Chytridiomycosis in amphibian populations in the Western Cape, South Africa

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Thesis submitted in fulfilment of the requirements for the degree of MSc in the Department of Zoology, University of the Western Cape.

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ii

Abstract

Chytridiomycosis in amphibian populations in the Western Cape, South Africa

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MSc thesis, Department of Zoology, University of the Western Cape

There have been many cases reported of amphibian populations declining. These are often due to anthropogenic factors such as habitat destruction and pollution. However, some declines have not had an obvious cause and many of these have been investigated and found to be due to pathogenic disease. *Batrachochytrium dendrobatidis* is a recently described pathogen of frogs.

The population declines that have been associated with chytridiomycosis have occurred in relatively undisturbed areas such as national parks. The declines tend to occur at higher altitudes or in colder climates. This is thought to be because of the frog immune system being slower at lower temperatures.

Chytrid fungus has been found in frog populations throughout the world. Little research has been carried out in Africa although, chytridiomycosis has already been seen in Kenya and South Africa.

In this project frogs were sampled from selected transects in the Western Cape and three sites in the Northern Cape. The effect of altitude on the occurrence of infection was tested in the Western Cape. It was found that 18 frogs were infected in the Western Cape and the effect of altitude was not significant. Large numbers of dead and dying frogs were found in two of the Northern Cape sites and the incidence of chytridiomycosis was high in these populations. Chytrid was found in two *Bufo gariepensis* from the Eastern Cape and in *Xenopus petersii* from Kasanka National Park, Zambia.

More research is needed on chytridiomycosis in these populations. The frogs in the Western Cape seem to survive with chytrid fungus infection whereas, the frogs in the Northern Cape are dying. This suggests another factor acting on the Northern Cape frog populations.

Declaration

I declare that *Chytridiomycosis in amphibian populations in the Western Cape, South Africa* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name………………

Date……………………..

Signed…………………..

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Contents

Introduction

Modern amphibians have been traced back for at least 100 million years (Wake 1991). The earliest known anuran is from the lower Triassic of Madagascar. There have been a number of fossils of anurans or pre-anurans found in the Jurassic fossils in North America (Pough et al. 2001). There are now over 5,504 extant species in about 310 genera. These occur throughout the world except in very cold or xeric climates (Frost 2002). Even though new species are being discovered and described each year, amphibians are thought to be declining (Hanken 1999). Many frogs have two life stages, which often occur in different habitats and eat different food. They have a permeable skin which makes them susceptible to changes in the environment. It has been suggested that because of these factors frogs make good bioindicators and their declines may show information about the environments in which they live (Wake 1991, Lips 1998).

Amphibian decline

Amphibian decline has been discussed for many years in the scientific literature. The declines were first noticed in the late 1970's in different areas worldwide. The worldwide declines cause concern because amphibians are often an essential part of the ecosystem in which they live. It is not known if these declines are due to one unknown factor or many local factors (Barinaga 1990). Many hypotheses have been suggested to explain these declines.

Reasons for amphibian declines

Anthropogenic factors such as habitat modification and pollution have been suggested as the main cause of decline. This is because humans are seen to affect many of the environments in which the declines have been seen. Examples of habitat modification and pollution that may affect amphibian populations are the draining of wetlands, acid rain, mining runoff, pesticides or fertilizers (Barinaga 1990, Alford and Richards 1999, Blaustein et al. 1994a and b, Corn and Fogleman 1984). All of these may have an adverse effect on amphibian populations.

However, some declines have been seen in sites considered pristine such as national parks. Examples are the Monteverde Forest in Costa Rica and montane tropical

1

rainforests in Australia (Barinaga 1990, Hero 1996). There is thought to be little human impact upon these sites. This means that there are possibly other reasons for the observed declines.

Natural population fluctuations have been mentioned because there is little known about many of the declining frog species. Pounds et al. (1997) tested the theory of natural population fluctuations on the frogs in the Monteverde region in Costa Rica. If the frogs' decline was a natural fluctuation then after the decline the frog populations should recover. However, only one of the species that declined increased in numbers afterwards. This suggested that the declines are not just natural fluctuations. To test if the decline in frog species was similar to the declines seen in other species the frog population fluctuations were compared to bird population fluctuations. It was found that more frog species disappeared than bird species over the study period. This suggests that the amphibian declines are not just part of the overall biodiversity crisis.

Each frog species reacts differently to the weather conditions and it is possible that the frogs cannot be found because of poor weather conditions rather than population declines (Hero 1996). Changes in rainfall in Costa Rica and Australia have been seen over the past eight years but the frogs which are declining are the species least likely to be affected by rainfall patterns (Lips 1998). The Monteverde forest has had rainfall since the large population declines but the frogs still seem to be declining (Pounds et al. 1997). As the populations are not seen to be recovering it may be that the changes in weather patterns may have caused the frogs to become susceptible to other factors such as disease. A change in the rainfall pattern may cause the frogs to come into contact with each other more frequently and this may make the transmission of disease more likely (Pounds et al. 1999).

The introduction of exotic species has been suggested as a reason for the decline. Exotic species may compete with the native amphibians, use them as food or introduce a new pathogen. These would cause a decline in amphibian numbers. In the tropical rainforests in Australia where declines have been seen animals such as the cane toad, *Bufo marinus,* have been introduced (Hero 1996) and in other areas fish have been introduced into fish-free rivers and streams. Many rivers in Central and South America contain introduced rainbow trout. These fish may compete with the

2

adult frogs for prey or eat the eggs and larvae of frogs (Lips 1998). Another type of introduction is seen when endangered species are captive-bred and then introduced back into the wild. These new individuals may carry new diseases that spread through the existing population (Cunningham 1996).

Although these sites are considered pristine local and global pollution cannot be ruled out as a factor in the frogs' decline, Blaustein (1994) suggests that no environment is truly pristine. An unknown pollutant may be adversely affecting the frogs. Even though most of these sites are protected they are not sheltered from airborne pollutants (Lips 1998).

An increase in UV-B radiation is thought to have occurred due to reductions in ozone in the atmosphere. This has been recorded since 1979 (Madronich and Gruijl 1993). It is not known if there has been an increase in UV-B radiation over the sites where the amphibian declines are occurring. Even if an increase is found over these sites no data exists on how an increase in UV-B radiation affects the declining species (Hero 1996). Tests have been carried out on some species of frog. These have shown that increases in UV-B radiation reduce hatching and survival rates of tadpoles. In adults UV-B damages eyes and increases tumours. An increase in UV-B will affect other components of the ecosystem. This may change water quality and predator-prey interaction, both of which would affect frogs (Alford and Richards 1999). UV-B radiation has been seen to work synergistically with other factors. Tests on *Rana cascadae, Bufo boreas* and *Hyla regilla* showed that UV-B radiation and the fungus *Saprolegnia ferax* affect the frogs eggs individually but have a greater effect when added together (Kiesecker and Blaustein 1995).

Disease was considered a factor because of the epidemic nature of some of the declines (Berger et al. 1998). There are a number of diseases known to affect amphibians such as *Aeromonas hydrophilia* which is an opportunistic bacteria that can be found in healthy frogs but is known to cause red leg disease (Bradford 1991, Rollins-Smith et al. 2002a). *A. hydrophilia* has been linked to the declines of *Bufo boreas boreas* in the Rocky Mountains in Colorado (Carey 1993). Other pathogens such as viruses have been found in declining frog populations. Viruses in the genus *Ranavirus* have been seen to infect *Rana temporaria, Ambystoma tigrinum* and

3

Limnodynastes ornatus (Cullen and Owens 2002, Rollins-Smith et al. 2002a). There are a number of fungi known to affect amphibians. *Saprolegnia ferax* has been found in *Bufo boreas* in Oregon. It infects the egg masses of these frogs (Blaustein et al. 1994a). *Basidiobolus ranarum* has been seen in the gut of a number of different species of amphibian (Nickerson and Hutchison 1971, Rollins-Smith et al. 2002a). However, tests are being carried out to see if this fungus is similar or the same as *Batrachochytrium dendrobatidis* (Carey et al. 1999). *B. dendrobatidis* is a recently described pathogen of frogs that has been found in many declining frog populations.

Chytrid fungus

When mass mortality events were seen in Big Tableland, Queensland, Australia a group of scientists collected some of the dead and dying animals and tested them thoroughly for disease. They looked at dead frogs from the Fortuna Forest Reserve in western Panama as well. A small spherical fungus was found in the stratum corneum and stratum granulosum of these dead and dying frogs and the deaths were linked to these fungi. This was a previously undescribed species of parasitic fungus that belonged to the phylum Chytridiomycota (Berger et al. 1998). This fungus was described and named *Batrachochytrium dendrobatidis* (Longcore et al. 1999).

Chytrids are small, spherical fungi. They are either free-living, known to decompose keratin, chitin, cellulose and other plant material (Berger and Speare 1998), or are parasitic on certain fungi, algae, higher plants, protozoa and invertebrates (Fellers 2000). *B. dendrobatidis* is the first chytrid fungus known to infect vertebrates. The zoospores insert their contents into keratinised skin cells where they become a spherical zoosporangium. This zoosporangium matures and cleaves into flagellated zoospores which are discharged through a discharge tubule that either opens to the outside of the skin or another part of the same frog's skin (Rollins-Smith 2002b, Pessier et al. 1999).

Chytridiomycosis only occurs in keratinised skin, therefore, it is found in the skin of post-metamorphic frogs and the keratinised jaw sheaths of tadpoles (Daszak et al. 1999, Berger et al. 1998). This indicates that the fungus uses keratin as a nutrient. The disease moves though the population causing local population extinctions. It is usual for virulent pathogens to lower the host numbers so that transmission becomes

4

impossible. This causes the pathogen to die out and the host population to recover. The extinctions seen due to chytridiomycosis suggest that the chytrid has a mechanism for existence at low host density. It could be that it uses a reservoir host such as another animal or the tadpole mouthparts. The tadpole can carry the fungus without any known effects. The fungus may be able to survive outside the host by becoming saprophytic or living on keratin in the water.

There are three ways chytridiomycosis may kill a frog. The first is that the presence of the fungi in the skin may affect cutaneous respiration or osmoregulation, or the fungus itself could excrete a toxin that the amphibian absorbs or it may be a combination of the two (Daszak et al. 1999). However, none of these theories have been proven. It has been noted that the organisms infecting frogs in Australia and Central America are indistinguishable. This has been concluded by looking at their ultrastucture, pathogenesis and preliminary genetic work (Cunningham 1998. Morell 1999). Chytridiomycosis has now been found in many amphibian populations throughout the world.

Worldwide distribution of chytridiomycosis
By testing museum records chytridiomycosis was found in frogs on the East Coast of Australia collected in 1978 (Speare and Berger 2000a). It is thought that the fungus came to Brisbane in the late 1970s and spread at about 100 km a year (Laurance 1996, Morell 1999). Chytridiomycosis has now been found in 46 species of amphibian in Australia (Speare and Berger 2000a) and some frogs seem to be developing a resistance. This has been seen in the green eyed tree frog in Queensland, which decreased in numbers when other species disappeared but is now recovering (Morell 1999).

In North America the fungus was first seen in captive arroyo toads in California. Chytrid fungus has been seen in the frogs in the Washington Zoo since 1991 (Milius 1998). Deaths of Boreal toads in the Rocky Mountains National park have been linked to the chytrid fungus. Two populations of the toads were being monitored when one suddenly declined in 1996 and the second in 1999. Only two of the frogs were examined to find the fungus present (Muths et al. 2000). Since then wild cricket frogs

5

in Illinois and common American toads in Maryland have been found to have low levels of chytrid fungi with no obvious effects (Milius 1998).

