Impact of gold mining on *Cordylus giganteus* and recommendations for conservation and management

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ABSTRACT

I investigated whether inorganic contaminants associated with gold mining waste discharges in the Free State Province, South Africa, were accumulated by a threatened species of lizard, *Cordylus giganteus*; if the route of exposure could be dietary, and whether accumulation of contaminants could be associated with potential physiological costs. I compared elemental concentrations in tissue and blood samples between populations of this species, from four sites around the province. Inorganic contaminants were known to be elevated in soils, water, sediments and vegetation of the first mining site, and to a lesser extent at the second mining site. The third site was not known to be contaminated by mining discharges, but was selected because of its potential to be contaminated by wind-blown contaminants. This site was also heavily overgrazed. The fourth site was both uncontaminated by mining and relatively undisturbed. Lizards from the most contaminated site had significantly higher blood concentrations of Li, Na, Al, S, Ca, P, Si, Cr, Mn, Fe, Ni, W and Bi when compared with all the other sites investigated. Based upon a comparison of elemental concentrations in selected lizard prey items found at these sites (Coleoptera, Tenebrionidae) I did not find conclusive evidence for a dietary route of exposure to contaminants. I tested for significant differences in body condition among populations. Lizards from the heavily grazed site were in similar condition to lizards from the most contaminated site, and all these lizards were in significantly poorer condition than lizards from the undisturbed site. The adult sex ratio of the population inhabiting the most contaminated site also deviated significantly from an expected 1:1 ratio in favour of females. The reason for this deviation is not understood, but may be a consequence of sexes being differentially affected by inorganic contaminants. My research demonstrates that the disposal of gold and uranium mine waste has resulted in the accumulation of contaminants by a representative resident vertebrate, and that this accumulation is potentially associated with poorer body condition which might affect fitness. It highlights the potential threat of mining-waste discharges to lizards, shows the need for site remediation measures,

and also highlights the need for further investigation into the potential effects of environmental contaminants from gold and uranium mine waste on exposed vertebrates in South Africa.

DECLARATION

I declare that this thesis is my own, unaided work unless specifically acknowledged in the text. It has not been submitted before for any degree or examination at any other university, nor has it been prepared under the aegis or the assistance of any other body or organisation or person outside the University of the Witwatersrand, Johannesburg.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INORGANIC CONTAMINATION OF A THREATENED LIZARD, *CORDYLUS GIGANTEUS*, IN SOUTH AFRICA

INTRODUCTION

South Africa is well known for its gold and uranium mining industry, which is often heavily criticised for the damage it causes to the environment (Smuts & Winter 2004). Potential effects from mining on the environment are numerous. The most serious of these effects include: changes in hydrogeological systems; hydrological transformations of soils and surficial flows; contamination of soils and surficial water reservoirs; and pollution of the atmosphere (Rybicka 1996).

The release of various organic and inorganic contaminants used during mining processes has been shown to be associated with the accumulation of contaminants by organisms inhabiting these environments. Examples of studies where the accumulation of inorganic contaminants have been documented in living organisms include the accumulation of cadmium, lead, copper and zinc in tissues of three spined stickelback fish sampled from various watercourses in Flanders (*Gasterosteus aculeatus*; Bervoets *et al.* 2001); the accumulation of lead, cadmium, mercury, manganese, selenium and chromium in feathers, liver, kidney and heart tissue of laughing gulls (*Larus atricilla*) sampled from an airport in New York (Gochfeld *et al.* 1996); elevated tissue levels of cadmium and nickel in muskrat (*Ondatra zibethica*) occurring in the vicinity of an ore-smelter in Canada (Parker 2004); and in South Africa, elevated concentrations of copper, nickel, chromium, iron and manganese in organs of red-knobbed coot (*Fulica cristata*) occurring in the vicinity of gold mining operations in Gauteng (Van Eeden 2003). The majority of these studies report only on the accumulation of inorganic contaminants in living organisms, but not the physiological (or fitness and survival) consequences thereof.

Nevertheless, some investigations into fitness effects of exposure to inorganic contaminants have reported a number of effects on various taxonomic groups. Haywood *et al.* (2004) and Haywood (2004) reported consequences for *Xenopus laevis* tadpoles during a seven-day exposure to elevated concentrations of Zn, Cu, Pb and Cd. These effects included reduced hatching success, reduced survival, reduced growth, and increases in the occurrence of malformations. Potential negative effects of ingestion of metal contaminants include reduced growth in fish (*Oncorhynchus mykiss*; Hansen *et al.* 2004). Exposure to elevated copper concentrations in water (up to 26µg Cu/L) was associated with immune system deficiencies in fish (*Oncorhynchus mykiss*; Dethloff & Bailey 1998). Also indicating immune system effects, Snoeijs *et al.* (2004) reported a decrease in immune responsiveness from birds (*Parus major*) sampled at a site with elevated levels of mainly lead and cadmium emanating from a metallurgic smelter. Tilapia larvae exposed to varying concentrations (30, 50, 100, 200 or 400 µg/L) of copper sulphate all expressed suppressed yolk absorption rates (Wu *et al*. 2003). Increases in levels of territorial aggressive behaviour and reductions in egg hatching success from great tit couples exposed to high environmental concentrations of arsenic, cadmium, copper, lead and zinc has also been reported (Janssens *et al*. 2003), as has damage to the DNA structure of fish (*Sparus aurata*) and molluscs (*Scapharca inaequivalvis*) due to exposure to elevated concentrations (0.1 ppm) of copper sulphate (Gabbianelli *et al.* 2003). Furthermore, exposure to metal contaminants also affects organisms at a population level. For example, skewed sex ratios in favour of adult female birds have been reported in some populations of white-tailed ptarmigan (*Lagopus leucurus*) exposed to elevated concentrations of cadmium in the Colorado Rocky Mountains ore belt (Larison *et al.* 2000).

On a global scale reptiles appear to be declining mainly due to habitat loss, introduced invasive species, diseases and parasitism, unsustainable harvesting, climate change and environmental pollution (Gibbons *et al.* 2000). Reptiles (and lizards in particular) are considered to be useful bioindicator organisms because they are mostly insectivorous (Bishop & Gendron 1998), are integral to many food webs (Lambert 1997; Campbell & Campbell 2000), and they show high site fidelity (Burger *et al*. 2004; Lambert 1993). These characteristics of lizards provide opportunities to compare contaminant effects on individuals and populations inhabiting contaminated and reference sites within a small geographical area (Hopkins 2000).

Despite these features, reptiles remain the least studied vertebrate group with regard to the accumulation and effects of inorganic contaminants (Hopkins 2000). This has been attributed to various reasons, including difficulty in sampling sufficient numbers, their relatively low economic value and their apparent difficulty in laboratory maintenance (Loumbourdis 1997). Among the reptiles studied, turtles have received far greater attention than crocodilians and squamates (lizards and snakes) (Hopkins *et al*. 2002). Furthermore, studies of squamates have mostly focused on organic contaminants such as DDT, dieldrin and PCB concentrations (Campbell & Campbell 2000; Campbell & Campbell 2001). Negative effects from organic contaminants on lizards include reductions in brain cholinesterase activity in green anoles exposed to organophosphorous pesticides (Hall & Clark 1982). A species of dwarf lizard (*Lacerta parva*) exhibited liver, kidney and intestinal damage after exposure to low doses of malathion, an organophosphorous pesticide (Ozelmas & Akay 1995). Exogenous estradiol caused ovary development in male leopard geckos (*Eublepharis macularius*) (Bull *et al.* 1988). Also, various studies have examined the lethal doses of organic contaminants in lizards (e.g. Kihara &Yamashita 1978; McIlroy *et al.* 1985; Twigg & Mead 1990).

The few studies on potential effects of inorganic pollution on reptiles have generally been in controlled environments, such as laboratories (Brasfield *et al.* 2004; Hopkins *et al.* 2001, 2002, 2005). These studies mostly measured the relevant inorganic uptake concentration by killing individuals and sampling tissues from various major organs. Some field studies investigating the effects of inorganic contaminants on reptiles have also been attempted. These have also mostly used destructive sampling techniques, requiring tissue samples from major organs such as kidneys and livers (Anan *et al.* 2001; Burger *et al.* 2004; Loumbourdis 1997). Thus, few studies have used non-destructive sampling in a field environment, to analyze potential effects of inorganic contaminants on reptiles (e.g. Jeffree *et al.* 2001).

Cordylus giganteus (giant girdled or sungazer lizard) is a species endemic to the highveld region of South Africa. The highveld also supports extensive farming activities (cropping), as well as gold and coal mining, and therefore populations may be exposed to inorganic contaminants. *Cordylus giganteus* is considered to be threatened and has been listed as vulnerable in the IUCN Red List of Threatened Species (see http://www.redlist.org). Until now, the main recorded threats to this species have been direct loss of habitat through agriculture and industrial development, as well as the illegal collecting of these animals for the pet and "muti" (traditional medicine) trades. However, large areas of Gauteng and the Free State Provinces (the latter is where most *C. giganteus* populations occur) have been reportedly affected by contaminated dust, seepage and groundwater emanating from gold mining activities (Coetzee 1995; Rosner & van Schalkwyk 2000; Weiersbye *et al.* 2003). These contaminants may provide an additional threat to populations of *C. giganteus* if the lizards are negatively affected by exposure to these contaminants.

