0.5
19/07/2004	12.2	0.5

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WESTERN CAPE

19/07/2004	12.3	0.6
19/07/2004	12.3	0.6
19/07/2004	12.3	0.6
19/07/2004	12.4	0.8
19/07/2004	12.4	0.8
19/07/2004	12.6	1.1
19/07/2004	12.6	1.1
19/07/2004	12.7	1.3
19/07/2004	13	1.7
19/07/2004	13.2	2.1
19/07/2004	13.5	2.5
19/07/2004	13.6	2.7
19/07/2004	13.7	2.9
19/07/2004	13.8	3.0
19/07/2004	14.1	3.5
19/07/2004	14.4	4.0
19/07/2004	14.4	4.0
19/07/2004	14.6	4.3
19/07/2004	14.6	4.3
19/07/2004	14.8	4.6
19/07/2004	14.9	4.8
19/07/2004	14.9	4.8
19/07/2004	15.1	5.1
19/07/2004	15.1	5.1
19/07/2004	15.2	5.2
19/07/2004	15.3	5.4
19/07/2004	15.4	5.6
19/07/2004	15.6	5.9
19/07/2004	15.7	6.0
19/07/2004	15.8	6.2
19/07/2004	15.9	6.3
19/07/2004	16	6.5
19/07/2004	16.2	6.8
19/07/2004	16.7	7.6
19/07/2004	16.7	7.6
19/07/2004	16.8	7.8
19/07/2004	16.8	7.8
19/07/2004	17.1	8.3
19/07/2004	17.2	8.4
19/07/2004	17.8	9.4
19/07/2004	18.2	10.0
19/07/2004	18.2	10.0
26/07/2004	12.1	0.0
26/07/2004	12.6	0.9
26/07/2004	12.6	0.9
26/07/2004	12.6	0.9
26/07/2004	12.8	1.2
26/07/2004	12.9	1.4
26/07/2004	12.9	1.4
26/07/2004	12.9	1.4
26/07/2004	13	1.6
26/07/2004	13	1.6

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26/07/2004	13.1	1.7
26/07/2004	13.3	2.1
26/07/2004	13.3	2.1
26/07/2004	13.5	2.4
26/07/2004	13.6	2.6
26/07/2004	13.6	2.6
26/07/2004	13.7	2.8
26/07/2004	13.7	2.8
26/07/2004	14	3.3
26/07/2004	14	3.3
26/07/2004	14	3.3
26/07/2004	14	3.3
26/07/2004	14.1	3.4
26/07/2004	14.1	3.4
26/07/2004	14.1	3.4
26/07/2004	14.1	3.4
26/07/2004	14.1	3.4
26/07/2004	14.2	3.6
26/07/2004	14.2	3.6
26/07/2004	14.2	3.6
26/07/2004	14.4	4.0
26/07/2004	14.5	4.1
26/07/2004	14.6	4.3
26/07/2004	14.6	4.3
26/07/2004	14.6	4.3
26/07/2004	14.6	4.3
26/07/2004	14.8	4.7
26/07/2004	15	5.0
26/07/2004	15	5.0
26/07/2004	15.1	5.2
26/07/2004	15.2	5.3
26/07/2004	15.2	5.3
26/07/2004	15.2	5.3
26/07/2004	15.3	5.5
26/07/2004	15.3	5.5
26/07/2004	15.5	5.9
26/07/2004	15.5	5.9
26/07/2004	16	6.7
26/07/2004	16.2	7.1
26/07/2004	16.3	7.2
26/07/2004	17.9	10.0
08/08/2004	12.9	0.0
08/08/2004	12.9	0.0
08/08/2004	13	0.2
08/08/2004	13	0.2
08/08/2004	13.1	0.5
08/08/2004	13.2	0.7
08/08/2004	13.2	0.7
08/08/2004	13.3	0.9
08/08/2004	13.3	0.9
08/08/2004	13.5	1.4
08/08/2004	13.5	1.4

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08/08/2004	13.5	1.4
08/08/2004	13.6	1.6
08/08/2004	13.6	1.6
08/08/2004	13.6	1.6
08/08/2004	13.6	1.6
08/08/2004	13.6	1.6
08/08/2004	13.6	1.6
08/08/2004	13.7	1.9
08/08/2004	13.7	1.9
08/08/2004	13.7	1.9
08/08/2004	13.7	1.9
08/08/2004	13.7	1.9
08/08/2004	13.7	1.9
08/08/2004	13.7	1.9
08/08/2004	13.8	2.1
08/08/2004	13.8	2.1
08/08/2004	13.8	2.1
08/08/2004	13.8	2.1
08/08/2004	13.8	2.1
08/08/2004	13.8	2.1
08/08/2004	13.8	2.1
08/08/2004	13.8	2.1
08/08/2004	13.9	2.3
08/08/2004	13.9	2.3
08/08/2004	13.9	2.3
08/08/2004	13.9	2.3
08/08/2004	13.9	2.3
08/08/2004	14	2.6
08/08/2004	14	2.6
08/08/2004	14.1	2.8
08/08/2004	14.1	2.8
08/08/2004	14.2	3.0
08/08/2004	14.2	3.0
08/08/2004	14.2	3.0
08/08/2004	14.2	3.0
08/08/2004	14.4	3.5
08/08/2004	14.5	3.7
08/08/2004	14.5	3.7
08/08/2004	14.7	4.2
08/08/2004	15.1	5.1
08/08/2004	15.1	5.1
08/08/2004	15.8	6.7
08/08/2004	17.2	10.0
15/08/2004	12.3	0.0
15/08/2004	12.3	0.0
15/08/2004	12.4	0.2
15/08/2004	12.4	0.2
15/08/2004	12.4	0.2
15/08/2004	12.5	0.5
15/08/2004	12.5	0.5
15/08/2004	12.5	0.5
15/08/2004	12.7	1.0