In Central America there have been reports of chytridiomycosis from Costa Rica and Panama. This has been seen in many montane rainforest species and has caused mass die offs, local extinctions and population declines. It is hypothesised that the Golden Toad may have been affected by chytrid infection. In Panama Lips (1999) found declines in 10 species and these are thought to be linked to chytrid infection. These declines moved in a wave from northwest to southeast. In Ecuador chytridiomycosis has been seen in *Atelopus* species, *Telmatobius niger* and *Gastrothecus pseustes* (Daszak et al. 1999).

Chytridiomycosis has been found in Europe by Bosch et al. (2001). It was found in *Alytes obstetricans* in the Penalara Natural Park. Before 1999 the toads were present in 35 of the ponds sampled. When these ponds were studied in 1999 they were present in only five. It is thought that the extended larval period of the midwife toad makes it highly susceptible to the infective spores of the fungi (Bosch et al. 2001).

The chytrid fungus has been discovered in Africa. Speare and Berger (2000b) reported chytridiomycosis in *Ptychadena anchietae* and *Afrana angolensis* from Kenya, and in *Xenopus laevis* from Grabouw, Bredasdorp, Hex River and Knysna in South Africa.

A colony of 241 *Xenopus tropicalis* was imported into North America from West Africa. Within four months 91% of them had died. When examined the frogs were seen to have *Chlamydia pneumoniae* and chytridiomycete fungus (Reed et al. 2000). This suggested that the fungus would be seen in West African frog populations if it were looked for.

There seem to be similarities between the declining frog populations that have been studied, related to chytridiomycosis. Most of these declines have occurred in relatively undisturbed areas such as national parks. These mass mortality events are geographically widespread, and the population loses 50-100% of its individuals. The die offs occur mainly at higher altitudes or in colder climates and not all of the

6

amphibian species at the site are affected (Carey 2000). It has been found that often the infected species in Eastern Australia have low clutch sizes and occupy a restricted geographic range (Berger et al. 1998). It is often seen in montane environments which receive higher levels of ultraviolet light and receive more air-borne contaminants. The possibility that the fungus is carried by insects means that the insect is deposited on the mountain slope because the air rises as it travels over the mountains. Higher temperatures are known to increase the immune response of an amphibian and colder temperatures decrease and even stop immune responses (Carey 2000). This would affect how the amphibians' immune system was capable of fighting the parasitic infection.

Amphibian immune defences

Little is known about the amphibian immune system in response to fungal pathogens. Most amphibians have an innate and adaptive immune system. The innate immune system is used until the adaptive response can be initiated. The first defence against a chytrid infection is probably anti-microbial peptides of the innate immune system. The anti-microbial peptides are non-specific and will work against a variety of microorganisms. They are produced in specialised granular glands in the skin. It is not known if external conditions affect the rate and onset of the innate immune system but it is likely that temperature, pH, hydration of the skin and environmental toxins all influence the synthesis of peptides. Each species is known to produce a unique set of peptides. Amphibians have phagocytic cells and serum complement that will work on certain pathogens. (Carey et al. 1999, Rollins-Smith et al. 2002a and b)

It is not known if the adaptive immune system has a defence against fungal pathogens in amphibians but it works by creating memory cells that can respond to another attack of the same disease.

The effects of chytridiomycosis on infected amphibians

The chytrid fungus can infect the amphibian although the amphibian may look healthy externally. The clinical signs of chytridiomycosis vary between species but death occurs a few days after signs of disease. The signs of disease include; lethargy, reddening of ventral skin, convulsions with extension of the hind limbs, accumulation of sloughed skin over the body, occasional ulcers, an abnormal posture, the loss of the

7

righting reflex, haemorrhages in the skin, muscles and eye, hyperaemia of digital skin and congestion of viscera (Berger and Spear 1998, Daszak et al. 1999).

Reasons and aims for this project

It is not known if Western Cape frog populations are in decline or where chytridiomycosis is present in the Western Cape. Therefore, this study looked at amphibian populations in the Western Cape and determined the presence of chytrid fungus. A number of populations of the same species were looked at from a wide area in the Western Cape.

Frogs from other areas in Eastern and Southern Africa were also checked for chytrid fungus infection. This gives an idea of the extent of chytridiomycosis in this area.

Hypothesis

Chytrid fungus has already been found in the Western Cape. It was reported in *Xenopus laevis* in 1999. Mendez, Speare and Cunningham found infected frogs in Grabouw, Bredasorp and Hex River in July and in Knysna in August 1999 (Speare and Berger 2000b) although no mass die offs have been seen. I hypothesise that chytridiomycosis is found at high altitude sites in the Western Cape in frog species with an aquatic lifestyle and a long larval period.

The importance of the Western Cape study area

Nine of the 15 family groups of amphibians in sub-Saharan Africa are endemic (Duellman 1993). The Western Cape is ecologically important with the dominant biome being fynbos. This ecosystem is very rich in plant species and the climate is considered Mediterranean with warm wet winters and hot dry summers (Meadows 1985). The loss of amphibian species from this area may have an effect on the prey of the frogs and tadpoles and the animals that use the frogs and tadpoles as food. Amphibians are often local top carnivores consuming many invertebrates (Wake 1991). However, most tadpoles are herbivores and feed on algal crusts and detritus (Passmore and Carruthers, 1995). If the tadpole numbers decrease the build up of algae and detritus will increase.

8

Candidate species list

Chytrid fungus is more likely to be found in frogs that are associated with an aquatic lifestyle, high altitude and a long period as a tadpole (Carey 2000). With these factors in mind, below is a list of some species that would be worthwhile studying for chytrid fungus. All these species occur in the Western Cape.

Bufonidae

Frogs in the family Bufonidae have thick skin and are often associated with temporary pools. *Bufo gariepensis* is found at high altitude in the Drakensberg Mountains but breeds in water-logged depressions and metamorphosis takes place within 20 days. *Bufo rangeri* exists in a large area and prefers to breed in deep pools. *Capensibufo rosei* is found in the mountains of the Cape Peninsula and surrounding mountains where water is available throughout the year, and their eggs are laid in shallow depressions with mossy substrate. Metamorphosis takes place after 51 days. *Capensibufo tradouwi* is another high altitude species and is found to the north and east of the south-western Cape.

Heleophrynidae

Species in the family Heleophrynidae all have tadpoles that spend at least 12 months in the water but few of them have upper or lower keratinised jaw sheaths. The chytrid fungus is found in the larval stage on these jaw sheaths. All species in this genus are considered difficult to collect. *Heleophryne orientalis* is found along the Langeberg Mountains. The eggs may be laid between boulders where water seeps over them rather than them being laid in water. *Heleophryne purcelli* is found at high altitude in the mountains of the south, southwest and western Cape. *Heleophryne regis* is found in the mountains east of the Gouritz River valley where it rains all year round. *Heleophryne rosei* is found on the eastern side of Table Mountain but it is critically endangered.

Hyperoliidae

There are two species in the genus *Hyperolius* found in the Western Cape. *Hyperolius horstockii* eggs are attached to submerged vegetation. *Hyperolius*

9

marmoratus lays its egg in submerged vegetation and metamorphosis takes place with 100 days.

Semnodactylus wealii is found at low and high altitudes where the eggs are attached to submerged vegetation. The jaw sheaths in the larvae are heavy and there is a hardened plate on each side of the lower sheath.

Microhylidae

In the family Microhylidae; *Breviceps* and *Probreviceps* do not have a tadpole stage, they are direct developers.

Pipidae

The frogs in the genus *Xenopus* spend most of their lives in water. *Xenopus* tadpoles have no hardened mouthparts and most species are found in lowlands although *Xenopus laevis* has a wide distribution and the fungus has been found in South Africa in this species (Speare and Berger 2000b). Work is being carried out at Potchefstroom University on the occurrence of chytrid fungus in populations of *Xenopus laevis* in South Africa.

Ranidae

All the frogs in the genus *Afrana* are associated with water. *A. fuscigula* and *A. vandijki* have populations living at high altitudes. *A. fuscigula* tadpoles can take anywhere from 81 days to a year to metamorphose and are widespread. The fungus has already been found in *A. angolensis* in Kenya (Speare and Berger 2000b).

The frogs in the genus *Arthroleptella* all have direct development and emerge as froglets.

In the genus *Strongylopus, Strongylopus bonaespei, S. fasciatus and S. grayii* occur in the Western Cape. *S. bonaespei* occurs in high altitude grasslands whereas *S. fasciatus* is more widely distributed and found in grasslands. *S. grayii* is widely distributed.

In conclusion, any of the species listed here could be tested for chytridiomycosis. A widely distributed animal such as *Xenopus laevis* may be a good subject as it is common and associated with the water. Chytridiomycosis has already been found in *X. laevis* and work is currently being carried out at Potchefstroom University. A species with a long larval life may be tested, such as a heleophrynid. The common river frog *Afrana fuscigula* may be a good subject as it is widespread with populations in high altitude sites and a long larval life (Channing 2001).

11

Materials and Methods

<u>Collection techniques</u>

Frogs were collected from sites around the Western Cape where transects had been chosen due to their accessibility. These transects covered areas of high and low altitude and different habitats such as nature reserves and towns (fig 1). Frogs were caught and a toe clip was taken and placed in 70% ethanol. The site where the frog was collected was recorded using a global positioning system (GPS) (appendix 1).

Three sites in the Northern Cape were visited. Two of these were in Goegap Nature reserve. Dead frogs had been seen here the previous year. Two dead frogs were collected from one of the ponds and all the live individuals that could be seen were sampled from the small ponds. The other site was in the Richtersveld. This was in a nature reserve and not on a major route through the reserve (appendix 1).

B. gariepensis was taken from one site in the Eastern Cape and these were tested for chytridiomycosis due to their different lifestyle (appendix 1).

Frogs were collected from Mozambique, Zambia and Tanzania as part of another study. A toe clip was taken from each preserved individual.
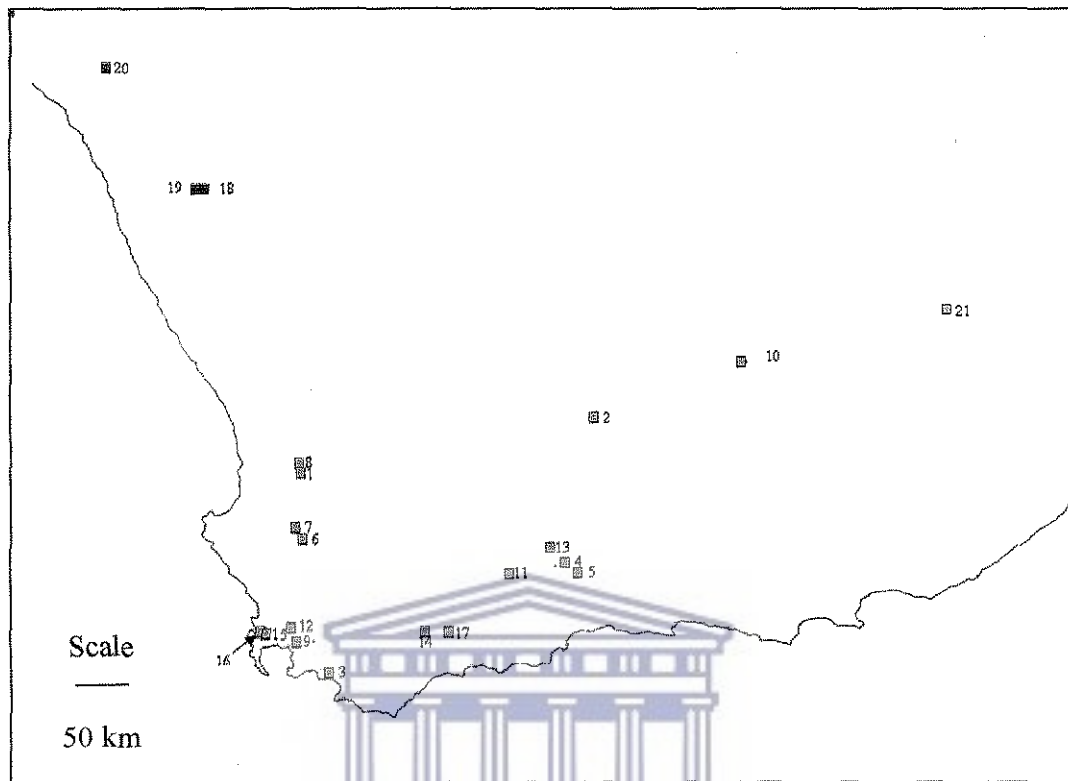
12
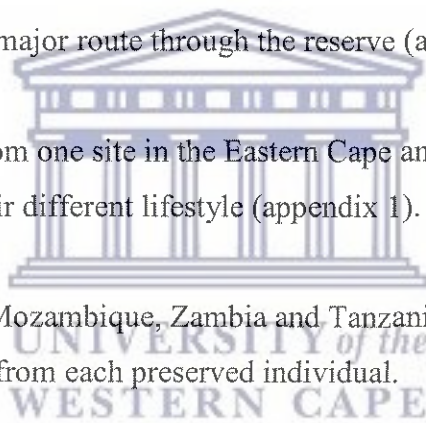
Map of sites

Fig 1. Sites in South Africa where frogs were sampled.