Sungazer lizards are unique among cordylids because they live in self-excavated burrows, normally in soil types that become very hard when dry (Branch 1998). Burrows are normally found in undulating grassland, dominated by *Themeda triandra* (Jacobsen *et al.* 1990; Ruddock 2000). *Cordylus giganteus* are partially opportunistic sit-and-wait predators, preferring beetles (van Wyk 2000). Their position in the food chain, and their tendency to high site fidelity (McIntyre *pers. obs.*), make these reptiles ideal for use in ecotoxicological investigations, especially when repeated sampling of the same individuals are an advantage.

I focused on the population health and conservation of *C. giganteus* in and around the Welkom area of the Free State Province, South Africa, an area of intensive mining. My aim was to investigate the possible uptake of inorganic contaminants by *C. giganteus* living in the mining area. For comparison, I had a control site away from mining activity. First, I asked whether lizards in mining areas contained significant levels of the inorganic contaminants known to be elevated on the study sites, and second, whether they could be accumulating these through dietary intake. Third, I asked whether *C. giganteus* from mining areas were in poorer body condition compared to the control site. Finally, I tested whether the sex ratios of the *C. giganteus* populations from the mining areas were significantly different to the other populations under investigation.

MATERIALS AND METHODS

Study area

I sampled four sites in the Free State Province (Fig. 1.1). Mining 1 and Mining 2 are contaminated sites, affected by mining effluents. Overgrazed Rangeland is not known to be a contaminated site, but is in the vicinity of mining areas. Thus, the possibility exists that this site is affected by wind-blown contaminants from nearby mining operations. The only known disturbance to this site, however, appears to be severe overgrazing. Undisturbed Rangeland is an undisturbed site, unaffected by any mining-related contaminants.

Mining 1 (Evaporation pan site)

This site (27°56’S; 26°33’E) containing a natural saltpan, is in the Welkom area of the Free State Province. The pan has been used over a period of 40 years for the legally licensed discharge and evaporation of gold mine process water and, to a lesser extent, purified sewage effluent (Weiersbye *et al.* 2003). The pan water and sediment, as well as the surrounding soils, were contaminated with a variety of elements. These elements are associated with acid mine drainage and mine process water discharge, and included sulphur (S), chloride (Cl), sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), aluminium (Al), manganese (Mn), strontium (Sr), titanium (Ti), zinc (Zn), chromium (Cr), cobalt (Co), nickel (Ni), uranium (U), gold (Au), mercury (Hg), copper (Cu), arsenic (As), lithium (Li), vanadium (V), yttrium (Y) and selenium (Se). Total dissolved solid concentrations were also reported to be significantly higher than other saltpans in the area (Weiersbye *et al.* 2003).

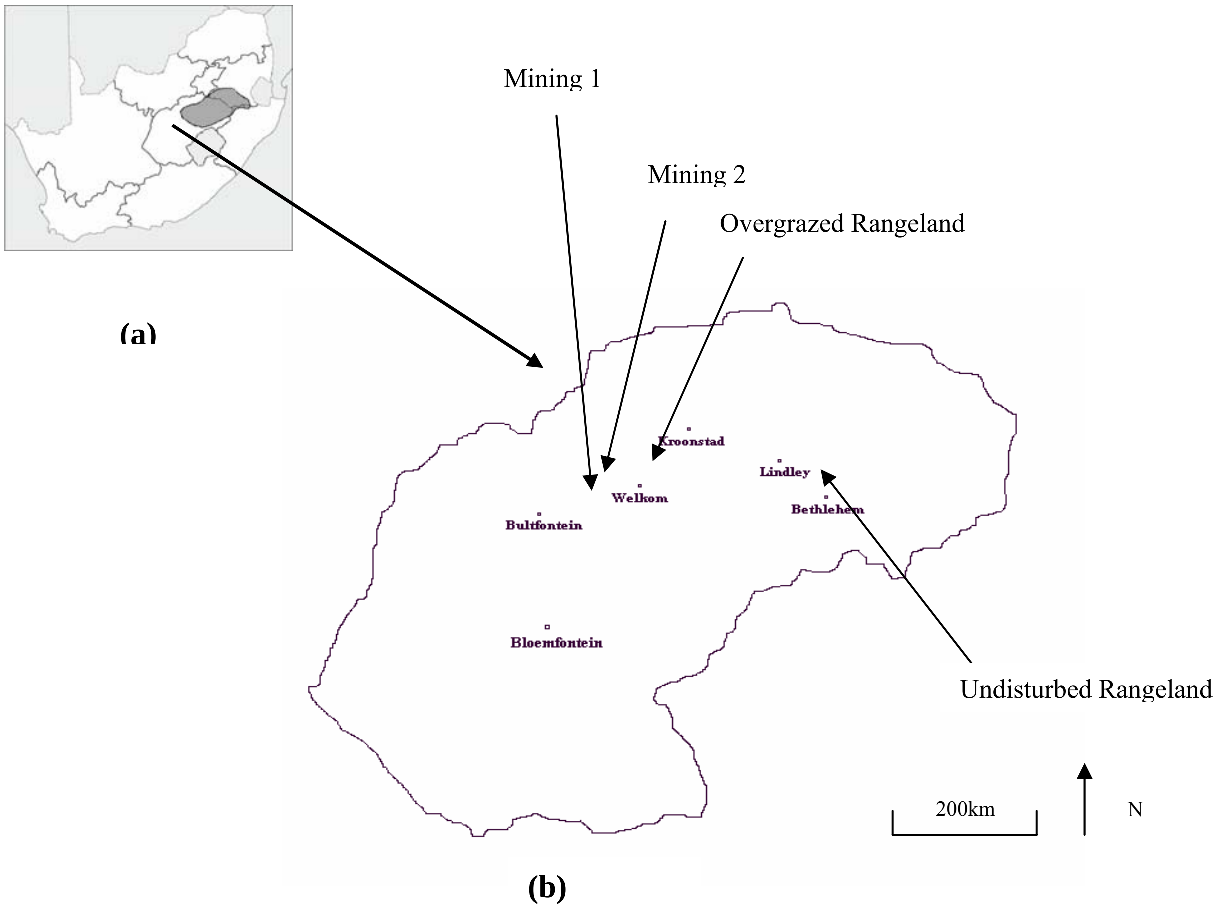

Figure 1.1: (a) Map of South Africa with provincial boundaries, showing the geographical distribution of *C. giganteus* (dark shading) based on de Waal (1978), Jacobsen *et al*. (1990) and Ruddock (2000). (b) Enlarged map of the Free State Province, showing the approximate locality of the four study sites.

A population of *C. giganteus* occurs in close proximity to the evaporation pan itself (within a 500 m radius). Anecdotal reports have speculated that this population has been in decline over the past few years (J. Hardy, Directorate of Nature Conservation, DTEEA, *pers. comm.*). The potential therefore exists that this population of *C. giganteus* are negatively affected by the exposure to an obviously contaminated environment. Potential pathways of exposure include direct contact through the soil in which these lizards live, as well as exposure through diet (invertebrates).

Mining 2 (Slimes dam site)

The second *C. giganteus* population potentially affected by mining related contaminants occurs on a farm (27°56'S; 26°40'E) that received acid mine drainage from the adjacent slimes dams. This farm (Slimes dam site) is roughly 10 km from the Evaporation pan site and the surface water streams and soils are contaminated by seepage from adjacent slimes dams (I.M. Weiersbye *pers. comm.*). Only a small population of *C. giganteus* appears to be persisting on this site.

Overgrazed Rangeland site

Overgrazed Rangeland is a severely overgrazed game ranch site situated about 15 km northeast of Welkom (27°52'S; 26°47'E). No mining activity has taken place in its immediate vicinity and it is believed to be largely free of any contaminants associated with mining. Nevertheless, pollution from gold mining has been shown to spread far beyond the actual mining footprint, and the possibility exists that this site may also have been affected in the form of contaminated dust (Weiersbye & Witkowski. 2003). This site presented the opportunity to investigate the potential of lizards being affected by wind-blown contaminants and the potential physiological costs associated with less vegetation cover (and an expected loss of prey availability).

Undisturbed Rangeland (Control) site

The control site, Undisturbed Rangeland, is situated near Lindley in the Free State Province (28°01'S; 28°05'E). The population of lizards on this farm has been fenced off and protected from most anthropogenic influences. The site is free of cattle grazing and the grassland around this population is undisturbed. This population thus served as a control, since no anthropogenic disturbance was expected to affect it. Unfortunately, no populations of *C. giganteus* in closer vicinity to the previous three sites in the Welkom area were considered to be sufficiently undisturbed for use in the study as a control population.

Climate can be expected to influence the amount of prey available to lizards (and hence the physiological condition of lizards). Thus, I obtained climate data for the two previous years (2003 and 2004) from the South African Weather Service. These data, along with the other main characteristics of each site is reported in Table 1.1.

Table 1.1: Main characteristics of sites where I investigated the accumulation and effects of mining contaminants and disturbance on populations of *C. giganteus*. Two types of disturbance effects are identified (mining emissions and overgrazing). X indicates sites affected by one or both of these disturbances.