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15/08/2004	12.7	1.0
15/08/2004	12.8	1.2
15/08/2004	12.8	1.2
15/08/2004	12.9	1.5
15/08/2004	12.9	1.5
15/08/2004	12.9	1.5
15/08/2004	13	1.7
15/08/2004	13.1	2.0
15/08/2004	13.1	2.0
15/08/2004	13.2	2.2
15/08/2004	13.2	2.2
15/08/2004	13.3	2.4
15/08/2004	13.3	2.4
15/08/2004	13.3	2.4
15/08/2004	13.4	2.7
15/08/2004	13.4	2.7
15/08/2004	13.5	2.9
15/08/2004	13.5	2.9
15/08/2004	13.5	2.9
15/08/2004	13.6	3.2
15/08/2004	13.6	3.2
15/08/2004	13.6	3.2
15/08/2004	13.7	3.4
15/08/2004	13.7	3.4
15/08/2004	13.7	3.4
15/08/2004	13.8	3.7
15/08/2004	13.8	3.7
15/08/2004	13.9	3.9
15/08/2004	14	4.1
15/08/2004	14.1	4.4
15/08/2004	14.4	5.1
15/08/2004	14.5	5.4
15/08/2004	14.5	5.4
15/08/2004	14.8	6.1
15/08/2004	15.1	6.8
15/08/2004	15.2	7.1
15/08/2004	15.6	8.0
15/08/2004	15.8	8.5
15/08/2004	16	9.0
15/08/2004	16	9.0
15/08/2004	16.1	9.3
15/08/2004	16.2	9.5
15/08/2004	16.3	9.8
15/08/2004	16.3	9.8
15/08/2004	16.4	10.0
22/08/2004	13.2	0.0
22/08/2004	13.5	0.6
22/08/2004	13.6	0.8
22/08/2004	13.7	1.0
22/08/2004	13.7	1.0
22/08/2004	13.7	1.0
22/08/2004	13.7	1.0

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22/08/2004	13.7	1.0
22/08/2004	13.8	1.2
22/08/2004	13.8	1.2
22/08/2004	13.9	1.4
22/08/2004	14	1.6
22/08/2004	14.1	1.8
22/08/2004	14.1	1.8
22/08/2004	14.2	2.0
22/08/2004	14.2	2.0
22/08/2004	14.2	2.0
22/08/2004	14.2	2.0
22/08/2004	14.3	2.2
22/08/2004	14.3	2.2
22/08/2004	14.3	2.2
22/08/2004	14.3	2.2
22/08/2004	14.4	2.4
22/08/2004	14.4	2.4
22/08/2004	14.4	2.4
22/08/2004	14.5	2.7
22/08/2004	14.5	2.7
22/08/2004	14.5	2.7
22/08/2004	14.6	2.9
22/08/2004	14.7	3.1
22/08/2004	14.8	3.3
22/08/2004	14.8	3.3
22/08/2004	14.8	3.3
22/08/2004	14.9	3.5
22/08/2004	14.9	3.5
22/08/2004	15	3.7
22/08/2004	15	3.7
22/08/2004	15.2	4.1
22/08/2004	15.2	4.1
22/08/2004	15.2	4.1
22/08/2004	15.3	4.3
22/08/2004	15.3	4.3
22/08/2004	15.3	4.3
22/08/2004	15.4	4.5
22/08/2004	15.5	4.7
22/08/2004	15.6	4.9
22/08/2004	15.6	4.9
22/08/2004	15.7	5.1
22/08/2004	15.8	5.3
22/08/2004	15.9	5.5
22/08/2004	16.4	6.5
22/08/2004	16.5	6.7
22/08/2004	16.9	7.6
22/08/2004	17.4	8.6
22/08/2004	18	9.8
22/08/2004	18.1	10.0
25/08/2004	13.2	0.0
25/08/2004	13.3	0.2
25/08/2004	13.3	0.2

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WESTERN CAPE

25/08/2004	13.3	0.2
25/08/2004	13.3	0.2
25/08/2004	13.6	0.8
25/08/2004	13.6	0.8
25/08/2004	13.7	0.9
25/08/2004	14.2	1.9
25/08/2004	14.2	1.9
25/08/2004	14.2	1.9
25/08/2004	14.3	2.1
25/08/2004	14.3	2.1
25/08/2004	14.3	2.1
25/08/2004	14.3	2.1
25/08/2004	14.4	2.3
25/08/2004	14.6	2.6
25/08/2004	14.7	2.8
25/08/2004	14.9	3.2
25/08/2004	14.9	3.2
25/08/2004	15.1	3.6
25/08/2004	15.1	3.6
25/08/2004	15.2	3.8
25/08/2004	15.3	4.0
25/08/2004	15.3	4.0
25/08/2004	15.9	5.1
25/08/2004	15.9	5.1
25/08/2004	15.9	5.1
25/08/2004	16	5.3
25/08/2004	16.1	5.5
25/08/2004	16.2	5.7
25/08/2004	16.3	5.8
25/08/2004	16.4	6.0
25/08/2004	16.4	6.0
25/08/2004	16.5	6.2
25/08/2004	16.5	6.2
25/08/2004	16.6	6.4
25/08/2004	16.7	6.6
25/08/2004	16.8	6.8
25/08/2004	16.8	6.8
25/08/2004	17	7.2
25/08/2004	17.2	7.5
25/08/2004	17.4	7.9
25/08/2004	17.5	8.1
25/08/2004	17.5	8.1
25/08/2004	17.6	8.3
25/08/2004	17.7	8.5
25/08/2004	17.7	8.5
25/08/2004	17.7	8.5
25/08/2004	18	9.1
25/08/2004	18.2	9.4
25/08/2004	18.2	9.4
25/08/2004	18.4	9.8
25/08/2004	18.5	10.0
03/09/2004	14.1	0.0

UNIVERSITY of the
WESTERN CAPE

03/09/2004	14.2	0.3
03/09/2004	14.2	0.3
03/09/2004	14.2	0.3
03/09/2004	14.2	0.3
03/09/2004	14.2	0.3
03/09/2004	14.3	0.5
03/09/2004	14.3	0.5
03/09/2004	14.4	0.8
03/09/2004	14.4	0.8
03/09/2004	14.4	0.8
03/09/2004	14.4	0.8
03/09/2004	14.4	0.8
03/09/2004	14.4	0.8
03/09/2004	14.5	1.0
03/09/2004	14.5	1.0
03/09/2004	14.5	1.0
03/09/2004	14.6	1.3
03/09/2004	14.6	1.3
03/09/2004	14.7	1.5
03/09/2004	14.7	1.5
03/09/2004	14.8	1.8
03/09/2004	14.8	1.8
03/09/2004	14.8	1.8
03/09/2004	14.8	1.8
03/09/2004	15	2.3
03/09/2004	15	2.3
03/09/2004	15.2	2.8
03/09/2004	15.2	2.8
03/09/2004	15.2	2.8
03/09/2004	15.3	3.1
03/09/2004	15.4	3.3
03/09/2004	15.4	3.3
03/09/2004	15.5	3.6
03/09/2004	15.5	3.6
03/09/2004	15.6	3.8
03/09/2004	15.6	3.8
03/09/2004	15.8	4.4
03/09/2004	15.9	4.6
03/09/2004	15.9	4.6
03/09/2004	16	4.9
03/09/2004	16	4.9
03/09/2004	16.1	5.1
03/09/2004	16.2	5.4
03/09/2004	16.2	5.4
03/09/2004	16.2	5.4
03/09/2004	16.3	5.6
03/09/2004	16.3	5.6
03/09/2004	16.4	5.9
03/09/2004	16.4	5.9
03/09/2004	16.5	6.2
03/09/2004	16.8	6.9
03/09/2004	16.9	7.2