Key to Sites

1. Algeria, Cederberg (Nature reserve)
2. Beaufort West
3. Fernkloof (Nature reserve)
4. Gamkakloof
5. Grobbelaars river
6. Groot Winterhoek (Nature reserve)
7. Groot Winterhoek (Nature reserve)
8. Jamaka Farm Pond, Cederberg
9. Landroskop (Nature reserve)
10. Richmond
11. Seweweekspoort

13

12. Stellenbosch

13. Swartberg

14. Swellendam

15. Table Mountain

16. Table Mountain

17. Tradouws Pass

18. Goegap Kraaifontein (Nature reserve)

19. Goegap Bloukokerboom-water (Nature reserve)

20. Paradise Kloof (Nature reserve)

21. Stormberg

Study species

A. fuscigula was chosen as the test species as it is widespread with populations at high and low altitude and a long larval life. Other species were tested such as *A. vandijki* and *S. grayii* when *A. fuscigula* could not be found. Some *B. gariepensis* were tested because they have a different lifestyle to the river species such as *A. fuscigula* (Channing 2001).

Histological techniques

The collected toe tips were removed from ethanol and placed in Perenyi fixative (appendix 2) for 24 hours. This fixes and decalcifies the toe. The toe was then washed in 70% ethanol and dehydrated using increasing concentrations of ethanol. The toe was left in each concentration for one hour with two changes of absolute ethanol. The toe was then washed in xylene and placed in 50:50 wax:xylene for one hour at 60 ^{o}C. The toe was then transferred to 100% wax for one hour and then into a second change of wax for 24 hours. Each toe was then embedded and sectioned into 6 µm thick sections. These were placed on slides and washed in xylene for five minutes. The slides were rehydrated in decreasing concentrations of ethanol for five minutes in each concentration. The last step was distilled water. The slides were then stained in haemotoxylin and eosin using the methods of Humason and dehydrated with the last step in xylene. The slides were then covered.

14

Scanning techniques

The slides were scanned at 400x and then checked at 1000x oil immersion using a binocular compound microscope. If chytrid was found a picture was taken of the slide. 100 sections were cut and stained for each toe unless the toe was too small. To speed up the process of scanning the slides the number of sections looked at was later reduced to 50 sections. Fig 2 shows that 94.9% of the chytrid infections were found within the first 50 sections.
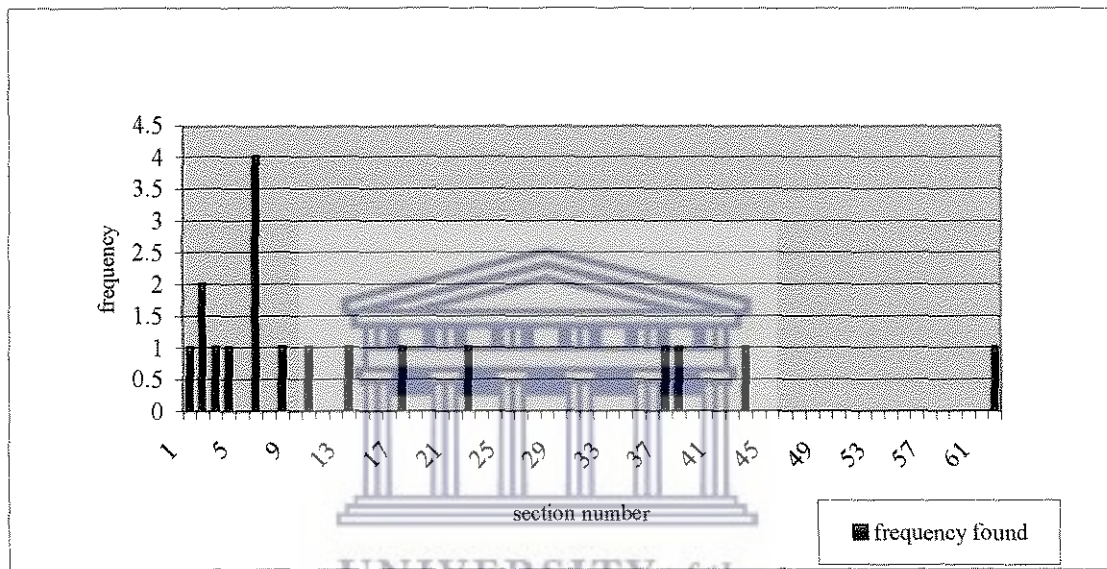

Fig 2. The number of sections scanned before chytrid infection was found

Analysis of data

Pictures of the skin were sent to Rick Speare for confirmation of chytrid infection. As this study generated presence-only data most of the analysis was descriptive. There have been many references to the altitude of the site affecting the presence of chytrid. (Carey 2000, Carey et al. 1999, Young et al. 2001). Carey et al. (1999) suggested that chytrid infection was found above 1500 m in temperate areas and 400 m in tropical regions. Altitude was used rather than temperature even though the temperature is thought to affect chytrid infection. This is because the temperature changes with altitude so it is a good measure and it is hard to get average climate data

15

for the microclimates that the frogs are found in (Bradford 1984, Snyder and Weathers 1975). A non-parametric difference of means test was carried out on the Western Cape data. This is equivalent to a Mann Whitney U test (Hamilton 1990, Salkind 2000). This tests for a significant difference between the altitudes where chytrid infected frogs were found and the altitudes where non-infected frogs were found.

16

Results

Illustrations of chytrid infections

The following are a sample of pictures taken of the infected sections. Not every positive result is represented here as most of the pictures show similar views of the empty thalli. Four forms of the chytrid can be seen on H and E sections. The first is uninucleate with homogenous basophilic cytoplasm. The second is multi nucleate separated by cytoplasm. There is a cyst-like form that contains multiple discrete round spores and finally empty thalli that are shown by the cyst wall that is left behind (Pessier et al. 1999).
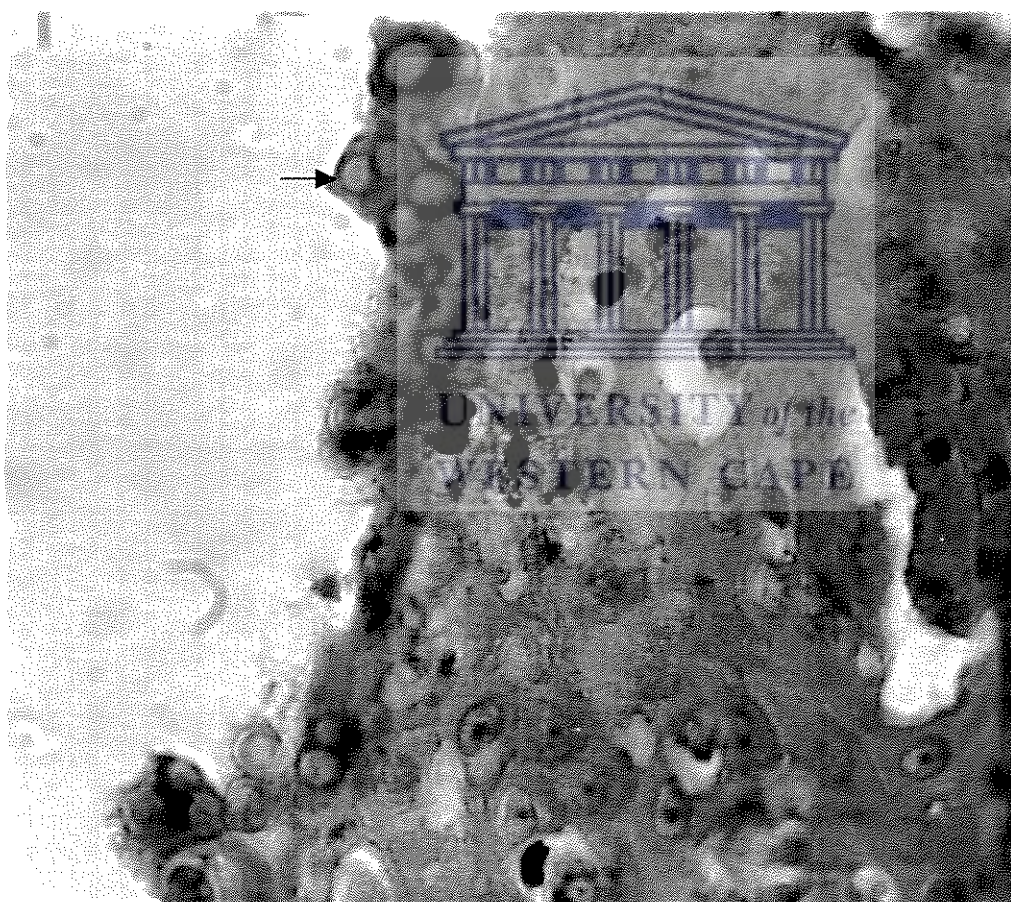