Site	Total rainfall (mm) 2003-4	Average monthly rainfall (±SD) for 2003-4	Mining emissions	Over-grazing
Mining 1 (Evaporation pan)	474.6	19.78 ± 23.99	X	
Mining 2 (Slimes dam)	474.6	19.78 ± 23.99	X	
Overgrazed Rangeland	474.6	19.78 ± 23.99		X
Undisturbed Rangeland (Control)	892.7	42.80 ± 53.66		

Study animal

Cordylus giganteus is the largest member of the Cordylidae, with adults reaching total lengths of almost 400 mm (Bates 1992; van Wyk 1992). The Cordylidae is the only lizard family restricted to the African continent and many of its members are endemic to the southern African sub region (Branch 1998). *Cordylus giganteus* are endemic to South Africa and restricted to the highveld grasslands of the Free State and Mpumalanga provinces (Fig. 1.1). This also happens to be an area impacted by gold and coal mining, as well as agriculture (grazing and cropping of maize and sunflowers). Females reproduce on average only once every second year, giving live birth to two or three young in late summer and autumn (van Wyk 1991, 1992). Van Wyk (2000) found that *C. giganteus* fed during eight months of the year, with foraging success peaking during warm spring and early summer months. He considered them to be partially opportunistic insectivores, preferring beetles, especially scarabaeids, curculionids and tenebrionids.

The listing of *C. giganteus* as vulnerable by the IUCN has been criticised in the past for not being well substantiated and apparently being based on charisma and unsubstantiated claims of over-exploitation for the pet trade (Branch 2001). The listing has, however, not changed recently, mainly because the population viability of this species has not been investigated. In addition, there is little information on the impact of changing land-use practices on the status of *C. giganteus*. Thus, the current conservation status of the lizard is simply not known. However, it is likely to be affected by the following parameters: (1) habitat destruction (van Wyk 1992; Jacobsen *et al.* 1990); (2) disturbance and contamination from mining (Weiersbye *et al.* 2003); and (3) illegal collecting for the pet and muti trade (Newbery *pers. comm.*).

Investigating potential accumulation of inorganic contaminants by *C. giganteus*

Lizard sampling

I obtained tissue and blood samples from adult lizards from Mining 1 and Overgrazed Rangeland during February 2004, as well as September-October 2004. Lizards from Mining 2 were sampled only during February 2004 and the Control population was sampled only during September/October 2004. I permanently marked individual lizards using passive integrated transponders (PIT tags; Identipet), with a unique alpha-numeric code. These tags were injected subcutaneously into the postero-femoral region of the hind legs. This is a widely used method for marking lizards and other animals that has previously been evaluated (Keck 1994; Perret & Joly

2002; Gibbons & Andrews 2004). This marking will also aid in future monitoring attempts of these populations.

I weighed lizards to the nearest 0.1 g on a digital scale, measured snout-vent length (SVL) and tail length to the nearest 1 mm with a ruler and head length, width and height to the nearest 0.1 mm using digital callipers. I sexed lizards by checking for the presence of generation glands in the femoral and forearm regions. These glands are only present in males (van Wyk 1992). I took a blood and/or tissue sample (see below), before releasing lizards at the point of capture within one day.

Lizard contaminant accumulation

Because *C. giganteus* is a species of conservation concern, I used non-lethal techniques for obtaining tissue and blood samples. The levels of elements in blood, tissue and urine are known to differ due to the half-life of each contaminant, and the affinity of particular cell types for different ions. Blood sampling holds obvious advantages over tissue sampling, being less invasive and repeatable on the same individual over time. However, tissue samples from tail clippings in reptiles may be more reliable than blood in documenting the accumulation of contaminants, due to the composite of blood, skin, bone and tissue found in tail clippings (Jackson *et al.* 2003). Thus, I obtained tissue and blood samples in order to ensure accumulated contaminants were detected and for comparative purposes between tail tissue and blood. I removed small amounts of tissue (5-10 mm) from the tips of *C. giganteus* tails (Hopkins *et al*. 2001; Jackson *et al.* 2003) for analysis of inorganic contaminants. I also obtained a 150 µl blood sample from the suborbital sinus of each lizard, using two 75 µl heparinised micro-capillary tubes. This procedure is a standard method for obtaining blood from free-ranging lizards (Mautz & Nagy 2000; Znari & Nagy 1997; Nagy 1993). Each blood sample was stored in polypropylene containers, centrifuged for seven minutes at 3000 rpm and the plasma removed using a micropipette. After removal, the plasma and red blood cells were frozen and returned to the laboratory for further analysis.

Inorganic concentrations were quantified for the blood and tissue samples in the laboratory. I cleaned all tail tissue and invertebrate samples (not red blood or plasma samples) thoroughly with toluene before analysis, to remove external soil and vegetative particles. All samples analyzed were dried and then underwent closed microwave digestion in aqua regia (55% solution of nitric acid, HNO_3 and 32% solution of Hydrochloric acid, HCl), using a Multiwave 3000 microwave (Anton Paar).

The digest solutions were cooled to room temperature, and then made up to volume in 50 ml graduated PVP volumetric flasks using double-distilled water (Milipore). The concentrations of 21 elements were analyzed in triplicate using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) on a Ciros-CCD analyzer (Spectro). Plasma power was set at 1300W; coolant flow at 13 l/min; auxiliary flow at 1 l/min; and nebulizer flow at 1 l/min.

By comparing elemental concentrations between lizards from the mining affected sites and lizards from areas not affected by mining activity (Rangeland 1 and Rangeland 2: Control) populations, I would be able to detect metal uptake in lizards from mining contaminated areas.

Dietary exposure of *C. giganteus* to inorganic contaminants

Because *C. giganteus* are insectivorous, an obvious pathway to exposure is through diet, i.e. invertebrates that feed on detrital or plant material that has acquired contaminants via the soil-water pathway, or predatory invertebrates. A previous study reported on the seasonal dietary composition of *C. giganteus* in some detail (Van Wyk, 2000). Accordingly, I hand-collected as many potential prey items as possible during the lizard sampling period. These invertebrates were then assessed for elemental contents. I froze all the invertebrates I collected before removing their abdomens (and thus abdominal contents), and drying the remaining tissues. The removal of the abdominal contents was done in accordance with standard practice in investigations involving accumulation of contaminants in actual tissues of food chain representatives (Sandoval *et al.* 2001). The remaining invertebrate samples (entire organisms minus abdominal contents) were then cleaned in toluene to remove the antifreeze used in the pitfall traps (see below), as well as dissolve surface cuticles and embedded dust or vegetation particles. Concentrations of inorganics were also quantified using ICP-OES after microwave digestion in aqua regia. By determining the concentrations of inorganics in the invertebrates found in the area, I investigated the potential pathway of contaminant transport between the two trophic levels represented by *C. giganteus* and the invertebrates respectively.

Potential effects of inorganic contaminants

Lizard body condition

I performed a regression of body weight (log-transformed) on SVL (log-transformed) to obtain an index of body condition (Anderholm *et al.* 2004; Cuadrada 1998; Dunlap & Mathies 2003; Jakob *et al.* 1996), for comparison among sites. Since lizards were sampled from the control site only during the final sampling period, I restricted the comparison only to adult lizards caught during this period, upon first capture, from all sites. Mining 2 was excluded from analysis due to small sample sizes.

Invertebrate abundance

Because food availability directly impacts body condition, I measured prey abundance at the three main sites under investigation [Mining 1, Overgrazed Rangeland and Undisturbed Rangeland (Control)]. Since lizards from Mining 2 were not used in comparisons of body condition due to a small sample size (n = 7), invertebrate abundance was not measured here. I used a nested-cross array of pitfall traps (Perner & Schueler, 2004) to sample invertebrate abundance. Accordingly, I buried 25 unbaited plastic bowls (diameter = 200 mm; depth 150 mm) with their open ends flush with the ground.

Individual traps were set out in a cross form, with distances of traps from the centre in increasing order: 0.5, 3, 6, 12, 24, and 48 m. Each trap was covered with a metal grid consisting of squares small enough to prevent the entry of any small vertebrates. Small amounts of automotive antifreeze were used to kill and preserve all invertebrates caught in the traps. I set 25 traps at each site, for a period of four weeks during summer. I checked each trap once a week and collected and preserved all trapped insects for later identification in the lab. All insects were sorted, identified to the lowest taxon possible (mostly family) and counted.

Grass cover

Sungazer lizards have been thought to be very sensitive to changes in general habitat characteristics including changes to vegetation type (Jacobsen *et al.* 1990). Changes in vegetation cover can be expected to influence the amount of prey available to the lizards, and thus indirectly, the body condition of lizards. Such vegetation cover changes can also be expected to influence lizard populations in other ways. For example, thermoregulatory behaviour of lizards may be influenced since less vegetation cover may provide more basking opportunity (a potential trade-off may be found in the potential increase of exposure to aerial predators). I therefore estimated the percentage of vegetation cover for each site under investigation. I did this by visually estimating the percentage of ground covered by vegetation (aerial cover) in ten 1 x 1 m quadrants for each site. An overhead digital photograph was also taken at head height over each quadrant in order to obtain a visual archival record.

Lizard population sex ratios

I tested for a significant departure from a 1:1 sex ratio using chi-square tests. Since the sample size for Mining 2 was considered too small (n = 7), I only tested the three remaining populations (Mining 1, Overgrazed Rangeland and Undisturbed Rangeland).

Data analysis

Statistical analyses were performed with Statistica (Version 6.1, StatSoft Inc.). Data are reported as means ± standard deviations (SD). Statistical significance was set at $\alpha = 0.05$.

RESULTS

I captured a total of 198 individual lizards (Mining 1 = 86; Mining 2 = 7; Overgrazed Rangeland = 73; Undisturbed Rangeland = 32).

Lizard elemental concentrations: Tail and blood elemental concentrations between sexes

I initially compared concentrations of 19 elements found in whole blood and tissue between lizard sexes (n = 12 males, 12 females). Two elements (Hg and Pb) were below minimum detection limits for most of the samples analyzed. None of the elements occurred in significantly different concentrations ($P = 0.05$) between sexes (see Appendix 1, Table A1.1.). Because no differences were observed in elemental concentrations between sexes, I subsequently used samples from both sexes for all further comparisons between tissue types and populations.