UNIVERSITY of the
WESTERN CAPE

03/09/2004	17.1	7.7
03/09/2004	17.2	7.9
03/09/2004	17.3	8.2
03/09/2004	18	10.0
09/09/2004	12.6	0.0
09/09/2004	12.6	0.0
09/09/2004	12.6	0.0
09/09/2004	12.6	0.0
09/09/2004	12.8	0.3
09/09/2004	12.8	0.3
09/09/2004	12.8	0.3
09/09/2004	12.8	0.3
09/09/2004	12.9	0.4
09/09/2004	12.9	0.4
09/09/2004	13.1	0.6
09/09/2004	13.4	1.0
09/09/2004	13.5	1.1
09/09/2004	13.5	1.1
09/09/2004	13.6	1.3
09/09/2004	13.6	1.3
09/09/2004	13.8	1.5
09/09/2004	13.8	1.5
09/09/2004	13.9	1.6
09/09/2004	14.2	2.0
09/09/2004	14.2	2.0
09/09/2004	14.2	2.0
09/09/2004	14.6	2.5
09/09/2004	14.7	2.6
09/09/2004	14.7	2.6
09/09/2004	14.7	2.6
09/09/2004	14.8	2.8
09/09/2004	15	3.0
09/09/2004	15.1	3.1
09/09/2004	15.8	4.0
09/09/2004	15.8	4.0
09/09/2004	15.9	4.1
09/09/2004	16	4.3
09/09/2004	16.3	4.6
09/09/2004	16.3	4.6
09/09/2004	16.4	4.8
09/09/2004	16.4	4.8
09/09/2004	16.5	4.9
09/09/2004	16.5	4.9
09/09/2004	16.7	5.1
09/09/2004	16.7	5.1
09/09/2004	16.8	5.3
09/09/2004	16.9	5.4
09/09/2004	17.4	6.0
09/09/2004	17.4	6.0
09/09/2004	18	6.8
09/09/2004	18.2	7.0
09/09/2004	18.2	7.0

UNIVERSITY of the
WESTERN CAPE

09/09/2004	18.2	7.0
09/09/2004	18.3	7.1
09/09/2004	18.3	7.1
09/09/2004	18.5	7.4
09/09/2004	19.7	8.9
09/09/2004	19.8	9.0
09/09/2004	20	9.3
09/09/2004	20.6	10.0
09/09/2004	20.6	10.0
19/09/2004	14	0.0
19/09/2004	14.2	0.4
19/09/2004	14.2	0.4
19/09/2004	14.3	0.5
19/09/2004	14.4	0.7
19/09/2004	14.4	0.7
19/09/2004	14.7	1.3
19/09/2004	14.8	1.4
19/09/2004	15.1	2.0
19/09/2004	15.1	2.0
19/09/2004	15.1	2.0
19/09/2004	15.2	2.1
19/09/2004	15.4	2.5
19/09/2004	15.6	2.9
19/09/2004	15.6	2.9
19/09/2004	15.6	2.9
19/09/2004	15.6	2.9
19/09/2004	15.6	2.9
19/09/2004	15.7	3.0
19/09/2004	15.7	3.0
19/09/2004	15.7	3.0
19/09/2004	15.8	3.2
19/09/2004	15.8	3.2
19/09/2004	15.9	3.4
19/09/2004	15.9	3.4
19/09/2004	16.1	3.8
19/09/2004	16.1	3.8
19/09/2004	16.1	3.8
19/09/2004	16.2	3.9
19/09/2004	16.3	4.1
19/09/2004	16.5	4.5
19/09/2004	16.6	4.6
19/09/2004	16.6	4.6
19/09/2004	17	5.4
19/09/2004	17.3	5.9
19/09/2004	17.3	5.9
19/09/2004	17.3	5.9
19/09/2004	17.4	6.1
19/09/2004	17.6	6.4
19/09/2004	17.8	6.8
19/09/2004	17.8	6.8
19/09/2004	18	7.1
19/09/2004	18.1	7.3

UNIVERSITY of the
WESTERN CAPE

19/09/2004	18.1	7.3
19/09/2004	18.4	7.9
19/09/2004	18.4	7.9
19/09/2004	18.5	8.0
19/09/2004	18.7	8.4
19/09/2004	18.7	8.4
19/09/2004	18.8	8.6
19/09/2004	19	8.9
19/09/2004	19.2	9.3
19/09/2004	19.3	9.5
19/09/2004	19.6	10.0
23/09/2004	14.1	0.0
23/09/2004	14.3	0.4
23/09/2004	14.7	1.1
23/09/2004	14.7	1.1
23/09/2004	14.9	1.5
23/09/2004	14.9	1.5
23/09/2004	15.2	2.0
23/09/2004	15.3	2.2
23/09/2004	15.3	2.2
23/09/2004	15.6	2.7
23/09/2004	15.6	2.7
23/09/2004	15.6	2.7
23/09/2004	15.6	2.7
23/09/2004	15.8	3.1
23/09/2004	15.8	3.1
23/09/2004	15.8	3.1
23/09/2004	15.9	3.3
23/09/2004	15.9	3.3
23/09/2004	16	3.5
23/09/2004	16.1	3.6
23/09/2004	16.2	3.8
23/09/2004	16.2	3.8
23/09/2004	16.3	4.0
23/09/2004	16.4	4.2
23/09/2004	16.4	4.2
23/09/2004	16.5	4.4
23/09/2004	16.6	4.5
23/09/2004	16.7	4.7
23/09/2004	16.7	4.7
23/09/2004	16.8	4.9
23/09/2004	16.8	4.9
23/09/2004	17	5.3
23/09/2004	17.1	5.5
23/09/2004	17.2	5.6
23/09/2004	17.2	5.6
23/09/2004	17.3	5.8
23/09/2004	17.4	6.0
23/09/2004	17.5	6.2
23/09/2004	17.7	6.5
23/09/2004	17.7	6.5
23/09/2004	17.7	6.5