Fig 3 shows the infection of the dead *A. fuscigula* from Groot Winterhoek. This was from the higher altitude site. The skin was sloughing off in many places due to the infection. Here at least 12 empty thalli can be seen (arrow).
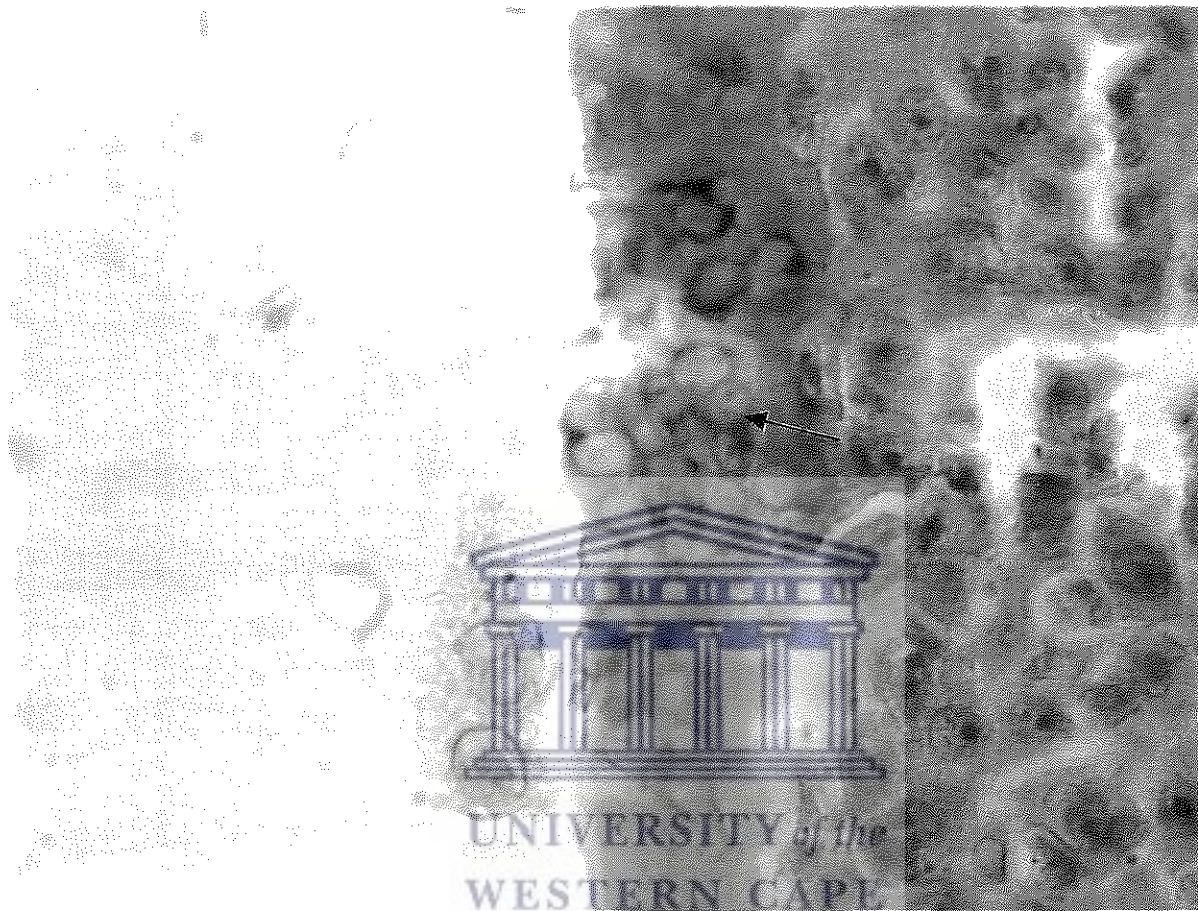
17

Fig 4 shows a heavy infection of chytrid fungus. This frog was collected from
Bloukokerboom-water in Goegap Nature Reserve and appeared healthy although there
were other frogs found dead in the same pool. At least 33 empty thalli can be seen in this
piece of skin. A nearly empty zoosporangium can be seen (arrow)
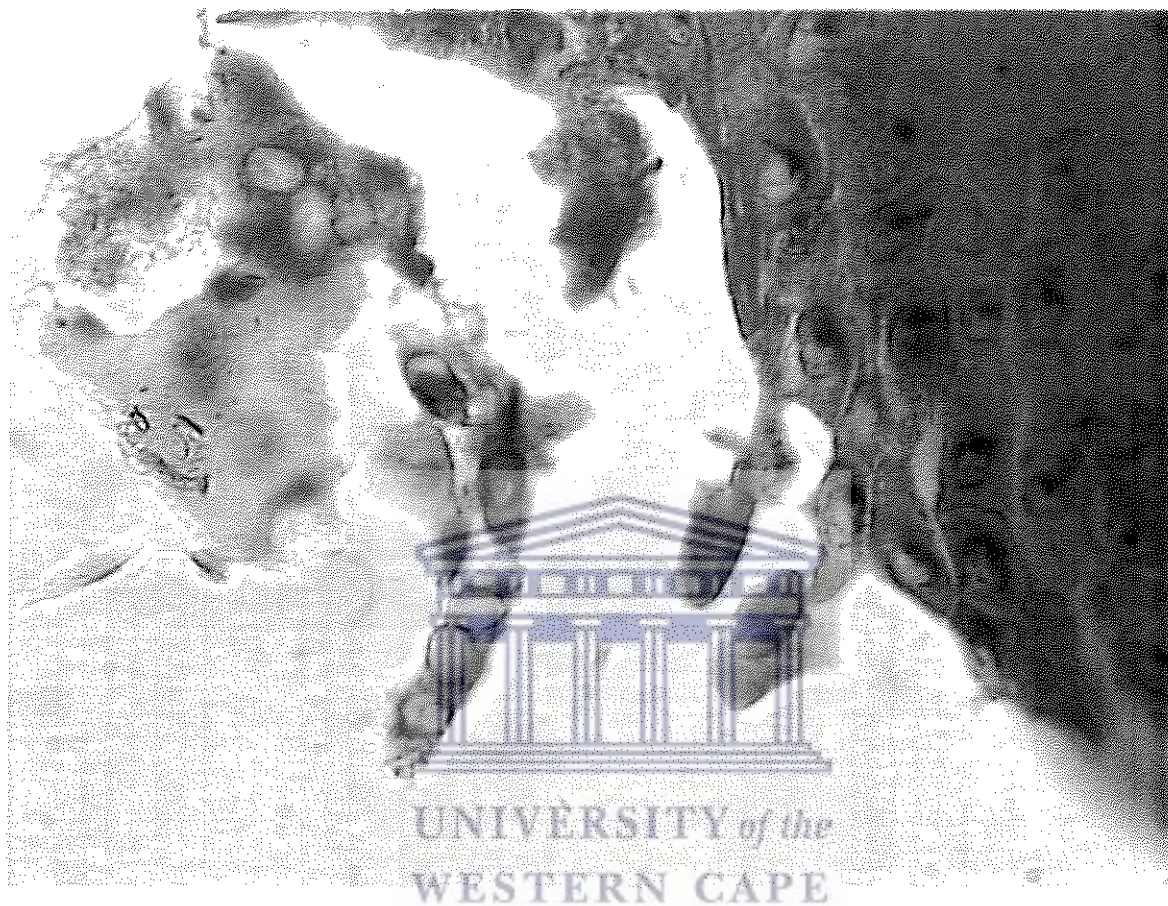
Fig 5 shows the skin of an *A. fuscigula* from Kraaifontein, Goegap Nature Reserve. There are at least seven empty thalli in the sloughed off skin. *B. dendrobatidis* is known to cause sloughing of the skin and hyperkeratosis.

Fig 6 shows a piece of sloughed-off skin from *A. fuscigula* from Kraaifontein, Goegap Nature Reserve, with zoosporangium full of zoospores (arrow). This means that it is an active infection. There are at least six full zoosporangia in this piece of skin. The discharge papillae can been seen on some of the zoosporangia (arrow head).

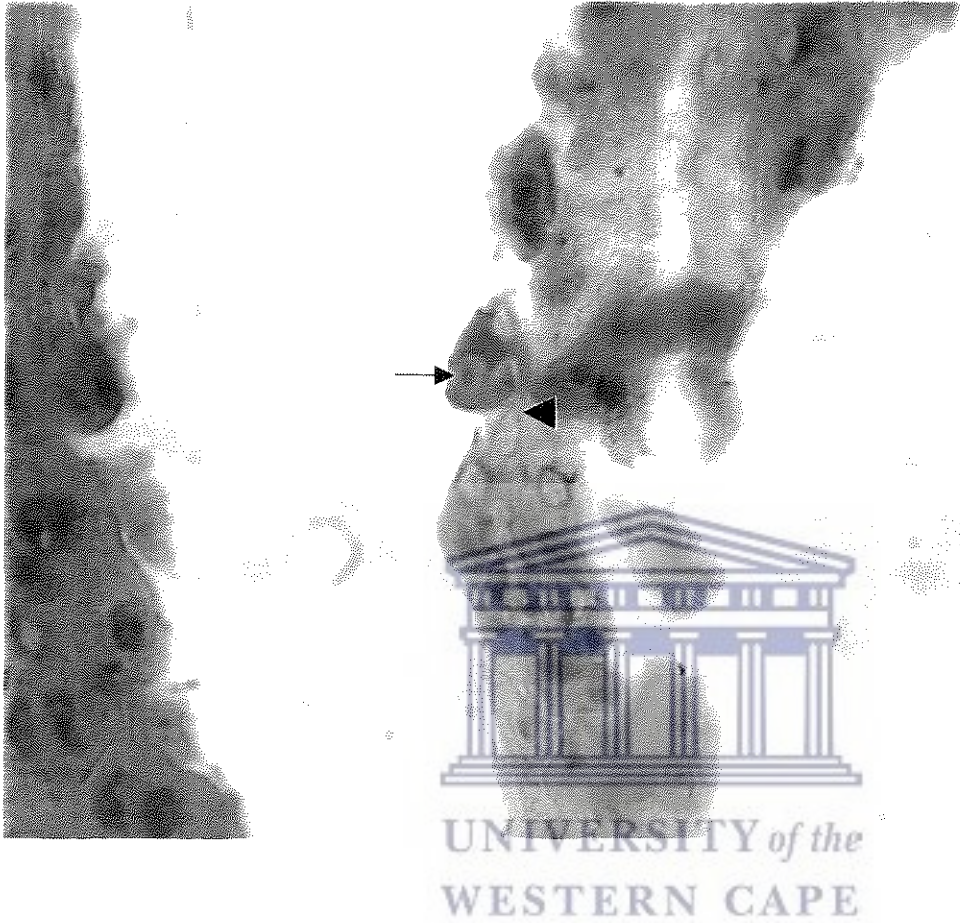The results are divided into three areas. The Western Cape is the main study area and this is where transects were planned and high and low altitude sites were tested. The Northern Cape was visited and three sites were sampled. Two of these sites had shown-die offs previously. Other samples taken from southern and eastern Africa were tested for chytridiomycosis to see if it is present in those areas.

Western Cape
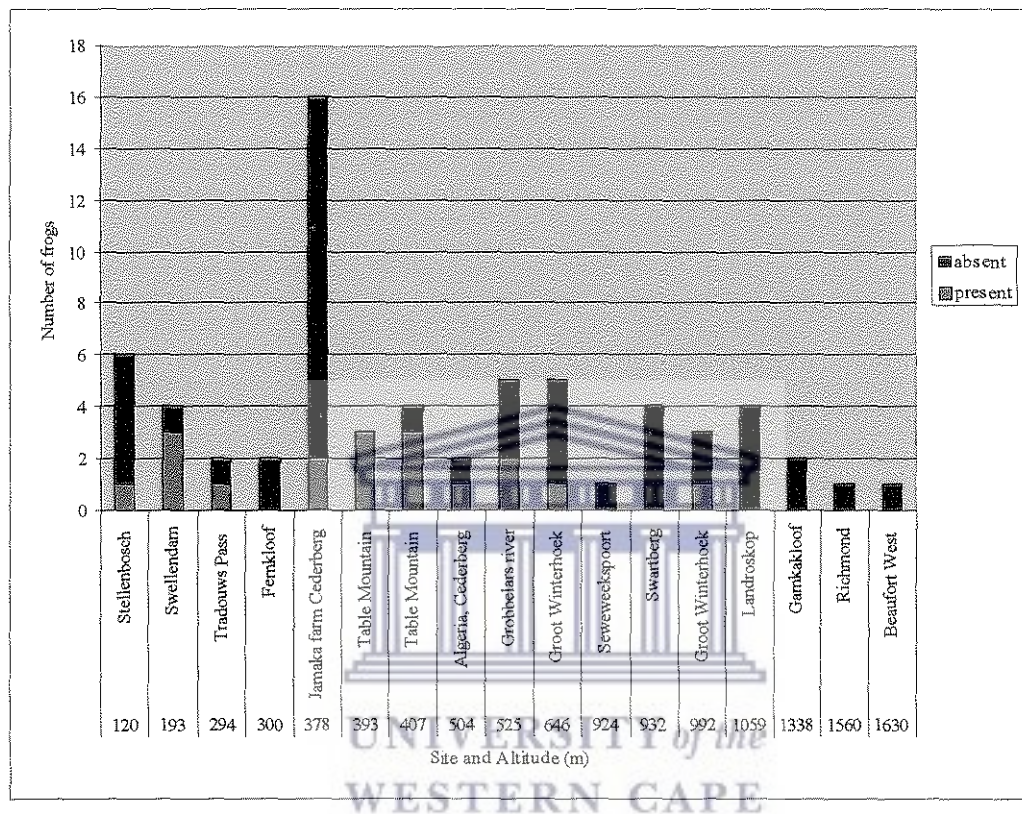
Fig 7 shows that chytrid was found in frogs at 10 of the 17 sites tested in the Western Cape.