Lizard elemental concentrations: Tail elemental concentrations between sites

Elemental concentrations found in tail tissue from lizards did not differ significantly between sites (see Appendix, Table A1.2). Measured elemental concentrations for lizard tail tissue are reported in Table A1.3 (see Appendix). Lead and mercury concentrations were largely below minimum detection limits for ICP-OES in the tail samples.

Lizard elemental concentrations: Whole blood elemental concentrations between sites

Significant differences between sites were observed for concentrations of the following elements in *C. giganteus* whole blood samples: Li, Na, Al, S, P, Si, Ca, Cr, Mn, Fe, Ni, Cu, W and Bi (Table 1.2; Fig. 1.2 – Fig 1.5). Lead and mercury concentrations were largely below minimum detection limits for ICP-OES in the whole blood samples. Samples from Mining 1 had significantly higher concentrations of Li, Na, Al, S, Ca, P, Si, Cr, Mn, Fe, Ni, W and Bi, when compared with all the other investigated sites. Cu concentrations were only significantly higher in individuals from Mining 1 when compared with individuals from Undisturbed Rangeland (Control). Tukey test results, confirming the above mentioned differences, are reported in Appendix (Table A1.4).

Table 1.2: Mean concentrations (μg/g dry mass) ± standard deviations (SD) and one-way analysis of variance (ANOVA) results comparing *C. giganteus* whole blood elemental concentrations between sites. Cu concentrations were log-transformed before analysis to meet the normal distribution requirements (Kolmogorov-Smirnov *D* = 0.35; df = 3; *P* = <0.05). All significant *P*-values are highlighted.

	Mining 1 (n = 3)		**Undisturbed Rangeland (n = 4)**		**Mining 2 (n = 4)**		**Overgrazed Rangeland (n = 4)**		**ANOVA**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	**df**	***P***
Li	8.4	0.9	2.5	0.4	2.9	1.2	3.2	1.5	20.99	3	**<0.001**
Na	1441	224.2	647.7	126.7	488.4	255.4	571.2	231.1	13.63	3	**<0.001**
Mg	247.1	74.8	118.4	42.1	143.4	107.6	128.7	60	2.01	3	0.17
Al	62	11.7	19.3	1.8	21.6	11.8	21.4	7.9	16.92	3	**<0.001**
Si	24	3.4	7	0.9	8.1	3.8	8.5	3.6	21.43	3	**<0.001**
P	22.4	2.8	6.3	1.1	7	3	7.8	3.3	26.07	3	**<0.001**
S	6276.3	418.4	1995.3	786.4	2782.4	1836.3	2620.8	1157.9	8.13	3	**<0.005**
K	3165.1	1092.9	1147.4	726.5	1932.2	1490.4	1733.4	712.9	2.16	3	0.15
Ca	266.3	34.9	136.6	56.3	154.8	88.3	87.4	32.5	5.41	3	**0.016**
Cr	137.4	15.2	40.6	9.2	44.7	19.6	50.1	22	22.78	3	**<0.001**
Mn	13.4	1.6	4	1	3.4	1.4	5.2	2.3	25.8	3	**<0.001**
Fe	1677.1	181.9	450.4	170.2	786.5	431.4	725.6	344.4	9.51	3	**0.002**
Ni	214	64.9	31.6	5.4	65.6	47.8	46.9	19.4	15.22	3	**<0.001**
Cu	87.9	43	6.2	1.6	34	52.3	12.6	5	6.250	3	**0.010**
Zn	20.3	8.1	7.6	1.6	12.8	11.5	7.7	4.7	2.15	3	0.151
W	82.3	6.8	23.9	6.9	27	8.9	28	10	35.76	3	**<0.001**
Bi	24	4.1	7.1	1.6	8	3.4	8.3	3.6	20.41	3	**<0.001**

Invertebrate elemental concentrations

Sample sizes allowed only for comparisons of elemental concentrations found in Tenebrionidae (beetles) between areas of different land use (areas known to be affected by mining pollutants vs. rangeland). Concentrations of most elements for Tenebrionidae did not differ significantly between areas of different land use. Ni and W concentrations were, however, significantly higher in beetles from mining areas, when compared with rangelands. Detailed results for the comparisons of all other elemental concentrations of beetles between land uses are reported in Appendix, Table A1.5.

Site effects on *C. giganteus*

Body condition

All the residual values from all sampled adult lizards were used in an initial t-test to quantify any potential differences in condition between sexes. I found no significant difference in body condition between sexes (t = -0.88; df = 111; P = 0.379) (see Appendix 1, Fig A1.1). For this reason, all adults were combined for subsequent comparisons of body condition between sites. Residual values were significantly different between sites (ANOVA F = 30.24; df = 2; P = <0.001; Fig. 1.2). A post-hoc Tukey test showed that lizards from Mining 1 and Overgrazed Rangeland were in significantly poorer condition than lizards from the Undisturbed Rangeland (Control) population. I did not detect any significant differences in condition between lizards from Mining 1 and Overgrazed Rangeland.

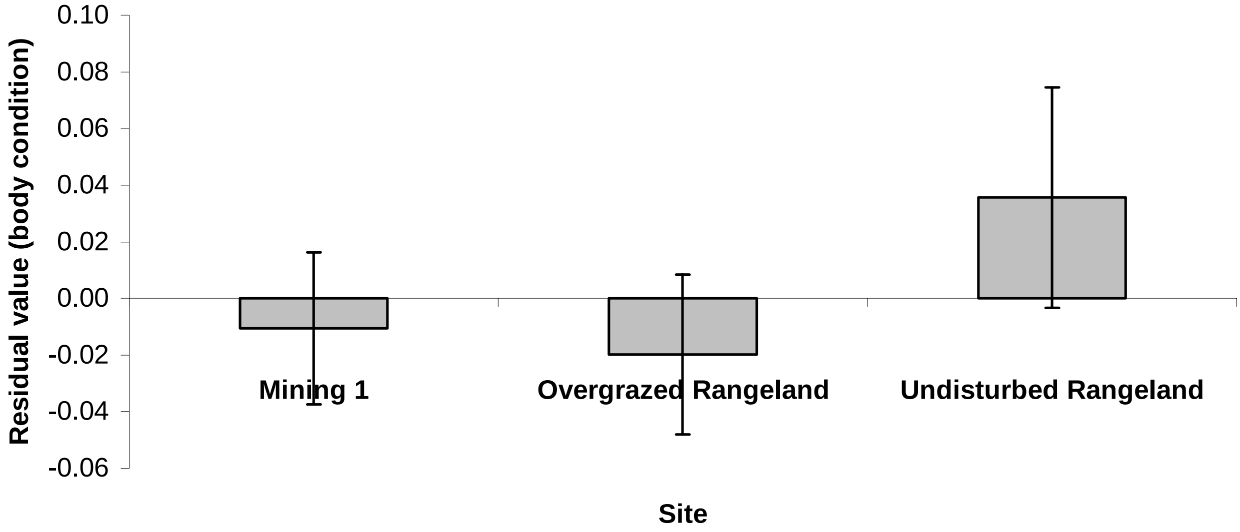

Figure 1.2: Plot of mean residual values (body condition), obtained after regressing log-transformed body weight over log-transformed SVL of *C. giganteus* from three localities. Mean (± SD) values are reported.

Prey availability

Total numbers of invertebrates sampled from the relevant orders and families are reported in Table A1.6 (see Appendix) and were used as an index of prey availability. Total numbers of relevant invertebrates sampled were significantly different between sites (χ^2 = 29.589; df = 2; P = <0.001). Invertebrate abundance was highest at Mining 1. Overgrazed Rangeland and Undisturbed Rangeland (Control) had similar abundances, but showed significantly less insect abundance than Mining 1 (Fig. 1.3).

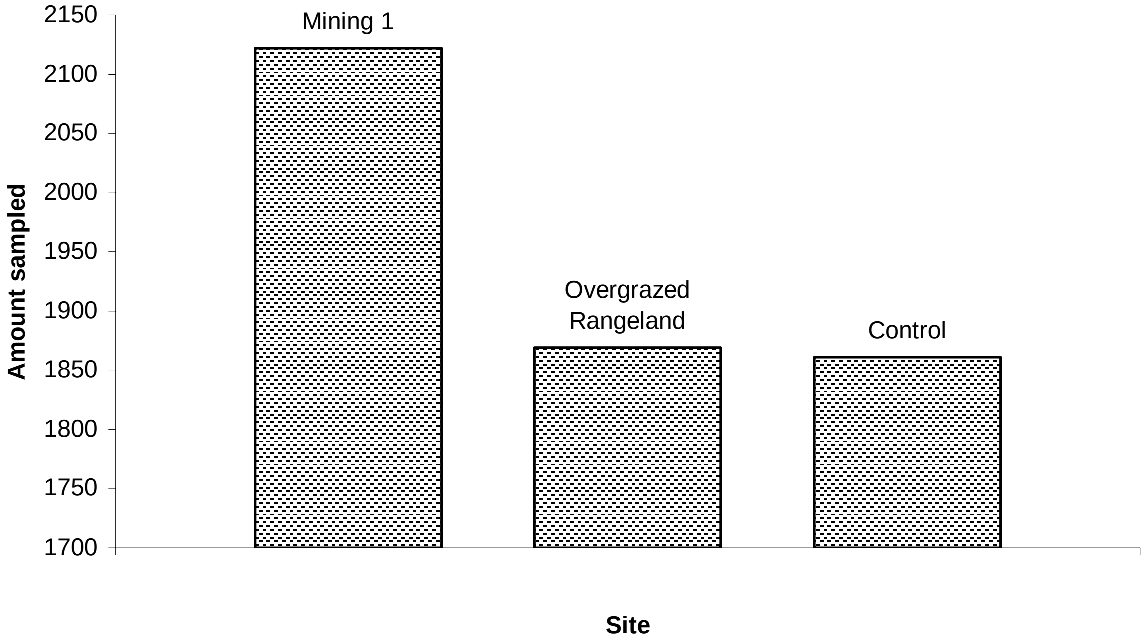

Figure 1.3: Total numbers of prey items (invertebrates) sampled at three of the sites. These invertebrates were sampled during a four-week period from September to October 2004.