UNIVERSITY of the
WESTERN CAPE

23/09/2004	17.7	6.5
23/09/2004	17.8	6.7
23/09/2004	17.8	6.7
23/09/2004	17.9	6.9
23/09/2004	17.9	6.9
23/09/2004	18	7.1
23/09/2004	18	7.1
23/09/2004	18.6	8.2
23/09/2004	18.8	8.5
23/09/2004	19	8.9
23/09/2004	19.2	9.3
23/09/2004	19.4	9.6
23/09/2004	19.6	10.0
30/09/2004	15.2	0.0
30/09/2004	15.3	0.1
30/09/2004	15.6	0.5
30/09/2004	15.6	0.5
30/09/2004	15.7	0.7
30/09/2004	15.9	1.0
30/09/2004	16.1	1.2
30/09/2004	16.7	2.1
30/09/2004	16.7	2.1
30/09/2004	16.7	2.1
30/09/2004	17.3	2.9
30/09/2004	17.3	2.9
30/09/2004	17.5	3.2
30/09/2004	17.9	3.7
30/09/2004	18.2	4.1
30/09/2004	18.2	4.1
30/09/2004	18.4	4.4
30/09/2004	18.6	4.7
30/09/2004	18.6	4.7
30/09/2004	18.8	4.9
30/09/2004	18.8	4.9
30/09/2004	18.8	4.9
30/09/2004	18.9	5.1
30/09/2004	19	5.2
30/09/2004	19.2	5.5
30/09/2004	19.2	5.5
30/09/2004	19.2	5.5
30/09/2004	19.3	5.6
30/09/2004	19.4	5.8
30/09/2004	19.4	5.8
30/09/2004	19.5	5.9
30/09/2004	19.5	5.9
30/09/2004	19.6	6.0
30/09/2004	19.7	6.2
30/09/2004	19.8	6.3
30/09/2004	19.8	6.3
30/09/2004	19.8	6.3
30/09/2004	19.8	6.3
30/09/2004	19.9	6.4

UNIVERSITY of the
WESTERN CAPE

30/09/2004	20.2	6.8
30/09/2004	20.7	7.5
30/09/2004	20.9	7.8
30/09/2004	20.9	7.8
30/09/2004	20.9	7.8
30/09/2004	20.9	7.8
30/09/2004	21.1	8.1
30/09/2004	21.1	8.1
30/09/2004	21.5	8.6
30/09/2004	21.9	9.2
30/09/2004	22.4	9.9
30/09/2004	22.4	9.9
30/09/2004	22.5	10.0
30/09/2004	22.5	10.0
30/09/2004	22.5	10.0
17/10/2004	16	0.0
17/10/2004	16.3	0.3
17/10/2004	16.5	0.5
17/10/2004	16.8	0.8
17/10/2004	16.9	0.9
17/10/2004	17	1.0
17/10/2004	17	1.0
17/10/2004	17	1.0
17/10/2004	17.1	1.1
17/10/2004	17.5	1.6
17/10/2004	17.7	1.8
17/10/2004	17.8	1.9
17/10/2004	17.9	2.0
17/10/2004	18	2.1
17/10/2004	18.2	2.3
17/10/2004	18.2	2.3
17/10/2004	18.5	2.6
17/10/2004	18.6	2.7
17/10/2004	18.6	2.7
17/10/2004	18.6	2.7
17/10/2004	18.8	2.9
17/10/2004	19	3.1
17/10/2004	19	3.1
17/10/2004	19	3.1
17/10/2004	19.1	3.2
17/10/2004	19.1	3.2
17/10/2004	19.6	3.8
17/10/2004	19.6	3.8
17/10/2004	19.7	3.9
17/10/2004	19.8	4.0
17/10/2004	19.8	4.0
17/10/2004	20.1	4.3
17/10/2004	20.1	4.3
17/10/2004	20.8	5.0
17/10/2004	20.8	5.0
17/10/2004	21	5.2
17/10/2004	21	5.2

UNIVERSITY of the
WESTERN CAPE

17/10/2004	21.1	5.3
17/10/2004	21.2	5.4
17/10/2004	21.5	5.7
17/10/2004	21.5	5.7
17/10/2004	21.6	5.8
17/10/2004	21.7	5.9
17/10/2004	21.7	5.9
17/10/2004	22	6.3
17/10/2004	22.4	6.7
17/10/2004	23.2	7.5
17/10/2004	23.3	7.6
17/10/2004	23.3	7.6
17/10/2004	23.5	7.8
17/10/2004	23.9	8.2
17/10/2004	24.1	8.4
17/10/2004	24.4	8.8
17/10/2004	24.4	8.8
17/10/2004	24.5	8.9
17/10/2004	25.3	9.7
17/10/2004	25.6	10.0
24/10/2004	17.3	0.0
24/10/2004	17.4	0.2
24/10/2004	17.4	0.2
24/10/2004	17.6	0.5
24/10/2004	17.8	0.8
24/10/2004	18	1.1
24/10/2004	18	1.1
24/10/2004	18.3	1.6
24/10/2004	18.6	2.0
24/10/2004	18.6	2.0
24/10/2004	18.7	2.2
24/10/2004	18.8	2.3
24/10/2004	18.9	2.5
24/10/2004	19	2.7
24/10/2004	19.2	3.0
24/10/2004	19.2	3.0
24/10/2004	19.4	3.3
24/10/2004	19.4	3.3
24/10/2004	19.5	3.4
24/10/2004	19.7	3.8
24/10/2004	19.7	3.8
24/10/2004	19.8	3.9
24/10/2004	19.9	4.1
24/10/2004	20	4.2
24/10/2004	20.2	4.5
24/10/2004	20.3	4.7
24/10/2004	20.3	4.7
24/10/2004	20.3	4.7
24/10/2004	20.8	5.5
24/10/2004	20.8	5.5
24/10/2004	20.9	5.6
24/10/2004	21	5.8