A total of 65 frogs were tested and chytrid fungus was found in 18 frogs. Infection was found at low altitude sites such as Stellenbosch and high altitude sites such as Groot Winterhoek (fig 7). Frogs infected with chytrid were found in rivers in town centres such as the Stellenbosch site where there is a lot of human activity as well as in nature reserves such as Cederberg where human impact upon sites should be minimised. At the sites where chytrid infection was found not every frog had the

21

infection. Only at one of the Table Mountain sites did all three of the frogs collected have chytridiomycosis. The number of frogs at a site with chytridiomycosis was not proportional to the number of frogs collected at a site. There were never more than three frogs at any one site found to be infected. At sites where large numbers of frogs were collected such as the Jamaka farm pond only two frogs were seen to be infected.
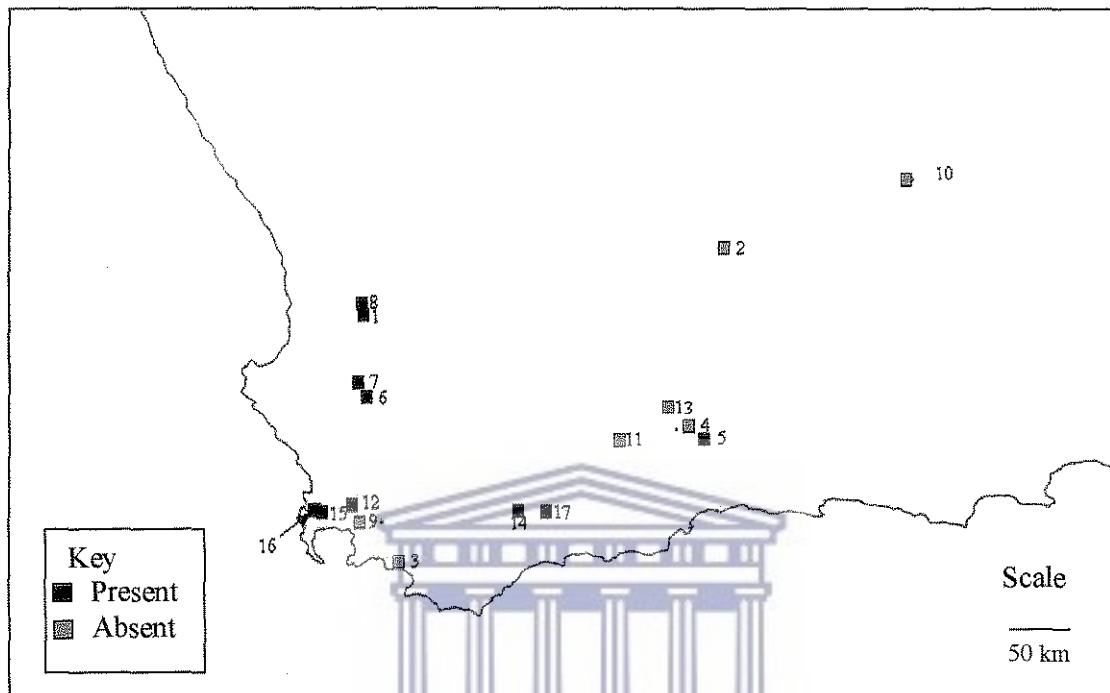
Fig 8 shows the distribution of the sites where chytridiomycosis was found in the Western Cape.

Fig 8 shows the distribution of the sites where chytridiomycosis was found in the Western Cape. There is no pattern shown in the locations. The chytrid fungus seems to be widely distributed. The sites where chytrid was not found in the frogs do not display a pattern either. If the fungus moves in a wave such as was seen in Australia and Central America the wave must have already covered the Western Cape. In Australia it could be seen that the declines moved northwards at about 100 km a year (Laurance et al.1996) and in Central America the disease progressed from northern Costa Rica to western Panama in about 2 years, between 1996 and 1998 (Daszak et al. 1999). However, Hero and Gillespie (1997) suggested that the amphibian declines in Australia were seen to move northwards according to when the decline was first discovered and reported rather than when it first occurred. There was no fungus found

22

in the two southern-most sites but nothing can be concluded from this as it cannot be proven that chytridiomycosis does not occur in other frogs at these sites or sites further south of these.

Table 1. Species in the Western Cape infected with chytrid fungus

Site	Species	Date	Altitude (m)	Number of individuals found infected	Notes
Algeria, Cederberg	*Afrana fuscigula*	19.7.02	504	1	
Grobbelaars River	*Afrana fuscigula*	19.6.02	525	2	
Groot Winterhoek	*Afrana fuscigula*	19.7.02	646	1	
Groot Winterhoek	*Afrana fuscigula*	19.7.02	992	1	Dead
Jamaka farm pond, Cederberg	*Afrana fuscigula*	18.7.02	378	1	
Jamaka farm pond, Cederberg	*Strongylopus grayii*	18.7.02	378	1	
Stellenbosch	*Afrana fuscigula*	31.5.02	120	1	Dead
Swellendam	*Afrana fuscigula*	26.8.02	193	3	
Table Mountain	*Afrana fuscigula*	30.6.02	407	3	
Table Mountain	*Afrana fuscigula*	30.6.02	393	3	
Tradouws pass	*Afrana fuscigula*	20.6.02	294	1	

Afrana fuscigula was chosen as the study species, therefore most of the toes sectioned were taken from *A. fuscigula*. When *A. fuscigula* could not be found or another species was found in the same site, a toe was taken from one of those individuals as well. At the Cederberg site chytrid fungus was found in *Strongylopus grayii* as well as *A. fuscigula* (table 1).

When looking at the sites where chytrid was present in the Western Cape there were no mass die offs, most of the frogs that were collected seemed healthy. There were

23

two dead frogs found, one in Groot Winterhoek and the other in Stellenbosch (table 1). Both of these did have chytrid infections but other frogs taken from the same area had no chytrid infection. Nine frogs from older collections were sampled and no chytrid was found. These were taken from sites across the Western Cape and were collected from 1973 to 1977 (appendix 3). Some of the frogs that were in this older collection were from sites visited for this study. One frog was taken from Groot Winterhoek in 1973 and others from Cederberg in 1977. These were all *A. fuscigula.* No infection was found in these. This could show that the fungus was introduced to the populations later than 1977 or the few frogs sampled may not have been infected while other frogs in the same populations could have been carrying the fungus.

The hypothesis that chytrid would only be found at high altitude sites was tested. Chytrid infected frogs were found at low altitude sites like Stellenbosch at 120 m and high altitude sites such as Groot Winterhoek at 992 m. This differs from other studies (Speare 1999) where infected frogs were only found above a certain altitude.

To test to see if there was a significant difference between chytrid infections at high and low altitude a Mann-Whitney U difference of means test was performed (Hamilton 1990, Salkind 2000). In this test the altitudes where infected frogs were found were compared to the altitudes where none-infected frogs were found (table 2).
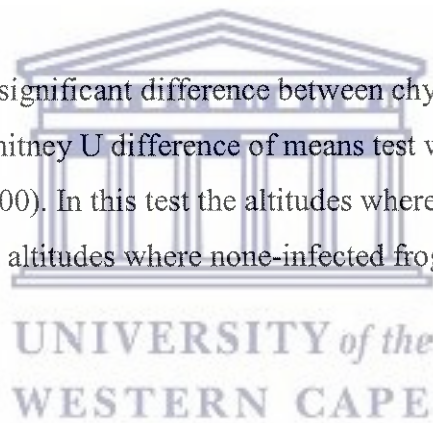
Table 2. Results of the Mann-Whitney U test to determine if there is a significant difference between the altitude of frogs with and without chytrid infection.

Chytrid infection	n	Mean	Standard deviation	Degrees of freedom	t	P
Present	18	40.64	18.71	63	1.38	Not significant
Absent	47	33.37	19.16			

In table 2 it can be seen that the mean rank of the altitude was higher for the frogs with chytrid fungus. When the t value was calculated it was found not to be significantly different from the mean rank of the altitudes of frogs that did not have chytrid infection.

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25

Fig 9. The presence of chytridiomycosis at sites in the Northern Cape

Table 3. Species in the Northern Cape infected with chytrid fungus.

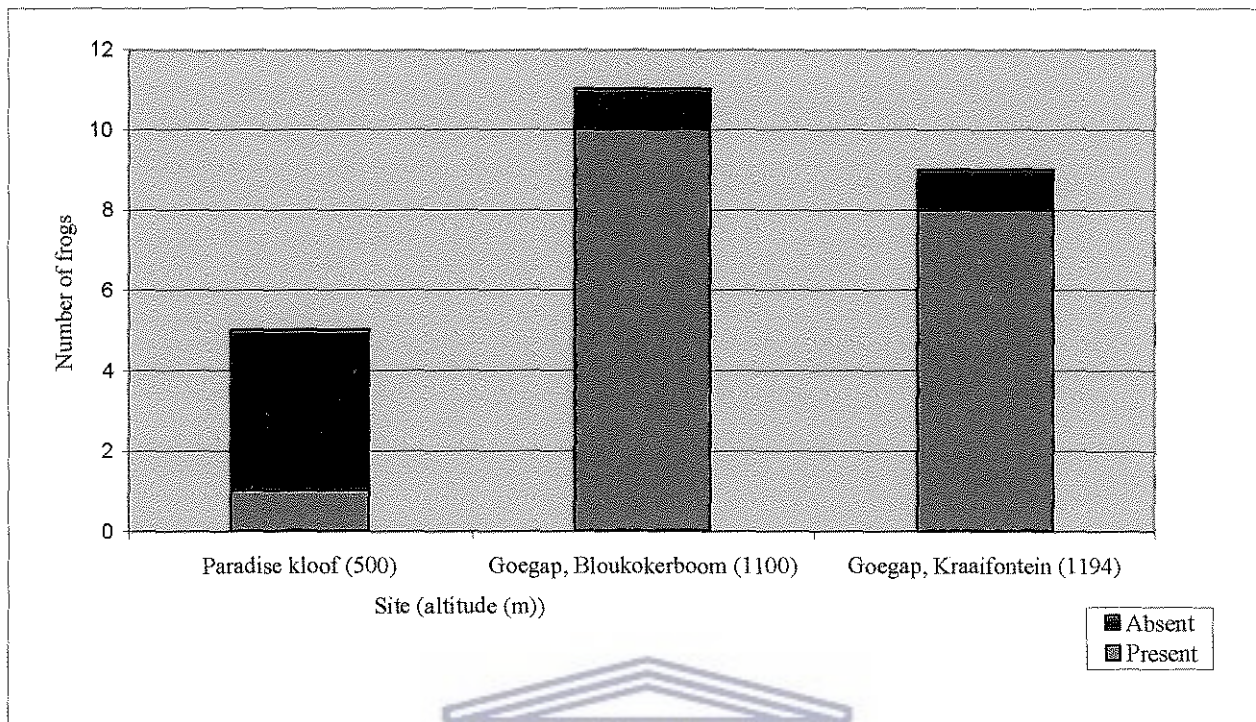| Place	Species	Date	Altitude (m)	Number infected	Notes
Bloukokerboom-water, Goegap	*Afrana fuscigula*	2.8.02	1100	10	1 dead
Kraaifontein, Goegap	*Afrana fuscigula*	1.8.02	1194	8	
Paradise Kloof, Richtersveld	*Strongylopus springbokensis*	6.8.02	500	1	

There were large die offs seen in Goegap Nature Reserve. At the Bloukokerboom-water site 12 dead and decaying frogs were found although apparently healthy frogs were seen. Fig 9 shows that 11 frogs were taken from the Bloukokerboom-water site, of which two were dead. One of the dead frogs had chytridiomycosis (table 3). The other frog's skin was too decomposed to find chytrid infection histologically. This is shown in the single absent record at the Bloukokerboom-water site (fig 9). Every other frog at the Bloukokerboom-water site was infected with chytridiomycosis. Nine

26

frogs were sampled from the Kraaifontein site. All of these were alive and appeared healthy. All but one frog that was sampled had chytrid infection. Both of the Goegap sites were at high altitude.