Comparing grass cover between sites

Significant differences exist in aerial vegetation cover between sites ($\chi^2 = 60.91$; df = 2; *P*-value = <0.001). Undisturbed Rangeland (Control) had the highest percentage aerial cover (90%), followed by Mining 1 (65%), then Overgrazed Rangeland (10%). Vegetation consisted primarily of grasses at all sites.

Population sex ratios

Neither the Overgrazed Rangeland population nor the Undisturbed Rangeland Control population deviated significantly from a 1:1 sex ratio (Overgrazed Rangeland: 32 males: 41 females; $\chi^2 = 0.555$; df = 1; $P = 0.758$) (Control: 14 males: 18 females; $\chi^2 = 0.25$; df = 1; $P = 0.882$). The observed sex ratio from the Mining 1 population did, however, differ significantly from an expected 1:1 ratio, in favour of females (25 males, 61 females; $\chi^2 = 7.535$; df = 1; $P = 0.023$).

DISCUSSION

Elemental concentrations in *Cordylus giganteus* tissue

Cordylus giganteus accumulates various inorganic contaminants in areas affected by mining effluents in the Free State Province. Lizards from Mining 1 (Evaporation Pan) showed elevated concentrations of the following elements in whole blood samples: Li, Na, Al, S; Ca, P, Si, Cr, Mn, Fe, Ni, W and Bi. These concentrations could rarely be compared with other reported values for metal concentrations, especially in reptiles, since very few studies have measured heavy metal accumulations in this group. A notable exception is Hopkins *et al.* (2002), who studied the accumulation of a number of metals by banded water snakes (*Nerodia fasciata*) in organs including gonads, kidneys and liver. They found high concentrations of As, S, Cd, Sr, Cu and V. All reported concentrations of Cu by Hopkins *et al.* (2002) were substantially lower than those reported here. The highest values reported for Cu in water snakes were less than 60 μg/g dry mass, found in the gonads of the snakes fed two years on a contaminated diet with elevated concentrations of Cu. Van Eeden (2003) found Cu concentrations of 14.4 μg/g dry mass in blood samples obtained from red-knobbed coot (*Fulica cristata*) occurring around some contaminated water sources in the Gauteng Province, South Africa. In contrast, I found mean Cu concentrations of 87.9 μg/g dry mass for *C. giganteus*, measured in blood samples obtained from lizards in the Evaporation Pan area.

Other metal concentrations found in blood samples of sungazer lizards from Mining 1 are also relatively high when compared with metal concentrations found in various organs of red-knobbed coot (*Fulica cristata*), collected from a contaminated water source in South Africa. Van Eeden & Schoonbee (1992) reported mean Ni concentrations of 40.9 μg/g dry mass in red-knobbed coot organs. The mean Ni concentration in sungazer lizard blood samples from Mining 1 was 214 μg/g dry mass. Also, Van Eeden & Schoonbee (1992) reported mean Cr concentrations of 8.9 μg/g dry mass for the same bird species. This value is also much lower than the mean concentration of 137.4 µg/g dry mass for *C. giganteus* blood samples from Mining 1. Fe and Mn concentrations compared more favourably between the two investigations. Coot organs were reported to have mean concentrations of 1566 µg/g dry mass and 45.2 µg/g dry mass for the two metals respectively. My investigation showed mean Fe and Mn concentrations of 1677.1 µg/g dry mass and 13.4 µg/g dry mass respectively for blood samples obtained from *C. giganteus* in the Mining 1 area.

Besides the above-mentioned metals, lizards from Mining 1 also had higher mean concentrations of Li, Na, Al, S, P, Si, W and Bi. Some of the elements which I found to be enriched in lizard blood from Mining 1, had also previously been reported as being elevated in the water or sediments of the salt pan used for evaporation of mine water on the Mining 1 site, i.e. Li, Na, Al, Cr, Mn, Fe and Ni (Weiersbye *et al.,* 2003). While I did not measure metal accumulation in the organs (i.e., liver, kidneys and gonads), the high metal concentrations in blood could indicate that these organs might also have high concentrations of heavy metals.

Tissue elemental concentrations in males and females

I found no significant differences in concentrations of all the elements investigated between sexes of *C. giganteus*. In contrast, Burger *et al.* (2004) documented differences in metal concentrations measured between sexes of *Anolis sagrei*. They discovered generally higher concentrations of most metals measured in females. These differences were attributed to differences in diet due to microhabitat differences in foraging locations (females tending to feed closer to the ground than males, which tend to feed on tree trunks and branches). The similar elemental concentrations for male and female *C. giganteus* is not surprising, since no known feeding or other behavioural differences exist between sexes of this lizard species (van Wyk 1992). It is thus expected that lizards from both sexes would be exposed to the same environmental contaminants, if exposure is dietary related, or through direct contact with contaminated soil.

Dietary exposure of *C. giganteus* to inorganic contaminants

Potential pathways of exposure to inorganic contaminants by *C. giganteus* include exposure through diet (i.e. the ingestion of contaminated prey items), as well as through direct contact with contaminants found in the soils inhabited by lizards. Most of the controlled ecotoxicological experiments on reptiles have focused on the obvious potential accumulation of contaminants through diet (see Hopkins *et al.* 2001; 2002; 2005). I only found significantly elevated concentrations of Ni and W in beetles from areas affected by mining contaminants (Mining 1 and Mining 2). The lack of elevated concentrations of the other elements analyzed (particularly Li, Na, Al, S; Ca, P, Si, Cr, Mn, Fe, and Bi) in beetles from mining affected areas does not exclude diet as a pathway of exposure for these elements. Rather, this may indicate that either the sampling was inadequate, or that the subsets of invertebrates analyzed for elemental content in this study are not accumulating significant concentrations of any of the inorganic contaminants investigated. Based on other studies (e.g. Hopkins *et al.* 2001; 2002; 2005), it is likely that *C. giganteus* may be exposed to inorganic contaminants through diet, and this aspect requires further investigation before diet can be excluded as a pathway of potential exposure.

It is possible that the lizards on Mining 1 (Evaporation Pan) and other areas affected by mining contaminants also accumulate inorganic contaminants through direct exposure. Van Wyk (2000) reported finding substantial amounts of soil and grass in the stomach contents of *C. giganteus*. It is thus possible that lizards accumulate contaminants through the accidental ingestion of soil and vegetation, during prey capture. Also, gut contents of the prey items (which were removed before measuring elemental concentrations in invertebrates) can be high in inorganic contaminants and the lizards would ingest these. I washed the insects and removed gut contents in accordance with best practise for food chain studies requiring knowledge of metal accumulation contents in actual invertebrate tissues. However, this probably resulted in an underestimation of the actual amount of total elements ingested by lizards (i.e. as soil contamination on prey items, and via gut contents). Direct exposure could also potentially include the direct absorption of any of these contaminants through the skins of lizards, although this is an unlikely pathway of exposure.

Physiological costs of exposure to inorganic contaminants

The effects of inorganic contaminants on reptiles are very poorly understood. In fact, no adult reptile mortality due to metal intoxication has ever been reported (Linder & Grillitsch 2000). A few studies have documented contaminant concentrations in certain reptiles (mostly turtles and alligators), but not the actual biological effects associated with high concentrations of such contaminants (Hopkins *et al.* 1999). A notable exception is Hopkins *et al.* (2002), who reported that high concentrations of six elements (As, Cd, Cu, Se, Sr and V), accumulated through contaminated diet, did not negatively affect food consumption, growth, condition factor, overwinter survival and mass loss, metabolic rate as well as gonadosomatic indices in banded water snakes (*Nerodia fasciata*). However, Brasfield *et al.* (2004) found acute mortality in fence lizard (*Sceloporus undulates*) eggs that had been exposed to high concentrations of Cd.

Cordylus giganteus lizards from Undisturbed Rangelands were in a significantly better condition than lizards sampled from Overgrazed Rangeland and Mining 1. The amount of prey items available to lizards at these sites may have influenced the condition of lizards. However, I found that the Mining 1 site had the highest prey availability and significantly more prey items than the other two sites (Overgrazed Rangeland and Undisturbed Rangeland). Since the vegetation was extremely sparse and the amount of grass cover very low (~10%) at the Overgrazed Rangeland site, it is not surprising that invertebrate abundance was lower at this site. In contrast, the Undisturbed Rangeland site had a very high percentage of grass cover (~90%), yet low prey availability. This may have been the result of some cold weather experienced during the invertebrate sampling period for this area, reducing invertebrate activity. Since my investigation did not reveal any inorganic contaminant accumulation in these lizards and no environmental pollution has been reported for the immediate area around the Overgrazed Rangeland site, it is unlikely that mining related environmental contamination affects the condition of these lizards. Rather, low prey availability is the most likely explanation of the relatively poor body condition of lizards on this site.