UNIVERSITY of the
WESTERN CAPE

24/10/2004	21	5.8
24/10/2004	21.1	5.9
24/10/2004	21.1	5.9
24/10/2004	21.1	5.9
24/10/2004	21.3	6.3
24/10/2004	21.3	6.3
24/10/2004	21.4	6.4
24/10/2004	21.4	6.4
24/10/2004	21.6	6.7
24/10/2004	21.6	6.7
24/10/2004	21.6	6.7
24/10/2004	21.8	7.0
24/10/2004	21.8	7.0
24/10/2004	22	7.3
24/10/2004	22.1	7.5
24/10/2004	22.2	7.7
24/10/2004	22.5	8.1
24/10/2004	22.5	8.1
24/10/2004	22.6	8.3
24/10/2004	22.7	8.4
24/10/2004	23.3	9.4
24/10/2004	23.3	9.4
24/10/2004	23.5	9.7
24/10/2004	23.6	9.8
24/10/2004	23.7	10.0
31/10/2004	15.6	0.0
31/10/2004	15.7	0.1
31/10/2004	15.9	0.3
31/10/2004	16.1	0.5
31/10/2004	16.1	0.5
31/10/2004	16.1	0.5
31/10/2004	16.3	0.6
31/10/2004	16.4	0.7
31/10/2004	16.6	0.9
31/10/2004	16.8	1.1
31/10/2004	16.8	1.1
31/10/2004	17	1.3
31/10/2004	17	1.3
31/10/2004	17.2	1.4
31/10/2004	17.3	1.5
31/10/2004	17.5	1.7
31/10/2004	17.6	1.8
31/10/2004	17.6	1.8
31/10/2004	17.7	1.9
31/10/2004	17.8	2.0
31/10/2004	17.9	2.1
31/10/2004	18.1	2.3
31/10/2004	18.2	2.3
31/10/2004	18.4	2.5
31/10/2004	18.5	2.6
31/10/2004	18.5	2.6
31/10/2004	18.7	2.8

UNIVERSITY of the
WESTERN CAPE

31/10/2004	19	3.1
31/10/2004	19.1	3.2
31/10/2004	19.2	3.2
31/10/2004	19.4	3.4
31/10/2004	19.6	3.6
31/10/2004	19.7	3.7
31/10/2004	19.8	3.8
31/10/2004	20.1	4.1
31/10/2004	20.1	4.1
31/10/2004	20.2	4.1
31/10/2004	20.5	4.4
31/10/2004	20.93	4.8
31/10/2004	21	4.9
31/10/2004	21.5	5.3
31/10/2004	21.6	5.4
31/10/2004	21.9	5.7
31/10/2004	22.1	5.9
31/10/2004	22.1	5.9
31/10/2004	22.3	6.0
31/10/2004	22.5	6.2
31/10/2004	22.7	6.4
31/10/2004	22.8	6.5
31/10/2004	23.1	6.8
31/10/2004	23.1	6.8
31/10/2004	23.7	7.3
31/10/2004	23.8	7.4
31/10/2004	24.7	8.2
31/10/2004	25.23	8.7
31/10/2004	25.3	8.7
31/10/2004	26.7	10.0
07/11/2004	16.6	0.0
07/11/2004	16.8	0.1
07/11/2004	16.9	0.2
07/11/2004	17	0.2
07/11/2004	17.1	0.3
07/11/2004	17.2	0.4
07/11/2004	17.3	0.4
07/11/2004	17.5	0.6
07/11/2004	17.6	0.6
07/11/2004	17.6	0.6
07/11/2004	17.6	0.6
07/11/2004	17.6	0.6
07/11/2004	17.7	0.7
07/11/2004	17.7	0.7
07/11/2004	17.8	0.7
07/11/2004	17.9	0.8
07/11/2004	18.1	0.9
07/11/2004	18.2	1.0
07/11/2004	18.2	1.0
07/11/2004	18.2	1.0
07/11/2004	18.3	1.1
07/11/2004	18.3	1.1

UNIVERSITY of the
WESTERN CAPE

07/11/2004	18.4	1.1
07/11/2004	18.4	1.1
07/11/2004	18.7	1.3
07/11/2004	18.9	1.4
07/11/2004	19	1.5
07/11/2004	19.6	1.9
07/11/2004	20.6	2.5
07/11/2004	21	2.7
07/11/2004	21	2.7
07/11/2004	21.1	2.8
07/11/2004	21.3	2.9
07/11/2004	21.5	3.0
07/11/2004	21.6	3.1
07/11/2004	21.7	3.2
07/11/2004	21.9	3.3
07/11/2004	22.3	3.5
07/11/2004	22.9	3.9
07/11/2004	23.3	4.2
07/11/2004	23.3	4.2
07/11/2004	24.1	4.7
07/11/2004	24.3	4.8
07/11/2004	24.4	4.8
07/11/2004	25.1	5.3
07/11/2004	25.4	5.5
07/11/2004	25.6	5.6
07/11/2004	25.6	5.6
07/11/2004	26.3	6.0
07/11/2004	26.5	6.1
07/11/2004	27.6	6.8
07/11/2004	28	7.1
07/11/2004	28.1	7.1
07/11/2004	28.9	7.6
07/11/2004	29.4	8.0
07/11/2004	32.3	9.8
07/11/2004	32.7	10.0
14/11/2004	19.5	0.0
14/11/2004	19.7	0.2
14/11/2004	19.9	0.4
14/11/2004	20	0.5
14/11/2004	20.2	0.7
14/11/2004	20.3	0.8
14/11/2004	20.7	1.3
14/11/2004	21	1.6
14/11/2004	21.1	1.7
14/11/2004	21.1	1.7
14/11/2004	21.2	1.8
14/11/2004	21.2	1.8
14/11/2004	21.3	1.9
14/11/2004	21.3	1.9
14/11/2004	21.4	2.0
14/11/2004	21.6	2.2
14/11/2004	22	2.6