Fig 9 shows that five frogs were sampled from Paradise Kloof in the Richtersveld. These were all *Strongylopus springbokensis* (table 3). One of these was found to have chytrid infection. All of the frogs appeared healthy. The Paradise Kloof site was in the Richtersveld National Park and relatively isolated from other water sources as it was a natural spring. The water that the frogs were found in would be connected up to other waterways infrequently when there is a heavy rainfall.

Other sites in southern and eastern Africa

Three *Bufo gariepensis* were sampled from Stormberg in the Eastern Cape and one from Golden Gate National Park in the Free State. Two of the frogs from the Eastern Cape were found to have chytridiomycosis.
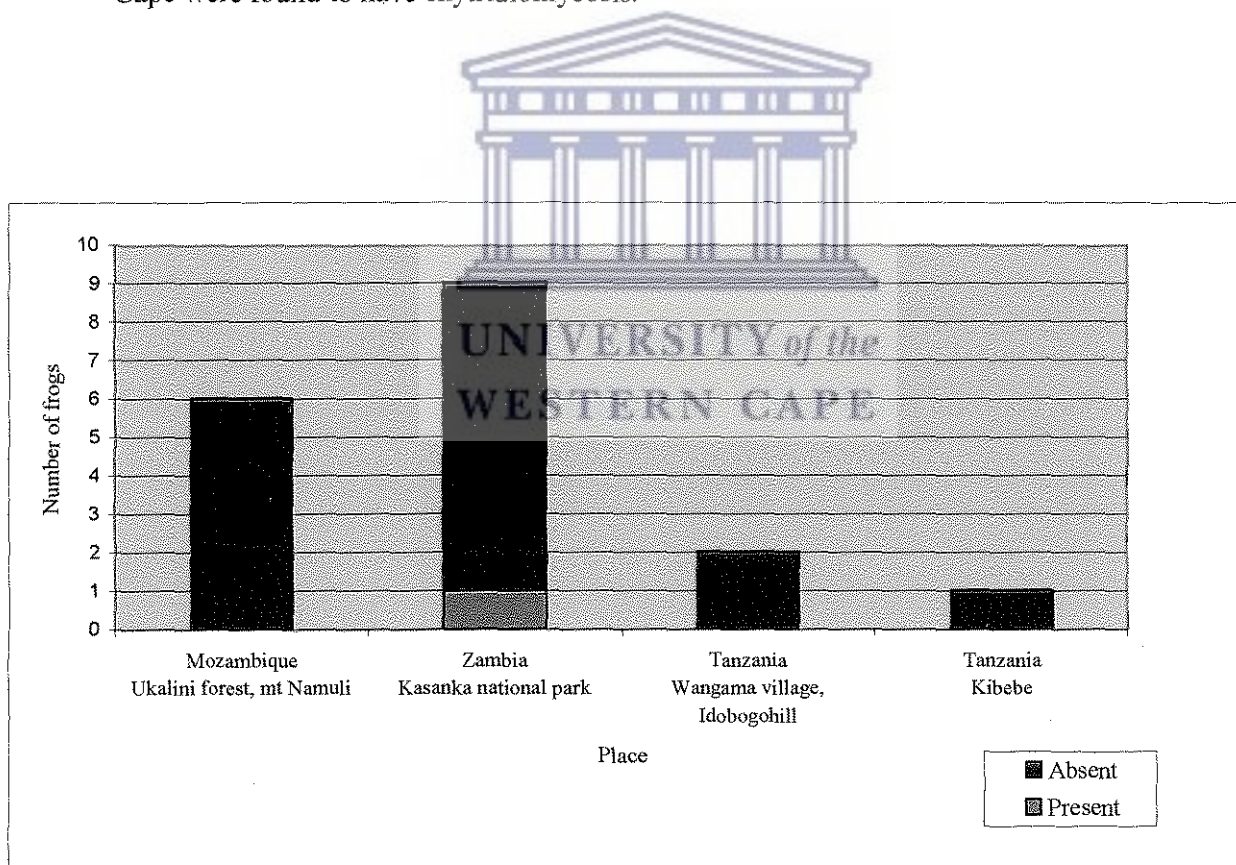
Fig 10. The presence of chytridiomycosis at sites in Southern and Eastern Africa.

Table 4. Species in Eastern and Southern Africa with chytrid fungus.

Site	Species	Country	Date
Kasanka national park	*Xenopus petersii*	Zambia	19.10.01

Fig 10 shows that 18 frogs were sampled from other collections and chytrid was found in *Xenopus petersii* from Kasanka National Park, Zambia (table 4). Other frogs taken from the same place were not infected.

Frogs were sampled from Mozambique, Zambia and Tanzania. There were many different species (appendix 4) and this does not show a representative sample from any of the areas.

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Discussion

The three areas will be discussed separately.

Western Cape.

In the Western Cape 18 infected frogs were found. Ten of the 17 sites tested had frogs with positive chytridiomycosis. This does not mean that the other sites do not have frogs with the disease, it may be that the frogs tested were clear of chytridiomycosis but others in the population were infected. At every site each frog that could be seen was caught although this is not always 100% of the population. Infected frogs were found at sites in nature reserves as well as sites in the centre of towns such as the Stellenbosch site.

Chytridiomycosis was found to be widespread throughout the population in the Western Cape. No pattern could be seen from the maps drawn. Chytrid infections were seen in two species in the Western Cape. *Afrana fuscigula* was chosen as the test species as it was widespread, has an aquatic lifestyle and a long larval stage. This was important as the chytrid fungus has a motile infective stage that is transmitted through the water. It was expected that if chytrid infection were to occur, it would be found in a species such as this. *Strongylopus grayii* is a stream frog found all over the Western Cape, however, the tadpoles are found in standing water rather than running rivers and streams. It is plausible to assume that the infection could be passed from frog to frog whilst the frogs group for breeding. The chytrid may even stay in the water to infect the hatched larvae, as it is still not known how long *B. dendrobatidis* can last outside its host.

The presence of chytrid was not affected by altitude. This was tested with a non-parametric difference of means test. The mean altitude where the infected frogs were found was compared to the mean altitude where the non-infected frogs were found. This showed no significant difference. It was hypothesised that the higher altitude sites would have frogs with chytrid infections rather than the low altitude sites. This has been seen in other cases and is thought to be due to the frog's immune system being less effective at low temperatures. It is thought that the first immune response shown by a frog would be anti-microbial peptides used by the innate immune system

29

(Carey et al. 1999). Rollins-Smith et al. (2002b) tested ten peptides found in different species of frogs and found that they were all effective in inhibiting mature *B. dendrobatidis* as well as the zoospores, which is the infective stage of the chytrid life cycle. It was seen that the peptides inhibited *B. dendrobatidis* effectively at 10 and 22 °C. This means that altitude would have little effect on the action of the peptides against the fungus. However, it is probable that the cold temperature decreases the rate of synthesis of the peptides by the skin. There is little known about peptide production in the skin of frogs and there may be other factors working on the immune system of the frogs such as UV-B radiation or pollution. Although none of the frogs found here were tested by Rollins-Smith (2002b) it is probable that the frogs here have an immune defence against the chytrid fungus. In a paper by Navas (1996) it was found that high altitude species carried out a series of behavioural characteristics to keep their body temperatures above a critical level. They did this by choosing specific microhabitats at certain times of the day. Nevas found that aquatic environments were less variable in temperature than terrestrial sites. The frogs at the high altitude sites in the Western Cape may carry out behavioural thermoregulation to keep their body temperatures similar to the low altitude populations. It is feasible that the temperature does not change enough between the high and low altitude sites in this area to detect a difference. It has been found that *B. dendrobatidis* optimum temperature is 23 °C with slower growth at 28 °C and growth stopping at 29 °C (Daszak et al. 1999).

In the Western Cape area the frogs sampled were all adults and all but two of the frogs appeared healthy. None of the symptoms associated with chytrid infection were seen. In most examples of chytrid infection the frog dies shortly after metamorphosis. However, in the National Zoological Park in Washington DC three Whites tree frogs (*Litoria caerulea*) from Australia were found to be infected with chytridiomycosis and all of these were over ten years old. Two of the frogs were found dead with no symptoms and the other was treated for the abnormal shedding of skin but died a day later. When the three dead frogs were studied other apparently unrelated diseases were found. These were considered the cause of death. These diseases may have made the frogs susceptible to chytrid infection or they may have co-existed with the chytrid infection for years (Pessier et al. 1999). In the Fortuna Forest Reserve in Panama frogs were found to be healthy with low levels of chytrid infection while frogs with high levels of infection were found dead (Lips 1999). The frogs from the Western

30

Cape seem to live with the disease although it is possible that the frogs found to be infected became sick and died after they had been sampled. The frogs with infection may be dying but no dead animals are found due to the low numbers that are infected. The dead frogs would be eaten by other animals or decay.

One frog from Stellenbosch was found dead, although the river was in the middle of the town and was polluted. It is possible that the frog could have died from any number of causes. However, this frog did have chytridiomycosis and five other frogs, taken from the same river, did not have chytrid infections and all five appeared healthy. If the conditions that the frogs were living in were causing the frogs to die then it would be expected that more frogs would have been found dead and dying. The only frog at the Stellenbosch site that was found dead was the only frog with chytridiomycosis from that site. The other dead frog was collected in Groot Winterhoek and was found to have chytrid infection. This site was in a nature reserve and had relatively little human interference. It is possible that another factor caused the frog to die. At this site, like the Stellenbosch site, other frogs taken from the same site were apparently healthy and, when tested, free from infection.

The frogs from this area do not follow the pattern of chytrid infection seen in other areas. The frogs here are not reported to be declining and seem to be able to mature while carrying chytrid infections or are only infected later in the life cycle. At sites where one frog was found to be infected other frogs from the same area were clear of infection. The only exception to this was one of the Table Mountain sites. Here all of the collected frogs had chytridiomycosis. The sites where these frogs were collected from were man-made concrete pools. They were situated by the roadside and collected the water coming off the mountain. The fact that not all of the frogs at one site were infected could show that the fungus is not as virulent in Western Cape populations or the frogs have a way of keeping away the infection. It is possible that the frogs with chytrid infections have been immunosupressed by another factor and this has made them susceptible to chytrid infection. This would explain the high incidence of chytrid infection at the Table Mountain site where the frogs were not in their natural environment. It may be that the frogs are not being infected from an early stage and an adult frog's immune system can cope with a new chytrid infection. It is thought that the rates of depopulation seen in areas in America and Australia suggest

31

an introduced virulent pathogen. The pattern seen in the Western Cape, where not all the population was infected may suggest a co-evolved host pathogen relationship. The chytrid is not host specific in this area, this indicates that the host and parasite did not evolve together (Daszak 1999 et al.). It is possible that the frogs from the same sites that appeared clear of infection were infected and the infection was missed in the laboratory process.