Lizards from Mining 1 have been shown to accumulate very high concentrations of inorganic contaminants when compared with lizards from all other sites. These include Li, Na, Al, S, Ca, P, Si, Cr, Mn, Fe, Ni, W and B in blood. Also, Cu concentrations measured in lizards from Mining 1 were significantly higher than the concentrations measured from populations unaffected by mining contaminants. Lizards from this site are also in comparatively poor condition (similar in condition to lizards from Overgrazed Rangeland). Since prey availability is significantly higher on this site than the other study sites, it is unlikely that a shortage of prey is affecting the condition of lizards on this site. A potential cause for the lower body condition of lizards at the Mining 1 (Evaporation Pan) would appear to be the high concentrations of inorganic contaminants they are exposed to (and are accumulating in blood). However, a direct link between the contaminant accumulation and the loss of physiological condition has not been demonstrated and it is possible that other environmental variables may be affecting the condition of lizards from this site.

Potential inorganic contaminant effects on populations

Mining 1 had significantly fewer male lizards when compared with the other two sites (29% male, compared with 44% for the other sites). Van Wyk (1992) and Ruddock (2000) did not report any significant gender based differences for feeding behaviour in these lizards that could potentially lead to higher levels of contaminant accumulation by either of the sexes. Furthermore, no significant differences were observed when concentrations of the various metals in blood and tail tissue were compared between sexes. Some studies have provided evidence that inorganic contaminants may affect individuals of a specific sex more than the other (see Burger *et al.* 2004 and Larison *et al.* 2000). While my study does not provide conclusive evidence, it does suggest the possibility that the lizard exposure to inorganic contaminants at Mining 1 has influenced the population sex ratio at that site. The lack of difference in elemental concentrations between sexes of this lizard, however, weakens this argument and further investigations into the potential endocrine disrupting influence of the inorganic contaminants need to be undertaken.

CONCLUSION

The results of my study indicate that inorganic contaminants emanating from gold mining effluents, as well as over-grazing, may have a negative impact on populations of *C. giganteus* in the Welkom area of the Free State Province. Lizards from a mining contaminated site have accumulated Li, Na, Al, S, Ca, P, Si, Cr, Mn, Fe, Cu, Ni, W and Bi in blood. The effects of accumulation of these contaminants on lizards are poorly understood, but a potential cost of this, and of overgrazing, could be a reduction of body condition in individual lizards. Also, the skewed sex ratio exhibited by the mining influenced population, indicates a potential population level effect of exposure to inorganic contaminants. Since *C. giganteus* is already considered to be a threatened species, further research into the exact effects of mining related contaminants on this species is critical. Also, the overall conservation status of this species on the highveld needs to be assessed, in order to quantify the threat posed to this species by land disturbance and the gold mine emissions, as well as to facilitate future monitoring of restoration efforts.

CHAPTER 2: CONSERVATION AND MANAGEMENT RECOMMENDATIONS FOR *CORDYLUS GIGANTEUS* IN THE WELKOM AREA OF THE FREE STATE PROVINCE

INTRODUCTION

Reptiles are considered to be important components of terrestrial ecosystems, being integral to many food webs (Lambert 1997). Lizards in particular, are predators and prey of vertebrates, as well as invertebrates. They are thus crucial to the proper functioning of many ecological processes (Campbell & Campbell 2000). For these reasons, it is very important on a community level, to understand the potential effects of contaminants on reptiles. Lizards, especially, are considered to be susceptible to the bioaccumulation of persistent environmental contaminants because they are often secondary and tertiary predators in the systems in which they occur (Bishop & Gendron 1998). Also, in contrast to birds and mammals, lizards have relatively weak dispersal abilities, making them good indicators of localized terrestrial habitat quality (Lambert 1993).

Here, I discuss the potential effects that inorganic contaminants have on populations of threatened sungazer lizards, *Cordylus giganteus*. Potential conservation measures for these populations of lizard are explored. I also report on the estimated population size of *C. giganteus* at Mining 1 (Evaporation Pan) – a preliminary result from a mark-recapture study. Furthermore, I highlight the need for relevant future research to aid in conservation related decisions.

INORGANIC POLLUTANT EFFECTS ON SUNGAZER LIZARDS

It is evident from my investigation that inorganic contaminants emanating from mining operations in the Welkom area of the Free State Province are affecting populations of the threatened lizard, *C. giganteus*. I showed that individuals from the Evaporation pan in particular, are accumulating abnormally high concentrations of Li, Na, Al, S, Ca, P, Si, Cr, Mn, Fe, Ni, Cu, W and Bi (Chapter 1). The potential effects of this accumulation are poorly understood. Nevertheless, exposures to high concentrations of some of these elements have been shown to have severe negative effects on other vertebrates. For example, rats have exhibited anaemia from exposure to high Al concentrations through drinking water (Farina *et al.* 2005). This included decreased red blood cells, blood haemoglobin and hematocrit. Unfortunately, no blood Al levels were reported in this study. Lizards from the Evaporation pan had far higher blood concentrations of Al (62 µg/g dry mass) when compared with lizards from any of the other populations sampled (Undisturbed Rangeland = 19.3 µg/g dry mass; Slimes dam = 21.6 µg/g dry mass; Overgrazed Rangeland = 21.4 µg/g dry mass).

Elevated concentrations of Cu have also been shown to have deleterious effects on vertebrates. Gaetke & Chow (2003) reported liver cirrhoses as well as severe gastrointestinal effects of Cu exposure in humans. Fish also suffered oxidative stress in liver tissue (Sanchez *et al.* 2005). Both the Evaporation pan and Slimes dam lizards (populations affected by mining effluents) had abnormally high blood concentrations of Cu, albeit the latter were still much lower than the former. The Evaporation Pan lizards displayed mean concentrations of 87.9 µg/g dry mass of Cu (compared with the control site concentrations of 6.2 µg/g dry mass).

Muralidhara (2005) reported oxidative stress in the testes of mice exposed to high concentrations of Fe. Evaporation pan lizards showed significantly elevated concentrations of Fe in blood samples (1677.1 µg/g dry mass, compared to control population concentrations of 450.4 µg/g dry mass). Exposures to high concentrations of Cr have also been shown to have definite negative effects on vertebrates. Elbetieha & Al-Hamood (1997) reported that mice displayed lowered fertility and reproductive potential when exposed to high Cr concentrations.

Evaporation pan lizards had the highest blood concentrations of this metal as well (137.4µg/g dry mass, compared to control concentrations of 40.6 µg/g dry mass).

While the physiological effects of exposure to inorganic contaminants are not fully understood, I have also demonstrated that lizards from the contaminated site, as well as the overgrazed site, are in poorer condition than lizards from a control population. Furthermore, the adult sex ratio of the Evaporation Pan population was significantly skewed towards females. This may be the result of male lizards being more vulnerable to negative effects associated with the high levels of inorganic contamination. The means whereby individuals are exposed to the contaminants at Evaporation Pan has not been identified conclusively, but is likely to be through (1) prey ingestion, (2) direct contact and/or (3) indirect ingestion of contaminated soil. Although the invertebrates analyzed from the Evaporation Pan did not contain abnormally high inorganic concentrations (Chapter 1), I excluded the possibility of surface contamination and gut contents, both of which would increase the elemental load consumed by *C. giganteus*. I also did not assess the amounts of metals excreted in the faeces of *C. giganteus* in order to determine the assimilation efficiencies for the various contaminants.

CONSERVATION MEASURES

Since very little is currently known regarding the overall health of *C. giganteus* populations across the Free State Province, the conservation value of the Mining 1 (Evaporation pan) population cannot be gauged. For the same reason, however, the value of the Evaporation Pan population cannot be underestimated. It is thus important that all possible conservation measures be carefully considered and implemented. These may include:

(1) *Site remediation:*

With much potential for further threat on an already threatened species of reptile, a precautionary approach would involve the removal of as much of the threat posed by inorganic contaminants as possible. Thus, the remediation of the Evaporation Pan site is a priority conservation measure. Whilst the exact manner of remediation is beyond the scope of this investigation, it is worth pointing out that some conventional techniques are not applicable. Excavation, compacting, ripping, grading and drainage of soils, alongside the addition of topsoil and organic wastes are all commonly employed methods of remediation (Wong 2003). These high disturbance techniques are not appropriate for the contaminated areas (two pans and a drainage line), because of the direct threat posed to existing lizard populations that live in burrows on the adjacent grasslands. Rather, the use of pollution control at source to prevent any further discharges, and low-disturbance evaporation and crust removal from the pans and drainage lines, followed by phytoremediation techniques on the pan itself are recommended (Weiersbye & Cukrowska 2005). Evaporation involves the removal of pollutant and salt crusts that form every winter on the pan (evaporation exceeding rainfall by 2.5x in this region), whereas phytoextraction uses tolerant green plants and their associated microbiota to remove and concentrate environmental contaminants (Cunningham & Owe 1996; Wong 2003).