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WESTERN CAPE

14/11/2004	22.2	2.8
14/11/2004	22.2	2.8
14/11/2004	22.3	2.9
14/11/2004	22.6	3.3
14/11/2004	22.7	3.4
14/11/2004	22.7	3.4
14/11/2004	22.9	3.6
14/11/2004	23.3	4.0
14/11/2004	24	4.7
14/11/2004	24	4.7
14/11/2004	24.1	4.8
14/11/2004	24.1	4.8
14/11/2004	24.2	4.9
14/11/2004	24.3	5.1
14/11/2004	24.3	5.1
14/11/2004	24.4	5.2
14/11/2004	24.7	5.5
14/11/2004	25	5.8
14/11/2004	25.1	5.9
14/11/2004	25.3	6.1
14/11/2004	25.5	6.3
14/11/2004	25.7	6.5
14/11/2004	25.9	6.7
14/11/2004	26	6.8
14/11/2004	26.1	6.9
14/11/2004	26.2	7.1
14/11/2004	26.6	7.5
14/11/2004	27.5	8.4
14/11/2004	28.5	9.5
14/11/2004	28.8	9.8
14/11/2004	29	10.0
22/11/2004	18.5	0.0
22/11/2004	18.5	0.0
22/11/2004	18.6	0.1
22/11/2004	18.8	0.3
22/11/2004	19.1	0.6
22/11/2004	19.1	0.6
22/11/2004	19.1	0.6
22/11/2004	19.1	0.6
22/11/2004	19.4	0.8
22/11/2004	19.4	0.8
22/11/2004	19.5	0.9
22/11/2004	19.6	1.0
22/11/2004	20.1	1.5
22/11/2004	20.7	2.1
22/11/2004	20.8	2.2
22/11/2004	21.2	2.5
22/11/2004	21.3	2.6
22/11/2004	21.5	2.8
22/11/2004	22.2	3.5
22/11/2004	22.3	3.6
22/11/2004	22.7	4.0

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22/11/2004	22.7	4.0
22/11/2004	23.1	4.3
22/11/2004	23.3	4.5
22/11/2004	23.3	4.5
22/11/2004	23.7	4.9
22/11/2004	23.9	5.1
22/11/2004	24.1	5.3
22/11/2004	24.4	5.6
22/11/2004	25	6.1
22/11/2004	25.5	6.6
22/11/2004	25.7	6.8
22/11/2004	26.1	7.2
22/11/2004	26.2	7.3
22/11/2004	26.2	7.3
22/11/2004	26.6	7.6
22/11/2004	26.6	7.6
22/11/2004	26.9	7.9
22/11/2004	27.2	8.2
22/11/2004	27.3	8.3
22/11/2004	28.2	9.2
22/11/2004	29.1	10.0

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Appendix 3: The numbers of tadpoles trapped per hour at different water depths.

Water depth (cm)	*C. platys* trapped per hour	*S. grayii* trapped per hour	*X. laevis* trapped per hour
5	0.0000	0.8056	0.0000
6	0.0000	0.1508	0.0000
7	0.0000	0.4724	0.0000
8	0.0000	0.2864	0.0000
9	0.0314	0.3363	0.0000
10	0.0118	0.0463	0.0000
11	0.0000	0.1203	0.0000
12	0.0103	0.0774	0.0000
13	0.0000	0.0605	0.0037
14	0.0000	0.0730	0.0039
15	0.0200	0.0658	0.0000
16	0.0025	0.0305	0.0000
17	0.0031	0.0144	0.0000
18	0.0040	0.0600	0.0000
19	0.0047	0.0385	0.0030
20	0.0036	0.0284	0.0018
21	0.0000	0.0368	0.0000
22	0.0000	0.1076	0.0000
23	0.0107	0.0958	0.0000
24	0.0000	0.0217	0.0000
25	0.0265	0.0150	0.0000
26	0.0000	0.0453	0.0000
27	0.0000	0.1933	0.0084
28	0.0021	0.0117	0.0000
29	0.0000	0.0061	0.0025
30	0.0033	0.0489	0.0000
31	0.0029	0.0242	0.0000
32	0.0114	0.0236	0.0000
33	0.0074	0.0253	0.0000
34	0.0041	0.0070	0.0020
35	0.0041	0.0212	0.0000
36	0.0045	0.0085	0.0090

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37	0.0000	0.0170	0.0000
38	0.0000	0.0062	0.0000
39	0.0000	0.0209	0.0045
40	0.0125	0.0133	0.0000
41	0.0278	0.0000	0.0000
42	0.0000	0.0306	0.0000
43	0.0000	0.0123	0.0123
44	0.0000	0.0190	0.0000
45	0.0000	0.0703	0.0042
46	0.0079	0.0000	0.0159
47	0.0000	0.0302	0.0257
48	0.0000	0.0154	0.0000
49	0.0021	0.0000	0.0000
50	0.0044	0.0081	0.0011
51	0.0000	0.0000	0.0123
52	0.0061	0.0024	0.0063
53	0.0022	0.0000	0.0000
54	0.0029	0.0056	0.0000
55	0.0000	0.0180	0.0000
56	0.0131	0.0033	0.0000
57	0.0000	0.0000	0.0015
58	0.0000	0.0024	0.0165
59	0.0000	0.0065	0.0000
60	0.0018	0.0102	0.0018
61	0.0000	0.0000	0.0074
62	0.0000	0.0069	0.0033
63	0.0000	0.0062	0.0033
64	0.0000	0.0060	0.0000
65	0.0000	0.0000	0.0189
66	0.0000	0.0068	0.0115
67	0.0019	0.0000	0.0000
68	0.0000	0.0000	0.0000
69	0.0000	0.0043	0.0045
70	0.0000	0.0000	0.0045
71	0.0000	0.0037	0.0078
72	0.0000	0.0065	0.0000
73	0.0000	0.0028	0.0000

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74	0.0000	0.0487	0.0000
75	0.0000	0.0000	0.0000
76	0.0000	0.0035	0.0000
77	0.0000	0.0000	0.0000
78	0.0000	0.0000	0.0000
79	0.0000	0.0079	0.0084
80	0.0044	0.0121	0.0000
81	0.0000	0.1351	0.0000
82	0.0000	0.0000	0.0000
83	0.0000	0.0000	0.0000
84	0.0000	0.0046	0.0000
85	0.0039	0.0039	0.0000
86	0.0000	0.0286	0.0000
87	0.0000	0.0179	0.0000
88	0.0000	0.0098	0.0000
89	0.0000	0.0000	0.0000
90	0.0104	0.0119	0.0000
91	0.0000	0.0000	0.0000
92	0.0000	0.0000	0.0000
93	0.0000	0.0000	0.0000
95	0.0000	0.0135	0.0000
96	0.0000	0.0000	0.0000
97	0.0000	0.0000	0.0000
98	0.0000	0.0000	0.0000
99	0.0000	0.0000	0.0196
100	0.0000	0.0865	0.0000

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Appendix 4: The numbers of tadpoles trapped at different trap depths.