Northern Cape

Three sites were visited in the Northern Cape. The first two were in Goegap Nature Reserve. All the live adults were collected from both the ponds and two of the dead frogs were sampled. These frogs were *A. fuscigula* like most of the frogs in the Western Cape that were investigated. Every frog except one tested for chytridiomycosis was infected. One of the dead frogs could not be confirmed with chytridiomycosis as it was too decomposed and all the skin had broken up with a secondary fungal infection. The frogs at the Goegap nature reserve were in a different habitat to the frogs in the Western Cape. The area was dry and the frogs were found in two small pools of water. In contrast to the Western Cape frog populations at least 12 frogs were found dead in these small pools. Many of these frogs could not be tested for chytridiomycosis as they were decomposing in the water. These die-offs seemed more than natural population fluctuations. The reason for this is that large numbers of dead frogs were found and if this were natural there would be a predator to clear up the dead animals. These findings were more similar to the findings from other sites with chytrid infections such as the ones reported in the paper by Berger et al. (1998) where many dying and dead frogs were found. The sites were both at high altitudes and they were in Goegap Nature Reserve, which has had nature reserve status since 1990. The chytrid probably was spread from one pool to the other when they were both cleaned out three years ago.

The other case of chytridiomycosis was seen in a *Strongylopus springbokensis*. This was found in Paradise Kloof in the Richtersveld. This site is in a nature reserve and off the 4x4 track meaning that there is very little human interference. There were large numbers of *Strongylopus springbokensis* as well as *Cacosternum namaquense* and *Bufo robinsoni*. Only five samples were taken owing to a lack of time and *S. springbokensis* was concentrated on, as it is associated with the water more than the

32

others. This area is dry semi-desert and the water that the frogs were living in came from an underground spring. It is unusual that chytrid fungus was found in one of the frogs here as the site is not often connected to a river system and means that the fungus is not likely to have been passed on down stream. It is unlikely that the fungus was brought in by humans due to the inaccessibility of the area. It is possible that the fungus was transported by one of the other animals in the reserve from another wet area. It is possible that the fungus can survive for long periods of time in the water cycle feeding on keratin in the water.

There are many theories as to why chytridiomycosis has emerged recently as a disease in amphibians. The first is that the chytrid has been introduced somehow and is spreading through naïve populations which are unprepared to defend themselves against it. Another is that *B. dendrobatidis* is a widespread organism that has either increased in virulence or that the amphibians are succumbing to the disease due to an increase in host susceptibility (Berger et al. 1998). In this study the chytrid was found at many sites in the same species and die offs were only seen at sites in the Northern Cape. This suggests another factor contributing to the die offs in the Northern Cape as individuals in the Western Cape from the same species have similar chytrid infections and there have been no mass die offs seen. It may be that the chytrid infection is not the cause of death and it has a synergistic effect with other factors. This was seen in *Rana* and *Bufo* embryos which were subjected to the fungus *Saprolegnia ferax* and UV-B radiation. It was found that the two factors together had a far greater effect than just one factor on its own (Kiesecker and Blaustein 1995). This may be an environmental factor. The pools in the Northern Cape, where the die offs were seen, were in semi desert so that there was little protection over the water and both the pools were exposed. This is seen in the paper by Bosch et al. (2001) where sites in the Penalara National Park were studied. Here high altitude small lakes surrounded by grassland in a temperate area were studied. Although in their study they rule out UV-B radiation due to the way *Alytes obstetricans* eggs are carried by the male until they are fully formed tadpoles. It may be that UV-B radiation affects the frogs in the Goegap sites. It has been suggested that UV-B radiation increases by 5 to 6% for every one km increase in altitude if the sky is clear and unpolluted. Low elevation sites may be more polluted with tropospheric pollutants, aerosols and fog (Davidson et al. 2001) and the Goegap sites were all at high altitudes suggesting that if UV-B

33

were increasing these frogs would be susceptible. An increase in UV-B radiation has been seen to reduce survival and hatching rates of amphibian embryos in certain species and is thought to be linked to photolyase activity and therefore, the ability to repair UV damage. However, this would affect the frog's larval stage and the frogs dying here were adult. It has been suggested that UV-B radiation may affect the chemistry of the water and food supply, changing predator prey interactions with other UV-B affected animals. It may affect the survival of adults through damage to eyes and increases in tumours and immunosupression (Alford and Richards 1999). There is no evidence to suggest that there has been an unnatural increase in UV-B radiation (Barinaga 1990). It is not known what effect an increase of UV-B radiation would have on the species of frogs tested here. Immunosupression suggested as a result of increases in UV-B radiation in some frog species would give the chytrid fungus opportunity to infect these frogs.

Even though the sites were in a nature reserve it is possible that there was an unknown pollutant in the pools affecting the frogs. Goegap has only been a nature reserve since 1990. Before 1960 most of the land was agricultural until donated for use as a flower reserve. Goegap farm was then added on to the protected site. Davidson et al. (2001) suggest upwind agricultural pollution as a cause in the decline of the Californian red legged frog, *Rana aurora draytonii;* it is possible that an unknown pollutant is affecting the frog populations in Goegap.

It may be that another disease causes the animal to become susceptible to chytrid infection. In the paper by Berger et al. (1998) *Aeromonas hydrophilia* and *Flavobacterium indologenes* were found in chytrid infected frogs but were not considered the cause of death for the whole population because no more than 44% of the sick and dead frogs had one of the bacteria.

It is possible that the frogs in the Western Cape have an immune defence against the chytrid fungus that the frogs from the same species do not have in the Northern Cape. The innate immune system is known to be able to produce peptides that kill the chytrid fungus in some frogs. It is not known if *A. fuscigula* has anti-microbial peptides that would be effective against chytrid fungus. It is not known at what concentrations frogs have anti-microbial peptides in their skin or what causes the

34

production of them by the specialised skin glands. The frogs in the Northern Cape may have something inhibiting their immune response. It is suggested that temperature, pH, hydration of the skin and environmental toxins may have an effect on immune response (Rollins-Smith 2002a). It is possible that the chytrid fungus does not cause an immune response. The presence of the chytrid in the frog skin causes little inflammation, which is a sign of immune response. The inflammation causes increased permeability of blood vessels and this releases immunoglobulins and complement. It is possible that the fungi produce compounds that inhibit the inflammatory response and that the neutrophils and macrophages do not recognise the chytrid as a pathogen. The fungus may be able to protect itself against any anti-microbial peptides that the frog's immune system produces (Carey 2000).

Perhaps an introduced animal has caused the frog's immune system to become compromised. In the two Goegap ponds there were a number of leaches that might affect the frogs.

Other sites in southern and eastern Africa

Chytrid was found in *Bufo gariepensis* in the Stormberg Mountains. These frogs are not highly associated with water. They live in rocky areas and in dry thorn bush. The eggs are laid in wet vegetation or water logged depressions and metamorphosis can take as little as 20 days (Channing 2001). This would make chytrid infection less likely because of the motile infective stage needing water to infect the animal. It is possible that the infection is passing from animal to animal in the water during the breeding season.

A small number of samples were taken from other areas in Eastern and Southern Africa from other collections. There were various species tested and chytrid infection was seen in *Xenopus petersii*. They are a truly aquatic species and are more likely to be affected with chytrid as they spend their whole lives in water. This frog was from Zambia, Kasanka National Park. This shows that chytridiomycosis is not restricted to South Africa and stretches further into Africa. The frogs tested from Mozambique and Tanzania were all clear of infection but this does not mean that chytrid infection would not be found in frogs in these countries.

35

Conclusions

This project gives an overview of chytrid infection in the Western Cape and at a small number of sites in the Northern Cape and Eastern Cape. It is presence-only data and the absence of chytrid at a site cannot be proven. The transects were chosen carefully to cover a wide area and differing altitudes. More sites could have been studied to give a better picture of the area but there were time constraints and the laboratory procedures were time consuming. Once the method had been perfected it was quick and easy to process a toe although slow at the beginning. One hundred sections were cut for each toe unless the toe was too small to get 100 sections. Later only 50 sections were scanned. This decision was taken after the number of sections that had to be examined before chytrid infection was found was plotted on a graph. It was found that infection was discovered within the first 50 sections 94.4% of the time.

There have been few studies carried out in Africa on amphibian decline. This project has shown that there is chytridiomycosis in Western Cape frog populations. The Western Cape data collected shows unusual results in the fact that there were no mass die offs seen. The frogs seemed to be living with their chytrid infection. It has shown that chytridiomycosis is widespread and not related to altitude, being found at high and low altitude sites. The Northern Cape data showed die-offs of chytrid infected frogs at two sites and a single case of chytridiomycosis in the Richtersveld. Chytrid was seen in *Bufo gariepensis* in the Eastern Cape and *Xenopus petersii* in Zambia. This shows that chytrid is widespread and more work needs to be carried out on African frog populations.

Future Work

The Northern Cape data is important as the mass dies offs there need to be investigated and the cause of them needs to be found. It would be interesting to infect a healthy Northern Cape and Western Cape *A. fuscigula*. It is important to find out if the chytrid is killing the Northern Cape frogs and to see if the fungus kills the Western Cape frog when they are in the same conditions. It would be useful to infect frogs from tadpole stage and see if they mature post metamorphosis. The UV-B levels of the sites need to be tested along with the pH of the water, as the pH of the water is known to affect the epidemiology of chytrid blooms. Bosch et al. (2001) found that

36

the levels of calcium, hydrogen, magnesium and NO3- were different in ponds with and without *A. obstetricans*. It would by interesting to see if the water with animals with chytridiomycosis differs chemically from the water with animals without chytridiomycosis. Davidson et al. (2001) found that declines in *Rana a. draytonii* were associated with wind borne agro-chemicals. The sites in this study, although many of them may be in protected areas, may be receiving pollution from the surrounding mining area.

It may be interesting to look for chytridiomycosis in a species with a different life style to the semi aquatic frogs studied here. Perhaps a study on a frog that does not have a larval stage to check that chytrid does not occur in them. This study concentrated on *A. fuscigula* because if chytrid were to occur it was more likely to occur in a species such as this. *B. gariepensis* was looked at apart from the main data set. This was due to the frogs being collected for another survey. Chytridiomycosis was found in two of the frogs and this suggests that it may be found in many different species in the Western Cape.

The few samples taken from outside South Africa showed one positive result. This indicates that more work is needed to establish where chytrid fungus is found in other parts of Africa. It is important to discover if the infection is following the same patterns as the infection in places such as Australia and Central America or whether it shows the same pattern as the infections in the Western Cape where no die-offs have been seen.

37
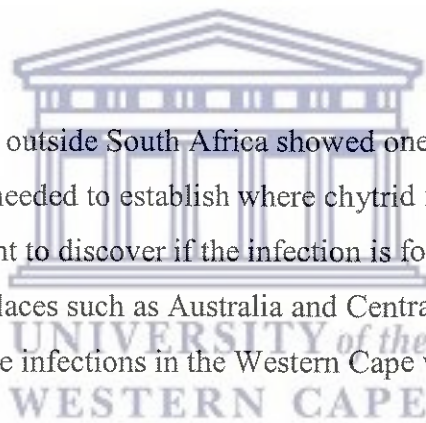
Appendix 1

Site names and GPS coordinates.