Evaporative transport, crust removal and phytoextraction appear to be the least invasive measures of reducing the concentrations of inorganic contaminants in the soils and groundwater at the Evaporation Pan. Phytoremedition, however, would have to exclude all species known to invade grasslands (indigenous as well as exotic invasives), and have to be carefully implemented on the pollution point sources only (i.e. on and around the base of the slimes dams, and only on the evaporation pans themselves) in order to prevent drastic changes in general habitat (e.g. more woody species in a grassland). *Cordylus giganteus* only occurs in highveld grasslands (Jacobsen *et al.* 1990). Thus, it is important that the choices of suitable vegetation be carefully considered, and strategically planted on the pollutant point sources only, so as not to alter major habitat characteristics. Furthermore, key microhabitat requirements for *C. giganteus* need to be identified in order to ensure that these are not negatively affected by potential rehabilitation techniques.

(2) *Applying a precautionary principle with current conservation tools, specifically translocations*

Many conservation biologists have debated the effectiveness of translocations as a conservation tool for reptiles and amphibians over the past decades (for detailed discussions see Burke 1991; Dodd & Seigel 1991; Griffith *et al.* 1989; Reinert 1991; Seigel & Dodd 1992; Taubes 1992 and Trenham & Marsh 2002). Some successes have been reported with translocations of lizards in New Zealand. These include translocations of specific skink species (Towns & Ferreira 2002) and notably, populations of tuatara (*Sphenodon guntheri*) (Nelson *et al.* 2002). However, there are risks involved in the translocations of any naturally occurring reptile populations. Some of these risks include the spreading of diseases and parasitic organisms to populations of previously unexposed reptiles (Cunningham 1996). Also, various known and unknown genetic impacts may be made on already existing populations of reptiles (Whiting 1997). In addition, the levels of contaminants found in the lizards on the most contaminated site could result in genetic damage, and therefore the Evaporation Pan populations should not be 'rescued' by translocation to clean populations, in case this impairs the latter population as well.

Translocations of *C. giganteus* have been attempted a number of times previously (Jacobsen *et al.* 1990; Groenewald 1992; J. Hardy, DTEEA Nature Conservation *pers. comm.*; Newbery 1992; Newbery *et al.* 1992). Many of these translocations have not been as successful as hoped (Groenewald 1992). Also, the successes of most translocation attempts have simply not been assessed (van Wyk *pers. comm.*). DTEEA Nature Conservation officials translocated lizards from other populations at Welkom (which were scheduled for mine construction activities) to augment an existing population at the evaporation pan (i.e. the most contaminated site), as well as to other sites in the Welkom area (J. Hardy *pers. comm.*). These translocation attempts were unfortunately not recorded or followed-up to ascertain success rates, and the translocation of lizards to the most contaminated mining site has resulted in their exposure to contaminants. This exposure may be resulting in impairment of the population, and hence increased liability for gold mining companies that manage the Mining 1 site. Also, many of these translocation operations seem to have taken place in somewhat haphazard fashion and operations have simply not been set up in ways to facilitate accurate monitoring.

With many potential risks involved in the translocation of populations of *C. giganteus* around mining sites, and no quantitative data available regarding the success of such attempts, it is advisable to avoid such operations until their effectiveness can be assessed. Further research into the microhabitat preferences of *C. giganteus* will also aid in the suitability assessments of sites for new populations.

POPULATION SIZE AT MINING 1
(EVAPORATION PAN)

In order to obtain baseline information on population ecology of *C. giganteus* at the mine evaporation pan (the most contaminated site), I instigated a mark-recapture study. Immediate estimates of the population size at this site will assist the mining company in ascertaining the success of their pan rehabilitation measures in the long term. A once-off population estimate is of limited value because no population trends can be estimated from this. Nevertheless, future population investigations at this site will be able to use these data as part of any long-term mark-recapture studies. Unfortunately, I only had sufficient sample sizes for the mine evaporation pan population; there were insufficient animals found at the second mining site (only seven animals were captured).

I used the Peterson method, as described in Krebs (1989). This specific method is applicable to closed populations. While I cannot be sure that the population at the evaporation pan is closed, the sedentary nature of these lizards make it unlikely that significant emigration or immigration takes place, particularly over the relatively brief duration of this study. Further underlying assumptions include that there is no heterogeneity between animals in their catchability, that there is no trap response, that catching and marking do not affect mortality or emigration rates, and that emigration is not permanent (Krebs 1989).

Two samples were taken at the evaporation pan. Each sample consisted of all the adult lizards captured during ten days of sampling, ensuring the entire study area was covered per sample. These samples were taken during 4 - 14 February 2004 and 20 - 30 September 2004. I caught lizards using noose traps, each consisting of a peg knocked into the ground next to each individual burrow and an attached nylon noose in the entrance of each burrow. Traps were monitored continuously to ensure that no lizards died while caught in traps. I set nooses at all known burrows. Trapping effort per burrow continued for two days or until an adult resident was caught. All captured lizards were permanently marked with Passive Integrated Transponder (P.I.T.) tags (each tag having a unique alpha-numeric code). These I injected subcutaneously into the postero-femoral region of the right hind leg of individual lizards.

Population size was estimated using the following parameters in Krebs (1989):

M = number of individuals marked in the first sample

C = total number of individuals captured in the second sample

R = number of individuals in second sample that were marked

From these three variables I was able to obtain an estimate of

N = size of the population at the time of marking

The estimator I used to calculate N was from Bailey (1952):

$N = M(C+1) / R+1$

Ninety-five percent confidence intervals were estimated using the normal equation as set out in Krebs (1989):

R/C +- $\{Z_a[\sqrt{[(1-f)(R/C)(1-R/C)]} / C-1] + 1/2C\}$

where Z_a = 1.96, for 95% confidence interval.

A total of 69 adult lizards from both sexes were marked during the first sampling period (February 2004). My trapping method excluded the capture of juveniles. Thus, while juveniles were present in the population, only the adult population size could be calculated. Subsequently, 34 of 50 adult lizards captured during the second sampling period (September 2004) were recaptures of marked individuals. A population size estimate (N) was calculated as 101 (95% CI: 88.2 - 118.4). The total population of *C. giganteus* in the Evaporation pan site is thus estimated as being between 88 and 119 adult individuals, with an unknown number of juveniles.

CONCLUSION: RESEARCH NEEDS & FUTURE DIRECTIONS

Reptile ecotoxicology, especially involving inorganic contaminants, is a relatively new field (Hopkins 2000; Linder & Grillitsch 2000). There is a general paucity of information regarding the accumulation of inorganic contaminants and the effects of such accumulation, on reptiles. One of the problems faced by reptile ecotoxicologists is that the effects of environmental contaminants on reptiles cannot be predicted with toxicity thresholds established from other vertebrates, due to reptiles' unique combinations of physiological and life history characteristics (Campbell & Campbell 2002). Thus, the need exists for very specific investigations using applicable reptile species and standardised investigation techniques. Linder & Grillitch (2000) made some recommendations regarding future research needs. Their recommendations include research into:

- The regulatory capacity of reptiles: This includes the ability of reptiles to acclimate and adapt to contaminated environments.
- The remobilization of metals during major stress phases, including hibernation and reproduction.
- Critical organ and tissue concentrations of individual elements.
- Biomarkers of adverse effects. These may include various physiological indicators such as hormonal levels and the effects of inorganic contaminants on these.

While we know that mining contaminants are being accumulated by (and probably negatively affect) populations of *C. giganteus*, more information is required to make adequate conservation recommendations. Future studies regarding pollutant effects on *C. giganteus* should include firstly, investigations into the fitness consequences of metal accumulation in this lizard. Well-controlled and standardized techniques should be applied here. Due to the conservation concern for this species, it is perhaps important to use a substitute (and not threatened) species of lizard for any lab-based studies into individual pollutant effects. Second, the manner in which environmental contaminants affect population viability and fitness of this threatened lizard needs to be investigated. Long-term monitoring of the population at the mine evaporation pan is recommended in order to determine potential population level effects.

The mine evaporation pan population of sungazer lizards presents an opportunity for further investigation into the apparent effects of contaminant exposure on reptiles. Lizards here have been shown to accumulate high concentrations of various inorganic contaminants, and the population size appears large enough for most field investigations. This study has provided a base-line for the long-term monitoring of sungazer lizard contaminant levels and population health in order to assist the mining company and the regulators in determining the success of their remediation measures.

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APPENDIX

Table A1.1: One-way Analysis of Variance (ANOVA) results comparing elemental concentrations measured in *C. giganteus* between sexes, using whole blood and tissue samples. There were no sex effects.

Element	*F*	df	*P*
Li	0.02	1	0.8796
Na	0.01	1	0.924
Mg	0.28	1	0.5953
Al	0.41	1	0.5237
Si	0.11	1	0.7436
S	0.06	1	0.8051
K	0.23	1	0.6367
Ca	0.04	1	0.8412
Cr	0.05	1	0.8294
Mn	0.36	1	0.5499
Fe	0.15	1	0.7015
Ni	0.07	1	0.79
Cu	0.1	1	0.7585
Zn	0.23	1	0.6298
W	0.04	1	0.8484
Bi	0.05	1	0.8298
P	0.22	1	0.6388
Ti	0.71	1	0.4056
Ba	0.04	1	0.8521

Table A1.2: One-way analysis of variance (ANOVA) results for elemental concentrations from lizard tissue samples. Concentrations of Li, Na, Si, P, Cu, Ba and Bi were log-tranformed before analysis to meet normal distribution requirements. No significant differences were found between sites.