Trap depth (cm)	*S. grayii* trapped per hour	*C. platys* trapped per hour	*X. leavis* trapped per hour
0	0.0000	0.0031	0.0026
1	0.0482	0.0083	0.0040
2	0.0177	0.0000	0.0147
3	0.1821	0.0064	0.0000
4	0.0489	0.0014	0.0000
5	0.0158	0.0049	0.0000
6	0.0326	0.0044	0.0000
7	0.0114	0.0038	0.0019
8	0.0110	0.0124	0.0000
9	0.0221	0.0032	0.0018
10	0.0480	0.0036	0.0009
11	0.0059	0.0020	0.0000
12	0.0179	0.0023	0.0045
13	0.0364	0.0000	0.0000
14	0.0130	0.0050	0.0000
15	0.0138	0.0035	0.0143
16	0.0415	0.0000	0.0057
17	0.0166	0.0063	0.0022
18	0.0305	0.0000	0.0021
19	0.0218	0.0036	0.0000
20	0.0135	0.0051	0.0000
21	0.0365	0.0000	0.0000
22	0.0021	0.0040	0.0021
23	0.0000	0.0000	0.0000
24	0.0207	0.0022	0.0022
25	0.0257	0.0000	0.0000
26	0.0147	0.0042	0.0042
27	0.0000	0.0000	0.0000
28	0.0097	0.0097	0.0000
29	0.0142	0.0000	0.0000
30	0.0000	0.0020	0.0020
31	0.0031	0.0000	0.0124
32	0.0095	0.0000	0.0039
33	0.0089	0.0000	0.0031
34	0.0175	0.0000	0.0000

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35	0.0057	0.0000	0.0020
36	0.0104	0.0000	0.0000
37	0.0000	0.0000	0.0000
38	0.0000	0.0000	0.0000
39	0.0000	0.0000	0.0000
40	0.0000	0.0000	0.0000
41	0.0168	0.0000	0.0000
42	0.0159	0.0000	0.0000
43	0.0000	0.0000	0.0000
44	0.0000	0.0049	0.0000
45	0.0000	0.0000	0.0000
46	0.0000	0.0000	0.0000
47	0.0000	0.0000	0.0000
48	0.0060	0.0000	0.0000
49	0.0000	0.0000	0.0000
50	0.0270	0.0000	0.0000
51	0.0000	0.0000	0.0074
52	0.0000	0.0000	0.0000
53	0.0000	0.0000	0.0000
54	0.0164	0.0000	0.0059
55	0.0000	0.0000	0.0000
56	0.0000	0.0000	0.0000
57	0.0000	0.0000	0.0000
58	0.0101	0.0000	0.0000
59	0.0000	0.0000	0.0000
60	0.0000	0.0000	0.0000
61	0.0000	0.0000	0.0000
62	0.0139	0.0000	0.0000
63	0.0411	0.0000	0.0000
64	0.0000	0.0000	0.0000
65	0.0062	0.0000	0.0065
66	0.0000	0.0000	0.0000
67	0.0180	0.0000	0.0000
68	0.0000	0.0000	0.0000
69	0.0000	0.0000	0.0000
70	0.2162	0.0000	0.0000
71	0.0000	0.0000	0.0000
72	0.0000	0.0000	0.0000
73	0.0000	0.0000	0.0000
74	0.0000	0.0000	0.0000
75	0.0000	0.0000	0.0000
76	0.0000	0.0000	0.0000

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77	0.0000	0.0000	0.0000
78	0.0000	0.0000	0.0000
79	0.0000	0.0000	0.0000
80	0.0000	0.0000	0.0000
81	0.0000	0.0000	0.0000
82	0.0000	0.0000	0.0000
83	0.0000	0.0000	0.0000
84	0.0000	0.0000	0.0000
87	0.0000	0.0000	0.0000
90	0.0000	0.0000	0.0000

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Appendix 5: The number of tadpoles trapped per water temperature.

Water temperature (degrees C)	*S. grayii* trapped per hour	*C. platys* trapped per hour	*X. leavis* trapped per hour
10	0.1563	0.0000	0.0000
11	0.0759	0.0000	0.0000
12	0.0233	0.0000	0.0000
13	0.0737	0.0000	0.0005
14	0.0244	0.0003	0.0014
15	0.0094	0.0004	0.0028
16	0.0208	0.0015	0.0030
17	0.0301	0.0019	0.0053
18	0.0243	0.0025	0.0038
19	0.0161	0.0096	0.0037
20	0.0205	0.0082	0.0007
21	0.0037	0.0039	0.0000
22	0.0176	0.0028	0.0009
23	0.0113	0.0026	0.0000
24	0.0117	0.0055	0.0000
25	0.0190	0.0063	0.0032
26	0.0000	0.0000	0.0000
27	0.0000	0.0039	0.0035
28	0.0000	0.0107	0.0000
29	0.0000	0.0000	0.0049
30	0.0000	0.0222	0.0000
32	0.0000	0.0000	0.0000
33	0.0000	0.0000	0.0000

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Appendix 6: The number of tadpoles trapped at each oxygen concentration.

Oxygen concentration (mg/l)	*S. grayii* trapped per hour	*C. platys* trapped per hour	*X. laevis* trapped per hour
0	0.00128	0.002717	0.00128
1	0.00861	0.005352	0.0034
2	0.01727	0.005746	0.00091
3	0.01214	0.002652	0.00096
4	0.01685	0.003937	0.0029
5	0.0099	0.001165	0.01173
6	0.0014	0.001401	0.0014
7	0.00368	0.003676	0.00368
8	0	0	0
9	0	0	0
10	0	0	0

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Appendix 7: The numbers of tadpoles caught in different water clarities.

Water clarity (cm)	*S. grayii* trapped per hour	*C. platys* trapped per hour	*X. laevis* trapped per hour
0	0.0000	0.0000	0.0000
1	0.0000	0.0588	0.0000
2	0.0588	0.0252	0.0000
3	0.0049	0.0000	0.0000
4	0.0843	0.0119	0.0000
5	0.0035	0.0052	0.0000
6	0.0243	0.0032	0.0011
7	0.0400	0.0062	0.0007
8	0.0141	0.0061	0.0020
9	0.0121	0.0026	0.0044
10	0.0081	0.0029	0.0008
11	0.0112	0.0051	0.0009
12	0.0127	0.0018	0.0013
13	0.0190	0.0026	0.0010
14	0.0115	0.0034	0.0024
15	0.0458	0.0011	0.0045
16	0.0489	0.0015	0.0031
17	0.0137	0.0014	0.0027
18	0.0138	0.0000	0.0029
19	0.0238	0.0048	0.0000
20	0.0035	0.0000	0.0000
21	0.0057	0.0000	0.0041
22	0.0182	0.0000	0.0458
23	0.0217	0.0000	0.0000
24	0.0184	0.0037	0.0184
25	0.0053	0.0000	0.0000
26	0.0163	0.0000	0.0000
27	0.0160	0.0000	0.0160
28	0.0286	0.0000	0.0000
29	0.0000	0.0000	0.0000
30	0.0000	0.0000	0.0000
31	0.0000	0.0000	0.0000
32	0.0278	0.0000	0.0000
33	0.3333	0.0000	0.0000
34	0.0000	0.0000	0.0000

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35	0.0000	0.0000	0.0000
37	0.0556	0.0000	0.0000
39	0.0000	0.0000	0.0000
40	0.0556	0.0000	0.0000
63	0.1111	0.0000	0.0000
73	0.0000	0.0000	0.0000

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Appendix 8: Weekly results of the gut contents and water analysis for the 2005 season from the study pond at Kenilworth racecourse. Significant differences indicated in bold. Counts were taken over 100 grid points, so the numbers below also represent percentages.