Site number	Site name	Latitude	Longitude	Altitude (m)
1	Algeria, Cederberg	32° 22' 27.4"	19 °03' 41.3"	504
2	Beaufort West	32° 00' 31.0"	22°25' 05.0"	1630
3	Fernkloof	34° 23' 45.0"	19° 16' 03.0"	300
4	Gamkakloof	33° 20' 41.0"	22° 01' 12.4"	1338
5	Grobbelaars river	33° 25' 11.2"	22° 14' 27.0"	525
6	Groot Winterhoek	33° 03' 14.1"	19° 04' 46.7"	646
7	Groot Winterhoek	32° 59' 54.8"	19° 03' 23.9"	992
8	Jamaka farm pond, Cederberg	32° 20' 14.9"	19° 01' 29.0"	378
9	Landroskop	34° 02' 55.6"	19° 00' 32.6"	1059
10	Richmond	31° 25' 48.0"	24° 19' 08.0"	1560
11	Seweweekspoort	33° 22' 57.2"	21° 24' 26.2"	924
12	Stellenbosch	33° 56' 00.0"	18° 52' 00.0"	120
13	Swartberg Nature Reserve	33° 22' 30.0"	22° 06' 03.0"	932
14	Swellendam	34 °00' 35.7"	20° 27' 33.1"	193
15	Table Mountain	33° 56' 52.5"	18° 26' 04.2"	407
16	Table Mountain	33° 56' 53.7"	18° 25' 59.9"	393
17	Tradouws pass	33° 57' 54.8"	20° 42' 16.6"	294
18	Goegap, Kraaifontein	29° 37' 45.0"	18° 02' 03.0"	1194
19	Goegap, Bloukokerboom	29° 38' 03.0"	18° 00' 25.0"	1100
20	Paradise Kloof	28° 19' 45.2"	17° 00' 14.0"	500
21	Stormberg	31° 08' 12.0"	26° 37' 32.0"	1904
22	Golden Gate National Park	28° 30' 31.0"	28° 39' 14.0"	2500

Appendix 2

Perenyi fixative (Humason 1979)

Chromic acid, 1% aqueous (1 g/100 ml water)	15.0 ml
Nitric acid, 10% aqueous (10 ml /90 ml water)	40.0 ml
95% ethyl alcohol	30.0 ml
distilled water	15.0 ml.

Appendix 3

Site and species list of samples from older collections across the Western Cape

Site	Species	Date
Groot Winterhoek	*A. fuscigula*	19.9.73
Groot Kouport	*Xenopus laevis*	11.2.77
Groot Kouport	*X. laevis*	11.2.77
Groot Kouport	*X. laevis*	11.2.77
Cederhout Kloof	*A. fuscigula*	1.2.77
Groot Kouport	*A. fuscigula*	11.2.77
Cederberg	*A. fuscigula*	2.2.77
Cederberg Groot Kouport Kloof	*A. fuscigula*	11.2.77
Groot Kouport	*A. fuscigula*	18.1.77

Appendix 4

Site and species list of Eastern and Southern African frogs.

Site	Species	Number of specimens
Mozambique, Mt Namuli	*Afrana angolensis*	3
	Phrynobatrachus acridoides	1
	Nothophryne broadleyi	1
	Tomopterna tuberculosa	1
Zambia, Kasanka National Park	*Ptychadena oxyrhynchus*	3
	Ptychadena taenioscelis	1
	Hildebrandtia ornata	1
	Xenopus laevis	1
	Xenopus petersii	3
Tanzania, Wangama village	*Kassina senegalensis*	1
	Tomopterna tuberculosis	1
Tanzania, Kibebe	*Afrana angolensis*	1

39

References

Alford, R. and S Richards. (1999). Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* **30**: 133-165.

Barinaga, M. (1990). Where have all the froggies gone? *Science* **247**: 1033-1034.

Berger, L., R. Speare, P. Daszak, D. Green, A. Cunningham, L. Goggin, R. Slocombe, M. Ragan, A. Hyatt, K. Mc Donald, H. Hines, K. Lips, G. Marantelli and H. Rarkes. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Population Biology* **95**: 9031-9036.

Berger, L. and R. Speare. (1998). *Chytridiomycosis: a new disease of wild and captive amphibians.* Anzccart newsletter **11**: 1-3. http:// www.jcu.edu.au/ school/ phtm/ PHTM/ frogs/ anzcarrt.htm.

Berger, L., R. Speare and A. Kent. (Nov 1999). *Diagnosis of chytridiomycosis in amphibians by histologic examination.* www.jcu.edu.au/ school/ phtm/ PHTM/ frogs/ histo/ chhisto.htm.

Blaustein, A. (1994). Chicken little or Neros fiddle? A perspective on declining amphibian populations. *Herpetologica* **50**: 85-97.

Blaustein, A., G. Hokit, R. O'Hara and R. Holt. (1994a). Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. *Biological Conservation* **67**: 251-254.

Blaustein, A., D. Wake and W. Sousa. (1994b). Amphibian declines: judging stability, persistence and susceptibility of populations to local and global extinctions. *Conservation Biology* **8**: 60-71.

Bosch, J., I. Martinez-Solano and M. Garcia-Paris. (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological Conservation* **97**: 331-337.

Bradford, D. (1984). Temperature modulation in a high-elevation amphibian, *Rana muscosa*. *Copeia* **4**: 966-976.

Bradford, D. (1991). Mass mortality and extinction in a high elevation population of *Rana muscosa*. *Journal of Herpetology* **25**: 174-177.

Carey, C. (1993). Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. *Conservation Biology* **7**: 355-362.

Carey, C., N. Cohen and L. Rollins-Smith. (1999) Amphibian declines: an immunological perspective. *Developmental and Comparative Immunology* **23**: 459-472.

Carey, C. (2000). Infectious diseases and worldwide declines of amphibian populations, with comments on emerging diseases in coral reef organisms and in humans. *Environmental Health Prospectives* **108**: 143-150.

Channing, A. (2001). *Amphibians of Central and Southern Africa.* New York. Cornell University Press.

Corn, P. and J. Fogleman. (1984). Extinction of montane populations of the Northern Leopard frog (*Rana pipiens*) in Colorado. *Journal of Herpetology* **18**: 147-152.

Cullen, B. and L. Owens. (2002). Experimental challenge and clinical cases of Bohle iridovirus (BIV) in native Australian anurans. *Diseases of aquatic organisms* **49**: 83-92.

Cunningham, A. (1996). Disease risks of wildlife tranlocations. *Conservation Biology* **10**: 349-353.

Cunningham, A. (1998). Disease and pathology working group report: a breakthrough in the hunt for a cause of amphibian declines. *Froglog* **30**: 3-4

Daszak, P., L. Berger, A. Cunningham, A. Hyatt, D. E. Green and R. Speare. (1999). Emerging infectious diseases and amphibian population decline. *Emerging Infectious Diseases* **5**: 735-748.

Davidson, C., B. Shaffer and M. Jennings. (2001). Declines of the California red-legged frog: climate, UV-B, habitat, and pesticides hypotheses. *Ecological Applications* **11**: 464-479.

Duellman, W. (1993). Amphibians in Africa and South America: evolutionary history and ecological comparisons. In Peter Goldblatt (Ed.) *Biological relationships between Africa and South America.* Yale University Press: New Haven: 200-243.

Fellers, G. (June 2000). Western Ecological Research Centre. USGS. http:// listproc.ucdavis.edu/ archives/ herp-1/ log0006/ 0012.html.

Frost, D. R. (2002). Amphibian species of the world: an online reference. V2.20 (15 July 2002). Electronic database available at http:// research.amnh.org/ herpetology/ amphibia/ index.html.

Hamilton, L. (1990). *Modern data analysis. A first course in applied statistics.* California: Wadswort Inc.

Hanken, J. (1999). Why are there so many new amphibian species when amphibians are declining? *Tree* **14**: 7.

Hero, J. and G. Gillespie. (1997). Epidemic disease and amphibian declines in Australia. *Conservation Biology* **11**: 1023-1025.

Hero, J. (winter 1996). Where are Queensland's missing frogs? *Wildlife Australia*: 8-13.

41

Pough, F., R. Andrews, J. Cadle, M. Crump, A. Savitzky, K. Wells. (2001). *Herpetology.* New Jersey: Prentice- Hall Inc.

Pounds, J., M. Fogden, J. Savage and G. Gorman. (1997). Tests of null models for amphibian declines on a tropical mountain. *Conservation Biology* **11**: 1307-1322.

Pounds, J., M. Fogden and J. Campbell. (1999). Biological response to climate change on a tropical mountain. *Nature* **398**: 611-615.

Reed, K. D., G. R. Ruth, J. A. Meyer and S. K. Shukla. (Jan 2000) *Chlamydia pneumoniae infection in a breeding colony of African Clawed frogs (Xenopus tropicalis).* Emerging Infectious Diseases **16**. http:// www.cdc.gov/ ncidod/ cid/ vol6no2/ reed.htm.

Rollins-Smith, L., J. Doersam, J. Longcore, S. Taylor, J. Shamblin, C. Carey and M. Zasloff. (2002a). Antimicrobial peptide defences against pathogens associated with global amphibian declines. *Developmental and Comparative Immunology* **26**: 63-72.

Rollins-Smith, L., C. Carey, J. Longcore, J. Doersam, A. Boutte, J. Bruzgal and M. Conlon. (2002b). Activity of antimicrobial skin peptides from ranid frogs against *Batrachochytrium dendrobatidis*, the chytrid fungus associated with global amphibian declines. *Developmental and Comparative Immunology* **26**: 471-479.

Salkind, N. (2000). *Statistics for people who think they hate statistics.* California: Sage publications.

Snyder, G. and W. Weathers. (1975) Temperature adaptations in amphibians. *American Naturalist* **109**: 93-101.

Speare, R. (1999). *Amphibian decline.* http:// www.jcu.edu.au/ school/ phtm/ PHTM/ frogs/ adhist.htm.

Speare, R. and L. Berger. (2000a). *Chytridiomycosis in amphibians in Australia.* http:// www.jcu.edu.au/ school/ phtm/ PHTM/ frogs/ chyspec.htm.

Speare, R. and L. Berger. (2000b). *Global distribution of chytridiomycosis in amphibians.* http:// www.jcu.edu.au/ school/ phtm/ PHTM/ frogs/ chyglob.htm.

Wake, D. (1991). Declining amphibian populations. *Science* **253**: 860.

Young, B., K. Lips, J. Reaser, R. Ibanez, A. Salas, R. Cedeno, L. Coloma, S. Ron, E. Marca, J. Meyer, A. Munoz, F. Bolanos, G. Chaves and D. Romo. (2001). Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* **15**: 1213-1223.

Humason, G. (1979). *Animal tissue techniques*. San Francisco California: Freeman.

Kiesecker, J. and A. Blaustein. (1995). Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. *Proceedings of the National Academy of Sciences USA* **92**: 11049-11052.

Laurance, W., K. McDonald and R. Speare. (1996). Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conservation Biology* **10**: 406-413.

Lips, K. (1998). Decline of a tropical montane amphibian fauna. *Conservation Biology* **12**: 106-117.

Lips, K. (1999). Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology* **13**: 117-125.

Longcore, J. E., A. P. Pessier and D. K. Nichols. (1999). *Batrachochytrium dendrobatidis* gen.et sp. Nov., a chytrid pathogenic to amphibians. *Mycologia* **91**: 219-227.

Madronich, S. and F. de Gruijl. (1993). Skin cancer and UV radiation. *Nature* **366**: 23.

Meadows, M. (1985). *Biogeography and ecosystems of South Africa*. Cape Town: Juta.

Milius, S. (4ᵗʰ July 1998). Fatal Skin Fungus Found In US Frogs. *Science News* 154: 7 http:// www.sciencenews.org/ sn_arc98/ 7_4_98/ fob9.htm.

Morell, V. (1999). Are pathogens felling frogs? *Science* **284**: 728-731.

Muths, E., D. Green and M. Banowetz. US Geological survey. (29.3.2000). http:// www.npwrc.usgs.gov/ narcam/ info/ news/ chytrid.htm.

Navas, C. (1996). Implications of microhabitat selection and patterns of activity on the thermal ecology of high elevation neotropical anurans. *Oecologia* **108**: 617-626.

Nickerson, M. and J. Hutchison. (1971). The distribution of the fungus *Basidiobolus ranarum* Eidam in Fish, Amphibians and Reptiles. *The American Midland Naturalist* **86**: 500-502.

Passmore, N. and V. Carruthers. (1995). *South African frogs*. Johannesburg: Witwaterstrand University Press.

Pessier, A., D. Nichols, J. Longcore and M. Fuller. (1999). Cutaneous Chytridiomycosis in Poison dart frogs (*Dendrobates* spp) and White's Tree Frogs (*Litoria caerulea*). *Journal of Veterinary Diagnostic Investigation* **11**: 194-199.