	Kolmogorov-Smirnov			**ANOVA**		
	D	**df**	***P***	***F***	**df**	***P***
Li	0.315	3	<0.05	1.045	3	0.383
Na	2.72	3	<0.05	2.655	3	0.064
Mg			n.s.	0.991	3	0.417
Al			n.s.	1.521	3	0.239
Si	0.290	3	<0.05	0.602	3	0.618
P	0.279	3	<0.05	1.804	3	0.165
S			n.s.	0.817	3	0.499
K			n.s.	1.599	3	0.23
Ca			n.s.	0.869	3	0.473
TI			n.s.	0.412	3	0.746
Mn			n.s.	1.715	3	0.196
Fe			n.s.	0.549	3	0.654
Cu	0.446	3	<0.01	2.182	3	0.108
Zn			n.s.	3.017	3	0.054
Ba	0.288	3	<0.05	0.903	3	0.457
Bi	0.281	3	<0.05	0.545	3	0.655

Table A1.3: Mean concentrations (μg/g dry mass) and standard deviations (SD) of all elements sampled in *C. giganteus* tail tissue for each of the four sites.

	Mining 1 (Evaporation pan) n=3		**Undisturbed Rangeland n=4**		**Mining 2 (slimes dam) n=4**		**Overgrazed Rangeland n=4**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Li	2.5	1.5	1.8	0.1	2.5	0.3	3.0	0.9
Na	703.4	318.5	643.8	94.2	562.4	43.5	747.7	199.0
Mg	2392.9	507.6	2618.3	393.3	2105.9	151.4	2351.1	268.8
Al	167.2	152.1	135.4	16.3	220.7	112.9	303.2	92.9
Si	22.7	19.0	30.1	6.4	25.0	10.4	74.4	67.7
P	11.8	18.2	15.1	6.8	12.3	10.3	58.3	63.5
S	8031.2	3804.7	10397.6	4603.1	6366.5	789.4	77900.0	4055.9
K	927.6	640.3	411.2	140.8	579.9	229.7	538.1	72.8
Ca	118530.4	21598.1	133002.8	22479.5	110850.1	5773.6	120916.3	20281.6
Ti	21.3	19.6	16.7	0.5	26.1	10.1	28.7	19.7
Cr	32.1	20.5	26.0	0.7	26.3	0.7	34.3	14.5
Mn	11.3	4.9	8.7	1.2	13.7	5.8	16.5	7.8
Fe	406.2	404.1	264.2	21.1	452.7	183.4	547.4	212.6
Ni	41.0	57.5	20.6	1.7	23.1	2.5	26.7	14.8
Cu	18.7	35.7	5.8	1.8	7.3	2.3	7.4	4.1
Zn	179.6	25.8	147.5	6.0	150.4	27.0	163.1	13.6
W	20.6	17.6	18.7	2.0	14.7	3.5	19.2	9.2
Bi	23.8	20.8	32.2	7.3	26.8	11.6	79.8	71.9
Ba	57.5	29.6	59.2	10.2	51.7	11.8	128.3	101.9

Table A1.4: Tukey test results indicating between which sites significant differences occurred in elemental concentrations obtained from whole blood samples. M1 = Mining 1; M2 = Mining 2; R = Overgrazed Rangeland; C = Undisturbed Rangeland (Control).

	M1-R	M1-M2	M1-C	R-M1	R-M2	R-C	M2-M1	M2-R	M2-C	C-M1	C-R	C-M2
Li	X	X	X	X			X			X		
Na	X	X		X			X					
Mg												
Al	X	X	X	X			X			X		
Si	X	X	X	X			X			X		
P	X	X	X	X			X			X		
S			X							X		
K												
Ca	X			X								
Cr	X	X	X	X			X			X		
Mn	X	X	X	X			X			X		
Fe			X							X		
Ni	X	X	X	X			X			X		
Cu	X		X	X						X		
Zn												
W	X	X	X	X			X			X		
Bi	X	X	X	X			X			X		

Table A1.5: Mean concentrations (µg/g dry mass), standard deviations (SD) and one-way analysis of variance (ANOVA) results of elements in all beetle samples between areas of differing land use (mining contaminant affected vs. rangeland). Concentrations of Al, Si, P, Ca, Ti, Fe and Bi were log-transformed before analysis to meet the normal distribution requirements. Significant p-values are highlighted.

	Mining (n = 5)		**Rangeland (n = 3)**		**Kolgorov-Smirnov**			**ANOVA**		
	Mean	**SD**	**Mean**	**SD**	**D**	**df**	***P***	**F**	**df**	***P***
Li	4.1	1.1	234.7	402.0		1	n.s.	1.85	1	0.223
Na	556.7	126.4	431.6	51.2		1	n.s.	2.55	1	0.162
Mg	1032.5	331.9	979.4	146.9		1	n.s.	0.07	1	0.807
Al	389.5	268.0	279.6	99.2	0.40	1	<0.01	0.45	1	0.525
Si	54.1	33.5	36.5	8.7	0.40	1	<0.01	0.93	1	0.371
P	49.9	32.5	33.3	8.5	0.39	1	0.012	0.87	1	0.388
S	8553.0	3957.8	4333.7	601.5		1	n.s.	3.16	1	0.126
K	4072.5	656.1	3387.4	699.6		1	n.s.	1.95	1	0.212
Ca	695.9	164.7	733.8	136.9	0.35	1	0.042	0.16	1	0.705
Ti	64.1	48.5	53.1	17.3	0.40	1	<0.01	0.03	1	0.868
Cr	56.9	14.7	35.7	4.1		1	n.s.	5.63	1	0.055
Mn	55.0	14.6	41.8	4.4		1	n.s.	2.19	1	0.189
Fe	1045.9	488.8	788.7	264.6	0.38	1	0.017	0.83	1	0.399
Ni	64.6	19.9	33.7	1.4		1	n.s.	6.77	1	**0.041**
Cu	35.0	10.8	20.9	2.8		1	n.s.	4.64	1	0.075
Zn	293.5	58.3	268.2	19.8		1	n.s.	0.50	1	0.506
W	29.5	7.9	13.1	1.5		1	n.s.	12.06	1	**0.013**
Bi	56.5	37.1	37.8	9.9	0.40	1	<0.01	0.83	1	0.396
Ba	9.2	9.1	1.6	1.2		1	n.s.	1.38	1	0.361

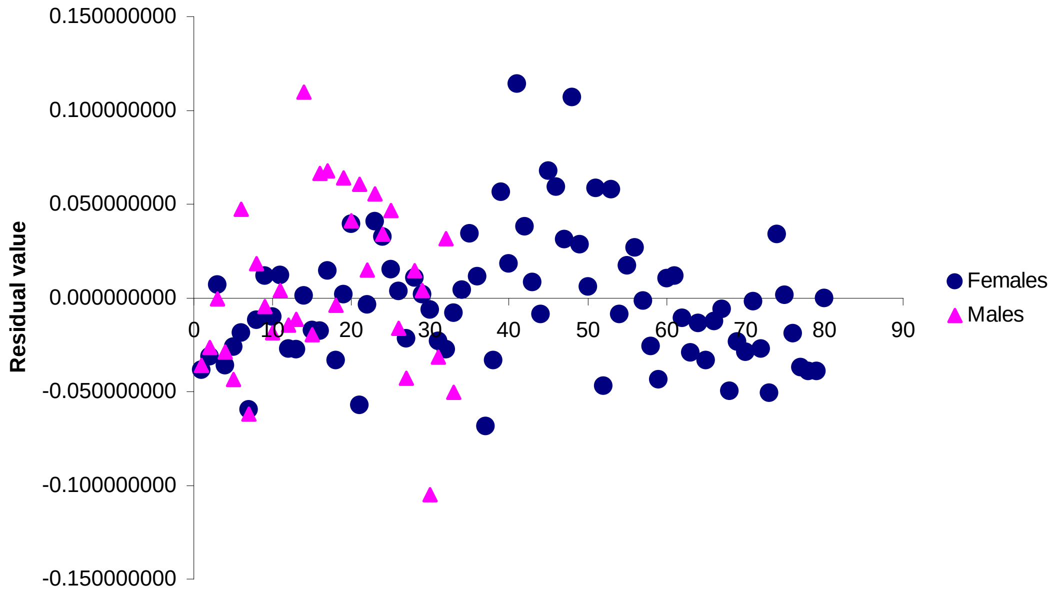

Figure A1.1: Residual values (indicating physiological body condition) for both sexes of *C. giganteus* lizards sampled from all sites.

Table A1.6: Total amounts of invertebrates sampled from study sites. M1 = Mining 1; R = Overgrazed Rangeland; C = Undisturbed Rangeland (Control).

	Site		
	M 1	**R**	**C**
ARACHNIDA			
Araneae	253	108	63
Solipugida	59	16	0
INSECTA			
COLEOPTERA			
Histeridae	372	264	238
Coccinelidae	1	0	0
Carabidae	1	0	0
Undetermined	175	57	195
HYMENOPTERA			
Eumenidae	5	1	3
Formicidae	757	989	724
Sphecidae	72	24	16
Undetermined	222	87	116
ISOPTERA			
Hodotermitidae	12	124	0
LEPIDOPTERA	95	169	257
MANTODEA			
Thespidae	0	1	0
Undetermined	28	0	0
ORTHOPTERA			
Acrididae	1	0	1
Undetermined	6	1	95
PHASMATODEA	7	1	0
BLATTODEA			
Blaberidae	0	0	7
Blatellidae	0	0	45
Blattidae	0	0	1
Blattodea Undetermined	6	0	52
HEMIPTERA			
Acanthosomidae	0	0	2
Pyrrhocoridae	0	0	2
Riduviidae	0	1	8
Hemiptera Undetermined	43	0	3
Undetermined Insecta	7	26	33
TOTAL	**2122**	**1869**	**1861**