Date (week starting)	Sample	N	Mean single celled algae	Mean colonial algae	Mean filamentous algae	Mean plant	Mean animal	Mean other
16/5/05	Water	1	15	0	63	20	1	1
	S. grayii	5	66	1	20	2	0	11
23/5/05	Water	2	27	0	67	2	0	5
	S. grayii	5	64	2	26	5	0	4
30/5/05	Water	2	30	9	47	1	0	15
	S. grayii	5	61	3	12	6	1	17
6/6/05	Water	2	58	5	13	4	0	21
	S. grayii	5	51	2	**23**	4	0	**20**
	X. laevis	5	38	1	**10 (p=0.03)**	1	0	**50 (p=0.03)**
13/6/05	Water	2	**18**	0	10	0	0	**73**
	S. grayii	5	**50 (p=0.026)**	2	15	4	0	**29 (p=0.046)**
	X. laevis	5	37	1	22	0	0	40
20/6/05	Water	2	52	8	18	4	1	**19**
	S. grayii	5	**60**	1	9	4	0	27
	X. laevis	5	**37 (p=0.01)**	1	14	0	0	**48 (p=0.05)**
27/6/05	Water	2	**68**	2	14	0	0	17
	S. grayii	5	46	0	14	7	0	32
	X. laevis	5	**28 (p=0.01)**	2	13	0	0	57
	Water	2	29	0	8	1	0	62

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4/7/05	*S. grayii*	5	63	2	19	**4**	0	12
	X. laevis	5	44	2	12	**0 (p=0.01)**	0	42
11/7/05	Water	2	46	2	5	1	0	47
	C. platys	5	40	0	10	3	2	55
	S. grayii	1	51	1	21	4	1	22
	X. laevis	5	31	1	14	1	0	54
18/7/05	Water	2	43	11	16	1	0	30
	S. grayii	5	**57**	0	23	**8**	0	**11**
	X. laevis	5	**26 (p=0.03)**	0	31	**0 (p=0.01)**	0	**42 (p=0.02)**
25/7/05	Water	2	30	35	28	1	0	18
	S. grayii	5	50	1	7	2	1	39
	X. laevis	5	38	2	21	0	0	38
1/8/05	Water	2	46	3	21	6	0	25
	S. grayii	5	65	0	14	3	0	17
	X. laevis	5	51	1	26	0	0	21
8/8/05	Water	2	61	3	22	2	0	14
	C. platys	4	52	0	7	1	0	41
	S. grayii	6	61	3	22	2	0	14
	X. laevis	5	46	0	6	2	0	46
15/8/05	Water	1	51	2	17	0	1	31
	C. platys	8	52	6	19	**1 (p=0.01)**	0	23
	S. grayii	8	58	7	9	**8**	0	19
	X. laevis	4	44	2	36	**1 (p=0.05)**	0	18
22/8/05	Water	2	56	2	22	6	0	16
	C. platys	4	65	1	15	2	0	19
	S. grayii	2	71	0	12	6	1	11
	X. laevis	4	46	1	30	0	0	24

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29/8/05	Water	2	55	2	21	7	0	16
	C. platys	2	63	1	24	2	0	11
	S. grayii	1	70	2	9	9	0	10
	X. laevis	7	65	1	10	2	0	23
5/9/05	Water	2	**24**	1	42	1	0	34
	C. platys	6	54	0	14	8	0	24
	S. grayii	5	**63 (p=0.03)**	0	12	7	0	**18**
	X. laevis	5	43	0	14	4	0	**38 (p=0.04)**
12/9/05	Water	2	59	2	14	2	1	23
	C. platys	5	69	0	14	3	0	14
	S. grayii	1	65	1	17	3	0	14
	X. laevis	5	74	0	10	3	0	12
19/9/05	Water	2	71	3	10	2	0	15
	C. platys	5	58	0	4	6	1	31
	S. grayii	4	66	0	10	4	0	20
	X. laevis	5	52	0	3	9	0	35
26/9/05	Water	2	68	2	20	4	2	7
	C. platys	5	64	0	12	3	0	20
	S. grayii	5	**70**	3	8	**7**	0	11
	X. laevis	5	**57 (p=0.05)**	22	25	**1 (p=0.02)**	0	15
3/10/05	Water	2	69	3	15	5	1	10
	C. platys	5	67	0	8	5	0	19
	S. grayii	4	58	0	13	**12**	1	17
	X. laevis	5	60	0	11	**1 (p=0.01)**	0	28
10/10/05	Water	2	62	0	18	4	0	17
	C. platys	5	68	0	19	5	0	8
	X. laevis	5	64	0	19	3	0	13

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17/10/05	Water	1	39	1	19	1	0	40
	C. platys	5	**55**	0	3	4	1	36
	S. grayii	2	53	0	3	13	0	**32**
	X. laevis	6	**34 (p=0.03)**	0	3	2	0	**61 (p=0.02)**
24/10/05	Water	2	59	2	8	4	0	27
	C. platys	3	68	0	4	4	1	23
	S. grayii	1	64	0	7	13	0	16
	X. laevis	4	50	1	12	1	0	37
31/10/05	Water	2	37	1	28	2	1	32
	C. platys	4	**64**	0	3	**8**	2	**23**
	X. laevis	5	**36 (p=0.04)**	0	6	**0 (p=0.02)**	0	**57 (p=0.02)**
7/11/05	Water	2	28	0	14	4	0	55
	C. platys	4	61	1	3	7	2	27
	X. laevis	4	49	0	4	2	0	46

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